Vision Changes after Spaceflight Are Related to Alterations in Folate– and Vitamin B-12–Dependent One-Carbon Metabolism


Abstract

Approximately 20% (7 of 38) of astronauts on International Space Station (ISS) missions have developed measurable ophthalmic changes after flight. This study was conducted to determine if the folate– and vitamin B-12–dependent 1-carbon metabolic pathway is altered in these individuals. Since 2006, we have conducted experiments on the ISS to evaluate nutritional status and related biochemical indices of astronauts before, during, and after flight. Data were modeled to evaluate differences between individuals with ophthalmic changes (n = 5) and those without them (n = 15), all of whom were on ISS missions of 48–215 d. We also determined whether mean preflight serum concentrations of the 1-carbon metabolites and changes in measured cycloplegic refraction after flight were associated. Serum homocysteine (Hcy), cystathionine, 2-methylcitric acid (2MCA), and methylmalonic acid concentrations were 25-45% higher (P < 0.001) in astronauts with ophthalmic changes than in those without them. These differences existed before, during, and after flight. Preflight serum concentrations of Hcy and cystathionine, and mean in-flight serum folate, were correlated with change (postflight relative to preflight) values in refraction (P < 0.05), and preflight serum concentrations of 2MCA tended to be associated (P = 0.06) with ophthalmic changes. The biochemical differences observed in crewmembers with vision issues strongly suggest that their folate– and vitamin B-12–dependent 1-carbon transfer metabolism was affected before and during flight. The consistent differences in markers of 1-carbon metabolism between those who did and those who did not develop changes in vision suggest that polymorphisms in enzymes of this pathway may interact with microgravity to cause these pathophysiologic changes.

Introduction

In what has been described as one of the most important clinical findings from spaceflight to date, several astronauts on long-duration ISS missions have had long-term (and potentially permanent) changes in vision during and after spaceflight (1). To date, 38 astronauts from the Canadian Space Agency, European Space Agency, Japan Aerospace Exploration Agency, and NASA have lived aboard the ISS for 3–6 mo. Seven of them have had measurable ophthalmic changes after flight, including optic disc edema, globe flattening, choroidal folds, hyperopic shifts, and cotton wool spots. These 7 cases have been described in detail (1).

Currently, the etiology of these ophthalmic changes is unknown, but it has been hypothesized that microgravity-induced cephalad fluid shifts and resultant increased intracranial pressure and/or localized introrroital changes may be involved (1). We present here evidence that the folate– and vitamin B-12–dependent 1-carbon metabolic pathway involving Hcy recycling and transsulfuration may be different in these individuals. This presents an alternative hypothesis that individuals with this altered pathway may be predisposed to anatomic and/or physiologic changes that render them susceptible to ophthalmologic damage during spaceflight.

Participants and Methods

All protocols were approved by the Johnson Space Center Committee for the Protection of Human Subjects, and informed consent was obtained from all participants. Participants (n = 20) were crewmembers on Expeditions 14–25 (missions of 48–215 d, 153 ± 52 d, mean ± SD;
flown between 2006 and 2011). These crewmembers were participating in an experiment to evaluate nutritional status and related biochemical indices. Five of the 7 individuals with ophthalmic changes (1) (designated here as OC+) participated in this study. The other 2 OC+ crewmembers flew before this experiment was initiated and thus only a limited set of nominal pre- and postflight medical data are available from them. All 7 OC+ were men. The other 15 crewmembers (designated as OC--; n = 9 men and 6 women) did not have any reported or measurable ophthalmic changes. Crewmembers were 46 ± 4 and 50 ± 4 y of age for OC- and OC+, respectively. BMI for the groups were 24 ± 3 and 28 ± 4 kg/m² for OC- and OC+, respectively. BMI for men only in the OC- group was 26 ± 1 kg/m².

Blood samples were collected by using standard phlebotomy techniques at ~180, 45, and 10 d before spaceflight (L-180, L-45, L-10); on flight days 15, 30, 60, 120, and 180; on landing day (R+0); and 30 d after landing (R+30). For L-10 and in-flight blood collections, the gel separator tubes were centrifuged within 20–30 min after collection and were soon (within minutes) transferred to the freezer where they were stored at –96°C on the ISS for 6–12 mo until they were transferred back to Earth on the space shuttle. Upon return to Houston, the samples were thawed and aliquots were made and then refrozen until they were batch-analyzed along with preflight samples. Except for samples collected on R+0, all blood samples were collected after an 8-h fast.

