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Nutritional Status Assessment During the Phase IIA and Phase III Lunar/Mars Life Support Test Project

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Introduction

Nutrition is a critical concern for extended-duration space missions (Smith and Lane, 1999). Loss of body weight is a primary consequence of altered nutrition, and is frequently observed during space flight (Smith and Lane, 1999). Other existing dietary concerns for space flight include excessive intakes of sodium and iron, and insufficient intakes of water and vitamin D (Smith and Lane, 1999). Furthermore, dependence on closed or semi-closed 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. We report here a study which was designed to assess nutritional status before, during, and after the 60-d and 91-d 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 (*Block95*, Block et al., 1994) 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-d Phase IIA study, and weeks 1, 4, 6, 9, and 12 of the 91-d Phase III study. The FFQ 7-d was administered once per week on weeks 1, 3, 6, and 8 of the 60-d study, and weeks 2, 5, 8, 10, and 13 of the 91-d study. Five-day weighed food records were completed on weeks 2 and 5 of the 60-d study and on weeks 3, 7 and 11 of the 91-d 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 pre-chamber 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-d 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-d 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-h, the other for the past 7 days. Responses for these questionnaires were hand-written.

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 semi-closed, space-like food system on crew nutritional status. Specific tests and analytical methods are shown in Table 1, and are described in more detail in JSC#28566 (Nutritional Status Assessment for Extended Duration Space Flight, Rev. 1, 2000), and have been reported elsewhere (Smith et al., *in review*).

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 (Schoeller et al., 1982). Sodium bromide

was used to measure extracellular fluid volume (ECF) (Drongowski, 1982). Body weight was determined weekly using a standard scale.

Biosample collection

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

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-d chamber study, a total of 52 mL of blood was collected over approximately 70 days. For the 91-d chamber study, a total of 98 mL of blood was collected over approximately 100 days.

Urine was collected for two 24-h periods before, every day during, and two 24-h periods after the chamber studies; pre- and post-chamber urine collections began on the day of blood collection. Complete urine analysis was conducted once (on CD32) during the 60-d study, and three times (CD30, CD40, CD60) during the 91-d 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 2-to-3 daily exchanges through the airlock. Urine samples were processed in the laboratory daily, 24-h 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-d, FFQ 7-D, Weighed Records), and the dependent variables were the nutrients of interest. Pre-chamber dietary intake data are presented, but these were not included in the statistical analyses, as the differences between pre-chamber and in-chamber intakes were not a primary research question.

Biochemical analyte data for the 60-d study were analyzed using paired t-tests, except when in-chamber analyses were available. In these cases, and for the 91-d chamber study, data were analyzed using repeated-measures analysis of variance. The class variable was study phase (pre-chamber, in-chamber, post-chamber), and dependent variables were the indices measured. This analysis identified effects of the semi-closed 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.

Results

Results of the dietary intake studies are shown in **Table 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 2% of their pre-test values.

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

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

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

Body weight did not change during the chamber studies (**Figure 1**). Markers of lean body mass, urinary creatinine (**Figure 3**) and 3-methylhistidine (data not presented), were unchanged during the chamber studies. No changes in total body water were observed in either chamber study (**Figure 2**). Extracellular fluid volume (ECFV) was measured using a 1.2 g dose of sodium bromide in capsule form for the 60-d 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 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-d study. This form of the dose was better tolerated, and ECFV was similarly unaffected during the longer chamber study (**Figure 2**).

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

There was a steady decline in serum 25-hydroxyvitamin D concentrations noted throughout the 91-d study, with final concentrations being significantly lower than pre-chamber values (**Table 4, Figure 5**). There was a tendency for both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations to decline in both studies (**Figure 5**). Vitamin D intake (**Figure 5**) was below the NASA recommendation of >10 mg/day in 6 of the 8 subjects, although dietary vitamin D intake was higher in the 60-d study compared to the 91-d study (**Figure 5**). There was also a small but

statistically significant decline in serum calcium at CD30, although all data during the 91-d study were within clinical normal ranges (**Table 4**). Bone-specific alkaline phosphatase was increased at the end of the 60-d study, but not the 91-day study (**Table 4**). Other indices of bone and calcium metabolism were unchanged (**Table 4**).

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

Folate status, as assessed by the concentration of RBC folate, increased by more than 16% in 3 subjects during the 60-d study, and increased by more than 17% in 3 subjects during the 91-d study (**Figure 7a**). Folate intake, as determined during the weighed diet sessions, was generally above standard recommendations (**Figure 7b**).

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

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 semi-closed food system can support nutritional requirements over a short period of time (i.e., 2-3 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 contribute 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 (Rambaut et al., 1977) 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 food frequency questionnaire 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-d study, however the determinations were higher than expected. ECFV, which is approximately 40% of total body water (Oh and Uribarri, 1999), was 62±4% of measured total body water in the 91-d study compared to 33±5% in the 60-d study. Although ECFV and total body water are typically highly correlated (Oh and Uribarri, 1999), 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 (Davis-Street et al., 2001). 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 calcium excretion (Smith et al., 1999, Smith et al., 1977). Hypercalciuria contributes to the increased risk of renal-stone formation associated with space flight (Whitson et al., 1997). Vitamin D is of concern during space flight due to absence of endogenous production related to the lack of ultraviolet light exposure (Holick, 1996), and also due to its importance in bone and calcium metabolism. Vitamin D stores were decreased in the 91-d chamber study, but were unchanged in the 60-d 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 semi-closed food system for truly extended periods (i.e., years).

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

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.

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Table 1. Analytical methods used for biochemical analyses.[1]

Protein status	
Retinol Binding Protein (S)	radial immunodiffusion
Transthyretin (S)	nepholometry
Protein Electrophoresis (S)	electrophoresis
3-methylhistidine (U)	ion exchange chromatography
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
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
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)[2]	antibody isolation, ICP-MS

Table 1. Continued.

Antioxidant status		Mineral status	
Total Antioxidant Capacity (S)	spectrophotometry	Iron (S)	ICP-MS
Superoxide Dismutase (WB)	spectrophotometry	Zinc (S,U)	ICP-MS
Glutathione Peroxidase (WB)	spectrophotometry	Selenium (S,U)	ICP-MS
Malondialdehyde (S)	spectrophotometry	Iodine (S,U)	ICP-MS
4-OH-alkenal (S)	spectrophotometry	Phosphorus (U)	spectrophotometry
8-OH deoxyguanosine (U)	HPLC	Magnesium (U)	spectrophotometry
Fat soluble vitamin status		**General**	
Retinol (S)	HPLC	Aspartate aminotransferase (S)	enzymatic rate reaction
Retinyl palmitate (S)	HPLC	Alanine aminotransferase (S)	enzymatic rate reaction
β-carotene (S)	HPLC	Sodium (S)	ISE
α-carotene (S)	HPLC	Potassium (S)	ISE
Serum phylloquinone (S)	HPLC	Chloride (S)	ISE
α-tocopherol (S)	HPLC	Cholesterol (S)	spectrophotometry
γ-tocopherol (S)	HPLC	Triglyceride (S)	spectrophotometry
γ-carboxyglutamic acid (U)	HPLC	Creatinine (S,U)	spectrophotometry
tocopherol:lipid ratio (S)	calculation		

[1] Sample types are indicated in parentheses, S = serum or plasma, WB = whole blood or erythrocytes, U = urine. RBC = Red blood cells.

