

(NASA-CR-188435) EXPERIMENT 305:
PATHOPHYSIOLOGY OF MINERAL LOSS
DURING SPACE FLIGHT Final Report
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**Final Science Report
SLS-2****Experiment 305: Pathophysiology of Mineral Loss During Spaceflight****Claude D. Arnaud, M.D.
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San Francisco, CA 94143****Objectives**

The objective of this SLS-2 experiment was to determine the pathophysiology of mineral loss during space flight. This was to be accomplished by (1) determining the concentrations of blood minerals and of calciotropic hormones (parathyroid hormone-PTH, vitamin D metabolites) before, during, and after a 14 day shuttle flight, and (2) determining, by calcium kinetic analysis (using stable calcium isotopes), the influence of space flight on intestinal calcium absorption.

History of Project

Part (1) of this experiment was also performed on SLS-1. The results of that investigation showed a dramatic increase of 30-40% in serum ionized calcium by day 2 in space which persisted until day 8, with a rapid return to baseline values (partially by day R+1, completely by R+6). Serum PTH showed a normal adaptive response to this increased serum ionized calcium, with a rapid decrease of 20-30% by day 2 persisting through day 8 and a slower return up to normal levels by day R+6. $1,25(\text{OH})_2$ vitamin D levels were reduced by day 8 in flight as an expected response to the lowered serum PTH. When data for PTH (all values were within the normal range) and serum Ca^{++} were plotted against each other, the expected negative correlation was found, validating the results from a biological perspective. The magnitude of the increase in serum calcium was surprising, being at the low end of the range seen in hypercalcemia of malignancy, and at a value which would be expected to cause some symptoms such as nausea and general malaise. A second unexpected finding was the much tighter negative correlation between PTH and Ca^{++} in males compared to females, a difference which had not been noted before in the literature (but had also not been investigated). The results of this SLS-1 study were consistent with our hypothesis of a primary increase in bone resorption, or the release of calcium from the bone, due to exposure to space flight, with a consequent

normal adaptive response from the parathyroid glands reducing PTH secretion and a reduction in 1,25(OH)₂ vitamin D production by the kidney.

The experiment performed on SLS-2 included both the endocrine response and intestinal calcium absorption objectives. Due to some of the unexpected findings from SLS-1, special care was taken for SLS-2 samples and measurement procedures to ensure maximum confidence in the results.

Methodology

Sampling

Serum samples were obtained for the hormonal analyses at the prescribed times, to correspond with those obtained for SLS-1, and all samples were obtained. Oral and intravenous administration of the stable calcium isotope tracers for the calcium absorption studies were accomplished as planned. There were no apparent anomalies in tracer administration based on experiment logs, and checks of the used and spare injection kits confirmed this. Blood samples were obtained within minutes of the prescribed times for the tracer study. The UMS functioned as expected, and the data for urine volumes have been provided to E-305 from JSC with appropriate corrections for dilution and loss using the LiCl and UMS inflight calibration data. These data were required in order to make 24-hour urine pools for measuring collagen crosslink concentrations. Preflight hormone blood draws and the calcium absorption study starting at L-15 (actually L-19 because of the launch slip) went as planned, with all samples obtained. Postflight, the hormone blood draws and urine collections were done as planned. All samples were maintained frozen until analysis (in the case of the serum specimens) or until aliquoting for further analysis (urine).

Tracer Administration

⁴⁶Ca and ⁴⁸Ca, stable isotopes of calcium, were used as tracers in this study. The double-isotope method for determination of intestinal calcium absorption was used, where an oral tracer is given (usually with a small calcium load), followed some time later by an intravenous injection of the other tracer. The ratio of the oral to intravenous tracer in the blood is taken as the fraction of the calcium load absorbed. For this experiment, the double-isotope study was done three times, once preflight (scheduled for L-15, done at L-19) and twice inflight (FD3 and FD12). ⁴⁶Ca was used as the IV tracer (0.35 mg ⁴⁶Ca as CaCl₂ in 1 cc sterile saline), and ⁴⁸Ca was given as an oral dose (2.50 mg ⁴⁸Ca in a total of 20 mg elemental calcium as CaCO₃). ⁴⁸Ca was ingested on awakening, followed by the ⁴⁶Ca injection 60-70 minutes later. Blood samples (6 ml, in serum separator tubes) were obtained just before the ⁴⁶Ca injection and at 4 hours, 10 hours, and 24 hours post-⁴⁶Ca.

Mineral and Hormone Analysis

The measurement of Ca^{++} and PTH in serum specimens were done to confirm the results obtained from SLS-1. In addition, because the serum ionized calcium measurement is influenced by the pH of the sample, we also added a measurement of serum pH. In order to maximize compatibility with our SLS-1 measurements, we elected to obtain an ionized calcium analysis instrument from the same manufacturer as the one we used for SLS-1, but a newer model which could also measure blood pH, Mg^{++} , Na^+ , and K^+ . The instrument (Nova, Model 8) uses the same selective ion electrode technology for Ca^{++} measurement as in the older model. At the time we made the measurements for the SLS-2 samples, we arranged to have a service representative from the company present with us in the laboratory in case any problems arose. Careful analytical handling procedures were used, and samples were measured for each astronaut as a set to maximize the internal consistency within each data set. Prior to analysis of the samples from the astronauts, a set of experiments was done using fresh and frozen/thawed serum from normal subjects, using all the procedures planned for handling and analysis including NASA-provided blood drawing tubes, to optimize the techniques and obtain baseline data.

Ca^{++} and pH were measured in a single sample at the same time, and PTH was measured on the same day using an aliquot of the same sample. Serum tubes were thawed rapidly and placed on ice, and opened in a glove box in a nitrogen atmosphere to eliminate any possibility of CO_2 contamination. The serum was aspirated into a nitrogen-filled syringe which was then capped and allowed to come to room temperature (22°C). Each serum sample was then aspirated directly from the syringe into the ionized calcium analyzer through a stainless steel probe without exposure to air. Duplicate measurements were made, using 180λ each. Standards were measured routinely during the analysis. Intact PTH was measured by the same sensitive and specific two-site radioimmunoassay used for SLS-1 samples. The remaining serum from each tube was aliquoted and refrozen for analysis of the vitamin D metabolites.

Serum samples were also analyzed for osteocalcin, $25(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ as specified. Total calcium values were obtained from data sharing with E192. Urine deoxypyridinoline was measured with a commercial assay kit (Metra Biosystems, Mountain View, CA).

Calcium Isotope Ratio Analysis

Calcium was isolated from samples by precipitation with oxalate and washing in ammoniated oxalate buffer, followed by resolution with nitric acid, and final purification on an ion-exchange column and extraction as calcium rhenate, as preparation for analysis by thermal ionization mass spectrometry (TIMS). ^{46}Ca and ^{48}Ca concentrations in the samples were determined by

reference to the isotope ^{44}Ca . Precision of the measurements was 1.4% for $^{46}\text{Ca}/^{44}\text{Ca}$ and 0.067% for $^{48}\text{Ca}/^{44}\text{Ca}$, as determined on reference samples of NBS Standard Reference Material 915, Calcium Carbonate. ^{46}Ca and ^{48}Ca serum concentrations were then calculated with reference to the administered dose and expressed at each time point as percent administered dose per gram circulating calcium in the blood (%ID/gm Ca). Intestinal calcium absorption values were determined as the ratio of %ID $^{48}\text{Ca}/\text{gm Ca}$ to %ID $^{46}\text{Ca}/\text{gm Ca}$. Where possible, the values from the 4, 10 and 24 hour samples were averaged to determine an effective fractional calcium absorption (α) for each subject.

Additional Serum Analyses

Based on the results we obtained from analysis of the serum samples with the Nova, and the very low pH results we obtained in a number of the flight samples, we enlisted the aid of an expert in acid-base metabolism at UCSF, Dr. Anthony Sebastian, to discuss these results. At that time we had not analyzed for serum osteocalcin and other measurements we planned to make, so we still had the 6 ml draw serum samples frozen in our laboratory. In order to confirm the Ca^{++} and pH results we had obtained from the selective ion electrode method, we arranged with Dr. Sebastian to remeasure pH, as well as pO_2 and pCO_2 in these samples using a blood gas analyzer. While we know that measurement of blood gases on frozen samples can cause some systematic errors, the samples we had were drawn to fill the tubes, had never been opened, and were therefore essentially anaerobic. In addition, we expected that freezing of the samples would only drive off blood gases, and therefore could only increase serum pH if we lost CO_2 rather than decrease pH. These samples were analyzed at the General Clinical Research Center at UCSF, in Dr. Sebastian's laboratories. Samples were processed about 6 at a time, taken at random from the sample pool. They were thawed rapidly, and the serum was aspirated anaerobically from the flight vacutainer into a syringe using an 18 gauge spinal tap needle. During this process it was noted that some of the samples were under positive pressure in the vacutainer, and while this was not quantified directly (it was unexpected and we had no pressure measuring equipment available), note was made of the approximate volume of gas forced into the syringe when the needle pierced the top of the vacutainer. Serum samples were then injected directly into a blood gas analyzer for measurement of pH, pCO_2 , and pO_2 , and a second aliquot analyzed for total CO_2 content using a different method. Thus, at the end of the analysis, we had obtained serum pH and pCO_2 each by two independent methods.

Results

Based on our SLS-1 results, we expected to see an increase in serum Ca^{++} and a corresponding decrease in PTH in flight. Figure 1 shows the change in serum ionized calcium relative to baseline. Similar to the results we obtained

from SLS-1, there is a rapid and persistent elevation of the Ca^{++} . Figure 2 shows the change in serum iPTH, again showing the decrease during spaceflight in response to the increased serum calcium. Because the increase in Ca^{++} was so rapid and large, we also evaluated the data for total plasma calcium provided to us from E192 (we were unable to measure this in the serum because of the presence of fibrin due to the serum clotting procedure used for our samples). The data from E192 (Figure 3) also show a rise in total serum calcium (about 5% compared to the 20% increase in Ca^{++}), which also persists throughout the mission. Thus, the possible influence of serum pH on our Ca^{++} measurements (discussed below) may contribute to some of the increase we see, but the increase in total calcium seen in a completely different data set confirms that the PTH response we see is of biological significance. As we did in SLS-1, we did a correlation of serum iPTH vs Ca^{++} to document the expected negative correlation between these two parameters in the subject population. These results are shown in Figure 4, where this negative correlation was seen for 3 out of 4 subjects. Of note in this SLS-2 experiment, the preflight baseline values of PTH were lower in this population compared to the SLS-1 subjects, and this probably contributed to the smaller decrease inflight when compared to the SLS-1 results.

Figures 5 and 6 show the results for measurement of serum $25(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$. The level of $25(\text{OH})\text{D}_3$ increases during flight by 15-20%. There is no biological explanation for this to occur, as this metabolite is in excess in the serum as a substrate for $1,25(\text{OH})_2\text{D}_3$. However, the increase may be related simply to the decrease in plasma volume seen in the astronauts, because the $25(\text{OH})\text{D}_3$ concentration in the serum is not tightly regulated, so it would not be excreted preferentially. Normal preflight serum $1,25(\text{OH})_2\text{D}_3$ values are very consistent and show about a 30-40% decrease by FD6, similar to the decrease seen in SLS-1. This level persists until day 12, but appears to begin to recover toward the end of the flight. The slight increase on R+1 was also seen on SLS-1.

Serum osteocalcin concentration is shown in Figure 7. Values for this bone-specific protein decline rapidly in flight and remain depressed. Osteocalcin concentration in serum is often taken as an indicator of bone formation, so this could signify a decrease in bone formation during spaceflight. However, serum osteocalcin is also depressed by cortisol, so a stress response cannot be ruled out in spaceflight.

