STATISTICAL APPROACES TO ANALYZING ENERGY EXPENDITURE DATA AMONG ZUCKER DIABETIC FATTY RATS

A Thesis

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN PUBLIC HEALTH

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December 2013

Major Subject: Biostatistics

ABSTRACT

Obesity is widely becoming a worldwide epidemic and often results from a combination of a sedentary lifestyle, inadequate food intake, and genetic predisposition. It is often of interest to scientists studying this epidemic to assess how much physical activity the study participants partake in or the amount of energy expenditure expended within a given time period. Energy expenditure is often used for this purpose where the study participants are subjected to devices which measure the amount of energy expended frequently within a specified time period. For example, in studying obesity among Zucker diabetic fatty (ZDF) rats, an animal model often used for studying obesity and the onset of diabetes, energy expenditure can be assessed by the use of an Oxymas instrument (an open circuit calorimeter; Columbus Instruments, Ohio, USA), a device which measures various components of energy expenditure every five to ten minutes.

The resulting data are often of the functional longitudinal form and several statistical techniques can be employed to analyze such data. In this paper, we apply various statistical approaches to analyze the energy expenditure data from the ZDF rats; we compare statistical models based on linear mixed effects models and functional mixed effects models with smoothing splines. We find that in our current analyses, the use of the mixed effects models with a quadratic term for the time of observation following a summary of the data from minutes to hours and a log transformation to achieve approximate normality perform adequately well in assessing the effects of the treatment on the energy expenditure variables. We also find that the functional mixed effects model with a quadratic spline can be used as an effective option for analyzing the data after summarizing the data per hour without applying any transformation techniques. We therefore recommend first summarizing the energy expenditure per hour to reduce the noise associated with the frequency of the data collection and using either linear mixed effects models with polynomial terms for time or functional mixed effects model with smoothing splines to analyze the data collected repeatedly over a 24-hour period, when a curve linear relationship is suspected between time and the various energy expenditure variables.

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CHAPTER I

INTRODUCTION

Obesity is becoming a growing public health concern world-wide, as the world population engages in more sedentary lifestyles. The growing trend in sedentary lifestyles such as sitting is increasing with developing technology [1]. Obesity often results from an imbalance between food intake and energy expenditure, genetic predisposition, consuming diets high in fat, and inflammation [2]. The rise in obesity contributes to the development of other adverse health outcomes such as insulin resistance, type II diabetes, obstructive sleep apnea, osteoarthritis, stroke, hypertension, and cancer [3]. With growing concerns regarding the treatment of this spreading epidemic is also a rise in scientific research in better understanding the underlying causal pathways for obesity and its related adverse health outcomes. Since energy expenditure is known to be in the pathway of obesity, there has been a growing interest in also better understanding how energy expenditure relates to obesity and obesity related health outcomes.

Ovine interferon tau (INFT) is a member of the type I INF family and is known to be non-toxic even at high concentrations and it has also been shown to play a regulatory role in sheep reproduction cycles [4-5]. It also has stable acid, antiviral, antiproliferative and immunomodulatory properties [6-8]. It has been used in previous experiments with health outcomes related to inflammation and autoimmune diseases such as multiple sclerosis among NZW mice [8] and diabetes in NOD mice [9]. The treatment was given orally in both experiments. Since obesity is related to inflammation and IFNT has been shown to possess anti-inflammatory properties, the scientific objective of this study was to assess the impact of INFT in delaying the onset of diabetes and obesity related adverse health outcomes among Zucker diabetic fatty (ZDF) rats, an animal model for studying obesity. It is also of interest to determine how INFT impacts energy expenditure among the ZDF rats.

Energy expenditure is defined as the amount of energy expended by the body to perform daily body tasks such as movement, breathing and digestion [10]. In studying obesity and obesity related health outcomes, scientists are often interested in also measuring energy expenditure. For example, in studying the effects of the oral treatment with IFNT [4] on obesity and obesity related health outcomes among the ZDF rats energy expenditure is measured using a computer-controlled Oxymas instrument (an open circuit calorimeter; Columbus Instruments, Ohio, USA). The Oxymas instrument measures the energy expenditure for each animal over a 24-hour period frequently, such as every five to ten minutes. Each animal's carbon dioxide production, oxygen consumption, total heat production and respiratory quotient are obtained by the instrument. The resulting data are often not only longitudinal in nature but can also be seen as non-linear functions of time (see Figure 1 in Appendix), often leading to confusion among scientists regarding the most appropriate method for analyzing such data.

Longitudinal data result from data collected repeatedly over time on subjects under observation [11-14] and are becoming more prevalent in biostatistical research. Several approaches can be taken to analyze longitudinal data depending on the nature and distribution of the variables in the data. Due to the repeated measures nature of the data, where data are collected repeatedly on subjects over a given time period, the resulting multiple data collected on each individual are often correlated. In other words, these data have a clustered structure per subject under observations and the data within each cluster or subject are correlated. The correlation within each subject or cluster can affect the variances of the variables by either increasing or decreasing the variance, depending on the nature of the correlation. In analyzing such data, it is important to account for any correlations which might exist between the observations. In the linear mixed effects setting, the data are longitudinal and continuous and it is often assumed that the relationships between the outcomes of interest and time or transformation of the time scale are linearly related. While polynomial terms for time such as the quadratic or cubic terms can also be included in the models when a curvilinear relationship is suspected.

In longitudinal data settings, data from certain experiments may appear as curves or functions of time and therefore, choosing statistical techniques which model the data using the curves as the basic unit of analysis might also be appealing [15]. Functional mixed effects models extend the linear mixed effects model to functional settings where the basic unit of analysis is curves [15]. In analyzing functional data, statistical smoothing techniques are often employed to smooth the curves and regression methods from either nonparametric or semi parametric statistics are applied [16].

In this manuscript, we focus on two different types of approaches to analyzing longitudinal data (1) linear mixed effects models with linear and polynomial terms for time; and (2) functional mixed effects models with smoothing spline to analyze the energy expenditure data obtained from the ZDF rats using the Oxymas instrument. The mixed effects models treats the longitudinal data as both linear and polynomial functions of time while the functional mixed effects models treat the data as arbitrary non-linear functions of times which can be approximated by smoothing splines.

The statistical objective of this study is to compare various statistical approaches to analyzing the energy expenditure data collected on the ZDF rats based on linear mixed effects models and functional mixed effects models. The various models are applied to the energy expenditure data. In Chapter 2 of the paper, we discuss the materials and methods employed and the statistical models considered. Chapter 4 provides the results and conclusions while the discussion section is provided in Chapter 3.

CHAPTER II

MATERIALS AND METHODS

ZDF Rats

Male ZDF rats were obtained from Charles River at the age of 23 days. The animals were fed a Purina 5008 diet throughout the study. The Purina 5008 diet consists of 23.5% crude protein, 6.0% fat, 34.9% starch, 2.6% sucrose, 0.5% glucose plus fructose, 6.8% minerals, 3.8% fiber and 17,364 kJ gross energy/kg [17-18]. The study animals were kept in a temperature- and humidity-controlled facility on a 12-h light: 12-h dark cycle and the study was approved by the Texas A&M University Animal Use and Care Committee [17-18].

At 28 days of age, six rats were randomly assigned to receive either drinking water (distilled and deionized H_2O) providing 0, 4 or 8 μg IFNT/kg body weight per day. Concentrations of IFNT in the drinking water were adjusted daily based on the volume of water consumed to provide the desired treatment dosages. The drinking water was changed every other day. During the eight-week study period, concentrations of IFNT in the drinking water averaged 0, 13.4 and 26.8 ng/ml, respectively, for rats receiving 0, 4 or 8 μg IFNT/kg body weight per day.

Each animal was placed in an Oxymas instrument for 24 hours to study the impact of IFNT on energy expenditure among the twelve weeks-old animals. The Oxymas instrument measures their energy expenditure data frequently. All the animals had free access to food and drinking water during the observation period and the following variables related to energy expenditure were measured: volumetric O_2 consumption (L/h/kg BW) (VO_2), volumetric CO_2 production (L/h/kg BW) (VCO_2), respiratory quotient (O_2 consumption/ CO_2 production) (RQ) and heat production (kcal/h) (Heat) [17-18,].

In analyzing longitudinal data, one needs to adequately account for all its characteristics. Unlike cross-sectional data, the response variables in longitudinal data settings are measured repeatedly over time. In such data, statistical models which allow scientists to assess the individual trajectory over time are often employed. When the response variables are continuous linear functions of time, the linear mixed effects models with a linear term for time can be applied however, when curvilinear relationships are suspected, polynomial terms for time such as the quadratic or cubic terms can be added to the model. When such data have responses which are non-linear functions of time or are best described as curves over time, semi-parametric models such as functional mixed effects models with smoothing splines can also be applied. In addition to adequately choosing models which best describe the relationships between the response variables and time, another important characteristic of longitudinal data is that the repeated measures collected on each subject are often correlated. Therefore, choosing models which allow one to either model the variances directly or adjust the variances to correct for the induced correlations by the repeated measures within each subject is also an important characteristic to be considered when analyzing longitudinal data. Another advantage of using longitudinal data analysis to analyze longitudinal data is that these models are able to adequately handle missing data [19]. In this paper, we consider models based on two types of mixed effects models, namely the linear mixed effects models and the functional mixed effects model with smoothing splines.

