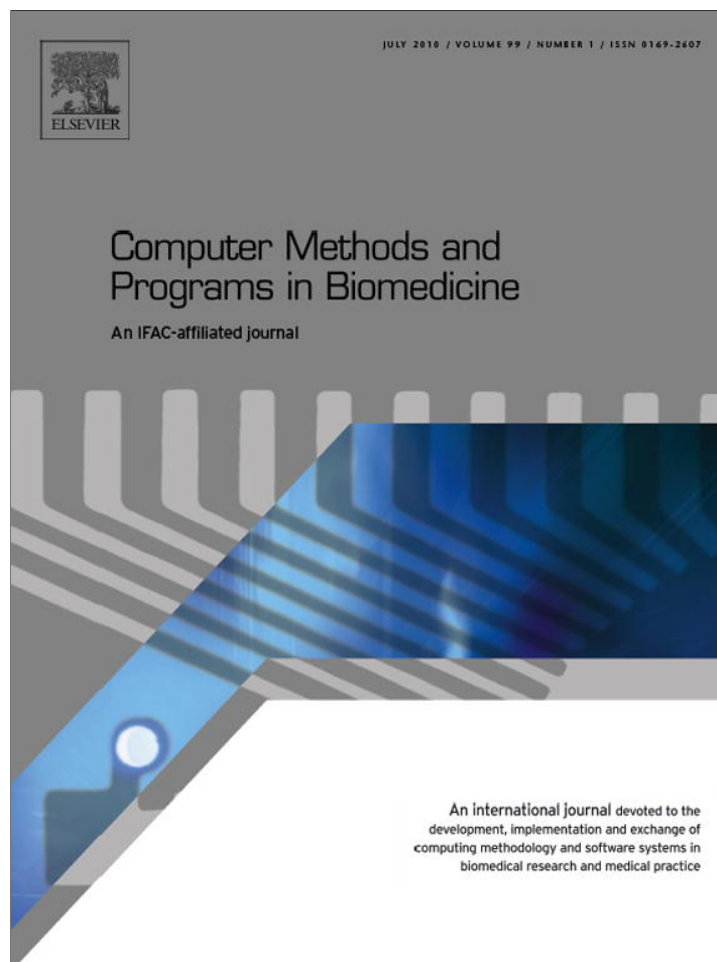


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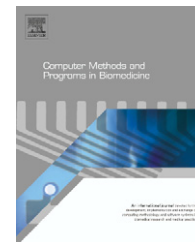
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## Assessing erectile neurogenic dysfunction from heart rate variability through a Generalized Linear Mixed Model framework

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### ABSTRACT

**Background:** The low (LF) vs. high (HF) frequency energy ratio, computed from the spectral decomposition of heart beat intervals, has become a major tool in cardiac autonomic system control and sympatho–vagal balance studies. The (statistical) distributions of response variables designed from ratios of two quantities, such as the LF/HF ratio, are likely to non-normal, hence preventing e.g., from a relevant use of the t-test. Even using a non-parametric formulation, the solution may be not appropriate as the test statistics do not account for correlation and heteroskedasticity, such as those that can be observed when several measures are taken from the same patient.

**Objectives:** The analyses for such type of data require the application of statistical models which do not assume a priori independence. In this spirit, the present contribution proposes the use of the Generalized Linear Mixed Models (GLMMs) framework to assess differences between groups of measures performed over classes of patients.

**Methods:** Statistical linear mixed models allow the inclusion of at least one random effect, besides the error term, which induces correlation between observations from the same subject. Moreover, by using GLMM, practitioners could assume any probability distribution, within the exponential family, for the data, and naturally model heteroskedasticity. Here, the sympatho–vagal balance expressed as LF/HF ratio of patients suffering neurogenic erectile dysfunction under three different body positions was analyzed in a case–control protocol by means of a GLMM under gamma and Gaussian distributed responses assumptions.

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**Results:** The gamma GLMM model was compared with the normal linear mixed model (LMM) approach conducted using raw and log transformed data. Both raw GLMM gamma and log transformed LMM allow better inference for factor effects, including correlations between observations from the same patient under different body position compared to the raw LMM. The gamma GLMM provides a more natural distribution assumption of a response expressed as a ratio.

**Conclusions:** A gamma distribution assumption intrinsically models quadratic relationships between the expected value and the variance of the data avoiding prior data transformation. SAS and R source code are available on request.