[2] Additional details are included in methods section for this test.

[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 sensitive electrode, RIA = radioimmunoassay.

Table 2. Dietary intake data.

	60-d Chamber Study				91-d Chamber Study			
	Pre[1]	FFQ 24-h	FFQ 7d	Weighed Records	Pre[1]	FFQ 24-h	FFQ 7d	Weighed Records
Energy								
MJ/d	9.38±1.45 [2]	10.51±0.45	9.97±0.65	10.76±0.43	8.57±2.03	8.72±0.46	7.41±0.32	9.20±0.83
kcal/d	2243±347	2511±108	2384±156	2571±102	2048±485	2083±109	1770±77	2199±198
Protein, g/d	104.9±18.9	80.5±4.6	70.4±6.3	75.8±3.7	84.4±21.9	59.4±2.5	51.8±4.3	58.5±3.2
Calcium, mg/d	907±185	910±145[a]	943±127[ab]	1120±112[b]	1116±374	1052±322	937±349	1126±162
Iron, mg/d	18.0±0.4	19.4±2.7[a]	23.6±4.3[ab]	26.7±4.2[b]	16.4±3.9	21.0±7.5	17.2±5.8	20.1±5.7
Sodium, mg/d	3603±580	4100±347	3752±287	3890±330	3252±902	3845±267[a]	2876±287[b]	3332±170[ab]
Water, ml/d	[3]	1689±232[a]	1953±277[b]	2430±232[c]	[3]	2730±721	2626±747	3217±471

[1] Pre-chamber data were not included in statistical analyses.

[2] Data are mean ± SEM, and represent the average of the 4 individual subject averages for each assessment technique. For each study, data in columns with different superscripts are significantly ($p<0.05$) different.

[3] Data not available, the pre-chamber questionnaire was not designed to estimate water intake.

Table 3. Hematological and iron status indices.

	60-d Chamber Study		91-d 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 (% PCV)	44±5	42±4	39±1	38±3	37±2	37±1
Mean Corpuscular Vol (fl)	93±3	92±3	90±4[ab]	90±3[ab]	91±3[a]	89±4[b]
Serum Ferritin (ng/ml)	119±20	98±31[2]	77±57[a]	68±53[ab]	66±56[ab]	49±36[b]
Ferritin Iron						
ng Fe/ml	20.7±6.2	16.6±4.5	[3]	[3]	[3]	[3]
% saturation	17.5±5.1	17.5±4.9				
Transferrin (mg/dl)	227±20	222±35	273±37	253±22	253±27	273±26
Transferrin receptors (µg/ml)	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 columns with different superscripts are significantly ($p<0.05$) different.

[2] $p=0.054$

[3] Analyses not available.

Table 4. Serum calcium and bone metabolism markers.

	60-d Chamber Study		91-d Chamber Study			
	Pre	Post	Pre	CD30	CD40	Post
Calcium						
Total (mmol/L)	2.54±0.06 [1]	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 (pg/ml)	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, U/L	50±10	51±12	59±13	66±21	67±19	63±19
Bone Specific (µg/L)	10.8±2.2[a]	14.4±3.8[b]	9.4±3.3	9.6±5.2	9.7±5.3	9.3±5.0
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 columns with different superscripts are significantly ($p<0.05$) different.

Table 5. General chemistry indices.

	60-d Chamber Study		91-d Chamber Study			
	Pre	Post	Pre	CD30	CD40	Post
Total protein (g/L)	72±3 [1]	69±1	71±4[a]	65±4[b]	65±5[b]	68±4[ab]
Albumin (g/dL)	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)	106±18	97±9	8±9	80±18	8±18	71±18
Cholesterol (mmol/L)	4.53±0.78	4.27±0.85	4.58±0.93	4.63±1.11	4.19±0.98	4.58±1.32
Triglycerides (mmol/L)	0.73±0.25	0.87±0.12	0.89±0.68	0.94±0.44	1.06±0.64	0.95±0.75
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±1	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 columns with different superscripts are significantly ($p<0.05$) different.

[2] Analyses not available.

Table 6. Vitamin status antioxidant/oxidative damage indices.

	60-d Chamber Study			91-d Chamber Study				
	Pre	CD32[1]	Post	Pre	CD30	CD40	CD60[2]	Post
Transaminase (% activation; vitamin B_6)	113±13		121±18	89.6±11.8	93.8±20.1	95.2±18.5		88.3±11.0
Glutathione Reductase (% activation; riboflavin)	17.8±6.5		10.9±1.5	31.6±24.8	32.8±29.3	28.9±20.2		25.2±18.7
RBC Superoxide Dismutase (U/g Hb)	592±40 [2]		659±43	986±143	943±122	986±90		1050±92
RBC Glutathione Peroxidase (U/g Hb)	26.3±3.1		25.2±1.9	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)	1.13±0.09		1.18±0.13	1.17±0.08	1.10±0.10	1.13±0.11		1.23±0.13
8-OH-2-deoxyguanosine (μg/g creatinine)	2.90±0.36	2.96±1.26	3.00±0.84	3.43±0.79	3.35±1.07	3.11±1.03	3.45±1.25	3.08±1.23

[1] Urine samples were collected and analyzed at CD32 of the 60-d study, and on CD60 of the 91-d study, however, blood samples were not.

[2] Data are mean ± SD. For each study, data in columns with different superscripts are significantly ($p<0.05$) different.

Figures

Figure 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 pre-chamber body weight.

Figure 2. Fluid compartments (TBW, ECF) for the 60-day and 91-day chamber tests. Data are expressed for each individual as a percentage of their body weight.

Figure 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 pre-chamber data.