Statistical Model Considered

Linear Mixed Effect Models (LMEM)

Linear mixed effects models are often employed by researchers conducting longitudinal studies where measurements are collected repeatedly over time on each subject. The models can be seen as extensions of linear regression models where the data are now collected repeatedly rather than cross-sectionally. In such models, the response variables are seen as either linear or curvilinear functions of time while the normality distributional assumption is often assumed for the random variables. The linear mixed effects model is written as:

$$y_{ij} = X_{ij}\beta + Z_{ij}b_i + \varepsilon_{ij}$$

where y_{ij} is the j^{th} response for the i^{th} subject, β is a $p \times 1$ vector of fixed coefficients and X_{ij} is the $1 \times p$ vector of fixed variables, b_i is a $q \times 1$ vector for the random effects included in the model while Z_{ij} is a $1 \times q$ vector for the random variables included in the model. The random error terms ε_{ij} represent the random variation associated with the $y_{ij}{}^{\text{th}}$ response. The following distributional assumptions are made:

$$\varepsilon_{ij} \sim N(0, \sigma^2);$$

$$b_i \sim N(0, \sigma_b^2).$$

The mean response of y_{ij} is $X_{ij}\beta$ and is considered the fixed component of the model while $Z_{ij}b_i$ represents the individual variation from the overall population mean. The term of $Z_{ij}b_i$ represent the random components of the model and allow one to model the individual trajectory or patterns over time for the data that change across individuals within the study.

In our current application, we have four different energy expenditure variables, namely $\mathrm{VO_2}$, $\mathrm{VCO_2}$, heat expenditure and RQ while the fixed covariate included in the models are time and INFT treatment group assignment. The random component of our model is a random intercept for each animal in the study. We also considered including a random term for time in our modeling strategies, however; no statistical advantage was found by the inclusion of the random term for time in addition to having a random intercept in the model.

The random intercept in the model allows us to assess how each subject's intercept deviates from the overall intercept included in the model after adjusting for time and INFT treatment group assignment. An unstructured (UN) form was assumed for the random intercept.

Four different approaches were considered under the linear mixed effects model for each energy expenditure variable included in the model. In the first approach, each outcome was analyzed without considering any transformations while the unit of time was minutes. In the second approach, the data were log transformed and the unit for time was also minutes. In the last two approaches, we obtained the mean of each outcome per hour while analyzing the outcomes with and without log transformations, respectively. The log transformations were applied to achieve approximate normality for the data. Under each approach, three types of terms were considered for time. We considered the mixed effects models with linear, quadratic and also cubic terms for time under all the approaches. All the statistical analyses were performed using SAS PROC MIXED (SAS Institute, Cary, NC).

Functional Mixed Effects Models (FMEM)

The Functional Mixed Effects Models (FMEM) is an extension of the linear mixed effects models to the functional setting, where the basic units of analyses are curves [15, 19]. In statistical modeling, assuming linearity when the linearity assumption is invalid or when the forms of the continuous predictors are arbitrary non-linear functions may bias the effect of the given predictor on the response variable [15, 19]. When the linearity assumption cannot be assumed or when one would rather consider arbitrary non-linear relationships, models in nonparametric regression or semi-parametric regression provide alternative approaches to modeling such data [20-22]. Such models allow greater flexibility by allowing the curves to follow arbitrary smooth functions, rather than restricting the curves to be linear [15]. The functional mixed effects model is specified as follows:

$$y_{ij} = X_{ij}\beta(t_{ij}) + Z_{ij}\alpha_i(t_{ij}) + e_{ij},$$

where y_{ij} is response on the i^{th} curve at t_{ij} ; $i = 1, \cdots, n$ and $j = 1, \cdots, mi$; $\beta(t) = \{\beta_1(t), \cdots, \beta_p(t)\}^T$ is a $p \times 1$ vector of fixed functions while $\alpha_i(t) = \{\alpha_{1i}(t), \cdots, \alpha_{qi}(t)\}^T$ is a $q \times 1$ vector of random functions assumed to be zero mean Gaussian processes [15]. The coefficients $\beta(t)$ represents the population average functions while $Z_{ij}\alpha_i(t_{ij})$ represents the deviation of the i^{th} subject-specific curve from the population average functions [15]. The term $X_{ij}\beta(t_{ij}) + Z_{ij}\alpha_i(t_{ij})$ represents the i^{th} subject-specific function [15]. Several approaches can be taken in modeling $\beta(t)$ and $\alpha_i(t_{ij})$ including nonparametric and parametric approaches. When parametric approaches are considered or when the linear or polynomial functions are considered for covariates included in the model, the model reduces to the random effects models [15]. In this paper, we consider modeling $\beta(t)$ and $\alpha_i(t)$ using smoothing splines [15].

The mixed effects model is closely related to the smoothing approaches employed in nonparametric and semi parametric statistics [19]. In nonparametric regression, smoothing splines are often used to fit smooth curves or functions to arbitrary nonlinear functions without restricting the function to be of a particular pattern [19]. An advantage of models such as the functional mixed effects models with smoothing spline is that they allow one to model the relationship between the continuous covariate and the mean response by approximating the nonlinear functions while also adjusting for any induced correlation between the multiple observations collected on each subject or within each cluster [19]. In our current application, we employed both linear and quadratic smoothing splines for modeling the energy expenditure data. These models are specified as

$$y_{ij} = \beta_o + \beta_1 time_i + \beta_2 treatment_i + \sum_{i=1}^{k} \mu_k (time_i - \kappa_k)_+ + b_{oi} + \varepsilon_{ij}$$

$$y_{ij} = \beta_o + \beta_1 time_i + \beta_2 time_i^2 + \beta_3 treatment_i + \sum_{i=1}^{k} \mu_k (time_i - \kappa_k)_+^2 + b_{oi} + \varepsilon_{ij}$$

respectively. In the model, the functions $(time_i - \kappa_k)_+$ and $(time_i - \kappa_k)_+^2$ are known as linear and quadratic spline basis, respectively, while κ_k known as linear as the knots. The smoothing splines and the random intercept, b_{oi}, are treated as random and we assume $\mu_k \sim N(0, \sigma_u^2)$ and $b_{oi} \sim N(0, \sigma_b^2)$. When $\sigma_u^2 = 0$, then above model reduces to the mixed effects models. A defining difference between the functional mixed effects model and the linear mixed effects model is the inclusion of the functional random effects, $(time_i - \kappa_k)_+^{\{p\}}$ which are modeled as normal random curves with mean zero [15]. The higher the degree of the polynomial, the smoother the smoothing spline regression [19]. The smoothness of the curve is controlled by $\lambda = \sqrt{\frac{\sigma_u^2}{\sigma_\varepsilon^2}}$, the smoothing parameter while the mean square error of the model increases with increasing λ [15].

In our application of the functional mixed effects models with smoothing splines, we also consider four modeling approaches. Two linear spline approaches were considered with the untransformed energy expenditure outcome variables observed every 5-10 minutes while the second approach was based on their means per hour over the twenty-four hour period. The same approach was used for the functional mixed effects models with quadratic smoothing splines. We considered 12 equally spaced knots for both the linear and quadratic splines. Since the smoothness of the curves is largely controlled by the smoothing parameter, the number of knots chosen is not crucial [19]. To account for the correlation between the repeated measures on each subject, we also included a random intercept in the model with an unstructured (UN) form for the form of the covariance matrix.

CHAPTER III

DISCUSSION

In this paper, we considered various statistical approaches to analyzing energy expenditure data. We recognize that the energy expenditure data collected by the Oxymas instrument are best described as curves or functions of time, rather than continuous linear repeated observations over time. We recommend either using polynomial terms for time and summarizing the data by hour and taking log transformation when using the linear mixed effect model or considering using a functional mixed effect model with smoothing splines to fit the ZDF rats' data.

CHAPTER IV

CONCLUSION

Linear Mixed Effects Models (LMEM)

Figure 1a provides the plot of $\mathrm{VO_2}$ consumption (L/h/kg BW) against time for the Zucker diabetic fatty rats by treatment group. The plot illustrates that the relationship between $\mathrm{VO_2}$ and time (minutes) is non-linear and there also appears to be an overall quadratic pattern over time. Similar patterns are also observed in Figure 2a where there appears to be a wavering pattern between increases in $\mathrm{VO_2}$ followed by decreases across the 24 hour observation period. The other plots (b, c and d) in Figure 1 and Figure 2 also are graphs of the mean trajectory of $\mathrm{VCO_2}$ (L/h/kg BW), RQ, heat expenditure (kcal/kg BW/h) versus time in minutes and hours respectively, among the ZDF rats at 28 days of age. These graphs also portray non-linear relationships with time as was observed for $\mathrm{VO_2}$ consumption. In addition the shape of the curves in Figure 2 are smoother than the shape of the curves in Figure 1 indicating that summarizing the data per hour seems to reduce some of the random noise associated with the frequency of the data collection.

