

Estimation of Amino Acid Requirement Adjusting for Carry-Over Effect Based on Approximate Change-Point Regression Model.

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Abstract

It is an important issue to determine amino acid requirement in human in nutritional science. The indicator amino acid oxidation technique is often employed to the issue. An indicator amino acid is measured repeatedly from each participant at intake of various dosage of the target amino acid to determine the requirement. Change-point models are often employed to determine the requirement. Carry-over effects due to repeated measurement may influence on estimation of the change-point. In this paper, a model is introduced to evaluate the carry-over effect and to estimate the change-point adjusting for the carry-over effect. A simple inference procedure is introduced based on an approximation of the change-point model. It can be easily executed using a standard software for the non-linear mixed effect models. We apply the model to a dataset analyzed in a previous study to estimate the requirement of lysine in human. A simulation study is also conducted to evaluate the performance of the proposed method with small sample size.

Keywords: Indicator amino acid oxidation technique, Indispensable amino acid, Non-linear mixed effect model, Wash-out period

Accepted January 27 2013

Introduction

In nutritional science, the determination of amino acid requirement in humans has been of surging interest and importance [1-4]. The amino acid requirement is needed in order to support activities such as the determination of food and nutrition adequacy of population food intakes, setting of national food and nutrition guidelines by countries worldwide [5]. One of major methods to estimate amino acid requirement in human is indicator amino acid oxidation (IAAO) technique, based on the concept that when the target amino acid does not reach at the required level, in another word, is limiting protein synthesis, other amino acids in excess must be, therefore oxidized. This is because amino acid cannot be stored and therefore as the dietary amount of the target amino acid is increased, the other (indicator) amino acid is used more for protein synthesis and the oxidation of the indicator amino acid decreases until the requirement of the target amino acid is reached at sufficient level. By using this characteristic of amino acid, the IAAO method is designed to determine the requirement of a target amino acid. The IAAO is inversely related to protein synthesis and the inflection point where the oxidation of the indicator amino acid

stops decreasing and reaches a plateau is referred to as the change-point, where the requirement of the target amino acid is reached is at which protein synthesis is maximized and thus the oxidation of the indicator amino acid is minimized. Zello *et al* [6] reported a study to determine the requirement of lysine. Phenylalanine, an indicator amino acid was evaluated by collecting expired breath. ¹³CO₂ samples, which were not used for protein synthesis, repeatedly for each participant with various dosage of the target amino acid (lysine). A change-point model to estimate the requirement of lysine was applied [6]. As summarized in next section, phenylalanine was repeatedly measured and thus the observations within a participant should be regarded as statistically dependent. Another issue arising from repeated measurement is carry-over effect. Estimate of the change-point may be influenced by carry-over effect due to an insufficient washout-period. To the best of our knowledge, the influence of carry-over effect have not been accounted for in previous studies for amino acid requirement [1,3]. In medical studies such as crossover trial for bioequivalence of pharmacokinetics, carry-over effects are often discussed [7,9]. Model-based adjustment of carry-over effects have been often applied [8,9]. In most of the literatures dealing with model-based

adjustment of the carry-over effect, effects of the carry-over are incorporated in the analysis of variance models. However, such an approach based on linear regression does not seem to be a natural approach for the estimation of a change-point. In this paper, we propose a model to estimate the amino acid requirement adjusting for the carry-over effect. We introduce a simple inference procedure based on an approximate change-point model. Statistical inference based on maximum likelihood theory is utilized to make inference of the change-point. Our method can be conducted with a standard statistical software package for the non-linear mixed effect model such as NLMIXED procedure of SAS (SAS Institute inc., Cary, NC) and *nlme* function in S-plus (Mathematical Systems inc., Tokyo) and R and thus has a potential of wide use for determining amino-acid requirement.