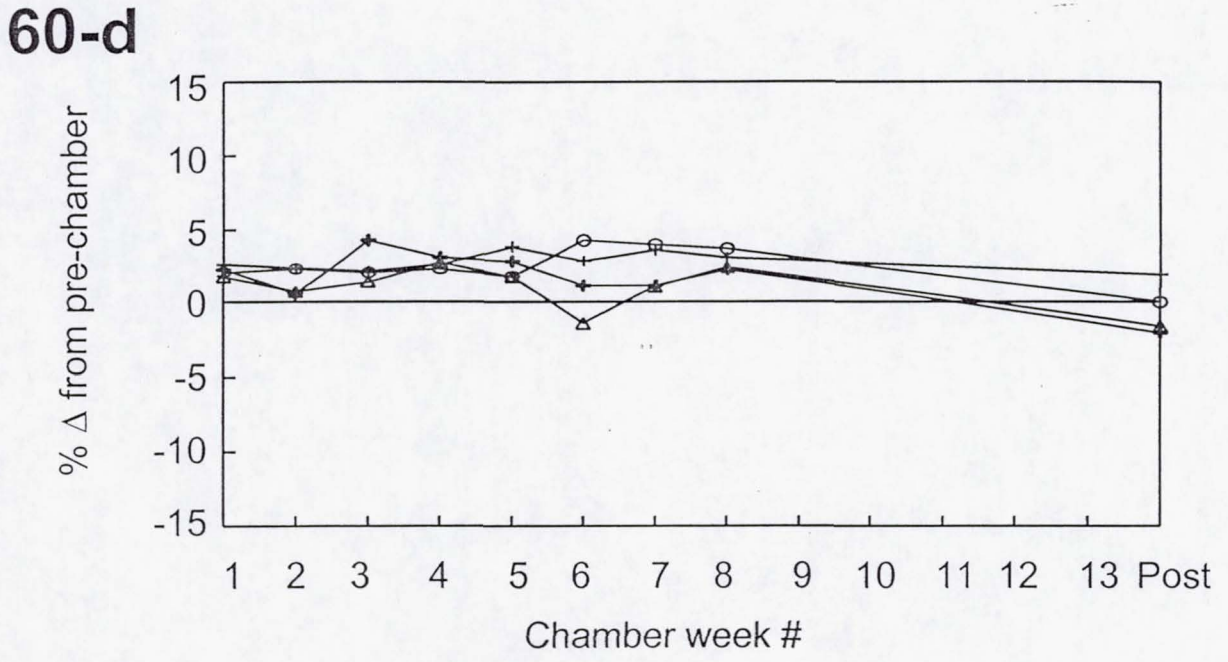
Figure 4. Serum ferritin concentration (Panel A) and dietary iron intake determined from weighed food records (Panel B) for the 60-day and 91-day chamber tests.

Figure 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.

Figure 6. Urine collagen crosslink excretion for the 60-day and 91-day chamber tests. Data are shown for n-telopeptide (Panel A), pyridinium crosslinks (Panel B), and deoxypyridinoline (Panel C).

Figure 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.

Figure 1


60-d
% Δ from pre-chamber
15
10
5
0
-5
-10
-15
1 2 3 4 5 6 7 8 9 10 11 12 13 Post
Chamber week #

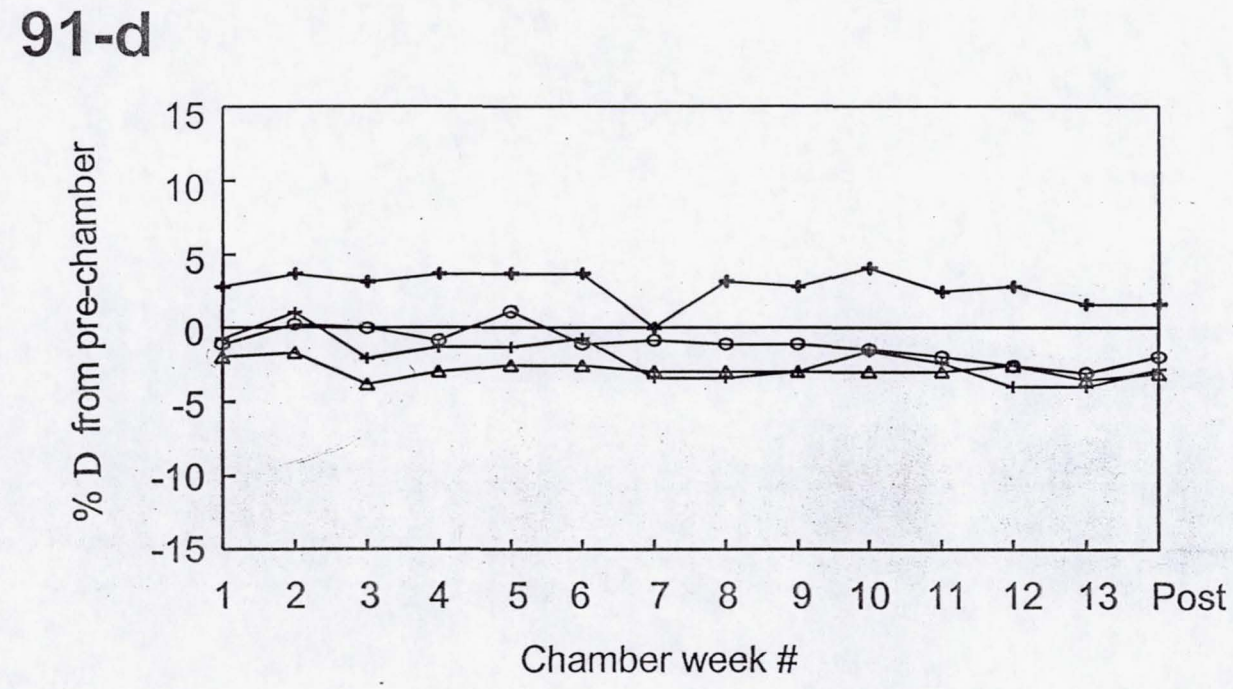
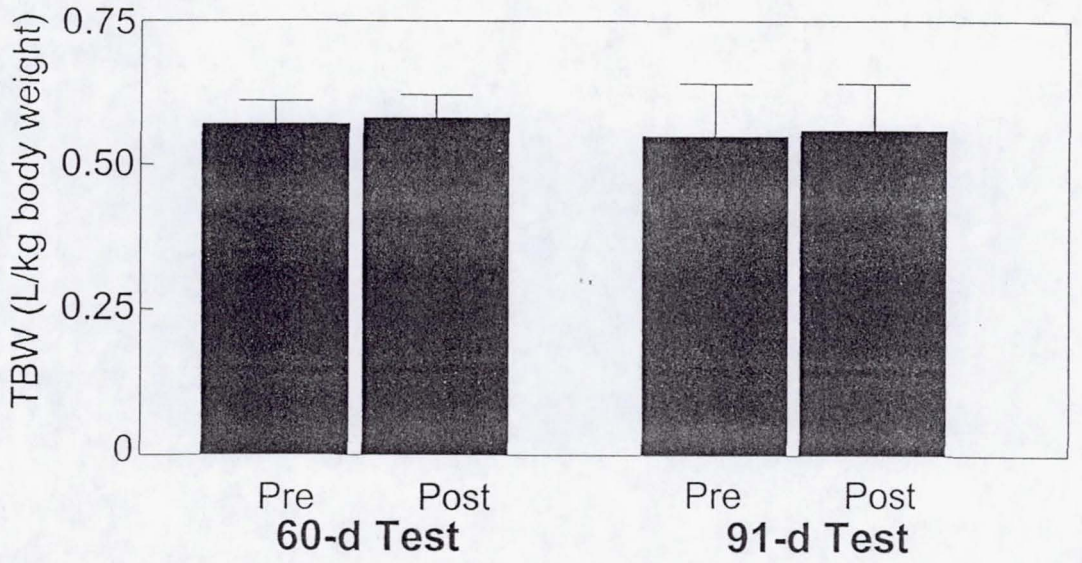

