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A Theoretical Modeling for Suggesting Unique Mechanism of Adolescent Calcium Metabolism

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Abstract

Purpose: Modeling has been used for elucidating the mechanism of complex biosystems. In spite of importance and uniqueness of adolescent calcium (Ca) metabolism characterized by a threshold Ca intake, its regulatory mechanism has not been covered and even not proposed. Hence, this study aims at model-based proposing potential mechanisms regulating adolescent Ca metabolism. **Methods:** Two different hypothetic mechanisms were proposed. The main mechanism is conceived based on Ca-protein binding which induces renal Ca filtration, while additional mechanism assumed that active renal Ca re-absorption regulated Ca metabolism in adolescents. Mathematical models were developed to represent the proposed mechanism and simulated them whether they could produce adolescent Ca profiles in serum and urine. **Results:** Simulation showed that both mechanisms resulted in the unique behavior of Ca metabolism in adolescents. Based on the simulation insulin-like growth factor-1 (IGF-1) is suggested as a potential regulator because it is related to both growth, a remarkable characteristic of adolescence, and Ca metabolism including absorption and bone accretion. Then, descriptive modeling is employed to conceptualize the hypothesized mechanisms governing adolescent Ca metabolism. **Conclusions:** This study demonstrated that modeling is a powerful tool for elucidating an unknown mechanism by simulating potential regulatory mechanisms in adolescent Ca metabolism. It is expected that various analytic applications would be plausible in the study of biosystems, particularly with combination of experimental and modeling approaches.

Keywords: Active Ca re-absorption, Adolescent Ca metabolism, Mechanism, Modeling, Ca-protein binding, Insulin-like growth factor-1

Introduction

Modeling has been used as one of the effective way of describing the mechanism of complex systems. The ability of models to explore scenarios offers an effective approach to propose unknown mechanisms in various biological systems and experimental designs (Kreutz and Timmer 2009). A novel modeling approach that assessed hundreds of possible mechanisms were employed to propose a mechanistic interaction of functional channels in regulating lignin biosynthesis which has been uncovered (Lee et al. 2012). A previous study reported the use of modeling in simulating Ca dynamics as a result of system perturbations to aid in study design and interpretation which assisted cost-effective experimental design (Lee et al. 2011).

Because of the above advantages in studying mechanisms in biosystems, various types of modeling approaches have been widely used to examine Ca metabolism. Conceptual models, which verbally describe the system, have been used to elucidate the regulating mechanism of Ca/bone metabolism by connecting supportive data or publications (Doty and Seagrave 2000; Fatayerji et al. 2000; Nordin 1990). The conceptual model is generally associated with a diagrammatic model, which graphically

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represents the objects and their relations, to visualize the verbally explained mechanism. This type of modeling is beneficial to visualize the intermediate step of the regulating mechanism. Mathematical modeling has become popular to depict the conceptually developed model and to evaluate and predict the metabolic variables involved in the mechanism. Mathematical modeling can be used to prove the conceptual model by simulating the model and comparing it to the actual data set (Komarova et al. 2003; Raposo et al. 2002). Also, it has been used to quantitatively estimate Ca parameters, which cannot be directly measured, by fitting the experimental data to examine the effect of regulators in Ca/bone metabolism (Hill et al. 2008; Wastney et al. 1996).

