

JSC Human Life Sciences Project

**E029 - Magnetic Resonance Imaging after Exposure to
Microgravity**

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Final Report for Experiment E029: Life and Microgravity Spacelab

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1. TITLE

Magnetic Resonance Imaging After Exposure To Microgravity

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INTRODUCTION

A number of physiological changes have been demonstrated in bone, muscle and blood from exposure of humans and animals to microgravity. Determining mechanisms and the development of effective countermeasures for long duration space missions is an important NASA goal. Historically, NASA has had to rely on tape measures, x-ray and metabolic balance studies with collection of excreta and blood specimens to obtain this information. The development of magnetic resonance imaging (MRI) offers the possibility of greatly extending these early studies in ways not previously possible; MRI is also non-invasive and safe, i.e., no radiation exposure. MRI provides both superb anatomical images for volume measurements of individual structures and quantification of chemical/physical changes induced in the examined tissues. DEXA is a noninvasive technique that employs low dose x-rays to measure bone, fat and lean tissue composition. This proposal will apply MRI and DEXA technology to examine changes resulting from exposure to microgravity in 5 different areas: muscle, intervertebral disc, bone marrow, body composition, and fluid distribution.

OBJECTIVES

1. Measure the muscle volumes of the calf, thigh, and back using MRI before and after flight. These data will be compared with pre and post flight muscle performance measurements. Determine the total body lean tissue mass using DEXA.
2. Determine the changes in the MRI muscle volumes and DEXA soft tissue distribution between R+0 and R+2 days. Determine the calf muscle transverse relaxation time (T₂) before and after flight.
3. Determine the change in intervertebral disc size and spine length in the lumbar spine after flight and determine the rate of recovery after return to one G.
4. Determine the bone marrow T₂ and cellularity of L3 before and after flight using MRI spectroscopy.

BACKGROUND AND PURPOSE

Space flight without effective countermeasures causes significant loss in muscle mass and strength, similar to horizontal bed rest (1-5). It is believed that these losses are at least partially responsible for degraded muscle performance observed following space flight (3). Our measurements on the crew of Spacelab mission SLJ/STS47 demonstrated significant muscle-specific atrophy after only 8 days in weightlessness (6). We repeated these MRI muscle measurements on the 17 day LMS mission and included more extensive recovery measurements after flight.

Research studies indicate that normal ambulation is necessary for intervertebral disc health

(7-11). Unloading of the spinal column for long periods of time, as in space flight, may result in adaptive changes which may require significant time for readaptation on return to one G and possibly in some degree of permanent change after prolonged weightlessness. The results of our 5 and 17 week horizontal bed rest studies demonstrated that overnight or longer bed rest causes expansion of the disc area, reaching an equilibrium value of about 22% (range 10-40%) above baseline within the first 4 days of bed rest (12). We have shown that following 8 days of space flight, disc area returns to baseline within a few days of ambulation (12). This proposal repeated these measurements on the longer LMS mission to determine if (1) longer exposure to weightlessness would result in post-flight changes and (2) whether measurements taken early after flight would demonstrate the expected disc expansion during flight. For this purpose, this proposal measured the cross-sectional area of intervertebral lumbar discs and vertebral length (L1-L5) before and at several time points after flight including recovery day.

Our bed rest studies demonstrated fluid accumulation in the lower limbs following reambulation which can result in significant error in the estimation of muscle atrophy depending on when measurements are performed-this misinterpretation of the degree of atrophy may impact muscle performance estimates (13). The physiological explanation for these fluid changes is not understood. Two possibilities are: (1) fluid redistribution from the upper body to the lower because of the change in the gravity vector and (2) fluid accumulation resulting from muscle repair caused by post-flight damage and/or rebuilding muscle lost during weightlessness (14-16). We examined this using DEXA and MRI on R+0 and R+2 days, an elapsed time during which significant muscle volume (MRI) or mass (DEXA) change would not be expected. In addition to volume and mass changes, we measured T2 of the calf muscles before and after flight. Exercise is known to cause a transient fluid shift which resolves in less than 45 minutes (17-18) while muscle damage causes changes that evolve more slowly and may last several days to weeks (19-20). We have previously reported that T2 values are elevated shortly after reambulation but not during bed rest, suggesting that fluid has been retained in the limbs compared to bed rest or pre-bed rest (21).

Several space experiments have documented altered hematopoietic activity which may be related to cellularity changes in the bone marrow (22-23). It is known from bone biopsies, for example, that marrow fat increases 100% during the first 20 weeks of paralysis (24). Rats flown aboard Cosmos (19-21 days) have shown increases in marrow fat of 150 - 200% in the tibia and humerus while rats undergoing rear limb suspension have shown even larger increases, +685 and +500% in marrow fat of the tibia and humerus respectively as well as decreases in bone formation (25). These studies may also have relevance to bone atrophy that occurs during space flight and bed rest. It is speculated that adipose cells serve as a store of energy required for hematopoiesis, it may serve a similar purpose for control of osteolytic or osteoblastic activity. One objective of our Life and Microgravity Spacelab (LMS) experiment was to document this expected change in the marrow composition using a noninvasive MRS technique developed in our laboratory(26). The percent fat and cellular

portion of the marrow was measured in L3 before and after flight.

Body composition (bone, fat, lean) are affected either directly or indirectly by space flight (27-30). DEXA derived body composition was determined to complement the fluid shift and muscle loss measurements described above.

METHODS

MRI (magnetic resonance imaging) and DEXA (dual energy x-ray absorptiometry) imaging of the crewmembers of the Spacelab mission, LMS, was performed prior to flight on days L-(50-51), L-(29-31) days and post flight on R+0, R+2, R+10, and R+30 days. Two hours and 15 minutes per session were required except on R+0 when an abbreviated MRI was used because of time restrictions. The total time on R+0 was about 45 minutes for MRI and 15 minutes for DEXA for a total time of about 1 hour. The MRI unit used was a 1.5 Tesla Siemens machine located at The Methodist Hospital in Houston except on R+0 when a portable 1.0 Tesla G.E. instrument was brought to the landing site. In addition to the nominal imaging protocol, additional abbreviated MRI sessions were requested (Astronaut office, IRB, LMS crew) for bone marrow spectroscopy measurements only. These sessions required about 20 minutes and were obtained at various times depending on crew availability and the differences in the observed MRI values from baseline. All crewmembers received a DEXA scan using a Hologic 2000 device located at The Methodist Hospital in Houston or at the Kennedy Space Center. For the bed rest control study conducted at Ames Research Center, 8 male subjects received whole body DEXA scans using a Lunar Corp, DPX scanner and MRI scans using a 1.5 Tesla Siemens magnet located at O'Conner Hospital. The O'Conner unit was located about 5 miles from Ames. The DEXA and MRI imaging were obtained during the week prior to bed rest, on the last day of bed rest and again 24 to 48 hours after reambulation. For the bed rest study, the subjects were transported by stretcher and ambulance to the imaging sites during the bed rest portion of the study or by automobile during the ambulatory phase.

A. MRI

In order to minimize the effects of fluid shifts caused by lying after standing upright, crewmembers are recumbent for a minimum of 10-15 minutes before the start of data acquisition and the order in which the measurements are made was kept the same from one imaging session to the next. The imaging sequence was spine and back followed by the limb measurements. For the limb imaging, the feet were positioned in a holder to minimize movement during image acquisition and to insure repositioning. The portion of the limb being imaged was suspended while the non-imaged portion was supported with foam supports. An RF shield was positioned caudally to the imaged portion of the leg. For the thigh and calf muscles 32, 1 cm slices are obtained using a $T_e=10$ (flight) or 20 (bed rest) msec, $T_r=800$ (flight) or 1500 (bed rest) msec and a 256x512 matrix. For the back muscles, a similar protocol is used except 20, 0.5 cm slices centered on L3 were obtained

using a spine coil. A phantom was imaged during each imaging session to correct for any changes in pixel size.

Calf muscle T2 was measured by repeating the calf region scan using the same receiver gain at $T_e=50$ msec. T2 values for each muscle pixel were calculated by comparing the image intensity at $T_e=10$ msec and $T_e=50$ msec. T2 values of each individual muscle were obtained by averaging the T2 for all the pixels within that muscle. Because of time constraints, T2 measurements were not made on the bed rest subjects.

For the spine imaging, the subjects were positioned so that the imaging area was centered within the L3 vertebra, determined from a sagittal scout view. A coronal scout image was used to position a 1 cm slice of interest through the center of the spinal column. A 3 slice gradient echo sequence, $T_e=7$ msec, $T_r=800$ msec with 1 acquisition and a 256 x 256 matrix was used. A phantom was imaged during each imaging session to correct for any changes in pixel size. Disc area was obtained for discs T12-L1, L1-2, L2-3, L3-4, L4-5 from the number of pixels in each image.

To measure the fat and water percentages in vertebral bone marrow, volume selective proton spectra were obtained using a surface receive coil. A cubic volume of interest of 15mm x 15mm x 15mm located in the center the L3 vertebral body was selected based on the initial scan of the spine region. A Gradient Inversion Spectroscopy technique was used to acquire spectra at $T_e = 12, 18, 24,$ and 30 ms with $T_r = 2$ s. The images were corrected for T2 weighting by exponential extrapolation of the spectra obtained at various TE. The intensities of fat and water were calculated by integrating the areas under the fat and water peaks after baseline correction. Bone marrow cellularity was determined from the fat to water peak areas. Since the spectroscopy software was not available on the O'Conner Hospital magnet at the time of the start of the study, pre-bed rest spectroscopy measurements were not obtained. Measurements however were made at end of bed rest and on R+2 and R+15 days.

B. DEXA

A whole body DEXA scan was performed at the time of the MRI scans. The DEXA scan required approximately 15 minutes and gives a radiation exposure of less than 1 mrem. The whole body scan was analyzed for bone mineral density (BMD), soft tissue, lean, and fat. In order to examine fluid redistribution between the upper body and the lower limbs, the soft tissue values of the legs was divided by the whole body soft tissue minus the legs. This ratio was used to compare pre-flight with post-flight.

RESULTS

A. STATISTICAL ANALYSIS

Unless otherwise noted the data were analyzed using repeated measures ANOVA with a Tukey-Kramer or Dunnett multiple comparison test when the ANOVA was significant. Significance was chosen as a $p < 0.05$.

B. MUSCLE VOLUME

There were 8 bed rest subjects, however, one subject was not able to enter the MRI magnet head forward because of claustrophobic reasons and therefore only leg imaging was done on this individual. Therefore, only 7 subjects were imaged for the back and spine measurements. The muscle volume for the 7-8 subjects were analyzed using repeated measures ANOVA for the 3 time points (pre-bed rest, last day of bed rest, and 1-2 days after reambulation). Table 1 gives the results as a percent change from baseline at the end of bed rest and during reambulation. These results show that all regions demonstrated significant loss during bed rest except the psoas. The gastrocnemius and anterior calf were no longer significant after 1-2 days of reambulation, presumably because of fluid shifting into the lower limbs-see later section on fluid shift for discussion.

The 4 flight crew received two baseline measurements. For this report the values for both time points were averaged and all individual data points were expressed as a percent change from the average baseline. The data were analyzed statistically as above for the 5 time points, pre, R+0, R+2, R+10, and R+30 days- table 2 gives these results. The R+0 data for the crew is not comparable to the bed rest subject's end of bed rest data since the flight crew were not recumbent after flight, but were in wheelchairs for one to several hours prior to imaging. The R+2 day data is however, comparable between the two studies. Overall, the changes at R+2 seen in the flight crew and bed rest subjects are quite similar, i.e., there are no changes in the psoas, anterior calf and gastrocnemius; the percent change in the soleus, quadriceps, hamstrings and intrinsic back muscles are significant and similar. By R+10 days there were no significant differences from baseline in any of the muscle groups. This is different than SLJ/STS47 (8 day mission) which showed some muscle deficit remaining at R+14 days. The R+0 volume changes after LMS are somewhat greater than SLJ/STS47 obtained on R+1 while the R+2 LMS data are somewhat less than the SLJ data obtained on R+1. These data demonstrate the importance of imaging time for post-flight measurements of muscle volume. We will correlate these muscle changes with muscle performance data from other investigators when this data becomes available. Table 3 shows the muscle volume data from the 8 day SLJ/STS47 Spacelab mission for comparison.

C. FLUID REDISTRIBUTION

The above muscle volume data at R+0 and R+2 days, clearly indicates that fluid moves into

the lower limbs and possibly other muscles after flight or bed rest. For the bed rest subjects above, there was little difference between the end of bed rest data and 1-2 days after reambulation for most muscles except the calf-these changes ranged from 3-6%. We have seen much larger changes, 10-15%, after longer duration bed rest, probably reflecting a response to a relatively overloaded condition after bed rest. The flight data, although showing a slightly different pattern, was in general similar to bed rest. The physiological explanation for these fluid changes is not understood. Two possibilities are: (1) Muscle is dehydrated during weightlessness or bed rest because of fluid redistribution. After return from spaceflight or bed rest, fluid redistributes from the upper body to the lower because of the change in the gravity vector. (2) A second possibility is that there is fluid accumulation resulting from muscle repair caused by post-flight damage and/or rebuilding muscle lost during weightlessness. In the first case, if muscle becomes dehydrated during space flight or bed rest and subsequently rehydrates following reambulation or return to one G, a key question is what is the most appropriate time to measure muscle atrophy, R+0, R+2, R+10 days or some other post-flight time. In second situation, R+0 is the most appropriate time for measurement.

We attempted to investigate possibility number 1 by examining the soft tissue ratio of the legs to upper body from the DEXA scan before during and after bed rest or flight. Table 4 and 5 give these ratios for the bed rest and flight data respectively. While there was a shift during bed rest, this returned to baseline within 1-2 days after bed rest since there is no apparent difference between R+2 and baseline. There was no significant change in the pre-flight and post-flight ratios indicating that the inflight shift to the upper body from the legs has already reverted back to baseline by the time measurements were taken on R+0. There is no evidence for an increased ratio after flight. This might indicate that gravity dependent fluid shift is not responsible for the muscle swelling seen post-flight.

In order to investigate possibility number 2, we measured T2 of the calf muscles. T2 is sensitive to changes in tissue water/protein content. The graphs 1-4 show the calf T2 for the 4 flight crew. Statistical testing showed that the R+2 and R+10, but not R+30 data for the anterior calf and soleus were significantly above baseline. The T2 from the gastrocnemius ANOVA did not reach significance, $p=0.07$. These results appear to show an elevated T2 at R+2 and sometimes at both R+2 and R+10 which returns toward baseline by R+30. This would indicate that fluid accumulation does occur during this time period after flight, but returns toward the baseline condition by R+30 days. We cannot rule out that T2 is elevated during flight or immediately thereafter, e.g., on R+0. We did not attempt to measure muscle T2 on R+0 because the magnet strength of the portable machine was different than the instrument used for the pre and post-flight measurements. However, it seems unlikely that T2 would be elevated during flight since fluid shifts out of the lower limbs during flight and not into the legs. Also, we have shown that measurements in bed rest do not change muscle T2, but that T2 is elevated following reambulation. The data from the present experiment are consistent with the bed rest data. Interestingly, in 2 individuals, the T2 was still elevated at R+10 when muscle volume differences are not apparent, but performance decrements are

still apparent (Narici). No T2 data was obtained during the bed rest study as previously mentioned. The muscle volume, soft tissue and the T2 data suggest that the most accurate time to obtain muscle atrophy measurements may be before or shortly after reambulation for bed rest and shortly after return to one G in the case of space flight. Reambulation causes a movement of fluid and swelling of muscle that lasts several weeks and is probably associated with muscle damage and/or repair.

D. INTERVERTEBRAL DISC

Table 6 gives the percent change from baseline in the intervertebral area for the bed rest subjects. During bed rest, prior to reambulation, the discs are expanded about 12% above baseline. This is considerably less than the 22% average that we observed previously during 5 and 17 weeks of bed rest (12). This difference may result from the difference in the two bed rest studies, i.e., 6 degree head-down at Ames versus horizontal bed rest in Houston. The head-down condition is likely to provide greater weightbearing on the discs compared to the horizontal condition. There were no residual effects after 1-2 days of reambulation similar to our 5 week bed rest studies. This is also reflected in the spine length measurements, Table 8, which showed about 3.5 mm increase in length during bed rest which normalizes to baseline values by 1-2 days post bed rest. Table 7 gives the cross-sectional disc areas for the flight crew. There is no evidence of disc expansion on R+0. There seems to be slight, but nonsignificant decrease in disc area post-flight. The spine length data of the flight crew is given in Table 9. Because of a missing data point, one way ANOVA was performed on this data instead of repeated measures ANOVA. This data shows a lengthening of the spine relative to baseline on R+0, which appears to be at variance with the disc area measurements. An explanation may be that the disc cross-sectional area reverts back to baseline more quickly than the spine curvature which contributes to spine length as well as disc size. The crew were seated rather than recumbent prior to imaging. Sitting loads the disc about the same as when standing, but might provide support for the back slowing the readaptation process for spinal curvature. There is a tendency for spine length to be decreased post-flight similar to the cross-sectional disc area measurements. These small post-flight changes which were not observed in the Ames or in our previous bed rest studies may have some relevance for longer space missions.

E. BONE MARROW

The proton spectroscopy data (T2 Water, T2 Fat, and Percent Water) were analyzed for three subgroups of subjects (four astronauts, seven normal controls, and seven bed rest subjects). The data available for the astronauts included two pre-flight measurements and 6-7 post-recovery measurements. For the bed rest subjects there were three measurements, end of bed rest, R+2 and R+15 days post reambulation. The normal controls had 4 to 11 observations over an 85 week period.

Immediately post-flight no significant change in the fraction of the water (cellular) component

was found although subsequent post-flight measurements may indicate some change, figure 5. There appeared to be a small decrease in the T2 of the cellular component post-flight, but what was surprising was the increase in T2 in all crewmembers that was clearly evident by the final data collection point at 30 days post-flight. We obtained IRB and astronaut permission to obtain additional measurements when the crewmember's time and schedule permitted. These data are shown in figure 6. For 3 of the 4 crewmembers, the T2 remains elevated above baseline for more than 4 months after landing. These data appeared to show a consistent pattern post-recovery: an increase followed by a reduction. The data were analyzed using non-linear regression models, e.g. a Farazdaghi and Harris growth curve model. Although the data pattern was consistent with this model, the limited number of data points for each astronaut precluded a good fit of the model to each individual. However, an exponential growth curve model fit to the data indicated a statistically significant change over elapsed time for the astronauts. In addition to these models, a linear-linear piecewise regression was fit to the data. This is a four parameter model since it requires two linear regressions to be fit. From a visual perspective, this model gave the best fit. Even though the estimated R^2 was greater than 0.80, the number of parameters for the model left only three degrees of freedom for error which produced 95% confidence intervals (CI) that included '0'. Because of the number of data points 90% CI's, would be a better choice. The same model was fit to all four astronauts simultaneously. This produced a good visual fit, but because of the different data patterns for each individual gave an R^2 of only 0.36. The astronaut data did not show a change in the T2 of the fat component, figure 7. Standard linear regression with the other variables for the controls and bed rest subjects indicated, in general, no statistically significant deviations from a non-time-related flat response figures 8-10.

Since the fraction of the cellular portion of the marrow is changing only slightly if at all, we believe that the observed T2 change in the cellular component represents a change in the cellular composition of the marrow. One explanation for this change might increased hematopoiesis to replace lost red cells following flight since the loss of red cell mass during short duration weightlessness is documented. However, the time frame of the T2 response is much longer than needed to replace lost red cells which should be completed within one month after flight. Another explanation for the post-flight T2 response might be increased osteoblastic activity which might be expected to have a longer time frame. In support of this, our 17 week bed rest studies demonstrated an increase in bone formation markers compared to pre-bed rest after reambulation; alkaline phosphatase by 50% and osteocalcin by 33% (31). These findings have significant implications for medical research on earth as well as microgravity. The results of this portion of the project are the most intriguing of our findings.

F. BODY COMPOSITION

Tables 10-11 give the whole body BMD for the bed rest subjects and flight crew. The BMD did not change with bed rest or flight although one point at R+10 appeared to show an increase. It was reported (12th Man in Space Symposium, Euro/Mir 97) that BMD was not

altered immediately after flight (n=1), but did show a decrease several weeks after flight. Our data (n=4) does not show a decrease after bed rest or flight. The R+10 day increase in BMD is presumably not significant since the R+0 and R+30 day values are the same as baseline. Also the increase at R+10 days, 2%, is within expected instrument precision. In any case, it is an increase not a decrease. Subregional analysis showed no significant change in the lumbar spine or pelvis BMD. These results indicate that the reported post-flight BMD decrease after the 20 day Euro/Mir 97 mission is probably not correct. The whole body lean tissue values are given in Tables 12-13. There was no significant change in whole body lean for the flight crew, but a small change was statistically evident for the bed rest study during bed rest and at R+2 days. It is important to point out that lean tissue measurements using DEXA cannot distinguish between fluid changes from actual changes in muscle mass. The bed rest subjects demonstrated no change in total body fat, Table 14, while the flight crew showed a loss in total body fat, Table 15, that appeared to persist up to at least 10 days after the flight. The differences between the flight and bed rest lean and fat results may reflect variation in the diet and or physical activity of the two groups and possibly sample size. Table 16 gives the reported flight crew body weights before and after flight which shows a loss during flight which is not significant by R+10 days although the mean weight is still about 2 Kg less than baseline. The DEXA data would suggest that most of this loss was due to fat.

CONCLUSIONS

Clearly, the most interesting findings of our experiment was the unexpected finding of the dramatic changes in bone marrow following flight. The significance of these findings needs further work, but we could speculate that if these changes represent a response of the trabecular skeleton to loading, measurements such as these may provide an early indicator of bone formation. This information might be useful in assessing early the response to countermeasures against space flight or osteoporosis associated with aging. Assuming that alterations that result in bone loss with aging or other causes are at least partially rooted in the bone marrow, these findings could lead to a better understanding of the basic physiology of the remodeling process with disease. It may be possible to investigate bone specific changes in remodeling in response to loading or from other types of interventions. We would like to verify the findings on LMS on another short-term (8-17 days) shuttle mission with a design to intentionally follow these changes after flight. It would also be interesting to investigate women entering the menopause and correlate ultimate changes in BMD over 1-2 years versus the changes occurring in the marrow.

We did not observe any residual expansion of the intervertebral discs as seen after long duration bed rest, rather we did observe some slight contraction in the discs following flight which might be important after long duration weightlessness.

As expected, our MRI measurements demonstrated small decreases in muscle volume in the calf, thigh and back similar to the changes after 17 days of bed rest. There was fluid

movement into the lower limb as evidenced by MRI volume changes between R+0 and R+2 and increases in T2 on R+2 and in some cases on both R+0 and R+10 days. Our data suggest that reambulation after flight or bed rest causes swelling of muscle that lasts several weeks and is probably associated with muscle damage and/or repair.

There were no significant changes in total body BMD or lean tissue after flight, but there appeared to be loss in total body fat which paralleled changes in total body weight.

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PRESENTATIONS

"Vertebral Bone Marrow Changes Following Space Flight" A LeBlanc, C Lin, H Evans, L Shackelford, S West, T Hedricks. 12th Man In Space Symposium, Washington, DC, June 8-13, 1997.

"T2 Vertebral Bone Marrow Changes After Space Flight" American Society for Bone Mineral Research, Cincinnati, Ohio, September 10-14, 1997.

"Magnetic Resonance Imaging After Exposure to Microgravity"

A LeBlanc, C Lin, H Evans, R Rowe, L Shackelford, T Hedricks. LMS One Year Science Review, Montreal, Quebec, August 20-21, 1997.

NON-TECHNICAL SUMMARY

Researchers have found a number of physiological changes in bone, muscle and blood from exposure of humans and animals to microgravity. Determining mechanisms and the development of effective countermeasures for long duration space missions is an important NASA goal. Magnetic resonance imaging (MRI) offers the possibility of greatly extending these early studies; MRI provides both superb anatomical images of individual structures and quantification of chemical/physical changes induced in the examined tissues. In addition, MRI is non-invasive and safe. Dual photon x-ray absorptiometry (DEXA), commonly used clinically to evaluate osteoporosis, is a noninvasive technique that employs low dose x-rays to measure bone, fat and lean tissue composition. This experiment used MRI and DEXA technology to examine changes resulting from exposure to microgravity in 5 different areas: muscle, intervertebral disc, bone marrow, body composition, and fluid distribution.

Our MRI measurements demonstrated decreases, 3-12%, in muscle volume in the calf, thigh and back similar to changes after 17 days of bed rest-within 10 days after flight these differences from baseline were no longer apparent. Our data suggest that reambulation after flight or bed rest causes swelling of muscle that lasts several weeks and is probably associated with muscle damage and/or repair.

It is generally believed that there is significant expansion of the intervertebral disc during weightlessness as observed during bed rest simulation. Research studies suggest that normal ambulation is necessary for intervertebral disc health and that extended time in microgravity may have deleterious effects on disc health. We did not observe any significant residual changes in the intervertebral discs after flight.

The most interesting finding of our experiment was the dramatic MRI change in bone marrow found following flight. The significance of these findings needs further work, but we speculate that if these changes represent a response of the trabecular skeleton to mechanical loading, measurements such as these may provide an early indicator of bone formation. This would be useful in assessing the early response of techniques designed to prevent bone loss from space flight, aging, or other causes. Assuming that bone marrow is an essential element in the process that leads to changes in bone mass, these findings could provide a better understanding of the basic physiology of the remodeling process in particular alterations caused by aging or disease. It may be possible to investigate region specific changes in bone remodeling in response to mechanical loading or other interventions.

TABLE 1
BED REST MUSCLE VOLUME - PERCENT CHANGE FROM BASELINE \pm SD

<u>MUSCLE</u>	<u>BED REST*</u>	<u>p</u>	<u>R+1/2 DAYS</u>	<u>p</u>
Anterior Calf (n=8)	-5.7 \pm 3.2	<0.001	-2.5 \pm 1.9	NS
Soleus (n=8)	-10.0 \pm 3.0	<0.001	-5.5 \pm 2.3	<0.001
Gastroc (n=8)	-8.4 \pm 3.1	<0.001	-2.2 \pm 3.1	NS
Soleus & Gastroc (n=8)	-9.4 \pm 2.9	<0.001	-4.3 \pm 2.1	<0.001
Quads (n=8)	-5.6 \pm 1.7	<0.001	-4.8 \pm 2.2	<0.001
Hams (n=8)	-3.7 \pm 3.1	<0.01	-3.7 \pm 1.5	<0.01
Intrinsic Back (n=7)	-6.3 \pm 2.9	<0.001	-6.2 \pm 1.8	<0.001
Psoas (n=7)	-1.0 \pm 1.3	NS	0.1 \pm 1.4	NS

* Measured on day 16 of bed rest, ANOVA with Tukey-Kramer multiple comparison test.
p = compared to baseline
n = number of subjects

TABLE 2

FLIGHT CREW MUSCLE VOLUME - PERCENT CHANGE FROM BASELINE \pm SD, n=4

MUSCLE	L-50/51	L-29/31	R+0	P	R+2	P	R+10	P	R+30	P
ANTERIOR CALF	-0.2 +0.6	0.2 +0.6	-3.3 +2.9	<0.05	-2.4 +2.2	NS	1.0 +3.2	NS	0.6 +1.7	NS
SOLEUS	-0.1 +1.3	0.1 +1.3	-9.8 +3.7	<0.01	-7.1 +2.5	<0.01	-3.2 +4.6	NS	-1.6 +2.0	NS
GASTROC	-0.9 +1.2	0.9 +1.2	-12.4 +3.6	<0.01	-2.5 +4.6	NS	4.0 +9.5	NS	0.3 +4.7	NS
SOLEUS & GASTROC	-0.4 +1.2	0.4 +1.2	-10.8 +3.0	<0.01	-5.4 +3.1	<0.05	-0.5 +6.3	NS	-0.9 +2.8	NS
QUADS	-0.4 +1.0	0.4 +1.0	-7.4 +5.0	<0.01	-4.1 +4.3	<0.05	-2.6 +3.7	NS	-0.9 +2.5	NS
HAMS	-0.3 +0.4	0.3 +0.4	-2.8 +5.5	NS	-4.2 +3.7	<0.05	0.4 +4.3	NS	1.1 +1.9	NS
INTRINSIC BACK	-0.6 +1.0	0.6 +1.0	-10.0 +5.6	<0.01	-7.9 +1.2	<0.01	-7.9 +1.2	NS	-0.3 +1.5	NS
PSOAS	-0.5 +1.1	0.5 +1.1	-5.0 +2.1	<0.01	0.6 +3.5	NS	0.6 +3.5	NS	1.2 +1.0	NS

Table 3

Change in Muscle Volume of Four Crewmembers of Shuttle Flight STS-47. Values are expressed as the Percent Change from the Average of Three Preflight Measurements.

Crewmember†	CALF		THIGH			LUMBAR	
	Anterior	Soleus+ Gastroc	Quadriceps	Hamstrings	Intrinsic	Psoas	
	Recovery +1 Day						
1	-4.3	-5.4	---	-7.2	-4.8	-0.9	
2	-4.3	-5.2	-4.1	-6.2	-14.5	-7.2	
3	-2.3	-6.9	-4.5	-10.1	-7.9	-0.8	
4	-4.6	-7.5	-9.4	-8.7	-14.2	-3.6	
Mean	-3.9	-6.3	-6.0	-8.0	-10.3	-3.1	
±SE	0.5	0.6	1.7	0.9	2.4	1.5	
*p	0.005	0.005	0.071	0.002	0.023	0.129	
	Recovery +15/16 Days						
1	-1.5	-0.1	---	-3.2	-2.0	2.1	
2	-1.3	-1.2	-0.3	-2.1	-7.4	-2.5	
3	-5.3	-8.2	-6.2	-6.6	-5.4	-4.6	
4	-5.0	-8.3	-7.9	-7.2	-9.0	-4.4	
Mean	-3.3	-4.4	-3.1	-4.8	-5.9	-2.4	
±SE	1.1	2.2	2.3	1.3	1.5	1.6	
*p	0.058	0.058	0.175	0.032	0.029	0.224	

* Repeated measures ANOVA using means contrasts and a Greenhouse-Geisser adjustment factor.

† All crewmembers took the salt/water orthostatic countermeasure prior to landing; crewmembers 3 and 4 underwent LBNP 1-2 days before landing; crewmember number 1 performed regular exercise during flight.

TABLE 4

Bed Rest - Ratio of Legs to Upper Body Soft Tissue

SUBJECT	PRE-BED REST	BED REST	R+1/2
249	0.55	0.52	.053
374	0.52	0.50	0.54
405	0.50	0.49	0.49
420	0.47	0.45	0.46
428	0.57	0.52	0.54
435	0.53	0.50	0.50
439	0.52	0.46	0.50
457	0.51	0.48	0.52
MEAN	0.52	0.49	0.51
SD	0.03	0.02	0.02
P VALUE		<0.01	NS

TABLE 5

Flight Crew - Ratio of Legs to Upper Body Soft Tissue

CREW MEMBER	PRE-FLT	R+0	R+2	R+10	R+30
LMS1	0.50	0.49	0.51	0.52	0.50
LMS2	0.54	0.54	0.55	0.56	0.55
LMS3	0.52	0.54	0.54	0.55	0.55
LMS4	0.59	0.56	0.57	0.57	0.58
MEAN	0.54	0.53	0.54	0.55	0.55
SD	0.04	0.04	0.03	0.02	0.03
P VALUE		NS	NS	NS	NS

TABLE 6
BED REST-INTERVERTEBRAL DISC CROSS-SECTIONAL AREA, PERCENT CHANGE
FROM BASELINE

	Disk Number					Average
	T12-L1	L1-L2	L2-L3	L3-L4	L4-L5	
Subject-249						
Bedrest	-3	7	-1	11	12	5
R+1	-12	5	-1	4	12	2
Subject-405						
Bedrest	1	11	32	35	19	19
R+2	-17	1	5	2	10	0
Subject-420						
Bedrest	10	32	8	11	21	16
R+2	-4	3	0	2	6	1
Subject-428						
Bedrest	4	-1	16	3	0	5
R+2	-1	1	8	-3	-6	-0
Subject-435						
Bedrest	17	11	10	8	15	12
R+1	-7	2	-4	-1	2	-2
Subject-439						
Bedrest	28	26	8	-1	13	15
R+1	5	7	1	1	1	3
Subject-457						
Bedrest	6	6	14	8	8	8
R+1	-3	-9	0	-4	-4	-4
All Subjects						
Bedrest	9	13	12	11	13	12
p	<0.05	<0.01	<0.01	<0.05	<0.01	<0.01
R+(1-2)	-6	2	1	0	3	0
p	NS	NS	NS	NS	NS	NS

TABLE 7

FLIGHT CREW-INTERVERTEBRAL DISC CROSS-SECTIONAL AREA,
PERCENT CHANGE FROM BASELINE

	T12-L1	L1-L2	L2-L3	L3-L4	L4-L5	AVERAGE	p
LMS-1							
L-50	1	-1	-0	-1	3	0	
L-29	-1	1	0	1	-3	-0	
R+0	-5	5	-4	1	-9	-2	
R+2	3	2	3	2	-4	1	
R+10	-6	-3	-7	-1	-17	-7	
R+30	2	6	-1	1	-4	1	
LMS-2							
L-51	6	1	-0	-1	-4	0	
L-29	-6	-1	0	1	4	-0	
R+0	-6	-4	-3	-1	0	-3	
R+2	-5	1	-5	-3	4	-1	
R+10	-15	-9	-11	-10	-2	-9	
R+30	-10	-8	-8	-2	-2	-6	
LMS-3							
L-50	-0	1	-0	*	1	0	
L-31	0	-1	0	*	-1	1	
R+0	-3	-6	-3	*	-1	-6	
R+2	-3	-3	-6	*	3	-1	
R+10	+	-4	-5	*	-1	-8	
R+30	0	-4	-3	*	-2	-5	
LMS-4							
L-50	-1	3	-1	2	-3	-0	
L-30	1	-3	1	-2	3	0	
R+0	-3	-3	-0	-2	4	-1	
R+2	-11	-7	-2	-3	-1	-5	
R+10	-13	-2	-5	-3	-5	-6	
R+30	2	3	-1	4	-0	2	
ALL SUBJECTS							
L-(50/51)	1	1	-1	-1	-1	0	
L-(29/31)	-1	-1	1	1	1	0	
R+0	-4	-2	-3	-5	-1	-2	NS
R+2	-4	-2	-2	-0	1	-2	NS
R+10	-9	-5	-7	-9	-6	-6	<0.01
R+30	-1	-1	-3	-4	-2	-1	NS

* Problem measuring disk.

+ Not available due to scan positioning

TABLE 8

BED REST - CHANGE IN SPINE LENGTH (mm), DISC T12-L1 TO DISC L4-L5

	<u>SUBJECTS</u>								P
	249	405	420	428	435	439	457	AVERAGE ALL SUBJECTS	
BEDREST	1.7	3.2	4.5	3.7	4.8	4.6	2.1	3.5	<0.001
R+1/2	-0.4	0.7	0.8	-0.6	0.6	-0.0	-0.1	0.2	NS

TABLE 9

FLIGHT CREW - CHANGE IN SPINE LENGTH (mm), DISC T12-L1 TO DISC L4-L5

	<u>CREWMEMBERS</u>								P*
	LMS-1	LMS-2	LMS-3	LMS-4	AVERAGE ALL CREWMEMBERS				
L-50/51	0.1	-0.3	0.1	0.4	0.1				
L-29/31	-0.1	0.3	-0.1	-0.4	-0.1				
R+0	2.7	2.0	1.5	1.4	1.9	<0.01			
R+2	0.3	-0.7	-1.2	-1.7	-0.8	NS			
R+10	-1.5	-2.0	-	-1.7	-1.7	<0.05			
R+30	0.3	-1.2	-2.0	-0.7	-0.9	NS			

* One way ANOVA was done because of missing data point.

TABLE 10

Bed Rest - Whole Body BMD (gm/cm²)

SUBJECT	PRE BED REST	BED REST	R+1/2
249	1.269	1.258	1.270
374	1.260	1.275	1.267
405	1.407	1.426	1.406
420	1.174	1.169	1.174
428	1.290	1.288	1.309
435	1.135	1.122	1.111
439	1.365	1.400	1.388
457	1.144	1.146	1.141
MEAN	1.255	1.260	1.258
SD	0.099	0.112	0.109
P VALUE		NS	NS

TABLE 11

Flight Crew - Whole Body BMD (gm/cm²)

FLIGHT CREW	PRE-FLT	R+2	R+10	R+30
LMS1	1.095	1.087	1.119	1.083
LMS2	1.256	1.259	1.277	1.262
LMS3	1.147	1.167	1.187	1.167
LMS4	1.171	1.164	1.197	1.164
MEAN	1.167	1.169	1.195	1.169
SD	0.067	0.070	0.064	0.073
P VALUE		NS	<0.01	NS

TABLE 12

Bed Rest - Whole Body Lean Tissue (kg)

SUBJECT	PRE-BED REST	BED REST	R+1/2
249	57.06	57.19	57.11
374	53.51	51.41	52.23
405	62.98	59.92	61.69
420	58.68	55.47	56.70
428	64.33	63.43	62.66
435	55.53	52.68	53.60
439	60.38	57.17	57.75
457	56.26	55.27	55.91
MEAN	58.59	56.56	57.20
SD	3.74	3.84	3.58
P VALUE		<0.01	<0.01

TABLE 13

Flight Crew - Whole Body Lean Tissue (kg)

FLIGHT CREW	PRE-FLT	R+0	R+2	R+10	R+30
LMS1	57.08	53.42	57.13	55.6	57.2
LMS2	58.28	57.21	56.86	60.42	58.31
LMS3	63.06	64.42	65.24	66.14	64.22
LMS4	58.18	58.71	59.19	58.84	59.8
MEAN	59.15	58.44	59.60	60.25	59.88
SD	2.66	4.56	3.89	4.41	3.08
P VALUE		NS	NS	NS	NS

TABLE 14

Bed Rest - Whole Body Fat Tissue (kg)

SUBJECT	PRE BED REST	BED REST	Δ	R+1/2	Δ
249	16.74	16.37	-0.37	15.86	-0.88
374	13.43	14.84	1.41	14.18	0.74
405	23.79	23.63	-0.16	22.87	-0.92
420	28.83	30.15	1.32	29.95	1.12
428	26.69	27.93	1.24	26.02	-0.67
435	20.69	23.05	2.35	22.59	1.89
439	33.48	34.15	0.67	34.08	0.60
457	8.30	7.88	-0.42	7.49	-0.81
MEAN	21.49	22.25	0.75	21.63	0.13
SD	8.38	8.74	1.00	8.74	1.09
P VALUE		NS		NS	

TABLE 15

Flight Crew - Whole Body Fat Tissue (kg)

CREW MEMBER	PRE-FLT	R+2	Δ	R+10	Δ	R+30	Δ
LMS1	25.99	22.52	-3.47	23.77	-2.22	24.45	-1.54
LMS2	19.80	18.48	-1.32	18.02	-1.78	20.11	0.30
LMS3	22.92	19.80	-3.12	19.48	-3.44	18.43	-4.49
LMS4	15.87	12.60	-3.27	13.77	-2.10	14.29	-1.58
MEAN	21.14	18.35	-2.79	18.76	-2.38	19.31	-1.83
SD	4.33	4.19	0.99	4.13	0.73	4.20	1.98
P VALUE		<0.01		<0.05		NS	

Δ = Difference from baseline

TABLE 16
Crew Body Weight (kg)*

	PRE-FLT ^o	R+0	A	R+2	A	R+9	A	R+30	A
LMS1	87.67	80.80	-6.87	83.18	-4.49	83.86	-3.81	86.82	-0.90
LMS2	84.88	80.34	-4.54	0.45	-4.43	83.41	-1.47	84.77	-0.11
LMS3	92.31	90.34	-1.97	90.00	-2.31	90.91	-1.40	88.64	-3.67
LMS4	79.16	74.55	-4.61	77.05	-2.11	7.95	-1.21	79.09	-0.07
Mean	86.00	81.51	-4.49	82.67	-3.33	84.03	-1.97	84.83	-1.19
SD	5.50	6.54	2.00	5.49	1.30	5.31	1.23	4.14	1.70
P		<0.01		<0.05		NS		NS	

* Weights taken with crewmen clothed without shoes. Scales were at various locations at JSC and KSC. Weights showing 2 decimal places were converted from pounds.

o Average of L-60 and L-30 except for L-60 of LMS1 which appeared aberrant-used L-90 in place of L-60 for this data point.

A Difference from baseline.

Figure 1. LMS-1 Calf Muscle T2

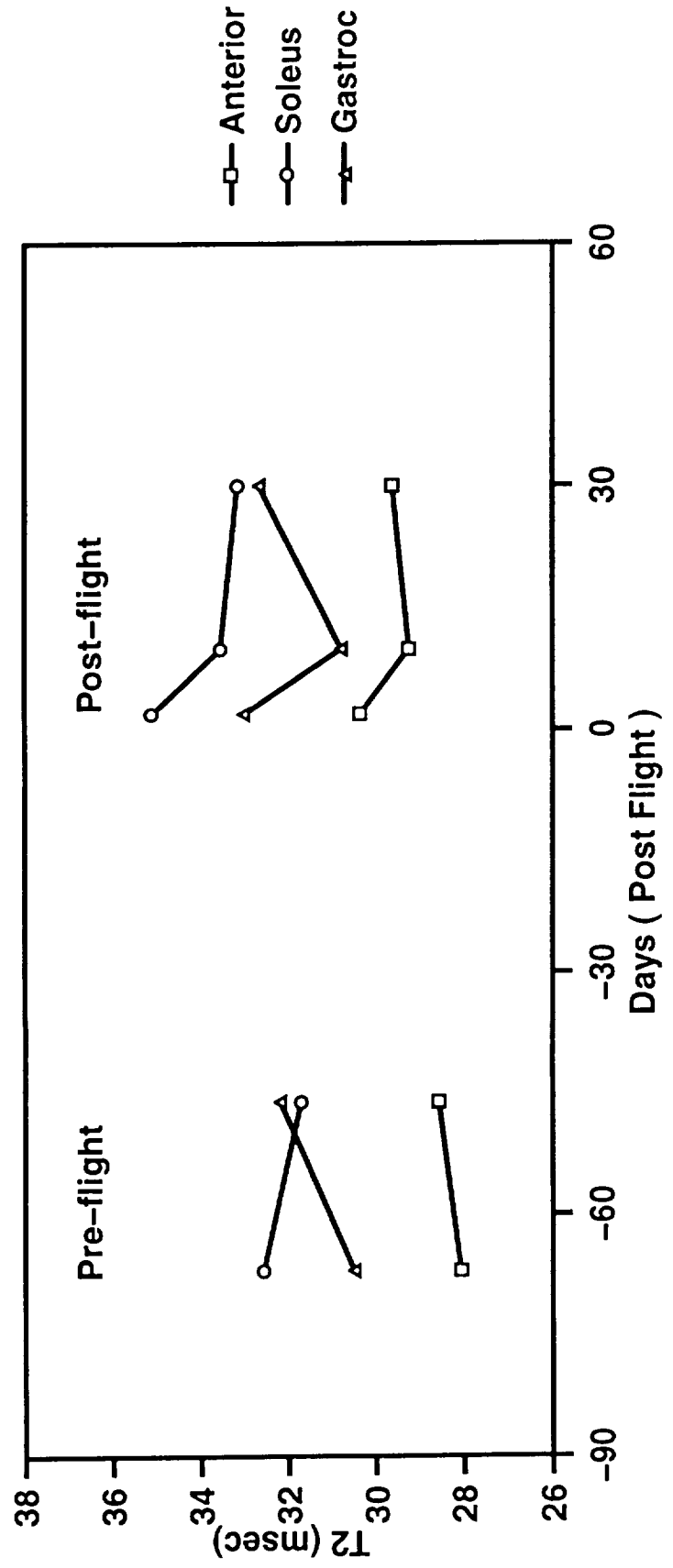


Figure 2. LMS-2 Calf Muscle T2

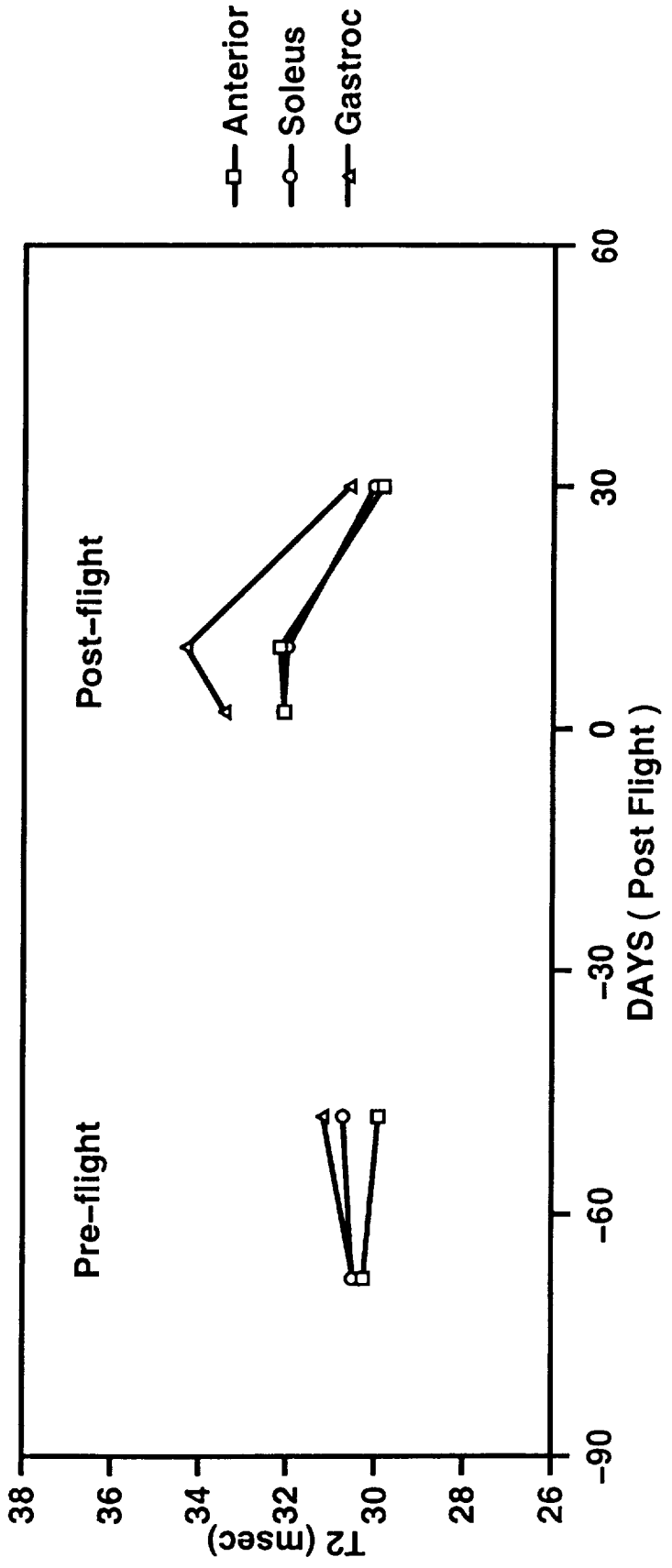


Figure 3. LMS-3 Calf Muscle T2

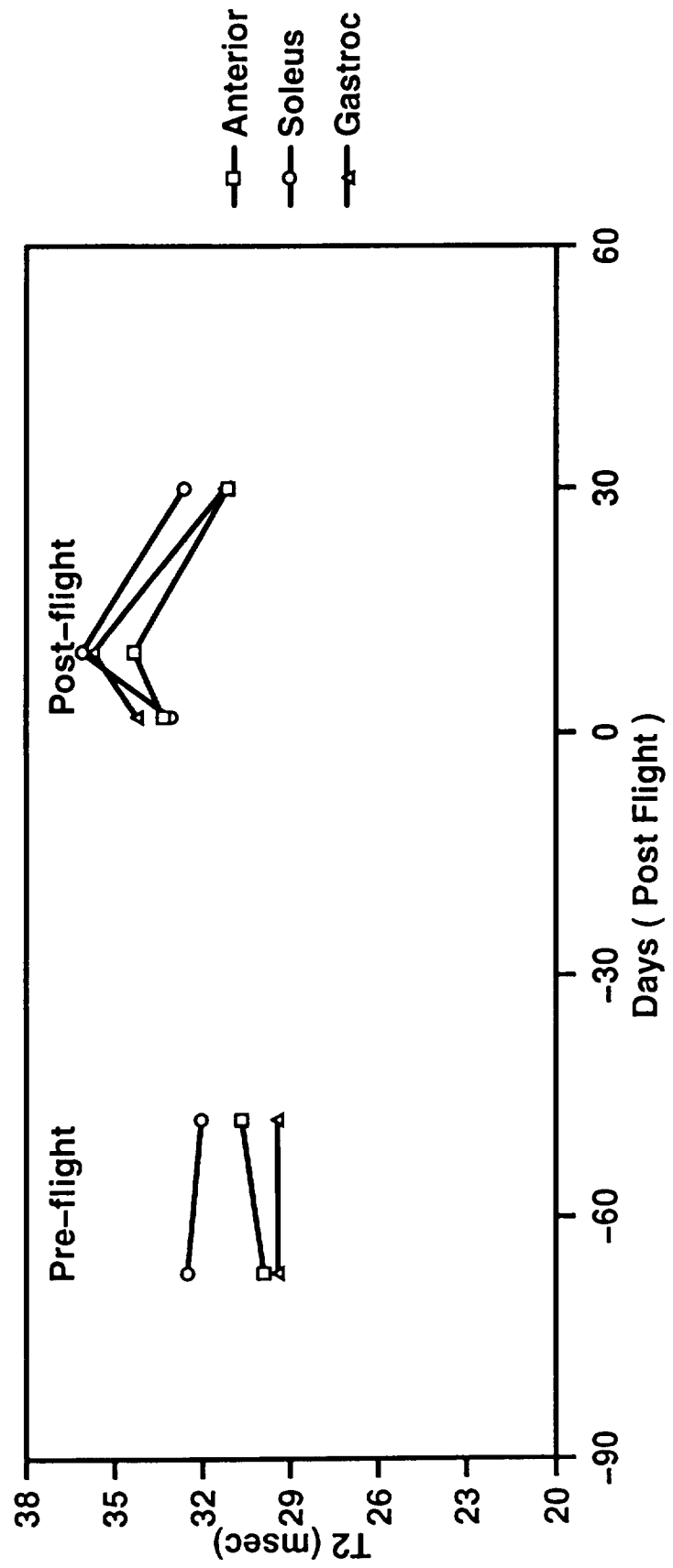


Figure 4. LMS-4 Calf Muscle T2

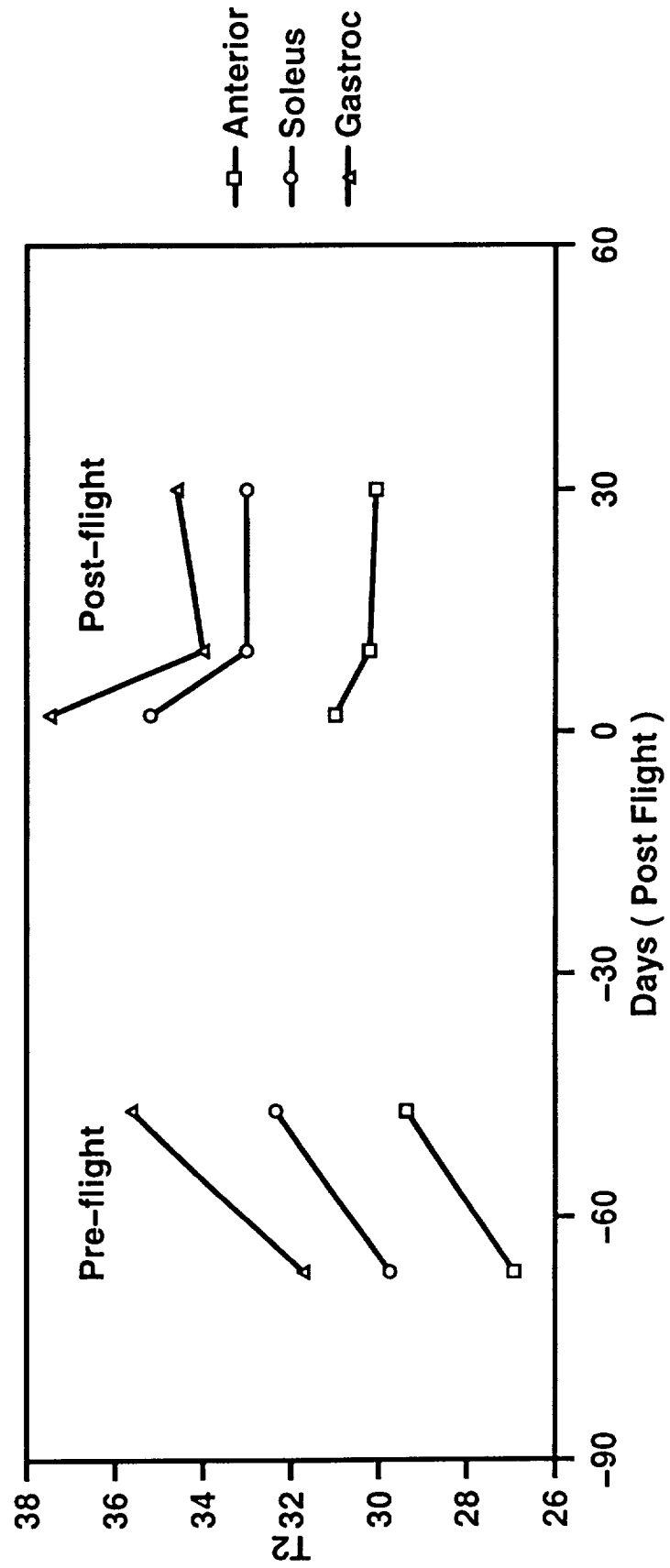


Figure 5. L3 Bone Marrow Water Percentage – Astronauts

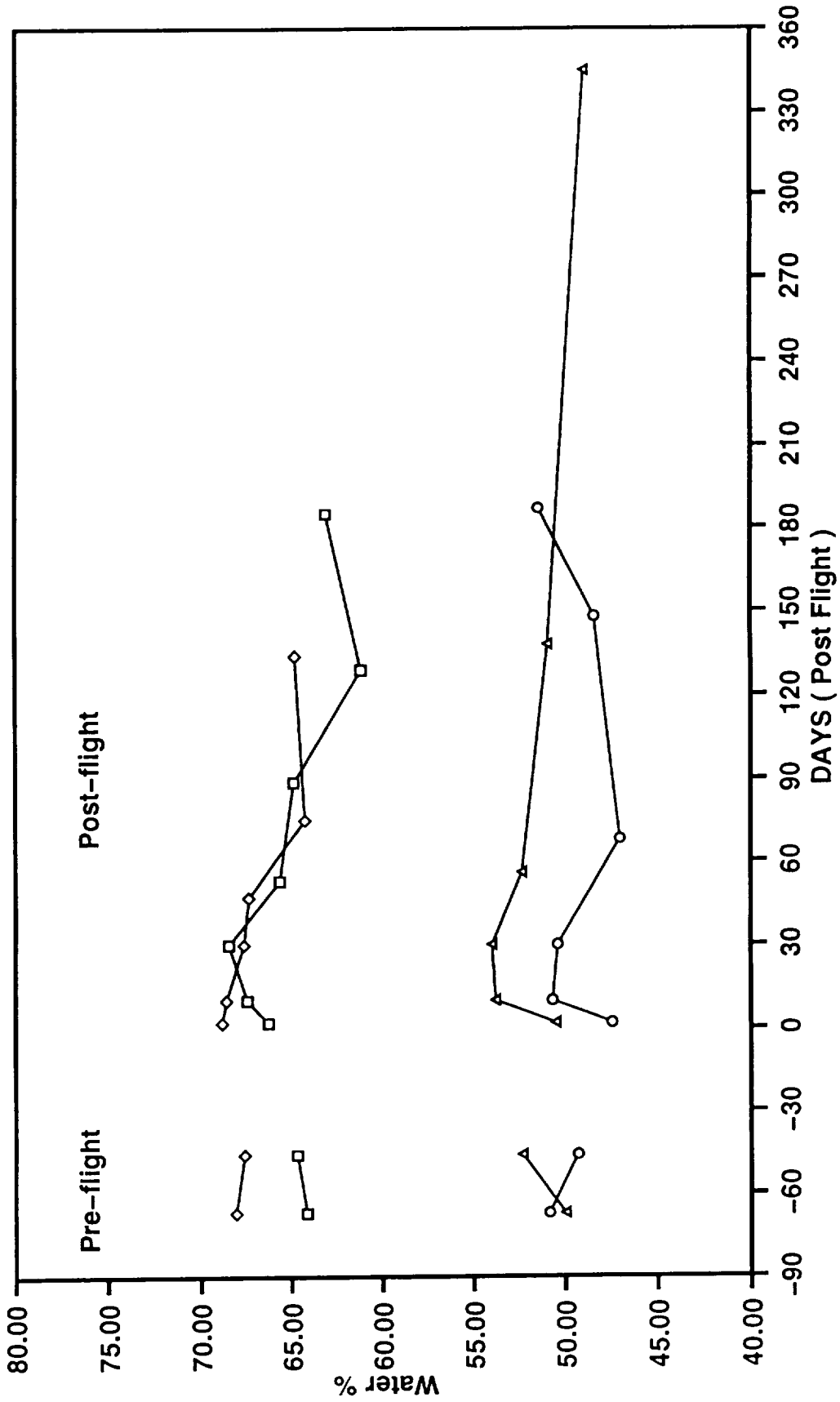


Figure 6. L3 Bone Marrow Water T2 - Astronauts

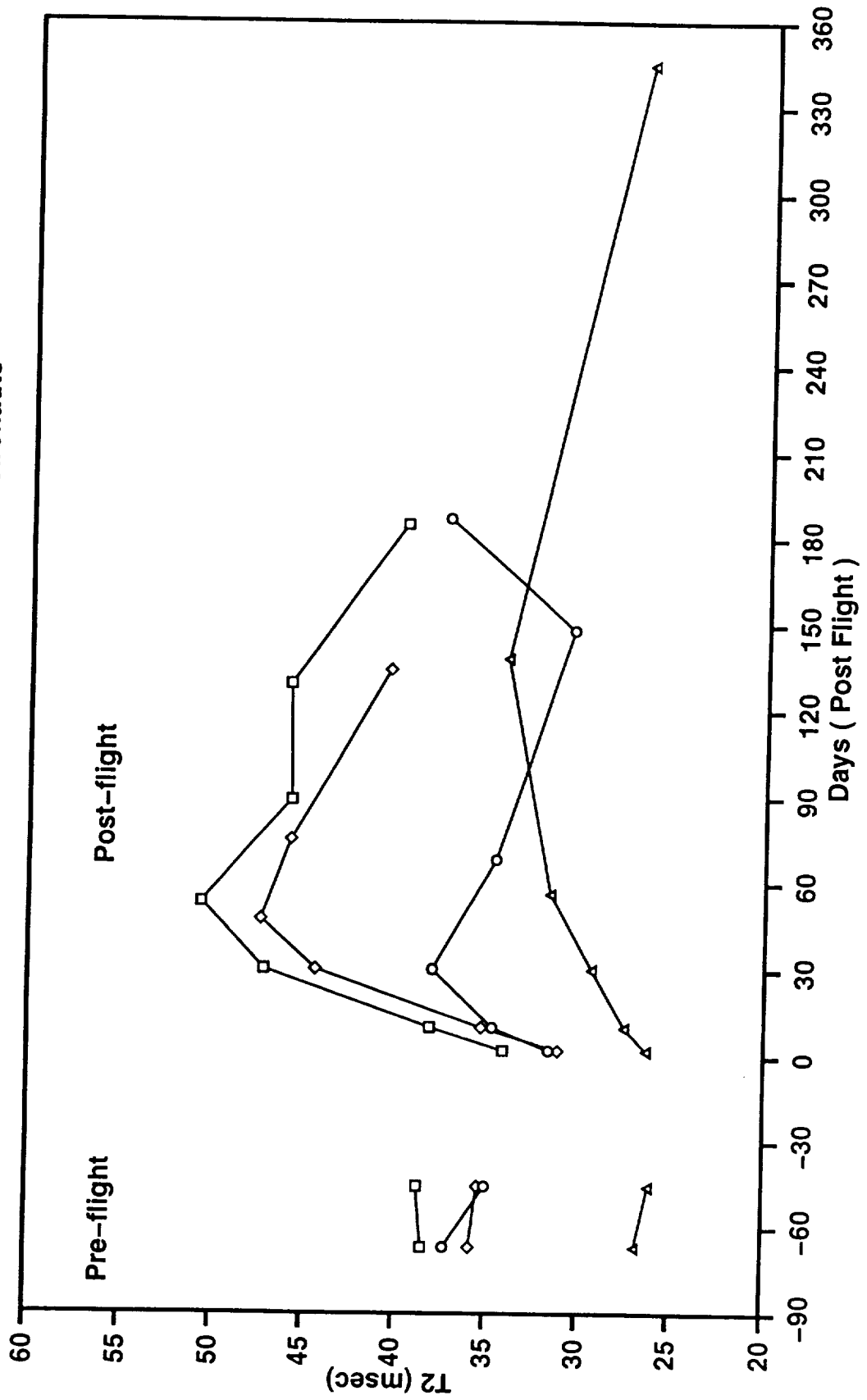


Figure 7. L3 Bone Marrow Fat T2 – Astronauts

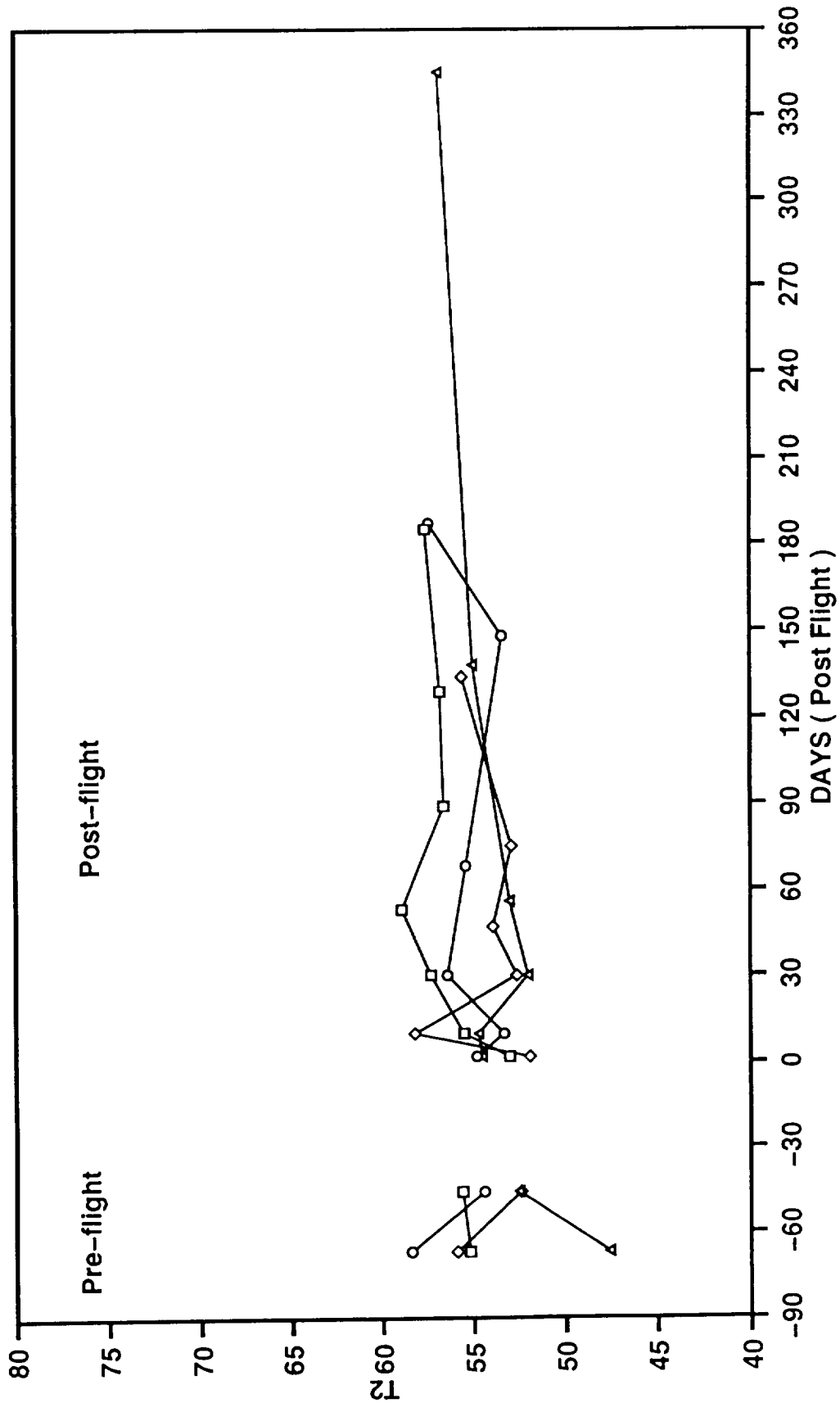


Figure 8. L3 Bone Marrow Water Percentage – Controls

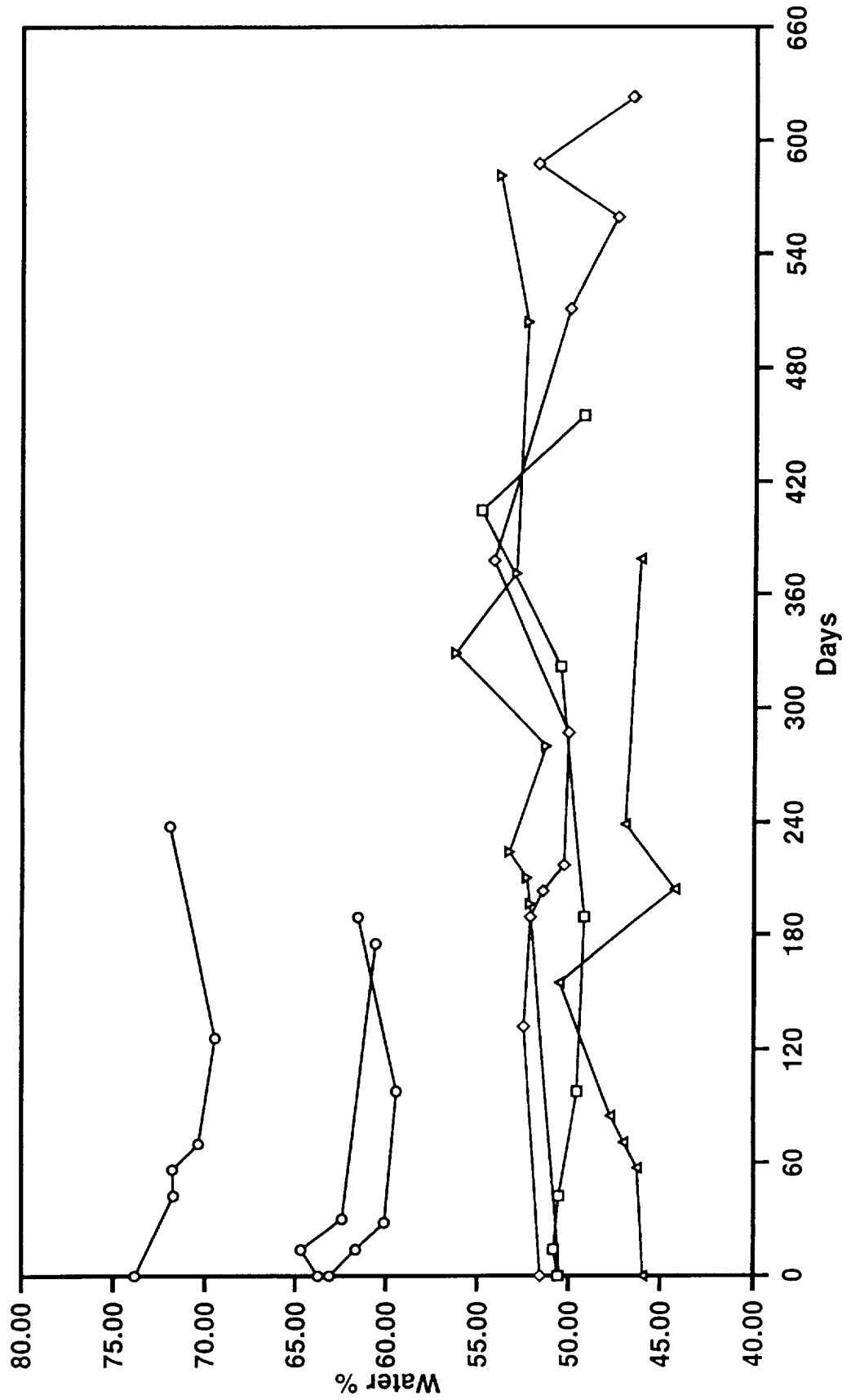


Figure 9. L3 Bone Marrow Water T2 – Controls

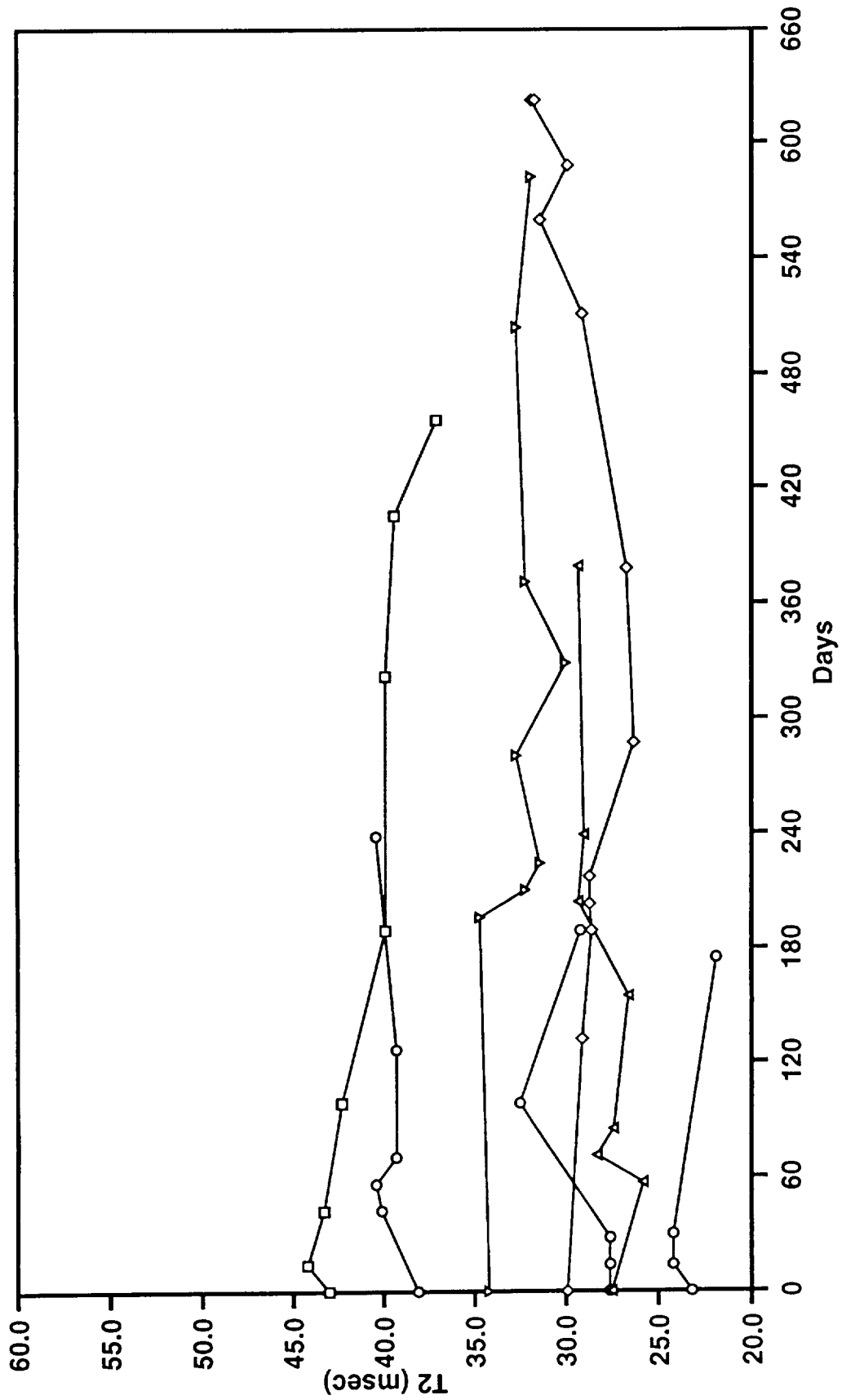
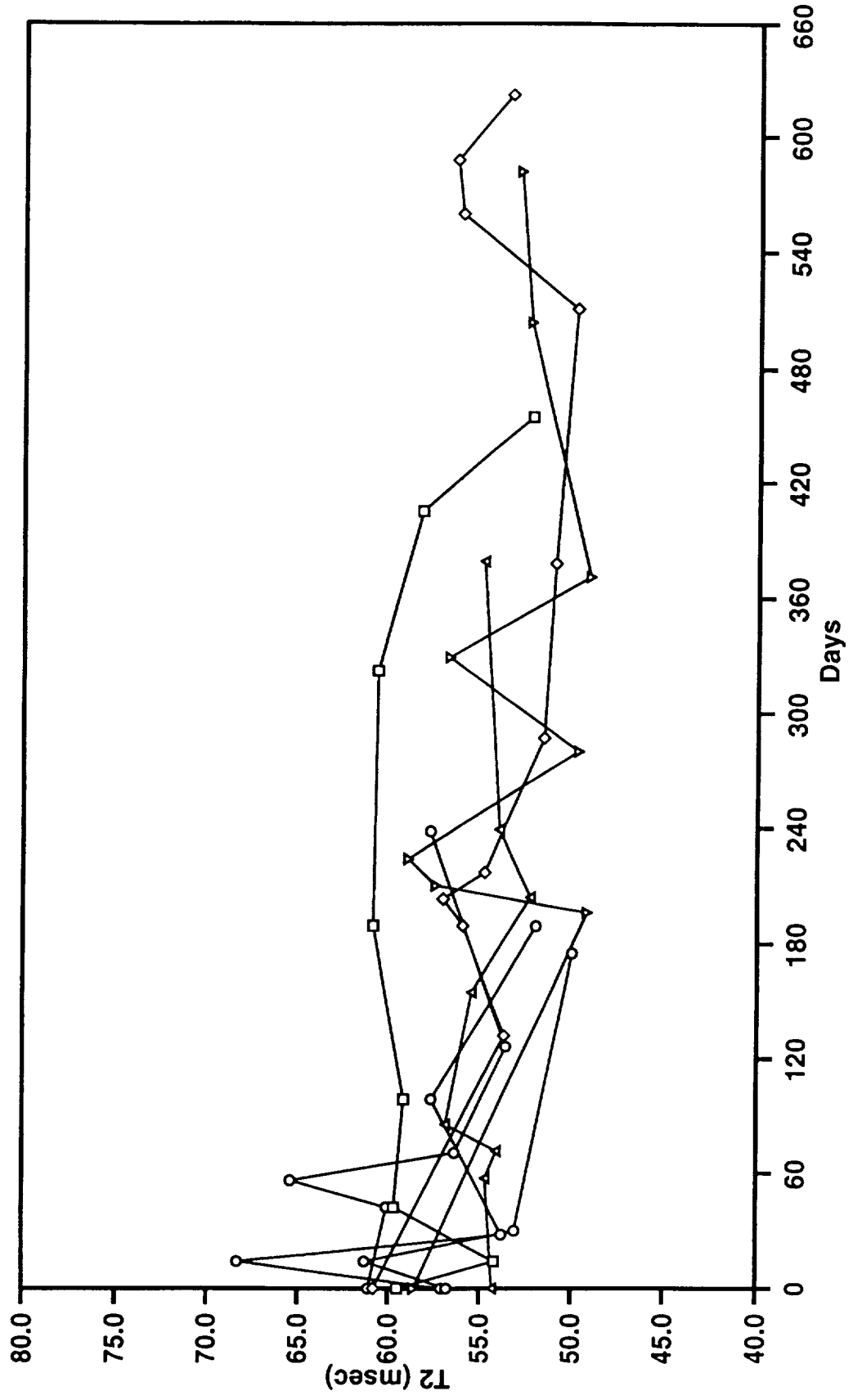


Figure 10. L3 Bone Marrow Fat T2 – Controls



JSC Human Life Sciences Project

**E030 - Extended Studies of Pulmonary Function in
Weightlessness**

Principal Investigator:

Dr. John B. West
University of California, San Diego
La Jolla, California

One Year Post-Flight Science Report
LMS One Year Science Review
Saint-Hubert, Quebec, Canada

**Extended Studies of Pulmonary Function in Weightlessness
Astronaut Lung Function Experiment -- ALFE
E-030**

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1. Objectives

On Life and Microgravity Spacelab (LMS), we extended our very productive studies in Spacelabs SLS-1, SLS-2, and D-2 of the changes in pulmonary function in microgravity (μG). This was done by studying the effects of exercise on pulmonary function in μG , and additionally by studying changes in chemoreceptive control of ventilation in μG . These measurements will clarify the ventilatory responses to both exercise and changes in inspired O_2 and CO_2 , and therefore give information on the function of the chest wall and respiratory muscles. We performed a comprehensive battery of pulmonary function tests both on the ground, and in flight, before and after 85% of maximal exercise testing performed by another experiment (E-920). These studies will shed light on the effects of the known alterations in the distribution of ventilation, perfusion, ventilation-perfusion ratio, and body fluids on the exercise stressed lung, on the mechanical and muscular behavior of the lung and chest wall, and on the chemoreceptors that may contribute to dyspnea in μG .

