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A model to analyse the postprandial nutrient concentration in the plasma of pigs

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ABSTRACT

Changes in the postprandial nutrient concentration in the plasma are the result of the combined effects of intake, digestion, absorption, and metabolism. The concentration typically follows an asymmetrical bell-shaped curve as a function of the time after the meal. Although differences between dietary treatments can be analysed using a pairwise comparison of the observed nutrient concentrations, this provides little insight in the possible underlying biological mechanisms. These mechanisms may be represented in a model that can be used in a regression analysis to summarise the observed data in a limited number of parameters. The objective of this study was to propose equations that can be used in the statistical analysis of postprandial nutrient concentrations. The equations were derived from the compartmental representation of the Erlang function in which the last of a series of compartments was assumed to represent the nutrient concentration in the plasma. The preceding compartments were used to represent the postprandial response provoked by ingestion of the meal. A homeostatic control mechanism was included based on a target nutrient concentration that the animal seeks to maintain. This target concentration may differ between the fasting state and after ingestion of a meal. The models were developed as differential equations, which were integrated analytically providing equations that can be used for data analysis. The fit of the equations was tested using the postprandial histidine concentration of a pig that received a diet that was either balanced or unbalanced in the amino acid supply. The unbalanced diet was also deficient in histidine. The observed data could be summarised in three or four parameters that describe the target histidine concentration after an overnight fast, the possible change in the target concentration due to ingestion of a meal, the area under curve of the postprandial response (i.e., the "metabolic exposure"), and a rate constant describing the dynamics of the response. The biological interpretation of these and derived parameters is discussed, including the potential pitfalls of interpreting nutrient concentrations as nutrient flows. In conclusion, the models developed here are based on biological concepts and allow to summarise time series of nutrient concentrations in a limited number of parameters. The concepts can be modified depending on how the biological mechanisms involved are perceived and on the type of available data.

Implications

There are different ways to analyse the effect of an experimental treatment on the postprandial nutrient concentration in the plasma. A pairwise comparison of observations between treatments ignores the relationship that exists between successive observations. Analysis of the data by a regression model requires to define the (supposed) underlying biological mechanisms and summarises the data as a set of parameters. Differences between experimental treatments can then be summarised in parameters and analysed in relation to the biological representation in the model and the experimental treatments.

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Specification table

Subject	Nutrition
Type of data	Maple V (release 5.1) code and output, data used as example, R code (version 4.1.2) of the non-linear regression models and output.
How data were acquired	Data were obtained from a study in which growing pigs were given, after an overnight fast, a meal with either a balanced or unbalanced supply of amino acids. Pigs had jugular catheters from which blood samples were taken at regular intervals. Plasma was obtained by centrifugation of the blood, and plasma amino acids were analysed by Ultra Performance Liquid Chromatography (UPLC).
Data format	Raw data, analysed data (i.e., estimated model parameters), and output from Maple with the analytical solutions obtained after integrating the differential equations.
Parameters for data collection	Data are recorded as the histidine concentration in the plasma as a function of time after ingestion of a meal.
Description of data collection	The data were collected from a growing pig that was given a meal with a balanced or unbalanced supply of amino acids. The unbalanced diet was deficient in histidine. The meal was given after an overnight fast.
Data accessibility	Repository name: Portail Data INRAE Data identification number: https://doi.org/10.15454/GKFOEH