Optimal bone mineral accretion during adolescence significantly influences peak bone mass, which is related to the life-time bone health. Adequate Ca intake during adolescence has been addressed as an effective strategy to optimize bone mineral accretion to prevent osteoporosis (Heaney et al. 2000). During adolescence, which is characterized by the rapid growth spurt, a threshold in Ca intake in regards to Ca retention has been observed from a range of Ca intakes. In other words, absorbed Ca is more likely to be retained in the bone than be excreted through urine. Urinary Ca is constant regardless of Ca intake until bone accretion potential is reached at the threshold Ca intake. Urinary Ca excretion rises rapidly after the bone accretion is saturated (Heaney et al. 2000; Matkovic et al. 1990; Matkovic and Ilich 1993). This is supported by a weak correlation between urine Ca excretion and Ca intake in adolescents (Braun et al. 2006; Matkovic 1991). It was estimated that a 100 mg increase in Ca intake resulted in only 2.5 mg of urinary Ca excretion in adolescent girls (Jackman et al. 1997). The relationship between Ca retention and Ca intake is well illustrated as an asymptotic function by non-linear regression (Jackman et al. 1997). The amount of bone accreted and the threshold Ca intake are dependent on the life stage of an individual and have maximal values during adolescence (Matkovic and Heaney 1992). In contrast, adult urine Ca excretion in adults was 2 times higher than adolescents, suggesting urine Ca increases with respect to an increase in Ca absorption, i.e., a close relationship between Ca intake and urine excretion (Matkovic 1991). Hence, Ca metabolism is more effective in adolescent in terms of utilizing increased Ca absorption for maximizing peak bone mass. However, the mechanism(s) resulting in this difference is unknown and no publications have proposed a possible mechanism.

The goal of this study is to propose potential mechanisms regulating constant urine Ca excretion and enhanced bone accretion associated with the threshold behavior of Ca intake in adolescents. A mathematical model is developed to systemically represent and simulate the proposed mechanisms. Then, descriptive modeling is following to conceptualize the hypothesized mechanisms for urine Ca excretion and bone accretion during adolescence.

Materials and Methods

The main proposed mechanism focuses on Ca-protein binding inducing renal Ca filtration, while additional hypothesis assumes active renal Ca re-absorption to regulate Ca metabolism in adolescents. Both conceptual and mathematical modeling techniques were main analytical tools for describing hypothesized mechanisms and for mechanistically testing them.

Proposed mechanism 1: Ca-protein binding in Ca filtration in the kidney

The first proposed mechanism is conceived based on the renal filtration, where macromolecules and its bound ions cannot cross the membrane and be excreted in urine, whereas free ions are filterable. Figure 1 shows the concept of urine Ca excretion regulation: high Ca intake results in increased serum Ca concentration but is regulated by enhanced Ca-protein binding, and thus the difference between serum Ca concentration and Ca-protein, which indicates filterable Ca is constant until Ca-protein binding is saturated.

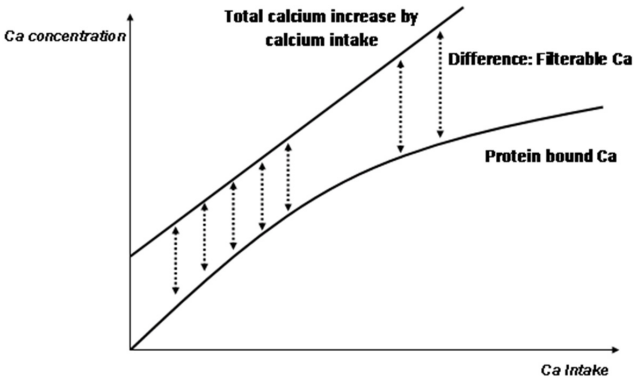

Figure 1. Graphical concept of proposed mechanism 1. Urine Ca excretion is regulated through Ca intake and Ca-protein binding.

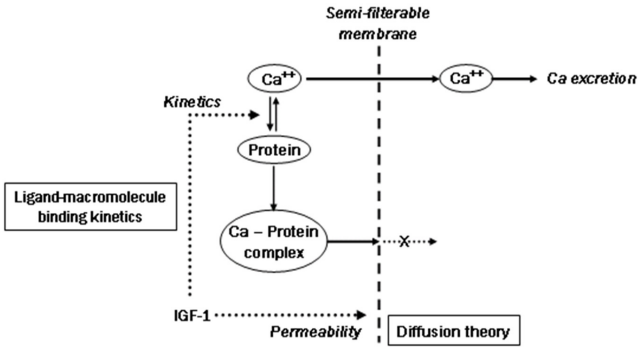

Figure 2. Proposed core idea of regulation of Ca filtration via the action of Ca-protein binding.

