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Modelling in nutrition: an introduction

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The purpose of the present paper is to provide an introduction to modelling, particularly mathematical modelling, for nutritional researchers with little or no experience of the modelling process. It aims to outline the function of modelling, and to give some guidance on factors to consider when designing protocols to generate data as part of the modelling process. It is not intended in any way to be a comprehensive guide to mathematical modelling. The paper discusses the uses of modelling, and presents a 'hydrodynamic analogy' to compartmental modelling, to explain the process to the non-mathematically-minded and to examine some of the pitfalls to be avoided when using stable-isotope tracers. Examples of the use of modelling in nutrition are presented, including methods for determining absorption, as well as a discussion of possible future avenues for nutritional modelling.

Nutritional modelling

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The modelling process

Before beginning a model-based study, it is useful to have an understanding of the role and function of modelling in scientific investigation. At its most basic level, modelling can be defined as the creation of a simplified representation of the world. As soon as we start to describe something, we are creating a linguistic model of the world, resulting in a simplification borne of a lack of knowledge. We may then (advisedly) decide to draw a picture or diagram of what we are trying to model. This pictorial model may be a further simplification, perhaps because we have started to decide what is important, or practical. For mathematical models, this picture is then translated into the language of mathematics, usually involving further simplifications resulting from considerations of tractability or the limitations of mathematics itself. This mathematical model is then often formalized as a computer simulation as an aid to prediction, or estimation of variables.

The art of effective modelling is to decide which features of the real world are to be included in the model, and to what extent they are to be simplified. These decisions must be based on an understanding of the required function of the model, as well as constraints imposed by our limited understanding of the world. We shall consider a range of functions of models in nutrition and elsewhere.

Data reduction

One of the most widespread uses of modelling is to compress a large amount of data into a smaller number of model variables. As soon as we take the mean of two numbers, or take the slope of a line through a data set, we are implicitly invoking a model. (This activity is so basic to science that we hardly recognize it as modelling, sometimes at our peril.) More complex data reduction exercises may, for example, summarize a large amount of experimental

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data from a study (such as nutrient concentrations in blood, faeces and urine etc.) as a simple percentage absorption.

Providing an overview

Many models, especially pictorial models, have a role in presenting a tractable overview of complex systems of mechanisms, perhaps showing the interrelationships between different organs and control systems, and how nutrients flow in, around and out of the body. These models are extremely useful for summarizing the complex knowledge we may have about a system, but may often be confused with models designed for data reduction or prediction. It is rare that sufficient data will be available from any one study to allow the estimation of all the variables in a mathematical model describing all known aspects of a nutrient's metabolism.

Observing the unobservable

One of the most powerful uses of modelling is its ability to infer information about systems or quantities which are unobservable on practical or ethical grounds. For example, it may be important to know the size of liver stores of a particular nutrient, but it would not be ethical to remove the liver from a cohort of human volunteers just to obtain such information. Modelling can provide the means to infer this information from other, more practical and ethical measurements. An example of this form of inference is given later (p. 136).

Raising questions

Probably the most important function of mathematical modelling is that it forces the scientists to ask questions about the system or process under study. If a model is to be coded into a computer simulation, then rate equations must be given form, and variables must be given values.

Compartmental modelling

Compartmental modelling is frequently used in human nutrition to describe the absorption, redistribution and disposal of nutrients in the body. In this approach, the system is broken down into a number of compartments, or 'pools'. The contents of each of these pools are assumed to be perfectly mixed, and material moves between the pools at a rate which depends on its concentration in the pool and on a characteristic rate constant. It is common in nutrition that the fluxes between the pools are 'donor-controlled', i.e. the flow out of a pool is dependent on the product of a rate-constant and the concentration in the donor pool.