Urine deoxypyridinoline results are shown in Figure 8. The individual data are graphed to show the differences we noted in 3 subjects compared to the fourth. During the preflight and inflight periods, there are peaks in DPD excretion which correspond to days when E192 experiments were done. It is not clear what factors in that experiment could have increased DPD excretion. However, when these high data points are eliminated and the data reanalyzed,

we see the expected increase in DPD with flight (Figure 9). The increase is about 40%, climbing rapidly from day 4 to day 8 and remaining at that level throughout the flight and up to 2 weeks postflight. This is the first evidence that there is a dramatic increase in bone resorption in humans during spaceflight, and supports our original hypothesis for this experiment.

Fractional intestinal calcium absorption was measured as the ratio of specific activity of orally-administered ^{48}Ca (in a 20 mg Ca load, fasting) to that of intravenously administered ^{46}Ca , given about 1 hour later. The preflight study was done on L-19, and the inflight studies done on FD3 and FD12. In two samples (one preflight, one inflight) the isotopic ratio of ^{46}Ca was inordinately high, suggesting contamination of the blood sample from residual isotope at the injection site, and these samples were eliminated from the analysis. On FD3, one crewmember had unmeasurable concentrations of ^{48}Ca from the oral dose, even though the ^{46}Ca injection was done. While confirmation of the ^{46}Ca tracer injections was possible because the used injectors were returned, the package with the ^{48}Ca capsules was not returned so no confirmation could be made that the crewmember took the capsule. The sensitivity of the ^{48}Ca analysis is such that if there was any absorption (even as low as 1-2%) it would be seen. Thus, it was assumed that the crewmember did not take the dose, and these data were eliminated from the analysis.

The data for fractional absorption (α) are shown in Figure 10. The preflight mean of 0.58 is higher than the normal true fractional absorption of calcium (0.3-0.5), but it is expected because of the design of the study, giving a small calcium load orally in the fasted condition. By FD3, the absorption fraction had decreased to about 0.4 (a 33% decrease), and the 3 crew for which data were available all showed about the same response. On FD12, two crewmembers showed one pattern, a very low fractional absorption, while the other two crew had a value higher than preflight. On further inspection of the data, it was found that the male crew had the high values and the female crew the lower values, so these results were plotted separately on Figure 10. A representative ^{46}Ca specific activity curve is shown in Figure 11, where the peak values preflight and for the two inflight studies represent the 4 hour data points. There is a suggestion of an increase in peak specific activity during flight, which may indicate a decrease in the size of the rapidly exchangeable calcium pool (blood, soft tissues).

Our primary serum analyses had identified an unexplained decrease in serum pH which could affect our measurement of serum Ca^{++} . Therefore, we undertook the additional analyses outlined previously, with the aid of Dr. A. Sebastian at UCSF. The results of measurement of blood CO_2 are shown in Figure 12, and pH in Figure 13. These data confirm that the decrease in serum pH seen with the Nova specific ion Ca^{++}/pH analyzer was due to an increase in the CO_2 content in the blood, and not another factor such as lactic acid. This decreased pH was only seen in flight samples. We have been unable to reproduce this

and using flight equipment to process the blood samples using the identical protocol used in flight. In addition, the preflight and postflight samples from the astronauts were processed using the flight protocol (clotting in the refrigerator, centrifuging in a non-refrigerated centrifuge, and immediately freezing the whole tube), and did not show this increase in CO₂. We have thus been unable to come up with a technical explanation for the increased CO₂ in our frozen blood samples. Some hypotheses for a physiological explanation will be considered in the discussion.

Discussion

The results we have obtained from SLS-1 and SLS-2 are consistent with our original hypothesis that increased release of calcium from bone would cause an increase in serum ionized calcium, and a subsequent adaptive decrease in the secretion of parathyroid hormone into the blood. Our results from SLS-1 were somewhat puzzling in that we did not expect to see such a rapid rise of Ca⁺⁺, nor one of such magnitude; instead we expected that the rise would be more gradual with an increase in osteoclastic recruitment leading to the increased resorption within a few days to a week of microgravity. We thus hypothesized that some other mechanism, such as an opening of the bone-blood barrier, could be leading to the early dramatic increase in Ca⁺⁺. Our observations from SLS-2 provide another possible mechanism for this increase, a direct effect of reduced serum pH on the equilibrium between free and protein-bound calcium. This effect is clearly due to an increase in the serum CO₂ in the samples we received from flight. We cannot rule out a technical source for this increased CO₂, but we cannot reproduce it in the laboratory on earth. Independent of any possible influences of pH on Ca⁺⁺, data from E192 show that serum total calcium is also increased, which supports our results showing an adaptive decrease in PTH during flight.

Our initial hypothesis that bone resorption increases early in flight and causes a rise in serum calcium is directly supported by the urine DPD results. By FD5, the output of this collagen crosslink starts to increase, to a new plateau value about 40% above baseline. This is consistent with the time needed to recruit new osteoclasts from the bone marrow and increase the number of bone resorbing sites. The fact that serum calcium rises earlier, with an adaptive decrease in PTH by day 2 means that there may be other factors influencing serum Ca⁺⁺, independent of the increase in bone resorption. The existence of an early, alternative mechanism to increase serum Ca⁺⁺ in no way diminishes the role of increased bone resorption a few days into flight, and the possible use of antiresorptive drugs in long-term flights is still a valid consideration. However, because the increase in blood calcium occurs earlier and sets off the adaptive hormonal regulatory processes, it is important to identify this other mechanism as well. Serum osteocalcin, generally considered to be a marker for bone formation, is decreased early in flight. If it is true that

bone formation, or mineralization, is decreased, then this could be another mechanism contributing to the increased serum calcium as its normal movement into bone from the serum is reduced.

The endocrine regulation of calcium metabolism in spaceflight appears to occur normally. PTH is decreased in response to the increased serum calcium, and a decrease in $1,25(\text{OH})_2\text{D}_3$ presumably secondary to decreased PTH occurs by day 6 and persists for the length of the mission. There is the expected decrease in intestinal calcium absorption, although it was not expected to decrease as early as FD3-4. In addition, the increase in calcium absorption seen in the two male crew by FD12 was unexpected, especially because there was no correlation for the crew as a whole between serum $1,25(\text{OH})_2\text{D}_3$ and the fractional calcium absorption. It is possible that other mechanisms influence calcium absorption as well, such as changes in intestinal motility due to the lack of gravity.

The gender differences noted in SLS-1 and now in SLS-2 also deserve further investigation. With the expected negative correlation between PTH and Ca^{++} only clearly apparent in males, and a significant difference in intestinal absorption late in the flight, there appear to be factors other than the ones we associate classically with calcium and bone homeostasis. The sex hormones are a clear starting point, as might be responses to stress.

The increased CO_2 we found in our blood samples from flight is puzzling, because we cannot reproduce it in the laboratory. While we cannot rule out a technical explanation, we may also consider some physiological explanations why this may occur. First, it is known that in microgravity there is increased blood in the periphery, and it is possible that there is considerable stasis in the capillary bed. If this was the case, the residence time of blood in the capillaries could be increased, allowing more CO_2 to diffuse into the blood and decreasing its pH. Because we obtain blood from venous puncture, it may be the case that this blood contains more CO_2 than central blood because of its increased capillary residence time. It may not show up in increased lung output of CO_2 because the peripheral blood is a small fraction of the total circulating blood, and this fraction may even decrease in space. A second possibility may be that the red cells are capable of carrying more CO_2 while in microgravity, so that even if the central blood contained more CO_2 it would not necessarily be transported across the lung membranes and exhaled. Both these hypotheses could be tested in microgravity. Before doing so, however, all technical explanations should be ruled out, and this necessitates measuring the blood gases immediately after obtaining a blood sample in space using a blood gas analyzer. Portable units are available and should be able to be flight qualified for this purpose.

Summary

Our hypothesis that increased bone resorption with exposure to microgravity causes an increase in serum calcium and that the body will try to return the serum calcium to normal through adaptive processes is supported by our results from the SLS-1 and SLS-2 missions. Increased serum calcium, decreased serum PTH, a later decrease in serum $1,25(\text{OH})_2\text{D}_3$, and decreased intestinal calcium absorption all occur. Urine deoxypyridinoline rises after 4 days of flight and remains elevated, consistent with recruitment of osteoclasts and the formation of new bone resorbing sites. Serum osteocalcin is decreased, a possible indicator of decreased bone formation.

The magnitude and timing of the increase in serum Ca^{++} indicate that processes other than bone resorption also influence the serum calcium. The rise in serum calcium occurs by day 2 along with the adaptive decrease in serum PTH, while the evidence for bone resorption does not come until several days later. This could be due to a direct release of calcium from the bone fluid compartment, or from other stores of rapidly exchangeable calcium in the body (eg mitochondria); it could also be influenced by a decrease in the rate of mineralization of bone while resorption and intestinal absorption are still at normal levels.

Gender differences in serum calcium regulation occur in spaceflight, and these should be considered further in the design and implementation of further studies of calcium and bone metabolism in spaceflight.

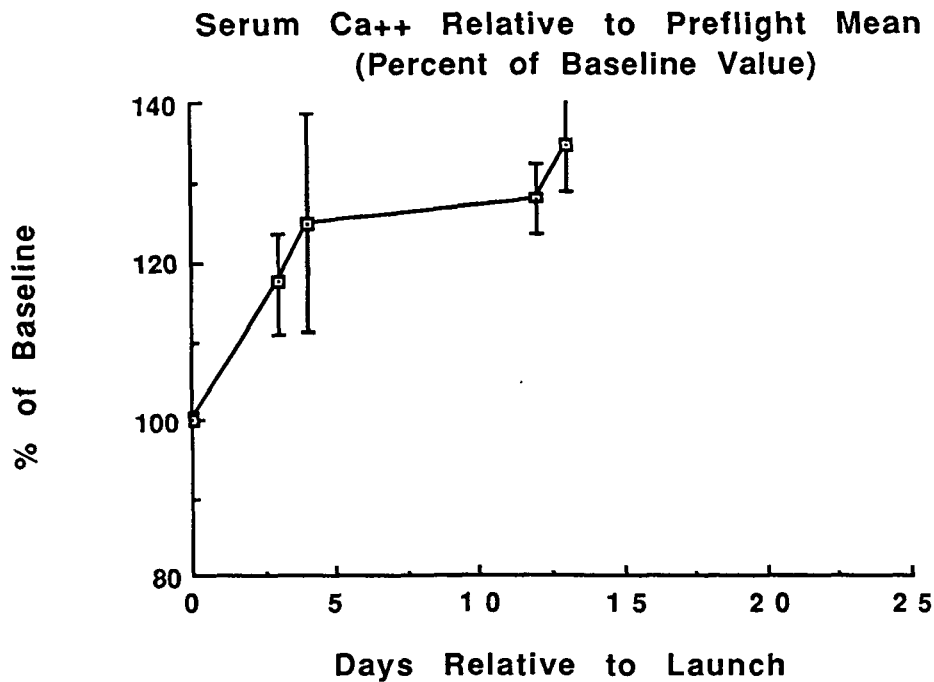


Figure 1. The rise in serum Ca⁺⁺ relative to baseline values is about 25% and persists throughout the flight. This is virtually identical to the results seen for SLS-1.