Materials and Methods

Lysine requirement study

We provide a brief summary of the study conducted by Zello *et al.* [6]. Following the IAAO framework, the study was designed to determine the requirement of indispensable amino acid, lysine in healthy adult humans using L-[1-¹³C] phenylalanine as an indicator amino acid. Seven participants were enrolled in the study. The study consisted of two parts. In the first dietary period (series 1), the participants received an intake of the target amino acid at day 3, 6, and 9. After a washout period of at least 14 days, the second dietary period (series 2) started and the participants received an intake at day 3, 6 and 9 again. Each participant received six different dosage of seven lysine intakes (5, 10, 20, 30, 40, 50 and 60 mg/kg/d) and the order of doses were determined by a Latin square. Phenylalanine oxidations at each lysine intake and order of dosage of lysine intake were presented in Table 7 and 3

of Zello *et al.*[6] respectively. Zello *et al*[6] applied a change-point regression model by using the estimating method presented in the section 6.5 of Seber and Lee [10]. The change-point was estimated as 36.9 mg/kg/d with the 95% confidence interval (CI): [-0.041, 0.040] by Fieller's method.

Change-point regression model and approximate inference.

Let y_{ij} and x_{ij} be the measurement of L-[1-¹³C] phenylalanine and the dose of lysine at the j^{th} intake of lysine. Individual profiles of phenylalanine over dosage of lysine intake are presented (Fig. 1). As outlined in the previous section, the participants received lysine repeatedly [6]. In each series, dosage of lysine at day 3 may influence the measurement of phenylalanine oxidation at day 6 if 3 days interval of dosage is not enough for washout period. Similarly the measurement at day 9 may be influenced by the amount of lysine intake at day 6 and at day 3. We here introduce a model taking into account the influence of previous dosages of lysine. Let x_{ij}^* be the actual dose of lysine in the i^{th} participant's body at the j^{th} intake of lysine and x_{ij} be the dose at the j^{th} intake. Define $w_{ij}^{(-3)}$ and $w_{ij}^{(-6)}$ as the dose at three days and six days before the j^{th} intake respectively; $(w_{i1}^{(-3)}, w_{i1}^{(-6)})=(0, 0)$, $(w_{i2}^{(-3)}, w_{i2}^{(-6)})=(x_{i1}, 0)$, $(w_{i3}^{(-3)}, w_{i3}^{(-6)})=(x_{i2}, x_{i1})$, $(w_{i4}^{(-3)}, w_{i4}^{(-6)})=(0,0)$, $(w_{i5}^{(-3)}, w_{i5}^{(-6)})=(x_{i4},0)$ and $(w_{i6}^{(-3)}, w_{i6}^{(-6)})=(x_{i5}, x_{i4})$. The order of the intake is given in Table 3 of Zello *et al.*[3]. Between x_{ij} and x_{ij}^* , a structural model $x_{ij}^* = x_{ij} + f_{-3} w_{ij}^{(-3)} + f_{-6} w_{ij}^{(-6)}$ (Eq1) is considered. Parameters f_{-3} and f_{-6} are regarded as a rate of dosage of intake at three days and six days before the j^{th} intake accumulating in body at j^{th} intake of lysine respectively.

We consider the following change-point model,

$$\begin{aligned}
 y_{ij} &= \mu + b_i + m(x_{ij}^* - x_{cp})I(x_{ij}^* < x_{cp}) + \varepsilon_{ij} \\
 &= \mu + b_i + m(x_{ij} + f_{-3} \omega_{ij}^{(-3)} + f_{-6} \omega_{ij}^{(-6)} - x_{cp})I(x_{ij} + f_{-3} \omega_{ij}^{(-3)} + f_{-6} \omega_{ij}^{(-6)} < x_{cp}) + \varepsilon_{ij}
 \end{aligned}
 \tag{Eq2}$$

for $i=1,2, \dots, N$ and $j=1,2, \dots, J$, where J denotes the number of lysine intake, N is the number of participants and $I(x_{ij}^* < x_{cp}) = 1$ if $x_{ij}^* < x_{cp}$ and 0 otherwise. Fixed effects are m , μ and x_{cp} , and b_i is a random effect following a normal distribution with mean 0 and variance σ_b^2 . A random error ε_{ij} follows a normal distribution with mean 0 and variance σ^2 and b_i and ε_{ij} are assumed statistically independent.

In the model (Eq2), multiple unknown parameters are included in the indicator function. Thus it seems difficult to apply the formerly-used methods [10]. One may employ the maximum profile likelihood method [11,12]. However it is difficult to obtain a confidence interval even with the profile likelihood method.

