

Nutritional Status Assessment in Semiclosed Environments: Ground-Based and Space Flight Studies in Humans^{1,2}

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ABSTRACT Adequate nutrition is critical during long-term spaceflight, as is the ability to easily monitor dietary intake. A comprehensive nutritional status assessment profile was designed for use before, during and after flight. It included assessment of both dietary intake and biochemical markers of nutritional status. A spaceflight food-frequency questionnaire (FFQ) was developed to evaluate intake of key nutrients during spaceflight. The nutritional status assessment protocol was evaluated during two ground-based closed-chamber studies (60 and 91 d; $n = 4$ /study), and was implemented for two astronauts during 4-mo stays on the Mir space station. Ground-based studies indicated that the FFQ, administered daily or weekly, adequately estimated intake of key nutrients. Chamber subjects maintained prechamber energy intake and body weight. Astronauts tended to eat 40–50% of WHO-predicted energy requirements, and lost >10% of preflight body mass. Serum ferritin levels were lower after the chamber stays, despite adequate iron intake. Red blood cell folate concentrations were increased after the chamber studies. Vitamin D stores were decreased by > 40% on chamber egress and after spaceflight. Mir crew members had decreased levels of most nutritional indices, but these are difficult to interpret given the insufficient energy intake and loss of body mass. Spaceflight food systems can provide adequate intake of macronutrients, although, as expected, micronutrient intake is a concern for any closed or semiclosed food system. These data demonstrate the utility and importance of nutritional status assessment during spaceflight and of the FFQ during extended-duration spaceflight. *J. Nutr.* 131: 2053–2061, 2001.

KEY WORDS: • *weightlessness* • *food-frequency questionnaire* • *dietary intake* • *humans*

Nutrition is a critical concern for extended-duration space missions (1,2) and is critical to maintaining crew health, safety and productivity. Monitoring of nutritional status before, during and after long (>30 d) space missions will help provide optimal nutritional support of astronauts.

Loss of body weight is a primary consequence of altered nutrition and is frequently observed during spaceflight (1,2). Other current dietary concerns for spaceflight include excessive intakes of sodium and iron, and insufficient intakes of water and vitamin D (1,2). Additionally, long-term 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, either in low Earth orbit (e.g., International Space Station) or beyond (e.g., missions to Mars).

Dietary intake during spaceflight is often inadequate, with

crew members typically consuming 60–70% of predicted energy requirements (1,2). The ability to identify crew members who are not eating or drinking enough while on orbit is necessary to mitigate undernutrition. Spaceflight research often includes detailed recording of all foods consumed. Although this yields extremely accurate data, this method requires considerable time and effort, and thus is not suitable for routine medical monitoring during spaceflight.

Many of the physiologic changes that occur during flight have nutritional implications (2). Loss of bone and muscle tissue, fluid shifts (3) and hematologic alterations (e.g., reduced RBC mass) occur in astronauts. Environmental factors such as radiation also play an important role in the ability of humans to live and work in space.

To ensure adequate nutritional support for astronauts, we developed a comprehensive nutritional assessment profile. It includes pre- and postflight assessment of a battery of biochemical markers of nutritional status, and a limited in-flight protocol, including dietary intake assessment and body mass measurement. The ground-based assessments were intended to be comprehensive (covering essentially all nutritional components, e.g., body composition, musculoskeletal status, vitamins or minerals). Due to resource constraints (e.g., crew time, freezer volume) on orbit, the in-flight assessment was limited

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to a dietary intake assessment and body mass determinations. The dietary intake assessment was implemented in the form of a food-frequency questionnaire (FFQ).⁴ The FFQ was designed to provide a quick and easy, yet reasonably accurate method for crew members to provide dietary intake information to the ground. It was targeted at specific nutrients (energy, protein, fluid, sodium, iron and calcium) to reduce complexity of the questionnaire.

We report here results from two types of studies, i.e., ground-based, semiclosed chamber studies (60- and 91-d durations) and spaceflight studies of astronauts residing on the Russian Mir space station (~4-mo durations). The ground studies had the following two key objectives: 1) to assess nutritional status of crew members consuming a space-like food system, and 2) to validate and use an FFQ designed specifically for use with semiclosed spaceflight food systems. The flight studies reported here represent the initial implementation of this nutritional assessment protocol.

SUBJECTS AND METHODS

Two types of studies were conducted, i.e., ground-based, semiclosed chamber studies and flight studies aboard the Mir space station. The semiclosed environment of each provided unique opportunities to examine the effect of a limited food system on dietary intake and nutritional status and to assess and implement means of monitoring dietary intake.

Environment

Chamber studies. Two ground-based studies that involved prolonged (60- and 91-d) stays in an enclosed chamber facility at the NASA Johnson Space Center in Houston were conducted. The cylindrical chamber was 20 ft (6.1 m) in diameter, with three levels, namely, a work/galley area, a mechanical area and living quarters. The primary objective of studies with this chamber was to test regenerative air and water system technology for use on potential planetary missions. A group of ~20 "supplemental" projects was included to maximize return from habitation of the semiclosed chamber environment. These projects tested objectives relevant to spaceflight or confinement, and included psychological studies, in situ training assessments, and sleep and behavioral studies. We report here the results of one such "supplemental" study, which was designed to assess the nutritional effect of a semiclosed space-like food system and validate a dietary intake questionnaire for semiclosed food systems.