Urine samples were collected during 24-h (during flight) or 48-h (pre- and postflight) periods. These were collected as individual voids and pooled appropriately as previously described (2).

Serum Hcy, cystathionine, 2MCA, and MMA were determined in serum by GC-MS in an external commercial laboratory (Metabolite Laboratories). Serum folate was measured using a radioassay (Solid Phase No Boil Dualcount, Siemens). These analytes are not affected by one freeze-thaw cycle, which is required for the in-flight samples. Urinary 4-pyridoxic acid was measured before, during, and after flight, whereas RBC folate was measured before and after flight only, as previously described (3).

Cycloplegic refractions were determined before and after flight as previously described (1). Cabin CO₂ was measured by the nominal ISS instruments. (38x413) Serum folate was measured before, during, and after flight, whereas RBC folate was measured during the flight cycle, which is required for the in-flight samples. Urinary 4-pyridoxic acid, plasma 5'-pyridoxal phosphate, and MMA were measured in serum by using standard phlebotomy techniques at ~180, 45, and 10 d before spaceflight (L-180, L-45, L-10); on flight days 15, 30, 60, 120, and 180; on landing day (R+0); and 30 d after landing (R+30). For L-10 and in-flight blood collections, the gel separator tubes were centrifuged within 20–30 min after collection and were soon (within minutes) transferred to the freezer where they were stored at –96°C on the ISS for 6–12 mo until they were transferred back to Earth on the space shuttle. Upon return to Houston, the samples were thawed and aliquots were made and then refrozen until they were batch-analyzed along with preflight samples. Except for samples collected on R+0, all blood samples were collected after an 8-h fast.

Urine samples were collected during 24-h (during flight) or 48-h (pre- and postflight) periods. These were collected as individual voids and pooled appropriately as previously described (2).

Serum Hcy, cystathionine, 2MCA, and MMA were determined in serum by GC-MS in an external commercial laboratory (Metabolite Laboratories). Serum folate was measured using a radioassay (Solid Phase No Boil Dualcount, Siemens). These analytes are not affected by one freeze-thaw cycle, which is required for the in-flight samples. Urinary 4-pyridoxic acid was measured before, during, and after flight, whereas RBC folate was measured before and after flight only, as previously described (3).

Cycloplegic refractions were determined before and after flight as previously described (1). Cabin CO₂ was measured by the nominal ISS cabin gas analyzer.

Statistical analyses. All statistical analyses were performed using Stata IC software (v 11.2, StataCorp) and setting 2-tailed α to reject the null hypothesis at 0.05 with no adjustments for multiple comparisons. To meet the assumptions required of the statistical analysis, some of the dependent variables required a log transformation: 2MCA, cystathionine, RBC folate, 4-pyridoxic acid, and 5’-pyridoxal phosphate. Additionally, the MMA data from one OC- participant was deemed an overly influential outlier in the analysis and was thus eliminated from the evaluation of the effects of spaceflight on ophthalmic changes and MMA.

As described earlier in “Methods,” our dependent variables were assessed three times before flight, five times during flight, and two times post landing. (Some crewmembers had fewer in-flight measurements, because their missions were shorter than 180 d.) Separate mixed-effects linear regression models were used to evaluate the effects of ophthalmic status (OC+, OC-) and spaceflight on our continuously scaled dependent variables. For each of these variables, our statistical model included dummy-coded β coefficients comparing in-flight, R+0, and R+30 data to preflight data, and a coefficient evaluating the linear change over time during flight. We also included an ophthalmic status × flight day interaction term to test the null hypothesis that OC+ astronauts experienced changes during flight similar to those of OC- and a flight day × ophthalmic status interaction term to test whether the preflight vs. flight differences were similar for OC+ and OC- participants.

As typical with mixed-effects modeling, these models included a random intercept to accommodate the longitudinal design of the experiment, allowing each participant to have their own off-set, and in these off-set data we included random slope coefficients for the R+0 indicator and the in-flight changes observed to accommodate observed heterogeneity of variance on the terms evaluating change from before flight to R+0 and change during flight. This accommodation incorporates the between-subject variability on these effects and provides better statistical inference (4).

Exceptions to the above methods. The model for the CO₂ data included fewer terms (these were terms evaluating the in-flight change and effects of OC status, their interaction term, and the random y-intercept and flight day terms), because only in-flight data were available.