It may be helpful for the newcomer to consider a hydrodynamic analogy to compartmental modelling, to understand both the process and some of the pitfalls to be avoided. A typical compartmental model (See Fig. 1) may be represented as a series of buckets each containing a hole for each of the flows out of the pool. The rate at which water flows out of the buckets depends not only on the amount in the bucket (the concentration) but also on the size of the holes (the rate-constants). A tap filling the first bucket may

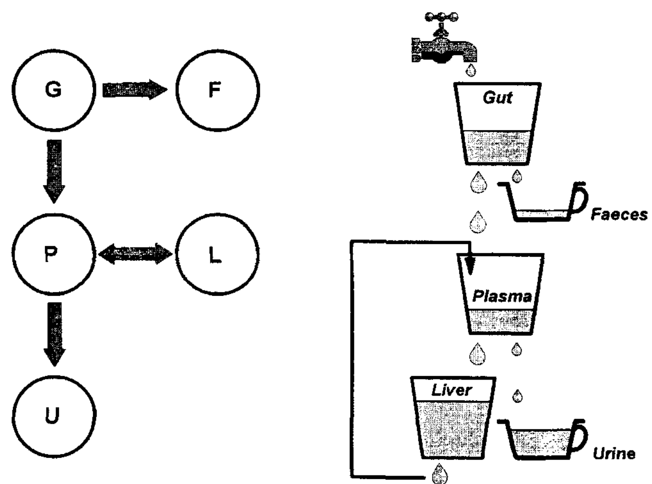

Fig. 1. Hydrodynamic analogy of a compartmental model.

be viewed as the diet, and over a long timescale the level in each of the buckets will stabilize. This is the steady-state solution to the model. (Strictly, only those pools with both inputs and outputs are able to stabilize, and in the example shown, the urinary and faecal pools will not reach a steady-state without the periodic emptying not explicitly described by the model.)

In order to probe the system, perhaps to estimate the size of pools we cannot easily measure, we may use tracers. Originally, radioactively-labelled analogues of the nutrients of interest were used, but ethical considerations have led to the widespread use of stable-isotope tracers, detected by mass spectrometry. The use of tracers is analogous to adding a dye to one of the pools. Measurement of the appearance and disappearance of the tracer in observable pools gives us the opportunity to infer something about the state of other pools.

Tracers can be added to the input pool, i.e. to the food, either as an extrinsic label where the labelled nutrient is simply mixed in with a test meal, or as an intrinsic label, where, for example, a plant has been grown such that the nutrient within it is isotopically labelled. Intrinsic labelling is clearly much more difficult and expensive than extrinsic labelling. However, most nutrients are consumed as part of a food matrix, and exogenously-added nutrients may not behave identically to those within a plant or animal structure. On occasions, biological and ethical considerations may allow tracers to be added to a plasma pool, providing further opportunities to probe the system.

A number of issues arise in the use of compartmental modelling and tracer studies.

Number of pools

Compartmental models are based on our biological view of human nutritional systems. Whilst this is both self-evident and also necessary, it can lead the unwary to build a compartmental model containing a pool for each of the known biological pools. This may be useful for building a simulation where both the pool sizes and exchange variables are well-characterized, but if the model is to be used to

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identify these variables, then there is a mathematical requirement to use as few pools as possible to describe the data collected.

When building models, the well-known Principle of Parsimony, or Occam's Razor should be applied liberally. The principle states that one should not increase, beyond what is necessary, the number of entities required to explain anything'. In other words, do not use any more pools in a model than are required to produce the observed behaviour. A number of objective tests exist for determining the complexity of models, such as the Akaike information criterion (Akaike, 1974) and the *F* test, and these should be used to avoid 'overfitting' of experimental data.

Linear independence of tracer

It is often the case in human studies that little material is available for analysis, and it is therefore essential to obtain as good discrimination as possible between the stable-isotope tracer and the naturally-abundant form of the nutrient. Continuing the analogy of dyes as tracers in the hydrodynamic model, this is equivalent to choosing a dye which is as different a colour as possible from the natural colour of the system, and from other dyes used in the same study. For example, if the 'natural colour' of the system were green, it would be unwise to choose blue and yellow tracers in the experimental design.

