A Negative Feedback Model for a Mechanism Based Description of Longitudinal Observations

Application for Bone Turnover Biomarkers

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Keywords
Second order model, negative feedback, clearance rate constant, bone mineral density, bone-specific alkaline phosphatase, osteoporosis

Summary
In modern medicine the diagnosis and prognosis of an abnormal metabolic condition is based on blood borne measurements involving one or more biomarker.

Objective: This paper reports the development of a minimal negative feedback model for the description of longitudinal biomarkers concentrations for treatment of osteoporosis in postmenopausal women.

Methods: Literature data were obtained from double-blind, placebo-controlled clinical trial over three years. There were four treatment groups: 1) Placebo, 2) Alendronate, 3) Conjugated Estrogen, and/or 4) Combination therapy. The negative feedback model consists of a biomarker and a companion controller. By considering the above basal biomarker values it is shown that the dynamics can be described by a second order differential equation without the involvement of biomarker production rate. The second order differential equation is also analogous to classical negative feedback servo mechanism model with two parameters $\omega_n$ and $\xi$. It was assumed that the rate constants defining the negative feedback model were equal which would set $\xi$ to 0.707 with only $\omega_n$ to be estimated.

Results: $\omega_n$ was estimated for both lumbar spine bone mineral density (BMD) and bone-specific alkaline phosphatase (BAP) in four treatments groups. The $t_{0.1}$ of BMD and BAP were estimated at 26.8 (0.30) and 9.4 (0.30) days respectively.

Conclusions: The negative feedback model of BMD supports the mechanism whereby Conjugated Estrogen and Alendronate decrease the clearance rate constant of BMD analogous to increased apoptosis of osteoclasts. The linked negative feedback models facilitate a mechanism based prediction of BMD using the concentrations of the bone turnover marker BAP.

1. Introduction

In modern medicine, the diagnosis and prognosis of an abnormal metabolic condition is based on blood borne measurements involving one or more biomarker [1]. The quantitative assessment of a biomarker concentration can lead to discrete qualitative measures or mappings such as stable, moderate or severe for describing the underlying disease condition and its progression [2]. However, a mechanism-based assessment of biomarkers in a longitudinal setting requires description in terms of production and clearance rates. The longitudinal measurements of a biomarker concentration itself however, would not be sufficient in estimating its clearance rate, for that, a mathematical model and an elaborate experimental setup is required. A mechanistic description would further enable analysis of longitudinal observations in terms of changes in production or clearance rates. A mechanistic description also enables cascade of metabolic events. In a simple cascade the whole or a proportion of clearance rate from one biomarker acts as production rate for the subsequent one [3]. A metabolic cascade could also be arranged in a way that one biomarker modulates a rate constant of another leading to a change in its clearance rate.

In its simplest form the process of an endogenous biomarker disappearance from the systemic circulation is governed by a first order disappearance rate constant $(1/time)$ together with a production rate $(amount/time)$ [4]. The dynamic change in a biomarker concentration can be brought about by changes in production rate, clearance rate or a combination of the two. However the essential requirement is that the clearance rate constant be known [5]. Knowing the clearance rate constant and production rate enable computation of concentration (i.e. convolution) or knowing the concentration and computing the production rate as deconvolution [6]. In longitudinal observations of biomarkers the clearance rate constant is almost never known unless from other experimental procedures. As a result the conventional method of choice for the description of
longitudinal observations is mixed-effect (or multilevel) models [7]. The multilevel method has advantages over simple linear regression among which the most important is the use of all observations "intention to treat". It also provides the natural hierarchy for nesting of observations [8]. Although the mixed-model is the ideal method for the description of longitudinal observations for providing an estimate of change with time (the regression coefficient for the elapsed time as independent variable) after correcting for other covariates it limitations for a mechanistic description. For a mechanistic description, biomarker’s clearance rate constant is prerequisite.

The objective of this paper is to derive an alternative mechanistic approach for obtaining clearance rate constant estimates for biomarkers in longitudinal observations using bone remodelling as an example. Bone remodelling is a complex metabolic process which involves bone forming and resorbing cells at different level of maturation in addition to paracrine and autocrine hormonal controls [9]. At the level of basic multicellular unit (BMU) of bone remodelling [10] the differential equations describing the rate of change of bone cells involve product of power functions representing their interactions. These interactions produce regions of unstable behaviour [9]. There are also detailed mathematical models, which describe the dynamics of bone modelling and remodelling processes with comprehensive description for hormonal controls [11, 12]. However, these mathematical models have many parameters and the abstraction does not include description for blood borne biomarkers. Current mathematical models for bone processes are generally used for bringing the current body of knowledge together, promoting hypothesis testing, and fostering innovative experimental designs [13]. Detailed bone models have also been used as a modiﬁer of hormone’s kinetics following i.v. infusion for example, of Parathyroid hormone (PTH) [12]. Although current bone models have the essential components of the process they lack inclusion of indirect bone biomarkers that correlate with absorption or resorption processes. In addition they do not take into account the exchange between different body compartments.