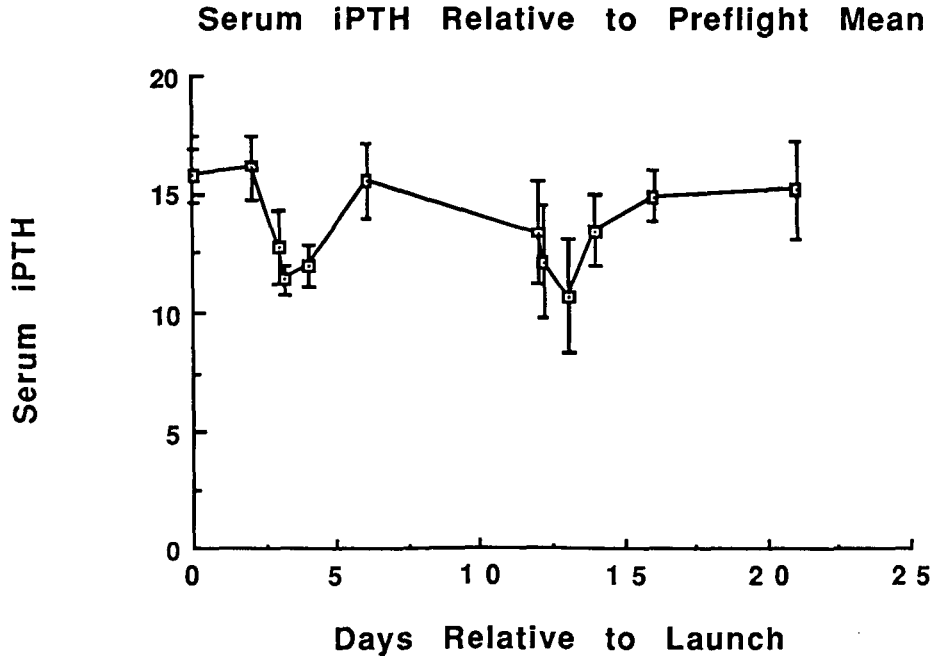


Figure 2. Depression of serum iPTH occurs early in flight and persists. Rise on FD6 is unexplained, but it should be noted that the baseline values for this population are at the low end of the normal range, so the result may be related simply to the variation in the data.

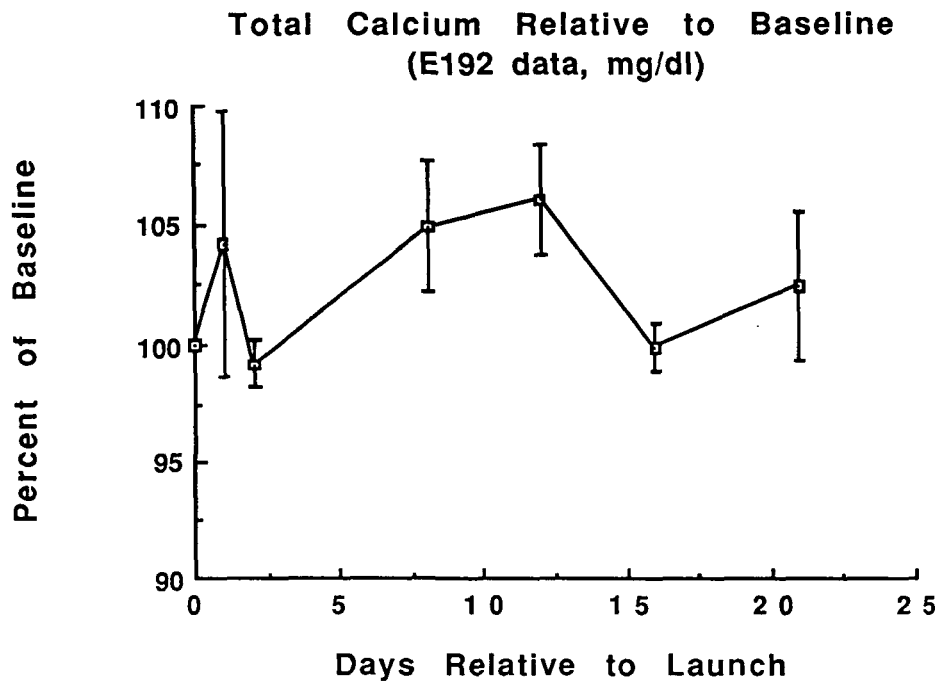


Figure 3. Results from E192 data sharing show a rise in total serum calcium after the first couple of days which persists throughout the mission, and returning back to normal quickly upon recovery.

Correlations for PTH vs Ca⁺⁺
Individual Crewmembers

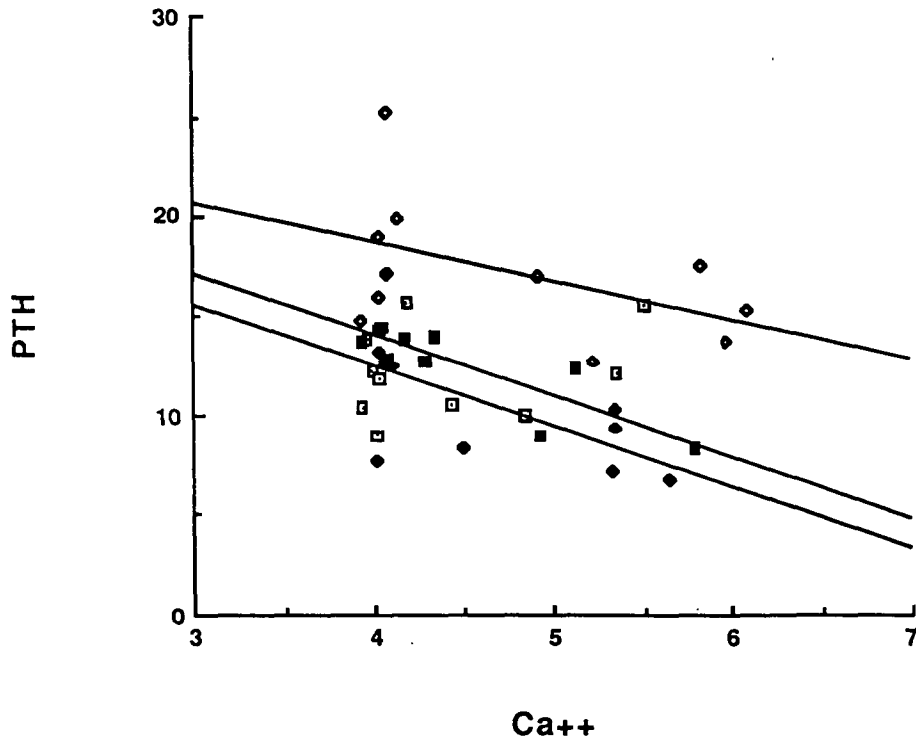


Figure 4. The individual correlations between the serum Ca⁺⁺ and PTH within the normal range for the individual crew are shown. The correlations were significant for 3 of the 4 crew, similar to the results seen on SLS-1. These correlations indicate that the results seen for Ca⁺⁺ are not an artifact, otherwise the normal depression of PTH should not occur.

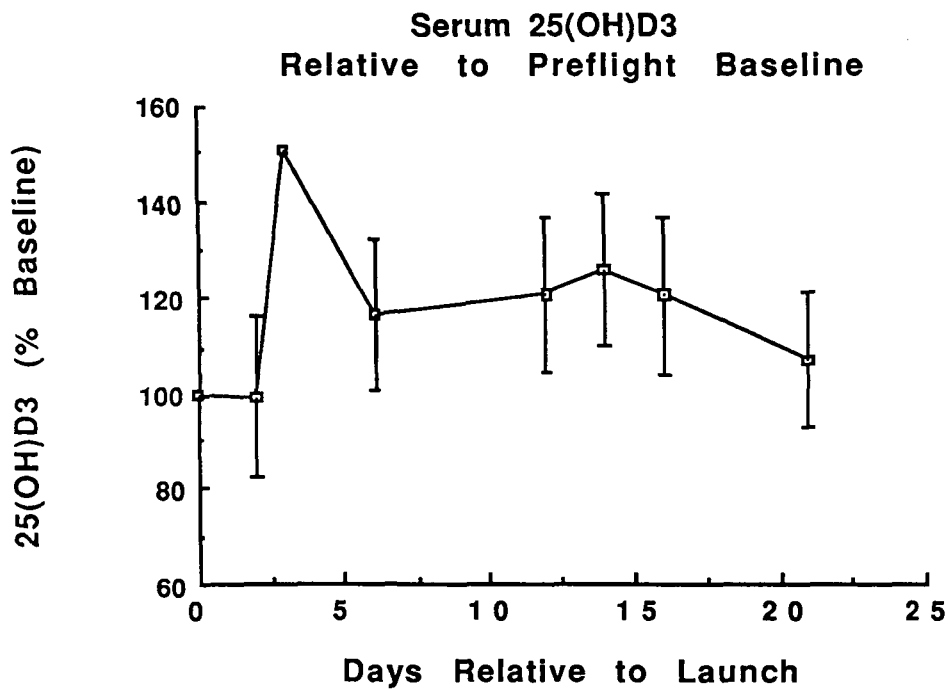


Figure 5. Change in serum 25-hydroxyvitamin-D₃ with spaceflight. The 15-20% rise may be related to reductions in plasma volume.

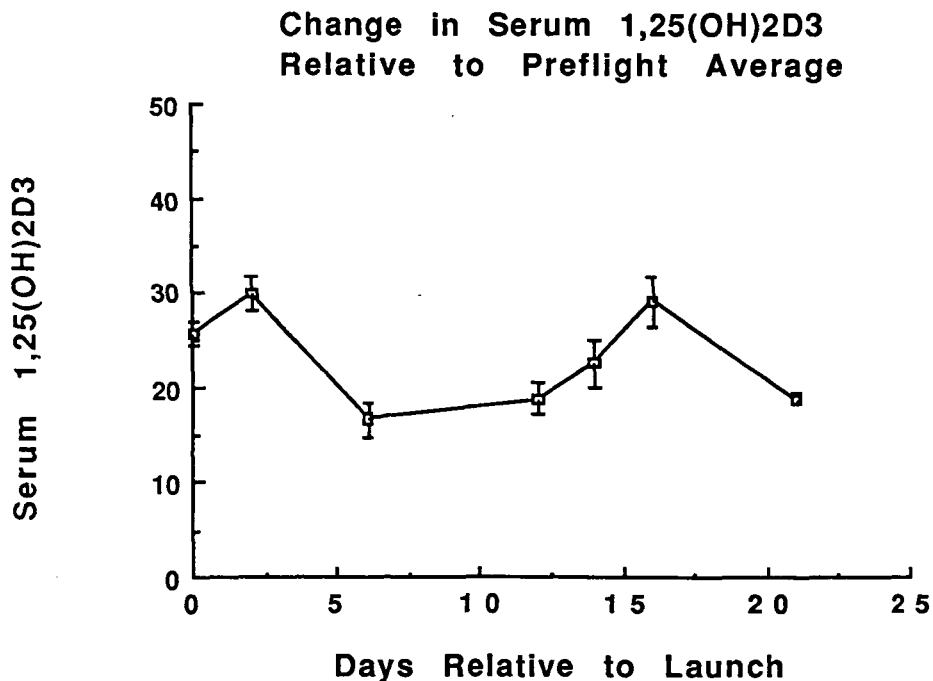


Figure 6. Serum 1,25-dihydroxyvitamin-D₃ relative to preflight average values (pg/ml). Normal preflight values are very consistent and show about a 30-40% decrease by FD6, similar to the decrease seen in SLS-1. This level persists until day 12, but appears to begin to recover toward the end of the flight. The slight increase on R+1 was also seen on SLS-1.

