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MIXED-EFFECTS MODELS FOR MODELING
CARDIAC FUNCTIONS AND TREATMENT EFFECTS

By

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B.S., Chonnam National University, KOREA, 1997

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Master of Science

Department of Bioinformatics and Biostatistics
University of Louisville
Louisville, Kentucky

May 2011

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ABSTRACT

MIXED-EFFECTS MODELS FOR MODELING CARDIAC FUNCTIONS AND TREATMENT EFFECTS

Hyejeong Jang

April 15, 2011

Mixed-effects model is an efficient tool for analyzing longitudinal data. The random effects in mixed-effects model can be used to capture the correlations among repeated measurements within a subject. The time points are not fixed and all available data can be used in mixed-effects model provided data are missing at random. For this reason, we focus on applying mixed-effects models to the repeated measurements of cardiac function including heart rate, left ventricle developed pressure, and coronary flow in the Glutathione-S-transferase P (GSTP) gene knockout and wild-type mice following ischemia/reperfusion injury performed in the isolated, Langendorff-perfused heart. Cardiac function is measured during three time periods: pre-ischemia, ischemia (no flow), and reperfusion. We developed piecewise nonlinear mixed-effects model to describe the different aspects of the cardiac function during each period. We applied nonlinear mixed-effects models and a changing point model to examine how cardiac function was altered by ischemia/reperfusion-induced injury and for comparison between mouse strains. These findings provide evidence of a new application for the mixed-effects model in physiological and pharmacological studies of the heart.

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CHAPTER 1

Introduction

1.1 Longitudinal data analysis

Longitudinal studies are defined by having an outcome variable that is repeatedly measured over time. In a longitudinal study, the outcome variable is measured in a subject on several different occasions [1]. Longitudinal studies have been used to study the impact of disease and treatment methods in medicine, to find the cause of the development of children and the clinical progress of patients in psychology, and to study the causes of the behavior change in the behavioral sciences [2].

A longitudinal study has several advantages. First, repeated measurements from the same subject are not completely correlated [3], thus, repeated measures increase statistical power for same or smaller sample size. For this reason, longitudinal studies have more power than cross-sectional studies for a fixed number of subjects [3]. Second, in a longitudinal study, each subject can serve as his or her own control [3]. The exclusion of between-subject variability from measurement error leads to more efficient estimators for parameters related to treatment effects [3]. Third, aging effects (i.e., changes over time within subjects) can be separated from cohort effects (i.e., differences among subjects at baseline) [3]. As a result, the individual change of a certain outcome

variable over time can be studied in longitudinal studies [1].

The key feature of longitudinal data is that repeated measures on the same subject are not independent. Therefore, the correlation within a subject must be accounted for using a specific statistical method. Repeated measures analysis of variance (repeated measures ANOVA) and multivariate ANOVA (MANOVA) are two traditional approaches for analyzing longitudinal data [3]. Repeated measures ANOVA is also called a mixed-effects ANOVA, where a single random subject effect is included to account for the correlation among the repeated measurements within the same subject [4]. However, the repeated measures ANOVA requires that all individuals have a complete balanced data set and a fixed time schedule, and treats time as a categorical variable [5]. The advantage of the MANOVA approach versus the ANOVA approach is that the MANOVA assumes a general form for the correlation of repeated measurements over time, while ANOVA assumes a much more restrictive compound-symmetric form [3]. However, MANOVA model has a limitation because it requires complete data [3]. In addition, both MANOVA and ANOVA models provide no information about subject-specific growth curve [3]. Due to these reasons above, covariance pattern models, generalized estimating equations (GEE) models, and mixed-effects models are generally used for longitudinal data [3].

1.2 Mixed-effects models

Mixed-effects models are widely used method for analyzing longitudinal data. Mixed-effects models have been developed and described under various names [3]: random

effects models [6], variance component models [7], multilevel models [8], hierarchical linear models [9], two-stage models [10], random coefficient models [11], mixed models [12], empirical Bayes models [13], and random regression models [14]. A mixed-effects model has a regression form as linear regression or nonlinear regression, where the coefficients are expressed as summation of fixed effects and random effects. The time points for the measurements of each subject may vary. In a mixed-effects model, random effects are often included to describe the subject-specific effect, and fixed effects are included to describe population-level effect. The correlation between the repeated measurements is captured by the random effects and their distribution assumption. The magnitude of the variance of the random effects captures the between-subject variation. Therefore, mixed-effects models provide a more flexible covariance structure for nonconstant within-subject correlation [15], and have the ability to accommodate missing and imbalanced data which are common in longitudinal data [5].

In many longitudinal analyses, change is assumed to be steady and incremental so that a linear model is appropriate [16]. However, in numerous situations, the change is not uniform but rather faster during some periods and slower in others [16]. In these cases, nonlinear mixed-effects (NLME) models are more appropriate than linear mixed-effects (LME) models to describe these nonlinear changes. A NLME model is usually established based on some mechanistic considerations, and its parameters usually are interpretable. NLME models are often used due to interpretability, parsimony, and validity beyond the observed range of the data [17]. Furthermore, a nonlinear model generally uses fewer parameters than a competitor linear model, providing a more parsimonious description of the data and more reliable predictions for the response

variables [17].

Based on Cudeck and Harring [16], the best way to select an appropriate NLME model is to graph data from an individual and examine the performance of candidate functions. In lieu of a theoretical rationale that favors a specific form, model choice is made based on the bases of goodness of fit, interpretability of the parameters, and appropriateness of the functional form to the situation in which it is applied.

In this thesis, we applied mixed-effects models and change point model to experimental longitudinal data. The experiments were carried out in Dr. Conklin's laboratory to examine the impact of Glutathione-S-transferase P (GSTP) on the recovery of postischemic cardiac function including heart rate, left ventricle (LV) developed pressure, and coronary flow in the Glutathione-S-transferase P (GSTP) gene knockout (KO) and wild-type (WT) mice following ischemia/reperfusion injury performed in the isolated, Langendorff-perfused heart. Cardiac function was measured during three time periods: pre-ischemia, ischemia (no flow), and reperfusion periods. For this study, we developed piecewise nonlinear function to describe the different aspects of the cardiac function during each period. We applied nonlinear mixed-effects models and changing point model to examine how cardiac function was altered by ischemia/reperfusion-induced injury and for comparison between mouse strains. The Wald test and the Akaike information criterion (AIC) were applied to obtain a parsimonious model for each aspect of cardiac function.

CHAPTER 2

Models and Methods

2.1 Linear mixed-effects (LME) models

The linear mixed-effects (LME) model can be written as:

$$y_i = X_i\beta + Z_ib_i + \varepsilon_i, \quad (\text{E } 2.1)$$

where (X_i, Z_i, y_i) ($i=1, \dots, N$) are the observations for i^{th} subject. Specifically, X_i is the $n_i \times p$ covariate matrix associated with the fixed effects β , where $\beta \in R^{p \times 1}$, Z_i is the $n_i \times r$ design matrix associated with the random effects b_i , where $b_i \in R^{r \times 1}$, y_i is the $n_i \times 1$ vector of response variable, and ε_i is $n_i \times 1$ within-subject error vector. The random effects $b_i (i=1, \dots, N)$ are assumed to be independent and identically distributed (i.i.d.) as $N(0, \mathbf{D})$, and $\varepsilon_i (i=1, \dots, N)$ are assumed to be i.i.d. as $\varepsilon_i \sim N(0, \sigma^2 I_{n_i})$, and b_i and $\varepsilon_i (i=1, \dots, N)$ are independent. As a result, it can be shown that the observation y_i and random effects b_i follow the joint multivariate normal distribution:

$$\begin{bmatrix} y_i \\ b_i \end{bmatrix} \sim N \left(\begin{bmatrix} X_i\beta \\ 0 \end{bmatrix}, \begin{bmatrix} Z_i\mathbf{D}Z_i' + \sigma^2 I_{n_i} & Z_i\mathbf{D} \\ \mathbf{D}Z_i' & \mathbf{D} \end{bmatrix} \right). \quad (\text{E } 2.2)$$

Estimation in LME models

The parameters in the LME models are generally estimated by maximum likelihood (ML) method or restricted maximum likelihood (REML) method. Under the distribution assumptions for random effects b_i and random errors ε_i ($i=1, \dots, N$), the response variable for i^{th} subject (i.e., y_i) has mean and variance as follows:

$$E(y_i) = X_i\beta,$$

$$Var(y_i) = \Sigma_i(\theta) = Z_i D Z_i' + \sigma^2 I_{n_i}.$$

In case $\Sigma_i(\theta) = \Sigma_i$ is known, the ML estimate of the fixed effects β is:

$$\hat{\beta} = \left\{ \sum_{i=1}^N (X_i' \Sigma_i^{-1} X_i) \right\}^{-1} \sum_{i=1}^N (X_i' \Sigma_i^{-1} y_i). \quad (E 2.3)$$

However, the variance component parameters, i.e., the parameters in the variance of b_i (say D) and the variance ε_i (say σ^2), are unknown and need to be estimated by ML method or REML method. Let us denote the variance component parameters as θ , and their estimator as $\hat{\theta}$. Then the ML estimate of the fixed effects β is estimated by the following equation (E 2.4), where Σ_i in (E 2.3) is replaced by $\Sigma_i(\hat{\theta})$:

$$\hat{\beta} = \left\{ \sum_{i=1}^N (X_i' \hat{\Sigma}_i^{-1} X_i) \right\}^{-1} \sum_{i=1}^N (X_i' \hat{\Sigma}_i^{-1} y_i). \quad (E 2.4)$$

Generally, the REML estimator for the variance component in Σ_i is recommended because the REML estimate provides unbiased estimators for variance components [18]. In this thesis, we use REML method to obtain estimates of variance components in LME model (E 2.1) and then obtain the estimates of the fixed-effect parameters as in (E 2.4) [19].

Statistical inference in LME models

Hypothesis tests are very important to examine whether certain covariates significantly contribute to the response variable, and whether certain parameters are the same, which may indicate no treatment effect. The associated statistic null hypothesis is $H_0: \lambda'\beta = 0$, and the alternative hypothesis is $H_1: \lambda'\beta \neq 0$.

If the underlying assumptions for the random effects b_i and within-subject errors ε_i ($i=1 \dots N$) hold, the ML estimator of β , say $\hat{\beta}$, follows the following normal distribution:

$$\hat{\beta} \sim N\left(\beta, \left\{\sum_{i=1}^N (X'_i \hat{\Sigma}_i^{-1} X_i)\right\}^{-1}\right).$$

The test statistic for testing $H_0: \lambda'\beta = 0$ versus $H_1: \lambda'\beta \neq 0$ can be carried out using the Wald test statistic:

$$Z = \frac{\lambda'\hat{\beta}}{\sqrt{\lambda' \widehat{Cov}(\hat{\beta}) \lambda}} \sim N(0,1).$$

A special case for testing $H_0: \beta_k = 0$ versus $H_1: \beta_k \neq 0$ can be carried out using the Wald test statistic:

$$\frac{\hat{\beta}_k}{\sqrt{Var(\hat{\beta}_k)}} \sim N(0,1).$$

An alternative to test for the hypothesis is the likelihood ratio test (LRT). The LRT for $H_0: \lambda'\beta = 0$ versus $H_1: \lambda'\beta \neq 0$ is obtained by comparing the maximized log-likelihoods for two models: the full model versus the reduced model. The reduced model incorporates the constraint that $\lambda'\beta = 0$, while the full model does not (i.e., without the

constraint $\lambda'\beta = 0$) [18]. The LRTs can also be used for hypothesis tests about the covariance components [18]. However, the REML method can be used to compare nested models only involving the reduction in covariance components but should not be used to compare nested regression models for reduction in fixed-effects terms [18].

