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Oxidant damage during and after spaceflight

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Stein, T. P., and M. J. Leskiw. Oxidant damage during and after spaceflight. *Am. J. Physiol. Endocrinol. Metab.* 278: E375–E382, 2000.—The objectives of this study were to assess oxidant damage during and after spaceflight and to compare the results against bed rest with 6° head-down tilt. We measured the urinary excretion of the F₂ isoprostane, 8-iso-prostaglandin (PG) F_{2α}, and 8-oxo-7,8-dihydro-2 deoxyguanosine (8-OH DG) before, during, and after long-duration spaceflight (4–9 mo) on the Russian space station MIR, short-duration spaceflight on the shuttle, and 17 days of bed rest. Sample collections on MIR were obtained between 88 and 186 days in orbit. 8-iso-PGF_{2α} and 8-OH DG are markers for oxidative damage to membrane lipids and DNA, respectively. Data are mean ± SE. On MIR, isoprostane levels were decreased inflight (96.9 ± 11.6 vs. 76.7 ± 14.9 ng·kg⁻¹·day⁻¹, *P* < 0.05, *n* = 6) due to decreased dietary intake secondary to impaired thermoregulation. Isoprostane excretion was increased postflight (245.7 ± 55.8 ng·kg⁻¹·day⁻¹, *P* < 0.01). 8-OH DG excretion was unchanged with spaceflight and increased postflight (269 ± 84 vs 442 ± 180 ng·kg⁻¹·day⁻¹, *P* < 0.05). On the shuttle, 8-OH DG excretion was unchanged in- and postflight, but 8-iso-PGF_{2α} excretion was decreased inflight (15.6 ± 4.3 vs 8.0 ± 2.7 ng·kg⁻¹·day⁻¹, *P* < 0.05). No changes were found with bed rest, but 8-iso-PGF_{2α} was increased during the recovery phase (48.9 ± 23.0 vs 65.4 ± 28.3 ng·kg⁻¹·day⁻¹, *P* < 0.05). The changes in isoprostane production were attributed to decreased production of oxygen radicals from the electron transport chain due to the reduced energy intake inflight. The postflight increases in the excretion of the products of oxidative damage were attributed to a combination of an increase in metabolic activity and the loss of some host antioxidant defenses inflight. We conclude that 1) oxidative damage was decreased inflight, and 2) oxidative damage was increased postflight.

isoprostanes; 8-hydroxydeoxyguanosine

THERE ARE A NUMBER OF REASONS for suspecting that oxidative stress may be increased with spaceflight. There is an increase in the exposure to high-energy radiation because of the absence of the protective effects of the earth's atmosphere, with the resultant generation of high-energy free radicals (25). Other possible causes for increased free-radical generation are altered oxygen metabolism from perturbed gas exchange within the lungs (38), or a change in intermediary metabolism. The Skylab investigators suggested that a possible reason for an apparent increase in

energy expenditure during spaceflight was an uncoupling of oxidative phosphorylation (28). A similar suggestion was made by Burakhova and Mailyan after examining the electron transport system in rat skeletal muscle after 3 wk in space (3). The body generates ~5 g of reactive oxygen species per day, mostly by leakage from the electron transport chain during oxidative phosphorylation (12).

Until recently, the quantification of oxidative stress has been difficult to assess in humans because of the lack of sensitive and reliable assays. This problem now appears to have been solved by the discovery of the F₂ isoprostanes to assess free-radical lipid oxidation and the development of methods for the analysis of the products of DNA oxidation, specifically 8-oxo-7,8-dihydro-2 deoxyguanosine (8-hydroxydeoxyguanosine, 8-OH DG) (20, 24, 29). Isoprostanes are derived from arachidonic acid containing phospholipids by autooxidation, leading to a series of PGF_{2α}-like compounds. The bicycloendoperoxide PG intermediates are reduced to four regioisomers, each of which can comprise eight racemic diastereoisomers. These 64 isomers are collectively called the PGF_{2α} isoprostanes [8-iso-prostaglandin (PG) F_{2α}].

Like lipids, DNA is also susceptible to oxidative damage (1, 7, 27); in fact, DNA is constantly being damaged and repaired in living cells. It has been estimated that the damage rate is ~10⁴ nucleotides·cell⁻¹·day⁻¹ (20). The most abundant of the nucleotide oxidation products is 8-hydroxydeoxyguanosine. Once produced, 8-hydroxydeoxyguanosine is not further degraded and is excreted in the urine without further metabolism (19). Measurements of urinary isoprostane and 8-hydroxydeoxyguanosine excretion have provided strong supporting evidence for a role for oxidative damage in the pathogenesis of a wide variety of human disorders, including atherosclerosis and cancer (11, 20, 21, 24).

The primary objectives of this experiment were to assess oxidant stress during and after long-duration spaceflight on the Russian space station, MIR. To assess oxidative damage, we measured the urinary excretion of 8-iso-PGF_{2α} and 8-hydroxydeoxyguanosine. Together the two assays can provide an assessment of any oxidative damage incurred. To facilitate interpretation of the data from MIR, we compared the MIR data against the results from a short-duration (17-day) shuttle mission and a 6° head-down tilt, bed-rest study.

METHODS

Informed consent forms were obtained in accordance with the policies of the United States National Aeronautics and

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Space Administration (NASA), the Russian Academy of Sciences Experiment Board, The Russian Space Agency (RSA), and the University of Medicine and Dentistry of New Jersey.

Long-Duration Flight on MIR

The subjects for this study were two astronauts (American) and four cosmonauts (Russian). Time in earth orbit varied with the subject; range was 4–9 mo.

Pre- and postflight. The preflight measurements consisted of between two and four sessions during the year before the mission. Each session lasted 2 days. Twenty-four-hour pools were obtained on *day 2*, and for most sessions 24-h pools were also collected on *day 1* of the session. The actual number of preflight sessions depended on crew availability. Sessions were conducted either at NASA facilities in the US (the Johnson and Kennedy Space Centers) or the RSA facility at Star City, near Moscow. During each of the two days, the subjects kept a detailed record of their dietary intake.

The American astronauts were launched and landed on the shuttle in Florida; however, before launch they lived in Russia, in RSA facilities in Star City. They were flown to Houston and the postflight studies were continued at the Johnson Space Center. The Russian cosmonauts returned on a Soyuz space vehicle and spent the first 2 wk postflight at Star City. The postflight studies followed the same protocol as the preflight, with sessions being either return (R)+0 and R+1, or R+1 and R+2, R+6 and R+7, and R+13 and R+14. On occasion, a session was displaced by a day due to a crew member's being unavailable. For the preflight period, each session entailed 2 days of dietary monitoring with 24-h urine collections on ≥ 1 , but usually 2, days.

Inflight. With two exceptions, a similar protocol to that of the preflight and postflight periods was performed inflight. All of the inflight data were collected between 88 and 147 days of spaceflight [mean 147 ± 8 days (33)]. Briefly, the food was bar coded, and the crew recorded the time and amount of food eaten. Opportunities for collecting 24-h urines on MIR were limited because of the need to conserve water; only one 24-h urine collection could be done at a time, because on MIR, urine water was recycled for future use. Inflight urine collections were done between 88 and 147 days after launch. The urines were collected in specially designed plastic bags. An aliquot was removed and put in a 10-ml syringe, which was placed in the on-board -20°C freezer. Samples were brought back to earth on the shuttle supply missions and stored at -70°C until analyzed (details given in Ref. 33).

Diet analysis. The dietary records were analyzed by NASA personnel from the Nutrition and Metabolism Laboratory of the Johnson Space Center by use of the Nutritionist 2.8 program (University of Minnesota, St. Paul, MN), together with some data especially collected by NASA personnel on Russian foods.

Space Shuttle

The urine samples used for this study were collected on the 17-day Life and Microgravity mission (LMS), which was flown in the summer of 1996. Details of the mission and the sample collection are given in Ref. 34. Briefly, the subjects were the four payload crew members of the LMS mission. There were two overall goals of the mission. The first was to conduct a series of experiments on the response of the human musculoskeletal system to spaceflight, and the second was to perform a series of material science experiments. The samples analyzed for this experiment were the urines collected as part of the nitrogen and energy balance studies (34). Daily dietary intake was monitored, and daily urine collections were made

for the period beginning 15 days before launch and ending 15 days after landing, as previously described (34). Samples were stored at -16°C inflight, and after return to earth they were transferred to a freezer at -70°C .

Bed Rest

A 17-day bed-rest study with 6° head-down tilt was conducted in the Clinical Research Center of the NASA-Ames Research Center with eight healthy adult males recruited from the local community. The objective of the bed-rest study was to compare bed rest and spaceflight, using the LMS mission as the flight study. Accordingly, the bed-rest study was designed to simulate the flight experiment as closely as possible. The bed-rest study included the full complement of exercise testing that was done on the shuttle payload (34).

As with the flight study, the bed-rest study was divided into three phases, a 15-day pre-bed-rest ambulatory period, followed by 17 days of bed rest, and ending with a 15-day recovery period. During the 47 days of the study, the subjects received all their food from the research center. An attempt was made to provide the subjects with a "controlled" ad libitum diet. Twelve daily menus were made up, comprised of 2,500 kcal/day and 90 g protein/day. In addition, subjects were allowed access to a snack basket containing fruit, cookies, some candy, and granola bars. Details of the bed-rest study are given in Refs. 31 and 34. Urine was collected continuously for the 47-day period and kept frozen at -70°C until analyzed.

Analytical methodology. In some cases where a 24-h pool was missing from the MIR studies, the missing pool was supplied by NASA as part of a sample-sharing agreement between investigators. Most of the urinary creatinine values for the MIR studies were supplied by NASA; the remainder were measured by us using the picric acid method with a kit marketed by Sigma-Aldrich (St. Louis, MO). The 24-h pools for the LMS mission were available from our previous experiments (31, 34). Accurate 24-h pools were not available for the bed-rest study because all urine voids were ad libitum; therefore, some of the potential "24"-h pools deviated quite significantly from 24 h, so either 48- or 72-h pools were made up by the fractional aliquot method. Where the number of periods was uneven (e.g., the bed-rest period was actually 17 days), the paired days were selected so that the 2-day exercise periods fell in the same pool (31).

Isoprostane analyses were done on unextracted urine (UN) and an organic extract of urine (EX), by use of an ELISA kit (Oxford Chemicals, Oxford, MI). The isoprostanes were extracted from the urine using the methodology recommended by the manufacturer. Urine (0.5–1 ml) was adjusted to pH 3.0 and loaded onto a C_{18} Sep-Pak (Waters, Milford, MA). After the sample was washed with water (10 ml), followed by heptane (10 ml), the isoprostanes were eluted with ethyl acetate (5 ml). Sodium sulfate (~ 1 g) was added, and the solution was applied to a silica Sep-Pak and the isoprostanes eluted with a 1:1 mixture of ethyl acetate and methanol. The solvent was removed with dry N_2 , and the residue was dissolved in a known volume of a dilution buffer supplied by the kit manufacturer and assayed by ELISA. For the MIR isoprostane samples, each sample (extracted or unextracted) was assayed at three different dilutions. For 8-OH DG we used the ELISA kit (Genox, Baltimore, MD). Each sample from MIR was assayed in triplicate at three dilutions. For financial reasons the LMS flight and bed-rest studies were analyzed only for 8-iso-PGF $_{2\alpha}$ and 8-OH DG in duplicate at one dilution. We did not have enough urine to do both extracted and unextracted isoprostane determinations on the

Table 1. *MIR data*

	Units	Preflight	Inflight	Postflight				F
				R-All	R1	R6-8	R12-14	
Weight	kg	81.9 ± 2.2	ND	77.3 ± 1.9	ND	ND	ND	18.65
Energy intake	kcal · kg ⁻¹ · day ⁻¹	34.7 ± 2.8	26.9 ± 2.2	32.3 ± 1.6	31.1 ± 3.2	34.4 ± 2.6	31.4 ± 3.0	5.38
N intake	mg N · kg ⁻¹ · day ⁻¹	211 ± 26	193 ± 21	184 ± 20	184 ± 23	187 ± 26	180 ± 24	NS
Creatinine	g/day	1.67 ± 0.13	1.62 ± 0.19	1.61 ± 0.13	1.41 ± 0.19	1.55 ± 0.13	1.56 ± 0.12	NS
8-OH DG	ng · kg ⁻¹ · day ⁻¹	269 ± 84	270 ± 69	442 ± 180*	683 ± 261	445 ± 187	605 ± 188	4.74
8-OH DG	ng · g creatinine ⁻¹ · day ⁻¹	13.2 ± 3.7	14.0 ± 2.3	33.6 ± 10.9*	25.4 ± 10.4	36.9 ± 14.9	23.9 ± 10.7	6.77
UN 8-iso-PGF _{2α}	ng · kg ⁻¹ · day ⁻¹	96.6 ± 11.6	76.7 ± 14.9	245.7 ± 55.8*	167.0 ± 45.6	281.2 ± 79.7	228.6 ± 59.1	12.98
UN 8-iso-PGF _{2α}	ng · g creatinine ⁻¹ · day ⁻¹	5.51 ± 1.27	4.39 ± 1.07	14.73 ± 4.25*	10.76 ± 3.41	17.80 ± 6.54†	13.05 ± 3.38	17.46
EX-8-iso-PGF _{2α}	ng · kg ⁻¹ · day ⁻¹	35.5 ± 5.9	20.2 ± 4.2*	87.1 ± 21.3*	55.1 ± 14.0	97.9 ± 30.3†	97.0 ± 20.7†	15.94
EX-8-iso-PGF _{2α}	ng · g creatinine ⁻¹ · day ⁻¹	2.10 ± 0.53	1.12 ± 0.21*	5.25 ± 1.55*	3.64 ± 1.25*	6.04 ± 2.61†	5.38 ± 0.82†	18.89

Diet, and isoprostane [8-iso-prostaglandin F_{2α} (8-iso-PGF_{2α})] and 8-oxo-7,8 dihydro-2 deoxyguanosine (8-OH DG) excretion before, during, and after spaceflight on MIR. Isoprostane data are for unextracted (UN) and extracted (EX) urine. Data have been normalized to either body wt or to creatinine. R(recovery)1, R6-8, and R12-14 refer to the number of days elapsed after landing. R-All is the mean value for the entire postflight period. Data are means ± SE for 6 astronauts and cosmonauts. Statistical analyses were by repeated-measures ANOVA (RMANOVA) on the mean preflight, inflight, and postflight values, **P* < 0.05 vs. preflight. If overall significance was found at the *P* < 0.01 level, paired *t*-tests were used to determine if any of the individual postflight time points were significantly different from the mean preflight value. Significance (†) was accepted at the *P* < 0.01 level. ND, not determined; NS, not significant.

LMS flight experiment urines, so we did only the unextracted assays.

Statistics. Data were analyzed by a repeated-measures design ANOVA (RMANOVA). For consistency all data sets were divided into three time periods, preflight (pre-bed-rest), flight (bed rest), and postflight (recovery), and the natural logarithms of the data were used for the RMANOVA. Significance was accepted at *P* < 0.05. If the RMANOVA indicated significance at *P* < 0.05 or better, group differences were identified by the Student-Newman-Keuls test. To investigate whether there was any time dependence in the postflight (bed-rest) period, we used paired *t*-tests of a specific postflight (bed-rest) period against the mean preflight value. Significance was accepted at *P* < 0.01. The Sigmasat Statistical System (SPSS, Chicago, IL) was used for the statistical computations. Data in the text, figures, and tables are means ± SE.

RESULTS

Regarding MIR, we have previously described the changes in nutrition and the accompanying weight changes for these astronauts before and after spaceflight (33). Briefly, the average weight loss was 4.6 ± 1.1 kg (range 2.4-8.0 kg, Table 1). Energy intake inflight was significantly less than either pre- or postflight (22 ± 9%, *P* < 0.05, Table 1). Postflight energy intake returned to, but was not increased over, the preflight levels. Likewise, dietary intake of the antioxidant vitamins (A, C, E, and selenium) were similar pre- and postflight (Table 2). We have no data on antioxidant intake inflight (Table 2). The urinary excretion of 8-iso-PGF_{2α} was decreased by 20% inflight (*P* < 0.05); 8-OH DG was unchanged (Table 1).

The postflight data collection schedule called for three sessions of 2 days per session, beginning on R+0 or R+1, R+6 or R+7 and R+13 or R+14. The sessions were combined to give a single data set for each subject. Isoprostane levels were decreased inflight by ~20% and increased postflight by 200% (Table 1, *P* < 0.01). These relationships applied to both UN and EX and

whether the results were expressed as per kilogram body wt or normalized to creatinine (Table 1). There was no change in 8-OH DG inflight, but as with 8-iso-PGF_{2α}, 8-OH DG excretion was substantially increased postflight (Table 1, *P* < 0.05). Figure 1 shows the postflight time course for 8-OH DG and 8-iso-PGF_{2α}. Excretion of 8-iso-PGF_{2α} was substantially increased for the duration of the postflight measurement period.

As for the shuttle, the dietary data in Table 3 are for the days corresponding to the urine samples analyzed, namely five samples from the week immediately preceding launch, the five last samples from the flight period, and the first five days after landing. Energy intake was decreased by 40% inflight. As with MIR, postflight energy intake returned to, but was not increased over, the preflight levels. No changes in 8-OH DG inflight or postflight were found; however, isoprostane levels in unextracted urine were decreased inflight by ~40% (Table 3, *P* < 0.01). No changes postflight were observed, although the error bars are much larger postflight than preflight (Fig. 2).

Energy intake was reduced by 10% during the bed-rest period (Table 4) (34). Excretion of 8-OH DG was unchanged during and after bed rest. Excretion of 8-iso-PGF_{2α} (extracted and unextracted) was un-

Table 2. *Antioxidant intakes before, during, and after spaceflight on MIR*

	Preflight	Inflight	Postflight
Selenium, µg/day	161 ± 22	NM	150 ± 20
Vitamin A, µg/day	1,237 ± 201	NM	1,427 ± 99
β-Carotene, µg/day	5,365 ± 1,052	NM	6,311 ± 731
Retinol, µg/day	344 ± 48	NM	373 ± 63
α-Tocopherol, mg/day	12.1 ± 1.4	NM	10.3 ± 0.8
Vitamin C, mg/day	166 ± 23	NM	169 ± 23

Values are means ± SE of 6 astronauts and cosmonauts. NM, not measured.

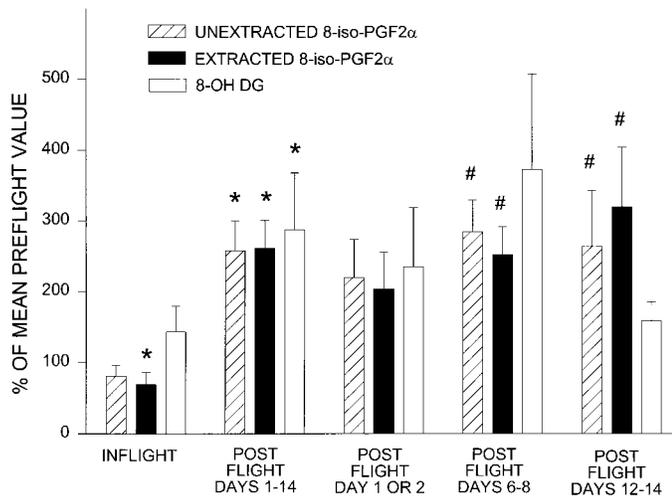


Fig. 1. Changes in unextracted (UN) and extracted (EX) isoprostanate [8-iso-prostaglandin $F_{2\alpha}$ (8-iso-PGF $_{2\alpha}$)] and 8-oxo-7,8 dihydro-2 deoxyguanosine (8-OH DG) excretion during and after spaceflight on MIR. There were 6 subjects. Data are expressed as percentage of mean preflight data normalized to creatinine. Statistical analyses for inflight and mean postflight against preflight mean were done by repeated-measures ANOVA (RMANOVA). * $P < 0.01$ vs. mean preflight for 8-iso-PGF $_{2\alpha}$ (EX or UN); $P < 0.05$ for 8-OH DG. To determine whether any of individual postflight isoprostanate data sets were significantly different from preflight, paired t -tests were used, with significance (#) accepted at $P < 0.01$.

changed during bed rest but was increased during the recovery phase by $\sim 30\%$ (Table 4). Statistical significance was found only with the data normalized to creatinine. As with the other two studies, the SEs are much greater, particularly for 8-iso-PGF $_{2\alpha}$ during the recovery phase (Fig. 3).

DISCUSSION

The database. Although there was considerable negative publicity in the lay press about the problems encountered with the Shuttle-MIR program, these flights (MIR 21 and 23) were less problematic than some of the earlier or later flights (4). As described in our previous paper, although the amount of data obtained was not large, the quality of the data was good (33). The principal reasons for the limited data were that 1) one of the Russian crew members was switched

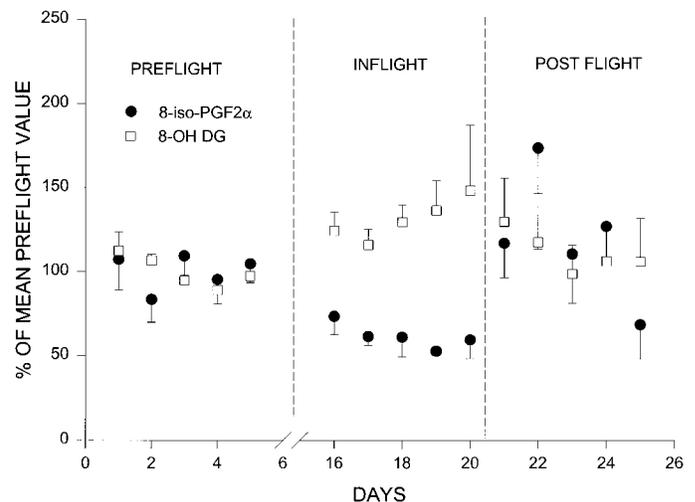


Fig. 2. Changes in UN isoprostanate and 8-OH DG excretion during and after spaceflight on the shuttle. Data are expressed as percentage of mean preflight data normalized to creatinine. Five urines from last week preflight, last 5 days inflight, and first 5 days postflight were analyzed. There were 4 subjects. No individual inflight or postflight data points are significantly different from mean preflight value, but overall mean inflight value is significantly different from either mean preflight or mean postflight ($P < 0.05$ by RMANOVA).

preflight for medical reasons; 2) technical problems on MIR precluded any measurements during the first three months in orbit; and 3) crew availability for all three phases of the experiment was limited (4).

There were also limitations for the shuttle and bed-rest studies. For the shuttle, the amount of urine available was inadequate to permit assays for both extracted and unextracted 8-iso-PGF $_{2\alpha}$ to be done. We elected to do the unextracted assay because the urine requirements were less. For both the shuttle and bed-rest analyses there were also financial limitations.

The ELISA for 8-iso-PGF $_{2\alpha}$ measures only one of several possible isomers. Although the values from extracted and unextracted urines correlate with each other ($r^2 = 0.74$, $P < 0.01$), the unextracted values with this kit are about three times greater than the extracted values. In the present study, $r = 3.4 \pm 0.2$. According to the manufacturer of the kits (Oxford Chemicals) only the extracted values correlate with

Table 3. LMS flight data

Units	Preflight	Inflight	Postflight			<i>F</i>	
			R-All	R0	R1		
Weight	kg	85.3 ± 2.3	ND	80.9 ± 1.5			
Energy intake	kcal · kg ⁻¹ · day ⁻¹	39.1 ± 2.6	23.3 ± 2.5*	35.0 ± 2.9	31.4 ± 4.9	30.9 ± 1.9	7.70
Nitrogen	mg N · kg ⁻¹ · day ⁻¹	228 ± 4	158 ± 14*	195 ± 15	155 ± 26	202 ± 25	5.23
Creatinine	g/day	1.97 ± 0.07	1.61 ± 0.11	1.84 ± 0.15	1.64 ± 0.03	1.75 ± 0.10	NS
8-OH DG	ng · kg ⁻¹ · day ⁻¹	178 ± 36	192 ± 16	179 ± 22	187 ± 25	183 ± 35	NS
8-OH DG	ng · g creatinine ⁻¹ · day ⁻¹	8.29 ± 1.79	10.28 ± 1.18	7.60 ± 0.71	9.80 ± 1.26	8.46 ± 1.25	NS
UN 8-iso-PGF $_{2\alpha}$	ng · kg ⁻¹ · day ⁻¹	15.6 ± 4.3	8.0 ± 2.7*	14.8 ± 1.8	13.8 ± 3.1	18.9 ± 2.1	8.63
UN 8-iso-PGF $_{2\alpha}$	ng · g creatinine ⁻¹ · day ⁻¹	0.70 ± 0.21	0.40 ± 0.12*	0.71 ± 0.14	0.74 ± 0.19	0.91 ± 0.14	7.48

Diet, and isoprostanate and 8-OH DG excretion before, during, and after spaceflight on the space shuttle. Isoprostanate data are from unextracted (UN) urine. Data have been normalized to either body wt or to creatinine. R0 and R1 refer to no. of days elapsed after landing. R-All is the mean value for the entire postflight period. There were four astronauts. Statistical analyses were by RMANOVA on the mean preflight, inflight, and postflight values. * $P < 0.05$ vs. preflight. Overall significance of the RMANOVA was not great enough to justify further analysis of the recovery period data. ND, not determined; NS, not significant.

Table 4. *Bed rest data*

	Units	Ambulatory	Bed Rest	R-All	R0	R1	F
Weight	kg	79.2 ± 5.7	77.5 ± 4.8	78.7 ± 4.9		77.6 ± 4.8	NS
Energy intake	kcal · kg ⁻¹ · day ⁻¹	36.1 ± 1.9	32.5 ± 1.7*	35.9 ± 2.1	33.3 ± 2.3	34.7 ± 1.7	20.92
Nitrogen	mg N · kg ⁻¹ · day ⁻¹	183 ± 9	176 ± 10*	186 ± 11	179 ± 11	177 ± 8	6.84
Creatinine	g/day	1.82 ± 0.09	1.81 ± 0.08	1.72 ± 0.08	1.91 ± 0.09	1.70 ± 0.06	NS
8-OH DG	ng · kg ⁻¹ · day ⁻¹	183 ± 17	192 ± 15	173 ± 16	192 ± 24	167 ± 16	NS
8-OH DG	ng · g creatinine ⁻¹ · day ⁻¹	7.98 ± 0.86	8.44 ± 0.66	7.87 ± 0.68	7.91 ± 0.88	7.76 ± 0.56	NS
UN 8-iso-PGF _{2α}	ng · kg ⁻¹ · day ⁻¹	48.9 ± 23.0	65.4 ± 28.3	74.7 ± 35.3	82.0 ± 53.4	88.0 ± 44.0	NS
UN 8-iso-PGF _{2α}	ng · g creatinine ⁻¹ · day ⁻¹	2.40 ± 1.06	3.25 ± 1.34	3.93 ± 1.76*	3.87 ± 2.34	4.46 ± 2.17	6.78
EX 8-iso-PGF _{2α}	ng · kg ⁻¹ · day ⁻¹	18.8 ± 5.0	23.0 ± 5.0	35.8 ± 13.4	40.9 ± 24.1	30.9 ± 8.0	NS
EX 8-iso-PGF _{2α}	ng · g creatinine ⁻¹ · day ⁻¹	0.89 ± 0.19	1.11 ± 0.17	1.76 ± 0.52	1.79 ± 0.92	1.54 ± 0.31	6.97

Diet, and isoprostane and 8-OH DG excretion before, during, and after bed rest. Isoprostane data are from UN and EX. Data have been normalized to either body wt or to creatinine. R-All refers to the mean value for the entire recovery period; R0 and R1 are days bed-rest phase was terminated and day after, respectively. There were 7 subjects. Statistical analyses were by RMANOVA on the mean pre-bed-rest, bed-rest, and recovery phase values. * $P < 0.05$ vs. pre-bed-rest. Overall significance of the RMANOVA was not great enough ($P < 0.01$) to justify further analysis of recovery period data. NS, not significant.

those found by mass spectroscopy. The values from UN are higher because of cross reactivity from other immunoreactive isoprostanes. Because the extracted method and the gas chromatography-mass spectrometry methods measure only one of the several possible 8-iso-PGF_{2α} isomers, the unextracted data are useful in that they provide information showing that the increase in isoprostane production is not limited to one specific isoprostane.

Some of the preflight and pre-bed-rest SEs for 8-iso-PGF_{2α} and 8-OH DG appear to be rather large. This was due to intersubject variability rather than to variability within the assay. The intra-assay coefficients of variability for 8-OH DG were $4.6 \pm 0.5\%$ for MIR, $4.5 \pm 0.3\%$ for shuttle, and $2.5 \pm 0.2\%$ for bed rest. For unextracted isoprostanes the corresponding values were 4.3 ± 0.4 , 7.4 ± 0.6 and $11.0 \pm 0.6\%$, respectively. Extracted isoprostane assays were done only on MIR and the bed-rest subjects. The coefficients of variation were 6.3 ± 0.7 and $7.6 \pm 0.5\%$, respectively. Intersubject variation was much greater (20–50%) and accounts for the high SEs for the mean prestudy values in the tables. If the data are normalized to percentage of the

mean preflight value, much of the variance in the preflight bed-rest period disappears (Figs. 1–3).

Inspection of the preflight isoprostane data suggests that the three groups of subjects (MIR, shuttle, and bed-rest) were different. One astronaut on MIR had very high 8-OH DG and isoprostane levels. Eliminating this subject from the preflight means gives 9.84 ± 1.89 ($n = 5$) ng · g creatinine⁻¹ · day⁻¹ for 8-OH DG, 4.42 ± 0.71 ng · g creatinine⁻¹ · day⁻¹ for unextracted isoprostane, and 1.65 ± 0.34 ng · g creatinine⁻¹ · day⁻¹ for extracted isoprostane. The postflight differences between the groups are still significant for either unextracted isoprostanes ($P < 0.05$, $F = 7.55$), extracted isoprostanes ($P < 0.05$, $F = 16.81$), and 8-OH DG ($P < 0.05$, $F = 5.46$) as is the inflight decrease for unextracted isoprostanes ($P < 0.05$, $F = 16.81$).

The differences most likely reflect those in the diets of the three groups. The differences in preflight status are unlikely to be the cause of the postflight increases in isoprostane excretion on MIR, because a similar increase was found after bed rest. The bed-rest subjects were on a metabolic balance study and thus on a constant diet for the duration of the 47-day study

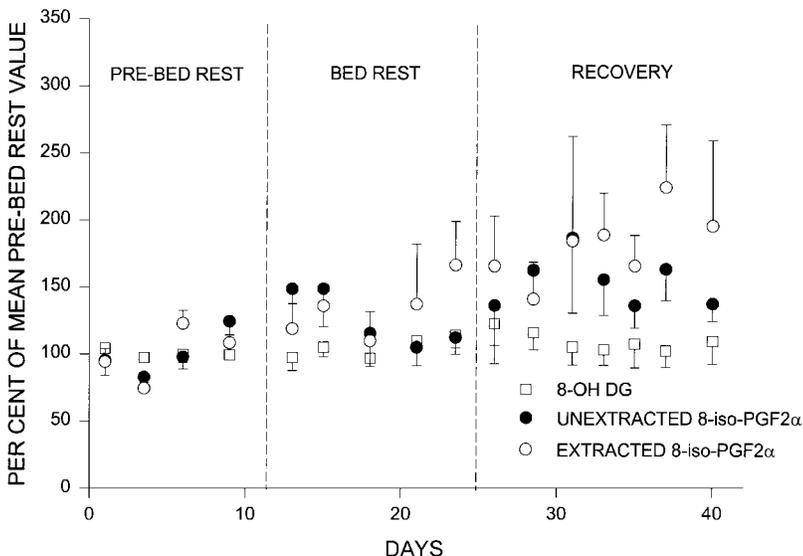


Fig. 3. Changes in UN and EX isoprostane and 8-OH DG excretion during and after bed rest. Data are expressed as percentage of mean preflight data normalized to creatinine. There were 7 subjects. No individual inflight or postflight data points are significantly different from mean preflight value, but overall mean for recovery phase is significantly different from mean pre-bed-rest value ($P < 0.05$ by RMANOVA).

period. For MIR, the two American astronauts lived in Russia before launch from the US and they landed in the US with all postflight measurements being made in the US. Some of the samples for the preflight measurements on the two American crew persons were collected while they were in Russia. The Russian crews ate the food available at Star City, Russia, pre- and postflight.

To determine whether the results could have been skewed by the differences between launch and landing procedures for astronauts and cosmonauts, we examined the data of the four Russians separately. The RMANOVA confirmed the overall postflight increases in 8-OH DG ($F = 8.39$, $P < 0.05$), unextracted 8-iso-PGF_{2α} ($F = 9.81$, $P < 0.05$), and extracted 8-iso-PGF_{2α} ($F = 11.69$, $P < 0.05$), but not the inflight decrease in isoprostane excretion. A small number of subjects precludes detecting any but the grossest differences.

Inflight. On both MIR and the space shuttle, 8-iso-PGF_{2α} was decreased inflight (Tables 1 and 3). The decrease was found with both EX and UN for MIR and for UN on the shuttle. With both missions, 8-OH DG showed a weak trend toward an increase (Figs. 1 and 2). The two conclusions that can be drawn with certainty from the inflight results are 1) that the decreased isoprostane excretion on MIR is not an artifact caused by possible environmental abnormalities on MIR, because the same result was found on the space shuttle, and 2) oxidative damage to lipid membranes was not increased during spaceflight. The discussion of the inflight data that follows is less certain but is reasonable.

The inflight decrease in 8-iso-PGF_{2α} production could be due to 1) increased quenching of free-radical chain reactions as a consequence of increased antioxidant intake, 2) increased production of endogenous antioxidants, or 3) decreased oxidant production and propagation. The first two are unlikely. Increased antioxidant intake inflight is improbable; dietary intake was decreased during spaceflight (Tables 2 and 3), and there was no specific requirement for the crew to take supplementary vitamins or antioxidants above their preflight amounts (personal communication, Dr. S. Lucid).

An increase in endogenous antioxidant production is also unlikely. The available evidence suggests that the MIR crew persons were in negative energy balance. Lane et al. showed that energy expenditure inflight was not different from that on the ground [35.2 ± 1.8 vs. 36.2 ± 5.8 kcal·kg⁻¹·day⁻¹ (15)]. Our value for a mission with heavy exercise requirements was 40.8 ± 0.6 kcal·kg⁻¹·day⁻¹ (34). Energy intake on both the MIR [26.9 ± 2.2 kcal·kg⁻¹·day⁻¹ (33)] and LMS [24.6 ± 3.3 kcal·kg⁻¹·day⁻¹ (34)] missions was substantially less than either of these two values, and the differences are statistically significant ($P < 0.05$). Moreover, energy intake on MIR was only slightly higher than complete bed rest with no activity [24.2 ± 0.8 kcal·kg⁻¹·day⁻¹ (10)] and substantially less than either bed rest with activity [this bed-rest study, 30.8 ± 1.3 kcal·kg⁻¹·day⁻¹ (34)] or sitting in a metabolic chamber at rest [29.7 ± 2.3 kcal·kg⁻¹·day⁻¹ (8)].

Therefore, it is highly improbable that the MIR crew persons were in energy balance.

The magnitude of the energy deficit was apparently sufficiently great to impact the whole body protein synthesis rate adversely (33). Increased production of antioxidants in the face of decreased intake, decreased total body protein synthesis, decreased oxidative metabolism, and decreased need for antioxidants is therefore highly improbable. Moreover, this argument does not explain the differences between 8-OH DG and 8-iso-PGF_{2α}. The third option, decreased endogenous free-radical production, is the expected result if there is a downregulation of intermediary metabolism in response to the decreased energy intake and no increase in free-radical generation from radiation.

Different aspects of oxidative stress are measured by 8-OH DG and 8-iso-PGF_{2α}, namely DNA damage and cell membrane damage, respectively. A decreased flux through the electron transport chain will generate fewer free radicals in the mitochondria. Mitochondria are exceptionally well endowed with membranes; most of the internal structure of mitochondria is membranous. Any diminution of oxidative phosphorylation is likely to decrease oxidative damage to the internal mitochondrial membrane arrays.

Other arguments support the decrease in isoprostane production being related to energy metabolism. First, on MIR, the percentage of decrease in inflight energy intake from the preflight intake showed a correlation with the percentage of decrease in isoprostane (unextracted) production inflight ($r^2 = 0.62$, $P = 0.065$, and $r^2 = 0.65$, $P = 0.060$ normalized to creatinine). Second, finding a decrease inflight in isoprostane excretion was not unique to MIR. A decrease in isoprostane excretion was also found with the LMS mission, where there was also a significant reduction in dietary intake inflight (Table 3). In contrast, with the bed-rest study, dietary intake was approximately the same before and during bed rest, and isoprostane excretion was unchanged (Table 4).

It is not known why dietary intake is decreased during spaceflight or why there is an apparent inverse relationship between exercise and intake (30, 34). We suggest that the adverse effects of exercise on energy intake during spaceflight are a consequence of decreased efficiency in disposing of the heat produced during exercise. Thermoregulatory mechanisms are less efficient in microgravity (and in bed rest) for a number of reasons (5, 6, 9). First, blood flow to the extremities is decreased, and this reduces the effective surface area available for convection cooling (5, 9, 26). Second, plasma volume is reduced during spaceflight (16), and this reduces the ability to transfer heat from the core to the periphery. To compensate, either the rate of blood flow perfusing the peripheral capillaries has to be increased, or it will take longer to dissipate the heat. The latter is more likely; cardiac output is less during exercise in microgravity than it is on the ground (5). Third, unlike on the ground, where sweat water forms into beads, which have a large surface area, in microgravity sweat water forms a coherent sheet on the body,

sweat losses after exercise are decreased in microgravity (17). Convertino (5) suggested that the high level of skin wetness suppresses sweating. Also, water is an excellent insulator, and this contributes to the decreased ability to dispose of excess heat. The cumulative result of these effects is that dissipating the excess heat generated during exercise takes longer than it does on the ground.

As long as heat disposal is in progress, blood will be diverted away from the gut to the periphery (2, 13). Diversion of blood away from the gut will lead to a suppression of intake, because signals to the appetite center from the gut (neural and endocrine) to the brain will act to reduce food intake because the gut is not ready to process food (2). The greater the amount of aerobic exercise, the more time is needed for heat dissipation and the longer blood flow is diverted away from the gut, resulting in the inverse relationship between exercise and energy intake. This hypothesis also explains the unexpected observation that astronauts on a given mission appear to synchronize their intake (Fig. 3 in Ref. 34). The amount and type of exercise done on these various missions (Skylab, Space Life Sciences missions 1 and 2, and the LMS mission) were part of the mission requirements and thus were the same for all the crew for a given mission, but differed between the missions. Improving heat dissipation after exercise by increasing airflow may attenuate the appetite loss.

Postflight. Oxidative damage was increased after spaceflight on MIR. Both 8-OH DG and 8-iso-PGF α were increased by more than twofold after 3+ mo on MIR (Table 1, Fig. 1). 8-iso-PGF $_{2\alpha}$ was also increased by a lesser amount after bed rest (Table 4), but not after 17 days on the space shuttle (Table 3).

Most of the other available evidence also supports an increase in oxidative damage after long-duration spaceflight. Spot plasma analyses by Russian investigators on cosmonauts after long-duration flights showed a nonstatistically significant trend for an increase in the accumulation of lipid oxidation products in the serum (22). The sample size was small and there is more "noise" in randomly collected blood samples than with the integrated value that is obtained from a 24-h urine pool. Most rodent studies (14, 23), but not all (18), showed increased production of lipid peroxidation products postflight, together with decreased antioxidant enzyme activity postflight. The rodent observations were attributed to the stress associated with reentry into earth's gravity (14, 23).

After the astronauts landed, their dietary intake and metabolic activity returned to preflight levels as they readjusted to gravity and began to replace the muscle and fat that had been lost during flight. With this increase in metabolic activity comes an increase in oxidative phosphorylation and the associated increase in free-radical generation.

After landing, astronauts' endogenous antioxidant capacity does not return immediately to its preflight level; on MIR, enhanced excretion of isoprostanes persisted until the end of the measurement period (Fig. 1).

In the period following return to earth, competition develops within the body for available resources, principally amino acids (32). We suggest that the synthesis of host antioxidant protein defenses is suboptimal because of competition for amino acids occurring between repleting muscle and other tissues. In two earlier publications, we presented evidence based on our observations and the studies of others that such competition does occur after long-duration spaceflight (33, 36, 37), short-term flights (30, 32), and on the ground with the hindlimb-suspended rat (35). Because of this combination of increased free-radical production and a shortfall in antioxidant capacity, free-radical propagation is more extensive, and greater amounts of the products of free-radical damage are recovered in the urine. The loss of host antioxidant proteins inflight is general; therefore, postflight the DNA is vulnerable, and 8-OH DG excretion is increased.

A reduction in antioxidant capacity inflight is of little consequence when oxidant production is decreased, but when the crew lands and metabolic activity increases, endogenous antioxidant defenses are at a low level. A diminution of astronaut antioxidant defenses is a potential problem both for returning astronauts and for a future mission to Mars. Even though the gravitational force on Mars is only one-third that on earth, the transition from nothing to one-third g will stimulate an anabolic response with an increase in free-radical production. A deficit in endogenous antioxidant capacity may be treatable with dietary antioxidant supplements.

Radiation. This study found no evidence for increased free-radical production from high-energy radiation. The radiation flux ranged between 30 and 100 mREM/day with a mean of 60 mREM/day. During these flights there were no bursts of high-energy radiation (personal communication, Dr. V. S. Schneider). Free-radical production from ionizing radiation is qualitatively and quantitatively different from that of metabolic origin. A "hit" from a high-energy particle is an infrequent event compared with the continuous flux of low-energy semistable free radicals generated from metabolic processes. But the collateral damage from the impact of a single high-energy incoming particle can be extensive because of the very high energies involved. The weak trend toward an increase in 8-OH DG production inflight can be accounted for by the decreased synthesis of host defense proteins secondary to the overall depression of protein synthesis.

Different results may be found with other missions in different orbits exposed to different degrees of solar and extragalactic radiation and where nutritional status is not a confounding variable. In the present study, any radiation-induced damage could have been obscured by the metabolic decrease in free-radical production. Any apparent benefit from the "protective effects" of undernutrition against oxidative damage is counterbalanced by the much more serious consequences of undernutrition.

In summary, 1) oxidative damage was decreased during long-duration spaceflight on MIR secondary to

an overall decrease in metabolic activity; 2) inflight, undernutrition may have a protective effect against oxidative damage; and 3) oxidative damage was increased after return from several months in earth orbit.

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