The objectives of the experiment were:

- I. Determine the effect of exercise on the lung in μG . Subjects performed a battery of pulmonary function tests immediately after exercise including measures of cardiac output and lung water, pulmonary diffusing capacity and its subdivisions, and measures of the inhomogeneity of pulmonary ventilation, perfusion, and ventilation-perfusion ratio, and measure metabolic rate via resting oxygen consumption and carbon dioxide production.
- II. Further investigate the alterations in intra-acinar gas mixing in μG . Following up on unexpected results from SLS-2 in the area of gas mixing alterations within the acinus of the lung in μG , the inhomogeneity of ventilation tests were modified by altering the inspiratory flow rates to increase the information return from them.
- III. Determine separately the pattern of chest wall and abdominal movement during normal ventilation, exercise ventilation and induced hyperventilation. While performing the studies, subjects were instrumented with a respiratory inductance plethysmograph (RIP), a non-invasive device that allows continuous monitoring of the motion and contribution of the rib cage and abdomen. While the subject is not on the mouthpiece, unperturbed measurements of inspiratory and expiratory times were made.
- IV. Determine the ventilatory response to carbon dioxide during μG , and pre- and post-flight. Subjects performed a hypercapnic response test in which they rebreathed from a bag containing some CO_2 in a hyperoxic gas mixture.
- V. Determine the ventilatory response to acute hypoxia pre- and post-flight. Subjects rebreathed a low O_2 gas mixture from a bag in which the CO_2 was actively removed to maintain isocapnia in the face of increased ventilation and decreasing O_2 .

2. Background

The LMS project originated from a proposal submitted in response to AO-84, and which was initially manifested on the SLS-3 flight. This proposal in turn stemmed from our (then

planned) SLS-1 and SLS-2 investigations. Those investigations are now complete, and many of the results have been reported. The publications to date from SLS-1, D-2, and SLS-2 are listed below. The mission team has been involved with the SLS-1, SLS-2, D-2, and EuroMir-95 flights.

3. Methods

Hardware

The hardware flew on both SLS-1 (STS-40) and SLS-2 (STS-58), and is documented in the publications arising from those missions. A complete description of the system is available in Guy et al. (1991a). Briefly, the system centers around a bag in box system. Gases are dispensed into the bags within the box, and the subject controls the breathing path based on prompts presented on an alphanumeric display. Flow was measured with a Fleisch #2 pneumotachograph in the wall of the bag in box which was coupled to a Validyne MP-45 differential pressure transducer. Gas concentrations were measured at the lips of the subject with a quadrupole mass spectrometer (GASMAP).

For LMS, a new microcomputer was used. This provided the ability to directly store data on board on magneto-optical disk cartridges, as well as providing better display capabilities for the crew member. Completely new software was developed and flown. The Respiratory Inductance Plethysmograph (RIP) was borrowed from its previous use on D-2 (STS-55) and packaged to operate as a stand-alone unit. New RIP suits were developed.

The flow measuring system was calibrated before and after measurements by integration of flow from a 3 liter calibration syringe. Alinearities of the flowmeter were determined preflight using the method of Yeh et al. (1982) and corrected for in the analysis. The mass spectrometer was calibrated according to manufacturer instructions preflight and the calibration checked daily by sampling all the gas mixtures carried on board. Mass spectrometer sample transport time was determined daily by sampling a sharp puff of CO₂ containing gas and correcting for the delay in the subsequent analysis.

Test Sequence and Data Collection Schedule

Subjects performed a predefined sequence of pulmonary function tests on themselves. The sequence was:

- REB – Rebreathing Cardiac Output and Diffusing Capacity
- SSB – Slow single breath test and high oxygen REB
- QDT – Distribution of pulmonary perfusion
- SBH – Slow single breath test with breathhold
- RGE – Resting Gas Exchange
- FSB – Fast Single Breath test
- RGE -- Resting Gas Exchange
- FBH – Fast Single Breath test with Breathhold
- COV – Control of Ventilation (CO₂ rebreathing response)

This sequence was performed prior to exercise, and 10 minutes after the completion of the 85% $\dot{V} O_2(\text{max})$ ramp exercise protocol of the E-920 experiment. In addition to the four payload crew, who each performed the protocol pre- and post-exercise, additional data were collected on two of the orbiter crew who performed the protocol pre-exercise only. Additional pre-exercise data was also collected on the payload crew on some flight days in which E-920 exercise was not scheduled. The actual sequence of data collection is described below. For

convenience, data collection sessions scheduled over two day periods (e.g., FD-8 and FD-9) in which the crew were split across the two days are referred to as a single session (FD-8). Data collected in 1G were with the subject seated on a tall chair, with the legs extended somewhat below the chair (a draftsman's chair)

In addition to the above PFT sequence, an isocapnic hypoxic rebreathing response test was performed pre-exercise only, in the pre- and post-flight periods only.

Day	Pre-Exercise PFT	Pre-Exercise Hypoxic Response	Post-Exercise PFT
L-90	X	X	
L-60	X	X	X
L-30	X	X	X
L-15	X	X	X
FD-2	X		
FD-4	X		X
FD-8	X		X
FD-12	X		
FD-15	X		X
FD-17	X		
R+0	X		
R+1	X	X	X
R+5	X	X	X
R+9	X	X	
R+15	X	X	

Test Details and Data Analysis

REB – Rebreathing Cardiac Output and Diffusing Capacity. Beginning at residual volume (RV), subjects inspired a test mixture containing 0.3% C¹⁸O, 0.6% C₂H₂ and 10% Argon. The test mixture also contained either 21% O₂ or 85% O₂, allowing determination of the pulmonary capillary blood volume and membrane diffusing capacity from the diffusing capacity measurements. High oxygen measurements were made immediately following the slow single breath test, and a 30 second period of oxygen pre-breathing. In flight, a loss of the oxygen mixture resulted in a contingency procedure of 30 seconds of pure oxygen pre-breathing using an emergency breathing mask to be implemented. In all cases however, an alveolar oxygen percentage of at least 80% was achieved for the high oxygen test.

Analysis of the rebreathing was performed using a modification of the method of Sackner et al. (1975). A linear least squares fit to the logarithmically corrected soluble gas concentration was performed to the alveolar plateau data from breath number 3 of the rebreathing period to the end.

SSB – Slow single breath test; SBH – Slow single breath test with breathhold; FSB – Fast Single Breath test; FBH – Fast Single Breath test with Breathhold. These four tests form a suite of single breath tests designed to shed light on previously unexpected findings from SLS-2. During that mission, helium and sulfur hexafluoride phase III slopes unexpectedly

reversed, with SF₆ slope actually becoming flatter than that of He in μ G after a breathhold. These results suggest that there is either some change in acinar conformation or in the propagation of the cardiac pressure wave through the lung (Prisk et. al, 1996). Interestingly the effect is not seen in the short periods of μ G in parabolic flight (Lauzon et. al, 1997).

All prior single breath tests were performed with inspiratory flow rates of 0.5 l/sec. In this suite of tests, we varied inspiratory flow rate between 0.25 l/sec (the slow tests) and 1.0 l/sec (the fast tests) altering the position of the stationary front between convective and diffusive gas transport in the lung periphery. The addition of a breathhold in some tests allows time for some diffusive equilibration to occur.

Because of the loss of the O₂ mixture which contained the He and SF₆, only pre-exercise single breath measurements on the four payload crew could be made on FD-2 and FD-4, after which the gas supply was exhausted.

QDT – Distribution of pulmonary perfusion. This test was a repeat of the hyperventilation breathhold study performed in SLS-1 and SLS-2. Subjects hyperventilate for 20 seconds to lower overall lung CO₂, and then breathhold at TLC for 15 seconds. During the breathhold, CO₂ evolves at a rate proportional to regional pulmonary blood flow, marking regions of different perfusion. Markers of inhomogeneity are cardiogenic oscillations and a terminal change in CO₂ concentration after airways closure (Prisk et. al, 1994). This test was included in the LMS mission to examine the effects of exercise on pulmonary blood flow in μ G.

RGE – Resting Gas Exchange. A measure of resting metabolic rate and gas exchange status. Subjects breathe quietly on the mouthpiece for 120 seconds during which time only inspired flow is measured (bag volume limitations preclude the collection of the exhalate during this period). This is followed by 60 seconds of breathing during which both inspiration and expiration are recorded. The test terminates with a slow controlled exhalation from TLC to RV during which time the range of intra-breath respiratory exchange ratio can be determined, providing an indication of the range of \dot{V}_A/\dot{Q} present in the lung (Prisk et al., 1995).

COV – Control of Ventilation (CO₂ rebreathing response). The method of Read (1967) was utilized. Subjects rebreathed from FRC from a bag filled with 60% O₂, 5% CO₂, the balance N₂. As O₂ was consumed, and CO₂ produced the bag concentrations changed, although even at the completion of the test, O₂ level in the bag was still hyperoxic (>21%) ensuring no stimulation of the hypoxic chemoreceptive system. Subjects rebreathed from the bag for 4 minutes, or until the CO₂ level reached 10%, or until the subject voluntarily terminated the test. Ventilation was recorded on a breath by breath basis and the plot of VE vs. CO₂ used to determine the CO₂ responsiveness.

RIP -- Respiratory Inductance Plethysmography. Throughout the sequence of tests, subjects wore a close fitting lycra suit with wires embedded at the level of the nipples and the umbilicus. The RIP system developed for the D-2 mission was used, and provided with an independent power supply for the LMS mission. The RIP was calibrated during the sequence by a series of iso-volume maneuvers where the subject made small inspiratory efforts against a closed glottis or airway. This provides a measure of the relative gains of the rib cage and abdominal bands of the system. Total gain determination was derived from the periods of quiet breathing on the mouthpiece during which time, total respired volume is measured by the flowmeter in the bag-in-box system.

Hypoxic Response. We used a modified form of the system of Rebeck and Campbell (1974). Using a separate system (Mackey, 1996) subjects rebreathed from FRC from a bag initially containing air. As subjects rebreathed from the bag, CO₂ rose and O₂ fell. A scrubber circuit containing a fan, whose speed was controlled by a computer, and a canister filled with soda lime drew air from one end of the bag, and returned it to the other. A computer measured the end tidal CO₂ as the subject exhaled and adjusted the fan speed to allow CO₂ to rise in the bag, or to remove CO₂ so as to maintain a constant end-tidal CO₂ from the subject. End-tidal CO₂ was held at a level approximately 0.3% above the resting end-tidal CO₂ level of the subject as determined in a 30 second period of quiet breathing prior to the start of rebreathing. Subjects continued to rebreathe until inspiratory oxygen in the bag fell below 7.5%. Blood oxygen levels were monitored with a Nellcor N-200 pulse oximeter on the finger of the subjects. The total system delay between SaO₂ and inspired gas concentrations was determined by measuring the delay between the first inspiration of air at the end of the rebreathing period, and the nadir of the SaO₂ recording, and was typically ~20 seconds. SaO₂ was then time shifted by this delay to correspond with the other signals and the response determined from the breath by breath \dot{V}_E vs. SaO₂ plot.

Statistical Treatment

We followed the same statistical approach used on prior spaceflights. Subjects acted as their own controls. For pre-exercise data only, we included all sessions detailed in table 1, and included data from 6 subjects (four payload crew plus the two orbiter crew on whom we obtained significant amounts of inflight data). Preflight data were tested for stability, and then pooled to form a single baseline. Measurements were then tested on a day by day basis, and as grouped periods of inflight and postflight.

When considering the effects of exercise, data were resampled to include only the four payload crew (who were the only subjects in which we have post-exercise data), and the number of days under consideration reduced to only those shown in table 1 when post-exercise data were collected. Preflight data were tested for stability, and then pooled to form a single baseline. Measurements were then tested on a day by day basis, and as grouped periods of inflight and postflight, as well as for pre-exercise or post-exercise. Differences between pre- and post-exercise were tested for in all cases. Note that because of the resampling necessary in this process, numbers for pre-exercise measurements from the "exercise" data set do not necessarily match numbers derived from the data set involving all 6 crew, as fewer subjects and days are involved.

In all cases, results are expressed as a percentage of preflight, pre-exercise baseline data and data are normalized on an individual basis to this value. Thus the preflight pre-exercise value is by definition 100%, and the SE is a measure of the intra-subject variability only. This normalization process allows pooling of data from subjects with a wide range of sizes.

4. Results and Discussion

REB – Rebreathing Cardiac Output and Diffusing Capacity. Lung volumes determined from argon dilution during the rebreathing tests show a consistently different pattern from that seen in SLS-1 and SLS-2. In this mission, both RV and FRC were elevated compared to preflight controls, RV by ~20%, and FRC by ~12% (figures 1 and 2), while in prior missions we observed a decrease (Prisk et al., 1993; Elliott et al., 1994). There are however significant differences in the data collected in the control period. In the ground data collected

during LMS, subjects were seated in a high chair, which likely prevented descent of the diaphragm; and as a consequence, lowered FRC measured in 1G. In the data collected in μG however, subjects were in more of an upright posture, without the attendant constraints placed on the diaphragm. Similarly, we hypothesized that the reduction in RV seen in μG in SLS-1 was as a result of the removal of the dependent weight of the lower lung present in 1G. It seems that when subjects are seated however, the support of the raised diaphragm raises FRC somewhat accounting for the increase we saw in μG . Of note in the RV data are the results from R+0 which show a significantly elevated RV, which is abolished in all subsequent postflight data which are constant from R+1 on. This likely stems from the cardiovascular instability of the subjects immediately upon return to 1G, with an unwillingness to fully exhale to RV with the attendant negative cardiovascular consequences of something approaching a valsalva maneuver. Examination of the lung volume data pre- and post-exercise shows no differences as a result of exercise, a result we expected in these data.

Figures 3, 4 and 5 respectively show the cardiac output, heart rate and cardiac stroke volume measured from acetylene uptake during the flight. Once again, the changes in cardiac output and stroke volume observed during LMS were smaller than those seen during SLS-1 (Prisk et al., 1993), and SLS-2 (unpublished observations). This difference is likely due to the different control conditions used in LMS and these other missions (sitting vs. standing), and emphasizes the importance of gravity on venous return from the lower extremities. Even a relatively modest change from standing to an erect sitting posture markedly increases the control cardiac output and thus lowers the apparent effect of microgravity. The modest increases that we see in cardiac output (which are not significant) and in stroke volume are intermediate between the changes we previously observed between standing and μG , and between supine and μG .

Of note is the observation that both cardiac output and stroke volume initially increase upon entry into μG , and thereafter begin to decrease. However, after day 12, there is a substantial increase in both these variables, which is not accompanied by a concomitant increase in heart rate (figure 4). We had previously noted a modest increase in cardiac output and stroke volume on day 9 of SLS-1, and attributed that to either an anticipatory effect as the end of the mission approached, or resulting from increased workload associated with the de-orbit preparations. A similar observation remains unexplained from the SLS-2 results. For comparison, the graphs of cardiac output from SLS-1 and SLS-2 are shown in figure 6. Since we again see an increase in both cardiac output and stroke volume late in flight during LMS, we are led to the conclusion that some competing adaptation mechanism is at work that becomes dominant to the reduction in cardiac output that occurs, presumably secondary to a continuing reduction in circulating blood volume during the early phase of adaptation to μG . Whether the late mission increase in cardiac output and stroke volume has a neurological origin is at present unknown, but the observation may be of considerable interest in the adaptation to long duration exposure to μG .

The determination of the changes in pulmonary tissue volume (V_t) following was confounded by the slow dynamic response of the GASMAP to acetylene. This precluded our ability to accurately resolve the initial inspired acetylene which is critical in the determination of V_t . Calculating V_t using an assumed inspired concentration produces a large increase in V_t that is unphysiological, leading us to believe this to be a measurement artifact (because we believe these data to be erroneous, they are not shown). Further, prior observations of V_t in μG made in the absence of exercise indicate a small decrease (Verbanck, 1997). Despite the questions surrounding the reliability of the V_t measurements in absolute terms, pre- and post-exercise measurements are subject to the same error, and can therefore be compared. There

were no differences in V_I measured pre- and post-exercise suggesting that the 85% exercise level used in the inflight protocol was not detrimental to the fluid balance of the lung.

Diffusing capacity measurements made under normoxic conditions show a pattern similar to that seen on previous missions (Prisk et al., 1993, Verbanck et al., 1997). D_L is raised in μG and remains constant for the duration of the flight (figure 7). The magnitude of the increase is greater in this mission than in SLS-1, but differences in both the choice of maneuver (in this case, a rebreathing maneuver, in SLS-1, a single breath technique), and in the choice of position for the ground control measurements (see above) contribute to this. The rapid change and return again suggest that a strictly mechanical process is involved in the changes in D_L , as was suggested from SLS-1. Pre- and post-exercise D_L measurements both preflight and inflight show no difference except on FD-15, when a small, but likely physiologically insignificant difference was seen. This again suggests that the 85% of $\dot{V} O_2$ max exercise protocol used in flight produces not deleterious consequences to the pulmonary system in terms of fluid balance in the lung. High oxygen measurements of diffusing capacity were very variable inflight, perhaps due to the off nominal procedures we were forced to employ following the loss of the high oxygen gas tank. As a consequence, they, and the subdivisions of D_L calculated from them will not be further discussed. Of interest is the incomplete return of D_L to baseline on R+0 (figure 7). These measurements were made rapidly following return to 1G, and without the subject's ambulating. The higher value on R+0, (which is statistically different from all other post flight days) suggests that the fluid balance state of the subject's is different on R+0, perhaps as a result of the fluid loading prior to reentry (which was not a feature of all crew on SLS-1). A similar effect is visible in the cardiac output data (figure 3) where \dot{Q}_c is actually elevated on R+0, and stroke volume (figure 5) is slightly above preflight baseline prior to large decreases in both variables on R+1.

Single Breath Tests: Changes in acinar inhomogeneity in μG . A preliminary analysis of the data relating to acinar inhomogeneity are available at this time. Only limited inflight data were available because of the loss of the O_2 Mix tank pressure during pre-launch activities or during launch. Preflight and postflight data were not different and have been combined. Because of limited inflight gas supplies, data collection was limited to pre-exercise studies only on the four payload crew only.

This experiment was designed to throw light on the possible mechanisms operating at the acinar level that cause inhomogeneity of ventilation. Data from both SLS-2 (Prisk et al., 1996), and D-2 (Paiva, unpublished observations) suggest that in μG , acinar gas mixing changes considerably, especially in smaller subjects, although the cause of this is at present completely unknown, and the result was unexpected. Data collected in periods of short-term μG in the KC-135 suggest that the effect is not present in only 25 seconds (Lauzon et al., 1997). The results point to changes in the manner in which the quasi-stationary diffusion front that forms between inspired and resident gas is spread in the lungs, and consequently how gas is mixed. By using gases of widely different diffusivity (He and SF_6), different breathhold times (0 and 10 seconds, allowing different times for mixing) and different inspiratory flow rates (0.25 l/sec and 1.0 l/sec) which alters the position of the diffusion front (faster inspiration, more peripheral) we hoped to be able to examine how these interaction between diffusive and convective gas mixing was altered by μG . This mixing occurs in regions of lung that were traditionally thought to be too small to be affected by gravity.

The results from this study show that the anomalous responses we observed in SLS-2 and in D-2, are not a universal event, and vary markedly between subjects. Unfortunately the limited number of inflight observations severely limits our ability to draw substantial conclusions

from this data set. Accordingly, the comments below are highly speculative and may not stand up to further scrutiny.

Helium data (figure 8) shows a marked effect of μG , with substantially lower phase III slopes in μG compared with 1G. Further, breathholding reduces phase III slopes considerably, except for slow inspirations in μG (figure 8A, right). In these tests, at slow inspirations the diffusion front is much more central than in the other tests, suggesting that even the highly diffusible helium is unable to equilibrate between units of differing concentration because they are too far apart. That this happens only in μG is puzzling, and suggests some alteration in gas transport may indeed occur in this situation.

Sulfur hexafluoride results (figure 9) again show a reduction in phase III slope in all circumstances, except that of slow inspirations in μG in which there is no change in phase III slope compared to 1G after a 10 second breathhold. In contrast, when the inspiration is faster, phase III slope is considerably reduced, suggesting that the determinants of phase III slope in μG are sufficiently close to that with a distally located diffusion front, the cardiogenic action during breathholding and the effects of diffusion are able to lower the existing concentration gradients between lung units.

The differences between He and SF₆ phase III slopes are shown in figures 10 and 11. These differences previously showed marked reductions in μG , that were even larger following a breathhold in μG (Prisk et al., 1996). In these subjects however, there are no reductions in μG . However an interesting difference exists between the results from the slow inspirations (0.25 l/sec, proximal diffusion front), which show no effect of breathhold or μG , and those performed at a fast inspiratory rate (1.0 l/sec, distal diffusion front) which show a marked reduction in the SF₆-He phase III slope difference following a breathhold. Whether this is an artifact introduced by the sparse nature of the data set is at present unknown, but if it holds up to closer scrutiny is suggestive of the theory that the changes we observed in SLS-2 and D-2 relate to conformational changes in the most peripheral parts of the lung.

QDT – Distribution of pulmonary perfusion. Data from this test are still under analysis.

RGE – Resting Gas Exchange. Analysis of resting gas exchange showed no clear and consistent trends in the data, either inflight or postflight. Accordingly, we present only average data from preflight, inflight and postflight.

Resting gas exchange may be changed by changes in the atmosphere the subjects are in. Figures 12 and 13 show inspired oxygen and CO₂ levels encountered during the mission. FIO₂ was slightly elevated inflight (by ~ 1.3%). Despite an apparent large increase in inspired CO₂, the absolute value of the increase was small (~0.2%, or 2 Torr). Such an increase is below the level likely to cause any alteration in resting ventilation based on data obtained in the CO₂ exposure study performed in a chamber in Germany (Elliott et al., 1997). The slight increase in inspired CO₂ post exercise inflight likely reflects the increased CO₂ load in the Spacelab resulting from the exercise itself.

Expired gas tensions show a slight increase in end tidal O₂ that inflight mirrors the changes in inspired levels (figure 14). Similarly CO₂ is slightly elevated inflight (figure 15). The absolute magnitude of these changes are small (O₂ ~ 2.3 mmHg, CO₂ ~1.6 mmHg). Postflight however, there is a reduction in expired O₂ and an increase in expired CO₂, possibly suggesting a slight change in the setting of the resting ventilation levels. A similar increase in end tidal CO₂ was seen in the SLS-1 and SLS-2 results (Prisk et al., 1995). Post exercise

there are modest reductions in both expired O_2 and CO_2 in all states, although the cause is at present unclear.

Neither total minute ventilation (figure 16), nor respiratory frequency (figure 17) show any significant changes either as a result of spaceflight, or as a result of exercise. Similarly, the SLS-1 and SLS-2 data show little or no change in these parameters. The absence of a change post exercise indicates that subjects had fully recovered from the modest bout of exercise to which they were subjected, and suggests that there was no residual effect of this exercise on resting ventilation. Ventilatory drive, as indicated by T_i/T_{TOT} was slightly elevated by microgravity and remained so postflight (figure 18), but the change was small. Ventilatory drive seemed unaffected by exercise.

Gas exchange ($\dot{V} O_2$, fig 19; $\dot{V} CO_2$ fig 20) was slightly elevated inflight, and clearly elevated postflight. This increase likely explains the changes in post flight end tidal O_2 and CO_2 (figures 14, 15), which occurred in the absence of large changes in ventilation. The cause of this increase is unknown. On previous missions, there was a modest increase in gas exchange inflight, but this returned to baseline postflight (Prisk et al., 1995).

Overall the data suggest only small or no changes in the resting gas exchange status of the subjects, and no adverse effects of the bout of exercise the subjects were required to perform.

Data from the slow controlled exhalation, during which time intra-breath respiratory exchange ratio (and subsequently the range of \dot{V}_A/\dot{Q} in the lung) is calculated are still under analysis.

COV – Control of Ventilation (CO_2 rebreathing response). There were no apparent changes in the hypercapnic control of ventilation due to μG per se as evidenced by either the slope of the CO_2 response curve (ventilation as a function of CO_2) (see figure 21), or in the Ventilation measured at a PCO_2 of 60 mmHg (figure 22). However, the maximum CO_2 reached at the end of the 4 minutes of CO_2 rebreathing figure 23) was significantly reduced by μG . The cause of this reduction is at present unknown and still under investigation.

Exercise increased the CO_2 responsiveness of the subjects both in 1G, and in μG , although the variability of the measurements makes these increases non-significant. In all cases exercise reduced the maximum PCO_2 reached at the end of the rebreathing period (figure 23), although the magnitude of this reduction in μG was much greater than in either the preflight or postflight periods. The decrease in CO_2 levels during the rebreathing is curious given the lack of any large change in $\dot{V} CO_2$ (see figure 20). Whether it indicates anything of physiological significance is yet to be determined.

RIP -- Respiratory Inductance Plethysmography. Data from the RIP system are still under analysis.

Hypoxic Response. The hypoxic ventilatory response measurements were performed preflight and postflight only, and were only performed during the pre-exercise PFT sessions, and we attempted to reduce their arterial oxygen saturation to 75%.

Hypoxic ventilatory response was elevated postflight compared to preflight (although the changes failed to reach statistical significance at the $p < 0.05$ level) when considering both the slope of the response (figure 24) and the ventilation at and arterial oxygen saturation of 75% (figure 25). Of particular note are the data collected on R+1 which show a marked increase in response, and a considerably larger variability in the results compared to all other

postflight and preflight measurements. The increase in the ventilatory response is mirrored in both tidal volume and frequency data (not shown) which both show substantial increases in both magnitude and variability on R+1.

These data suggest that there are likely substantial changes in the hypoxic ventilatory response as a result of μG . Because of the logistical constraints imposed by the postflight data collection scenario, we were unable to obtain HVR data on R+0, immediately following spaceflight. However it appears likely that had we been able to perform these measurements, an even higher response would have been seen. Similarly, the data are suggestive of an increased sensitivity to hypoxia in μG . Further, the data were collected under nearly normocapnic conditions, with end tidal CO_2 being controlled to only very slightly above the subject's normal end tidal value. It is known that there is synergism between the hypoxic and hypercapnic ventilatory responses. Thus, it seems likely that the inflight studies of the HVR which will be performed during Neurolab, during which we will control CO_2 to an elevated isocapnic level will yield interesting and provocative results.

5. Conclusions

Despite the loss of the O_2 Mix tank and limited data collection capability, significant data were obtained during LMS. There are few differences to be found between the pre-exercise and post-exercise data. We attribute much of this to the severe descoping that was forced upon the experiment by the inflight restriction of an exercise level of only 85% of maximum $\dot{V}\text{O}_2$. We are therefore unable to provide any substantial conclusions regarding the effect of heavy exercise on the lung in μG . However it appears that modest exercise has no adverse effects on lung function in μG . Further data interpretation will have to wait for more complete analyses and publication of detailed papers.

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7. Summary

The Astronaut Lung Function Experiment (ALFE) studied the effects of moderate exercise in μG on the lung and investigated changes in the chemical control of ventilation that are hypothesized to occur upon exposure to μG . The analysis completed to date suggests that moderate exercise in μG has no adverse consequences on the lung, although the effects of extreme exercise remain unknown. There were no changes in the ventilatory response to inhaled carbon dioxide caused by μG . However, based on measurements that were only able to be performed before and after the LMS mission, it seems likely that the ventilatory response to lowered oxygen levels in the blood (in this case caused by breathing a low oxygen mixture during a portion of the experiment) is increased in μG . Such a heightened response has the potential to disrupt sleep, and sleep is known to be of poor quality during μG . This effect will be directly investigated during the upcoming Neurolab Spacelab mission in 1998.

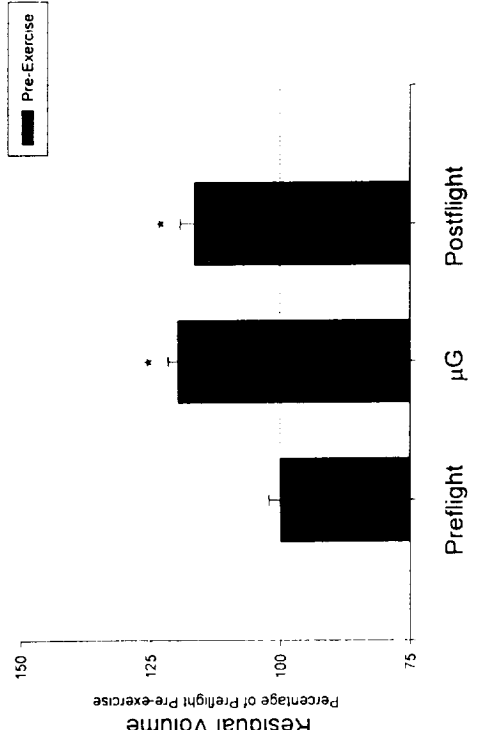
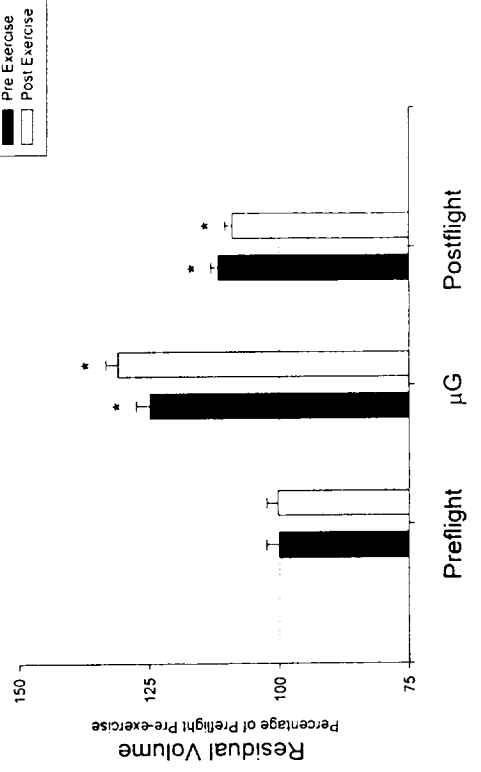
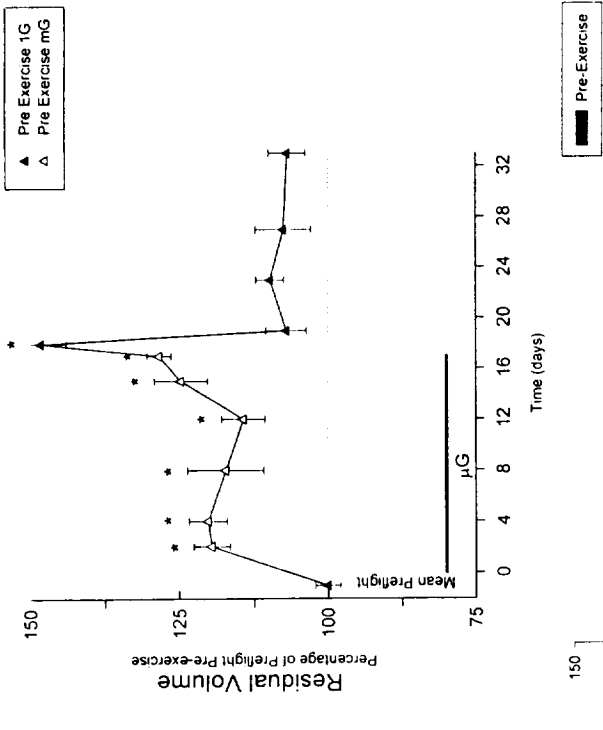
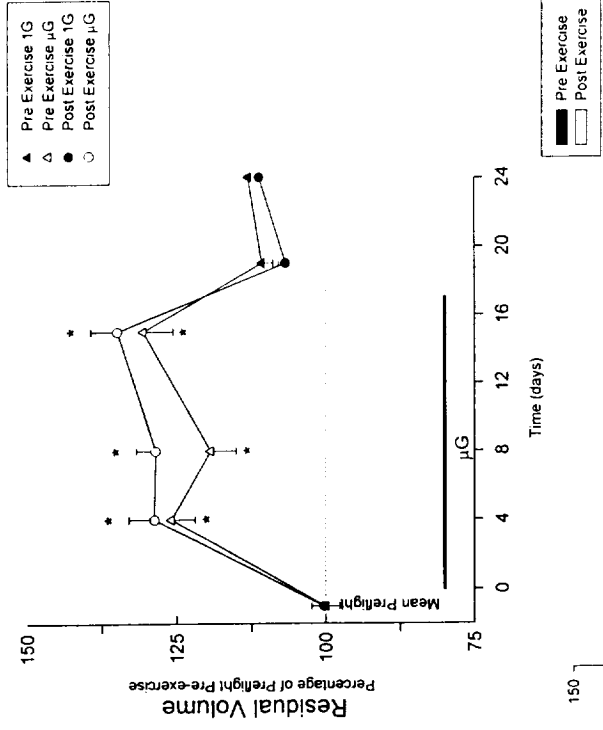


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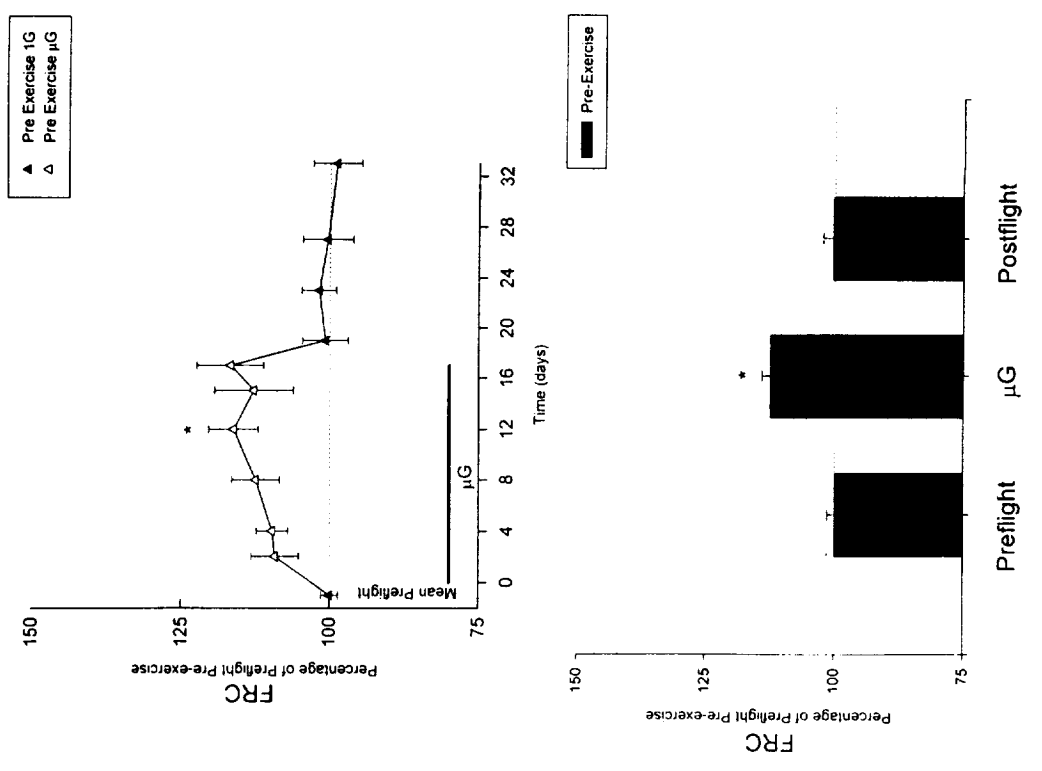
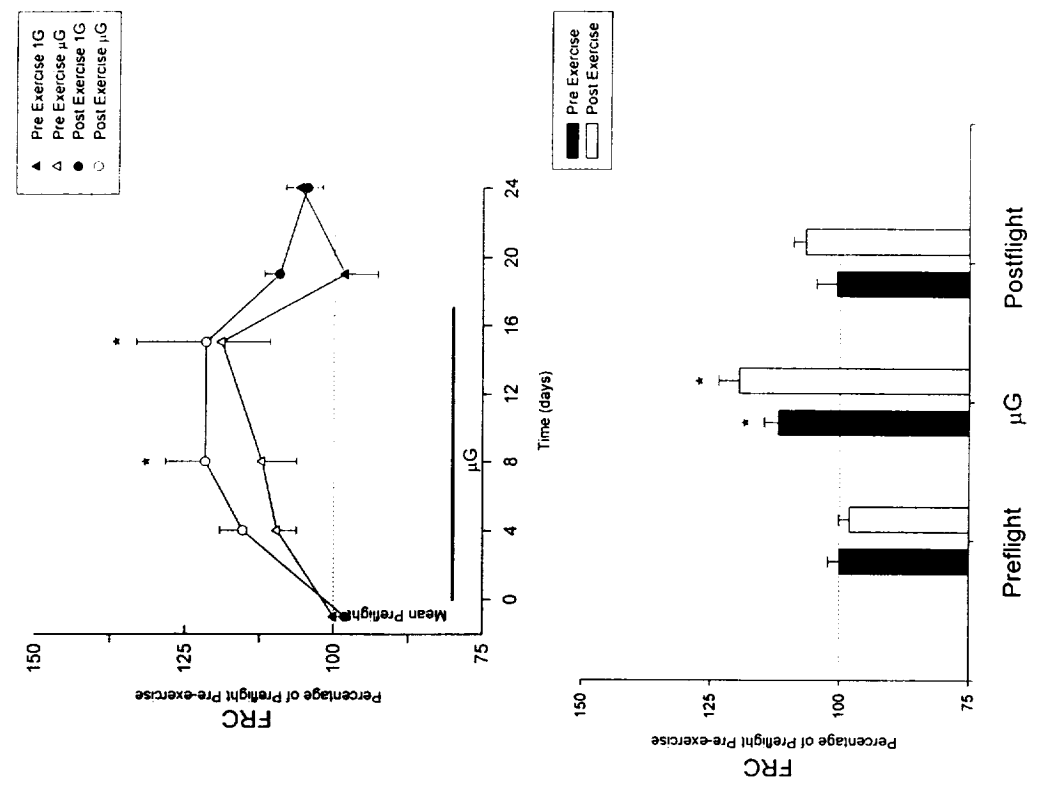


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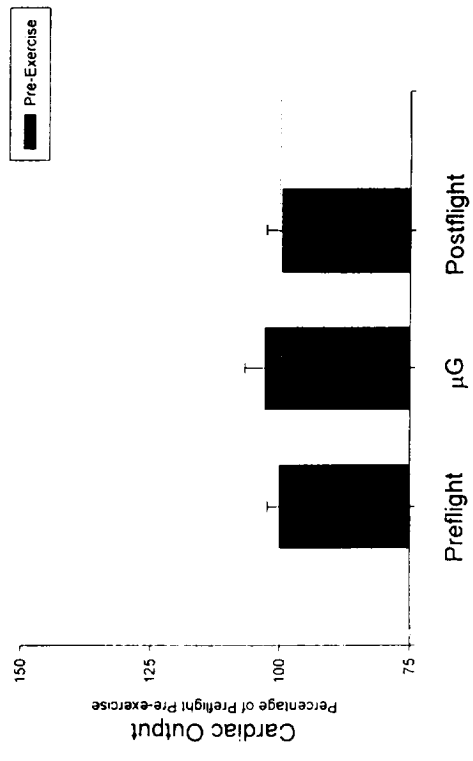
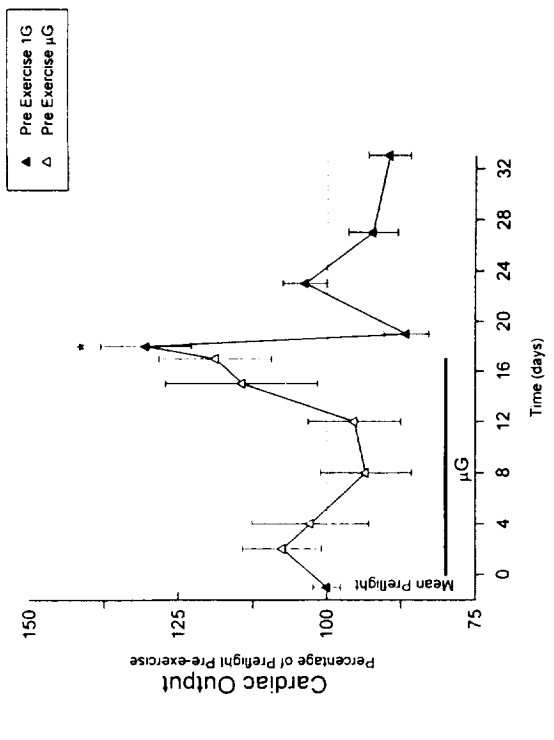
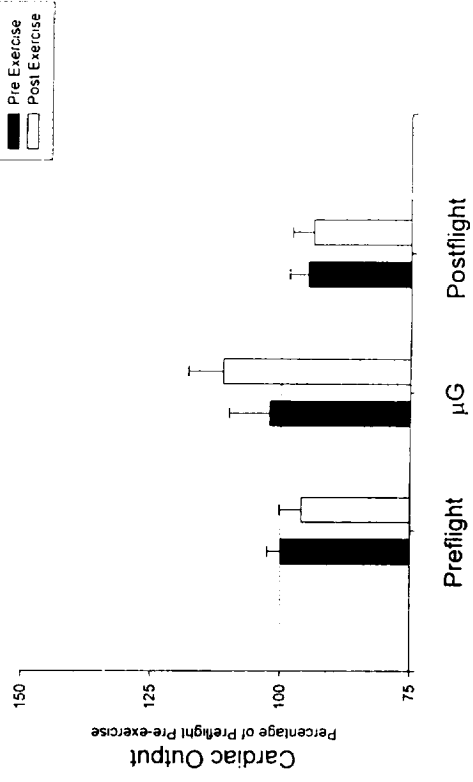
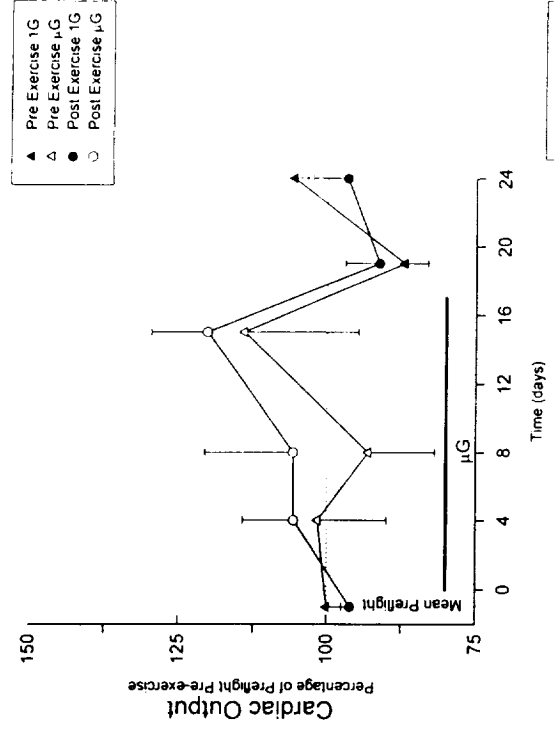


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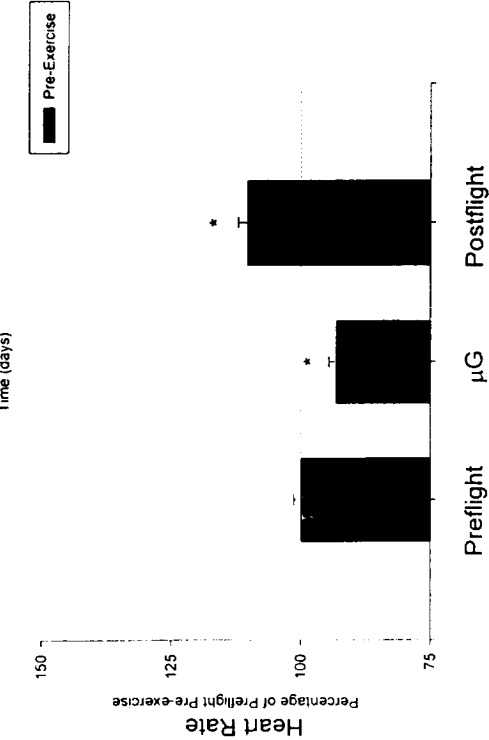
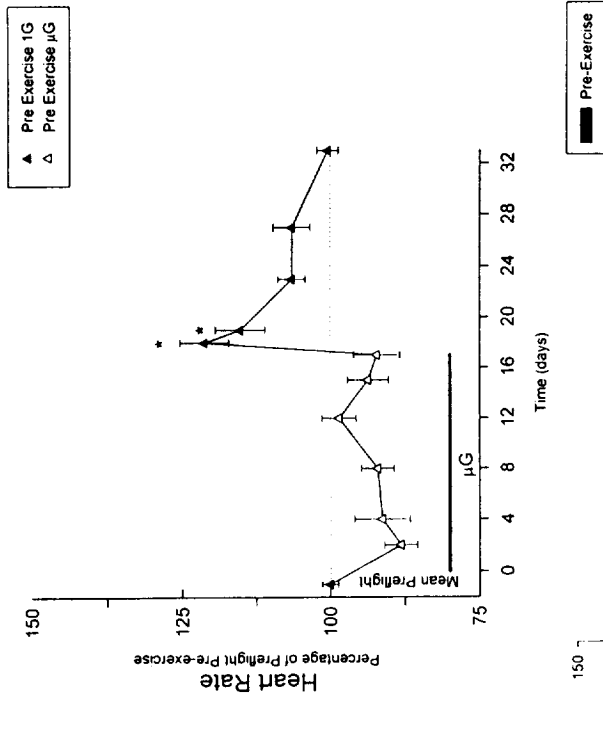
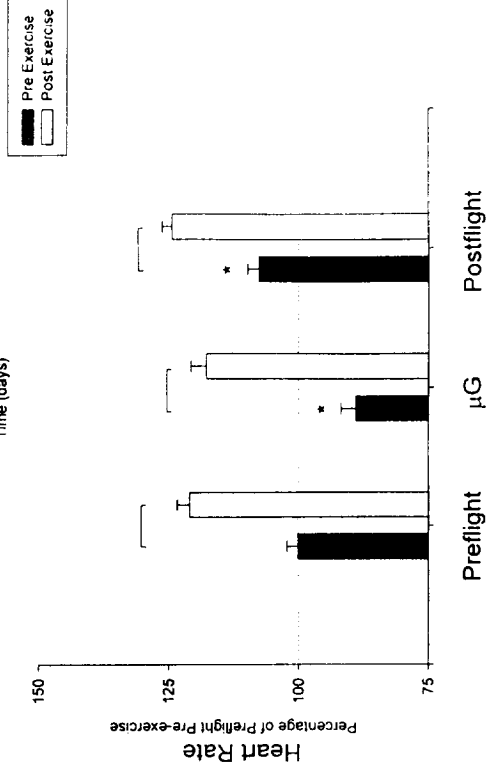
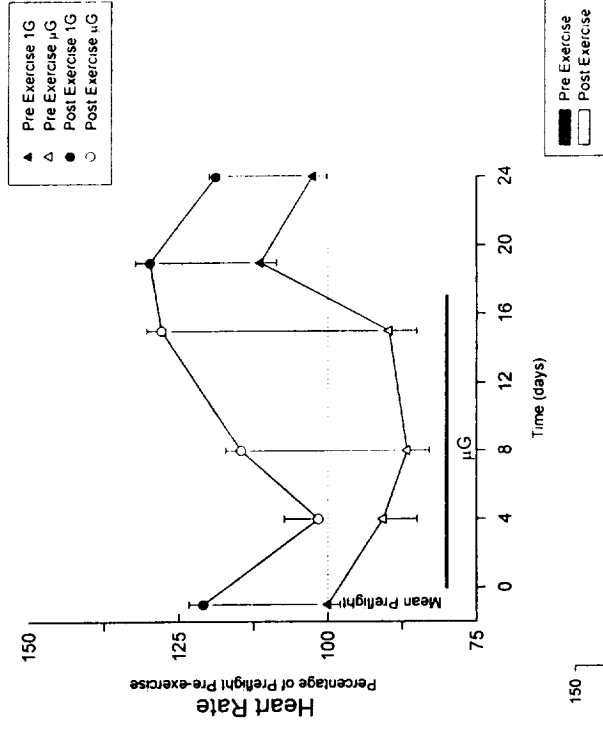


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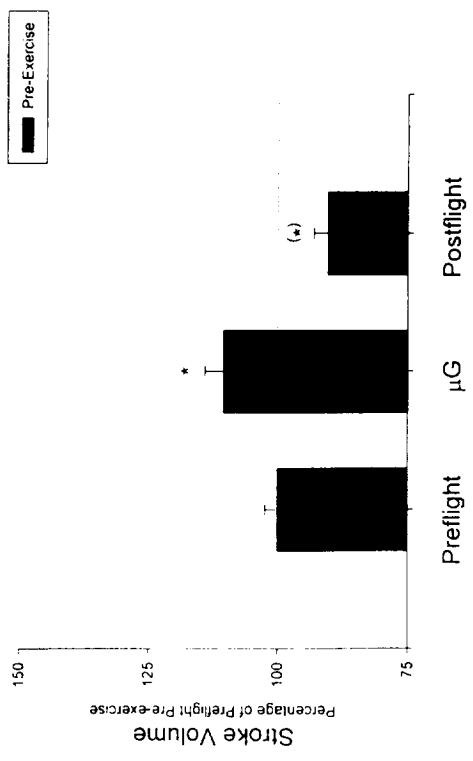
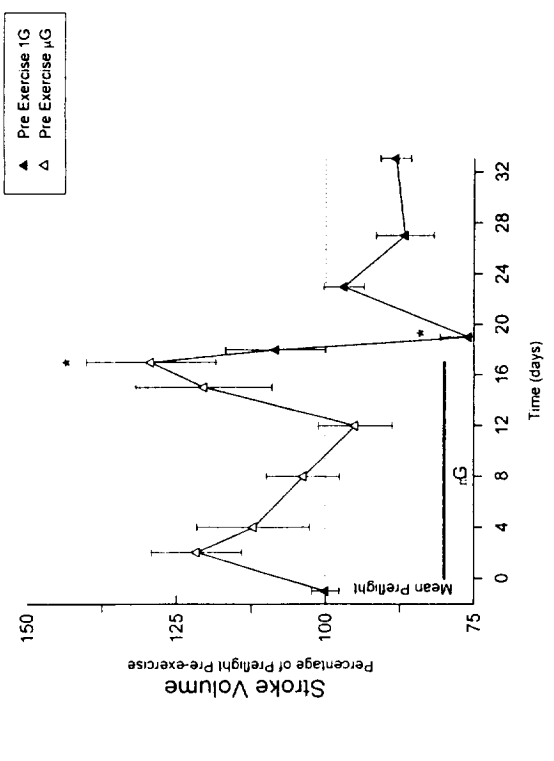
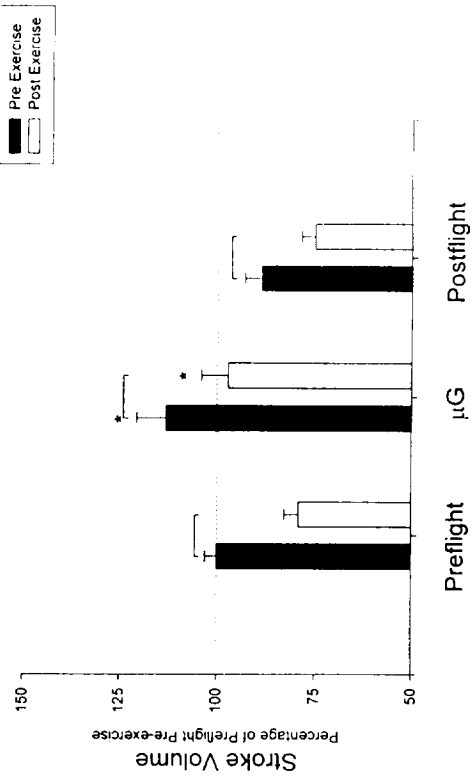
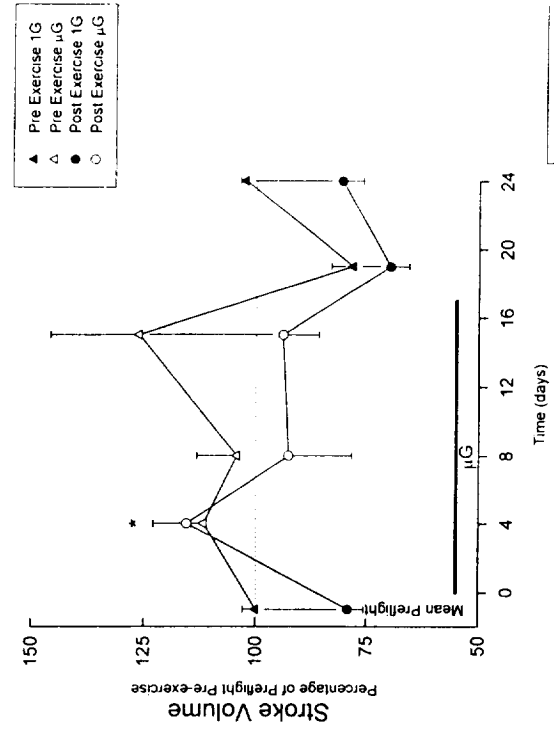
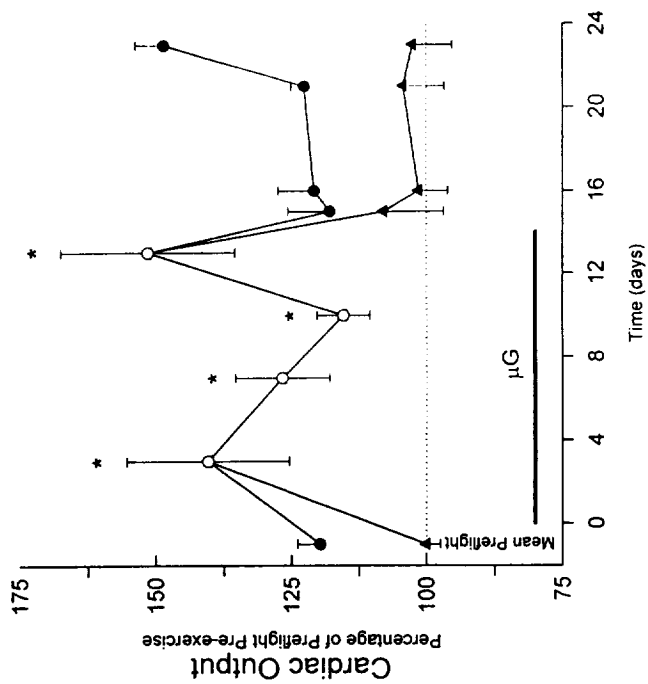


figure 5

Q_c -- SLS-2



Q_c -- SLS-1

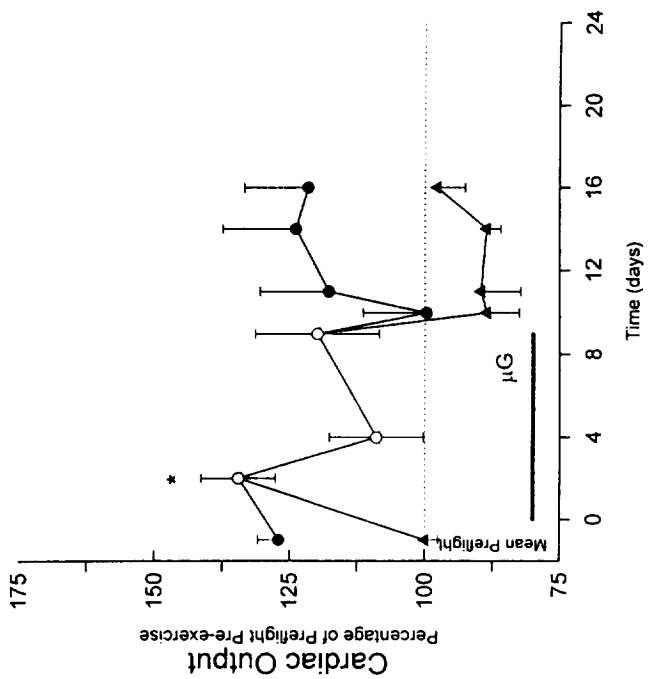


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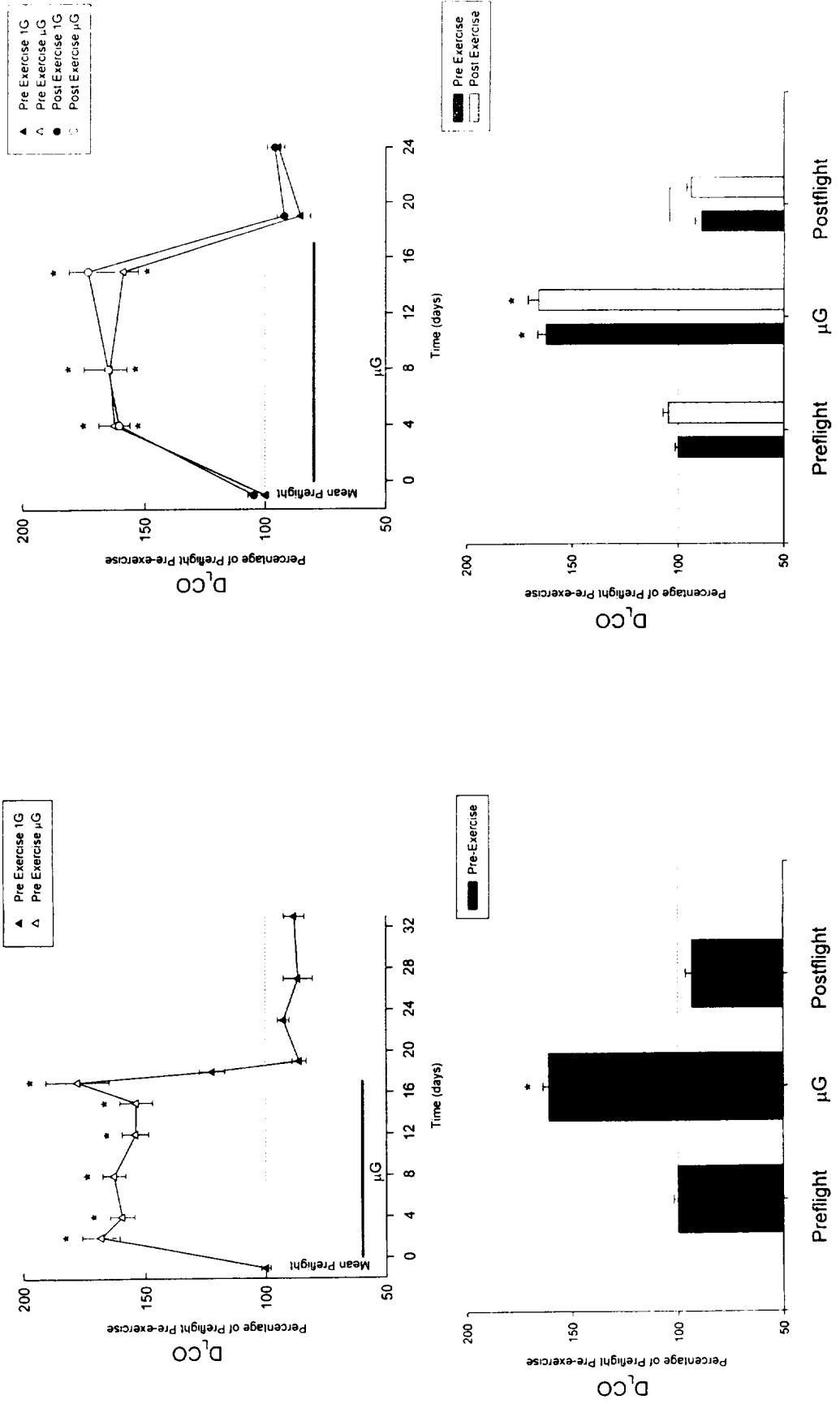


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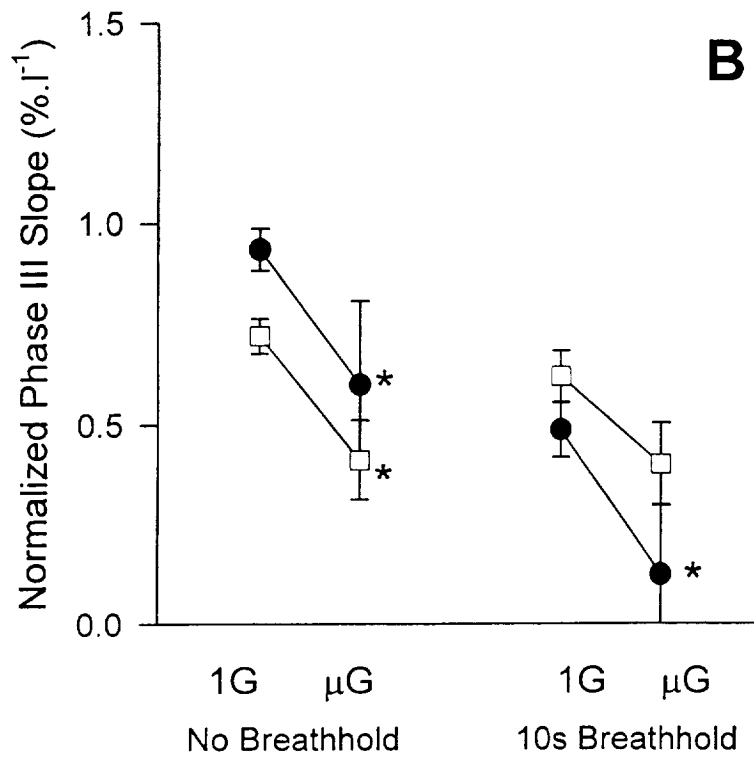
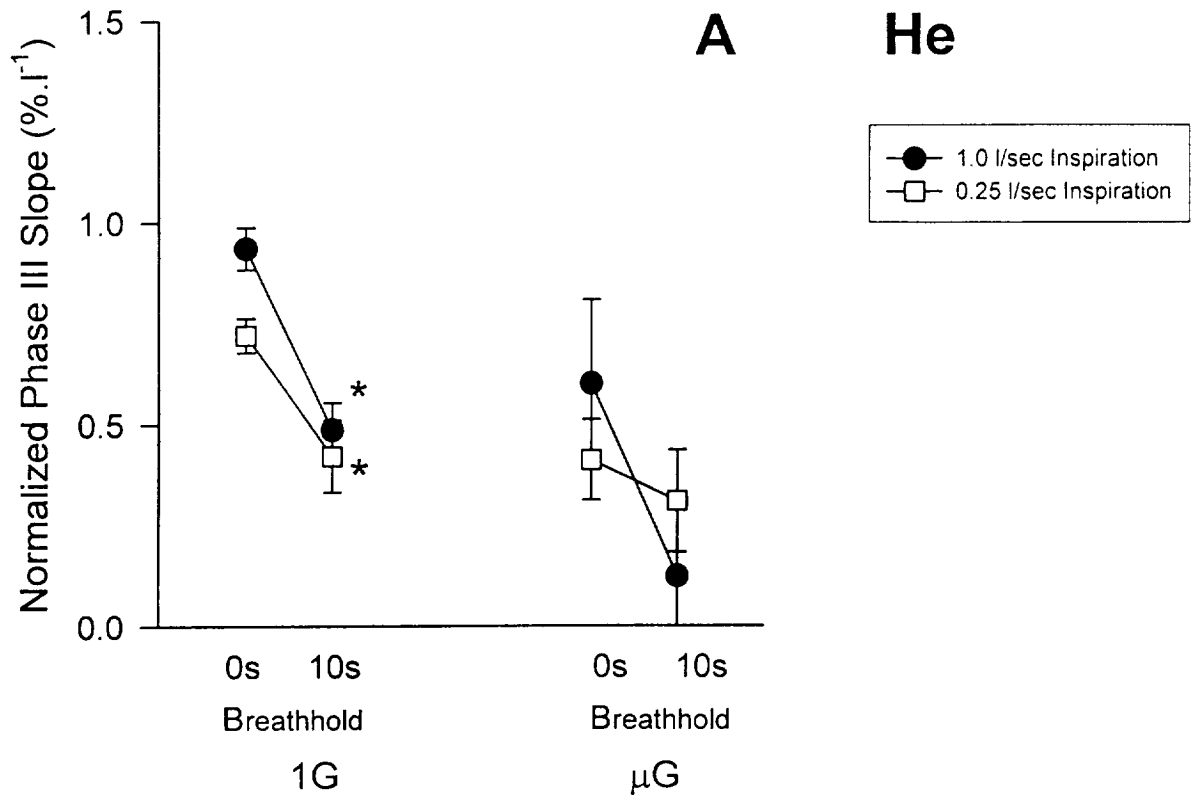


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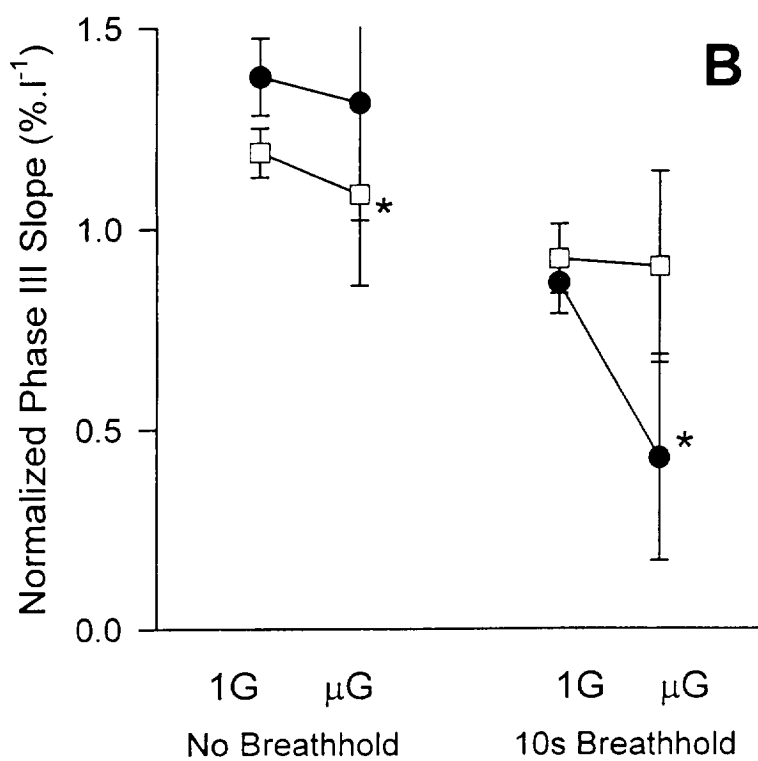
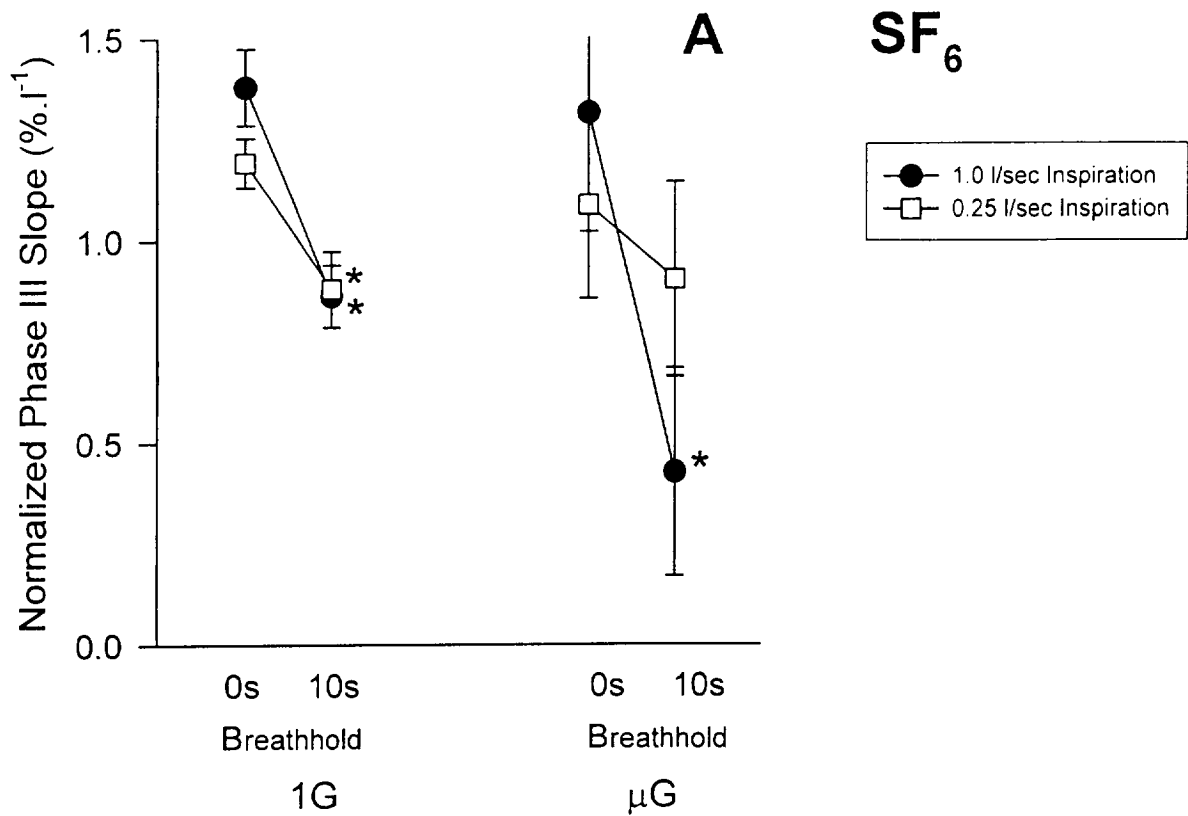