We propose an approximate inference procedure. We introduce a model

$$\begin{aligned}
 y_{ij} &= \mu + b_i + \log[1 + \exp\{x_{ij}^* - x_{cp}\}] + \varepsilon_{ij} \\
 &= \mu + b_i + \log[1 + \exp\{x_{ij} + f_{-3}\omega_{ij}^{(-3)} + f_{-6}\omega_{ij}^{(-6)} - x_{cp}\}] + \varepsilon_{ij}
 \end{aligned}
 \tag{Eq3}$$

Suppose $m < 0$. If $x_{ij}^* < x_{cp}$, $\exp\{m(x_{ij}^* - x_{cp})\} \gg 1$ and then the model (Eq3) provides an approximation $y_{ij} \approx \mu + b_i + m(x_{ij}^* - x_{cp}) + \varepsilon_{ij}$. If $x_{ij}^* \gg x_{cp}$, $\exp\{m(x_{ij}^* - x_{cp})\} \approx 0$ and then $y_{ij} \approx \mu + b_i + \varepsilon_{ij}$.

Therefore the model (Eq3) is expected to approximate the model (Eq2). If the model (Eq3) approximates the model (Eq2) well, one can estimate unknown parameters of the model (Eq2) by maximum likelihood method based on the model (Eq3). The model (Eq3) is a special case of the standard non-linear mixed effect models [13] and unknown parameters in the model (Eq3) can be easily estimated by using standard software packages for nonlinear

mixed effect models such as NLMIXED procedure in SAS and *nlme* function in S-plus and R. Confidence intervals are obtained following the standard asymptotic theory of the maximum likelihood estimation [13].

The mean functions of the model (Eq2) and that of the model (Eq3) are plotted with $m = -0.1$ (A) and $m = -0.5$ (B) (Fig. 2). These plots indicate that the appropriateness of approximation of the model (Eq3) to the model (Eq2) depends on the absolute value of m ; if the absolute value of m is not sufficiently large, the approximation may be poor. By multiplying some positive constant to y_{ij} , the absolute value of m can be large. By the transformation, the parameters x_{cp} , f_{-3} and f_{-6} do not change. Based on this property, we propose to apply the model (Eq3) after multiplying a large positive constant ρ to y_{ij} .

