Section 2

Regulatory Physiology

2

Regulatory Physiology

Helen W. Lane, Peggy A. Whitson, Lakshmi Putcha, Ellen Baker, Scott M. Smith, and Karen Stewart of the Johnson Space Center, Houston, TX; Randall J. Gretebeck and R. R. Nimmagudda of the Universities Space Research Association; Dale A. Schoeller of The Committee on Human Health Nutrition and Nutritional Biology, University of Chicago Department of Medicine, Chicago, IL; Janis Davis-Street, Robert A. Pietrzyk, and Diane E. DeKerlegand of KRUG Life Sciences, Houston, TX; Charles Y. C. Pak of Southwestern Medical School, Dallas, TX; D.W.A. Bourne of the University of Oklahoma, Oklahoma City, OK

BACKGROUND

As noted elsewhere in this report, a central goal of the Extended Duration Orbiter Medical Project (EDOMP) was to ensure that cardiovascular and muscle function were adequate to perform an emergency egress after 16 days of spaceflight. The goals of the Regulatory Physiology component of the EDOMP were to identify and subsequently ameliorate those biochemical and nutritional factors that deplete physiological reserves or increase risk for disease, and to facilitate the development of effective muscle, exercise, and cardiovascular countermeasures. The component investigations designed to meet these goals focused on biochemical and physiological aspects of nutrition and metabolism, the risk of renal (kidney) stone formation, gastrointestinal function, and sleep in space. Investigations involved both ground-based protocols to validate proposed methods and flight studies to test those methods. Two hardware tests were also completed.

The first of the Regulatory Physiology studies was designed to relate nutritional status to the definition and maintenance of energy balance, with the primary focus on determining energy requirements during flight. Maintaining energy balance is an integral part of meeting the goals of the EDOMP because any crew member who is in negative energy balance (i.e., whose energy expenditure exceeds energy intake) will lose lean body mass regardless of the type, frequency, or intensity of exercise regimens [1,2] or protein consumption [3].

Results from Skylab suggest that crew members can and do lose weight during flight despite the consumption of adequate calories (energy) and protein [4]. The weight lost as a result of microgravity exposure generally consists of body fluids and electrolytes [5], red blood cells [6], and muscle or lean tissue [7]. A significant portion of in-flight weight loss, even during relatively brief missions (up to 10 days) is thought to be due to loss of lean body mass [4]. One important cause of loss of lean body mass is being in negative energy balance [1]. Loss of lean body mass reduces muscle work capacity and promotes loss of electrolytes, especially potassium, both of which affect muscle and cardiovascular function. Detailed Supplementary Objective (DSO) 612 was designed to (1) measure total energy expenditure (TEE) during spaceflight, (2) compare those measurements with calculations of energy intake, and (3) compare those results with traditional estimates of energy requirements for healthy, active adults. The ultimate goal of this effort was to prevent loss of lean body mass and electrolytes by ensuring that crew members' energy intake was equivalent to their energy utilization.

A second aim of the Regulatory Physiology investigations was to assess how the physiological reactions to microgravity exposure affect the risk of forming renal stones. Renal stone risk can be assessed by characterizing the key factors that contribute to stone formation, including metabolic, environmental, and physicochemical factors [8]. Metabolic factors, so named because a change in their excretion is usually of metabolic origin, include urinary calcium, oxalate, uric acid, citrate, and pH. Risk factors that can be influenced by environmental factors include total urine volume and urinary sodium, sulfate, phosphorus, and magnesium. These metabolic and environmental factors are used to calculate physicochemical risk factors, e.g., the supersaturation of calcium oxalate, brushite (calcium phosphate), monosodium urate, undissociated uric acid, and struvite. These factors are compared to values from normal populations to assess the risk of stone formation relative to those populations.

Several of the physiological changes that take place during human exposure to microgravity are thought to affect the factors that contribute to the risk of renal stone formation, especially changes in urine volume or urinary calcium, phosphate, potassium, and sodium excretion. For example, urinary calcium concentrations begin to increase within 24 hours of exposure to microgravity [9]; urinary phosphate levels also tend to be higher during flight than

before. Both of these imbalances probably reflect the bone-demineralization process, and both increase the urinary saturation of calcium oxalate and calcium phosphate. Additional factors that could aggravate the potential for stone formation in astronauts include diets high in animal protein, frequent exercise, loss of lean body mass, and varying degrees of dehydration.

A precursor to DSO 610 involved analyzing urine samples from crew members before and immediately after spaceflight, and using those results to estimate risk profiles [10]. Although those results were limited by the lack of in-flight measurements, they did indicate that some metabolic reactions to spaceflight could increase the risk of renal stone formation, and that the risk could increase as the duration of flight increased [10]. DSO 610 was undertaken to characterize the risk of stone formation directly during flight. A secondary goal was to assess the influence of diet on the risk factors for stone formation.

The third segment of the Regulatory Physiology investigations concerned gastrointestinal (GI) function. The GI tract plays a central role in maintaining energy balance by absorbing nutrients from food and other consumed substances in forms that the body can use. Changes in GI motility and gastric secretion can result in decreased appetite [11] as well as malabsorption of amino acids, fats, vitamins, fluids, electrolytes, and many medications, which in turn affects the bioavailability of these substances [12-15]. GI motility, a central aspect of GI function, therefore plays a key role in the absorption and disposition of nutrients and drugs The absence of a gravity vector in spaceflight, coupled with the corresponding changes in body posture and fluid distribution, may reduce GI motility.