Flight studies. These studies were conducted with two astronauts on missions to Mir as part of the NASA Mir Science Program. The missions included launch from and return to Earth on board a U.S. space shuttle and residence for ~4 mo on Mir.

Subjects

Chamber studies. Subjects for the 60-d study were 1 woman and 3 men; subjects for the 91-d study were 2 women and 2 men. The ages of the 5 male subjects ranged from 26 to 36 y, and prechamber body mass ranged from 56.8 to 83.4 kg [body mass index (BMI) = 23.0 ± 3.4 kg/m², mean \pm SD]. The ages of the 3 female subjects ranged from 28 to 41 y, and prechamber body mass ranged from 57.4 to 69.4 kg (BMI = 22.4 ± 3.3 kg/m²). All subjects were required to pass an Air Force Class III physical examination for clearance to participate in the study.

Flight studies. Two men aged 40 to 54 y with preflight body mass in the range from 70.5 to 88.6 kg participated in these studies. These ranges reflect data for all male astronauts ($n = 6$) who resided on Mir as part of the NASA Mir Science Program (see subject confidentiality, below).

Subject confidentiality. Because the number of subjects in these studies is small and their participation in the chamber studies and NASA Mir missions has been highly publicized, additional restrictions are required to maintain subject confidentiality. Specifically, data from the chamber studies are not presented by gender because only one woman participated in the 60-d study. Only two crew members participated in the flight studies. Because individual results are reported here, details of individual subject characteristics are minimized, and data in the figures have been truncated for one subject to eliminate identification of subjects based on flight duration.

All procedures for both the ground-based and flight studies were reviewed by the Johnson Space Center Institutional Review Board to ensure ethical use of human subjects. Informed consent was obtained from all subjects.

Food systems

Chamber studies. The food system for the 60-d study was designed to be similar to that planned for use on the International Space Station. Commercial products comparable to foods on the International Space Station Daily Menu Food List were located in local grocery stores and incorporated into a standardized menu that included fresh, frozen and thermostabilized items. Energy requirements were calculated for each subject based on the WHO equation (4), adjusted for moderate activity (specifically 1.7 for men, 1.6 for women). Macronutrient contents of the standardized menu were calculated using the Daily Nutritional Requirements for Spaceflight (2,5).

A 20-d cycle menu was repeated throughout each chamber test period. Although only foods from the menu were allowed, subjects were not required to eat exactly the planned menu. The menu was adjusted only when an item could not be supplied due to seasonal availability or some other reason. Food preparation equipment for this study consisted of two microwave ovens. A side-by-side refrigerator/freezer was available for food storage.

The food system for the 91-d study was developed in a similar manner, but it was designed to be similar to that planned for use on a planetary (e.g., Moon, Mars) base. Accordingly, during the 91-d study, the 20-d cycle menu consisted of a 50% vegetarian diet defined as ≤ 4 servings of meat/wk. Additionally, an experimental diet was used for 10 d of the 91-d study (d 31–40). It consisted entirely of food items that could be produced in a regenerative food system.

During the 91-d study, food preparation equipment included a combination microwave/convection oven, a bread-making machine, a blender and a portable stove-top burner. A side-by-side refrigerator/freezer was also available for food storage.

Flight studies. The food system used on board Mir consisted of about half U.S. space foods and half Russian space foods (6). Because refrigeration was not available for food items, all foods were shelf-stable—dehydrated, thermostabilized (e.g., canned) or in natural form. Although a 6-d cycle menu was planned, actual eating patterns during flight rarely followed the scheduled menu. About once per mission, a cargo vehicle arrived with a limited number of fresh food items (e.g., fruits, vegetables). These items typically are edible for < 1 wk.

Dietary intake assessment

Chamber studies. Before entering the chamber, the subjects completed a standard dietary assessment questionnaire (7) to assess their usual diet over the past year. During their chamber stay, subjects completed a specialized FFQ (described below) to assess intake over 24-h (24-h FFQ) or 7-d (7-d FFQ) periods. The 24-h FFQ was administered 3 times/wk during wk 4 and 7 of the 60-d study, and wk 1, 4, 6, 9 and 12 of the 91-d study. The 7-d FFQ was administered once per week during wk 1, 3, 6 and 8 of the 60-d study, and wk 2, 5, 8, 10 and 13 of the 91-d study. Five-day weighed food records were completed for wk 2 and 5 of the 60-d study and wk 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 and mineral supplements, and medi-

⁴ Abbreviations used: BMI, body mass index; CD, chamber day; FFQ, food-frequency questionnaire.

cines consumed. A research dietitian (B.L.R.) met with the subjects before the prechamber data collection session to provide training for all diet intake assessment methods.

Three of the 60-d chamber subjects reported occasional use of vitamin and mineral supplements, and one 91-d study subject reported daily supplement use. Intake data herein represent total nutrient intake from the foods consumed as well as supplements.

Flight studies. About 6 mo before flight, crew members completed the same standard dietary assessment questionnaire (7) as the chamber subjects. During the flight, crew members filled out a specialized spaceflight FFQ (see below) once per week, and the data were transmitted to mission control in Moscow via telemetry. The files were then encrypted, and sent to the NASA Johnson Space Center in Houston. A nutrient estimation algorithm was applied to the data, and the resulting nutrient intake information was reported to the flight surgeon.

