5.1

Nutritional Status Assessment During Phases IIa and III of the Lunar-Mars Life Support Test Project

Scott M. Smith, Ph.D., Gladys Block, Ph.D., Janis E. Davis-Street, Diane E. DeKerlegand, Stephanie A. Fanselow, J. Vernell Fesperman, Patricia L. Gillman, Jeannie L. Nillen, Barbara L. Rice, Myra D. Smith

SUMMARY

The studies described here were designed to assess nutritional status during the chamber stays and to validate a new tool for estimating dietary intake during space flight. Comprehensive nutritional assessments were conducted before, during, and after the chamber studies. Dietary intake was assessed using three techniques: traditional weighed dietary records, and two Food Frequency Questionnaires both designed for use with space food systems but administered to obtain either daily or weekly intake estimates. These were compared with each other to assess variability between techniques.

Introduction

Nutrition is a critical concern for extended-duration space missions (11). Loss of body weight is a primary consequence of altered nutrition and is frequently observed during space flight (11). Other existing dietary concerns for space flight include excessive intakes of sodium and iron and insufficient intakes of water and vitamin D (11). Furthermore, dependence on closed or semiclosed food systems increases the likelihood of inadequate intakes of key nutrients. This is a significant concern for extended-duration space missions.

Space nutrition research often necessitates detailed recording of all food consumption. While this yields extremely accurate data, it requires considerable time and effort, and thus is not suitable for routine medical monitoring during space flight. To alleviate this problem, a food frequency questionnaire (FFQ) was designed to provide a quick and easy, yet reasonably accurate, method for crewmembers to provide dietary intake information to the ground support crew.

We report here a study which was designed to assess nutritional status before, during, and after the 60-day and 91-day chamber stays. An additional goal of the study was to validate a food frequency questionnaire designed specifically for use with space flight food systems.

Subjects and Methods

Subjects

Subject characteristics are described elsewhere. All procedures were reviewed by the Johnson Space Center Institutional Review Board to ensure ethical use of human subjects. Informed consent was obtained from all subjects.

Dietary Intake Assessment

The subjects completed a standard food frequency questionnaire, entitled Block95 (1), prior to entering the chamber to assess usual diet over the past year. During the chamber stay, a specialized food frequency questionnaire (described below) was completed to assess intake either over 24-hour (FFQ 24-h) or seven-day (FFQ 7-d) periods. The FFQ 24-h was administered three times per week on weeks 4 and 7 of the 60-day Phase IIa study, and weeks 1, 4, 6, 9, and 12 of the 91-day Phase III study. The FFQ 7-d was administered once per week on weeks 1, 3, 6, and 8 of the 60-day study, and weeks 2, 5, 8, 10, and 13 of the 91-day study. Five-day weighed food records were completed on weeks 2 and 5 of the 60-day study and on weeks 3, 7, and 11 of the 91-day study. During the weighed record sessions, subjects were provided a digital scale and log book and were instructed to weigh and record all food, fluids, vitamin/mineral supplements, and medicines consumed. A research dietitian (BLR) met with the subjects before the prechamber data collection session to provide training for all diet intake assessment methods.

Three of the Phase IIa subjects reported occasional use of vitamin/mineral supplements, while one Phase III subject reported daily supplement use. Intake data contained herein represent total nutrient intake (i.e., intake from both the foods consumed as well as supplements).

Food Frequency Questionnaire (FFQ)

The food frequency questionnaire used in the chamber was constructed by one of the authors (GB) based on the key nutrient contents of the more than 200 food items on the menu list. Nutrient data for all foods (except milk and dried cereals for the 60-day study, see below) were obtained using the Nutrition Data System (NDS-R, Version 4.01/29 developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, Food and Nutrient Database 29 released Dec. 1996). For the 60-day study, nutrients in milk and dried cereal were obtained using values provided by Block et al. Specific nutrients studied included energy, protein,

calcium, sodium, iron, and water. Two versions of the chamber food frequency questionnaire were presented, one asking about dietary intake for the past 24 hours, the other for the past seven days. Responses for these questionnaires were handwritten.

Biochemical Assessment of Nutritional Status

A complete biochemical nutritional assessment profile was developed for use with flight crews on extended-duration space missions. This assessment profile was used in these ground-based studies to determine the impact of the semiclosed, space-like food system on crew nutritional status. Specific tests and analytical methods are shown in Table 5.1-1, are described in more detail in JSC#28566 (Nutritional Status Assessment for Extended Duration Space Flight, Rev. 1, 2000), and have been reported elsewhere (13).

Table 5.1-1 *Analytical methods used for biochemical analyses*[1]

Protein status	
Retinol binding protein (S)[2]	*radial immunodiffusion*
Transthyretin (S)	*nepholometry*
Protein electrophoresis (S)	*electrophoresis*
3-methylhistidine (U)	*ion exchange chromatography*
Water-soluble vitamin status	
RBC transketolase stimulation (WB)	*spectrophotometric*
RBC glutathione reductase (WB)	*spectrophotometric*
RBC NAD/NADP (WB)	*spectrophotometric*
N-methyl nicotinamide (U)	*HPLC*
2-pyridone (U)	*HPLC*
RBC transaminase (WB)	*spectrophotometric*
4-pyridoxic acid (U)	*HPLC*
Red cell folate (WB)	*radioreceptor assay*
Vitamin C (S)	*HPLC*

[1]Details of most methods have been published in reference 13. Detailed descriptions of all tests are available in JSC #28566 (Nutritional Status Assessment for Extended-Duration Space Flight, Rev 1, 2000)

[2]Sample types are indicated in parentheses: S = serum or plasma, WB = whole blood or erythrocytes, U = urine, RBC = red blood cells

[3]Abbreviations of analytical methods: ELISA = enzyme-linked immunosorbent assay, HPLC = high-performance liquid chromatography, ICP-MS = inductively coupled plasma emission mass spectrometer, IRMA = immunoradiometric assay, ISE = ion-selective electrode, RIA = radioimmunoassay

Table 5.1-1 continued *Analytical methods used for biochemical analyses*[1]

Calcium/bone status	
25-hydroxyvitamin D (S)	*RIA*[3]
1,25-dihydroxyvitamin D (S)	*RIA*
Parathyroid hormone, intact (S)	*IRMA*
Osteocalcin (S)	*RIA*
Calcium (S)	*ISE*
Alkaline phosphatase:	
Total (S)	*spectrophotometry*
Bone-specific (S)	*ELISA*
Ionized calcium (S)	*ISE*
N-telopeptide (U)	*ELISA*
Pyridinoline (U)	*ELISA*
Deoxypyridinoline (U)	*ELISA*
Hematology	
Hemoglobin (WB)	*spectrophotometry*
Hematocrit (WB)	*calculation*
Mean corpuscular vol. (WB)	*electronic pulse measurement*
Transferrin receptors (S)	*ELISA*
Transferrin (S)	*microparticle immunoassay*
Ferritin (S)	*enzyme immunoassay*
Ferritin iron (S)	*antibody isolation, ICP-MS*
Antioxidant status	
Total antioxidant capacity (S)	*spectrophotometry*
Superoxide dismutase (WB)	*spectrophotometry*
Glutathione peroxidase (WB)	*spectrophotometry*
Malondialdehyde (S)	*spectrophotometry*
4-OH-alkenal (S)	*spectrophotometry*
8-OH-deoxyguanosine (U)	*HPLC*