2.2 Nonlinear mixed-effects (NLME) models

The general formula of the single level NLME models is proposed by Lindstrom and Bates [6]. At first level the j^{th} observation on the i^{th} subject is modeled as:

$$y_{ij} = f(\phi_{ij}, v_{ij}) + \epsilon_{ij} \quad i = 1, \dots, N, \quad j = 1, \dots, n_i, \quad \epsilon_{ij} \sim N(0, \sigma^2),$$

where N is the number of subjects, n_i is the number of observations on the i^{th} subject, f is a real-valued differentiable function of a subject specific parameter vector ϕ_{ij} , and covariate vector v_{ij} , and ϵ_{ij} is a normally distributed within-subject error term. The function f is nonlinear in at least one component of the group-specific parameter vector ϕ_{ij} , which is modeled as:

$$\phi_{ij} = A_{ij}\beta + B_{ij}b_i, \quad b_i \sim N(0, \Psi),$$

where β is a p -dimensional vector of fixed effects, and b_i is a q -dimensional random effects vector associated with the i^{th} subject with variance-covariance matrix Ψ . The matrices A_{ij} and B_{ij} are of appropriate dimensions and depend on the i^{th} subject and possibly on the values of some covariates at the j^{th} observation. Again here, b_i and $\epsilon_{ij} = (\epsilon_{i1}, \dots, \epsilon_{in_i})^T$ are assumed to be normally distributed with mean zero and variance D and Ψ , respectively.

Estimation in NLME models

Different parameter estimations have been proposed in the NLME model [17]. In this thesis, we will describe methods based on the likelihood function. There are three methods for approximating the likelihood function in the NLME model; the LME approximation proposed by Lindstrom and Bates, the method of using Laplacian approximation to the likelihood function, and the method of using an adaptive Gaussian quadrature rule to improve the Laplacian approximation [17]. A REML method using the alternating algorithm was also proposed by Lindstrom and Bates [15]. Of these methods, the alternating algorithm of Lindstrom and Bates is used for estimation and inference in our nonlinear models. The estimation algorithm of Lindstrom and Bates for the single-level NLME model alternates two steps, a penalized nonlinear least square (PNLS) step and a linear mixed effects (LME) step.

In the PNLS step, the current estimate of Δ (the precision factor) is held fixed, and the conditional modes of the random effects b_i and conditional estimates of the fixed effects β are obtained by minimizing a penalized nonlinear least squares objective function:

$$\sum_{i=1}^M ||y_i - f_i(\beta, b_i)||^2 + ||\Delta b_i||^2.$$

The LME step updates the estimate of Δ based on a first-order Taylor expansion of the model function f around the current estimates of β and the conditional modes of the random effects b_i , which will be denoted by $\hat{\beta}^{(w)}$ and $\hat{b}_i^{(w)}$, respectively.

Letting

$$\hat{X}_i^{(\omega)} = \frac{\partial f_i}{\partial \beta^T} \Big|_{\hat{\beta}^{(\omega)}, \hat{b}_i^{(\omega)}}, \quad \hat{Z}_i^{(\omega)} = \frac{\partial f_i}{\partial b^T} \Big|_{\hat{\beta}^{(\omega)}, \hat{b}_i^{(\omega)}},$$

$$\hat{\omega}_i^{(\omega)} = y_i - f_i(\hat{\beta}^{(\omega)}, \hat{b}_i^{(\omega)}) + \hat{X}_i^{(\omega)} \hat{\beta}^{(\omega)} + \hat{Z}_i^{(\omega)} \hat{b}_i^{(\omega)},$$

the appropriate log-likelihood function used to estimate Δ is:

$$\begin{aligned} \ell_{LME}(\beta, \sigma^2, \Delta | y) = & -\frac{N}{2} \log(2\pi\sigma^2) - \frac{1}{2} \sum_{i=1}^M \{ \log |\Sigma_i(\Delta)| \\ & + \sigma^{-2} [\hat{\omega}_i^{(\omega)} - \hat{X}_i^{(\omega)} \beta]^T \Sigma_i^{-1}(\Delta) [\hat{\omega}_i^{(\omega)} - \hat{X}_i^{(\omega)} \beta] \}, \end{aligned}$$

where $\Sigma_i(\Delta) = I + \hat{Z}_i^{(\omega)} \Delta^{-1} \Delta^{-T} \hat{Z}_i^{(\omega)T}$.

Lindstrom and Bates also proposed a REML estimation method for Δ , which consists of replacing the log-likelihood in the LME step of the alternation algorithm by the log-restricted-likelihood. The algorithm alternates between the PNLs and LME steps until a convergence criterion is met. The algorithm has been implemented by Lindstrom and Bates [15] in R, which can be called by function nlme in R.

Statistical Inference in NLME models

Inference on the parameters of an NLME model estimated via the alternating algorithm is based on the LME approximation to the log-likelihood function. At the convergence, under the normality assumption for the random effects and within-subject errors, the estimator β has the following distribution:

$$\hat{\beta} \sim N \left(\beta, \sigma^2 \left[\sum_{i=1}^M \hat{X}_i^T \Sigma_i^{-1} \hat{X}_i \right]^{-1} \right),$$

where $\Sigma_i(\hat{\Delta}) = I + \hat{Z}_i^{(\omega)} \hat{\Delta}^{-1} \hat{\Delta}^{-T} \hat{Z}_i^{(\omega)T}$, $\hat{X}_i^{(\omega)} = \frac{\partial f_i}{\partial \beta^T} \Big|_{\hat{\beta}^{(\omega)}, \hat{b}_i^{(\omega)}}$, and $\hat{Z}_i^{(\omega)} = \frac{\partial f_i}{\partial b^T} \Big|_{\hat{\beta}^{(\omega)}, \hat{b}_i^{(\omega)}}$.

The test statistic for testing $H_0: \lambda' \beta = 0$ versus $H_1: \lambda' \beta \neq 0$ can be carried out using the

Wald test statistic:

$$z = \frac{\lambda' \hat{\beta}}{\sqrt{\lambda' \widehat{Cov}(\hat{\beta}) \lambda}} \sim N(0,1).$$

A special case for testing $H_0: \beta_k = 0$ versus $H_1: \beta_k \neq 0$ can be carried out using the

Wald test statistic:

$$z = \frac{\hat{\beta}_k}{\sqrt{Var(\hat{\beta}_k)}} \sim N(0,1).$$

2.3 Method of estimation of change point in two phase growth models

Suppose the growth model contains two growth phases: $f_1(t; \beta_1)$ at the first phase where $t \leq \gamma$ and $f_2(t; \beta_2)$ at the second phase where $t \geq \gamma$. Let us denote $y(t)$ as the response variable at time, then $y(t)$ is expressed as:

$$y(t) = \begin{cases} f_1(t; \beta_1) & \text{if } t \leq \gamma \\ f_2(t; \beta_2) & \text{if } t \geq \gamma. \end{cases}$$

γ is the unknown time where the growth curve from the first phase shifts to the secondary phase. γ is called the change point [22]. β_1 and β_2 are unknown parameters in the phase 1 and phase 2 curves, respectively. The continuous change implies $f_1(\gamma; \beta_1) = f_2(\gamma; \beta_2)$.

To search for the change point, the least squares estimate (LSE) is obtained.

An algorithm for calculating a least squares estimate (LSE)

Let us denote (t_i, y_i) ($i = 1, \dots, n$) as n observations, ordered in such a way that $t_1 \leq t_2 \leq \dots \leq t_n$. We assume that the unknown change point γ belongs to a given interval $\Gamma = [\bar{\gamma}, \underline{\gamma}]$. For the change point $\gamma \in \Gamma$, there exists a natural number $m(\gamma)$ with $2 \leq m(\gamma) \leq n - 1$ such that the first $m(\gamma)$ observations (t_i, y_i) , ($i = 1, \dots, m(\gamma)$) belong to phase 1 and the last $(n - m(\gamma))$ observations (t_i, y_i) , ($i = m(\gamma) + 1, \dots, n$) belong to the phase 2. $m(\gamma)$ depends on the unknown change point γ and γ satisfies $t_{m(\gamma)} \leq \gamma \leq t_{m(\gamma)+1}$, where $m(\gamma)$ is called change index. The LSE for model parameters $(\beta_1, \beta_2, \gamma)$ is defined as the solution of minimizing the following sum of squares:

$$S(\beta_1, \beta_2, \gamma) = \sum_{j=1}^{m(\gamma)} (y(t_i) - f_1(t_i; \beta_1))^2 + \sum_{i=m(\gamma)+1}^n (y(t_i) - f_2(t_i; \beta_2))^2,$$

under the restriction $f_1(\gamma; \beta_1) = f_2(\gamma; \beta_2)$.

The detailed descriptions for the estimation change point are found in Schulze [19].

2.4 Model selection

Likelihood Ratio Test (LRT)

The likelihood ratio test (LRT) can be used to compare nested models. The LRT statistic is obtained as:

$$LRT = 2(\log L_{full} - \log L_{reduced}),$$

where $\log L_{full}$ and $\log L_{reduced}$ are the maximized log-likelihood values for the full and reduced models, respectively. LRT is compared to the chi-square distribution with degrees of freedom being the number of additional parameters in the full model versus the reduced model. In this thesis, we start from a full model. Then we apply the LRT and the following Akaike Information Criterion (AIC) to remove the least significant term using a backward procedure, and obtain a parsimonious model for the final interpretation.

Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC)

Akaike Information Criterion (AIC) is used to compare models that are not nested and defined for a given model as [20]:

$$AIC = -2\log L + 2p,$$

where p is the number of parameters in the model, and the L is the likelihood function at the estimated parameters.

The model with the smallest AIC value is preferred. The adjustment in AIC to the deviance is often called the penalty for using additional parameters in model fitting.

Another criterion for model selection is the Bayesian Information Criterion (BIC) [21]. This criterion is defined as:

$$BIC = -2\log L + p\log N,$$

where N is the number of observations, p is the number of parameters in the model, and the L is the likelihood function at the estimated parameters.

As in AIC, the model with the lowest BIC value is preferred. The detailed descriptions

about model selection are referred to Donald and Robert [3].

CHAPTER 3

Applications

In this section, we applied mixed-effects models and change point model to investigate the contribution of Glutathione-S-transferase P (GSTP) gene on the recovery of post-ischemic cardiac function. As a member of a large family of GSTs [23], GSTP is a major cardiovascular GST isoform and accounts for 20-50% of the total GST activity in mouse aorta [24] and heart [25]. To examine the impact of GSTP on postischemic cardiac function, the experiments were carried out in Dr. Conklin's laboratory. Cardiac function was assessed by measurements of heart rate, coronary flow, and left ventricle (LV) developed pressure. To examine the effect of the deletion of the GSTP gene in myocardial ischemia-reperfusion (I/R) injury, the cardiac function of knockout (KO) mice was compared with wild-type (WT) mice. Each aspect of the cardiac function data was collected during three time periods: pre-ischemia, ischemia, and reperfusion periods. We developed piecewise nonlinear mixed-effects model to describe the different aspects of the cardiac function, and applied the nonlinear mixed-effects models, change point model, and model reduction techniques to obtain a parsimonious model for the final interpretation. We performed hypothesis tests to examine the impact of deletion of GSTP for different aspects of cardiac function including testing whether cardiac function

including testing whether cardiac performance eventually recovers during reperfusion period.

3.1 Experimental data and graphical presentation

Experimental Data

Data were obtained from Glutathione-S-transferase P (GSTP) gene KO mice and WT mice hearts. Three different datasets were obtained for three response variables: namely, measurements of heart rate, coronary flow, and LV developed pressure. Cardiac function was measured in hearts exposed to ischemia/reperfusion injury by subjecting isolated hearts to 5 minutes of pre-ischemia, 30 minutes of global ischemia and 45 minutes of reperfusion in six KO and seven WT hearts. The measurements of each one of the three response variables are presented in Figures 1, 2, and 3, respectively.

