Modeling Calcium Loss from Bones during Space Flight

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Calcium loss from bones during space flight creates a risk for astronauts who travel into space, and may prohibit space flights to other planets. The problem of calcium loss during space flight has been studied using animal models, bed rest (as a ground-based model), and humans in-flight. In-flight studies have typically documented bone loss by comparing bone mass before and after flight. To identify changes in metabolism leading to bone loss, we have performed kinetic studies using stable isotopes of calcium. Oral (^{43}Ca) and intravenous (^{46}Ca) tracers were administered to subjects (n=3), three-times before flight, once in-flight (after 110 days), and three times post-flight (on landing day, and 9 days and 3 months after flight). Samples of blood, saliva, urine, and feces were collected for up to 5 days after isotope administration, and were analyzed for tracer enrichment. Tracer data in tissues were analyzed using a compartmental model for calcium metabolism and the WinSAAM software. The model was used to: account for carryover of tracer between studies, fit data for all studies using the minimal number of changes between studies, and calculate calcium absorption, excretion, bone calcium deposition and bone calcium resorption. Results showed that fractional absorption decreased by 50% during flight and that bone resorption and urinary excretion increased by 50%. Results were supported by changes in biochemical markers of bone metabolism. In-flight bone loss of approximately 250 mg Ca/d resulted from decreased calcium absorption combined with increased bone resorption and excretion. Further studies will assess the time course of these changes during flight, and the effectiveness of countermeasures to mitigate flight-induced bone loss. The overall goal is to enable human travel beyond low-Earth orbit, and to allow for better understanding and treatment of bone diseases on Earth.

1. Introduction

Loss of calcium from bones during space flight remains a significant challenge. Bone loss poses a barrier to flights of several years duration that will be necessary for human exploration of other planets. Calcium loss has been documented in animals and humans through increased urinary calcium excretion and by decreases in bone mass determined through radiological imaging. The loss is site specific, with weight bearing bones (such as the hip) exhibiting greater loss than non-weight bearing bones, for example, the skull. In addition, loss varies greatly between individuals. Bone mass, or amount of calcium in bones, is determined by genetics, level of exercise, diet and other possible physiological factors and these factors may influence an individual's ability to retain calcium in zero gravity. Bone loss can be measured by imaging, through biochemical markers (e.g., compounds released when bone is resorbed), and by balance studies (as the difference between calcium absorbed and calcium excreted). This latter approach was used on Skylab and described by Whedon et al. [1977]. Imaging methods and balance studies are descriptive in that they define the net loss, while biochemical markers provide only indirect measures of bone loss. To examine the rate of loss more directly, and to investigate the mechanisms resulting in the changes in calcium metabolism, we have performed kinetic studies with stable isotopes, as described by Smith et al. [1999].

Stable isotopes are naturally-occurring forms of an element that can be differentiated based on their mass. Some forms are normally present at low levels and these can be used as tracers to study metabolism. We have used two forms of calcium, administered one orally and one intravenously and then measured their levels in serum, saliva, urine and feces over time. Data were analyzed by compartmental modeling. With this approach it is assumed that calcium in the body exists in compartments that turnover at distinct rates. Transfer between compartments can be determined by adjusting the rates until the model calculated value fits the observed data. Once the model fits the observed data

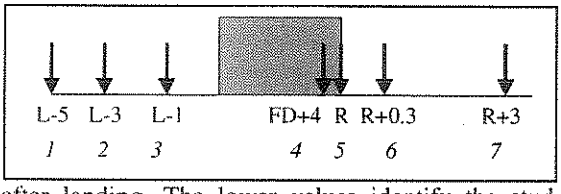
obtained under one condition, specific parameters are changed to fit data obtained under a different condition. In this way the model can be used to calculate rates of metabolism, and determine the sites and rates of change under different conditions.

This approach was used to study rates of calcium metabolism in astronauts and cosmonauts before going into space, after a prolonged period in space, and after they returned to earth. The aim of the studies was to determine rates of calcium metabolism, identify sites of change during space flight, and calculate the time required for metabolism to return to preflight levels after returning to earth. The studies were conducted at the Johnson Space Center, Houston, TX, Star City, Russia, on the space station Mir, and on the Shuttle.

2. Experimental Methods

Smith et al. [1999] has described the experiments in detail. Briefly, three subjects were studied and seven studies were performed (Fig 1); three preflight (approximately 5, 3 and 1 month before launch), one in-flight after 4 months, and three post-flight (landing day, after 9 days and after 3 months). Each study consisted of measurement of calcium intake in the diet and administration of ^{43}Ca orally and ^{46}Ca intravenously. Serum, saliva, urine and feces were sampled over the following 5 days. Isotope enrichment and total calcium were determined in each sample. The amount of isotope was expressed as % of administered dose.