91-d
% D from pre-chamber
15
10
5
0
-5
-10
-15
1 2 3 4 5 6 7 8 9 10 11 12 13 Post
Chamber week #

Figure 2


TBW (L/kg body weight)
0.75
0.50
0.25
0
Pre
Post
60-d Test
Pre
Post
91-d Test

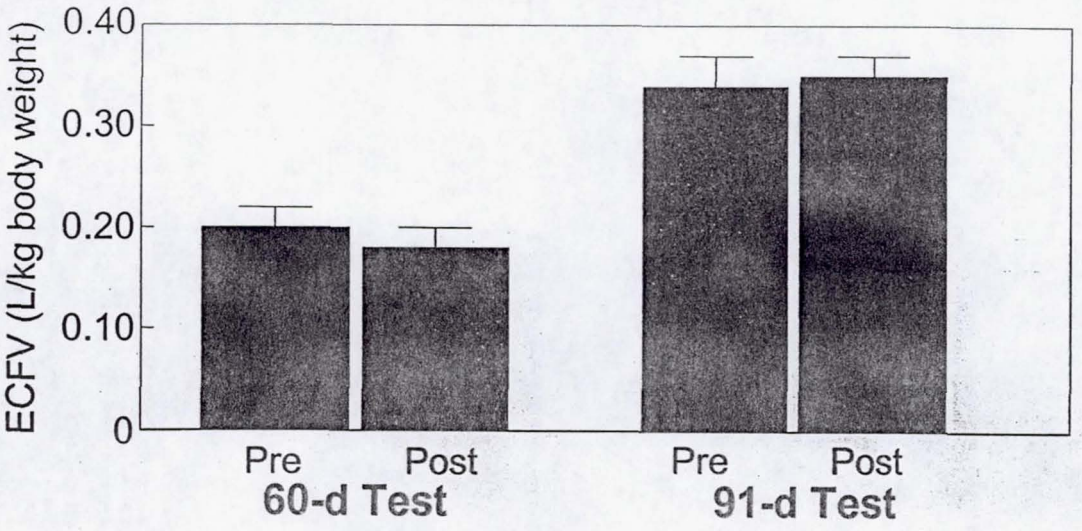

ECFV (L/kg body weight)
0.40
0.30
0.20
0.10
0
Pre
Post
60-d Test
Pre
Post
91-d Test

Figure 3

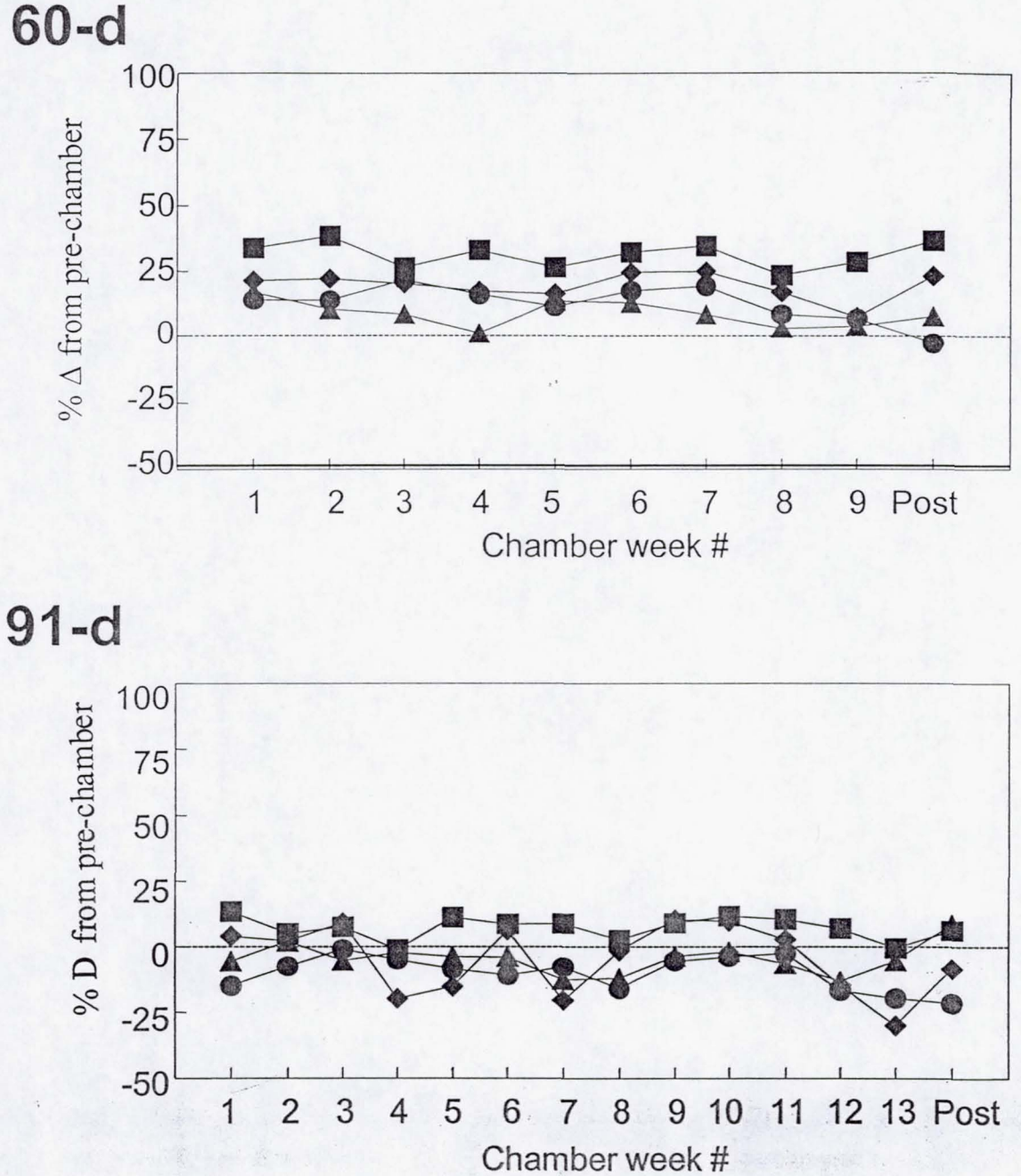

60-d
100
75
50
25
0
-25
-50
% Δ from pre-chamber
1 2 3 4 5 6 7 8 9 Post
Chamber week #
91-d
100
75
50
25
0
-25
-50
% D from pre-chamber
1 2 3 4 5 6 7 8 9 10 11 12 13 Post
Chamber week #