In principle, the number of isotopes available for a particular element or nutrient determines the number of tracers it is possible to use. For example, Se has six stable isotopes (atomic masses 74, 76, 77, 78, 80 and 82) and so five tracers could be possible; one isotope is required to 'trace' the naturally-occurring Se. Cu, however, has only two stable isotopes (masses 63 and 65) and so only one tracer is possible. In practice, however, errors in determination by mass spectrometry would make it impossible in practice to distinguish the theoretical maximum number of tracers.

The mass spectrum of a sample from a stable-isotope tracer experiment is given by:

$$\underline{m} = \underline{\underline{T}}\,\underline{x},$$

where $\underline{m}$ is the column vector whose elements are the molar proportions of each isotope in the sample (the 'isotopic spectrum'), the matrix $\underline{\underline{T}}$ contains the isotopic spectra of the tracers, and the naturally-abundant spectrum, and $\underline{x}$ is a vector containing the molar proportions of each source of the compound (i.e. how much is naturally abundant and how much is from each of the tracers).

For modelling, we need to know how much of the measured nutrient is from each of the possible sources, i.e. we need to determine $\underline{x}$. For uniquely determined systems where we have the same number of tracers as isotopes, this may be solved by matrix inversion as:

$$\underline{x} = \underline{\underline{T}}^{-1}\,\underline{m}.$$

The error in the determination of $\underline{x}$ depends both on the error in $\underline{m}$ and on the 'closeness' of tracers, embodied in the inverse matrix $\underline{\underline{T}}^{-1}$. For square matrices, i.e. when using the same number of tracers as isotopes, this can be characterized by the condition number of the system, given by:

$$cond(A) = \|T\|\,\|T^{-1}\|,$$

where $\|T\|$ is the matrix-norm of T. Details of this calculation can be found in standard texts on numerical analysis (for example, see Gerald, 1978). It is important that we choose tracers to make the condition number as small as possible, as it can be shown that the error in $\underline{x}$ may be as great as the condition number multiplied by the error in $\underline{m}$.

For non-square matrices, i.e. when fewer tracers are used (as is usually the case), a similar analysis may be performed by considering the corresponding number of isotopes with the highest abundance.

Interference

Another issue which limits the number of tracers which can be used in a stable-isotope study is interference from other entities with the same atomic or molecular mass. For example, in the study of Se metabolism, ^{80}Se cannot be distinguished in the mass spectrometer from $^{40}Ar_2$, or $^{40}Ar^{40}Ca$, which are both likely to be abundant in an argon plasma of an inductively-coupled plasma mass spectrometer. A table of common interferents is given by Crews *et al.* (1996).

Resolution

The closeness of the isotopic spectrum to natural abundance also affects the expected error in determination. From a study of human Se metabolism (SJ Fairweather-Tait, HM Crews, TE Fox, JR Dainty, C Atherton, M Baxter, DJ Lewis and P Strutt, unpublished results) Fig. 2 shows predictions of the expected error in determination of stable isotopes from three labelled sources of Se when present at a range of concentration in faecal samples. The data were generated assuming a mass spectrometer measurement error of 5 % in the most abundant isotope, and demonstrate the strong link between enrichment and precision. In this example, the intrinsically-labelled cod (*Gadus morrhua*) has an isotopic

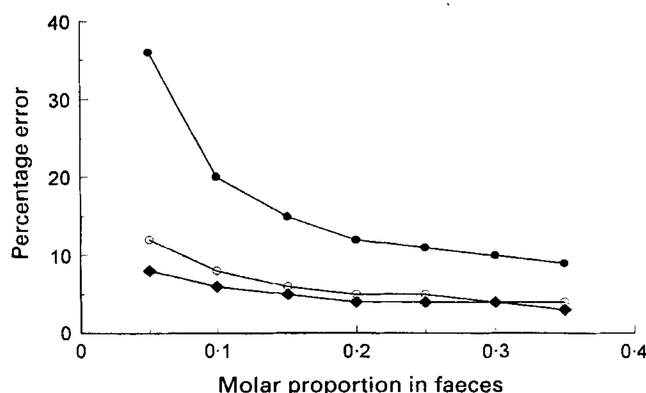

Fig. 2. Predicted error in stable-isotope determination using three labelled sources of selenium when present at a range of concentrations in faecal samples. (●), Intrinsically-labelled cod (*Gadus marrhua*); (○), selenium salt; (◆), intrinsically-labelled garlic (*Allium sativum*). (From SJ Fairweather-Tait, HM Crews, TE Fox, JR Dainty, C Atherton, M Baxter, DJ Lewis and P Strutt, unpublished results.)