[1]Details of most methods have been published in reference 13. Detailed descriptions of all tests are available in JSC #28566 (Nutritional Status Assessment for Extended-Duration Space Flight, Rev 1, 2000)
[2]Sample types are indicated in parentheses: S = serum or plasma, WB = whole blood or erythrocytes, U = urine, RBC = red blood cells
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Table 5.1-1 continued *Analytical methods used for biochemical analyses*[1]

Mineral status	
Iron (S)	*ICP-MS*
Zinc (S,U)	*ICP-MS*
Selenium (S,U)	*ICP-MS*
Iodine (S,U)	*ICP-MS*
Phosphorus (U)	*spectrophotometry*
Magnesium (U)	*spectrophotometry*
Fat-soluble vitamin status	
Retinol (S)	*HPLC*
Retinyl palmitate (S)	*HPLC*
ß-carotene (S)	*HPLC*
∝-carotene (S)	*HPLC*
Serum phylloquinone (S)	*HPLC*
∝-tocopherol (S)	*HPLC*
γ-tocopherol (S)	*HPLC*
γ -carboxyglutamic acid (U)	*HPLC*
tocopherol:lipid ratio (S)	*calculation*
General	
Aspartate aminotransferase (S)	*enzymatic rate reaction*
Alanine aminotransferase (S)	*enzymatic rate reaction*
Sodium (S)	*ISE*
Potassium (S)	*ISE*
Chloride (S)	*ISE*
Cholesterol (S)	*spectrophotometry*
Triglyceride (S)	*spectrophotometry*
Creatinine (S,U)	*spectrophotometry*

[1]Details of most methods have been published in reference 13. Detailed descriptions of all tests are available in JSC #28566 (Nutritional Status Assessment for Extended-Duration Space Flight, Rev 1, 2000)
[2]Sample types are indicated in parentheses: S = serum or plasma, WB = whole blood or erythrocytes, U = urine, RBC = red blood cells
[3]Abbreviations of analytical methods: ELISA = enzyme-linked immunosorbent assay, HPLC = high-performance liquid chromatography, ICP-MS = inductively coupled plasma emission mass spectrometer, IRMA = immunoradiometric assay, ISE = ion-selective electrode, RIA = radioimmunoassay

Bone densitometry and body composition were determined using dual energy X-ray absorptiometry techniques (Hologic QDR 2000). Total body water (TBW) was determined using isotope (^{18}O) dilution, as described previously (8). Sodium bromide was used to measure extracellular fluid volume (ECF) (3). Body weight was determined weekly using a standard scale.

Biosample Collection

For the 60-day test, blood samples were collected six days prior to entering the chamber (designated CD-6) and four days after completion of the chamber stay (designated R+4). For the 91-day study, blood samples were collected once before (CD-9), twice during (chamber day 30, designated CD30, and CD40), and once after (R+4) the chamber stay. The CD30 and CD40 blood collections were immediately before and after implementation of the BIO-Plex diet (described in Chapter 4.4).

Fasting blood samples were collected immediately after awakening, at the same time of day, in order to minimize the effect of diurnal changes in endocrine and biochemical markers. For the 60-day chamber study, a total of 52 mL of blood were collected over approximately 70 days. For the 91-day chamber study, a total of 98 mL of blood were collected over approximately 100 days.

Urine was collected for two 24-hour periods before, every day during, and two 24-hour periods after the chamber studies; pre- and postchamber urine collections began on the day of blood collection. Complete urine analysis was conducted once (on CD32) during the 60-day study and three times (CD30, CD40, and CD60) during the 91-day chamber study.

All urine samples were collected as individual voids. During the chamber studies, urine samples were stored in a refrigerator in the chamber and were transferred to the outside in one of the two to three daily exchanges through the airlock. Urine samples were processed in the laboratory daily, 24-hour pools were created, and aliquots were either analyzed immediately or were frozen for batch analysis upon completion of the study.

Statistical Analysis

Dietary data were analyzed using repeated-measures analysis of variance. The class variable was assessment tool (FFQ 24-h, FFQ 7-d, Weighed Records), and the dependent variables were the nutrients of interest. Prechamber dietary intake data are presented, but these were not included in the statistical analyses, as the differences between prechamber and in-chamber intakes were not a primary research question.

Biochemical analyte data for the 60-day study were analyzed using paired t-tests, except when in-chamber analyses were available. In these cases, and for the 91-day chamber study, data were analyzed using repeated-measures analysis of variance. The class variable was study phase (prechamber, in-chamber, postchamber), and

dependent variables were the indices measured. This analysis identified effects of the semiclosed food system on indices of nutritional status. Because of the repeated-measures design of this study, each subject served as his or her own control. The only exception to this analysis was for the RBC transketolase assay for thiamin status. Since this is qualitative rather than quantitative, statistical analyses were not performed.

Findings

Results of the dietary intake studies are shown in Table 5.1-2. Energy and protein intakes were similar for the three intake assessment techniques during both studies. Caloric intakes were 94 ± 16% and 85 ± 16% of the World Health Organization (WHO) recommendations for the subjects in the 60-day and 91-day tests, respectively. Subjects in both tests maintained their body weights within 3% of their pretest values on exit from the chamber.

During the 60-day study, questionnaire estimates of calcium and iron intakes were lower than those of the weighed diet records (Table 5.1-2). Subsequent analysis revealed that these differences were related to differences in the nutrient content

Table 5.1-2 *Dietary intake data*[1]

	60-Day Chamber Study			
	Pre[2]	FFQ 24-h	FFQ 7-d	Weighed Records
Energy				
MJ/d	9.38 ± 1.45	10.51 ± 0.45	9.97 ± 0.65	10.76 ± 0.43
kcal/d	2243 ± 347	2511 ± 108	2384 ± 156	2571 ± 102
Protein, g/d	104.9 ± 18.9	80.5 ± 4.6	70.4 ± 6.3	75.8 ± 3.7
Calcium, mg/d	907 ± 185	910 ± 145[a]	943 ± 127[ab]	1120 ± 112[b]
Iron, mg/d	18.0 ± 0.4	19.4 ± 2.7[a]	23.6 ± 4.3[ab]	26.7 ± 4.2[b]
Sodium, mg/d	3603 ± 580	4100 ± 347	3752 ± 287	3890 ± 330
Water, mL/d	[3]	1689 ± 232[a]	1953 ± 277[b]	2430 ± 232[c]

[1]Data are mean ± SEM and represent the average of the four individual subject averages for each assessment technique. For each study, data in the same row with different letter superscripts are significantly ($p < 0.05$) different from each other

[2]Prechamber data were not included in statistical analyses

[3]Data not available – the prechamber questionnaire was not designed to estimate water intake

Table 5.1-2 continued *Dietary intake data*[1]