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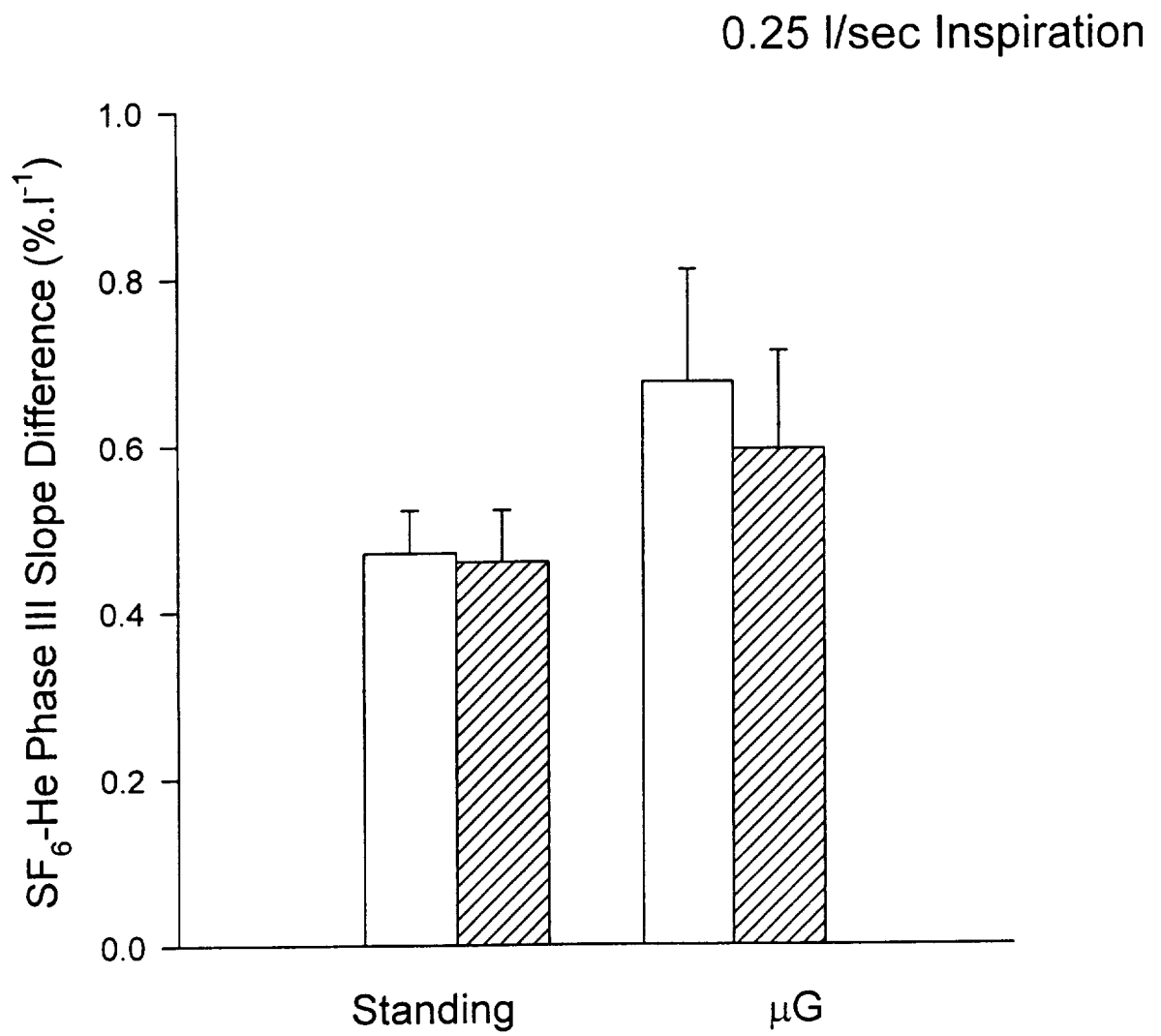


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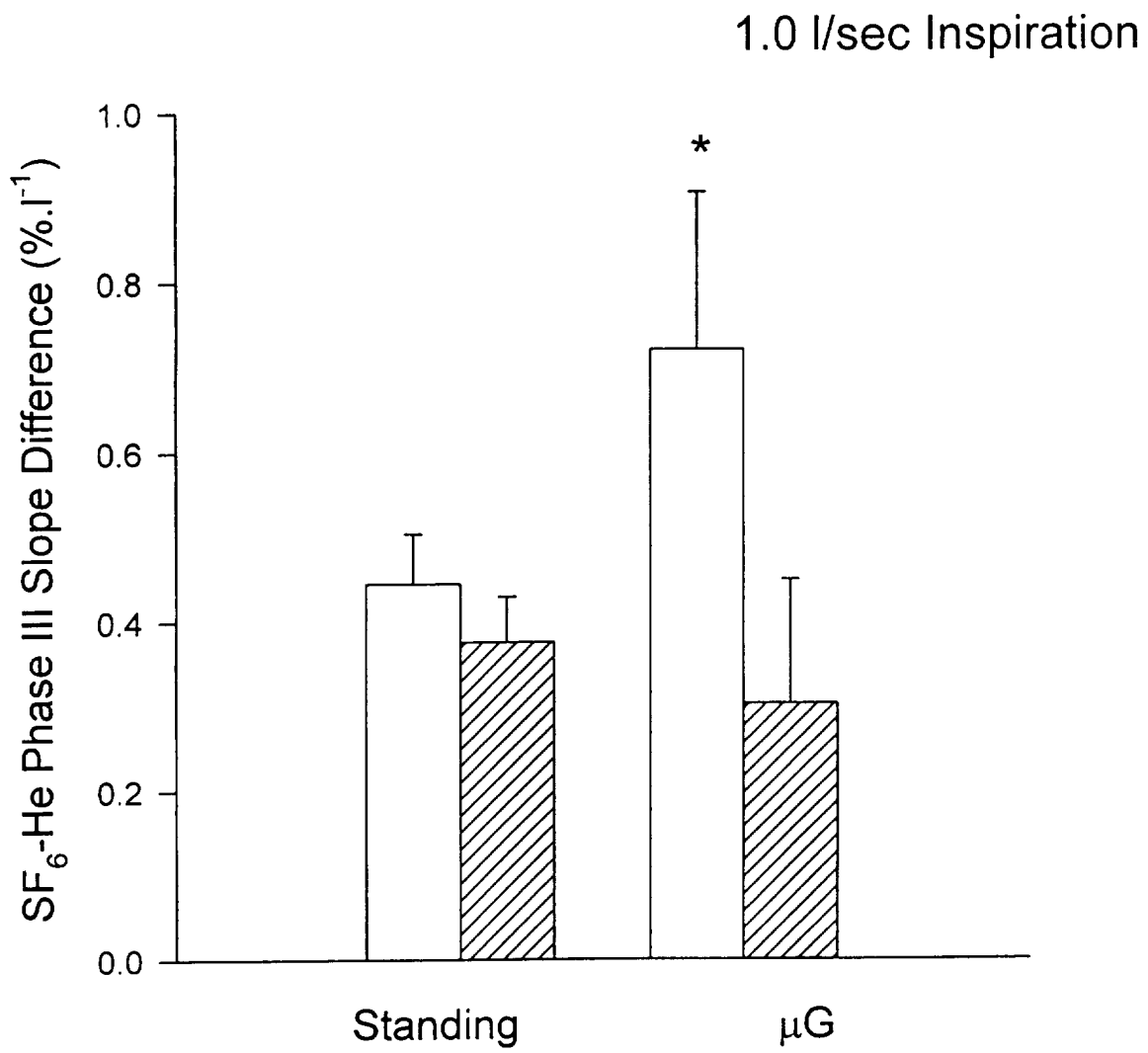


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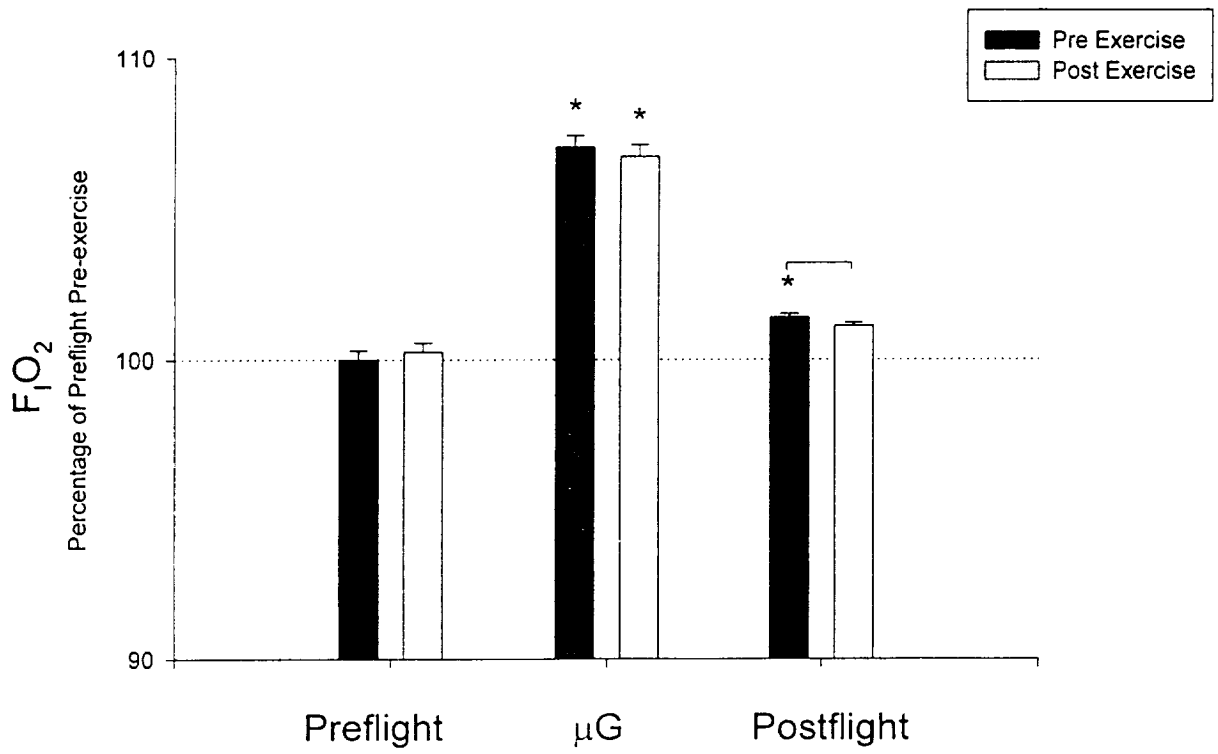
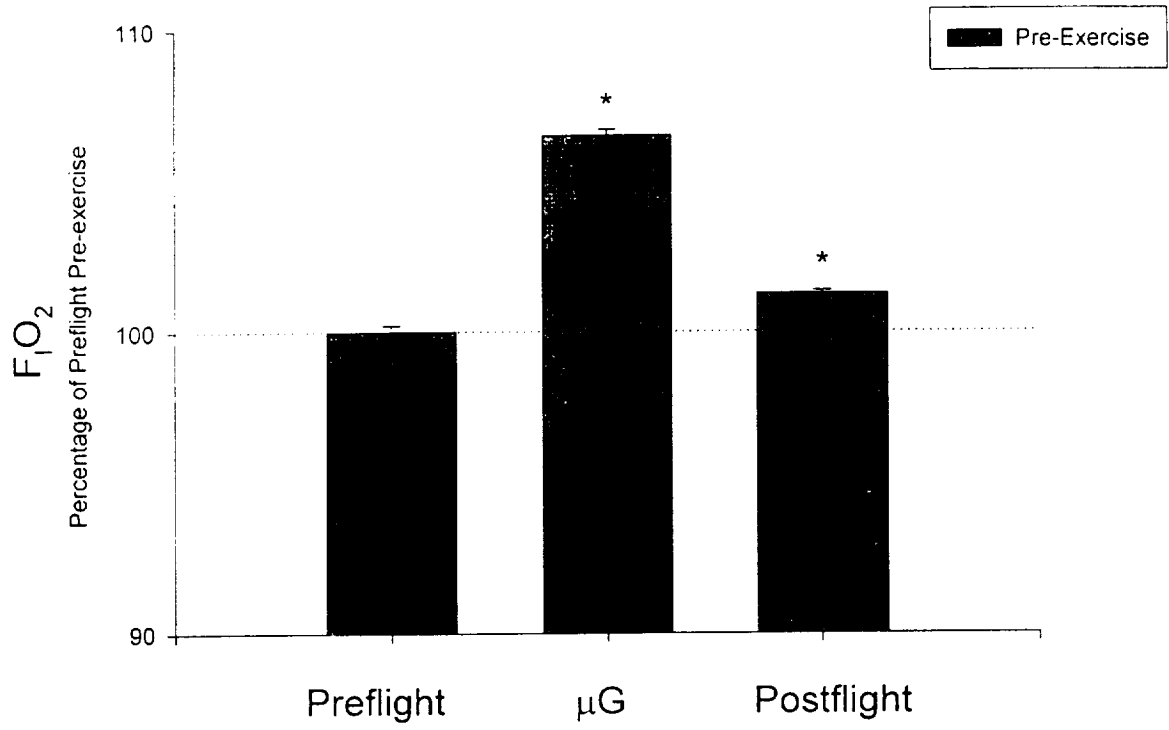


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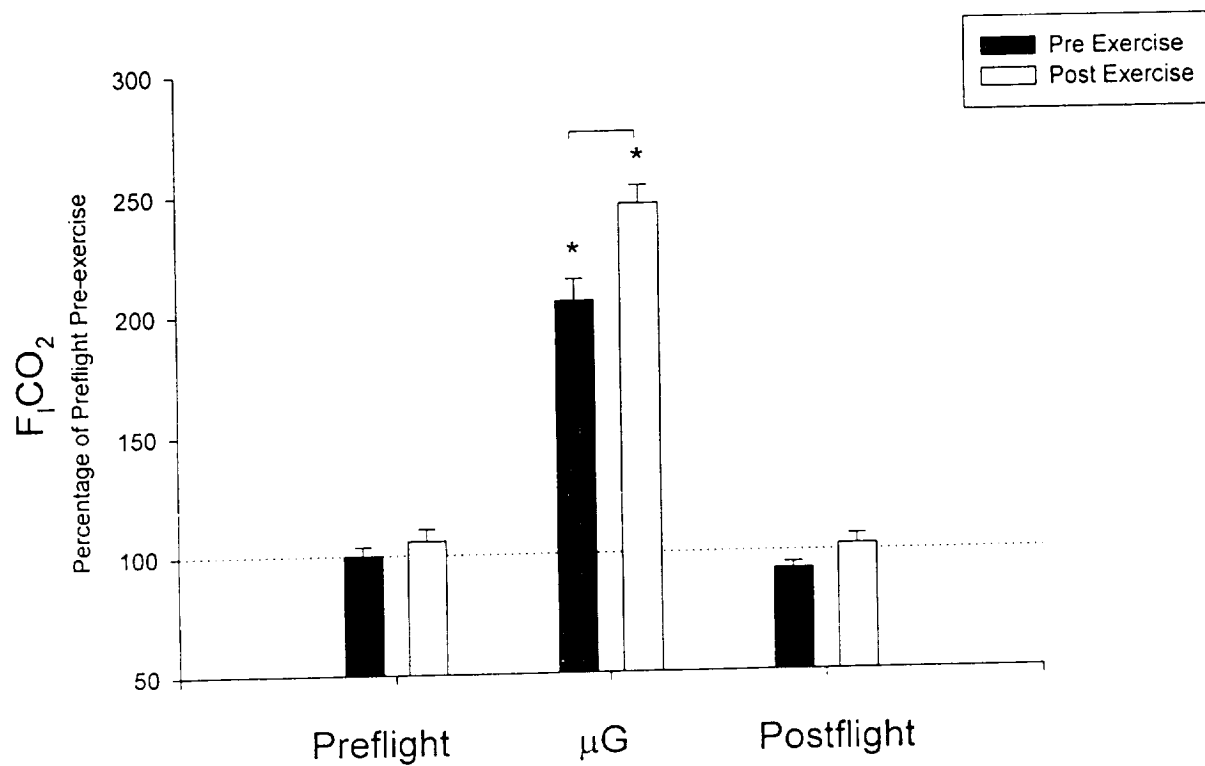
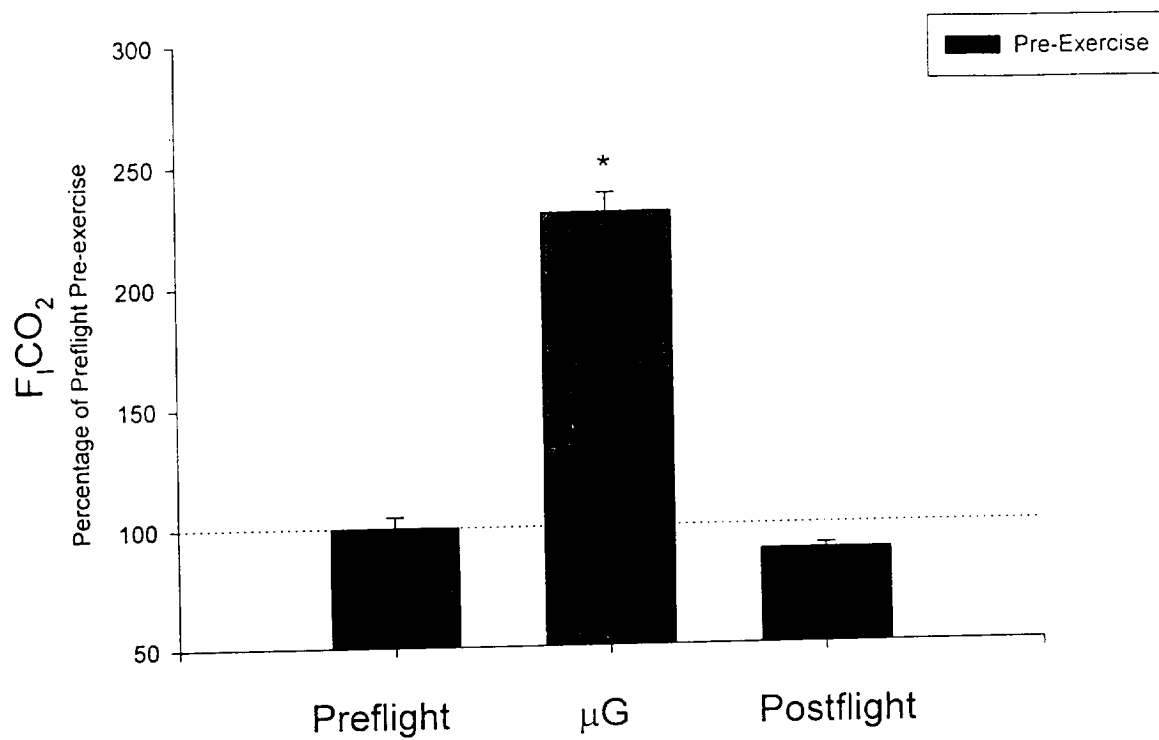


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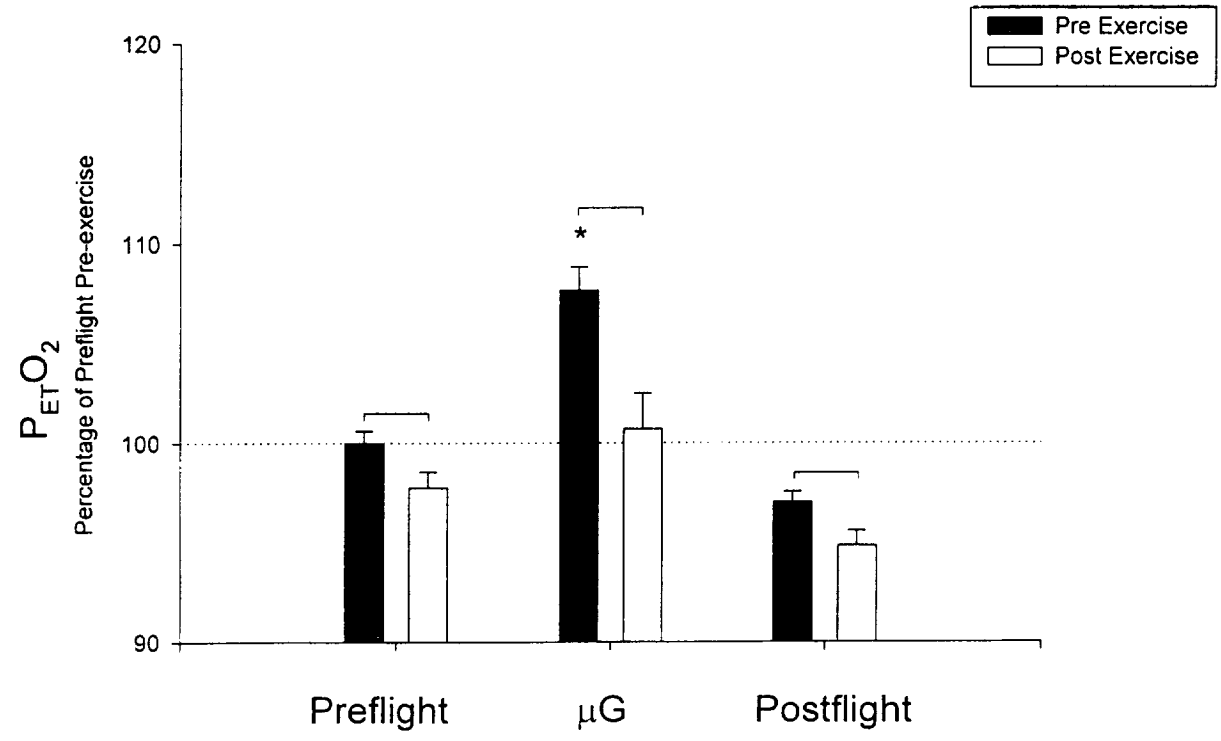
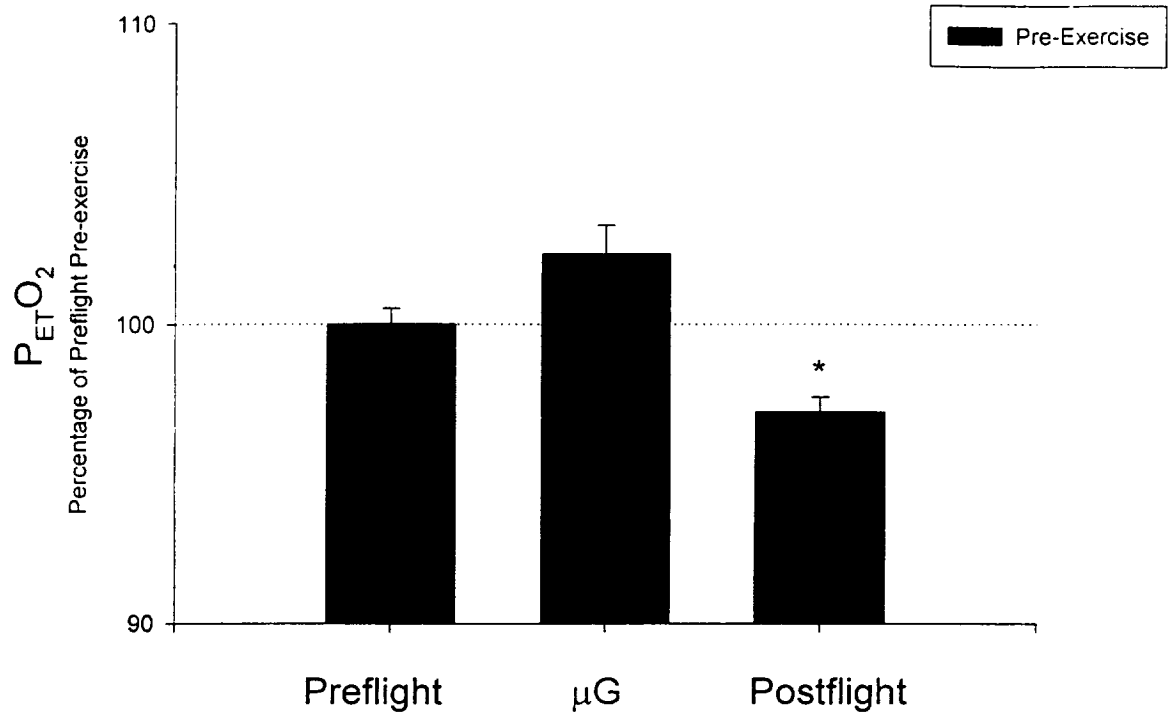


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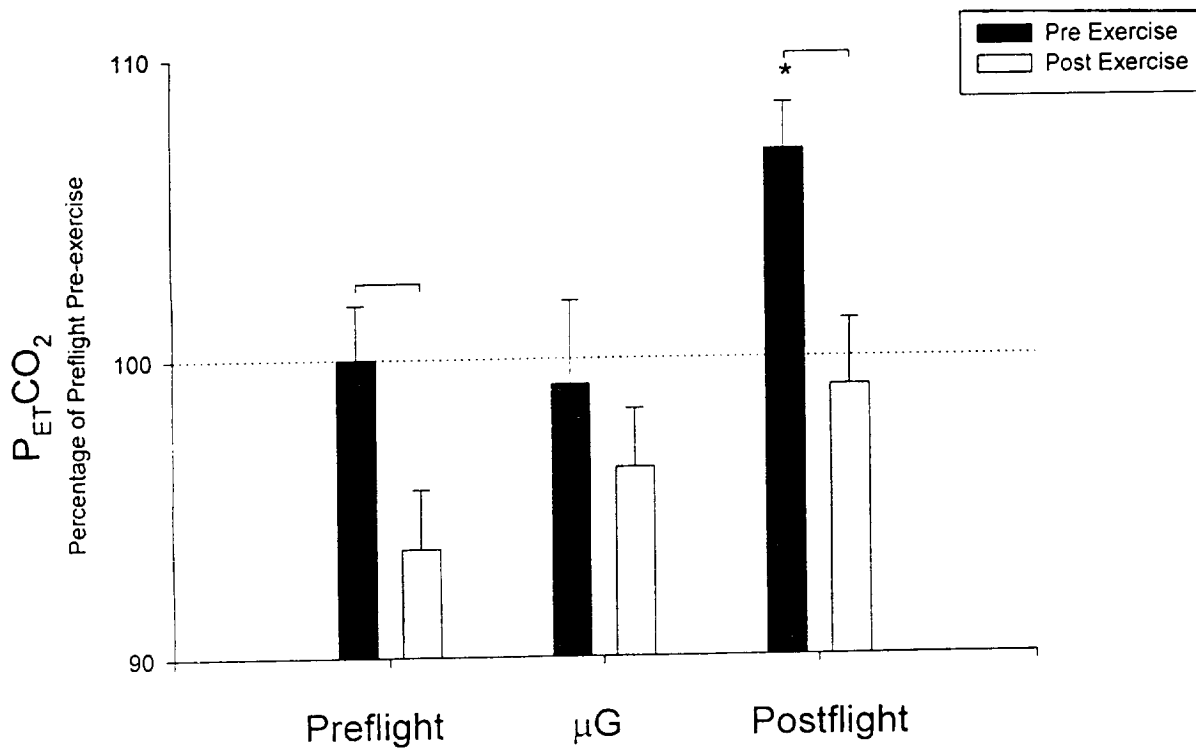
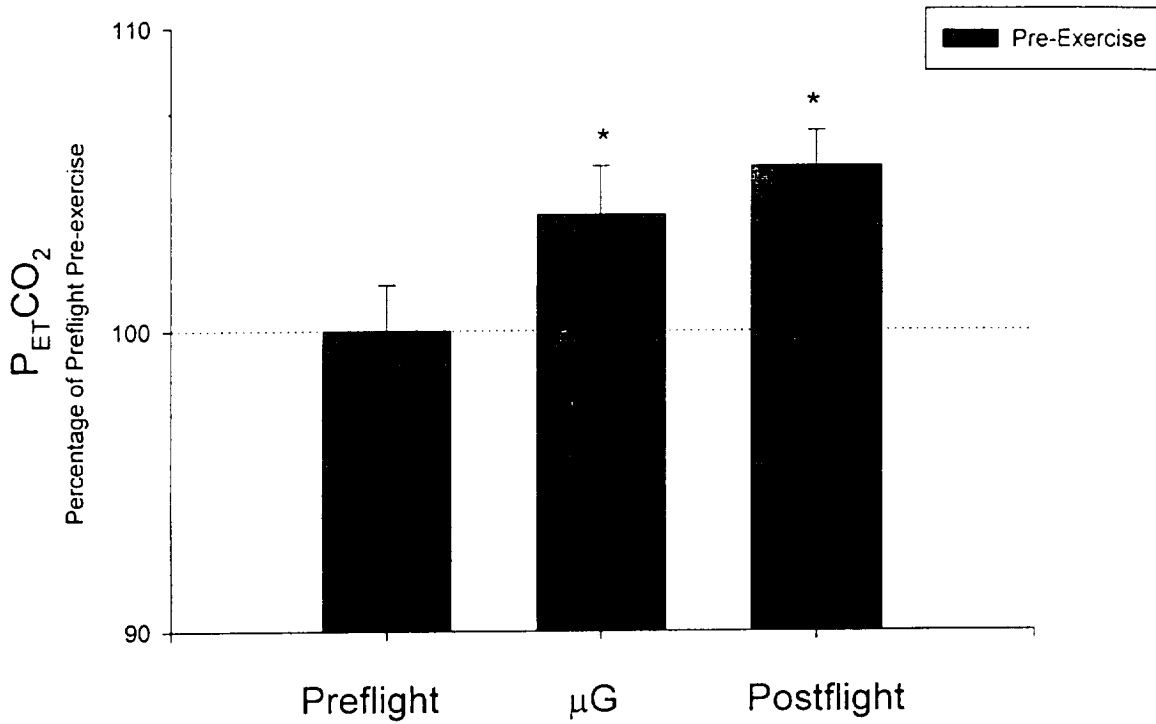


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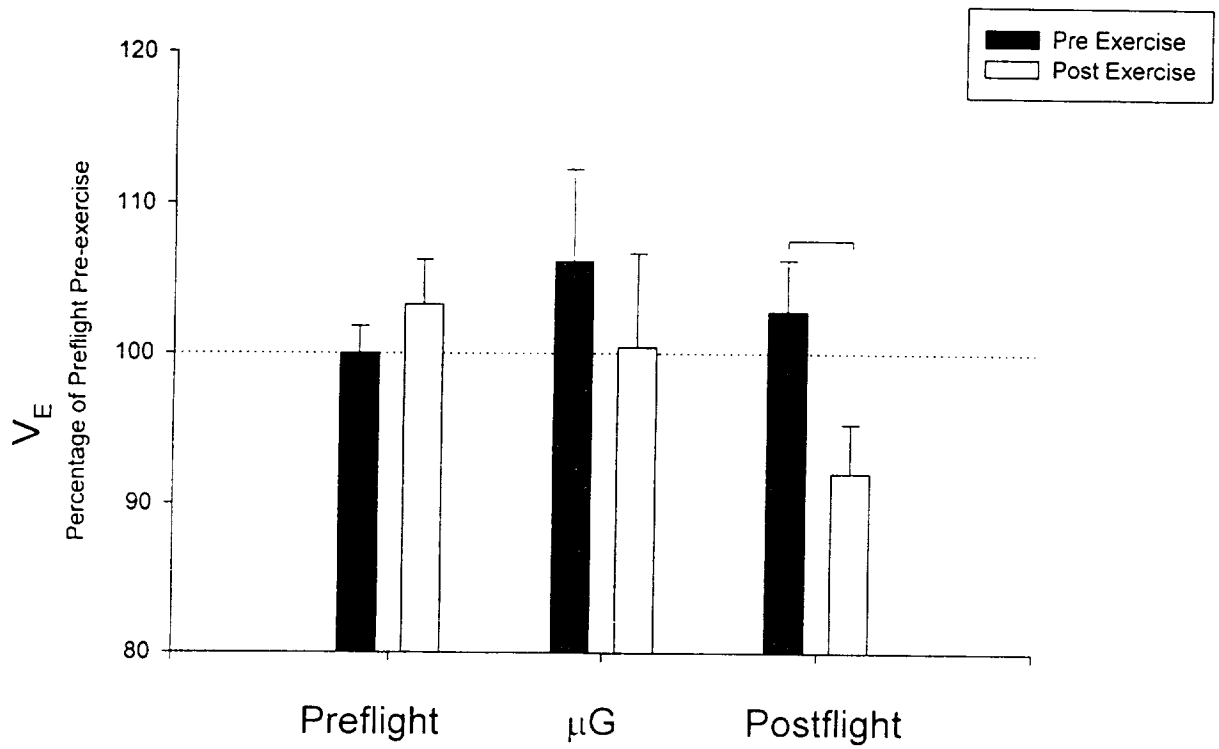
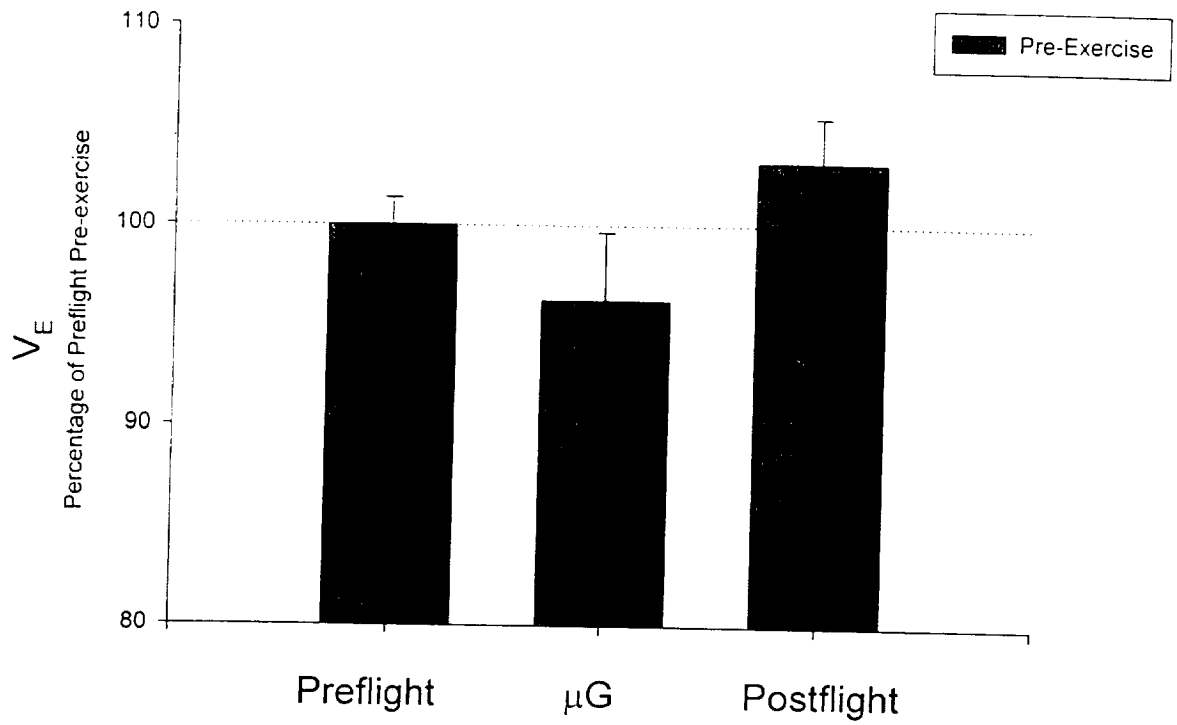


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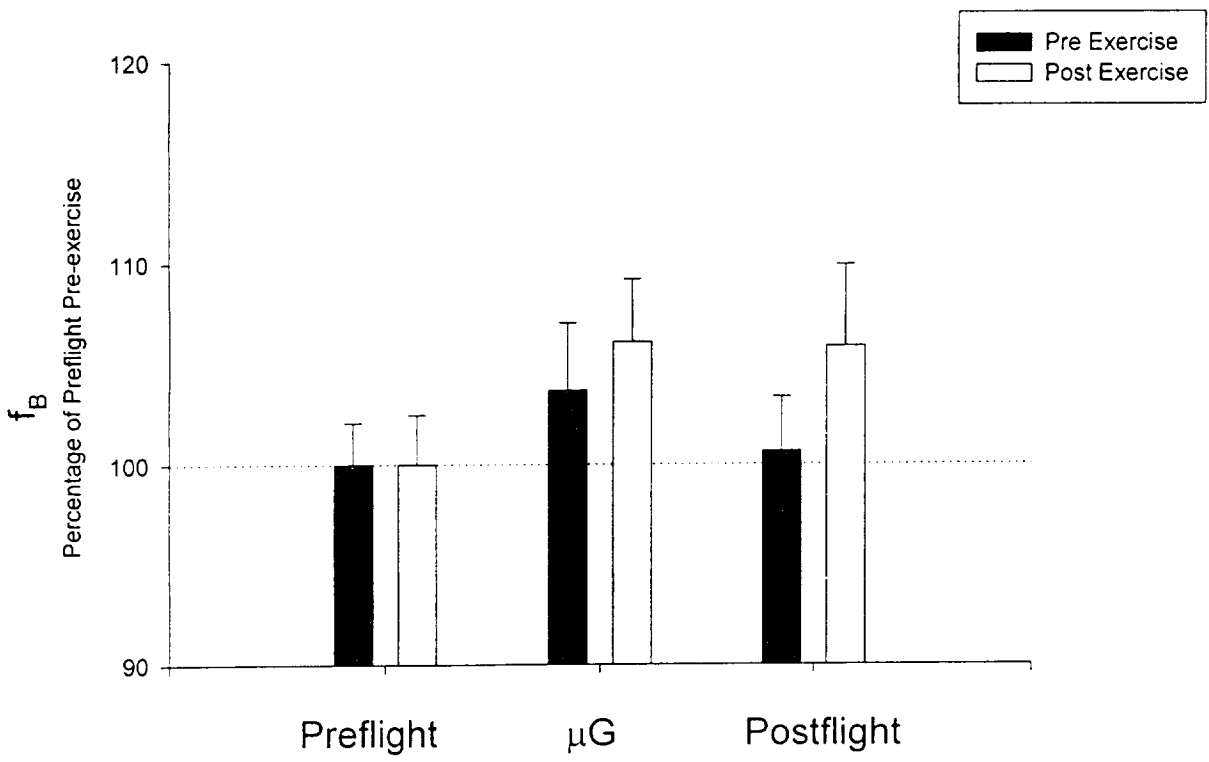
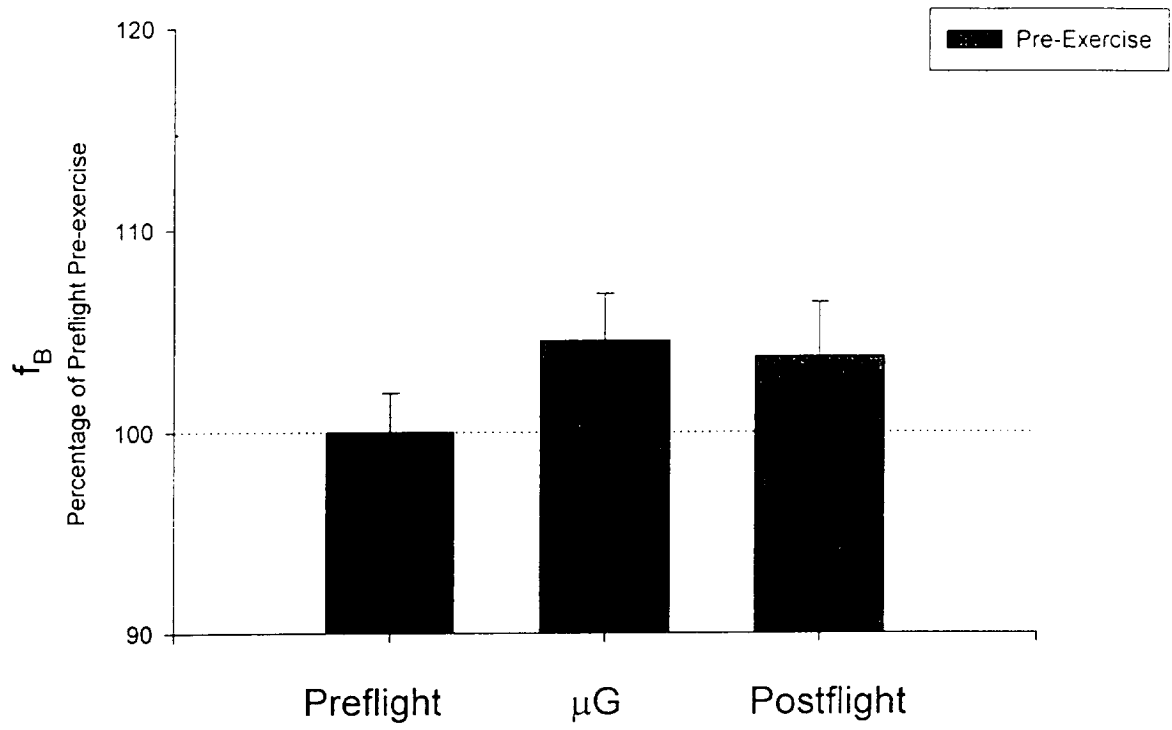


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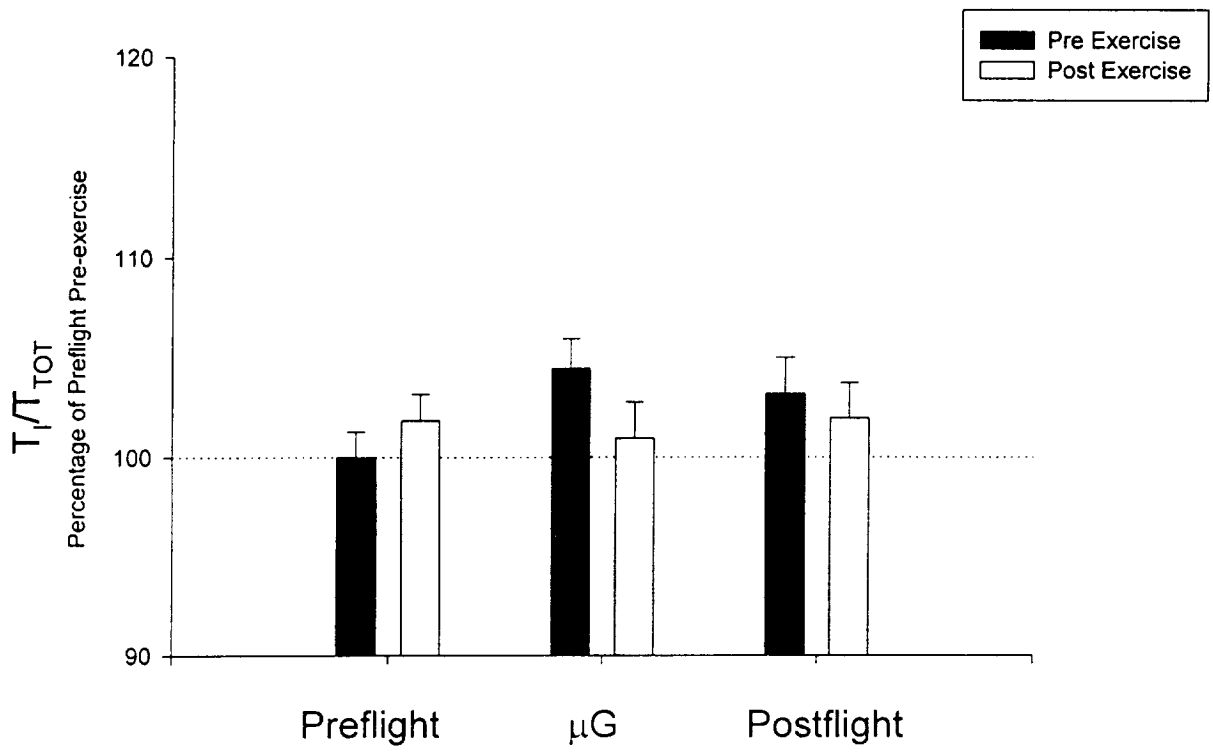
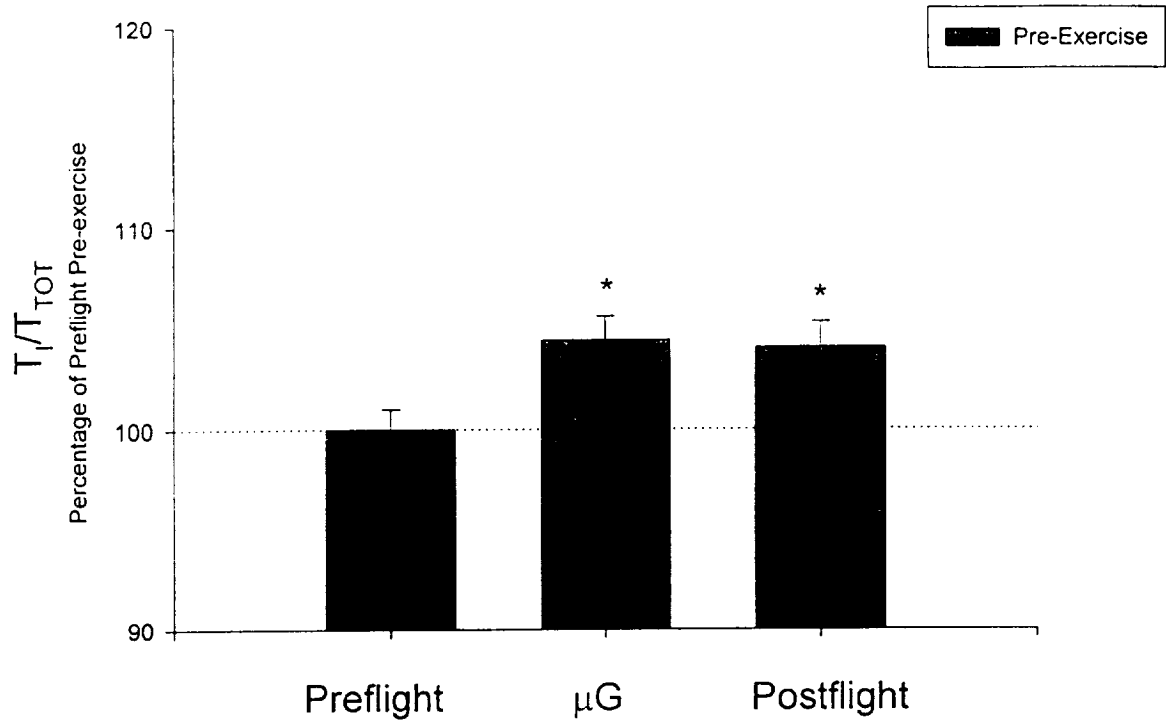


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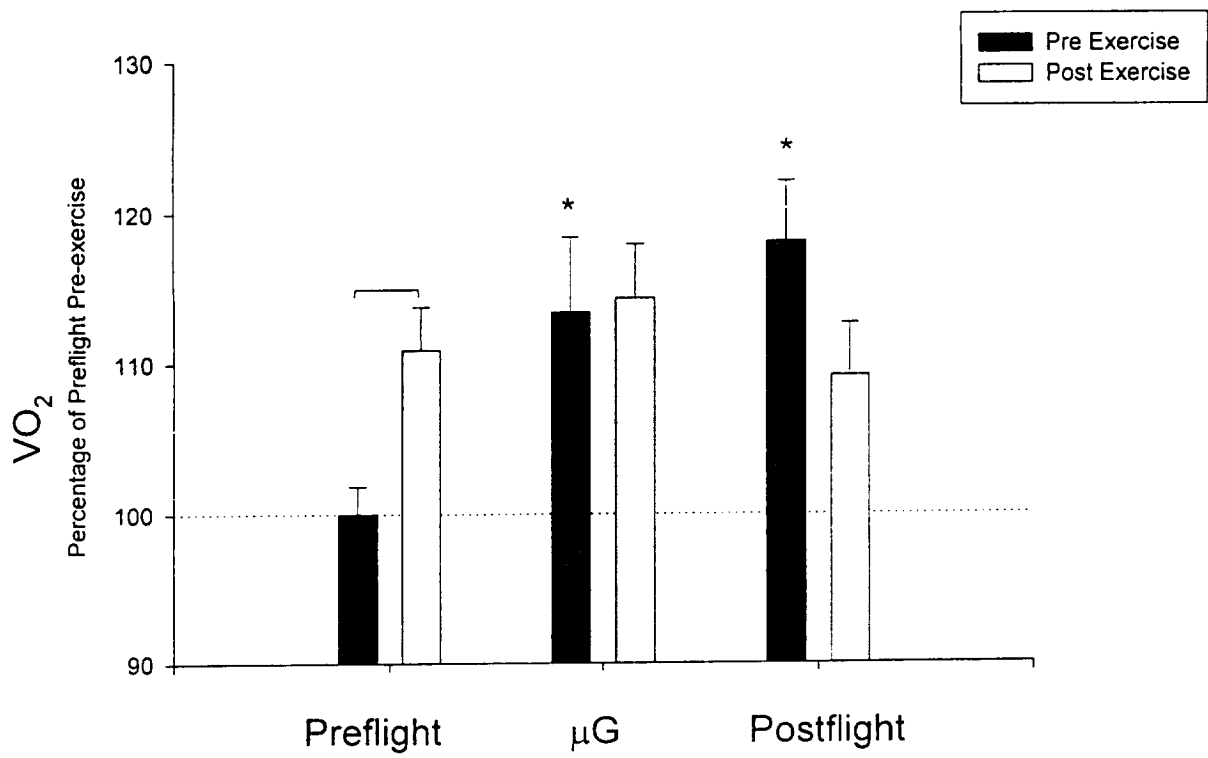
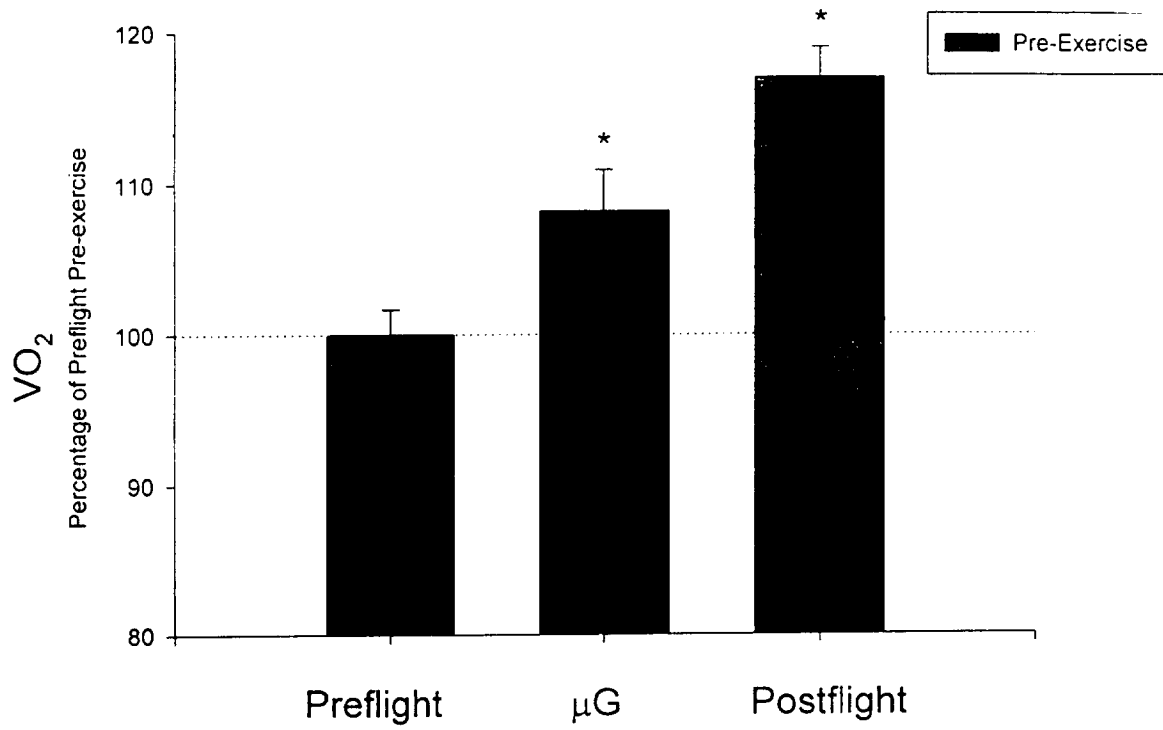


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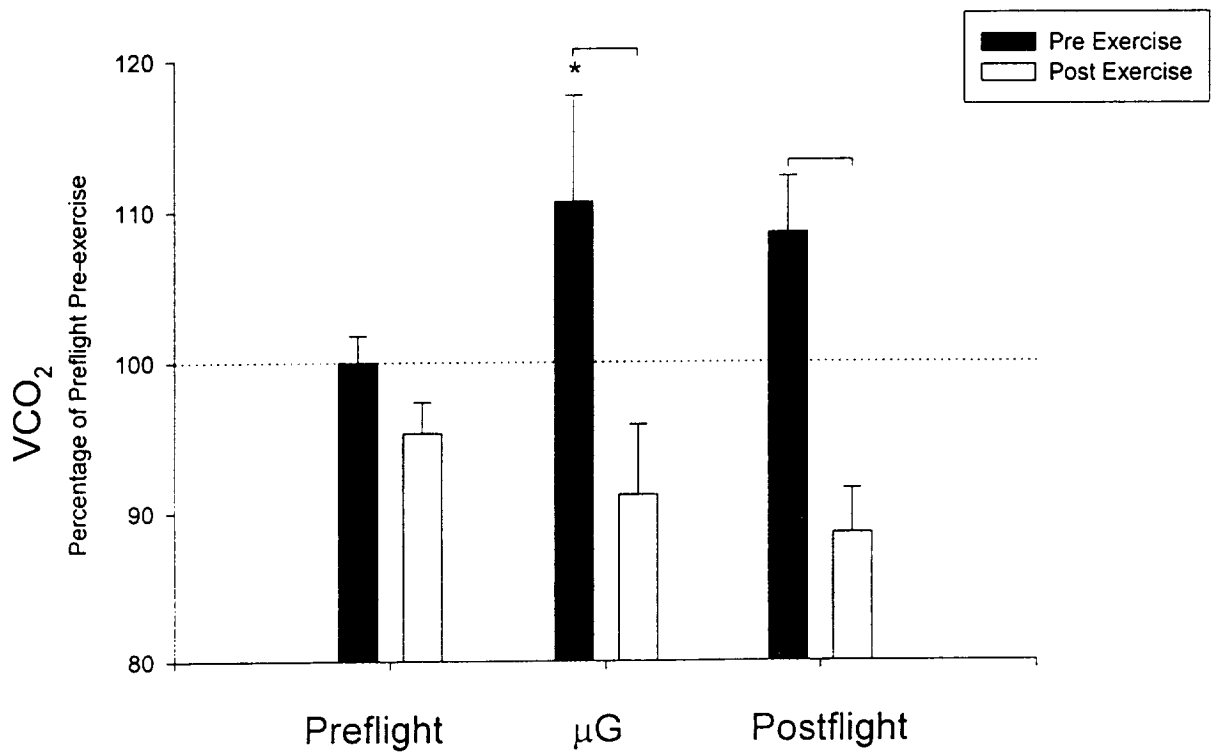
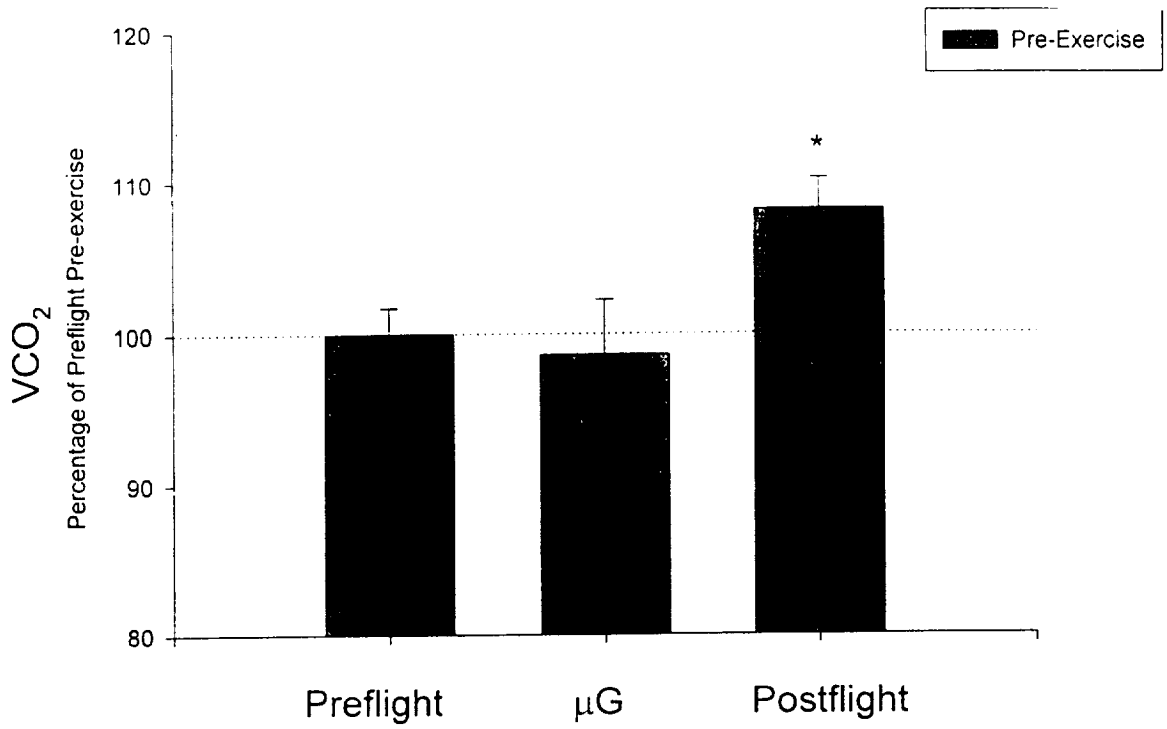


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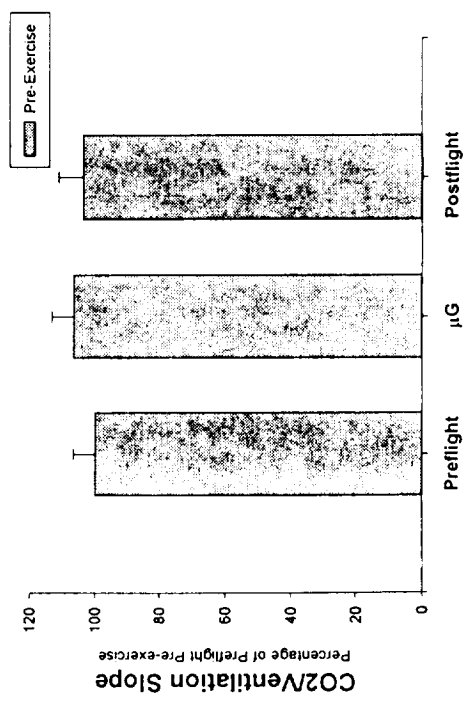
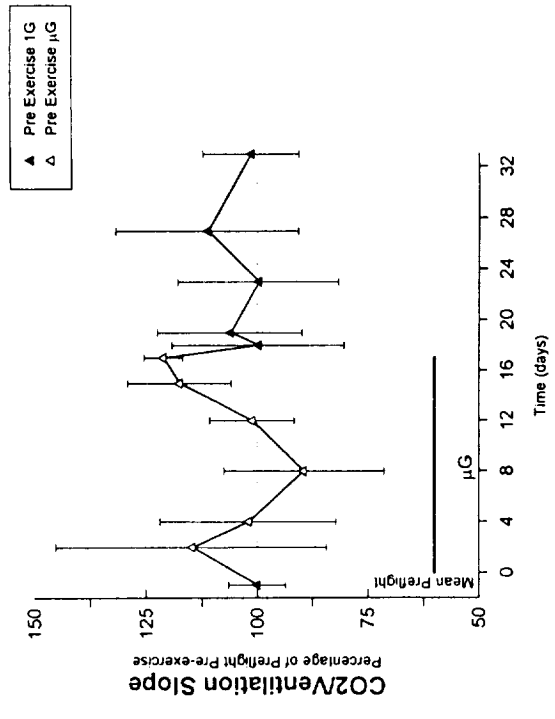
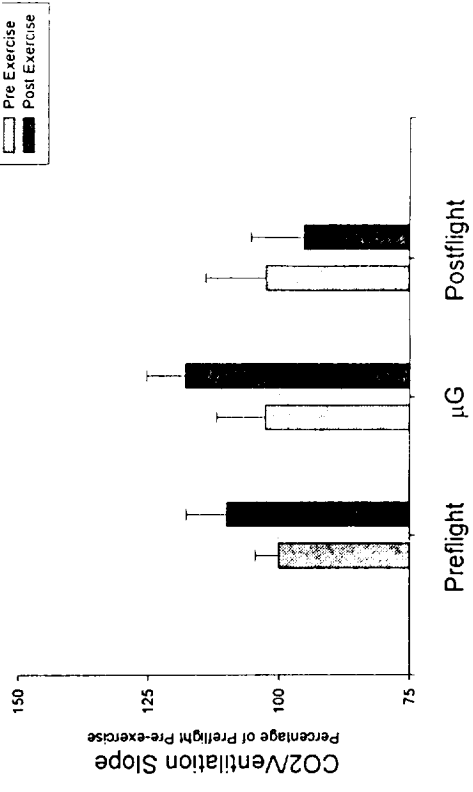
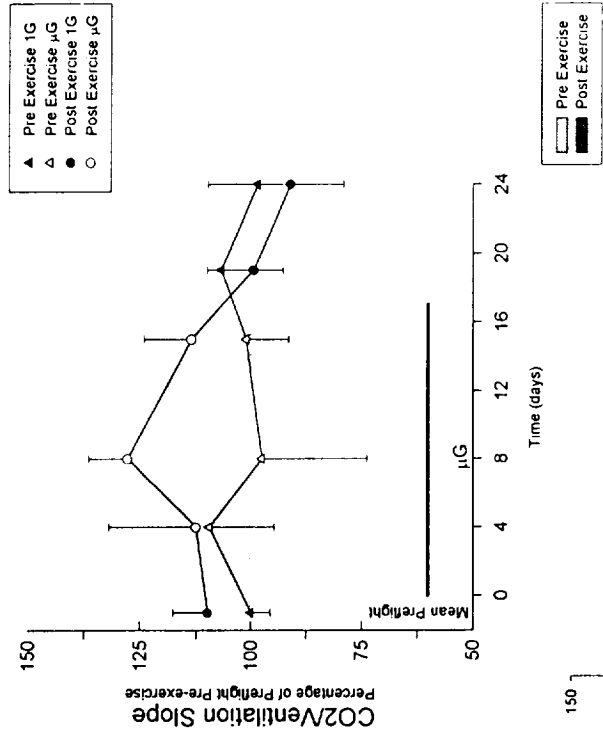


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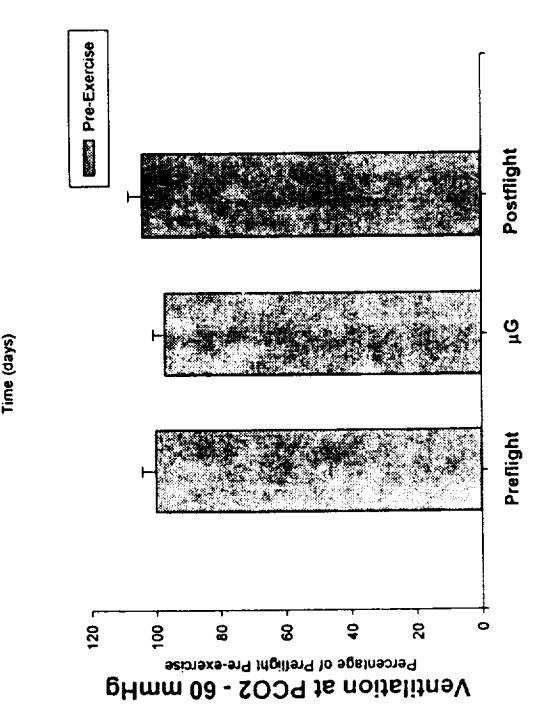
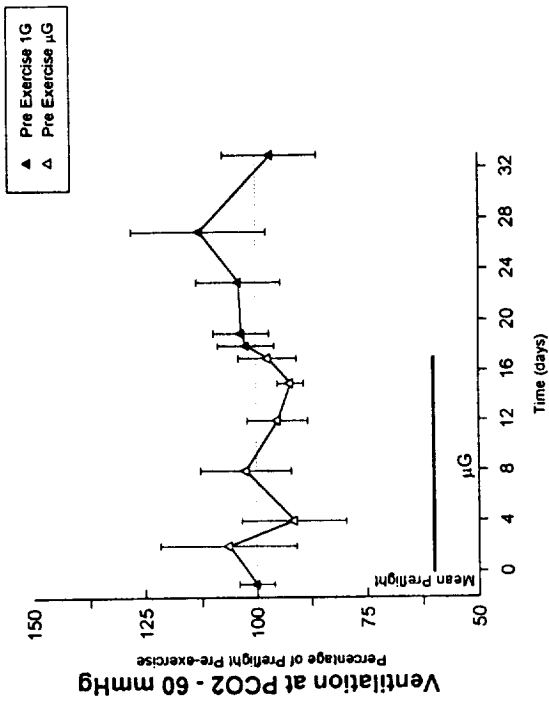
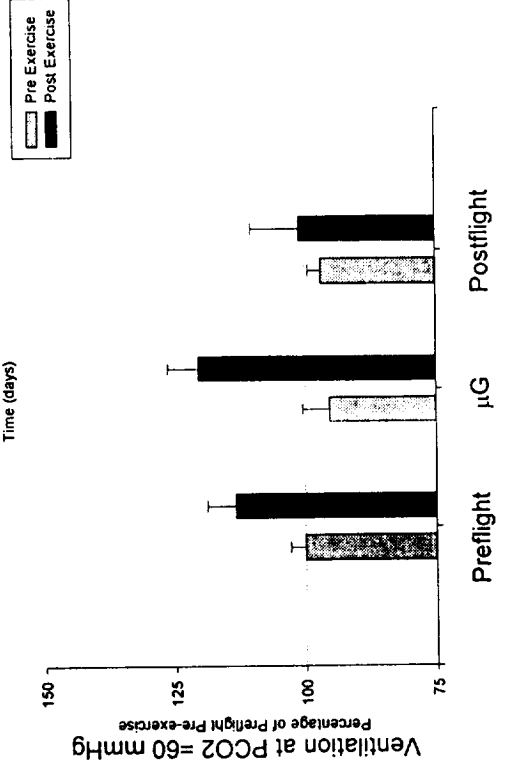
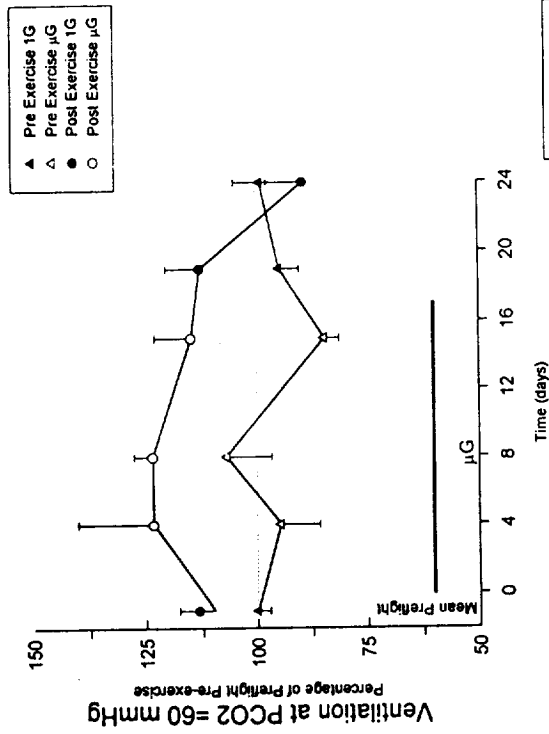