Graphical presentation

Figures 1 to 3 show how the heart rate, coronary flow, and LV developed pressure change over different time periods for GSTP KO mice and WT mice. As illustrated in Figures 1 to 3, there was no significant difference between WT mice and KO mice during pre-ischemia for each one of the three endpoints. Figure 1 indicates that during reperfusion, heart rate returned to the pre-ischemic range in WT mice but was greater than the pre-ischemic range in KO mice. Figure 2 indicates that the recovery of coronary flow was better in WT mice than KO mice during reperfusion. Figure 3 shows that LV developed pressure started to increase after a few minutes of

perfusion in both WT and KO mice and recovered to a much greater extent in WT mice than KO mice. Figure 3 also indicates that ischemia/reperfusion injury significantly decreased LV developed pressure.

3.2 Nonlinear model and statistical analysis for heart rate

The measurements of heart rate over time in different periods for each individual are presented in Figure 1. We first carry out the analysis for each one of the three time period separately, then we build an integrated model to describe the heart rate over time for the entire experiment. Hypothesis tests are carried out to examine whether the heart rate in WT mice is significantly different from that in GSTP KO mice, and whether the heart rate during reperfusion recovers to the level of pre-ischemia.

Figure 1. Individual profile for heart rate.

Figure 2. Individual profile for coronary flow.

Figure 3. Individual profile for LV developed pressure.

Model and analysis of heart rate during pre-ischemia

To examine the heart rate during pre-ischemia, we started with a full linear mixed-effects model with the following form:

$$y_{ij} = \beta_0 + \beta_1 t_{ij} + \beta_2 \text{type}_i + \beta_3 t_{ij} \text{type}_i + b_{0i} + b_{1i} t_{ij} + \varepsilon_{ij}, \quad (\text{E } 3.1)$$

where y_{ij} is the measurement of heart rate for subject i at time t_{ij} ($i = 1, \dots, N$),

$$\begin{bmatrix} b_{0i} \\ b_{1i} \end{bmatrix} = N \left(\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_0^2 & \rho \sigma_0 \sigma_1 \\ \rho \sigma_0 \sigma_1 & \sigma_1^2 \end{pmatrix} \right), \quad \varepsilon_{ij} \sim N(0, \sigma^2),$$

$$\text{type}_i = \begin{cases} 1 & \text{if mouse } i \text{ is knockout} \\ 0 & \text{if mouse } i \text{ is wild - type.} \end{cases}$$

The fixed effect parameters are β_0 , β_1 , β_2 , and β_3 . β_0 is the mean of the heart rate of WT mice at the starting point (i.e., $t_{ij} = 0$); β_1 describes the change rate of the heart rate for WT mice during pre-ischemia; β_2 is the mean difference of the heart rates of the KO mice versus WT mice at the starting time of the experiment; β_3 is the slope difference of the heart rates between KO mice versus WT mice. The random effect b_{0i} ($i = 1, \dots, N$) describes the deviation of the heart rate of i^{th} subject away from its group average at time $t_{ij} = 0$, and b_{1i} ($i = 1, \dots, N$) is the deviation of the slopes for i^{th} subject away from its group-level slope.

We start from the full model (E 3.1), and then the least significant term was removed using the Wald test and AIC. The process was repeated until the AIC reaches its minimum. As a result, we could remove time and interaction terms in the fixed effects but could not remove the two random effects. Type in fixed effects (say, β_2) was not significant. However, we kept this parameter for testing whether or not heart rate is significantly different between the pre-ischemia and reperfusion period for both WT mice

TABLE 1

Estimates of fixed-effects and variance components for linear mixed-effects model for heart rate during pre-ischemia

Parameter	Estimate	95% C.I.	Z	P- value
β_0	295.61	[260.67, 330.55]	18.17	0.00
β_2	14.72	[-41.69, 71.13]	0.61	0.55
σ_0	78.73	[51.87, 119.49]		
σ_1	12.49	[8.23, 18.95]		
ρ	-0.82	[-0.94, -0.49]		
σ	11.45	[9.17, 14.30]		

and KO mice. Table 1 shows the estimates of the fixed-effects and the variance components for the final model for pre-ischemia period.

The linear mixed-effects model (E 3.1) was applied to pre-ischemia, ischemia, and reperfusion periods, respectively, for each aspect of cardiac function. The results are presented in Appendix A.

Model and analysis of heart rate during ischemia

Figure 1 shows that the pattern of heart rate change over time during ischemia is not linear, and resembles exponential decline. Therefore, nonlinear mixed-effects model was considered for accurate approximation of the response variable. We used exponential function of the form $C_1 \exp(-C_2 (t_{ij} - T_1)_+)$ to describe the dynamic change of the heart rate during ischemia, where C_1 describes the heart rate at the beginning of ischemia, $C_2(> 0)$ describes the exponential decline rate, $(t - T_1)_+$ takes $t - T_1$ if $t - T_1 > 0$ and 0 otherwise, where T_1 is a known constant, representing the time to start ischemia injury. We fit a nonlinear mixed-effects model of the following form for heart rate during ischemia:

$$y_{ij} = C_{1i} \exp(-C_{2i} (t_{ij} - T_1)_+) + \epsilon_{ij}, \quad (\text{E } 3.2)$$

where

$$C_{ki} = \beta_{1k} + \beta_{2k} \text{type}_i + b_{ki} \quad k = 1, 2,$$

$$\begin{bmatrix} b_{1i} \\ b_{2i} \end{bmatrix} = N \left(\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_1^2 & \rho\sigma_1\sigma_2 \\ \rho\sigma_1\sigma_2 & \sigma_2^2 \end{pmatrix} \right), \quad \epsilon_{ij} \sim N(0, \sigma^2),$$

$$\text{type}_i = \begin{cases} 1 & \text{if mouse } i \text{ is knockout} \\ 0 & \text{if mouse } i \text{ is wild-type.} \end{cases}$$

C_{1i} is the value of y_{ij} at $t_{ij} = T_1$ and C_{2i} is the constant exponential decline rate.

The fixed effect β_{11} represents the mean of the heart rate for WT mice at the beginning of ischemia, and $\beta_{11} + \beta_{21}$ represents the mean of heart rate for KO mice at the beginning of ischemia. The random effect b_{1i} ($i = 1, \dots, N$) describes the deviation of the heart rate for i^{th} subject away from its group mean. Similarly, β_{12} and $\beta_{12} + \beta_{22}$ represent the exponential decline rate for WT mice and KO mice, respectively, and b_{2i} describes the deviation of the exponential decline rate for i^{th} subject from group-level decline rate.

Nonlinear mixed-effects model (E 3.2) was fitted to the ischemia data and the results are given in Table 2. From Table 2, it is clear that there is no significant difference for the heart rate at the beginning of ischemia between the two types of mice. However, the exponential decline rate for WT mice is significantly larger than for KO mice at significant level 0.1. The fitted heart rates at group-level and the predicted heart rates at subject-level, as well as the observed heart rates are presented in Figure 4. As can be seen in Figure 4, the subject-level predictions closely matched the observed heart rates during ischemia indicating the goodness of fit of the model.

Model and analysis of heart rate during reperfusion

TABLE 2

Estimates of fixed-effects and variance components for nonlinear mixed-effects model (E 3.2) for heart rate during ischemia

Parameter	Estimate	S.E.	95% C.I	Z	P- value
β_{11}	458.23	26.46	[406.48, 509.97]	17.32	0.00
β_{21}	-15.59	40.34	[-94.49, 63.31]	-0.39	0.70
β_{12}	0.50	0.03	[0.45, 0.55]	18.50	0.00
β_{22}	-0.07	0.04	[-0.15, 0.00]	-1.83	0.07
σ_1	62.68		[38.02 , 103.35]		
σ_2	0.06		[0.04, 0.10]		
ρ	0.5		[-0.17, 0.85]		
σ	12.02		[11.11, 13.00]

Figure 4. Plots of the fitted heart rates at group-level and the predicted heart rates at subject-level, as well as the observed heart rates (circles) versus time during ischemia.

Figure 1 shows that the pattern of heart rate during reperfusion is nonlinear.

The heart rate during reperfusion increases and reaches a plateau. The nonlinear of the

the form $f(t; C_3, C_4) = C_3[1 - \exp(-C_4(t - T_2)_+)]$ describes this scenario, where C_3 is the plateau of heart rate when the time for reperfusion goes to large, and C_4 is related to the increasing rate of heart rate during reperfusion. The nonlinear mixed-effects model to describe the heart rate during reperfusion can be expressed as:

$$y_{ij} = C_{3i}[1 - \exp(-C_{4i}(t_{ij} - T_2)_+)] + \epsilon_{ij}, \quad (\text{E } 3.3)$$

where

$$C_{ki} = \beta_{1k} + \beta_{2k} \text{type}_i + b_{ki} \quad k = 3, 4,$$

$$\begin{bmatrix} b_{3i} \\ b_{4i} \end{bmatrix} = N \left(\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_3^2 & \rho\sigma_3\sigma_4 \\ \rho\sigma_3\sigma_4 & \sigma_4^2 \end{pmatrix} \right), \quad \epsilon_{ij} \sim N(0, \sigma^2),$$

$$\text{type}_i = \begin{cases} 1 & \text{if mouse } i \text{ is knockout} \\ 0 & \text{if mouse } i \text{ is wild-type} \end{cases}.$$

C_{3i} is the asymptote as $t_{ij} \rightarrow \infty$ and C_{4i} is the constant exponential increasing rate.

Model (E 3.3) can be fitted by using the function `SSasymOrig` in R [17]. Figure 5 shows that the fitted heart rates at group-level and the prediction heart rates at subject-level. Nonlinear mixed-effects model (E 3.3) was fitted and the results are given in Table 3. An overall assessment of the quality of the nonlinear mixed-effects model (E 3.3) is shown in Figure 5. As can be seen in Figure 5, subject-level predictions closely matched the observed heart rates during reperfusion indicating the goodness of fit of the model.

An integrated mixed-effects model for heart rate during the entire experiment

The preliminary analyses of heart rate data indicate that linear mixed-effects model is appropriate to describe the heart rate during pre-ischemia and nonlinear

Figure 5. Plots of the fitted heart rates at group-level and the predicted heart rates at subject-level, as well as the observed heart rates versus time during reperfusion.

TABLE 3

Estimates of fixed-effects and variance components for nonlinear mixed-effects model (E 3.3) for heart rate during reperfusion

Parameter	Estimate	S.E.	95% C.I.	Z	P- value
β_{13}	276.49	23.68	[230.14, 322.85]	11.67	0.00
β_{23}	107.35	39.81	[29.43, 185.26]	2.70	0.01
β_{14}	-1.66	0.33	[-2.31, -1.01]	-4.97	0.00
β_{24}	-1.09	0.52	[-2.10, -0.08]	-2.11	0.04
σ_3	61.39		[21.02, 179.29]		
σ_4	0.86		[0.54, 1.38]		
ρ	-0.77		[-0.93, -0.34]		
σ	38.62		[36.29, 41.10]		

mixed-effects models are preferred to describe the heart rate for ischemia and reperfusion.