	90-Day Chamber Study			
	Pre[2]	FFQ 24-h	FFQ 7-d	Weighed Records
Energy				
MJ/d	8.57 ± 2.03	8.72 ± 0.46	7.41 ± 0.32	9.20 ± 0.83
kcal/d	2048 ± 485	2083 ± 109	1770 ± 77	2199 ± 198
Protein, g/d	84.4 ± 21.9	59.4 ± 2.5	51.8 ± 4.3	58.5 ± 3.2
Calcium, mg/d	1116 ± 374	1052 ± 322	937 ± 349	1126 ± 162
Iron, mg/d	16.4 ± 3.9	21.0 ± 7.5	17.2 ± 5.8	20.1 ± 5.7
Sodium, mg/d	3252 ± 902	3845 ± 267[a]	2876 ± 287[b]	3332 ± 170[ab]
Water, mL/d	[3]	2730 ± 721	2626 ± 747	3217 ± 471

[1]Data are mean ± SEM and represent the average of the four individual subject averages for each assessment technique. For each study, data in the same row with different letter superscripts are significantly ($p < 0.05$) different from each other
[2]Prechamber data were not included in statistical analyses
[3]Data not available – the prechamber questionnaire was not designed to estimate water intake

data used for two foods (milk and cereal) between the nutrient databases used to analyze the weighed diet records and the food frequency questionnaire. When the databases were synchronized for nutrient content of these food items, no differences were observed (data not presented). This problem was identified prior to the initiation of the 91-day study and was thus avoided in that study.

Sodium intake assessment yielded similar results for the three techniques during the 60-day chamber study. However, the FFQ 24-h sodium intakes were higher than those for FFQ 7-d questionnaires during the 91-day study.

Water intake assessment during the 60-day study was different for all three assessment techniques. Conversely, no differences were observed during the 91-day study.

Body weight did not change during the chamber studies (Figure 5.1-1). No changes in total body water were observed in either chamber study (Figure 5.1-2). Markers of lean body mass, urinary creatinine (Figure 5.1-3) and 3-methylhistidine (data not presented) were unchanged during the chamber studies. Extracellular fluid volume (ECFV) was measured using a 1.2 g dose of sodium bromide in capsule form for the 60-day study. One subject experienced gastric distress and subsequently did not receive the bromide dose after the chamber. ECFV did not change in the other three subjects (Figure 5.1-2). Modifications to the ECFV protocol resulted in administration of 1.5 g of sodium bromide as a ~50 mL liquid solution for the 91-day study. This form of the dose was better tolerated, and ECFV was similarly unaffected during the longer chamber study (Figure 5.1-2).

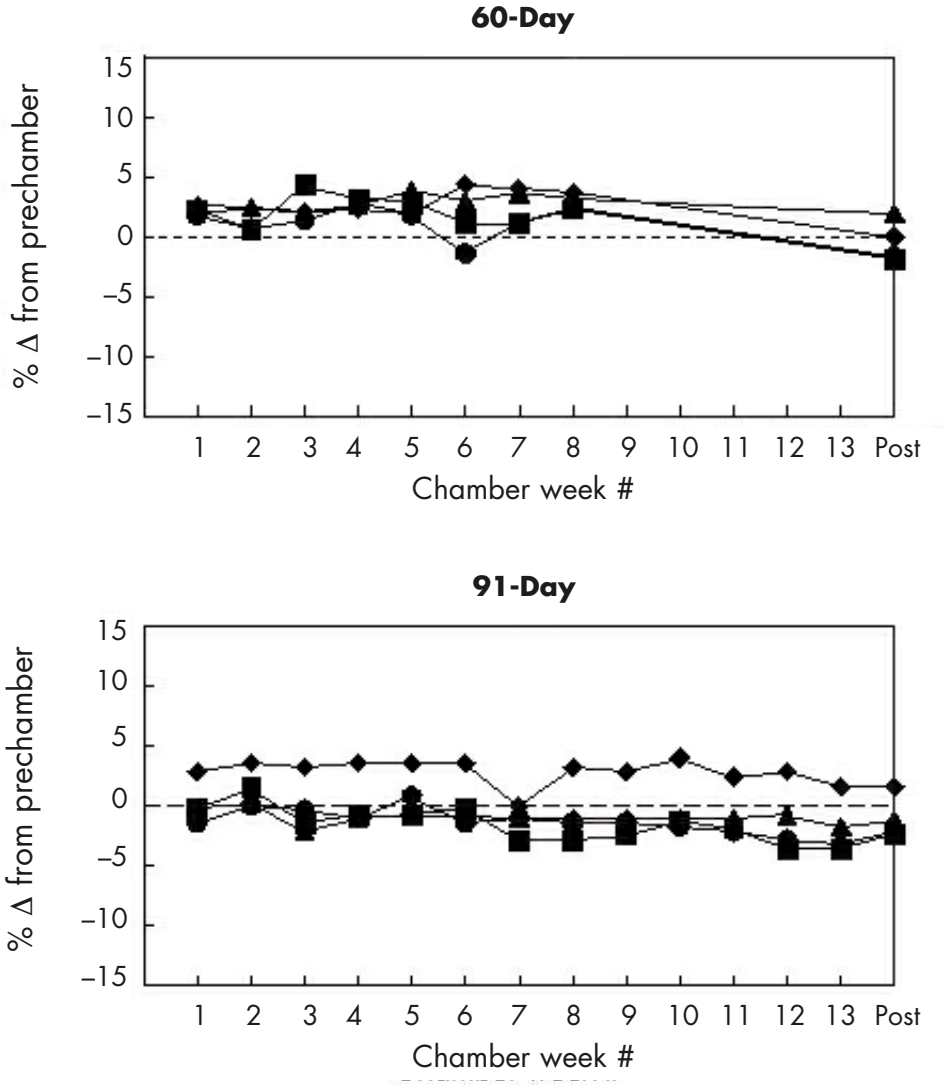

Figure 5.1-1 *Body weight data for the 60-day and 91-day chamber tests. Data are expressed for each individual as a percent change from their prechamber body weight*

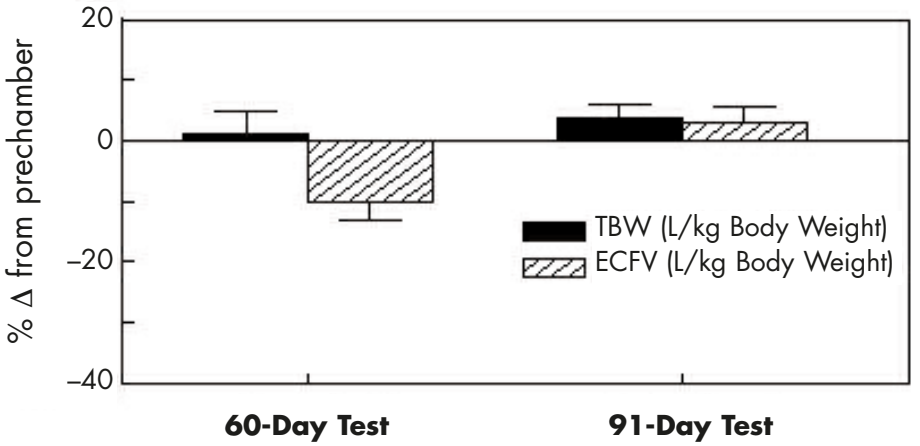

Figure 5.1-2 *Fluid compartments (TBW, ECVF) for the 60-day and 91-day chamber tests. Data are expressed for each individual as a percent change from their prechamber measurement*