One subject reported use of a vitamin A, C and E supplement during the preflight study period. During flight, the other subject reported occasional use of a multivitamin and mineral supplement. The intake data presented herein include total nutrient intake from both food and supplements.

Food-frequency questionnaire (FFQ)

Chamber studies. The FFQ used in the chamber was constructed by one of the authors (G.B.) using the key nutrient contents of the >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. (7). Specific nutrients targeted by the FFQ were energy, protein, calcium, sodium, iron and water. Two versions of the chamber FFQ were presented, one asking about dietary intake for the past 24 h, the other asking about the past 7 d. Questionnaire responses for these ground-based studies were handwritten.

Flight studies. The spaceflight FFQ, based on the key nutrient contents of the food items available from the U.S. and Russian space food lists, was constructed by one of the authors (G.B.). Nutrient content of foods was obtained via proximate analysis performed by the NASA Johnson Space Center Water and Food Analytical Laboratory. Nutrients studied were energy, protein, calcium, sodium, iron and water. A computerized FFQ was developed and was included on the laptop computers on board Mir. Completion of this questionnaire required ~10 min/wk.

Biochemical assessment of nutritional status (chamber and flight studies)

A complete biochemical nutritional assessment profile was developed for use with crew members before and after extended-duration space missions. This profile was intended to be comprehensive and to provide information on virtually all aspects of nutritional status (e.g., body composition, bone and muscle markers, vitamins, minerals). Due to technical (e.g., tests not operational at the time) and manuscript length limitations, not all tests are reported herein. A comprehensive data set from these studies will be published in a future NASA technical memorandum.

Most analytical determinations were completed using standard, commercial techniques. Serum total protein (3.0% CV), calcium (3.0% CV), cholesterol (4.5% CV), triglycerides (4.5% CV), electrolytes (sodium, 1.5% CV; potassium, 3.0% CV; chloride, 3.0% CV), aspartate aminotransferase (5.3% CV), alanine aminotransferase (5.3% CV) and total alkaline phosphatase (5.3% CV) were analyzed using a Beckman SYNCHRON CX7 automated clinical chemistry system (Beckman Coulter, Brea, CA). Serum albumin (<5.0% CV) and transthyretin (1.5% CV) were analyzed using the Beckman Appraise and Array 360 instruments, respectively (Beckman Coulter). Urine creatinine (4.5% CV) was analyzed on the Beckman CX3 system (Beckman Coulter).

Hemoglobin (<1.5% CV), hematocrit (calculated) and mean corpuscular volume (< 2% CV) were determined using a Coulter MaxM instrument (Beckman Coulter). Serum ferritin (<10% CV) and transferrin (3.63% CV) were analyzed using the Beckman Access and Array 360 instruments, respectively (Beckman Coulter). Transferrin receptors (5.7% CV) were measured using a commercially available ELISA (Ramco Laboratories, Houston, TX). RBC folate (6.4% CV) was measured using a commercially available radioreceptor assay (Diagnostic Products, Los Angeles, CA).

For the 60-d study and the flight studies, ferritin iron content was also determined by a modified version of the procedure developed by Herbert et al. (8). Briefly, the iron content of ferritin was determined after separation of ferritin from other iron-containing proteins. This was accomplished by the immunoprecipitation of serum ferritin with rabbit antihuman polyclonal antibody (Accurate Scientific, Westbury, NY) bound to immobilized rProtein A cross-linked to agarose beads (RepliGen, Cambridge, MA). Iron was released from the ferritin by digestion with 1 mol/L HNO₃ (GFS Chemicals, Columbus, OH) in a 75°C water bath. The hydrolysate was then diluted with deionized water (Milli-Q UF Water System, Millipore Corp., Bedford, MA) and analyzed for iron content by an Elan 6000 inductively coupled plasma mass spectrometer (Perkin Elmer, Norwalk, CT) equipped with a microconcentric nebulizer (Cetac Technologies, Omaha, NE). The intra-assay CV for this assay was 9.3%, and the interassay CV was 10.5%.

Ionized calcium (1.5% CV) was determined using ion-sensitive electrode techniques (i-STAT, Princeton, NJ). Serum intact parathyroid hormone (5.85% CV) was measured by RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA). Vitamin D metabolites 25-hydroxyvitamin D (9.1% CV) and 1,25-dihydroxyvitamin D (16.2% CV) were also determined using commercially available kits (DiaSorin, Stillwater, MN). Bone-specific alkaline phosphatase (5.6% CV) was measured by ELISA (Metra Biosystems, Palo Alto, CA).

RBC superoxide dismutase (<9% CV), glutathione peroxidase (<9% CV) and serum oxygen-radical absorbance capacity (<7% CV) were measured spectrophotometrically using commercially available kits (Randox Laboratories, Crumlin, Antrim, UK). HPLC techniques (9) were used to determine 8-hydroxy-2'-deoxyguanosine (5.13% CV) in urine.

Biosample collection

Chamber studies. Blood samples were collected before (entry - 6 d) and after (egress + 4 d) the 60-d test. For the 91-d study, blood samples were collected before (entry - 9 d), twice during [immediately before and after the 10-d regenerative food system test, i.e., chamber d 30 (CD30) and 40], and after (egress + 4 d) the chamber stay. Urine was collected for 48 h before, every day during and 2 d after the chamber studies. Pre- and postchamber urine collections began on the day of blood collection. All urine voids were collected during the chamber studies, but few analytes were measured in all samples. However, complete urine analysis was conducted once (on CD32) during the 60-d chamber study and 3 times during the 91-d chamber study (CD30, CD40, CD60).