Figure 1. Time-line of studies performed on each of the three astronauts and cosmonauts; at launch (L) minus 5 mo, 3 mo and 1 mo, in-flight (FD) after 4 mo while on the Shuttle, on landing (R), and at 0.3 mo and 3 mo



after landing. The lower values identify the study number. The shaded area indicates the time in-flight aboard the Mir space station.

3. Kinetic Analysis

3.1 Modeling Software

Tracer data were analyzed by compartmental analysis using WinSAAM (Windows version of the Simulation, Analysis, And Modeling software, available on the web at www.winsaam.com. The software is described by Grief et al. [1998]. The program is a general equation solving package that is designed for analyzing biological systems where flexibility is required to represent varied experimental conditions, different inputs (e.g., infusions, bolus doses, and combinations), changes in experimental conditions during a study, and features are required to represent delays, discontinuous sampling etc. Use of the program is described in Wastney et al. [1998]. WinSAAM is designed to expedite the development and testing of compartmental models. By specifying transfer coefficients (L(i,j) representing transport into compartment i from compartment j, fraction/unit time), the program will assemble the corresponding set of differential equations. This allows models to be readily set up and changed to test various configurations against the data. In addition to adjusting parameters by least squares minimization to fit observed data, the program provides statistical measures of the fit and parameter estimates. A new feature of the software called project management allows multiple studies as described by Lyne et al. [1992] to be fitted simultaneously to provide population estimates for parameters.

3.2 Compartmental Model

Compartmental models are useful for studying biological systems because compartments are often used to represent material within a component such as an organelle or tissue. They have been used to study metabolism from the cellular to population level. Calcium has been modeled using 3- or 4-compartments to represent exchange with calcium in blood. The number of compartments is the minimum required to fit the data. This approach to modeling is called reverse engineering, or the inverse problem.

We used a 3-compartment model, where the first compartment, turns over in about 1 hr, the second in 2 hr and the third in 3 days (Fig 2). The compartments are considered to represent calcium in serum and some extravascular fluid, interstitial fluid and exchangeable calcium on bone. There is loss from the third compartment that represents deposition of calcium into bone. Other loss pathways are into urine, and into the gastrointestinal tract. Absorption occurs by a single pathway into the sampled compartment. Entry of natural calcium occurs into the gastrointestinal tract via the diet and into serum via bone resorption. These data

are used to determine the steady state (e.g., pool sizes, M(i) and transport rates, R(i,j)).

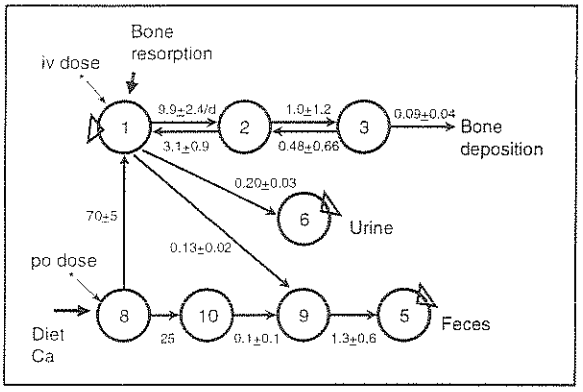


Figure 2. Model of calcium metabolism in humans (modified from Smith et al. [1999]). Compartment 1 includes blood, 2 includes intracellular calcium and 3 includes exchangeable calcium on bone. Arrows with asterisk indicate site of tracer entry (by intravenous dose, iv or orally, po). The triangles indicate sampling sites (serum, urine and feces). Saliva data were fitted as a fraction (0.41) of compartment 1. Compartments 8, 10, and 9 are in the gastrointestinal tract. Values next to the arrows are fractional transfer coefficients (mean±SD, n=3). The larger arrows indicate site of calcium entry, via the diet or from bone resorption.

3.3 Modeling Calcium Space Flight Data

Assumptions in modeling the data were that subjects were in steady state during each 5-day study (i.e., pool sizes did not change). The model was first fitted to all data (oral and iv tracers in serum, saliva, urine and feces) for each subject, for preflight study 1. The model was then solved out until the start of the second study, to account for carryover of tracer in all of the compartments. A minimum number of changes, considered necessary and sufficient to fit data from the next study, were then introduced into the model. The process was continued until kinetic data from all seven studies were fitted. The model was used to calculate absorption as the ratio of L(1,8)/(L(1,8)+L(10,8)), excretion, bone deposition, and bone resorption.

There were several challenges in fitting the data;

- Isotopes were administered by two routes, orally and intravenously. This was accounted for in the modeling by setting up two models with identical structure and parameter values but different initial conditions (either compartment 1, for the iv dose or compartment 8 for the oral dose).
- Studies were repeated at varying time intervals with repeated dosing. Carryover of tracer was accounted for in the modeling, by simulating the model through the same time-line as the experiments and 'adding' tracer at the start of each new study. With this approach tracer remaining in each compartment from the previous study was present as background when the next study was started, and therefore automatically accounted for in the modeling.
- Data were relatively sparse. Due to the limitations of space flight, the number of samples and frequency of sampling were not ideal. To interpret the data, some parameters (i.e., slower parameters such as loss from compartment 3) were weighted by the population values from previous studies in adults. The program only adjusted these parameters away from the mean if necessary to fit all of the data.
- Tracer was administered as an oral solution and therefore all calcium was ionized and available for absorption. Values may be higher than absorption of calcium from the diet, where some of the calcium is probably complexed with food. Absorption of calcium from food is about 40% and so this value was used for calculating the amount of calcium absorbed from diet, in studies where tracer absorption exceeded this value.