Linear Term for Time in the LMEM

Table 1 provides the results from the application of the linear mixed effects models with linear time under the various modeling strategies for volume of $\mathrm{O_2}$ consumption in the ZDF rats at 28 days of age. The raw data indicates that the data are untransformed and in the original scale while log $\mathrm{VO_2}$ indicates that the data were log transformed. From Table 1, all the time variables in the four models are statistically significant ($p < 0.05$)

indicating that time is an important predictor or O_2 consumption. We find no statistical significant differences between the low dose IFNT (4 µg IFNT/kg BW/day) and the control group (0 µg IFNT/kg BW/day) and high dose IFNT (8 µg IFNT/kg BW/day) and the control group (0 µg IFNT/kg BW/day).

Figure 3 shows the residuals plots for the volume of O_2 consumption for the various LMEM with linear time term. The residuals vary around zero in Figure 3(a) and 3(b) and appear to be less dispersed and smoother when the data are summarized by hour following a log transformations when compared to the residuals from the models for the raw data with time in units of minutes in Figures 3(c) and 3(d). Therefore, the residual plots 3(a) and 3(b) indicate that the LMEM based on the summarized data per hour and log transformations seem to fit the data better when compared to the raw data analyzed at the minute level for time.

Quadratic Term for Time

Table 2 represents the results from fitting the LMEM with quadratic time term for volume of O_2 consumption (L/h/kg BW) in the ZDF rats at 28 days of age. Time was included in both units of hours and minutes and were all found to be statistically significant $(p < 0.0001)$. The standard errors for the quadratic terms for time were estimated to be very small.

Figure 4 has the corresponding residuals plots for volume of O_2 consumption for the models in Table 2. Figures 4(a) and 4(b) indicate that summarizing the data per hour would also be better for modeling the volume of O_2 consumption when compared to the models based on the non-summarized data in Figures 4(c) and 4(d). Thus, the summarized data per hour seem to fit the data better when compared to the data analyzed at the minute level for time.

Cubic Term for Time

Table 3 provides the results from fitting the LMEM with a cubic time term for volume of O_2 consumption (L/h/kg BW) in the ZDF rats at 28 days of age. For the minutes data with log transformed and non-transformed models, all the polynomial terms for time (linear, quadratic and cubic time) are statistically significant. However, only the linear and quadratic terms for time are statistically significant based on the models where the data are summarized by hour. In other words, the cubic terms for the time effects were not statistically significant based on the data where time is summarized per hour ($p > 0.05$). The standard errors for the quadratic and cubic terms were also estimated to be very small in these models.

Figure 5 provides the residual plots for VO_2 (L/h/kg BW) from the LMEM with a cubic term for time for the ZDF rats at 28 days of age. The residual plots also indicate that it is more beneficial to analyze the data after summarizing the data by hour rather than using the models based on units of minutes for time.

For the other energy expenditure variables namely, $\mathrm{VCO_2}$, RQ and heat expenditure, similar results as reported for $\mathrm{VO_2}$ were also observed.

Functional Mixed Effects Models (FMEM)

Linear Spline and Quadratic Spline

Table 4 shows the result from fitting the FMEM with linear and quadratic splines for volume of $\mathrm{O_2}$ consumption (L/h/kg BW) in the ZDF rats at 28 days of age. None of the variables in the FMEM with the linear smoothing spline were statistically significant (p > 0.05). Similarly, none of the variables in the FMEM with a quadratic spline based on the untransformed data with time in minutes were also found to be significant (p > 0.05). However, the FMEM with quadratic smoothing spline has a significant p-value for time as well as time squared based on the data summarized per hour. The residual plots for the models are in Figure 6. The residual plots based on the FMEM also confirm that summarizing the data by hour prior to analyzing the data seem to fit the data better than not summarizing the data.

According to the results of fitting the LMEM and the FMEM, we find that fitting polynomial terms for time and summarizing the data per hour after taking a log transformation worked better for the LMEMs while fitting a quadratic spline model for the FMEMs seemed to fit the data better than the linear spline model.

The functional mixed effects models considered allow us to treat the data as curves. Also, we find that by taking the average of the energy expenditure variables prior to analyzing them using the functional mixed effects methods, we are able to reduce the random noise in the data which allow the curves to be better approximated with the reduction of the random noise associated with the frequency of measuring the energy expenditure variables.

In analyzing energy expenditure data collected by devices such as the Oxymas instrument where the data are collected frequently, the first step is to evaluate plots of the variables against time where time is in minutes. If there appears to be a lot of random noise or wavering pattern in the plots, we find that summarizing the data by hour or any other desired method of data summary helps to reduce the random noise associated with the frequency of the data collection. If the data can be best described as curves over time, rather than linear, we recommend either using a linear mixed effects model with appropriate polynomial terms for time or the functional mixed effects models with smoothing splines. The use of the smoothing spline is to approximate the shape of the curves and there are several approaches for smoothing a curve. In our current application, linear and quadratic smoothing splines were applied.

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APPENDIX

Table 1. Results from fitting the LMEM with a linear time term for volume of O_2 consumption (L/h/kg BW) in the ZDF rats at 28 days of age

Model	**Outcome**	**Variable**	$\hat{\boldsymbol{\beta}}$	**S.E.**	**P-value**
LMEM (Raw data)	VO_2, L/h/kg BW	Low dose vs. control	0.02670	0.04082	0.5131
		High dose vs. control	-0.02682	0.04082	0.5111
		Time (minutes)	-0.00020	0.00001	<.0001
LMEM (Log transformed)	Log VO_2, L/h/kg BW	Low dose vs. control	0.01827	0.02810	0.5155
		High dose vs. control	-0.01887	0.02810	0.5020
		Time (minutes)	-0.00014	0.00000	<.0001
LMEM (Mean VO_2 per hour)	Mean VO_2, L/h/kg BW	Low dose vs. control	-0.03768	0.09715	0.6981
		High dose vs. control	0.02854	0.09715	0.7689
		Time (hours)	-0.00113	0.00029	0.0001
LMEM (Mean log VO_2 per hour)	Mean log VO_2, L/h/kg BW	Low dose vs. control	-0.02652	0.06044	0.6609
		High dose vs. control	0.01616	0.06044	0.7892
		Time (hours)	-0.00074	0.00019	0.0002

LMEM: Linear mixed effects model; SE: standard error;VO_2: volumetric oxygen consumption; $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log VO_2 indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 2. Results from fitting the LMEM with a quadratic time term for volume of O_2 consumption (L/h/kg BW) in the ZDF rats at 28 days of age

Model	**Outcome**	**Variable**	$\hat{\beta}$	**S.E.**	**P-value**
LMEM (Raw data)	VO_2, L/h/kg BW	Low dose vs. control	0.02551	0.04115	0.5353
		High dose vs. control	-0.02638	0.04115	0.5215
		Time (minutes)	-0.00131	0.00004	<.0001
		Time ∗ Time	7.838E-7	0	<.0001
LMEM (Log transformed)	Log VO_2, L/h/kg BW	Low dose vs. control	0.01748	0.02832	0.5371
		High dose vs. control	-0.01857	0.02832	0.5121
		Time (minutes)	-0.00088	0.00003	<.0001
		Time ∗ Time	5.272E-7	0	<.0001
LMEM (Mean VO_2 per hour)	Mean VO_2, L/h/kg BW	Low dose vs. control	-0.03780	0.09716	0.6972
		High dose vs. control	0.02860	0.09716	0.7685
		Time (hours)	-0.01316	0.00142	<.0001
		Time ∗ Time	0.000448	0.00005	<.0001
LMEM (Mean log VO_2 per hour)	Mean log VO_2, L/h/kg BW	Low dose vs. control	-0.02660	0.06044	0.6599
		High dose vs. control	0.01620	0.06044	0.7887
		Time (hours)	-0.00877	0.00096	<.0001
		Time ∗ Time	0.000299	0.00004	<.0001

LMEM: Linear mixed effects model; SE: standard error; VO_2: volumetric oxygen consumption; $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log VO_2 indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 3. Results from fitting the LMEM with a cubic time term for volume of O_2 consumption (L/h/kg BW) in the ZDF rats at 28 days of age

Model	**Outcome**	**Variable**	$\hat{\beta}$	**S.E.**	**P-value**
LMEM (Raw data)	VO_2, L/h/kg BW	Low dose vs. control	0.02499	0.04128	0.5450
		High dose vs. control	-0.02631	0.04129	0.5240
		Time (minutes)	-0.00061	0.000099	<.0001
		Time ∗ Time	-4.48E-7	0	<.0001
		Time ∗ Time ∗ Time	5.84E-10	0	<.0001
LMEM (Log transformed)	Log VO_2, L/h/kg BW	Low dose vs. control	0.01702	0.02843	0.5495
		High dose vs. control	-0.01851	0.02843	0.5152
		Time (minutes)	-0.00028	0.000066	<.0001
		Time ∗ Time	-5.46E-7	0	<.0001
		Time ∗ Time ∗ Time	5.09E-10	0	<.0001
LMEM (Mean VO_2 per hour)	Mean VO_2, L/h/kg BW	Low dose vs. control	-0.03780	0.09716	0.6973
		High dose vs. control	0.02859	0.09716	0.7686
		Time (hours)	-0.01837	0.004349	<.0001
		Time ∗ Time	0.000898	0.000359	0.0124
		Time ∗ Time ∗ Time	-0.00001	8.898E-6	0.2051
LMEM (Mean log VO_2 per hour)	Mean log VO_2, L/h/kg BW	Low dose vs. control	-0.02660	0.06045	0.6599
		High dose vs. control	0.01620	0.06045	0.7887
		Time (hours)	-0.00994	0.002933	0.0007
		Time ∗ Time	0.000400	0.000242	0.0982
		Time ∗ Time ∗ Time	-2.54E-6	6.001E-6	0.6718