An integrated mixed-effects model for heart rate is expressed as:

$$y_{ij} = \begin{cases} C_{1i} & 0 \leq t_{ij} \leq 5 \\ C_{1i} \exp(-C_{2i}(t_{ij} - 5)_+) & 5 < t_{ij} \leq 33 \\ C_{3i}[1 - \exp(-C_{4i}(t_{ij} - 33)_+)] & 33 < t_{ij} \leq 78 \end{cases} \quad (\text{E } 3.4)$$

where

$$C_{ki} = \beta_{1k} + \beta_{2k} \text{type}_i + b_{ki} \quad , \quad k = 1, \dots, 4,$$

$$\text{type}_i = \begin{cases} 1 & \text{if mouse } i \text{ is knockout} \\ 0 & \text{if mouse } i \text{ is wild-type} \end{cases}.$$

The interpretations of the parameters are exactly the same as those in the separated models. We start from the full model (E 3.4) with general variance-covariance on random effects. The approximate 95% confidence intervals on variance-covariance components for random effects indicate diagonal variance-covariance structure which covariances between the random effects are zero. To determine which variance-covariance structure is needed for random effects, AIC and LRT are used. As a result, the simpler variance-covariance structure for random effects (say, diagonal variance-covariance) was preferred. Table 4 shows the estimates of fixed-effects and the variance components for the integrated mixed-effects model (E 3.4) with diagonal variance-covariance structure on random effects for the entire experimental data. Figure 6 shows the plot of the standardized residuals versus the subject-level predicted values, which shows no evidence for the violation of the homogeneity of variance. An overall assessment of the integrated model (E 3.4) is provided by Figure 7, which indicates

TABLE 4

Estimates of fixed-effects and variance components for the integrated mixed-effects model (E 3.4) for heart rate

Parameter	Estimate	S.E.	95% C.I.	Z	P- value
β_{11}	289.69	18.11	[254.30, 325.09]	15.99	0.00
β_{21}	27.59	28.06	[-27.23, 82.42]	0.98	0.33
β_{12}	-1.10	0.05	[-1.20, -1.00]	-21.55	0.00
β_{22}	-0.06	0.07	[-0.21, 0.08]	-0.84	0.49
β_{13}	275.85	24.44	[228.08, 323.62]	11.28	0.00
β_{23}	110.79	41.86	[28.99, 192.59]	2.65	0.01
β_{14}	-1.64	0.34	[-2.30, -0.98]	-4.86	0.00
β_{24}	-1.12	0.53	[-2.15, -0.09]	-2.14	0.03
σ_1	45.65				
σ_2	0.00				
σ_3	63.71				
σ_4	0.88				
σ	32.18				

that the proposed model fit this experimental data very well. Figure 8 shows that the recovery of heart rate in WT mice is more stable than in KO mice during reperfusion. Rigorous statistic tests and inference are provided in the following subsection for the entire experiment.

Statistical test and inference for heart rate

To test whether the heart rate during reperfusion recovers to the level at pre-ischemia for WT mice, the underlying null hypothesis is $H_0: \beta_{11} - \beta_{13} = 0$ and the alternative hypothesis is $H_1: \beta_{11} - \beta_{13} \neq 0$.

The Wald test statistic is:

$$z = \frac{\lambda' \hat{\beta}}{\sqrt{\lambda' \widehat{Cov}(\hat{\beta}) \lambda}} = 0.389,$$

Figure 6. Diagnostic plot: Standardized residuals versus subject-level fitted heart rates.

Figure 7. Plots of the fitted heart rates at group-level and the predicted heart rates at subject-level, as well as observed heart rates (circles) versus time for the entire experimental data.

Figure 8. Fitted and observed heart rates versus time.

where $\lambda' = (1, 0, 0, 0, -1, 0, 0, 0)$.

P -value is $2P(z \geq 0.389) = 0.697$, which indicates that we cannot reject H_0 at the 5% significance level. Therefore, we conclude that heart rate for WT mice during reperfusion recovered to the level at pre-ischemia.

To test whether the heart rate during reperfusion recovers to the level at pre-ischemia for KO mice, the underlying null hypothesis is $H_0: \beta_{11} + \beta_{21} - \beta_{13} - \beta_{23} = 0$ and the alternative hypothesis is $H_1: \beta_{11} + \beta_{21} - \beta_{13} - \beta_{23} \neq 0$.

The Wald test statistic is:

$$z = \frac{\lambda' \hat{\beta}}{\sqrt{\lambda' \widehat{Cov}(\hat{\beta}) \lambda}} = -1.772,$$

where $\lambda' = (1, 1, 0, 0, -1, -1, 0, 0)$ and P -value is $2P(z \geq | -1.772 |) = 0.077$.

Since P -value is 0.077, we cannot reject H_0 at the 5% significance level. Therefore, we conclude that heart rate for KO mice during reperfusion recovered to the level at pre-ischemia.

3.3 Nonlinear model and statistical analysis for coronary flow

An integrated mixed-effects model for coronary flow during the entire experiment

The preliminary analysis of coronary flow data during reperfusion indicated that the change in coronary flow was significantly different in KO mice compared with WT mice. In addition, linear mixed-effects models are appropriate to describe coronary flow during pre-ischemia and ischemia and nonlinear mixed-effects model is preferred to describe coronary flow during reperfusion period. The integrated mixed-effects model for coronary flow is expressed as:

$$y_{ij} = \begin{cases} C_{1i} & 0 \leq t_{ij} \leq 5 \\ C_{2i} & 5 < t_{ij} \leq 33 \\ C_{3i} + (C_{4i} - C_{3i}) \exp(-C_{5i}(t_{ij} - 34)_+) - C_{7i}[\exp(-C_{5i}(t_{ij} - 34)_+) - \exp(-C_{6i}(t_{ij} - 34)_+)] & 33 < t_{ij} \leq 78 \end{cases} \quad (\text{E 3.5})$$

where

$$C_{ki} = \beta_{1k} + \beta_{2k} \text{type}_i + b_{ki} \quad , \quad k = 1, \dots, 7,$$

$$\text{type}_i = \begin{cases} 1 & \text{if mouse } i \text{ is knockout} \\ 0 & \text{if mouse } i \text{ is wild - type} \end{cases} .$$

C_{3i} is the asymptote as $t_{ij} \rightarrow \infty$, C_{4i} is the coronary flow immediately after ischemia at

$t_{ij} = 34$ and C_{5i} and C_{6i} are non-negative values and the logarithm of the rate constants. ϵ_{ij} is a normally distributed within-subject random errors. b_{ki} ($k=1,\dots,6$) are random effect associated with i^{th} subject. The fixed effect β_{11} and $\beta_{11} + \beta_{21}$ are the mean of the coronary flows for WT mice and KO mice during pre-ischemia, respectively; β_{12} and $\beta_{12} + \beta_{22}$ are the mean of coronary flows for WT mice and KO mice during ischemia, respectively; β_{13} and $\beta_{13} + \beta_{23}$ represent the mean of coronary flows for WT mice and KO mice when the time for reperfusion goes to large, respectively; β_{14} and $\beta_{14} + \beta_{24}$ are the mean coronary flows of WT mice and KO mice at the beginning of reperfusion; The random effect $b_{ki}(i = 1, \dots, N)$ describes the deviation of coronary flow for i^{th} subject away from its group mean.

The integrated model (E 3.5) was fitted to the entire experimental data and the analysis results are given in Table 5 for estimates for fixed effects and Table 6 for estimates for variance components. Figure 9 shows the plot of the standardized residuals versus subject-level predicted values, which does not indicate any systematic deviation from the homogeneity of variance. An overall assessment of the integrated mixed-effects model (E 3.5) is shown in Figure 10. Both the fitted coronary flows at group-level and the predicted coronary flows at subject-level are displayed to show how individual effects are accounted for in the integrated mixed-effects model. As can be seen in Figure 10, the subject-level predictions matched the observed coronary flows very well for the entire experimental data indicating the goodness of fit of the model. Figure 11 shows that the recovery of coronary flow in WT is faster than that in KO mice during reperfusion. Rigorous statistical test and inference are provided in the following subsection.

Statistical test and inference for coronary flow

To test whether the coronary flow during reperfusion recovers to the level of pre-ischemia for WT, the underlying null hypothesis is $H_0: \beta_{11} - \beta_{13} = 0$ and the alternative hypothesis is $H_1: \beta_{11} - \beta_{13} \neq 0$.

The Wald test statistic is:

$$z = \frac{\lambda' \hat{\beta}}{\sqrt{\lambda' \widehat{Cov}(\hat{\beta}) \lambda}} = -0.346,$$

where $\lambda' = (1, 0, 0, 0, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0)$, and P -value is $2P(z \geq |-0.346|) = 0.730$.

Because P -value is 0.730, we cannot reject H_0 at the 5% significance level. Therefore, we conclude that the coronary flow for WT mice during reperfusion recovered to the level at pre-ischemia.

TABLE 5

Estimates of fixed-effects for the integrated mixed-effects model (E 3.5) for coronary flow

Parameter	Estimate	S.E.	95% C.I.	Z	P- value
β_{11}	2.86	0.24	[2.37, 3.35]	11.47	0.00
β_{21}	-0.28	0.36	[-0.99, 0.44]	-0.75	0.44
β_{12}	0.00	0.01	[-0.02, 0.03]	0.27	0.79
β_{22}	0.02	0.02	[-0.02, 0.05]	1.02	0.31
β_{13}	2.95	0.28	[2.40, 3.50]	10.51	0.00
β_{23}	-1.04	0.41	[-1.84, -0.23]	-2.52	0.01
β_{14}	1.66	0.42	[0.84, 2.48]	3.94	0.00
β_{24}	-0.23	0.61	[-0.97, 1.43]	0.38	0.71
β_{15}	-1.08	0.12	[-1.31, -0.84]	-8.91	0.00
β_{25}	-0.19	0.19	[-0.56, 0.18]	-1.00	0.32
β_{16}	-1.09	0.12	[-1.33, -0.85]	-9.02	0.00
β_{26}	-0.18	0.19	[-0.55, 0.19]	-0.96	0.34
β_{17}	250.54	44.76	[163.31, 3337.76]	5.60	0.00
β_{27}	-32.95	83.24	[-195.16, 129.26]	-0.40	0.69

TABLE 6

Estimates of variance components for the integrated mixed-effects model (E 3.5) for coronary flow

Parameter	Estimate	Parameter	Estimate	Parameter	Estimate
σ_1	0.65	ρ_{15}	-0.30	ρ_{35}	0.17
σ_2	0.02	ρ_{16}	-0.30	ρ_{36}	0.16
σ_3	0.74	ρ_{17}	0.07	ρ_{37}	0.73
σ_4	1.06	σ_{23}	-0.16	σ_{45}	-0.46
σ_5	0.31	ρ_{24}	-0.66	ρ_{46}	-0.46
σ_6	0.31	ρ_{25}	0.66	ρ_{47}	0.02
σ_7	38.61	ρ_{26}	0.66	ρ_{56}	1.00
ρ_{12}	-0.08	ρ_{27}	0.24	ρ_{57}	0.71
ρ_{13}	0.57	ρ_{34}	0.63	ρ_{67}	0.71
ρ_{14}	0.44

Figure 9. Diagnostic plot: Standardized residuals versus subject-level fitted

Figure 10. Plots of the fitted coronary flows at group-level and the predicted coronary flows at subject-level, as well as the observed coronary flows (circles) versus time for the entire experimental data.

Figure 11. Fitted and observed coronary flows versus time.

To test whether the coronary flow during reperfusion recovers to the level at pre-ischemia for KO mice, the underlying null hypothesis is $H_0: \beta_{11} + \beta_{21} - \beta_{13} - \beta_{23} = 0$ and the alternative hypothesis is $H_1: \beta_{11} + \beta_{21} - \beta_{13} - \beta_{23} \neq 0$.

The Wald test statistic is:

$$z = \frac{\lambda' \hat{\beta}}{\sqrt{\lambda' \widehat{Cov}(\hat{\beta}) \lambda}} = 2.526,$$

where $\lambda' = (1, 1, 0, 0, 0, 0, -1, -1, 0, 0, 0, 0, 0, 0)$ and P -value is $2P(z \geq 2.526) = 0.012$.