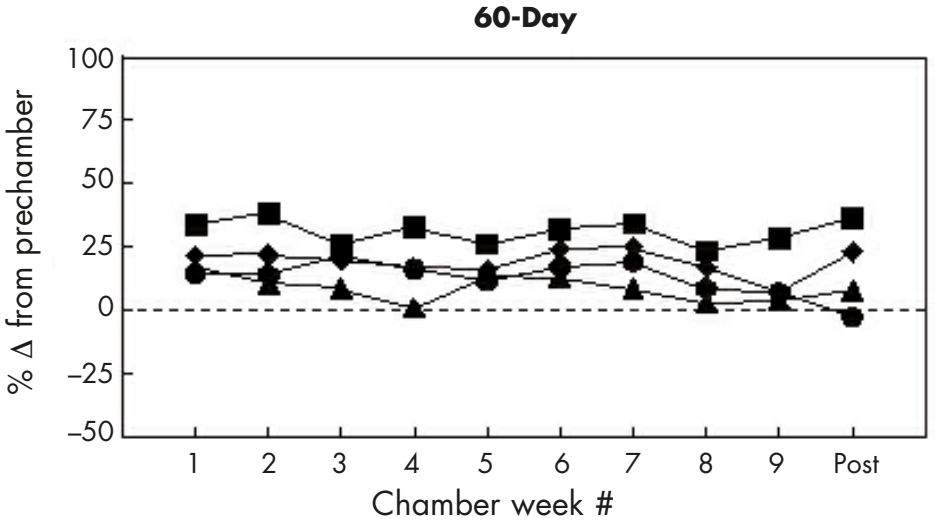

Figure 5.1-3 *Urinary creatinine excretion for the 60-day and 91-day chamber tests. Data are expressed for each individual as a percent change from their prechamber data*

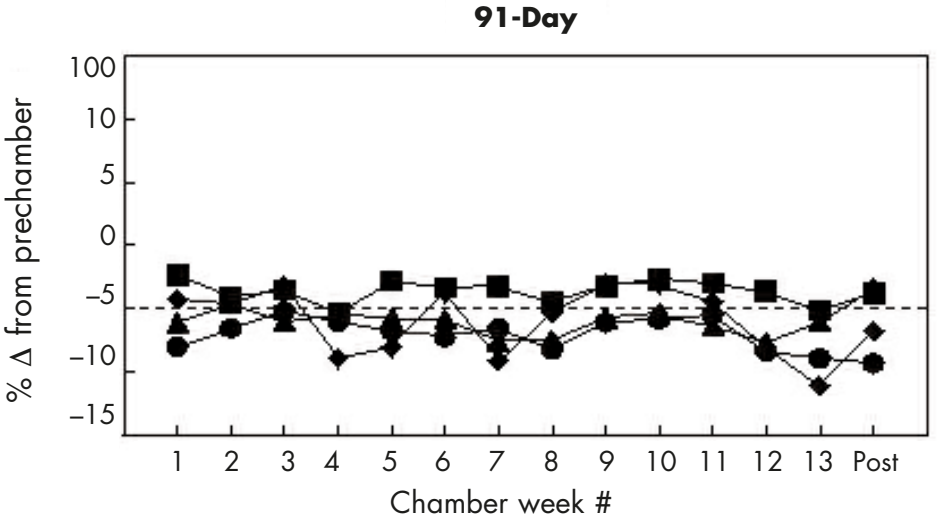

Figure 5.1-3 continued *Urinary creatinine excretion for the 60-day and 91-day chamber tests. Data are expressed for each individual as a percent change from their prechamber data*

Iron status tended to be negatively influenced throughout both studies (Table 5.1-3, Figure 5.1-4), despite high dietary iron intake (Table 5.1-2, Figure 5.1-4). Serum ferritin decreased by 21 ± 13 μg/L ($p = 0.054$) after the 60-day test, and by 29 ± 22 μg/L ($p < 0.05$) after the 91-day test. All subjects had iron intakes in excess of NASA recommendations. Most other hematological parameters (Table 5.1-3) tended to decrease.

There was a steady decline in serum 25-hydroxyvitamin D concentrations noted throughout the 91-day study, with final concentrations being significantly lower than prechamber values (Table 5.1-4, Figure 5.1-5). There was a tendency for both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations to decline in both studies (Figure 5.1-5). Vitamin D intake (Figure 5.1-5) was below the NASA recommendation of greater than 10 mg/day in six of the eight subjects, although dietary vitamin D intake was higher in the 60-day study compared to the 91-day study (Figure 5.1-5). There was also a small but statistically significant decline in serum calcium at CD30, although all data during the 91-day study were within clinical normal ranges (Table 5.1-4). Bone-specific alkaline phosphatase was increased at the end of the 60-day study but not the 91-day study (Table 5.1-4). Other indices of bone and calcium metabolism were unchanged (Table 5.1-4).

Table 5.1-3 *Hematological and iron status indices*[1]

	60-Day Chamber Study		91-Day Chamber Study			
	Pre	Post	Pre	CD30	CD40	Post
Hemoglobin (g/L)	149 ± 13[2]	146 ± 11	134 ± 4	130 ± 8	127 ± 7	126 ± 5
Hematocrit	0.44 ± 0.05	0.42 ± 0.04	0.39 ± 0.01	0.38 ± 0.03	0.37 ± 0.02	0.37 ± 0.01
Mean corpuscular vol (fL)	93 ± 3	92 ± 3	90 ± 4[ab]	90 ± 3[ab]	91 ± 3[a]	89 ± 4[b]
Serum ferritin (µg/L)	119 ± 20	98 ± 31[2]	77 ± 57[a]	68 ± 53[ab]	66 ± 56[ab]	49 ± 36[b]
Ferritin iron µg Fe/L % saturation	 20.7 ± 6.2 17.5 ± 5.1	 16.6 ± 4.5 17.5 ± 4.9	 [3] 	 [3] 	 [3] 	 [3]
Transferrin (g/L)	2.27 ± 0.20	2.22 ± 0.35	2.73 ± 0.37	2.53 ± 0.22	2.53 ± 0.27	2.73 ± 0.26
Transferrin receptors (mg/L)	3.6 ± 0.9	3.5 ± 1.6	3.8 ± 0.9	4.0 ± 1.1	4.2 ± 0.8	3.4 ± 0.5

[1]Data are mean ± SD. For each study, data in the same row with different letter superscripts are significantly ($p < 0.05$) different from each other
[2]$p = 0.054$
[3]Analyses not available