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## 1. Introduction

Heart rate variability (HRV) consists of the fluctuation between the intervals of consecutive normal heartbeats (RR intervals). It has become increasingly important in physiological studies. A signal derived from the RR intervals could provide meaningful information regarding the neural regulation of the cardiovascular system [1]. The RR signals can be studied either in the time or frequency domains [2]. For the latter case, the RR spectra are usually split in (at least) three frequency bands: the Very Low Frequency (VLF), low frequency (LF) and high frequency (HF) bands. A number of studies (cf. e.g. [2–4]) suggest that the LF band reflects sympathetic and vagal modulations while the HF band consists of a marker for vagal modulation. Consequently, the LF/HF ratio is considered a mirror of the sympatho/vagal balance, hence characterizing their relationships and commonly used as a non-invasive way of studying the health state of the cardiovascular system [2–5].

Erectile dysfunction is defined as the inability to achieve and maintain an erection sufficient to permit satisfactory sexual intercourse [1,6,7]. Neurogenic erectile dysfunctions are an important group of organic etiologies probably because a deficiency of neurotransmitters is the final common pathway in many diseases and conditions [1,6,8]. A research area of increasing interest consists of studying the benefits of using spectral analysis to screen neurogenic erectile dysfunction by means of the heart rate variability [9–11].

Dynamical state modifications such as those provoked by body position changes (i.e., supine, seated and standing) are usually used as tools to analyze the sympatho-vagal balance by means of the LF/HF index under different treatments or health conditions [12]. In these experiments the LF/HF ratio and the Normalized LF and HF values are calculated at each of the body position states for the same patient. Usually, the data are analyzed by means of a *t*-test (hence assuming independence amongst data) or of its non-parametric counterpart, the Mann-Whitney test [13]. In any case, prior to analyze the statistical significance of treatment group differences, some distributional properties must be assessed, such as independently distributed normal data (i.i.d.) for the classical *t*-test. If the normal distribution assumption cannot be considered valid, non-parametric methods are usually employed. Yet, i.i.d. and variance homogeneity assumptions are still required [14]. Several experimental situations can lead to unfulfilling these required assumptions. For example, when several measures are taken on the same patient under different conditions and/or at different time intervals, correlated data structures

are expected over the response data. Also, different variances between subjects belonging to different groups or sampling times could be expected. The use of classical statistical tests is not longer appropriate in these cases [15,16].

Nowadays, the statistical theory as well as commercial statistical software has been significantly enhanced, allowing researchers to better fit experimental data even under more complex situations. One of these new approaches is known as Generalized Linear Mixed Models [14]. This kind of models allows the exploration of different effects that could impact the data as well as the consideration of more appropriate distributional assumptions for the observed data and, at the same time, considering different types of variation and correlations by using random effects.

In the present work we show that for the assessment of the neurogenic erectile dysfunction using the LF/HF ratio, a model assuming the classical independent and normal distributed observations does not properly explain the underlying data structure, leading to a poor inference and thus yielding inadequate conclusions. To overcome this limitation two new approaches using random effects are suggested. The first one is based on a log transformation of the responses, which deals with the non-normal distribution and the observed heteroskedasticity. The second approach is based on directly assuming a gamma (non-normal) distribution for the LF/HF ratio data.

## 2. Subjects and data

The study was approved by the local Ethics Committee (Hospices Civil de Lyon, France). After explanation of the experimental procedure, written informed consent was obtained from each subject. The diagnosis and recruited patients with an erectile dysfunction, defined as insufficient rigidity of the penis for penetration, was made by a single urologist. Staffs of Hospices Civils de Lyon, Université Claude Bernard and École Normale Supérieure de Lyon were recruited as a control group. All subjects underwent neurological, physical, urologic and psychiatric examinations. Subjects were allocated into two groups: control, no erectile dysfunction ( $n=17$ ), and patients with erectile dysfunction ( $n=15$ ). The mean duration of erectile dysfunction in this group was 4 years. Erectile dysfunction was determined with the International Index of Erectile Function (IIEF) questionnaire; 53% ( $n=8$ ) of the patients had a mild erectile dysfunction (IIEF score between 17 and 25), 34% ( $n=5$ ) had a moderate erectile dysfunction (IIEF score between 11 and 16), and 13% ( $n=2$ )

had severe erectile dysfunction (IIEF score between 10 and 1). All patients of the control group had an IIEF score above 26.

