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Construction of the Gamma-tocopherol centile during the Gestational Period: A Comparison of Smoothing Methods

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Abstract

In this paper, we construct centile curves for $\gamma-$tocopherol as well as the normal range during the gestational period with two statistical smoothing methods and reasonable goodness of fit tests has been included, respectively.

The LMS model of Cole and Green (1992) is a widely used method to fit a centile curve. Fitting a suitable model, we have the measurement in the reference sample following a standard normal distribution on all ages after a chosen Box-Cox transformation. The coefficients of this transformation are modeled as smooth age dependent parameter curves for the median, variation and skewness, respectively. The drawback of LMS is that the kurtosis (leptokurtosis or platykurtosis) might not be eliminated.

Further, we briefly introduce the Box-Cox power exponential (BCPE) distribution, which is the basis of generalizing LMS model into LMSP method, proposed by Rigby and Stasinopoulos (2004). It provides a model for a dependent variable, which shows both skewness and kurtosis. The centile curves are obtained by modeling each of the parameters of the distribution as a smooth non-parametric function of an explanatory variable.

In conclusion, we obtain similar centile curves with above two methods. Comparing the two methods, we find LMSP is more flexible while LMS is easier. Besides, the former one is useful for kurtosis, robust to outliers not LMS.

Content

Introduction

Age related reference ranges are a commonly used tool in the medical field. The last decade has witnessed an upsurge in methods for constructing age-related centile curves (Wright and Royston, 1997),and 30 existing methods are summarized in **appendix A** (Borghi and Onis, 2006).

We mainly focus on the methods, which handle the age continuously with the distributional assumptions; because it met our dataset and research purpose most and give more convinced result. Our paper could be considered as a supplement of research paper (Palm et al.) provided by Obstetrics and Gynecology, Uppsala University.

Tocopherol, or vitamin E, is a fat-soluble vitamin in eight forms that is an important antioxidant. Recent studies into the use of the single isomer vitamin E esters as possible help in preventing oxidative stress, which would relate to pre-eclampsia. Therefore, a reference range is needed, and the centile curves are more necessary and direct way to find the abnormal values.

The normal range, that is, reference range is a critical definition in our paper. The common definition of it is that the normal range is set to cover ninety-five percent (95%) of all values from the general population. Five percent (5%) of results therefore fall outside the normal range. For example, if we have the simplest standardized normal distribution, the shadow part (**Figure 1**) will be the normal range:

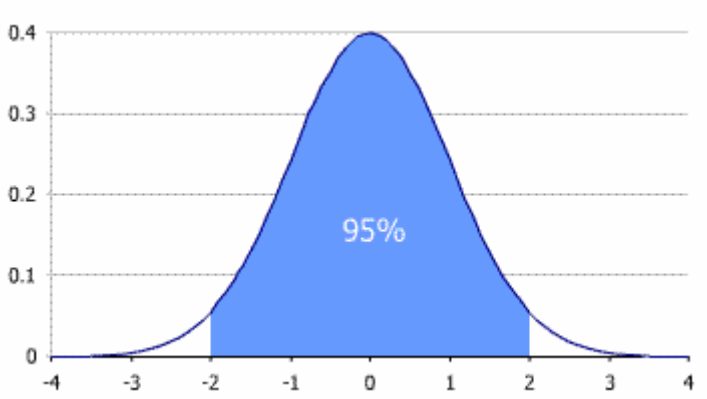


Figure 1 The normal range under standardized normal distribution

There are other kinds of methods in defining the normal range as well. Let us take the blood pressure as an example. The reference range to check the normal is as follows.

Table 1 Classifying blood pressure values (units: mmHg) (WHO, 2003)

Range	systolic blood pressure	diastolic blood pressure
Hypotension	lower than 100	lower than 60
Normal range	between 100 and 140	between 60 and 90
Mild hypertension	between 140 and 160	between 90 and 100
Moderate hypertension	between 160 and 180	between 100 and 110
Severe hypertension	higher than 180	higher than 110

The above way is simple and reasonable for the hypotension normal range; however, under most situations, measurements could change depending on some variables, such as age, gender, etc. Therefore, we construct the centiles related to gestational time.

In our paper, we construct the centiles of the gamma-tocopherol during the gestational period. Two methods, that is, LMS and LMSP are utilized to smooth the centile curves, the percentile values of two methods are given respectively. In the end, the comparison of two models is summarized.

Data

Fifty-nine pregnant women from one outpatient antenatal clinic in Uppsala City were consecutively recruited into the study during 2003-2004 (Palm et al.). The data includes all healthy women at least 18 years old with normal, spontaneous pregnancy at booking. The exclusion criteria were non-Swedish speaking women and women taking drugs other than iron medication or folic acid. Because of the miscarriage, moving, and withdrawing the study, the remaining 52 women are our data set.

The gestational period was divided into two weeks intervals, and if a woman had more than one measurement taken within a two week interval, the last value is used. This situation happens in the data of the 8th, 10th, 11th, 29th, 33rd, 34th, 37th, 39th patient during some time periods. To make the sample size not too small on every age, the biweekly data is preferred. To make our dataset more reliable and effectiveness, we eliminate the age groups which are too small. (Sample size smaller than 10). The summary of the remaining data are given in **Appendix B**.

We want to know the general information about all of serum γ – tocopherol firstly. The distribution of γ – tocopherol is right-skewed from the histogram of all data, and the most observations are less than 0.2mg/mmol intuitively.

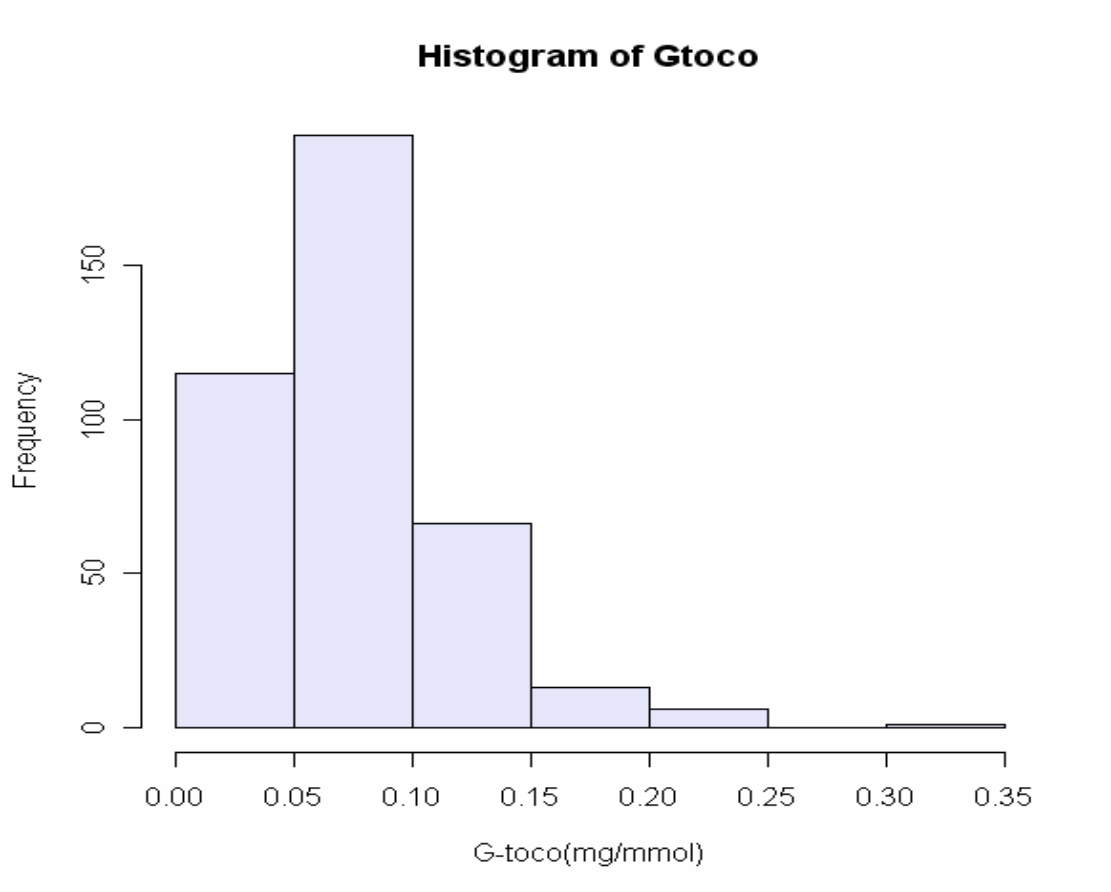


Figure 2 Histogram of Gamma-tocopherol

Further, we check the box-plot (**Figure 3**) of $\gamma-$tocopherol (Gtoco) to see the details of every age (twoweek) distribution. The distribution of biweekly data should be considered, because the sample size will not too small for all the time points, on the other hand, the weekly data involves many blank time point. What's more, we need to keep consistence with the data interval of Palm's research. We should fix attention to the names of our variables: "Gtoco" and "twoweek". Gtoco is our measurement, and "twoweek" is just a symbol of the biweekly data, e.g., if we have the value 12 on the twoweek axis, that is, 12th week, not 24th week.