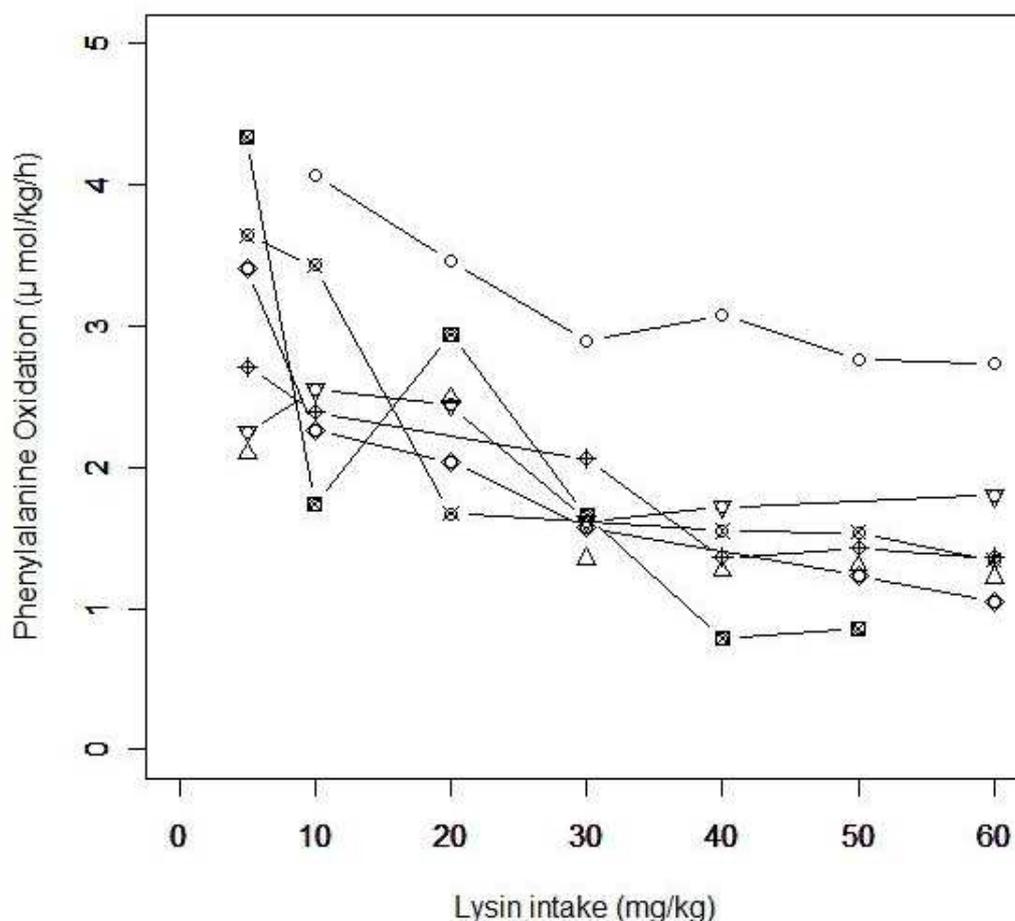


Figure 1. Effect of lysine intake on production of $^{13}\text{CO}_2$ from the oxidation of L-[1- ^{13}C] phenylalanine ($F^{13}\text{CO}_2$) [1] Illustrated is the oxidation response of phenylalanine at varying dietary intakes of lysine.

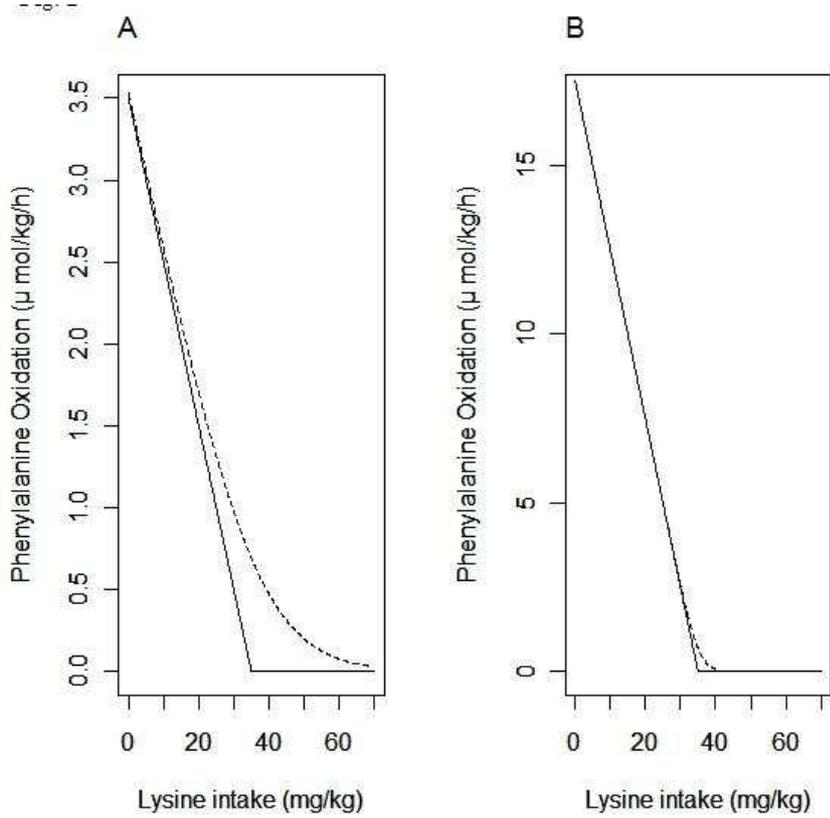


Figure 2. Mean profiles of the change-point model (Eq2) and the approximate change-point model (Eq3) with $m = -0.1$ (A) and $m = -0.5$ (B).