The core idea of the proposed mechanism is Ca-protein binding inducible by hormonal systems of Ca metabolism, which blocks the filtration of bound Ca (Fig 2). A few researchers have investigated the effect of protein on ion binding (Besarab et al. 1981; Linse et al. 1995; Pedersen 1972; Saroff 1963; Wills and Lewin 1971).

Mathematical modeling of Ca-protein binding kinetics

Binding kinetics of ligands to macromolecules can be used to estimate Ca-protein binding, resulting in hyperbolic or sigmoidal saturating curves according to ligand concentrations (Hammes 2000). The simple reaction equation can be written as:

$$L + P \leftrightarrow PL \tag{1}$$

where P and L indicate the protein and ligand, respectively

Thus, the kinetic equation is represented by the following equation which results in a hyperbolic curve (Equation 2). If there are *n* identical binding sites on the protein, the binding kinetics are represented by the sum of the binding sites and the kinetics show sigmoidal function (Equation 3).

$$r = \frac{[PL]}{[P]+[PL]} = \frac{K[L]}{1+K[L]} \tag{2}$$

$$r = \frac{nK[L]}{1+nK[L]} \tag{3}$$

where $K = [PL]/[P][L]$, r is the moles of ligand bound per mole of protein, and n is identical binding sites

Based on the ligand-macromolecule binding kinetic theory, the Ca-protein interaction can be mechanistically represented by replacing the ligand with Ca and the macromolecule with serum protein. In normal physiological conditions, one mole of protein can bind to 1 or 2 Ca ions (Saroff 1963). Thus, while serum Ca concentration increases with Ca intake, the Ca-protein binding capacity will reach a plateau according to the sigmoidal function (when n = 2). Therefore, the proposed mechanism of controlling filterable Ca can be proved by the kinetics of Ca-protein binding. In detail, the reaction equation for Ca and protein is written in Equation 4 with $K_d = [Ca]^2 \cdot [P] / Ca_2P$]. Total Ca and protein in serum are represented by Equation 5 and 6, respectively. Thus, the amount of protein-bound Ca is $[CaP] + [Ca_2P]$ and filterable Ca is $[Ca] = [Ca]_T - ([CaP]+[Ca_2P])$. By re-arranging the above equations as a function of $[Ca]_T$ and X which represents protein-bound Ca($[CaP] + [Ca_2P]$), the following equation is derived with the assumption that $[CaP]$:$[Ca_2P]$=1:1.

$$Ca + Ca + P \leftrightarrow Ca + CaP \leftrightarrow Ca_2P \tag{4}$$

$$[Ca]_T = [Ca]+[CaP]+[Ca_2P] \tag{5}$$

$$[P]_T = [P]+[CaP]+[Ca_2P] \tag{6}$$

$$X^3 - (2\cdot[Ca]_T + [P]_T)\cdot X^2 + (0.5K_d + [Ca]_T{}^2 + 2\cdot[P]_T[Ca]_T)\cdot X - [P]_T[Ca]_T{}^2 = 0 \tag{7}$$

Proposed mechanism 2: Renal Ca re-absorption

An alternative mechanism is that renal Ca re-absorption regulates urine Ca excretion and consequently influences bone accretion. Renal Ca re-absorption consists of passive and active Ca transport. Active Ca re-absorption may be regulated by a protein carrier, similar to phosphate re-absorption since these two nutrients are closely related. The *de novo* synthesis of phosphate transporting proteins is suggested to regulate renal phosphate re-absorption (Caverzasio and Bonjour 1993). Therefore, we speculate that active Ca transport in the kidney regulates urine Ca excretion.

The basic concept is the same as the previously suggested mechanism shown in Fig 1. The increased amount of filtered Ca is compensated by the hyperbolic pattern of renal Ca re-absorption. The difference between filtered and re-absorbed Ca, indicating urine Ca excretion,

is constantly maintained due to the compensation before re-absorption is saturated. Constant urine Ca excretion resulting from the regulation of renal Ca re-absorption enables adolescents to deposit absorbed and re-absorbed Ca into bone. This results in high bone accretion during adolescence. Above the threshold intake, saturated bone accretion allows greater amounts of Ca to be filtered which exceeds the kidney's ability to re-absorb Ca. Therefore, urine Ca rapidly rises to excrete the excess Ca.