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spectrum much closer to natural abundance than did either the Se salt or intrinsically-labelled garlic (*Allium sativum*).

Examples

A number of examples of various uses of modelling in human nutrition are now presented. The first example shows the use of a model to make predictions of expected experimental findings from a biological hypothesis, and concerns the absorption of the organic micronutrient, β-carotene.

It has been observed that plasma β-carotene concentrations are variable after administration of oral β-carotene, either remaining unchanged (Johnson & Russell, 1992) or increasing (for example, see Kostic *et al.* 1995). In a study using human ileostomy volunteers, Faulks *et al.* (1997) showed (by a mass balance on ileal effluent) that 90 % of administered β-carotene was absorbed without measurable changes in plasma β-carotene concentration. This observation led to the generation of a hypothesis that the β-carotene was absorbed into the plasma, but that it exchanged so quickly with other stores, that it would not be observable within the limits of detection available. A mathematical model was developed to explore the hypothesis. The purpose of the model was to predict the expected plasma β-carotene concentrations for a number of different assumptions of exchange kinetics.

For simplicity, it was assumed that the β-carotene was absorbed at a constant rate over a given period, and that it equilibrated with other, unobservable body stores. The structure of the model is shown in Fig. 3, together with the range of predictions. It can be seen that the model predicts increasing plasma excursions for increasing estimates of the half-life of the plasma pool. Comparing these predictions with the limits of detection indicated that if the β-carotene had a half-life as short as 2 min, then the plasma excursion would not be detectable. The half-life was comparable with estimates for the turnover rate of chylomicron triacylglycerol, and demonstrated that the absence of a plasma response does not necessarily imply non-absorption.

The use of models in this predictive sense is also of value in estimating the likely enrichments which may be seen in human studies, and so acting as an aid to experimental design and tracer selection.

The second example shows the use of a model to estimate absorption, as an alternative to the method often referred to as 'area under the curve' (for example, see Gibaldi & Perrier, 1982). The area-under-the-curve calculation is model-independent, and relies on the administration of both an oral and an intravenous dose, with the assumption that they are both subject to the same rate of removal from the plasma. In this case, the fractional absorption, F, of the oral dose is given by:

$$F = \frac{\int_0^\infty C_{oral}}{dose_{oral}} \frac{dose_{iv}}{\int_0^\infty C_{iv}} .$$

This is frequently rewritten as:

$$F = AUC_{oral} \times dose_{iv}/(AUC_{iv} \times dose_{oral}),$$

where AUC is the area under the plasma curve for either the oral or the intravenous (iv) tracer. Unfortunately, it is easy to forget that the original equation has integrals to infinite time, which implies that the measurements must be taken until the tracers are negligible. Although the model-independence of the method is especially useful, it may not be possible to design a study when samples can be taken at sufficiently long time periods.

It is possible, however, to make an estimation of the extent of absorption of a nutrient using a single tracer experiment, if the form of an appropriate kinetic model is well-established. For example, if it can be assumed that a nutrient is absorbed into a pool of known volume, V, at essentially a constant rate, R, over a fixed time, t_{abs}, and that it is cleared from the plasma following first-order kinetics with a rate-constant, k, then the following equations can be written:

during the absorption period, $t < t_{abs}$

$$C = R(1 - e^{-kt})/kV,$$

and in the post-absorptive period, $t > t_{abs}$

$$C = C_{max}e^{-kt},$$

where C_{max} is the plasma concentration at t_{abs}. In this case, plasma data following the absorptive phase (i.e. when plasma concentrations start to decline) may be used to estimate a value for k, and the data in the absorption period used to estimate R and t_{abs}. The product of R and t_{abs} then gives an estimate of the amount of nutrient absorbed. Fig. 4 shows the application of this methodology to the absorption of an organic micronutrient.