Results

The results of statistical analyses of lysine data are presented. We fit the model (Eq3) with sufficiently large ρ for estimation to have better approximation. Fitted mean functions over dosage of lysine based on the model (Eq3) and that of corresponding the model (Eq2) with $\rho=1$ and

$\rho=3$ are presented (Fig. 3).with observations of a participant (idno=3 as an example). Approximation of the model (Eq3) to the model (Eq2) is not good with $\rho=1$, whereas the mean function of the model (Eq2) is close to that of the model (Eq3) with $\rho = 3$. To evaluate goodness-of-fit, we introduce a measure called the standardized mean absolute difference (SAAD). Let

$$\hat{y}_{ij} = \hat{\mu} + \hat{m} (x_{ij} + \hat{f}_{-3}\omega_{ij}^{(-3)} + \hat{f}_{-6}\omega_{ij}^{(-6)} - \hat{x}_{cp}) I(x_{ij} + \hat{f}_{-3}\omega_{ij}^{(-3)} + \hat{f}_{-6}\omega_{ij}^{(-6)} < \hat{x}_{cp})$$

$\hat{\mu}$, \hat{f}_{-3} , \hat{f}_{-6} and \hat{x}_{cp} are estimators by applying the approximate model (Eq3). SAAD is defined as

$$SAAD(\rho) = \frac{1}{\rho n} \sum_{i=1}^N \sum_{j=1}^J |y_{ij} - \hat{y}_{ij}|$$

which is regarded as an average absolute difference between the observed and the predicted value standardized to the scale of original observations. Minimum of SAAD is attained at $\rho = 3$. With $\rho = 3$, the change-point is estimated as 35.43 with 95% CI: [22.32, 48.53], whereas, with $\rho=1$, it is estimated very differently as 25.66 with 95% CI : [13.92-37.40] (Table 1). By taking account into

within-participant correlation, the width of the confidence interval of the change-point is much narrower than previously-reported one [6]. With $\rho = 3$, f_{-3} and f_{-6} are estimated 0.15 of 95% CI: [-0.01, 0.30] and 0.04 of 95% CI: (-0.18, 0.26) respectively (Table 2). This result indicates that about 15% intake at three days ago and 4% one at six days ago are remaining in participant's body and thus washout period seems not to be enough. Although the washout period is not necessarily enough, the estimate without the lysine effect of previous accumulation is close to that in our approach which takes account into carry-over effect [6]. With $\rho > 1$, the estimates of x_{cp} , f_{-3} and f_{-6} are not strongly affected by p

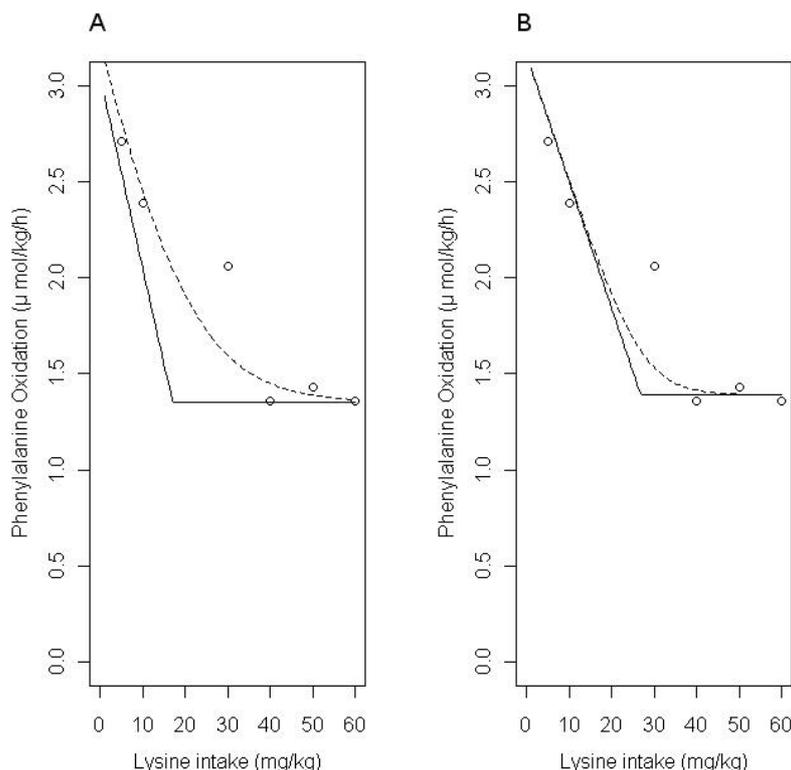


Figure 3. Estimated mean profile of a participant (*idno=3*) by the approximate change-point model (Eq3) and that of the corresponding change-point model (Eq2) with $\rho = 1$ (A) and $\rho = 3$ (B): the mean profile of the model with $\rho = 3$ is standardized to the original scale divided by ρ .