Each participant was asked to refrain from drinking coffee and tea in the 24 h preceding the experimental session. Recording sessions took place between 9:00 a.m. and 12:00 a.m. in a quiet room. Data acquisition was done on a beat-by-beat basis. Collected data consist of: (1) the RR interval (RRI) time in ms between two R-peaks on the electrocardiogram (ECG), obtained from a standard bipolar ECG lead and an R-peak detection circuit with precision of 1 ms, (2) systolic blood pressure (SBP) and diastolic blood pressure (DBP) obtained by finger photoplethysmography (Finapres™ TNO, Biomedical Instrumentation Research Unit, Amsterdam, The Netherlands).

During test, heart rate and blood pressure were monitored with an ECG monitor and an automated oscillometer (Dynamap™ Criticon Inc.™, Tampa, FL, USA).

Breathing was quantified by a device constructed in the Laboratoire de Physiologie de l'Environnement (Faculté de Médecine Lyon Grange-Blanche). This device uses comfortable elastic bands, which do not restrict breathing movements, wrapped around the rib cage and abdomen to measure thoracic and abdominal displacements during breathing.

Continuous data acquisition (ECG, blood pressure and respiration) at a sampling rate of 1000 Hz (ECG, blood pressure) and 500 Hz (respiration), were done on a PC Pentium™ 133 MHz with a 12-bit analogue-to-digital converter (AT-MIO-16E-10; National Instruments™, Austin, TX, USA) equipped with a software developed with LabVIEW 4.0.1 software™ (National Instruments™, Austin, TX, USA). RRI, blood pressure and respiratory frequency records were performed over three body positions: supine position (15 min), followed by seated position (15 min), and finally standing position (15 min). These postural changes provoke instantaneous changes in heart rate and blood pressure mainly resulting of autonomic modifications [10,17]. The body position sequence was fixed and was the same for all subjects. The test was interrupted before the end of the 15 min if presyncopal or syncopal symptoms occurred (a feeling of faintness), rapid drop in systolic blood pressure (more than 25 mmHg) or tachycardia (more than 160 beat/min).

### 3. Data pre-processing

A sliding-window median filter was applied to the recorded RRI and SBP data to replace outliers and/or abnormal values with a local average.

RRI and SBP data were independently re-sampled on a regular grid, at sampling frequency:  $f_e = 10$  Hz, and hence transformed into time series to which spectral analysis can be applied. A standard linear detrending procedure was systematically applied to each time series. To finish with, the data were high pass filtered. The cut-off frequency was:  $f_c = 0.025$  Hz. The spectral powers were split into 3 frequency bands: very low frequency (VLF, 0.025–0.040 Hz), low frequency (LF, 0.040–0.150 Hz), and high frequency (HF, 0.150–0.400 Hz).

The frequency raw ratio for subject “s” under condition “c” (control, disease) and body position “p” (supine, seated, stand-

ing) was expressed as

$$Y_{scp} = FR_{scp} = \frac{LF_{scp}}{HF_{scp}}. \quad (1)$$

One sample for each subject and per body position was calculated from the frequency bands. In Table 1, the summary statistics of the LF/HF ratio for each health and body position are shown. In 3 cases, ratios could not be computed due to too poor data quality.

### 4. Statistical modeling

The main objective was to evaluate differences between the groups of patients under all body position changes. The first attempt to model this type of experimental data was the application of a general linear model, which incorporates all the factors that could produce a change in the LF/HF ratio, such as Health condition, body position and their interaction. In the General Linear Model the error term, which accounts for extraneous variability, is assumed to be independent and homoskedastic with Gaussian distribution, i.e.  $\varepsilon_{scp}$  i.i.d.  $N(0, \sigma^2)$ . For example, the observed value for a healthy subject “s” in supine position can be expressed as follows:

$$\begin{aligned} FR_{s=s,c=healthy,p=supine} &= \beta_0 + 1 \times \beta_{c=healthy} + 0 \times \beta_{c=disease} + 1 \times \beta_{p=supine} + 0 \\ &\quad \times \beta_{p=seated} + 0 \times \beta_{p=standing} + 1 \times \beta_{c=healthy,p=supine} + 0 \\ &\quad \times \beta_{c=healthy,p=seated} + 0 \times \beta_{c=healthy,p=standing} \\ &\quad + \varepsilon_{s=s,c=healthy,p=supine} \end{aligned} \quad (2)$$

Then the complete dataset can be modeled as  $\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\varepsilon}$ , where  $\mathbf{Y}$  is a  $N \times 1$  vector of observed LF/HF ratios,  $\mathbf{X}$  is an  $N \times p$  matrix indicating the design effects in each subject, and  $\boldsymbol{\beta}$  is a  $p \times 1$  vector of unknown constants to be estimated.