LMEM: Linear mixed effects model; SE: standard error; VO_2: volumetric oxygen consumption; $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log VO_2 indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 4. Results from fitting the FMEM with the linear and the quadratic spline for volume of O_2 consumption (L/h/kg BW) in the ZDF rats at 28 days of age

Model	**Outcome**	**Variable**	$\hat{\beta}$	**S.E.**	**P-value**
FMEM (Linear spline)	VO_2, L/h/kg BW	Low dose vs. control	0.02669	0.04082	0.5133
		High dose vs. control	-0.02682	0.04082	0.5112
		Time (minutes)	-0.1233	0.06751	0.0679
	Mean VO_2, L/h/kg BW	Low dose vs. control	-0.03782	0.09715	0.6971
		High dose vs. control	0.02866	0.09715	0.7680
		Time (hours)	0.005148	0.004072	0.2062
FMEM (Quadratic spline)	VO_2, L/h/kg BW	Low dose vs. control	0.02552	0.04115	0.5352
		High dose vs. control	-0.02638	0.04115	0.5215
		Time (minutes)	-0.04059	0.03112	0.1922
		Time $*$ Time	0.006202	0.007797	0.4264
	Mean VO_2, L/h/kg BW	Low dose vs. control	-0.03763	0.09715	0.6985
		High dose vs. control	0.02858	0.09715	0.7686
		Time (hours)	0.02841	0.007354	0.0001
		Time $*$ Time	-0.00088	0.000314	0.0051

FMEM: Functional mixed effects model; SE: standard error; VO_2: volumetric oxygen consumption; $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log VO_2 indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 5. Results from fitting the LMEM with a linear time term for volume of CO_2 production (L/h/kg BW) in the ZDF rats at 28 days of age

Model	**Outcome**	**Variable**	$\boldsymbol{\widehat{\beta}}$	**S.E.**	**P-value**
LMEM (Raw data)	VCO_2, L/h/kg BW	Low dose vs. control	-0.01733	0.04364	0.6913
		High dose vs. control	-0.02207	0.04364	0.6131
		Time (minutes)	-0.00009	8.92E-6	<.0001
LMEM (Log transformed)	Log VCO_2, L/h/kg BW	Low dose vs. control	-0.01738	0.03785	0.6462
		High dose vs. control	-0.01661	0.03785	0.6608
		Time (minutes)	-0.00008	7.357E-6	<.0001
LMEM (Mean VCO_2 per hour)	Mean VCO_2, L/h/kg BW	Low dose vs. control	-0.09255	0.09210	0.3150
		High dose vs. control	0.01432	0.09210	0.8764
		Time (hours)	-0.00019	0.000237	0.4331
LMEM (Mean log VCO_2 per hour)	Mean log VCO_2, L/h/kg BW	Low dose vs. control	-0.08092	0.07386	0.2734
		High dose vs. control	0.009173	0.07386	0.9012
		Time (hours)	-0.00018	0.000196	0.3663

LMEM: Linear mixed effects model; SE: standard error; VCO_2: volumetric carbon dioxide production; $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log VCO_2 indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 6. Results from fitting the LMEM with a quadratic time term for volume of CO_2 production (L/h/kg BW) in the ZDF rats at 28 days of age

Model	Outcome	Variable	$\hat{\beta}$	S.E.	P-value
LMEM (Raw data)	VCO_2, L/h/kg BW	Low dose vs. control	-0.01813	0.04358	0.6775
		High dose vs. control	-0.02177	0.04358	0.6174
		Time (minutes)	-0.00082	0.000033	<.0001
		Time ∗ Time	5.233E-7	0	<.0001
LMEM (Log transformed)	Log VCO_2, L/h/kg BW	Low dose vs. control	-0.01804	0.03776	0.6329
		High dose vs. control	-0.01637	0.03776	0.6647
		Time (minutes)	-0.00069	0.000027	<.0001
		Time ∗ Time	4.344E-7	0	<.0001
LMEM (Mean VCO_2 per hour)	Mean VCO_2, L/h/kg BW	Low dose vs. control	-0.09262	0.09209	0.3146
		High dose vs. control	0.01435	0.09209	0.8762
		Time (hours)	-0.00641	0.001154	<.0001
		Time ∗ Time	0.000232	0.000042	<.0001
LMEM (Mean log VCO_2 per hour)	Mean log VCO_2, L/h/kg BW	Low dose vs. control	-0.08097	0.07384	0.2729
		High dose vs. control	0.009198	0.07384	0.9009
		Time (hours)	-0.00541	0.000952	<.0001
		Time ∗ Time	0.000195	0.000035	<.0001

LMEM: Linear mixed effects model; SE: standard error; VCO_2: volumetric oxygen consumption; $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log VCO_2 indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 7. Results from fitting the LMEM with a cubic time term for volume of CO_2 production (L/h/kg BW) in the ZDF rats at 28 days of age

Model	Outcome	Variable	$\hat{\beta}$	S.E.	P-value
LMEM (Raw data)	VCO_2, L/h/kg BW	Low dose vs. control	-0.01850	0.04358	0.6712
		High dose vs. control	-0.02172	0.04358	0.6182
		Time (minutes)	-0.00033	0.000081	<.0001
		Time ∗ Time	-3.52E-7	0	<.0001
		Time ∗ Time ∗ Time	4.15E-10	0	<.0001
LMEM (Log transformed)	Log VCO_2, L/h/kg BW	Low dose vs. control	-0.01841	0.03774	0.6257
		High dose vs. control	-0.01632	0.03774	0.6655
		Time (minutes)	-0.00019	0.000067	0.0041
		Time ∗ Time	-4.45E-7	0	<.0001
		Time ∗ Time ∗ Time	4.17E-10	0	<.0001
LMEM (Mean VCO_2 per hour)	Mean VCO_2, L/h/kg BW	Low dose vs. control	-0.09262	0.09209	0.3146
		High dose vs. control	0.01436	0.09209	0.8761
		Time (hours)	-0.00248	0.003536	0.4837
		Time ∗ Time	-0.00011	0.000292	0.7118
		Time ∗ Time ∗ Time	8.506E-6	7.235E-6	0.2398
LMEM (Mean log VCO_2 per hour)	Mean log VCO_2, L/h/kg BW	Low dose vs. control	-0.08098	0.07384	0.2728
		High dose vs. control	0.00920	0.07384	0.9008
		Time (hours)	-0.00112	0.002919	0.7010
		Time ∗ Time	-0.00018	0.000241	0.4661
		Time ∗ Time ∗ Time	9.283E-6	5.972E-6	0.1202

LMEM: Linear mixed effects model; SE: standard error; VCO_2: volumetric oxygen consumption; $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log VCO_2 indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 8. Results from fitting the FMEM with the linear and the quadratic spline for volume of CO_2 production (L/h/kg BW) in the ZDF rats at 28 days of age

Model	Outcome	Variable	$\hat{\beta}$	S.E.	P-value
FMEM (Linear spline)	VCO_2, L/h/kg BW	Low dose vs. control	-0.01734	0.04364	0.6912
		High dose vs. control	-0.02207	0.04364	0.6131
		Time (minutes)	-0.07630	0.04358	0.0801
	Mean VCO_2, L/h/kg BW	Low dose vs. control	-0.09270	0.09205	0.3140
		High dose vs. control	0.01438	0.09205	0.8758
		Time (hours)	0.002107	0.003504	0.5476
FMEM (Quadratic spline)	VCO_2, L/h/kg BW	Low dose vs. control	-0.01813	0.04358	0.6775
		High dose vs. control	-0.02177	0.04358	0.6174
		Time (minutes)	-0.00691	0.01305	0.5963
		Time $*$ Time	0.000960	0.002688	0.7210
	Mean VCO_2, L/h/kg BW	Low dose vs. control	-0.09249	0.09211	0.3154
		High dose vs. control	0.01432	0.09211	0.8765
		Time (hours)	0.01843	0.005797	0.0015
		Time $*$ Time	-0.00056	0.000247	0.0237

FMEM: Functional mixed effects model; SE: standard error; VCO_2: volumetric oxygen consumption; $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log VCO_2 indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 9. Results from fitting the LMEM with a linear time term for respiratory quotient in the ZDF rats at 28 days of age