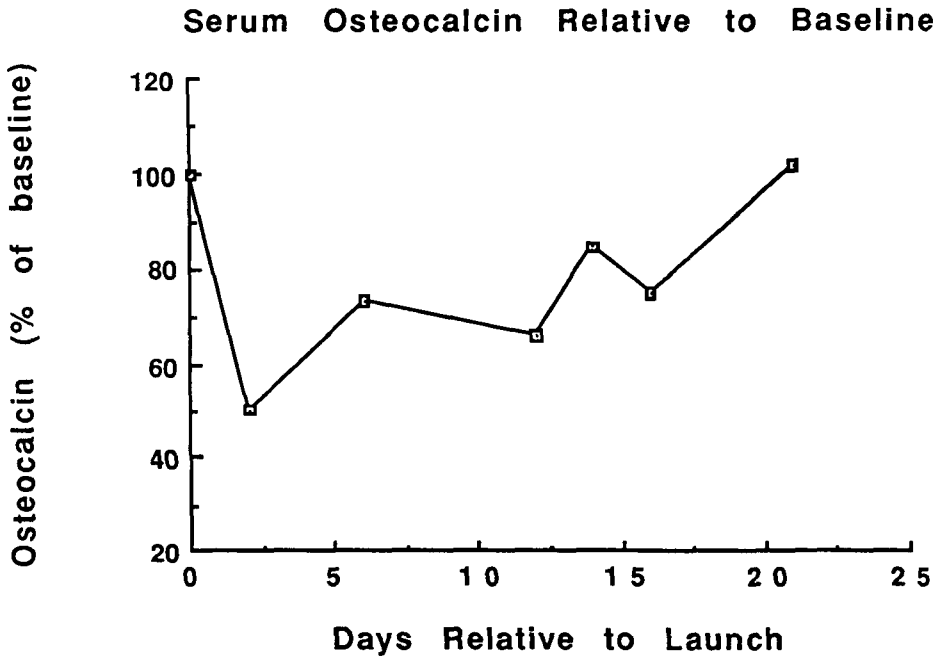


Figure 7. Serum osteocalcin relative to baseline values. Decrease by FD2 persists throughout flight, indicating possible decrease in bone formation.

Individual data for Urine DPD
Preflight, Inflight, Postflight

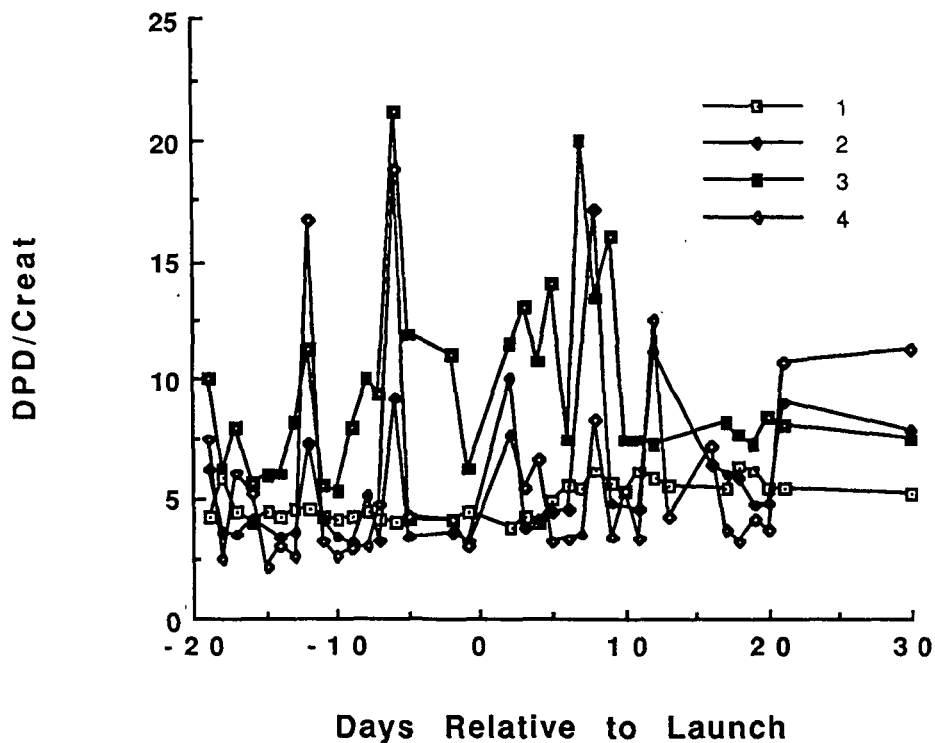


Figure 8. Individual data for urinary deoxyypyridinoline. Note that in 3 subjects there are peaks in the data on the days when other experiments were being done for E192.

Urine Deoxypyridinoline
Preflight, Inflight, Postflight

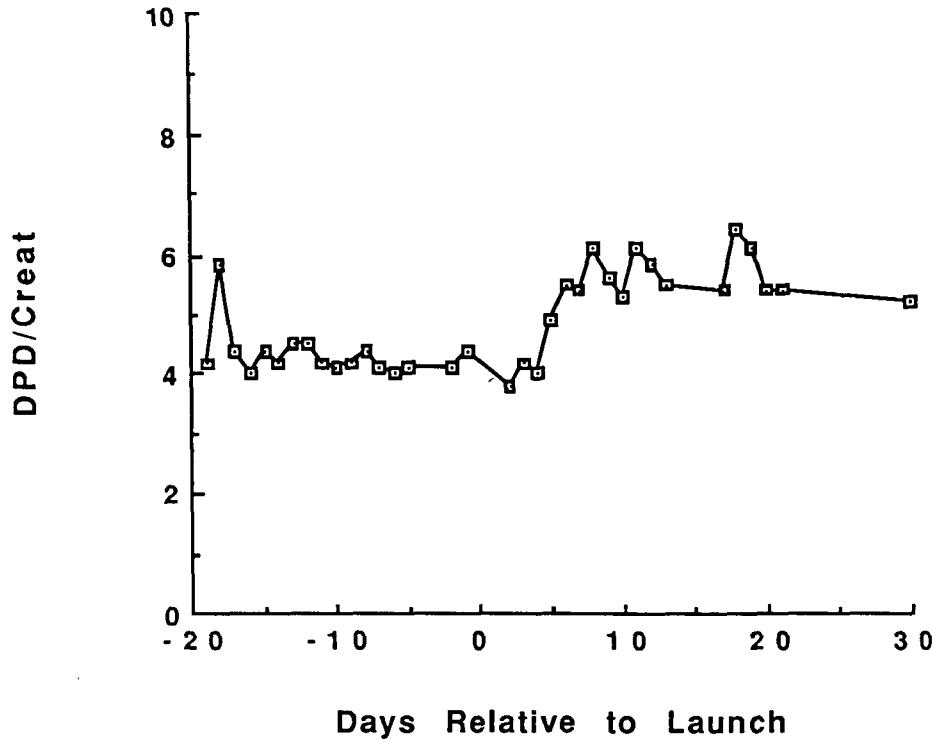


Figure 9. Urine deoxypyridinoline in one crewmember. The preflight baseline is stable. Within 5 days after launch the value increases about 40% and remains at this level throughout the flight and up to 2 weeks postflight.

Fractional Calcium Absorption $^{48}\text{Ca}/^{46}\text{Ca}$
Preflight, FD3, FD12

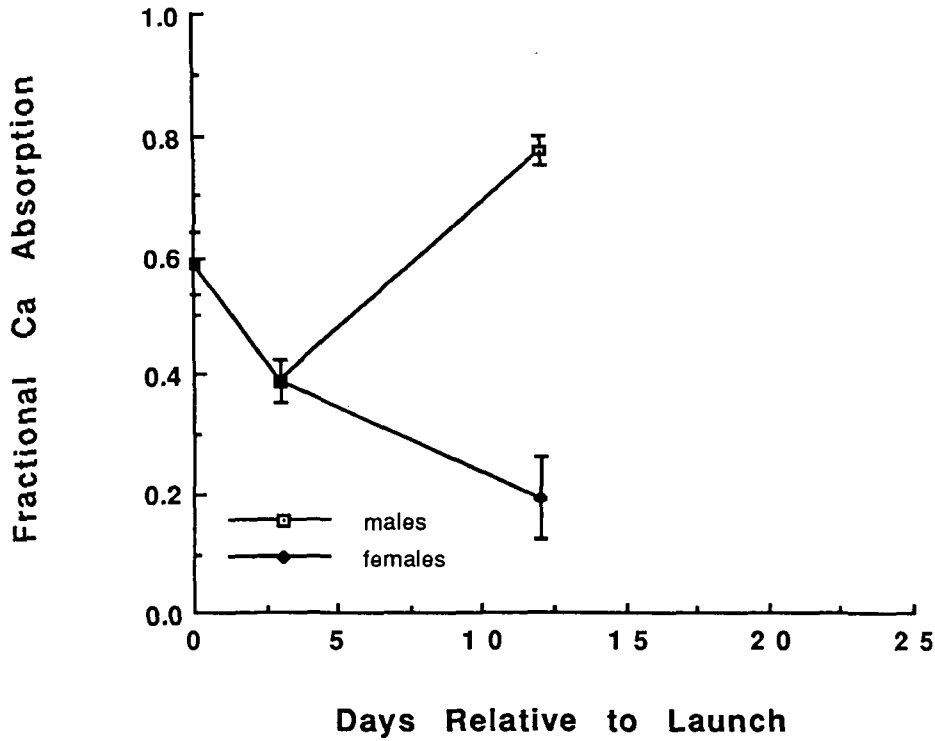


Figure 10. Fractional calcium absorption preflight and on FD3 and FD12. Data for FD12 are divided into male and female crew because of large differences noted. No such differences were noted preflight or on FD3.

**Specific Activity of ^{46}Ca After Injection
Preflight, FD3, FD12 Studies in 1 Subject**

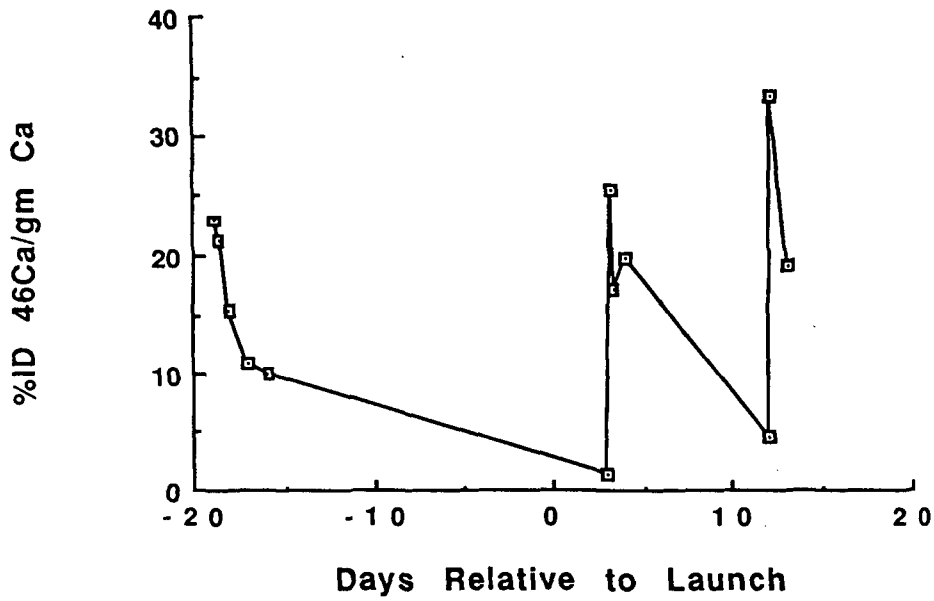


Figure 11. Specific activity for ^{46}Ca following intravenous administration. Data shown from one subject for illustration. Apparent increase in peak values could be related to a shrinking of the miscible calcium pool (serum, soft tissues).