Figure 4

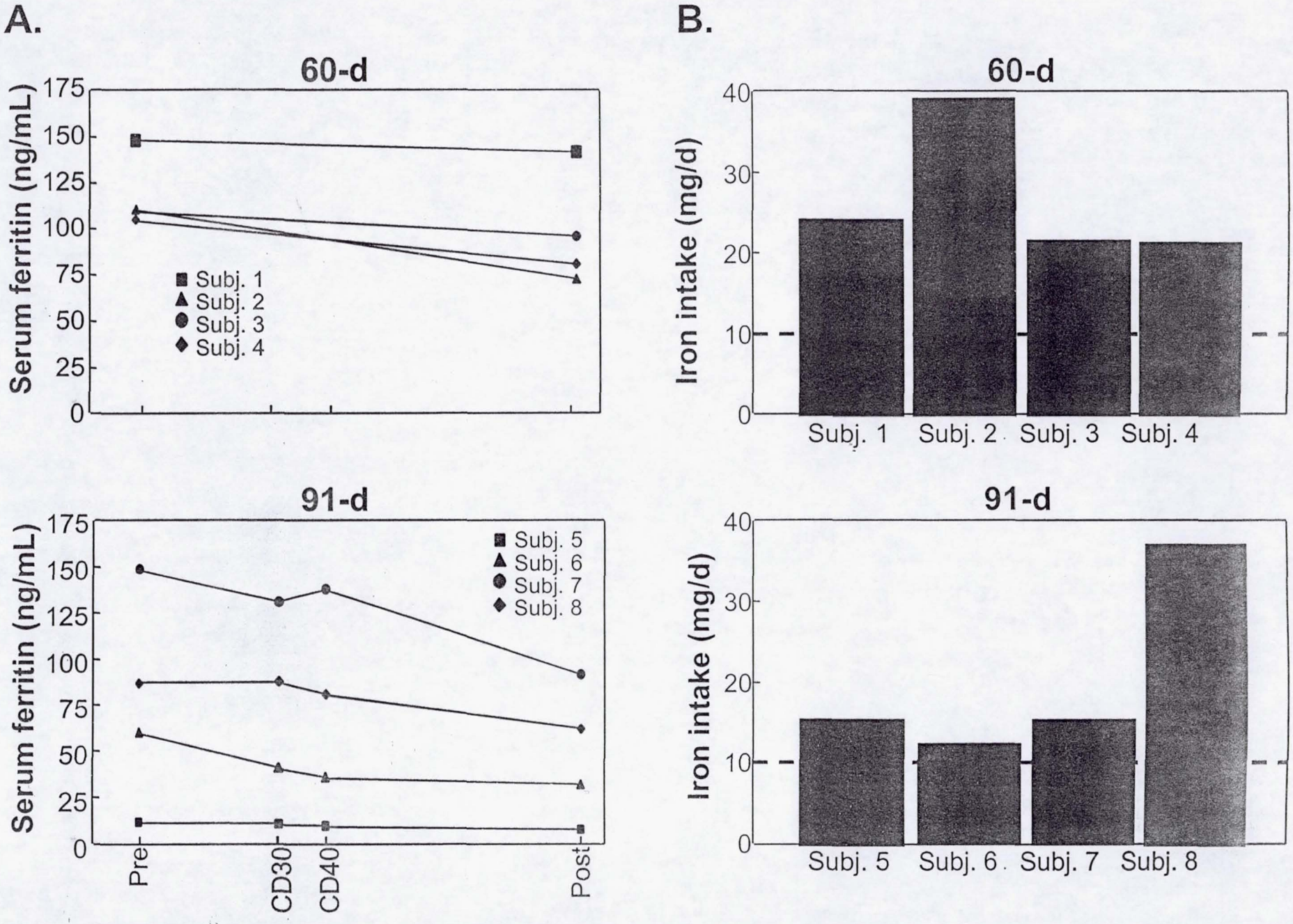

A.
60-d
Serum ferritin (ng/mL)
175
150
125
100
75
50
25
0
Subj. 1
Subj. 2
Subj. 3
Subj. 4
91-d
Serum ferritin (ng/mL)
Subj. 5
Subj. 6
Subj. 7
Subj. 8
Pre
CD30
CD40
Post
B.
60-d
Iron intake (mg/d)
40
30
20
10
0
Subj. 1
Subj. 2
Subj. 3
Subj. 4
91-d
Iron intake (mg/d)
Subj. 5
Subj. 6
Subj. 7
Subj. 8