Blood samples from fasting subjects were collected immediately after awakening, at the same time of day, 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 ~70 d; for the 91-d chamber study, a total of 98 mL of blood was collected over ~100 d.

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 or 3 daily exchanges of equipment and other material through an airlock. Urine samples were processed in the laboratory daily as follows: 24-h pools were created, and aliquots were either analyzed immediately or frozen for batch analysis upon completion of the study.

Flight studies. The nutritional assessment protocol was conducted twice before flight (~6 mo and 2 wk before launch), and within hours of landing after the flight. Blood samples were collected before (twice) and after the flight; urine was collected over two 48-h periods before and one 48-h period after the flight.

TABLE 1

Dietary intake data from the ground-based chamber studies¹

	60-d chamber study				91-d chamber study			
	Pre ²	24-h FFQ	7d FFQ	Weighed records	Pre ²	24-h FFQ	7d FFQ	Weighed records
Energy,								
MJ/d	9.38 ± 1.45	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 ^b	2876 ± 287 ^a	3332 ± 170 ^{ab}
Water, mL/d	³	1689 ± 232 ^a	1953 ± 277 ^b	2430 ± 232 ^c	³	2730 ± 721	2626 ± 747	3217 ± 471

¹ Before subjects' entry into the chamber, intake data were assessed using a standardized questionnaire (7). During the chamber studies, intake was assessed using a specialized food-frequency questionnaire (FFQ) for 1 d (24-h FFQ) or 7 d (7-d FFQ), or by weighing all food items (see Subjects and Methods section for details of the questionnaires and schedules). Data are means ± SEM, and represent the average of the 4 individual subject averages for each assessment technique. For each study, within a row, data with different letter superscripts are significantly ($P < 0.05$) different.

² Prechamber data were not included in statistical analyses.

³ Data not available: the prechamber questionnaire was not designed to estimate water intake.

Blood samples were collected by standard phlebotomy techniques. With the exception of samples collected on the day of landing, all blood samples were collected in the morning after an 8-h fast. Blood samples were processed for individual analytes and stored at -20°C until analysis. Before and after flight, urine voids were collected into individual containers and stored with ice packs or refrigerated until processing, which occurred within 24 h of collection. Twenty-four-hour pools were created, and aliquots were prepared and stored at -20°C until analysis.

Body mass

Pre- and postflight body mass measurements of astronauts and all body mass measurements for the chamber studies were performed using a calibrated scale. During flight, body mass was determined biweekly using the Mir body mass measuring device. Pre- and postchamber and pre- and postflight anthropometric measurements were also completed, although those data are not reported here.

Statistical analysis

Chamber studies. Data are expressed as means ± SD, except in cases in which data represent means of means, for which SEM is used (see table footnotes for indications). Dietary data were analyzed using repeated-measures ANOVA. The class variable was assessment tool (24-h FFQ, 7-d FFQ, weighed records), and the dependent variables were the nutrients. Post-hoc Tukey tests were performed to assess specific differences between sessions. Significance was assigned to differences of $P < 0.05$. Statistical analyses were performed using SigmaStat (SPSS, Chicago, IL). Prechamber dietary intake data are presented, but these were not included in the statistical analyses because the differences between prechamber and in-chamber intakes were not the 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 ANOVA. The class variable was study phase (pre-chamber, in-chamber, postchamber phases), 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. Data from the RBC transketolase assay for thiamin status were not subjected to statistical analysis because this assay is qualitative rather than quantitative.

Flight studies. Because only two crew members participated in the flight studies, statistical analyses were not performed on these data. Data from individual subjects are presented.

RESULTS

Dietary assessment

Chamber studies. Energy and protein intakes were similar for the 3 intake assessment techniques during both studies (Table 1). Week-by-week energy intake data are shown in Figure 1. Body weight did not change during the chamber studies (data not shown).

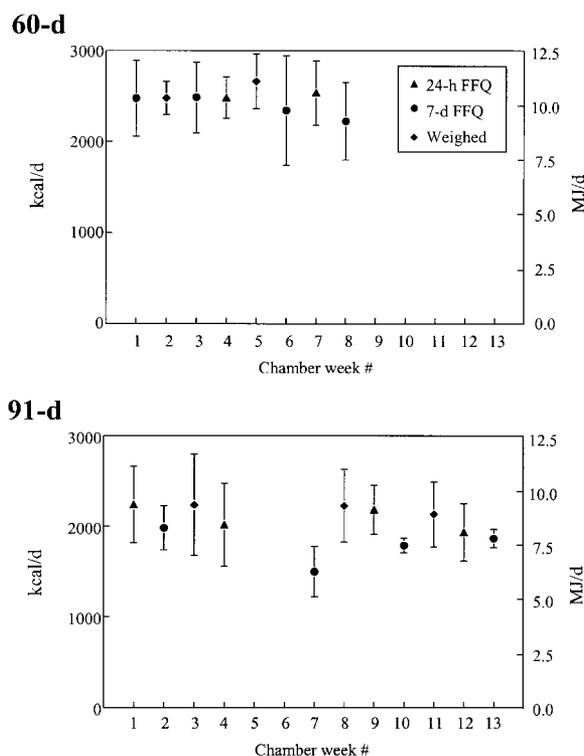


FIGURE 1 Energy intake estimated by various techniques throughout the two chamber studies. Data for each point are means ± SD, $n = 4$ subjects/session. Data were not collected during wk 5 and 6 of the 91-d study because this was during the experimental diet phase (see Subjects and Methods). FFQ, food-frequency questionnaire.