This paper addresses how to interpret biomarker data from patients suffering from bone diseases such as osteoporosis in a mechanistic manner to determine whether a change in biomarker concentration is brought about by changes in its production or clearance rates. The primary biomarker for osteoporosis is bone mineral density (BMD). The blood BAP (bone specific alkaline phosphatise) representing bone formation biomarker and cross linked N-telopeptide of bone collagen normalised to creatinine (NTX) depicting bone resorption biomarker [14]. The characteristic response time or clearance rate constants of bone biomarkers are different [14]. These differences in clearance rates have to be taken into account when biomarkers time course are used for inferences about drug effectiveness. The clearance rate based models would also play an important role for assessment of detailed bone cell models [13] by providing guidance on the likely mechanistic characteristics that is required of them. For example, it may be a requirement that, the detailed model possess the capability of producing an increase in BMD primarily by reducing its clearance rate following a therapeutic intervention. In addition to a mechanism-based interpretation of data, clearance based models have the inherent ability to provide dynamic flux patterns as input or modulating signals to mathematical models representing systems biology in information systems, such as DMPS (Database for Modelling Signalling Pathways) [15]. The clearance rate models have also a role in long term drug administration by quantifying changes in clearance rate constant sensitivity.

2. Material and Methods

2.1 Database

Data for this work was obtained from the published literature [14]. The study’s subjects were women 42 to 82 years of age who all had had a hysterectomy (removal of reproductive organs). All women participated in double-blind, placebo-controlled clinical trial over two years, which was extended for an extra year. There were four treatment groups, placebo (n = 27), alendronate (10 mg/day, n = 39), conjugated estrogens (CE, 0.625 mg/day, n = 74) and alendronate (10 mg/day) plus CE (0.625 mg/day, n = 72). At the beginning of the third year the treatments were switched to placebo except for alendronate plus CE which either did not change or switched to placebo. The primary aim of these studies was to directly examine the differential effect of the various treatments. The mean of the base line values for groups was obtained from Table 1 of [14]. The mean change in bone mineral density (BMD) and serum bone-specific alkaline phosphatase (BAP) as percentage change from baseline were obtained from Figures 3 and 4 in [14]. The percentage changes from baseline values together with the mean basal values were used to construct the mean profiles for BMD and BAP over three years. The sampling times for BAP were 0, 1, 3, 6, 12, 18, 24, 27, 30 and 36 months (n = 10). The sampling times for BMD were 0, 3, 6, 12, 18, 24, 30 and 36 months (n = 8).

2.2 Methods

The simplest form of a biomarker clearance from systemic circulation is a one compartment model shown in Figure 1a [4]. As an extension the biomarker S in Figure 1a is assumed to have an intrinsic companion controller. This companion controller aims to keep the set level of S constant by using a closed loop negative feedback control. The value of C is inherently unknown unless it is explicitly defined. It is assumed that the controller C has the same value as S at the steady state. The differential equations describing the second order negative feedback model in Figure 1b are:

\[
\frac{dS}{dt} = R - k_S S - k_C C
\]

(1)

\[
\frac{dC}{dt} = -k_C C + k_S S
\]

(2)

In Figure 1(b) the fluxes \(k_S S\) and \(k_C C\) are control fluxes and they only appear in the receiving compartment differential equations not the source [16].
The external or internal controls for the second order model are related through $k_4$ since the source $S$ for the control flux is known. By replacing both $S$ and $C$ in Equations 1 and 2 with their respective differences from basal levels $S_b$ and $C_b$ that is $s + S_b = S$, $c + C_b = C$ and assuming steady state condition $R_1$ will disappear from Equation 1 yielding the following differential equations in terms of only the above basal values.

\[
\begin{align*}
\frac{ds}{dt} &= -k_1 s - k_2 c \\
\frac{dc}{dt} &= -k_3 c + k_4 s
\end{align*}
\]  

(3)

(4)

By differentiating both sides of Equation 3 and replacing the derivative of $c$ from (4) and $c$ from (3) a second order differential equation in $s$ is obtained.