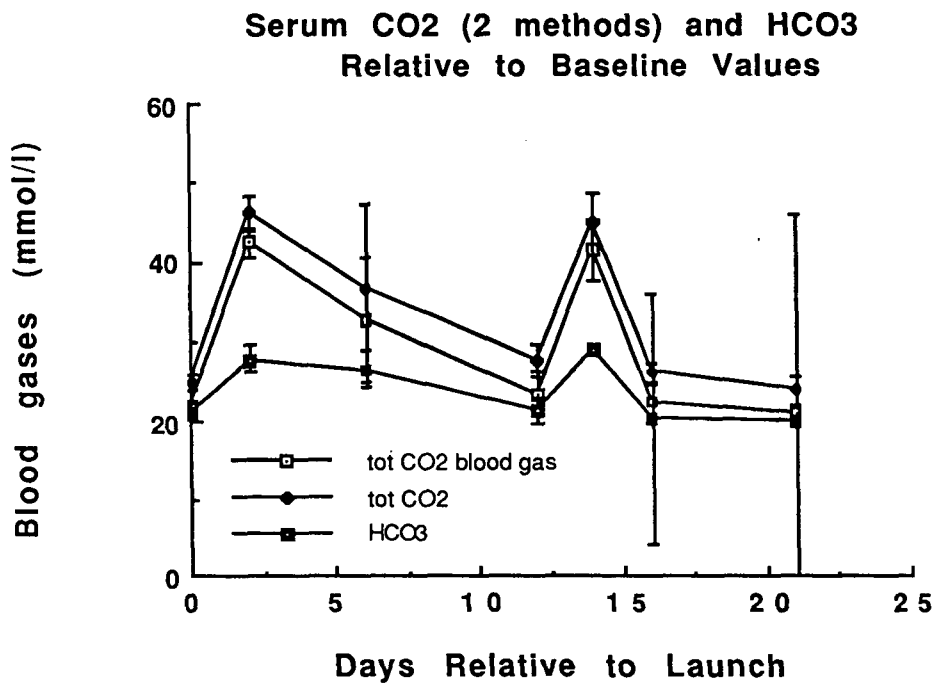


Figure 12. Blood CO₂ measurements relative to preflight values. Virtually all flight samples have elevated concentration of CO₂, as determined by two different methods. This corresponds to the lowered pH seen in these samples.

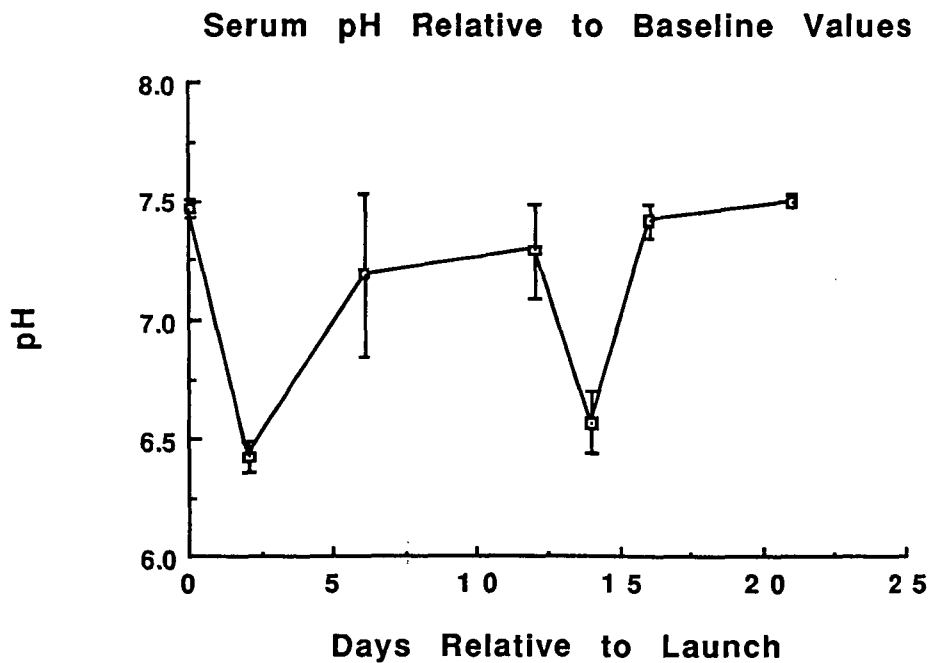


Figure 13. Serum pH, as measured by blood gas analyzer system, confirming results obtained using the Nova specific ion Ca⁺⁺/pH measurement system. Very low values could not be reproduced in the laboratory even under identical blood processing and handling conditions.

Supplemental Report - SLS-1 Experiment 305
"Pathophysiology of Mineral Loss During Space Flight"
Claude D. Arnaud, M.D. and Christopher E. Cann, Ph.D.
Co-Principal Investigators

This report supplements the final report submitted previously for E305 for the SLS-1 mission. It contains the previously reported material, but in addition it contains results from the analysis of urine obtained by special request, provided by Dr. Carolyn Huntoon from residual samples from E192. The methodology, results, and discussion sections have been revised to include these analyses in the overall mission results.

Objective

The objective of this SLS-1 experiment was to determine the pathophysiology of mineral loss during space flight. This was to be accomplished by (1) determining the concentrations of blood minerals and of calciotropic hormones (parathyroid hormone, vitamin D metabolites) before, during and after a 7-10 day shuttle flight and (2) determining, by calcium kinetic analysis (using stable calcium isotopes), the influence of space flight on calcium absorption and bone turnover.

History of Project

Due to priority considerations, part (2) of the experimental package was deleted from the composite of SLS-1 experiments, leaving part (1) which involved simple blood sampling at L-15, L-7, L-2, FD 2, FD 8, R+1 and R+6. Both parts (1) and (2) will be performed on SLS-2.

At this writing, all serum collected during SLS-1 for E305 has been consumed in the measurement of ionized calcium, immunoreactive intact parathyroid hormone, 25 hydroxyvitamin D and 1,25 dihydroxyvitamin D.

In order to further interpret the preliminary results we found in the serum samples (detailed below), we requested and obtained small amounts of urine which had been collected for other experiments (E192) and for which all other analyses had been completed. The volumes of urine obtained were small, and samples were not available for all time points; however, enough samples were analyzed to provide useful information to help in the interpretation of the serum results we obtained.

Methodology

Serum ionized calcium was measured by selective ion electrode, intact immunoreactive parathyroid hormone (intact PTH) by a sensitive and specific two-site radioimmuno-metric assay, and 25 hydroxyvitamin D and 1,25 hydroxyvitamin D by receptor binding assays. The full complement of 28 serum intact PTH measurements were performed but the volumes remaining were inadequate to measure ionized calcium in two serum samples (MS3 for R+1 and PS2 for FD 8), 25 hydroxyvitamin D in two serum samples (MS1 for L-2 and FD 8); and 1,25 dihydroxyvitamin D in one serum sample (MS1 for R+1). In general, the consistency with which blood was collected was surprisingly good, but the quantity of serum recovered was considerably less than the 2.0 ml that was anticipated, i.e. approximately 1.5 ml in most cases.

The volume of most urine samples obtained was small (100-200 μ l), so the analyses we could do were limited. Our primary goal was to determine the concentrations of the collagen crosslinks pyridinoline (PYD) and deoxypyridinoline (DPD) released during bone resorption. With the urine volumes available, we were only able to measure the concentrations of PYD, using a specific immunometric assay. Samples were obtained and analyzed from individual urine voids, from preflight, inflight, and postflight voids. 80-100 samples per crewmember were obtained. Where possible, 24-hour analyses were done by mathematically pooling the results from the individual voids.

Results

We expected that among the astronaut crew the biologically normal negative correlation between serum ionized calcium and intact PTH would be exhibited because we expected that any changes due to space flight would be in the normal physiological range of responses. We therefore performed a statistical analysis of the relationship of these variables across all measurements in all subjects. The correlation coefficient for this relationship was negative (0.52) and significant ($p < 0.01$) as expected (Figure 1). Based on this analysis, we concluded that the responses of the crew to perturbations in extracellular calcium homeostasis reflected appropriate physiologic adaptive responses.

Serum ionized calcium increased dramatically on FD 2 to levels 40% above control, levels that are normally considered to be representative of severe hypercalcemia (Figure 2). By FD 8, serum ionized calcium levels were still 35% above normal, indicating that clinically significant hypercalcemia was maintained throughout the flight. Mean serum intact

PTH declined appropriately to about 50% of control throughout the flight, a finding that biologically validates the increase in serum ionized calcium and discounts the possibility that PTH is responsible for the hypercalcemia. Serum calcium returned toward control values on R+1 and was no different from control on R+6. Serum intact PTH returned more slowly to control values.

Serum 1,25 dihydroxyvitamin D (Figure 2) was within the range of control on FD 2 but had declined by 40% on FD 8. Postflight, 1,25 dihydroxyvitamin D values increased to 25% above control on R+1 and then decreased to control values by R+6. No changes were observed in serum 25 hydroxyvitamin D levels inflight or postflight.

We sought cause for the relatively low correlation coefficient between serum ionized calcium and intact PTH by examining the correlation coefficients for these variables in individual subjects (Figure 3). MS1 and PS1 fit one pattern with a correlation coefficient of 0.8, while MS3 and PS2 exhibited a much lower correlation (0.3) (Figure 4). On decoding the data, we found that the male crew members showed the highest correlation (Figure 5). This is an unexpected finding that deserves further investigation, especially relative to the possible influence of reproductive hormones on calcium metabolism in space flight. Preliminary assessment of sex differences in the responses of the vitamin D metabolites show no correlations.

Results for the analyses of PYD in urine were mixed, with some crew showing the expected increase of PYD in urine from increased bone resorption and others not. This also appeared to be related to gender. Results from one female and one male crewmember are shown in Figure 6 for morning fasted values and in Figure 7 for total daily output. Figure 8 shows the comparison of the first morning urine void and the total 24 hour PYD output relative to urinary creatinine. During flight, the normal nocturnal increase in PYD is suppressed.

Discussion

The data we report herein support our hypothesis concerning the bone and mineral abnormalities caused by space flight. The data suggest that our measurements are biologically valid, that they qualitatively parallel those observed in immobilization osteoporosis except that hypercalcemia is more severe during acute microgravity exposure, and that they are consistent with increased bone resorption playing a major role in the pathogenesis of mineral loss during space flight. Based on

these results, anti-resorptive drugs could be useful in preventing the bone loss of space flight.

The evidence from these data that exposure to microgravity causes increased bone resorption with consequent hypercalcemia and decreased serum is suggestive. The magnitude of the increase in serum ionized calcium in the presence of decreased 1,25-dihydroxyvitamin D suggests that the calcium is being released from bone rather than coming from an increase in intestinal calcium absorption. We expect to test this directly with experiments on SLS-2. However, because we were unable to measure total serum calcium in these samples (due to low volumes and the presence of fibrin in the serum), and the evidence from other experiments (E192) for an increase in total calcium is limited, we cannot rule out that the increase in ionized calcium is due to a change in the free to bound fraction of calcium in the serum. This could be due to a change in serum protein concentration or a change in serum pH altering the free to bound ratio.

The increase in total urinary PYD during flight and the recovery period is consistent with the hypothesis that there is increased osteoclastic resorption during spaceflight which persists after return to 1-g. When normalized to urinary creatinine, the 24 hour PYD output also rises inflight in females, similar to the increase in total PYD seen in males, although the gender differences, similar to those seen in the serum results, need to be clarified. In terrestrial clinical studies, the first morning urine PYD is higher because of the nocturnal increase in bone resorption, and so this void is often used for routine clinical diagnosis because of greater measurement sensitivity and ease of collection. We expected to see this increase in these studies, and during the preflight period this was the case, with a 65% increase in the first morning samples. This appeared to be the case for the postflight samples as well, but the number of postflight samples available was small. Inflight, this nocturnal increase was eliminated, with AM concentrations $98 \pm 1.5\%$ of 24 hour levels. This was an unexpected finding, and clearly warrants further study in the context of the effects of spaceflight on diurnal variations in bone turnover.

All our results and interpretation of the PYD analyses must be done in the context of possible nonspecificity. Pyridinoline crosslinks are produced when collagen is formed, and they are released and excreted when collagen is broken down. In bone, this occurs during osteoclastic resorption of the bone matrix. For routine clinical studies, PYD excretion is a relatively sensitive marker for bone turnover, because any changes in

PYD excretion are normally due to changes in bone resorption. In spaceflight, however, there is also breakdown of collagen from other sources such as muscle, and the elevated PYD in the urine may come partly from these other sources. Deoxypyridinoline is specific for bone collagen, but the volumes of urine available to us did not permit DPD analysis. We expect to do this with urine samples from SLS-2 to confirm our PYD findings from SLS-1.