Introduction

The postprandial nutrient concentration in the plasma is the result of the combined effects of intake, digestion, absorption, and metabolism of the nutrient. After an overnight fast and ingestion of a meal, the concentration typically follows an asymmetrical bell-shaped curve, characterised by a rapid increase from the baseline, to reach a maximum and gradually decline to the baseline again. Differences between experimental treatments can be reported as the time points at which the concentration of one treatment differs from another treatment, using a repeated-measures model (Bos et al., 2003). Also, the area under the curve (**AUC**) can be calculated, which is typically done by dividing the response curve in trapezoids between two successive observations. The AUC is then the sum of the areas of each trapezoid. The trapezoid method is frequently used in pharmacokinetics to study the fate of an administered drug, because the plasma concentration of the drug starts at zero (before the administration of the drug) and ends at zero (once the drug is fully metabolised). The interpretation of the AUC is more difficult for nutrients, which are not only digested, absorbed, and catabolised but may also be synthesised from other nutrients, and stored in and mobilised from body reserves. Alternatively, regression models can be used to describe the change in the nutrient concentration in the plasma as a function of time. McKnight et al. (2013) developed a framework of conceptual models to analyse the change in nutrient concentration after ingestion of a meal or provision of a marked nutrient. The nutrients in these models were represented as compartments with different inflows (e.g., through nutrient absorption, *de novo* synthesis, or marker supply) and outflows (e.g., through uptake of the nutrient by tissues or catabolism). Gloaguen et al. (2012) used an Erlang function to describe the change in amino acid concentration in the plasma. The model was mainly used to express the plasma kinetics using a limited number of parameters but without a biological interpretation of the model parameters. The goal of this paper was to further develop the Erlang function to make the underlying biological assumptions more explicit.

Material and methods

The Erlang function

The Erlang function can be seen as a series of n compartments in which each compartment receives input from the previous compartment and where the output is described by first-order kinetics (Matis et al., 1989):

$$\frac{\partial X_n}{\partial t} = \lambda\, X_{n-1} - \lambda\, X_n$$

where X_n is the size of compartment n (μmol/L), $\partial X_n/\partial t$ the change in size of the compartment (μmol/L per h), t the time (h), and λ the fractional outflow rate (1/h). For this study, it was assumed that $n = 3$, but this has no consequence for the rationale developed further. The last compartment (i.e., X_3) may be seen as the nutrient concentration in the plasma (C, μmol/L; top panel of Fig. 1). The differential equations describing the system are as follows:

$$\frac{\partial X_1}{\partial t} = -\lambda\, X_1$$

$$\frac{\partial X_2}{\partial t} = \lambda\, X_1 - \lambda\, X_2$$

$$\frac{\partial X_3}{\partial t} = \frac{\partial C}{\partial t} = \lambda\, X_2 - \lambda\, X_3$$

with initial conditions when time = 0: $X_1(0) = Q$, $X_2(0) = 0$, and $X_3(0) = C(0) = 0$. The Q (μmol/L) is the apparent quantity of the dietary nutrient arriving in the plasma provoking the change in the nutrient concentration. The *AUC* for each of the three compartments equals Q/λ. Integrating the system of differential equations with Maple (version 5.1, Waterloo Maple Inc., Waterloo, Canada) results in the following equation for the last compartment $C(t)$:

$$C(t) = \frac{1}{2}\, AUC\, \lambda^3\, t^2\, \exp(-\lambda\, t) \qquad (1)$$

where *AUC* μmol·h/L is the area under the curve, λ the fractional outflow rate from C (1/h), and t the time (h).

The modified Erlang function

In the model depicted in the top panel of Fig. 1 and Eq. (1), the concentration C(t) will initially be zero, but will gradually increase and decrease again to zero due to the flow of Q (i.e., the initial value of X_1) through X_2 and C. This may be sufficient to describe the fate of a drug, but is not appropriate to describe the metabolism of a nutrient because it is unlikely that the nutrient concentration will equal zero, even during fasting. To include the concept of a basal nutrient concentration in the model, the Erlang function was modified as depicted in the bottom panel of Fig. 1, where a target nutrient concentration is included that the animal seeks to maintain (C_{target}; μmol/L). The control of C is bidirectional and a nutrient concentration greater than C_{target} results in a net outflow from C,