\[
\frac{d^2s}{dt^2} + (k_1 + k_3) \frac{ds}{dt} + (k_2 + k_4) s = 0
\]  

(5)

Similarly Equation 4 can be differentiated to obtain the same second order differential equation in terms of $c$. It is noted that in the above second order differential equation for the event of both $k_1$ and $k_2$ being zero. The solution for $s$ will be a simple harmonic motion $s(t) = s(0) \sin(\omega_n t)$ with $\omega_n = \sqrt{k_1 k_4}$. The production rate of the biomarker ($R_1(0)$) in Figure 1b is computed by the following equation using steady state assumption (i.e. equating left hand side of Eqs. 1 and 2 to zero).

\[
R_1(0) = (k_1 + \frac{k_3}{k_4})S(0)
\]  

(6)

In Equation 6 the coefficient of $S$ is termed the clearance rate constant which also accounts for clearance rate of the controller. The differential equation in (5) can be re-parameterized such that the left hand side is analogous to a basic closed loop negative feedback servomechanism with two parameters $\omega_n$ (the natural frequency) and $\zeta$ (the damping ratio).

\[
\frac{d^2s}{dt^2} + 2\zeta \omega_n \frac{ds}{dt} + \omega_n^2 s = (\text{forcing - term}) \omega_n^2
\]  

(7)

The analytical solution for Equation 7 with $\zeta < 1$ (under damped condition) is

\[
s(t) = (\text{forcing - term}) \left[1 - e^{-\omega_n t} \left(\cos(\omega_n t) - \frac{\zeta}{\sqrt{1-\zeta^2}} \sin(\omega_n t)\right)\right].
\]  

(8)

With $\omega = \omega_n \sqrt{1-\zeta^2}$.

The basic servomechanism can follow a step input (the forcing term) without error, which means that $s$ in the analytical solution (Eq. 8) will approach the same value as the forcing term as time progresses [17].

In theory, the parameters of the servo model ($\omega_n$ and $\zeta$) can be estimated for each individual depending on the number and timings of observations in a number of ways. The simplest method assumes that the dynamic between two consecutive observations follows a step input similar to a basic servo mechanism. A minimisation algorithm was implemented for estimation of servo model parameters. The minimization algorithm used the notion that at each observation point ($p_j$) the basic servomechanism model has already reached the previous observation ($p_{j-1}$) before the next forcing term ($p_j - p_{j-1}$) was applied, where the forcing term is the difference of two consecutive above basal concentrations as shown in Figure 9 (Figure 2), where $s$ is the solution (basic servomechanism response, Eq. 8) of the corresponding observation ($p$).

\[
\text{sum-of-squares} = \sum_{j=2}^{n} (s_j - p_j)^2
\]  

(10)

The sums of squares formed by Equation 10 can be minimized using a nonlinear minimization algorithm in R [18] using the function nlm() [19].

The comparison of Equations 5 and 7 reveal the following identities

\[
k_1 + k_2 = 2 \zeta \omega_n
\]  

(11)

\[
k_1 k_2 + k_3 k_4 = \omega_n^2
\]  

(12)
†Equations 11 and 12 reveal that we have four rate constants and two equations with known right hand sides. Therefore, the parameters of the extended model cannot be identified. We have already made the assumption that at steady state the controller has the same value as the biomarker itself. It is further assumed that the controller has the same clearance rate constant as the biomarker itself that is $k_1 = k_2$. This assumption allows to fix the product of $k_3$ and $k_4$ with the most parsimonious solution where $k_3 = k_4$ and all rate constants being equal. With all the rate constants of the extended model being equal by using †Equations 11 and 12 $\zeta$ becomes $0.707 (\sqrt{2})$.

Thus the minimisation of sum of squares (†Eq. 10) reduces to estimating one parameter namely $\omega_n$.

The observed biomarkers concentrations can also be described by changes in the controller rate constant $k_4$ keeping the initial production rate ($R_j(0)$) constant. The fold change in controller rate constant $k_4$ can be computed at each measurement interval assuming steady state condition using the following equation.

$$\alpha_j = \frac{k_4 R_j(0) - k_5 k_j S_j - k_6 k_j S_j}{k_5 k_j S_j} \quad \text{(13)}$$

where $j$ is a time interval (e.g. between 0 and 3 months) and $i$ is a specific response (subject).

The term forward simulation indicates that, the biomarker production rate ($R_j$) or the fold change in $k_4$ computed with the steady state assumption at each time point was used as step input (or step change) brought forward by one time interval. The differential equations were solved numerically using lsoda() function [20].