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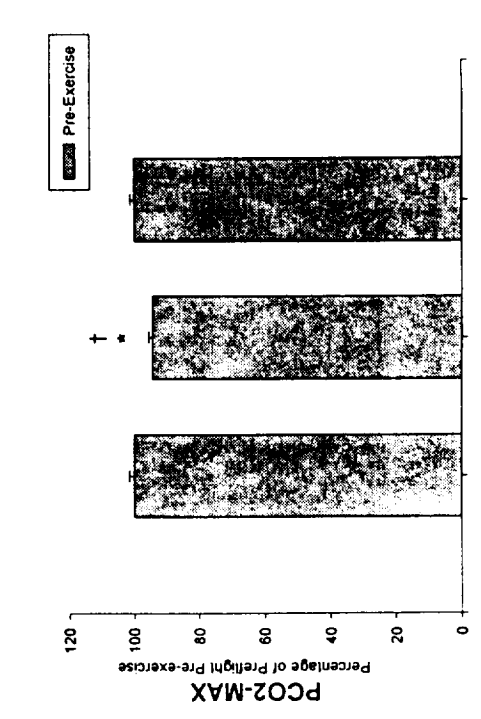
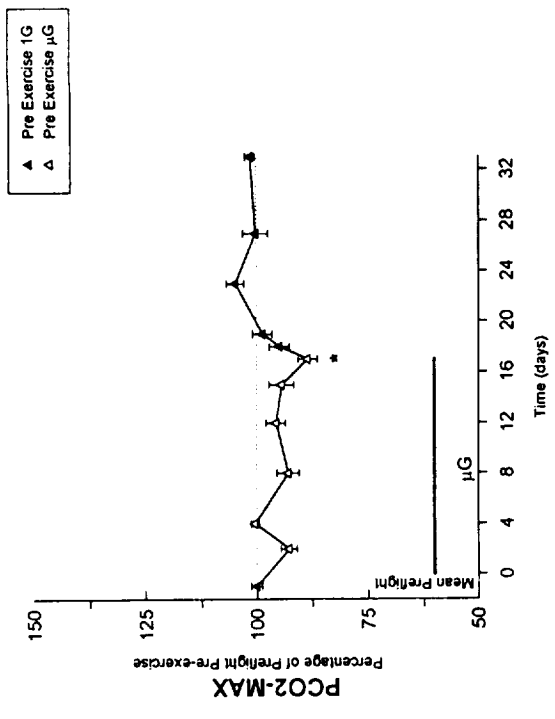
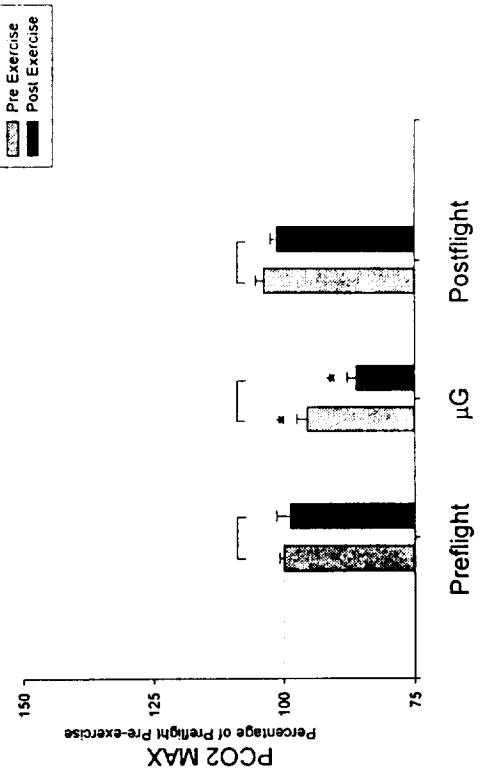
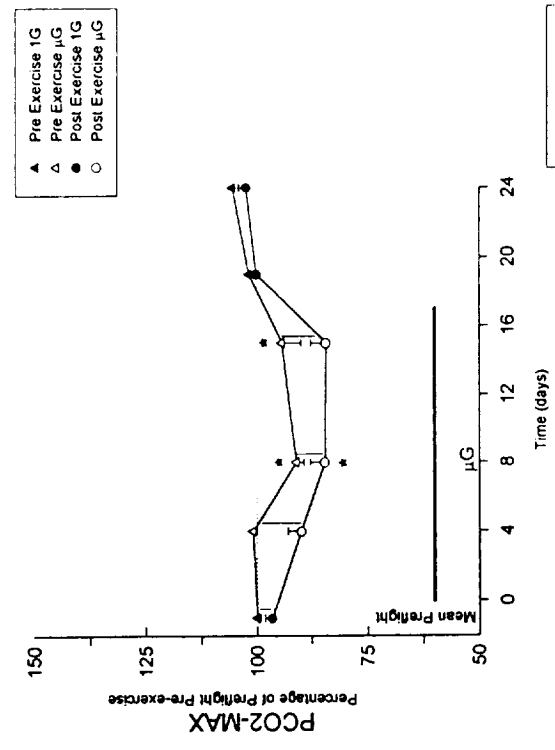


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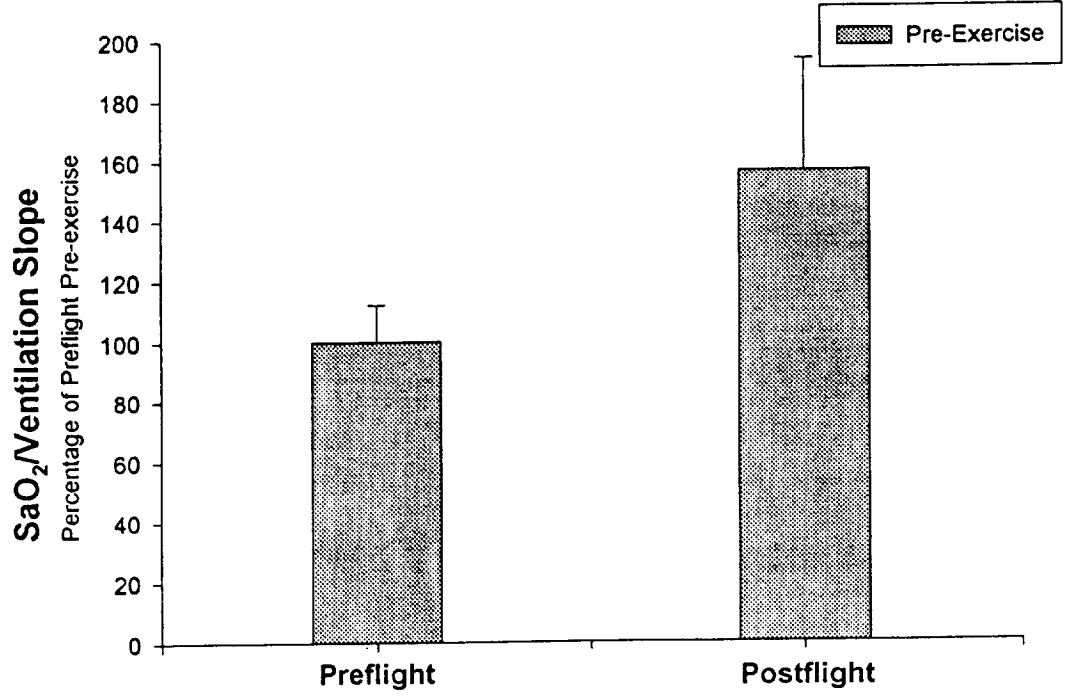
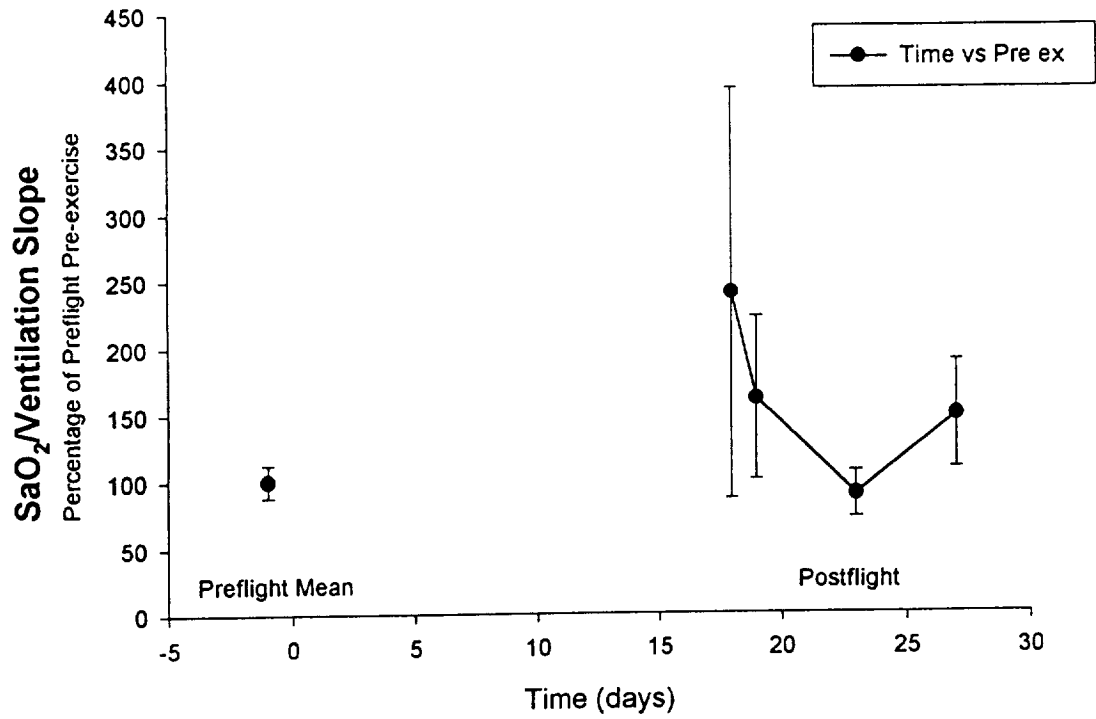


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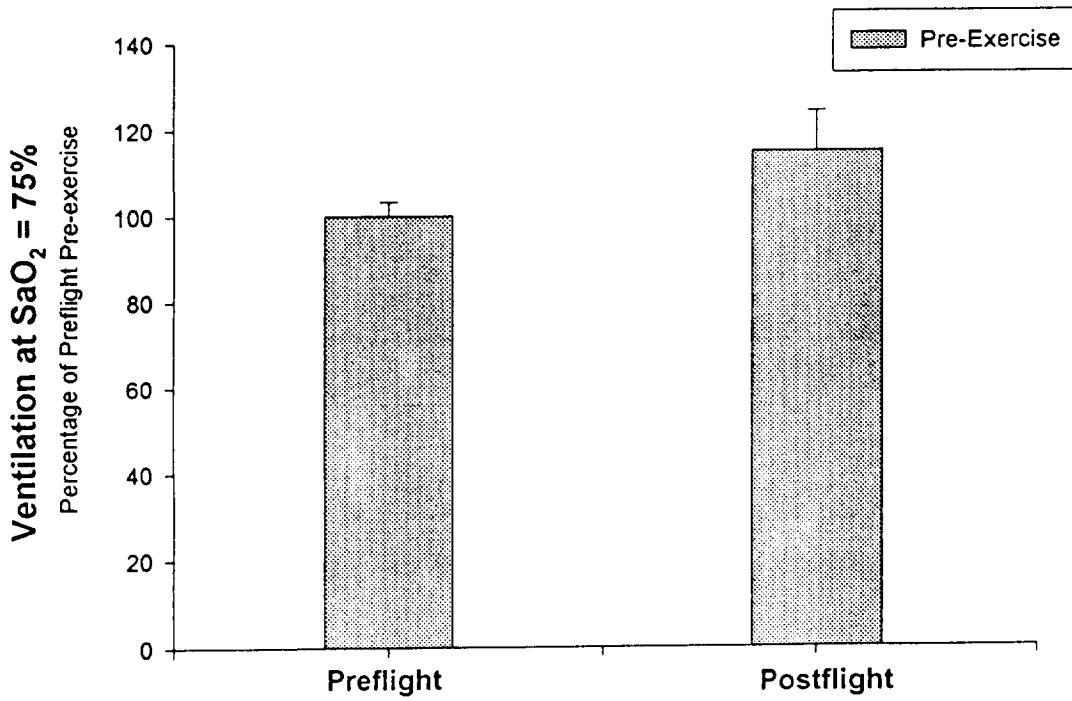
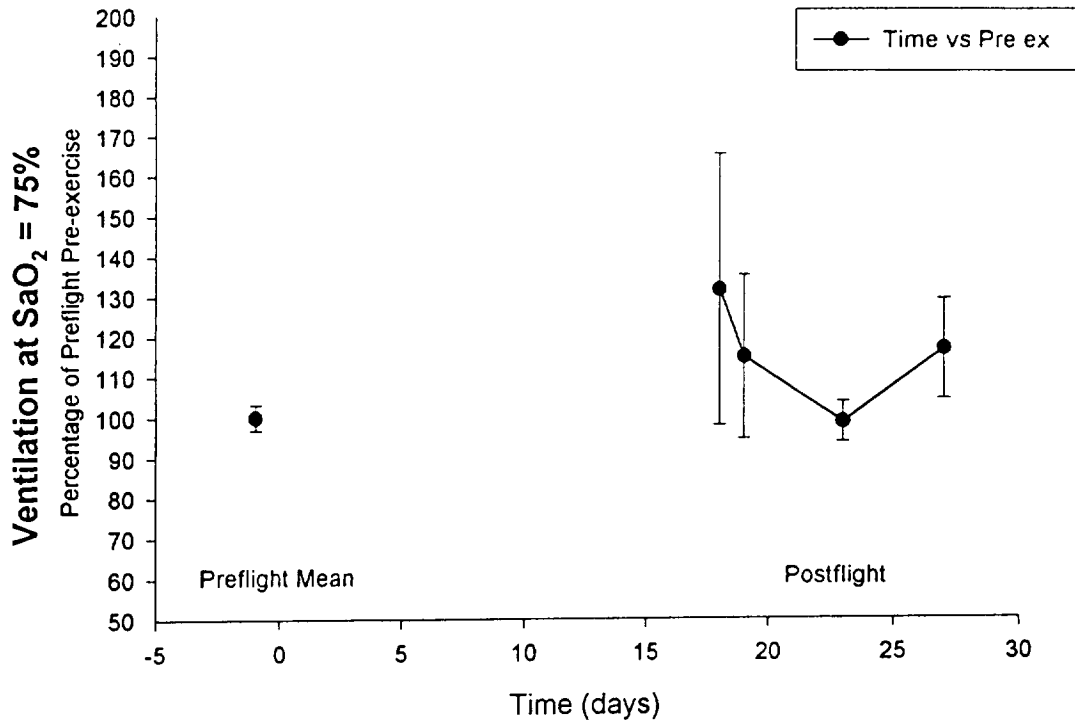


figure 25

JSC Human Life Sciences Project

**E074 - Direct Measurement of the Initial Bone Response to
Spaceflight in Humans**

Principal Investigator:

Dr. Christopher Cann
University of California, San Francisco
San Francisco, California

One Year Symposium Report: LMS E074

Christopher E. Cann, Ph.D.
University of California, San Francisco

Introduction and Objectives

Changes in calcium metabolism during spaceflight are well documented, but the exact mechanism which initiates this process still is not understood. E074, Direct Measurement of the Initial Bone Response to Spaceflight in Humans, was designed to determine which of several physiologic processes could be responsible for the calcium and bone changes seen in flight. While the changes seen in short term flight are of little clinical significance, understanding these processes is key to developing rational countermeasures for skeletal alterations in long term spaceflight, and may also be useful in the evaluation of individual astronaut skeletal responses to microgravity.

The design of E074 was optimized to evaluate the transient adaptive responses of the human calcium and skeletal homeostatic systems to microgravity exposure. Previous investigations on SLS-1 and SLS-2 (E305, Pathophysiology of Mineral Loss in Spaceflight) addressed the specific questions 1) does serum calcium increase in flight, 2) if so is it primary or secondary to an increase in parathyroid hormone, and 3) are there adaptive effects of the system to any hormonal changes which might occur? The results from SLS-1 and SLS-2 suggested that there was a primary increase in serum calcium and compensatory decreases in PTH and later the active vitamin D hormones and intestinal calcium absorption, but technical problems prevented a good answer about the magnitude of this increase and the adaptations to it. The lessons learned from SLS-1 and SLS-2 were, however, incorporated in the planning for the LMS mission and led to a very good final experimental protocol.

The adaptation of calcium metabolism and skeletal homeostasis to a stimulus can occur over several time frames. We addressed all 3 time frames during which we expected to see alterations: immediately on insertion to microgravity (response at the cellular level), 1-5 days into flight (response at the bone tissue level), and weeks to months after flight (response at the bone organ level).

Hypotheses

Hypothesis 1. Exposure to microgravity causes a rapid and sustained release of calcium from the bone, leading to hypercalcemia. The early release of calcium from bone may not be due to an increase in osteoclastic activity or number, but could come from a leak in the membrane separating the "bone fluid compartment" from the blood. Osteoclastic resorption probably also increases, but at a later time.

Hypothesis 2. Exposure to microgravity will cause an increase in the activation frequency for osteoclasts, increasing bone resorption on a time scale of days with sustained hypercalcemia. The calcium homeostatic system will attempt to adapt to this hypercalcemia with a series of hormonal responses, but because the hypercalcemia is primary, these normal adaptive responses will lead to a net decrease in bone balance.

Hypothesis 3. The increase in osteoclast activation and subsequent bone resorption will lead to a transient in bone balance, with resorption remaining elevated weeks or possibly months after flight.

Methods

Hypothesis 1. Serum ionized and total calcium, pH, and parathyroid hormone were measured in blood samples obtained preflight and during the first 4 days in flight. All processing of blood, whether on the ground or in flight, was done with identical procedures to minimize the possibility of artifacts. If the serum ionized calcium is increased and it is a primary increase, the serum PTH should decrease. This is tested by comparing the time course of Ca^{++} vs PTH early in flight, and by testing to see that the relationship follows that expected for normal individuals. The origin of any increase in serum ionized calcium is identified by using a stable calcium isotope ($Ca-48$) as a tracer.

Hypothesis 2. Serum calcium, PTH, and vitamin D metabolites, urine deoxypyridinoline (a bone collagen breakdown product) and calcium and tracer calcium were measured as a function of time. An increase in osteoclastic resorption several days into flight due to an increased number of osteoclasts should be evident by an increase in the excretion of deoxypyridinoline and calcium in the urine and a decrease in the ratio of $Ca-48$ to total calcium in the urine. Serum PTH should be depressed and remain low as long as the calcium-releasing stimulus persists. Later in flight (>8-10 days), 1,25-dihydroxyvitamin D production in the kidney should be depressed as a result of reduced 1-alpha-hydroxylase activity secondary to PTH suppression.

Hypothesis 3. 24-hour urine calcium and deoxypyridinoline excretion were measured along with serum markers of bone metabolism for 3 months prior to flight until approximately 2 months postflight. If the spaceflight induced an increase in bone resorption which followed a normal bone remodeling cycle, markers of bone resorption and formation would still be elevated 2 months postflight, indicating that the skeleton had not yet readapted to a 1-G steady state.

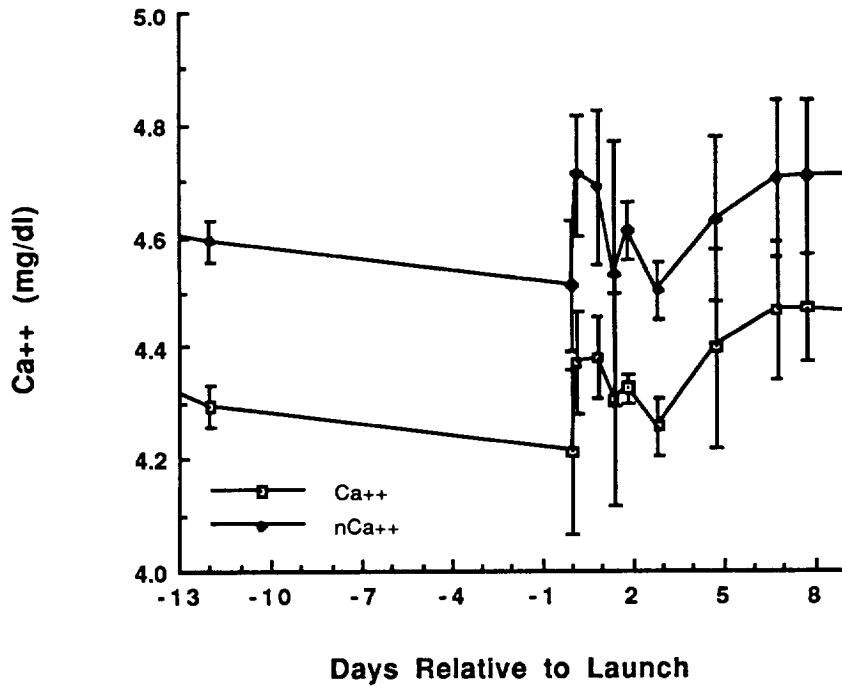
Results

Hypothesis 1

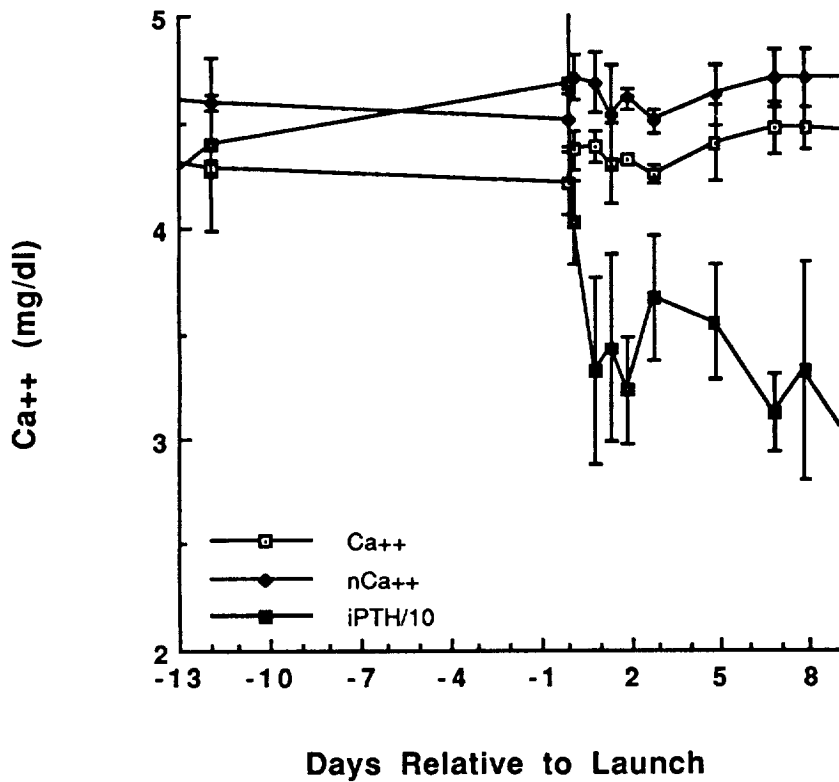
Figure 1 shows the results of the early response of serum ionized calcium to spaceflight. The first data point on launch day (day 0) is taken before breakfast and represents the last preflight sample. Because sample processing (freezing) could result in some alterations to blood gases, both raw results for Ca^{++} and those normalized to pH 7.4 are shown. By 3 hours into flight, Ca^{++} was elevated over baseline values, then decreased back near baseline by day 3. The later rise (days 5-8) can be explained by an increase in bone resorption due to increased numbers of osteoclasts whose proliferation was stimulated by spaceflight.

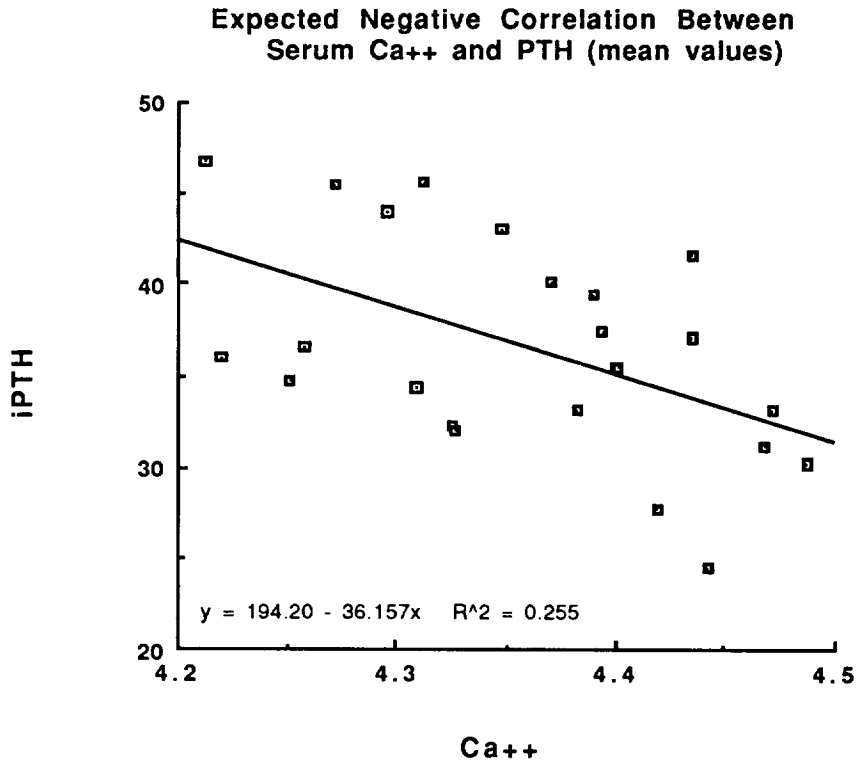
Figure 2 shows the time course of parathyroid hormone concentration relative to the serum ionized calcium. PTH is reduced in response to elevated serum Ca^{++} even at the 3 hour time point inflight and shows the expected negative relationship over the first 8 days of flight. This indicates that the calcium homeostatic system is responding normally as expected, as is seen in Figure 3 where there is a close negative correlation between Ca^{++} and PTH over the preflight, inflight and postflight periods.

Serum Ionized Calcium Preflight and Early Inflight (n=4, mean +/- sd)



Serum Ionized Calcium and PTH Preflight and Early Inflight (n=4, mean +/- sd)



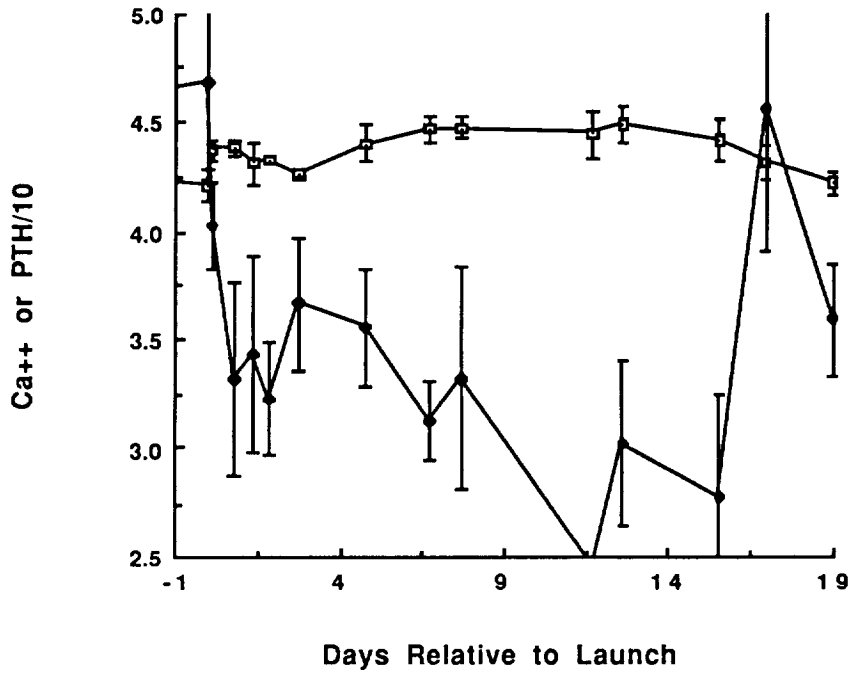


Hypothesis 2

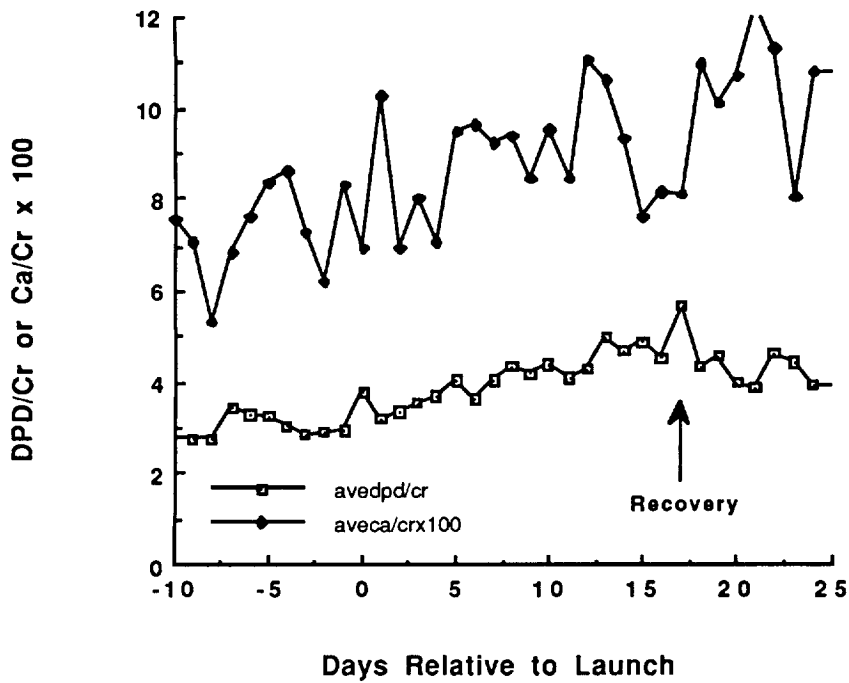
Figure 4 shows the relationship between serum ionized calcium for the flight and early recovery periods, demonstrating the same strong negative relationship as was seen in the early flight period. Again, this indicates that the adaptation of the calcium metabolic system to an increase in serum calcium is normal, continues throughout flight, and is not impaired at recovery.

Urine deoxypyridinoline and calcium are relatively stable during the preflight period while the astronauts are eating a consistent diet, start to rise a few days into flight, and remain elevated after recovery. Limited data are shown in this graph (Figure 5), so no error bars are given.

Relationship Between Serum Ca⁺⁺ and PTH
L-1 to R+2

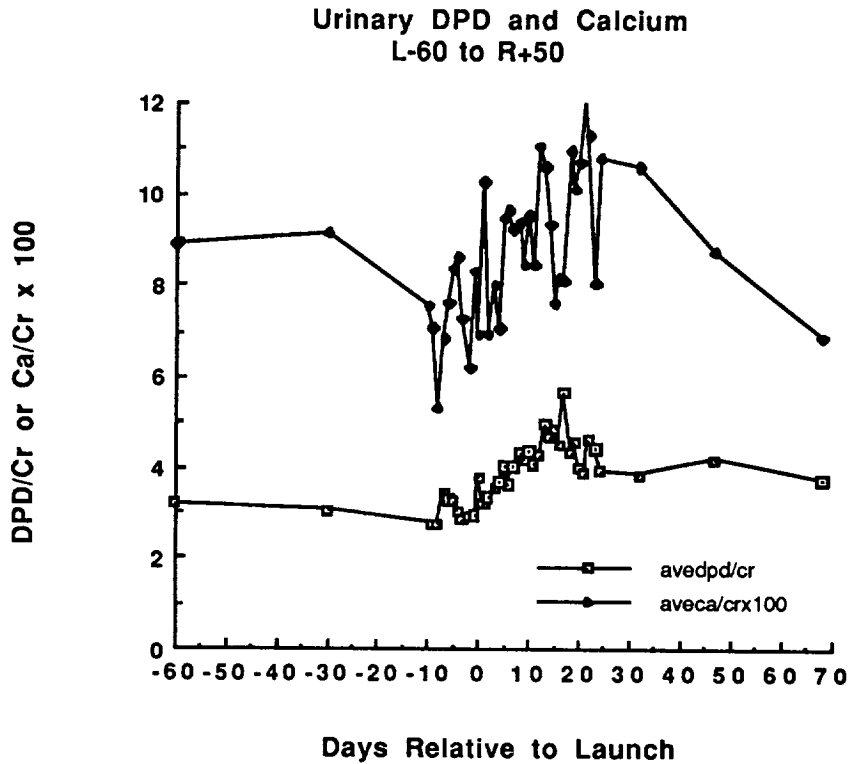


Urinary DPD and Calcium
L-10 to R+7

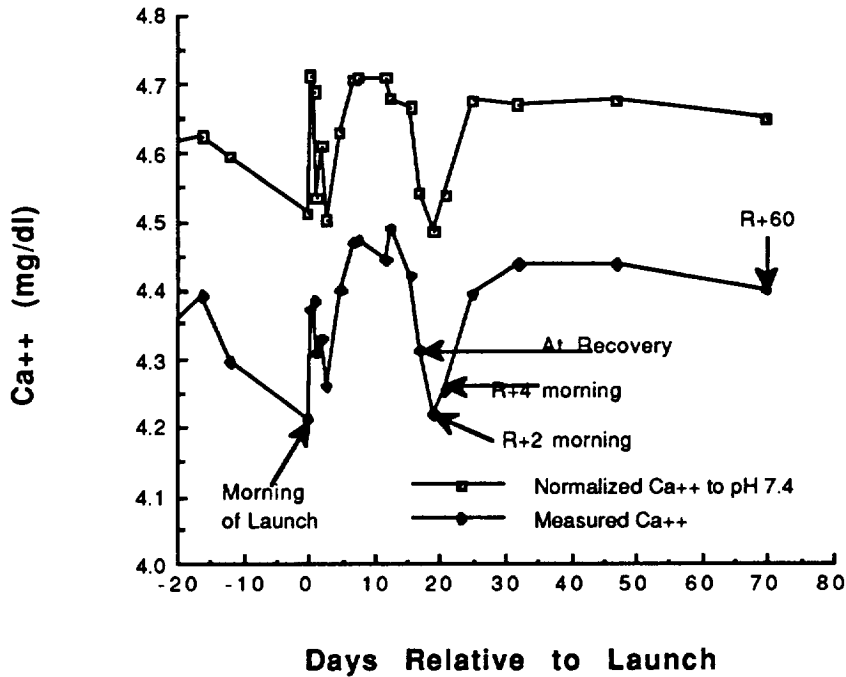


Hypothesis 3

An increase in bone resorption induced by microgravity should persist through at least one bone remodeling cycle in adults. The time taken to complete one such cycle is in the range of 4 months to 2 years in humans. Figure 6 and 7 indicate that for at least 2 months postflight there are alterations in calcium metabolism which can be measured. The quantitative elevation of deoxypyridinoline can be used to estimate the bone resorption rate in mg/day. Such a measurement may be useful in predicting the long term net change in bone mass in an individual astronaut due to single or multiple short term exposures to spaceflight.



Average Serum Ca⁺⁺ Pre-, In-, Postflight for 4 Astronauts

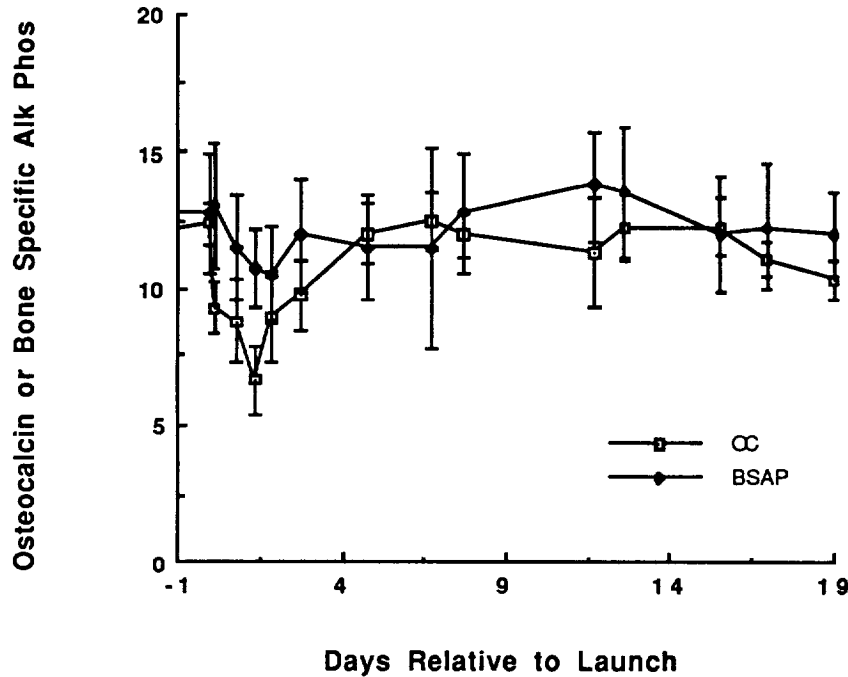


Ancillary Findings

One hypothesis for the decrease in bone following spaceflight has been the possibility that in addition to bone resorption or breakdown being increased, formation of new bone to replace that lost may not occur normally. The basis of this hypothesis comes from animal studies, primarily in rats but with some small amount of data from primates as well, where mineralization of bone is impaired and new osteoblasts are not produced. However, it is also well known that the increased endogenous corticosteroid production from stress will reduce bone formation, so a reduction in formation or indices of formation could be due either to a direct effect on osteoblasts through unloading or mediated by humoral agents.

We measured two markers in the serum which are considered to be specific for bone formation under most circumstances, bone specific alkaline phosphatase and osteocalcin (Figure 8). We found no effect of flight on BSAP. Osteocalcin was reduced for the first few days of flight, then increased back to baseline levels and remained there during flight and the first few days of recovery. This response is more consistent with a cause of stress of flight than of a bone response, although the bone response cannot be ruled out.

Response of Serum Bone Formation Markers
Osteocalcin and Bone Specific Alk Phos
L-1 to R+2 (mean/sem, n=4)



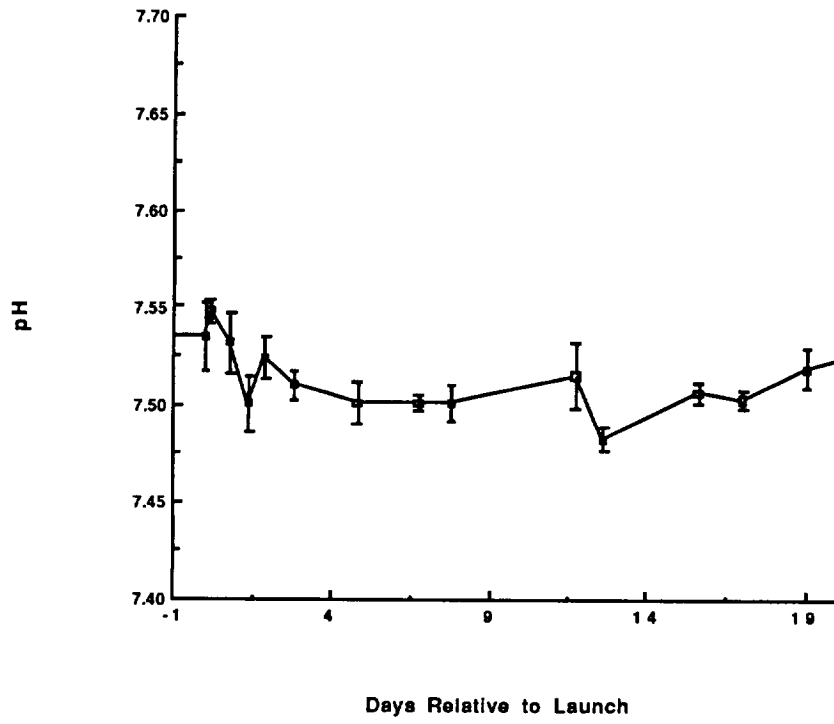
Unexpected Findings

Possible Metabolic Acidosis

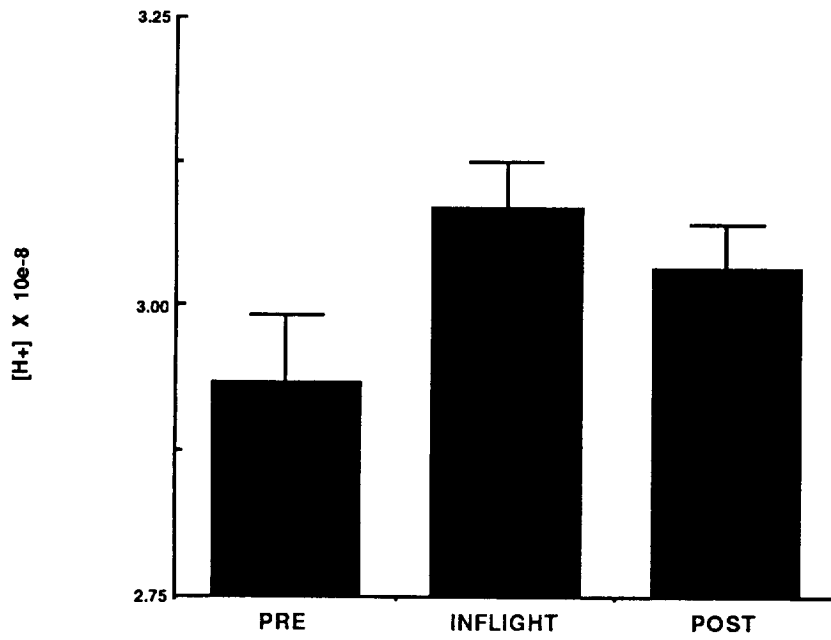
Early findings of increased serum ionized calcium and significantly decreased blood pH in samples from SLS-2 led us to investigate the possibility that there were changes in blood acid-base balance which could be contributing to the metabolic changes we saw. Through comprehensive laboratory investigations, we concluded that most if not all of the increase in blood CO₂ that we saw in those samples was an artifact of blood processing (although we have still not identified the source of the increased CO₂). Because of this, special care was taken to obtain and process blood samples for LMS with a procedure where any such artifacts would be minimized, using the same hardware, processing time and temperatures expected inflight. Through the diligent efforts of the crew and ground support personnel, we believe that the samples we obtained for LMS are free of artifact.

Figures 9 and 10 show the serum pH and calculated hydrogen ion concentration pre, in and postflight. There is approximately a 10% increase in the blood H⁺ concentration inflight, which returns toward baseline postflight. This is paralleled by a decrease in urine chloride inflight (Figure 11), which is consistent with the hypothesis that metabolic acidosis exists during spaceflight. This could occur if there is a decrease in peripheral vascular resistance, pooling of capillary blood increasing its residence in the periphery, and increased serum chloride to balance the H⁺ ions. This is an exciting hypothesis which we hope to explore further.

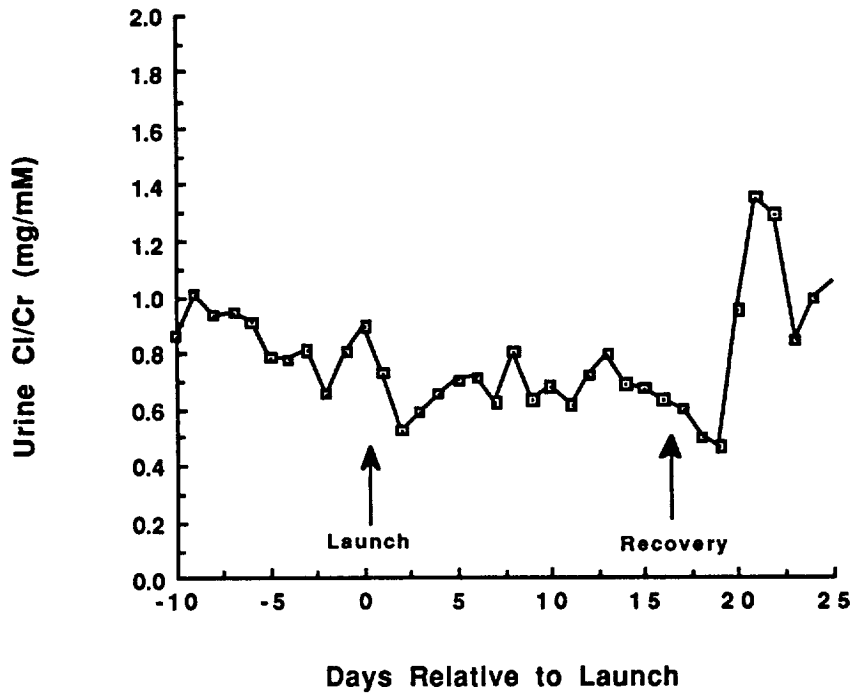
Response of Serum pH to Flight
L-1 to R+2 (mean/sem, n=4)



Serum H⁺ Concentration
Pre, In, and Postflight



Urine Chloride Excretion
L-10 to R+7



Conclusions and Speculation

Bone resorption increases relatively early in spaceflight (3-5 days). Serum ionized calcium is increased as a result and remains elevated throughout the duration of the flight.

The normal adaptive responses of a decrease in serum PTH as ionized calcium is increased occur.

The effect of a short term exposure to spaceflight is still manifest 2 months postflight with increased bone turnover and possibly elevated serum calcium.

Serum ionized calcium is elevated as early as 2 hours into flight, and PTH is already decreased as an adaptive response. This is too early for an increase in osteoclastic bone resorption due to recruitment of new cells, which occurs as expected by 3-5 days. In addition, urine DPD is not increased early, but occurs on the same expected time scale of 3-5 days. There may be another mechanism for the early increase in serum ionized calcium.

Serum pH is decreased, with a slight (5%) increase in blood H⁺ concentration as early as 2 hours. Urine pH does not change significantly, but urine chloride excretion is decreased early. These findings are consistent with a slight metabolic acidosis occurring immediately upon exposure to microgravity. However, the serum ionized calcium is increased even when normalized to pH 7.4, so the increase in H⁺ cannot explain completely the increased Ca⁺⁺.

Speculation: Decrease in peripheral capillary resistance and pooling of blood in the extremities leads to increased residence time for the blood in the capillary beds. Blood acid content is increased slightly, and may lead to alterations in renal handling of calcium. This should be investigated further to see if the response is sufficient to warrant prophylactic treatment with bicarbonate or other agents.

Bottom Line: Two and a half weeks of spaceflight induces a bone remodeling transient which lasts at least 2 months after return to earth. The increase in bone resorption occurs within 3-5 days as expected, and there is no evidence for a depression of bone formation at this point, except for a transient decrease possibly related to a stress response. Calcium metabolism is altered within 2 hours of launch, possibly due to a slight metabolic acidosis caused by the shift of blood to the peripheral capillary beds, and there may be renal effects in response to this as well.

JSC Human Life Sciences Project

**E401 - The Effects of Microgravity on Skeletal Muscle
Contractile Properties**

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The Effects of Microgravity on Skeletal Muscle Contractile Properties

Investigation E401

Final report

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Date: July 21st, 1997

CMU Ref: E401-PEMS PF02

**The Effects of Microgravity
on Skeletal Muscle Contractile Properties
Experiment E401 STS-78**

Final Report

I. Introduction

Exposure of humans and lower mammals to either actual or simulated spaceflight has been shown to lead to muscle wasting and weakness (Berg et al. 1991, Dudley et al. 1989, Hather et al. 1992, LeBlanc et al. 1988, Thomason and Booth, 1990). By and large, the data on lower mammals and humans indicate that postural muscles are less resistant to atrophy than non-postural muscles, (c.f. Thomason and Booth, 1990). The functional implications of atrophy may be evaluated by studying changes in skeletal muscle contractile features. Muscle function of humans exposed to spaceflight had so far been assessed through strength measurements performed under conditions of voluntary activation by the nervous system. Changes in voluntary contraction force may therefore arise from either muscular or neural factors or both. On the contrary, by stimulating the muscle directly it is possible to measure force beyond the volitional control of the subject and assess aspects of muscle contractility, which cannot possibly be determined from voluntary contractions. The use of this technique thus provides an objective measure of the functional status of skeletal muscle since contractions are at a precise level of activation. These contractile parameters, as studied by means of percutaneous muscle stimulation, have been shown to be fibre type-specific, resulting in changes of twitch characteristics, loss of force at specific frequencies of stimulation and changes in fatigability (Russel *et al.* 1983; Lopes *et al.* 1982, Russel *et al.* 1984; Church *et al.* 1984, Davies et al. 1987). Percutaneous muscle stimulation has previously been used to investigate the changes in human muscle contractile properties induced by bedrest deconditioning (Davies et al. 1987, Cerretelli et al. 1996), but never by spaceflight. This investigation was therefore set out to evaluate changes in muscle function of the human triceps surae induced by spaceflight, by-passing neural control, through direct muscle electrical stimulation.

A. Hypothesis

The hypothesis of the present study was that muscle atrophy induced by spaceflight would alter the contractile features of the human plantar flexors. Spaceflight was expected to lead to a decrease in electrically elicited contraction force as well as in muscle size. Given the difference in fibre composition of the soleus and gastrocnemius (88% type I for the SOL and 48% for the GAS, 16) it was expected that a greater atrophy of the soleus, apparently being more vulnerable to atrophy than the gastrocnemius (Thomason

and Booth, 1990), would lead to: i) faster contraction and relaxation times, ii) tetanization at higher stimulation frequencies, iii) and an increase in fatiguability. If instead, both the soleus and gastrocnemius atrophied to the same extent, no change in twitch characteristics, a non frequency-dependent loss of torque, and no change in fatiguability would be expected.

In addition, the comparison of the results of the voluntary versus the electrically induced torque would allow assessing changes in voluntary drive induced by spaceflight.

B. Objectives of the experiment

The aim of experiment E401 was to assess the changes in the electrically evoked isometric contractions of the human triceps surae (TS) during 17-day spaceflight.

Specifically, this study was set out to determine if spaceflight modified:

1. The relation between stimulus intensity and maximum twitch-torque
2. The relation between the TS twitch-torque and ankle joint angle
3. The maximum voluntary strength of the TS as well as the ability to voluntarily activate all TS motor units
4. The relationship between the frequency of stimulation and torque
5. The resistance to fatigue during repeated electrically evoked contractions
6. The force per cross-sectional area of the TS muscle group (evaluated in collaboration with experiment E029).

II. Methods of data acquisition and analysis

The contractile characteristics of the triceps surae were investigated during both voluntary and electrically-evoked isometric contractions using the percutaneous electrical muscle stimulator (PEMS, ESA instrument, developed by C.I.R., Switzerland) and recording the torque generated with the torque-velocity dynamometer (TVD, ESA instrument, developed by E.T.H., Zurich, Switzerland). In each testing session five PEMS protocols were performed according to the following order:

1. Supramaximal Current Intensity (SMCI) determination. The TS was stimulated with 50 μ s pulses at an ankle angle of 15 degrees dorsiflexion, increasing the stimulation intensity from an initial 100 mA up to a maximum of 800 mA (in 50 mA steps), and monitoring the twitch torque developed with the TVD, until no further increase in torque was observed.
2. Angle-torque relationship (ATR). Twitches were measured at 7 different angles of the ankle joint (30, 25, 15, 5 degrees plantar flexion (PF), and 5, 15, and 20 degrees

dorsi flexion (DF) (degrees relative to the TVD foot plate), two twitches per angle, 5 seconds apart.

3. Maximum voluntary contraction (MVC) with super-imposed twitches. Three isometric MVCs (at 20 degrees DF), each lasting 4 seconds and interspaced by 20 seconds were performed. During each contraction, the degree of muscle activation was assessed using the twitch super-imposition technique (Merton 1954). This consists of single stimuli (two) at SMCI, in response to which, if the contraction is submaximal, an increase in force on top of the voluntarily contraction is obtained. It thus allows to identify submaximal central neural drive during MVC.
4. Frequency-torque relationship (FTR). This was obtained by stimulating the muscle at varying frequencies. Three twitches (5 seconds between each twitch) were given followed by consecutive trains of pulses, each 1 second in duration at 10, 20, 30, and 50 Hz. This was performed with a current intensity of 60% SMCI. This lower SMCI had to be used to minimize the subject discomfort at the higher frequencies. The stimulation intensity of 60% of SMCI, referred to the muscle twitch. At 50 Hz, because of temporal summation, the torque generated was about 85% of that produced at the same frequency using 100% SMCI. Furthermore, previous tests conducted in the author's laboratory, showed that the shape of the FTR curve obtained at 60% SMCI matches that obtained at 100% SMCI. This showed that the portion of the muscle reached by the stimulation is maximally activated and behaves as the whole muscle. So, 60% SMCI were regarded as a good compromise between subject's tolerance to the test and scientific outcome.
5. Fatigue test. During this protocol the fatigability of the TS was tested during 120 s stimulation at 60% SMCI using trains of stimuli at 20 Hz lasting 350 ms repeated once per second. From this test a fatigue index (F.I.) was calculated as the ratio between the torque of the last (120th) over that of the 1st contraction.
6. Calf muscle plus bone CSA (CSA_{m+b}). CSA_{m+b} was determined anthropometrically from circumference (measured at the site of maximum circumference obtained before the flight) after subtraction of subcutaneous fat by conversion of lateral and medial calf skinfolds into adipose tissue thickness using the equations of Jones *et al.* (1986).

Experimental schedule

Before the flight, PEMS protocols 1 to 5 were on L-90 (actual L-87,-86, -85, -84, -83), L-60 (actual L-50, and L-45), L-30 (actual L-31 and L-30). On L-15 (actual L-12)

protocols 1 to 5 were also repeated.

Calf CSA_{m+b} was measured on all subjects on L-30 (L-31 and L-30)

During the flight (FD1=20 June '96) PEMS protocols 1 to 5 were performed on FD3/4, FD8/9, FD13/14 and FD16.

During the recovery period PEMS protocols 1 to 5 were carried out on R+2 (actual R+2), R+4 (actual R+4), R+8 (actual R+8), R+15 (actual R+15), R+30 (actual R+30).

Calf CSA_{m+b} was measured on R+2, R+8, R+15 and R+30

III. Results

Twitch angle-torque relationship (ATR)

During the flight, no significant changes in ATR relation were observed. However, during recovery, significant decreases in twitch peak torque (PT_w) at joint angles comprised between 20 deg of dorsiflexion (DF) and 5 deg of plantar flexion (PF) were found on days R+4, R+8, R+15 and R+30 (Fig. 1a). The largest decreases occurred on R+8 at which PT_w was reduced by about 20% ($p < 0.01-0.05$), irrespective of joint angle, across angles from 20 deg DF to 5 deg PF (Fig. 1b). No significant changes in PT_w were present beyond 5 deg PF.

Twitch characteristics

Time to peak tension showed no significant changes during spaceflight and recovery. Half-relaxation time (1/2RT) significantly decreased during the flight (-18% on FD8 and -8% on FD16), and on recovery day R+2 (-7%); thereafter, 1/2RT values were not different from pre-flight.

Frequency torque relation

No changes in FTR were found during the flight, while a progressive downward-shift in the ATR curves occurred during recovery. For all stimulation frequencies (1, 10, 20, 30 and 50 Hz) the nadir of the decrease in peak torque (PT) was reached on R+8 and was on average 24.0% (< 0.01 , Fig. 2a). On R+15, PT values from 1 to 50 Hz were still below (-13%, $P < 0.05$) those obtained before the flight. The loss of PT was not frequency dependent since no significant differences were found between the percent PT loss at each frequency. This effect was clearly shown by normalizing torques at all frequencies for those developed at 50 Hz, as a result of which all FTR curves matched with that obtained before the flight (Fig. 2b).

Maximum voluntary contraction and tetanic torque

No change in MVC was found during the flight, but a progressive increase up to 17% ($P<0.01$) was observed throughout recovery (Fig. 3). The increase in MVC was accompanied by an increased capability, in all crewmembers, in twitch-occlusion, showing an improved ability in motor unit recruitment.

By contrast, the tetanic torque at 50 Hz (PT50) showed a significant decrease throughout recovery, reaching its nadir (-22%, $P<0.01$) on R+8 (Fig. 3).

CSAm+b and Torque/CSAm+b

Calf CSA_{m+b} was significantly reduced (-7%, $P<0.01$) after the flight on days R+2 and R+4. Thereafter, a progressive recovery was observed and on R+30 CSA_{m+b} was back to its pre-flight values.

A significant decrease (-19%, $P<0.01$) in tetanic torque at 50Hz normalized for calf CSA_{m+b} (PT50/CSA_{m+b}) was found during recovery on R+8.

Fatigability

A significant increase in the fatigability (decrease in F.I.) was found during recovery on days R+8, R+15 and R+30. This increase in fatigability, showing a nadir on R+15 (16%, $P<0.01$, Fig. 4), was due to a greater decrease in the torque values at the end than that at the beginning of the fatigue test.

IV. Conclusions

The present results on electrically evoked human gastrocnemius muscle function assessed during the NASA-LMS mission (Shuttle flight STS-78), show a significant impairment in contractile parameters during recovery, but not during spaceflight. The observed decrease in muscle tetanic torque (24%) was greater than the decrease in calf CSA_{m+b} (7%) as shown by a significant decrease in tetanic torque/CSA. The latter finding is in agreement with a reported decrease in soleus fibres specific tension observed by Widrick et al. (1996) in the same study and may indicate the presence of muscle damage during the re-loading phase in 1-G. Fatigability of the triceps surae was found to increase by 16% after the flight. This seems partly explained by the decrease in half-relaxation time, but could also be due to muscle damage. It seems noteworthy that also for in the quadriceps, of the same crewmembers, Tesch and Berg (1996) also found fatigability to be increased. The paradoxical increase in MVC during recovery is likely explained by a submaximal neural activation at the beginning of the study, however, other neural factors such as antagonist muscles activation or a training effect may be involved too.

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Fig 1. a) Angle torque relation before and after spaceflight (during not shown because n.s.), (b) Percent decrease in torque on R+8 (mean±SE, **=P<0.01)

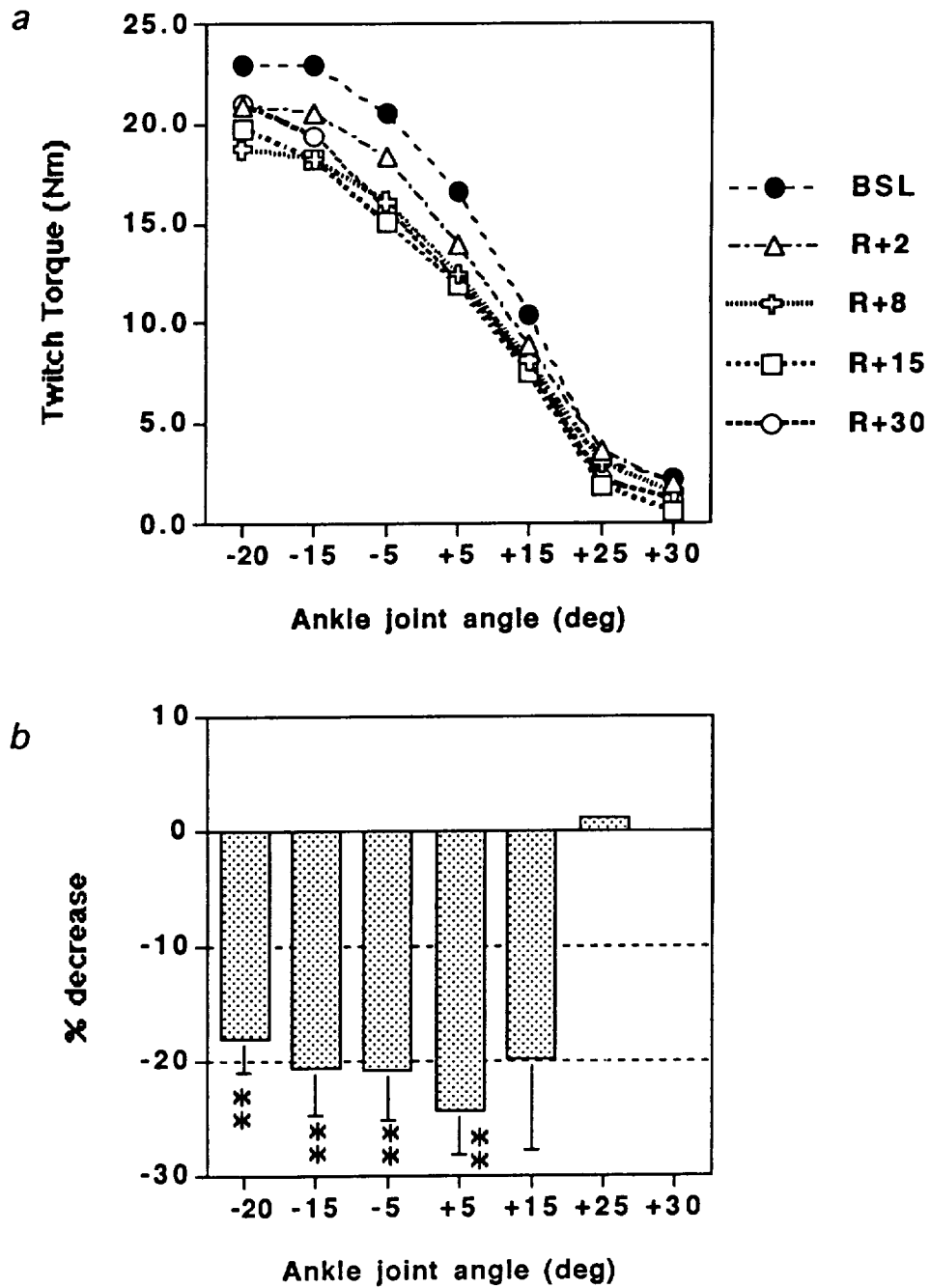


Fig. 2. Frequency-Torque relation in absolute values (a), and normalized for the torque at 50 Hz (b), before, during and after spaceflight. (*=P<0.01)

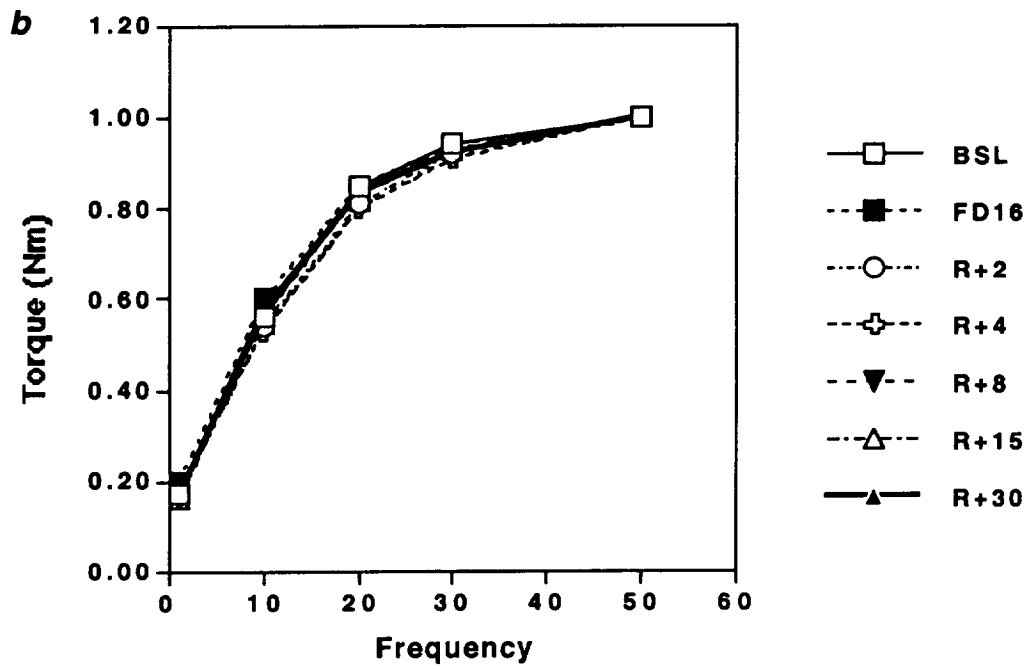
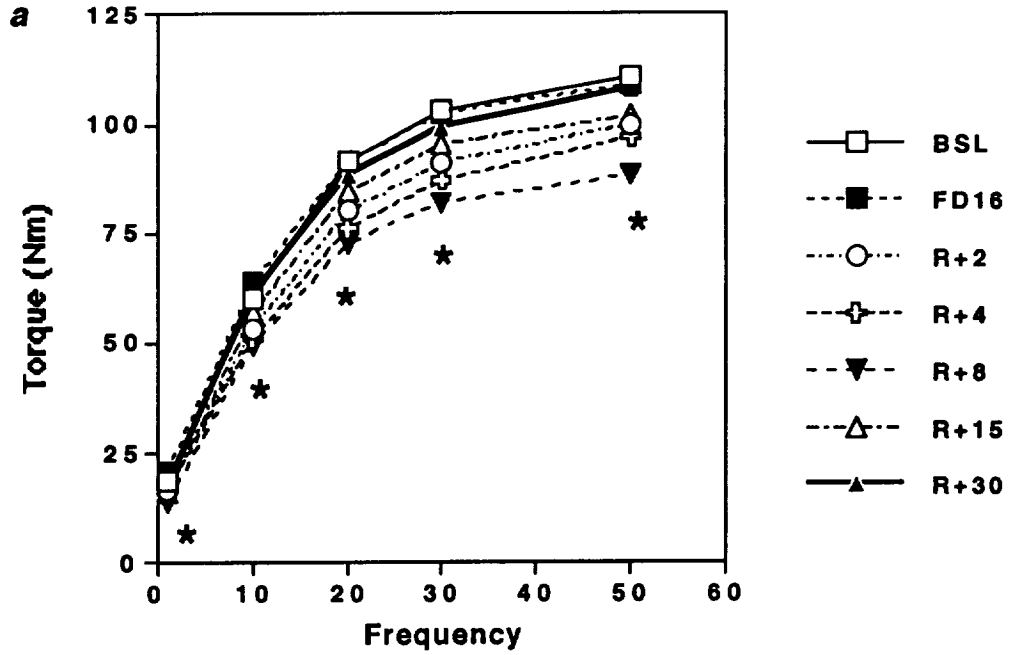


Fig. 3: Maximum voluntary contraction (MVC) and tetanic torque at 50 Hz (T50), before, during and after spaceflight (means±SE)

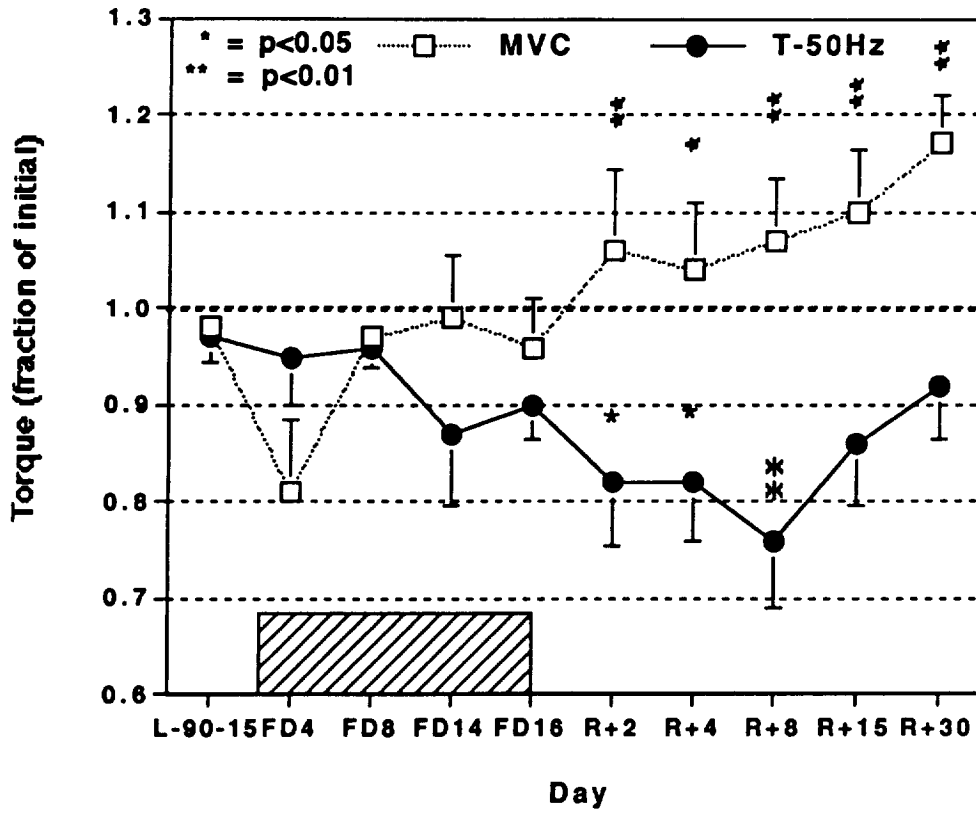
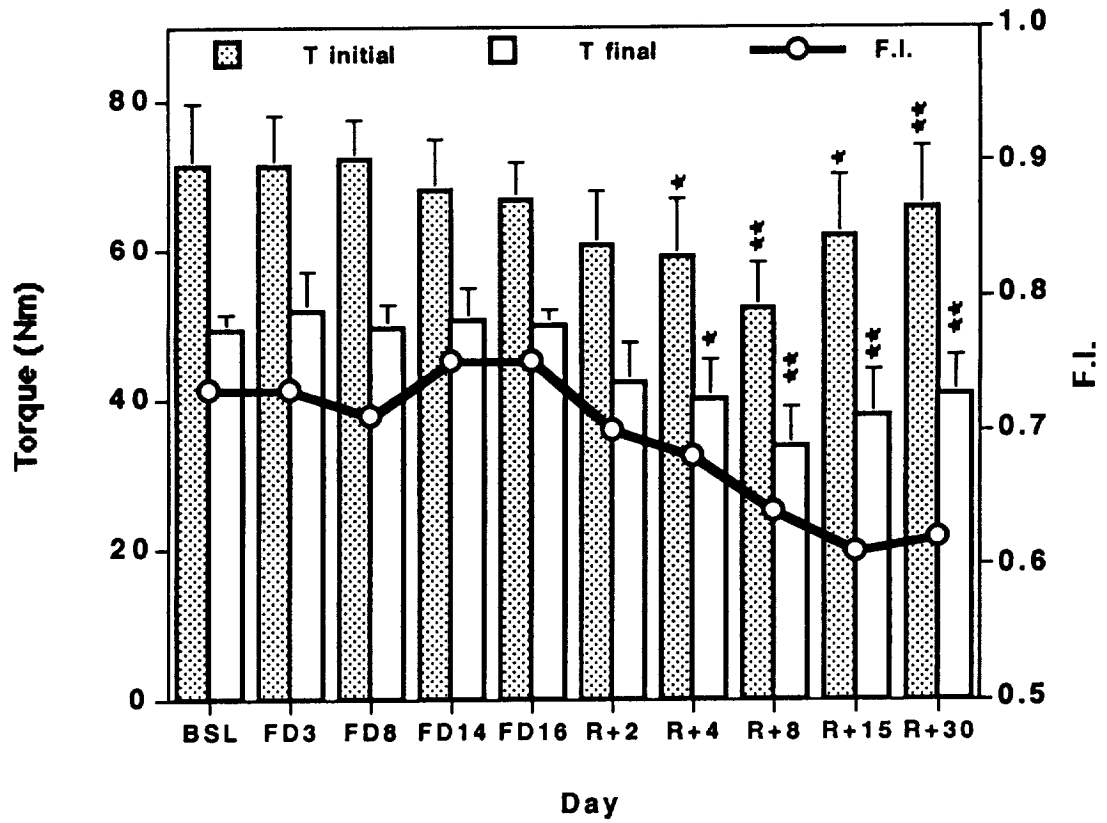


Fig. 4: Triceps surae fatigability before, during and after spaceflight. T initial and T final is the torque produced by the first and last contractions in the fatigue test; F.I. is the fatigue index (ratio of T final / T Initial). Values are mean±SE.



JSC Human Life Sciences Project

E407 - Effects of Microgravity on the Biochemical and Bioenergetic Characteristics of Human Skeletal Muscle

Principal Investigator:

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E 407: Final Report

Effects of Microgravity on the Biomechanical and Bioenergetic Characteristics of Human Skeletal Muscle

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Theoretical Backgrounds

Long term space flights are known to affect muscle structure and function, essentially because of the absence of the constant pull of gravity. However, the extent and the ultimate reasons thereof are only partially understood. Therefore, investigations concerning the muscular activity as a function of the time spent in microgravity are of great interest from both the theoretical and practical points of view. Indeed, on the one hand they will allow us to gain a deeper understanding of the basic aspects of muscle contraction. On the other, they will provide useful suggestions on how to improve the countermeasures for preventing microgravity deconditioning.

Skeletal muscle transforms chemical energy into mechanical energy. This takes place during muscle contraction which is normally triggered by voluntarily induced nerve stimulation. Muscle contractions can be subdivided into three general categories: i) isometric, wherein the muscle is not permitted to shorten, so that the result of the contraction is the generation of force only, ii) concentric, wherein the force applied to the muscle is lower than the maximal the muscle is able to produce; as a consequence the muscle shortens, thus performing external mechanical work (force times distance shortened), iii) eccentric, wherein the force applied to the muscle is greater than the maximal; the muscle is therefore forcibly lengthened, thus absorbing mechanical work.

The maximal force the muscle can produce under isometric conditions depends on its length; it attains a peak at a length approximately equal to the "physiological" length of the muscle in vivo; it is less at greater or smaller lengths.

The speed at which a muscle shortens is a function of the force imposed on the muscle. For concentric contractions, the force velocity relationship is hyperbolic, the velocity increasing from zero (when the force is equal to the maximal isometric) to a maximum when the force is zero (unloaded contraction). For eccentric contractions, the velocity of shortening is negative (the muscle becomes

longer); it increases (becomes more negative) only slightly for forces not much larger than the isometric; for greater forces the increase in speed becomes much larger.

As a first approximation, skeletal muscles can be viewed as being constituted by the contractile machinery and by an elastic system in series transmitting the force generated by the machine to the appropriate lever arms, thus smoothing and damping the responses of the system.

The mechanical events of the contraction are elicited and preceded by electric signals which can be recorded by appropriately positioned electrodes applied to the skin. These electric signals, defined Electromyogram (EMG) yield information concerning the activity of the different motor units.

Phenomenologically, muscle function is completely characterised when the following basic aspects are known:

- i. force length characteristics of the contractile component;
- ii. force length characteristics of the series elastic component;
- iii. force velocity characteristics of the contractile component;
- iv. activation pattern of the motor units

Present knowledge of the biomechanics of isolated muscles is rather satisfactory (e.g. see Carlson and Wilkie, 1974; Woledge, Curtin, and Homsher, 1985). However, our understanding of the functional characteristics of human muscles in vivo is less complete. In addition, long term space flights are known to affect muscle structure and function (Nicogossian, 1989; Grigoriev and Egorov, 1991), to an extent and in a manner, as yet only partially understood. Therefore, investigations concerning the biomechanics of human muscle function on Earth and in microgravity are of great interest from both the theoretical and practical points of view.