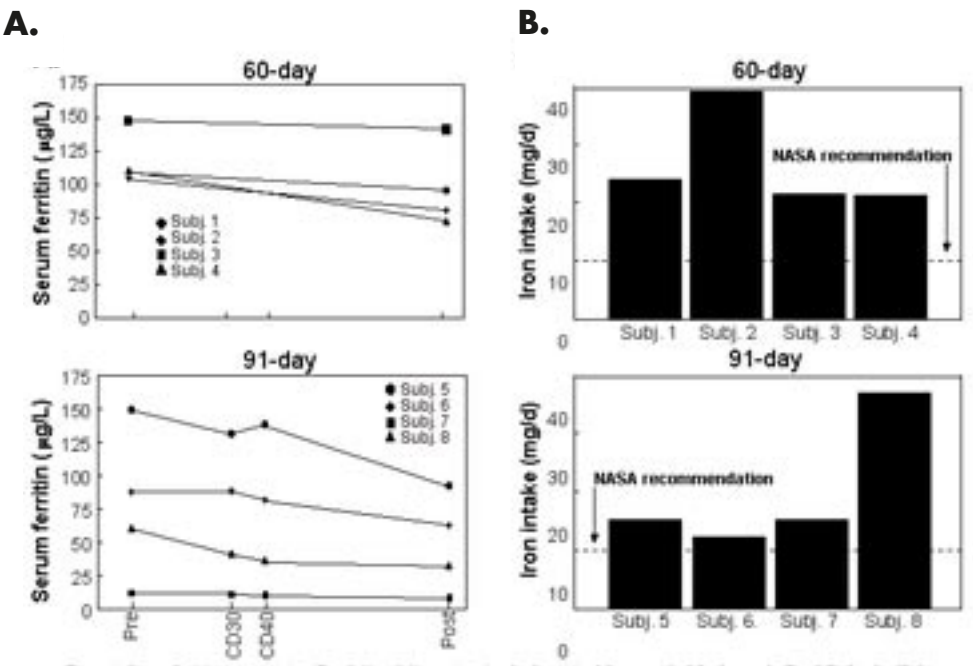

Figure 5.1-4 *Serum ferritin concentration (Panel A) and dietary iron intake determined from weighed food records (Panel B) for the 60-day and 91-day chamber tests*

Table 5.1-4 *Serum calcium and bone metabolism markers*[1]

	60-Day Chamber Study		91-Day Chamber Study			
	Pre	Post	Pre	CD30	CD40	Post
Calcium						
Total (mmol/L)	2.54 ± 0.06	2.54 ± 0.12	2.43 ± 0.11[a]	2.26 ± 0.09[b]	2.35 ± 0.14[ab]	2.35 ± 0.07[ab]
Ionized (mmol/L)	1.27 ± 0.01	1.27 ± 0.02	1.27 ± 0.04	1.26 ± 0.04	1.26 ± 0.05	1.27 ± 0.02
Parathyroid hormone (ng/L)	26.9 ± 9.3	25.8 ± 7.3	21.8 ± 12.9	18.6 ± 9.1	28.6 ± 16.5	22.3 ± 7.5
25-(OH)-vitamin D (nmol/L)	45.9 ± 6.3	43.5 ± 6.3	76.3 ± 14.4[a]	58.9 ± 13.2[ab]	54.9 ± 17.1[ab]	44.2 ± 23.1[b]
1,25-$(OH)_2$-vitamin D (pmol/L)	56.2 ± 38.5	60.9 ± 31.2	74.1 ± 29.0	59.2 ± 20.2	65.7 ± 22.3	47.0 ± 30.3
Alkaline phosphatase						
Total (μkat/L)	0.8 ± 0.2	0.8 ± 0.2	1.0 ± 0.2	1.1 ± 0.4	1.1 ± 0.3	1.1 ± 0.3
Bone-specific (μkat/L)	0.18 ± 0.04[a]	0.24 ± 0.06[b]	0.16 ± 0.06	0.16 ± 0.09	0.16 ± 0.09	0.16 ± 0.08
Osteocalcin (ng/mL)	12 ± 3	11 ± 4	10.3 ± 4.8	12.1 ± 5.3	12.9 ± 5.4	11.3 ± 6.7

[1]Data are mean ± SD. For each study, data in the same row with different letter superscripts are significantly ($p < 0.05$) different from each other

Table 5.1-5 *General chemistry indices*[1]

	60-Day Chamber Study		91-Day Chamber Study			
	Pre	Post	Pre	CD30	CD40	Post
Total protein (g/L)	72 ± 3	69 ± 1	71 ± 4[a]	65 ± 4[b]	65 ± 5[b]	68 ± 4[ab]
Albumin (g/L)	44 ± 2	43 ± 4	45 ± 3	43 ± 3	44 ± 4	45 ± 3
Transthyretin (mg/L)	[2]	[2]	274 ± 45	250 ± 55	255 ± 85	240 ± 67
Creatinine (μmol/L)	104 ± 15	97 ± 13	82 ± 11	77 ± 15	75 ± 17	73 ± 20
Cholesterol (mmol/L)	4.53 ± 0.76	4.25 ± 0.84	4.56 ± 0.94	4.63 ± 1.12	4.20 ± 0.97	4.56 ± 1.31
Triglycerides (mmol/L)	0.7 ± 0.2	0.87 ± 0.13	0.89 ± 0.67	0.94 ± 0.44	1.06 ± 0.64	0.95 ± 0.74
Sodium (mmol/L)	142 ± 1[a]	140 ± 1[b]	139 ± 2[a]	140 ± 0[ab]	141 ± 1[b]	139 ± 0[a]
Potassium (mmol/L)	3.9 ± 0.3	3.7 ± 0.1	3.9 ± 0.4	3.7 ± 0.1	3.7 ± 0.2	3.5 ± 0.1
Chloride (mmol/L)	108 ± 3	104 ±	106 ± 1	107 ± 3	107 ± 2	107 ± 2
Aspartate transaminase (U/L)	25 ± 3	26 ± 6	20 ± 3	20 ± 4	19 ± 1	18 ± 3
Alanine transaminase (U/L)	18 ± 4	22 ± 10	17 ± 6	16 ± 2	13 ± 2	13 ± 3

[1]Data are mean ± SD. For each study, data in the same row with different letter superscripts are significantly ($p < 0.05$) different from each other
[2]Analyses not available