Further issues

A number of other issues of importance in the modelling process, often overlooked, are now briefly considered.

Timescales

The timescale over which a process occurs is crucial to our ability to observe it. Processes could be so fast that we are unable to measure them in our experiment, such as the mixing of tracers in the plasma pool. They could also be so slow that we cannot see any changes during the time-course of our experiment. An example of this could be the exchange of bone stores of minerals. Finally, two biologically-distinct processes could also occur at the same rate, such that we cannot differentiate them. We therefore need to ensure that our experimental approaches and models act over the timescale appropriate to the phenomena we wish to study.

Non-linear models

All the compartmental models we have discussed so far are classified mathematically as linear models. This terminology is used, not because the responses are linear, but because the concentrations appear in the equations only in linear combinations, i.e. they are not squared, or multiplied by each other in a complicated fashion. Linear models are particularly useful because they exhibit a wide variety of

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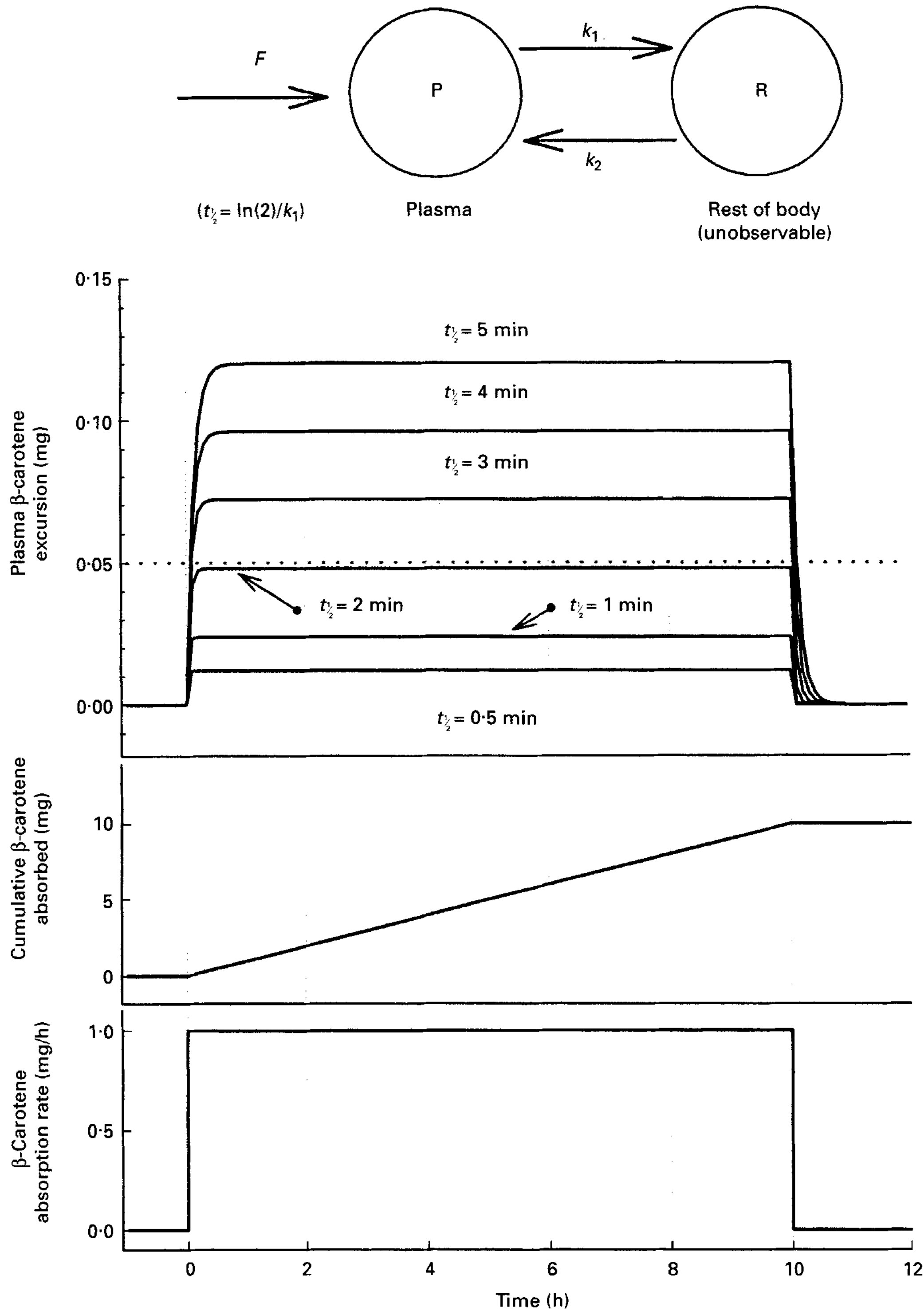