Serum folate was measured before, during, and after flight, but unlike the dependent variables described above, only in-flight data were evaluated, because diet was standardized during the missions. During flight, the standardized menu has room for some variability to accommodate taste preferences but clearly does not have as many choices as the open food system used by crewmembers before flight. Serum folate analysis proved to be unreliably estimated by these methods because of non-normally distributed residuals with many overly influential outliers, so for this dependent variable we resorted to a simpler (and conservative) nonparametric measure of association, Somers’ D, between OC (+/−) and serum folate concentrations observed during flight. Jackknife SE adjustment for the multiple in-flight observations per participant (5,6).

The association of preflight concentrations (the mean of data from all preflight sessions) of Hcy, MMA, 2MCA, and cystathionine, and mean in-flight serum folate with cycloplegic refractive changes observed post flight were evaluated by Somers’ D measure of association.

Results

Serum Hcy, cystathionine, 2MCA, and MMA concentrations were 25–45% higher (P < 0.001) in astronauts with ophthalmic changes than in those without them (Table 1). In-flight cabin CO₂ concentrations were higher (P < 0.05) for OC+ than OC- astronauts. None of the interaction effects in our model were significant, suggesting that the differences between OC+ and OC- were consistent before, during, and after flight. The 2MCA model also indicated that concentrations were lower during flight (P < 0.05) and at R+0 (P < 0.05) relative to before flight.

Urinary 4-pyridoxic acid, plasma 5'-pyridoxal phosphate, and RBC folate were significantly lower at R+0 than before flight (P < 0.05), with no effect of OC status (data not shown). We did not find a significant association between serum folate concentration and OC status, but upon examination of the individual data (Fig. 1), 4 of the 5 OC+ astronauts had serum folate concentrations clustered at the lower end of the distribution of values, with one OC+ participant showing higher values. When the one OC+ outlier was removed, the in-flight serum folate means were consistently lower for OC+ crewmembers than for OC- crewmembers. The one OC+ crewmember with the high serum folate concentration did not experience any refractive changes after flight but presented the worst grade of optic disc edema. That same crewmember had the highest preflight serum Hcy concentration of any crewmember in this study and was the only OC+ crewmember who reported taking multivitamin supplements during flight.

Preflight Hcy and cystathionine were positively associated (P < 0.05) and in-flight serum folate was negatively associated (P < 0.02) with the absolute change in refractive index (postflight relative to preflight). In-flight serum folate was negatively associated (P = 0.02) and preflight serum 2MCA tended to be positively associated (P = 0.06) with the absolute change in refractive index (postflight relative to preflight).

Of the 5 OC+ crewmembers participating in this experiment, only 1 crewmember reported taking multivitamin supplements daily during flight. Eight of the OC- crewmembers reported taking multivitamin supplements during flight (3.7 ± 2.7 multivitamins/wk).

Discussion

Factors that could contribute to the ophthalmic changes observed in some crewmembers after long-duration spaceflight include microgravity-induced fluid shifts (7–9), increased intra-
craniocerebral pressure, optic nerve sheath changes, and/or changes in intraocular pressure (1,10). At this point, the unifying pathologic mechanism is hypothesized to be prolonged exposure to the effects of cephalic fluid shifts that occur during microgravity exposure. The question remains, however: why are only ~20% of crewmembers affected when all crewmembers presumably experienced the fluid shifts on exposure to microgravity? Furthermore, why would one crewmember be affected during a particular mission when a fellow crewmember on the same mission (and exposed to the same environment) did not have ophthalmic changes? The evidence provided here suggests that this phenomenon could be explained by crewmembers who have ophthalmic changes involving an altered metabolic pathway involving Hcy, cystathionine, 2MCA, and MMA. Our data show that an association exists between ophthalmic changes and higher concentrations of intermediates of the pathway involving these enzymes. Differences in this pathway may influence anatomic or physiologic susceptibility to environmental stressors such as fluid shifts or response to cabin CO2.

Exposure to microgravity provides a common stress, but the spectrum of individual response is large. Factors associated with spaceflight, from fluid shifts, cabin CO2 concentrations, and dietary sodium or coffee consumption to intense resistive exercise, have been suggested as potential causes of the ophthalmic changes. If the changes were strictly due to environmental factors, then no difference would be expected in the concentration of Hcy (or other metabolites) between crewmembers with ophthalmic changes and those without them, because ISS environmental factors (including food system and air quality) are similar for all crewmembers.

