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Review on Application of Biosystem Modeling: Introducing 3 Model-based Approaches in Studying Ca Metabolism

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Abstract

Purpose: This review aims at introducing 3 modeling approaches classified into 3 categories based on the purpose (estimation or prediction), structure (linear or non-linear) and phase (steady-state or dynamic-state); 1) statistical approaches, 2) kinetic modeling and 3) mechanistic modeling. We hope that this review can be a useful guide in the model-based approach of calcium metabolism as well as illustrates an application of engineering tools in studying biosystems. **Background:** The meaning of biosystems has been expanded, including agricultural/food system as well as biological systems like genes, cells and metabolisms. This expansion has required a useful tool for assessing the biosystems and modeling has arisen as a method that satisfies the current inquiry. To suit for the flow of the era, examining the system which is a little bit far from the traditional biosystems may be interesting issue, which can enlarge our insights and provide new ideas for prospective biosystem-researches. Herein, calcium metabolic models reviewed as an example of application of modeling approaches into the biosystems. **Review:** Calcium is an essential nutrient widely involved in animal and human metabolism including bone mineralization and signaling pathways. For this reason, the calcium metabolic system has been studied in various research fields of academia and industries. To study calcium metabolism, model-based system analyses have been utilized according to the purpose, subject characteristics, metabolic sites of interest, and experimental design. Either individual metabolic pathways or a whole homeostasis has been modeled in a number of studies.

Keywords: Biosystems modeling, Calcium metabolism, Kinetic modeling, Mechanistic modeling, Statistical modeling

Introduction

Expansion in the range of biosystems now includes various biological systems such as genes, cells, and metabolism, besides agricultural and food systems, requiring effective methods for evaluating the system. As a result, mathematical modeling has been launched as one of powerful tool for studying biosystems as biological complexity propels interdisciplinary application of engineering methods to solve questions in biosystems. The most powerful function of model-based analysis is to explore various scenarios that may be limited in the laboratory, offering an effective approach to test hypotheses, evaluate underlying bio-mechanisms, predict bio-system dynamics, and assist in experimental designs (Kreutz and Timmer, 2009).

In this review, calcium (Ca) metabolism has been selected as an example biosystem which is not a traditional field of own. Calcium metabolism has been an important topic due to its essentiality in human health (Weaver and Heaney, 1999). Calcium metabolism involves 4 major organs (intestine, serum, kidney, and bone) and 3 metabolic pathways (absorption occurring in the intestine, excretion involving re-absorption in the kidney, and bone resorption and formation, called bone turnover). Bone formation deposits calcium into bone, while bone resorption extracts Ca from bone to compensate the decreased calcium level in serum. A complex coordination of biochemical regulators and organic actions balances the 3 main calcium metabolic pathways to maintain serum calcium concentration according

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to internal and external conditions, indicating the mathematical modeling is suitable for analyzing the system (Nordin, 1990; Weaver and Heaney, 2006). In fact, cross-linkage between calcium metabolism and modeling approaches has offered effective evaluation and better understanding of calcium metabolism and its regulation. For this reason, the model-based approaches have been applied to analyze the calcium metabolism including calcium absorption, excretion, bone remodeling and whole calcium homeostasis.

Mathematical modeling approaches in the study of calcium and bone metabolism are reviewed by 3 categories of statistical, kinetic, and mechanistic modeling even though they are interchangeably used, and thus cannot be completely separated. This study defines the characteristics of 3 types of modeling and discussed advantages/disadvantages with a few example models, offering a guideline to use an adequate modeling type in the future studies of biosystems engineering.

Three modeling approaches in the study of calcium metabolism

For convenience, this review distinguishes the 3 modeling approaches by the following definitions;

1) Statistical modeling focuses on identifying relationships among variables in calcium metabolism to explain it as a function of significant predictors.
2) Calcium kinetic modeling quantitatively estimates calcium metabolic pathways at steady-state, based on models fitted to experimental data. The calcium kinetic model is mathematically represented by the sum of linear functions.
3) Mechanistic modeling predicts the dynamics of calcium metabolism in response to various inputs or perturbations. This model does not have a specific form, indicating that non-linear functions are the typical mathematical expression, in contrast to calcium kinetic modeling.

In practice, three types of modeling approaches are hard to completely separate as they are interchangeably used.