During the 60-d study, questionnaire estimates of calcium and iron intakes were lower than the intakes determined from weighed diet records (Table 1). Subsequent analysis revealed that these differences were related to differences in nutrient content data for two foods (milk and cereal) between the nutrient databases used to analyze the weighed diet records and the FFQ. When the databases were synchronized for nutrient content of these food items, no differences were observed (data not presented). This problem was identified before the 91-d study began and was thus avoided in that study.

Sodium intake assessment yielded similar results for the three techniques during the 60-d chamber study. However, during the 91-d study, the 24-h FFQ sodium intake estimates were higher than those for the 7-d FFQ (Table 1).

Water intake estimates during the 60-d study were different ($P < 0.001$) for all three assessment techniques. Conversely, no differences were observed during the 91-d study.

Flight studies. Energy intake estimated by the spaceflight FFQ was $< 50\%$ of predicted energy requirements for the two crew members studied (Fig. 2). These results are supported by data showing that postflight body weight was $> 10\%$ lower than preflight body weight (Fig. 3). Intake of other nutrients was also below recommended levels for spaceflight (Fig. 2), as would be expected from the reduced energy intake.

Biochemical assessment

Chamber studies. Biochemical results from the chamber studies are shown in Tables 2–4. Iron status tended to be negatively influenced throughout both studies, i.e., values for most hematologic variables (Table 2) tended to decrease. Serum ferritin was significantly ($P < 0.05$) lower at the end of

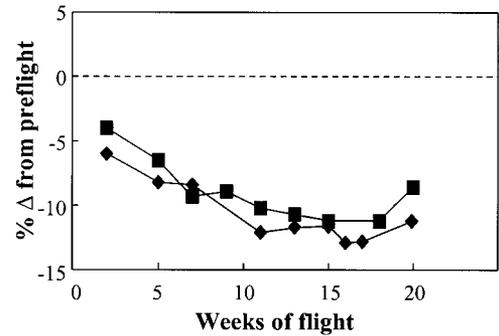


FIGURE 3 Body weight during spaceflight. Data are expressed as mean percentage change from individuals' preflight weights.

the 91-d study than before the subjects entered the chamber; a similar trend was seen ($P = 0.054$) in the 60-d study. Folate levels, as assessed by the concentration of RBC folate did not change ($P = 0.13$) during the 60-d study and increased significantly during the 91-d study (Table 2). Vitamin B-6 and riboflavin markers were unchanged during the chamber studies.

Serum 25-hydroxyvitamin D declined steadily throughout the 91-d study; final concentrations were significantly lower than prechamber values (Table 3). A small but significant ($P < 0.05$) decline in serum calcium was noted at CD30, although all data obtained during the 91-d study were within clinical norms. Other indices of bone and calcium metabolism were generally unchanged in both studies (Table 3).

During the 91-d study, thiamin status, as assessed by erythrocyte stimulation of transketolase by thiamin pyrophosphate, did not change from prechamber levels. These data were not available for the 60-d study.

General clinical chemistry and antioxidant-related measurements (Table 4) were relatively unchanged during the two chamber studies. For most of these variables, statistically significant differences generally were not clinically important. A very small, albeit significant decrease in serum sodium concentration occurred during the 60-d study, and serum sodium was elevated on CD40 during the 91-d study (Table 4). Serum total protein concentrations were decreased on CD30 and CD40, and returned to prechamber levels after the 91-d study. Glutathione peroxidase activity was elevated during the 91-d chamber study, but not during the 60-d study. There were no differences in serum albumin, creatinine, chloride, aspartate aminotransferase or alanine aminotransferase. Urinary calcium and collagen crosslink excretion did not change during either of the chamber studies (data not presented).

Flight studies. Biochemical results from the flight subjects are shown in Tables 2–4. The observed hematologic changes indicated a nominal response to spaceflight, with reduced hemoglobin and hematocrit, and increased serum ferritin (Table 2). Ferritin iron saturation was reduced after landing. Serum transferrin receptor concentrations tended to be lower after flight.

No difference was observed between preflight and postflight measurements of serum calcium (either total or ionized) (Table 3). The level of vitamin D stores increased between the first and second preflight sessions, but was decreased after landing. Postflight urinary calcium and collagen crosslink excretions were higher than preflight excretions (data not presented).