Fig. 3. Prediction of plasma response after administration of oral β-carotene. $t_{½}$, Half-life; *F*, fractional absorption; k_1, k_2, rate-constants. (Reproduced with permission, from Faulks *et al.* 1997.)

behaviour, there is a large wealth of mathematical theory available to solve them, and, for small perturbations at least, non-linear systems can be approximated by linear ones. A feature of linear models, however, is that the output of the system scales directly with the input. In other words, a doubling of an input results merely in a doubling of the output. In biological systems, however, large deviations from linearity occur due, for example, to saturation of carrier systems or the action of homeostatic control systems.

Talking about tracers

When discussing the labelling of foods with stable isotopes, for example when a plant food such as garlic is enriched

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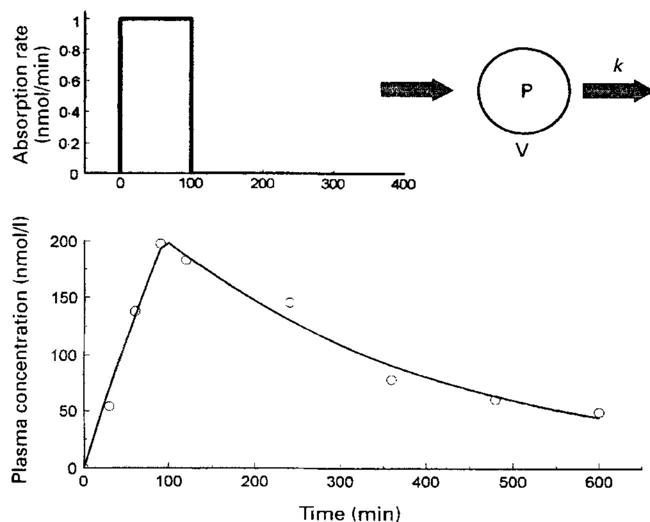

Fig. 4. Estimating absorption of an organic micronutrient using a single tracer. P, Plasma; V, volume of pool; *k*, rate-constant.

with ^{82}Se, individuals are often heard to call the tracer ^{82}Se, when strictly the tracer is the whole isotopic spectrum. Whilst this may be a convenient shorthand, it can lead to confusion when discussing the measured mass spectrum when the real ^{82}Se is measured, which of course contains contributions from the ^{82}Se in all the other isotopic sources. It is strongly recommended that the researcher refers to 'garlic–Se', for example, as the tracer, and calculates this quantity by deconstruction of the raw mass-spectrometry counts as discussed previously, i.e. the $\underline{x}$ values. Furthermore, expressing the quantity in molar units rather than masses will avoid the confusion which can be caused by each of the tracers and samples having different molecular weights.

Future opportunities and challenges

By comparison with the engineering sciences, modelling in nutrition is in its infancy. Practitioners of modelling in the nutritional sciences will find great benefit in exploring the mathematical and modelling techniques in use elsewhere. As examples, the field of engineering process control theory could have application in the understanding of biological control mechanisms; time series analysis may have application in probing nutritional systems by less interventionistic means, and non-linear kinetics will be required to fully model and interpret some of the more complex and interesting fields of 'over-nutrition', where excess intake of nutrients causes accumulation in dysfunctional pools.

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