Because P -value is 0.012, we reject H_0 at the 5% significance level. Therefore, we conclude that the coronary flow for KO mice after reperfusion does not recover to the level of pre-ischemia.

Based on the two hypothesis tests, we conclude that the recovery rate of the coronary flow is better in WT mice than in KO mice.

3.4 Left ventricle (LV) developed pressure

Figure 3 illustrates the LV developed pressure over different period of time. Particularly, the LV developed pressure during reperfusion was constant and increased exponentially. The onset point to exponential growth is called a change point. The onset of LV developed pressure is important since it indicates how rapidly the LV developed pressure starts to recover. The asymptotic value for the LV developed pressure is also important since it indicates the degree of recovery. We propose the following model:

$$y(t) = \begin{cases} C_1 & t \leq C_2 \\ C_1 + C_3(1 - e^{-C_4(t-C_2)}) & t \geq C_2 \end{cases}$$

to describe the LV developed pressure during reperfusion, where C_2 is the change point, and $C_1 + C_3$ is the asymptotic LV developed pressure. Note that the true time of transition from the constant to the exponential growth is unknown for each mouse and is influenced by random factors, so the change point was estimated by including a random effect in the model [20]. We propose the following nonlinear mixed-effects change point model:

$$y_{ij} = \begin{cases} C_{1i} & t_{ij} \leq C_{2i} \\ C_{1i} + C_{3i}[1 - \exp(-C_{4i}(t_{ij} - C_{2i}))] & t_{ij} \geq C_{2i} \end{cases}, \quad (\text{E } 3.6)$$

where

$$C_{ki} = \beta_{1k} + \beta_{2k}type_i + b_{ki}, \quad k = 1, \dots, 4,$$

$$type_i = \begin{cases} 1 & \text{if mouse } i \text{ is knockout} \\ 0 & \text{if mouse } i \text{ is wild-type} \end{cases}.$$

C_{1i} is constant LV developed pressure before the onset of exponential growth, C_{2i} represents the unknown transition time between the constant LV developed pressure and the exponential growth, $C_{1i} + C_{3i}$ is the asymptotic LV developed pressure as $t_{ij} \rightarrow \infty$, and C_{4i} is the exponential constant growth rate for the exponential LV developed pressure. Table 7 shows the estimated parameters and Figure 12 shows the fitted results for LV developed pressure during reperfusion. LV developed pressure recovered to a much greater extent in WT hearts than in KO hearts and there was no significant difference in transition time between the two types of mice. An overall assessment of the nonlinear mixed-effects change point model (E 3.6) is shown in Figure 13. Both the fitted LV developed pressures at group-level and the predicted LV developed pressures at

subject-level during reperfusion are displayed to show how individual effects are accounted for the nonlinear mixed-effects change point model (E 3.6). As can be seen Figure 13, the subject-level predictions matched the observed LV developed pressures very well during reperfusion, indicating the goodness of fit of the model.

Statistical test and inference for LV developed pressure

$\hat{\beta}_{12} = 49.67$ with p-value < 0.01 indicates that the transition time is about $49.67 - 33 = 16.67$ minutes from starting reperfusion. To test whether there is a significant difference between WT mice and KO mice in time at the onset of the recovery in LV

TABLE 7

Estimates of fixed-effects and variance components for nonlinear mixed-effects change point model (E3.6) for left ventricle (LV) developed pressure during reperfusion

Parameter	Estimate	S.E.	95% C.I.	Z	P- value
β_{11}	3.22	0.35	[2.54, 3.90]	9.24	0.00
β_{21}	-0.17	0.51	[-1.16, 0.83]	-0.33	0.74
β_{12}	49.67	1.24	[47.26, 52.09]	40.15	0.00
β_{22}	0.55	1.90	[-3.15, 4.25]	0.29	0.77
β_{13}	25.10	3.40	[18.47, 31.73]	7.39	0.00
β_{23}	-10.48	5.01	[-20.26, -0.70]	-2.09	0.03
β_{14}	-2.36	0.10	[-2.56, -2.16]	-22.80	0.00
β_{24}	-0.31	0.19	[-0.68, 0.05]	-1.67	0.10
σ_1	0.79		[0.48, 1.32]		
σ_2	3.18		[2.05, 4.95]		
σ_3	8.76		[5.90, 13.01]		
σ_4	0.20		[0.06, 0.65]		
ρ_{12}	-0.60		[-0.91, 0.16]		
ρ_{13}	0.17		[-0.44, 0.68]		
ρ_{14}	0.49		[-0.54, 0.93]		
ρ_{23}	-0.73		[-0.93, -0.22]		
ρ_{24}	-0.55		[-0.91, 0.30]		
ρ_{34}	0.04		[-0.51, 0.58]

Figure 12. Fitted and observed LV developed pressures versus time during reperfusion.

Figure 13. Plots of the fitted LV developed pressures at group-level and the predicted LV developed pressures at subject-level, as well as observed LV developed pressures (circles) versus time during reperfusion.

developed pressure during reperfusion, the underlying null hypothesis is $H_0: \beta_{22} = 0$ and the alternative hypothesis is $H_1: \beta_{22} \neq 0$.

The Wald test statistic is:

$$z = \frac{\hat{\beta}_{22}}{\sqrt{Var(\hat{\beta}_{22})}} = 0.29,$$

P -value is $2P(z \geq 0.29) = 0.77$, which indicates that we cannot reject H_0 at the 5% significance level. Therefore, we conclude that there is no significant difference in time at the onset of the recovery in LV developed pressure during reperfusion between KO-type and WT mice.

To test whether the LV developed pressures between WT mice and KO mice after reperfusion are significantly different, the null hypothesis is $H_0: \beta_{23} = 0$, and the alternative is $H_1: \beta_{23} \neq 0$.

The Wald test statistic is:

$$z = \frac{\hat{\beta}_{23}}{\sqrt{Var(\hat{\beta}_{23})}} = -2.09,$$

P -value = $2P(z \geq |-2.09|) = 0.03$, which indicates that we reject H_0 at the 5% significance level. The results show that the asymptotic LV developed pressure during reperfusion is significantly higher in WT mice than that in KO mice.

CHAPTER 4

Discussion and conclusions

The defining feature of a longitudinal study is that subjects are measured repeatedly over time. As repeated observations made on the same subject tend to be correlated, longitudinal data require specific statistical methods to account for the correlation. Mixed-effects model is an efficient tool for analyzing longitudinal data. It provides covariance structure for correlated repeated measurements. Unlike traditional repeated measures ANOVA, the time points are not fixed and all available data can be used in mixed-effects model, provided data are missing at random.

In this study, we focus on applying the mixed-effects models to the repeated measurements of cardiac function including heart rate, coronary flow, and left ventricle developed pressure in the isolated, Langendorff perfused hearts of the Glutathione-S-transferase (GSTP) gene KO and WT mice following ischemia/ reperfusion injury. Each aspect of the cardiac function data consists of three time periods: pre-ischemia, ischemia, and reperfusion periods. We developed piecewise nonlinear mixed-effects model to describe the different aspects of cardiac function. We examined how cardiac function was altered by ischemia/reperfusion injury for 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 aspect of cardiac function. The goodness of fit is examined, and the proposed models fit the experimental data very well. 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. Therefore, nonlinear mixed-effects models are highly recommended for the assessment of the dynamic change of the cardiac function and the assessment of the treatment effect on the cardiac function.

REFERENCES

- [1] J. Twisk, *Applied longitudinal data analysis for epidemiology: a practical guide*, Cambridge University Press, New York, 2003.
- [2] R. D. Wimmer and J. R. Dominick, *Mass Media Research: An Introduction*, Thomson Wadsworth, Belmont, 2005.
- [3] D. Hedeker and R. D. Gibbons, *Longitudinal data analysis*, John Wiley and Sons, New Jersey, 2006.
- [4] G. Fitzmaurice, M. Davidian M, G. Verbeke, and G. Molenberghs, *Longitudinal Data Analysis: A Handbook of Modern Statistical Methods*, Chapman & Hall/CRC, 2008.
- [5] C. Krueger and L. Tian, "A comparison of the general linear mixed model and repeated measures ANOVA using a dataset with multiple missing data points," *Biological Research Nursing*, Vol. 6, pp. 151-157, 2004.
- [6] N. M. Laird and J. H. Ware, "Random-effects models for longitudinal data," *Biometrics*, vol. 38, pp. 963-974, 1982.
- [7] A. P. Dempster, D. B. Rubin, and R. K. Tsutakawa, "Estimation in covariance component models," *Journal of the American Statistical Society*, vol. 76, pp.341-353, 1981.
- [8] H. Goldstein, *Multilevel Statistical Models*, 2nd edition, Halstead Press, New York, 1995.
- [9] S. W. Raudenbush and A. S. Bryk, *Hierarchical Linear Models*, 2nd edition, Sage, Thousand Oaks, CA, 2002.
- [10] R. D. Bock, *Measurement of human variation: a two stage model*, in *Multilevel Analysis of Educational Data*, ed. R.D. Bock, Academic Press, New York, pp. 319-342, 1989.
- [11] J. D. Leeuw and I. Kreft, "Random coefficient models for multilevel analysis," *Journal of Educational Statistics*, vol. 11, pp. 57-85, 1986.
- [12] J. S. Long, *Regression Models for Categorical and Limited Dependent Variables*, Sage Publications, Thousand Oaks, CA, 1997.

- [13] S. L. Hui and J. O. Berger, "Empirical Bayes estimation of rates in longitudinal studies," *Journal of American Statistical Association*, vol. 78, pp. 753-759, 1983.
- [14] R. D. Bock, "Within-subject experimentation in psychiatric research," In R. D. Gibbons and M. W. Dysken, editors, *Statistical and Methodological Advances in Psychiatric Research*, pp. 59-90, Spectrum, New York, 1983b.
- [15] M. J. Lindstrom and D. M. Bates, "Nonlinear mixed effects models for repeated measures data," *Biometrics*, vol. 46, pp.673-687, 1990.
- [16] R. Cudeck and J. R. Harring, "Analysis of nonlinear patterns of change with random coefficient models," *Annual Review of Psychology*, vol. 58, pp. 615-637, 2007.
- [17] J. C. Pinheiro and M. B. Douglas, *Mixed-Effects Models in S and S-PLUS*, Springer, New York, 2000.
- [18] G. M. Fitzmaurice, N. M. Laird, and J. H. Ware, *Applied Longitudinal Analysis*, John Wiley, New York 2004.
- [19] B. T. West, K. B. Welch, and A. T. Andrzej, *Linear Mixed Models: A practical Guide Using Statistical Software*, Chapman & Hall/CRC, 2007.
- [20] H. Akaike, "Information theory and an extension of the maximum likelihood principle," *2nd International Symposium on Information Theory*, ed. B.N. Petrov and F. Csaki, pp. 267-281, Akademiai Kiado, Budapest, 1973.
- [21] G. Schwarz, "Estimating the dimension of a model," *Annals of statistics*, vol. 6, pp. 461-464, 1978.
- [22] U. Schulze, "A method of Estimation of Change Points in Multiphasic Growth Models," *Biometrical Journal*, vol. 26, pp. 495-504, 1984.
- [23] K. D. Tew and D. M. Townsend, "Regulatory functions of glutathione S-transferase P1-1 unrelated to detoxification," *drug metabolism reviews*, pp. 1-15, 2011.
- [24] D. J. Conklin, P. Habetzettl, and A. Bhatnagar, "Glutathione-S-transferase P protects against endothelial dysfunction induced by tobacco smoke," *American Journal of Physiology: Heart and Circulatory Physiology*, vol. 296, pp. H1586-1597, 2009.
- [25] D. J. Conklin, B. G. Hill, K. Kaiserova, Y. Guo, R. Bolli, and A. Bhatnagar, "Deficiency of Glutathione-S-transferase P (GSTP)-mediated Detoxification of Lipid Peroxidation Products Increases Myocardial Ischemia-Reperfusion Injury," *Circulation*, vol. 118, pp. S546 – S547, 2008.

