Astronaut ophthalmic syndrome

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ABSTRACT: During and after missions on the International Space Station, some astronauts experience ophthalmic changes, including choroidal folds, optic disc edema, cotton-wool spots, globe flattening, and refraction changes. Astronauts with ophthalmic issues had significantly higher plasma concentrations of metabolites that are associated with the 1-carbon metabolic pathway than those without ophthalmic issues. We hypothesized that genetic differences might explain the metabolite differences. Indeed, genetics and B vitamin status were significant predictors of ophthalmic issues. We now have developed a hypothesis regarding the mechanisms that link 1-carbon pathway genetics and the condition that we suggest calling, "astronaut ophthalmic syndrome." We maintain that this condition is genetically predisposed and is associated with endothelial dysfunction that is induced by oxidative stress. Subsequent edema can hinder cerebrospinal fluid efflux and can lead to locally increased pressures in the subarachnoid space within the orbit, which impinges on the optic nerve and/or eye in affected individuals. Confirming this hypothesis will help characterize the genetics of 1-carbon pathway metabolism, homocysteine, oxidative stress, endothelial dysfunction, and cardiovascular and potentially other diseases.—Zwart, S. R., Gibson, C. R., Gregory, J. F., Mader, T. H., Stover, P. J., Zeisel, S. H., Smith, S. M. Astronaut ophthalmic syndrome. FASEB J. 31, 3746–3756 (2017). www.fasebj.org

KEY WORDS: B vitamins · cerebrospinal fluid · endothelial dysfunction · oxidative stress · microgravity

Only recently has it been recognized that some astronauts have experienced ophthalmic changes during and after returning from 4- to 6-mo missions on the International Space Station (ISS) (1–3). These changes include choroidal folds, optic disc edema, cotton-wool spots, globe flattening, and changes in refraction (1, 2, 4).

Several theories quickly surfaced regarding the etiology of these spaceflight-induced effects. They tended to focus on the possibility that fluid shifts as a result of exposure to microgravity could increase the intracranial pressure (ICP) that impinges on the optic nerve and/or eye (1–3, 5); however, to date, no evidence exists for an increase in ICP

during spaceflight (1), and data from parabolic flights demonstrate that ICP decreases during transient microgravity exposure (6). Nonetheless, optic disc swelling may result from a rise in ICP from cephalad fluid shifts during spaceflight that lead to venous stasis in the head and neck. This stasis could cause the impairment of cerebrospinal fluid (CSF) drainage into the venous system and cerebral venous congestion, leading to elevated ICP (7). Elevated ICP could result in optic nerve sheath (ONS) distention, globe flattening, stasis of axoplasmic flow, and swelling of axon and optic disc, all of which are similar to what occurs in patients with terrestrial idiopathic intracranial hypertension (7). Although in-flight CSF pressures have never been measured, lumbar puncture opening pressures (LPOPs) of 28 and 28.5 cmH$_2$O have been documented in astronauts at 12 and 57 d, respectively, after long-duration spaceflight and may have been higher during the mission (7). Unfortunately, no preflight data were available. In other astronauts, postflight MRI changes that suggested increased ICP have included pituitary concavity, empty sella, and changes in pituitary stalk configuration (8).

A second possible mechanism for the etiology of ophthalmic changes is that the disc edema, ONS distention, globe flattening, and resultant visual changes were caused by an elevation in ONS CSF pressures as a result of a