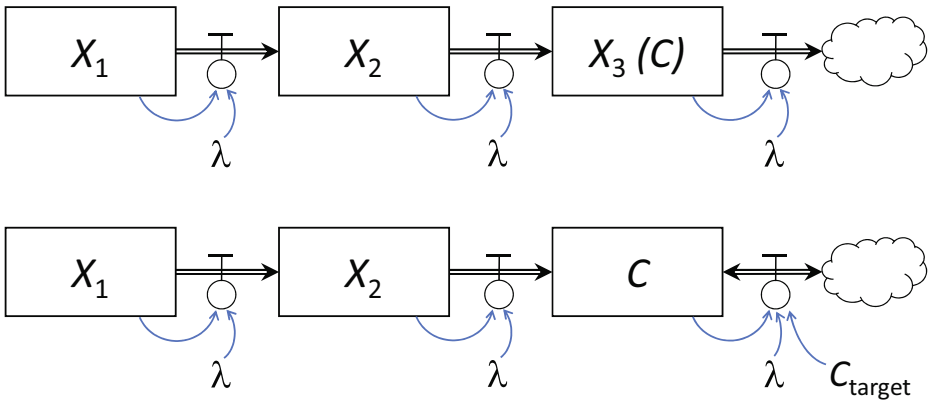

Fig. 1. Top panel: Forester diagram of the Erlang function described as a series of n sequential compartments (n = 3). The last compartment (C) is assumed to represent the nutrient concentration in the plasma (μmol/L). Ingestion of a meal provokes a change in C via two compartments (X_1 and X_2). The λ (1/h) is the fractional outflow rate. Bottom panel: Modified Erlang function where the nutrient concentration in the plasma is controlled by a target concentration (C_{target}; μmol/L) that the animal seeks to maintain.

whereas a lower concentration results in a net inflow into C. The differential equation describing the change in C is as follows:

$$\frac{\partial C}{\partial t} = \lambda\, X_2 \; - \lambda(C - C_{target})$$

Integrating this equation with $C(0) = C_{target}$ results in:

$$C(t) = \frac{1}{2}\, AUC\, \lambda^3\, t^2\, \exp(-\lambda\, t) + C_{target} \qquad (2)$$

Eq. (2) equals Eq. (1) with a constant term (i.e., C_{target}) added to it. The AUC is the area under the curve of $C(t)$ over and above C_{target}, and can be considered as the "metabolic exposure" due to ingestion of the meal. The top panel of Fig. 2 illustrates the nutrient concentration as described by Eq. (2). The maximum of $C(t)$ occurs at $t_{max} = 2/\lambda$ and equals $C_{max} = C_{target} + 2\, AUC\, \lambda \exp(-2)$.

There are experimental situations in which the postprandial nutrient concentration does not return to the target concentration, at least not during the period of measurements. This can be represented by assuming that the target concentration changes due to ingestion of a meal to become $C_{target} + C_\delta$, so that:

$$\frac{\partial C}{\partial t} = \lambda\, X_2 - \lambda(C - (C_{target} + C_\delta))$$

Integrating the system of equations with $C(0) = C_{target}$ results in:

$$C(t) = \frac{1}{2}\, AUC\, \lambda^3\, t^2\, \exp(-\lambda\, t) + C_{target} + C_\delta(1 - \exp(-\lambda\, t)) \qquad (3)$$

Eq. (3) equals Eq. (2) with an extra term (i.e., $C_\delta\,(1 - \exp(-\lambda\, t))$ that describes the dynamics of the change in the target concentration from C_{target} to $C_{target} + C_\delta$. An example of Eq. (3) is given in the bottom panel of Fig. 2, illustrating that the observed nutrient concentration in the plasma is the result of two modes of action provoked by the ingestion of a meal: a change in the nutrient concentration related to the supply and further metabolism of the nutrient from the meal, and a change in the target concentration during the measurement period. The dynamics of both modes of action are determined by λ.

Fitting the model to experimental data

The use of the equations is illustrated by fitting these to the histidine concentration in the plasma of a growing pig that received, after an overnight fast, a diet with a balanced or unbalanced supply of amino acids. The supply of some amino acids, including histidine, was deficient in the unbalanced diet. Fitting the model to the data was done using the nls procedure of R (version 4.1.2). The example data and the R code are available from https://doi.org/10.15454/GKFOEH.

Results

Fig. 3 illustrates the fit of Eq. (2) to the histidine concentration in the plasma of a pig that received diet balanced in the amino acid supply. The estimated parameter values were C_{target} = 44.4 μmol/L, AUC = 116 μmol·h/L, and λ = 1.83/h, with a residual standard deviation of 6.4 μmol/L. The maximum concentration (C_{max}) of 102 μmol/L was attained at 1.09 h.