There was no apparent change (postflight compared with preflight) for serum albumin, creatinine, chloride, aspartate

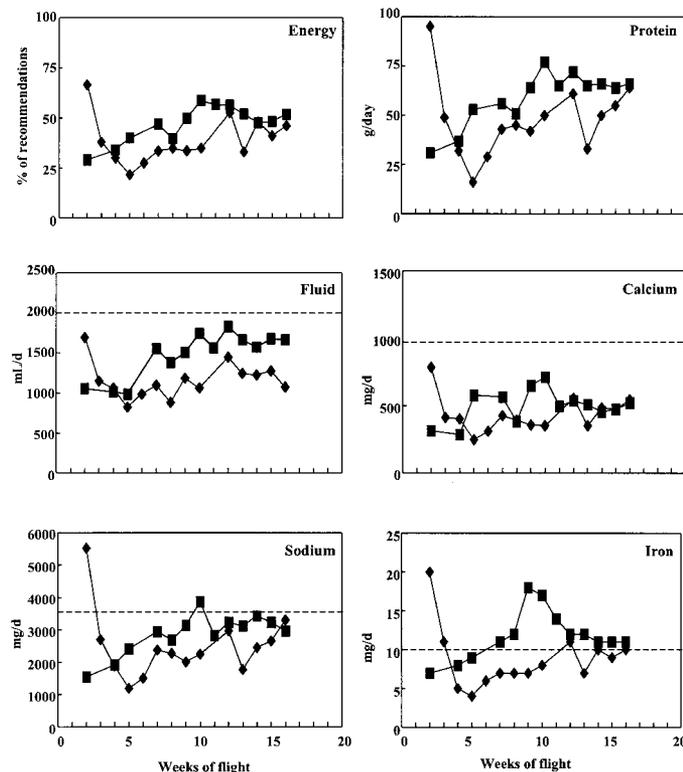


FIGURE 2 Dietary intake during spaceflight for two Mir astronauts. Energy intake is expressed as a percentage of the WHO recommendations (4, see Subjects and Methods for details). Dotted lines represent NASA intake recommendations.

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TABLE 2

Hematologic, iron and folate status indicators from ground-based chamber studies and Mir space station crew members¹

	Ground-based studies ²						Flight studies ³					
	60-d chamber study		91-d chamber study				Subject 1			Subject 2		
	Pre	Post	Pre	CD30	CD40	Post	L - 180 d	L - 10 d	R + 0	L - 180 d	L - 10 d	R + 0
Hemoglobin, g/L	149 ± 13	146 ± 11	134 ± 4	130 ± 8	127 ± 7	126 ± 5	151	151	127	162	163	135
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	0.45	0.45	0.40	0.46	0.47	0.40
MCV, fL	93 ± 3	92 ± 3	90 ± 4 ^{ab}	90 ± 3 ^{ab}	91 ± 3 ^b	89 ± 4 ^a	91	93	91	89	89	87
Ferritin, µg/L	119 ± 20	98 ± 31 ⁴	77 ± 57 ^b	68 ± 53 ^{ab}	66 ± 56 ^{ab}	49 ± 36 ^a	93	74	208	132	155	212
Ferritin iron, µg Fe/L	20.7 ± 6.2	16.6 ± 4.5	— ⁵	—	—	—	23.0	16.4	19.9	23.6	23.9	21.0
% saturation	17.5 ± 5.1	17.5 ± 4.9					24.7	22.1	9.6	17.9	15.4	9.9
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	2.59	2.79	2.17	2.22	1.97	1.80
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	5.2	3.1	2.6	3.7	6.5	4.0
Folate, nmol/L	928 ± 54	1092 ± 167	1662 ± 532 ^a	1763 ± 571 ^{ab}	1796 ± 531 ^{ab}	1907 ± 610 ^b	1171	1493	1031	1106	1552	1027

¹ CDx, chamber day x; L - x, launch minus x d; R + 0, return + 0 d (i.e., landing day); MCV, mean corpuscular volume.
² Data are means ± SD, n = 4 for each chamber study. For each study, data in the same row with different letter superscripts are significantly (P < 0.05) different.
³ For flight studies, individual data are presented. Statistical analyses were not conducted on flight study data.
⁴ P = 0.054
⁵ Analyses not available.

aminotransferase or alanine aminotransferase (data not presented).

DISCUSSION

The ground-based study described here provided a valuable opportunity to test a nutritional assessment profile and a unique FFQ, in an environment similar to that found on a space station, without the constraints of an actual space mis-

sion. The results indicate that a specially designed FFQ can be used to obtain a reliable estimate of individual dietary intake. These studies confirm that a semiclosed food system, as used in the chamber studies, can support nutritional requirements over a relatively short period of time (i.e., 2–3 mo).

The comprehensive nutritional status assessment profile described here (with minor modifications) has been implemented by NASA as a requirement for extended-duration (i.e., International Space Station) space travelers. The anthro-

TABLE 3

Serum calcium and bone metabolism markers from ground-based chamber studies and Mir space station crew members¹

	Ground-based studies ²						Flight studies ³					
	60-d chamber study		91-d chamber study				Subject 1			Subject 2		
	Pre	Post	Pre	CD30	CD40	Post	L - 180 d	L - 10 d	R + 0	L - 180 d	L - 10 d	R + 0
Calcium												
Total, mmol/L	2.54 ± 0.06	2.54 ± 0.12	2.43 ± 0.11 ^b	2.26 ± 0.09 ^a	2.35 ± 0.14 ^{ab}	2.35 ± 0.07 ^{ab}	2.40	2.37	2.35	2.47	2.35	2.40
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	1.23	1.30	1.25	1.22	1.21	1.16
PTH, 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	17.9	27.2	25.0	24.1	24.5	12.2
25(OH) vitamin D, nmol/L	45.9 ± 6.3	43.5 ± 6.3	76.3 ± 14.4 ^b	58.9 ± 13.2 ^{ab}	54.9 ± 17.1 ^{ab}	44.2 ± 23.1 ^a	36.4	76.9	28.5	44.7	73.6	50.9
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	77.3	77.3	54.0	69.8	153.1	111.6
Alk. PO ₄ ase												
Total, µkat/L	0.8 ± 0.2	0.8 ± 0.2	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.0	1.1	1.4	1.3	1.2	1.3
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	0.34	0.26	0.33	0.25	0.25	0.20