Since each patient is observed more than once (under three different body positions), data from the same patient are expected to be correlated. The lack of independence in the observed values can be modeled by the inclusion of an extra observed random term representing a subject effect. This is the linear mixed model formulation:

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\boldsymbol{\gamma} + \boldsymbol{\varepsilon} \quad (3)$$

where the new term includes  $\mathbf{Z}^{N \times q}$ , an  $N \times q$  design matrix of 0s or 1s indexing the observations coming from the same patient and  $\boldsymbol{\gamma} \sim N(\mathbf{0}, \mathbf{G})$  is a  $q \times 1$  vector of normal random terms with a covariance matrix  $\mathbf{G}$ . In this context, for a subject “s” we have the conditional mean (subject specific)  $E[Y_s | \gamma_s] = \mathbf{x}'_s \boldsymbol{\beta} + \mathbf{z}'_s \boldsymbol{\gamma}$  and the marginal mean (or population average)  $E[Y_s] = \mathbf{x}'_s \boldsymbol{\beta}$ . The estimation of the covariance parameters can be done by the Restricted Maximum Likelihood (REML) [18].

Under the assumption of normal distribution of the data, two linear mixed models were fitted (Eq. (3)). The first one (model M1) used the raw data,  $Y_{scp} = FR_{scp} = (LF_{scp}/HF_{scp})$ . The corresponding boxplots are shown in Fig. 1 (top raw panels) and show that distributions are skewed and also that

**Table 1 – Summary statistics for each health x body position combination for LF/HF ratio.**

	Min	1Q	Median	Mean	3Q	Max	N	Missing values
<b>Control</b>								
Supine	1.12	1.47	1.62	2.27	2.37	7.48	17	
Seated	0.99	1.67	2.69	3.45	3.85	11.99	16	1
Standing	2.26	5.12	6.27	7.56	9.00	16.51	17	
<b>Disease</b>								
Supine	0.96	1.78	3.23	3.88	4.17	11.42	14	1
Seated	0.63	2.82	4.32	5.34	6.84	14.62	15	
Standing	1.04	4.87	7.74	7.85	11.06	15.05	14	1

variances (as measured by the width of the central box) are heterogeneous. The second model (model M2) is similar to model M1, yet applied to log transformed data:  $LY_{scp} = \log(LF_{scp}/HF_{scp}) = \log(LF_{scp}) - \log(HF_{scp})$ . The log transformation has the property of changing a ratio into a difference and can produce variance stabilization. It also permits to interpret a ratio as a difference. In Fig. 1 (bottom row panels), the boxplots of the transformed data are displayed. They are more symmetric but still display variance heterogeneity.

The observed skewed distribution of the raw data (Fig. 1, left panel) suggests a non-normal behavior. Thus, we propose a third model (M3) in which the observed LF/HF ratios are assumed to follow a gamma distribution [19], which is a skewed distribution not having homogeneous variances: the variance–mean dependence is quadratic. Under non-normal distribution in the exponential family we can use the Generalized Linear Mixed Model (GLMM) [12] approach. In the GLMM the mean is linked to the linear predictor by an invertible “link” function “g” in such a way that [19]:

$$E(Y|\gamma) = g^{-1}(X\beta + Z\gamma) \tag{4}$$

When the chosen link is the log function, then  $\log(E(Y|\gamma)) = X\beta + Z\gamma$  or  $E(Y|\gamma) = e^{X\beta + Z\gamma}$  (It should be stressed that we are not transforming the observed data as in model M2,

but the conditional expected value, i.e. the mean). To fit such a GLMM a pseudo-likelihood approach based on linearization [20] can be applied.