GI motility has two distinct components: (1) gastric emptying (GE) rate, which is the rate at which the stomach contents empty into the small intestine, and (2) intestinal transit time, which is the rate at which the intestinal contents move from the small bowel to the cecum. GE rate is known to be slower in supine subjects [16,17]. A head-down bed-rest study was performed to determine whether the associated fluid redistribution, which mimics that of spaceflight, would alter GI motility; DSO 622 focused on measuring GE rate and intestinal transit time before and during spaceflight.

The fourth component of the Regulatory Physiology investigations focused on circadian rhythms, an important component of performance, during spaceflight. Efforts to maximize crew time during flight have included shifting work-rest schedules before flight to allow around-the-clock operations during missions. People who work in 24-hour operations consistently demonstrate drowsiness, fatigue, sleep disturbances, and impaired performance and mood [18-21]. The complexity and demanding nature of many spaceflight tasks dictate that any performance decrements arising from shift-change work must be minimized whenever possible.

Human circadian patterns can be altered by different methods: modifying the sleep period gradually over time with corresponding shifts in the work schedule or shifting by periodic timed exposures to bright light (7,000-12,000 lux). Bright light can shift cycles of body temperature, cortisol and melatonin release, markers of circadian patterns [22].

Plasma melatonin is a good indicator of shifts in human circadian rhythms [23-25]. The endogenous melatonin cycle is known to be sensitive to light and darkness, and seems to cycle in synchrony with core body temperature as well [26]. DSO 484 was designed to test the effectiveness of a timed bright-light treatment in combination with sleep shifting over a 7-day period before flight and during flight. The effectiveness of this shift was monitored daily by measuring melatonin and cortisol in saliva and urine samples.

Two new hardware devices also were tested as part of this effort: a Portable Clinical Blood Analyzer (PCBA) manufactured by the i-STAT Corporation of Princeton, NJ (DSO 492), and an In-flight Urine Collection Absorber (IUCA) device (DSO 328). Research on human adaptation to weightlessness often involves collecting biological samples, which typically are stored during flight and analyzed on return to Earth. This process presents several problems, including the inability to analyze and interpret data during the mission, the need for in-flight refrigeration or freezing, and the potential instability of the samples during storage. Real-time analysis of electrolytes, pH, and ionized calcium in blood samples would provide valuable information for physicians who provide health care to astronauts or other remote populations.

We assessed several performance characteristics of the PCBA on the ground and during spaceflight. A ground-based study involved comparisons of capillary (fingerstick) and venous blood, control solutions vs. blood samples, and PCBA vs. traditional laboratory methods. The flight study (DSO 492), on the other hand, involved real-time analyses of control solutions and capillary samples with the PCBA, and comparisons among preflight, in-flight, and postflight sample periods.

Also developed and tested was a prototype device for collecting and storing small volumes of urine during flight for experiments involving excretion of stable (non-radioactive) isotopes. The urine collection devices used for non-Spacelab missions were cumbersome, tended to leak, could not be used by women, and required considerable storage space. The IUCA was developed as an alternative that would facilitate the collection of small-volume urine samples for metabolic studies that did not require measurements of void volume.

The IUCA consisted of a small, conical piece of high-absorbency filter paper that could be placed in urine-collection funnels, which themselves were attached to the Shuttle waste collection system. This assembly could be used by men and women, and could be used on the

Table 2-1. EDOMP Regulatory Physiology investigations

DSO Number	*Title*	*PI*
612	Energy and Metabolic Requirements for Extended-Duration Space Flight Assessment	H.W. Lane
610	In-Flight Assessment of Renal Stones	P.A. Whitson
622	Gastrointestinal Function during Extended-Duration Spaceflight	L. Putcha
484	Assessment of Circadian Shifting in Astronauts by Bright Light	L. Putcha
492*	Evaluation of a Portable Clinical Blood Analyzer	H.W. Lane
328*	Evaluation of an In-Flight Urine Collection Absorber	H.W. Lane

*Assessment of flight hardware

Shuttle middeck. Ground-based evaluations focused on potential effects of the absorbent filter on the analysis of deuterium (^{2}H) and "heavy oxygen" (^{18}O), two stable isotopes that are used to measure energy expenditure, water metabolism, and body composition. DSO 328 was performed to evaluate the ease with which men and women could use this system during flight.

Table 2-1 lists the DSOs that were flown for the Regulatory Physiology section of the EDOMP. Methods and results from investigations of nutritional status, especially energy and hydration deficits, renal-stone formation risk, gastrointestinal changes, and circadian-rhythm shifting, are reviewed below.

METHODS AND RESULTS

Energy and Metabolic Requirements for Extended-Duration Space Flight (DSO 612)

The specific aim of this study was to measure energy intake and expenditure in healthy men during brief (<14-day) spaceflights, and to compare these measurements with estimates of energy requirements for healthy, active adults. Earlier estimates of energy requirements for spaceflight relied on results from metabolic balance studies and food intake records [4,27]. For this study, total energy expenditure was calculated by indirect calorimetry from a modified version of a doubly labeled water protocol [28], from records of food and fluid intake [29], and from estimates of energy requirements published by the World Health Organization (WHO) [30].