Summary

The results of this study of calcium regulation and bone metabolism during SLS-1 are consistent with our hypothesis that spaceflight causes a primary increase in bone resorption, leading to normal adaptive hormonal responses to reduce the increased serum calcium. These adaptive responses are not pathologic, but can be predicted based on our model of calcium homeostasis. We saw an early and sustained increase in serum ionized calcium with a rapid decrease in serum parathyroid hormone in response. Both these parameters normalized upon return to 1-g, with the PTH response lagging the ionized calcium. 1,25-dihydroxyvitamin D concentrations were reduced by 6 days into flight, probably from reduced synthesis due to the reduced serum PTH, an effect which should reduce intestinal calcium absorption as part of the adaptive response to reduce serum calcium. This parameter also normalized on return to 1-g. Urinary pyridinoline output increased during spaceflight, most likely due to an increase in bone resorption. These values did not return to normal by day 6 postflight, but limited data are available.

Several findings were unexpected.

The magnitude of the increase in serum ionized calcium was close to that seen in hypercalcemia of malignancy, but apparently was not accompanied by a similar increase in total serum calcium (based on data from E192). This suggests that factors other than simple bone resorption may come into play, such as variations in serum proteins or serum pH which will affect the ratio of free to bound calcium in the blood.

As a group, the blood samples from the crew showed the expected negative correlation between serum ionized calcium and PTH, with a correlation coefficient of about -0.5. This is similar to that seen in other population studies and validated the measurements as being biologically appropriate. Further inspection of these data, however, revealed individual differences, and these were traced to a gender difference; males had a correlation of about -0.8 between PTH and Ca^{++} , while there was no

correlation for females. This unexpected difference may be due to the influence of sex steroids, but this could not be tested with the small volumes of serum available.

Gender differences were also noted in the results for urinary PYD, with both sexes showing a rise with spaceflight, but which was only apparent in females when data were normalized to urine creatinine. However, the most striking finding in the PYD results was the suppression of the normal nocturnal rise in PYD excretion, from the normal 65% excess relative to creatinine to no excess. This suggests that spaceflight may affect the diurnal variations in bone turnover.

While in general the results of this study support our hypothesis of a primary increase in bone resorption during spaceflight, the unexpected findings suggest that this may not be the only process affecting calcium and bone metabolism in microgravity. The gender differences suggest that sex steroids may have a significant influence, not unexpected given the existence of such an influence on earth, and this can be investigated further with appropriate attention to these hormones. Clearly the disproportionate magnitude of the ionized calcium increase needs to be investigated, with special attention to those other moieties known to affect it. The suppression of the nocturnal elevation of PYD excretion must be confirmed, preferably using the more specific bone marker DPD, and hypotheses put forth to try to explain this. We will attempt to explore these unexpected findings during SLS-2 as possible within the constraints of that experiment.

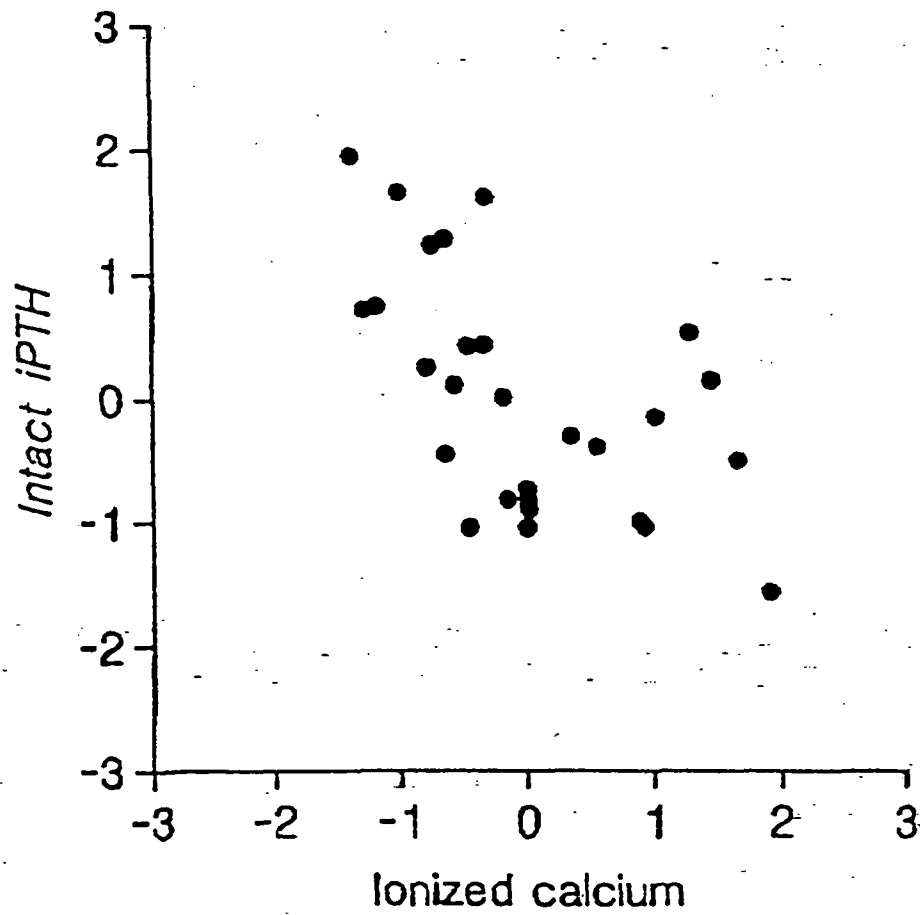


FIGURE 1. Relationship Between Serum Ionized Calcium and Intact iPTH

(All Values)

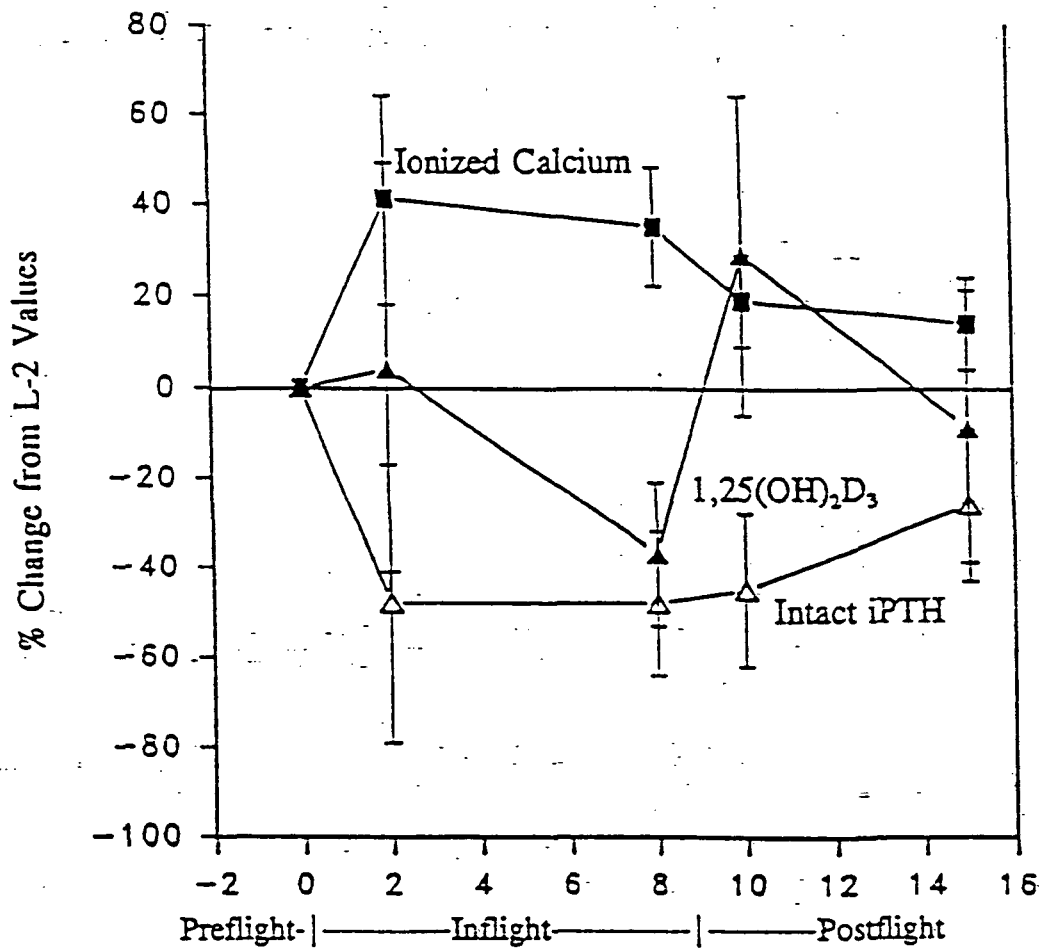


FIGURE 2. The Effect of Microgravity on Serum Ionized Calcium, Intact iPTH and 1,25 (OH)₂D₃.

(Values are mean ± S. D.)

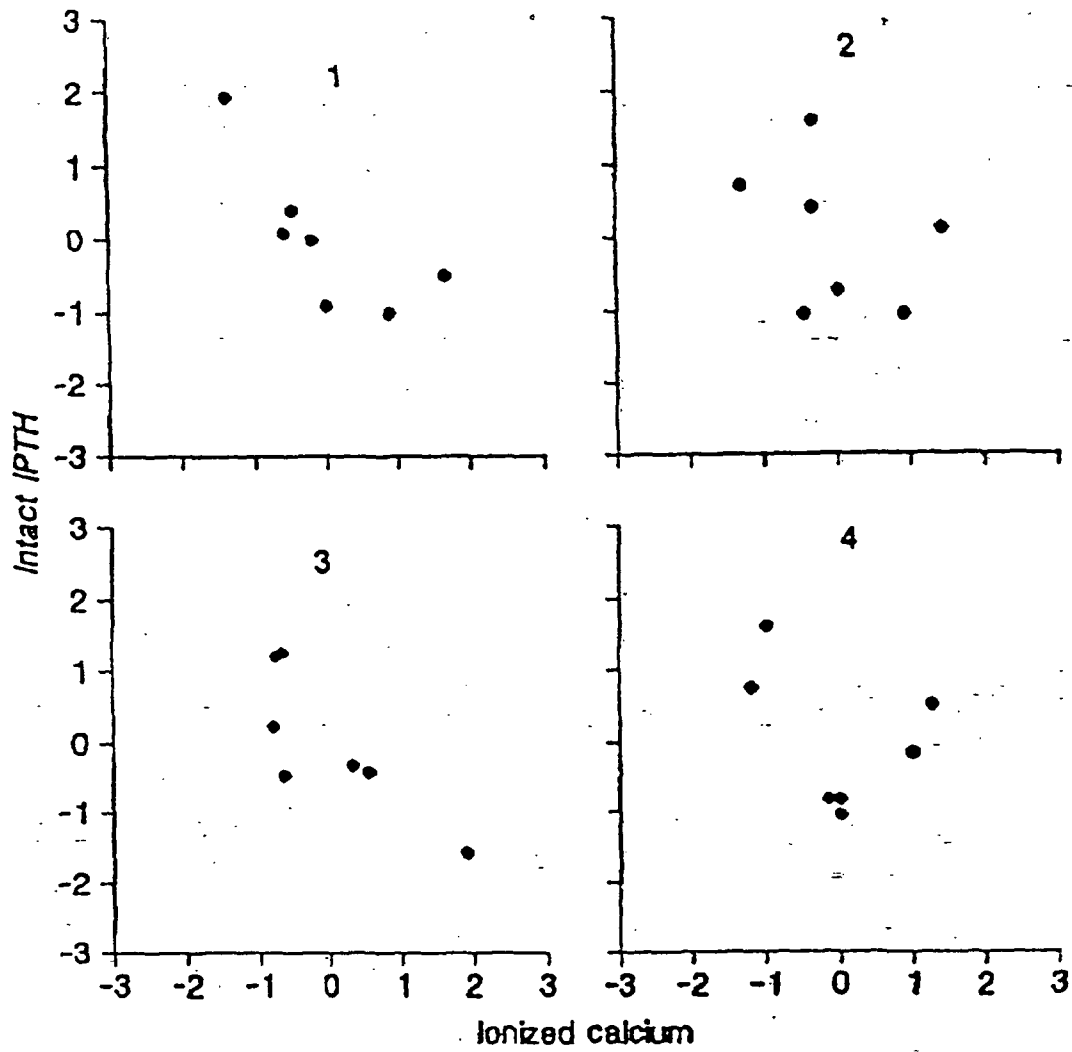


FIGURE 3. Relationship between Serum Ionized Calcium and Intact iPTH
(Individual Crewmembers)