A concern has also been raised about CO2 exposure, given the ability of elevated ambient CO2 to increase cerebral blood flow. However, many of the crewmembers’ mission times overlapped and therefore ISS cabin CO2 exposure was the same for several crewmembers during particular missions, but the ophthalmic changes were not necessarily similar for such crewmembers. Furthermore, although one may speculate that ISS cabin CO2 exposure caused differences in Hcy and other metabolites, there remains no explanation for these differences before flight.

Nutritional deficits could cause elevated Hcy, but if they were present, the circulating concentrations of Hcy would be higher than those observed here. Nutritional deficits are also unlikely, because astronauts must meet strict flight standards and have generally excellent health and nutritional status. On orbit, the spaceflight food system provides folate, vitamin B-6, and vitamin B-12 in excess of the defined ISS requirements and the Earth-based Institute of Medicine’s RDA (11), and one of the affected individuals even took multivitamins.

The fact that significant differences existed before flight as well as during flight discounts environmental effects on Hcy and related metabolites. Furthermore, the consistently higher concentrations of metabolites in the 1-carbon transfer pathway before, during, and after flight among crewmembers who experienced ophthalmic changes suggest there may be a genetic polymorphism in these crewmembers in one or more enzymes in that pathway and warrants further study.

Mildly elevated Hcy may be produced by the existence of genetic variants, caused by single-nucleotide polymorphisms, of one or more of a number of enzymes involved in folate– and vitamin B-12–dependent 1-carbon metabolism. These polymorphisms are quite common and result in a moderate elevation of Hcy as opposed to rare mutations in the same enzymes that involve these enzymes. Differences in this pathway may influence anatomic or physiologic susceptibility to environmental stressors such as fluid shifts or response to cabin CO2.

Exposure to microgravity provides a common stress, but the spectrum of individual response is large. Factors associated with spaceflight, from fluid shifts, cabin CO2 concentrations, and dietary sodium or coffee consumption to intense resistive exercise, have been suggested as potential causes of the ophthalmic changes. If the changes were strictly due to environmental factors, then no difference would be expected in the concentration of Hcy (or other metabolites) between crewmembers with ophthalmic changes and those without them, because ISS environmental factors (including food system and air quality) are similar for all crewmembers.

A concern has also been raised about CO2 exposure, given the ability of elevated ambient CO2 to increase cerebral blood flow. However, many of the crewmembers’ mission times overlapped and therefore ISS cabin CO2 exposure was the same for several crewmembers during particular missions, but the ophthalmic changes were not necessarily similar for such crewmembers. Furthermore, although one may speculate that ISS cabin CO2 exposure caused differences in Hcy and other metabolites, there remains no explanation for these differences before flight.

Nutritional deficits could cause elevated Hcy, but if they were present, the circulating concentrations of Hcy would be higher than those observed here. Nutritional deficits are also unlikely, because astronauts must meet strict flight standards and have generally excellent health and nutritional status. On orbit, the spaceflight food system provides folate, vitamin B-6, and vitamin B-12 in excess of the defined ISS requirements and the Earth-based Institute of Medicine’s RDA (11), and one of the affected individuals even took multivitamins.

The fact that significant differences existed before flight as well as during flight discounts environmental effects on Hcy and related metabolites. Furthermore, the consistently higher concentrations of metabolites in the 1-carbon transfer pathway before, during, and after flight among crewmembers who experienced ophthalmic changes suggest there may be a genetic polymorphism in these crewmembers in one or more enzymes in that pathway and warrants further study.

Mildly elevated Hcy may be produced by the existence of genetic variants, caused by single-nucleotide polymorphisms, of one or more of a number of enzymes involved in folate– and vitamin B-12–dependent 1-carbon metabolism. These polymorphisms are quite common and result in a moderate elevation of Hcy as opposed to rare mutations in the same enzymes that cause severe inherited hyperhomocysteinemia. One of the most studied polymorphisms in this pathway is MTHFR 677C→T, which has an allele frequency of ~30% in many ethnic groups.
Heterozygotes (C/T) have a 30% decrease in the activity of the enzyme MTHFR and homozygotes (T/T) a 60% decrease in this critical enzyme. The metabolic consequences of this reduced enzyme activity are most frequently observed in situations of moderately low folate nutritional status (14). Similarly, the amount of dietary folate needed to maintain normal folate status may be greater in individuals with the MTHFR C677T polymorphism (15). Because recent folate intake greatly affects serum folate concentration, we looked at in-flight vitamin status, which is similar for all crewmembers (11). Crewmembers with ophthalmic changes had consistently lower serum folate during spaceflight than crewmembers without changes, a finding consistent with the notion that individuals with polymorphisms in the discussed pathway may require more dietary folate to maintain folate status.