Subjects were 13 men on six Shuttle flights between 1992 and 1994. Details of the study protocol are described elsewhere [31]. Briefly, each subject completed two 5-day test sessions, one before and the other during flight. Test sessions involved consuming a dose of doubly labeled water ($^{2}H^{18}O$), providing urine and saliva samples periodically, and recording food and fluid intake, as well as exercise and use of medications. These data were used to calculate water turnover, amount and nutrient content of consumed food, and total energy expenditure. Each crew member served as his own control, and the preflight assessment was the control period. One-way repeated-measures ANOVA was used to identify any differences between energy intake and expenditure in the preflight vs. in-flight periods and the WHO determination of energy requirements. The Student-Newman-Keuls test was used for post hoc comparisons. Paired t-tests were used to detect any differences in dietary intake between the preflight and in-flight periods. TEE was corrected for body weight and fat-free mass, and was tested similarly to detect differences between reported intake and expenditure.

Results

Results from this study are summarized in Table 2-2. Energy expenditures calculated by using the doubly labeled water technique were similar before and during flight, and also were similar to the WHO estimates for required energy. Thus, the WHO estimates were predictive of dietary energy requirements for this group of subjects, both before and during spaceflight. Both energy and fluid intake were lower during flight than before, and probably contributed to the overall weight loss for this group (mean -1.5 kg, range +1.0 to -3.9 kg) (mean -3.3 lb, range +2.2 to -8.6 lb).

Table 2-2. Energy intake and expenditure, and fluid intake and turnover, from 13 men before and during 8- to 14-day spaceflights

	Before Flight	*During Flight*
Energy intake, MJ/d	11.38±2.06[a]	8.76±2.26[b]
Energy expenditure, MJ/d	12.40±2.83[a]	11.70±1.89[a]
WHO-predicted energy requirement, MJ/d	12.64±0.51[a]	12.68±0.49[a]
Fluid intake, l/d	2.7±0.4	2.2±0.5*
Water turnover, l/d	3.8±0.5	2.7±0.6*

[a]Values are means ± s.d.

[b]Energy results with different superscripts are significantly different from one another, $p < 0.001$.

*Significantly different from preflight values, $p < 0.05$.

In-flight Assessment of Renal Stones (DSO 610)

The specific aim of this study was to assess urinary components and dietary factors in order to clarify which variables contribute to a putative increase in the risk of renal stone formation associated with microgravity exposure. Details of this flight study and a previous ground-based investigation, are available elsewhere [10,32].

Six male astronauts, who flew on Shuttle missions lasting 11 to 16 days, participated in this study. Each subject collected urine during two 24-hour periods before flight, the last within 10 days of launch. During flight, void-by-void samples were collected over two 24-hour periods and aliquots were placed into tubes containing either 0.05% thymol or 0.1% thimerosol, once between flight days 3 and 4 and again within 4 days of landing. Preflight and postflight samples were collected into containers and stored at about 4°C. At the end of each period, the contents were mixed and decanted into graduated cylinders for total volume and pH measurements. A 10-ml aliquot was removed for biochemical analyses. A second 10-ml aliquot was acidified with 6 molar (M) hydrochloric acid (HCl) for the analysis of citrate, oxalate, and sulfate; two additional aliquots were removed and thymol and thimerosal added to serve as controls for the room temperature storage of the in-flight controls.

Before and after flight, crew members also maintained daily handwritten logs of food and fluid consumption for four 48-hour periods, beginning 24 hours before the urine collection periods and continuing throughout those 24-hour periods. During flight, food and fluid intake was tracked with an automated barcode scanning system. Neither diet nor activity level differed from the crew members' usual routines. Fluid intake was ad lib during all sampling periods; however, all crew members consumed the equivalent of normal saline about 90 minutes before landing as part of an established countermeasure against orthostatic intolerance. Urinary factors associated with renal stone formation were analyzed according to the methods described in Ref. 10.

Results

Urinary risk factors for the six crew members in this study are listed in Table 2-3. Changes in the chemical environment of the urine increased the risk of forming calcium oxalate and calcium phosphate stones in this group. Urine volume after landing was equivalent to that before flight; in-flight volume may have been less than preflight volume, but this difference was not significant at the 0.05 level. Urinary pH was no different during flight than before, but did drop significantly ($p < 0.05$) on landing day, as did landing day values for a previous study of 150 astronauts [10]. Urinary calcium excretion increased slightly by the late in-flight period and was still greater than preflight values on landing day. Urinary potassium levels dropped during flight ($p < 0.05$) and returned to preflight levels after landing. Citrate excretion tended to decline during flight, but the difference was not statistically significant for this small group. However, previous studies have indicated that landing day citrate levels tend to be lower than preflight values [10].

Relative supersaturation ratios for calcium oxalate, brushite (calcium phosphate), sodium urate, and uric acid indicated increased-risk range (>2.0) for renal-stone formation during the early in-flight period, and calcium-oxalate remained significantly elevated throughout the remainder of the flight. Calcium-oxalate supersaturation remained in the high-risk range (>2.0) on landing day, but was not statistically different from preflight. The risk of brushite stone formation followed the same pattern as that of calcium oxalate. The risk of sodium urate and uric acid stone formation reached the high-risk range during flight, but the change was not statistically significant.

Dietary intake of fluid, energy, protein, potassium, phosphorus, and magnesium all were significantly less during flight than before or after flight (data not shown). However, records on landing day may have been incomplete because of scheduling constraints on those days.