Figure 5

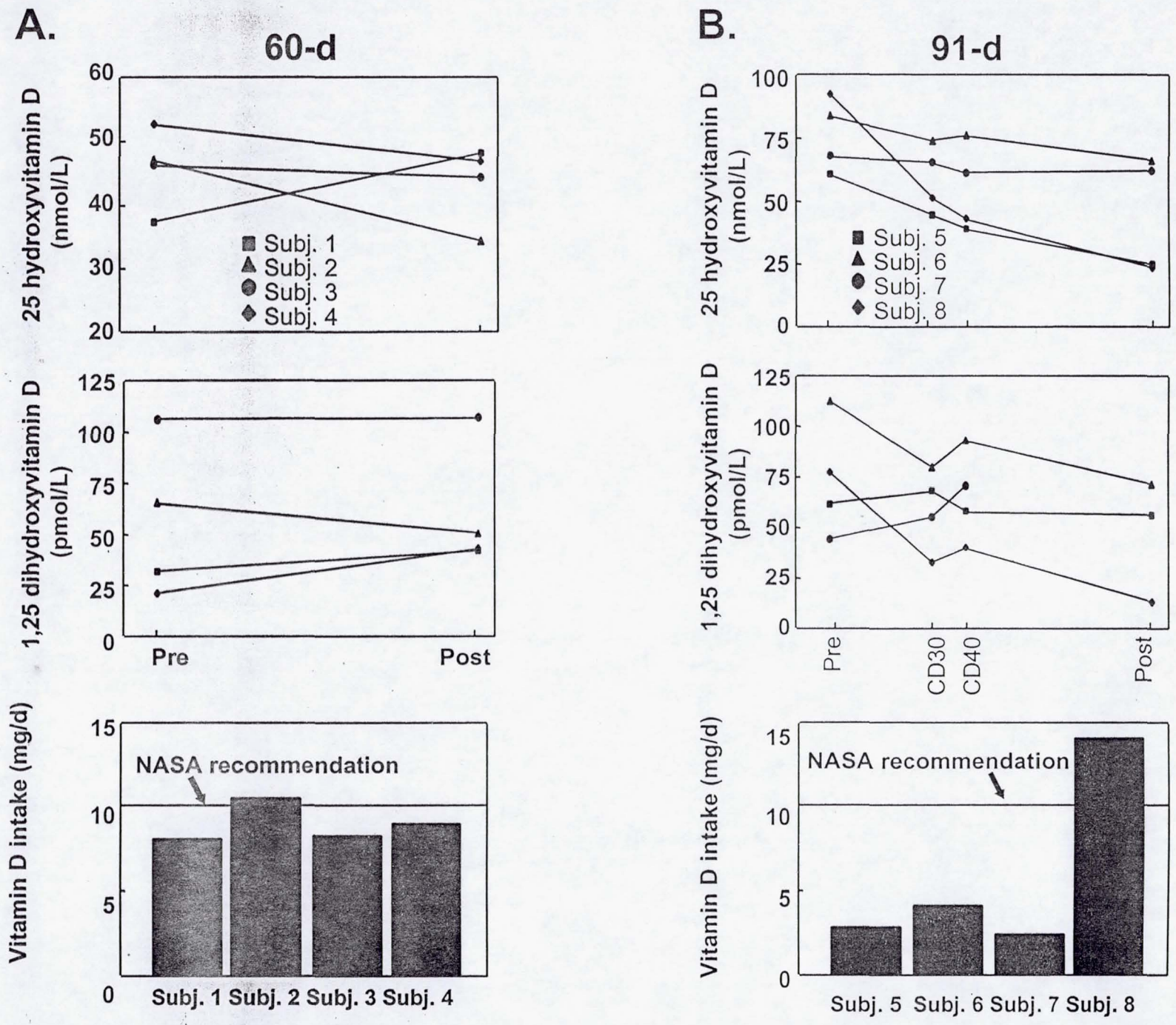
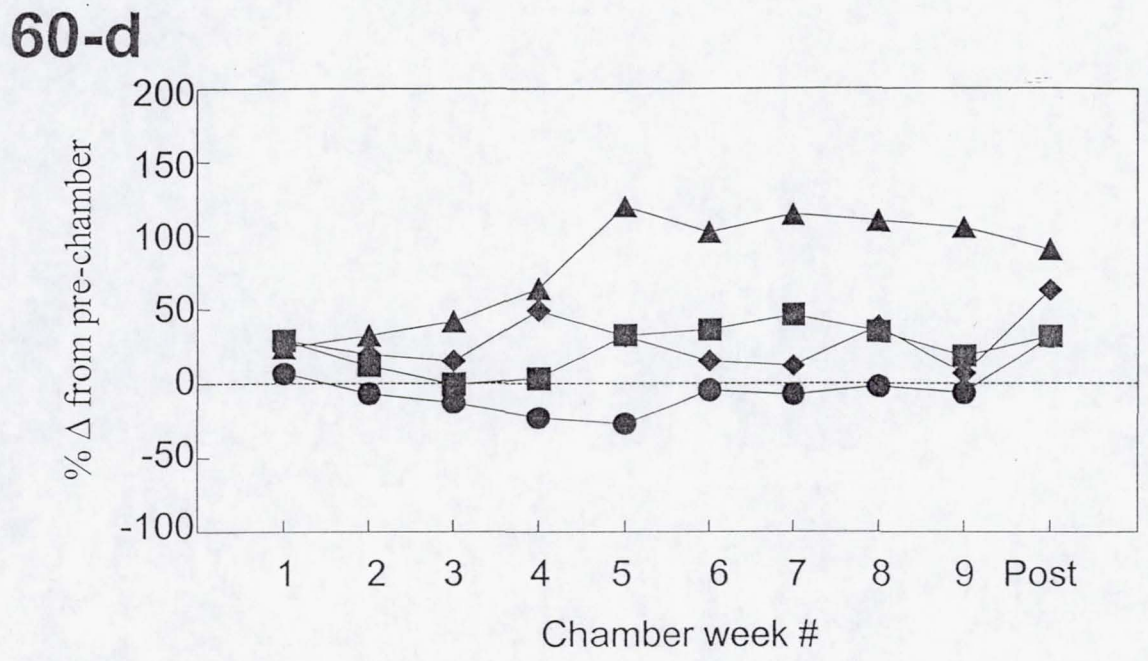

A.
60-d
25 hydroxyvitamin D (nmol/L)
60
50
40
30
20
Subj. 1
Subj. 2
Subj. 3
Subj. 4
1,25 dihydroxyvitamin D (pmol/L)
125
100
75
50
25
0
Pre
Post
Vitamin D intake (mg/d)
15
10
5
0
NASA recommendation
Subj. 1 Subj. 2 Subj. 3 Subj. 4
B.
91-d
25 hydroxyvitamin D (nmol/L)
100
75
50
25
0
Subj. 5
Subj. 6
Subj. 7
Subj. 8
1,25 dihydroxyvitamin D (pmol/L)
125
100
75
50
25
0
Pre
CD30
CD40
Post
Vitamin D intake (mg/d)
15
10
5
0
NASA recommendation
Subj. 5 Subj. 6 Subj. 7 Subj. 8

Figure 6A


60-d
200
150
100
50
0
-50
-100
% Δ from pre-chamber
1
2
3
4
5
6
7
8
9
Post
Chamber week #

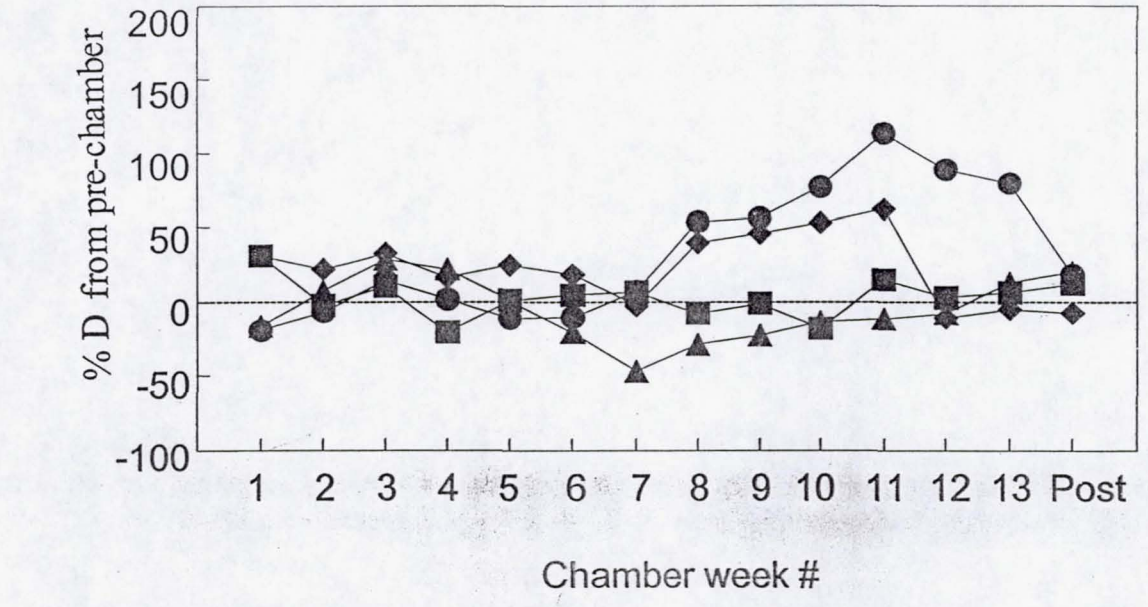

91-d
200
150
100
50
0
-50
-100
% D from pre-chamber
1
2
3
4
5
6
7
8
9
10
11
12
13
Post
Chamber week #