Mathematical modeling of renal Ca re-absorption

Mathematical modeling of active Ca absorption has been modeled using Michaelis-Menten type equation (Feher et al. 1992). Because renal Ca re-absorption has the similar mechanism, Ca re-absorption is represented by following asymptotic equation, producing a hyperbolic curve according to Ca load.

$$\text{Re}-absorption = \frac{V_{\max} \cdot [Filtered \quad Ca]}{K_M + [Filtered \quad Ca]} \tag{8}$$

where K_M is constant and V_{max} is maximal Ca re-absorption

Results and Discussions

The protein-bound Ca, filterable Ca, and total Ca can be plotted at a given K_d and total serum protein concentration (Fig 3). The simulated plot describes the proposed mechanism: protein-bound Ca increases and reaches a plateau, while filterable Ca stays constant and rises only after protein-bound Ca plateaus. Lower protein concentration results in more rapid saturation, which is consistent with the hypothesis. Therefore, our proposed mechanism seems to work for regulating filterable and protein-bound Ca in renal filtration process.

Simulated plots of alternative mechanism using arbitrary values for parameters are shown in Figure 4. Increased amounts of filtered Ca are compensated by re-absorbed Ca, which consequently results in constant Ca excretion in urine. The regulation in this model is based on parametric change, which is designed to influence parameters in the model. For example, we can deduce that high K_M value delays the Ca re-absorption saturation point. Saturation plateau is not as clear as the mechanism in the first hypothesis, suggesting that the first mechanism is more plausible than this one.

There is a potential regulator that can handle the both hypothesis. IGF-1 is involved in Ca metabolism including Ca-protein binding and renal Ca re-absorption. In addition, it is related to growth which is one of the main characteristics observed during adolescence. IGF-1 increases $1,25(OH)_2D_3$ by stimulating renal 1-α-hydroxylase activity (Nesbitt and Drezner 1993; Wright et al. 1997) and consequently enhances intestinal Ca absorption and renal phosphate re-absorption (Caverzasio and Bonjour 1993; Mauras et al. 2000; Nesbitt and Drezner 1993; Rosen 2003; Wright et al. 1997). Thus, high serum Ca and phosphate concentration can be maintained to be incor-

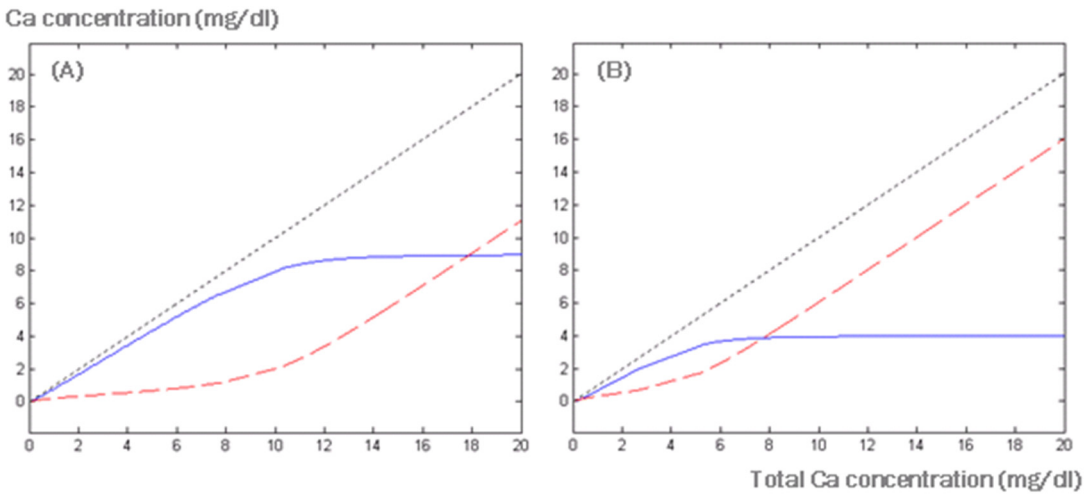

Figure 3. Simulation of proposed binding equation for serum Ca. Solid, dashed, and dotted lines indicate protein bound, filterable, and total Ca, respectively. The serum protein concentration of (A) is 9 g/dl and 4g/dl for (B). Arbitrary values were selected for serum Ca, serum protein concentration and K_d.

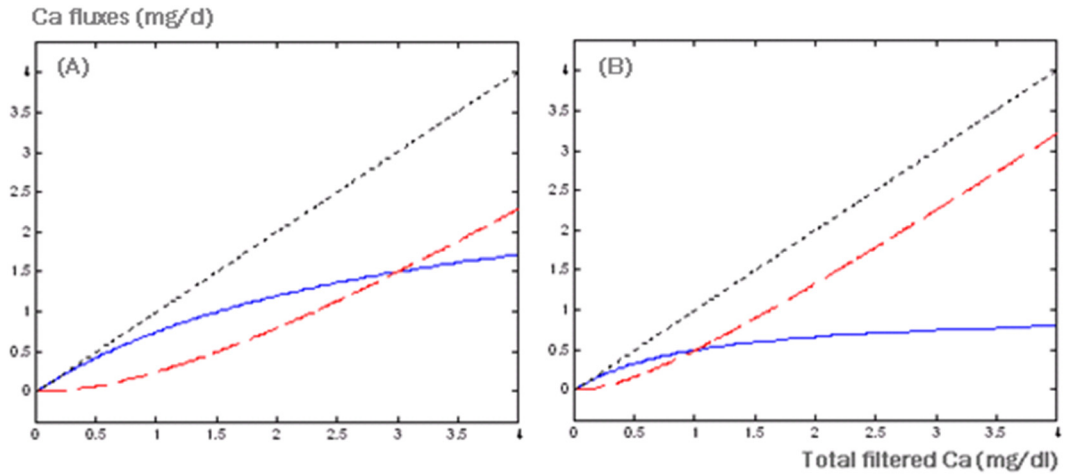

Figure 4. Simulation of Ca re-absorption. Solid, dashed, and dotted lines indicate re-absorptive, excreted, and total filtered Ca fluxes, respectively. Given values are arbitrary. $K_M = 1$ and $V_{max} = 1$ for (A), and $K_M = 0.5$ and $V_{max} = 0.5$ for (B).

porated in bone (Krabbe et al. 1982).

IGF-1 is positively correlated to the synthesis and the concentration of serum albumin (Kaysen et al. 1995), the major Ca binding protein in serum. This indicates that IGF-1 stimulates the synthesis of serum albumin (Binnerts et al. 1992; Mauras et al. 2000). Enhanced protein concentration in serum can alter Ca-protein binding kinetics by approximating ligand and macromolecular binding (Hammes 2000). Therefore IGF-1 induced albumin synthesis may result in increased Ca-protein binding rather than increased serum ionized Ca. This may occur until the Ca-protein binding capacity is saturated and reaches a plateau due to high Ca intake and consequent high serum Ca concentration. Renal filtration of free ions can be blocked by binding them to macromolecules, since macromolecules cannot pass the filtrating membrane in the kidney due to their size or charge. For this reason, protein bound Ca is not filterable during the renal filtration process. In addition, serum protein induced by IGF-1 can affect the permeability of filtering membrane in kidney (Lund et al. 2003; Rippe 2004). Thus, filtering ability may be affected and consequently Ca filtration is altered. Therefore, it can be postulated that the increased serum protein induced by high IGF-1 alters the amount of filterable Ca in kidney by changing binding kinetics of Ca to protein, and consequently the amount of filterable Ca maintains in constant. Urine Ca excretion is constant regardless of Ca intake before reaching the Ca-protein binding and bone accretion plateau, while serum Ca concentration drastically increases with intake.

As previously noted, IGF-1 is highly correlated with bone formation by stimulating the activity of osteoblasts (Yakar and Rosen 2003). High serum Ca and phosphate caused by high Ca absorption, renal phosphate re-absorption, and constant urine Ca excretion provide the favorable environment for bone formation. Thus, the collective effects of high IGF-1 and high serum Ca and phosphate concentrations enable the rapid deposit of Ca into bone during adolescence. It is suggested that incorporation of serum albumin into the bone matrix during bone formation occurs as a result of its strong interaction with bone minerals (Triffitt and Owen 1977). The uptake of Ca-protein complexes, which results from tight ligand binding, in the bone are higher compared to that of ionized Ca alone (Wortsman and Traycoff 1980). Other researches have also suggested that albumin bound lipids increase osteoblast growth which consequently results in bone formation (39), and albumin is used to adsorb Ca and phosphate in implant material for human body (Tsai et al. 2007; Zeng et al. 1999). Thus, we may expect that serum protein helps Ca and phosphate to be incorporated into the bone matrix, resulting in high bone accretion.

Above the threshold intake, bone accretion reaches a plateau and further increases in Ca intake do not result in increased Ca retention (Hill et al. 2008; Matkovic and Heaney 1992). The high influx of Ca to bone or autocrine and paracrine regulatory interactions between osteoblasts and osteoclasts may cause the saturation of osteoblastic activity(Komarova et al. 2003; Yakar and Rosen 2003). Consequently, the saturation of bone accretion induces serum Ca concentrations beyond the protein binding

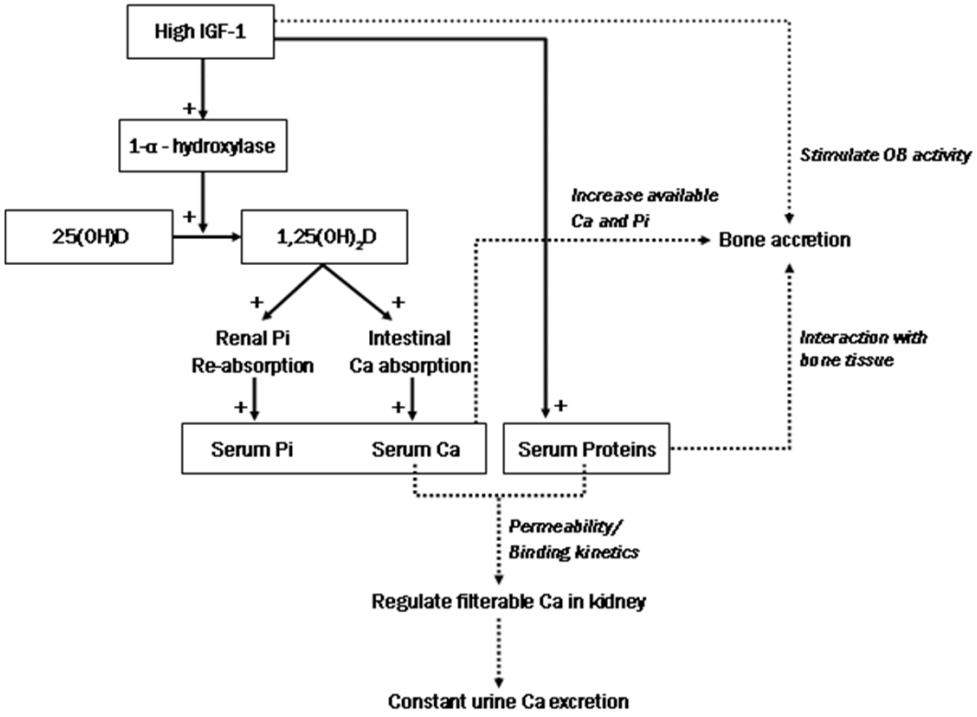

Figure 5. Proposed conceptual mechanism. Regulation of urine Ca and bone accretion by IGF-1 concentration in adolescents. Plus signs associated with solid arrows indicate stimulations, while dotted arrows with descriptions (italics) indicate interactions.

saturation point, and thus urine Ca rapidly rises to excrete the excess Ca. . Figure 5 visualizes the proposed mechanism.

Alternatively, adsorption isotherms may explain protein-Ca binding since Ca can be regarded to be adsorbed to protein. Adsorption isotherm is the function which relates the amount of absorbed substances at absorptive surface at constant temperature. The equilibrium relationship between the ligand and the protein is similar to equilibrium adsorption isotherms based on the assumption that protein-bound and free Ca are equilibrated in serum. Of adsorption isotherms, Langmuir adsorption isotherm produces a hyperbolic function which is consistent with the basic concept shown in Figure 1 (Smith 1981). The simplest equilibrium state of free and protein bound Ca is written in Equation 9. At equilibrium, the rate of adsorption (r_a) of Ca to protein will be equal to the rate of desorption (r_d). Rate of adsorption and desorption are expressed as a function of Ca, protein and Ca-protein complex (Equation 10 and 11). By re-arranging equations 9, 10, and 11 at equilibrium state, Langmuir equation is achieved and the amount of adsorbed Ca to protein, i.e., Ca-protein complex, is expressed as a free Ca concentration (Equation 12).

$$Ca + P \leftrightarrow CaP \tag{9}$$

$$r_a = k_c Ca(P - CaP) \tag{10}$$

$$r_d = k_{c'} CaP \tag{11}$$

$$CaP = \frac{K_c P \times Ca}{1 + K_c \times Ca} \tag{12}$$

Where k_c = rate constant of adsorption, $k_{c'}$ = rate constant of desorption, and $K_c = k_c / k_{c'}$

The analysis should be differently interpreted with the binding kinetics. Langmuir equation is function of free Ca, while binding kinetics expresses the amount of Ca-protein complex by total Ca. Langmuir equation explains that the increased free Ca is rapidly bound to protein and consequently the amount of free Ca constantly maintains until the binding saturation. Simulation shows that K_c affects saturating concentration of free Ca, and protein concentration (P) controls the maximal amount of protein bound Ca (Figure 6). This suggests that the change in serum protein level induced by IGF-1 may be related to binding affinity and may suppress the amount of Ca

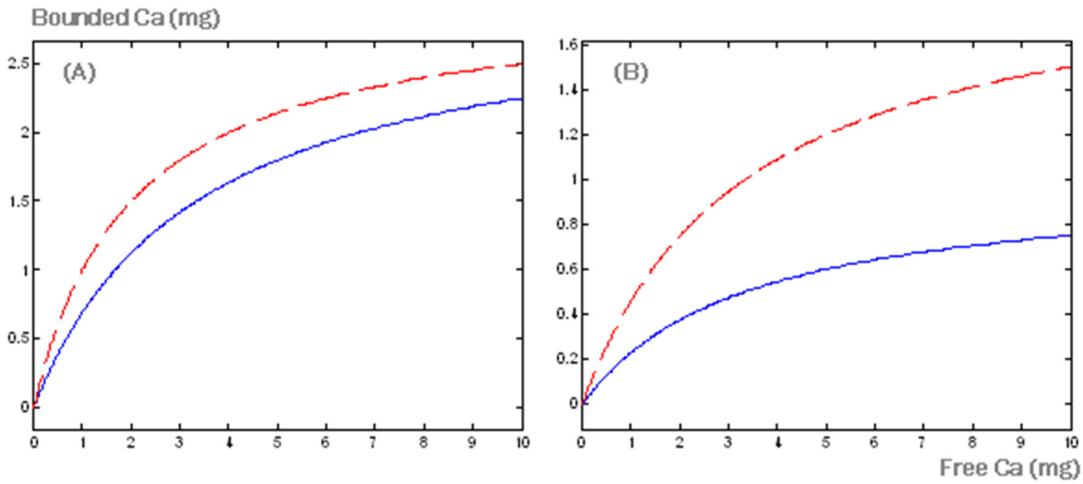

Figure 6. Simulation of the proposed Langmuir adsorption equation. (A) is simulated at different K_c values and different protein concentration for (B). Dashed and solid lines are for high and low values for each parameter, respectively. Values are arbitrary.