Fig. 3 also gives the fit of Eq. (3) to the experimental data in which the same pig was given a diet unbalanced in different amino acids and deficient in histidine. The estimated parameter values were C_{target} = 48.3 μmol/L, C_δ = −17.7 μmol/L, AUC = 34.1 μmol·h/L, and λ = 2.47/h, with a residual standard deviation of 2.7 μmol/L. The C_δ differed significantly from zero ($P < 0.001$).

The histidine concentrations were very different when the pig was given a diet balanced or unbalanced in amino acids, which is reflected by the estimated parameters. The C_{target} after an overnight fast was similar in both situations, but the deficient histidine supply in the unbalanced diet resulted in a lower target histidine concentration (i.e. $C_{target} + C_\delta$ = 30.6 μmol/L) compared to the balanced diet (C_{target} = 44.4 μmol/L). Also, the maximum histidine concentration was achieved earlier and attained a lower maximum value when the pig received the unbalanced diet compared to the balanced diet. The histidine concentration in the unbalanced diet was approximately 70% lower than in the balanced diet (1.00 vs 3.43 g/kg diet), which also resulted in a 70% reduction in the AUC (i.e., 34.1 vs 116 μmol·h/L).

Authors' points of view

The models and parameters

The models used here allow to summarise a series of observed nutrient concentrations in three or four model parameters. The parameters C_{target}, AUC, λ, and C_δ were chosen to parameterise the models, because of their biological interpretation and because initial values are easy to obtain "visually" from the observed data (i.e., $\lambda = 2/t_{max}$ and $AUC = (t_{max}\,(C_{max} - C_{target}))/(4\exp(-2)$ for the 3-parameter model), ensuring rapid convergence in the nonlinear parameter estimation procedure.

The models were derived from the Erlang function, to which several modifications were made in relation to the control of C. However, the same fractional rate (λ) was used to determine the

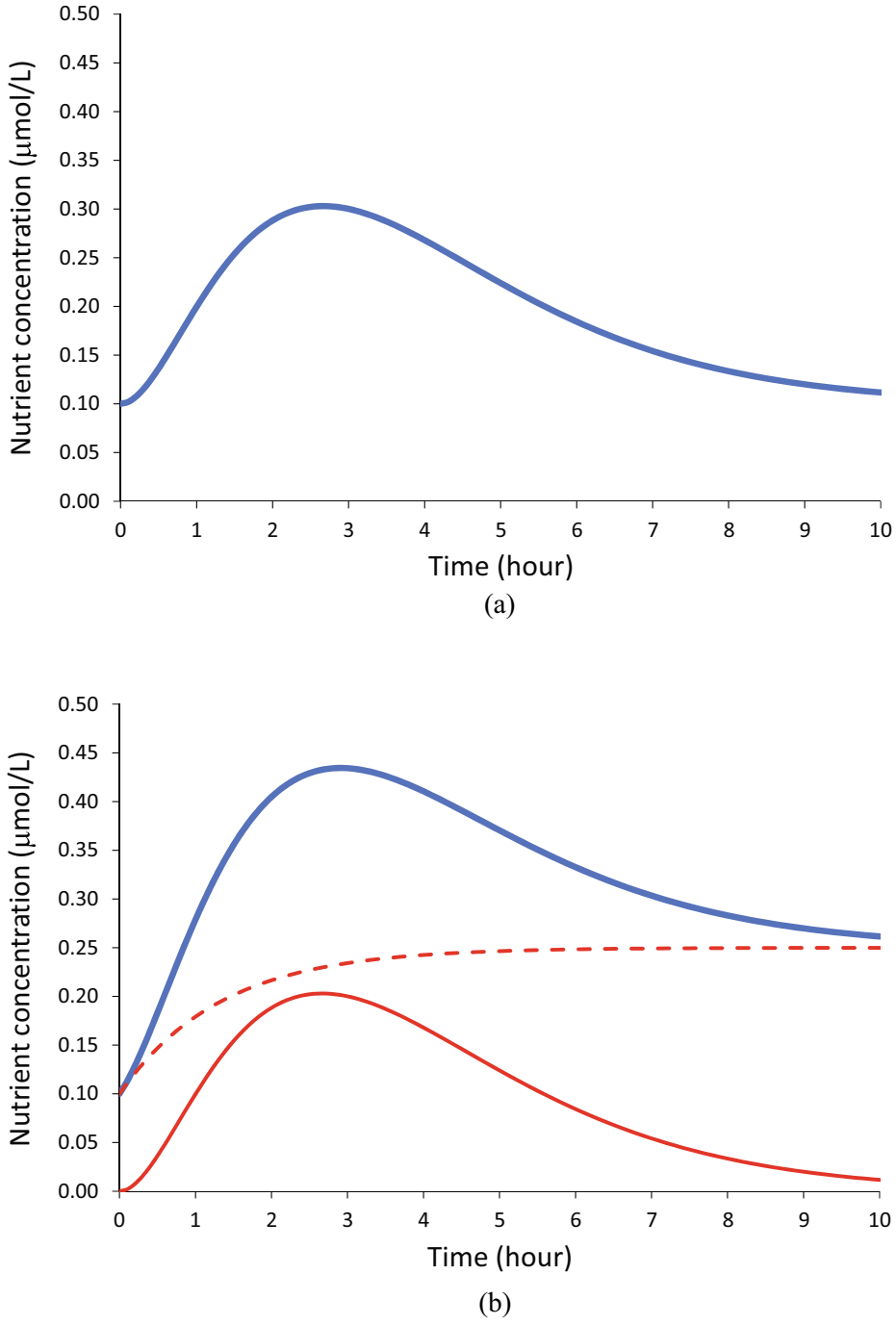

Fig. 2. Top panel: Postprandial nutrient concentration in the plasma of the modified Erlang function in which concentration is controlled by a constant target concentration (Eq. (2)). Bottom panel: Postprandial nutrient concentration in the plasma of the modified Erlang function in which the concentration is controlled by a changing target concentration due to the ingestion of a meal. The solid red line represents the change in the nutrient concentration provoked by ingestion of a meal (i.e., the first term of Eq. (3)) and the dashed red line the change in the target concentration from C_{target} to $C_{target} + C_{\delta}$ (i.e., the last two terms of Eq. (3)). The blue line is the sum of the two red lines and describes the nutrient concentration in the plasma. The C_{target} = 0.1 µmol/L, C_{δ} = 0.15 µmol/L, *AUC* = 1 µmol·h/L, and λ = 0.75 per hour (see text for details).

inflow into C from the diet (through X_1 and X_2) and to control C (through C_{target} and C_{δ}). An alternative approach would be to differentiate these fractional flow rates, but such an approach would require an additional parameter to be estimated.

Nutrient concentrations and nutrient flows

Changes in nutrient concentration are related to changes in nutrient flows but should not be interpreted as absolute values of flows. In steady-state conditions, the net nutrient flow through C equals zero but this does not mean that the actual flow through nutrient mobilisation and further metabolism is also zero. Likewise, Q is an "apparent" quantity and cannot be compared directly with the histidine supply by the meal.

The assumption that the nutrient concentration is controlled by nutrient homeostasis in the plasma is a rather strong assumption, because nutrient homeostasis can also be the result of the difference between inflow and outflow, each of which may be controlled independently without an explicit control of C. This has consequences on the interpretation of certain model concepts