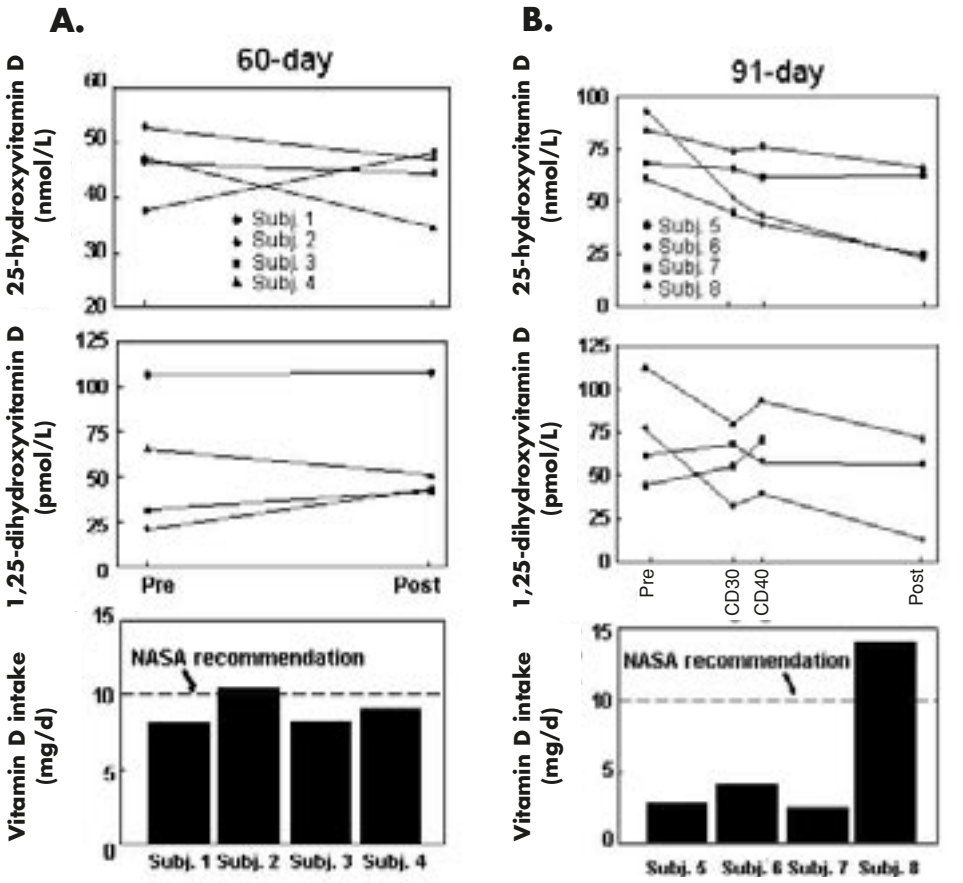

Figure 5.1-5 *Serum vitamin D metabolite concentrations and dietary vitamin D intake determined from weighed food records for the 60-day (Panel A) and 91-day (Panel B) chamber tests*

Note: There was insufficient sample to complete 1, 25-dihydroxyvitiamin D determinations on Subject 5

General clinical chemistry (Table 5.1-5) and antioxidant-related measurements (Table 5.1-6) were relatively unchanged during the two chamber studies. There was a negligible, albeit statistically significant, decrease in serum sodium concentration during the 60-day study. Serum sodium was slightly elevated on CD40 during the 91-day study. Serum total protein concentrations were slightly decreased on CD30 and CD40 and returned to prechamber levels after the 91-day study. Glutathione peroxidase activity was slightly elevated during the 91-day chamber study. Urinary calcium and collagen crosslink (n-telopeptide, pyridinium crosslinks, and deoxypyridinoline) excretion did not change during either of the chamber studies (Figure 5.1-6).

Table 5.1-6 *Vitamin status antioxidant/oxidative damage indices*[1]

60-Day Chamber Study			
	Pre	CD32[2]	Post
RBC transaminase (% activation; vitamin B_6)	113 ± 13		121 ± 18
RBC glutathione reductase (% activation; riboflavin)	17.8 ± 6.5		10.9 ± 1.5
RBC folate (nmol/L)	928 ± 54		1092 ± 167
RBC superoxide dismutase (U/g Hb)	592 ± 40		659 ± 43
RBC glutathione peroxidase (U/g Hb)	26.3 ± 3.1		25.2 ± 1.9
Oxygen radical absorbance capacity (mmol/L)	1.13 ± 0.09		1.18 ± 0.13
8-OH-2'-deoxyguanosine (µmol/mol creatinine)	1.16 ± 0.14	1.18 ± 0.50	1.20 ± 0.34

90-Day Chamber Study					
	Pre	CD30	CD40	CD60[2]	Post
RBC transaminase (% activation; vitamin B_6)	89.6 ± 11.8	93.8 ± 20.1	95.2 ± 18.5		88.3 ± 11.0
RBC glutathione reductase (% activation; riboflavin)	31.6 ± 24.8	32.8 ± 29.3	28.9 ± 20.2		25.2 ± 18.7
RBC folate (nmol/L)	1662 ± 532[a]	1763 ± 571[ab]	1796 ± 531[ab]		1907 ± 610[b]
RBC superoxide dismutase (U/g Hb)	986 ± 143	943 ± 122	986 ± 90		1050 ± 92
RBC glutathione peroxidase (U/g Hb)	46.6 ± 14.9[ab]	56.8 ± 11.9[a]	53.6 ± 15.8[ab]		44.3 ± 14.3[b]
Oxygen radical absorbance capacity (mmol/L)	1.17 ± 0.08	1.10 ± 0.10	1.13 ± 0.11		1.23 ± 0.13
8-OH-2'-deoxyguanosine (µmol/mol creatinine)	1.37 ± 0.32	1.34 ± 0.43	1.24 ± 0.41	1.38 ± 0.50	1.23 ± 0.49

[1]Data are mean ± SD. For each study, data in the same row with different letter superscripts are significantly ($p < 0.05$) different from each other

[2]Urine samples were collected and analyzed at CD32 of the 60-day study and on CD60 of the 91-day study; however, blood samples were not

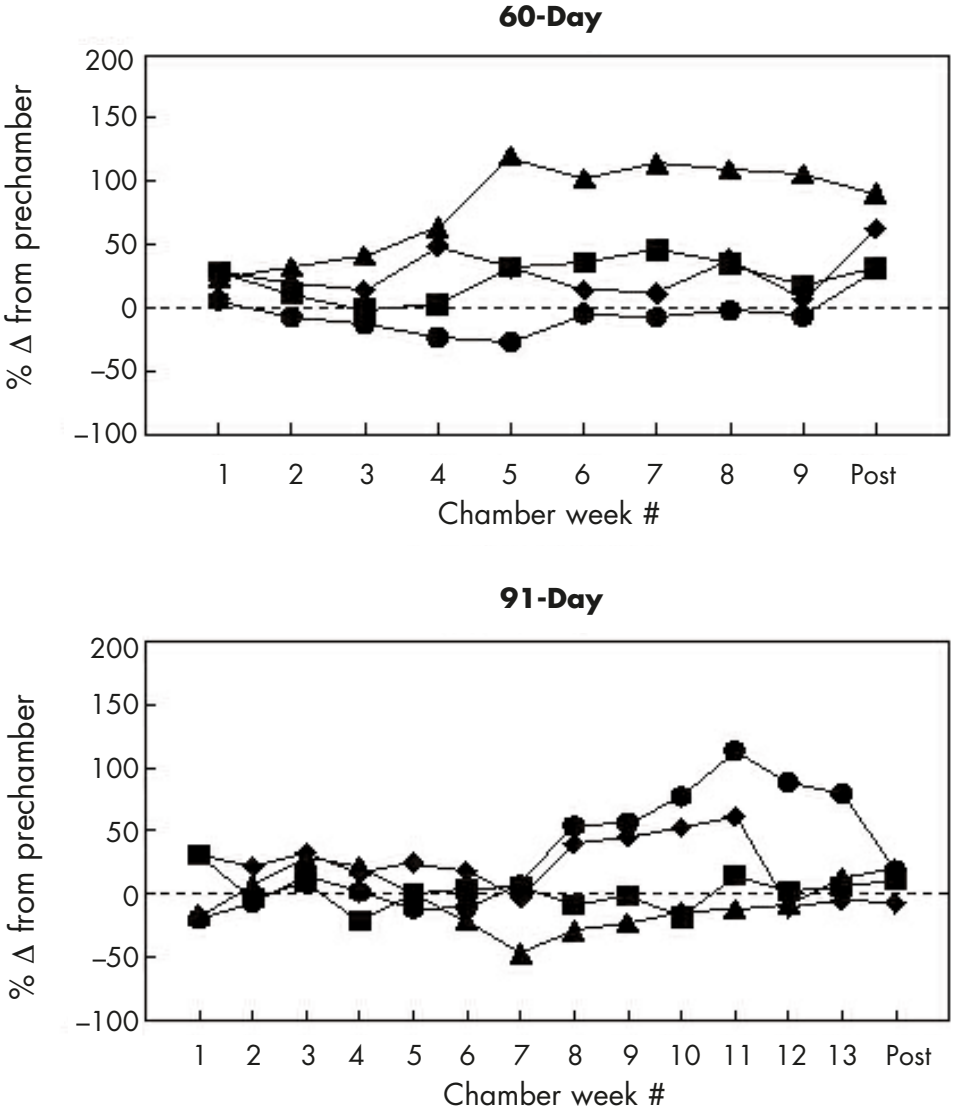

Figure 5.1-6 *Urinary collagen crosslink excretion for n-telopeptide for the 60-day and 91-day chamber tests*