4. Results

Data from one of the seven studies, for one subject are shown in Fig 3. Results of the changes in calcium metabolism during space flight are summarized in Table 1. There was a marked decrease in dietary calcium intake. Normally, this would initiate an increase in fractional absorption. However, during space flight fractional absorption decreased. The subjects were therefore absorbing less calcium. At the same time, they excreted almost twice as much calcium in urine. The combined result was a loss of calcium of up to 250 mg/d per subject.

Table 1. Changes in Calcium Metabolism during Space Flight

Parameter	Space Flight
Calcium	Decrease in dietary intake
	Decrease in fractional absorption
	Increase in urinary excretion
	Increase in bone resorption
	Net loss of up to 250 mg ca/d
PTH	Tended to decrease.

Vitamin D	Tended to decrease.
Bone Formation	Bone-specific alkaline phosphatase and osteocalcin tended to decrease.
Bone resorption	Increase in collagen-crosslinks during flight.

The studies showed that calcium loss is serious (up to 250 mg/d) and prolonged. Upon returning to earth subjects regained bone at approximately 100 mg/d. Therefore it was predicted that it would take twice as long as the length of the flight, in order for the subjects to fully regain their bone loss.

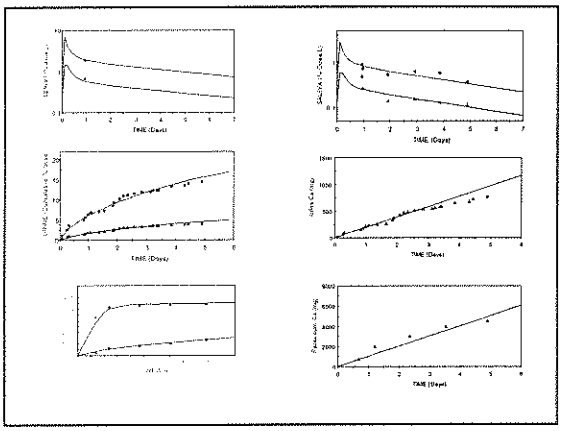


Figure 3. Observed data following oral (triangle) or intravenous (square) tracer and model (Fig 2)-calculated fits (lines). Data shown are one of seven studies from one of the subjects. Serum, saliva, urine calcium, feces and feces calcium. Cum, cumulative. Reprinted from Smith et al. [1999].

5. Conclusions

These studies analyzed changes in calcium metabolism in astronauts and cosmonauts after 3 mo in space. The results confirmed results of rate of bone loss from earlier missions of Whedon et al. [1977] and Rambaut and Johnson [1979] and in addition showed that the rate of recovery is slow. The current studies were limited by length of data collection. New studies planned for the International Space Station will extend the current studies by obtaining data earlier in the flight as well as for a longer period after tracer administration. These data will help to define the rate of change in metabolism during space flight.

To more fully define the changes in calcium metabolism during space flight it will be necessary to expand the current linear model, to a dynamic model that incorporates the regulatory mechanisms that are known to affect calcium metabolism (Fig 4). By integrating information on hormonal effects and biochemical changes a better understanding will be obtained on the sites and mechanisms where changes occur. This information will be useful for developing and testing countermeasures of bone loss in space and for diseases on Earth.

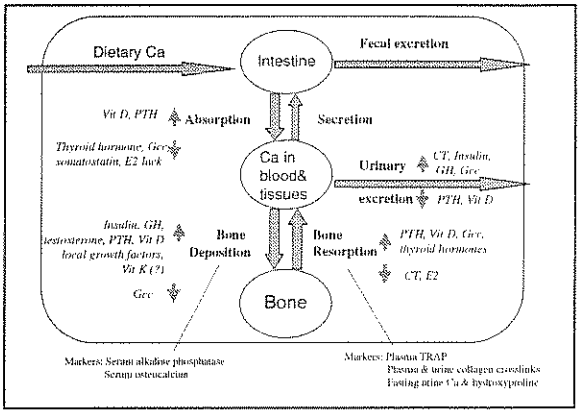


Figure 4. Processes of calcium absorption, deposition and resorption from bone, and excretion. Factors regulating calcium metabolism are shown next to the large arrows as increasing or decreasing the process (shown by smaller arrows). CT-calcitonin, E2-estrogen, Gcc-glucocorticoid hormones, GH-growth hormone, PTH-parathyroid hormone, Vit D-active vitamin D. Reference: Favus et al. [1993].

6. Acknowledgements

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7. References

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