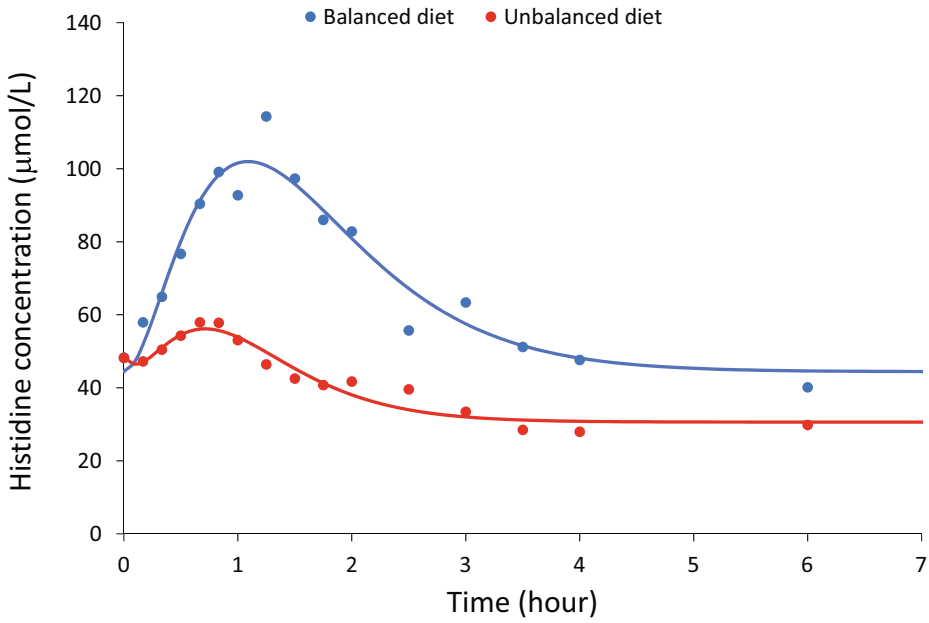

Fig. 3. Observed (dots) and estimated (line) histidine concentration in the plasma of a pig given either a diet balanced or unbalanced in the amino acid supply after an overnight fast.

and derived model parameters. There are several instances where the unknown nutrient inflow in C equals the unknown outflow (i.e., at C_{target}, $C_{target} + C_{\delta}$, and C_{max}) , but they result from different metabolic conditions. For example, the increase in concentration from C_{target} to C_{max} likely resulted from an increased inflow of nutrients provided by the meal, followed by an increased outflow, to become equal again at t_{max}. With this sequence of events, the nutrient flow is thus greater at C_{max} than at C_{target}, and the ratio between C_{max} and C_{target} is indicative for the nutrient flow at t_{max} relative to that at t_0. (i.e., for the balanced amino acid supply data, the histidine flow is 102/44.4 = 2.30 times greater at t_{max} than at t_0). However, such a reasoning must be used with caution, because opposing attributions of cause and effect can result in the same plasma concentration. For example, for the unbalanced amino acid supply, ingestion of the meal resulted in a lower target nutrient concentration (i.e., $C_{\delta} < 0$). This could be the result of an increased outflow followed by an increased inflow, but also from a decreased inflow followed by a decreased outflow. The ratio between $C_{target} + C_{\delta}$ and C_{target} of (i.e., (48.3 − 17.7)/48.3 = 0.63 may be indicative of an increased postprandial histidine utilisation resulting in an increased histidine mobilisation (compared to the situation during fasting), because the histidine concentration in the meal was limiting. However, the same response could also be the result of a reduced postprandial histidine mobilisation, followed by reduced histidine utilisation. Opposing mechanisms of cause and effect may thus result in the same response in nutrient concentration.

Conclusion

Modelling approaches can be used to analyse and interpret the postprandial kinetics of nutrients. These approaches allow to summarise the observed data in a limited number of parameters. These parameters can be interpreted biologically as target nutrient concentrations that the animal seeks to maintain (during fasting and after ingestion of a meal), metabolic exposure, and the dynamics of the response of the animal due to ingestion of a meal. The ratios between concentrations where the nutrient inflow equals the outflow (i.e., at minimum, maximum, and steady-state concentrations) are indicative of ratios in nutrient flows. These changes cannot be assigned to an explicit cause or effect, even though certain interpretations are more plausible than others.

Ethics approval

The experiment from which data were derived was authorised by the French Ministry of Agriculture (APAFiS #2019041610073508).

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Author contributions

JvM: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing.
FE: Statistical analysis, Writing – review & editing.
NLF: Writing – review & editing.

Declaration of interest

None.

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