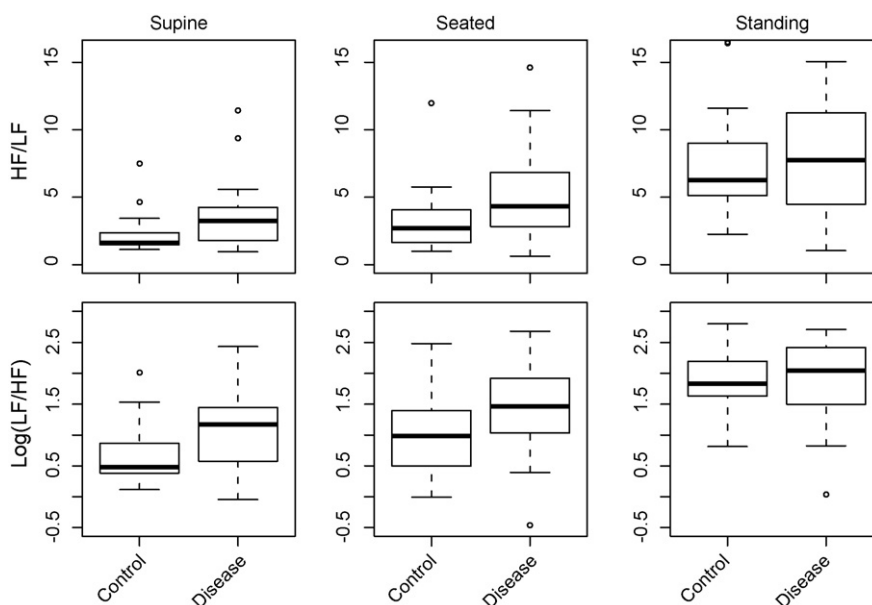
To evaluate the appropriateness of the different approaches we use the residual vs. predicted plots (residuals vs. fitted) and the quantile–quantile plot (qq plot) for residuals from each model. In all cases Pearson residuals  $r_p = y_i - \hat{\mu}_i / \sqrt{\hat{V}_{ar}(y_i)}$  were used (here the hat  $\hat{\cdot}$  denotes an estimate). These residuals tend to be approximately normally distributed with zero mean and unit variance in the link scale [19].

In order to evaluate the goodness of fit (GOF) for specific models the Generalized Chi-Square (GCS) value normalized by the number of degree of freedom (GCSn) was used in all the tested models. A value close to one implies that the variability in the data has been properly accounted for by the model [14].

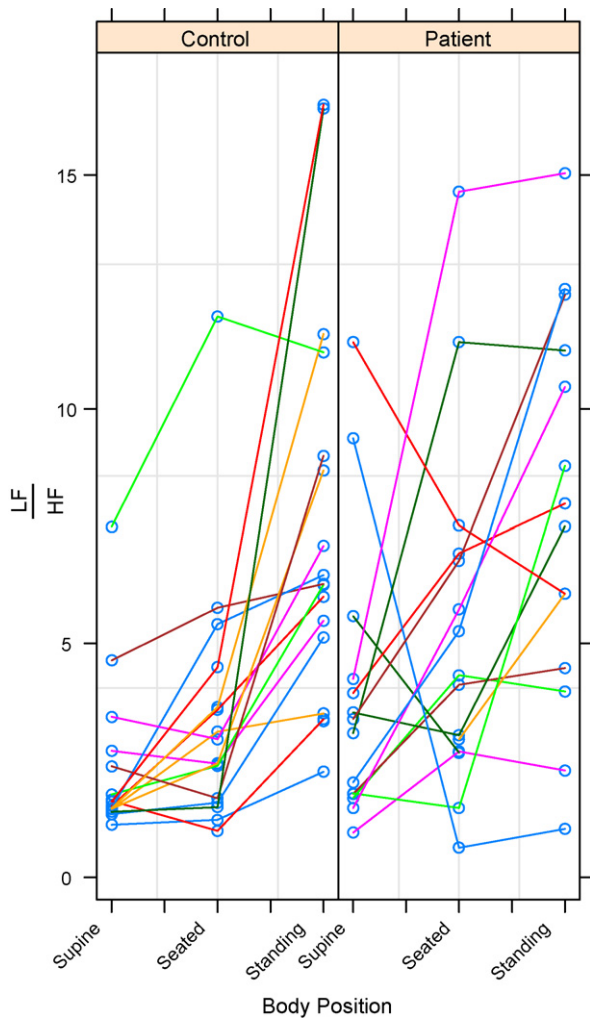
The models under Gaussian assumptions were fitted in R (<http://www.r-project.org>) and SAS (<http://www.sas.com>). Models under the gamma assumption were fitted with procedure GLIMMIX in SAS.