Model	Outcome	Variable	$\hat{\beta}$	S.E.	P-value
LMEM (Raw data)	RQ, L/h/kg BW	Low dose vs. control	-0.02857	0.01473	0.0525
		High dose vs. control	0.001814	0.01473	0.9020
		Time (minutes)	0.000050	1.374E-6	<.0001
LMEM (Log transformed)	Log RQ, L/h/kg BW	Low dose vs. control	-0.03581	0.01890	0.0583
		High dose vs. control	0.002247	0.01890	0.9054
		Time (minutes)	0.000062	1.72E-6	<.0001
LMEM (Mean RQ per hour)	Mean RQ, L/h/kg BW	Low dose vs. control	-0.04360	0.01998	0.0292
		High dose vs. control	-0.00695	0.01998	0.7281
		Time (hours)	0.000460	0.000035	<.0001
LMEM (Mean log RQ per hour)	Mean log RQ, L/h/kg BW	Low dose vs. control	-0.05548	0.02595	0.0326
		High dose vs. control	-0.00808	0.02595	0.7555
		Time (hours)	0.000571	0.000045	<.0001

LMEM: Linear mixed effects model; SE: standard error; RQ: respiratory quotient (O_2 consumption/ CO_2 production); $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log RQ indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 10. Results from fitting the LMEM with a quadratic time term for respiratory quotient in the ZDF rats at 28 days of age

Model	Outcome	Variable	$\hat{\beta}$	S.E.	P-value
LMEM (Raw data)	RQ, L/h/kg BW	Low dose vs. control	-0.02846	0.01480	0.0547
		High dose vs. control	0.001772	0.01480	0.9047
		Time (minutes)	0.000154	5.151E-6	<.0001
		Time ∗ Time	-7.43E-8	0	<.0001
LMEM (Log RQ transformed)	Log RQ, L/h/kg BW	Low dose vs. control	-0.03567	0.01900	0.0606
		High dose vs. control	0.002195	0.01900	0.9080
		Time (minutes)	0.000192	6.452E-6	<.0001
		Time ∗ Time	-9.26E-8	0	<.0001
LMEM (Mean RQ per hour)	Mean RQ, L/h/kg BW	Low dose vs. control	-0.04358	0.02000	0.0295
		High dose vs. control	-0.00696	0.02000	0.7280
		Time (hours)	0.002716	0.000167	<.0001
		Time ∗ Time	-0.00008	6.09E-6	<.0001
LMEM (Mean log RQ per hour)	Mean log RQ, L/h/kg BW	Low dose vs. control	-0.05545	0.02598	0.0329
		High dose vs. control	-0.00810	0.02598	0.7553
		Time (hours)	0.003380	0.000212	<.0001
		Time ∗ Time	-0.00010	7.743E-6	<.0001

LMEM: Linear mixed effects model; SE: standard error; RQ: respiratory quotient (O_2 consumption/ CO_2 production); $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log RQ indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 11. Results from fitting the LMEM with a cubic time term for respiratory quotient in the ZDF rats at 28 days of age

Model	Outcome	Variable	$\hat{\beta}$	S.E.	P-value
LMEM (Raw data)	RQ, L/h/kg BW	Low dose vs. control	-0.02838	0.01484	0.0558
		High dose vs. control	0.001763	0.01484	0.9054
		Time (minutes)	0.000059	0.000013	<.0001
		Time ∗ Time	9.631E-8	0	<.0001
		Time ∗ Time ∗ Time	-808E-13	0	<.0001
LMEM (Log RQ transformed)	Log RQ, L/h/kg BW	Low dose vs. control	-0.03558	0.01904	0.0617
		High dose vs. control	0.002184	0.01904	0.9087
		Time (minutes)	0.000082	0.000016	<.0001
		Time ∗ Time	1.035E-7	0	<.0001
		Time ∗ Time ∗ Time	-929E-13	0	<.0001
LMEM (Mean RQ per hour)	Mean RQ, L/h/kg BW	Low dose vs. control	-0.04358	0.01998	0.0292
		High dose vs. control	-0.00695	0.01998	0.7280
		Time (hours)	0.007038	0.000507	<.0001
		Time ∗ Time	-0.00046	0.000042	<.0001
		Time ∗ Time ∗ Time	9.355E-6	0	<.0001
LMEM (Mean log RQ per hour)	Mean log RQ, L/h/kg BW	Low dose vs. control	-0.05546	0.02595	0.0326
		High dose vs. control	-0.00809	0.02595	0.7553
		Time (hours)	0.008908	0.000644	<.0001
		Time ∗ Time	-0.00058	0.000053	<.0001
		Time ∗ Time ∗ Time	0.000012	1.318E-6	<.0001

LMEM: Linear mixed effects model; SE: standard error; RQ: respiratory quotient (O_2 consumption/ CO_2 production); $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log RQ indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 12. Results from fitting the FMEM with the linear and the quadratic spline for respiratory quotient in the ZDF rats at 28 days of age

Model	Outcome	Variable	$\hat{\beta}$	S.E.	P-value
FMEM (Linear spline)	RQ, L/h/kg BW	Low dose vs. control	-0.02857	0.01473	0.0525
		High dose vs. control	0.001814	0.01473	0.9020
		Time (minutes)	0.007767	0.004892	0.1125
	Mean RQ, L/h/kg BW	Low dose vs. control	-0.04362	0.02000	0.0292
		High dose vs. control	-0.00698	0.02000	0.7272
		Time (hours)	-0.00230	0.000722	0.0014
FMEM (Quadratic spline)	RQ, L/h/kg BW	Low dose vs. control	-0.02846	0.01480	0.0547
		High dose vs. control	0.001772	0.01480	0.9047
		Time (minutes)	0.000046	0.003512	0.9907
		Time ∗ Time	0.000018	0.000817	0.9826
	Mean RQ, L/h/kg BW	Low dose vs. control	-0.04359	0.02001	0.0295
		High dose vs. control	-0.00696	0.02001	0.7278
		Time (hours)	-0.00279	0.000947	0.0032
		Time ∗ Time	0.000093	0.000040	0.0214

FMEM: Functional mixed effects model; SE: standard error; RQ: respiratory quotient (O_2 consumption/ CO_2 production); $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log RQ indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 13. Results from fitting the LMEM with a linear time term for heat production (kcal/kg BW/h) in the ZDF rats at 28 days of age

Model	Outcome	Variable	$\hat{\beta}$	S.E.	P-value
LMEM (Raw data)	Heat, L/h/kg BW	Low dose vs. control	-0.1064	0.1403	0.4483
		High dose vs. control	-0.1989	0.1403	0.1562
		Time (minutes)	-0.00030	0.000018	<.0001
LMEM (Log transformed)	Log Heat, L/h/kg BW	Low dose vs. control	-0.05660	0.06559	0.3882
		High dose vs. control	-0.08460	0.06560	0.1972
		Time (minutes)	-0.00012	7.479E-6	<.0001
LMEM (Mean Heat per hour)	Mean Heat, L/h/kg BW	Low dose vs. control	-0.2113	0.2022	0.2960
		High dose vs. control	-0.1214	0.2022	0.5482
		Time (hours)	-0.00154	0.000486	0.0015
LMEM (Mean log Heat per hour)	Mean log Heat, L/h/kg BW	Low dose vs. control	-0.1044	0.08938	0.2431
		High dose vs. control	-0.05104	0.08938	0.5680
		Time (hours)	-0.00063	0.000203	0.0020

LMEM: Linear mixed effects model; SE: standard error; Heat: heat production; $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log heat production indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 14. Results from fitting the LMEM with a quadratic time term for heat production in the ZDF rats at 28 days of age

Model	**Outcome**	**Variable**	$\hat{\beta}$	**S.E.**	**P-value**
LMEM (Raw data)	Heat, L/h/kg BW	Low dose vs. control	-0.1082	0.1390	0.4363
		High dose vs. control	-0.1982	0.1390	0.1539
		Time (minutes)	-0.00201	0.000066	<.0001
		Time ∗ Time	1.218E-6	0	<.0001
LMEM (Log transformed)	Log Heat, L/h/kg BW	Low dose vs. control	-0.05738	0.06501	0.3775
		High dose vs. control	-0.08432	0.06501	0.1947
		Time (minutes)	-0.00084	0.000027	<.0001
		Time ∗ Time	5.084E-7	0	<.0001
LMEM (Mean Heat per hour)	Mean Heat, L/h/kg BW	Low dose vs. control	-0.2115	0.2021	0.2953
		High dose vs. control	-0.1213	0.2021	0.5482
		Time (hours)	-0.01944	0.002352	<.0001
		Time ∗ Time	0.000667	0.000086	<.0001
LMEM (Mean log Heat per hour)	Mean log Heat, L/h/kg BW	Low dose vs. control	-0.1044	0.08932	0.2424
		High dose vs. control	-0.05101	0.08932	0.5680
		Time (hours)	-0.00809	0.000983	<.0001
		Time ∗ Time	0.000278	0.000036	<.0001

LMEM: Linear mixed effects model; SE: standard error; Heat: heat production; $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log heat production indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 15. Results from fitting the LMEM with a cubic time term for heat production in the ZDF rats at 28 days of age