microgravity-induced compartmentalization of orbital subarachnoid space (SAS) with or without increased ICP (1, 4, 7, 9, 10). Perhaps asymmetric anatomy in the tightly confined, densely septated anatomic connection between the intracranial and orbital SAS, in conjunction with microgravity-induced venous and lymphatic stasis, caused a localized decrease in CSF absorption within the SAS of the optic nerve and a rise in local pressure (4, 9). In addition, variable ONS anatomy might lead to asymmetric CSF flow down the ONS cul-de-sac in a 1-way, ball valve–like fashion, adding to the elevation of SAS pressure in the orbital optic nerve (7). A case report that described an astronaut who returned from long-duration spaceflight with a normal LPOP that was associated with unilateral disc swelling (4) and unilateral loss of spontaneous venous pulsations for >2 yr in the same eye (10) further supports the compartment syndrome hypothesis. In another case, persistent asymmetric disc swelling and globe flattening for 6 mo after long-duration spaceflight were found, with LPOPs of 22 and 16 cmH$_2$O when lumbar punctures were performed 7 d and 12 mo, respectively, postmission (9), which again suggests asymmetric increases in local SAS pressures (4).

Regardless of the mechanism that causes astronaut ophthalmologic issues, the fact remains that not all astronauts are affected. In 2012, we reported that astronauts who experienced ophthalmic issues after flight had higher plasma concentrations of metabolites that are associated with the 1-carbon metabolic pathway—homocysteine, cystathionine, 2-methylcitric acid, and methylmalonic acid—than did astronauts who were not considered cases (11). Not only were the concentrations of these metabolites higher in the affected individuals during flight, they were also higher before and after flight (11). In evaluating these data, we ruled out several possible confounding factors: sex differences, vitamin deficiency, kidney function, and coffee consumption (12).

From these data, we hypothesized that genetic differences—single-nucleotide polymorphisms (SNPs) in genes for enzymes in the 1-carbon pathway—might be responsible for these admittedly subtle, but statistically significant, differences in cellular 1-carbon metabolism in affected astronauts. The affected astronauts may have had metabolic inefficiencies in the pathways of 1-carbon metabolism as a result of genetic variation that caused different gene expression or enzyme structures. Additional evidence that supports this hypothesis included lower serum folate concentrations in affected astronauts during flight (11). Serum folate is also lower in individuals with minor—less frequent—forms of certain SNPs in genes for 1-carbon pathway enzymes (13).

In 2016, we published ophthalmic and biochemical data sets from 49 astronauts who had flown missions to the ISS, and had data from extensive eye exams before and after flight, biochemical and metabolomic assessments, and results from 5 SNP assessments (14). Statistical modeling revealed that genetics and B vitamin status were significant predictors of the incidence of ophthalmic issues. Significant differences between the group of astronauts who developed ophthalmic abnormalities and the group of those who experienced no such problems occurred with respect to which of the alleles of 2 SNPs they possessed, the SNPs being methionine synthase reductase, rs1801394 (MTRR A66G), and serine hydroxymethyltransferase-1, rs1979227 (SHMT1 C1420T). That is, G alleles of MTRR 66 and C alleles of SHMT1 1420 were both associated with an increased incidence of ophthalmic issues (14). Adding B vitamin status—specifically serum concentrations of vitamin B$_6$, folate, and riboflavin—to the block regression model significantly improved the model (14).

Thus, we have established an association of 1-carbon pathway genetics and B vitamin status with the development of ophthalmic issues in some astronauts. Here, we hypothesize a potential physiologic mechanism that provides end-to-end linkage between SNPs and the condition that we suggest designating, "astronaut ophthalmic syndrome (AOS)."

THE HYPOTHESIS

We propose a multiple-hit hypothesis—along the lines of the hypothesis that cancer results from accumulated mutations in a cell's DNA—of how ocular changes occur during and after spaceflight in some astronauts but not in others. We hypothesize that a number of contributing factors, including genetic and environmental influences, result in endothelial dysfunction, which, combined with fluid shifts during spaceflight, can ultimately increase pressure in the SAS space that surrounds the optic nerve, and that this increased pressure could be exacerbated by structural variations of the area that surrounds the optic canal.