Statistical modeling

Statistics is a common method in calcium studies, particularly centered on comparing experimental and control groups. For example, the effects of dietary intervention, the action of specific regulators, and different physiological and environmental conditions have been examined by typical statistics such as paired t-test and multiple comparisons. Besides the classical statistics, statistical modeling has been used to estimate calcium metabolism based on relationships between target pathways and explanatory variables (e.g., experimental outcomes). Multiple regression analysis is an especially popular method to develop new models/methods while validating it against conventional methods. This approach is useful when developing a simple equation to estimate a specific area of calcium metabolism which is expensive and difficult to assess in experiments using potential predictors which are easily measurable (Hasling et al., 1992; Tomera and Harakal, 1997). Multiple regression analysis is a technique used to identify the relationship between a dependent variable and explanatory variable(s) (Kutner, 2005). Models developed by multiple regression analysis are functions of one or several explanatory variables and error terms that describe a dependent variable. Through minimizing the error term, generally by the least square method, we can develop and validate equations which predict dependent variables as a function of potential predictors (Kutner, 2005). The least square means (LSD) are widely used method in estimating parameter values when developing a model, which minimizes the square of difference between real data (y_i) and predicted value by the model ($f(x_i)$) (Equation 1).

$$\text{LSE} = \min \sum_{i=1}^{n} [y_i - f(x_i)]^2 \quad (1)$$

In calcium metabolic studies, multiple linear regression modeling was used to develop a simpler and less demanding alternative to estimate calcium metabolic pathways. For instance, single oral isotopic methods used to estimate fractional calcium absorption have been validated against the classical double isotope methods in adults (Heaney et al., 2002; Heaney and Recker, 1985; Heaney and Recker, 1988; Nordin et al., 1998) and children (Lee et al., 2011a). Moreover, a predictive equation of bone calcium retention in white adolescent boys was developed using multiple non-linear regression as a function of calcium intake and insulin-like growth factor I (IGF-1) (Hill et al., 2008).

However, statistical modeling including multiple regression analysis is confined to the availability of data, which is often limited in biological systems. Moreover, statistical models in calcium studies focus on identifying the relationship among variables obtained by experiments. Thus, it is not suitable to describe the regulatory mechanism

in calcium metabolism nor applicable to predict system dynamics in response to perturbations.

The statistical approach is useful to investigate the effect of treatments on calcium metabolism, or when validating a new method against traditional techniques. In contrast, its generic feature is not well-established for non-linear dynamic modeling to explore the mechanistic behaviors in calcium metabolism.

Calcium kinetic modeling

Calcium kinetic modeling is a useful tool for studying calcium kinetics, one of the main outcomes in calcium studies, particularly in the field of nutrition. Calcium kinetic modeling has been exploited using calcium isotopes to estimate steady-state calcium metabolism including fractional absorption, urine and fecal excretion, bone turnover and body calcium pool size. The model assumes that body calcium behaves as a single compartment with 3 routes of loss; urine, feces, and bone deposition (Heaney and Whedon, 1958).

Compartmental modeling in calcium kinetics is based on a series of first order differential equations to represent a rate change in each compartment (Jung et al., 1978). By solving the model, calcium kinetic modeling ends up with multi-exponential function and this model is normally used to fit the isotopic calcium disappearance curve. Consequently, it is generally associated with tracer studies which follow a metabolic change in calcium using isotopes, and compartmental modeling that describes the system as a flow of calcium between physical or biological storage compartments. Equation 2 indicates the general form of first order differential equations in the compartmental model and Equation 3 is the form of solution, where λ is the rate constant.

$$\frac{df_i(t)}{dt} = \sum_{j=1}^{n} \lambda_{ij} f_j(t) - \lambda_{ii} f_i(t) \quad (2)$$

$$f_i = \sum_i A_i e^{-\alpha_i t} \quad (3)$$

This model is well-established for calcium kinetics, visualizing the calcium pathways and calculating the rates of calcium transfer and the size of calcium pools. The numbers of compartments, which graphically represent each term of exponential functions, largely influence the shape of the calculated curve. The site of inflow or outflow is another influencing factor in compartmental modeling of calcium kinetics (Jung et al., 1978).

Calcium kinetic modeling associated with the compartmental model can estimate the amount of calcium transfer among compartments and size and turnover rates of each calcium pool (Figure 1). For example, by fitting the model curve to experimental data, such as isotopic data, it can estimate calcium absorption (% absorption and the actual amount of absorbed calcium) and bone parameters (the amount of bone formation and resorption, bone turnover rate, and bone balance). Because of these advantages, calcium kinetic modeling has been used in studying the effect of specific conditions on calcium metabolism such as subject characteristics (Bryant et al., 2003; Wastney et al., 1996), dietary intervention (Spence et al., 2005), and calcium source (Shahnazari et al., 2010; Weaver et al., 2009). As long-term assessment of calcium and bone metabolism became possible with ^{41}Ca, a calcium kinetic model that fits ^{41}Ca data collected over years were developed (Denk et al., 2006) and used to estimating bone resorption rates (Lee et al., 2011b). The procedures and contexts of calcium kinetic modeling are well summarized with suitable software, WinSAAM, by Wastney et al. (Wastney, 1999).