The variability of fold changes in $k_4$ from zero was examined using student t-test. Since we are using mean observations straight line (linear regression) was used to quantify the trend in magnitude of fold changes in $k_4$ with time. For situation where individual observations are available mixed model with random intercept and slope should be used to quantify the trend with time and also the relationship between two set of $k_4$ [7].

### 3. Results

The parameter of the servo model ($\omega_n$) was estimated for lumbar spine BMD and serum BAP. †Table 1 shows the $\omega_n$ estimates for lumbar spine BMD and serum BAP together with the corresponding $t_{1/2}$ of the biomarker $(k_j)$ for the second order negative feedback model. †Figure 3 shows the servomechanism model step responses following the above basal lumbar spine BMD and serum BAP as step inputs in each time interval for the four therapeutic interventions.

†Figure 4 shows the forward simulations for the negative feedback model (†Figure 1b) to step inputs computed for the above basal values in addition to the steady state production rate $R_j(0)$ using †Equation 6.

†Table 2 summarizes the fold change (as percentage) in $k_4$ of BMD in different time intervals. †Figure 5 (a and b) shows the simulation of BMD and BAP following percentage changes in $k_4$ as shown in †Tables 2 and 3. Note that there are minor differences in †Figures 4 and 5 essentially highlighting the differences in simulated measurement with changes in production rate or clearance rate constant. †Table 4 summarizes the mean percentage changes in $k_4$ during the first two years of therapies, the subsequent switch over year and the whole three years. The percentage changes in $k_4$ were regressed with time over three years for both BMD and BAP. †Table 5 summarizes the results. Note that coefficient of time $(\beta_1)$ in therapies with Alendronate for BMD were significant.

The implication for describing BMD with changes in the controller rate constant is that

### Table 1 Model parameter estimates for bone mineral density and bone specific alkaline phosphatase, the accuracies of $\omega_n$ estimates are shown in parenthesis expressed as coefficient of variation (%). The $\zeta$ is 0.707.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Therapy</th>
<th>Baseline level</th>
<th>$\omega_n$ (radian/month)</th>
<th>$k_4$ ($t_{1/2}$) (month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD (g/cm$^2$), n = 8</td>
<td>Placebo / Placebo</td>
<td>0.76</td>
<td>1.081 (3.1)</td>
<td>0.906</td>
</tr>
<tr>
<td>BMD (g/cm$^2$), n = 8</td>
<td>CE / Placebo</td>
<td>0.8</td>
<td>1.127 (1.7)</td>
<td>0.869</td>
</tr>
<tr>
<td>BMD (g/cm$^2$), n = 8</td>
<td>Alendronate/Placebo</td>
<td>0.83</td>
<td>1.109 (0.76)</td>
<td>0.883</td>
</tr>
<tr>
<td>BMD (g/cm$^2$), n = 8</td>
<td>Alendronate + CE/Placebo</td>
<td>0.85</td>
<td>1.116 (1.2)</td>
<td>0.878</td>
</tr>
<tr>
<td>BMD (g/cm$^2$), n = 8</td>
<td>Alendronate + CE/Alen-</td>
<td>0.83</td>
<td>1.114 (0.93)</td>
<td>0.879</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td>0.81 (0.03)</td>
<td>1.109 (0.017)</td>
<td>0.883 (0.013)</td>
</tr>
<tr>
<td>Bone Specific Alkaline Phosphatase (ng/ml), n = 10</td>
<td>Placebo /Placebo</td>
<td>12.7</td>
<td>3.015 (2.8)</td>
<td>0.325</td>
</tr>
<tr>
<td>Bone Specific Alkaline Phosphatase (ng/ml), n = 10</td>
<td>CE / Placebo</td>
<td>8</td>
<td>3.037 (3.1)</td>
<td>0.322</td>
</tr>
<tr>
<td>Bone Specific Alkaline Phosphatase (ng/ml), n = 10</td>
<td>Alendronate / Placebo</td>
<td>7.1</td>
<td>3.017 (4.4)</td>
<td>0.324</td>
</tr>
<tr>
<td>Bone Specific Alkaline Phosphatase (ng/ml), n = 10</td>
<td>Alendronate + CE / Placebo</td>
<td>6</td>
<td>3.355 (2.5)</td>
<td>0.293</td>
</tr>
<tr>
<td>Bone Specific Alkaline Phosphatase (ng/ml), n = 10</td>
<td>Alendronate + CE / A</td>
<td>en-)onate + CE</td>
<td>5.4</td>
<td>3.139 (2.5)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td>7.84 (2.89)</td>
<td>3.108 (0.136)</td>
<td>0.315 (0.013)</td>
</tr>
</tbody>
</table>
the decreases in clearance rates causing the change rather than increases in production rate. ▶ Figure 6a shows the production rate profile and ▶ Figure 6b the clearance rate profile necessary for the description of BMD. ▶ Figure 7a shows the production rate profile necessary for the description of serum BAP keeping the clearance rate constant. ▶ Figure 7b shows the dynamic changes in clearance rate constant profile necessary for the description of BAP serum concentration. The absolute values of changes in $k_4$ for BMD and BAP are positively correlated. The regression coefficient without inclusion of intercept is 15.96 (1.131) with $P < 0.0001$. The latter regression coefficient implies that on average the fold change in $k_4$ of BAP is 15.95 times that for BMD.