Moreover, previous experience accumulated by this group of researchers (Antonutto et al., 1995) demonstrated that the maximal explosive power of the lower limbs, i.e. the maximal power developed during a very short (0.25 - 0.30 s) all-out effort of the lower limbs, such as a maximal standing high jump off both feet, was substantially reduced after space flight. Indeed, on the occasion of the Euromir 94 and Euromir 95 missions it was observed that the maximal explosive power was reduced to about 70 % of the preflight value after one month in microgravity (one subject) and to about 50 % after six months (three subjects). Indirect but convincing arguments suggest that a large fraction of this decrease is due to a modification of muscle control and co-ordination in space, tending to favour smooth and slow motor patterns, as opposed to forceful, explosive ones.

The results of the present study will allow us to gain a deeper understanding of the effects of microgravity on muscle function per se. It will therefore become possible to better disentangle the extent to which the decline of the explosive power after space flight is due to changes of motor control and co-ordination and to changes of muscle activity per se.

Objectives of Experiment

The fundamental questions are:

- i. the force length characteristics of the contractile component;
- ii. the force length characteristics of the series elastic component;
- iii. the force velocity characteristics of the contractile component;
- iv. the motor units activation pattern

of the biceps and triceps brachii and of the triceps surae of four crew members before, during, and after the space flight. In a first step these fundamental characteristics are described by:

- i. the isometric torque - angle relationship;
- ii. iEMG - isometric torque relationship;
- iii. torque - angular velocity;
- iv. iEMG - isokinetic torque relationship.

Methods, Experimental Protocol and Data Analysis

Subjects. The experiments were performed on four male astronauts (43 ± 2 yr; 183 ± 4 cm; 86 ± 3 kg) who participated in the LMS -STS 78 mission which took place from June 20th 1996 to July 7th 1996. The subjects were tested four times before the flight to obtain base line data and three times during the flight. After the flight, measurements were taken on four occasions (see Figure 1).

Torque. Muscular torque, as given by the product of the force generated by the muscles times the lever arm, was assessed by means of the Torque Velocity Dynamometer (TVD) constructed in the Laboratory of Biomechanics of the Swiss Federal Institute of Technology (Zurich, Switzerland). The TVD allows one to measure the angular velocity, the angular position and the torque during predefined types of contractions of the flexors and extensors of the elbow and of the ankle. The following contraction types have been studied: i) isometric, wherein the position of the joint was fixed and the torque measured, and ii) isovelocitity, wherein the angular velocity was imposed and the torque measured; iii) the same as under ii), but during eccentric contractions, i.e. where the force exceeded the maximal isometric and the muscle was forcibly lengthened. Since the axis of rotation of the TVD was aligned with that of the ankle or of the elbow and since the activity of all accessory agonist and antagonist muscle groups was essentially negligible, the conclusions derived from the experiments apply to the tested muscle groups only, and define the characteristics thereof.

EMG. Surface myoelectric activity (EMG) of the *biceps brachii*, *triceps brachii*, *gastrocnemius lateralis*, *soleus* and *tibialis anterior* were recorded during the experiments placing silver - silver chloride electrodes on predefined positions (Zipp, 1982). The day before launch the locations contours were marked on the skin with a permanent ink pen and, to maintain consistent placements of the electrodes during the experiments performed in flight, the subjects were asked to refresh these contours daily.

EMG signals were fed to a physiological signal conditioner (PSC, NASA). Together with Torque and angular position (α) signals, they were then converted by means of an A/D converter (500/1000 Hz) (National Instruments, USA) interfaced to a portable computer (Think Pad, IBM, USA) and operating at a sampling frequency of 500 Hz (for angular position, velocity and torque) and of 1000 Hz (for EMG signal). During experiments on flight, data were recorded on optical disk and, in parallel, on a tape running on a portable tape recorder (HR 30, TEAC). When possible, they were also down linked to the Space Monitoring Area at the L. Johnson Space Centre in Houston to allow investigators to check the quality of the traces.

Experimental protocol. The experimental protocol pre-, in- and post-flight consisted in a series of predetermined profiles repeated at regular intervals on the TVD to form an experimental block. A single profile had a duration of about 8 s, wherein several TVD modes were combined as indicated in Table 1. The EMG was recorded throughout.

1. After placing the surface electrodes for EMG recording on the agonist and antagonist muscle groups, the subject entered the TVD and the necessary strapping and safety controls were performed. The lever arm of the TVD was automatically positioned to the appropriate angle (see Table 1) and the subject was asked to exert a Maximal Voluntary Contraction (MVC) and to maintain it for the whole duration of the profile.

2. The profile was started in the Isometric (IM) mode and the torque (T) was measured. Duration of this phase: 2 s plus a random interval ranging from 0 to 0.5 s. Independent variable: angle; dependent variable: T.
3. The TVD switched automatically to the Isokinetic (IK) mode. The joint angle was decreased (or increased) by a given amount at a predetermined angular velocity. The duration of this phase was 0.2 s for the Isokinetic concentric (IKc) mode and of 0.4 s for the Isokinetic eccentric (IKe) mode. The angle attained was maintained for 1.8 s plus a random time ranging from 0 to 0.5 s. An angular velocity equal to 50 % of concentric velocity, and in opposite direction, was then applied for the same duration (IKc: 0.2 s; IKe: 0.4 s). Thus, after 2.4 s plus the random time (0 to 0.5 s) from the onset of movement the original joint angle was reached again. Duration of this phase: 2.4 s + random time. Independent variable: angular velocity; dependent variable: T.
4. The TVD switched again to the IM mode. The original position was maintained for 2 s. Independent variable: angle; dependent variable: T.
5. The profile was followed by a pause of 50 s.
6. This single profile was followed by a second one wherein phases 2 to 4 were repeated at a sub maximal, pre-set Torque level, corresponding to 50 % of the individually established pre-flight MVC. In this case a screen appropriately positioned in front of the subject allowed him to check that, in phase 2 and 4, the exerted torque was indeed close to the required 50 % level.
7. A pause of 30 s followed.

The above described two profiles set had a total duration of about 96 s, subdivided into about 8 s of activity at 100 % MVC, 50 s pause, about 8 s at sub maximal level, 30 s pause. Angular velocities (concentric and eccentric) and angles are reported in Table 1. The torque corresponding to the 50 % MVC sub maximal level of muscular activation was set, for each individual and each muscle group, on the basis of the isometric torque attained by the subject during a

preliminary series of maximal voluntary contractions performed shortly before the first experimental session. Afterwards, this level was kept constant throughout the study and applied for all the tested angles.

As indicated in Table 1, we investigated 7 double profile sets and since each double profile was repeated twice, an experimental block on a given muscle lasted about 22.5 min.

The experiments were performed four times before flight (at days L-90, L-60, L-30 and L-15), three times in flight (FD 2, 3, 4; FD 7, 8, 9; FD 13, 14, 15) and four times after flight (R+1, 2; R+4, 5; R+8, 9; R+15, 16) (see Figure 1).

To run the experiments, a computerised procedure, developed by NASA according to the requirements of the investigators, was utilised. This procedure, running on the portable PC, made it possible to load via serial port the profiles into the TVD, to execute the profiles in the proper order while acquiring and storing EMG, torque, velocity and angle signals and to provide on the screen a visual feedback of the Torque and angle outputs to the subject.

Data were analysed off-line by means of automatic, computerised routines developed for this aim at the Laboratory of Biomechanics of ETH in Zurich according to the following methods.

a. Isometric Torque (T_{im} , N m): T_{im} values at 100 % of MVC and at sub maximal level were always obtained as the mean of the Torque signal over an interval of 500 ms. In each profile, the three values of T_{im} corresponding to the two angles α_0 and α_1 were determined: i) at α_0 and at α_1 just before the TVD lever arm moved to the new angle; ii) at α_0 again, 100 ms after the completion of the second movement (Fig. 2A).

b. iEMG during isometric contraction (iEMG, mV): the EMG raw signal corresponding to the time interval (Δt) over which T_{im} was calculated, was band-pass filtered (20 - 200 Hz), rectified, smoothed by means of a moving average with a time window of 75 ms, integrated and finally divided by Δt to obtain the average values in mV of the integrated, rectified myoelectric surface signal

(iEMG) representative of the electrical activity of the muscles (Basmajian and De Luca, 1985).

c. Isokinetic Torque (T_{ik} , N m): T_{ik} in either eccentric or concentric contractions was calculated as follows. The Torque signal corresponding to the first 40 ms after the onset of the movement was omitted. T_{ik} was then calculated as the average over the following 50 ms together with the corresponding mean angular velocity (ω , degrees s^{-1}). (Fig. 2B).

d. iEMG during isokinetic contraction (iEMG, mV): raw EMG signal was time-shifted by 30 ms so as to take into account the electro-mechanical delay (EMD) existing from muscular activation and corresponding mechanical output. Then, iEMG was calculated over the same time interval wherein T_{ik} was obtained applying the same procedure as for the iEMG of T_{im} .

Analysis of the data during isometric contractions has been completed both for Torque and EMG signals, whereas it is still in progress for the isokinetic protocols.

For each experimental session and tested muscular group, the individual T_{im} values at 100 % and 50 % of maximal activation were plotted as a function of α and fitted by means of a second order polynomial: $T_{im} = a_0 + a_1 \alpha + a_2 \alpha^2$. This allowed us to calculate, for elbow flexors and extensors and for ankle plantar flexors, the interpolated values of T_{im} at $\alpha = 0$, i.e. at the angular positions of 100 and 90 degrees of the elbow and ankle joints, respectively. This method, rather than considering the single T_{im} values corresponding to each angle, allowed us to obtain a reliable index of the individual muscular performance resulting from a statistical interpolation calculated on a broad set of data. Furthermore, it must be underlined that T_{im} at $\alpha = 0$ does not correspond to the maximal torque the subject can exert with muscles at that given joint. As such, its value may change as consequence of changes in the force-length relationship. The same kind of analysis was then applied to the iEMG values calculated during isometric

contraction so as to obtain the interpolated iEMG at the same angular position as for T_{im} .

As an example of the applied method, T_{im} values at 100 % of MVC of the elbow flexors and ankle plantar flexors in one subject are plotted as a function of the angular position α in Fig. 3A and 3B along with the regression equation fitting the data. The large full dot corresponds to the T_{im} obtained setting $\alpha = 0$ in the regression equation: it indicates T_{im} at 100 degrees for the elbow or at 90 degrees for the ankle. In the same Figures, the iEMGs of the *biceps brachii* and of *gastrocnemius lateralis* during the same set of measurements are also reported along with the equations fitting the data and the interpolated values at 100 or 90 degrees.

Statistics. Differences among the T_{im} and iEMG average values obtained in each single session were evaluated by means of the Friedman test, i.e. the analogous of the 2 - ways ANOVA in the non parametric domain. Pairwise differences were evaluated by means of the Wilcoxon non parametric test (Daniel, 1991). Average values are reported with their standard deviations, if not otherwise specified.

Results

Individual T_{im} and iEMG values were first normalised dividing them by the average of the corresponding values obtained on all sessions after flight.

In Fig 4A the normalised T_{im} at 100 % MVC of the flexors and extensors of the elbow and that of the plantar flexors is reported as a function of the day from launch. The average value of T_{im} of elbow extensors turned out to be significantly larger ($P < 0.05$) during flight than before flight. On the contrary, the average value of T_{im} of the plantar flexors was significantly smaller in flight than before ($P < 0.05$). Moreover, T_{im} of the plantar flexors post-flight was larger than before flight, although not significantly so ($0.05 < P < 0.1$). T_{im} assessed at sub

maximal level of muscular activity did not show, as expected, any significant variation during the whole period (Fig. 4B).

The normalised values of the *biceps and triceps brachii* iEMG during elbow flexion at 100 % MVC and at sub maximal level of activation are reported in Fig. 5A and 5B as a function of day from launch. No significant differences were detected either for agonist (*biceps brachii*) or for the antagonist (*triceps brachii*). On the contrary, iEMG assessed from *triceps brachii* during elbow extension at 100 % MVC and at sub maximal level increased significantly in the early phases of the flight. The increase of neuromuscular activity gradually subsided during the following days to attain the pre - flight level on the fifteenth/sixteenth day of microgravity exposure (upper panels of Fig. 6A and 6B). Also in the case of elbow extension, no significant variations of the antagonist (*biceps brachii*) iEMG was detected throughout the study (lower panels of Fig 6A and 6B).

The iEMGs during plantar flexion were calculated from surface EMG of *gastrocnemius lateralis* and of *soleus*; they showed significant variations in flight ($P < 0.05$) both at 100 % of MVC and at sub maximal activation (Fig 7A and 7B). iEMG assessed at 100 % MVC kept increasing during flight (Fig 7A), whereas those corresponding to the sub maximal level of activation very early in flight attained a value higher than that prevailing in pre-flight condition and declined slightly thereafter (Fig. 7B).

Discussion

Because of the low number of the subjects and in view of the fact that the results obtained during isokinetic contractions have not yet been completely analysed, the comments that follow must be considered with some caution. Within these limits, a few findings are worth underlining.

During flight, the neuromuscular system seemed able to maintain an unchanged capability of maximal recruitment of motor units. In fact, iEMG at 100 % of MVC of *biceps brachii*, *gastrocnemius lateralis* and *soleus* remained almost stable throughout the study, whereas that of *triceps brachii* increased in the early phases of flight and remained high thereafter until return on Earth. In this connection, it is worth pointing out that the observed increase of the iEMG of the latter muscle could not have been caused by cephalic fluids shift taking place at the onset of microgravity exposure (Greenleaf, 1977) since this phenomenon should have affected to the same extent all the EMG signals recorded from both the agonists and antagonists of the upper limbs.

The increase of *triceps brachii* iEMG was accompanied by a consensual and significant increase of the T_{im} of the elbow extensors. As a consequence, the ratio between mechanical output (T_{im}) and electrical activity, i.e. iEMG of *triceps brachii*, remained almost constant over the period of the study both at 100 % of MVC and at sub maximal level (Fig. 8A and 8B). Thus, early in flight there seemed to occur a sort of dishinibition of this muscle group which, however, gradually subsided in the course of the mission. When considering that *triceps brachii* is a "non antigravity" muscle, this phenomenon is similar to that reported by Edgerton and Roy (1996, 1997) who showed an increase of the electrical activity of the flexors (non antigravity), as compared to the extensors (antigravity) muscles of the lower limbs early in flight. Furthermore, a significant reduction of the activation of the extensors muscle and an increase of that concerning the flexors were in fact consistently reported in the resting and standing conditions during microgravity exposure. This was thought to reflect a

generalised flexor bias of muscle activation which, however, was progressively restored in the course of the flight (Edgerton and Roy, 1996, 1997). The present data suggest that a similar release of inhibitory inputs, whose causes are still unknown, may also occur at the level of the forearm extensors.

In the early phases of flight, T_{im} of the plantar flexors attained a value significantly lower than pre-flight; however, it progressively recovered in orbit to attain, after fifteen days of mission, a value close to control (Fig. 4B) without any further change upon return on Earth. In this case, however, there was a clear dissociation between muscular performances in terms of T_{im} and iEMG: the neuromuscular activity of *soleus* and of *gastrocnemius lateralis*, in fact, was higher in the early phases of the flight as compared to the control situation. This phenomenon was reflected by the consensual drop of the Torque to iEMG ratio (Fig 9A and Fig 9B) particularly evident in the *soleus* muscle at 100 % of MVC and, for both the *soleus* and *gastrocnemius*, at sub maximal level of contraction. In fact, the drop of electrical activation at the two level of voluntary contraction may be well explained by the dramatic activation of the antigravity, extensor muscles of the lower limb commonly found after the onset of microgravity (Edgerton and Roy, 1996, 1997). Also the gradual recover of the iEMG taking place during flight agrees with the progressive recovery of the activity level of the extensors described by others and taking place in the course of the mission (Edgerton and Roy, 1996). However, the drops of plantar flexors T_{im} to *soleus* and *gastrocnemius lateralis* iEMGs ratios were clearly unexpected and it does deserve some words of comment. The drop of the T_{im} to iEMG ratios indicates the sudden occurrence of a microgravity effect negatively affecting the electromechanical coupling of this muscle. In fact, a drop of the T_{im} to iEMG ratio indicates that the involved muscle is able to develop a lower level of tension in response of the same electrical activation. This finding agrees well with the data recently reported by others and concerning the relationship between maximal isometric torque of lower limb extensors and surface electromechanical activity of

quadriceps muscles (Berg and Tesch, 1996). In that case, a significant decrease of maximal T_{im} of quadriceps was documented after only ten days of lower limb unilateral unloading and it was paralleled by a consensual drop of the corresponding Torque to iEMG ratio. This marked drop of maximal force, as well as its fast recovery after reambulation, was not accompanied neither by a proportional decrease of muscle mass nor by the impairment of maximal recruitment capability. In addition, it has been recently documented by this group that significant and progressive drop of the T_{im} to EMG ratio of the plantar flexors was progressively developing during seventeen days of head - down tilt bed rest (Milesi et al., 1997).

Hence, an unidentified factor (or a series of them) may contribute to the drop of maximal force affecting some specific antigravity muscle of the lower limb and taking place very early in flight and during lower limb unloading. Moreover, the muscle functions seem to recover in a time span shorter than the period of exposure to microgravity.

In conclusion, and within the limits mentioned at the beginning of the discussion, we think that future research in microgravity should aim at: i) a better description of the time course of the effects on muscle tension and motor units recruitment and; ii) a clear understanding of the specific factor (s) underlying this phenomenon.

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Figure Legend

- Figure 1: Schedule of the experiments performed pre-flight, in-flight and post-flight. Numbers in the boxes indicate the subjects evaluated in each experimental session.
- Figure 2A: Experimental traces obtained during elbow flexion at 100 % of MVC (profile # 3) are reported in Figure 2A. In the upper panel, the trace of the angular position α is plotted as function of the time. ω_0 (concentric, 57.3 degrees s⁻¹) and ω_1 (eccentric, -28.6 deg s⁻¹) refer to the two angular velocities tested in this profile. In the middle panel, the thick parts of the Torque trace indicate the intervals over which T_{im} was calculated. In the lower panel, raw EMG of *biceps brachii* is plotted after band-pass filtering.
- Figure 2B: Typical traces obtained during isokinetic - eccentric action of the ankle plantar flexors. Thick parts of the traces indicate the intervals over which T and ω (angular velocity) were calculated during isokinetic experiments.
- Figure 3A: T_{im} (N m) and iEMG (mV) as a function of angular position (degrees) obtained during at 100 % MVC the elbow flexors in one subject. The lower X axis refer to angles measured from an elbow angle of 100 degrees and defined to be the reference position (0 degree); upper X axis refer to the TVD angle. Large full dots correspond to T_{im} and iEMG obtained substituting 0 for α in the regression equations (see text for details).
- Figure 3B: Same as in Figure 3A, but for T_{im} and iEMG assessed during sub maximal activation of the ankle plantar flexors. PF = plantar flexors; DF = dorsiflexion.
- Figure 4A: Normalised T_{im} of the elbow flexors (upper panel) and extensors (middle panel) and of the plantar flexors (lower panel) at 100 % of MVC is plotted as function of the days from launch.
- Figure 4B: The same as in Figure 4A, but referring to sub maximal activation of the three muscle groups.
- Figure 5A: Normalised iEMGs of the *biceps brachii* (agonist during elbow flexion) at 100 % MVC, and of the antagonist *triceps brachii* are plotted as function of the days from launch. Points without SD were obtained from less than three values and were not considered in the statistical analysis.
- Figure 5B: The same as in Figure 5A, but referring to sub maximal activation of the elbow flexors. Points without SD were obtained from less than three values and were not considered in the statistical analysis.

- Figure 6A: Normalised iEMGs of the *triceps brachii* (agonist during elbow extension) at 100 % MVC and of the antagonist *biceps brachii*, are plotted as function of the days from launch. Points without SD were obtained from less than three values and were not considered in the statistical analysis.
- Figure 6B: The same as in Figure 6A, but referring to sub maximal involvement of the elbow extensors. Points without SD were obtained from less than three values and were not considered in the statistical analysis.
- Figure 7A: Normalised iEMGs of the *gastrocnemius lateralis* and of *soleus* (agonists during ankle plantar flexion) at 100 % of MVC and of the antagonist *tibialis anterior* are presented as a function of days from launch.
- Figure 7B: The same as in Figure 7A, but referring to sub maximal activation of the plantar flexors. Values of the *tibialis anterior* are not presented because of the paucity of the available data.
- Figure 8A: Normalised T_{im} to iEMG ratios at 100 % of MVC of the elbow are plotted as function of the days from launch. Upper panel reports T_{im} to iEMG ratio obtained dividing T_{im} of the flexors of the elbow to the *biceps brachii* iEMG; lower panel refers to elbow extensors and *triceps brachii* iEMG. Points without SD were obtained from less than three values and were not considered in the statistical analysis.
- Figure 8B: The same as in Figure 8A, but referring to sub maximal activation of the two muscle groups. Points without SD were obtained from less than three values and were not considered in the statistical analysis.
- Figure 9A: Normalised T_{im} to iEMG ratios at 100 % of MVC of the ankle are plotted as function of the days from launch. Upper panel refers to T_{im} to iEMG ratio obtained dividing T_{im} of the plantar flexors to iEMG of *soleus*; lower panel was obtained dividing T_{im} to iEMG of *gastrocnemius lateralis*. Points without SD were obtained from less than three values and were not considered in the statistical analysis.
- Figure 9B: The same as in Figure 9A, but referring to sub maximal activation of the plantar flexors.