Gastrointestinal Function During Extended Duration Space Flight (DSO 622)

The specific aim of this study was to evaluate the effect of spaceflight on two components of GI motility, gastric-emptying (GE) rate and intestinal-transit time. GE rate can be measured by following blood or saliva concentrations of acetaminophen as it is absorbed through the intestinal wall; the absorption rate of acetaminophen after oral administration has been shown to be directly proportional to GE rate [33]. GE rate was determined by salivary concentration of acetaminophen, and the urinary concentration of its metabolites, after an oral dose. Intestinal transit time was measured indirectly by using a noninvasive technique, the GE rate was combined with the breath hydrogen test [15,34,39]. In this test, subjects consume lactulose, a nondigestible sugar, that passes undigested through the small intestine. Bacteria in the colon ferment the lactulose, producing hydrogen that is exhaled by the subject. The period between the ingestion of lactulose and peak rise in breath hydrogen represents mouth-to-cecum transit time [40,41] Since gastrointestinal motility depends on the rate of gastric emptying, performing both tests (breath-hydrogen for GI motility and aceta

intestinal transit time to be assessed indirectly by subtracting GE time from the overall GI transit time.

These methods were validated with six subjects in a 10-day head-down bed-rest study, and then tested with two crew members. Both subjects completed the protocol 3 times before launch (L-60, L-45, and L-30 days), once during flight (flight day 5), and once after landing (R+9 days).

Table 2-3. Renal stone risk-assessment profile for six male astronauts before, during, and after 11- to 16-day space shuttle missions†

	Before Flight		*Early in Flight*		*Late in Flight*		*Landing Day*		*7-10 Days after Landing*	
Total volume (l/d)	1.676	(0.09)	0.797	(0.08)	1.303	(0.17)	1.524	(0.35)	1.572	(0.31)
pH	6.01	(0.18)	5.95	(0.19)	5.92	(0.13)	5.14	(0.05)*	6.09	(0.14)
Calcium (mg/d)	166.2	(32.7)	131.8	(22.0)	221.8	(34.4)	245.2	(70.2)*	227.1	(50.0)
Phosphate (mg/d)	884.8	(110)	822.8	(170)	920.5	(104)	678.0	(165)	933.2	(166)
Oxalate (mg/d)	32.1	(4.6)	27.2	(2.7)	31.0	(2.4)	23.4	(3.6)	28.5	(5.4)
Sodium (mg/d)	2597	(443)	1807	(104)	2127	(285)	1704	(410)	4029	(654)*
Potassium (mg/d)	2620	(308)	1439	(256)*	1675	(177)*	1713	(395)	2157	(331)
Magnesium (mg/d)	93.5	(6.6)	89.2	(6.0)	101.8	(10.3)	71.2	(13.3)	97.8	(9.7)
Citrate (mg/d)	717.7	(115)	468.8	(109)	522.3	(66)	456.3	(82)	671.3	(148)
Sulfate (mmol/d)	20.8	(1.7)	13.3	(1.8)	20.7	(2.4)	21.9	(3.9)	17.0	(2.2)
Uric Acid (mg/d)	593.5	(49.0)	321.6	(32.3)	454.3	(72.4)	267.2	(74.2)*	556.2	(63.7)
Creatinine (mg/d)	1621	(159)	1237	(182)	1408	(61)	1598	(195)	1710	(152)
RELATIVE SUPERSATURATION VALUES										
Calcium Oxalate	1.52	(0.40)	2.95	(0.65)*	2.78	(0.47)*	2.07	(0.40)	1.60	(0.28)
Brushite	1.11	(0.32)	2.20	(0.32)*	2.10	(0.43)*	0.32	(0.07)	1.92	(0.31)
Sodium Urate	1.83	(0.47)	2.66	(0.50)	1.77	(0.42)	0.66	(0.42)	3.59	(0.88)
Struvite	1.29	(0.78)	3.46	(1.44)	0.99	(0.28)	0.05	(0.04)	1.05	(0.32)
Uric Acid Saturation	1.96	(0.69)	2.31	(0.75)	1.68	(0.31)	2.84	(0.76)	1.21	(0.38)

Asterisks indicate significant differences relative to before flight, $p<0.05$.
Values for before flight are the means (± SEM) from two separate preflight urine-collection sessions. All other values are the average of 6 subjects (except for early in flight, when n=5).
†Data were published in Whitson, et al. [32].

After the sleep period, crew members provided baseline breath, saliva, and urine samples, next consumed a prescribed low-fiber breakfast, and then ingested 20 g of lactulose and 650 mg of acetaminophen, both in liquid dosage forms. After ingesting the doses, crew members provided breath samples every 15 minutes for 4 hours, and saliva samples every 15 minutes for the first hour and then at 2, 3, 5, 4, 6, and 8 hours.

Saliva samples were analyzed for acetaminophen, and urine samples for acetaminophen, acetaminophen glucuronide, and acetaminophen sulfate, by high performance liquid chromatography [42]. The saliva and urine concentrations with respect to time were analyzed with the BOOMER pharmacokinetic program [43], which provided an indication of gastric emptying rate. Mouth-to-cecum transit time (MCTT) was calculated from peak hydrogen concentrations in the breath samples [15].