More specifically, we hypothesize that variations in the genetics of enzymes of the 1-carbon metabolic pathway lead to localized tissue insufficiency of B vitamins, with associated increased homocysteine and depleted reserves of antioxidant precursors. Oxidative stress, in combination with other physiologic (e.g., fluid shift, altered testosterone, or carbohydrate metabolism) or environmental (e.g., radiation or ambient CO$_2$) influences, contributes to a multiple-hit effect that results in endothelial dysfunction. Endothelial dysfunction, either alone or combined with inflammation, results in leaky blood vessels. Subsequent edema impinges upon arachnoid villi, which reduces (or blocks) efflux of CSF. This leads to an increase in CSF pressure in the SAS around the optic nerve, which, in turn, impinges on the optic nerve and exerts pressure on the posterior globe. This posterior pressure may result in globe flattening and a corresponding hyperopic shift in refraction. Furthermore, we hypothesize that common benign structural variations of the paranasal sinuses (e.g., intraorbital ethmoidal cells, such as Haller or Onodi air cells) that are adjacent to the optic canal can contribute to or exacerbate a compartmentalized pressure gradient in the optic canal region. It is likely that a number of these factors collectively contribute to AOS. **Figure 1** depicts this hypothesis.

Below, we dissect each piece of this hypothesis in more detail and provide supporting citations along with data from spaceflight and ground studies.
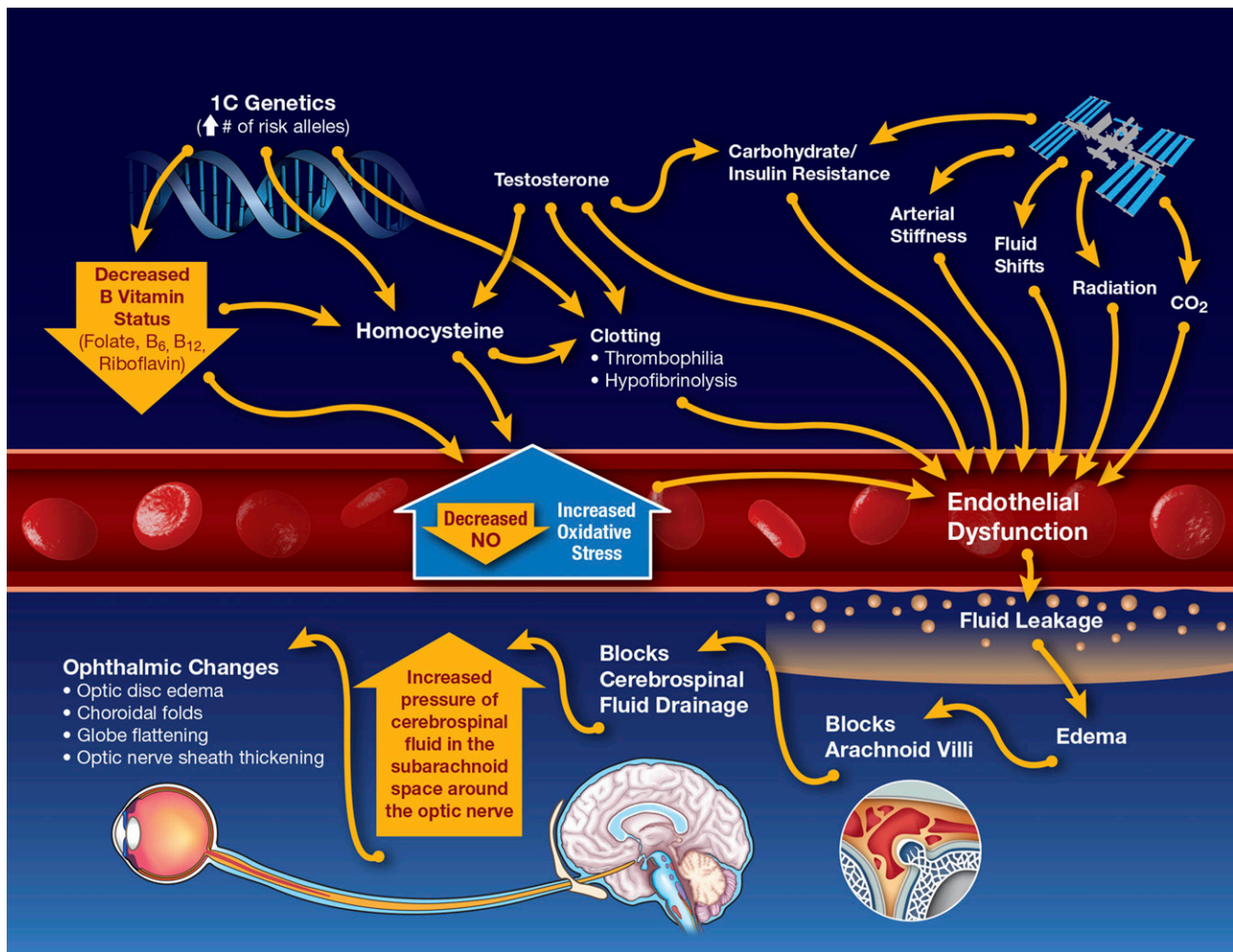
Figure 1. Depiction of the multiple-hit hypothesis showing how genetics of the enzymes of the 1-carbon metabolic pathway are proposed to be associated with astronaut ophthalmic syndrome. 1C, 1-carbon.