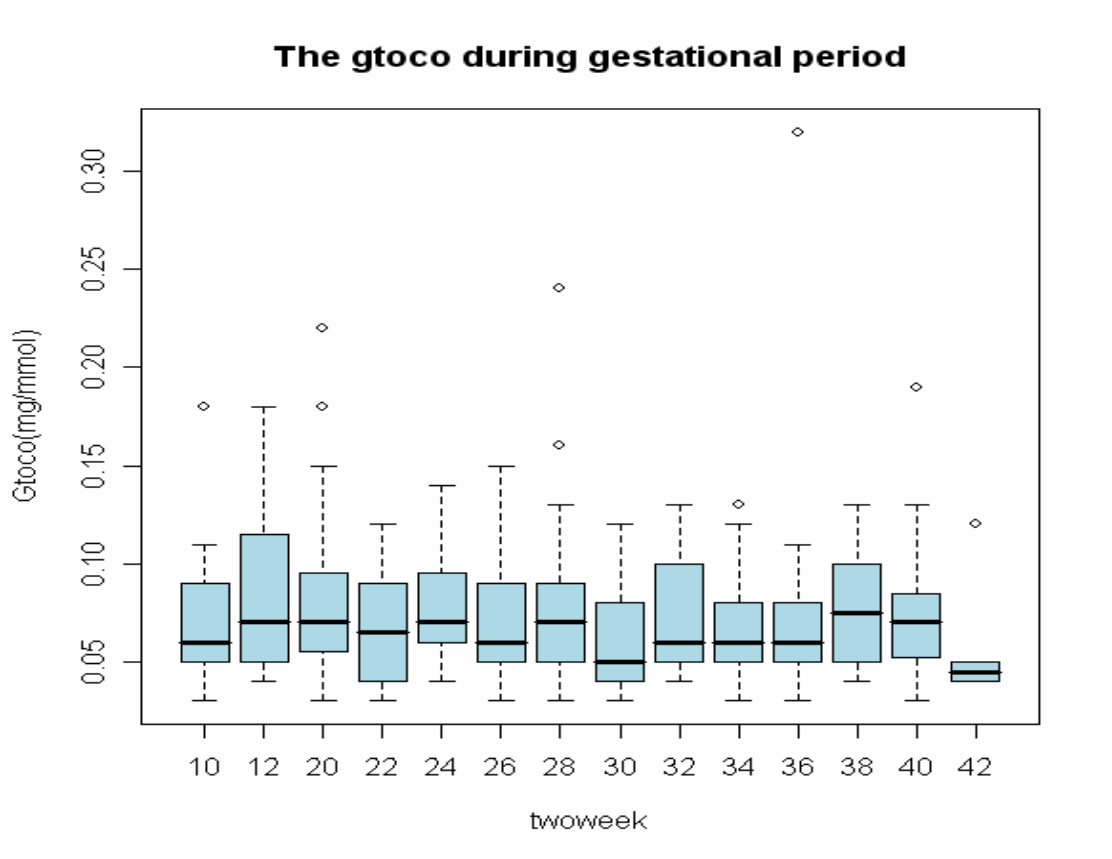


Figure 3 Box-plot of Gamma-tocopherol for different weeks

There are two ways to calculate the centiles, that is, for different women or for different time as our exploratory variable.

Following the different time (**Figure 3**), we can see the distribution of symmetric age groups are infrequency of occurrence, so we may guess the distribution of every group should not be normal distributed. We need test the normality of the data statistically. We check every group with the Shapiro-Wilk Normality Test. Just three groups can be considered as normally distributed. (**Appendix C**) It leads to the intuitive method, which give normal range of every age directly by (mean-1.96SD, mean+1.96SD) void.

On the other hand, we take 5 women's measurements during the gestational period as an example. The gamma-tocopherol for every woman is shown below; however, if we fit all women's lines in one graph, it will be too messy to tell the objects, so just first 5 women to be given for a general concept of our data. (**Figure 4**)

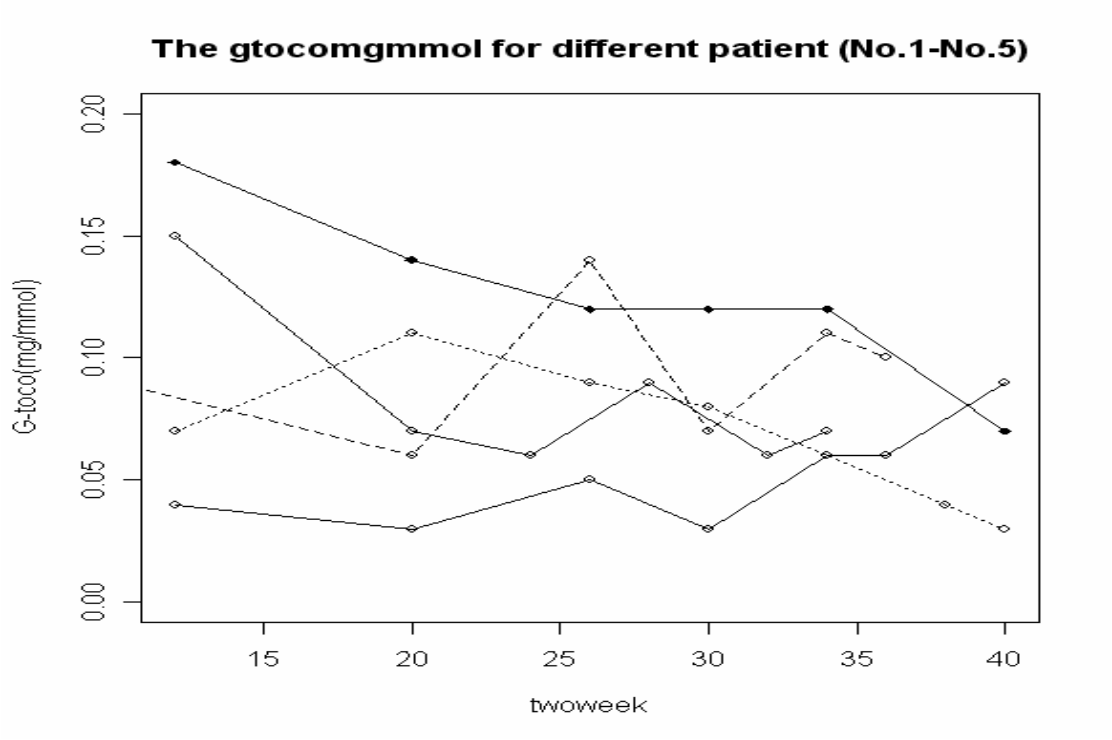


Figure 4 The gamma-tocoperol for different women

The "twoweek" is more suitable for exploratory variable, because the different women have their particular style, which is not so interesting. Therefore, the smooth methods based on transformation at different age ("twoweek") should come to rescue.

Methodology

1. LMS model

The LMS method provides a general method for smoothing centiles (Cole and Green, 1992). Each centile curve is summarized by 3 curves representing the median (M), the coefficient of variation (S) and the skewness (L) as they change with the independent variable (age).

The basic idea of it is the Box-Cox (1964) power family transformation, with the unknown parameter λ and δ.(where $y > 0$, $y > -\delta$)

(a) $y^{(\lambda)} = \begin{cases} (y^{\lambda} - 1)/\lambda & (\lambda \neq 0) \\ \log y & (\lambda = 0) \end{cases}$ and $y^{(\lambda)} = \begin{cases} [(y+\delta)^{\lambda} - 1]/\lambda & (\lambda \neq 0) \\ \log(y+\delta) & (\lambda = 0) \end{cases}$

Based on **(a)**, y can be mapped to x, which is distributed normally, through the following way.

(b) $x = \begin{cases} \dfrac{(y/\mu)^{\lambda}-1}{\lambda} (\lambda \neq 0) \\ \log\dfrac{y}{\mu} \quad (\lambda = 0) \end{cases}$

The rule of choosing λ is to minimize the SD of x.