**Figure 3**  a) The servomechanism step responses (solid lines) to the above basal lumbar spine BMD, b) the servomechanism step responses (solid lines) to the above basal serum BAP. The symbols are the mean values. The arrow indicates the switch over in treatment at 24 months.

**Figure 4**  a) Forward simulations of the second order negative feedback model for the lumbar spine bone mineral density, b) forward simulations of the second order negative feedback model for the serum bone-specific alkaline phosphatase; the step inputs to the models are the steady state and above basal production rates computed using Equation 6, the arrow indicates the switch over in treatment at 24 months.
4. Discussion

We have described a method for estimating clearance rate constants of biomarkers from observational studies. Although the trend and comparison between the biomarkers over time could be performed in a model-free context the current method provide a mechanistic understanding of the system. The method is similar to the deconvolution approach, where the biological signal together with a construct for its clearance rate are used to derive its input secretion pattern but only at steady state [6]. The use of negative feedback model was inspired by its original application in glucose and insulin regulation [16]. Although the actual order (or the number of state variables) of the bone remodelling process is higher than two, the second order model attempts to capture the dominant states. For general applicability of the second order negative feedback model and the fact that we have used mean datasets, we made the assumption that all rate constants were identical since our main focus was the estimation of clearance rate constant. Or equally the processes involved are so fast that differences between the parameters become relatively unimportant and can be neglected. The second major assumption for the estimation of servomechanism model parameter (ω_n) was that the input to the model between any two consecutive measurements was a step. Although the step input is the common input form for a servomechanism model, the same would not necessarily be the case for the second order negative feedback model. The step input over two consecutive measurements also imply that shorter intervals together with high concentration changes would produce higher clearance rate constant (or natural frequency). One way of relaxing the step input over two consecutive measurements would be, the extension by considering every three consecutive measurement occasions, for example, 1st, 2nd and 3rd; 2nd, 3rd and 4th etc. In such an algorithm, the difference between the first and third measurements would be the magnitude of step applied over the first and third time intervals and predictions would be for the second (the transient) and third measurements and subsequently third and fourth etc. In the latter algorithm the estimates of ω_n and ζ could be different to those estimated from two consecutive measurements.

As an aside and a demonstration for helping with the discussion without repeating the same analyses as in the main text, the above procedure was used for estimation of both ω_n and ζ for BMD and BAP.

Table 2  Fold changes (in percentage) in k_4 of BMD from baseline in different therapeutic intervention during various time intervals

<table>
<thead>
<tr>
<th>Therapy/time Interval (months)</th>
<th>0–3</th>
<th>3–6</th>
<th>6–12</th>
<th>12–18</th>
<th>18–24</th>
<th>24–30</th>
<th>30–36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo/placebo</td>
<td>–0.8</td>
<td>–0.8</td>
<td>–0.2</td>
<td>1</td>
<td>1</td>
<td>–0.5</td>
<td>1</td>
</tr>
<tr>
<td>CE/placebo</td>
<td>–4</td>
<td>–7</td>
<td>–10</td>
<td>–11</td>
<td>–12</td>
<td>–6</td>
<td>–3</td>
</tr>
<tr>
<td>Alendronate/placebo</td>
<td>–6</td>
<td>–7</td>
<td>–9</td>
<td>–12</td>
<td>–13</td>
<td>–12</td>
<td>–11</td>
</tr>
<tr>
<td>Alendronate + CE/Alendronate</td>
<td>–7</td>
<td>–8</td>
<td>–12</td>
<td>–14</td>
<td>–16</td>
<td>–18</td>
<td>–19</td>
</tr>
</tbody>
</table>