Fig. 1

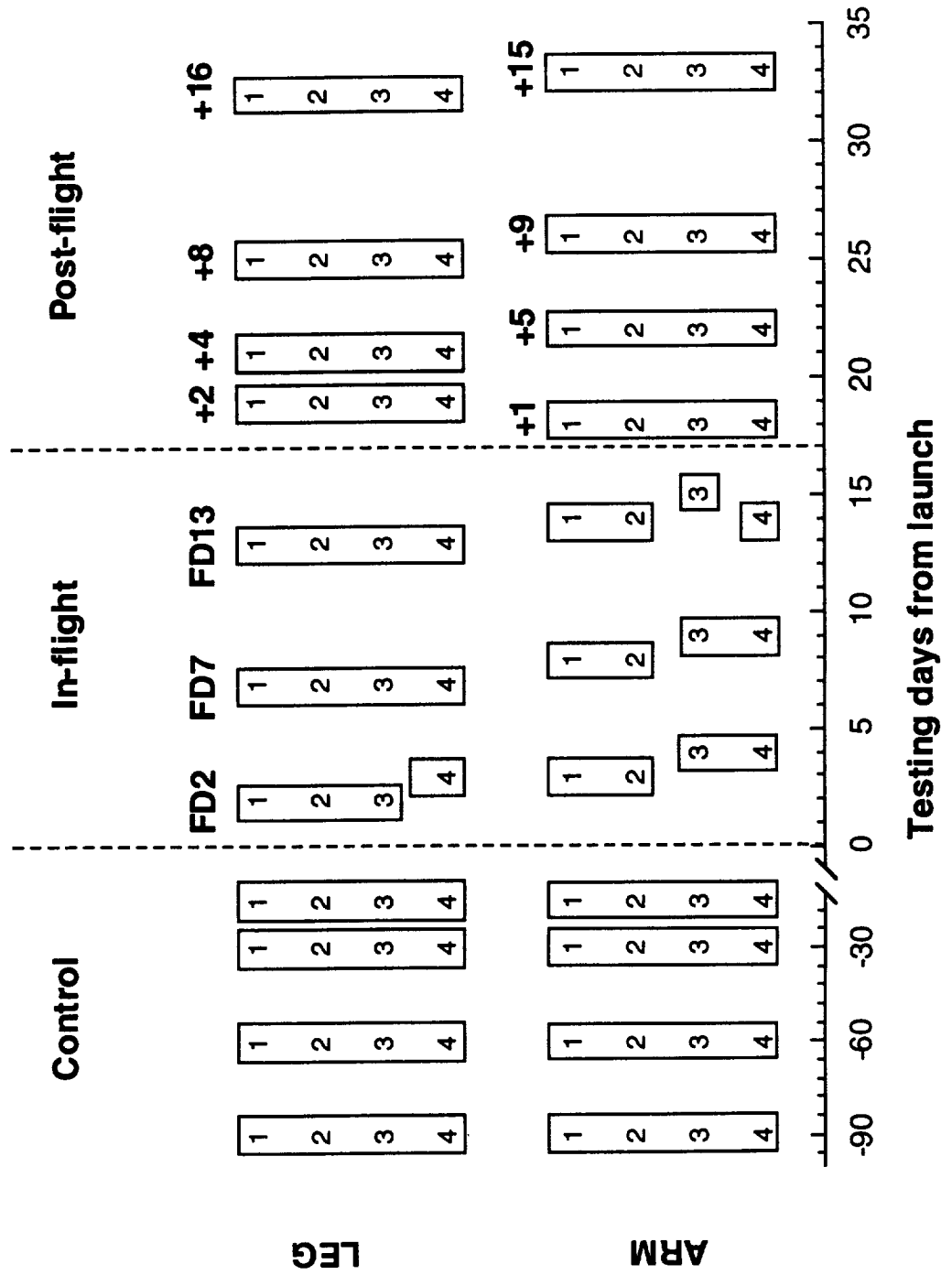


Fig. 2A

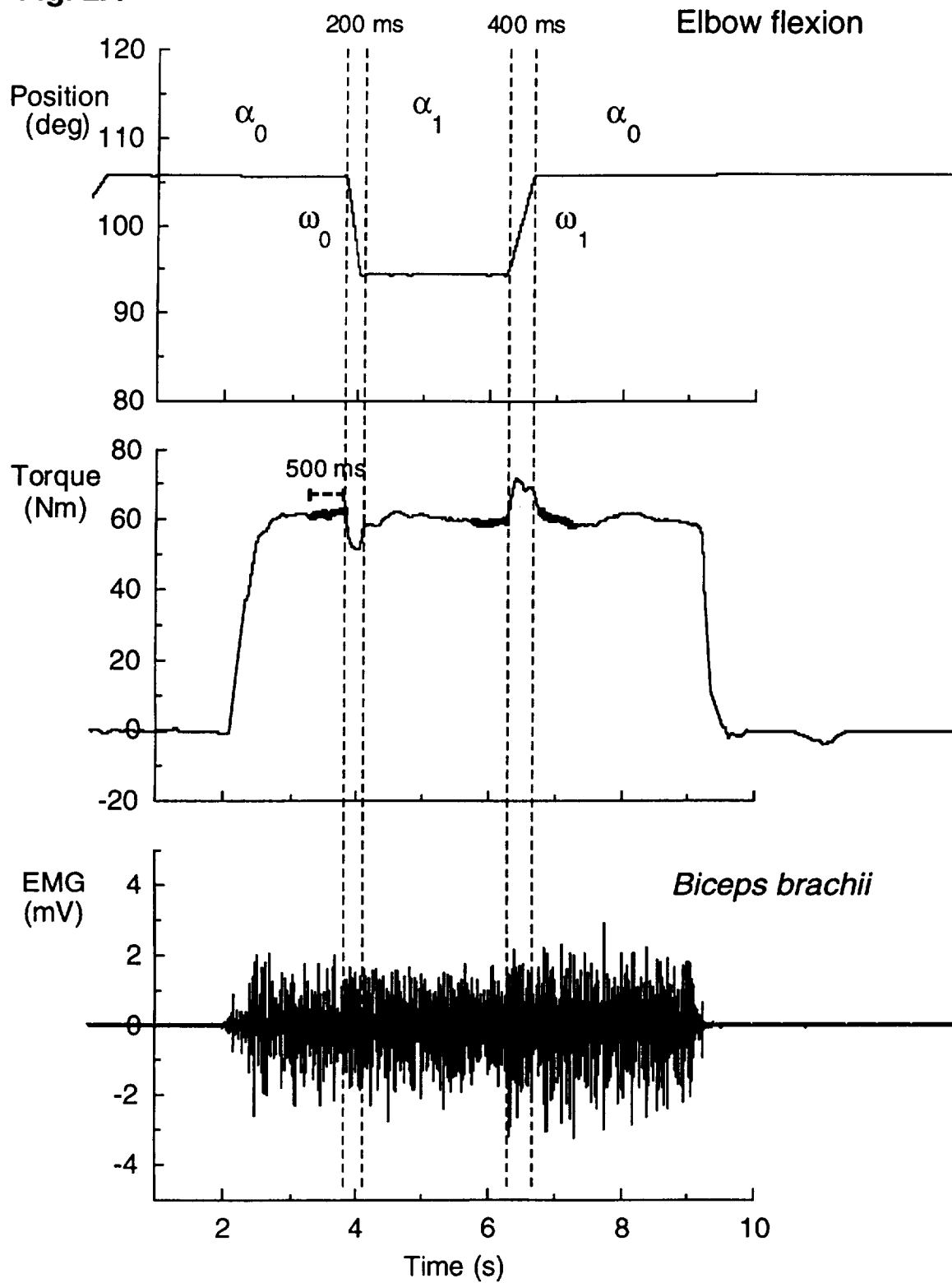


Fig. 2B

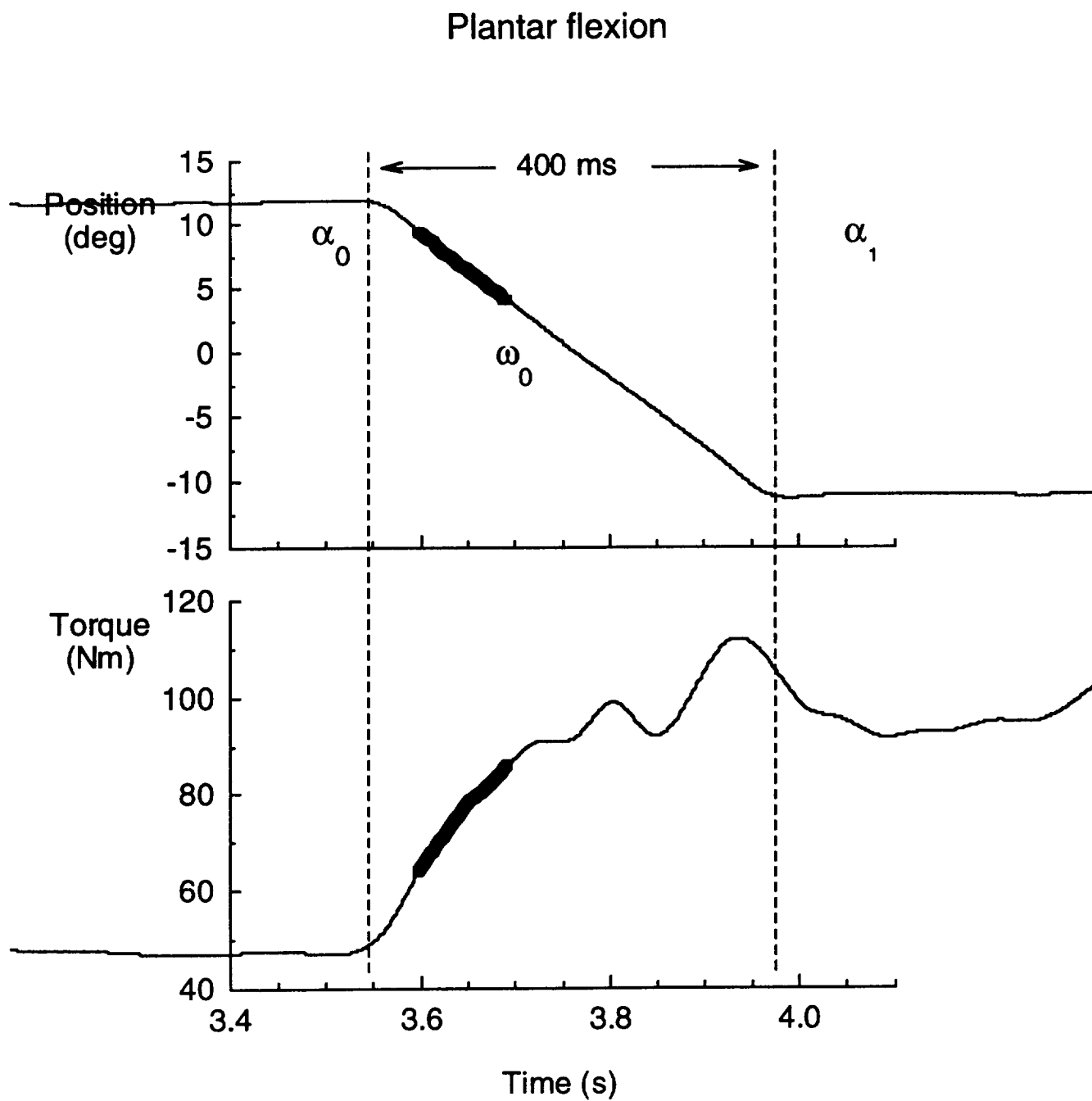


Fig. 3A

Elbow flexion at FD14 interpolation = $a_0 + a_1 \alpha + a_2 \alpha^2$

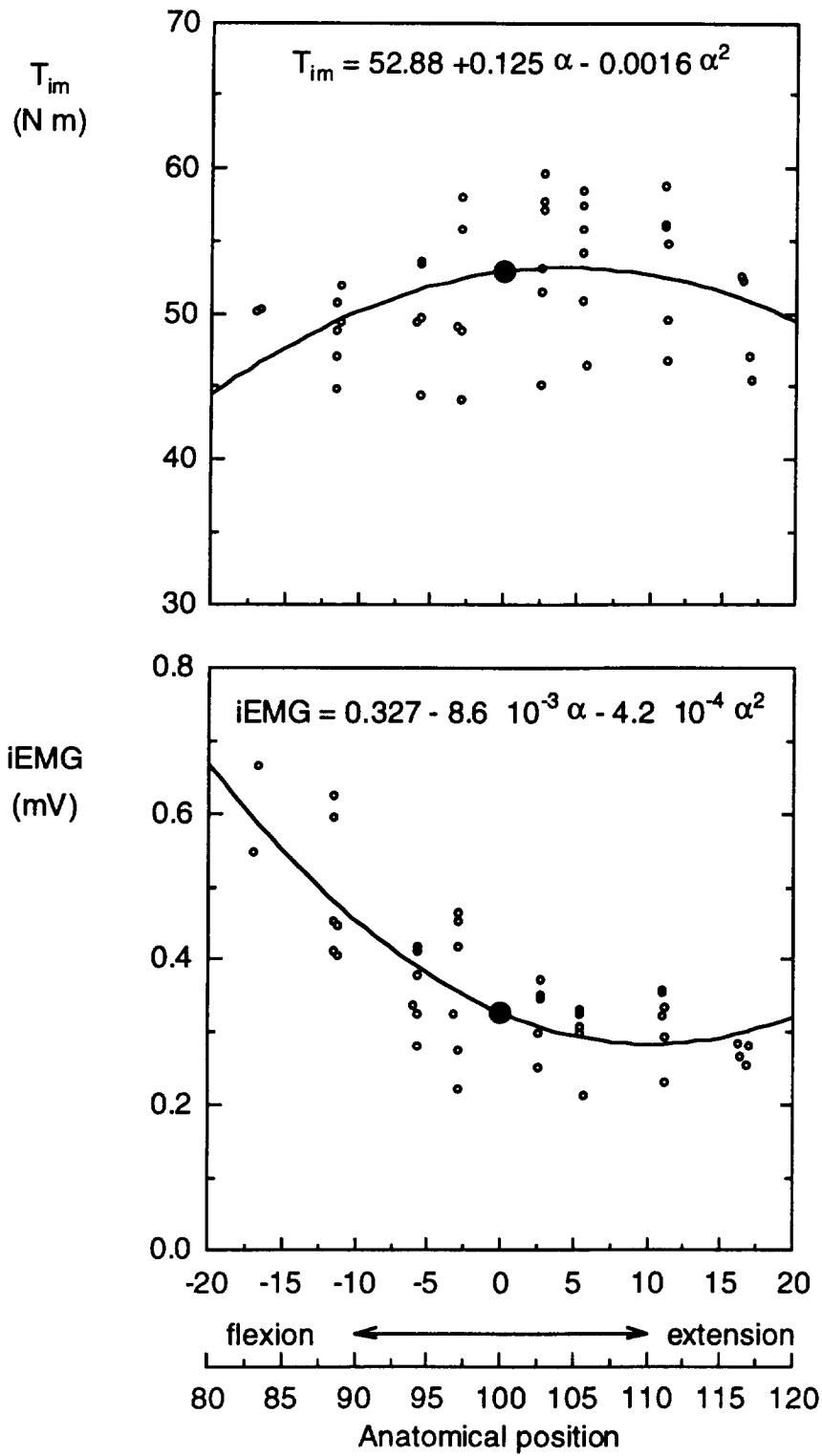


Fig. 3B

Plantar flexion at L-15 interpolation = $a_0 + a_1 \alpha + a_2 \alpha^2$

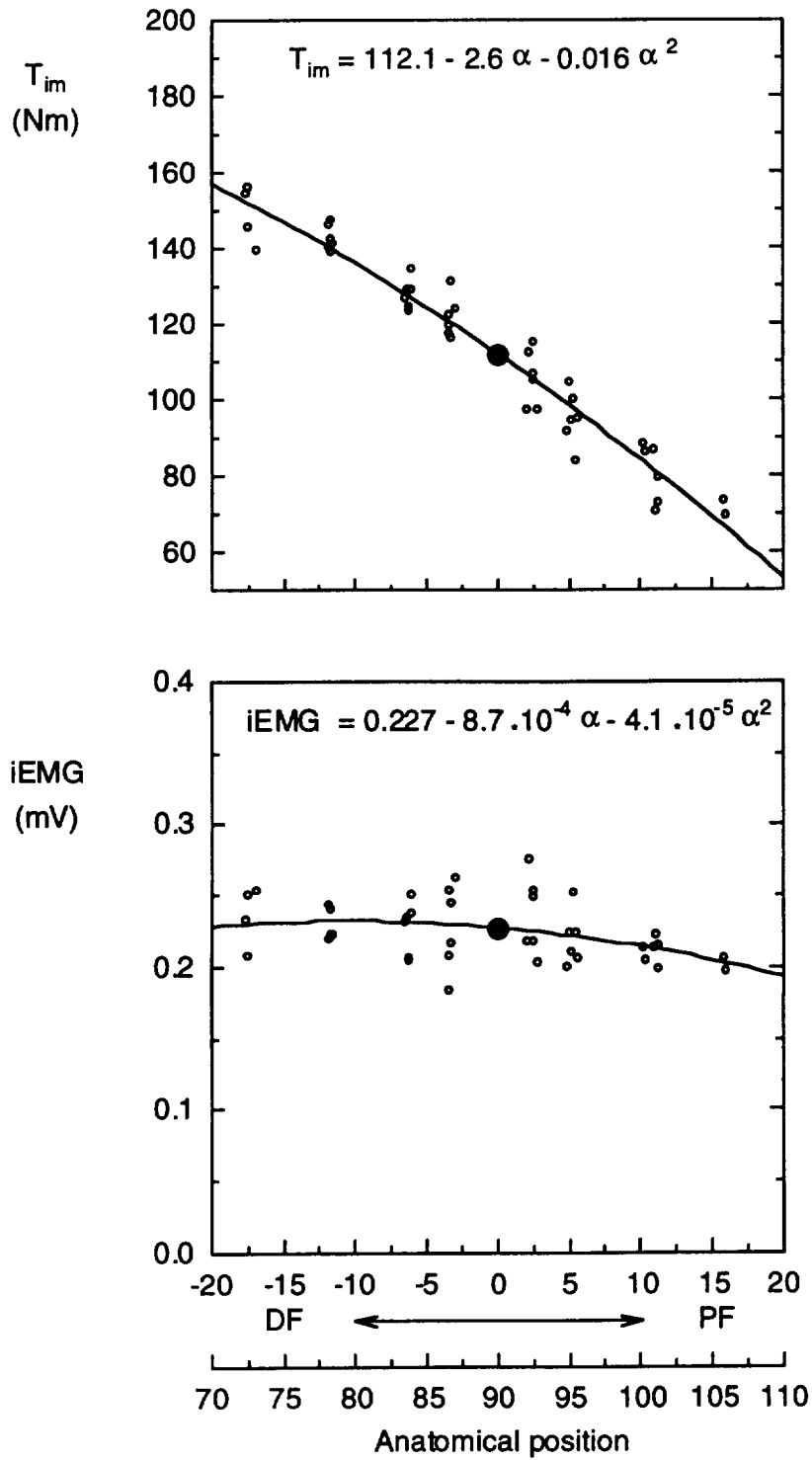


Fig. 4A

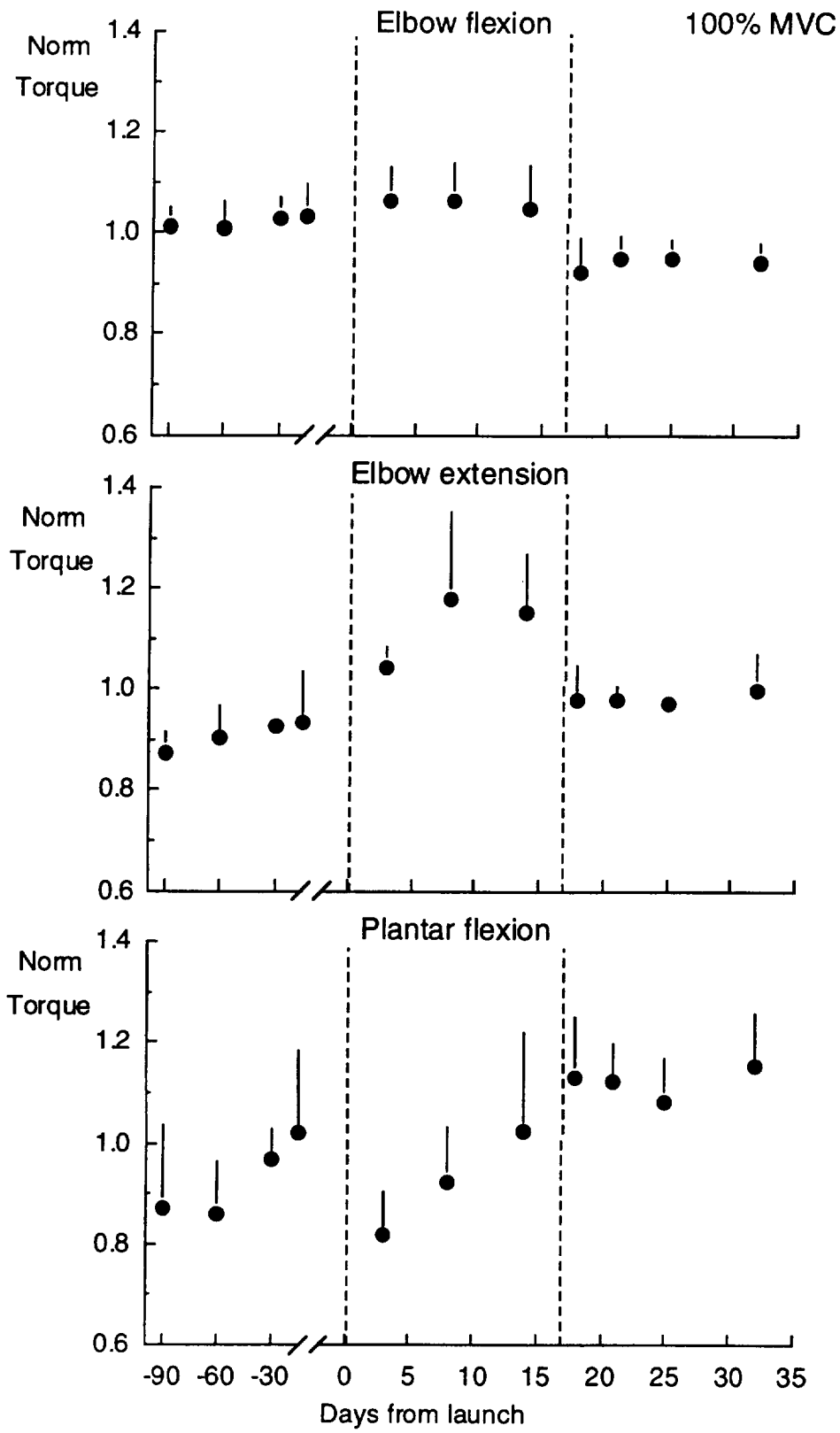


Fig. 4B

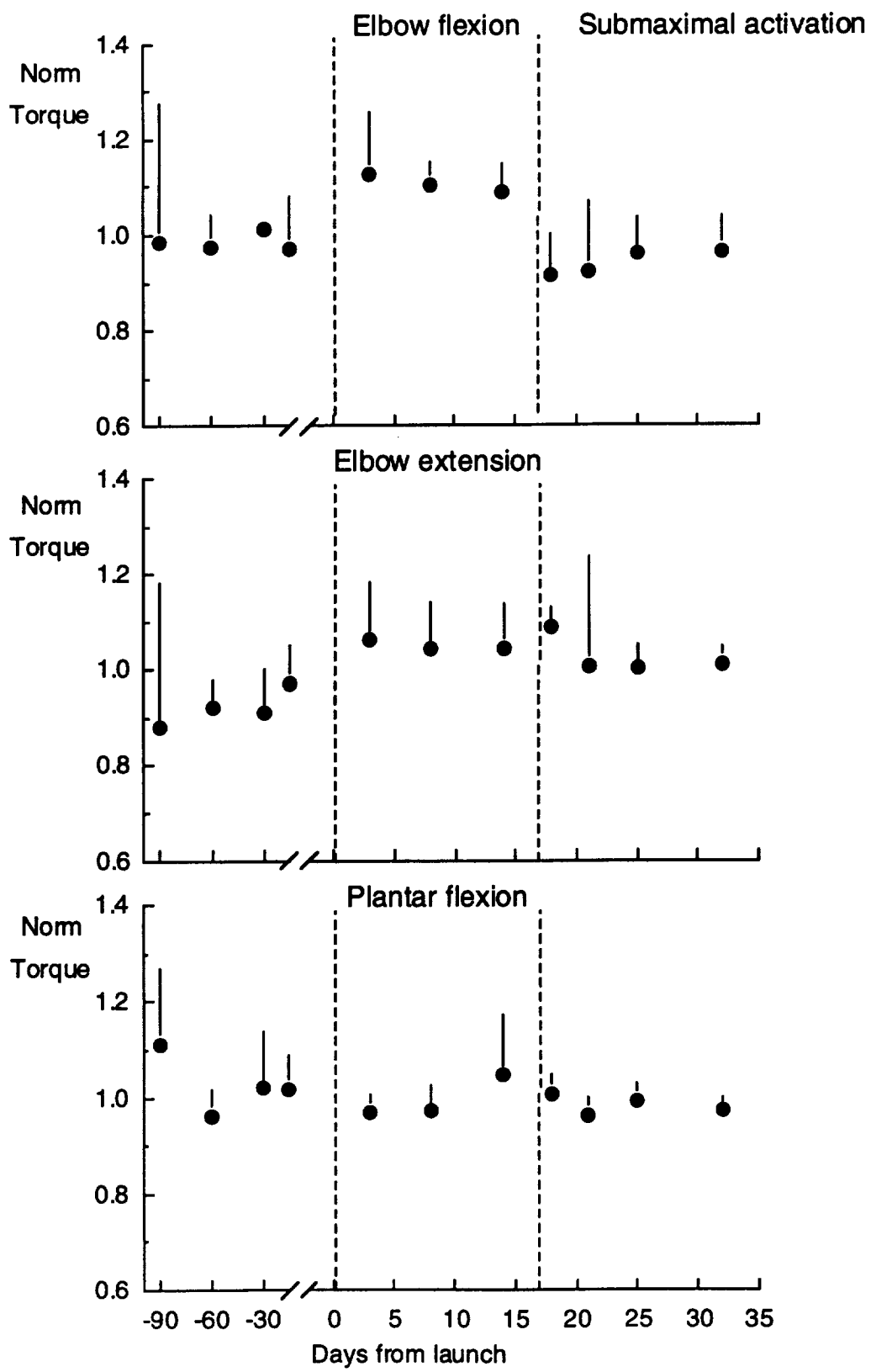


Fig. 5A

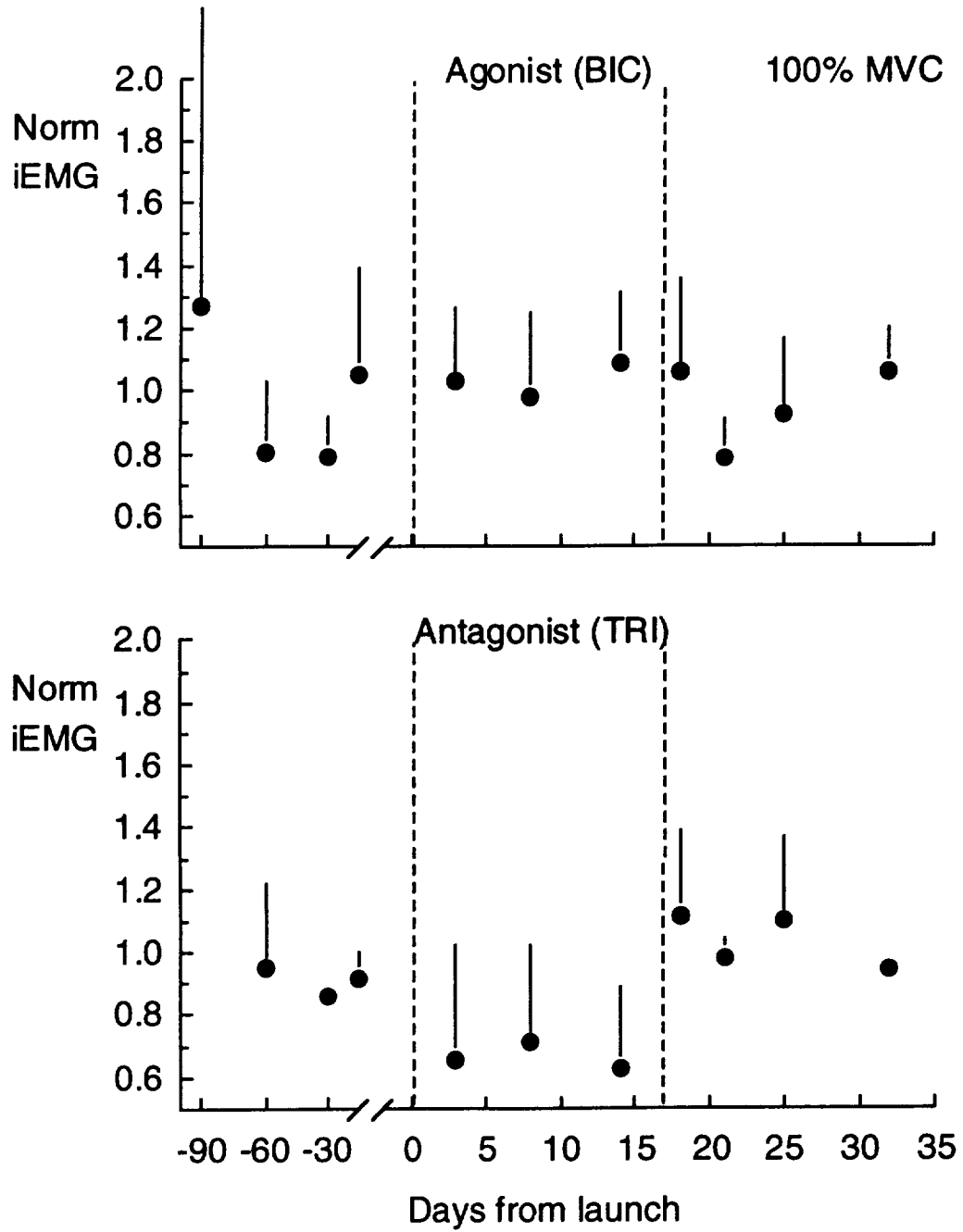


Fig. 5B

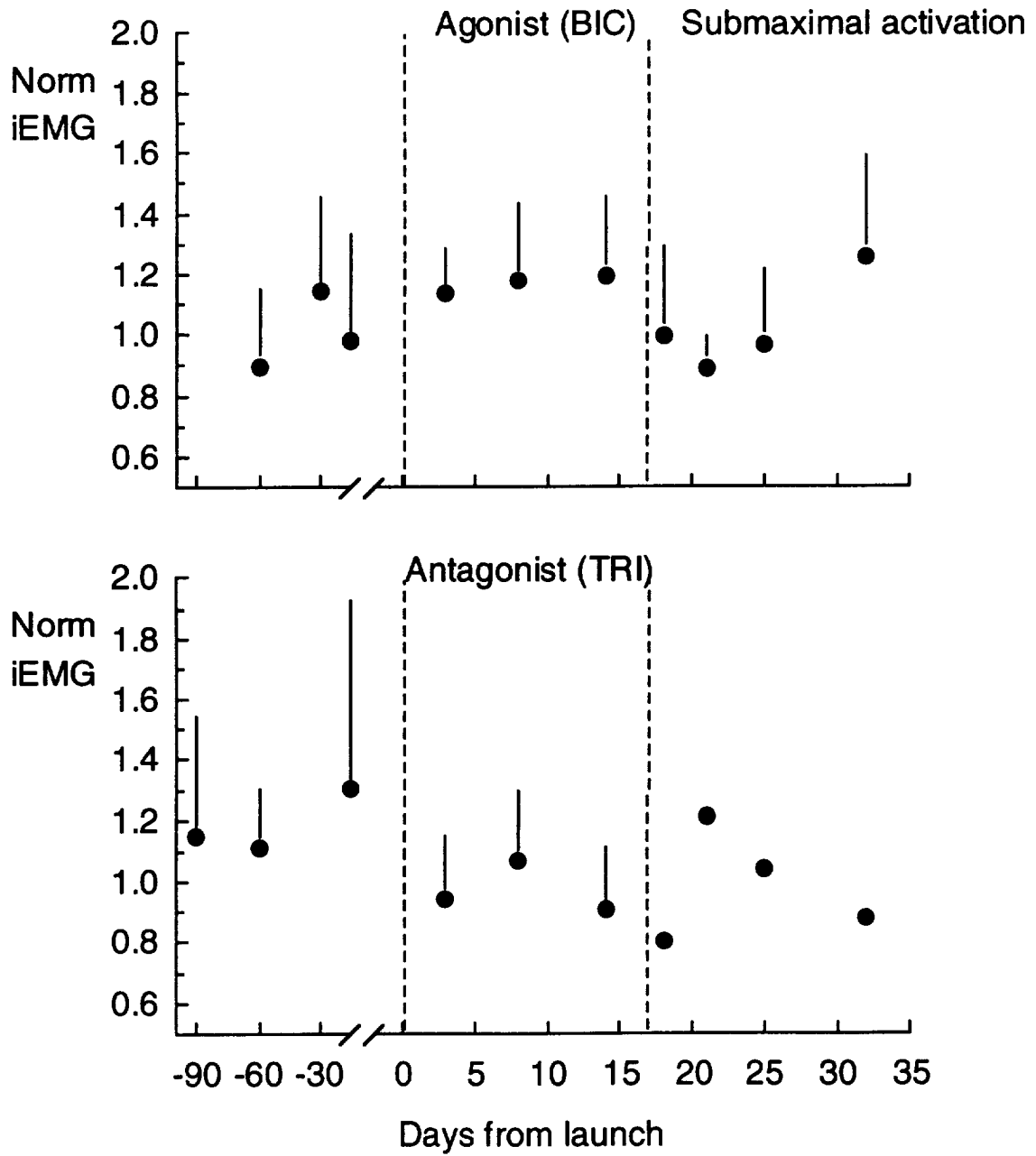


Fig. 6A

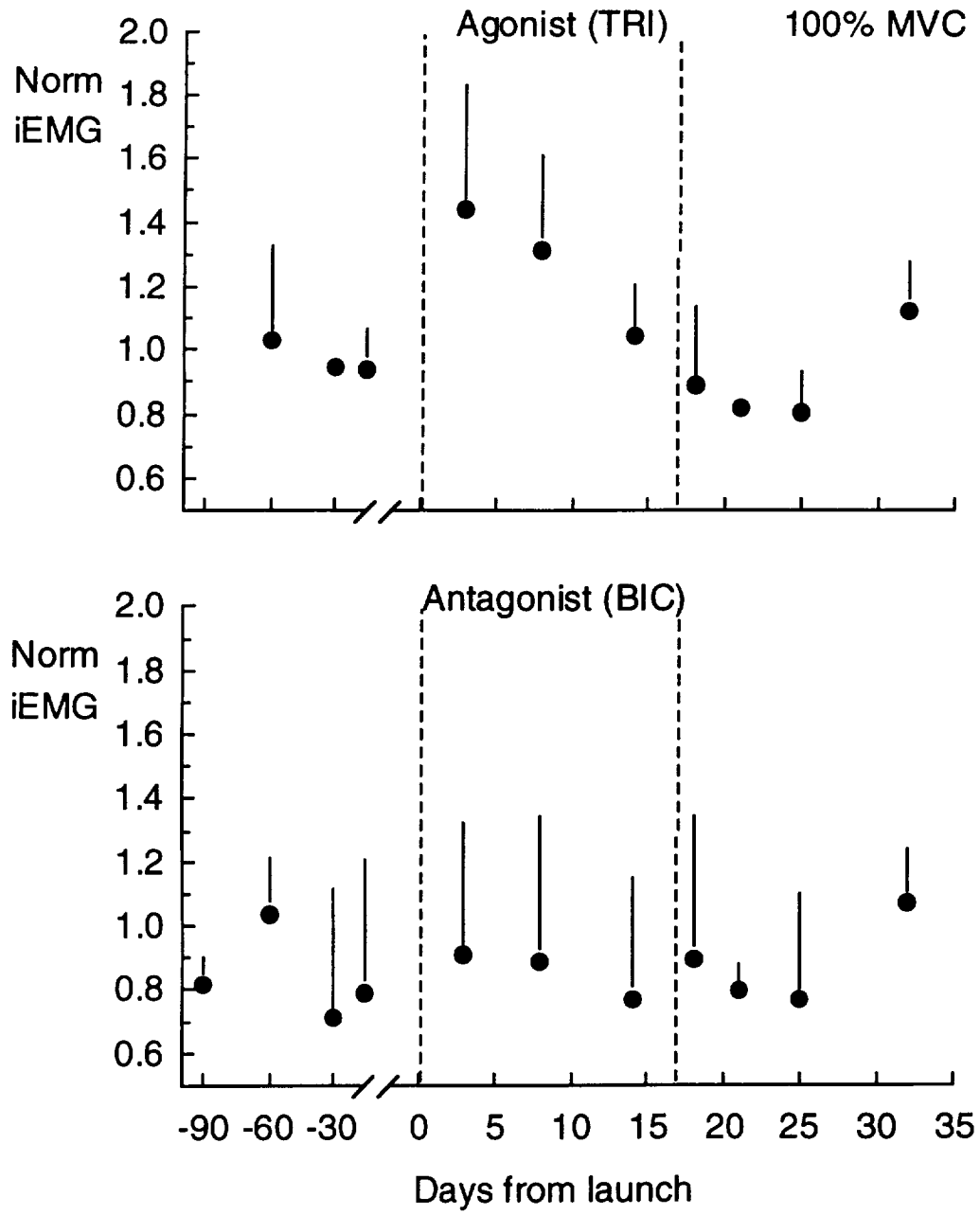


Fig. 6B

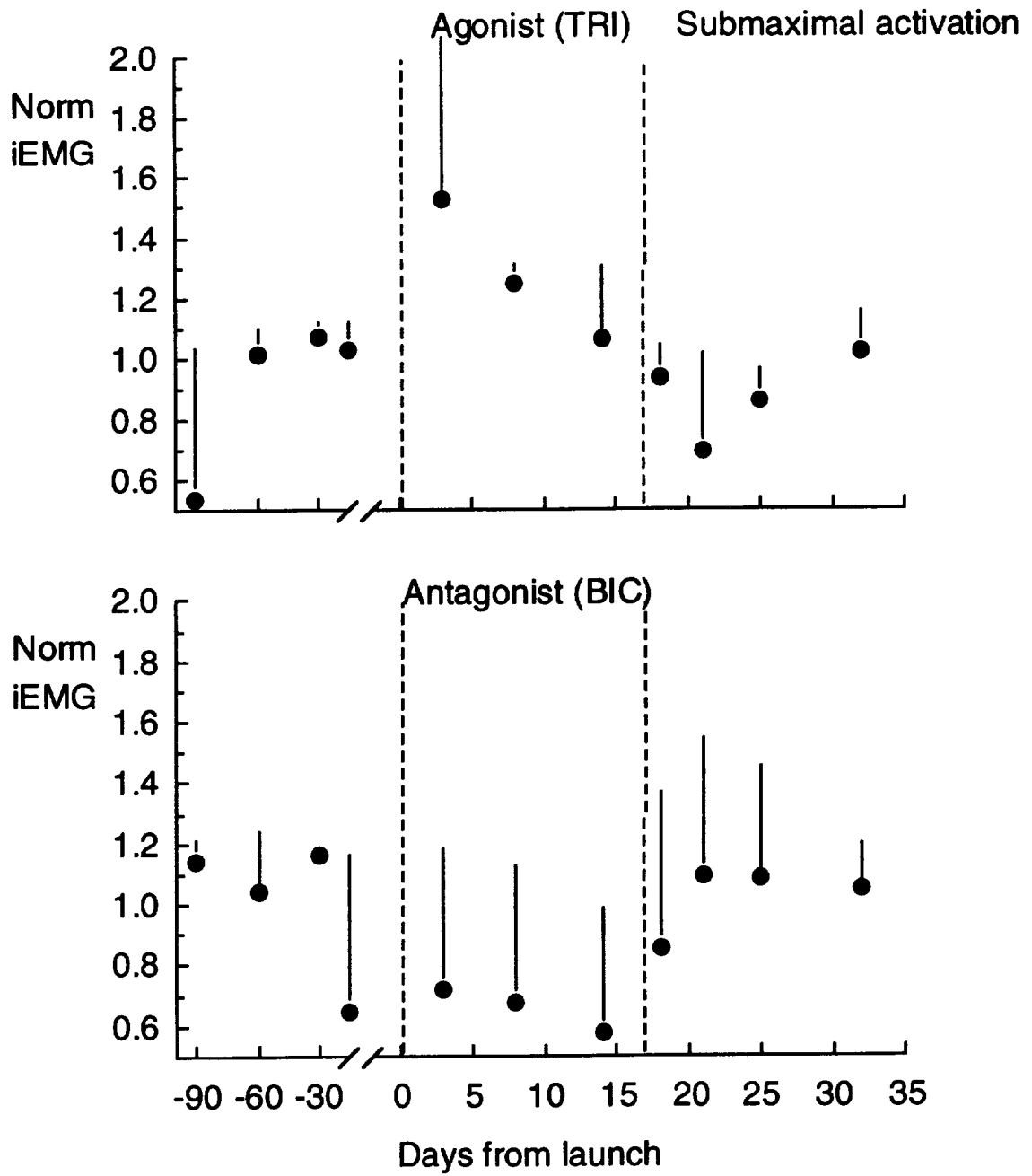


Fig. 7A

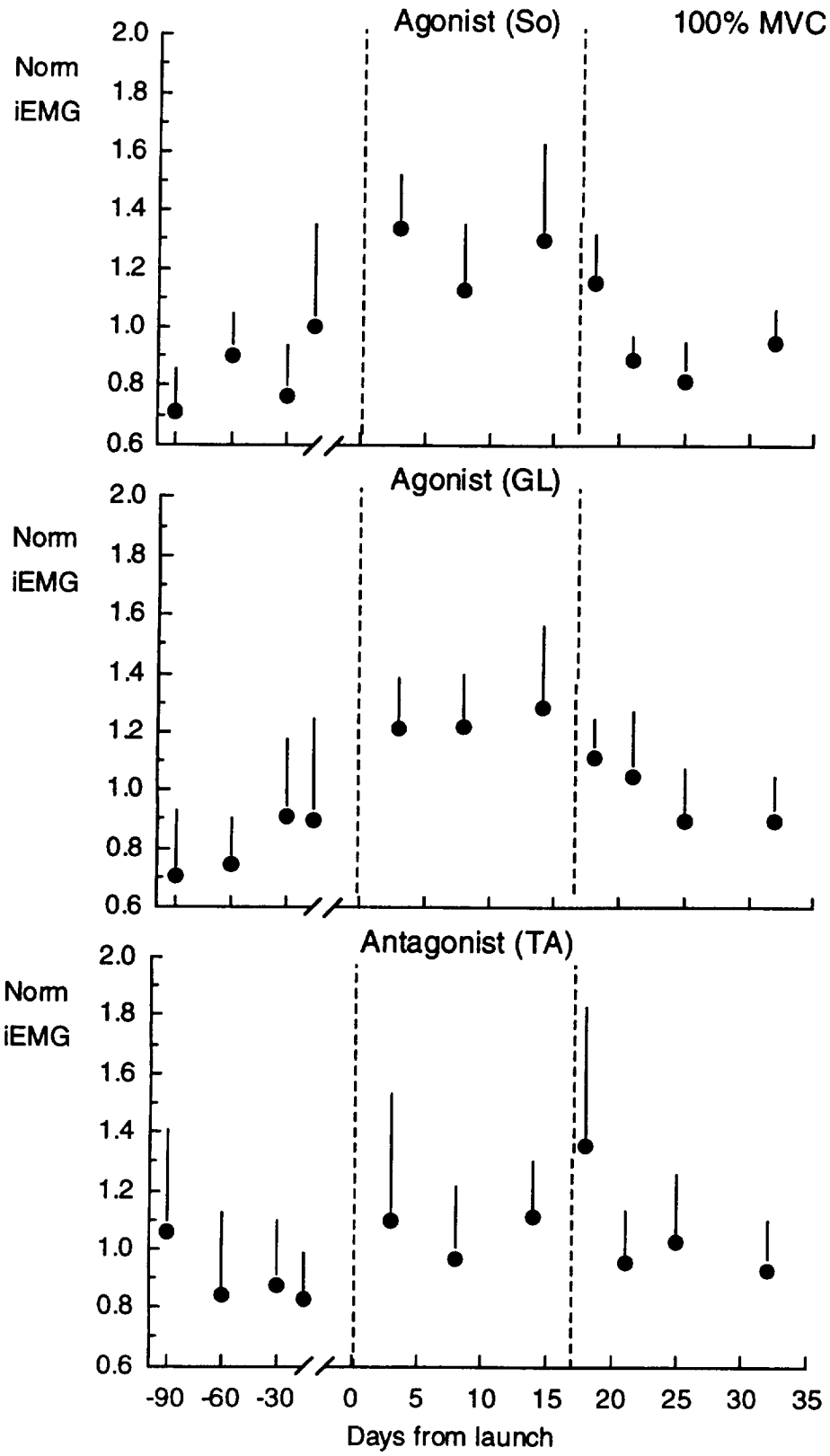


Fig. 7B

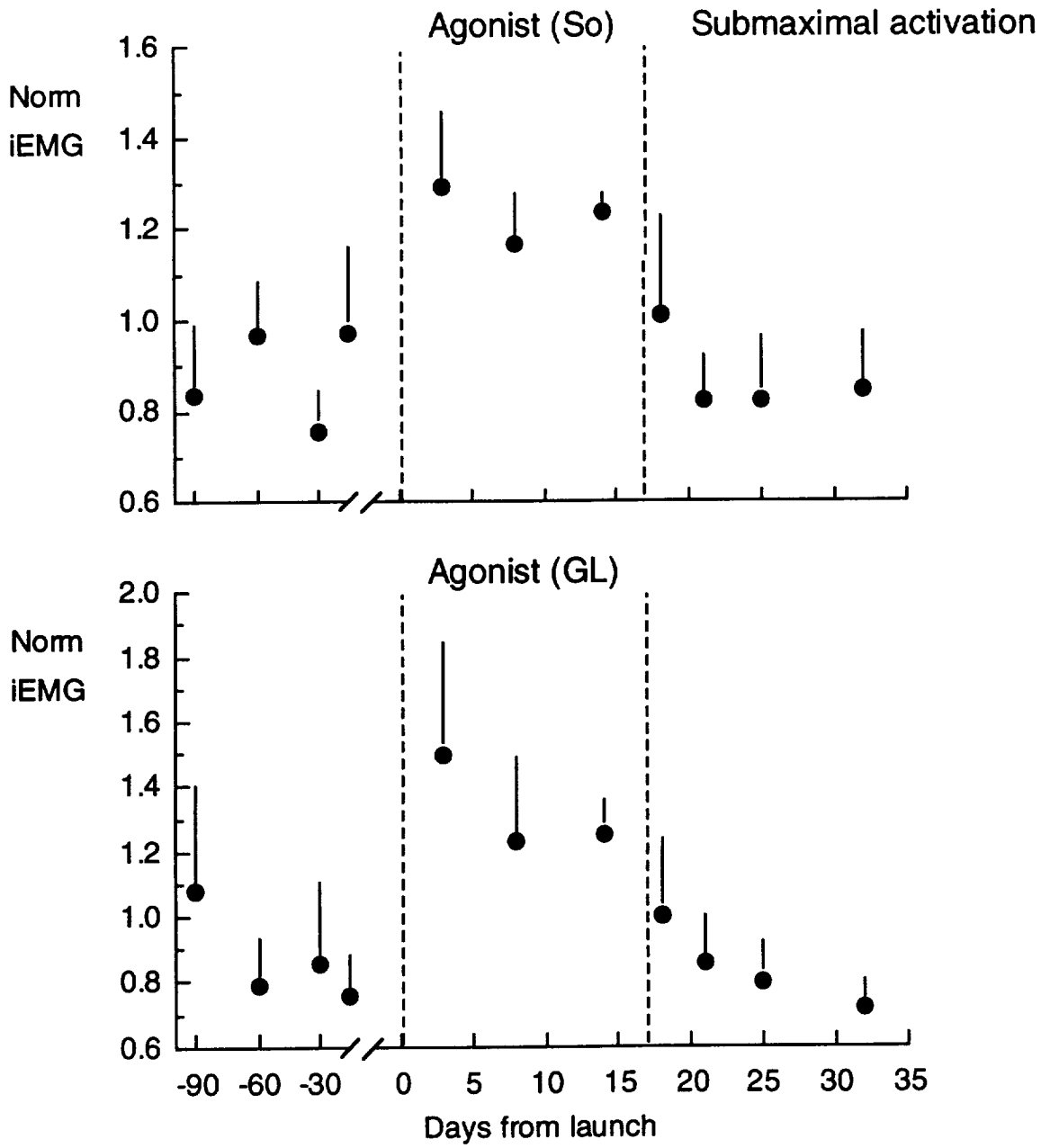


Fig. 8A

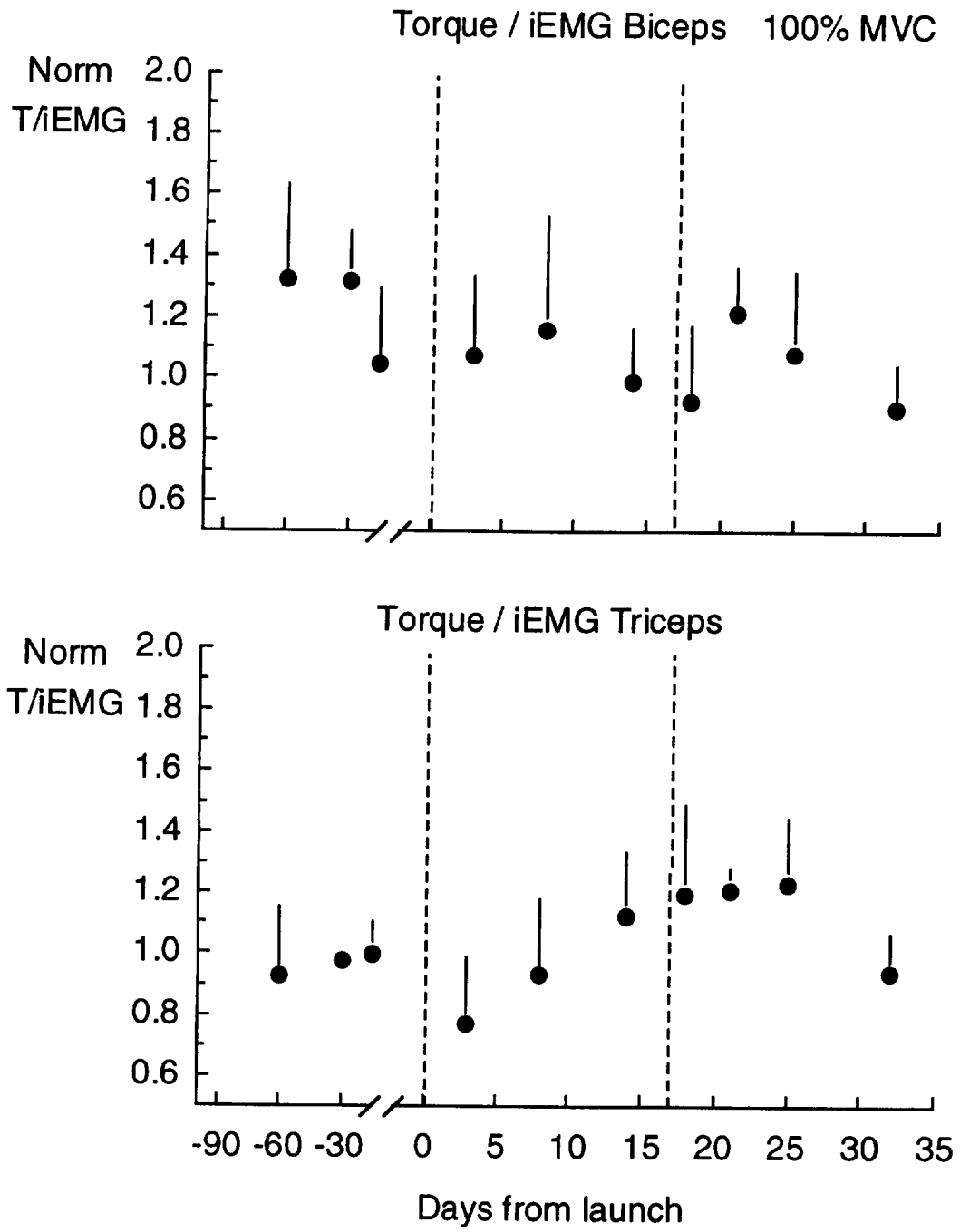


Fig. 8B

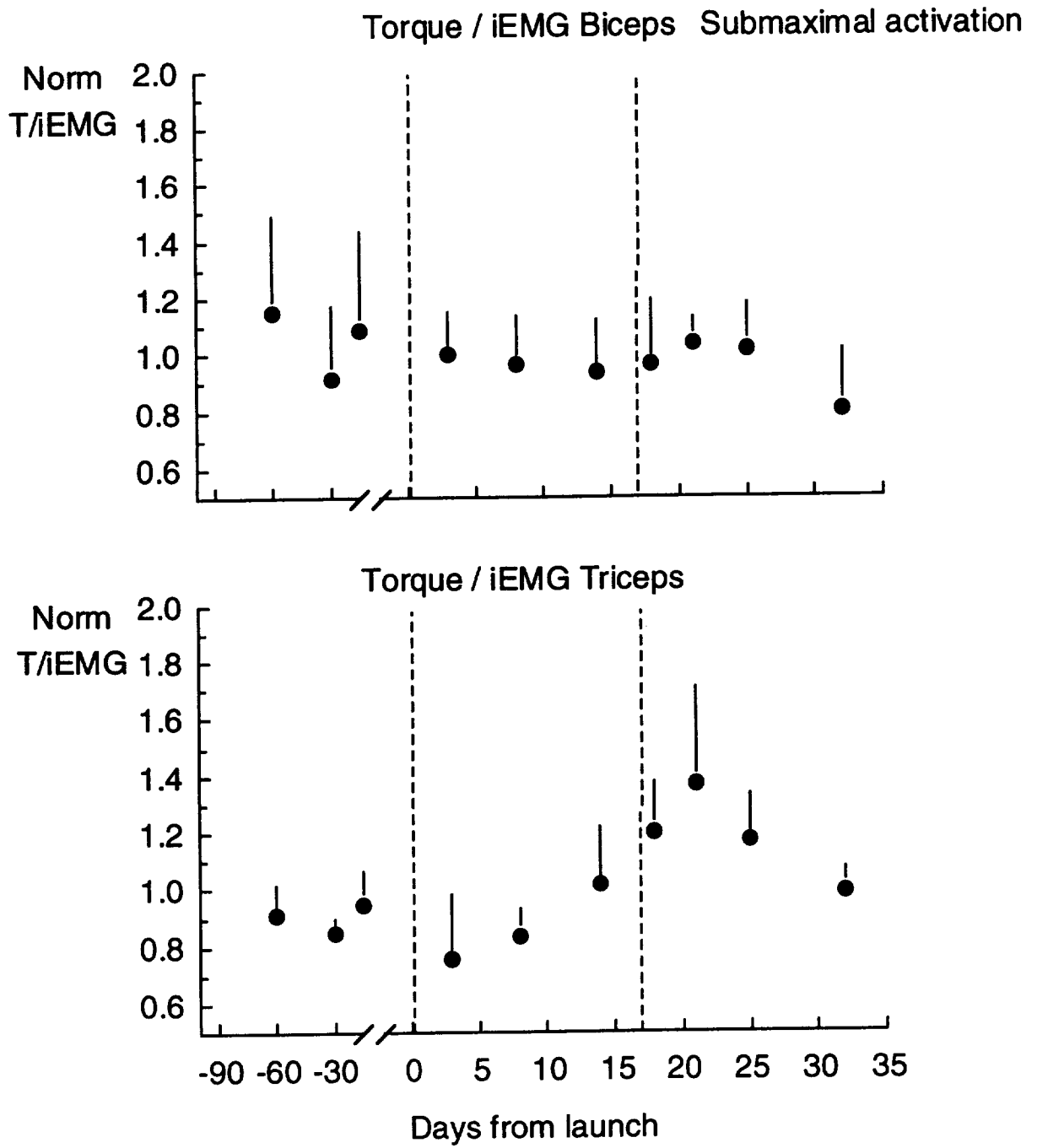


Fig. 9A

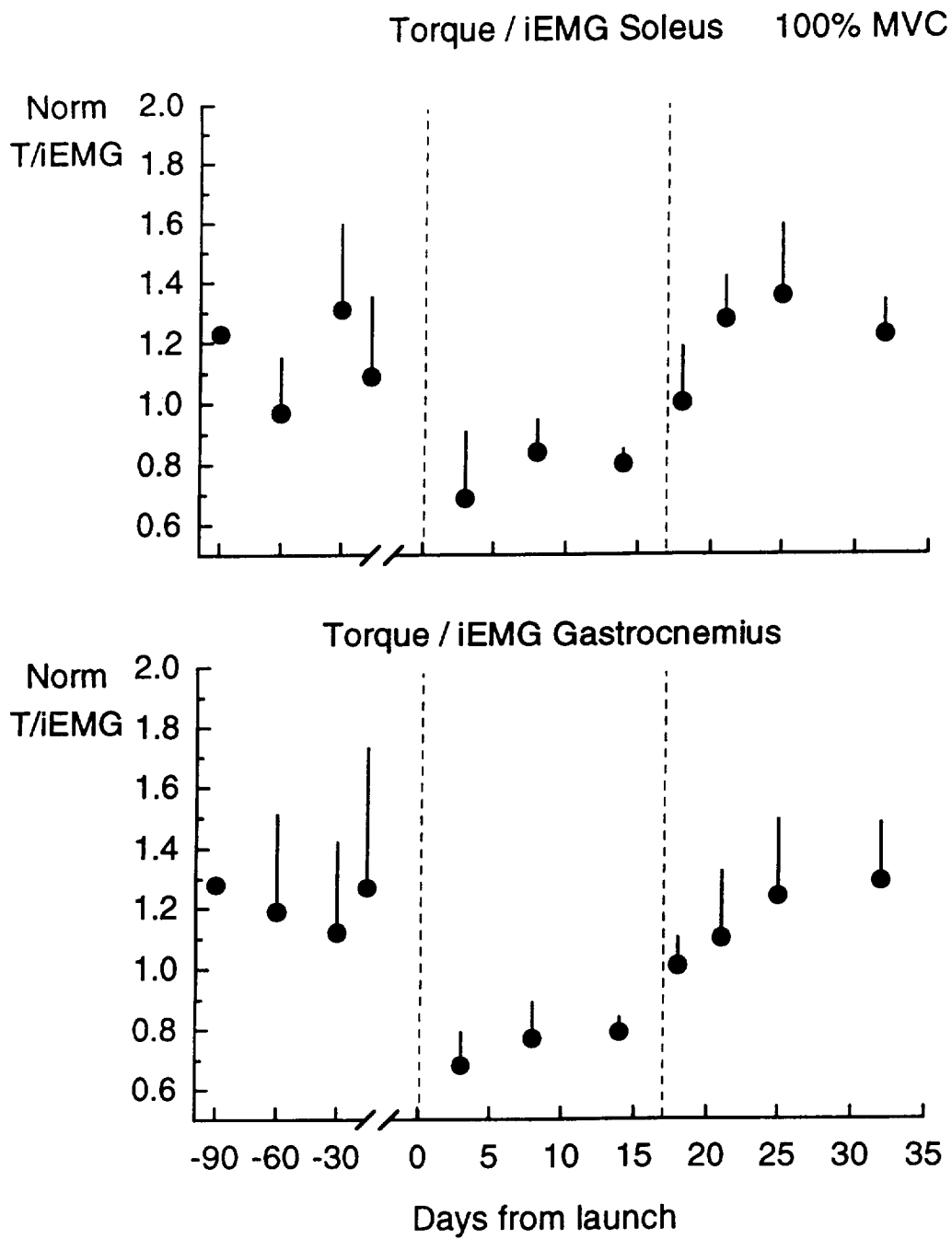
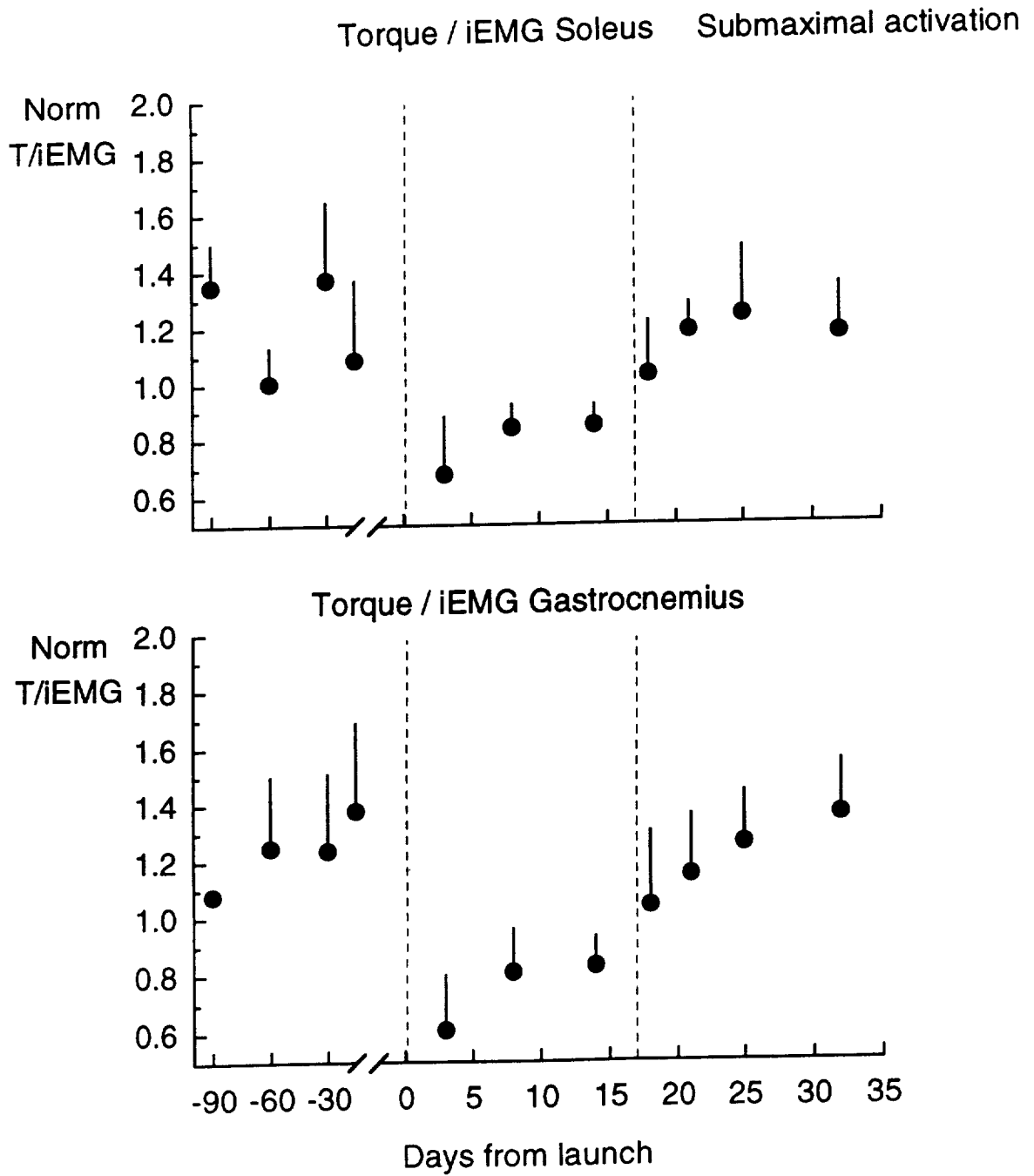


Fig. 9B



E 407: Non Technical Report
Effects of Microgravity on the Biomechanical and Bioenergetic Characteristics of Human Skeletal Muscle.

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Long term space flights are known to impair muscle function mainly because of the loss of muscle mass. This loss of muscle mass is an early event upon microgravity exposure; it affects especially the antigravity muscles of the lower limbs. In addition, a substantial remodelling of the muscle structure and a reorganisation of the neuromuscular control systems also occur during space flight and may contribute to the impairment of muscle maximal performances. Furthermore, recent findings show that short term lower limb unloading simulating microgravity may bring about a substantial drop of the maximal force of the leg extensors which is not accompanied neither by loss of muscle mass nor by changes of the maximal recruitment capability of muscle motor units. Hence, an unidentified factor (or a series of them) may contribute to the drop of maximal force of some antigravity muscles of the lower limbs and taking place very early upon lower limb unloading. Hence, the aim of the present study was: i) to quantify the extent as to which short term space flight affects mechanical muscle performance and neuromuscular recruitment and; ii) to describe the time course with which these phenomena occur.

To this aim, maximal and sub maximal force during static and dynamic contractions of the extensors and flexors of the elbow and of the plantar flexors of the ankle were studied in four astronauts who participated in the 17 days long LMS mission which took place from June 20th 1996 to July 7th 1996. The electric surface activity (integrated electromyogram, iEMG) of some of these muscles was also simultaneously recorded. Measurements were taken four times before flights, three times in-flights and four times after flight.

The iEMG at maximal voluntary contraction remained stable or even increased on flight, thus showing that the neuromuscular system was able to maintain an unchanged capability of maximal motor units recruitment.

Maximal static force of the elbow extensors was significantly larger during than before flight. This was due to the higher electrical activity with which these muscles were activated and suggests a drop of the inhibitory inputs normally prevailing in normogravity on this muscle groups of the upper limb.

Maximal static force of the plantar flexors, especially in the early phases of flight, was lower than pre-flight. In this case, however, the surface electrical activity of the activated muscles remained high and, as a consequence, the ratios of the maximal force to iEMG were lower. This indicates the sudden occurrence of microgravity effect negatively affecting the electromechanical coupling of this muscle groups and agrees with several, recent findings obtained during short-term lower limb unloading.

Future research in microgravity should aim at: i) a better description of the time course of the effects on muscle tension and motor units recruitment and; ii) a clear understanding of the specific factor (s) underlying this phenomenon.

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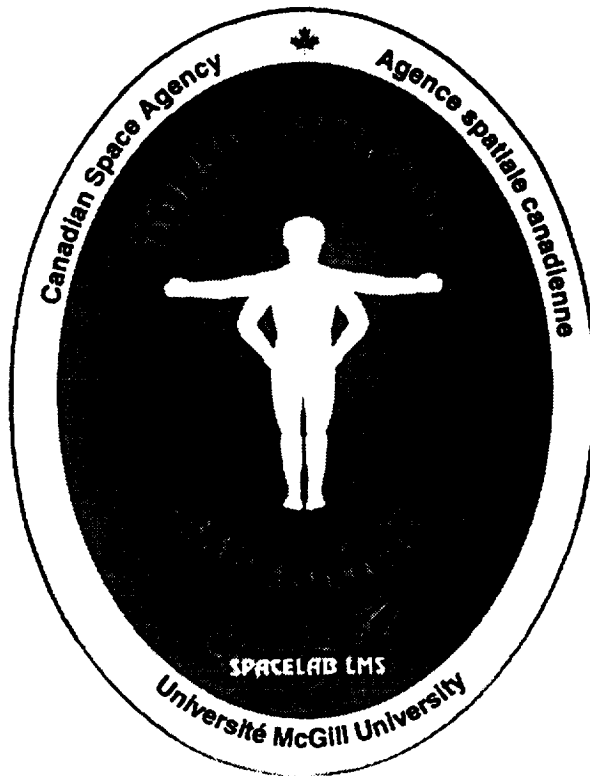
E410 - Torso Rotation Experiment

Principal Investigator:

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Final Science Report

Spacelab LMS Investigation E410



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BACKGROUND

Space motion sickness occurs in about 50% of all astronauts and cosmonauts. It is certainly unpleasant, can lower crew efficiency and thus the amount of work that can be done on a given flight, and could conceivably be hazardous, particularly during an extra-vehicular activity or urgent re-entry within a short time of launch. To date, there are no reliable ground-based tests to predict who will be affected, little knowledge of how to prevent the disorder once in space, and no fully acceptable means of treating the symptoms once they appear.

Torso rotation (TR) is a motor strategy in which the head is turned by rotating the torso and the eyes move with the head. Continuous torso rotation on the ground often leads to motion sickness. In the weightlessness of parabolic or orbital flight, many individuals have been seen to move their heads and upper bodies as a unit. Sometimes this is intended to combat motion sickness by reducing head movements, but often it precedes symptoms. Regardless, if deliberate TR on the ground causes motion sickness, inadvertent TR in space should have the same effect. If symptoms are already present, it would actually make them worse, but with a large enough delay that the cause-effect relationship might not be noticed.

Based on a series of inter-related, ground-based experiments (summarized in Bouyer, 1995), the underlying mechanism of torso-rotation-induced motion sickness seems to be excessive vestibular suppression leading to altered central processing of vestibular inputs. While transient cancellation of the vestibulo-ocular and other reflexes is a normal part of every gaze refixation that includes head movement, torso rotation increases the "duty cycle" of this process to an abnormally high level. This leads to changes that are not immediately reversible and that seem to be interpreted as a temporary vestibular malfunction. Vestibular pathology in general usually leads to severe motion sickness.

OBJECTIVES

The broad goal of the Torso Rotation Experiment (TRE) was to determine if astronauts adopt torso-rotation-like motor strategies while in weightlessness and if these changes carry over to the immediate post-flight period. Specifically, we wanted to learn if the eyes were stabilized relative to the outside world during spontaneous, self-generated head rotations (normal behaviour, utilizing vestibular reflexes) or relative to the head (requiring suppression).

METHODS

Data Acquisition

The equipment used to monitor eye, head and torso rotations is shown in Figure 1. It consisted of surface electrodes for yaw-axis electro-oculogram (EOG) recording and two separate electronics units that were mounted securely on the head and upper back, all interconnected with flexible cables. Removable data packs were used to store the results of each experiment session. There were no external controls on the electronics units as they were turned on, self-tested and programmed each time a data pack was inserted. Cues to the operator were provided by a single LED and a beeper and included codes for normal operation, the need for an EOG calibration, the end of the experiment, a low battery condition or an electronic problem.

The head-mounted unit contained a 3-axis angular velocity transducer (maximum 1000°/sec), an EOG pre-amplifier, an isolation amplifier for electroshock protection and a thermistor to monitor transducer temperature. The back-mounted unit contained another 3-axis rate sensor (maximum 300°/sec), a temperature sensor, a calendar clock, an auto-resetting EOG amplifier, other signal

conditioning amplifiers, anti-aliasing filters, a 12 bit analog to digital converter, a Motorola 68000 series microprocessor and a battery pack. Each data module contained 5 megabytes of flash EPROM data storage and 32 kilobytes of conventional EPROM program storage. This was sufficient for 43 minutes of continuous data acquisition.



Figure 1. Free-floating subject performing Torso Rotation Experiment during the Life and Microgravity Spacelab mission.

EOG signals were filtered (low pass below 200 Hz using 8 pole Bessel filter) and sampled at 512 Hz. Head yaw velocity was filtered (low pass below 50 Hz using 2 pole Bessel filter) and sampled at 128 Hz. All other rate sensor channels were filtered in a similar fashion but sampled at 64 Hz. It should be noted that measured head and upper torso angular velocities were almost totally restricted to less than 10Hz.

In addition to the direct measurement of eye, head and body rotations, subjects were videotaped whenever they were wearing the TRE equipment. Full coverage was obtained during pre and post-flight test sessions and most in-flight activity was also recorded. These data assisted interpretation of the EOG and rate sensor signals and were used to determine what the subject had been doing when unusual movement patterns were detected.

Pre and Post-Flight Procedures

Ground-based testing used the same type of data acquisition system as the flight experiment. The subject began by standing in the middle of a 3 metre diameter circle. EOG calibration was carried out by staring at a single, distant target and rotating the head to both sides in a series of short steps. Pure yaw and pure pitch rotations were also performed to provide information necessary for transforming rate sensor information to the anatomical axes during data analysis. Then, following tape-recorded cues, each subject performed a pseudo-random series of gaze refixations to eye-level targets located every 22.5° along the circle. During 6 minutes, 19 refixations of 22.5° to the left were performed, 14 of 22.5° to the right, 19 of 45° to the left, 27 of 45° to the right, 22 of 90° to the left and 20 of 90° to the right. Instructions were to look at each of the indicated targets in turn, using whatever combination of eye, head and body movements felt most comfortable and natural. The session ended with another series of calibration maneuvers.

In-Flight Procedures

Test sessions in weightlessness were less structured, consisting of passive monitoring of eye, head and body rotations during spontaneous, ad lib crew activity. This was possible because a relatively large proportion of naturally occurring gaze refixations occur about the yaw axis (i.e. to the left or right), so an acceptable data yield could be achieved even though precise EOG recordings can only be obtained for lateral eye movements. Each session began with the subject unstowing and putting on the TRE equipment and inserting a data module into the back-mounted unit to begin data acquisition. This was accomplished with the assistance of a second crewmember. For the following 43 minutes, the subject performed other activities, stopping only briefly every 5 minutes to re-calibrate the EOG and rate sensor recordings using methods similar to those employed on the ground. When the TRE equipment signalled that the data module was full, it was pulled out and the equipment was removed.

Motion Sickness Reports

After the completion of each experimental session, subjects were requested to complete a motion sickness report. This consisted of a list of seventeen signs and symptoms of motion sickness which could be rated on a none / mild / moderate / severe basis. It also included a numerical rating of overall discomfort level, ranging from 0 (all is well) through 10 (unhappy but can control) to 20 (out of control and vomiting). Both individual symptoms and overall discomfort were to be described as they had occurred before, during and after the Torso Rotation Experiment.

Data Reduction

For technical reasons, the net gain of the circuits used to amplify EOG signals was 2361 in three TRE units and 1000 in the remaining one. In the case of data obtained with the latter unit, all recorded eye movements were multiplied by 2.361 and appropriate amplifier re-sets were added to make the results conform to the more general standard.

The data from each individual experimental session were then computer-processed to reduce noise and to automatically identify saccades, amplifier resets, higher frequency motion artifacts, eyelid blinks and noise in the form of electromyographic activity and electromagnetic interference. The digital signal processing technique employed was a quadrature mirror filter pair with thresholding. The result was that smaller, high frequency signals were considered to be noise and were removed. Larger, high frequency signals were considered to be events and were flagged for future exclusion. Lower frequency signals were generally accepted by the processing. As a consequence, temporary drifts such as those caused by disturbances of EOG electrode half-cell potentials had to be identified and extracted manually at a later stage of data reduction. Figures 2 and 3 are examples of ground-based and flight results respectively, after processing. Figure 4 illustrates how saccades that occurred with the head fixed, and eye blink artifacts, were removed from the data.

After the first phase of automated analysis was complete, human pattern recognition ability was used to identify all EOG and rate sensor calibrations in the data files. These distinctive movements, partly illustrated in Figure 5, occurred at the beginning and end of each ground-based session and on nine or ten occasions during the in-flight experiments. In all cases, data recorded before the first calibrations were then flagged for future exclusion. For ground-based experiments only, all results after the last calibrations were also marked for trimming. Finally, a few short-duration, low frequency drifts were identified in the EOG signal and excluded from further analysis. These were usually the result of the subject rubbing or adjusting the surface electrodes.

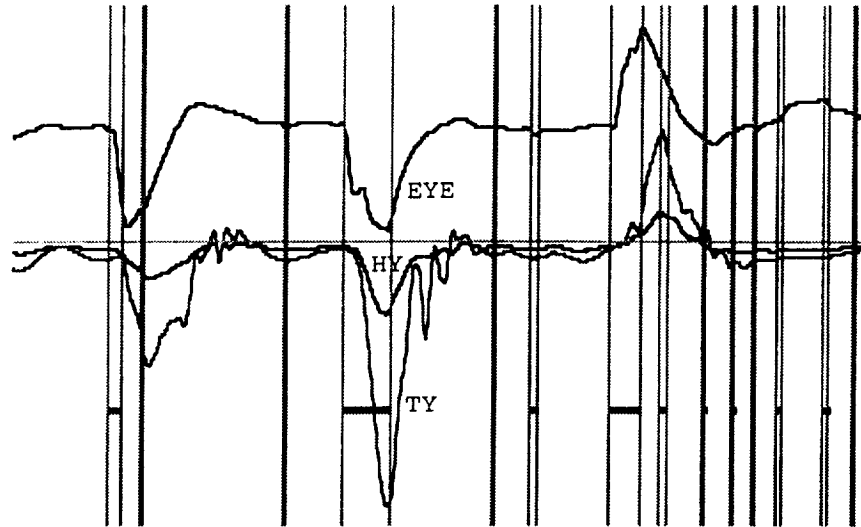


Figure 2. These data were recorded 30 days before launch and consist of three consecutive gaze refixations of about 45° . In this and the following 3 figures, eye position, head yaw-axis velocity and torso yaw-axis velocity have been labelled EYE, HY and TY respectively. TY always appears bigger than HY because a more sensitive rate sensor was used to record upper torso rotations. The vertical lines connected by horizontal bars represent sections trimmed out by the computer. Each record is 10 seconds in duration.

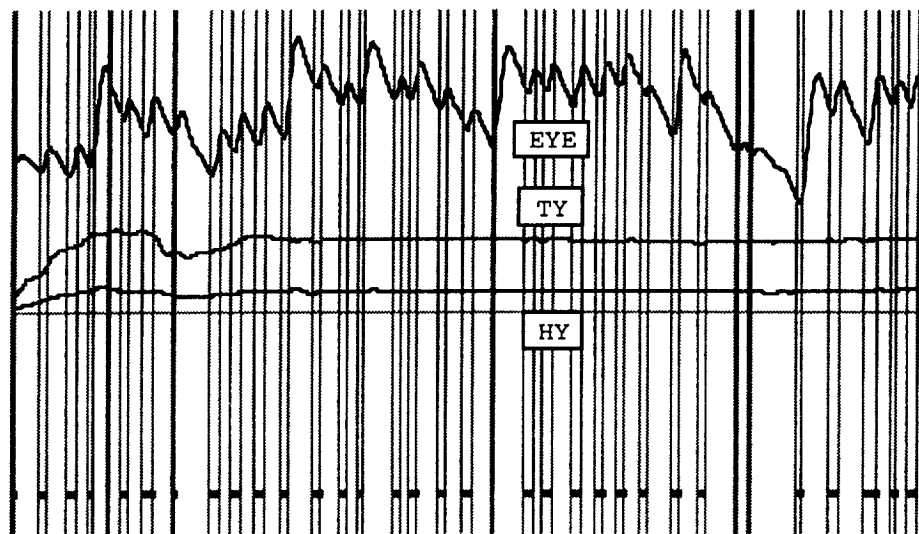


Figure 3. This data segment was recorded on Flight Day 15 and is an example of visual tracking during a prolonged, yaw-axis rotation. Rotation rate is approximately $90^\circ/\text{sec}$ and is particularly steady during the last 7 seconds. This is easily accomplished in weightlessness but would require a servo-controlled rotator on the ground.

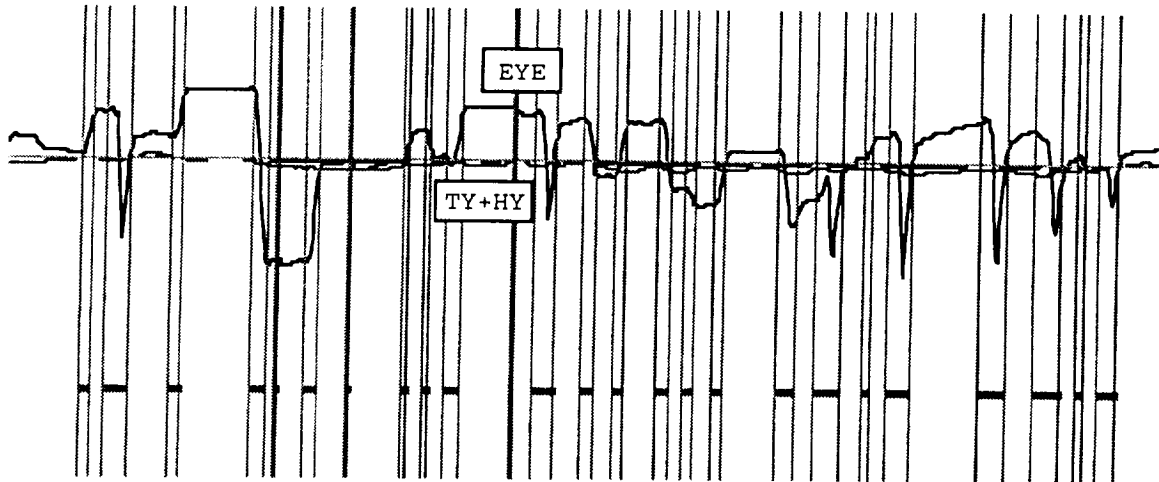


Figure 4. This is an example of saccadic eye movements and eye blink artifacts recorded when the head was not moving. The computer has identified and extracted all of them.

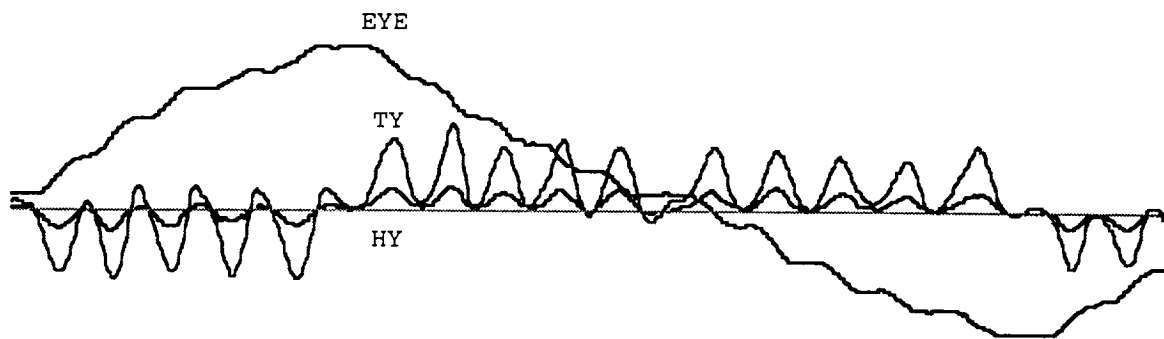


Figure 5. The step-wise pattern of torso, head and eye movements associated with each EOG calibration maneuver was easy to identify. Beyond their obvious use in relating eye to head movements, these segments had to be excluded from further analysis because the subjects were deliberately staring at an external target.

Digital signal processing continued with the definition of the three dimensional unit vector generated as the head was rotated during the EOG calibration maneuvers. On the basis of this vector, a small amount of error tolerance and a velocity threshold of $15^\circ/\text{sec}$, all periods of "pure" head yaw rotation were identified. Eye position as recorded by electro-oculography was then converted to eye velocity using a 151 term ideal differentiator with windowing and the resulting signal re-filtered using the same noise extraction procedure as before. Finally, EOG gain was calculated for each session by plotting eye velocity as a function of head velocity during EOG calibration maneuvers and by performing a linear regression fit to the resulting graph (Figure 6). The average R^2 value across all pre, in and post-flight sessions was 0.87 ± 0.17 .

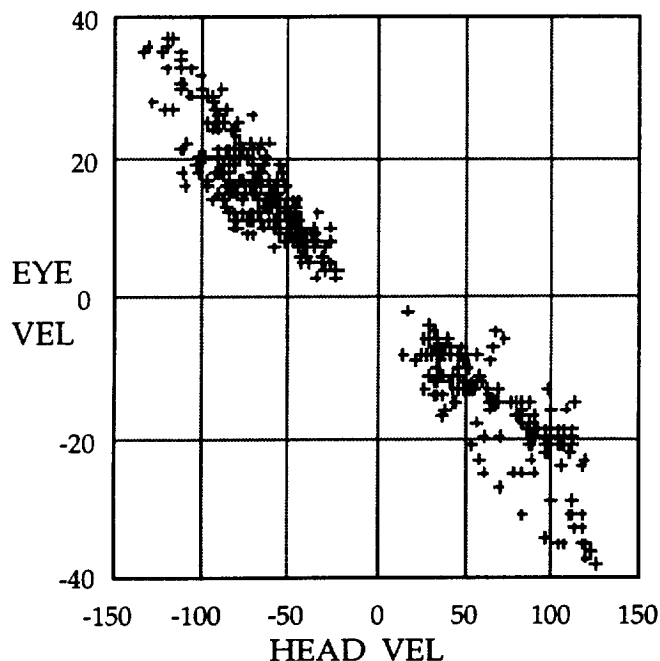


Figure 6. Eye velocity versus head velocity during an EOG calibration maneuver. Both measures are plotted in engineering units, but the clustering of points along a -45° slope indicates the eyes were pointing at a fixed target during the step-wise head movements.

RESULTS

General

The Life and Microgravity Spacelab mission (STS-78) launched on June 20, 1996 and landed on July 7. During the flight, four crewmembers participated in the Torso Rotation Experiment, acting as subjects and providing assistance to each other as required. None had been in space before. Their test schedule has been summarized in Table 1. All four were also tested on the ground at 90, 60 and 30 days before flight, on landing day, and 2 and 5 days after landing.

<u>Flight Day</u>	<u>Subjects Tested</u>
1	1,2,3,4
7	1,2,3,4
12	2,3
13	1
15	2,3,4

Table 1. In-flight testing schedule for TRE subjects.

In-flight testing was carried out as planned with only two minor exceptions. Subject 4 had to install a new battery pack after 16 minutes of his Flight Day 15 test. Subject 2 had to use the alternate set of TRE equipment when he received an "electronic failure" message at the beginning of his Flight Day 15 experiment. Neither problem could be reproduced after landing but the latter one was probably related to a reversible flash memory anomaly. In any case, they had no impact on the experiment. All ground-based sessions were completed without incident.

Data yield following the reduction process was consistent and as anticipated. On average, 0.73 ± 0.19 minutes remained out of the 6 minute ground-based tests and 0.84 ± 0.27 minutes out of the 43 minute in-flight experiments. The much lower in-flight yield (on a percentage basis) was expected because of the less structured nature of the task.

Pre-Flight

Following the data reduction process described above, the remaining results consisted of periods during which the subject was performing pure yaw-axis head movements, with all calibration maneuvers, saccades, artifacts and periods of inactivity excluded. It was then possible to determine if the eyes were being stabilizing relative to the outside world or relative to the body during self-generated head rotations by plotting eye velocity relative to head velocity on a point-by-point basis.

As an aid to interpreting these graphs, Figure 7a summarizes where data points would lie under various conditions. If the subject's eyes were always stabilized relative to the outside world, eye velocity (relative to the head) and head velocity (relative to space) would be equal but in opposite directions. By contrast, if the eyes were always stabilized relative to the body, only head velocity would change. In real life, one might expect a tendency to move back and forth between the two strategies (arrows), with a change in weighting towards the latter in the presence of increased vestibular suppression. To detect these potential changes, the plots have been divided into seven sectors as shown in Figure 7b and the number of data points calculated for each sector.

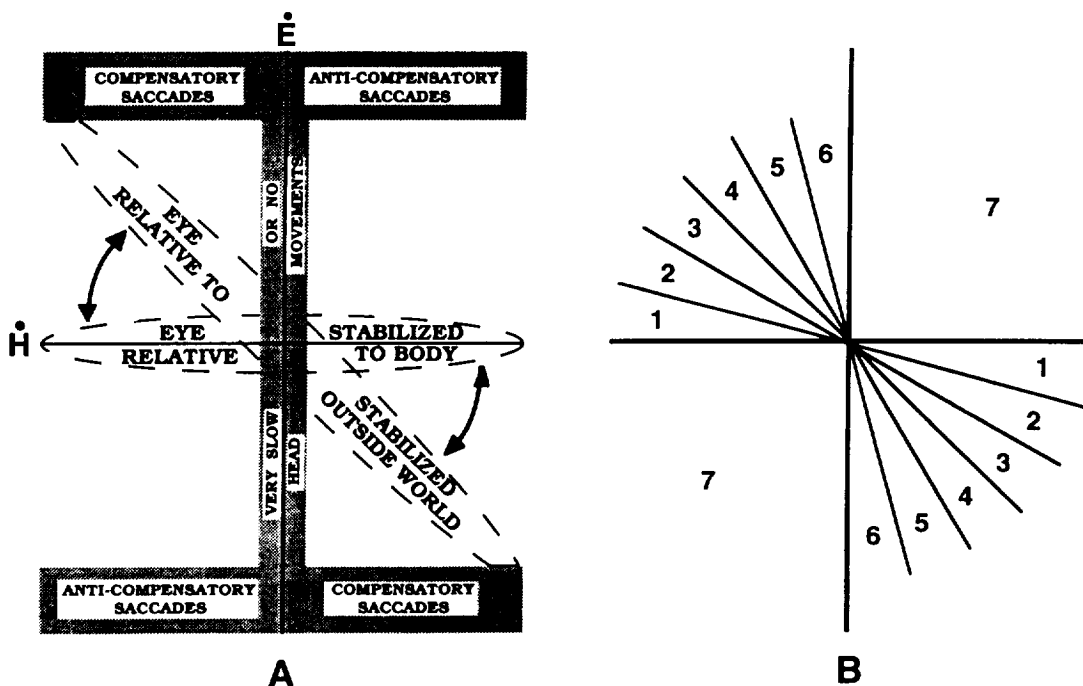


Figure 7. (a) Theoretical scatter diagrams of eye velocity as a function of head velocity. The shaded area indicates where the computer would have identified and trimmed compensatory, anti-compensatory and head-fixed saccades, and periods of very little or no head movement. (b) Division into sectors for further analysis.

Figure 8 is an example of results obtained 30 days before flight from Subject 3. Eye velocity has been plotted as a function of head velocity, up to a maximum of 400°/sec. A quick inspection of the graph suggests that the subject was usually stabilizing his eyes relative to the outside world as he performed the required series of gaze refixations. Indeed, 72% of the 3049 data points plotted here fall into sectors 3 and 4, close to the 45° slope that would indicate perfect stabilization. Note also that less than 16% of the data points lie in sector 7. These results are typical of Subjects 2, 3 and 4. Subject 1 was similar to the others in most respects, but he seemed less inclined to stabilize his eyes relative to the outside world.

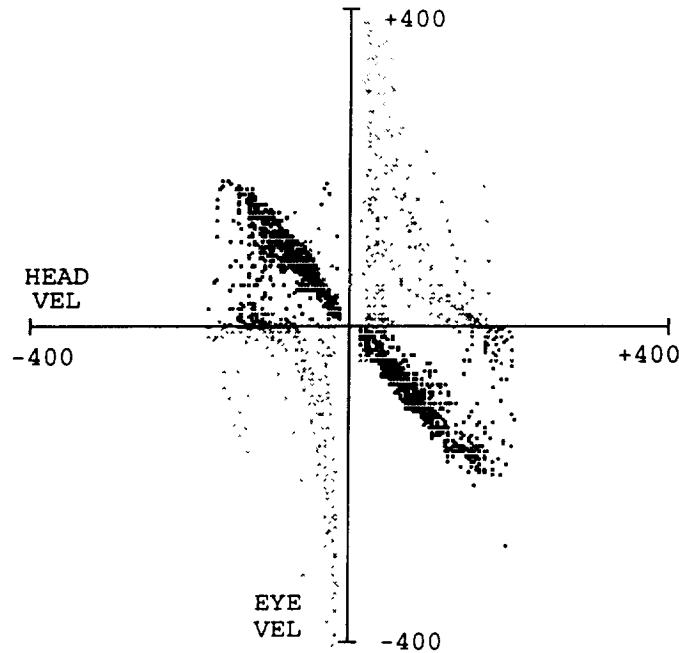


Figure 8. Eye velocity versus head velocity during a gaze refixation task performed before flight.

In-Flight

The data plotted in Figure 9 were obtained from Subject 3 on the first day of the LMS flight. Two changes are immediately obvious. First, maximum head and eye velocities are less than those seen pre-flight. Second, the subject was no longer stabilizing his eyes relative to the outside world, as demonstrated by the fact that only 11% of the 2419 data points now lie in sectors 3 and 4. However, 69% are now in sectors 1 and 2, indicating a great deal of suppression of the vestibular system in general and the vestibulo-ocular reflex in particular. These results are typical of all 4 subjects on Flight Day 1. Over a period of days, Subjects 2, 3 and 4 tended to return to a more ground-like eye stabilization strategy, but Subject 1 did not. All subjects increased their maximum head velocities after Flight Day 1.

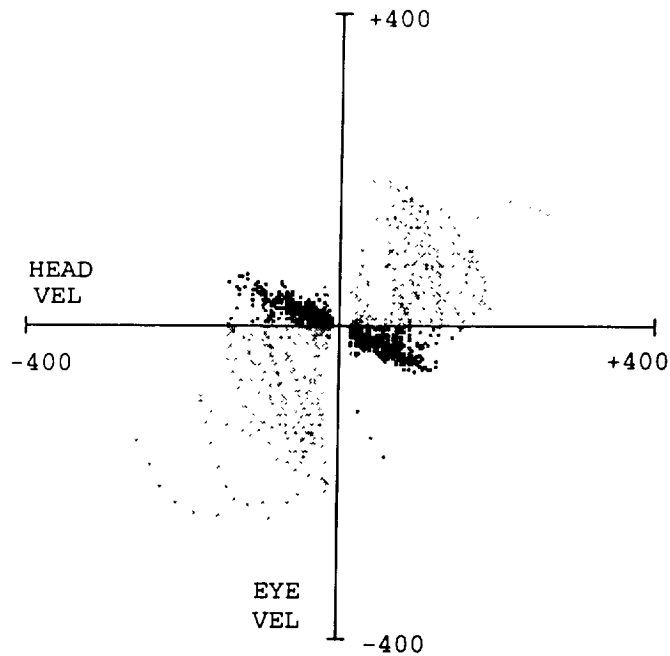


Figure 9. Eye velocity versus head velocity during ad lib, in-flight activity.

Post-Flight

Figure 10 is an example of results obtained from Subject 3 on landing day. The data are similar in every respect to those obtained pre-flight from the same subject (Figure 8), indicating a rapid return to a normal pattern of eye stabilization. This was true of all subjects, although the strategy adopted by Subject 1 was still a bit unusual, as had been noted pre-flight.

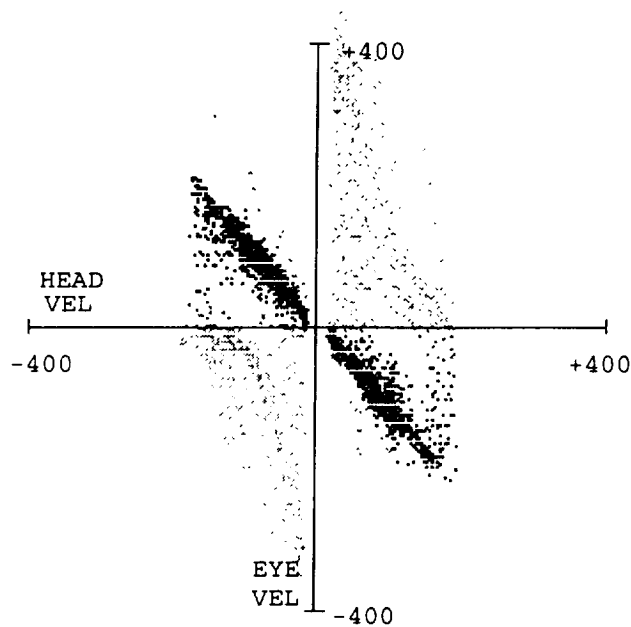


Figure 10. Eye velocity versus head velocity during a gaze refixation task performed after flight.

Combined

Figure 11 combines all results obtained from the 4 test subjects. The number of data points falling in each of sectors 1 to 4 has been expressed as a percentage of the total points for that subject and session, and plotted as a function of time before, during and after flight. A different symbol has been used for each subject, as noted in the figure legend. Almost no data points ever fell in sectors 5 and 6, so these results have not been included.

Although each individual had his own unique style during pre-flight testing, the control results were quite consistent both within and across subjects, with no obvious effect of learning. The majority of data points usually fell in sectors 3 and 4, indicating that the eyes were being stabilized relative to the outside world during most head movements.

The situation changed dramatically on Flight Day 1. When the subjects were compared to the means of their 3 pre-flight trials using the Mann-Whitney Rank Sum Test, there was a significant increase in the number of points in sector 1 ($P=0.014$), a slightly less significant increase in sector 2 ($P=0.029$), a statistically insignificant decrease in sector 3 ($P=0.100$) and a significant decrease in sector 4 ($P=0.029$). Taken together, this indicates a shift away from stabilizing the eyes relative to the outside world and toward suppression of vestibular reflexes. As anticipated, Subjects 2, 3 and 4 returned to a more normal strategy later in the flight but Subject 1 did not.

Also as expected, the post-landing patterns of all subjects were closely similar to those recorded pre-flight. This was true even for Subject 1, who continued to demonstrate more movements falling in sectors 1 and 2 and fewer in sector 4, indicating consistently lower usage of vestibular stabilization mechanisms.

Motion Sickness

The crewmembers taking part in this experiment were only minimally affected by space motion sickness. Subject 1 reported no specific symptoms but indicated that he felt worse when head "down" on Flight Day 1. Subject 2 was the most affected, experiencing mild cold sweating and malaise and moderate stomach awareness before performing the Torso Rotation Experiment on Flight Day 1. Subject 3 indicated that he developed a headache each time he wore the TRE head unit. On Flight Day 1, however, his discomfort preceded putting on the equipment. Subject 4 did not develop symptoms at any time during the flight.

DISCUSSION AND CONCLUSIONS

The present experiment has demonstrated a significant and consistent increase in gaze slip during spontaneous, self-generated head movements performed during the first day of space flight. This implies a less effective use of visual and vestibular inputs at that time and is not normal behaviour, regardless of the task being performed by the subject.

It is important to note that head velocities measured on Flight Day 1 were mostly below $100^\circ/\text{sec}$, suggesting a torso-rotation-like suppression of visual and vestibular mechanisms rather than a simple decrease of VOR gain resulting from altered otolith stimulation. In the latter case, visual tracking should have been effective in maintaining gaze on target. It is also significant that all three subjects who demonstrated strong eye stabilization before flight returned to this strategy within seven days of launch, whereas the single subject who showed the most pre-flight slip did not. Furthermore, all subjects reverted to their normal strategies immediately after landing with no need for re-adaptation. Taken together, these facts suggest that the mechanism responsible for the increased gaze slip is behavioral as opposed to a fundamental change in reflex function.

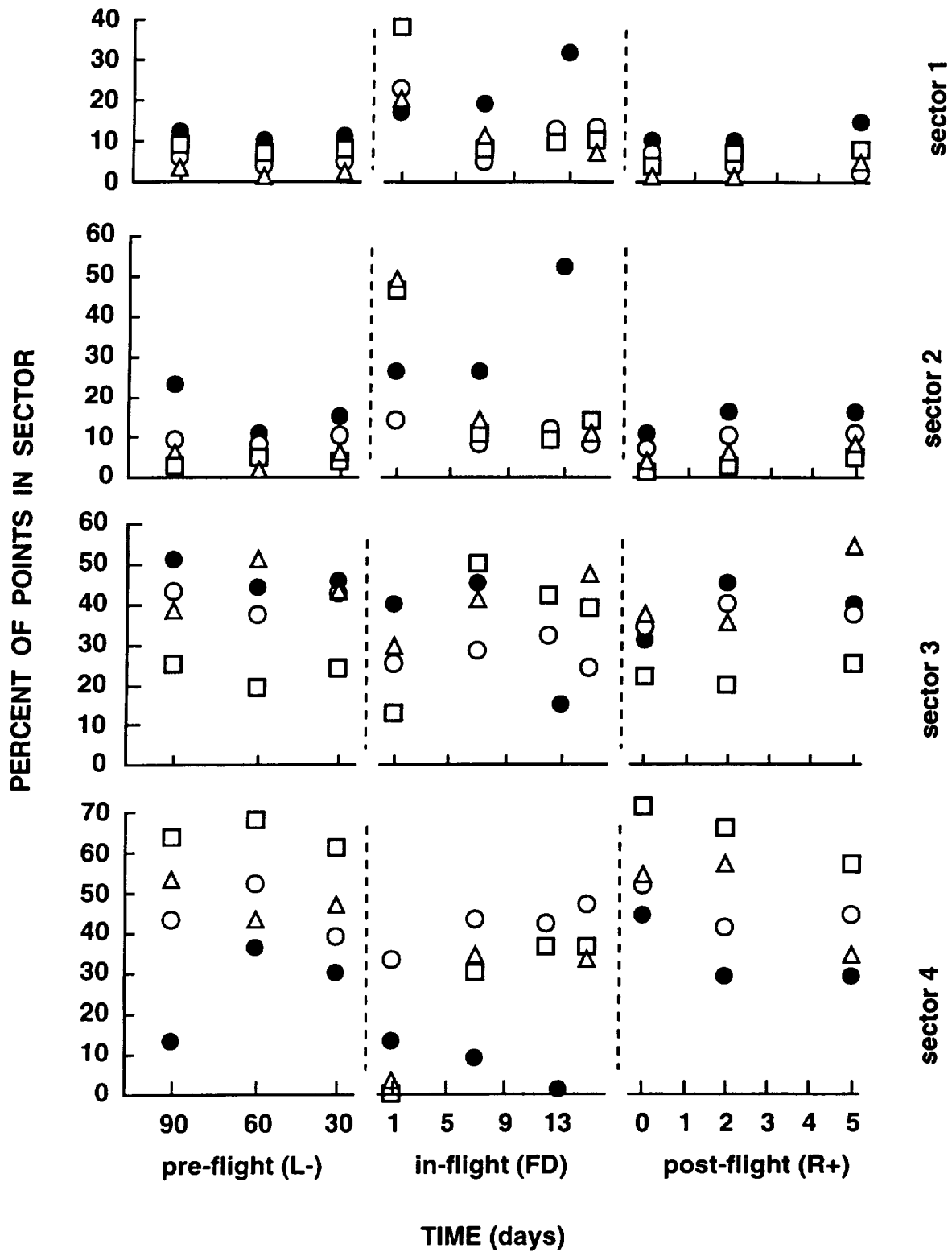


Figure 11. Percentage of data points falling into sectors 1 through 4 as a function of pre-flight, in-flight and post-flight time. Individual subjects have been identified by different symbols: Subject 1 (filled circles), Subject 2 (unfilled circles), Subject 3 (squares), Subject 4 (triangles).

The results of this experiment also support a modified interpretation of why motion sickness occurs and what might be done about it. According to this hypothesis, the astronaut has much in common with a passenger reading in the back seat of a car. In both cases, their vestibular systems are operating in altered acceleration environments that are beyond the normal range defined by evolution. This will produce motion sickness.

In addition, both seem to be suppressing vestibular function. The passenger is doing this to keep his or her gaze stable relative to the book, not gravitoinertial space as would be the goal of vestibular reflexes. It is not entirely clear why crewmembers might adopt torso-rotation-like motor strategies. Certainly the weightless environment is novel from a biomechanical point of view, and it may be that one response to this challenge is to simplify the control problem by reducing body degrees of freedom, i.e. stop using as many joints as possible. In some cases it could also be an attempt to control motion sickness by reducing head movements, although as pointed out earlier, this could be counter-productive. In any case, excessive and prolonged suppression will lead to temporary changes in vestibular function that will also produce motion sickness. In either case, it should be possible to train or instruct the individual to stop doing it.

In ground-based experiments utilizing an exaggerated version of torso rotation, it has also been shown that repeated or prolonged exposure causes symptoms to disappear. Adaptation to motion sickness probably consists of two, quite separate parts. First, updating of internal models as a consequence of the new acceleration environment will allow better prediction of sensory inputs and so reduce sensory conflict. This will produce a highly specific, non-transferable protection. Second, de-emphasizing the vestibular reference as a consequence of repeated suppression will produce a global reduction of the provocative input. This will produce a generalized, transferable protection.

Most motion sickness research has focused upon the highly specific type of adaptation. Unfortunately, this requires exposure to the provocative stimulus itself, which can be a problem when that environment cannot be duplicated for any significant length of time on or near the ground. The more generalized type of adaptation may be easier to deal with, however. Induced by a previously unstudied form of vestibular suppression, it has been shown to transfer from the laboratory to the real world of cars, aircraft and other vehicles. Perhaps it could also serve as a low-cost, portable and efficient means of reducing space motion sickness through ground-based pre-adaptation.

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NON-TECHNICAL SUMMARY

Torso rotation (TR) is a style of moving in which the head is turned by rotating the torso and the eyes move with the head. Continuous TR on the ground usually leads to motion sickness, probably because of excessive suppression of the balance organs (vestibular system). In weightlessness, many individuals also move their heads and upper bodies as a unit. If deliberate TR on the ground causes motion sickness, inadvertent TR in space should have the same effect.

Using equipment attached to the head and upper back, rotational movements of the eyes, head and upper torso were monitored while four astronauts performed other, active tasks. Post-flight analysis demonstrated an abnormally large amount of gaze slip on the first day of flight, indicative of balance organ suppression. A more normal pattern was seen 6 days later.

The results suggest that the astronaut has much in common with the passenger reading in the back seat of a car. In both cases, their balance organs are operating in altered acceleration environments that are beyond the normal range defined by evolution. This will produce motion sickness. In addition, both seem to be suppressing the balance organs. (The passenger is doing this to keep his or her gaze stable relative to the book, not the outside world as would be the goal of inner ear reflexes). Excessive and prolonged suppression will lead to temporary changes in balance organ function that will also produce motion sickness.

Potentially, astronauts could be trained to avoid balance organ suppression, just as car sickness can be reduced by not reading while moving. Alternatively, repeated exposure to exercises that deliberately suppress balance organ function on the ground might be used as a means of pre-adapting before flight.

JSC Human Life Sciences Project

**E920 - Effect of Weightlessness on Human Single Muscle Fiber
Function**

Principal Investigator:

Dr. Robert Fitts
Marquette University
Milwaukee, Wisconsin

FINAL REPORT LMS Flight Experiment E920

TITLE: Effect of Weightlessness on Human Single Muscle Fiber Function

Principal Investigator: Robert H. Fitts, Ph.D., Marquette University, Milwaukee, WI

Co-Investigator: D.L. Costill, Ball State University, Muncie, IN

Co-Authors: S.W. Trappe, T.A. Trappe, D.A. Riley, and J.J. Widrick

(1) Experiment Objectives. The primary objectives of this experiment were to: determine the extent to which zero-g alters human performance and skeletal muscle function; study the effects of zero-g on single fiber function to include an evaluation of the physiological and biochemical properties of the individual slow- and fast-twitch fibers of human skeletal muscle; elucidate the cellular mechanisms of the observed zero g-induced alterations in single fiber function; and assess the role of an altered cellular capacity in eliciting the zero g-induced reduction in both skeletal muscle and whole body performance and physical work capacity.

(2) Background. Previous studies on Skylab, Spacelab (SLS-1 and SLS-2), and the Russian Cosmos and Mir missions have documented that weightlessness produces significant muscle wasting particularly in the antigravity muscles. However a comprehensive description of microgravity induced changes in human skeletal muscle structure and function and the underlying cellular mechanisms contributing to the altered function have not been established. Consequently, the prime objectives of this project was to study of the effects of weightlessness on limb skeletal muscle function, and establish the cellular causes of the reduced functional capacity of skeletal muscle.

(3) Methods of Data Acquisition and Analysis.

Subjects. Four male crew members from STS-78 (LMS) were tested as part of this research. The crews average age, height, and weight were 42.8 ± 3.8 yr., 85.7 ± 6.2 kg, and 182.9 ± 7.5 cm, respectively. They were all healthy individuals and participated in moderate physical activities; i.e., running, swimming, or weight lifting. All subjects were informed of the risks and benefits associated with the research and gave their written consent in accordance with the Institutional Review Board's at Ball State University, Marquette University, and National Aeronautical and Space Administration.

Measurement of Oxygen Consumption. Submaximal and maximal exercise was performed on a semi-recumbent electronically resisted cycle ergometer. Two separate incremental exercise protocols were used to study these men. The first was a continuous exercise test to volitional exhaustion (VO_2 max), which consisted of four 3 min stages at 50, 100, 150, and 175 watts(w) followed by 25 w increments every two minutes until exhaustion. The second protocol, a submaximal exercise test, followed the same incremental sequence, but was terminated when the subjects attained a workload that was calculated to demand 85% of the VO_2 max determined during the L-60 testing. The VO_2 max tests were performed on L-60 and L-15, and R+4 and R+8. While the submaximal tests were conducted on L-30, flight days 2, 8, and 13, and R+1 and R+5.

During the ground based testing respiratory exchange was monitored throughout the test using a computer based system that incorporated a low-resistance gas meter, a 3.5 liter mixing chamber, 3.49 cm I.D. hoses, a Hans Rudolph breathing valve (2700 series) and electronic O_2 and CO_2 analyzers. The analyzers were calibrated before each test with gases of known concentrations. Heart rates were measured throughout the tests using radiotelemetry. In-flight testing utilized an on-board computer (LSLE Micro), mass spectrometer (GASMAP), a Hans Ruldolph breathing valve, Fleisch pneumotach #3, 3.5 liter mixing chamber, and 3.49 cm I.D. hoses. The mass spectrometer was calibrated before each test with O_2 and CO_2 of known concentrations. The Haldane transformation was used to calculate respiratory exchange using the predetermined cabin O_2 , CO_2 , N_2 , and H_2O data. Oxygen uptakes and heart rates during the submaximal exercise bouts were used to calculate each crew member's oxygen uptake at maximal heart rate ($VO_2@HR_{max}$). As anticipated, these values were closely related to the subject's measured VO_2 max ($r = 0.98$).

Calf Muscle Testing. Calf muscle performance was determined using a Torque-Velocity Dynamometer (TVD) designed by ESA specifically for the LMS mission. During testing, the subject was in the supine position using a platform/seat and harness to prevent extraneous movement. The lower leg was immobilized at 160 degrees with the foot secured to the foot plate using Velcro straps. The shaft of the dynamometer (Laboratory of Biomechanics, Zurich, Switzerland) was aligned with the axis of rotation about the ankle. The length of the leg restraint lever was adjusted to accommodate different limb lengths.

The right calf muscle of each crew member was tested. The calf muscle strength protocol consisted of three parts: (1) maximal isometric strength at ankle angles of 80, 90, and 100 degrees, (2) force-velocity measurements at 0.52, 1.05, 2.09, 3.14, 4.19, and 5.24 rad/s, and (3) a fatigue test consisting of 30 maximal contractions at 3.14 rad/s. With rest periods included, the total test time was approximately 30 minutes. At each isometric ankle angle, subject's performed two 50% efforts for warm-up followed by one maximal effort lasting 5 seconds. At isokinetic test velocity, a series of four warm-up contractions at approximately 50% effort were performed to familiarize the subjects to the test velocity and movement. Following this warm-up, subjects were asked to perform four maximal plantar flexion contractions at the selected angular velocity. A two-minute rest period occurred between each test velocity. Peak torque was taken as the highest value obtained for each of the four contractions, with the highest value used for analysis. Following a five-minute rest period, subjects performed the 30 maximal contractions without interruption (fatigue test). A contraction was performed at a rate of approximately 1 per s.

Pre-flight testing included four sessions conducted at L-90, L-60, L-30, and L-15. In-flight measurements of calf muscle contractile characteristics were conducted on flight day (FD) 2/3, 8/9, and 12/13. Due to the mission timeline, some crew members performed the TVD protocol on FD 2, while other crew members performed the protocol on FD 3 (the same scenario occurred on FD 8/9 and FD 12/13). These two-day testing sessions were considered together as one session. Post-flight (recovery) TVD testing was conducted on R+2, and R+8.

Cellular Analysis of Single Fiber Function:

- (a) Biopsy Procedure. Muscle biopsy samples were obtained from the gastrocnemius and soleus muscles 45 days before launch (L-45) and within 3 hours after landing. Pre- and post-flight muscle biopsies were placed on saline soaked gauze and divided into several portions. One portion, used for the single fiber functional experiments was immediately submerged in cold skinning solution and shipped overnight at 4°C to Marquette University where upon arrival it was stored at -20°C. A second portion was pinned at a mild stretch and immersion fixed in a 0.1 M cacodylate buffer (pH 7.2) consisting of 4% glutaraldehyde and 2% paraformaldehyde with 5mM calcium chloride. This sample was shipped overnight at 4°C to the Medical College of Wisconsin for osmium post fixation and embedding for electron microscopy.
- (b) Single Fiber Functional Experiments. The free Ca^{2+} concentration of the relaxing and activating solutions were pCa 9 (where $\text{pCa} = -\log \text{free } [\text{Ca}^{2+}]$) and pCa 4.5, respectively. A single fiber segment was isolated from a muscle bundle, transferred to an experimental chamber, and mounted between an isometric force transducer (Cambridge Model 400; Cambridge Technology, Inc., Watertown MA) and a DC position motor (Cambridge Model 300B; Cambridge Technology, Inc.). The experimental apparatus was attached to the stage of an inverted microscope during the experiments. Sarcomere length was adjusted to 2.5 μm and the fiber segment length (FL) was

recorded. A Polaroid photograph was taken of the fiber while it was briefly suspended in air and fiber width was measured at three points along the photo. The mean of these measurements was defined as fiber diameter by assuming the fiber takes on a circular cross-section when suspended in air. The fiber was activated by transfer from relaxing solution into an adjacent chamber containing activating solution. These solutions were maintained at 15°C throughout data collection. Output from the force transducer and position motor were directed to a digital storage oscilloscope before being amplified and interfaced to a personal computer. Custom software performed on-line analysis and stored data to disk.

Peak Force. Absolute peak force (in mN) was calculated as the difference between resting force, measured while the fiber was in relaxing solution, and the maximal force obtained during activation at pCa 4.5. Normalized peak force (in kN/m²) was defined as the absolute peak force divided by the fibers cross-sectional area.

Maximal Shortening Velocity. Maximal shortening velocity (V_o) was determined by the slack test procedure as previously performed in this laboratory. The times required for the redevelopment of force following 5-6 imposed slack steps (each $\leq 20\%$ of FL) were plotted against the corresponding slack length and the points fit with a linear least squares regression line. The slope of this line was V_o which was normalized to the length of the fiber and expressed as fiber length/s (FL/s).

Force-Velocity and Force-Power Relationship. To determine the force-velocity relationship, the fiber was fully activated and the position motor stepped to three sub-maximal force loads. After completion of the final step, the fiber was slacked to a length $\leq 20\%$ of FL, transferred back into relaxing solution, and re-extended to its original FL. The entire procedure was repeated at various loads in order to produce a total of 15-18 force-velocity data pairs for each fiber. The computer calculated peak force and the average force and shortening velocity over the last half of each isotonic step. The force and velocity data points for an individual fiber were fit with the hyperbolic Hill equation using an iterative curve fitting algorithm.

Fiber Enzyme Analysis. Individual freeze-dried fibers (approx. 2mm long segments) were dissected from the freeze-dried fiber bundles. The fibers were divided into three sections, and used for the enzyme and substrate assays (0.5 ug), and fiber type determination on SDS gels (0.5 ug), respectively. Each segment was weighed on a quartz fiber balance, and the segment used for the enzyme analyses (0.5 ug) was added to 5 ul of a glycerol-KCL-detergent medium under mineral oil. After incubation for 2 hr at room temperature, the samples were transferred to a -80°C freezer and stored under vacuum. Since each assay required only 0.1-0.2 μ l of extract, the 5 μ l extract provided enough sample for duplicate assays for all enzymes of interest. We used enzymatic methods based on the fluorometric determination of pyridine nucleotides to assay the activity of the glucose handling and/or glycolytic enzymes phosphorylase, hexokinase, glycogen synthase, phosphofructokinase (PFK), and lactate dehydrogenase (LDH), and the oxidative enzymes citrate synthase, and β -hydroxyacyl-CoA dehydrogenase (β OAC). We also assayed the rate limiting enzyme in free fatty acid metabolism CoA-carnitine acyl transferase (CAT). The sensitivity of each reaction was increased by enzymatic cycling

Substrate Assays of ATP, Phosphocreatine (PC), Glycogen, and Lactate. As with the enzyme assays, the selected substrates were assayed using enzyme methods based on the fluorometric determination of pyridine nucleotides, and the sensitivity was increased by enzymatic cycling. The metabolic assays were made on 0.1 N NaOH extracts of each fiber segment. The 0.5 μ g fiber sample was extracted in the NaOH for 30 min at 90°C, and the extract stored at 4°C under vacuum. For all substrates 0.1 μ l of the

fiber extract was added to the appropriate reaction buffers. In the case of glycogen, ATP, and PC the reagent volume is 1 μ l, while for lactate the reagent volume is 0.1 μ l.

Fiber Type Determination. Following each experiment, fiber type was determined by myosin heavy chain identification of SDS-PAGE gels. A flat bed scanner with a transparency adapter was used to store an image of each gel on computer disk. Image analysis software (SigmaGel, Jandel Scientific Software) was used to quantify the relative levels of MLC₁, MLC₂, and MLC₃ in each fiber.

Electron Microscopy. Longitudinal and cross thin sections (~ 60 nm) were cut from bundles of fibers and stained with uranyl acetate and lead citrate before examination and photographing in a JEOL 100 CXII electron microscope. Myofibril densities and sarcomere length were determined from cross and longitudinal sections, respectively. Final myofibril density values were adjusted for variations in sarcomere length on an individual subject basis.

Data Analysis. Results are presented as mean \pm SE. Data were analyzed using an ANOVA in which the treatment, i.e. the flight effect, was nested within subjects. When a significant treatment effect was observed, pre- to post-flight differences within an individual subject were evaluated with a two-tailed t-test. Statistical significance was accepted at $p < 0.05$.

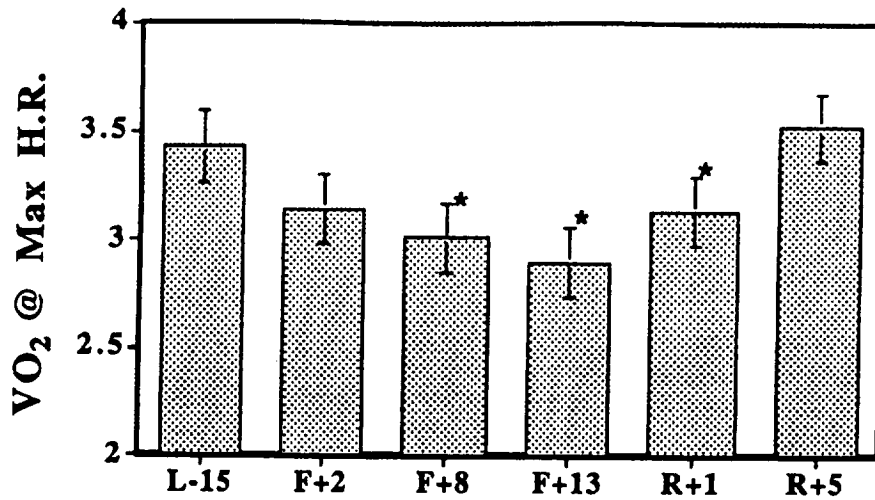
(4) Flight Results.

Aerobic Capacity. Body weights of the crew averaged 4.1 kg less on R+0 than preflight (L-15). This represents a 4.7% decline in body weight. At R+4 and R+8 the average crew weight was still 2.5% and 1% below the pre-flight value, respectively. Oxygen uptake during submaximal exercise of 50, 100, 150, and 175 watts was similar during tests performed pre-flight, in-flight, and post-flight suggesting that cycling efficiency was unchanged by space flight. Maximal oxygen consumption (VO₂ max) was 10.3% and 4.8% lower on R+4 and R+8 compared to the pre-flight value (Table 1). Although in-flight testing was limited to 85% of VO₂ max, we used the oxygen uptakes and heart rates during the submaximal exercise bouts to calculate each crew member's oxygen uptake at maximal heart rate (VO₂ @ HRmax). As anticipated, these values were closely related to the subject's measured VO₂ max ($r=0.98$). Figure 1 illustrates the means (\pm SE) for VO₂ @ HRmax before, during, and after the flight. The crew's calculated VO₂ max was significantly lower on FD 8, FD 13, and R+1 compared to the pre-flight value.

TABLE 1. Maximal Oxygen Consumption (VO₂ max)

CREW	L-15	R+4	R+8
A	3.212	2.811	2.949
B	3.795	3.487	3.542
C	3.687	3.386	3.682
D	3.664	3.193	3.450
Mean \pm S.D.	3.590 \pm 0.258	3.219 \pm 0.298	3.406 \pm 0.319

FIGURE 1. VO₂ @ H_{max}

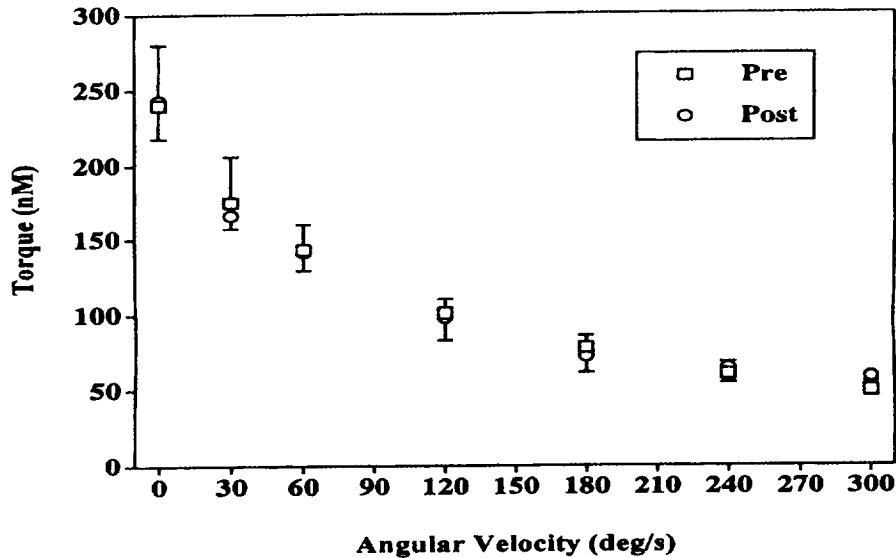


Calf Muscle Testing. Isometric calf strength values are shown in Table 2. The strength measurements on flight day (FD) 2/3 and FD 8/9 were considerably lower than all other testing session. Since the isometric values from FD 12/13 were equal to or slightly greater than pre-flight values, we conclude that technical difficulties with the TVD baseplate were responsible for the subpar performance of muscular strength on FD 2/3 and 8/9. Three of the four crew members had increased isometric strength on flight day 12/13 and R+2, and on R+8 all 4 crew members had isometric strength values that were greater than preflight values. No differences were observed in the torque-velocity relationship during or after the flight, and the pre-to-post relationship shown in figure 2 is representative of all testing sessions.