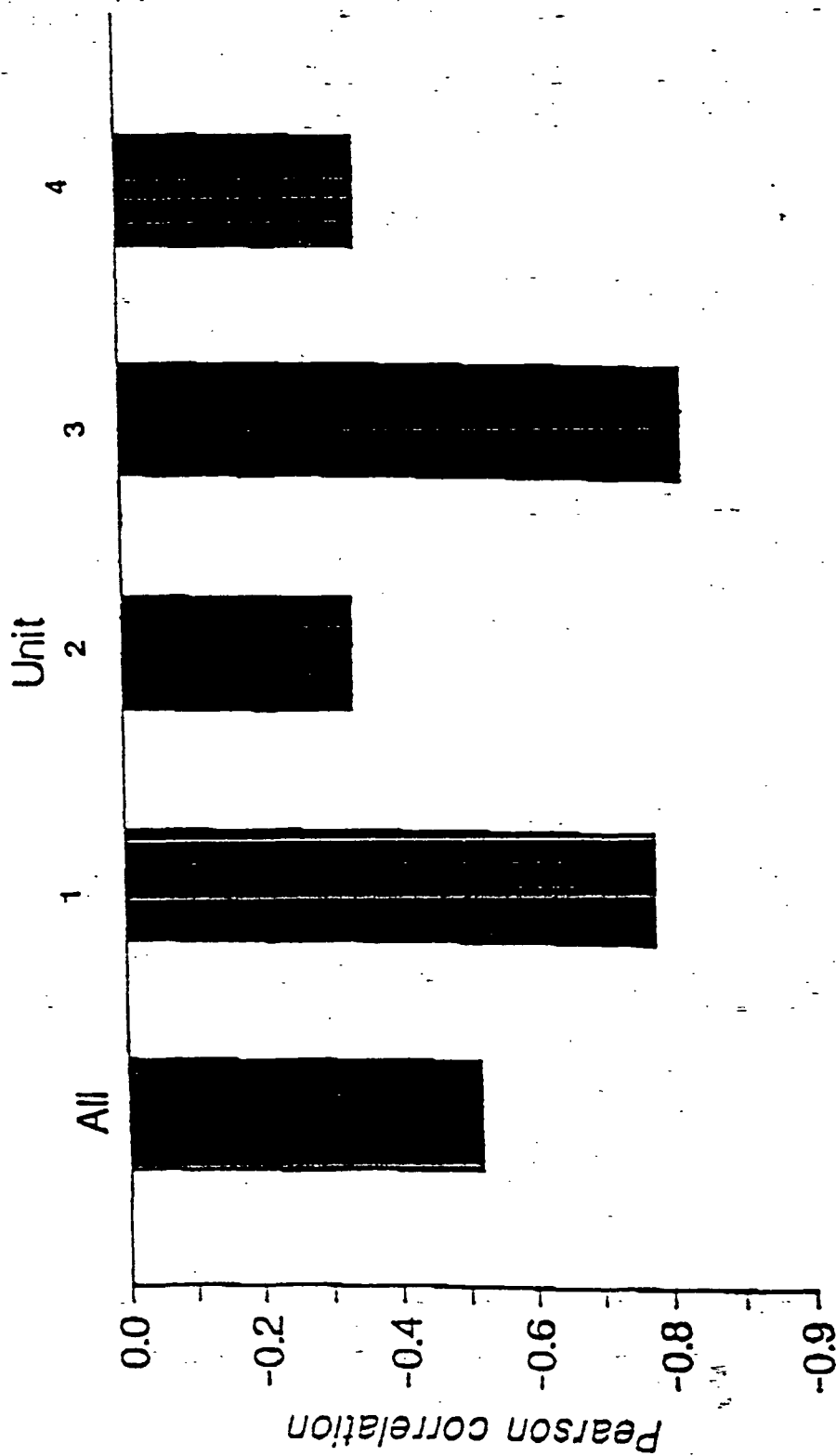


FIGURE 4. Correlations for Ionized Ca and PTH for Individual Subjects

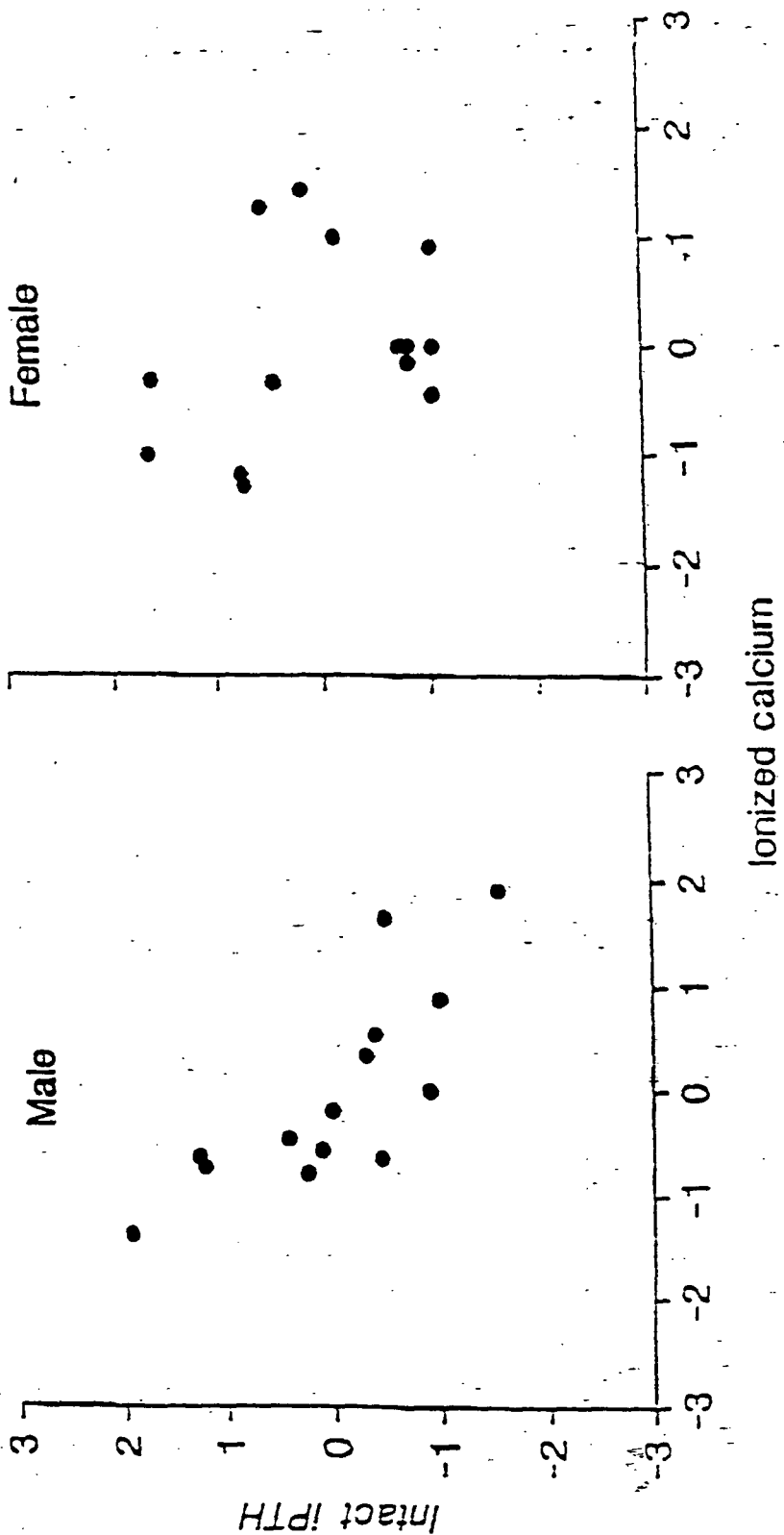


FIGURE 5. Relationship Between Serum Ionized Calcium and Intact iPTH
(Individual Crewmembers)

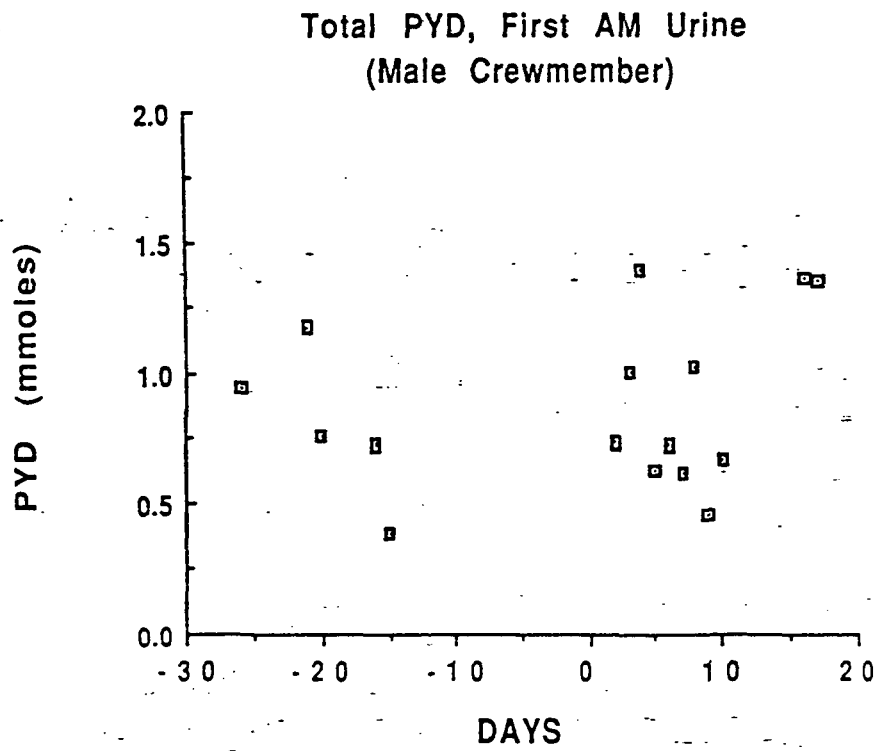
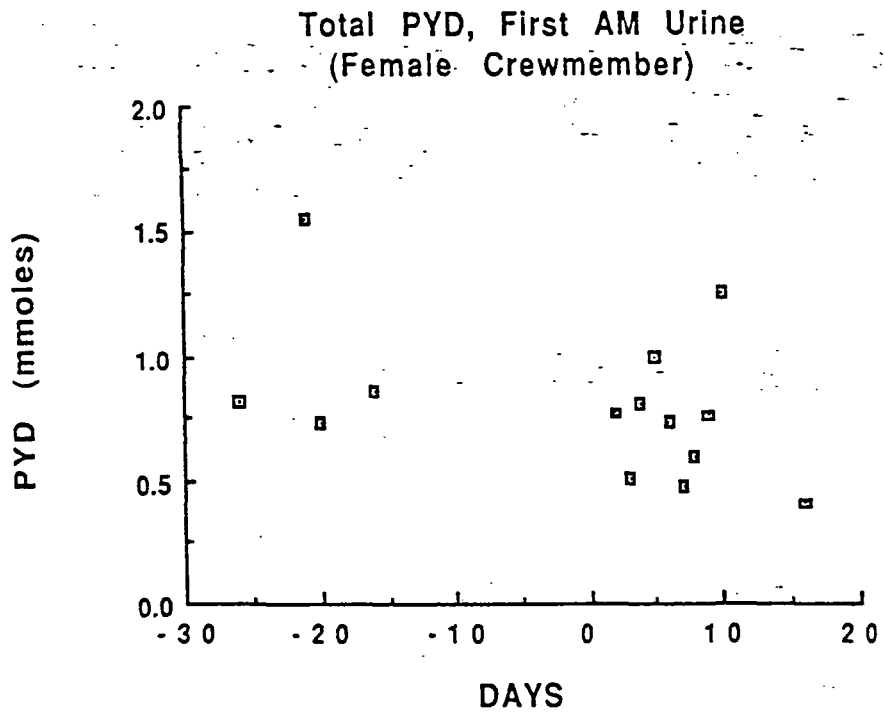


Figure 6. Total PYD in first morning fasted urine for female (top) and male (bottom) crewmembers. There are no significant differences noted inflight from preflight values.