Folate status, as assessed by the concentration of RBC folate, increased by more than 16% in three subjects during the 60-day study and increased by more than 17% in three subjects during the 91-day study (Figure 5.1-7a, Table 5.1-6). Folate intake, as determined during the weighed diet sessions, was generally above standard recommendations (Figure 5.1-7b).

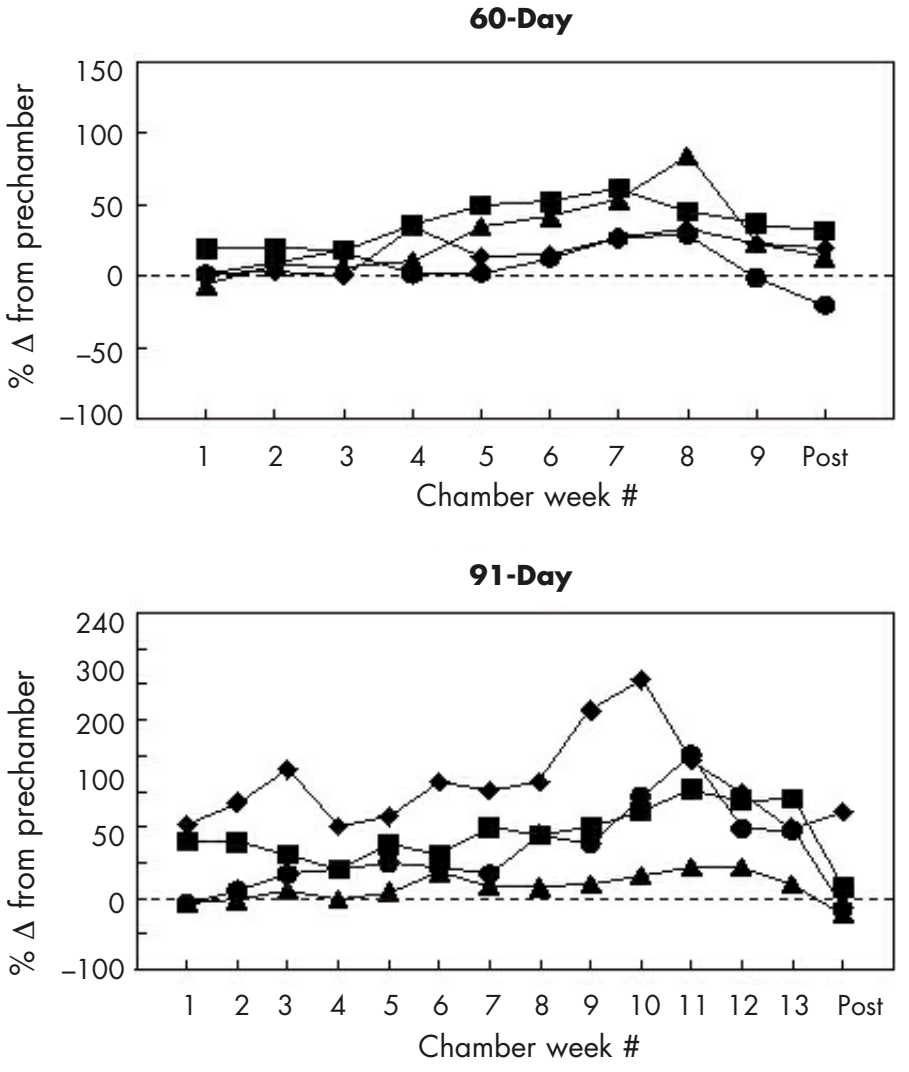

Figure 5.1-6 continued *Urinary collagen crosslink excretion for pyridinium crosslinks for the 60-day and 91-day chamber tests*

Vitamin B_6 and riboflavin status were unchanged during the chamber studies (Table 5.1-6). Thiamin status, as assessed by erythrocyte stimulation of transketolase by thiamin pyrophosphate, did not change from prechamber levels during the 91-day study (data not presented). Thiamin data were not available for the 60-day study.

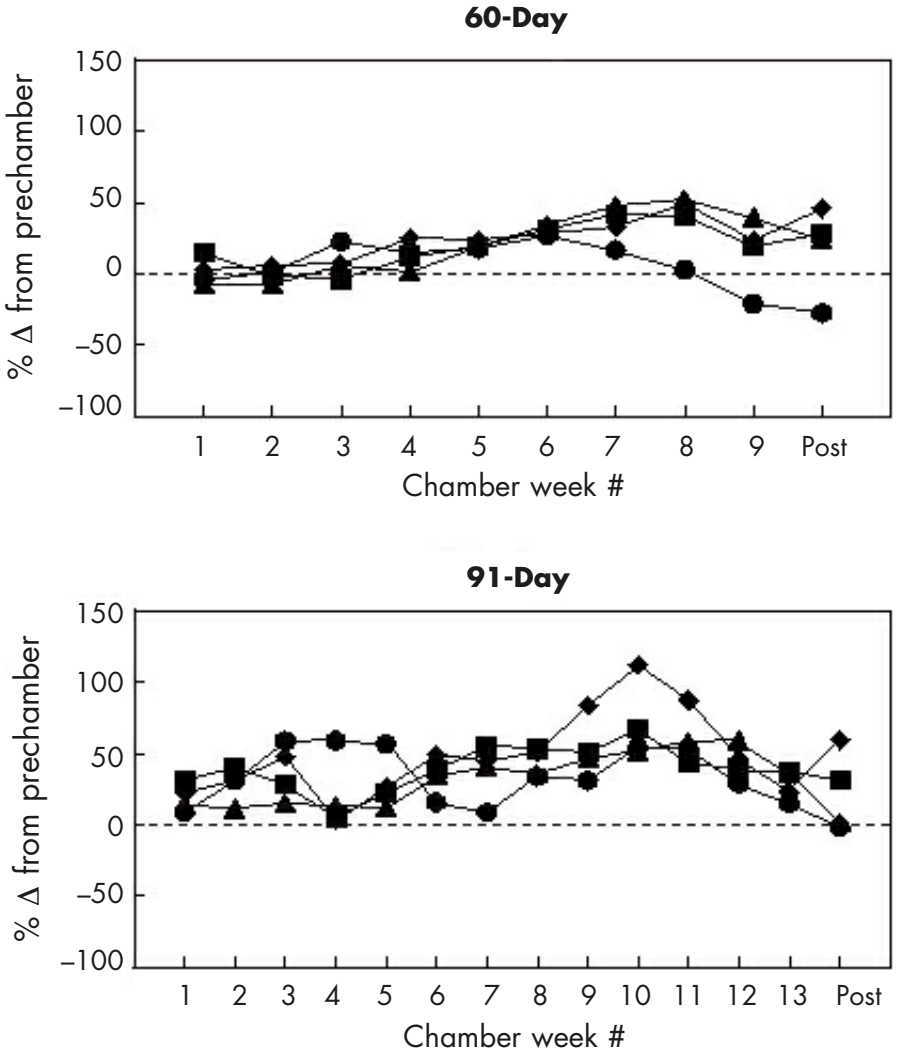

Figure 5.1-6 continued *Urinary collagen crosslink excretion for deoxypyridinoline for the 60-day and 91-day chamber tests*