Figure 6B

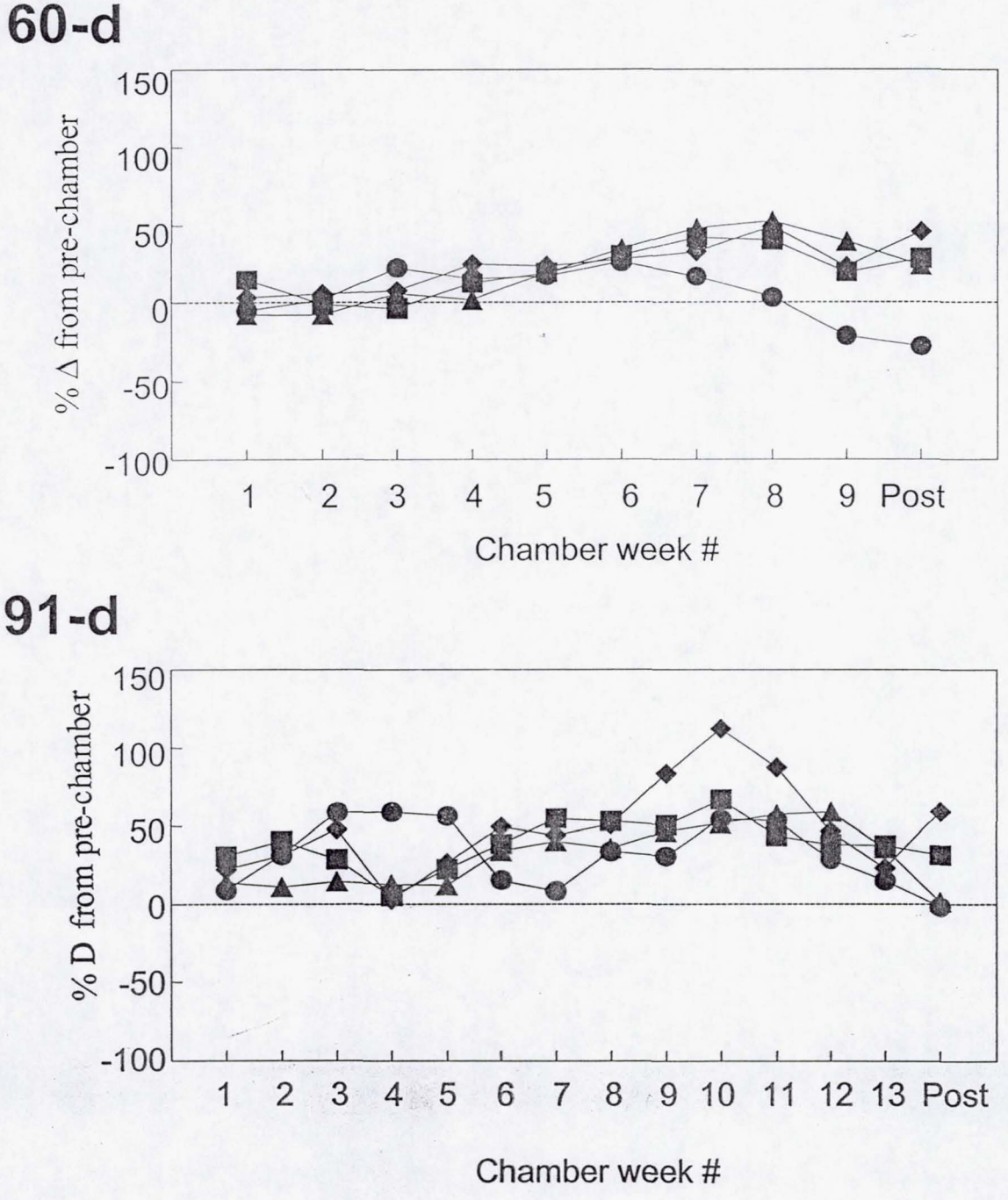

60-d
% Δ from pre-chamber
150
100
50
0
-50
-100
1 2 3 4 5 6 7 8 9 Post
Chamber week #
91-d
% D from pre-chamber
150
100
50
0
-50
-100
1 2 3 4 5 6 7 8 9 10 11 12 13 Post
Chamber week #