Appendix A: Linear mixed-effects model for single period

Figure 14. Heart rates versus time during pre-ischemia.

Figure 15. Heart rates versus time during ischemia.

Figure 16. Heart rates versus time during reperfusion.

Figure 17. Coronary flows versus time during pre-ischemia.

Figure 18. Coronary flows versus time during reperfusion.

Figure 19. Left ventricle (LV) developed pressures versus time during pre-ischemia.

Figure 20. LV developed pressures versus time during ischemia.

Figure 21. LV developed pressures versus time during reperfusion.

TABLE 8

Estimates of fixed-effects for linear mixed-effects model (E 3.1) for heart rate during pre-ischemia

Parameter	Estimate	S.E.	DF	Z	P-value
β_0	257.12	29.66	50	8.67	0.00
β_1	7.71	4.81	50	1.60	0.12
β_2	49.59	43.66	11	1.14	0.28
β_3	-6.98	7.08	50	-0.99	0.33

TABLE 9

Estimates of fixed-effects for linear mixed-effects model (E 3.1) for heart rate during ischemia

Parameter	Estimate	S.E.	DF	Z	P-value
β_0	114.58	9.56	322	11.99	0.00
β_1	- 4.38	0.45	322	- 9.67	0.00
β_2	17.00	14.81	10	1.15	0.28
β_3	- 0.62	0.70	322	- 0.89	0.37

TABLE 10

Estimates of fixed-effects for linear mixed-effects model (E 3.1) for heart rate during reperfusion

Parameter	Estimate	S.E.	DF	Z	P- value
β_0	167.62	25.85	526	6.48	0.00
β_1	3.02	0.71	526	4.28	0.00
β_2	- 65.31	40.06	10	-1.99	0.07
β_3	2.76	1.09	526	2.85	0.00

TABLE 11

Estimates of fixed-effects for linear mixed-effects model (E 3.1) for coronary flow during pre-ischemia

Parameter	Estimate	S.E.	DF	Z	P-value
β_0	2.86	0.27	50	10.27	0.00
β_1	0.00	0.03	50	0.01	0.99
β_2	-0.26	0.41	11	-0.64	0.54
β_3	-0.00	0.05	50	-0.10	0.92

TABLE 12

Estimates of fixed-effects for linear mixed-effects model (E 3.1) for coronary flow during reperfusion

Parameter	Estimate	S.E.	DF	Z	P- value
β_0	4.07	0.48	570	8.52	0.00
β_1	- 0.02	0.03	570	- 4.42	0.00
β_2	- 1.43	0.70	11	- 2.04	0.07
β_3	0.01	0.01	570	0.99	0.32

TABLE 13

Estimates of fixed-effects for linear mixed-effects model (E 3.1) for LV developed pressure during pre-ischemia

Parameter	Estimate	S.E.	DF	Z	P-value
β_0	67.52	5.47	50	12.33	0.00
β_1	-1.13	0.88	50	-1.29	0.20
β_2	10.48	8.06	11	1.30	0.22
β_3	1.76	1.30	50	1.36	0.18

TABLE 14

Estimates of fixed-effects for linear mixed-effects model (E 3.1) for LV developed pressure during reperfusion

Parameter	Estimate	S.E.	DF	Z	P- value
β_0	-24.01	4.07	570	-5.90	0.00
β_1	0.68	0.11	570	6.32	0.00
β_2	12.70	5.99	11	2.12	0.06
β_3	-0.32	0.16	570	-2.02	0.04

Appendix B: R code for the thesis

```
#####  
##### Heart rate #####  
#####  
  
mice<-function(a){  
  if(a==1){  
    fname=dir(pattern="WTmouse*")  
  }else { fname=dir(pattern="KOmouse*")  
    }  
  fnum=length(fname)  
  subject.mean=c()  
  
  val=4  
  #val=val+1  
  
  for(i in 1:fnum){  
    mytemp=read.csv(file=fname[i],header=T)  
    temp=mytemp[as.character(mytemp$period)!="",]  
    index1=c(rep(seq((dim(temp)[1]-5)/6),each=6),rep(((dim(temp)[1]-5)/6)+1,5))  
    temp.m=tapply(temp[,val],index1,mean)  
    subject.mean=cbind(subject.mean,temp.m)  
  
    nm=names(mytemp)  
  }  
  
  list(mean=subject.mean,nm=nm[val])  
}  
  
val.name<-mice(1)[[2]]  
wt<-mice(1)[[1]]  
ko<-mice(2)[[1]]  
wt<-as.numeric(round(wt,3))  
ko<-as.numeric(round(ko,3))  
wk<-c(wt,ko)  
length(wk)
```

```

dim(wk)<-c(1014,1)

colnames(wk)<-val.name

index2=c(rep("preisch",5),rep("isch",28),rep("reper",45))

sub.mean=data.frame(round(wt,3),index2)

type2<-c(rep("Wild",7*78),rep("Knockout",6*78))

wt2.subject<-paste(rep("WT",7*78),rep(1:7,each=78),sep="")

ko2.subject<-paste(rep("KT",3*78),rep(1:3,each=78),sep="")

ko3.subject<-paste(rep("KT",3*78),rep(5:7,each=78),sep="")

subject2<-c(wt2.subject,ko2.subject,ko3.subject)

time2<-rep(1:78,13)

mydata=data.frame(type=type2,subject=subject2,time=time2,wk,period=index2)

library(nlme)

mydata.g<-groupedData(Rate~time|subject,mydata)

type<-factor(mydata.g$type)

contrasts(mydata.g$type)

mydata.g$type <- relevel(mydata.g$type, ref="Wild")

contrasts(mydata.g$type)

###Preischemic period###

pre<-subset(mydata.g,period=="preisch")

pre.lis<-lmList(pre,na.action=na.omit)

pairs(pre.lis,id=0.01,adj=-0.5)

plot(intervals(pre.lis))

plot(pre,ylab="Heart Rate(BPM)",
      xlab="Time(min)-pre-ischemia period")

##Fitting linear mixed-effects model##

pre.lme<-lme(pre.lis,random=~1)

pre1.lme<-update(pre.lme,random=~time)

anova(pre.lme,pre1.lme) # pre1.lme is better

summary(pre1.lme)

pre.inter.lme<-lme(Rate~time*type,random=~time,pre)

pre1.inter.lme<-lme(Rate~time*type,random=~time,pre,method="ML")

pre2.lme<-lme(Rate~type,random=~time,pre,method="ML")

anova(pre2.lme,pre1.inter.lme) # pre2.lme is better

pre2.reml.lme<-lme(Rate~type,random=~time,pre)

summary(pre2.reml.lme)

```

```

plot(augPred(pre.inter.lme,level=0:1),ylab="Heat Rate(BPM)",
     xlab="Time(min)-pre-ischemia period")

###Ischemic period###

isch<-subset(mydata.g,period=="isch")

isch.lis<-lmList(isch,na.action=na.omit)

pairs(isch.lis,id=0.01,adj=-0.5)

plot(intervals(isch.lis))

plot(isch)

##Fitting linear mixed-effects model##

isch.lme<-lme(Rate~time*type,isch,na.action=na.omit,random=~1|subject)

summary(isch.lme)

isch1.lme<-lme(Rate~time*type,isch,na.action=na.omit,random=~time|subject)

summary(isch1.lme)

anova(isch.lme,isch1.lme) # isch1.lme is better

## Fitting nonlinear mixed-effects model##

rm.isch<-isch[as.character(isch$subject)!="KT5",]

#Finding initial value

pd <- function(para,time){

  para[1]*exp(-exp(para[2])*time)

}

vstart = c(1,-15)

td = na.omit(isch)

id = unique(td$subj)

allpar = c()

for(i in 1:length(id)){

  aa = td[td$subj==id[i],]

  aa$time = aa$time-6

  fun <- function(par){

    #par = exp(par)

    fit = pd(para=par,time=aa$time)

    ll = sum( (aa$Rate-fit)^2 )

    ll

  }

  fit = nlminb(start=(vstart),fun)

  pred = pd(fit$par,time=seq(0,50,by=.1))

```

```

dev.new()

plot(seq(0,50,by=.1),pred,col=2,type="l",ylim=c(0,341))

points(aa$time,aa$Rate)

title(paste("Subj:",id[i],sep=""))

allpar = rbind(allpar,fit$par)

}

nlstart = apply(allpar,2,mean)

isch.type.nlm<-nlme(Rate~Asym1*exp(-exp(xmid)*time),

                    fixed= Asym1 + xmid ~type,

                    random = Asym1 + xmid ~1,

                    #groups=~type,

                    start=c(Asym1=nlstart[1],0,xmid=nlstart[2],0),

                    control=list(maxIter=10,pnlsMaxIter=10,tolerance=0.1,niterEM=10,pnlsTol=0.1,msTol=0.01),

                    data=rm.isch)

summary(isch.type.nlm)

plot(augPred(isch.type.nlm,level = 0:1),

      xlab="Tme (min) - Ischemic period",ylab="Heart Rate (BPM)")

###Reperfusion period###

per<-subset(mydata.g,period=="reper")

a=min(per$time[per$period=="reper"])

per$time[per$period=="reper"]=per$time[per$period=="reper"]-a+1

per

plot(per,xlab="Tme(min)-Reperfusion period",ylab="Heart Rate(BPM)")

##Fitting linear mixed-effects model##

per1.lme<-lme(Rate~time*type,per,na.action=na.omit,random=~time|subject)

summary(per1.lme)

plot(augPred(per1.lme),grid = T)

plot(per)

##Fitting nonlinear mixed-effects model##

#remove KO5

rm.per<-per[as.character(per$subject)!="KT5",]

rm.per = na.omit(rm.per)

names(rm.per)

#Finding initial value

pd <- function(para,time){

```



```

SSasympOrig(time, para[1],para[2])
}
vstart = c(287,-2)
td = rm.per
id = unique(td$subj)
allpar = c()
for(i in 1:length(id)){
  aa = td[td$subj==id[i],]
  aa$time = aa$time
  fun <- function(par){
    fit = pd(para=par,time=aa$time)
    ll = sum( (aa$Rate-fit)^2 )
    ll
  }
  fit = nlminb(start=(vstart),fun)
  pred = pd(fit$par,time=seq(0,50,by=.1))
  dev.new()
  plot(seq(0,50,by=.1),pred,col=2,type="l",ylim=c(0,500))
  points(aa$time,aa$Rate)
  title(paste("Subj:",id[i],sep=""))
  allpar = rbind(allpar,fit$par)
}
n2start = apply(allpar,2,mean)
n3.type.rm.nlme<-nlme(Rate~SSasympOrig(time, Asym,lrc),
  data=rm.per,
  fixed = Asym + lrc ~ type,
  random = Asym + lrc ~ 1,
  start=c(Asym = n2start[1],0,lrc = n2start[2],0),
  control=list(pnlsMaxIter=10)
)
summary(n3.type.rm.nlme)
plot(n3.rm.nlme)
plot(augPred(n3.rm.nlme,level = 0:1),
  ylab="Heat Rate(BPM)",
  xlab="Time(min)-reperfusion period")