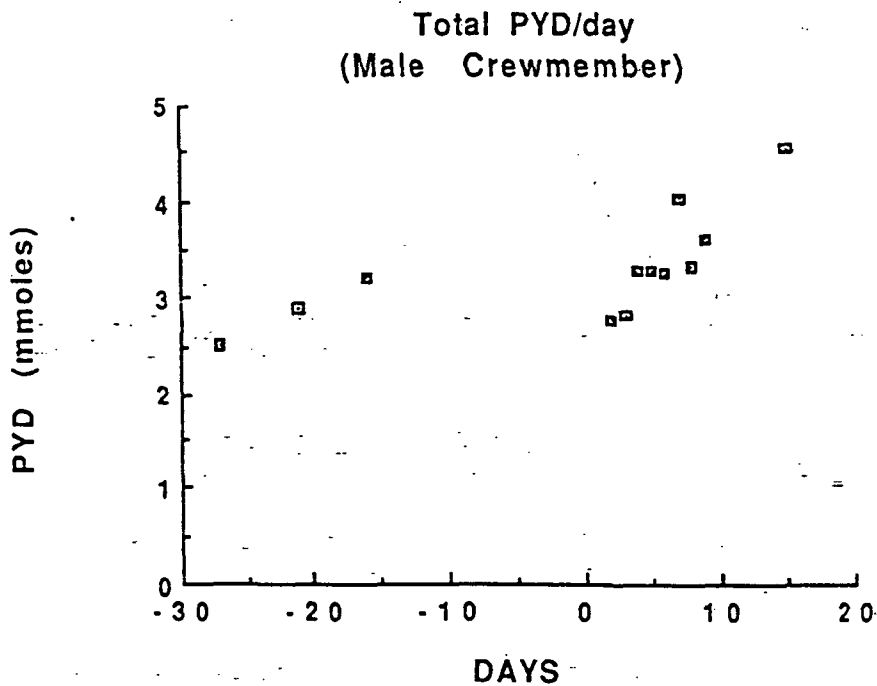
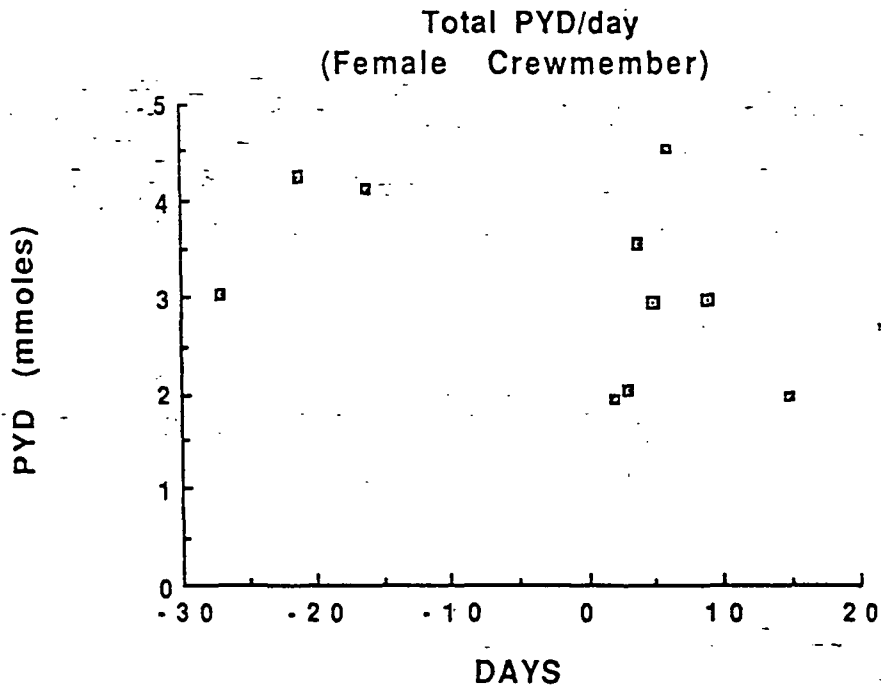


Figure 7. Total PYD output per day in female (top) and male (bottom) crewmembers. While female crew show little effect of spaceflight on total PYD output, male crew shows significant increase with flight, as expected if bone resorption is increased.

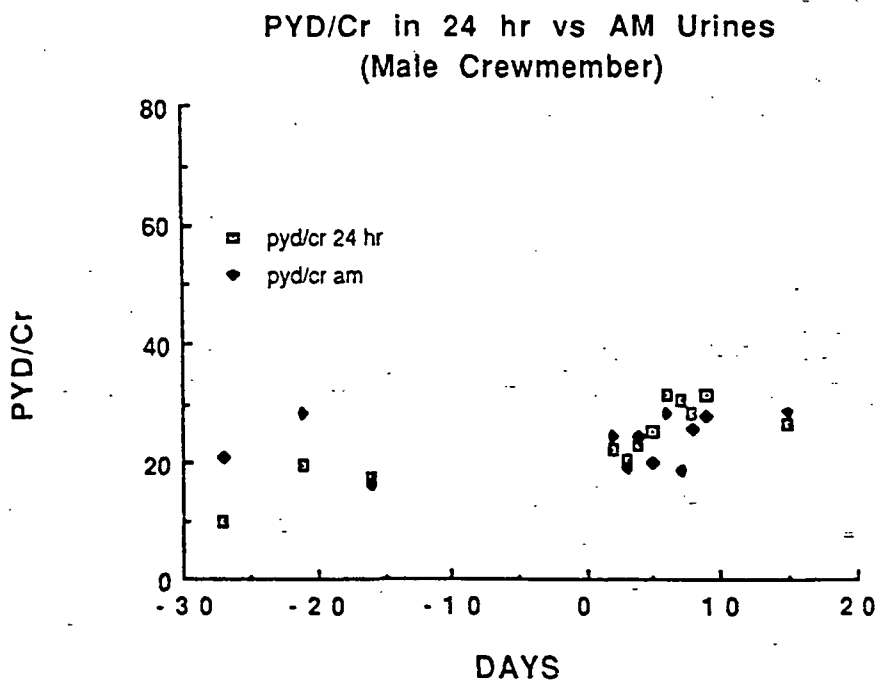
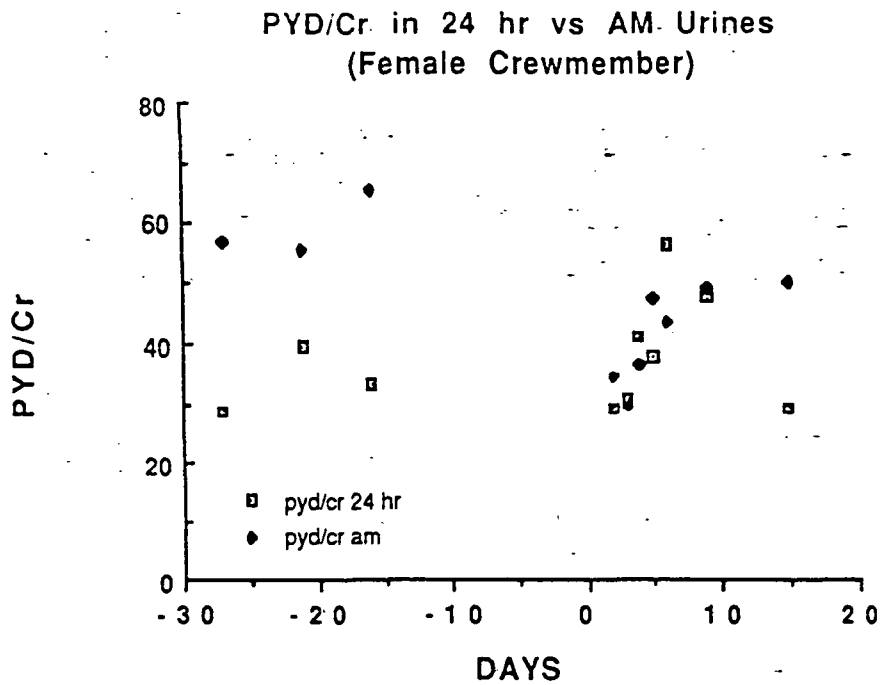


Figure 8. Urine PYD/Cr ratios in female (top) and male (bottom) crewmembers for first morning void and 24 hour pooled urines. Note that inflight, ratios are very similar for first morning and whole day collections, while preflight and postflight the expected nocturnal increase is seen.

Ratio of Urine PYD in
First Morning vs 24 Hour

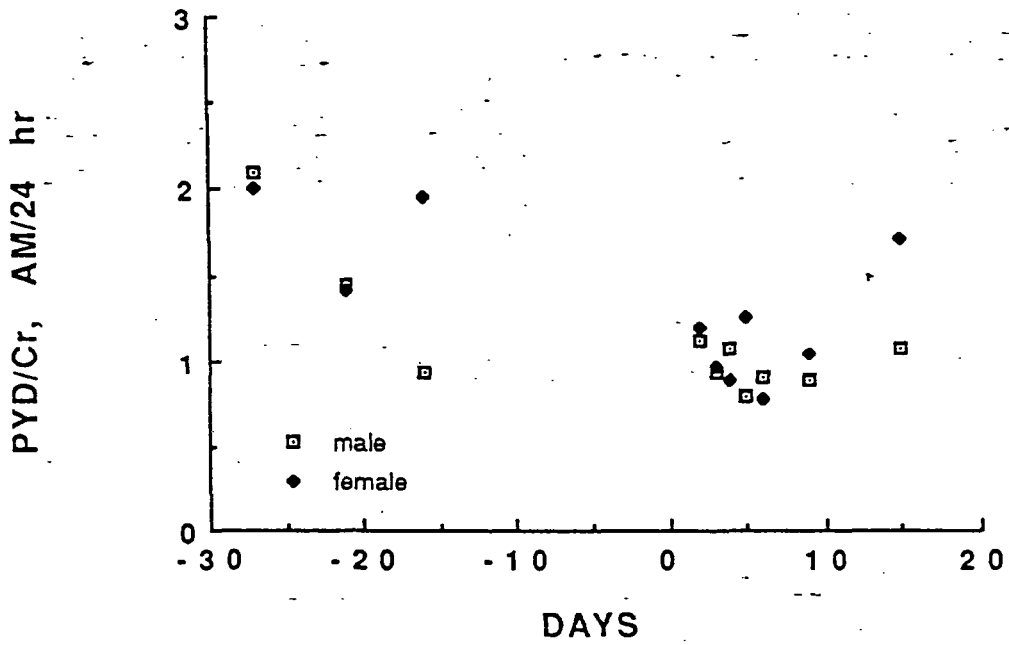


Figure 9. Ratio of PYD output in first morning void compared to 24 hour pooled urine collection. The normal 50-80% increase seen preflight is eliminated during the flight period.