TABLE 2. Isometric Calf Muscle Strength in Newton-Meters (N·m)

Crew Member	Preflight	FD 2/3	FD 8/9	FD 12/13	R+2	R+8
A	207	99	113	211	230	229
B	193	175	187	201	207	206
C	249	145	221	220	213	270
D	159	102	177	208	171	160
Mean ± SE	202 ± 37	130 ± 37	175 ± 45	210 ± 8	205 ± 25	216 ± 46

FIGURE 2. Calf Torque Velocity Relationship



Fiber Type Composition. Enzyme histochemical analysis of the biopsies showed space flight to have no significant effect on the fiber type composition of either the gastrocnemius or the soleus muscles. The composition of the soleus was 85% slow type I and 15% fast type IIa, while the gastrocnemius was 62% type I, 23% type IIa, and 15% fast type IIx. In the fibers isolated for single fiber analysis, we did observe an increase in the percentage of hybrid (fibers containing both slow and fast myosin) and fast type IIa fibers and a decreased percentage of slow type I fibers in the soleus following space flight (Table 3).

Table 3. Myosin Heavy Chain Composition of Pre- and Post-Space Flight Soleus Fibers.

subject	type I		type I/IIa		type IIa	
	pre	post	pre	post	pre	post
A	97% (36)	93% (27)	0%	7% (2)	3% (1)	0%
B	91% (20)	76% (28)	0%	14% (5)	9% (2)	11% (4)
C	88% (37)	65% (24)	0%	0%	12% (5)	35% (13)
D	88% (22)	84% (27)	12% (3)	3% (1)	0%	13% (4)
mean	91% (115)	79% (106)	2% (3)	6% (8)	6% (8)	16% (21)

Values are the percent of the total number of fibers for each individual subject/treatment combination. Number of fibers in parentheses.

Fiber Diameter and Cross-sectional Area. Space flight induced a decline in the mean fiber diameter and cross-sectional area of the slow- and fast-twitch fibers in both the soleus and the gastrocnemius muscles. In 3 of the 4 crew members the decline in the diameter of the soleus slow type I fibers post flight was significant (Table 4). Subject B showed the greatest decline in this variable and in fiber cross-sectional area. In the gastrocnemius muscle, subjects B and D showed a decline in fiber diameter and cross-sectional area for both the slow type I and fast type IIa fibers, while subjects A and C showed either no change or a slight increase in these variables (Table 4).

Table 4. Diameter (μm) of pre- and post-spaceflight single muscle fibers.

subject	soleus type I			gastrocnemius type I			gastrocnemius type IIa		
	pre	post	Δ	pre	post	Δ	pre	post	Δ
A	98 \pm 2	95 \pm 2	-3	79 \pm	84 \pm 1	+6	86 \pm 2	87 \pm 3	+1
B	107 \pm 3	87 \pm 2	-19 *	90 \pm	85 \pm 2	-6	103 \pm 3	95 \pm 2	-8
C	92 \pm 2	83 \pm 2	-10 *	73 \pm	74 \pm 3	+1	62 \pm 0	71 \pm 2	+15
D	93 \pm 2	86 \pm 2	-8 *	80 \pm	70 \pm 2	-13	88 \pm 1	82 \pm 2	-7 *
mean	97 \pm 1	88 \pm 1		80 \pm	79 \pm 1		89 \pm 2	83 \pm 2	
subject	p < 0.05			p < 0.05			p < 0.05		
treatment	p < 0.05			p < 0.05			p < 0.05		

Values are μm (mean \pm SE). Δ indicates percent difference between pre- and post-flight values. p * < 0.05; † 0.10 > p > 0.05.

Peak Force of Individual Fast and Slow Fibers. Space flight induced a 22% and 17% decline in the peak force capacity of the soleus type I and gastrocnemius type IIa fibers. For the slow type I fibers of the soleus, all subjects showed a significant drop in force (Table 5). The primary reason for the decline in peak force (P_o) for the soleus and the gastrocnemius was the reduced fiber size, and for the gastrocnemius muscle the fiber atrophy explained the entire force loss. Consequently, for the gastrocnemius type IIa fibers the force loss was restricted to subjects B and D (Table 5) as these were the only crew to show atrophy of this fiber type (Table 4).

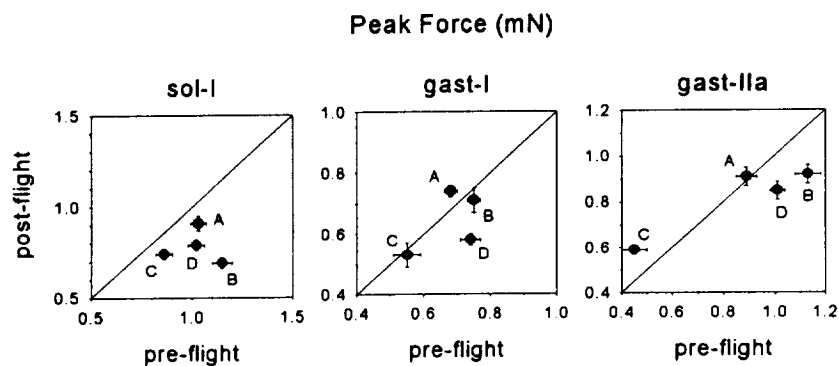
The subject variability in the response to the zero-g induced decline in force is perhaps best observed in Figure 3 which plots the average pre-flight force for each subject and fiber type versus the post-flight value. The figure also clearly shows that the force loss in the slow type I soleus fiber occurred in all 4 subjects, while for the gastrocnemius muscle fibers (type I and IIa) the decline in force was observed in subjects B and D but not A and C.

Table 5. Peak absolute force (mN) of pre- and post-spaceflight single muscle fibers.

subject	soleus type I			gastrocnemius type I			gastrocnemius type IIa		
	pre	post	Δ	pre	post	Δ	pre	post	Δ
A	1.03 \pm 0.04	0.91 \pm 0.04	-12 *	0.68 \pm 0.02	0.74 \pm 0.02	+9	0.89 \pm 0.04	0.91 \pm 0.04	+2
B	1.15 \pm 0.05	0.69 \pm 0.03	-40 *	0.75 \pm 0.02	0.71 \pm 0.04	-5	1.13 \pm 0.05	0.92 \pm 0.04	-19 *
C	0.86 \pm 0.04	0.74 \pm 0.03	-14 †	0.55 \pm 0.04	0.53 \pm 0.04	-4	0.45 \pm 0.05	0.59 \pm 0.02	+31
D	1.02 \pm 0.04	0.79 \pm 0.03	-23 *	0.74 \pm 0.03	0.58 \pm 0.02	-22 *	1.01 \pm 0.03	0.85 \pm 0.04	-16 *
mean	1.00 \pm 0.02	0.78 \pm 0.02		0.66 \pm 0.02	0.66 \pm 0.02		0.96 \pm 0.04	0.80 \pm 0.03	
subject	p < 0.05			p < 0.05			p < 0.05		
treatment	p < 0.05			p < 0.05			p < 0.05		

Values are mN (mean \pm SE). Number of fibers per mean same as in Table 1. Δ indicates percent difference between pre- and post-flight values. * p < 0.05; † 0.10 > p > 0.05.

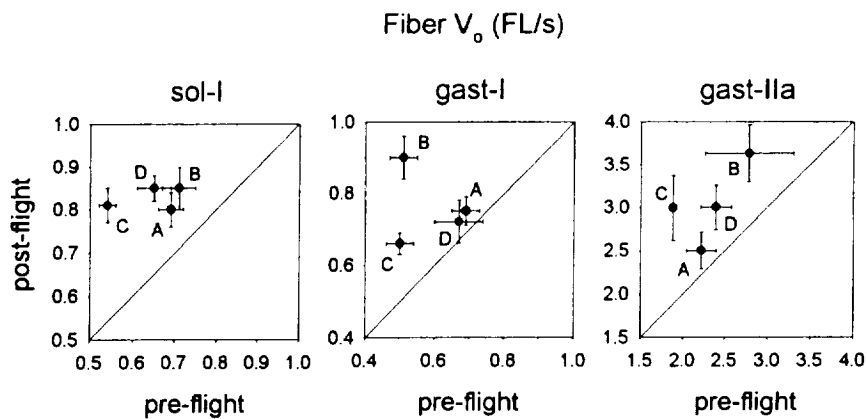
FIGURE 3.



When force was normalized for force per cross-sectional area (kN/m^2) there were no significant changes in the gastrocnemius fibers (slow- or fast-twitch) for any of the four crew members studied. However, for the slow-twitch type I fibers of the soleus, subjects B and D still showed a significant 9% decline in peak force. This indicates that for these subjects the space flight induced decline in peak force of the slow type I fiber could not be explained entirely by the fiber atrophy, but that additional factors most likely a disproportionate loss of contractile protein contributed to the decline.

Maximal Velocity of Fiber Shortening (V_o). Space flight induced an increase in the fiber V_o (fiber lengths/s) in all fiber types in both muscles studied. Furthermore this effect was observed in all 4 crew. This increase is shown in Figure 4 which plots the average pre-flight value for each subject versus their post-flight value. All of the post-flight values are above the line of identity indicating a post-flight increase in V_o for all subjects.

FIGURE 4.



The increased V_o was not caused by an altered myosin heavy or light chain composition (MHC or MLC) as no significant changes were observed in the myosin isozyme pattern. The elevated V_o was associated with an increase in the distance between the actin and myosin filaments. For all subjects, the actin-myosin distance in the slow type I fiber of the soleus increased (Table 6). The increased spacing was caused by a selective loss of actin filaments producing a 25% decrease in the actin density in the A band.

Table 6. Thin and thick myofilament spacing for pre- and post-spaceflight soleus fibers.

subject	actin-actin distance, nm			myosin-myosin distance, nm			actin-myosin distance, nm		
	pre	post	Δ	pre	post	Δ	pre	post	Δ
A	15.0 \pm 0.4	17.2 \pm 0.2	+15	31.9 \pm 0.4	32.0 \pm 0.6	0	17.2 \pm 0.3	18.6 \pm 0.2	+8
B	13.2 \pm 0.3	19.2 \pm 0.5	+45	35.2 \pm 0.5	37.4 \pm 1.5	+6	17.6 \pm 0.3	21.7 \pm 0.2	+23
C	10.9 \pm 0.2	16.3 \pm 0.3	+50	38.3 \pm 1.2	38.1 \pm 0.4	0	14.9 \pm 0.2	17.7 \pm 0.2	+19
D	13.7 \pm 0.4	16.9 \pm 0.3	+23	39.3 \pm 0.7	39.3 \pm 1.3	0	16.5 \pm 0.3	17.9 \pm 0.2	+8
mean	13.2 \pm 0.9	17.4 \pm 0.6		36.2 \pm 0.7	36.0 \pm 0.8		16.6 \pm 0.6	19.0 \pm 0.9	

Values are mean \pm SE. Five pre-flight and five post-flight fibers analyzed per subject. Actin-actin distance measured in A band. Δ indicates percent change from pre-flight value.

Peak Power. As a result of the decline in peak force, the peak power of the soleus type I and gastrocnemius type IIa fibers declined with space flight. In the soleus type I fibers the decline was significant for subject B and D, but not A and C (Table 7). This result is consistent with the observation that subjects B and D showed the greatest fall in P_o . In all cases, the post-flight peak power would have been considerably lower had fiber V_o not have increased.

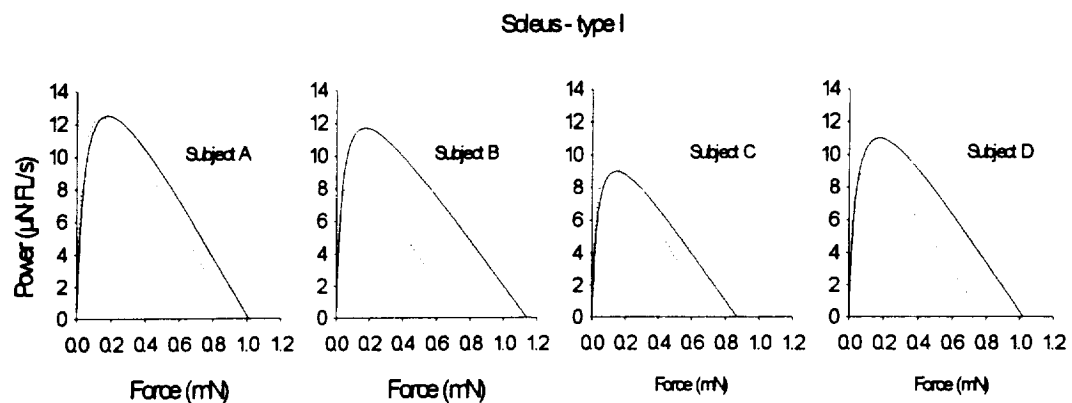
Table 7. Peak power ($\mu\text{N}\cdot\text{fiber length}\cdot\text{s}^{-1}$) of pre- and post-spaceflight single muscle fibers.

subject	soleus type I			gastrocnemius type I			gastrocnemius type IIa		
	pre	post	Δ	pre	post	Δ	pre	post	Δ
A	12.2 \pm 0.5	12.4 \pm 0.5	-2	8.7 \pm 0.4	10.0 \pm 0.4	+15 *	41.6 \pm 2.5	51.1 \pm 3.6	+23
B	10.9 \pm 0.6	8.2 \pm 0.3	-25 *	6.3 \pm 0.5	8.9 \pm 0.6	+41 *	41.2 \pm 3.8	42.1 \pm 3.8	+2
C	9.2 \pm 0.5	9.6 \pm 0.8	+4	7.3 \pm 0.8	6.1 \pm 0.5	-16	23.9	26.6 \pm 2.2	+11
D	11.0 \pm 0.6	9.1 \pm 0.4	-17 *	8.6 \pm 0.7	7.0 \pm 0.3	-19 *	51.9 \pm 4.5	40.3 \pm 1.8	-22 *
mean	10.9 \pm 0.3	9.9 \pm 0.3		7.9 \pm 0.3	8.4 \pm 0.3		45.7 \pm 2.7	39.6 \pm 2.0	
subject	p < 0.05			p < 0.05			p < 0.05		
treatment	p < 0.05			p < 0.05			p < 0.05		

Values are $\mu\text{N}\cdot\text{fiber length}\cdot\text{s}^{-1}$ (mean \pm SE). Number of fibers per mean in parentheses. Δ indicates percent difference between pre- and post-flight values. * p < 0.05.

Figure 5 shows the power-force relationship for each subject for the soleus slow type I fiber. From this figure it is clear that all subjects showed a drop in peak force, but only subjects B and D showed a reduced peak power. In the case of subjects A and C, the decline in force was less and the increased shortening velocity compensated, such that peak power was unaltered.

FIGURE 5.



Single Fiber Biochemistry. The experiments analyzing the effects of space flight on muscle enzyme and substrate levels in individual slow- and fast-twitch fibers are still in progress. Consequently, the data presented here are preliminary. The enzyme profile of the slow and fast fibers pre- and post-flight are shown in Table 8.

TABLE 8. Enzymatic Profile of Fast and Slow Fibers

Fiber Type	β OAC	CAT	CS	GP	GS	HK	LDH	PFK
Soleus Type I								
Pre	4.71 \pm .36	1.33 \pm .17	2.83 \pm .22	1.73 \pm .24	.269 \pm .03	.32 \pm .04	6.80 \pm .59	.239 \pm .03
Post	5.71 \pm .48	1.92 \pm .17*	3.64 \pm .25*	1.85 \pm .30	.503 \pm .07*	.32 \pm .03	6.08 \pm .51	.291 \pm .04
Gastrocnemius								
Type I								
Pre	3.96 \pm .45	1.75 \pm .40	3.06 \pm .27	1.91 \pm .49	.353 \pm .05	.39 \pm .07	6.19 \pm .91	.169 \pm .04
Post	4.89 \pm .88	3.25 \pm .79	3.94 \pm .44	1.90 \pm .30	.522 \pm .10	.47 \pm .08	5.98 \pm 1.1	.311 \pm .08
Type IIa								
Pre	3.29 \pm .41	1.53 \pm .39	3.10 \pm .48	2.18 \pm .67	.269 \pm .06	.29 \pm .05	16.5 \pm 1.8	.337 \pm .06
Post	2.70 \pm .49	1.67 \pm .44	4.43 \pm .49	3.36 \pm .63	.642 \pm .19	.53 \pm .10	21.5 \pm 2.3	.394 \pm .07
Type IIx								
Pre	3.37 \pm .71	1.12 \pm .27	3.53 \pm .44	1.34 \pm .32	.339 \pm .12	.74 \pm .27	22.6 \pm 3.3	.325 \pm .09
Post	2.68 \pm .37	1.76 \pm .65	2.83 \pm .50	3.59 \pm .1.3	.612 \pm .19	.59 \pm .20	25.3 \pm 5.3	.487 \pm .09

Data are means \pm S.E. in mol/kg/hr. Table abbreviations are: GP = glycogen phosphorylase, HK = hexokinase, GS = glycogen synthase PFK = phosphofructokinase, LDH = lactate dehydrogenase, CS = citrate synthase, β OAC = β -hydroxyacyl-CoA dehydrogenase, and CAT = CoA-carnitine acyl transferase. * indicates P < 0.05

TABLE 9. Substrate Profile of Single Fast and Slow Fibers (Data Presented as Means \pm SE in mol/kg dry wt)

Fiber Type	ATP	PCr	Glycogen	Lactate
Soleus Type I				
Pre	20.3 \pm 1.1	71.8 \pm 4.4	584 \pm 34	21.1 \pm 1.8
Post	21.9 \pm 1.9	76.1 \pm 4.9	637 \pm 50	22.3 \pm 1.9
Gastrocnemius				
Type I				
Pre	16.8 \pm 1.5	62.5 \pm 5.9	581 \pm 58	18.8 \pm 2.9
Post	23.9 \pm 3.2	73.2 \pm 9.1	795 \pm 110	24.5 \pm 2.7
Type IIa				
Pre	26.6 \pm 3.5	83.6 \pm 7.6	678 \pm 50	20.1 \pm 2.8
Post	25.7 \pm 3.2	72.6 \pm 6.1	650 \pm 65	27.2 \pm 4.2
Type IIx				
Pre	20.8 \pm 2.9	61.4 \pm 5.9	558 \pm 105	21.2 \pm 6.7
Post	24.5 \pm 3.1	75.7 \pm 3.4	485 \pm 133	14.4 \pm 3.8

In slow type I fibers the oxidative and glycolytic enzymes increased with space flight in both the soleus and the gastrocnemius. The only exception was LDH in the soleus and LDH and GP in the gastrocnemius which showed no change. However, the only significant increases occurred in CAT, CS, and GS in the slow fibers of the soleus. The substrate profile for the pre- and post-flight fibers are shown in Table 9. Microgravity had no significant effect on the high energy phosphate profile (ATP and PCr), cell glycogen or lactate in either the fast or the slow fiber types.

Comparison with Ground Based Results. As part of the LMS project, we conducted a 17 day bed rest study designed to duplicate the exact duration and testing sequence of the LMS flight. The studies and assays were identical to those carried out on the LMS crew. The results were for the most part qualitatively similar to the LMS flight results. The bed rest subjects showed a similar decline in VO_2 max, and the whole calf muscle function was essentially unaltered. Similar to flight, the single fiber studies revealed fiber atrophy, and a loss of force. From a quantitative perspective, the cell changes with bed rest were not as great as flight. For example, the soleus type I fibers showed a 5% and 13% decline in fiber size and force, respectively compared to the 10% and 20% change in these parameters following space flight. With bed rest 7 of the 8 subjects showed a significant drop in the peak power of the slow soleus type I fiber, while only 2 of the 4 astronauts showed significant declines in this parameter. With space flight, the large increase in shortening velocity help protect against the loss of power. In both studies, we observed a significant increase in glycogen synthase. The glycogen content of the slow type I fiber of the soleus increased in both studies, however, the increase was small and non-significant in the LMS flight study (Table 9). In contrast, type I cell glycogen increased 66% (from 524 to 875 mol/kg dry wt) with bed rest.

(5) Conclusion

The 17 day LMS space flight resulted in significant decline in the whole body aerobic capacity of the crew, and in the functional performance of individual slow- and fast-twitch fibers. The fiber atrophy and decline in function (force and power) was greatest in the slow type I fibers of the antigravity soleus muscle. Considerable subject variability occurred for example, subject D showed a 40% decline in peak power of the soleus type I fiber, while this parameter was reduced by only 12% in subject A. The reason for the different responsiveness of the crew to microgravity is unknown, but could relate to the degree of exercise countermeasure performed.

Cell atrophy was the primary cause of the decline in fiber force and power as the force per cross-sectional area showed only minimal changes. In the slow type I fibers of the soleus, 2 of the 4 crew members showed a significant drop in peak power. All 4 subjects showed an increased shortening speed, and this partially protected against the loss of power. The increased velocity appears to have resulted from a selective loss of filamentous actin. This increased the actin-myosin filament spacing which in turn allowed a faster cross-bridge cycling rate and increased fiber speed. The structural rearrangement of the filaments represents an important adaptation to preserve cell power. One expects that this strategy is considerable more efficient than switching the myosin phenotype from the slow to the fast isozyme. The latter strategy occurs in rats flown in space, and results in a faster but less efficient muscle.

The reduced force and power of the individual fibers of the soleus and gastrocnemius did not result in a compromised calf muscle function. This suggests that the cellular changes were either too small to be detected by the TVD or that the crew compensated for the decline in cell function by altering their motor unit recruitment pattern.

In future studies, it will be important to determine if the deleterious changes in cell function get progressively worse with increasing duration of flight or if a new steady state is obtained. If the latter occurs, we need to know at what point the new steady state is obtained. Animal studies suggest that microgravity induces an altered cell metabolism, such that, skeletal muscle fibers show an increased reliance on carbohydrates and a reduced ability to oxidize fats. In the bed rest study, we observed an increase in the enzymes of carbohydrate metabolism and cell glycogen concentration post bed rest. These data were consistent with an increased reliance on carbohydrate metabolism. In the LMS flight, cell glycogen increased in the slow type I fibers, but the change was not significant. However, data from others indicate that the crew was in negative caloric balance, and this lack of sufficient caloric intake may have reduced the microgravity induced increase in cell glycogen. In future studies, it will be important to determine if fat utilization is inhibited in humans during exercise in space as reliance on cell glycogen and blood glucose will lead to an increased fatigability and reduced physical work capacity. Finally, future studies most rigorously test high resistance exercise as a countermeasure to the microgravity induced cell atrophy.

Non-technical Summary Project E920
Effect of Weightlessness on Human Single Muscle Fiber Function

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Co-Authors. S.W. Trappe, T.A. Trappe, D.A. Riley, and J.J. Widrick

The 17 day LMS space flight resulted in significant decline in the whole body aerobic capacity of the crew, and in the functional performance of individual slow- and fast-twitch muscle fibers. The reduced cell size and function (force and power) was greatest in the slow type I fibers of the antigravity soleus muscle. Considerable subject variability occurred for example, subject D showed a 40% decline in peak power of the soleus type I fiber, while this parameter was reduced by only 12% in subject A. The reason for the different responsiveness of the crew to microgravity is unknown, but could relate to the degree of exercise countermeasure performed.

The loss of cell size was the primary cause of the decline in fiber force and power as the force per cross-sectional area showed only minimal changes. In the slow type I fibers of the soleus, 2 of the 4 crew members showed a significant drop in peak power. All 4 subjects showed an increased shortening speed, and this partially protected against the loss of power. The increased velocity appears to have resulted from a selective loss of one of the proteins involved in the contractile process. This structural rearrangement of the muscle represents an important adaptation to preserve cell power.

The reduced force and power of the individual fibers of the tested muscles did not result in a compromised calf muscle function. This suggests that the cellular changes were either too small to be detected by the whole leg measurement system or that the crew compensated for the decline in cell function by altering their strategy for muscle activation.

In future studies, it will be important to determine if the deleterious changes in cell function get progressively worse with increasing duration of flight or if a new steady state is obtained. If the latter occurs, we need to know at what point the new steady state is obtained. Animal studies suggest that microgravity induces an altered cell metabolism, such that, skeletal muscle fibers show an increased reliance on carbohydrates and a reduced ability to oxidize fats. In the LMS flight, cell glycogen increased in the slow type I fibers, but the change was not significant. However, data from others indicate that the crew was in negative caloric balance, and this lack of sufficient caloric intake may have reduced the microgravity induced increase in cell glycogen. In future studies, it will be important to determine if fat utilization is inhibited in humans during exercise in space as reliance on cell glycogen and blood glucose and the reduced muscle size will lead to an increased fatigability and reduced physical work capacity. Finally, future studies must rigorously test high resistance exercise as a countermeasure to the microgravity induced loss of muscle mass and physical work capacity.

JSC Human Life Sciences Project

E948 - Human Sleep, Circadian Rhythms and Performance in
Space

Principal Investigator:

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Pittsburgh, Pennsylvania

**FINAL REPORT OF NASA CONTRACT NAS 9-18404
"Human sleep, circadian rhythms and performance in space"
comprising Experiment E 948 (SACS) of the LMS mission aboard STS-78
SLEEP AND CIRCADIAN RHYTHMS IN FOUR ORBITING ASTRONAUTS**

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Note: the University of Lyon work will be reported separately.

INTRODUCTION

The study of human sleep, circadian rhythms and performance in space has both operational and scientific significance. Operationally, U.S. Spaceflight is moving away from brief missions with durations of less than one week. Most space shuttle missions now last two weeks or more, and future plans involving space stations, lunar bases and interplanetary missions all presume that people will be living away from the gravity and time cues of earth for months at a time. Thus, missions are moving away from situations where astronauts can "tough it out" for an acute limited-time mission, to situations where sleep and circadian disruptions are likely to become chronic, and thus resistant to short term pharmacological or behavioral manipulations. It, thus, becomes imperative that sleep and circadian rhythms in space be understood, so that long term countermeasures can be employed to ensure that crew performance and well-being are not compromised.

As well as the operational significance, there is a strong theoretical imperative for studying the sleep and circadian rhythms of people who are removed from the gravity and time cues of earth. Homo sapiens' development on a planet with a 24h rotation period has had a profound influence on human biology. Like most other organisms on the planet, our species is endowed with a circadian "clock" or pacemaker. This pacemaker is located in the suprachiasmatic nucleus of the hypothalamus (Moore, 1982). It comprises an endogenous, self-sustaining circadian timekeeping system (CTS) which drives circadian rhythms in vital signs, blood chemistry, mood and performance variables. These circadian rhythms "set the stage" for restful sleep at night and active wakefulness during the day. The CTS is entrained to the correct period (24h) and temporal orientation by various time cues ("zeitgebers"), the most powerful of which is the alternation of daylight and darkness. Importantly, the circadian system of our species appears unique in being particularly sensitive to daylight, as compared to the lower light levels characteristic of artificial illumination (Lewy et al., 1980; Wever et al., 1983; Czeisler et al., 1986, but see also Boivin et al., 1996). Thus, in leaving Earth, astronauts are also removing themselves from the prime zeitgeber of their circadian system -- the 24h alternation of daylight and darkness.

Although the present study comprised the first integrated U.S. study of sleep, circadian rhythms and performance in space on more than a single subject, there have been a few investigations of the various components, studied individually. Graeber (1987) gives the best review of sleep in space, drawing upon both the Russian and the American experience. Frost et al. (1976) conducted a series of experiments on Skylab astronauts engaged in long duration missions. Although profound changes in sleep architecture during the mission were not observed, there did appear to be patterns of sleep disruption commensurate with an inappropriately entrained CTS. Moreover, Frost et al.'s subjects noted that in space a given sleep disruption interfered more with "daytime" performance, than would an equivalent sleep disruption on Earth. Santy (1988) reviewed the sleep of 58 crew members from 9 space shuttle flights using a debriefing form and found significant sleep disruptions, especially on dual-shift missions where 50% of subject used a hypnotic in-flight at least once.

Circadian rhythms have also been studied in space. Hanley and Adey (1971) and Gazenko and Ilyin (1987) have studied mammals in various space environments and have shown that there is a tendency in microgravity for the CTS to exhibit aberrant phase relationships and period lengths. It appeared that although circadian rhythms were still generated, entrainment mechanisms did not work as well in the microgravity environment.

Recent support for such a view using human subjects comes from work of Gundel and co-workers (1994) who studied a brief mission of a single German astronaut to the Mir Space Station. This investigation found that the circadian rhythms of rectal temperature and alertness were phase delayed in space, relative to baseline, suggesting a possible transition to free-running. Sleep disruptions were also observed, with shortening and change in architecture during space flight.

The aim of the present study was to test the following 9 hypotheses:

- 1) Body temperature rhythms would be of lower amplitude in flight than on the ground, and would show further reductions from early flight to late flight;
- 2) Body temperature phase would be more labile in flight than on the ground, and/or would show changes from early flight to late flight (towards aberrant phases with respect to the sleep wake cycle), perhaps indicating free-running;
- 3) Circadian rhythms in urinary free cortisol and melatonin sulphate would be of lower amplitude and would be more phase labile in-flight than on the ground;
- 4) Sleep (as assessed both polygraphically and by diary) would be more disrupted in flight than on the ground, showing less SWS and aberrant patterns of REM sleep (e.g. short REM latencies); particularly late flight.
- 5) Sleep in the late flight block would be worse than that in the early flight block.
- 6) Hypnotic use would be more prevalent late flight than early flight.
- 7) Daily levels of mood, activation and objective performance would be influenced by sleep disruption and circadian dysfunction, taking the form of a deterioration from early flight to late flight;
- 8) End of shift questionnaires would reflect an increase in subjective fatigue and required effort as the flight progressed.
- 9) Ambient light levels as detected by the Actillum 8 would be lower in flight than on the ground.

METHODS

The Space Mission

Strenuous efforts were made to ensure that the timing and trajectory of the mission was as least disruptive as possible to the astronauts' circadian rhythms. Lift-off was in daylight (10:50 EDT), requiring

only a slightly earlier than usual waketime (05:50 EDT). For operational reasons, there were changes in bedtime and waketime (and thus the whole routine) throughout the flight, but these were distributed equally throughout the mission leading to a phase advance of 25 minutes per day for all but one day of the mission. Thus, for most of the mission, the astronauts lived, essentially on a 23.58h day. Strict rules ("Appendix K") governed the astronaut's time availability for work. An eight hour sleep opportunity was mandated for each day, as well as rest times at either end of the sleep period and time off for meals. For the present experiment, time was set aside during the work shift to "pay back" for time spent in EEG wiring etc close to the sleep episode.

Subjects

The subjects were four of the seven astronauts aboard the mission. All were males, ages ranged from 38y to 47y (mean= 42.5y). Two of the subjects were members of the NASA astronaut corps, two were payload specialists recruited specifically for this mission. All were extremely healthy with no reported sleep abnormalities. Arbitrary subject code numbers (S1 through S4) will be used throughout this report. The study was approved by both University of Pittsburgh and NASA Internal Review Boards for the ethical treatment of human subjects.

Measurement Block

The experiment was structured in terms of 72h measurement blocks within which all sleep, circadian rhythm, mood and performance measures were taken. There was one measurement block before the mission "pre-flight" (starting 7d before launch), one after the mission "post-flight" (starting 18d after landing), and two in-flight. The two in-flight measurement blocks were towards the beginning "early flight" (starting 2d after launch) and towards the end "late flight" (starting 12d after launch) of the 17-day mission. Each measurement block ran for exactly 72h. In-flight measurement blocks started and ended at the beginning of the workday, pre-flight and post-flight measurement blocks started and ended at the end of the workday.

Core Body Temperature

For the duration of each 72h measurement block, each subject wore a rectal thermistor connected to a Minilogger 8 belt-pack recorder. Probes were only removed for defecation. Rectal temperature was sampled automatically every six minutes around the clock, allowing 3-cycle circadian temperature rhythms to be plotted.

Urinary Variables

Urine samples were collected and urine volumes measured throughout each 72h measurement blocks. Samples were assayed for melatonin sulphate and free cortisol. Using the volume measure of each void, the assay results were then expressed as a rate per hour of melatonin or cortisol production, considering the bladder as an integrator. Also plotted was the volume of urine generated (water) per minute.

Objective Sleep Recording

Each sleep during the 72h measurement block was recorded using the Medilog Sleep Research

Recorder (MSRR) system. Electrodes recording EEG, EOG, and EMG were positioned on the subject's head using a cap-based system with extra disposable electrodes. Signals were recorded onto magnetic tape. After recovery, the tapes were played back in order to: 1) visually score each minute of sleep into conventionally defined sleep stages (0, 1, 2, 3, 4 and REM); and 2) run the signals through a computer-based analysis system allowing the measurement of: a) a count of delta waves, and b) a count of eye movements. These procedures allowed a detailed characterization of the duration, depth and architecture of each sleep episode.

Subjective Sleep Diary

After each sleep episode during the 72h measurement block, each subject completed a modified computer-based version of the Pittsburgh Sleep Diary (PghSD) (Monk et al., 1994). This brief instrument assesses the estimated timings of sleep onset and offset, records the number and nature of within-night awakenings, the use of space motion sickness and hypnotic medications, and concludes with visual analogue scale ratings of: 1) subjective sleep quality, 2) mood on awakening (tense vs. relaxed), and 3) alertness on awakening (sleepy vs. alert). The Pittsburgh Sleep Diary has been used in our laboratory for more than 7 years in well over 700 different subjects and patients and has been shown to be a useful adjunct to objective sleep recording.

Actillum 8

For the duration of each 72h measurement block, each subject wore an Actillum 8 on the non-dominant arm. Actigraphy involves wearing an electronic device which counts the number of arm movements per minute recording each minute's total individually in a memory chip. In the Actillum 8 device, this is supplemented by the simultaneous recording of ambient light levels. At the end of the study, the data from the device is dumped into a computer for subsequent analysis and storage.

Mood and Alertness

Five times per day (within each measurement block) spread throughout the waking interval, a very brief computer-based visual analogue scale technique (Monk 1989) was used to measure subjective alertness (global vigor) and overall mood (global affect). These ratings were used to create daily mean levels of mood and alertness.

Performance Tests

At three times per day (within each measurement block), before breakfast, lunch and dinner, each subject completed a 6-minute performance battery using the computer. The battery has been used in our laboratories for more than 12 years and is described in Monk et al. (1985). The two component tests (the speed and accuracy of which are evaluated) comprise 32 trials of a simple serial search task (searching for the letter "E" in 30 random uppercase letters) and 32 trials of a modified form of the Baddeley (1968) reasoning test (determining the truth or falsity of statements such as "M IS NOT BEFORE C - CM"). Results of these tests were used to create: 1) time of day functions in performance, and 2) mean daily levels, for each of the two tasks.

End-of-Shift Questionnaire

At the end of each work shift in the 72h measurement block, subjects completed a brief questionnaire regarding stresses such as EVAs, equipment malfunction and changes in procedure, as well as ratings of how long the shift lasted and how satisfied the subjects were with their work on it.

Data Analysis

Circadian rhythms in core temperature, urinary variables and subjective alertness were analyzed by sinusoidal technique involving the fitting of both a 24h fundamental and a 12h harmonic (Monk and Fort 1983) in order to derive an estimate of phase (time of fitted minimum), amplitude (fitted maximum minus fitted minimum, divided by two) and mean level for each subject within each 72h measurement block. Sleep variables were likewise reduced to conventional measures of objective sleep quality and architecture for each sleep "night." Sleep diary and end-of-shift questionnaires variables were used both as measures in their own right, and also as modifiers of the other sleep indices (e.g., "tagging" nights that may have been influenced by space motion sickness or hypnotic medications, or excessive work load).

RESULTS

Deviations from protocol

By and large, the quality of data collection was extremely good, especially inflight. Ground measurement blocks often suffered from competing demands upon the astronauts' time which occasionally lead to lost data. Battery problems meant that all pre-flight Actillum 8 data were lost. Technical problems with electrodes and/or tapes meant that three partial subject-nights of sleep were lost in the pre-flight measurement block, but some of these were made up with additional recordings. Similar technical problems meant that late flight and post-flight polysomnographic data were not available for one astronaut. There were fairly long gaps in the temperature record for one subject pre-flight. Two of the astronauts finished the post-flight measurement block 12h early. Occasional missing mood and performance sessions also occurred, but not enough to compromise the ability to test hypotheses.

Hypothesis 1 ("Body temperature amplitude will be reduced")

The mean rectal temperature rhythms of the four astronauts (expressing each datum a deviation from that subject's 72h mean) with standard errors is plotted in Figure 1 using the same axes throughout. Apart from a slight change in rhythm shape (discussed below), temperature rhythms under flight and ground conditions differed little. In particular, the similarity of amplitude between the four curves was quite striking. Figure 2 further illustrates this with comparisons between pre-flight and early flight (upper panel), and between early flight and late flight (lower panel), with appropriate changes of the abscissa to represent differences in wake time. Even when individual curves were compared separately for each subject (Figure 3), there was a fairly striking similarity between early flight and late flight curves, with no evidence of a diminution of temperature rhythm amplitude which would have occurred had the circadian pacemaker moved out of phase with the imposed routine. Table 1 also reveals that when amplitude measures (half-range) were calculated using 24h and 12h sinusoidal fits to each time series separately, the mean temperature rhythm amplitudes for the four measurement blocks showed only a slight diminution in-flight (pre-flight: 0.57, early flight 0.43, late flight 0.47, post-flight 0.52 deg.C). Clearly, there was no general confirmation of Hypothesis 1.

Hypothesis 2 ("Body temperature phase will be labile and/or indicate free-running")

As stated in the introduction, a feature of space flight is that it frees astronauts from the shackles of earth time. The depth of this freedom only became really apparent to the authors when they monitored the mission at the Science Monitoring Area at Johnson Space Center, Houston. All activities connected

with the flight were timed according to "Mission Elapsed Time" (MET). MET is a notional "time zone" that starts at day 0, 00:00 at the moment of lift-off (whenever that happens to be). There were two clocks in the Science Monitoring Area, one on MET the other on Greenwich Mean Time. Further, as described above, for operational reasons, each bedtime and waketime was advanced by 25 minutes per day during the mission. This made defining phase markers for the circadian rhythms difficult. For this reason we chose the most powerful earth zeitgeber the astronauts experienced, namely the playing of loud music from Huntsville to wake them up each morning (referred to here as "Reveille"). Arbitrarily we defined reveille on the first morning of the measurement block as "zero time", measuring phases from this temporal marker for all three circadian cycles. In terms of GMT, reveille was at 09:29 on 1996/6/22 for the early flight measurement block, and 05:39 on 1996/7/2 for the late flight block. For simplicity, we ignored the fact that actual day length (as measured by the sleep/wake cycle) was 23.58h rather than 24h (re-doing the analyses at a T of 23.58 made no difference to the general conclusions but prevented the use of clock time in reporting results). Unfortunately, there was no equivalent powerful zeitgeber for the ground baselines. For these we simply took the scheduled waketime on Day 1. Because of this, in flight versus ground differences in phase are best considered suspect.

The circadian phase marker was taken as the time of fitted (24h + 12h) minimum. On the ground, in healthy day workers, this typically occurs 2 to 4h before waketime (Czeisler et al, 1992; Monk et al, 1995). As is clear from Figure 2, there was no apparent difference in temperature curves between early flight and late flight measurement blocks, with both inflight blocks showing the same sort of phase relationships normally observed on the ground. Thus, the sinusoidal analysis (Table 1), revealed average minimum phase timings of 3.13h before reveille early flight, and 2.68h before reveille late flight. Two subjects showed a slight phase advance, two a slight phase delay (Table 1), but in no case was the phase difference more than 85 minutes, despite a delay of almost ten calendar days between the start of each 72h block. Thus there was no evidence to support Hypothesis 2.

Hypothesis 3 ("Urinary circadian rhythms will be disrupted in flight")

Figures 4, 5 and 6 plot the circadian rhythms (averaged across the three days of a measurement block) for each subject separately for water (urine volume), melatonin sulphate and free cortisol. These data were, as expected quite noisy, rendering it difficult to properly test Hypothesis 3. However, in melatonin sulphate, the two subjects showing a clear "standard" pattern on the ground (with a melatonin onset about 12h-15h after waking), also showed the same pattern in-flight. Thus, what evidence there was favored rejection of Hypothesis 3.

Hypothesis 4 ("Sleep will be more disrupted in-flight than on the ground")

For operational reasons, the pre-flight baseline sleeps took place within the week before the flight. This was an extremely busy and stressful time for the astronauts, rendering it unsuitable as a true "on ground" comparison. In the three subjects (S1,S2,S4) for whom, this was possible, the post-flight baseline, two weeks after the end of the mission was used as a comparison, for the fourth subject (S3), comparisons were made with the pre-flight baseline. In all cases, summary statistics were obtained from the average of nights 2 and 3 of each 72h block.

Statistics from polysomnography are given in Table 2 and Figures 7 - 10. Two main findings emerged from the data: 1) Time spent asleep was reduced from an average of 392 mins. in baseline to an average of 362 mins. in-flight, with three of the four subjects showing a reduction; and 2) Slow wave sleep (from both hand-scored and automated measures) was markedly reduced in flight (Stages 3&4: baseline= 37 mins., in-flight= 11 mins.), with all four subjects showing a reduction. Measures of sleep continuity and those connected with REM sleep failed to show any consistent pattern.

These findings were broadly confirmed by the diary findings (Table 2). Reported time in bed was reduced for all four subjects. It should be noted that the diary study did not reveal any major sleep disruption as a function of space flight. Rather, the extent to which Hypothesis 4 was confirmed appeared to be limited to reduced sleep durations and a suppression of delta sleep.

Hypothesis 5 ("Sleep will deteriorate from early flight to late flight")

As is illustrated in Figures 7 - 10, there was no consistent trend for sleep late flight to be inferior to early flight, or to have a different sleep architecture. Hypothesis 4 was thus not confirmed.

Hypothesis 6 ("More hypnotic use in flight")

No hypnotics (or anti-space adaptation sickness medications) were reported as being used in space, so Hypothesis 5 was not confirmed.

Hypothesis 7 ("Mood and performance more impaired late flight than early flight")

Because the mechanics of actually doing the performance tasks are very different in microgravity, performance comparisons could only be made between the two in flight measurement blocks, whereas all four measurement blocks could be used in the assessment of mood and activation. Unfortunately there were still residual practice effects which made the interpretation of the performance data difficult. The main consistent finding was that subjective alertness did show a decline from early flight to late flight in all four subjects, suggesting some confirmation of Hypothesis 7. However, some of this apparent deterioration may have been simply that the early flight readings were artificially inflated from the initial exhilaration of being up in space

Hypothesis 8 ("More effort needed late flight than early flight")

The in flight end of shift questionnaire data indicated that 88% of astronaut-shifts (21/24) were reported as having passed "very quickly" or "fairly quickly". One subject reported himself as being tired both at the beginning and end of shifts both early flight and late flight, the other three showed no particular trend. Features of the shift that affected astronauts were: equipment problems (7/24), timeline problems (6/24), physically strenuous work (5/24), and space motion sickness (1/24). Hypothesis 7 was not confirmed; three of the four subjects reported themselves as requiring less effort to carry out their work late flight than early flight.

Hypothesis 9 ("Less light exposure in flight")

Hypothesis 8 was confirmed, light levels averaged 70 Lux in flight versus 350 Lux post-flight. Actigraphy revealed no systematic change in sleep restlessness when comparing early flight to late flight.

DISCUSSION

While some of the present results speak to the resiliency of the human circadian system, even when faced with something as profound as orbiting the Earth in a weightless environment, there were some major effects observed, particularly in the various stages of sleep observed, and in the duration and depth of sleep actually obtained. Considering first the circadian temperature rhythm findings, it should

again be emphasized that this mission was unique in attempting to minimize any "mission operations" causes of circadian desynchrony. Thus, this was a single shift mission which took off in daylight, and which followed a trajectory that minimized the required phase shift between take off and landing. Further, instead of accomplishing this phase shift in abrupt one-hour shifts (as is often done), the required phase advance was instead trickled in at a rate of 25 minutes per day. This yielded a day length of 23.58h, which appeared to be well within the circadian range of entrainment of the present subjects. Without doubt, had a mission with a less benign schedule been studied, the much greater disruptions may have been observed. Having noted this, however, the authors must admit surprise that the human circadian system appears to be remarkably resilient to something as profound as that of orbiting the earth at 17,198 miles per hour, with no gravity, and sunrises and sunsets appearing every 90 minutes. Certainly, when designing the experiment we fully expected at the very least reduced rhythm amplitude and increased phase lability, with perhaps, even, evidence of free-running. The absence of such effects in the present data confirm the conclusions of Boivin et al., (1996) that daylight/darkness cycles are not a prerequisite for circadian entrainment, and also recent animal work suggesting that non-photic zeitgebers may be important (Mrosovsky, 1988).

Although the circadian temperature rhythm changes we observed were not as profound as we had expected, there were baseline versus in-flight differences in circadian temperature rhythm shape that were of interest. In particular, the rhythm took more of a "saw tooth" shape, than the more usual "tilted inverted U" shaped function usually observed on earth. Interestingly, this parallels findings made by Fuller and colleagues in orbiting monkeys. It is noteworthy that this saw tooth pattern is not the same rhythm shape as is observed in 6 deg. head-down tilt bedrest, a model of microgravity that mimics many of the fluid shift and cardiovascular effects. As we have demonstrated in a recent 17d bedrest study incorporating many of the experiments flown in the present LMS mission, bedrest appears to lead to a reduction in rhythm amplitude, and delay in phase (timing), but all within a rhythm shape that remains essentially sinusoidal.

The urinary circadian measures broadly confirmed the story given by the temperature rhythms, though with much less clarity. In the subjects showing clear rhythms on the ground (e.g., S1 and S2 in melatonin sulphate), there was a continuation of such well-ordered rhythms in both early flight and late flight measurement blocks.

Time of day effects in mood and performance efficiency were sometimes hard to interpret because of the strong practice ("learning curve") effect that was still observed in performance. Also, performance while floating in microgravity was undoubtedly very different to that while seated comfortably at a desk on Earth. For that reason, performance comparisons within the mission, are probably more justified than those comparing in-flight to ground. Here however, there was no compelling case for a build up in performance decrements as indicated by late flight versus early flight comparisons. Indeed (probably due to practice effects), performance appeared to be, if anything, better late flight than early flight. This appeared to part of a general trend, observable in other measures such as sleep diaries, end of shift questionnaires, polysomnography and nocturnal actigraph counts, which all failed to show increasing disruptions in either sleep or daytime performance as the flight progressed. Indeed, the only measure to show an early flight superiority was subjective alertness (global vigor) where the effect may have been simply due to an elevation of early flight readings above baseline levels.

The end-of-shift questionnaire data was useful in giving an insight into some of the features of space flight that might lead to a difficult workday in space. In the present mission, only one of our subjects reported experiencing space motion sickness, and that for only one day (the first in our early flight measurement block). Thus, the main challenges appeared to be those related to equipment malfunction and time-line (schedule) over-runs which are perennial problems in manned space flight. Having made that point, however, it would appear that the careful timeline planning constraints that are in place to protect the astronauts ("Appendix K") did their job in ensuring that excessive fatigue did not build up,

even towards the end of the mission.

With regard to sleep, it is clear that when scheduling is careful to avoid circadian disruptions, sleep in space need not be a major problem. This confirms the early findings of Frost et al. from the Skylab missions in the 1970s. What is clear from the present findings, however, is that without doubt, sleep is shorter and shallower in space than it is on the ground. As Table 1 reveals, actual sleep durations in our study averaged 5.9h early flight 6.3h late flight, compared with 7.0h post-flight (pre-flight sleeps were too close to the mission (L-7d) to be undisturbed). Our overall in-flight average figure of 6.1h of total sleep obtained (Stages 1-4 plus REM) is very close to the figure of 6.03h reported by Santy from retrospective reports. Using PSG on selected nights, Frost reported a figure of 6.00h and 6.30h for the 28d and 59 day Skylab studies, and 6.69h for the 84 day mission, although the figure fell to 5.87h when only the first 19 days of the 84 day mission were considered. While this figure may be acceptable for certain individuals, and for missions lasting less than three weeks, it would certainly seem to be problematical if much longer missions, involving a manned space station or a trip to Mars are involved, which would require people to live in space for months or years at a time.

When considerations of sleep depth are added, the effect becomes much more profound. All four of our subjects showed a quite dramatic diminution in delta sleep, as measured either by hand scored or automated techniques (Table 2, Figures 7 - 10). Since delta sleep represents the stages of sleep considered to be the deepest and most restorative (Horne, 1988), this flight-related effect is clearly of great concern. Since the circadian disruption of the flight was shown to be minimal, we must conclude that the delta sleep effect is not a function of the circadian system, but, rather an effect of the microgravity per se. Future experiments should focus upon this effect, which in many ways mimics the decline in delta sleep observed in advancing age (Reynolds, et al., 1991).

The lack of inappropriate phasing of the astronauts' circadian timekeeping systems was confirmed by the normal distribution of REM sleep over the night, and by the fairly normal REM latencies (52 minutes) shown in space relative to the same subjects' REM latencies on the ground (59 minutes). Again, these findings did not appear to differ when early flight and late flight measurement blocks were compared.

Although the sleep diary findings broadly confirmed the objective PSG data with regard to sleep timing and WASO, it failed to reflect the decrease in sleep depth evidenced by the delta sleep disruptions. Indeed, there was no evidence of a general decrease of subjective sleep quality when in-flight sleeps were compared with ground baselines, or when early flight and late flight sleeps were compared. The sleep diary was useful in providing insights into what factors typically disrupt sleep in space. The diary questions were developed in consultation with the astronauts, whose colleagues with flight experience interacted with them, thus rendering the questions appropriate. Again, one should note the caveats that this was a single shift mission specifically designed to minimize circadian disruptions, but it appeared that neither sleep medications (hypnotics) nor space adaptation sickness medications were used in flight, thus avoiding this potential confound. The main sources of sleep disruption appeared to be ambient temperature and noise.

CONCLUSIONS

When careful steps are taken to ensure that the astronauts' work/rest schedule does not lead to circadian desynchrony, there is no evidence that microgravity per se will disrupt the human circadian timekeeping system, or that such rhythms will degrade over a 17d mission. In particular, there was no evidence of amplitude reduction, phase lability or "free-running" behavior. Despite the astronauts' circadian rhythms being intact, there were some effects on sleep, notably a reduction in sleep duration, and a suppression of the deepest stages of sleep (Stages 3 and 4). However, there were no other

consistent effects of flight in sleep architecture. The only systematic effect on mood and performance appeared to be an increase in alertness in the early flight measurement block. End of shift questionnaires revealed timeline problems and equipment malfunction to be the major impacts on the astronauts' work.

PAO SUMMARY

The aims of this experiment are to find out how the human biological clock reacts to weightlessness and the removal of earth-based time cues such as sunrise and sunset, and how sleep and daytime mood and performance are affected by these changes. Four astronauts recorded daily rhythms in their body temperature, wrist movements, and urinary constituents, recorded their sleep by electrodes placed on their head, and completed computer-based diaries and tests of mood, activation and performance. These measurements were all taken in four 72-hour measurement blocks, one before and one well after the mission, and two during the space flight itself (one towards the beginning, and one towards the end). In order to get a relatively "pure" idea of what effects were due directly to space flight, rather than to changes in routine, the space shuttle mission was especially designed to take off during daylight, to avoid shift work, and to minimize the changes in work/rest schedule required by the flight.

When we plotted the daily ("circadian") rhythms collected by the astronauts in variables such as body temperature, we found the rhythms in orbit to be very similar to the ones we measured from the astronauts when on the ground. Also, there was no change in rhythms collected early on in the flight, as compared with those collected towards the end of the flight. In all cases, the rhythms indicated that the biological clock was successfully doing its job of preparing the body for sleep at "night" and wakefulness during the "day". This success was also reflected in mood, activation and performance scores, which showed no deterioration during the flight. However, there were some reliable changes in the sleep of the astronauts. Although the patterning or "architecture" of the various stages and depths of sleep was normal in space, there was less of the deepest stages of sleep (called "delta" sleep). Also, the total amount of sleep obtained (an average of 5.9 hours early in the flight, 6.3 hours later on in the flight) was below what is considered to be adequate (and below the average of 7 hours obtained well after the flight was over). The results of this experiment are important in telling us how we should schedule work and rest in future space missions, and in identifying a sleep problem that must be solved before we embark on long duration missions associated with Space Station and Manned Mars Missions.

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PUBLICATIONS RESULTING FROM CONTRACT

There are currently no papers resulting directly from the LMS flight. However, the following manuscript is under review:

Monk, T.H. Buysse, D.J. Billy, B.D. Kennedy, K.S. & Kupfer, D.J.(under review). The effects on human sleep and circadian rhythms of 17 days of continuous bedrest in the absence of daylight. *Sleep*.

In addition the following list of papers cite support from the NAS 9 - 18404 contract:

Alward, R.R. & Monk, T.H. (1993). *The nurses shiftwork handbook*. Washington, D.C.: American Nurses Publishing.

Carrier, J. And Monk, T.H. (1997) Estimating the endogenous circadian temperature rhythm without keeping people awake. *Journal of Biological Rhythms*, 12, 266-277.

Monk, T.H.(ed.) (1991). *Sleep, sleepiness and performance*. Chichester and New York: John Wiley & Sons Ltd.

Monk, T.H. & Carrier, J. (In press). Speed of mental processing in the middle of the night. *Sleep*, 20.

Monk, T.H., Buysse, D.J., Reynolds, C.F.III, Kupfer, D.J. & Houck, P.R. (1995) Circadian temperature rhythms of older people. *Experimental Gerontology*, 30(5), 455-474.

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Monk, T.H., Reynolds, C.F.III, Kupfer, D.J., Buysse, D.J., Coble, P.A., Hayes, A.J., Machen, M.A., Petrie, S.R. & Ritenour, A.M. (1994). The Pittsburgh Sleep Diary (PsgSD). *Journal of Sleep Research*, 3, 111-120.

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TABLE 1
Results of sinusoidal analyses of body temperature data

		Pre-flight	Early flight	Late flight	Post-flight
Amplitude	S1	0.68	0.47	0.51	0.56
	S2	0.61	0.56	0.71	0.58
	S3	N/A	0.31	0.23	0.54
	S4	0.42	0.39	0.44	0.39
	Mean	0.57	0.43	0.47	0.52
Phase	S1	1.10	3.00	2.10	1.00
	S2	2.70	3.00	3.20	3.60
	S3	N/A	3.70	2.30	2.30
	S4	3.10	2.80	3.10	3.20
	Mean	2.30	3.13	2.68	2.53
Mean Level EC	S1	36.84	36.80	36.91	36.95
	S2	37.03	36.89	36.97	36.99
	S3	N/A	36.74	36.73	36.89
	S4	36.81	36.51	36.54	36.90
	Mean	36.83	36.73	36.79	36.93
Percent Rhythm	S1	84.42	67.60	82.62	68.39
	S2	67.45	81.11	84.69	77.63
	S3	N/A	75.36	57.96	79.55
	S4	73.31	71.51	80.29	56.13
	Mean	75.06	73.89	76.39	70.42

TABLE 2
Results from PSG sleep recording.
All data are in minutes except Average delta counts per minute

		Baseline	Early flight	Late flight
Time Spent Asleep	S1	364	396	390
	S2	411	345	366
	S3	312	322	-
	S4	480	347	369
	Mean	392	353	375
Delta	S1	49	11	2
	S2	25	6	19
	S3	64	42	-
	S4	8	0	0
	Mean	37	15	7
REM	S1	68	103	110
	S2	144	97	81
	S3	70	63	-
	S4	118	99	81
	Mean	100	91	91
REM Latency	S1	61	40	50
	S2	40	37	40
	S3	76	70	-
	S4	57	53	59
	Mean	59	50	50
Average Delta Counts	S1	15.30	9.73	11.23

S2	15.90	15.92	13.15
S3	26.67	15.63	—
S4	9.20	3.18	3.01
Mean	16.77	11.12	9.13

TABLE 3
Sleep Diary Results

		Baseline	Early flight	Late flight
Estimated Time Asleep	S1	293	385	352
	S2	427	402	400
	S3	438	395	425
	S4	497	368	372
	Mean	414	388	387
Estimated Sleep Latency	S1	32	22	15
	S2	2	5	6
	S3	13	7	12
	S4	12	12	5
	Mean	15	12	10
Estimated WASO	S1	37	20	12
	S2	3	3	5
	S3	7	8	10
	S4	0	0	0
	Mean	12	8	7
Sleep Quality	S1	33	56	56
	S2	89	90	89
	S3	68	79	69
	S4	86	95	89
	Mean	69	80	76
Alert	S1	46	42	48
	S2	73	88	66
	S3	48	48	30
	S4	25	41	16

	Mean	48	55	40
Mood	S1	58	49	85
	S2	87	90	61
	S3	73	79	72
	S4	91	92	94
	Mean	77	78	78

FIGURE CAPTIONS

Figure 1

Mean core body temperatures (n=4) expressed as deviation from each subject's 72h mean. Mean +/- one s.e.m. is plotted as a function of time into 72h measurement block for each of the four measurement blocks. Pre-flight and post-flight measurement blocks started in the evening, in-flight measurement blocks in the subjective "morning".

Figure 2

Mean core body temperature (n=4) expressed as a deviation from each subject's 72h mean and plotted as a function of time. Upper panel: pre-flight baseline versus early flight, with the abscissa changed to line up to subjective "morning" (see text). Lower panel: early flight versus late flight measurement blocks, with both plotted simply as a function of hours into measurement block.

Figure 3

Core body temperature plotted as a 24h curve averaged across the three cycles comprising each measurement block. In all cases the abscissa is expressed as time from wake time on day 1 of the measurement block, and the ordinate uses the same size of scale for temperature.

Figure 4

Urinary water volume (mls per minute) regarding the bladder as an integrator. The data are plotted as a 24h curve averaged across the three cycles comprising each measurement block separately for each subject in each measurement block. In all cases the abscissa is expressed as time from wake time on day 1 of the measurement block, and the ordinate uses the same size of scale for volume.

Figure 5

Urinary melatonin sulphate excretion (ng per minute) regarding the bladder as an integrator. The data are plotted as a 24h curve averaged across the three cycles comprising each measurement block separately for each subject in each measurement block. In all cases the abscissa is expressed as time from wake time on day 1 of the measurement block, and the ordinate uses the same size of scale for melatonin sulphate.

Figure 6

Urinary free cortisol excretion (ug per minute) regarding the bladder as an integrator. The data are plotted as a 24h curve averaged across the three cycles comprising each measurement block separately for each subject in each measurement block. In all cases the abscissa is expressed as time from wake time on day 1 of the measurement block, and the ordinate uses the same size of scale for free cortisol.

Figure 7

Astronaut S1: Sleep architecture from baseline (post-flight), early flight and late flight measurement blocks. Plotted is the average of nights 2 and 3.

Figure 8

Astronaut S2: Sleep architecture from baseline (post-flight), early flight and late flight measurement blocks. Plotted is the average of nights 2 and 3.

Figure 9

Astronaut S3: Sleep architecture from baseline (pre-flight) and early flight measurement blocks (technical problems led to the loss of late flight and post-flight data). Plotted is the average of nights 2 and 3.

Figure 10

Astronaut S4: Sleep architecture from baseline (post-flight), early flight and late flight measurement blocks. Plotted is the average of nights 2 and 3.

Figure 1

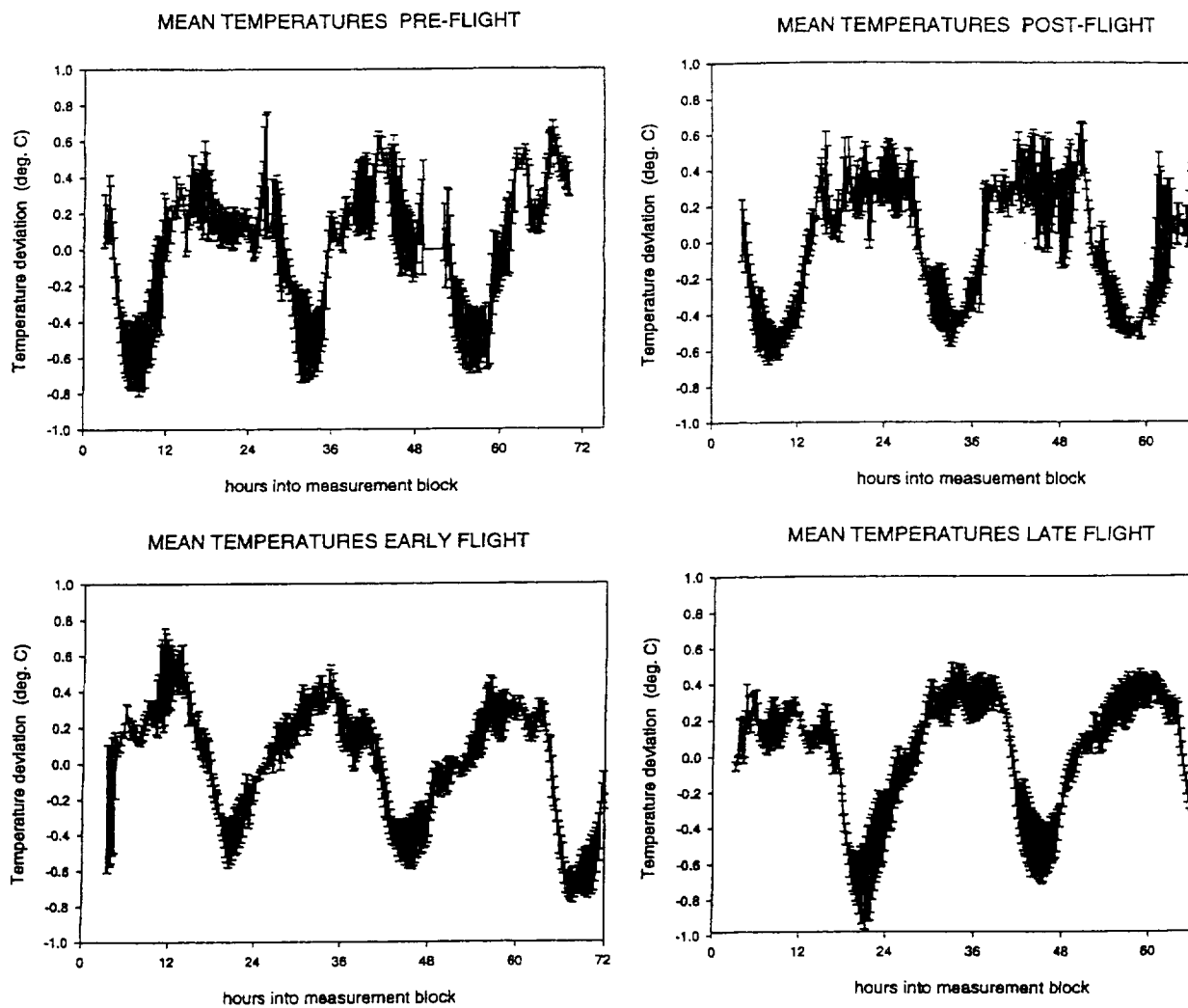
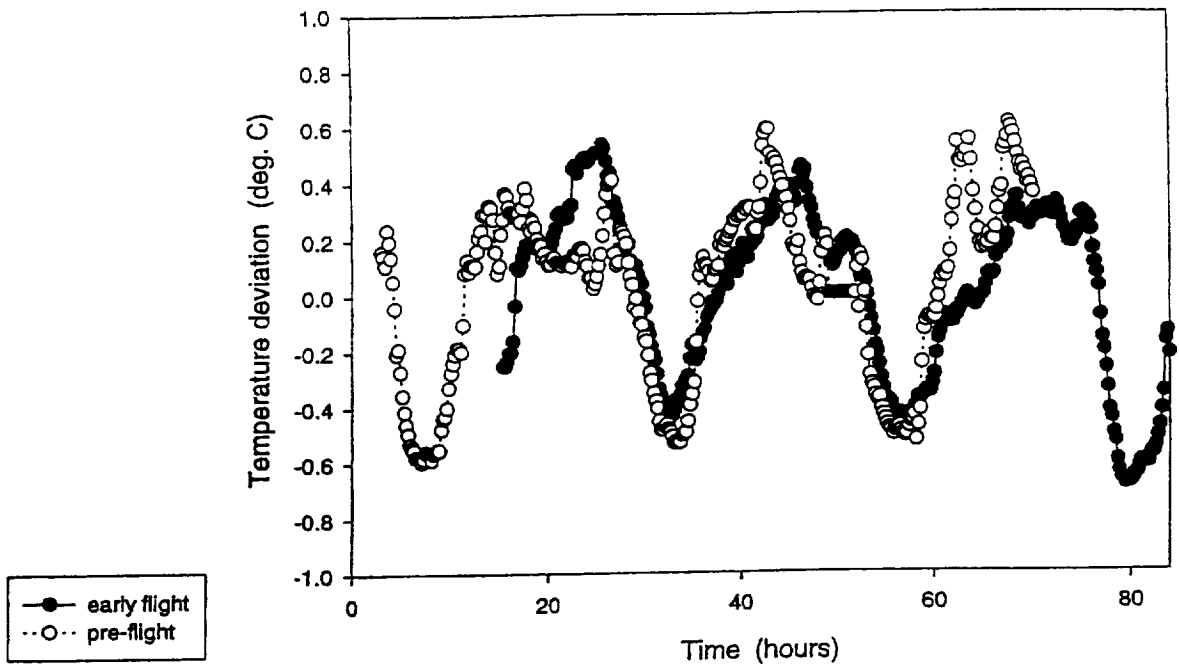