Model	Outcome	Variable	$\hat{\beta}$	S.E.	P-value
LMEM (Raw data)	Heat, L/h/kg BW	Low dose vs. control	-0.1091	0.1386	0.4311
		High dose vs. control	-0.1981	0.1386	0.1529
		Time (minutes)	-0.00082	0.000163	<.0001
		Time ∗ Time	-9.02E-7	0	<.0001
		Time ∗ Time ∗ Time	1.005E-9	0	<.0001
LMEM (Log transformed)	Log Heat, L/h/kg BW	Low dose vs. control	-0.05782	0.06479	0.3723
		High dose vs. control	-0.08426	0.06479	0.1935
		Time (minutes)	-0.00026	0.000066	<.0001
		Time ∗ Time	-5.22E-7	0	<.0001
		Time ∗ Time ∗ Time	4.88E-10	0	<.0001
LMEM (Mean Heat per hour)	Mean Heat, L/h/kg BW	Low dose vs. control	-0.2115	0.2021	0.2953
		High dose vs. control	-0.1213	0.2021	0.5482
		Time (hours)	-0.02276	0.007213	0.0016
		Time ∗ Time	0.000953	0.000595	0.1093
		Time ∗ Time ∗ Time	-7.18E-6	0.000015	0.6264
LMEM (Mean log Heat per hour)	Mean log Heat, L/h/kg BW	Low dose vs. control	-0.1044	0.08931	0.2423
		High dose vs. control	-0.05101	0.08931	0.5679
		Time (hours)	-0.00822	0.003014	0.0064
		Time ∗ Time	0.000289	0.000249	0.2448
		Time ∗ Time ∗ Time	-2.81E-7	6.166E-6	0.9636

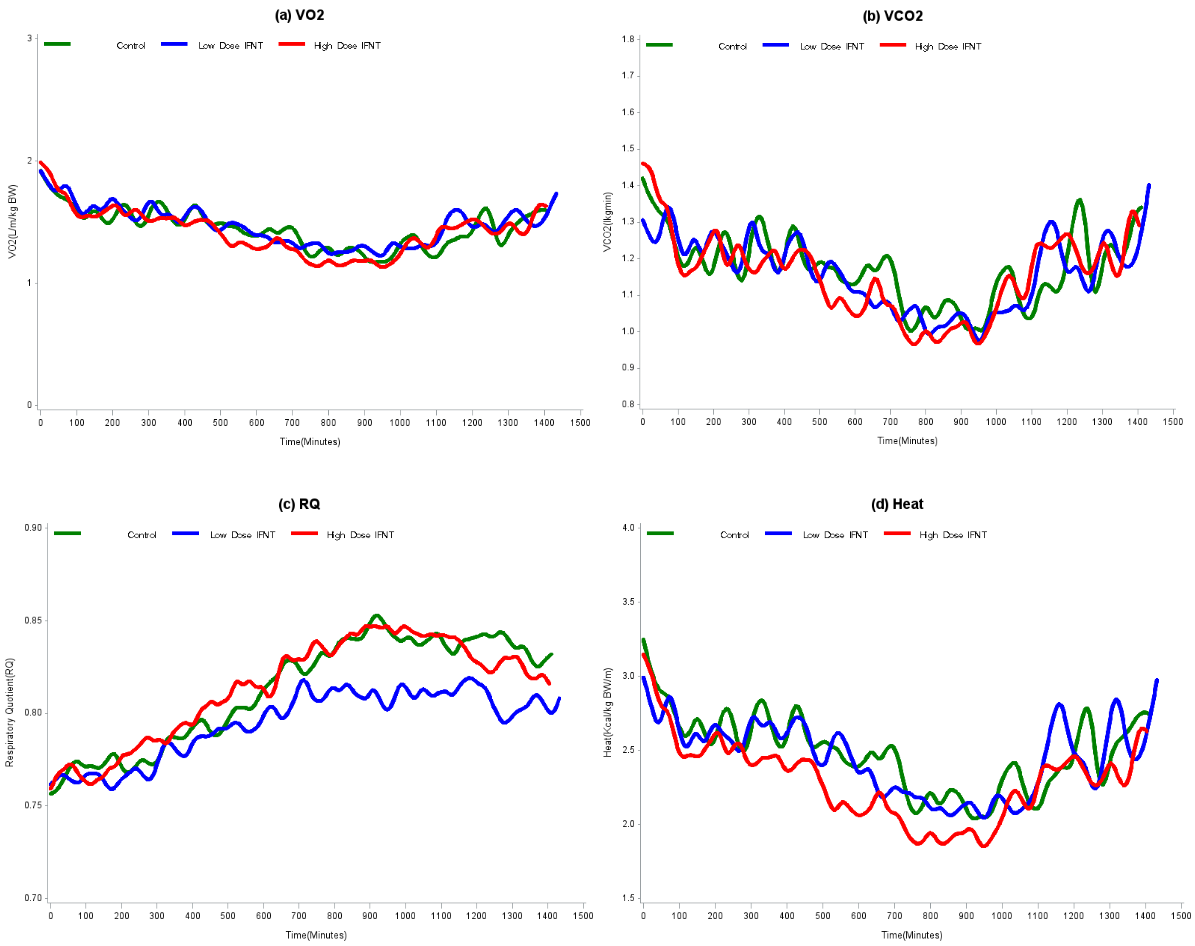
LMEM: Linear mixed effects model; SE: standard error; Heat: heat production; $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log heat production indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Table 16. Results from fitting the FMEM with the linear and the quadratic spline for heat production in the ZDF rats at 28 days of age

Model	Outcome	Variable	$\hat{\beta}$	S.E.	P-value
FMEM (Linear spline)	Heat, L/h/kg BW	Low dose vs. control	-0.1064	0.1403	0.4482
		High dose vs. control	-0.1989	0.1403	0.1562
		Time (minutes)	-0.1843	0.1026	0.0725
	Mean Heat, L/h/kg BW	Low dose vs. control	-0.2115	0.2019	0.2949
		High dose vs. control	-0.1213	0.2019	0.5482
		Time (hours)	0.007203	0.006321	0.2545
FMEM (Quadratic spline)	Heat, L/h/kg BW	Low dose vs. control	-0.1082	0.1390	0.4363
		High dose vs. control	-0.1982	0.1390	0.1539
		Time (minutes)	-0.04108	0.04237	0.3323
		Time $*$ Time	0.006160	0.009578	0.5202
	Mean Heat, L/h/kg BW	Low dose vs. control	-0.2112	0.2021	0.2962
		High dose vs. control	-0.1214	0.2021	0.5482
		Time (hours)	0.04342	0.01211	0.0003
		Time $*$ Time	-0.00134	0.000516	0.0095

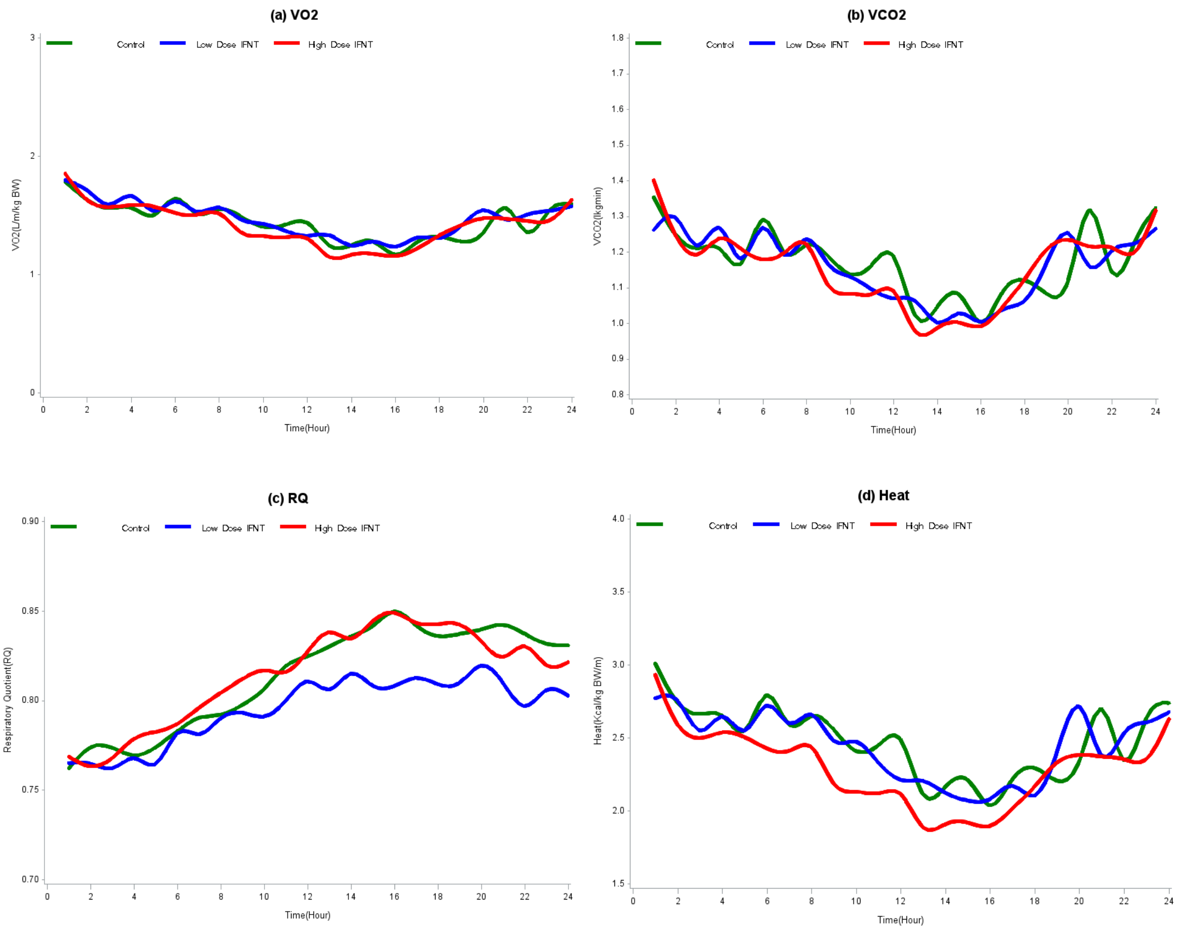
FMEM: Functional mixed effects model; SE: standard error; Heat: heat production; $\hat{\beta}$: estimated coefficient; ZDF: Zucker diabetic fatty rats. There are three treatment groups (control, low, and high doses) in the study. The results in the table indicate the comparison of low dose treatment group to the control group and the comparison of the high dose treatment group to the control group. Raw data indicates that the data are untransformed and on the original scale while log heat production indicates that the data were log transformed. The units of time considered are minutes and hour for the data summarized by hour.