¹ Abbreviations: CDx, chamber day x; L - x, launch minus x days, R + 0, return + 0 d (i.e., landing day); PTH, parathyroid hormone; Alk. PO₄ase, alkaline phosphatase.
² Data are means ± SD, n = 4 for each chamber study. For each study, data in a row with different letter superscripts are significantly (P < 0.05) different.
³ For flight studies, individual data are presented. Statistical analyses were not conducted on flight study data.

TABLE 4

General chemistry and antioxidant/oxidative damage indices from ground-based chamber studies and Mir space station crew members¹

	Ground-based studies ²								Flight studies ³					
	60-d chamber study			91-d chamber study					Subject 1			Subject 2		
	Pre	CD32 ⁴	Post	Pre	CD30	CD40	CD60 ⁴	Post	L - 180d	L - 10d	R + 0	L - 180d	L - 10d	R + 0
Total protein, g/L	72 ± 3		69 ± 1	71 ± 4 ^b	65 ± 4 ^a	65 ± 5 ^a		68 ± 4 ^{ab}	72	69	66	73	63	67
Transferrin, mg/L	— ⁵		—	274 ± 45	250 ± 55	255 ± 85		240 ± 67	270	270	219	300	300	290
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	4.06	4.27	3.39	5.51	4.94	4.09
Triglycerides, mmol/L	0.74 ± 0.24		0.87 ± 0.13	0.89 ± 0.67	0.94 ± 0.44	1.06 ± 0.64		0.95 ± 0.74	0.90	0.80	0.45	1.21	0.77	1.30
Sodium, mmol/L	142 ± 1 ^b		140 ± 1 ^a	139 ± 2 ^a	140 ± 0 ^{ab}	141 ± 1 ^b		139 ± 0 ^a	142	139	142	142	141	142
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	3.9	4.4	3.7	4.3	3.8	3.5
RBC SOD, U/g Hb	592 ± 40		659 ± 43	986 ± 143	943 ± 122	986 ± 90		1050 ± 92	472	869	974	650	1083	1028
RBC GPX, U/g Hb	26.3 ± 3.1		25.2 ± 1.9	46.6 ± 14.9 ^{ab}	56.8 ± 11.9 ^b	53.6 ± 15.8 ^{ab}		44.3 ± 14.3 ^a	26.9	76.6	78.8	16.2	52.1	71.7
ORAC, 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	1.47	1.31	1.23	1.33	1.31	1.36
8(OH)dG, μmol/mol creatinine	1.16 ± 0.14	1.18 ± 0.50	1.20 ± 0.34	1.37 ± 0.32	1.34 ± 0.43	1.24 ± 0.41	1.38 ± 0.50	1.23 ± 0.49	1.30	1.12	1.98	1.02	1.10	1.52

¹ Abbreviations: CDx, chamber day x; L - x, launch minus x days, R + 0, return + 0 d (i.e., landing day); SOD, superoxide dismutase; Hb, hemoglobin; GPX, glutathione peroxidase; ORAC, oxygen radical absorbance capacity; 8(OH)dG, 8-hydroxy-2'-deoxyguanosine.

² Data are means ± sd, n = 4 for each chamber study. For each study, data in a row with different letter superscripts are significantly (P < 0.05) different.

³ For flight studies, individual data are presented. Statistical analyses were not conducted on flight study data.

⁴ Urine samples were collected and analyzed on CD32 of the 60-d study, and on CD60 of the 91-d study; however, blood samples were not.

⁵ Analyses not available.

pometric, biochemical, clinical and dietary assessment components contribute valuable information to the total picture of nutritional status. The intent is to provide a preflight assessment of crew nutritional status to ensure optimal status before flight, a real-time means of monitoring dietary intake during flight and a nutritional component for the postflight rehabilitation program.

Body weight loss is a consistent finding during and after spaceflight and has been observed in both the Russian and U.S. space programs (1,2,10). The maintenance of body mass and composition in the ground-based chamber studies demonstrates that a food system like the one planned for the International Space Station can provide required energy and nutrients, assuming that the foods are consumed.

Inadequate dietary intake is a significant concern during spaceflight and has been seen on Apollo, Shuttle and Mir missions (1,2,11). The two Mir crew members who participated in the flight studies reported here were found to have an energy intake <50% of predicted requirements. This decrease appeared to be the result of a generally reduced dietary intake, and not simply the lack of selection of a few high energy items. The implications of reduced food intake on essentially all nutrients are important; clearly, crew dietary intake must be maintained to ensure crew health and safety.

Crew members on Skylab, the first U.S. space station (operating in the early 1970s), consumed the amount of energy prescribed (12). Although experimental constraints required these crew members to consume adequate food, their dietary intake clearly demonstrates that it is possible to meet the dietary recommendations. The data presented herein represent perhaps the most basic principle of nutrition, i.e., inadequate dietary intake leads to loss of body mass. The FFQ developed and tested here will provide the ability to monitor the crew

members and make recommendations to them about dietary intake while they are on orbit.