Figure 2

Temperature - pre-flight vs. early flight



Temperature - early flight vs. late flight

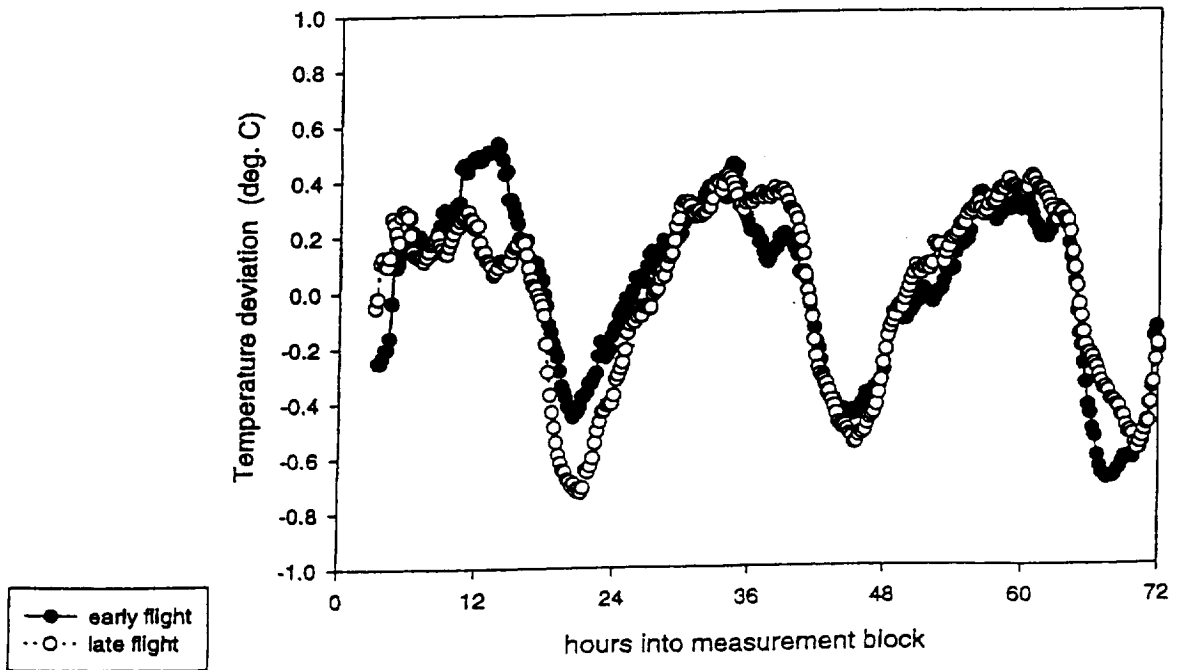


Figure 3

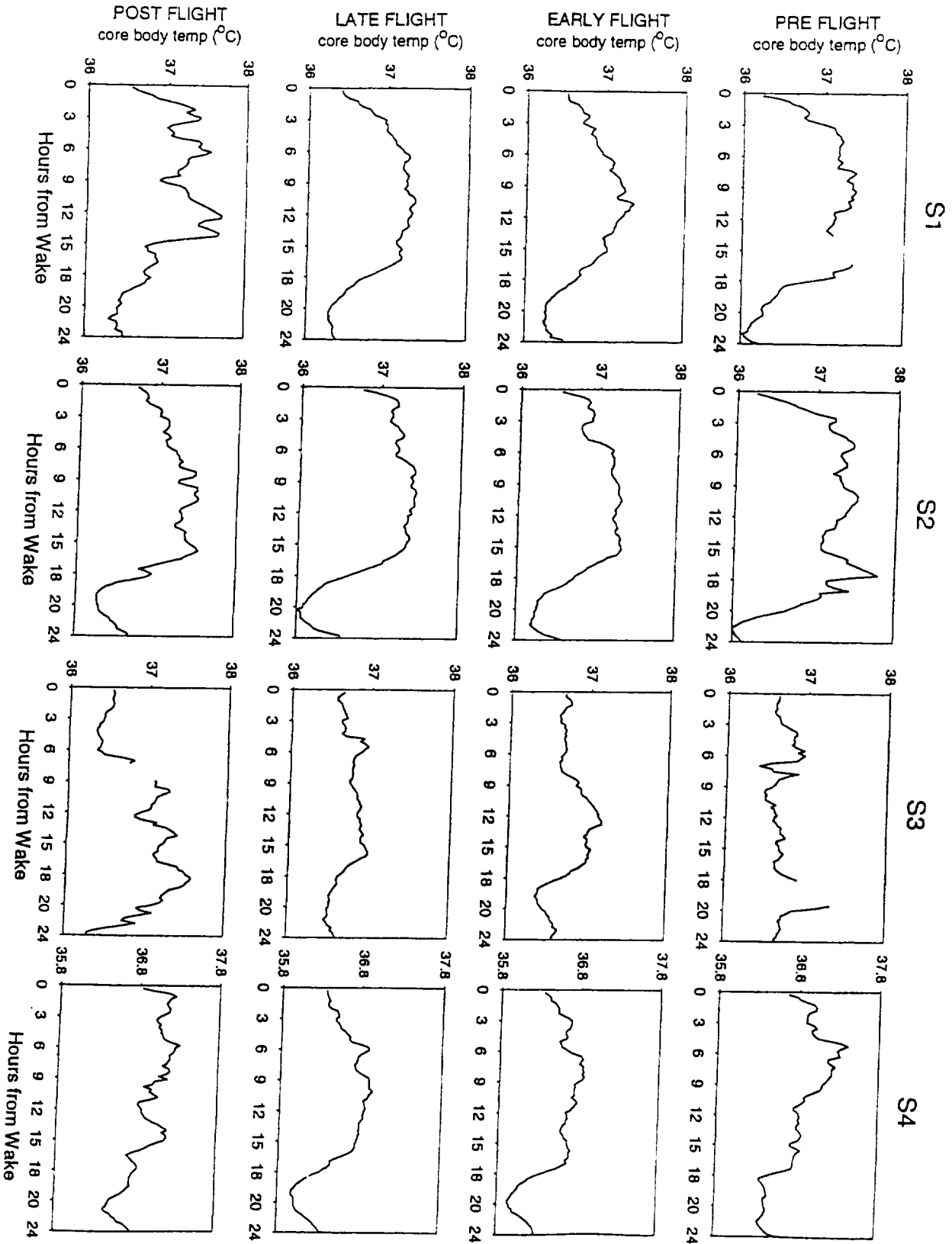


Figure 4

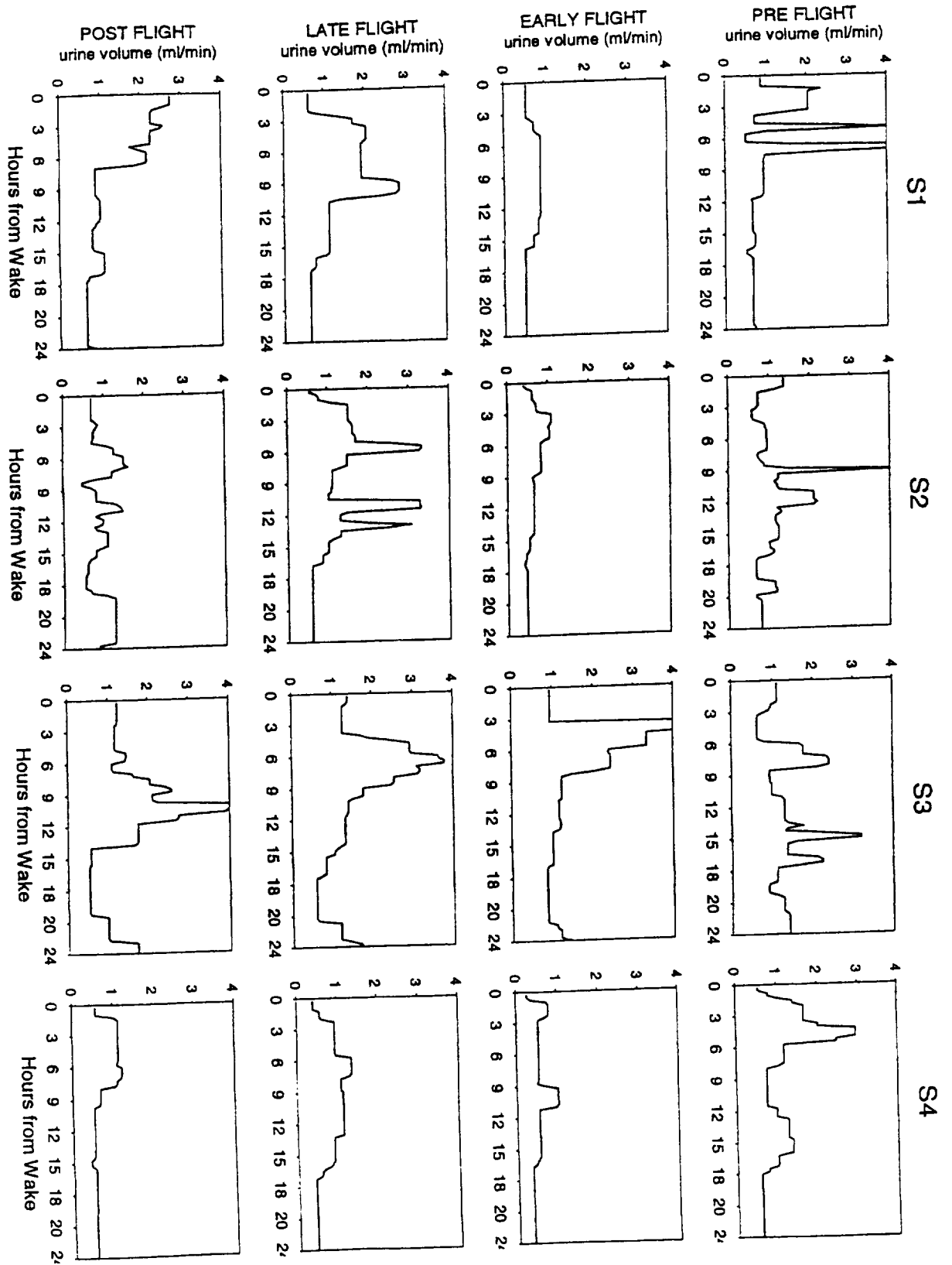


Figure 5

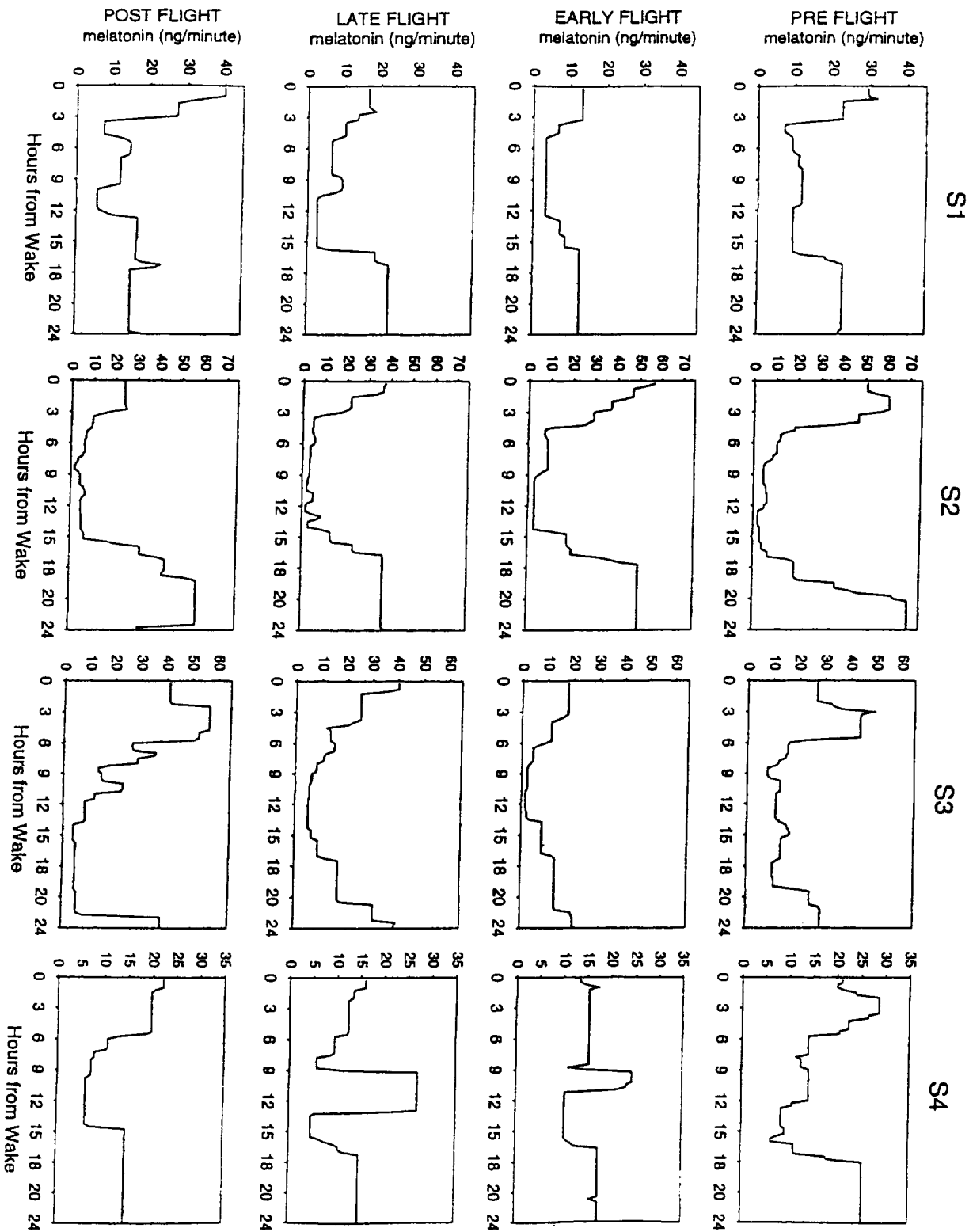


Figure 6

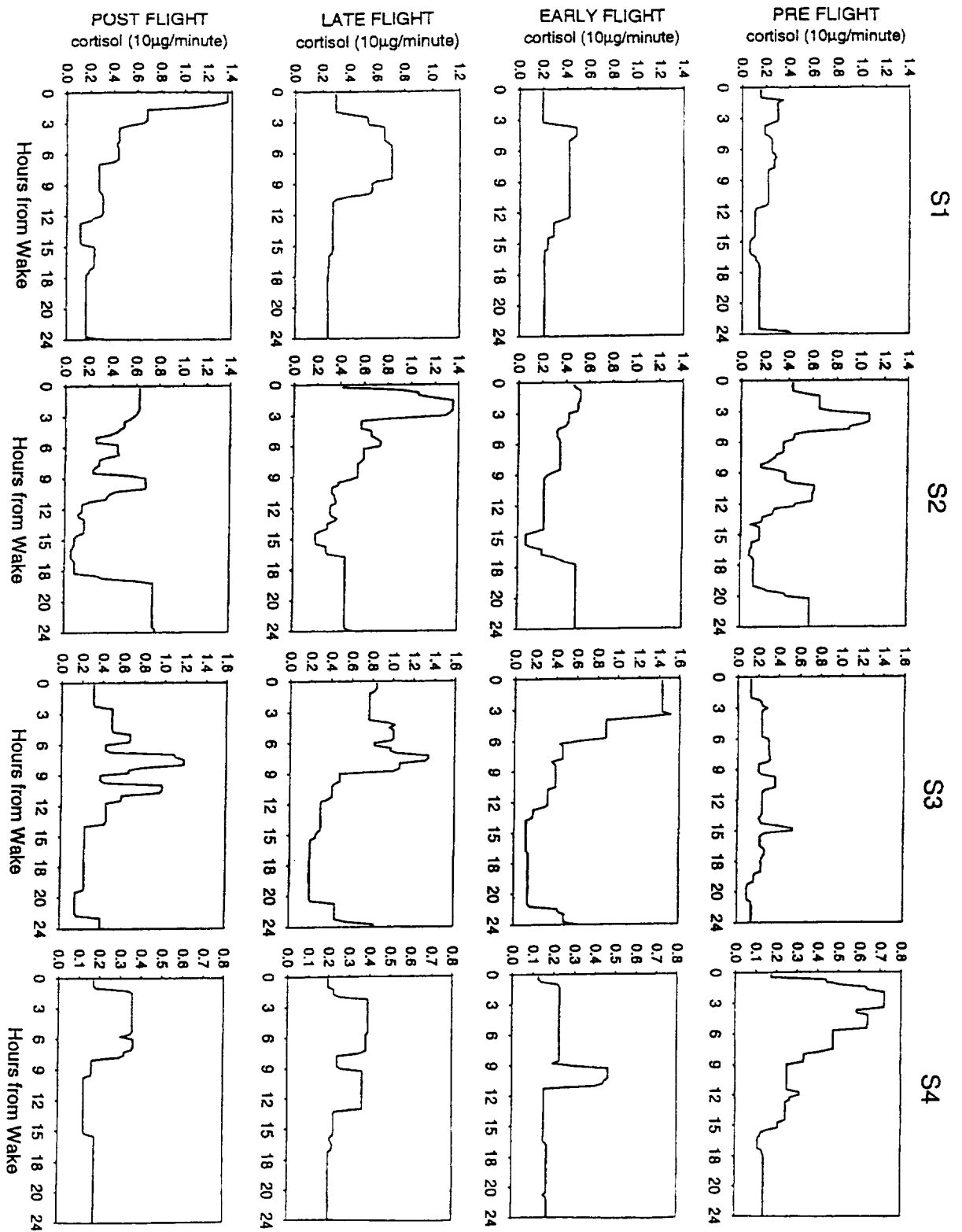
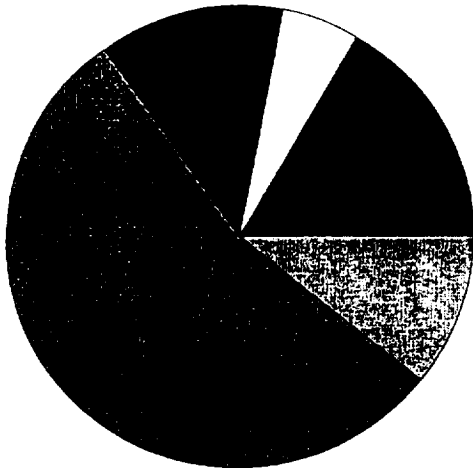
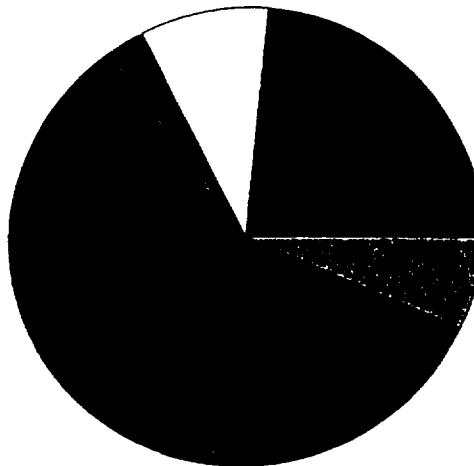


Figure 7

ASTRONAUT S1 BASELINE



ASTRONAUT S1 EARLY FLIGHT



ASTRONAUT S1 LATE FLIGHT

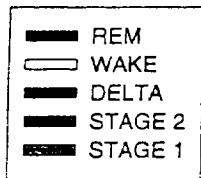
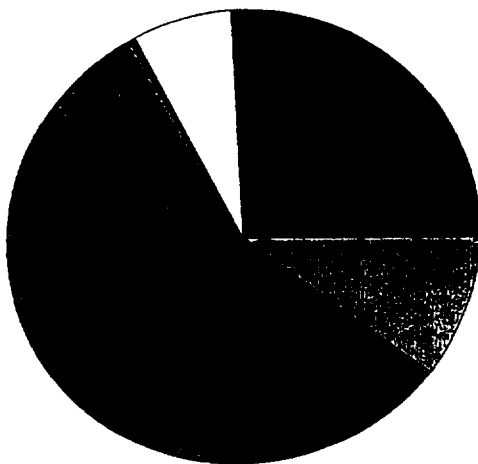
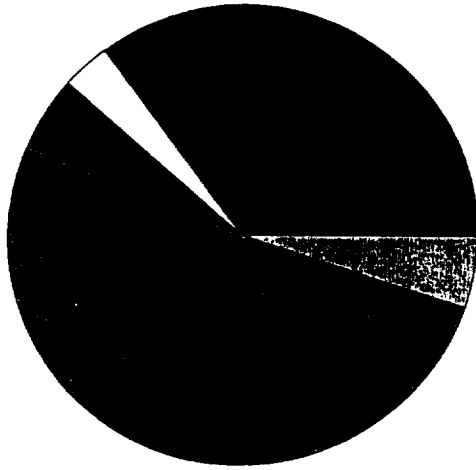
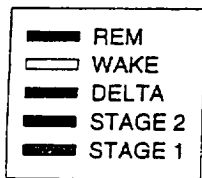
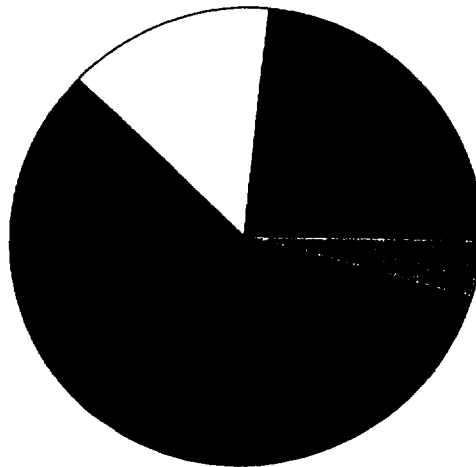


Figure 8

ASTRONAUT S2 BASELINE



ASTRONAUT S2 EARLY FLIGHT



ASTRONAUT S2 LATE FLIGHT

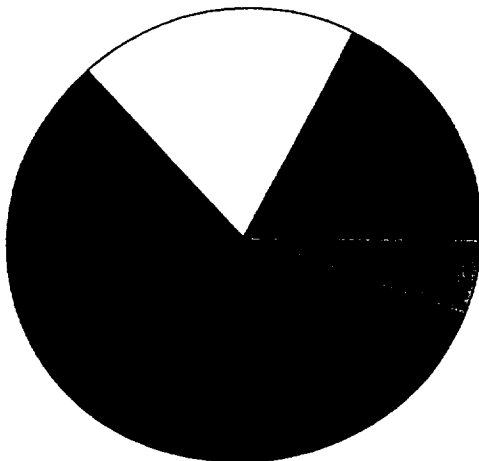
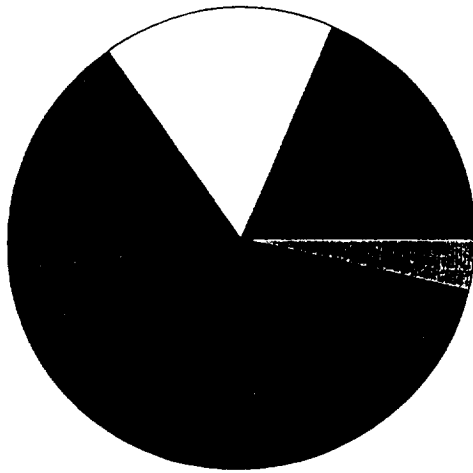


Figure 9

ASTRONAUT S3 BASELINE *



ASTRONAUT S3 EARLY FLIGHT

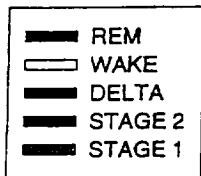
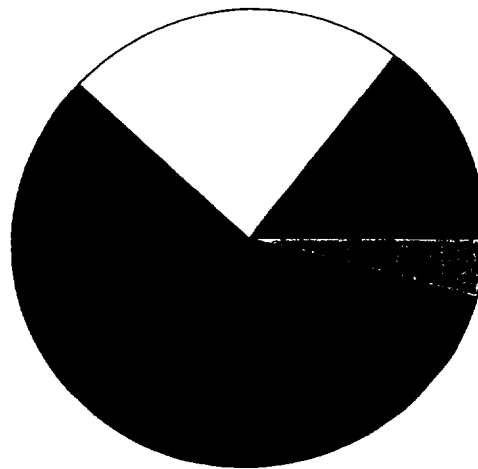
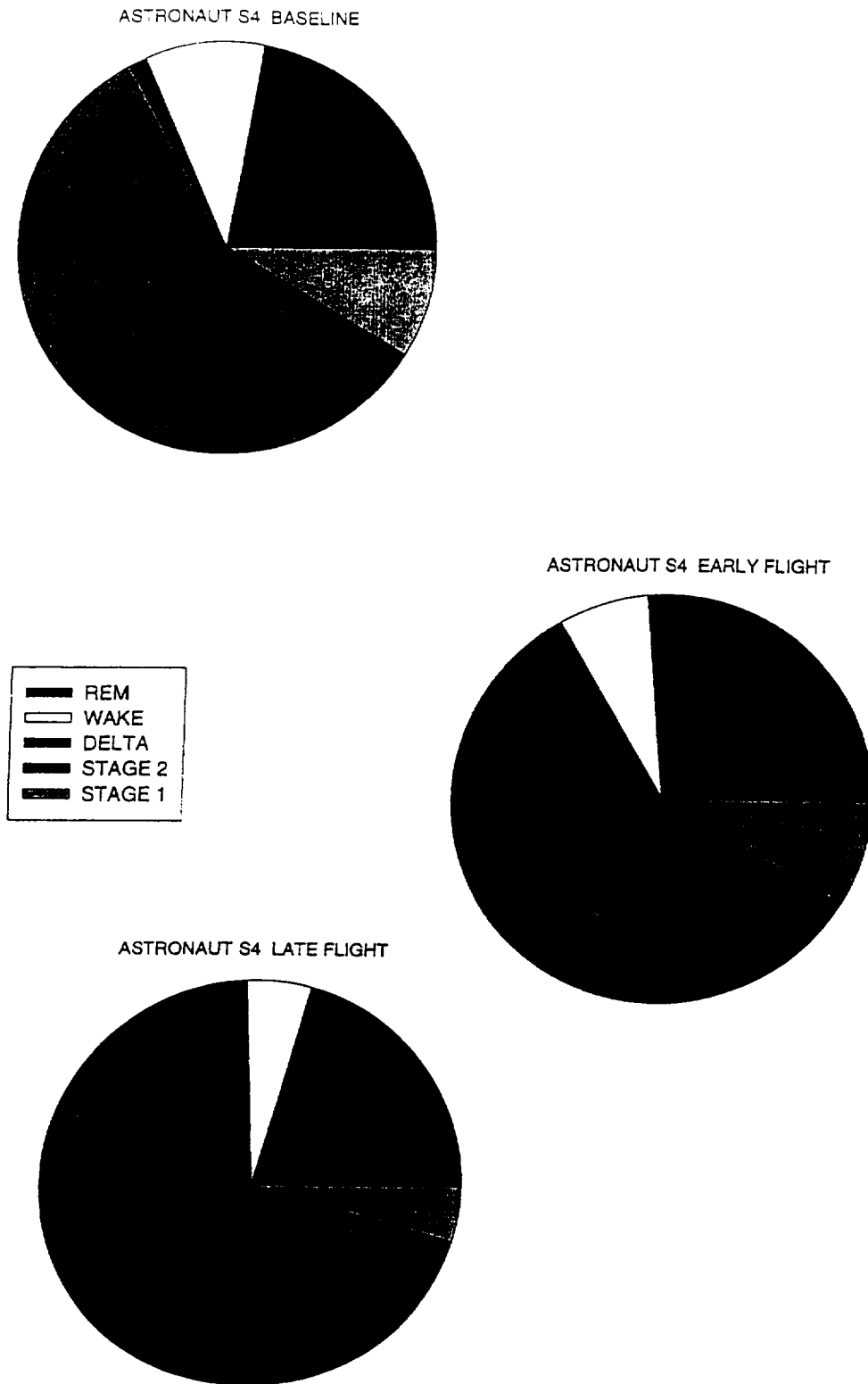


Figure 10



JSC Human Life Sciences Project

E963 - Microgravity Effects on Standardized Cognitive
Performance Measures

Principal Investigator:

Dr. Sam Schiflett
USAF Armstrong Lab
Brooks Air Force Base, Texas

LMS-PAWS

Microgravity Effects on Standardized Cognitive Performance Measures

Principal Investigator:
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Co-Investigators:
Douglas Eddy, NTI, Incorporated
Robert E. Schlegel, University of Oklahoma
Randa Shehab, University of Oklahoma

OBJECTIVE

The objective of this experiment was to determine the effects of microgravity upon cognitive skills critical to the success of operational tasks in short-term space flight.

BACKGROUND

Previous In-orbit Research

Astronauts are subject to a variety of stresses during space flight. These stresses include microgravity, physical isolation, confinement, lack of privacy, fatigue, and changing work/rest cycles [1]. Any one or a combination of these stressors could degrade the cognitive skills required to perform tasks essential to the success of a mission. Of these potential stressors, fatigue and changing work/rest cycles are known to cause deterioration in astronaut productivity [2]. However, over the past 20 years, only limited attempts have been made to systematically determine the effects of space flight on cognitive skills [3, 4, 5, 6].

In a previous cooperative USAF/NASA experiment [7,8], the Performance Assessment Workstation (PAWS) was flown as part of the payload for the Second International Microgravity Laboratory (IML-2) on-board the Space Shuttle Columbia (STS-65) in July 1994. The experiment studied the interactive effects of microgravity and fatigue on cognitive functioning of three astronauts for 13 days on a dual-shift mission. The same PAWS battery of performance tests used on the IML-2 flight was reflown on LMS. The tests measured short-term memory, spatial processing, attention, tracking, and dual task timesharing. All three astronauts completed 40, 20-minute sessions of the PAWS battery containing 6 cognitive performance tests and 2 subjective scales (mood and fatigue) on a laptop computer. Twenty-four sessions were preflight, 13 sessions were in-orbit, and 3 sessions were postflight.

In general, performance patterns of the astronauts in-orbit and during ground-based periods were comparable after learning of the task had stabilized, with the exception of Continuous Recognition and Attention Switching-Manikin. One subject showed an improvement in both tasks and the other two subjects each had a decrement in rate of responding (time/item) and reaction time for a single task. The remaining decrements in performance found in-orbit compared to ground-based learning predictions of modeled performance occurred in only one subject for Attention Switching-Mathematical Processing, Unstable Tracking (λ) and Dual Tracking (control losses). The results showing deviations from single-subject predicted performance levels occurred mainly during the first and last few days in space related to adaptation and accumulative fatigue, respectively. These trends of early and late performance decrements, especially in Tracking Tasks, are similar to the findings in Manzey, *et al.* [5].

Even if one considers the converging evidence from all of the Shuttle and MIR in-flight experiments reviewed, the currently available database about human performance in space is still too small to warrant final conclusions about cognition and visuomotor performance while living and working in microgravity. Also, isolation of microgravity as the single stressor causing in-orbit performance deterioration cannot be fully determined from the results as reported to date. Therefore, additional ground-based control research studies

and more in-orbit subjects are required before these results can be generalized to future space travelers. This LMS mission was flown to add to the performance database and gain additional insights into the cognitive functioning of astronauts in space.

Performance Test Selection Criteria

The Performance Assessment Workstation was assembled from tests contained within the Unified Tri-Service Cognitive Performance Assessment Battery (UTC-PAB) [9]. Upon examination of past payload and mission specialists' tasks and insights gained at NASA workshops held at Johnson Space Center on Human Factors requirements, several functional task areas potentially affected by microgravity were identified. Of these, spatial information-processing, fine motor control, directed attention, and time-sharing play a disproportionately important role in the success of a variety of Spacelab and Space Station tasks. An additional test selection criterion was the information provided by a specific test should aid in identifying the locus of the performance decrement potentially affected by living and working in microgravity.

METHODS

Data Acquisition

Subjects. The four male astronaut subjects were members of the seven person crew on Columbia Space Shuttle flight STS-78. Two of the astronauts had previous flight experience. Subject anonymity in data collection, analysis, and reporting of results was protected by randomly assigning subject numbers to the four astronauts. During the recovery phase all debriefing sessions of results were done on an individual basis.

Equipment. The Performance Assessment Workstation consists of a NASA modified IBM ThinkPad 755C laptop computer with a color display and a NASA 2" trackball (MSI Model 622).

Description of Tests and Subjective Scales. The performance tests are described in the order of presentation to the subjects. Unless otherwise noted the dependent measures for each test included: the percentage of correct responses, the mean correct reaction-time, and throughput. The duration of each test was three minutes unless stated otherwise. More detailed descriptions of all the PAWS tests and subjective scales as related to the ground-based reference group training schedules, test reliability and differential stability can be found in Schlegel, Shehab, Gilliland, Eddy, and Schiflett[10].

Visual Analogue Mood Scale. This scale samples the subject's mood using eight descriptors: alert, sad, tense, level of effort, happy, weary, calm, and sleepy. The subject indicates on a horizontal line how much each mood descriptor applies to him/her by placing a cursor between end points labeled "very little" and "very much" [11].

Unstable Tracking. This test, requires the subject to maintain a target (vertical line) in the center between two vertical lines on the left and right of the screen. A forcing function of increasing difficulty (λ level) is used to displace the target. The subject must manipulate a control device, trackball, to null this input disturbance. The dependent measure was the mean of the difficulty level (λ) the subject was capable of achieving before each control loss during a two-minute session.

Matrix Rotation. The Matrix test developed by Damos and Lyall [12] was used to assess spatial processing. This 90 second test displays a series of patterns. Each pattern is a 5 by 5 matrix with five illuminated cells that have been selected at random. The subject indicates whether successive matrices are similar or different with key responses. For "same" responses, the two patterns are never presented in exactly the same orientation; the second pattern is always rotated either 90 degrees clockwise or counter clockwise relative to the preceding pattern.

Sternberg Memory Search. The short-term memory test paradigm requires subjects to recognize visually presented letters and respond as rapidly and accurately as possible. At the beginning of the test, a set of four letters drawn randomly from a restricted alphabet is presented to the subject for memorization. Individual letters are presented next. If the presented letter matches one of the letters in the previously memorized set, the subject responds with a key press indicating "same" otherwise "different."

Continuous Recognition. In this working memory test, the subject is presented with two numbers, one above the other. The first task is to remember the bottom number. When the next two numbers appear, the subject determines if the new top number is the same as the previous bottom number. However, before responding, one must note the new bottom number because as soon as a response is made, the numbers are replaced by a new pair.

Directed Attention--Manikin and Mathematical Processing. In this four minute test, the subject has two distinct and discrete tasks to perform. One task is spatially-based and the other is mathematically-based. Both appear side-by-side in every display. An indicator shows which task is "active" (i.e., must be responded to) and each task has separate response keys. The switching from task to task is random (within constraints). The first key press for either task is taken as the response. Reaction times after a switch are recorded separately. The spatial processing task, manikin, appears on the left, the mathematical processing task is on the right of the display. A manikin "stick figure" is presented facing either forward or backward, upright or upside-down. The figure stands on a box and inside the box is either a rectangle or a circle symbol. Each of the figure's hands contains one of the symbols. The subject's task is to indicate which hand, left or right, holds the matching symbol. The mathematical processing task presents three single-digit numbers that must be added or subtracted. The subject indicates if the answer is greater or less than five with different response keys.

Dual Task. This test combines the Unstable Tracking and Sternberg Memory Search tests into a single time sharing task. In this implementation, the tracking task is presented in the center of the screen and the letters of the memory search task appear in a fixed location directly above the center null point. The tracking task was individually adapted after the first nine training trials and presented in the subcritical mode, with a "fixed lambda" empirically determined using the results of the single Unstable Tracking test. The dependent measure for tracking was the number of control losses during the trial.

Fatigue Scale. The subject keys a number from one to seven indicating which of seven statements best describes his momentary fatigue state. The statements range from fully alert (1) to completely exhausted (7).

Experimental Procedure. An orientation session provided subjects with background information about the experiment and a demonstration of the PAWS approximately one year prior to launch. Data were collected from each subject over a period of approximately two months. All four astronauts took the tests on roughly the same schedule. They completed 37 sessions that consisted of 24 sessions preflight (8 training sessions over 2 days and 16 practice sessions over approximately 3 weeks), 9 sessions approximately every other day in-orbit, and 4 sessions postflight every other day. After each test battery was completed the subjects wrote down or voiced into a micro-cassette recorder a data summary display containing 11 measures.

With the exception of the Dual Task, all task parameters remained constant throughout the experiment. Initially, the tracking component of the Dual Task was fixed at a constant lambda (difficulty level) of 2.0 for all subjects. However, the lambda value was individualized for each subject at a specific point during practice in order to equate task difficulty among subjects with different tracking abilities. The final lambda value for each subject was set at 70% of the mean of the maximum lambda values for the Unstable Tracking test on training Trials 7 and 8. The first exposure to the individualized fixed lambda level for each subject was at Trial 10. After being individualized, the lambda value remained constant for the Dual Task for the remaining practice, in-orbit, and recovery periods.

Analysis

Approach. Hypothesis testing is usually performed with standard statistical tests that require 8 to 12 subjects per group or test condition. On the LMS mission, normal inferential statistics for repeated measures would be woefully under powered to find significant effects with only four subjects. Therefore, single-subject analyses were used and the findings are only applicable to the actual subjects tested. The approach used with these data was a variation on the one presented at the 66th Annual Meeting of the Aerospace Medical Association [7] in conjunction with the results of IML-2.

Previous research by Newell and Rosenbloom enables the development of mathematical models of learning for each subject's performance data [13]. Once the "best" model is identified, appropriate transforms can be used to linearize both the model and data thus allowing standard regression predictions with known confidence limits. The predictions of the learning equations serve as "the baseline" for comparing the in-orbit data. This "baseline" contains the expected improvement (learning) that occurs with each administration.

Modeling Procedure. Three families of mathematical equations were fit to each subject's preflight data. They were power, hyperbolic, and exponential. These nonlinear equations have been shown to provide good fits to cognitive performance measures in controlled environments [13]. The following PAWS data measures were selected for modeling from each subject: Mean Lambda (1), Throughput (5), Mean Correct Reaction Time (RT) (5), Switching Time (2), and Control Losses (1). The number in parentheses is the number of measures of that type. Only the training and practice data (Trials 1 through 24) were used to determine the models of learning. The generalized models and their equations follow:

Power	$Y = A + B(X+E)^C$	(1)
Hyperbolic	$Y = A + B/(X+E)$	(2)
Exponential	$Y = A + Be^{CX}$	(3)

Y is the dependent measure of interest, for example, the number of tracking control losses. X is the trial or session number. A is the asymptote for the curve. E is the number of sessions that were theoretically missed before data collection commenced. B and C are parameters related to the rate of learning or growth of the function. The A and E values are occasionally zero giving the simpler forms of the equations. Each subject was allowed to have their own model family for each dependent measure. A common best model was not selected for all subjects. All data manipulation and statistical procedures were accomplished with the SAS Statistical Package, Version 6.1.

To make predictions from the models and estimate the confidence interval for their predictions, the data and estimates had to be linearized. This was accomplished with a log/log transform in the case of the power and hyperbolic functions and a log transform in the case of the exponential function. A linear regression was performed on the linearized data (session number and dependent measure). The statistic was the difference between the means of the predicted points and the mean of the actual in-orbit data values divided by the standard deviation for the regression of the baseline values. This approach tests for a shift in the mean of the in-orbit data from the mean of the data predicted by the model.

The approach was validated on the data of 96 ground-based subjects [10] who should not have had any "in-orbit" effect. The statistical test gave from three to eight significant effects out of the 96 subjects with alpha set at 0.05. Most dependent measures were best fit by a mixture of models: 40-50% exponential, 30-50% power, and 10-15% hyperbolic. In summary, the learning models allowed the assessment of the in-orbit effects while removing the expected, small effects of continued performance improvement with each testing session.

FLIGHT RESULTS COMPARED WITH GROUND RESULTS

Single Subject Modeling Analyses

The results of modeling each astronaut's baseline data are shown in the table. Each test is listed with its measures. The mean R^2 is listed summarizing the model fits for all subjects.

LMS Statistical Results by Task

Task/Dep. Measure	Mean R^2	Significant* In-orbit Effects
Tracking		
Mean Lambda	0.73	1 ^a
Matrix		
Time/Item	0.78	0
Mean RT	0.80	1
Memory Search		
Time/Item	0.71	0
Mean RT	0.79	0
Continuous Recognition		
Time/Item	0.87	0
Mean RT	0.88	0
Directed Attention - Manikin		
Time/Item	0.92	0
Mean RT	0.84	0
Switching time	0.88	0
Directed Attention - Math. Processing		
Time/Item	0.85	1
Mean RT	0.81	2
Switching time	0.62	2
Dual		
Control Losses	0.32	0

* $p < 0.05$

^a performance exceeded model predictions

Comparison of In-orbit Performance with Learning Model Projections

The table summarizes the statistically significant in-orbit effects for each dependent measure. The number in the last column indicates how many subjects of the four showed a significant effect ($p < 0.05$). Using this single-subject, modeling approach, the performance measures of most tests were not significantly affected in-orbit. No subject's mean in-orbit performance showed a significant deviation from model projections for the following tests: Memory Search, Continuous Recognition, Manikin, and Dual. Each test showing a significant effect is discussed and graphically illustrated with the subject's data in the following subsections. All the models were based on the first 24 trials and projected through the in-orbit and postflight trials. All significant effects were confined to the same two subjects designated as one (1) and two (2).

Critical Tracking Test. The most unexpected in-orbit effect was found for the Tracking test. Based on a generalized exponential model of preflight performance, Subject 1 showed improved in-orbit performance, $t(4)$

= -2.99, $p = .041$. The dependent variable was Lambda, the difficulty level of the unstable element that the subject was able to tolerate before losing control. Figure 1 shows the reciprocal of his Lambda ($1/\lambda$) plotted against trials. Apparently this subject found a better strategy for tracking in the last two sessions prior to launch and continued to use the approach thereafter.

Matrix Test. Figure 2 shows the second subject's RT for the Matrix Test improving until the fourth session after launch, Trial 28. Thereafter, RT appears to level off until recovery where it slows to a level comparable to pre-launch. The difference between the predicted RT and the actual was 143 msec, $t(4) = 3.17$. This subject's percentage of correct responses fluctuated from 84-97% during practice making the throughput measure difficult to model and it did not show a statistically significant effect. The one very slow RT on Trial 28 was unexplained.

Directed Attention Test. Performance on the mathematical portion of the Directed Attention Test showed significant degradation for two subjects. Figure 3 shows the RT measure from the mathematical processing task for the same two subjects shown in earlier figures. Actual RT and model predictions are plotted against trials on the left axis. Figure 3a also shows the subjective fatigue ratings of Subject 1 plotted against the right axis. For Subject 1, there was a pronounced increase in RT latency starting on the first session after launch, Trial 25, that was associated with his highest fatigue rating in-orbit. The difference between the predicted mean RT for all in-orbit sessions and the actual was 207 msec, $t(4) = 3.62$. This effect persisted through 7 days of recovery in which four postflight testing sessions were recorded. As shown in the figure, this subject gave another high fatigue rating on the first session of recovery, Trial 34. Dr. Lawrence Young, an astronaut selectee, commented at the 12th IAA Man in Space Symposium presentation of these data that the subject appears to have a new and reduced performance level resulting from the in-orbit experience.

In Figure 3b, Subject 2 appears to be responding slower starting on Trial 30 and never regains his speed even after recovery. Recall that the Matrix task showed this trend beginning on Trial 28 for this subject. The difference between the predicted mean RT and the actual was 129 msec, $t(4) = 2.98$.

Figure 4 shows the time per item measure for Subject 1 in the mathematical processing task. Again, starting on the first in-orbit testing session, Mission Day 2, performance was significantly degraded. Performance was highly variable on succeeding in-orbit days. The difference between the mean predicted time per item values and the actual was 220 msec, $t(4) = 2.85$. The time per item measure is the reciprocal of throughput, a combined measure of speed and accuracy. This subject appears to improve performance in the last three testing sessions after recovery; however, his best performance remains two sessions before launch.

Switching time is the reaction time for the mathematical processing task after responding to the manikin task in a previous display. It requires the subject to change cognitive processing from a spatial context to a logical symbol manipulation context. Figure 5 shows mean switching time and the model predicted values plotted against trials for the two significantly affected subjects. It appears for these two subjects that, in orbit, it was more difficult to transition from manikin to math than it was preflight. Figure 5a shows a switching time increase of nearly 400 msec when comparing performance pre-launch baseline with the first in-orbit testing session. A corresponding fatigue rating from 2 to 5 may explain some of the performance decrement. This performance level is equivalent to that of Trial 6 in early training. The difference between the predicted in-orbit mean switching time and the actual was 166 msec, $t(4) = 3.11$. In Figure 5b, Subject 2 appears to be having trouble as early as Trial 27 or 28 on this measure. The difference between the predicted mean switching time and the actual was also 166 msec, $t(4) = 3.05$. Seven days after recovery this subject had not yet recovered his speed on this task.

The statistical approach taken in this paper is very conservative. However, Subject 1 showed non-significant degradation in Manikin, Continuous Recognition and Matrix. Subject 2 showed non-significant degradation in Manikin, Continuous Recognition and the Dual Task. For a third subject with high variability, his data showed degradation in the plots of all tasks except the Tracking and Dual tests, but none were statistically

significant. The fourth subject showed non significant reduced performance in plots of Memory Search and Manikin, but actually showed improvement in the mathematical processing task.

CONCLUSIONS

Significance

With only four subjects it is not possible to generalize results to a population of future space travelers. However, if one confines their analysis and interpretation to the four subjects tested in this 16-day mission, some tentative conclusions can be drawn. Generally, in-orbit subjects were able to respond to the tests without significant impairment. The mathematical processing task, a part of the Directed Attention Test, showed significantly degraded performance in two out of the four subjects. The Matrix test performance was degraded in one subject. The improvement seen in the Tracking test for one subject appeared to be the result of an improved tracking strategy developed in the last two practice sessions prior to launch.

The current available database on human performance in space is too small to warrant final definitive conclusions about specific processes of cognition and psychomotor performance affected by living and working in microgravity. However, the data from this flight support the following preliminary conclusions.

1. Cognitive and psychomotor performance can be reliably measured in the microgravity.
2. Performance measures are sensitive to the combined stressor effects of the microgravity environment.
3. Predicted performance levels based on preflight data were not achieved in-orbit for mathematical processing for two-out-of-four subjects. The same was true of one subject on the Matrix Test.
4. A single stressor, such as microgravity or fatigue causing the in-orbit performance deterioration on the two tests cannot be fully determined from these results.

Future Plans

More research controlling other variables that are confounded with these results is needed. Some of the other variables include: experience (number of previous flights), work shift (red or blue), sleep quality and quantity, medications, fatigue, and subject motivation. A research proposal is currently under review at NASA that proposes a shuttle mission simulation that could remove many of the potential confounded causes of performance degradation observed in orbit.

The data from LMS will be combined with those from IML-2 for a future publication. Although there were many differences between the experiments on the two missions, the modeling approach taken with these data will permit a combined analysis with conclusions based on all seven subjects.

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ABSTRACT

The impact of microgravity and other stressors on cognitive performance need to be quantified before long duration space flights are planned or attempted since countermeasures may be required. Four astronauts completed 38 sessions of a 20-minute battery of six cognitive performance tests on a laptop computer. Twenty-four sessions were preflight, 9 sessions were in-orbit, and 5 sessions were postflight. Mathematical models of learning were fit to each subject's preflight data for each of 14 dependent variables. Assuming continued improvement, expected values were generated from the models for in-orbit comparison. Using single subject designs, two subjects showed statistically significant in-orbit effects. One subject was degraded in two tests, the other was degraded in one test and exceeded performance expectations in another. Other subjects showed no statistically significant effects on the tests. The factors causing the deterioration in the two subjects can not be determined without appropriate ground-based control groups.

Critical Tracking

$$R^2 = .75, Y = 0.18 + 0.08e^{-0.20X}$$

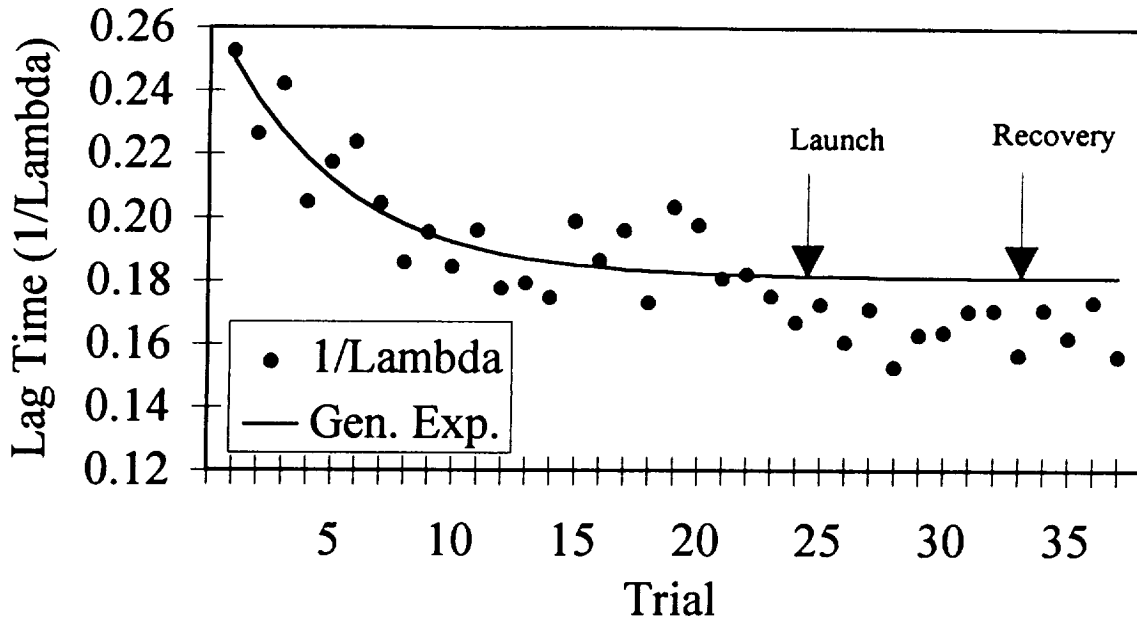


Figure 1. Lag time (1/Lambda) is plotted against trials for Subject 1. The solid line shows the generalized exponential model predictions; the circles are the data values.

Matrix Task

$$R^2 = .91, Y = 196 + 1527e^{-.05X}$$

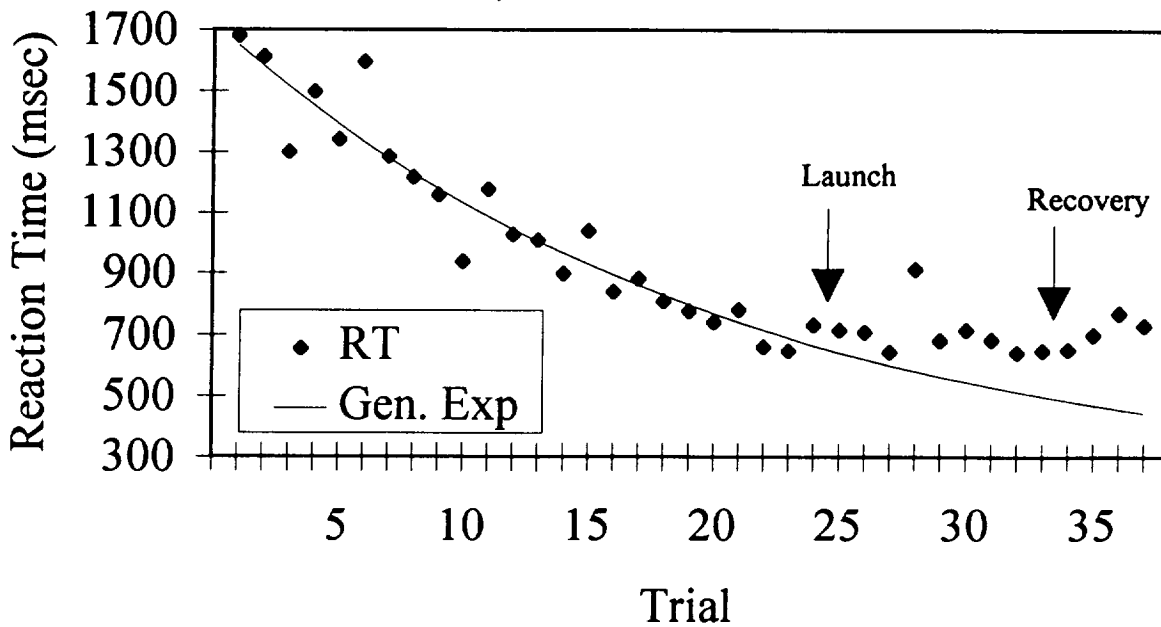
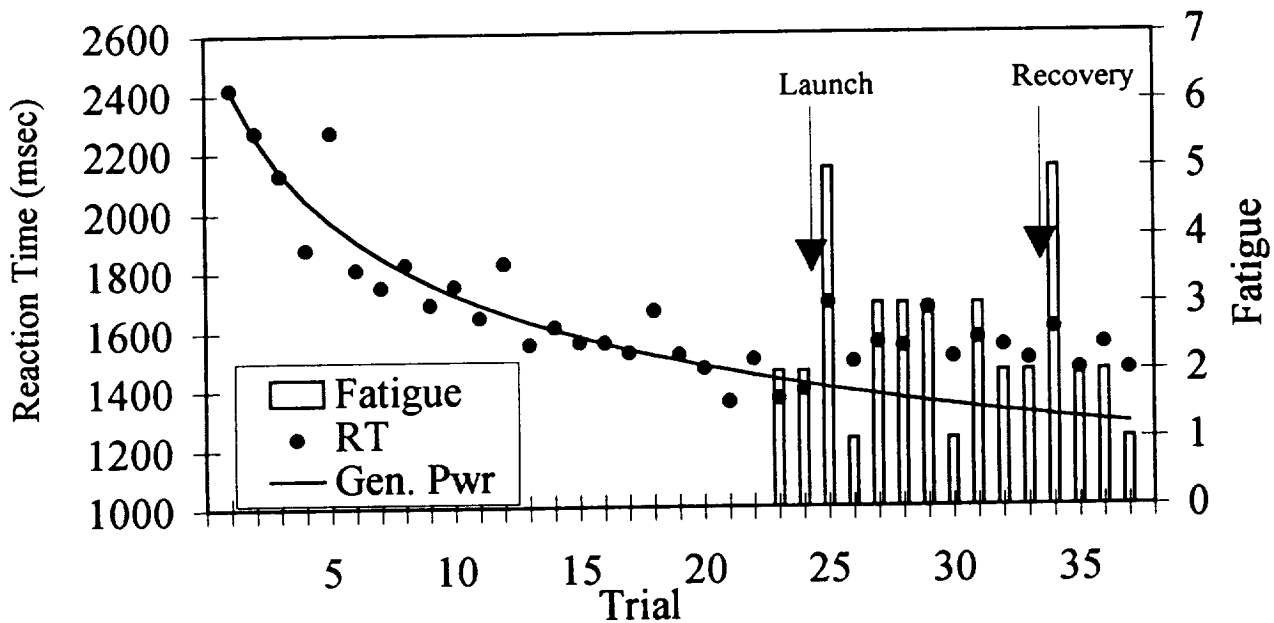


Figure 2. Mean correct RT plotted against trials for Subject 2 showing performance degradation in-orbit. The diamonds show actual data values.

(a) Directed Attention, Mathematical Processing

$$R^2 = .89, Y = 3270(X+2.27)^{-0.256}$$



(b) Directed Attention, Mathematical Processing

$$R^2 = .92, Y = 2209X^{-.18}$$

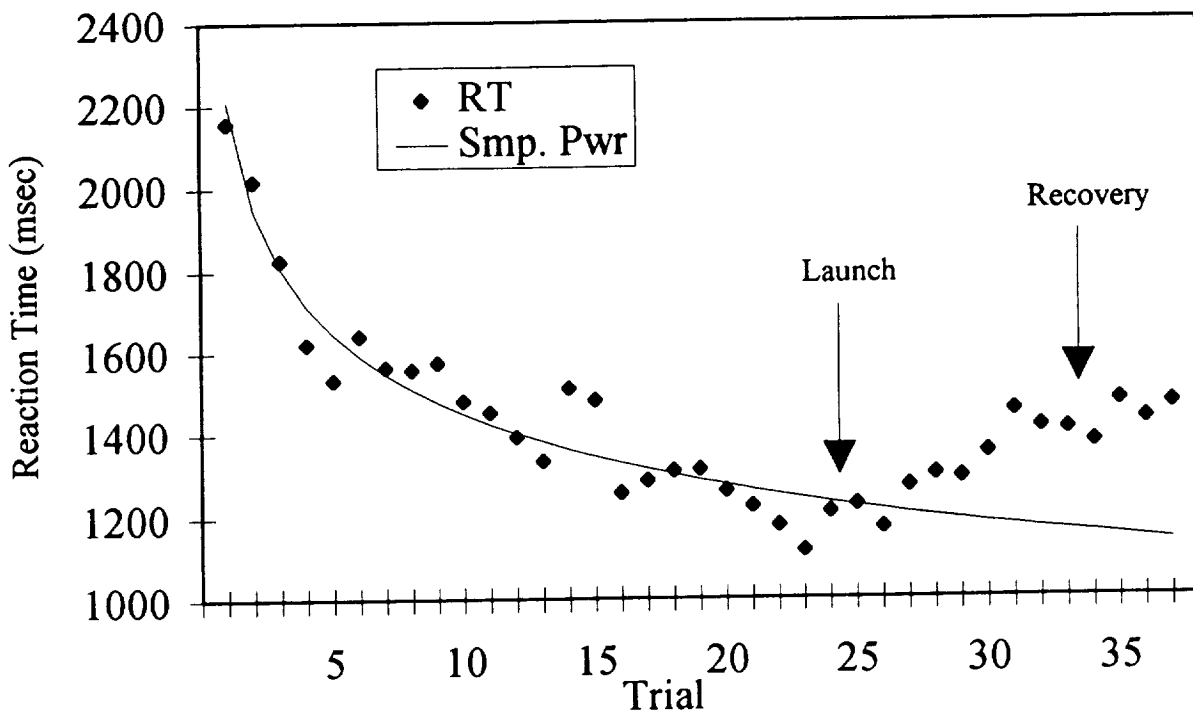


Figure 3. Mean correct RT plotted against trials. (a) Fatigue ratings for Subject 1 start two trials before launch, (b) data from Subject 2.

Directed Attention, Mathematical Processing

$$R^2 = .84, Y = 5.4(X+6.8)^{-0.37}$$

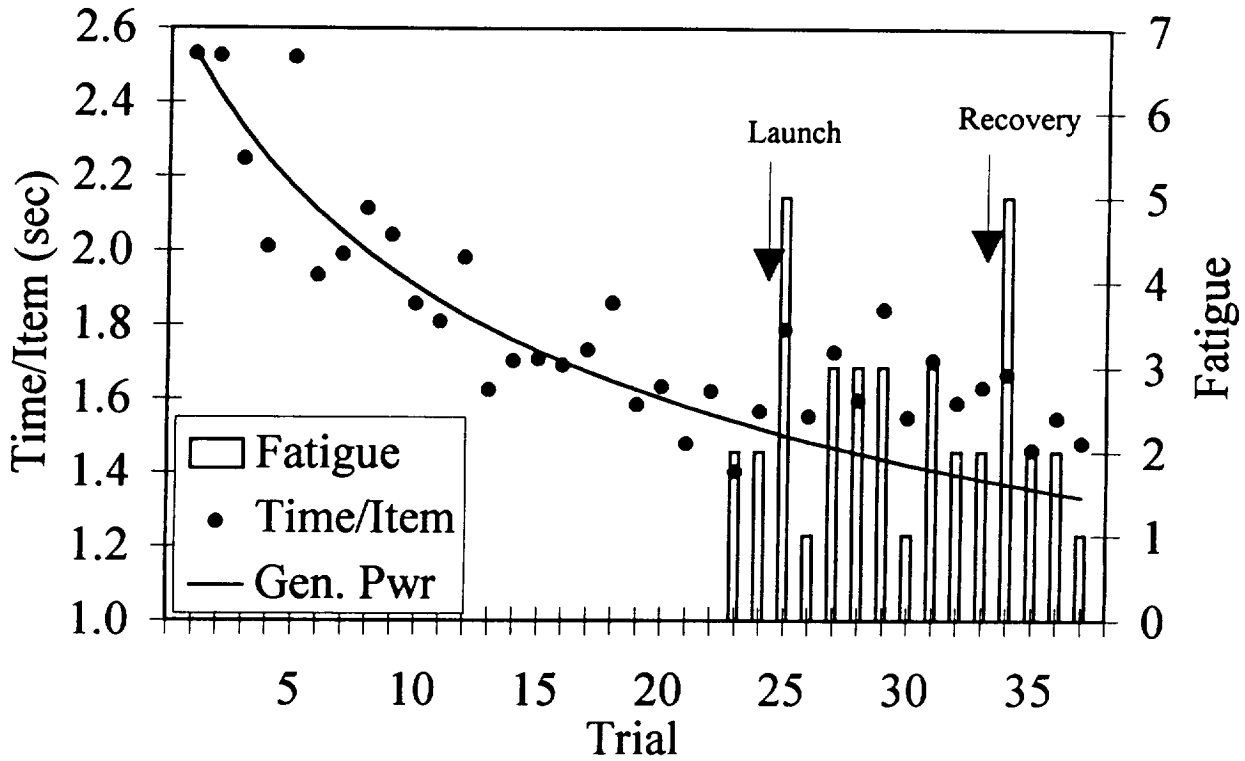
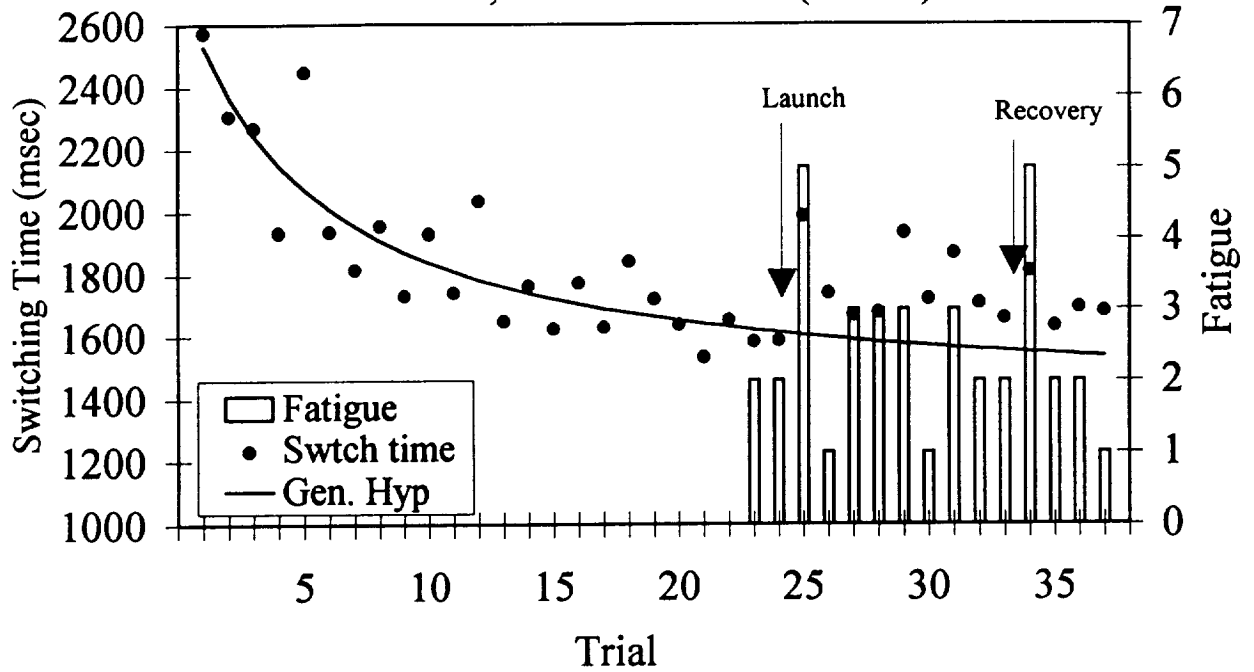


Figure 4. Time-per-item plotted against trials for Subject 1. Fatigue ratings are shown in the histogram plotted against the right axis starting two trials before launch.

(a) Directed Attention, Mathematical Processing

$$R^2 = .79, Y = 1363 + 7232 / (X + 5.2)$$



(b) Directed Attention, Mathematical Processing

$$R^2 = .89, Y = 2357(X + 0.3)^{-0.20}$$

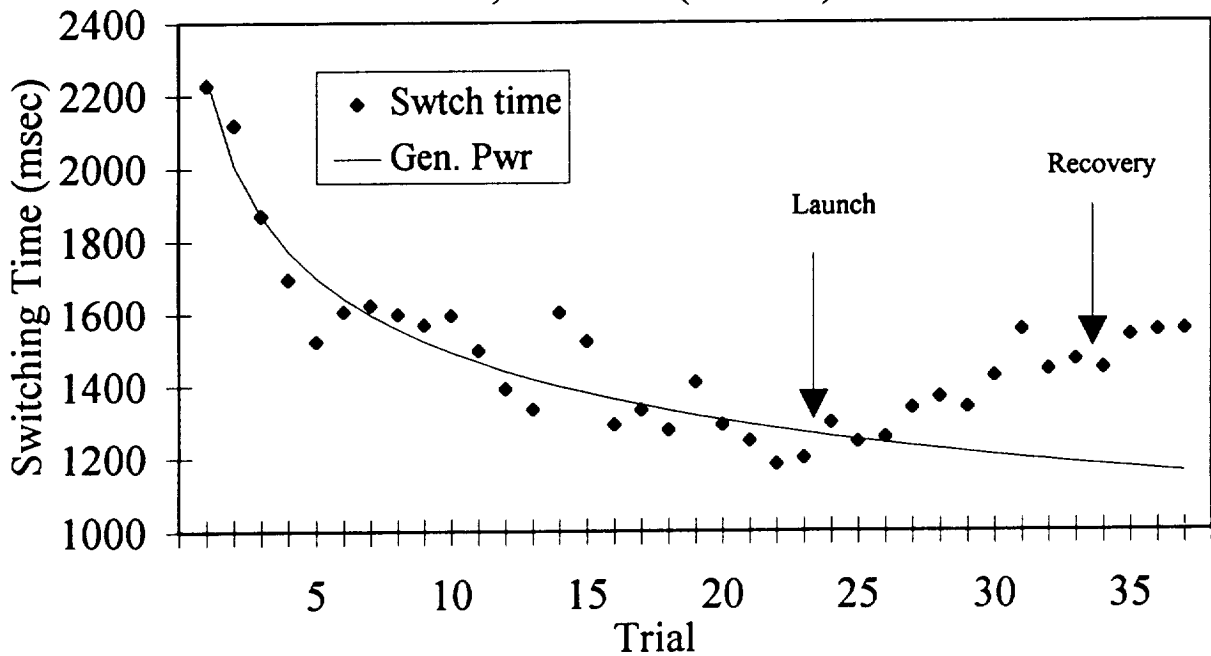


Figure 5. Mean switching time plotted against trials. Model predictions are shown with solid lines, (a) data from Subject 1 including Fatigue ratings, (b) data from Subject 2.

JSC Human Life Sciences Project

**E971 - Measurement of Energy Expenditures During
Spaceflight Using the Doubly Labeled Water Method**

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E971. MEASUREMENT OF ENERGY EXPENDITURE DURING SPACE FLIGHT USING THE DOUBLY LABELED WATER (DLW) METHOD

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A. SPECIFIC AIMS

1. To measure human energy expenditure during flight on the space shuttle by using the doubly labeled water method for measuring energy expenditure.
2. To determine whether astronauts are in negative energy balance during space flight on the shuttle.
3. To compare energy expenditure during spaceflight against that found with bedrest.

B. BACKGROUND

Human spaceflight is associated with a loss of body protein. Studies of nitrogen balance during spaceflight have reported a persistent negative nitrogen balance (5,18,24). Specific changes include a loss of lean body mass, decreased muscle mass in the calves, and decreased muscle strength (5,10,22). The major muscle loss is believed to be associated with the anti-gravity (postural) muscles.

The problem of protein wasting is of considerable practical importance because it may result in 'impaired performance during flight' (14). On the ground, chronic protein wasting has serious consequences. Apart from decreased physical performance, there is progressively increasing susceptibility to infection (1,23). Other processes that are compromised include wound healing, which may be a problem if injury ever occurs during spaceflight. It is interesting to note that decreased immunocompetence during spaceflight has been reported (6,7).

As yet, the mechanism of protein wasting during space flight is unknown and the protein wasting persists in being a problem particularly with long term missions. There are a number of viable hypotheses based on ground control analogies which can account for the muscle atrophy and protein loss. The principal ones are:

(1) Muscle atrophy secondary to disuse. Muscle atrophy can occur from less work being required of certain muscles at '0' g.- e.g. the postural muscles. The corresponding ground based analogy is bed rest (13).

(2) A stress response. A characteristic response to a metabolic stress (e.g. trauma) is a transient protein loss, which is usually self limiting (12,25). Conceivably the body responds to the acute change to '0' g as a stress. As part of the response to stress, protein turnover is increased with the increase in protein breakdown being greater than the increase in protein synthesis, hence the protein loss. Stress hormone levels (glucocorticoids, catecholamines) were found to be increased in the Skylab missions (9). We have shown on the SLS1 and SLS2 missions that a metabolic stress response is a factor in at least the early protein loss on the shuttle (18,19,20).

(3) An energy deficit. A characteristic response at '1' g to an energy deficit is a loss of body protein. An energy deficit can occur either from decreased intake or increased energy expenditure. An energy deficit was a factor in the muscle loss found on the Skylab missions. By using the dietary intake, nitrogen excretion, body composition data and computer modeling, the Skylab investigators concluded that the crew were in an energy deficit state for most of the flight (11,15).

The primary objective of this study was to determine if the payload crew were in energy balance. A secondary objective was to address the question of whether human energy expenditure is increased or decreased in flight for comparable activity. This issue was addressed by comparing the inflight energy expenditure and balance measurements against those obtained on the ground from the LMS bed rest study.

C. APPROACH TO PROBLEM

Energy expenditure for both studies was determined by the doubly labeled water (DLW, $^2\text{H}_2^{18}\text{O}$) method. Energy intake was measured by dietary monitoring and energy balance from the difference between intake and expenditure.

The doubly labeled water method is a highly accurate means of measuring energy expenditure in a safe, non-invasive, time-efficient manner using only urine or saliva specimens for

analysis. The accuracy is better than 5% and the reproducibility between repeat studies on the same subject is about 9%. The method consists of giving water labeled with the stable (non-radioactive) isotopes deuterium (^2H) and ^{18}O . The two isotopes leave the body water at different rates. Deuterium leaves as water, mainly as urine, whereas ^{18}O leaves as both water and exhaled CO_2 . The difference in loss rates is therefore equal to the rate of CO_2 production. The CO_2 production rate is directly related to the rate of energy expenditure.

The principal requirements from the subject is that he/she drink water labeled with the non-radioactive, naturally occurring isotopes ^{18}O and ^2H and the body water sampled at suitable time intervals (~3 - 20d, 2,17). The body water can be sampled either as blood, urine or saliva. We used saliva. Other measurements necessary to achieve these objectives included dietary intake, nitrogen balance, 3-methyl histidine excretion and body composition.

We measured energy expenditure and body composition before flight, inflight and postflight. Energy expenditure and balance were measured over blocks of 6 days. There were two consecutive 6 day blocks preflight (days L-15 to L-9 and L-9 to L-3), two similar consecutive 6 day blocks inflight (days FD-3 to FD-9 and FD-9 to FD-15) and a similar schedule postflight (R+3 to R+6 and R+9 to R+15). L-X refers to launch day minus X days, FD-X, flight day X and R+X to return + X days. Using such a block scheme increases the sensitivity of the measurements by allowing for duplicate measurements on each subject, an important factor when the number of test subjects is small. The space flight study was restricted to the four payload crew.

Table 1. PERIODS FOR MEASUREMENT OF ENERGY EXPENDITURE

<u>PREFLIGHT</u>	<u>INFLIGHT</u>	<u>POSTFLIGHT</u>
BLOCK 1 PRE L-15 to L-9	BLOCK 3 IN FD-3 to FD-9	BLOCK 5 POST R+3 to R+9
BLOCK 2 PRE L-9 to L-3	BLOCK 4 IN FD-9 to FD-15	BLOCK 6 POST R+9 to R+15

Figure 1. Energy expenditure was measured during the six time blocks shown above. For the flight experiment the blocks are space flight, preflight, inflight and post flight. For the bed rest study the blocks are pre-bed rest, bed rest and recovery.

In order to compare the flight results with that of the ground based LMS bed rest study we used the same protocol to study the bed rest subjects. Eight subjects were studied in the bed rest phase of this program. An important point about the bed rest study is that it included the full complement of exercise testing that was done on the payload crew. By doing so enables us to compare (bed rest + exercise) against (space flight + exercise + working in space) thereby providing an estimate of the energy costs of working in space.

A 17 day bedrest with 6° head down tilt was conducted in the Clinical research Center of the NASA-AMES Research Center. 8 healthy adult males were recruited from the local community.

The study was divided into three phases, a 15 day pre bedrest ambulatory period followed by 17 days of bedrest and ending with a fifteen day recovery period. During the 47 days of the study the subjects received all their nutrition from the research center. An attempt was made to provide the subjects with a 'controlled' ad libidum diet. Twelve daily menus were made up comprising of 2500 kcal/d and 90 g protein/d. In addition subjects were allowed access to a snack basket which contained fruit, cookies, some candy and granola bars. An accurate record of dietary intake was kept for the entire period. Urines were collected continuously for the 47 day period.

D-1. RESULTS: BED REST EXPERIMENT.

The combination of providing the subjects with access to a snack basket and not compelling them to eat all the food offered introduced enough latitude for them to adjust their intake needs to the situation. The difference was primarily in the two ambulatory phases where the subjects made more extensive use of the snack basket, although food items were also declined from the set menus. There was a significant decrease in intake for the bedrest phase (table 2). It is interesting to note that this intake value (33 kcal. kg⁻¹.d⁻¹) is higher than the energy expenditure reported for bedrest without activity (24 kcal. kg⁻¹.d⁻¹, 4) but about the same as that found for minimal activity (confined to a GCRC, 30 kcal. kg⁻¹.d⁻¹, 3). Since the subjects in this study had several days of quite intensive exercise one would expect their energy expenditure value to be above that of bedrest alone.

As expected, the subjects were in negative nitrogen balance during the bedrest phase of the experiment. Closer inspection of the N balance data shows that N balance on bedrest days 13-14 was significantly less than on any of the other bedrest days. These two days encompassed the last of the pulmonary function tests/exercise protocols (21). One possible explanation is that some of the muscles had been weakened by the bedrest to such an extent that the imposition of a strenuous exercise regimen caused some actual muscle damage.

<u>PERIOD</u>	<u>CONTROL</u>	<u>BED REST</u>	<u>RECOVERY</u>
n =	7	7	6
Energy intake, kcal. kg ⁻¹ .d ⁻¹	35.9 ± 2.0*	32.8 ± 1.6	36.3 ± 2.0*
N intake, mg N. kg ⁻¹ .d ⁻¹	181.8 ± 9.6	177.9 ± 8.8	186.5 ± 10.4
N balance, mg N. kg ⁻¹ .d ⁻¹	21.8 ± 3.0	1.1 ± 4.6*	27.2 ± 4.8

Table 2. Data from the NASA-LMS bed rest study. * p<0.05 vs control period.

The 3-MeH data from the bed rest study enabled us to close an important gap in our SLS1/2 studies. Specifically it permitted the comparison of 3-MeH excretion from two Life Sciences Shuttle missions (duration 9.5 and 16 d, n=9) against 17 days of bed rest (n=7) with 6° head down tilt against the pre-existing Skylab data. The Skylab studies had shown a marked elevation of 3-MeH excretion in flight; we found no increase on SLS1/2 and most bed rest studies did not show an increase. However no bed rest study except for the present bed rest study incorporated an exercise component specifically designed to emulate a shuttle flight. 3-MeH excretion was unchanged with either bed rest, (pre bed rest 5.30 ± 0.29 (7) vs bed rest 5.71 ± 0.30 (7) μmol 3-MeH kg⁻¹.d⁻¹, p=ns) or spaceflight, (preflight 4.98 ± 0.37 (9) vs 4.59 ± 0.39 (9) μmol 3-MeH kg⁻¹.d⁻¹ inflight p=ns). From these comparisons we concluded: (i) 3-MeH excretion was unaffected by spaceflight on the

Shuttle or bed rest plus exercise. (ii) Since protein breakdown (elevated 3-MeH) was increased on Skylab but not on Shuttle, it follows that muscle protein breakdown is not an inevitable consequence of space flight (21).

At time of writing we have completed all of the ^{18}O measurements and this enables us to calculate the total body water values. The ^2H measurements are in progress. The total body water data is summarized in table 3. The values look reasonable. However close inspection of the data indicates a potential problem. The body water tracked the body weight changes body weight. The mean change in TBW was -0.03 ± 0.21 liters and the decrease in body weight was -0.63 ± 0.22 kg.

PERIOD/SUBJECT	1	2	3	4	5	6	MEAN	SEM
CONTROL, C-1	44.17	45.11	41.04	44.34	49.49	48.31	45.41	1.25
CONTROL, C-6	42.91	46.18	41.01	46.00	51.11	50.83	46.34	1.67
BEDREST, BR-4	44.02	45.61	39.76	44.75	49.88	49.95	45.66	1.58
BEDREST, BR-10	44.02	46.62	41.56	44.65	49.34	49.88	46.01	1.32
RECOVERY, R+5	43.69	46.66	41.13	45.98	50.61	50.85	46.48	1.56
RECOVERY, R+10	45.63	44.56	45.23	47.56	51.90	52.28	47.86	1.40
BED REST CHANGE	0.48	0.47	-0.36	-0.47	-0.69	0.35	-0.04	0.22
RECOVERY CHANGE	1.12	-0.03	2.15	1.60	0.95	2.00	1.30	0.33

Table 3. Summary of the total body water changes with bed rest. Bed rest change is the difference between the bed rest values and the pre bed rest values; recovery changes is the difference between recovery and bed rest.

D-2. RESULTS: FLIGHT EXPERIMENT

The flight analyses are at present only partially completed. The principal result that we have to date is determination of the energy expenditure rates and hence energy balance for the two in-flight periods. These are summarized in table 4. While this data is still in preliminary form some conclusions can be drawn. Firstly energy intake was very low on this mission. The level is the same as that expended by a subject at complete rest. Energy expenditure was about where we had predicted it would be (20). It is apparent that the subjects were in marked negative energy balance throughout the mission.

INFLIGHT PERIOD	Days 3-9	Days 9-16
Energy intake (kcal. $\text{kg}^{-1} \cdot \text{d}^{-1}$.)	22.8 ± 1.8	24.4 ± 3.0
Energy expenditure (kcal. $\text{kg}^{-1} \cdot \text{d}^{-1}$.)	$28.7 \pm 1.4^*$	$28.5 \pm 2.0^*$
Energy balance (kcal. $\text{kg}^{-1} \cdot \text{d}^{-1}$.)	$-6.4 \pm 0.6^{\#}$	$-5.0 \pm 2.0^{\#}$

Table 4. Inflight energy intake, expenditure and balance for the four payload crew members. * $p < 0.05$ vs intake or # balance.

This negative energy balance led to a substantial loss of N in the urine. Because energy balance was so negative in flight it makes the comparison of the bed rest data against the flight data

somewhat problematic because during bed rest the subjects were not in energy balance. While we have considerably more data than is presented here, we feel that it would be premature to comment on it further in print at this point.

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E. PUBLICATIONS

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