Figure 1. Plots of the mean trajectories of VO_2 (L/h/kg BW), VCO_2 (L/h/kg BW), RQ, heat expenditure (kcal/kg BW/h) against time (minutes) over a 24 hour period among the ZDF rats at 28 days of age.



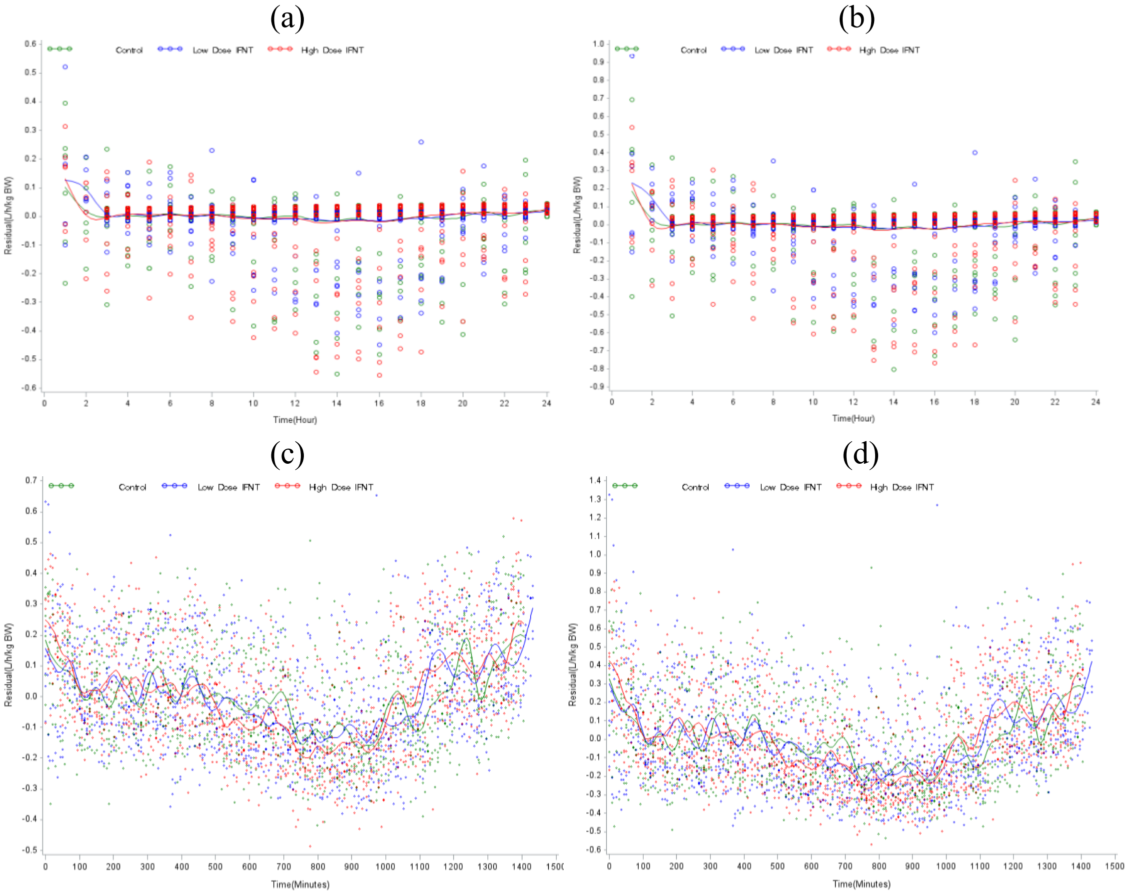
(a) Plot of untransformed VO_2 over time in minutes; (b) Plot of untransformed VCO_2 over time in minutes; (c) Plot of untransformed RQ over time in minutes; (d) Plot of untransformed heat over time in minutes. The plots indicate that all the energy expenditure variables considered in the study are not linearly related to time and are best represented as curves or non-linear functions of time. Quadratic patterns are observed for the energy expenditure variables. (VO_2: volume of oxygen consumption, VCO_2: volume of carbon dioxide production, RQ: respiratory quotient, heat: heat expenditure, ZDF: Zucker diabetic fatty rats)

Figure 2. Plots of the mean trajectories of $\mathrm{VO_2}$ (L/h/kg BW), $\mathrm{VCO_2}$ (L/h/kg BW), RQ, heat expenditure (kcal/kg BW/h) against time (hours) in a 24 hour period among the ZDF rats at 28 days of age.



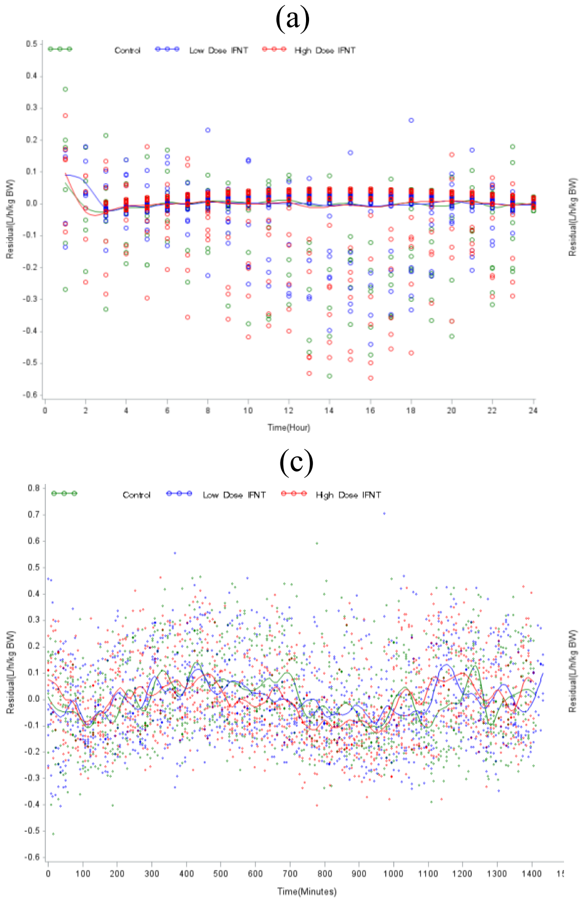
(a) Plot of untransformed $\mathrm{VO_2}$ over time in hours; (b) Plot of untransformed $\mathrm{VCO_2}$ over time in hours; (c) Plot of untransformed RQ over time in hours; (d) Plot of untransformed heat over time in hours. The plots indicate that all the energy expenditure variables considered in the study are not linearly related to time and are best represented as curves or non-linear functions of time. Quadratic patterns are observed for the energy expenditure variables. Moreover the shape of the curves in Figure 2 are smoother than the shape of the curves in Figure 1 indicating that summarizing the data per hours reduce some of the random noise associated with the frequency of the data collection. ($\mathrm{VO_2}$: volume of oxygen consumption, $\mathrm{VCO_2}$: volume of carbon dioxide production, RQ: respiratory quotient, heat: heat expenditure, ZDF: Zucker diabetic fatty rats)

Figure 3. Residual plots for VO_2 (L/h/kg BW) from the LMEM with a linear term for time for the ZDF rats at 28 days of age.