Table 2

Figure 5  a) BMD estimation following step changes in controller rate constant k_4, b) BAP simulations following step changes in controller rate constant k_4, see Tables 2 and 3 for the corresponding percentage changes in each interval. The arrow indicates therapy switchover at 24 months.
For this situation the parameters of the negative feedback model would not all be identical and a solution can be arrived at by making the assumption that \( k_3 = k_4 \) and \( k_4 = k_5 \) (Eqs. 11 and 12). The estimates of \( \omega_n \) and \( \zeta \) for BAP in treatment groups Placebo/Placebo, CE/Placebo, Alendronate/Placebo, Alendronate + CE/Placebo and Alendronate + CE/Alendronate + CE were (2.148, 2.295, 1.848, 1.816, 1.830) and (0.090, 0.084, 0.653, 0.641, 0.654) respectively. For BMD the corresponding estimates were (0.155, 0.253, 0.334, 0.353, 0.308) and (0.085, 0.782, 0.624, 0.648, 0.631) respectively. Figure 8 shows the estimates of the transient and the end measurements (second and third) using a moving sequence of three consecutive measurements for estimation of \( \omega_n \) and \( \zeta \).

As shown in Figure 8 for BMD the three-month estimates are under predicted due to lower estimates of natural frequencies compared to when using consecutive measurements (Table 1). The same is true for BAP as the estimates of natural frequencies are also lower. Close examination of Figure 8 shows that overall for both BMD and BAP there is at least one sequence of measurements, for example 1, 2, 3 or 2, 3, 4, that provides an adequate prediction.

There are also other algorithms whereby both \( \omega_n \) and \( \zeta \) can be estimated with the most pertinent being a consecutive three measurements sequences rather than a moving sequence. Overall these latter methods highlight the inherent flexibility and richness of second order negative feedback model for describing dynamical changes in longitudinal observations in a variety of ways. Each of these methods will also provide a different interpretation for the underlying change, for example, for BAP the step change over three measurements sequence can be from three to twelve months. Similarly for BMD the step change over three measurements sequence can be from six to twelve months.

The implication of using the negative feedback model for dynamic description is that each biomarker will have a known or as yet undiscovered intrinsic controller locked to it. Or the controller may also be an aggregate of more complex process as is the case for bone remodelling process. At the level of organ like bone it is biomechanical feedback that warrants that bone is not completely lost or excessively deposited as termed “mechanoStat” in [10]. Whereas at the species scale with different organ being involved it is calcium that is tightly regulated via PTH biochemical feedback [21]. In situations where there is no obvious feedback control for biomarkers other than changes in

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**Table 3** Fold changes (in percentage) in \( k_4 \) of BAP from baseline in different therapeutic interventions during various time intervals

<table>
<thead>
<tr>
<th>Therapy/time interval (months)</th>
<th>0–1</th>
<th>1–3</th>
<th>3–6</th>
<th>6–12</th>
<th>12–18</th>
<th>18–24</th>
<th>24–27</th>
<th>27–30</th>
<th>30–36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo/placebo</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>–1</td>
<td>14</td>
<td>13</td>
<td>1</td>
<td>–12</td>
<td>–16</td>
</tr>
<tr>
<td>CE/placebo</td>
<td>11</td>
<td>31</td>
<td>65</td>
<td>114</td>
<td>154</td>
<td>214</td>
<td>154</td>
<td>68</td>
<td>53</td>
</tr>
<tr>
<td>Alendronate/placebo</td>
<td>11</td>
<td>84</td>
<td>148</td>
<td>182</td>
<td>248</td>
<td>186</td>
<td>98</td>
<td>74</td>
<td>54</td>
</tr>
<tr>
<td>Alendronate + CE/placebo</td>
<td>14</td>
<td>127</td>
<td>197</td>
<td>243</td>
<td>264</td>
<td>253</td>
<td>130</td>
<td>122</td>
<td>61</td>
</tr>
<tr>
<td>Alendronate + CE/Alendronage + CE</td>
<td>39</td>
<td>171</td>
<td>281</td>
<td>313</td>
<td>341</td>
<td>341</td>
<td>306</td>
<td>293</td>
<td>248</td>
</tr>
</tbody>
</table>

*not different from 0

**Table 4** Mean (SD) fold changes (in percentage) in \( k_3 \) during various time intervals for BMD and BAP

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo/placebo</td>
<td>–0.21* (0.9)</td>
<td>0.64* (1)</td>
<td>0.15* (0.98)</td>
<td>4* (6)</td>
<td>–3* (13)</td>
<td>0.68* (10)</td>
</tr>
<tr>
<td>CE/placebo</td>
<td>–8 (3)</td>
<td>–7 (4)</td>
<td>–7 (3)</td>
<td>75 (59)</td>
<td>122 (75)</td>
<td>96 (67)</td>
</tr>
<tr>
<td>Alendronate/placebo</td>
<td>–8 (2)</td>
<td>–12 (0.84)</td>
<td>–10 (2)</td>
<td>130 (91)</td>
<td>103 (57)</td>
<td>120 (75)</td>
</tr>
<tr>
<td>Alendronate + CE/placebo</td>
<td>–10 (4)</td>
<td>–15 (1)</td>
<td>–12 (4)</td>
<td>169 (101)</td>
<td>142 (80)</td>
<td>157 (88)</td>
</tr>
<tr>
<td>Alendronate + CE/Alendronage + CE</td>
<td>–10 (3)</td>
<td>–17 (1)</td>
<td>–13 (4)</td>
<td>229 (124)</td>
<td>297 (38)</td>
<td>259 (97)</td>
</tr>
</tbody>
</table>

*not different from 0

**Table 5** Regression coefficients (\( s_2 \)) for percentage changes in \( k_4 \) with time (in months) over three years

<table>
<thead>
<tr>
<th>Therapy/regression coefficient</th>
<th>( \beta_0 )</th>
<th>( \beta_1 )</th>
<th>( \beta_0 )</th>
<th>( \beta_1 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo/placebo</td>
<td>–0.75 (0.60)</td>
<td>0.04 (0.02)</td>
<td>6.9 (5.6)</td>
<td>–0.35 (0.27)</td>
</tr>
<tr>
<td>CE/placebo</td>
<td>–8.1 (2.6)</td>
<td>0.02 (0.1)</td>
<td>57.6 (38.7)</td>
<td>2.2 (1.8)</td>
</tr>
<tr>
<td>Alendronate/placebo</td>
<td>–7.1 (1.1)</td>
<td>–0.2 (0.05)*</td>
<td>118.7 (47.6)</td>
<td>0.12 (2.2)</td>
</tr>
<tr>
<td>Alendronate + CE/placebo</td>
<td>–7.1 (1.8)</td>
<td>–0.3 (0.08)*</td>
<td>156.3 (55.6)</td>
<td>0.05 (2.63)</td>
</tr>
<tr>
<td>Alendronate + CE/Alendronage + CE</td>
<td>–6.7 (0.7)</td>
<td>–0.3 (0.03)**</td>
<td>183.3 (50.9)</td>
<td>4.3 (2.4)</td>
</tr>
</tbody>
</table>

\*P = 0.05, ***P = 0.0001
production rate or disappearance rate, the proposed method provide a possible explanation for the interpretation of experimental data.

In addition the negative feedback model provides a means by which such construct can be extended as cascades whereby changes in the controller (C) of one entity for example BMD modulates the controller rate constant ($k_4$) of the other such as BAP. Furthermore the controlling action through changes in (C) is mandatory as the value of biomarker (S) itself is known and cannot be altered.

The estimates of natural frequencies for BMD and BAP had low variances (high accuracies) with the clearance rate value for BAP being 2.8 times higher. The mean value of $t_{1/2}$ for BMD is approximately 0.88 months and for BAP is 0.31 month. We are not aware of any clearance rate constant

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**Figure 6** a) Changes in BMD production rate necessary for the description of BMD, b) changes in clearance rate constant of BMD brought about by changes in $k_4$ necessary for the description of BMD

**Figure 7** a) Changes in BAP production rate necessary for the description of BAP, b) changes in clearance rate constant of BAP brought about by changes in $k_4$ necessary for the description of BAP with production rate set to that of basal value
(t_{1/2}) estimates for BMD or SAP in the literature for comparison other than in two subjects with Paget’s disease following plasmapheresis. Alkaline Phosphatase activities of serum declined sharply but returned to preplasmapheresis values within 8 to 10 days with \( t_{1/2} \) estimated as 1.12 to 2.15 days [22].

The primary data for this work was based on the mean value extracted from the literature, although the mean values can be regarded as not being the representative of any individual nevertheless it is universally used in sciences literature for depiction of differences. The nature of current method that is estimating one parameter, the natural frequency of the simple servomechanism will ensure that it would be applicable at individual level as well.

It has been shown that the estimation of clearance rate constant (/time) was the first step in defining a dynamic model. The product of clearance rate constant and the measured dependent variable (▶Eq. 6 for BMD or BAP) yielded production rate and this value was subsequently fed back to the models in forward simulation mode to reproduce the measured values. It has also been shown that for the current time scale (ten measurements over three years) forward simulation reproduces the measured values adequately. The latter is expected for BMD since its \( t_{1/2} \) is well below quarter of measurement interval (three month). For BAP the shortest sampling interval (one month) is almost three times its \( t_{1/2} \). The other interesting feature of the negative feedback model was that the measured (dependent) values can be reproduced (simulated) by inducing computed changes in the controller rate constant (namely \( k_4 \)) at each measurement time under steady state assumption. The implication of decreasing \( k_4 \) (▶Figure 5) was that the measured BMD can be reproduced by decreasing clearance rate alone (▶Figure 6B). Or alternatively, by only increasing the production rates (▶Figure 6A). The decreasing \( k_4 \) for BMD would imply that the controller values (C) are decreasing whereas for the increasing production rate (\( R_k \)) would be the opposite.

Returning to the question whether it is the reduction in clearance rate of BMD or increase in its production that causes it to increase. Bone remodelling is a complex and tightly controlled process. The cells involved in bone remodelling are osteoblasts (bone forming cells) and osteoclasts in both active and responding forms together with various hormonal controls [11, 12]. Alendronate (a bisphosphonate) action on bone cells is by increasing apoptosis of osteoclasts thus reducing available bone resorption cells [23] or reduced osteoclast recruitment [21]. The decrease of bone resorption cells would result in a reduction of BMD clearance rate (or decrease disappearance of BMD flux) which was one of scenarios highlighted here. On the other hand an increase in BMD could also be achieved by increasing its production rate which was also one of the solution (scenarios). It is the reduction in clearance rate of BMD brought about by decreasing \( k_4 \) in the negative feedback model which is the preferred option. The inferred notion of decreasing clearance rate constant can be verified using a more detailed mathematical model which takes into account bone cells together with hormonal controls at various body compartments.
The linked negative feedback models may support a mechanism based simulation of drug efficacy whereby therapeutic outcome can be anticipated akin to a real time control system. At first a value for the clearance rate constant ($k_4$) of measured biomarkers are assumed. The initial values for the clearance rate constants are from the literature or exploratory observations. The basal concentration of biomarkers would then initialise the negative feedback biomarker models (Figure 1b). Using the second observation of a biomarker following a drug therapeutic intervention the fold change in $k_4$ (Equation 13) can be computed (Table 2 for BMD). In a similar manner the fold changes for $k_4$ of the second biomarker can be computed (for example, see Table 3 for BAP). In our example, the absolute fold changes in $k_4$ for BMD and BAP were correlated and they could be described by a simple straight line (see Results). In addition knowing the fold change in $k_4$ of a biomarker can lead directly to simulating its concentration as in Figure 5. Since the fold changes in $k_4$ of BMD and BAP were linearly related knowing fold changes in $k_4$ of BAP will lead to computing the corresponding values for $k_4$ of BMD. The BMD can then be predicted having estimated $k_4$ changes in the model. In many other situations it is expected that the fold changes in $k_4$ of one biomarker would not be linearly related to the corresponding changes in $k_4$ of other biomarkers. In such situations saturating functions depicting maximum drug effect may be useful [4]. The longitudinal fold changes in $k_4$ instead of biomarker’s value itself are more sensitive to external stimuli (drug effect) as the clearance rate of biomarkers has been taken into account.

The data analysis contribution of this paper has been the description of consequential above basal values by a second order servo system. As stated earlier the requirement for using the consecutive measurements can be relaxed gradually depending on the variability and the nature of the underlying mechanism. At one extreme depending on the physiology even the first and last values can be used and the objective function is formed from the predictions of the transient measurements. The simulation aspect of this paper will arise from the possible linked systems via respective $k_4$ whereby linear relationship between them would be the simplest and at population level mixed models would play an important role in terms of accurate predictions [7]. It is envisaged that the clearance rate models will facilitate the feedback from the Bedside to Bench in translational medicine by identifying the relationship between various biomarkers in a mechanistic framework [24, 25].

Acknowledgments

The authors would like to thank Drs. Oscar E. Della Pasqua and Meinidt Danhof for their valuable comments and suggestions. This study was performed within the framework of the Dutch Top Institute Pharma, Mechanism-based PK/PD modeling platform (project number D2–14).

References