Table 1. Results of statistical analysis of lysine data by the approximate change-point model (Eq5) with $\rho = 1, 3$.

	Estimate	$\rho = 1$			$\rho = 3$			
		SE	P	95% CI	Estimate	SE	P	95% CI
x_{cp}	25.66	4.80	0.002	(13.92, 37.40)	35.43	5.36	<0.001	(22.32, 48.53)
μ	1.47	0.23	<0.001	(0.90, 2.03)	4.54	0.65	<0.001	(0.98, 2.05)
m	-0.10	0.02	0.005	(-0.15, -0.04)	-0.2	0.04	0.003	(-0.99, 0.10)
f_1	0.14	0.06	0.052	(-0.00, 0.29)	0.15	0.06	0.061	(-0.01, 0.30)
f_2	0.05	0.10	0.638	(-0.19, 0.28)	0.04	0.09	0.663	(-0.18, 0.26)

Table 2. Estimate of the change-point by the approximate change-point model (Eq4).

ρ	Change-point	95% CI	SAAD
1	25.66	(13.92, 37.4)	0.394
2	32.98	(19.95, 46.01)	0.322
3	35.43	(22.32, 48.53)	0.314
4	36.49	(23.40, 49.58)	0.314
5	37.00	(23.88, 50.12)	0.314
6	37.24	(24.05, 50.43)	0.314
7	37.34	(24.08, 50.59)	0.314

Table 3. Empirical mean and coverage of the estimator by the approximate change-point model (Eq4) in the case of the true $f_1 = 0.15$.

ρ	Change-point model with carryover-effect			
	Change-point (true:34.5)		Carryover effect	
	Mean	Coverage	Mean	Coverage
1	24.047	0.385	0.134	0.966
2	30.467	0.851	0.142	0.977
3	32.539	0.939	0.146	0.977
4	33.434	0.953	0.148	0.973
5	33.887	0.954	0.150	0.973
6	34.144	0.956	0.150	0.974
7	34.285	0.951	0.151	0.975
8	34.395	0.946	0.158	0.971
9	34.481	0.938	0.157	0.972
10	34.657	0.932	0.145	0.969

Simulation

We present here the results of a simulation study. The objective of the simulation study is to evaluate bias and coverage probability of the estimators for the change-point in the presence of carry-over effect. Same as the

$$y_{ij} = \mu + b_i + m(x_{ij} + f_1 \omega_{ij}^{(1)} - x_{cp})I(x_{ij} + f_1 \omega_{ij}^{(1)} < x_{cp}) + \epsilon_{ij}. \tag{Eq5}$$

Parameters are set as $x_{cp}=34.5$, $m=0.2$, $f_1=0.15$, $V(b_i)=\exp\{0.7\}$ and $V(\epsilon_{ij}) = \exp(0.5)$. One thousand datasets were generated and the model [6] was fitted to them after multiplying a constant $\rho=1, 2, \dots, 10$. For the comparison, a model without the carry-over effect term $f_1 \omega_{ij}^{(1)}$ was also applied. Empirical biases and coverage probabilities for $f_1=0.15$ with various ρ s are summarized (Table 3). Biases in the estimate of the change-point by the model with the carry-over effect are negligible with $\rho > 3$. The carry-over effect also can be estimated with considerable biases. For both parameters, coverage probabilities are very close to the nominal level if an appropriate ρ is employed. On the other hand, the model without the carry-over effect provides the biases estimate for the change-point and the coverage probabilities are less than

situation in the article [6], sample size was set at $n = 7$ and the order of dosage of lysine was determined by a Latin square. Based on the data [6], we generated y_{ij} from the model

the nominal level. We conducted simulation experiments with the other set of parameters and obtained similar results to the above setting. These results indicate that in studies to determine the amino acid requirement, the estimation without adjusting for the carry-over effect may lead downwardly biased estimate of the change-point.

Extension

The slope of the model (Eq2) after the change-point is set at 0 since it seems to be natural for determining amino acid requirement than allowing any slope parameters from biological point of view. It is useful to consider a model with arbitrary slope parameter after the change-point to evaluate the assumption of zero slope after the change-point in the determination of amino acid requirement. Such models are often investigated for other issues in nutrition research [13,14]. Consider a model,

$$\begin{aligned} y_{ij} &= \mu + b_i + m_1(x_{ij}^* - x_{cp})I(x_{ij}^* < x_{cp}) + m_2(x_{ij}^* - x_{cp}) + \epsilon_{ij}, \\ &= \mu + b_i + m_1(x_{ij} + f_1 \omega_{ij}^{(1)} + f_2 \omega_{ij}^{(2)} - x_{cp})I(x_{ij} + f_1 \omega_{ij}^{(1)} + f_2 \omega_{ij}^{(2)} < x_{cp}) \\ &\quad + m_2(x_{ij} + f_1 \omega_{ij}^{(1)} + f_2 \omega_{ij}^{(2)} - x_{cp}) + \epsilon_{ij}. \end{aligned} \tag{Eq6}$$

With a similar approximate model, one can estimate unknown parameters using standard software for the non-linear mixed effect model. With $\rho = 3$, the slope parameter m_2 was estimated -0.0007 (95% CI: [-0.041, 0.040], $P=0.96$) indicating that the slope is flat after the change-point. The estimate of the change-point was not different from the one using the model (Eq2).

As another extension, one may consider the change-point is participant-specific. Let the participant-specific change

point denoted by $x_{cp,i}$ and the following structural model $x_{cp,i} = x_{cp} \exp(\eta^T Z_i)$ is considered, where Z_i is a covariate vector for the i^{th} participant. The unknown parameter η can be estimated by the method we proposed. Let $Z_i^{(1)}$ and $Z_i^{(2)}$ be BMI minus 1850 and RMI (resting metabolic rate, kcal/day) of the i^{th} participant in the data of Zello *et al.*[6]. We apply the model,

$$y_{ij} = \mu + b_i + m_1(x_{ij}^* - x_{cp})I(x_{ij}^* < x_{cp} \exp(\eta_1 Z_i^{(1)} + \eta_2 Z_i^{(2)})) + \epsilon_{ij}.$$

The parameters η_1 and η_2 were estimated 0.02 (95% CI: [-0.086, 0.135]) and -0.001 (95% CI: [-0.0002, 0.0016]) respectively and neither was statistically significant. The study was not designed to examine the effect of covariates on the change-point and the ranges of BMI and RMI were very narrow. It might cause non-significance of BMI and RMI.

Discussion

In this manuscript, we introduced a change-point model adjusting for carry-over effects due to repeated measurements in determining amino acid requirement based on the IAAO technique. A simple method for parameter estimation is proposed based on approximating the discon-

tinuous mean function with a smooth one. Our method can be easily conducted with a standard statistical software package such as R software. R is a free software developed by the R Foundation for Statistical Computing (<http://www.r-project.org/>). A program code in R is provided for our proposed method (Appendix I). Some researches considered to estimate the change-point from repeated measurements with alternative approximation [15,16]. Although they did not account for the carry-over effect, their approximations are also applicable to our models. Through the simulation study, we found that estimation based on approximation works well in the presence of the carry-over effect with small sample size arising in studies for amino acid requirement. Furthermore we found that estimate of the change-point with adjusting for the carry-over effect may be less biased when the order of the dosage of the target amino acid is determined by a Latin square. Thus here we recommend applying our proposed method in estimating the amino acid requirement based on the IAAO technique.

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Appendix I: R code to fit the approximate change-point model (Eq3)

```
#--including R package for non-linear mixed effect model-
library(nlme)

#--input dataset--
setwd("E:\\")
data<-read.csv("E:\\data_zello2.csv",header=T)

=====
#--multiplying constant to improve approximation--
rho<-1
data$fe_rho<-data$fe*rho

#--transforming data into repeated measurement format---
data2<-groupedData(fe~lys|id,data=as.data.frame(data))

#--Fitting approximate change-point mixed model----
result1<-
nlme(fe_rho~log(1+exp(m*(lys+f1*prelys1+f2*prelys2-
a)))+b+eb,
fixed=c(f1~1,f2~1,a~1,b~1,m~1),
random=eb~1,
data=data2,
start=c(f1=0.1,f2=0.1,a=30,b=3,m=-0.3),
method="REML")
summary(result1)
```