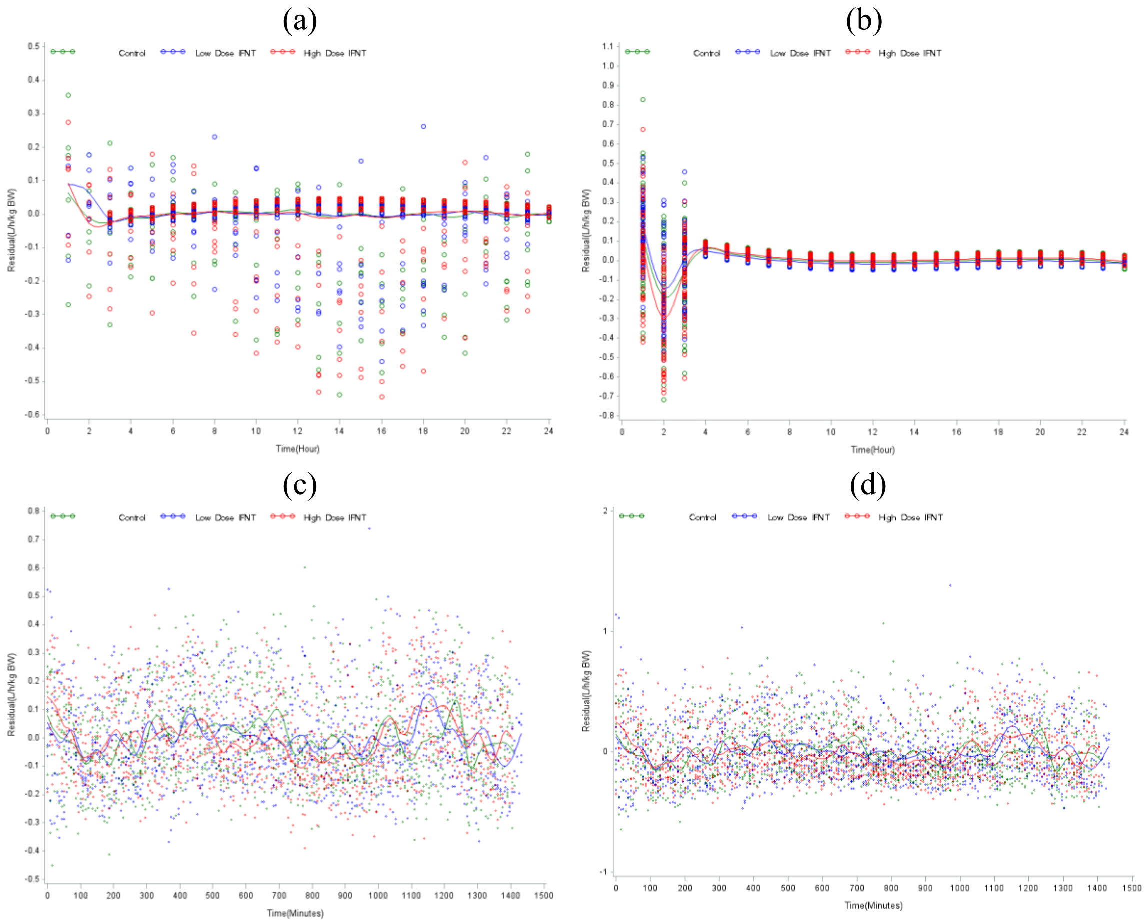
(a) Plot of the residuals from the LMEM with linear time effect for log transformed VO_2 vs. time (hours); (b) Plot of the residuals from the LMEM with linear time effect for VO_2 vs. time (hours); (c) Plot of the residuals from the LMEM with linear time effect for log transformed VO_2 vs. time (minutes); (d) Plot of the residuals from the LMEM with linear time effect for VO_2 vs. time (minutes). The residual plots (a) and (b) indicate that the LMEM based on the summarized data per hour seem to fit the data better when compared to the data analyzed at the minute level for time. (VO_2: volume of oxygen consumption, ZDF: Zucker diabetic fatty rats)

Figure 4. Residual plots for VO_2 (L/h/kg BW) from the LMEM with a quadratic term for time for the ZDF rats at 28 days of age.



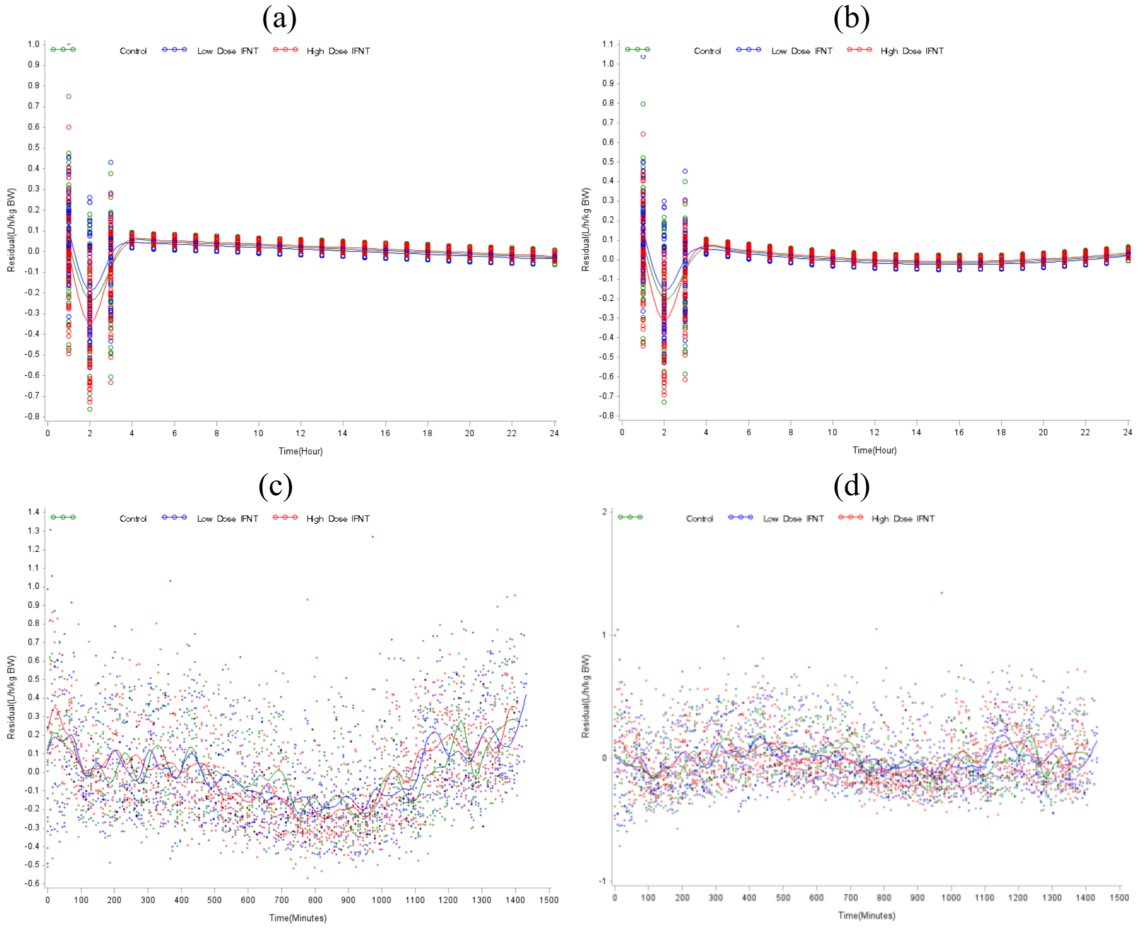
(a) Plot of the residuals from the LMEM with quadratic time effect for log transformed VO_2 vs. time (hours); (b) Plot of the residuals from the LMEM with quadratic time effect for VO_2 vs. time (hours); (c) Plot of the residuals from the LMEM with quadratic time effect for log transformed VO_2 vs. time (minutes); (d) Plot of the residuals from the LMEM with quadratic time effect for VO_2 vs. time (minutes). The residual plots (a) and (b) indicate that the LMEM based on the summarized data per hour seem to fit the data better when compared to the data analyzed at the minute level for time. (VO_2: volume of oxygen consumption, ZDF: Zucker diabetic fatty rats)

Figure 5. Residual plots for VO_2 (L/h/kg BW) from the LMEM with a cubic term for time for the ZDF rats at 28 days of age.



(a) Plot of the residuals from the LMEM with cubic time effect for log transformed VO_2 vs. time (hours); (b) Plot of the residuals from the LMEM with cubic time effect for VO_2 vs. time (hours); (c) Plot of the residuals from the LMEM with cubic time effect for log transformed VO_2 vs. time (minutes); (d) Plot of the residuals from the LMEM with cubic time effect for VO_2 vs. time (minutes). The residual plots (a) and (b) indicate that the LMEM based on the summarized data per hour seem to fit the data better when compared to the data analyzed at the minute level for time. (VO_2: volume of oxygen consumption, ZDF: Zucker diabetic fatty rats)

Figure 6. Residual plots for VO_2 (L/h/kg BW) from the FMEM with linear and quadratic spline for time for the ZDF rats at 28 days of age.



(a) Plot of the residuals from the FMEM with linear spline for VO_2 vs. time (hours); (b) Plot of the residuals from the FMEM with quadratic spline for VO_2 vs. time (hours); (c) Plot of the residuals from the FMEM with linear spline for VO_2 vs. time (minutes); (d) Plot of the residuals from the FMEM with quadratic spline for VO_2 vs. time (minutes). The residual plots (a) and (b) indicate that the FMEM based on the summarized data per hour seem to fit the data better when compared to the data analyzed at the minute level for time. (VO_2: volume of oxygen consumption, ZDF: Zucker diabetic fatty rats)