One possibility to explain how an altered 1-carbon transfer pathway could be linked to ophthalmic changes is that the affected individuals may be more susceptible to either the fluid shifts of microgravity or slight shifts in cabin CO₂ possibly associated with increased vascular permeability. Although cabin CO₂ is tightly regulated, some variation occurs between and within missions, and overall the crewmembers with ophthalmic changes had a higher mean cabin CO₂ concentration than those without changes (P < 0.05). Mild hyperhomocysteinemia evoked by folate deficiency or by a heterozygous mutation in the cystathionine β-synthase gene can increase arterial permeability and rigidity in animal models (16). With a MTHFR polymorphism, the blood concentration of the predominant active form of folate (that is, 5-methyltetrahydrofolate) is lower, promoting an increase in vascular permeability because of folate’s role in endothelial production of NO and also in the reduction of oxygen radical formation and scavenging of oxygen radicals (17). If vascular permeability of OC+ and OC- crewmembers was different before flight, then it is plausible that environmental insults such as slight elevations in CO₂ during spaceflight may have had more of an impact on intracranial pressure of OC+ than on intracranial pressure of OC- crewmembers exposed to similar CO₂ concentrations. In support of this idea, other studies in extreme environments have documented that the response of divers with polymorphisms in the folate- and vitamin B-12-dependent 1-carbon transfer pathway, when they are exposed to increased atmospheric pressure, is different from that of divers without such polymorphisms (18).

Altered Hcy metabolism is a risk factor for several vascularopathies, including ischemic stroke, intracranial aneurysms, and migraine headaches, and some studies have demonstrated that Hcy may be a risk factor for occlusive retinal vascular disease and some types of glaucoma (19–24). Case studies have also indicated that the visual acuity of individuals with vision loss accompanied by retinal hemorrhages, venous dilation, retinal edema, visual field scotoma, and elevated Hcy was fully resolved with folic acid treatment (25).

In summary, preexisting chemical differences, which have little or no demonstrable effect under Earth-gravity conditions, may set the stage for pathologic changes in affected astronauts during prolonged microgravity exposure. The existing data suggest that vision issues during spaceflight are associated with a difference in the folate- and vitamin B-12-dependent 1-carbon transfer pathway. Given the magnitude of this issue, follow-up with genetic analyses to examine the potential for polymorphisms in the pathway is required to provide a definitive answer. This association has important implications for future space travelers. Beyond that, these findings, taken together with the documented relationship between polymorphisms in the folate- and vitamin B-12-dependent 1-carbon pathway and predisposition to risk of clinical outcomes related to vascular events...
found in clinical practice, could have profound implications for a sizeable population of individuals on Earth.

Acknowledgments

The authors thank the astronauts who participated in the flight studies. Spaceflight studies are complex and complicated and require teams of individuals to ensure that detailed plans are carried out. Although we cannot thank each individual, we thank and recognize NASA’s Human Research Program, Human Health and Countermeasures Element, International Space Station Medical Project, Flight Analogs Project, and Lifetime Surveillance of Astronaut Health Program. The Johnson Space Center Nutritional Biochemistry Laboratory was responsible for protocol coordination, sample collection and processing, and most of the analyses. We thank Stephen Coburn, Ph.D. (Vitamin B6 Laboratory, Indiana University-Purdue University Fort Wayne, Fort Wayne, IN) for his counsel over the years and on this project in particular. We also thank Jane Krauhs, Ph.D. (Wyle, Houston, TX) for editing assistance. S.R.Z., K.E., M.H., and S.M.S. designed research and conducted the primary study; S.R.Z. and S.M.S. oversaw data collection and management; C.R.G. and T.H.M. collected the data and prepared the manuscript; and S.R.Z., K.E., M.H., and S.M.S. interpreted the data and prepared the manuscript; and S.M.S. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited