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Iron status and its relations with oxidative damage and bone loss during long-duration space flight on the International Space Station[1–3]

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ABSTRACT

Background: Increases in stored iron and dietary intake of iron during space flight have raised concern about the risk of excess iron and oxidative damage, particularly in bone.

Objectives: The objectives of this study were to perform a comprehensive assessment of iron status in men and women before, during, and after long-duration space flight and to quantify the association of iron status with oxidative damage and bone loss.

Design: Fasting blood and 24-h urine samples were collected from 23 crew members before, during, and after missions lasting 50 to 247 d to the International Space Station.

Results: Serum ferritin and body iron increased early in flight, and transferrin and transferrin receptors decreased later, which indicated that early increases in body iron stores occurred through the mobilization of iron to storage tissues. Acute phase proteins indicated no evidence of an inflammatory response during flight. Serum ferritin was positively correlated with the oxidative damage markers 8-hydroxy-2′-deoxyguanosine ($r = 0.53$, $P < 0.001$) and prostaglandin F2α ($r = 0.26$, $P < 0.001$), and the greater the area under the curve for ferritin during flight, the greater the decrease in bone mineral density in the total hip ($P = 0.031$), trochanter ($P = 0.006$), hip neck ($P = 0.044$), and pelvis ($P = 0.049$) after flight.

Conclusion: Increased iron stores may be a risk factor for oxidative damage and bone resorption. *Am J Clin Nutr* doi: 10.3945/ajcn.112.056465.

INTRODUCTION

Iron is an essential nutrient; its biological functions include oxygen binding, electron transport, and serving as a catalyst for literally hundreds of enzymes (1). Although iron deficiency can be detrimental, excess iron can be toxic. In addition to increased cardiovascular disease and cancer risk, iron overload can impair immune function and bone metabolism, increase sensitivity to radiation injury, and increase the risk of optic neuropathies (2–6). Thus, maintenance of iron homeostasis is extremely important for human health.

Evidence from short-duration (wk) and long-duration (mo) space missions shows that red blood cell mass decreases during space flight because of neocytolysis (7, 8). A consequence of this destruction of red blood cells is the transfer of the iron from those cells into storage proteins and processes. Evidence of this includes increased circulating concentrations of serum ferritin, an index of iron storage, after short- and long-duration space flights (9–11). In addition to the occurrence of physiologic changes that can increase tissue iron stores during space flight, the dietary iron content is very high in the International Space Station (ISS)[4] food system, largely because many of the commercial food items in the ISS menu are fortified with iron (12). The mean (±SD) iron content of the standard 16-d ISS menu is 20 ± 6 mg/d, and individual crew members have had intakes in excess of 47 mg/d for some weeks during long-duration missions. For reference, the defined space flight requirement for iron is 8–10 mg/d for both men and women (12, 13), and the current US Dietary Reference Intakes for iron are 8 mg/d for men and 10 mg/d for women (14). The tolerable upper intake limit for iron as defined by the Institute of Medicine is 45 mg/d; this number is based on symptoms of gastrointestinal distress (14).

Excess iron has been documented to be associated with health concerns secondary to oxidative damage—another concern for space travelers. The objective of this study was to complete a comprehensive assessment of iron status in men and women before, during, and after long-duration space flight and to investigate the association of iron status with oxidative damage and bone loss.

SUBJECTS AND METHODS

Participants

Subjects ($n = 23$; 16 men and 7 women) were astronauts on ISS expeditions 14–27 (missions of 50–247 d in duration; mean ± SD duration: 157 ± 46), which were flown between 2006 and 2011. The male and female subjects were 48 ± 4 and 45 ± 4 y of age and weighed 85 ± 9 and 62 ± 4 kg, respectively, at the

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[4] Abbreviations used: BMD, bone mineral density; CRP, C-reactive protein; FD, flight day; ISS, International Space Station; L−, launch minus (days before launch); NTX, *N*-telopeptide; PGF2α, prostaglandin F2α; 8OHdG, 8-hydroxy-2′-deoxyguanosine.

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time of launch. The protocol was reviewed and approved by the National Aeronautics and Space Administration Johnson Space Center Institutional Review Board and the Japanese Aerospace Exploration Agency and European Space Agency Medical Boards. Written informed consent was obtained from all subjects before they participated in the study.

Sample collection

Blood and 2 consecutive 24-h urine samples were collected at ~180, 45, and 10 d [designated as launch minus (L−)180, L−45, and L−10, respectively] and blood only at L−10 before launch and again on landing day (return) and 30 d after landing. During flight, the crew members provided blood and 24-h urine samples around flight day (FD) 15 (FD15), FD30, FD60, FD120, and FD180. Because flight durations varied, not all crews had 5 in-flight sessions. Blood samples were collected and processed by using standard techniques, as described previously (15, 16). Except for samples collected on landing day, all blood samples were collected after an 8-h fast. Urine collected before and after flight was collected into single-void urine containers (Cole-Parmer). Pooled 24-h urine aliquots were prepared and stored frozen at –80°C until analyzed. In-flight urine voids were collected into urine collection devices containing 1 mL of a lithium chloride solution as a volume marker, as previously described (17, 18). The blood collected at L−10 was centrifuged and immediately stored at –80°C until analyzed. The L−10 sample was treated exactly like the space flight samples; ie, the serum and plasma were not portioned into aliquots but rather remained on top of the separator gel in the blood collection tubes. The L−10 samples were frozen at –80°C until in-flight samples were available for analysis. The L−180, L−45, landing day, and 30 d after landing samples were analyzed within 5–10 d of collection. For urine analyses, a separate aliquot was made at each of the pre- and postflight time points, and all of the time points (including in-flight samples) for each subject were analyzed as a batch. The L−10 sample collection did not start until midway through the study; thus, not all of the subjects have data from that time point. A food-frequency questionnaire was used as previously described to estimate iron intake during flight (19, 20).

Biochemical analyses

Serum ferritin, transferrin, transferrin receptors, iron, whole-blood hematocrit, hemoglobin, superoxide dismutase, and plasma lipid peroxides were measured as previously described (21, 22). Urinary 8-hydroxy-2′-deoxyguanosine (8OHdG), prostaglandin F2α (PGF2α), heme, and helical peptide were also measured as previously described (16, 23). Total body iron was estimated by using an equation from Cook et al (24) that is based on the ratio of serum transferrin receptors to ferritin as follows: body iron (mg Fe/kg body weight) = −[log(transferrin receptor/ferritin) – 2.8229]/0.1207. The transferrin index was calculated as an estimate of iron saturation of transferrin and is the ratio of serum iron to transferrin; both analytes are expressed in μmol/L (25). The typical range for the transferrin index is 0.1–1 μmol Fe/μmol transferrin, and a value >1 is considered to indicate iron overload (25). Prealbumin and ceruloplasmin were measured by nephelometry (BN II System; Siemens Corporation). C-reactive protein (CRP) was measured by using a high-sensitivity immunoturbidometric method (BN II System). Serum hepcidin was measured by using a commercially available ELISA kit (DRG International). Red cell distribution width, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and mean corpuscular volume were measured by using standard clinical chemistry techniques (Beckman Coulter LH 750).

Correlations between markers of iron status and bone densitometry or bone biochemical measurements are presented for 18 subjects (12 men and 6 women; subjects taking a bisphosphonate during flight were excluded from these correlations). Measurements of bone mineral density (BMD), urinary *N*-telopeptide (NTX), and urinary calcium were made as previously described (16). A subset of these bone data (n = 13) were reported elsewhere (16), although not in relation to iron status as included here.

Statistical analyses

Mixed-effects linear regression modeling setting 2-tailed α to reject the null hypothesis at 0.05 was used to evaluate the effects of sex and flight on markers of iron status (Stata IC software version 12.1; StataCorp). For each outcome, our statistical model included dummy-coded β coefficients comparing the data from immediately before flight (L−10) with all other time periods, except in cases when assays were not performed on L−10; a comparison was made with data from L−45. All models included a random intercept to accommodate the longitudinal (within subject) experimental design. Some dependent variables required log transformation to achieve equal variance and normality. Overly influential data (statistical outliers) were excluded from analysis if the standardized residual exceeded ± 1.96 units away from the mean. However, the analyses were also done with the entire data set (including statistical outliers) to ensure that there were no major outcome differences.

A priori contrasts were determined along with a single post hoc Bonferroni multiple-test correction containing all hypothesis tests; this correction was used to adjust P values, resulting in a conservative critical P value rejection of 0.0004. Tests that remained significant after the adjustment for multiple comparisons are indicated. The area under the curve for ferritin and Pearson correlation coefficients was determined by using GraphPad Prism (version 5.04).

RESULTS

As determined with a food-frequency questionnaire completed once weekly, the overall iron intakes during the missions were 18 ± 5 and 16 ± 2 mg/d for the men and women, respectively.

Effect of space flight on biomarkers of iron status, acute phase proteins, and hematology

A significant interaction was found between sex and space flight effects on serum ferritin. Serum ferritin was higher early during space flight (FD15: $P < 0.0004$; FD30: $P = 0.005$; **Table 1**) than it was before flight, and the increase was greater among women. Only on FD15 did ferritin remain significantly higher than it was before flight after adjustment for multiple comparisons. Body iron estimates (total body iron) followed the same pattern as ferritin. The iron-transporting protein transferrin was lower at all time points during space flight and on landing day,

TABLE 1
Biomarkers of iron status in men and women before, during, and after long-duration space flight on the International Space Station[1]

	L−180	L−45	L−10	FD15	FD30	FD60	FD120	FD180	R+0	R+30
No. of subjects										
Women	7	7	5	7	7	7	5	5	7	7
Men	16	16	14	16	16	15	13	11	16	16
Ferritin (ng/mL)[2–4*]										
Women	42 ± 19	42 ± 31	30 ± 20	95 ± 46[c*]	84 ± 56[b]	64 ± 57	56 ± 50	54 ± 55	46 ± 43	25 ± 25
Men	164 ± 90	152 ± 69	135 ± 72	227 ± 131[c*]	227 ± 124[b]	212 ± 103	180 ± 114	176 ± 129	169 ± 74	104 ± 88
Transferrin (mg/dL)[3,5,6*]										
Women	264 ± 40	268 ± 58	272 ± 51	246 ± 24[b]	241 ± 41[c*]	249 ± 49[c*]	263 ± 75[c*]	254 ± 57[b]	247 ± 51[b]	287 ± 57
Men	250 ± 24	254 ± 24	251 ± 24	227 ± 19[b]	219 ± 21[c*]	215 ± 19[c*]	219 ± 22[c*]	224 ± 33[b]	229 ± 27[b]	251 ± 26
Transferrin receptors (μg/mL)[2,3,5*]										
Women	4.3 ± 0.7	4.4 ± 1.5	4.4 ± 0.6	3.3 ± 0.9[b]	3.3 ± 0.9[c*]	3.8 ± 0.7[c]	4.0 ± 1.0	4.1 ± 0.9[a]	3.7 ± 0.7[b]	5.2 ± 1.2
Men	4.6 ± 0.7	4.8 ± 1.0	4.8 ± 1.2	4.1 ± 1.1[b]	3.8 ± 1.0[c*]	4.1 ± 1.1[c]	4.7 ± 1.3	4.0 ± 0.8[a]	4.2 ± 0.9[b]	5.3 ± 1.0
Heme (μmol/L)[2,3,7]										
Women	24 ± 4	21 ± 5	24 ± 4	21 ± 7	18 ± 5	21 ± 3	21 ± 4	21 ± 3	22 ± 6[b]	22 ± 4
Men	32 ± 10	37 ± 12	33 ± 11	25 ± 6	33 ± 14	30 ± 13	27 ± 7	29 ± 9	47 ± 16[b]	31 ± 7
Hepcidin (ng/mL)[3]										
Women	21 ± 12	17 ± 10	20 ± 8	22 ± 10	22 ± 8	23 ± 11	23 ± 8	25 ± 10	23 ± 10	23 ± 10
Men	24 ± 12	22 ± 10	22 ± 7	22 ± 9	25 ± 11	22 ± 7	24 ± 9	25 ± 6	22 ± 7	24 ± 7
Iron (μmol/L)[2,3,5*]										
Women	22 ± 5	26 ± 10	30 ± 7	30 ± 7	28 ± 3	31 ± 10	27 ± 4	25 ± 10	18 ± 7[c,*]	23 ± 8[b]
Men	27 ± 7	25 ± 5	28 ± 5	33 ± 9	27 ± 5	26 ± 7	30 ± 11	26 ± 5	16 ± 5[c,*]	21 ± 5[b]
Body iron (mg/kg BW)[3,4*]										
Women	31 ± 2	31 ± 2	30 ± 3	35 ± 2[c*]	34 ± 3[b]	32 ± 3	32 ± 4	31 ± 4	31 ± 4	28 ± 3
Men	36 ± 2	36 ± 1	35 ± 2	37 ± 2[c*]	38 ± 2[b]	37 ± 2	36 ± 2	36 ± 2	36 ± 2	33 ± 3
Transferrin index[3,5*]										
Women	0.70 ± 0.21	0.83 ± 0.44	0.88 ± 0.17	0.97 ± 0.25[b]	0.96 ± 0.24	1.02 ± 0.33	0.88 ± 0.18	0.77 ± 0.24	0.57 ± 0.13[c*]	0.66 ± 0.27[b]
Men	0.89 ± 0.26	0.75 ± 0.14	0.89 ± 0.2	1.17 ± 0.39[b]	1.00 ± 0.24	0.99 ± 0.26	1.12 ± 0.41	0.90 ± 0.15	0.57 ± 0.22[c*]	0.65 ± 0.15[b]
CRP (mg/L)[2,3,5*]										
Women	1.8 ± 1.9	1.6 ± 0.9	2.3 ± 2.6	0.7 ± 0.6[a]	1.7 ± 2.5[b]	1.3 ± 1.5[a]	1.8 ± 2.7[a]	0.8 ± 0.8[a]	1.7 ± 1.9	2.0 ± 1.8
Men	1.3 ± 1.6	0.8 ± 0.9	1.2 ± 1.8	0.5 ± 0.2[a]	0.5 ± 0.2[b]	0.5 ± 0.2[a]	0.6 ± 0.4[a]	0.5 ± 0.3[a]	1.6 ± 1.3	0.8 ± 0.5
Ceruloplasmin (mg/dL)[2,3,8*]										
Women	32 ± 9	33 ± 9	34 ± 10	35 ± 9	35 ± 11	34 ± 9	36 ± 8	33 ± 9	32 ± 8	33 ± 9
Men	23 ± 3	24 ± 3	24 ± 3	24 ± 4	23 ± 4	22 ± 3	22 ± 2	22 ± 2	22 ± 3	23 ± 3
Prealbumin (mg/dL)[8,9*]										
Women	23 ± 3	22 ± 2	25 ± 4	25 ± 4	26 ± 4	27 ± 4	26 ± 4	26 ± 5	23 ± 3	22 ± 3
Men	30 ± 3	32 ± 4	31 ± 5	30 ± 5	30 ± 5	31 ± 5	32 ± 4	30 ± 4	30 ± 3	29 ± 4
Hct (%)[5,8]										
Women	39 ± 2	38 ± 2	ND	ND	ND	ND	ND	ND	36 ± 3[c*]	38 ± 3[b]
Men	42 ± 3	42 ± 3	ND	ND	ND	ND	ND	ND	40 ± 4[c*]	40 ± 3[b]
Hb (g/dL)[8,9*]										
Women	13 ± 1	13 ± 1	ND	ND	ND	ND	ND	ND	12 ± 1[b]	13 ± 1[b]
Men	14 ± 1	15 ± 1	ND	ND	ND	ND	ND	ND	14 ± 1[b]	14 ± 1[b]
MCH (pg)										
Women	31 ± 2	31 ± 2	ND	ND	ND	ND	ND	ND	31 ± 3	31 ± 2
Men	31 ± 1	31 ± 1	ND	ND	ND	ND	ND	ND	31 ± 1	31 ± 1

(Continued)

TABLE 1 (*Continued*)

	L−180	L−45	L−10	FD15	FD30	FD60	FD120	FD180	R+0	R+30
MCHC (g/dL)[3,6,9]										
Women	34 ± 1	34 ± 1	ND	ND	ND	ND	ND	ND	34 ± 0[b]	34 ± 0
Men	34 ± 1	34 ± 1	ND	ND	ND	ND	ND	ND	35 ± 1[b]	34 ± 0
MCV (fL)[9]										
Women	91 ± 4	92 ± 5	ND	ND	ND	ND	ND	ND	91 ± 6[c]	92 ± 6
Men	91 ± 3	91 ± 3	ND	ND	ND	ND	ND	ND	89 ± 2[c]	91 ± 3
RDW (%)[5*]										
Women	13.7 ± 0.8	13.8 ± 0.7	ND	ND	ND	ND	ND	ND	12.9 ± 0.5[c,*]	13.9 ± 1.1[c,*]
Men	13.6 ± 0.8	13.6 ± 0.3	ND	ND	ND	ND	ND	ND	12.6 ± 0.6[c,*]	14.4 ± 0.6[c,*]

[1] All values are means ± SDs. * P value remained significant after a Bonferroni post hoc adjustment for multiple comparisons ($P < 0.0004$). [a,b,c]A priori contrasts indicated a significant difference from L−10: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$. BW, body weight; CRP, C-reactive protein; FD, flight day; Hb, hemoglobin; Hct, hematocrit; L−, launch minus (days before launch); MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; ND, not determined; R+0, landing day; R+30, 30 d after landing; RDW, red blood cell distribution width.

[2] Log or skew log transformed to achieve normality and equal variance. Mixed-effects linear regression modeling setting 2-tailed α to reject the null hypothesis at 0.05 was used to evaluate the effects of sex and flight on markers of iron status.

[3] Statistical outliers were removed (ceruloplasmin: 3; ferritin: 2; heme: 6; high-sensitivity CRP: 5; iron: 1; MCHC: 2; transferrin: 1; transferrin receptors: 3; hepcidin: 6; body iron: 2; transferrin index: 1).

[4,7] Significant interaction between sex and time: [4]$P < 0.001$, [7]$P < 0.01$.

[5,9] Main effect of time: [5]$P < 0.001$, [9]$P < 0.01$.

[6,8] Main effect of sex: [6]$P < 0.05$, [8]$P < 0.001$.

but concentrations were significantly higher in women than in men before and during flight. After adjustment for multiple comparisons, transferrin values on FD30, 60, and 120 remained significantly greater than preflight values. The transferrin index, which is an estimate of transferrin saturation, increased significantly during flight; at times during flight, it was >1 μmol Fe/μmol transferrin, which indicated iron overload. The concentration of transferrin receptors was lower during flight on FD15, 30, 60, and 180 (only the value on FD30 remained significant after the post hoc adjustment) and on landing day. A significant interaction between space flight and sex occurred for heme (P = 0.005), in that women had a slightly lower heme concentration on landing day and men had a higher concentration, but these differences did not remain significant after the post hoc adjustment. When the data were analyzed with the statistical outliers in the model, the P value increased to 0.090.

Time had a significant main effect on serum iron (Table 1). Although this seemed to be related mostly to a decrease at landing ($P < 0.0004$), this effect remained significant (P = 0.03), even when only preflight and in-flight data were included in the analysis. However, this effect of time was not significant after post hoc adjustment.

No effects of sex or space flight on hepcidin, an important regulator of iron homeostasis, were detected. Ceruloplasmin, which is an acute phase protein, was significantly higher in women ($P < 0.0004$), but space flight had no effect on ceruloplasmin in either sex. CRP, an indicator of inflammation, was generally lower during flight in both sexes ($P < 0.0004$), but no significant differences remained on specific days compared with L−10 after the post hoc adjustment. Prealbumin, a negative acute phase protein, was significantly higher in men than in women ($P < 0.0004$). Prealbumin tended to increase during flight (P = 0.006), but after post hoc testing these values were not significantly different from the L−10 value.

Both hemoglobin and hematocrit were lower on landing day and 30 d after landing than on L−45, but only the decrease in hematocrit remained significant after adjustment for multiple comparisons. Hemoglobin was higher in men before and after flight ($P < 0.0004$), but mean corpuscular (red blood cell) hemoglobin did not change in response to space flight (Table 1). Mean corpuscular volume and mean corpuscular hemoglobin concentration were significantly lower on landing day (P = 0.0006 and P = 0.007, respectively). These changes were not significant after post hoc analyses. Red blood cell distribution width was also lower on landing day and 30 d after landing ($P <$ 0.0004).

Correlation of iron-status markers with markers of oxidative damage and bone loss

Ferritin was negatively correlated with superoxide dismutase ($r = -0.32$, P = 0.008; **Table 2**), which meant that a higher ferritin concentration was associated with a lower superoxide dismutase activity. Ferritin was positively correlated with urinary 8OHdG (r = 0.54, $P < 0.0004$), PGF2α (r = 0.27, $P <$ 0.0004), helical peptide (r = 0.52, $P < 0.0004$), NTX (r = 0.42, $P < 0.0004$), and calcium (r = 0.42, $P < 0.0004$). The percentage changes in ferritin and 8OHdG have remarkably similar patterns (**Figure 1**). Serum iron was positively correlated with total lipid peroxides (r = 0.37, P = 0.003), urinary 8OHdG

TABLE 2
Correlation coefficients (r) between biomarkers of iron status, oxidative damage, and bone resorption of crew members before and during long-duration space flight on the International Space Station[1]

	Superoxide dismutase	Lipid peroxides	Urinary 8OHdG	Urinary prostaglandin F2α	Urinary helical peptide	Urinary *N*-telopeptide	Urinary calcium
	U/g Hb	*μmol/L*	*μg/d*	*ng/d*	*μg/d*	*nmol/d*	*mmol/d*
Ferritin (ng/mL)	−0.32[2]	—	0.54[3]	0.27[3]	0.52[3]	0.42[3]	0.42[3]
Serum iron (μmol/L)	—	0.37[2]	0.21[4]	0.16[4]	—	—	0.18[4]
Transferrin index[5]	—	0.49[3]	0.33[3]	0.24[2]	0.28[2]	0.29[3]	0.36[3]

[1] n = 23 for all analyses except for helical peptide, *N*-telopeptide, and calcium, where n = 18. Hb, hemoglobin; 8OHdG, 8-hydroxy-2′-deoxyguanosine.
[2–4] Significant Pearson correlation: [2]$P < 0.01$, [3]$P < 0.001$, [4]$P < 0.05$.
[5] Calculated as a ratio of serum iron to transferrin, with both analytes expressed as μmol/L, and is used as an estimate for calculating the iron saturation of transferrin (25).

(r = 0.21, P = 0.010), and PGF2α (r = 0.16, P = 0.040). The transferrin index was positively correlated with plasma lipid peroxides ($P < 0.0004$) and urinary 8OHdG ($P < 0.0004$), PGF2α (P = 0.002), helical peptide (P = 0.002), NTX ($P < 0.001$), and calcium ($P < 0.0004$).

The AUC for the increase in ferritin during space flight was generated to quantify how much and how long ferritin was elevated during flight for each subject, and this was compared with changes in measures of BMD (**Table 3**). Negative correlations occurred between the AUC for ferritin and the percentage change from preflight in the BMD of hip (P = 0.031), trochanter (P = 0.006), hip neck (P = 0.044), and pelvis (P = 0.049). The higher the ferritin AUC, the more negative the percentage change from before flight, regardless of sex or type of exercise used during flight. Total BMD and lumbar spine BMD were not correlated with ferritin AUC.

DISCUSSION

Iron stores increased early during space flight and then returned to preflight concentrations by the end of 6-mo missions. Transferrin and transferrin receptors decreased later during flight, which supports the idea that mobilization of iron to storage in tissues increased. The transferrin index tended to increase early during flight (FD15), which indicated that transferrin was more saturated during flight. At several time points during flight (FD15, FD60, and FD120), the transferrin index was >1 μmol Fe/μmol transferrin, which is considered to indicate iron overload (25). Overall, the data support the idea that tissue iron stores increased during space flight, but an important question remains: are transient increases in iron stores a cause for concern? Although mean ferritin concentrations during flight were not outside the normal clinical range, the increase in ferritin was associated with evidence of oxidative damage and bone resorption.

Ferritin is well known to be an acute phase protein upregulated during an inflammatory response. The increase in ferritin early in space flight could have been caused by several factors, and one that cannot be ignored is inflammation. At the start of an inflammatory response (26), a rise in ferritin parallels a rise in CRP, and such a rise in CRP was not observed during flight; in fact, CRP decreased. Ceruloplasmin typically increases by 30–60% during an inflammatory response (26), but it did not change during flight. Prealbumin is a negative acute phase protein (27), which tends to decrease during infection and inflammation. Our space flight data indicate that prealbumin tended to increase slightly during flight. A main effect of time was seen, but no time points were different from L−10 on post hoc testing. Finally, the soluble transferrin receptor concentration is a sensitive indicator of tissue iron availability, and several studies indicate that it is not affected by chronic disease or inflammation (26, 28). In pregnant women with tissue iron deficiency, with or without infection, the circulating transferrin receptor concentration increased (29). During space flight, transferrin receptors decreased early and continued to decrease later, which further suggests that inflammation was not responsible for the observed increase in serum ferritin.

In this healthy astronaut population, ferritin, serum iron, and transferrin index were associated with markers of oxidative damage to DNA (8OHdG) and lipids (lipid peroxides and urinary PGF2α). The change in ferritin over the course of a 6-mo mission was strikingly similar to the change in urinary 8OHdG during space flight (Figure 1). Astronauts are exposed to an environment in which multiple oxidative stressors are present (radiation, changes in oxygen, and rigorous exercise). Others have documented similar associations between iron status (ferritin) and markers of oxidative damage, including malondialdehyde, urinary 8OHdG, advanced oxidation protein products, and oxidized LDL in healthy individuals in the general population (30–32). Furthermore, in an intervention study in

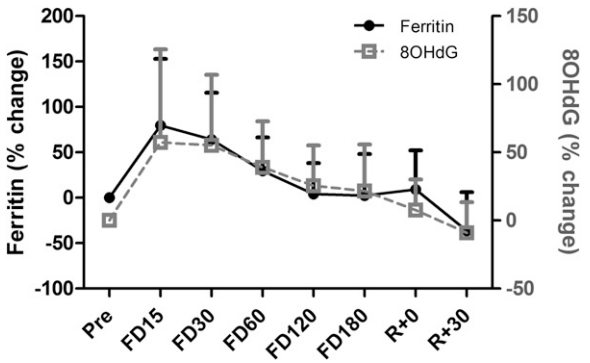

FIGURE 1. Mean (±SD) percentage change in serum ferritin and urinary 8OHdG in crew members before, during, and after long-duration space flight (n = 23). The preflight mean was determined from the mean of Pre data points (3 for ferritin, 4 for 8OHdG), and percentage change was calculated from that average. Other points represent the mean percentage change from Pre. Mixed-effects linear regression modeling setting 2-tailed α to reject the null hypothesis at 0.05 was used to evaluate the effects of sex and flight on markers of iron status. FD, flight day; Pre, preflight; R+0, landing day; R+30, 30 d after landing; 8OHdG, 8-hydroxy-2′-deoxyguanosine.

TABLE 3
Correlation coefficients (r) for the association between percentage change in bone mineral density of crew members after long-duration space flight and the increase in ferritin during flight expressed as the AUC[1]

	Ferritin AUC
Total left hip BMD	−0.51*
Left hip trochanter BMD	−0.62**
Left hip neck BMD	−0.48*
Pelvis BMD	−0.47*
Total BMD	—
Total lumbar spine BMD	—

[1] The 18 long-duration crew members presented in this analysis were not participating in any countermeasure studies in which bisphosphonates were administered to mitigate bone loss. The ferritin AUC was determined for each crew member and was associated with the percentage change in bone mineral density measured within 5 d of landing relative to preflight measurements. ***Significant Pearson correlation: *$P < 0.05$, **$P < 0.01$. BMD, bone mineral density.

which iron fortification of flour was tested for 8 and 16 mo, iron-related oxidative stress increased after exposure to a relatively small increase in dietary iron (33). Iron can be liberated from ferritin during periods of oxidative stress or exposure to some types of radiation (34–36). The observations that serum iron and transferrin index were also related to markers of oxidative damage support the involvement of iron in the types of oxidative damage represented by these markers, but we do not know whether the changes in oxidative damage are a cause or effect of the increase in ferritin.

The changes in iron stores during space flight were also related to markers of bone resorption and changes in BMD after flight. The greater the increase in ferritin during flight (or the longer it was elevated—either case would result in a higher AUC), the greater the decrease in BMD in the hip trochanter, hip neck, and pelvis after long-duration space flight. In support of this result are the correlations between ferritin or transferrin index and biochemical markers of bone resorption (helical peptide and NTX) or urinary calcium. Several other human and animal studies have also shown that iron overload is associated with bone loss by a mechanism believed to be related to oxidative stress (37–39). Kim et al (39) found that, in healthy subjects aged ≥40 y, increased body iron stores (assessed by measuring serum ferritin) were related to the rate of change in regional bone loss over 3 y. Except for the highest quartile (ferritin: 187–419 ng/mL), the ferritin concentrations in that study were not in the range expected with iron-overload diseases, but rather were within a normal clinical range. Furthermore, the overall range was similar to the range for the 23 crew members (7–631 ng/mL), the data for whom are presented here. Although we know that consuming enough calories and exercising with the advanced resistive exercise device during space flight mitigate changes in BMD (16), it appears that the change in ferritin and overall iron stores during flight may be another important factor to consider when defining dietary intake recommendations for iron, and potentially for antioxidants, during space flight.

Another implication for changes in iron status during flight is related to immunity and infection. The virulence of an extensive list of pathogens and other microorganisms is enhanced by iron (40). In one study, women with the highest ferritin concentrations (>120 ng/mL) were less likely to clear human papillomavirus infection than were individuals with lower ferritin concentrations (<120 mg/mL), which suggests that elevated iron stores may increase the risk of persistent infections (41). This could have significant consequences for astronauts, considering that the documented dysregulation of the immune system (42), a known increase in bacterial pathogenicity and virulence during space flight (43), and the increase in iron stores could combine and lead to an overall increase in disease incidence.

The hepcidin peptide was recently characterized as one of the main regulatory hormones of iron absorption and recirculation, and it is induced during inflammatory processes and infections (44, 45). An increase in circulating hepcidin is associated with a decrease in iron absorption and a decrease in release of iron from macrophages. Circulating hepcidin did not change during or after flight, which suggests that, despite high iron intakes by astronauts, iron absorption may not have changed. However, we cannot rule out the potential for a change in iron absorption during flight because microgravity may affect the hepcidin response. One factor contributing to the increase in ferritin early during flight could be the high dietary intake of iron. A possible mechanism for reducing the elevated iron stores that occur early in flight is to decrease absorption until new red blood cell production and turnover normalize iron status. Another possibility is a shift to long-term iron storage in the liver or other tissues. This question of the mechanism responsible for the return to normal iron status cannot be answered with the current data set. Until further iron kinetics studies are conducted, it is recommended that iron intake during flight be lowered to levels closer to the current Recommended Dietary Allowance for iron on Earth.

The changes in hematocrit, mean corpuscular volume, and red blood cell distribution width observed at landing were most likely not direct effects of space flight. They were probably related to either dilution effects, in response to the replacement of lost plasma volume much faster than lost red blood cells, or to saline infusions received by many crew members soon after landing (to help mitigate orthostatic intolerance) and before collection of blood samples.

In summary, greater ferritin concentrations were associated with greater concentrations of biomarkers of oxidative damage and with greater decreases in BMD in some regions after long-duration space flight. The data from this study and previous studies show that even transient increases in iron stores may have a detrimental effect because of the association of iron status with oxidative damage and bone loss. Even when iron stores are within a clinically normal range, as they are in astronauts in space and in healthy populations on Earth, a prolonged increase in iron intake with an increase in ferritin may exacerbate oxidative stress and impair bone metabolism and perhaps other aspects of health.

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analyzed the data; and SMS: had primary responsibility for the final content. All authors contributed to the design of the study, interpreted the results of the experiment, and edited, revised, and approved the final version of the manuscript. All authors declared that they had no conflict of interest.

REFERENCES

1. Beard JL. Iron. In: Bowman BA, Russell RM, eds. Present knowledge in nutrition. 9th ed. Washington, DC: International Life Sciences Institute, 2006:430–44.
2. Crichton RR. Iron metabolism from molecular mechanisms to clinical consequences. 3rd ed. West Sussex, United Kingdom: John Wiley & Sons Ltd, 2009.
3. Morlière P, Salmon S, Aubailly M, Risler A, Santus R. Sensitization of skin fibroblasts to UVA by excess iron. Biochim Biophys Acta 1997; 1334:283–90.
4. Nelson JM, Stevens RG. Ferritin-iron increases killing of Chinese hamster ovary cells by X-irradiation. Cell Prolif 1992;25:579–85.
5. Loh A, Hadziahmetovic M, Dunaief JL. Iron homeostasis and eye disease. Biochim Biophys Acta 2009;1790:637–49.
6. Deleon E, Lederman M, Berenstein E, Meir T, Chevion M, Chowers I. Alteration in iron metabolism during retinal degeneration in rd10 mouse. Invest Ophthalmol Vis Sci 2009;50:1360–5.
7. Alfrey CP, Rice L, Udden MM, Driscoll TB. Neocytolysis: physiological down-regulator of red-cell mass. Lancet 1997;349:1389–90.
8. Alfrey CP, Udden MM, Leach-Huntoon C, Driscoll T, Pickett MH. Control of red blood cell mass in spaceflight. J Appl Physiol 1996;81: 98–104.
9. Leach CS. Biochemical and hematologic changes after short-term space flight. Microgravity Q 1992;2:69–75.
10. Smith SM. Red blood cell and iron metabolism during space flight. Nutrition 2002;18:864–6.
11. Smith SM, Zwart SR, Block G, Rice BL, Davis-Street JE. The nutritional status of astronauts is altered after long-term space flight aboard the International Space Station. J Nutr 2005;135:437–43.
12. Smith SM, Zwart SR, Kloeris V, Heer M. Nutritional biochemistry of space flight. New York, NY: Nova Science Publishers, 2009.
13. National Aeronautics and Space Administration. Nutrition requirements, standards, and operating bands for exploration missions. Houston, TX: Lyndon B. Johnson Space Center, 2005 (JSC Document no. 63555).
14. Institute of Medicine. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press, 2001.
15. Smith SM, Heer M, Wang Z, Huntoon CL, Zwart SR. Long-duration space flight and bed rest effects on testosterone and other steroids. J Clin Endocrinol Metab 2012;97:270–8.
16. Smith SM, Heer MA, Shackelford L, Sibonga JD, Ploutz-Snyder L, Zwart SR. Benefits for bone from resistance exercise and nutrition in long-duration spaceflight: evidence from biochemistry and densitometry. J Bone Miner Res 2012;27:1896–906.
17. Smith SM, Wastney ME, Morukov BV, Larina IM, Nyquist LE, Abrams SA, Taran EN, Shih CY, Nillen JL, Davis-Street JE, et al. Calcium metabolism before, during, and after a 3-mo spaceflight: kinetic and biochemical changes. Am J Physiol 1999;277:R1–10.
18. Smith SM, Wastney ME, O'Brien KO, Morukov BV, Larina IM, Abrams SA, Davis-Street JE, Oganov V, Shackelford LC. Bone markers, calcium metabolism, and calcium kinetics during extended-duration space flight on the Mir space station. J Bone Miner Res 2005; 20:208–18.
19. Smith SM, Davis-Street JE, Rice BL, Nillen JL, Gillman PL, Block G. Nutritional status assessment in semiclosed environments: ground-based and space flight studies in humans. J Nutr 2001;131:2053–61.
20. Soller BR, Cabrera M, Smith SM, Sutton JP. Smart medical systems with application to nutrition and fitness in space. Nutrition 2002;18: 930–6.
21. Zwart SR, Jessup JM, Ji J, Smith SM. Saturation diving alters folate status and biomarkers of DNA damage and repair. PLoS ONE 2012;7: e31058.
22. Zwart SR, Kala G, Smith SM. Body iron stores and oxidative damage in humans increased during and after a 10- to 12-day undersea dive. J Nutr 2009;139:90–5.
23. Smith SM, Davis-Street JE, Fesperman JV, Smith MD, Rice BL, Zwart SR. Nutritional assessment during a 14-d saturation dive: the NASA Extreme Environment Mission Operations V Project. J Nutr 2004;134: 1765–71.
24. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood 2003;101:3359–64.
25. Beilby J, Olynyk J, Ching S, Prins A, Swanson N, Reed W, Harley H, Garcia-Webb P. Transferrin index: an alternative method for calculating the iron saturation of transferrin. Clin Chem 1992;38:2078–81.
26. Northrop-Clewes CA. Interpreting indicators of iron status during an acute phase response–lessons from malaria and human immunodeficiency virus. Ann Clin Biochem 2008;45:18–32.
27. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448–54.
28. Beguin Y. The soluble transferrin receptor: biological aspects and clinical usefulness as quantitative measure of erythropoiesis. Haematologica 1992;77:1–10.
29. Semba RD, Kumwenda N, Hoover DR, Taha TE, Mtimavalye L, Broadhead R, Eisinger W, Miotti PG, Chiphangwi JD. Assessment of iron status using plasma transferrin receptor in pregnant women with and without human immunodeficiency virus infection in Malawi. Eur J Clin Nutr 2000;54:872–7.
30. Mendes JFR, Arrruda SF, de Almeida Siquira EM, Ito MK, da Silva EF. Iron status and oxidative stress biomarkers in adults: a preliminary study. Nutrition 2009;25:379–84.
31. Tuomainen TP, Loft S, Nyyssonen K, Punnonen K, Salonen JT, Poulsen HE. Body iron is a contributor to oxidative damage of DNA. Free Radic Res 2007;41:324–8.
32. Syrovatka P, Kraml P, Potockova J, Fialova L, Vejrazka M, Crkovska J, Andel M. Relationship between increased body iron stores, oxidative stress and insulin resistance in healthy men. Ann Nutr Metab 2009;54:268–74.
33. Pouraram H, Elmadfa I, Dorosty AR, Abtahi M, Neyestani TR, Sadeghian S. Long-term consequences of iron-fortified flour consumption in nonanemic men. Ann Nutr Metab 2012;60:115–21.
34. Biemond P, van Eijk HG, Swaak AJ, Koster JF. Iron mobilization from ferritin by superoxide derived from stimulated polymorphonuclear leukocytes. Possible mechanism in inflammation diseases. J Clin Invest 1984;73:1576–9.
35. Aubailly M, Santus R, Salmon S. Ferrous ion release from ferritin by ultraviolet-A radiations. Photochem Photobiol 1991;54:769–73.
36. Reif DW, Schubert J, Aust SD. Iron release from ferritin and lipid peroxidation by radiolytically generated reducing radicals. Arch Biochem Biophys 1988;264:238–43.
37. Tsay J, Yang Z, Ross FP, Cunningham-Rundles S, Lin H, Coleman R, Mayer-Kuckuk P, Doty SB, Grady RW, Giardina PJ, et al. Bone loss caused by iron overload in a murine model: importance of oxidative stress. Blood 2010;116:2582–9.
38. Isomura H, Fujie K, Shibata K, Inoue N, Iizuka T, Takebe G, Takahashi K, Nishihira J, Izumi H, Sakamoto W. Bone metabolism and oxidative stress in postmenopausal rats with iron overload. Toxicology 2004;197: 93–100.
39. Kim BJ, Ahn SH, Bae SJ, Kim EH, Lee SH, Kim HK, Choe JW, Koh JM, Kim GS. Iron overload accelerates bone loss in healthy postmenopausal women and middle-aged men: a 3-year retrospective longitudinal study. J Bone Miner Res 2012;27:2279–90
40. Ashrafian H. Hepcidin: the missing link between hemochromatosis and infections. Infect Immun 2003;71:6693–700.
41. Siegel EM, Patel N, Lu B, Lee JH, Nyitray AG, Huang X, Villa LL, Franco EL, Giuliano AR. Circulating biomarkers of iron storage and clearance of incident human papillomavirus infection. Cancer Epidemiol Biomarkers Prev 2012;21:859–65.
42. Crucian BE, Stowe RP, Pierson DL, Sams CF. Immune system dysregulation following short- vs long-duration spaceflight. Aviat Space Environ Med 2008;79:835–43.
43. Wilson JW, Ott CM, Honer zu Bentrup K, Ramamurthy R, Quick L, Porwollik S, Cheng P, McClelland M, Tsaprailis G, Radabaugh T, et al. Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. Proc Natl Acad Sci USA 2007;104: 16299–304.
44. Ganz T. Hepcidin–a peptide hormone at the interface of innate immunity and iron metabolism. Curr Top Microbiol Immunol 2006;306: 183–98.
45. Ganz T. Hepcidin and iron regulation, 10 years later. Blood 2011;117: 4425–33.