SD of x is the coefficient of variation of y for the entire moderate λ approximately. We define the SD as σ, so the Z-score of y can be induced as follows,

(c) $z = \dfrac{x}{\sigma} = \begin{cases} \dfrac{(y/\mu)^{\lambda}-1}{\lambda\sigma} (\lambda \neq 0) \\ \dfrac{\log\dfrac{y}{\mu}}{\sigma} \quad (\lambda = 0) \end{cases}$

If we express the Z-score with $L(t)$, $M(t)$ and $S(t)$, it can be written as

(d) $z = \begin{cases} \dfrac{(\dfrac{y}{M(t)})^{L(t)}-1}{L(t)S(t)} \quad (L(t) \neq 0) \\ \dfrac{\log(\dfrac{y}{M(t)})}{S(t)} \quad (L(t) = 0) \end{cases}$

We calculate centile 100α of y at t utilizing the below formula:

(e) $C_{100\alpha}(t) = \begin{cases} M(t)[1+L(t)S(t)z_{\alpha}]^{\frac{1}{L(t)}} (L(t) \neq 0), \\ M(t)\exp(S(t)z_{\alpha}) \qquad (L(t)=0) \end{cases}$

where z_{α} is the normal equivalent deviate of size α. This shows that if L, M and S are smooth, then so are the centile curves.

Cole and Green (1992) propose the penalized likelihood function defined as

(f) $l - \frac{1}{2}\alpha_L \int \{L''(t)\}^2 dt - \frac{1}{2}\alpha_M \int \{M''(t)\}^2 dt - \frac{1}{2}\alpha_S \int \{S''(t)\}^2 dt$,

where likelihood **(g)** $l = \sum_{i=1}^{n} \left(L(t_i)\log\frac{y_i}{M(t_i)} - \log S(t_i) - \frac{1}{2}z_i^2 \right)$ and α_L, α_M and α_S are parameters for smoothness, i.e. edf (e.d.f.)(Cole and Green,1992), which stands for 'equivalent degrees of freedom'. The edf of each L, M and S curve is a measure of its complexity. 1 edf means a constant, and 2 edf corresponds to a straight line, 3 edf gives a simple curve like a quadratic, and 4 or more edf indicates progressively more

complex curve shapes.

2. LMSP model

LMSP method, which is a generalization of the LMS method, contains one more parameter than LMS model, as well as one more edf to modify the smoothness of the model. It is based on Box-Cox power exponential (BCPE) distribution.

The model introduces the fourth parameters τ (power exponential parameter), which is remove kurtosis, into the location parameter μ (median), scale parameter σ (approximate coefficient of variation), skewness ν (transformation to symmetry). The distribution can be defined as $\mathrm{BCPE}(\mu,\sigma,\nu,\tau)$. The details have been given in the paper. (Rigby and Stasinopoulos, 2004)

As α_{L}, α_{M} and α_{S} in LMS method, LMSP method also modifies the effective degrees of freedom (eg: df_{μ}) that are defined by the trace of the corresponding smoothing matrix in the fitting algorithm (Cole and Green, 1992), which is in turn directly related to the corresponding smoothing parameter (eg: λ_1). Therefore, we should find the best edf to set up the model.

In next part, the applications of our two smoothing methods are given, in order to find the centiles of our data set during the gestational period.

Result

1. Construct the centile with LMS model

The lmsChartMaker (Cole and Pan, 2005) is specific software to carry out the LMS method, so we use it in this part.

The difficulty of construction centile curves lies in deciding whether a bump or dip observed on a centile curve at a particular age is a real feature of the data, or whether it is simply sampling error. (Cole and Green, 1992) Lack of smoothing leads to irregular centile curves, especially for small sample size. However, over-smoothing centile results in losing information. On the other hand, the “lack of smoothing”

always gives better goodness of fit.

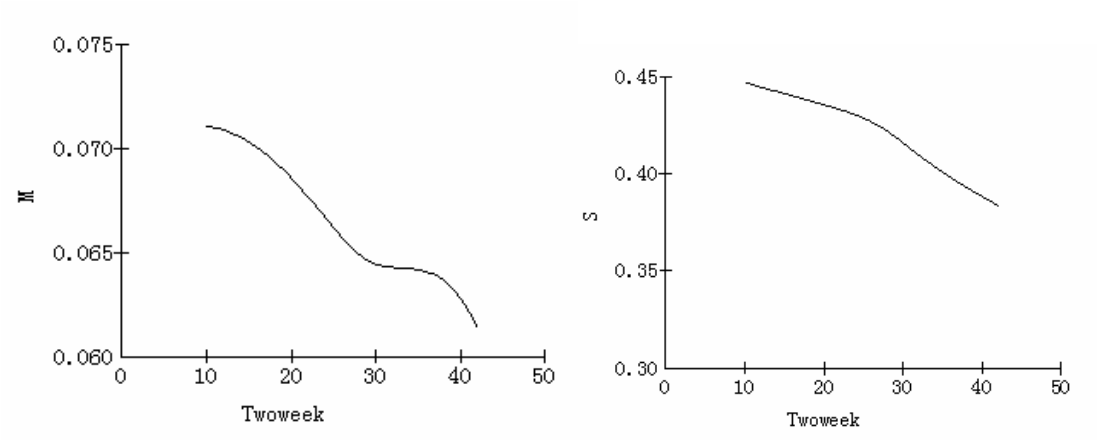
The LMSChartMaker software provides some rules to find the best edf. In our dataset, we use the rules of small samples. The order for choosing edf values should be M curve firstly, by increasing or decreasing the edf by 1 until the change in deviance is small. The default edf is 5, which is always adequate for our small dataset. The S and L curves are with 3 edf are default sufficient. In general, edf of M is not larger than S, however, edf of L should be not smaller than S. The smaller edf are welcome when it is no big difference.

Involving bigger edf to other new models, the deviance all show non-significant difference statistically (1 edf for 4 units decrease is significant), although a little better than the smaller ones. **(Table 2)**

Table 2 The deviance for different edf values

		edf		
Deviance	L	M	S	Cycles
-1405.5	3.0	3.0	3.0	4
-1405.9	3.0	4.0	3.0	4
-1406.6	3.0	5.0	3.0	4
-1407.5	3.0	6.0	3.0	4
-1407.5	3.0	6.0	3.0	3
-1408.5	3.0	7.0	3.0	3
-1409.5	3.0	8.0	3.0	4
-1410.6	3.0	9.0	3.0	4

After balancing the goodness and smoothness, we choose LMS (3/4/3).



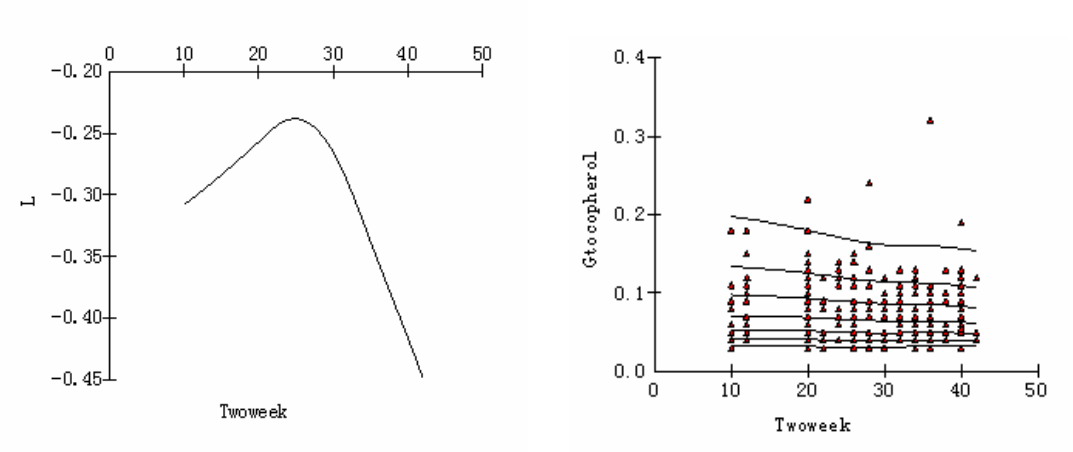


Figure 5 LMS (3/4/3) centile curves and L M S curves

We have noticed that all of the deviances are negative in our model fit. In fact, if we change the unit of the gamma-tocopherol, for example, multiplied all measurements by 1000, and change the unit to μg/mmol , the deviances all become positive. That is to say, the real value of the deviance is not very interesting, we just need the difference to select model. So we should know the difference between the deviances of penalized likelihood, global deviances of generalized additive model for location, scale and shape model (GAMLSS). (Stasinopoulos et al., 2006)

The degree of uncertainty in the estimated L, M and S curves and derived centile curves is obtained by simulation. “The underlying assumption of the LMS method is that after normalization, the data are converted to standard normal deviates. Therefore, on this assumption it is possible to generate a set of random normal deviates, at the same ages as the original data, and use the fitted LMS curves and calculate the original values. This simulated data set can be fitted from scratch, using the edf values chosen for the original data, leading to a new set of LMS curves.” (Cole, 1998a)

Subjectivity can not be avoided over the choice of models, due to change the complexity (edf) of the model. However, for our limited sample size, smoothness is more important than the goodness of fit if the difference is not significant.

“Repeating the process leads to a family of such curves and the values of the curves at each age can be ranked. Centile curves other than the median are calculated from each set of simulated LMS curves, and the process described above is applied to the ranked values of the centile curves at each age to obtain confidence intervals.” (Cole, 1998a)

With LMSChartMaker, the L , M and S can be obtained directly. (**Table 3**)

Table 3 The fitted values of L M S

week	L	M	S
10	-0.31	0.07	0.45

12	-0.30	7.09E-02	0.44
14	-0.29	7.06E-02	0.44
16	-0.28	7.01E-02	0.44
18	-0.27	6.94E-02	0.44
20	-0.26	6.86E-02	0.44
22	-0.25	6.77E-02	0.43
24	-0.24	6.67E-02	0.43
26	-0.24	6.57E-02	0.43
28	-0.25	6.49E-02	0.42
30	-0.27	6.44E-02	0.45
32	-0.29	0.064308	0.41
34	-0.32	6.42E-02	0.40
36	-0.36	6.41E-02	0.40
38	-0.39	6.37E-02	0.39
40	-0.42	6.28E-02	0.39
42	-0.45	6.14E-02	0.38

So we can calculate any percentile with the formula **(e)** we have.

After selecting the model, we test the goodness of fit of our model with Z-score test. SD is short for standard deviation and SDS for standard deviation score or Z score. The SDS or Z-score is calculated from the L, M and S curves, for different time we need.

A normal Q-Q plot of the residuals is commonly used to assess the normality assumption of the data. “The Shapiro-Wilk W-test may be too conservative.” (Pan and Cole, 2004) When we use this method to test the normality of z score, we find the null hypothesis is rejected, that is z score is not normal distributed. However, we use the D’Agostino-Pearson omnibus test instead, which is a recommended method using the software GraphPad Prism. The K2 statistic is default one for testing the normality. The result shows $K2=2.99$, $p\text{-value}=0.22$, that is, we can not reject the null hypothesis of the SDS’s normality. We can say our model is correct, because the Z-scores are normally distributed. The QQ plot confirms this result (**Figure 6**)

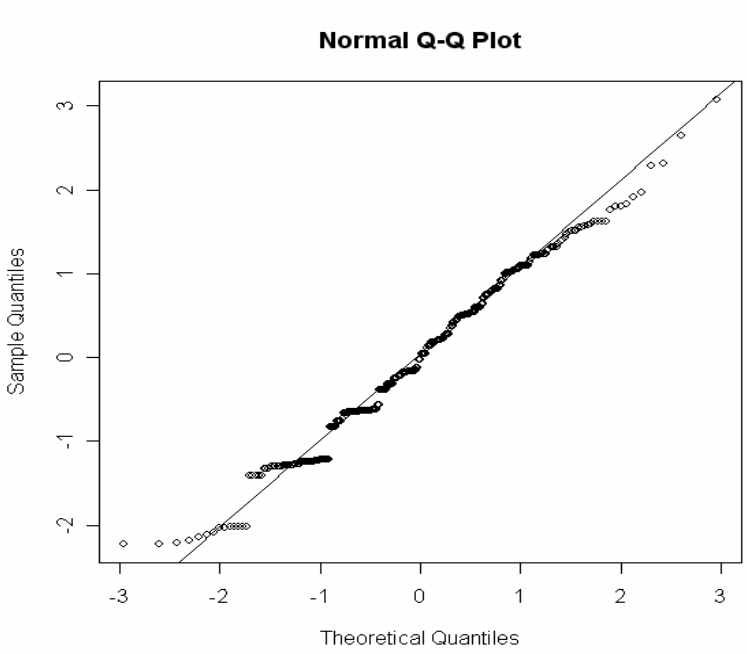


Figure 6 QQ plot of SDS

We test the moment values of Z score (**c**), and find the possible problem obstacle to pass Shapiro-Wilk normality test is the kurtosis, because the Gtoco exists kurtosis problem, when we use the Anscombe-Glynn kurtosis test, the p-value is just 1.82e-14, that is, we reject the null hypothesis, and the kurtosis of the Gtoco is not equal to 3. We know the original data has kurtosis significantly. Therefore, the following LMSP with Box-Cox power exponential (BCPE) transformation is a supplement.

2. Construct the centile curves with LMSP method

A. The best goodness of fit model

In this part, we use the LMPS method to fit the dataset of gamma-tocopherol. Because it is a complex procedure, the result is given directly.

Firstly, we choose the BCPE as the assumption method, and using the hyperparameter function in GAMLSS package to find the best degrees of freedom (df) of the $\mathrm{BCPE}(\mathrm{df}_{\mu},\mathrm{df}_{\sigma},\mathrm{df}_{\nu},\mathrm{df}_{\tau})$, and the worm plot of $\mathrm{BCPE}(8.23,4.72,1,1)$ is shown below, and $\lambda = 0.26$.

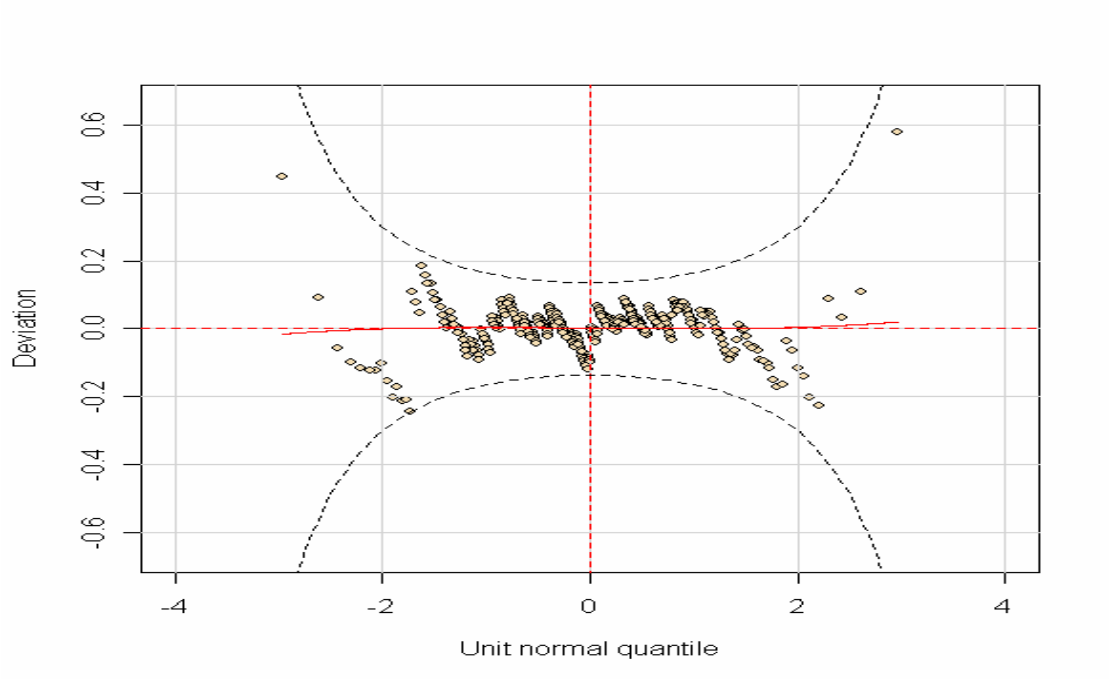


Figure 7 Worm plot from the Normal fitted model with BCPE(8.23, 4.72, 1, 1)

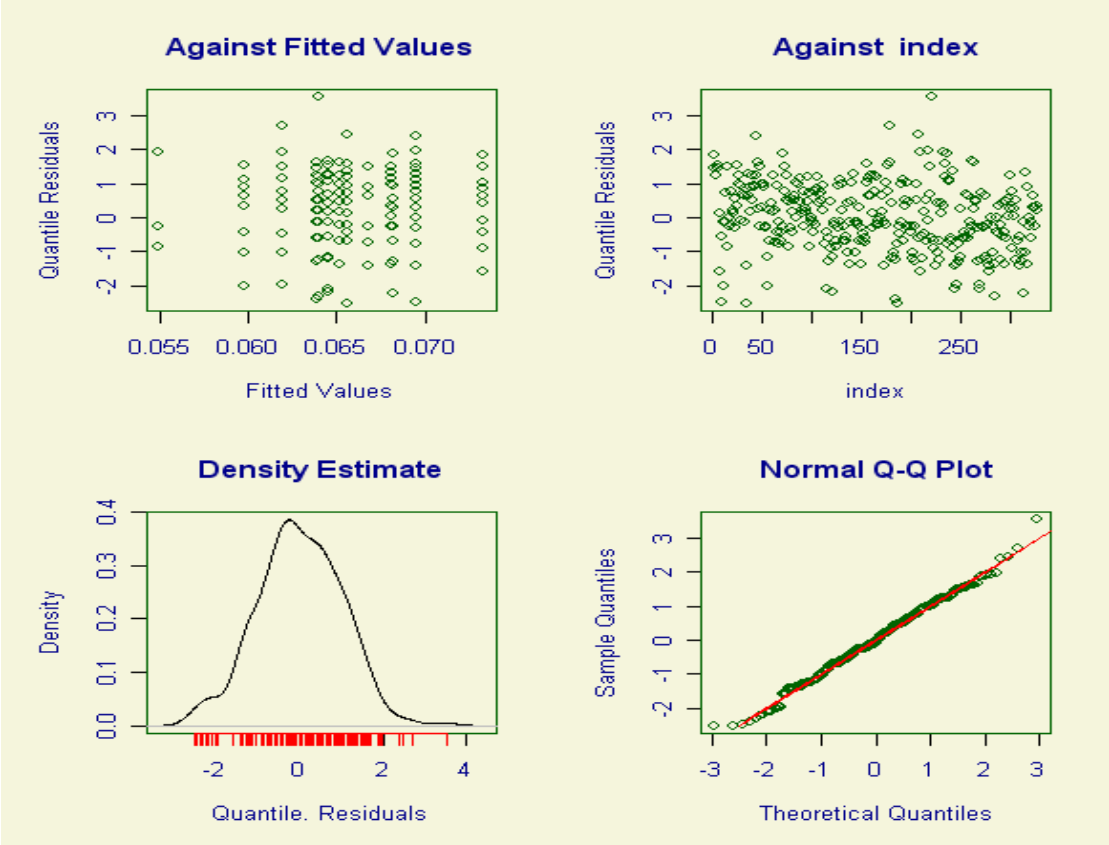


Figure 8 Residual plots from the Normal fitted model with BCPE(8.23, 4.72, 1, 1)

The curves of the worm plot are approximately straight line, which is a signal of

correct model. The summary of quantile residuals is as follows: mean is 0.003, and variance equals to 1.00, coefficient of skewness and kurtosis are 0.003 and 3.05 respective. It is approximately shows that the residual is standard normal distributed, which implies $BCPE(8.23, 4.72, 1, 1)$ is a suitable assumption.

We have mentioned the worm plot without giving any explanation, and next we remedy it. The worm plot is developed by Stef and Miranda (2001), and it consists of a collection of detrended Q-Q plots, each of which applies for one of the successive age groups. A flat worm indicates that the data met assumed distribution well. And some criteria for this visible test are given as follows.

Table 4 Interpretation of various patterns in the worm plot (Stef and Miranda , 2001)

Shape	Moment	If	Then
Intercept	Mean	Worm passes above the origin	Fitted mean is too small
		Worm passes above the origin	Fitted mean is too large
Slope	Variance	Worm has a positive slope	Fitted variance is too small
		Worm has a negative slope	Fitted variance is too large
Parabola	Skewness	Worm has a U-shape	Fitting distribution is too skew to the left
		Worm has an inverted U-shape	Fitted distribution is too skew to the right
S-curve	Kurtosis	Worm has an S-shape on the left bent down	Tails of the fitted distribution are too light
		Worm has an S-shape on the left bent up	Tails of the fitted distribution are too heavy

After fixing the $BCPE(8.23, 4.72, 1, 1)$ as the result, the worm plot above show the goodness of fit is very well under 95% confidence interval. So we are able to get the function of $\mu(t), \sigma(t), \nu(t),$ and $\tau(t)$. The plots of four parameters are shown below **(Figure 9)**

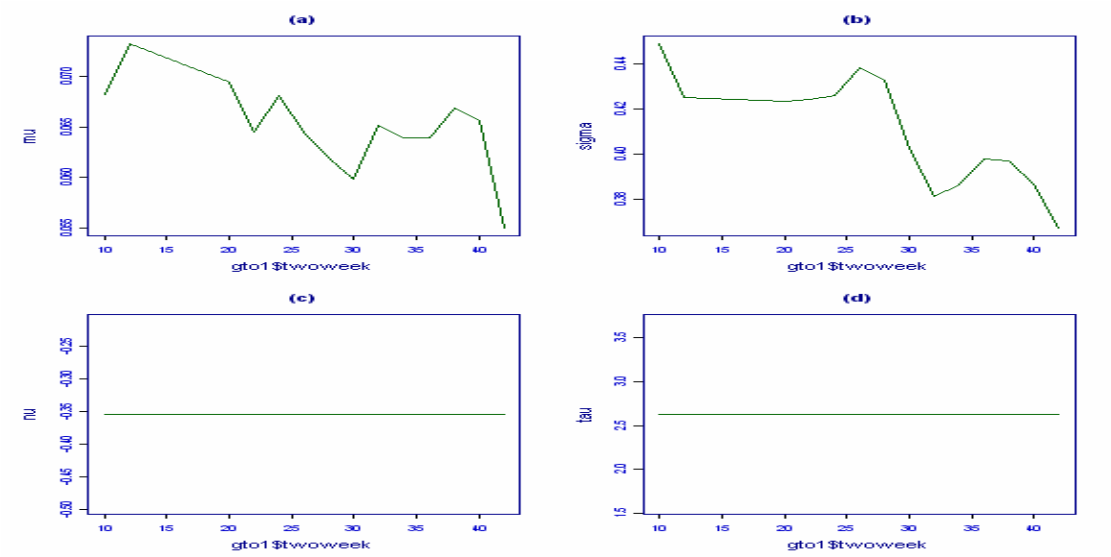


Figure 9 Fitted $\mu(t),\sigma(t),\nu(t),\tau(t)$ for the gamma-tocoperol

We can see $\nu(t)$ and $\tau(t)$ are constants, because the edf of them are equal to 1.Besides, we use the Q test to test the good-ness of fit. Testing the each age group is similar to check the worm. So-called 'Q-tests' are computed which are sensitive to age dependencies in the first four moments and non-normality of the Z-scores globally. The aim of Q test is whether the Z-scores are normally distributed independent of age. The Q test was described by Royston and Wright (2000). The p value of Q test result Q_M, Q_S, Q_L, Q_K, and Z are 8.16e-06, 0.00037, 0.11, 0.51, and 0.20 respectively, that is, the residual of the fitted model is normal distributed, and however, the mean and variance may be not 0, 1. For diagnostics of the model $BCPE(8.23, 4.72, 1, 1)$, we should add the degrees of freedom, i.e. df_μ and df_σ. The centile curves of $BCPE(8.23, 4.72, 1, 1)$ are given below (**Figure 10**), which is not smooth enough due to the fixed high edf. So we give up improving the model, because we prefer to smoothness and a regular model. And we choose a common reasonable model $BCPE(3,1,1,1)$ to discuss further.

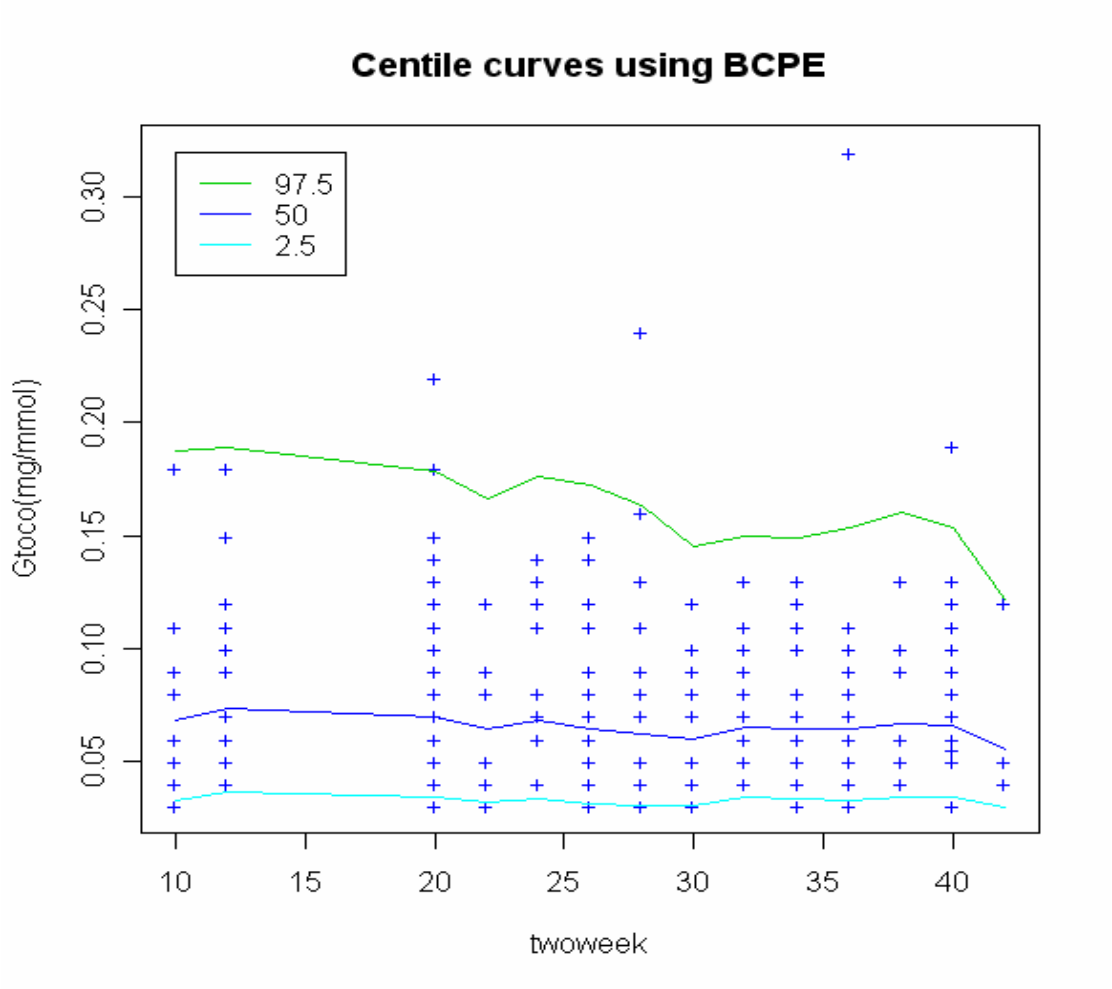


Figure 10 From the top to bottom are 97.5th, 50th, 2.5th centile curves with $BCPE(8.23, 4.72, 1, 1)$

B. Compare the smooth model with "best-fit" one

In the LMS part, we have said that the rule of our model should make smoothness come first and then the goodness of fit if reasonable. Therefore, we try the most common model, which is $BCPE(3,1,1,1)$ to compare with the $BCPE(8.23, 4.72, 1, 1)$.

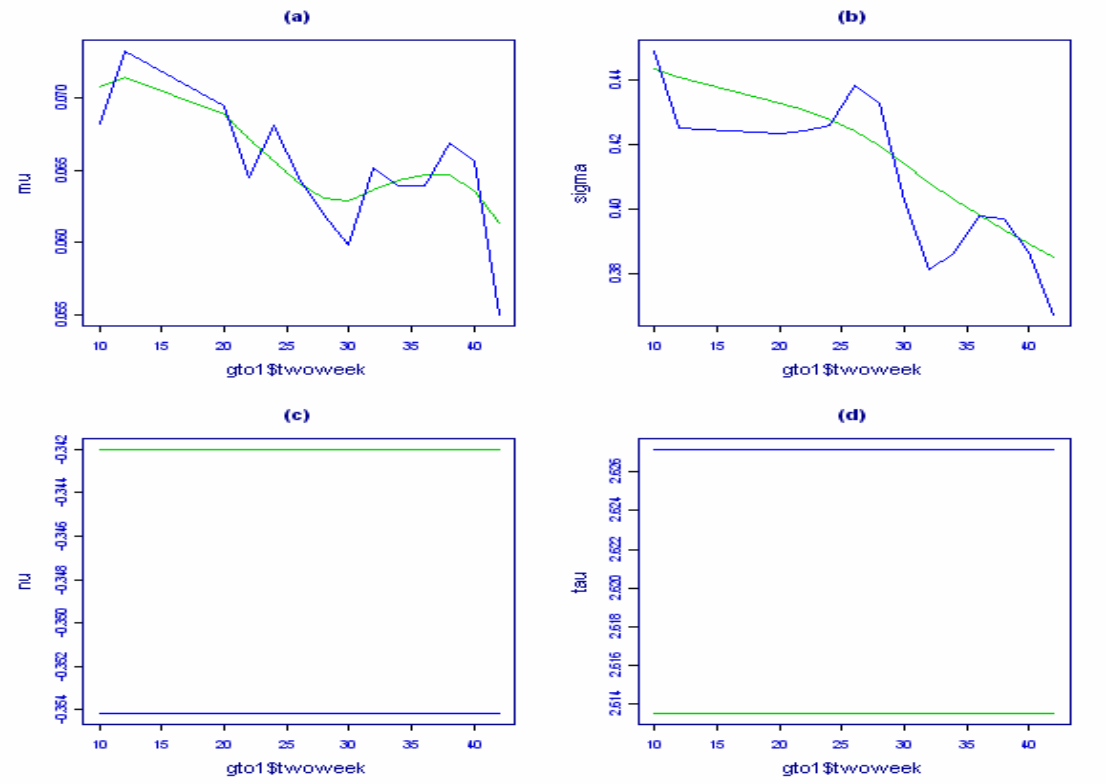


Figure 11 Comparing the four parameters curves of BCPE(3,1,1,1) BCPE(8.23, 4.72,1,1) **The simpler lines for mu and sigma are from** BCPE(3,1,1,1) .

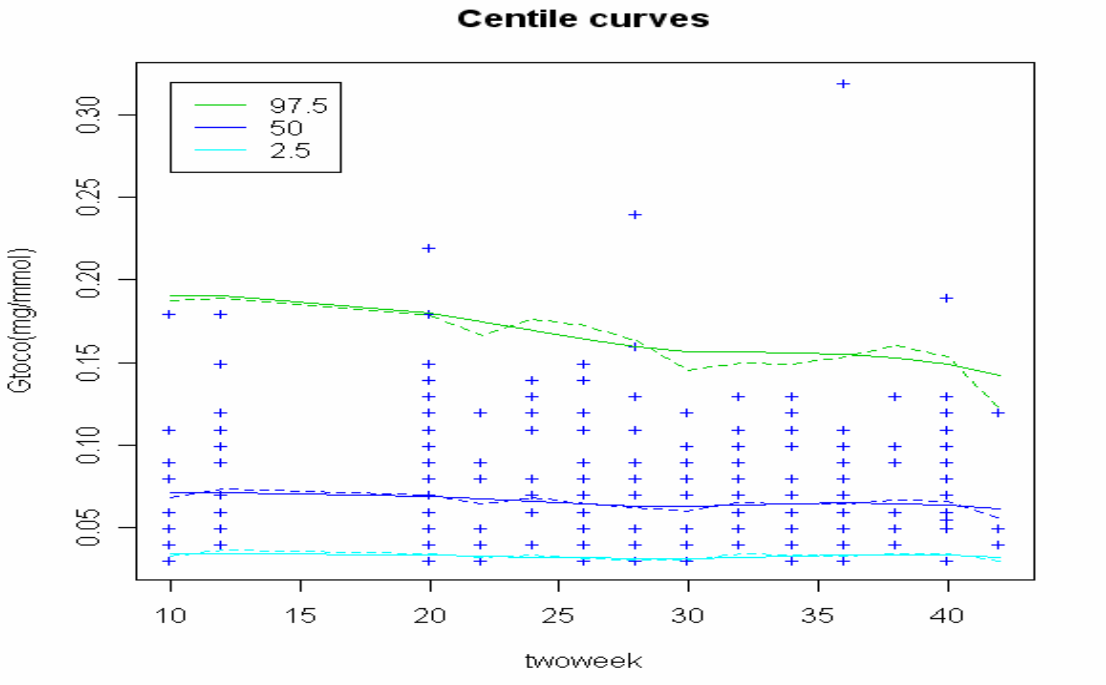


Figure 12 Comparing centile curves of two model (dash line for BCPE(8.23, 4.72,1,1) , solid line for BCPE(3,1,1,1))

To test the difference between 2 models, we have the BCPE(8.23, 4.72,1,1) Global deviance (19) =-1418.85(BCPE(8.23, 4.72,1,1)), and BCPE(3,1,1,1) Global deviance (10) =-1411.17 (BCPE(3,1,1,1)), therefore, the difference between the two models are not significant under 95% confident size, because the 1 unit for 4 is decrease of deviance is rough standard, and decrease in our model is less than 1 for one unit degree of freedom, because the Chi-squared statistic.

The worm plot is given (**Figure 13**), which shows the model is almost correct and acceptable due to the straight line. Therefore, I decide to select BCPE(3,1,1,1) as our final choice. Depending on our choice, we will compare the LMS (3/4/3) with the LMSP model based on BCPE(3,1,1,1) .

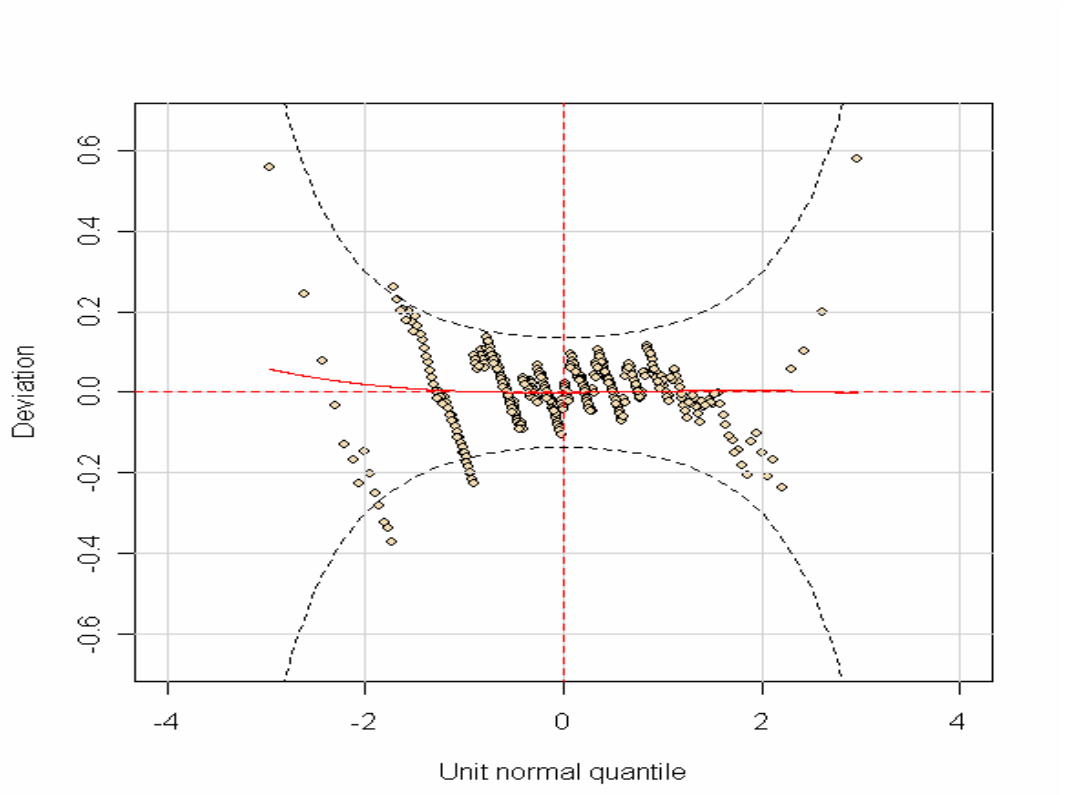


Figure 13 Worm plot from the Normal fitted model with BCPE(3,1,1,1)

Conclusion

1. Comparison of the LMS and LMSP method

The centile curves of LMS(3/4/3) and LMSP based on BCPE(3,1,1,1) are shown below (**Figure 14**). We can see the two models give very similar 2.5th and 50th centile curves, but the 97.5th centile curves from the 2 methods have big difference.

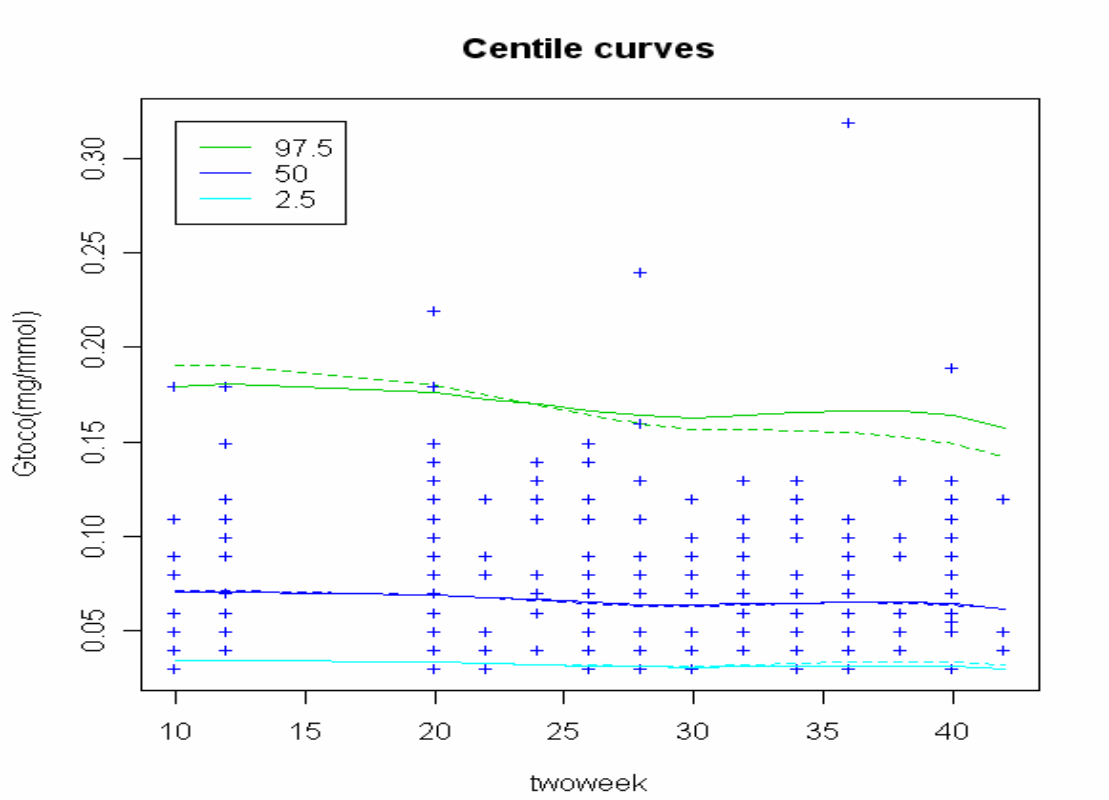


Figure 14 Comparing LMS and LMPS model. (Solid line for LMS; dashed line for LMSP)

From the plot of all the measurements, we pay attention to the values which are larger than 0.22, especially 0.32 at 36th week, and assuming them to be outliers, we can find the LMSP model (dashed line) robust to the outliers, and the LMS can not. Since the LMS model tries to use its skewness parameter as a proxy for modeling the kurtosis in the dataset. In our paper, a few outliers all happen to be in the upper tail, then the LMS tries to fit them using positive skewness, while the LMSP model views these outliers as representing kurtosis. “The fitted skewness parameter in LMS model is highly sensitive to outliers, while the LMSP is more robust to outliers” (Rigby and Stasinopoulos, 2004). Therefore, LMSP model give more convinced result because the kurtosis elimination and robust to outliers.

We should also note the LMS is a special case of LMSP when tau equals to two.

Further, we find it is flexible to use LMS and LMSP with R, and LMS is easily controlled by lmsChartMaker software.

2. The reference limits with different methods

Generally, when we come to the (lower and upper) reference limits, we can use age-specific method to get the 2.5th, 50th, and 97.5th percentile. For example, we use nonparametric method to calculate them in our paper for the ages which are not normally distributed with type 8th quantile method in R. (Hyndman and Yanan, 1996). Because we have 3 age groups correspond to the normal distribution, we give the lower and upper bounds with “mean-1.96SD” and “mean+1.96SD”. The reference limits of this intuitive method are shown in **Appendix D**.

For LMS and LMSP model, we can give any cenlile at any age, because we consider the “twoweek” as a continuous variable. So if we want to give the reference limits from 10th to 40th weekly, we give them as an example in **Appendix E** and **Appendix F**.

The intuitive method, LMS, and LMSP methods are all helpful to output the centiles we need, but the LMS and LMSP deal the age could predict any centile and centile curves. Besides, if we use the first method, the centile curves would be significant when it is a linear model, which is too weak to meet our smoothing aim. So the LMS and LMSP have advantages in this field, what’s more, LMSP is more convinced and reliable.

Discussion

“Fitting smooth centile curves has always been something of a subjective exercise, or even a black art.” (Cole and Green, 1992) The sample size of data in our paper is small, and it limits our reliability of the model. As a result, we have to take care of the irregular model; even they have perfect goodness of fit.

The smoothness trades off the goodness-of-fit for both LMS and LMSP models, and the standard to select the model is too flexible to control. Besides, we can not avoid the problem that find more developed smoothing methods for this kind longitudinal dataset.

What’s more, there are many discussions on LMS method to fit the longitudinal data when Cole proposed that relatively small longitudinal studies can provide well-defined estimates for the L, M and S curves, which as a conclusion from the study of the Cambridge infant growth study. (Cole, 1998a) The datasets were required

to be recent, cross-sectional ideally, however, in practical, most but not all of these aims have to be met. There are some papers with longitudinal data have been modeled by LMS method (Cole, 1988, 1998a).

For our data set, we will find the gamma-tocopherol is a longitudinal measurement, however, it is not so regular, that is during the period, every woman can have values at some of the ages. For example, the 1st woman tocopherol has measured in some of the weeks, and 2nd have values in other weeks. That leads to difficulty to calculate the correlation coefficient, even the dependence test. There is a method of conditional velocity proposed by Cole (1998b) expressing that difference between correlation data and independent data comes from the correlation coefficient; however, we have to get the same length for same women's measurement for every age to get the correlation coefficient in this method. It is impossible or too difficult in our model.

Although there has been little development towards the fitting of growth curves with correlated measurements (Borghi et al., 2006), the conditional standard deviation scores (Owen and Burton, 2000), multivariate centile charts (Thompson and Fatti, 1997), multilevel models (Pan and Goldstein, 1997) are all presented for solving this kind of problem to some extent. However, we should enlarge our data set and give more consistent measurements first, before we further our research.

For more computational development, the bootstrap technique can remedy our LMS, LMSP method, which are more suitable for cross sectional data. If we have large enough samples, we can deal with this by resampling of the measurements, to crate the new samples of same size in the bootstrap procedure, which will lead us to correctly estimating the standard errors of the centile estimates. (Borghi et al., 2006)

In later research, we will try to make the confidence intervals of centile curves to modify the reliability of the model. The method for longitudinal data should be pursued in the further study.

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Appendix

Appendix A: Methods for the construction of attained growth curves (Borghi and Onis, 2006)

Method	Centiles estimation	Curve-fitting method	Distributional assumptions
Bin methods, no smoothing Raw centiles, estimated separately	Separately	None	None
Bin and smooth methods, without distributional assumptions			
Fixed knot splines	Separately	Fixed knot splines	None
Eye fitting	Separately	Eye fitting	None
Kernel regression	Separately	Kernel estimation	None
CDC	Separately or Together	3-parameter linear Cubic splines	None Box-Cox normal
Bin and smooth methods, with distributional assumptions			
Eye fitting	Together	Eye fitting	Normal
Box-Cox normalization	Together	Eye fitting	Box-Cox normal
LMS1	Together	Cubic splines or others	Box-Cox normal
Variance stabilization	Together	Polynomials	Normal
Polynomial fitting	Together	Polynomials	Normal
Age handled continuously, without distributional assumptions			
Quantile regression, estimated separately	Separately	Quantile regression	None
HRY method	Together	Polynomials	None
Adapted HRY method	Together	Grafted polynomials	None
Kernel density estimation	Together	Kernel density estimation	None
Non-Gaussian quantile curves	Together	Nearest-neighbour kernel density of	None

		conditional cdf	
Regression quantiles, estimated together	Together	Natural splines	None
Age handled continuously, with distributional assumptions			
Multilevel models	Together	ML estimation of linear and non-linear models	Normal
Aitkin	Together	Linear models	Normal
Thompson and Theron	Together	Polynomials	Johnson system
LMS2	Together	Cubic splines	Box-Cox normal
Wade and Ades	Together	Exponential functions	Box-Cox normal
Wade and Ades (with correlations)	Together	ML exponential (spread, skewness); polynomial (mean)	Box-Cox
FPET method	Together	Fractional polynomials	(Modulus)-exponential-normal
Additivity and variance stabilization (AVAS)	Together	Non-parametric regression AVAS	Normal
Mean and dispersion additive models	Together	Parametric or non-parametric functions (MADAM)	Normal
S-distribution	Together	Polynomials	Normal
GAMLSS	Together	Linear parametric or additive non-parametric	Various
LSMT	Together	Cubic splines or (fractional) polynomials	Box-Cox-t
LSMP	Together	Cubic splines or (fractional) polynomials	Box-Cox-power-exponential

Appendix B: The summary of γ – tocopherol for different time (every two weeks)

week	min	median	mean	max	N
10	0.03000	0.06000	0.07944	0.18000	18
12	0.0400	0.0700	0.0825	0.1800	24
20	0.03000	0.07000	0.08027	0.22000	36
22	0.0300	0.0650	0.0660	0.1200	10
24	0.04000	0.07000	0.08133	0.14000	15
26	0.03000	0.06000	0.07314	0.15000	35
28	0.030	0.070	0.078	0.240	25
30	0.03000	0.05000	0.06348	0.12000	23
32	0.04000	0.06000	0.07424	0.13000	32
34	0.03	0.06	0.07	0.13	21
36	0.03000	0.06000	0.07351	0.32000	36
38	0.04000	0.09000	0.07615	0.13000	11
40	0.0300	0.0700	0.0760	0.1900	28

Appendix C The normality test result

week	n	W	p-value	Result
a10	18	0.828	0.003885	
a12	24	0.8964	0.01805	
a20	36	0.8184	3.869e-05	
a22	10	0.8968	0.2021	Normal
a24	15	0.8725	0.03666	
a26	35	0.9021	0.004504	
a28	25	0.7889	0.000149	
a30	23	0.9168	0.05704	
a32	32	0.9036	0.007645	
a34	21	0.9154	0.07015	
a36	36	0.5811	5.97e-09	
a38	11	0.9134	0.2676	Normal
a40	28	0.8834	0.004787	

Appendix D Find the limits of the reference interval with basic method

week	2.5Percentile	median	97.5Percentile	mean	Mean-1.96SD	Mean+1.96SD
10	0.03	0.06	0.18	0.08		
12	0.04	0.07	0.18	0.08		
20	0.03	0.07	0.21	0.08		
22		0.07		0.07	0.01	0.13

24	0.05	0.07	0.14	0.08		
26	0.03	0.06	0.15	0.07		
28	0.03	0.07	0.24	0.08		
30	0.03	0.05	0.12	0.06		
32	0.04	0.06	0.13	0.07		
34	0.03	0.06	0.13	0.07		
36	0.03	0.06	0.27	0.07		
38		0.09		0.08	0.019	0.134
40	0.03	0.08	0.19	0.08		

Appendix E Upper bound, median and lower bound (97.5th, 50th , 2.5th centiles) with LMS

twoweek	97.5^{th}	50^{th}	2.5^{th}
10	0.1792373	0.06997826	0.03373619
11	0.1801206	0.07032313	0.03390245
12	0.1808225	0.07059716	0.03403456
13	0.1811956	0.07074282	0.03410478
14	0.1812308	0.07075656	0.03411141
15	0.1809536	0.07064835	0.03405924
16	0.1803896	0.07042814	0.03395308
17	0.1795642	0.07010589	0.03379772
18	0.1785030	0.06969158	0.03359798
19	0.1772315	0.06919515	0.03335866
20	0.1757752	0.06862657	0.03308455
21	0.1741870	0.06800651	0.03278562
22	0.1726294	0.06739840	0.03249245
23	0.1712141	0.06684582	0.03222606
24	0.1697391	0.06626996	0.03194844
25	0.1680245	0.06560053	0.03162571
26	0.1662906	0.06492360	0.03129936
27	0.1648102	0.06434560	0.03102071
28	0.1636641	0.06389812	0.03080499
29	0.1629156	0.06360591	0.03066411
30	0.1627497	0.06354114	0.03063289
31	0.1632664	0.06374288	0.03073014
32	0.1641053	0.06407038	0.03088803
33	0.1648779	0.06437203	0.03103345
34	0.1655453	0.06463259	0.03115907
35	0.1661377	0.06486390	0.03127059
36	0.1666139	0.06504979	0.03136020
37	0.1668720	0.06515056	0.03140878
38	0.1666413	0.06506049	0.03136536
39	0.1656351	0.06466767	0.03117598

40	0.1636726	0.06390148	0.03080660

Appendix F Upper bound, median and lower bound (97.5th , 50th , 2.5th centiles) with LMSP

twoweek	97.5^{th}	50^{th}	2.5^{th}
10	0.1904712	0.07076776	0.03382181
11	0.1907488	0.07110538	0.03404542
12	0.1908566	0.07137180	0.03423327
13	0.1906562	0.07150826	0.03435524
14	0.1901233	0.07150642	0.03440745
15	0.1892616	0.07137050	0.03439265
16	0.1880747	0.07110475	0.03431361
17	0.1865665	0.07071342	0.03417313
18	0.1847408	0.07020073	0.03397398
19	0.1826020	0.06957094	0.03371895
20	0.1801549	0.06882828	0.03341075
21	0.1774487	0.06799444	0.03306072
22	0.1747080	0.06716083	0.03271487
23	0.1721142	0.06640224	0.03241111
24	0.1695080	0.06565705	0.03211945
25	0.1667345	0.06486551	0.03181090
26	0.1639853	0.06411175	0.03152950
27	0.1614999	0.06349928	0.03132949
28	0.1593700	0.06306537	0.03122980
29	0.1576749	0.06283821	0.03124405
30	0.1565953	0.06287570	0.03139746
31	0.1562129	0.06319629	0.03169529
32	0.1561421	0.06363061	0.03204872
33	0.1559824	0.06400290	0.03236534
34	0.1556998	0.06429965	0.03263811
35	0.1553278	0.06453937	0.03287757
36	0.1548134	0.06470658	0.03307757
37	0.1540605	0.06476598	0.03322143
38	0.1528829	0.06463711	0.03326716
39	0.1510971	0.06423833	0.03317139
40	0.1486113	0.06352826	0.03291195