Results

Pharmacokinetic variables, calculated from the levels of acetaminophen in saliva and its metabolites in urine, are shown in Table 2-4. The substantial variability and the small number of subjects preclude reaching any conclusions until more subjects can be tested. However, acetaminophen seemed to have been absorbed quickly during flight, since peak saliva concentrations were reached by 15 minutes after the dose was taken (data not shown). With regard to intestinal transit time, peak breath-hydrogen levels were greater during flight than before (data not shown); however, a trend of increased transit time during flight was noticed.

Assessment Of Circadian Shifting In Astronauts By Bright Light (DSO 484)

The specific aim of this study was to test whether bright-light treatment over a 7-day preflight period could produce a 9- to 12-hour phase shift in crew member circadian rhythms. Details of this study can be found in Ref. 44. The ultimate goal was to optimize sleep patterns and performance in preparation for a launch

Table 2-4. Pharmacokinetics of acetaminophen after a 650-mg oral dose in two crew members

	Subject 1			*Subject 2*		
	Before Flight	*During Flight*	*After Flight*	*Before Flight*	*During Flight*	*After Flight*
C_{max}, µg/ml	16.40	10.58	26.90	8.91	16.10	7.68
AUC, µg/ml/h	30.35	24.10	37.50	46.37	63.50	46.90
Elimination half-life, h	3.18	2.10	1.95	4.07	6.24	4.71

C_{max}, peak concentration of acetaminophen (in saliva)

AUC, area under the [saliva-acetaminophen concentration over time] curve

Subjects for this study were eight Shuttle astronauts (5 men and 3 women) from three missions. All subjects underwent a combination of sleep shifting and bright-light treatment; all provided saliva and urine samples before and during the treatment for analysis of cortisol and melatonin rhythms.

Two types of sleep shifts were attempted. The first group (n = 3) was instructed to sleep on treatment days 2 through 7 from 10:00 a.m. to 6:00 p.m. The second group (n = 5) was instructed to go to bed progressively later, and sleep later, over the same 6-day period. All subjects were exposed to bright light (7,000 - 12,000 lux), delivered via special ceiling lights in the crew quarters at JSC and KSC, for 2 to 6 hours daily before their scheduled sleep periods. Crew members in the first group (the 'abrupt' sleep shift group) were instructed to wear dark goggles, or remain in a dimly lit area, for several hours before bedtime so that their light-dark cycles were the same as those for the other sleep-shift group. Compliance with the treatment protocols was neither monitored nor recorded. Melatonin and cortisol rhythms were measured in saliva and urine as described in Ref. 44, and compared to results from 15 control subjects with normal sleep-wake cycles (data not shown).

After this initial study was completed, two additional crew members were tested with an expanded protocol that included in-flight assessments of ambient light, sleep duration, and sleep quality. These variables were measured with the Actillume device (described in the Hardware chapter), a wristwatch-type device worn continuously during flight. The activity data generated by the Actillume were autoscored for sleep, and illumination levels were analyzed for patterns of light exposure on all flight days. The participating crew members also kept manual logs of sleep and medication use throughout the flight period.

Results

At the beginning of the sleep-shift protocol, cortisol and melatonin acrophases (peaks) in the original group of eight subjects were within 2.5 hours of those of the control group. During the first 4 days of bright-light exposure, melatonin production was suppressed, but cortisol did not change; moreover, the melatonin rhythm (as a function of time of day) diminished during this period, but the cortisol rhythm was preserved. By the end of the 7-day treatment period, the peak melatonin concentrations were the same as they had been before the treatment, and they appeared within 2 hours of the expected 12-hour phase delay. The more gradual change in the melatonin acrophrase of the second group paralleled the more gradual change in that group's prescribed sleep-wake times. Cortisol acrophases were similar to melatonin acrophases except for one subject in the second sleep-shift group. With this one exception, all subjects achieved the expected circadian shift within the 7-day treatment period. Phase delays were 11-15 hours for subjects in the first group, and 7-12 hours for those in the second.

With regard to the later in-flight tests, results generated by the Actillume indicated that the ambient light in the cabin was much lower than the ambient light on the ground. Saliva melatonin peaks were higher during flight than before. Performance during shift work in flight was comparable to that during a preflight normal (non-shift) work schedule.

Evaluation of a Portable Clinical Blood Analyzer (DSO 492)

Details of the entire evaluation, which included verification of instrument calibration, assessments of precision, comparisons of methods, and statistical analyses, are given in Ref. 45.

As noted in the introduction to this chapter, the ground-based portion of this study involved comparisons of capillary (finger-stick), venous blood, and control solutions measured by PCBA vs. by traditional laboratory methods. The flight study involved only PCBA analysis of control solutions and capillary samples, with comparisons made across test periods (before, during, and after flight).

The PCBA, a hand-held, battery-powered device, was tested with approximately 85 µl of whole blood to analyze electrolytes, glucose, and hematocrit. Electrolytes (sodium, potassium, and ionized calcium) are determined by ion-selective electrode potentiometry; glucose by amperometry; hematocrit by conductometrics; and pH through direct potentiometry. Hemoglobin is calculated from hematocrit, and is not measured directly.

Subjects for the flight studies were 21 astronauts (18 men, 3 women) on five Space Shuttle missions. The subjects collected capillary samples from each other by finger-stick with a lancing device and balanced heparin

capillary tubes (radiometer). Blood was transferred quickly to the cartridge for PCBA analysis. Samples obtained before and after flight were collected either by the crew members or by medical technologists. Blood samples were scheduled to be collected 3 times before flight, twice during flight, and 3 times after flight (the latter on R+0, R+3, and R+6 days). Control samples were run every day that blood samples were collected.

Differences between ground and flight values (control samples) and data obtained before, during, and after flight (subject data) were investigated with repeated-measures ANOVA with *a priori* contrasts (i.e., contrasts planned before data analysis was undertaken). Data were missing on one flight day from 5 subjects. To maximize sample size, the in-flight data were averaged, and *a priori* contrasts were used to compare data obtained before, during (average in flight), and after flight (3 postflight days).

Results

PCBA performance in microgravity was similar between ground vs. in-flight for control solutions of all analytes except sodium, which was lower in flight than before [45]. However, this difference was within the performance limits set by the Clinical Laboratories Improvement Act (CLIA), and probably does not reflect any analytical problems in microgravity. Thus, measurements provided by the PCBA in microgravity, for the analytes tested, are probably no different from those obtained on the ground.

With regard to subject data, in-flight values of potassium were greater and ionized calcium were lesser during flight relative to preflight. In-flight glucose results were variable, probably because fasting state was not controlled in this experiment. Landing-day measurements of potassium, ionized calcium, and pH were lower than preflight values. However, these values returned to preflight levels by R+3 days (pH and ionized calcium) or by R+6 days (potassium).

Evaluation of an In-Flight Urine Collection Absorber (DSO 328)

The purpose of this project was to develop and evaluate a means of collecting small-volume urine samples that could be used in flight (on the Shuttle middeck or in other constrained areas) by male and female astronauts.

The In-flight Urine Collection Absorber (IUCA) was a conical, 75 cm^2 filter that fit into the urine-collection funnel. The filter material could absorb 20.4 gm of water per 100 cm^2, and weighed approximately 20 gm empty and 35 gm full. By comparison, the urine-collection device (UCD) assembly, which could be used only by men, weighed 65 gm empty and 300-400 gm when full. Theoretically, as the crew member voided, the vortex action created by the Shuttle's vacuum system would allow urine to saturate the IUCA. When the void was complete, the IUCA could be removed, placed in a double-ziplock bag, and stored in an absorber containment bag for return. This technology could be used only for experiments that did not require void volume to be measured.

Two ground-based tests were conducted to assess whether the absorbent material would affect the analysis of deuterium (^2H) and heavy oxygen (^{18}O). In the first test, tap water samples were spiked with known amounts of ^2H and ^{18}O, and aliquots were placed in UCDs and in unassembled IUCAs. The UCDs and IUCAs were stored at room temperature and sampled after 1, 2, and 3 weeks. Each sample was either processed (extracted with charcoal then filtered) or unprocessed (filtered only). Samples were weighed periodically to monitor gross evaporative loss. Processed and unprocessed samples were stored at –20°C until mass-spectrometric analysis of the stable isotopes. Results from this test indicated that evaporative losses were minimal from the IUCA samples and were far less than those from the samples stored in the UCD. Stable isotope enrichment was similar in both the IUCA and UCD samples.

For the second ground-based test, urine samples obtained from a subject who had ingested ^2H- and ^{18}O-labeled water as part of a protocol to measure energy expenditure and total body water, were collected and stored in either standard containers, UCDs, or IUCAs. Again, samples were processed and stored at –20°C until mass spectrometric analysis. Results from this test indicated little difference among the three storage conditions in terms of energy expenditure and total body water measurements. For a follow-up study in which two subjects collected urine, the variance among urine collected in a cup, in a flight urine-collection bag, or in an IUCA was within the 5% error characteristic of calculating energy expenditure and total body water from doubly labeled water.

The flight study involved asking male and female crew members to verify the performance of the IUCA during flight. Five crew members (3 men and 2 women) used the device on STS-67 and STS-70 to collect random urine samples over 2- to 4-day periods. The volumes recovered during flight ranged from 11 to 13 ml, with no difference between the male vs. female subjects.

DISCUSSION

Results from DSO 612 indicate that most of the 13 astronauts tested were in negative energy balance (a catabolic state) during spaceflight, and consumed inadequate fluid.

Although energy utilization was unchanged during spaceflight, energy (caloric) intake was lower during flight than before. The resulting negative energy balance probably was responsible for some of the weight loss present at landing day in almost all of the crew members

tested. The similarity between measures of energy utilization and the WHO calculation for predicting energy utilization was remarkable, and suggests that the WHO calculation can be used to estimate the energy (dietary) needs for space crews.

The reduction in fluid intake observed during flight (Table 2-2) may have produced dehydration, which in terms of urine concentration may foster renal stone formation. The importance of maintaining adequate food and fluid intake should be emphasized to avoid conditions that contribute to flight-induced deconditioning. This information also will be important for identifying requirements for the food system(s) on board future missions.

With regard to renal stone risk (DSO 610), this preliminary report of six male crew members revealed that renal stone risk increased as a result of changes in urinary composition during flight. Factors that contributed to increased potential for stone formation during flight were significant reductions in urinary pH and increases in urinary calcium. Urinary output (volume) and citrate, a potent inhibitor of calcium-containing stones, were slightly reduced during flight. Additional subjects should be assessed, with particular attention directed toward fluid, food, and medication intake, to minimize the risk of forming renal stones during and after flights. With regard to potential countermeasures, one logical recommendation would be for crew members to ingest at least 2.5 liters of water per day. In this way, the urinary concentration of stone-forming salts could be diluted, and the potential for crystal nucleation and renal-stone formation may well be reduced.

Results from the gastrointestinal-function investigation (DSO 622), which involved only two subjects, are too few to draw definitive conclusions. However, the acetaminophen results suggest that liquid dosage forms may be better absorbed during flight than solid forms such as tablets. Recommendations for future evaluations include (1) simultaneously measuring hydrogen concentrations in the spacecraft air and in the subject's breath, (2) collecting several baseline breath samples before the subject ingests the lactulose, and (3) continuing to collect breath samples for longer than 4 hours after lactulose ingestion. Further studies are needed to evaluate the effect of prolonged exposure to microgravity on gastrointestinal function. This information will be useful in developing pharmaceutical and nutritional countermeasures for future missions.

With regard to in-flight circadian rhythms (DSO 484), the number of participants in the study was too small and the number of treatments too large to generate definitive recommendations. However, a 7-day period of sleep-shifting and bright light treatment before flight successfully shifted melatonin and cortisol rhythms in the eight crew members tested. Data from two subjects who were tested before and during flight suggested that sleep quality was poor before flight, even after the bright-light treatment. Anecdotal reports suggest that sleep quality also was poor during the first few days after return. Future studies should include measurements of body-temperature cycles as well as cognitive performance and alertness over time. Rest-activity cycles and absolute illumination levels also should be recorded systematically during flight.

The PCBA (DSO 492) was highly reliable for measuring sodium, potassium, ionized calcium, pH, and glucose in real time, both on Earth and during spaceflight. Some of the variability noted between capillary (fingerstick) blood vs. venous (whole) blood in the ground-based comparisons probably reflects the fact that capillary blood more closely resembles arterial rather than venous blood. The success of the PCBA in measuring ionized calcium is of particular interest, given the importance of being able to follow changes in bone and calcium homeostasis during long spaceflight. In summary, the PCBA is a reliable device with which to measure most analytes and is being flown routinely to assist flight surgeons in evaluating crew health.

Finally, the IUCA (DSO 328) offered a lightweight means of collecting urine samples, from either men or women, during flight when urine volume measurements were not required. These devices can be used on Shuttle flights or on International Space Station.

REFERENCES

1. Woo R, Daniels-Kush R, Horton ES. Regulation of energy balance. Ann Rev Nutr 1985; 5: 411-33.
2. Rasvussin E, Bogardies C. Relationship of genetics, age, and physical fitness to daily energy expenditure and fuel utilization. Am J Clin Nutr 1989; 49:968-75.
3. Anderson HL, Heindel MB, Linksweiler H. Effect on nitrogen balance of adult man of varying sources of nitrogen and level of caloric intake. J Nutr 1969; 99:82-87.
4. Leonard JI, Leach CS, Rambaut, PC. Quantitation of tissue loss during prolonged space flight. Am J Clin Nutr 1983; 38:667-79.
5. Leach CS, Alfrey CP, Suki WN, et al. Regulation of body fluid compartments during short-term space flight. J Appl Physiol 1996; 81(1):105-16.
6. Alfrey CP, Udden MM, Leach-Huntoon C, et al. Control of red blood cell mass in space flight. J Appl Physiol 1996; 81(1):98-104.
7. Rambaut PC, Smith MC, Leach CS, et al. Nutrition and responses to zero gravity. Federation Proceedings 1977; 36:1678-82.
8. Pak CYC. Medical management of nephrolithiasis. J Urol 1982; 128(6):1157-64.

9. Whedon GD, Lutwak L, Rambaut PC, Whittle MW, Smith MC, Reid J, Leach C, Stadler CR, Sanford DD. Mineral and nitrogen metabolic studies–experiment M071. In: Johnston RS, Dietlein LF, editors. Biomedical Results from Skylab, NASA SP-377. Washington DC: NASA; 1977. p 165-74.
10. Whitson PA, Pietrzyk RA, Pak CYC, Cintron NM. Alterations in renal risk factors after space flight. J Urol 1993; 150:803-07.
11. Nicholl CG, Polak JM, Bloom SR. The hormonal regulation of food intake, digestion, and absorption. Ann Rev Nutr 1985; 5:213-39.
12. Holgate AM, Read NW. Relationship between small bowel transit time and absorption of a solid meal. Influence of metachlorpromide, magnesium sulfate, and lactulose. Dig Dis Sci 1983; 28:812-19.
13. Meyer JH, Gu YG, Elashoff J, Reedy T, Dressman J, Amidon G. Effect of viscosity and flowrate on gastric emptying of solids. Am J Physiol 1986; 250:G161-G164.
14. Meyer JH, Mayer EA, Jehn D, Gu Y, Fink AS, Fried M. Gastric processing and emptying of fat. Gastroenterology 1986; 90:1176-87.
15. Bond JH, Levitt MD. Investigation of a small bowel transit time in man utilizing pulmonary hydrogen measurements. J Lab Clin Invest 1972; 85:546-55.
16. Nimmo WS, Prescott LF. The influence of posture on paracetamol absorption. Br J Clin Pharm 1978; 5:348.
17. Thomas JE. Mechanics and regulation of gastric emptying. Physiol Rev 1957; 37:453-74.
18. Akerstedt T, Torsvall L, Gillberg M. Sleepiness and shift work: field studies. Sleep 1982; 5:S95-S106.
19. Gold DR, Rogacz S, Bock N, Tosteston TD, Baum TM, Speizer FE, Czeisler CA. Rotating shift work, sleep, and accidents related to sleepiness in hospital nurses. Am J Public Health 1992; 82:1011-14.
20. Englund CE, Ryman DH, Naitoh P, Hodgdon JA. Cognitive performance during successive sustained physical work episodes. Behav Res Methods Instru Computers 1985; 17:75-85.
21. Reinberg A, Migraine C, Apfelbaum M, et al. Circadian and ultradian rhythms in the feeding and nutrient intakes of oil-refinery operators with shift work every 3-4 days. Diabete Metab 1979; 5:33-41.
22. Czeisler CA, Allan JS, Strogatz SH, Ronda JM, Sanchez R, Rios CD, Freitag WO, Richardson GS, Kronauer RE. Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. Science 1986; 233:667-71.
23. Lewy AJ, Wehr TA, Goodwin FK, Newsom DA, Markey SP. Light suppresses melatonin secretion in humans. Science 1980; 210:1267-69.
24. McIntyre IM, Norman TR, Burrows GD, Armstrong SM. Melatonin rhythm in human plasma and saliva. J Pineal Res 1987; 4(2):177-83.
25. Arendt J. Melatonin and rhythmic functions in mammals: Therapeutic and commercial potential. In: Reinberg A, Smolensky MH, Labrecque G, editors. Annual Review of Chronopharmacology Vol 6. London: Pergamon Press; 1990. p 137-53.
26. Shanahan TL, Czeisler CA. Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core temperature in men. J Clin Endocrinol Metab 1991; 73:227-35.
27. Lane HW. Energy requirements for space flight. J Nutr 1992; 122:13-18.
28. Schoeller DA, Leitch CA, Brown C. Doubly labeled water method: in vivo oxygen and hydrogen isotope fractionation. Am J Physiol 1986; 251:R1137-R1143.
29. Gretebeck RJ, Schoeller DA, Gibson EK, Lane HW. Energy expenditure during antiorthostatic bed rest (simulated microgravity). J Appl Physiol 1995; 78:2207-11.
30. WHO. Energy and protein requirements. Report of a joint FAO/WHO/UNU expert consultation. World Health Organization Tech Report Services 1985; p 724.
31. Lane HW, Gretebeck RJ, Schoeller DA, et al. Comparison of ground-based and space flight energy expenditure and water turnover in middle-aged healthy male U.S. astronauts. Am J Clin Nutr 1997; 65:4-12.
32. Whitson PA, Pietrzyk RA, Pak CYC. Renal-stone risk assessments during Space Shuttle flights. J Urol 1997; 158:2305-2310.
33. Clements JA, Heading RC, Nimmo WS, Prescott LF. Kinetics of acetaminophen absorption and gastric emptying in man. Clin Pharmacol Ther 1978; 24:420-31.
34. Read NW, Miles CA, Fisher D, Holgate AM, Kime ND, Mitchell MA, Reeve AM, Roche TB, Walker M. Transit of a meal through the stomach, small intestine, and colon in normal subjects and its role in the pathogenesis of diarrhea. Gastroenterology 1980; 79:1272-82.
35. Read NW, Al-Janabi MN, Bates TE, Holgate AM, Cann PA, Kinsman RI, McFarlane A, Brown CH. Interpretation of the breath hydrogen profile obtained after ingesting a solid meal containing unabsorbable carbohydrate. Gut 1985; 26:839-42.
36. Scarpello JHB, Greaves M, Sladen GE. Small intestinal transit in diabetes. Br J Med 1976; 2:1225-26.

37. Shafer RB, Prentiss RA, Bond JH. Gastrointestinal transit in thyroid disease. Gastroenterology 1984; 86:852-55.

38. Metz G, Gassull MA, Leeds AR, Blendis LM, Jenkins DJA. A simple method of measuring breath hydrogen in carbohydrate malabsorption by end-respiratory sampling. Clin Sci Mol Med 1976; 50:237-40.

39. Corbett CL, Thomas S, Read NW, Hobson N, Bergman I, Holdsworth CD. Electrochemical detector for breath hydrogen determination measurement of small bowel transit time in normal subjects and in irritable bowel syndrome. Gut 1981; 22(10): 836-40.

40. Van Wyk M, Sommers DK, Steyn AGW. Evaluation of gastrointestinal motility using the hydrogen breath test. Br J Clin Pharmacol 1985; 20:479-81.

41. Ladas SD, Latoufis C, Giannopoulou H, et al. Reproducible lactulose hydrogen breath test as a measure of mouth-to-cecum transit time. Dig Dis Sci 1989; 34:919-24.

42. Jung D, Zafar NL. Micro high performance liquid chromatographic assay of acetaminophen and its metabolite in plasma and urine. J Chromatogr 1985; 339:199-202.

43. Bourne DWA. BOOMER, a simulation and modeling program for pharmacokinetic and pharmacodynamic data analysis. Computer Meth Prog Biomed 1989; 29:191-95.

44. Whitson PA, Putcha L, Chen YM, et al. Melatonin and cortisol assessment of circadian shifts in astronauts before flight. J Pineal Res 1995; 81:141-47.

45. Smith SM, Davis-Street JE, Fontenot TB, et al. Assessment of a portable clinical blood analyzer during space flight. Clin Chem 1997; 43:1056-65.