Figure 6C

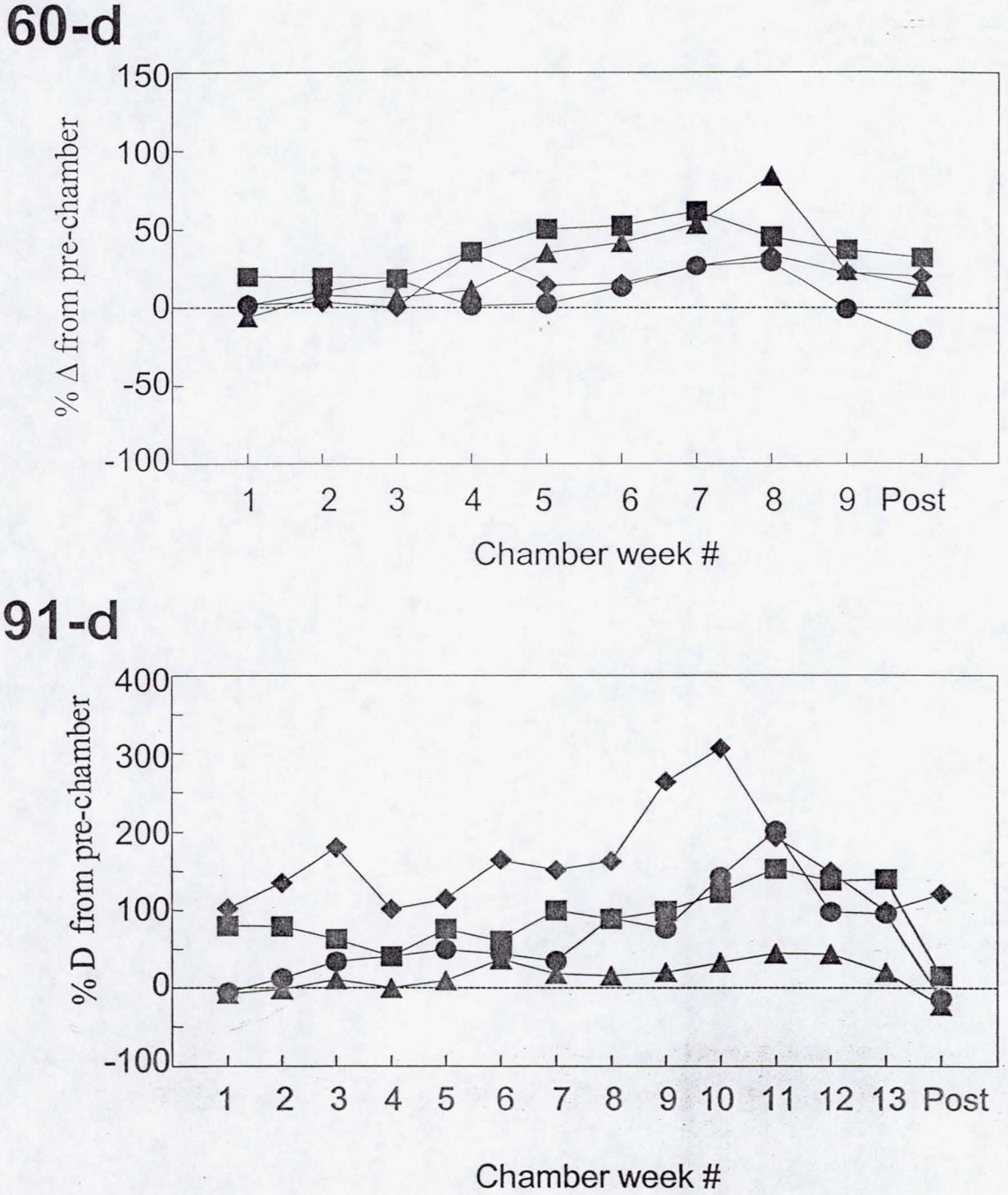
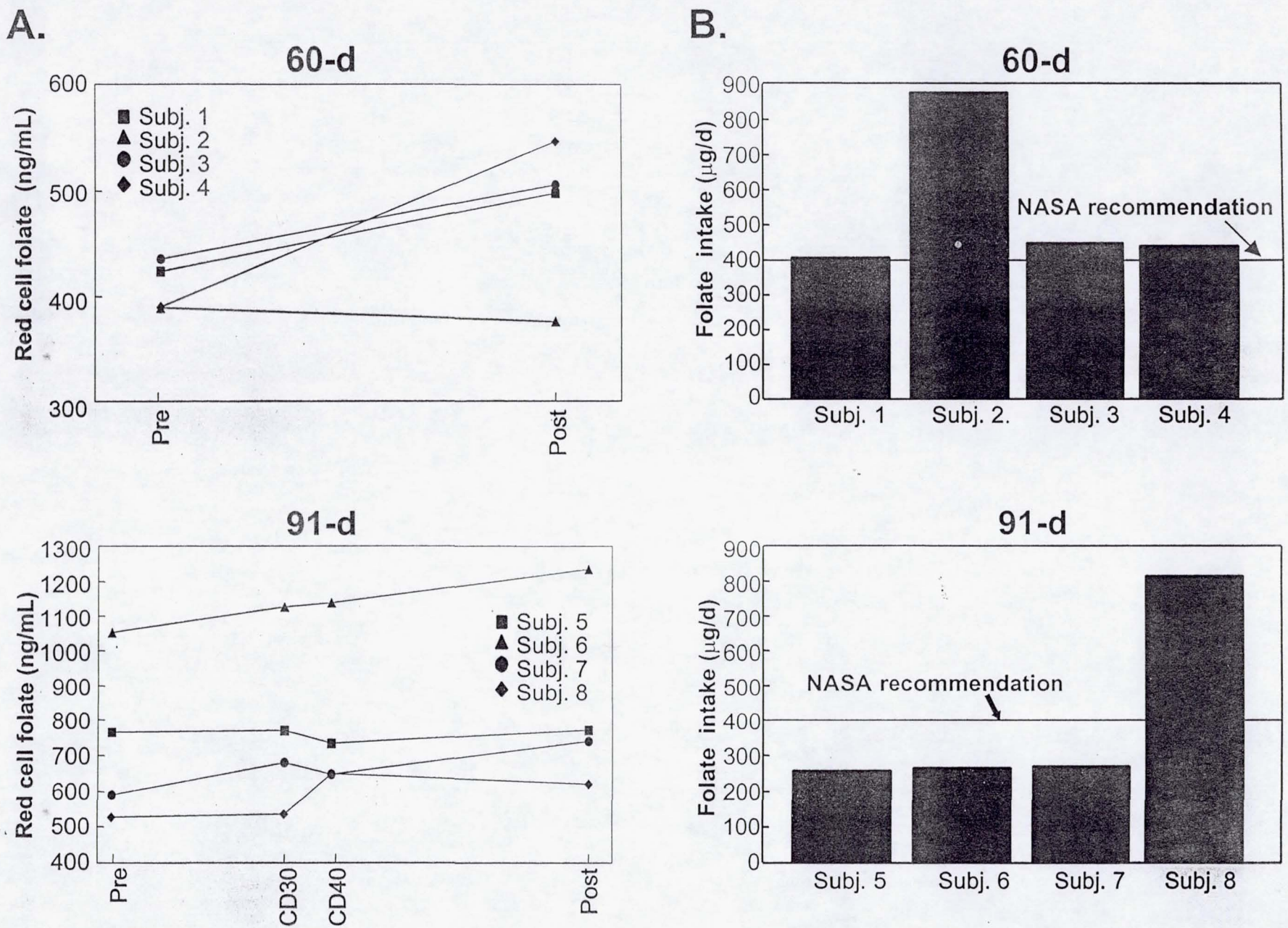

60-d
150
100
50
0
-50
-100
% Δ from pre-chamber
1 2 3 4 5 6 7 8 9 Post
Chamber week #
91-d
400
300
200
100
0
-100
% D from pre-chamber
1 2 3 4 5 6 7 8 9 10 11 12 13 Post
Chamber week #

Figure 7


A.
60-d
Red cell folate (ng/mL)
600
500
400
300
Subj. 1
Subj. 2
Subj. 3
Subj. 4
Pre
Post
B.
60-d
Folate intake (μg/d)
900
800
700
600
500
400
300
200
100
0
NASA recommendation
Subj. 1
Subj. 2.
Subj. 3
Subj. 4
91-d
Red cell folate (ng/mL)
1300
1200
1100
1000
900
800
700
600
500
400
Subj. 5
Subj. 6
Subj. 7
Subj. 8
Pre
CD30
CD40
Post
91-d
Folate intake (μg/d)
900
800
700
600
500
400
300
200
100
0
NASA recommendation
Subj. 5
Subj. 6
Subj. 7
Subj. 8