```

```

### The integrated model for heart rate ###

pre.isch.per <- mydata.g[as.character(mydata.g$subject)!="KT5",]

names(pre.isch.per)

IntModel2 <- function(time,C1,C2,C3,C4){
  C1*(time<=5)+ C1*exp(-exp(C2)*(time-5))*(time>5 & time<=33)+as.numeric(SSasympOrig((time-33),
  Asym=C3,lrc=C4))*(time>33)
}

integrate2.nlme<-nlme(Rate~IntModel2(time,C1,C2,C3,C4),
  na.action=na.omit,
  fixed = C1+C2+C3+C4 ~ type,
  random = C1+C2+C3+C4 ~ 1,
  start=c(C1=257.12,0,C2=n1start[2],0,C3=n2start[1],0,C4=n2start[2],0),
  data=pre.isch.per,
  #groups=~type,
  control=list(maxIter=50,pnlsMaxIter=10,tolerance=0.1,niterEM=10,pnlsTol=0.1,msTol=0.0001),
  verbose=T
)

summary(integrate2.nlme)

plot(augPred(integrate2.nlme,level = 0:1),
  ylab="Heat Rate(BPM)",
  xlab="Time(min)")

pre.isch.per.mat<-asTable(pre.isch.per)

bb=rownames(pre.isch.per.mat)

wi=which(unlist(gregexpr("W",bb))!=-1)

ki=which(unlist(gregexpr("K",bb))!=-1)

wrate.mat=pre.isch.per.mat[wi,]

krate.mat=pre.isch.per.mat[ki,]

predict.in<-predict(integrate2.nlme, level = 0:1)

wt.p<-predict.in[predict.in$subject=="WT1",]

wt.pp<-wt.p[,2]

kt.p<-predict.in[predict.in$subject=="KT1",]

kt.pp<-kt.p[,2]

plot(pre.isch.per$time,pre.isch.per[,5],ylab="Heat Rate (BPM)",

```

```

xlab="Time (min)",type="n",xlim=c(1,78))
for(i in 1:7){
  points(c(1:78),wrate.mat[i,],col=2,type="p",lwd=1,pch=1)
  points(c(1:78),krate.mat[i,],col=4,type="p",lwd=1,pch=3)
  legend("bottomright",c("WT: observation","WT: prediction","KO: observation","KO: prediction"),
        col=c(2,2,4,4),pch=c(1,-1,3,-1),lty=c(-1,1,-1,2),lwd=c(-1,3,-1,3))
  lines (c(1:78),wt.pp,lwd=3,col=2)
  lines (c(1:78),kt.pp,lwd=3,lty=2, col=4)
}
abline(v=c(5,33),lty=2)
arrows(13,450, 5,450, length = 0.1)
arrows(27,450, 32.5,450,length = 0.1)
text(20,450, "Ischemia")
arrows(45,450, 33.5,450, length = 0.1)
arrows(66,450, 80,450,length = 0.1)
text(56,450, "Reperfusion")

plot(integrate2.nlm)
qqnorm(integrate2.nlm)

###Hypothesis test###
vc<-integrate2.nlm$varFix
#wild-type
ramda.w=c(1,0,0,0,-1,0,0,0)
numer=t(ramda.w)%*%as.numeric(integrate2.nlm$coefficients$fixed)
denomi=sqrt(t(ramda.w)%*%vc)%*%ramda.w)
z.value=numer/denomi
z.value
df.w=as.numeric(integrate2.nlm$fixDF$X[1])
2*pt(abs(z.value), df=df.w , lower.tail=F)
#knockout-type
ramda.w=c(1,1,0,0,-1,-1,0,0)
numer=t(ramda.w)%*%as.numeric(integrate2.nlm$coefficients$fixed)
denomi=sqrt(t(ramda.w)%*%vc)%*%ramda.w)
z.value=numer/denomi

```

```

z.value

df.w=as.numeric(integrate2.nlme$fixDF$X[1])

2*pt(abs(z.value), df=df.w , lower.tail=F)

###Graphical presentation###

##overall graph##

mydata.mat<-asTable(mydata.g)

aa=rownames(mydata.mat)

wi=which(unlist(gregexpr("W",aa))!=-1)

ki=which(unlist(gregexpr("K",aa))!=-1)

wrate.mat=mydata.mat[wi,]

krate.mat=mydata.mat[ki,]

plot(mydata.g$time,mydata.g[,5],ylab="Heat Rate (BPM)",
      xlab="Time (min)",type="n",xlim=c(1,79))

for(i in 1:7){
  points(c(1:78),wrate.mat[i,col=2,type="o",lwd=1,pch=1)
  points(c(1:78),krate.mat[i,col=4,type="o",lwd=2,pch=1)
  legend("bottomright",c("WT", "KO"),col=c(2,4),lty=c(1,1))
  # lines (c(1:78),wrate.mat[i,col=2)
}

abline(v=c(5,33),lty=2)
abline(v=c(5,33),lty=2)

arrows(13,450, 5,450, length = 0.1)
arrows(27,450, 32.5,450,length = 0.1)
text(20,450, "Ischemia")
arrows(45,450, 33.5,450, length = 0.1)
arrows(66,450, 80,450,length = 0.1)
text(56,450, "Reperfusion")

##preischemic period##

pre.isch<-subset(mydata.g,period=="preisch")

mydata.preisch.g<-groupedData(Rate~time|subject,pre.isch)

plot(mydata.preisch.g,outer=~type,aspect=1,
      xlab="Time (min)-preischemic period",ylab="Heart Rate (BPM)")

mydata.mat<-asTable(mydata.preisch.g)

a1=rownames(mydata.mat)

wi=which(unlist(gregexpr("W",a1))!=-1)

```

```

ki=which(unlist(gregexpr("K",a1))!= -1)

wrate.mat=mydata.mat[wi,]
krate.mat=mydata.mat[ki,]

plot(mydata.preisch.g$time,mydata.preisch.g$Rate,
ylab="Heart Rate(BPM)",
      xlab="Time (min)",type="n")

for(i in 1:7){
  points(c(1:5),wrate.mat[i,],col=2,type="o",lwd=1,pch=1)
  points(c(1:5),krate.mat[i,],col=4,type="o",lwd=2,pch=1)
  # lines (c(1:5),wrate.mat[i,],col=2)
  legend("topright",c("WT", "KO"),col=c(2,4),lwd=c(1,2),lty=c(1,1))
}

##ischemic period##

isch<-subset(mydata.g,period=="isch")
#write.csv(isch,file="rate_isch.csv")
mydata.isch.g<-groupedData(Rate~time|subject,isch)
plot(mydata.isch.g,outer=~type,aspect=1,
xlab="Time(min)-Ischemic period",ylab="Heart Rate(BPM)")
mydata.mat<-asTable(mydata.isch.g)
a2=rownames(mydata.mat)
wi=which(unlist(gregexpr("W",a2))!= -1)
ki=which(unlist(gregexpr("K",a2))!= -1)
wrate.mat=mydata.mat[wi,]
krate.mat=mydata.mat[ki,]

plot(mydata.isch.g$time,mydata.isch.g$Flow,xlab="Time(min)-Ischemic period",
      ylab="Heart Rate(BPM)",type="n")

for(i in 1:7){
  points(c(6:33),wrate.mat[i,],col=2,type="o",lwd=1,pch=1)
  points(c(6:33),krate.mat[i,],col=4,type="o",lwd=2,pch=1)
  # lines (c(6:33),wrate.mat[i,],col=2)
  legend("topright",c("WT", "KO"),col=c(2,4),lwd=c(1,2),lty=c(1,1))
}

##reperfuion period##

reper<-subset(mydata.g,period=="reper")
mydata.reper.g<-groupedData(Rate~time|subject,reper)

```

```

plot(mydata.reper.g,outer=~type,aspect=1,
      xlab="Time (min)-reperfusion period",ylab="Heart Rate (BPM)",
    )
mydata.mat<-asTable(mydata.reper.g)
a3=rownames(mydata.mat)
wi=which(unlist(gregexpr("W",a3))!=-1)
ki=which(unlist(gregexpr("K",a3))!=-1)
wrate.mat=mydata.mat[wi,]
krate.mat=mydata.mat[ki,]
plot(mydata.reper.g$time,mydata.reper.g$Flow,xlab="Time (min)-reperfusion period",ylab="Heart Rate(BPM)",type="n")
for(i in 1:7){
  points(c(34:78),wrate.mat[i,],col=2,type="o",lwd=1,pch=1)
  points(c(34:78),krate.mat[i,],col=4,type="o",lwd=2,pch=1)
  legend("bottomright",c("WT", "KO"),col=c(2,4),lwd=c(1,2),lty=c(1,1))
}

#####
#####   Coronary flow   #####
#####

mydata.g<-groupedData(Flow~time|subject,mydata)
type<-factor(mydata.g$type)
mydata.g$type <- relevel(mydata.g$type, ref="Wild")
contrasts(mydata.g$type)
###Preischemic period###
pre<-subset(mydata.g,period=="preisch")
pre.lme<-lme(Flow~time*type,pre,na.action=na.omit,random=~1|subject)
summary(pre.lme)
pre1.lme<-lme(Flow~time*type,pre,na.action=na.omit,random=~time|subject)
summary(pre1.lme)
anova(pre.lme,pre1.lme)
plot(augPred(pre1.lme),grid = T,
      xlab="Time(min)- Preischemic period",ylab="Flow (mL/min)")
###Ischemic period###
isch<-subset(mydata.g,period=="isch")
isch.lis<-lmList(isch,na.action=na.omit)
pairs(isch.lis,id=0.01,adj=-0.5)

```

```

plot(intervals(isch.lis))

plot(isch)

##Fitting linear mixed-effects model##

isch.lme<-lme(Flow~time*type,isch,na.action=na.omit,random=~1|subject)

summary(isch.lme)

isch1.lme<-lme(Flow~time*type,isch,na.action=na.omit,random=~time|subject)

summary(isch1.lme)

anova(isch.lme,isch1.lme)

###Reperfusion period###

per<-subset(mydata.g,period=="reper")

##Fitting linear mixed-effects model##

per.lme<-lme(Flow~time*type,per,na.action=na.omit,random=~1|subject)

summary(per.lme)

per1.lme<-lme(Flow~time*type,per,na.action=na.omit,random=~time|subject)

summary(per1.lme)

anova(per.lme,per1.lme)

plot(augPred(per1.lme),grid = T,
      xlab="Time(min)- Reperfusion period",ylab="Flow (mL/min)")

plot(per)

### The integrated model for coronary flow ###

pre.isch.per<-mydata.g

pre.isch.per = pre.isch.per[-970,]

plot(pre.isch.per)

plot(pre.isch.per,outer=~type,aspect=1,xlab="Time (min)",
      ylab="Flow (mL/min)")

flow.pd <- function(C1,C2,C3,C4,C5,C6,C7,time){
  C1*(time<=5)+C2*(time>5 & time<=33)+(C3+(C4-C3)*exp(-exp(C5)*(time-34))-C7*(exp(-exp(C5)*(time-34))-exp(-
exp(C6)*(time-34))))*(time>33)
}

pd <- function(para,time=0:45){
  flow.pd(C1=para[1],C2=para[2],C3=para[3],C4=para[4],C5=para[5],C6=para[6],C7=para[7],time=time)
}

vstart = c(4,0,2.48,4,-0.92,-0.86,2.5)

td = na.omit(pre.isch.per)

id = unique(td$subj)