Calcium kinetic modeling is relatively simple, but has a power to estimate calcium metabolism at steady-state. However, it is not suitable for illustrating dynamic behavior of calcium metabolism neither mechanistic point of view. Because the basis of calcium kinetic modeling is to fit a simulated curve to experimental data, the generic mathematical function is fixed (multi-exponential function) with the underlying assumption of steady-state. Moreover, kinetic modeling requires a specific type of controlled

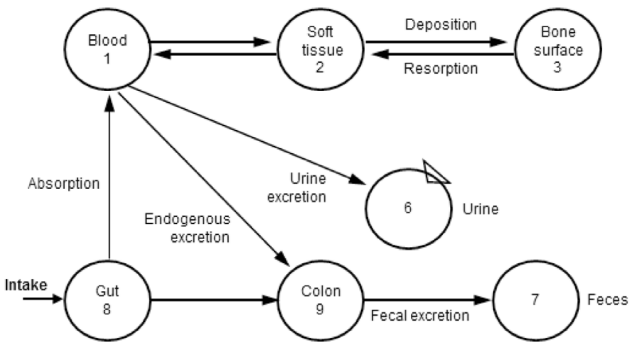

Figure 1. General compartmental model structure in Ca kinetic modeling. Numbers are arbitrarily assigned and each compartment represents a physical component in Ca metabolism. Ca intake is absorbed in the small intestine or excreted by feces. Absorbed Ca in the intestine can either deposited into bone through the soft tissue or excreted by urine with the action of the kidney and through endogenous excretion.

experiments which is costly and time consuming.

Mechanistic modeling in calcium studies

Mechanistic modeling is commonly associated with explicit mathematical equations. It is able to predict system responses and analyze mechanistic behaviors, providing better understanding of complex biological system (Epstein 1994; Wastney et al., 1997). Also, dynamic responses with respect to system variations can be tested using this type of modeling (Wastney et al., 1997). These advantages offer wide application of mechanistic modeling to various biological systems. As a result, mechanistic models have been used in calcium metabolism research to examine the regulatory mechanisms of calcium pathways and homeostasis. The model is associated with various physical and/or engineering theories such as the mass conservation and control theory.

Active calcium absorption has been modeled with a Michaelis-Menten (M-M) equation (Equation 4) to represent its saturable feature. Bronner and his colleagues (Bronner et al., 1986) used the M-M model to fit in situ experimental data in rats. This model was further used to examine 3 steps in active calcium absorption, i.e., Ca entry, facilitated diffusion and Ca extrusion, and showed enhanced Ca absorption by calbindinD_{9K} (Feher et al., 1992; Slepchenko and Bronner, 2001). Urine excretion has not been individually modeled, but only as a part of the calcium balance model (Hurwitz et al., 1987a; Peterson and Riggs, 2010; Raposo et al., 2002). The bone remodeling process has been modeled as a function of bone cells and their regulators (Komarova, 2005; Komarova et al., 2003; Kroll, 2000; Lemaire et al., 2004; Rattanakul et al., 2003). The model predicted relative changes in bone volume or mass in response to bone cell dynamics. In contrast to the bone cell models, bone mineralization was modeled as a function of mineral transport, diffusion and precipitation (Martin, 1994). However, few studies have linked calcium deposition/ resorption into bone with bone cell activity (Peterson and Riggs, 2010).

$$v = \frac{V_{max}[M]}{K_m + [M]} \quad (4)$$

where, v = rate of target metabolite, V_{max} = maximum rate of metabolite, K_m = metabolite concentration at the half-maximal rate, and [M] = metabolite concentration