EVALUATION OF THE HYPOTHESIS

Variations in genetics of enzymes in the 1-carbon metabolic pathway lead to localized tissue insufficiency of B vitamins

Literally hundreds of polymorphisms are associated with enzymes of the 1-carbon pathway. Whereas substantial literature exists on a few of these (*e.g.*, rs1801133, *MTHFR* C677T), far fewer researchers have evaluated the broader set when examining relationships between genetics, homocysteine, and disease incidence. In our research of astronaut ophthalmic issues, we started with a small set of SNPs and are currently working to expand to a much broader examination.

Here, we will use 1-carbon genetics as a generic term that refers to the amalgam of SNPs in DNA that codes for enzymes of the 1-carbon metabolic pathway, realizing that multiple genetic sites contribute to individual differences and that not only does the literature not clearly point to one or a few, but the discordance of the literature is likely a result of this common diversity. That is, an individual who has a preponderance of certain SNPs would be expected to have lower B vitamin status, higher serum homocysteine

concentrations, and the downstream path of events that we have hypothesized. We do not expect to find one SNP as the cause, but rather a pattern or grouping such that the overarching impact is one that affects the flow through this pathway. Nonetheless, to date, the data support the fact that a greater incidence of risk alleles in DNA for enzymes of this pathway is associated with ophthalmic issues during and after spaceflight. What we propose here is the mechanism by which an individual's genetics can lead to these issues.

Risk alleles of SNPs in genes for enzymes of the 1-carbon pathway are typically associated with altered enzyme structure, which yields a lower functional activity of the enzyme. Using the example of the *MTHFR* C677T SNP, the presence of the risk allele (T) can lead to downstream biochemical effects, including increased circulating homocysteine and decreased serum folate concentrations (15–19). Nutritionally, a lower functional activity of an enzyme in the 1-carbon pathway can increase B vitamin requirements—and the risk of vitamin insufficiency (13, 20, 21).

Tissue-specific disruptions in 1-carbon metabolism will have only a minor impact on serum or blood biomarkers, whereas the affected tissue can have severe disruptions

(22). The brain is one such place where a tissue-specific disruption can occur. The blood–brain barrier (BBB) provides a functional barrier that allows the transport of nutrients from the CSF into the brain interstitial space (23). When the BBB is compromised, such as in cases of chronic inflammation, hypoxia, hypertension, or viral infection, nutrient transport can be insufficient and can lead to localized cerebral nutrient deficiencies [reviewed previously (22)]. Folate is an example of a B vitamin that is concentrated in the CSF as it is transported across the BBB, and a disruption of the BBB can lead to localized cerebral folate deficiency without whole-body folate deficiency (24). Spaceflight effects on the BBB are not known, but it is known that the endothelial cells of the BBB are sensitive to injury by ionizing radiation (25, 26).