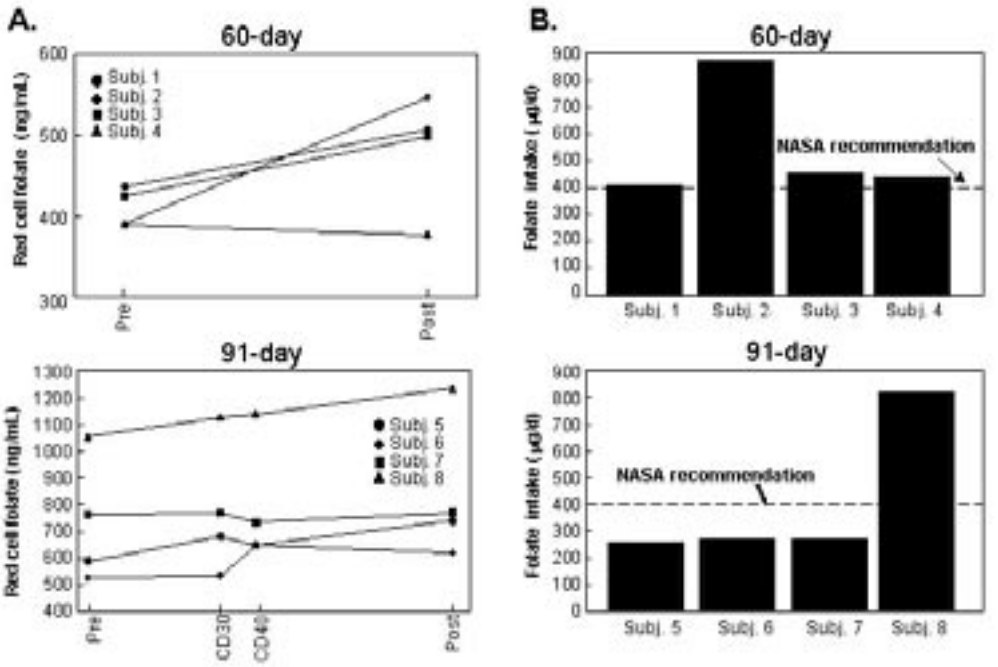

Figure 5.1-7 *Red blood cell folate concentration (Panel A) and dietary folate intake determined from weighed food records (Panel B) for the 60-day and 91-day chamber tests*

Discussion

The study described here provided a valuable opportunity to test a nutritional assessment profile and a unique food frequency questionnaire in an environment similar to that found on a space station, without the constraints of an actual space mission. The results indicate that a specially designed food frequency questionnaire can be used to reliably estimate individual dietary intake. These studies confirm that a semiclosed food system can support nutritional requirements over a short period of time (i.e., two to three months).

The comprehensive nutritional status assessment profile described here (with minor modifications) has been implemented by NASA as a medical requirement for extended-duration (i.e., International Space Station) space travelers. The anthropometric, biochemical, clinical, and dietary assessment components each contributes valuable information to the total picture of nutritional status. The intent is to provide a preflight assessment of crew nutritional status to assure optimal status prior to flight, a real-time means of monitoring dietary intake during flight, and a nutritional component for the postflight rehabilitation program.

Inadequate dietary intake is a significant concern during space flight. Skylab crewmembers consumed the amount of energy prescribed (7) due to experimental constraints which required adequate intake. This demonstrated that it is indeed possible to meet the dietary recommendations during space flight. Subjects in the studies provided here consumed adequate amounts of energy and maintained body mass. The FFQ developed and tested here will provide the ability to monitor and make recommendations to the crewmembers about dietary intake while on orbit.

Fluid compartments were unaffected after both chamber studies as determined by isotope dilution methods. ECFV determined using the liquid bromide dose was better tolerated in the 91-day study, however the determinations were higher than expected. ECFV, which is approximately 40% of total body water (6), was 62 ± 4% of measured total body water in the 91-day study compared to 33 ± 5% in the 60-day study. Although ECFV and total body water are typically highly correlated (6), neither the capsule nor liquid forms of the sodium bromide correlated well with total body water measurements (R = 0.42 and 0.18, respectively) in these studies. A previous evaluation of the liquid dosing regimen was conducted with 10 subjects, where ECFV was determined by both bromide dilution and bioimpedance techniques (2). These ECFV measurements were similar (bromide: 20.9 ± 5.1 L, BIA: 20.3 ± 4.5 L) and correlated well with BIA determination of total body water (R = 0.89). These observations suggest that additional modifications may be needed for routine determination of ECFV by bromide dilution.

Bone mineral loss during space flight results in increased urinary crosslink (12) and calcium excretion (9, 10). Hypercalciuria contributes to the increased risk of renal stone formation associated with space flight (14). Vitamin D is of concern during space flight due to absence of endogenous production related to the lack of ultraviolet light exposure (4) and also due to its importance in bone and calcium metabolism. Vitamin D stores were decreased in the 91-day chamber study but were unchanged in the 60-day study.

Iron status appeared to decline during the course of the studies (e.g., decreased ferritin, and a tendency for decreased hemoglobin and hematocrit). This occurred despite relatively high iron intakes. However, in examining individual diet records for the source of this iron, much of the intake was associated with (low bioavailability) fortified cereals. Conversely, limited intakes of other micronutrients may be of concern when individuals are dependent upon a closed or semiclosed food system for truly extended periods (i.e., years).

Although nutritional status was generally adequate in the 60-day and 91-day tests, micronutrient status is of concern in a semiclosed food system. Three subjects in the 91-day test had inadequate folate intakes, and three subjects in each test had inadequate vitamin D intakes. However, 10 days of the vegetarian BIO-Plex diet did not affect any of the biochemical indices examined during the 90-day test.

SIGNIFICANCE

This study was important for evaluating the space flight food frequency questionnaire and also for assessing a food system similar to that planned for the International Space Station. The International Space Station food system is still in development, and the data collected here will be important in further defining and refining this system in order to assure optimal health during long-duration flights.

Acknowledgments

We thank the Lunar-Mars Life Support Test Project crewmembers for their time, effort, and dedication to the success of these studies. The Nutritional Status Assessment profile reported here was defined and refined with the help of a panel of extramural experts. We gratefully acknowledge the advice and guidance offered by Gladys Block, Stephen Coburn, Michael Holick, Carl Keen, Donald McCormick, James Olson, and Connie Weaver.

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