```

```

min.time = min(td$time)

allpar = c()

for(i in 1:length(id)){

  aa = td[td$subj==id[i],]

  #aa$time = aa$time - min.time

  fun <- function(par){

    fit = pd(para=par,time=aa$time)

    ll = sum( (aa$Flow-fit)^2 )

    ll

  }

  fit = nlminb(start=(vstart),fun)

  pred = pd(fit$par,time=seq(0,100,by=.1))

  dev.new()

  plot(seq(0,100,by=.1),pred,col=2,type="l",ylim=c(0,max(td$Flow)))

  points(aa$time,aa$Flow)

  title(paste("Subj:",id[i],sep=""))

  allpar = rbind(allpar,fit$par)

}

nstart = apply(allpar,2,mean)

integrate.r<-nlme(Flow~flow.pd(C1,C2,C3,C4,C5,C6,C7,time),

                  fixed = C1+C2+C3+C4+C5+C6+C7~type,

                  random = (C1+C2+C3+C4+C5+C6+C7~1),

                  start=c(C1=nstart[1],0,C2=nstart[2],0,C3=nstart[3],0,C4=nstart[4],0,C5=nstart[5],0,C6=nstart[6],0,C7=nstart[7],0),

                  verbose=T,

                  #groups=~type,

                  control=list(maxIter=5,pnlsMaxIter=5,tolerance=1,niterEM=10,pnlsTol=1,msTol=0.5),

                  data=td

                  )

summary(integrate.r)

plot(augPred(integrate.r,level = 0:1), xlab="Time (min)",

      ylab="Flow (mL/min)")

plot(integrate.r)

qqnorm(integrate.r)

plot(augPred(pre.isch.per.type.nlme,level = 0:1), xlab="Time (min)",

```



```

      ylab="Flow (mL/min)")

###Hypothesis test###

vc<-integrate.r$varFix

#wild-type

ramda.w=c(1,0,0,0,-1,0,0,0,0,0,0,0,0)

numer=t(ramda.w)%*%as.numeric(integrate.r$coefficients$fixed)

denomi=sqrt(t(ramda.w)%*%vc%*%ramda.w)

z.value=numer/denomi

z.value

df.w=as.numeric(integrate.r$fixDF$X[1])

2*pt(abs(z.value), df=df.w , lower.tail=F)

#knockout-type

ramda.w=c(1,1,0,0,-1,-1,0,0,0,0,0,0,0)

numer=t(ramda.w)%*%as.numeric(integrate.r$coefficients$fixed)

denomi=sqrt(t(ramda.w)%*%vc%*%ramda.w)

z.value=numer/denomi

z.value

df.w=as.numeric(integrate.r$fixDF$X[1])

2*pt(abs(z.value), df=df.w , lower.tail=F)

###Graphical presentation###

##overall graph##

mydata.mat<-asTable(mydata.g)

aa=rownames(mydata.mat)

wi=which(unlist(gregexpr("W",aa))!=-1)

ki=which(unlist(gregexpr("K",aa))!=-1)

wrate.mat=mydata.mat[wi,]

krate.mat=mydata.mat[ki,]

plot(mydata.g$time,mydata.g[,5],xlab="Time (min)",
ylab="Flow (mL/min)",type="n",xlim=c(1,79))

for(i in 1:7){

  points(c(1:78),wrate.mat[i,col=2,type="o",lwd=1,pch=1)

  points(c(1:78),krate.mat[i,col=4,type="o",lwd=2,pch=1)

  legend("bottomright",c("WT","KO"),col=c(2,4),lty=c(1,1))

  # lines (c(1:78),wrate.mat[i,col=2)

}

```

```

abline(v=c(5,33),lty=2)

##preischemic period##

pre.isch<-subset(mydata.g,period=="preisch")

mydata.preisch.g<-groupedData(Flow~time|subject,pre.isch)

plot(mydata.preisch.g,outer=~type,aspect=1,xlab="Time (min)-Preischemic period",
ylab="Flow (mL/min)")

mydata.mat<-asTable(mydata.preisch.g)

a1=rownames(mydata.mat)

wi=which(unlist(gregexpr("W",a1))!= -1)

ki=which(unlist(gregexpr("K",a1))!= -1)

wrate.mat=mydata.mat[wi,]

krate.mat=mydata.mat[ki,]

plot(mydata.preisch.g$time,mydata.preisch.g$Flow,
xlab="Time (min) - Preischemic period",
ylab="Flow (mL/min)",type="n")

for(i in 1:7){

  points(c(1:5),wrate.mat[i,],col=2,type="o",lwd=1,pch=1)

  points(c(1:5),krate.mat[i,],col=4,type="o",lwd=2,pch=1)

  # lines (c(1:5),wrate.mat[i,],col=2)

  legend("topright",c("WT","KO"),col=c(2,4),lwd=c(1,2),lty=c(1,1))

}

##ischemic period##

isch<-subset(mydata.g,period=="isch")

mydata.isch.g<-groupedData(Flow~time|subject,isch)

plot(mydata.isch.g,outer=~type,aspect=1,xlab="Time (min) - Ischemic period",
ylab="Flow (mL/min)")

mydata.mat<-asTable(mydata.isch.g)

a2=rownames(mydata.mat)

wi=which(unlist(gregexpr("W",a2))!= -1)

ki=which(unlist(gregexpr("K",a2))!= -1)

wrate.mat=mydata.mat[wi,]

krate.mat=mydata.mat[ki,]

plot(mydata.isch.g$time,mydata.isch.g$Flow,xlab="Time (min)- Ischemic period",
ylab="Flow (mL/min)",type="n")

for(i in 1:7){

```

```

    points(c(6:33),wrate.mat[i,],col=2,type="o",lwd=1,pch=1)
    points(c(6:33),krate.mat[i,],col=4,type="o",lwd=2,pch=1)
# lines (c(6:33),wrate.mat[i,],col=2)
    legend("topright",c("WT","KO"),col=c(2,4),lwd=c(1,2),lty=c(1,1))
}

##reperfuiaon period
reper<-subset(mydata.g,period=="reper")
mydata.reper.g<-groupedData(Flow~time|subject,reper)
plot(mydata.reper.g,outer=~type,aspect=1,xlab="Time (min)- Reperfusion period",
ylab="Flow (mL/min)")
mydata.mat<-asTable(mydata.reper.g)
a3=rownames(mydata.mat)
wi=which(unlist(gregexpr("W",a3))!= -1)
ki=which(unlist(gregexpr("K",a3))!= -1)
wrate.mat=mydata.mat[wi,]
krate.mat=mydata.mat[ki,]
plot(mydata.reper.g$time,mydata.reper.g$Flow,xlab="Time (min)- Reperfusion period",
ylab="Flow (mL/min)",type="n")
for(i in 1:7){
    points(c(34:78),wrate.mat[i,],col=2,type="o",lwd=1,pch=1)
    points(c(34:78),krate.mat[i,],col=4,type="o",lwd=2,pch=1)
    legend("bottomright",c("WT","KO"),col=c(2,4),lwd=c(1,2),lty=c(1,1))
}

#####
##### LV depressed pressure #####
#####

mydata.g<-groupedData(DEVELOPED~time|subject,mydata)
type<-factor(mydata.g$type)
mydata.g$type <- relevel(mydata.g$type, ref="Wild")
contrasts(mydata.g$type)
###Reperfusion period###
per<-subset(mydata.g,period=="reper")
plot(per,outer=~type,aspect=1,xlab="Time (min)- Reperfusion period",
ylab="DEVELOPED (mmHg)")

```

```

t.pd <- function(C1,C2,C3,C4,time){
  C1*(time<=C2) + (C1+C3*(1-exp(-exp(C4)*(time-C2))))*(time>=C2)
}

pd <- function(para,time){
  t.pd(para[1],para[2],para[3],para[4],time=time)
}

vstart = c(0,43,1,0)
td = na.omit(per)
id = unique(td$subj)
allpar = c()

for(i in 1:length(id)){
  aa = td[td$subj==id[i],]
  fun <- function(par){
    fit = pd(para=par,time=aa$time)
    ll = sum( (aa$DEVELOPED-fit)^2 )
    ll
  }
  fit = nlminb(start=(vstart),fun)
  pred = pd(fit$par,time=seq(34,78,by=.1))
  dev.new()
  plot(seq(34,78,by=.1),pred,col=2,type="l",ylim=c(0,44))
  points(aa$time,aa$DEVELOPED)
  title(paste("Subj:",id[i],sep=""))
  allpar = rbind(allpar,fit$par)
}

n1start = apply(allpar,2,mean)
cpt.nlme<-nlme(DEVELOPED~t.pd(C1,C2,C3,C4,time),
  fixed= C1+C2+C3+C4 ~type,
  random = C1+C2+C3+C4 ~1,
  start=c(C1=n1start[1],0,C2=n1start[2],0,C3=n1start[3],0,C4=n1start[4],0),
  #groups=~type,
  control=list(maxIter=10,pnlMaxIter=10,tolerance=0.1,niterEM=10,pnlTol=1,msTol=0.1),
  data=td)

summary(cpt.nlme)

plot(augPred(cpt.nlme,level = 0:1),grid = T,ylab="DEVELOPED (mmHg)",

```

```

xlab="Time(min)- Reperfusion period")

###Hypothesis test###
vc<-cpt.nlme$varFix[4,4]
beta.hat<-cpt.nlme$coefficient$fixed[4]
t.value= beta.hat/sqrt(vc)

#wild-type
ramda.w=c(0,0,0,1,0,0,0,0)
numer=t(ramda.w)%*%as.numeric(integrate.r$coefficients$fixed)
denomi=sqrt(t(ramda.w)%*%vc)%*%ramda.w)
t.value=numer/denomi

t.value

df.w=as.numeric(integrate.r$fixDF$X[1])
2*pt(abs(t.value), df=df.w , lower.tail=F)

#knockout-type
ramda.w=c(0,0,0,0,0,1,0,0)
numer=t(ramda.w)%*%as.numeric(integrate.r$coefficients$fixed)
denomi=sqrt(t(ramda.w)%*%vc)%*%ramda.w)
z.value=numer/denomi

z.value

df.w=as.numeric(integrate.r$fixDF$X[1])
2*pt(abs(z.value), df=df.w , lower.tail=F)

###Graphical presentation###
##overall graph##
mydata.mat<-asTable(mydata.g)
aa=rownames(mydata.mat)
wi=which(unlist(gregexpr("W",aa))!=-1)
ki=which(unlist(gregexpr("K",aa))!=-1)
wrate.mat=mydata.mat[wi,]
krate.mat=mydata.mat[ki,]
plot(mydata.g$time,mydata.g[,5],xlab="Time (min)",
ylab="LV developed pressure (mmHg)",type="n",xlim=c(1,79))
for(i in 1:7){
  points(c(1:78),wrate.mat[i,],col=2,type="o",lwd=1,pch=1)
  points(c(1:78),krate.mat[i,],col=4,type="o",lwd=2,pch=1)
  legend("topright",c("WT","KO"),col=c(2,4),lty=c(1,1))
}

```

```

# lines (c(1:78),wrate.mat[i,],col=2)
}
abline(v=c(5,33),lty=2)
##reperfusion period##
reper<-subset(mydata.g,period=="reper")
mydata.reper.g<-groupedData(DEVELOPED~time|subject,reper)
plot(mydata.reper.g,outer=~type,aspect=1,xlab="Time (min)- Reperfusion period",
ylab="LV developed pressure (mmHg)")
mydata.mat<-asTable(mydata.reper.g)
a3=rownames(mydata.mat)
wi=which(unlist(gregexpr("W",a3))!=-1)
ki=which(unlist(gregexpr("K",a3))!=-1)
wrate.mat=mydata.mat[wi,]
krate.mat=mydata.mat[ki,]
plot(mydata.reper.g$time,mydata.reper.g$Flow,xlab="Time (min)- Reperfusion period",
ylab="LV developed pressure (mmHg)",type="n")
for(i in 1:7){
  points(c(34:78),wrate.mat[i,],col=2,type="o",lwd=1,pch=1)
  points(c(34:78),krate.mat[i,],col=4,type="o",lwd=2,pch=1)
  # lines (c(34:78),wrate.mat[i,],col=2)
  legend("bottomright",c("WT", "KO"),col=c(2,4),lwd=c(1,2),lty=c(1,1))
}

```

CURRICULUM VITAE

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Kong M, Jang H, Conklin DJ. Mixed-effects models for evaluating cardiac function and treatment effect. ICSA Symposium June 26- 29, 2011, New York.

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