## 5. Results

In order to show the asymmetric nature of the LF/HF ratio and show the effect of the log transformation on the data distribu-



**Fig. 1 – Box plots for each combination health by body position. Left panels: raw LF/HF ratio. Right panels log transformed ratios.**



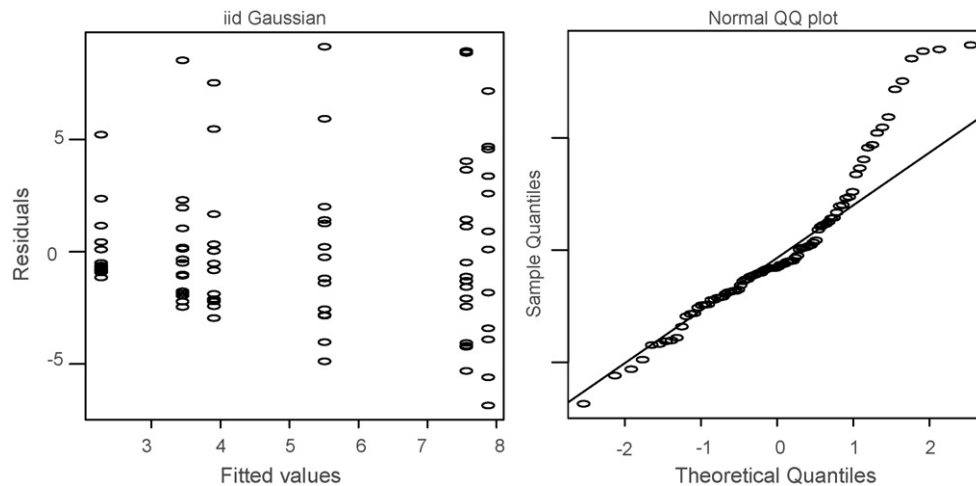
**Fig. 2 – Raw LF/HF ratios for all patients under each health condition and body position.**

tion, the boxplots of raw and transformed data for each health condition and body position are shown in Fig. 1. The asymmetric distribution of the raw ratios suggests that the response variable does not follow a normal distribution (left panels). It is also possible to see that the variance seems not to be equal in all health-body position combinations. One may notice on the right panels of Fig. 1 that transforming the observed variable by means of the log transformation produces a more symmetric distribution, but still the variance heterogeneity remains. Fig. 2 shows the raw LF/HF ratios for all subjects (control and disease patients) under each health condition and body position.

In Fig. 3 the residual vs. fitted and QQ plots are shown for the model assuming observed i.i.d. Gaussian raw data, i.e. fixed effects model (Eq. (2)). From Fig. 3 it is possible to see that the residuals do not follow a normal distribution and the variance increases as the predicted values increase. The QQ plot shows that the residuals do not follow a normal distribution since they tend to depart from the expected identity line.

The residuals and QQ plots for all fitted mixed models are shown in Fig. 4. In model M1 (top left panels in Fig. 3) the variance of the residuals is not homogeneous, it increases as the predicted values increase. In the corresponding QQ plot (top right panel) it is possible to see that residuals have a skewed distribution. Model 2 residuals are shown in the middle panel, they display a random pattern around zero. In the QQ plot the residuals match the straight line as expected under normal assumption. Modeling the log transformed data instead of the raw data approximately satisfies the required assumptions of the linear mixed model approach. In the bottom panels, the model under gamma distribution assumption with a log link function relating the expected value (model M3) is shown. The left panel shows Pearson residuals against predicted values in the linear predictor scale. This model also attains good results. The residuals in the linear scale were also evenly spread around zero. The QQ plot of the residuals also matches approximately the straight line.

The normalized Generalized Chi-Square value for model M1 ( $GSCn = 7.54$ ) was greater than one, suggesting that the raw data do not meet the normal and variance homogeneity. The log transformed data were better modeled in this



**Fig. 3 – Residuals vs. predicted values and residuals QQ plots for model in Eq. (2) under the assumption of  $\varepsilon \sim N(0, \sigma^2)$ .**

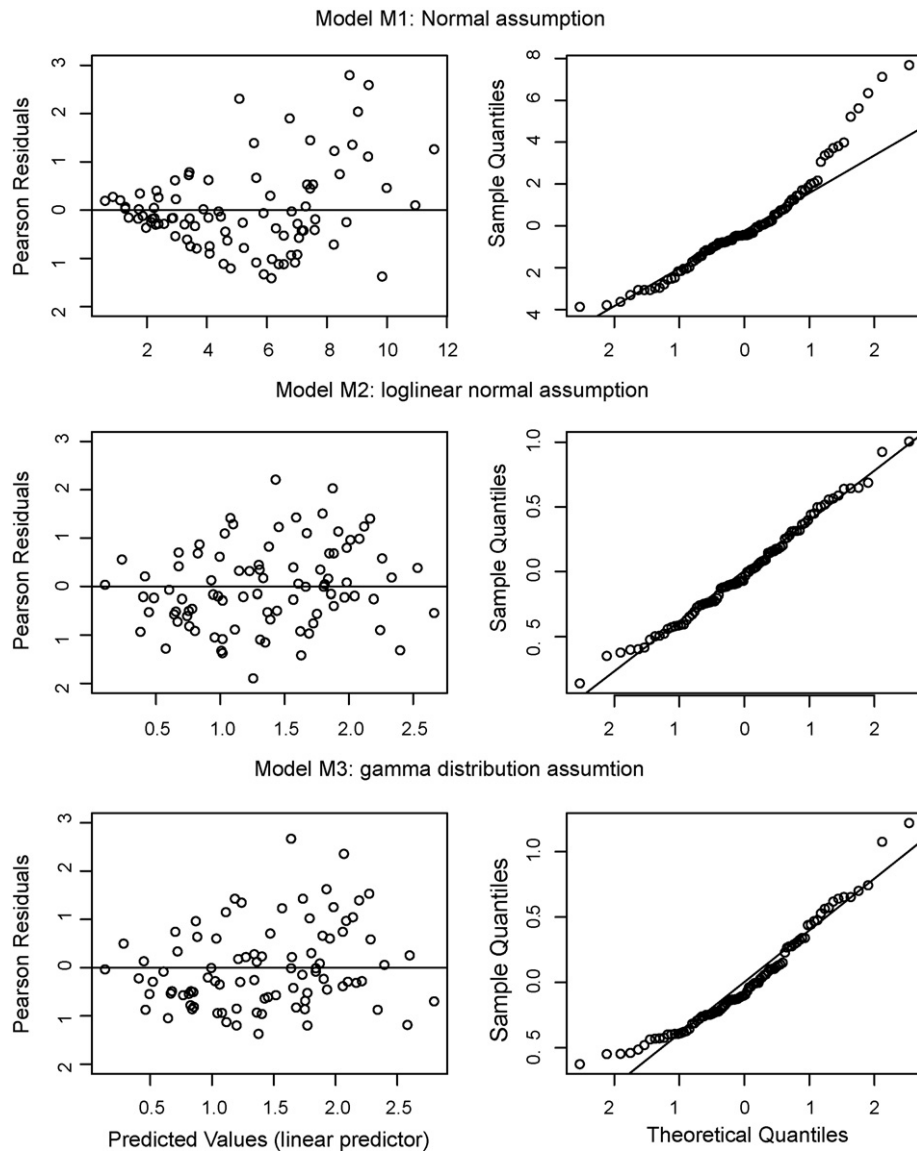


Fig. 4 – In the left panels the residuals vs. estimated values are shown. The QQ plots for residuals are shown in the right panels.

Table 2 – Estimated differences between least squared means of health condition and body position combinations under models M2 and M3. SE: Standard error, t: t-value, Pr > |t|: p-value.

Condition by Body position combination comparison	Model M2				Model M3			
	Estimate	SE	t	Pr >  t	Estimate	SE	t	Pr >  t
Healthy-Seated vs. Healthy-Standing	-0.866	0.159	-5.440	<.001	-0.871	0.159	-5.450	<.001
Healthy-Seated vs. Healthy-Supine	0.342	0.159	2.150	0.036	0.359	0.159	2.250	0.028
Healthy-Standing vs. Healthy-Supine	1.208	0.156	7.740	<.001	1.231	0.157	7.850	<.001
Disease-Seated vs. Disease-Standing	-0.417	0.183	-2.280	0.027	-0.411	0.184	-2.230	0.030
Disease-Seated vs. Disease-Supine	0.551	0.181	3.040	0.004	0.531	0.182	2.920	0.005
Disease-Standing vs. Disease-Supine	0.968	0.186	5.200	<.001	0.941	0.187	5.030	<.001
Healthy-Seated vs. Disease-Seated	-0.621	0.225	-2.750	0.008	-0.628	0.230	-2.720	0.009
Healthy-Standing vs. Disease-Standing	-0.172	0.227	-0.750	0.455	-0.167	0.232	-0.720	0.474
Healthy-Supine vs. Disease-Supine	-0.410	0.223	-1.840	0.071	-0.457	0.228	-2.00	0.050

context ( $GCSn=0.21$ ) and the model under gamma distribution assumptions for the raw data shows the same goodness of fit ( $GCSn=0.21$ ).

Based on the previous results, the appropriate models for statistical inference were M2 and M3. The least squares mean differences of the combination of “health condition” and “body position” ( $C \times P$ ) for models fitted with all data are shown in Table 2.

Results suggest that the inference was equivalent for models M2 and M3. For the healthy subjects (first three rows of Table 2) an increase in the LF/HF ratio from seating to standing position was found. The same happened for the diseased patients. When the control against disease patients were compared at a given body position (last three rows in Table 2), the only position for which the LF/HF ratio appears to be statistically different ( $p$ -value  $< 0.05$ ) was the seated position. Models M2 and M3 shows similar  $t$  statistics for the important parameters and comparisons.

## 6. Discussion

Under the Generalized Linear Mixed Model framework, it is possible to fit models with different distributional assumptions. In this work, a model built assuming a gamma distribution over the raw data allowed us to analyze and infer the effects of neurogenic erectile dysfunction disease. The GLMM framework allows us to fit correlated data under non-normal distribution assumptions, yielding a more flexible modeling strategy for these data. An alternative approach based on a linear mixed model (Gaussian context) of log transformed data yielded similar results.

Common approaches to the statistical analysis of such data are the application of  $t$ -test and/or non-parametric tests like Wilcoxon. The approach proposed here is superior to both, since it provides more information, for instance the effects of interest can be explicitly modeled and inferences are done in a more general context. Furthermore, the verification of the underlying assumptions can be done in a straightforward way. The decomposition of the expected mean into a linear combination (or an invertible function of them) of experimental effects gives us the chance of incorporating several factors and covariates believed to affect the responses as well as incorporating meaningful correlations and heterogeneous variances. After the fitting process the effects of these factors can be statistically assessed under an appropriate theoretical background. This process is not feasible for the classical or non-parametric approach.

The use of appropriate models, such as models M2 and M3, provided more powerful inferences than model M1. In particular, the difference between supine and seated body positions was not found to be statistically significant neither for healthy nor diseased subjects under model M1.

In the current work, both alternatives, the log transformation under normal distribution and raw data under gamma distribution, satisfied model assumptions, yielding valid inference of the expected effects and thus providing valid conclusions. Model M3 is appealing and realistic since we do not need to apply any explicit transformation to the observed data in order to force them to fulfill the assumptions. We just

need to define the expected relationship between the mean and the linear predictor and let the data speak for themselves. In addition the interpretation is straightforward since we are analyzing the sympatho-vagal balance as it is and not as a difference between frequency bands.

When comparing the sympatho-vagal balance ratio LF/HF between the healthy and diseased subjects we found significant differences only in the seated position with an increase in the LF/HF ratio for the disease subject. When the health condition is held constant, in both cases the LF/HF ratio increases from supine to standing (this conclusion is missed with model M1). The increase in the ratio from supine to seated body position was close to 50% higher for subjects with erectile dysfunction compared to healthy ones. These results are similar to the findings, reported by Lavie et al., stating that patients complaining of daytime sexual dysfunction have altered cardiac autonomic balance [21]. Lavie et al. showed that in patients with organic erectile dysfunction there is a relative decrease in the activity of the parasympathetic division combined with a dramatic increase in the activity of the sympathetic division during sleep [21]. Our results show appropriate statistical approach for the analysis of spectral cardiac sympatho-vagal parameters may be a valuable non-invasive and relatively simple method to study neurogenic erectile dysfunction in patients.

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