The reasons for inadequate dietary intake are anecdotal and varied. Time constraints, lack of appetite and inadequate menu selection are among the typical comments. Menu fatigue is one concern, although this is not supported by the ground-based data presented here. Suboptimal nutritional intakes have been documented among individuals in confined environments, including hospitals, military field operations and nursing homes (13-15). Although it is possible that the inadequate intake is an artifact of FFQ underestimation during the flight studies, the loss of body mass supports the dietary data. Minimizing the risk of undernutrition is a critical issue for NASA, and this will clearly be a cornerstone for ensuring crew health and safety during spaceflight.

The use of vitamin and/or mineral supplements is often debated in light of inadequate dietary intake. However, the energy deficit is clearly the highest priority issue, and if this can be mitigated through increased dietary intake, the intake of all nutrients will increase. No data suggest that increasing specific nutrient intake mitigates the physiologic changes observed during spaceflight (e.g., taking supplemental calcium does not stop weightlessness-induced bone loss). Nutrient intake must be maintained at adequate levels, but the space food system can provide all requisite nutrients. In isolated cases in which this is not true, supplementation will have to be considered as an alternative.

Bone mineral loss during spaceflight results in increased urinary calcium excretion (11,16). Hypercalciuria contributes to the increased risk of renal stone formation associated with spaceflight (17). Urinary calcium and collagen crosslinks were elevated in the flight studies, but not in the ground studies (data not presented), as was expected (18). The increased

renal stone risk during spaceflight is exacerbated by the low fluid intakes observed. High salt intakes and their relationship to hypercalciuria are additional issues of concern during spaceflight. Although sodium intakes were within the desired limits for the two subjects presented here, this was largely due to their overall inadequate food intake.

Vitamin D is a related subject of concern during spaceflight because the lack of ultraviolet light exposure curtails endogenous production of this vitamin (19), which plays a critical role in bone and calcium metabolism. In both crew members participating in the present study, the preflight increase in vitamin D stores is likely related to seasonal and location changes between the two preflight data sessions. Studies with other crews have shown similar seasonal effects on 25-hydroxyvitamin D (11). Reduced vitamin D stores of astronauts have been noted previously during flight (11) and were observed here at landing. Vitamin D stores of subjects in the 91-d chamber study were also decreased, but they were unchanged in the 60-d study. The cause of this difference between studies is unknown.

Radiation exposure during spaceflight is also of concern for crew health. With the exception of glutathione peroxidase, data from the ground-based study showed no appreciable change in the antioxidant markers. However, markers of oxidative damage to DNA were increased after spaceflight. Although small fluctuations in antioxidant markers tended to occur after spaceflight, these trends may have been related to reduced dietary intake rather than to increased utilization. The question remains, however: would diets rich in antioxidants mitigate some of the risk of radiation-induced cellular damage? The evidence supporting a role for nutrition in reducing mortality and morbidity from diseases linked to oxidative stress (e.g., cancer, cardiovascular disease) is increasing (20). The role of antioxidants in crew health during and after spaceflight remains to be fully elucidated.

Red blood cell mass and iron metabolism are altered during spaceflight (21,22). Iron stores and tissue iron tended to increase during spaceflight, as indicated by increased serum ferritin concentrations and reduced transferrin receptors, respectively, in the present study.

The ferritin iron results reported here are novel and intriguing. Ferritin iron saturation did not change in the 60-d chamber study, as expected (these analyses were not available for the 91-d chamber study). In the flight studies, however, ferritin iron saturation was reduced at landing. This suggests that the increase in ferritin observed after spaceflight may be related to an acute phase reaction and not necessarily to increased iron storage. Other indices of iron availability increased, confirming that the RBC mass reduction during flight (21) is indeed not an iron-deficiency anemia. This phenomenon, observed in only two subjects, clearly requires further validation with additional subjects before conclusions may be drawn.

Despite recommendations for both men and women to limit iron intake during spaceflight to <10 mg/d, both the U.S. and Russian space food systems currently provide excessive (>20 mg/d) amounts of dietary iron (6). The FFQ data obtained during spaceflight confirmed that iron intake by crew members during flight was excessive. The involvement of iron in the formation of potentially toxic free radicals has been described (23,24), and the risks of increased iron stores in a high radiation environment are a concern for spaceflight.

During the course of the chamber studies, ferritin decreased, and hemoglobin and hematocrit tended to decrease. This occurred despite relatively high iron intakes (Table 1). However, examination of individual diet records showed that

much of the iron had low bioavailability because it came from fortified cereals. Although this helped prevent subjects from ingesting too much iron, limited intakes of other micronutrients may be a concern when individuals depend on a closed or semiclosed food system for truly extended periods (i.e., years).

This study was important for evaluating the spaceflight FFQ and 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 to ensure optimal health during long-duration flights. The questionnaire will also provide important information for development of food systems for potential planetary exploration missions. Human spaceflight is physically and physiologically challenging, and thus demands that crew members be in peak condition before flight and that all possible means of ameliorating decrements be used during and after flight. Further, these findings confirm that diet and nutrition will continue to play a critical role in crew health and safety during spaceflight.

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