In addition to individual calcium metabolic models, homeostatic regulation of calcium metabolism has been modeled. In the 1970's, Powell's group presented a linear model which described a theoretical response of serum calcium with respect to PTH and calcitonin (Powell, 1972; Powell and Valentinuzzi, 1974). A complete homeostatic system of calcium metabolism was proposed by Hurwitz and his colleagues. They used a feedback control loop to model chicken calcium homeostasis (Hurwitz et al., 1983) and growth-associated calcium dynamics (Hurwitz et al., 1987a; Hurwitz et al., 1987b) in response to PTH, 1, α -hydroxlyase and vitamin D. The integral feedback control system was re-employed to model the calcium homeostasis system which was subjected to extreme perturbations (parturient hypocalcemia) in dairy cows (El-Samad et al., 2002). In 2000, Doty and Seagrave (2000) inserted calcium regulation into a model that denoted internal redistribution of water and electrolytes (sodium) during the space flight. A minimal but completed model was developed for calcium and phosphate homeostasis regulated by PTH and vitamin D (Raposo et al., 2002). The model was able to simulate the response of the calcium homeostasis system to a variety of extrinsic perturbations. In 2005, Wastney et al. (Wastney et al., 2005) developed a dynamic model of short-term Ca homeostasis in healthy men to predict dynamics in calcium and PTH in response to citrate infusion. Mechanism-based pharmacokinetics and pharmacodynamics (PK/PD) were used to model the PTH-regulated calcium homeostasis in rats and humans (Abraham et al., 2009). Most recently, a complete model that links calcium homeostasis and bone remodeling has been presented (Peterson and Riggs, 2010).

As shown in the above models, mechanistic modeling is a powerful tool to investigate the mechanism of the dynamic response in calcium metabolism. Explicit mathematical formula in the model offers simple testing methods in system dynamics, which may require huge costs and effort to examine through experiments. However, the complexity, randomness, and variety in calcium metabolic systems, which show large variations to different internal/external conditions, are challenges in developing the mechanistic model.

All the introduced model types are summarized in Table 1 in terms of usage and advantages, and limitations.

Conclusion

The power of modeling has been emphasized because of its ability to effectively evaluate the biosystems. In this

Table 1. Summary of introduced three types of modeling

Model type	Description
Statistical modeling	►Usage and advantages Identify the relationship among effective factors Investigate the effect of treatments on calcium metabolism Validate a new method against traditional techniques. ►Limitations Hard to apply for non-linear dynamic system Confined to the availability of data Not suitable to explore the regulatory mechanism nor applicable to predict system dynamics in Ca metabolism
Kinetic modeling	►Usage and advantages Estimates calcium fluxes in the metabolic pathways, which is hard to experimentally measure. Simple, but powerful for steady-state Ca metabolism ►Limitations Not suitable for dynamic behavior of Ca metabolism Versatility is limited due to fixed generic mathematical functions Requires a specific type of controlled experiments which is costly and time consuming
Mechanistic modeling	►Usage and advantages Investigate the mechanism of the dynamic response in calcium metabolism (with respect to system perturbations) Offers various simulations, which may require huge costs and efforts in experiments, due to explicit mathematical formula ►Limitations Complexity, randomness, and variety in calcium systems Large variations caused by different internal/external conditions

review, calcium metabolism was selected as a broadened biosystems, which is beyond the classical field of agricultural and food systems, and introduced how the mathematical modeling has been used in assessing calcium metabolism.

In summary, statistical modeling has been commonly used to establish function(s) to estimate a target system based on the identified relationships between calcium metabolism and/or mechanistic variables (e.g., dietary components, physical characteristics, and environments etc.). Calcium kinetic modeling is able to quantify steady-state calcium metabolic pathways and is capable to examine different calcium kinetics at the different conditions. It is generally associated with compartmental models and tracer study because the model fits experimental data. Both statistical and kinetic modeling techniques focus on the identification and estimation of calcium metabolism. Alternatively, mechanistic modeling highlights how calcium metabolic pathways work and how they are regulated. The established mechanistic models predict the dynamics in calcium metabolism in response to metabolic perturbations, offering the ability to do systemic analysis of target systems. Most mechanistic models rely on previous publications as experiments cannot estimate all the model parameters which vary with model assumptions. Complexities in modeling, prerequisites of fundamental backgrounds and high computational loads are great challenges in this type of model.

As new experimental results have been currently reported, available data for modeling will be larger and we can improve the previous models by integrating new findings to the models. Also, global connection of different types of models (i.e., statistical, kinetic and mechanistic models) may be an option for future modeling to compensate for lack of available data for model validation and ultimately to develop a comprehensive model which would aid in studying unknown mechanisms in calcium and bone metabolism. For example, an integrative model may be able to test various scenarios of nutritional treatment (e.g., high calcium intake) or therapeutic regimes (e.g., estrogen replacement) to prevent or reduce osteoporosis.

As each modeling technique has a different power in system analysis, the selection of an adequate model-based approaches and an effective analytical tool is an important issue. Introduced modeling approaches in this study are expected to suggest a guideline for a prospective model-based research in the field of biosytems engineering. In addition, it is believed that this review illustrates a good example of applying engineering method for analyzing biosystems.

Conflict of Interest

The authors have no conflicting financial or other interests.

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