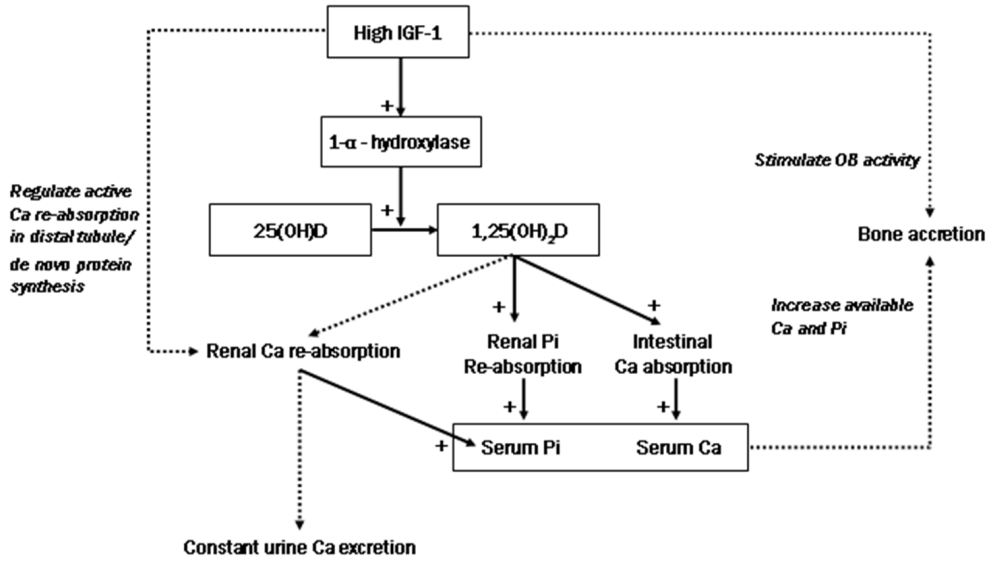

Figure 7. Proposed mechanism of urine Ca regulation and bone accretion in adolescents at peak IGF-1 concentration. Plus signs associated with solid arrows indicate stimulation, while dotted arrows with descriptions (italics) indicate interaction.

filtration.

For the second hypothesis, the initial conditions are the same as the previous model. That is, serum Ca and phosphate are high due to the enhanced intestinal Ca absorption and renal phosphate re-absorption is induced by high IGF-1 concentration.

Since it has been suggested that IGF-1 affects renal Ca re-absorption in distal tubule (Halhali et al. 2007), IGF-1 is designed to influence parameters in the model. For example, we can deduce that high IGF-1 results in high K_M value, and consequently it delays the Ca re-absorption saturation point. Therefore, the mechanism can be described by the renal Ca re-absorption model.

IGF-1 regulates renal phosphate re-absorption which is closely correlated to renal Ca re-absorption (Caverzasio and Bonjour 1988; Caverzasio and Bonjour 1993). The regulation of renal phosphate re-absorption has been suggested to be through the synthesis of *de novo* protein which transports phosphate (Caverzasio and Bonjour 1993). IGF-1 may affect renal Ca re-absorption in the distal tubule where transport is active and affected by hormonal systems (Halhali et al. 2007). Therefore, we hypothesize that renal Ca re-absorption is altered by the effect of IGF-1 and regulates urine Ca excretion.

Similar to the previously suggested mechanism, the high level of IGF-1 directly affects bone metabolism by

stimulating osteoblasts activity and allowing rapid deposit of Ca into bone until the threshold intake is reached. Figure 7 visualizes the proposed mechanism.

Conclusions

In this study, modeling was utilized as a tool for elucidating an unknown mechanism, which is one of powerful ability of modeling technique. We proposed two possible mechanisms explaining adolescent Ca metabolism characterized by a threshold in Ca intake in regards to Ca retention. Both mechanisms, i.e., selective renal filtration induced by Ca-protein binding in serum and active renal Ca re-absorption, successfully simulated that urine Ca excretion stays constant and rises after Ca is saturated in serum, meaning that absorbed Ca is likely to be retained in the bone than be excreted through urine. Nevertheless, it should be noted that the model only suggested the potential mechanism which may not be true and further experimental studies are necessary to uncover the mechanism.

Conflict of interests

The authors have no conflicting financial or other interests

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