Whereas circulating concentrations of homocysteine typically reflect the release of homocysteine from the liver (with the kidneys being the primary site of clearance) (27), they likely do not always reflect tissue concentrations of constituents and metabolites of the 1-carbon pathway. Antoniades *et al.* (19) showed that the *MTHFR* C677T genotype affected plasma homocysteine and the vascular tissue concentration of 5-methyl tetrahydrofolate (5-MTHF), but not tissue homocysteine content. They found that the 5-MTHF concentration in vascular tissue was a more important determinant of endothelial function and vascular oxidative stress than circulating or even tissue homocysteine. The *MTHFR* 677T allele was associated with less acetylcholine-induced vasorelaxation, and vascular 5-MTHF was associated with vasomotor responses to acetylcholine, but these associations were not true for homocysteine or circulating 5-MTHF. The authors suggested that the effects of the *MTHFR* C677T SNP on endothelial function are mediated *via* the vascular concentration of 5-MTHF and not plasma or vascular homocysteine (19).

Tissue concentration of B vitamins is rarely determined in population studies, but common variants of genes that are involved in 1-carbon metabolism can clearly lower circulating concentrations of B vitamins, including vitamin B_{12}, folate, and vitamin B_6 (28, 29). In many cases, it is a haplotype block of bases within genes that leads to lower B vitamin status. For example, Zinck *et al.* (29) found that the *SHMT1* TA haplotype, which includes the SNP at base pair 1420, was associated with higher red blood cell folate status, and that the *SHMT1* CG haplotype was associated with lower red blood cell folate status. Heterozygous *SHMT1*-knockout mice have higher concentrations of tissue folate than homozygous *SHMT1*-knockout mice (30). Of interest, we found that the *SHMT1* 1420T SNP was protective and associated with a lower risk of optic disc edema after flight than the *SHMT1* 1420C SNP (14).

Additive effects, which occur when multiple 1-carbon pathway SNPs are present or when an SNP combines with another hit, such as low riboflavin status or with another mutation, can exacerbate the changes in pathway intermediates and clinical outcomes (31, 32). Leber's hereditary optic neuropathy is caused by a disruption of mitochondrial metabolism from a mutation in mitochondrial DNA, but the severity of the neuropathy is affected by *MTRR* A66G and *MTHFR* C677T genetics (31). The effects of the SNP risk alleles in this case are thought to be increased sensitivity to oxidative stress and the enhancement of mitochondrial degeneration (31). The presence of multiple SNPs of 1-carbon pathway genes, including *MTRR* and *MTHFR* variants, can have an additive effect on the lowering of folate status (13, 33) and the synergistic effects on serum homocysteine concentrations in models of neurocognitive disorders, such as autism (34).

In our study of 1-carbon genetics and astronaut ophthalmic issues, we found that genetics (*i.e.,* the number of risk alleles for *MTHFR* 677 and 1298, *MTRR* 66, *SHMT1* 1420, and *CBS* 844ins68) and the status of B vitamins (*i.e.,* B_6, folate, and riboflavin) were significant contributors to the statistical model predicting ocular outcomes after flight (14). It should be noted that vitamin B_{12} status could not be included in that statistical model because the data set was limited. Nonetheless, we speculate that B_{12} is as important as other B vitamins in this process. Astronauts with ophthalmic changes after flight had higher circulating concentrations of homocysteine than did astronauts without ophthalmic changes, but the homocysteine concentrations were still within the clinically normal range (11). Although differences in homocysteine concentration were not large, they were of the same magnitude as the differences observed in studies of homocysteine between individuals with the risk (TT) form of the *MTHFR* C677T SNP (17, 35, 36) and those with the CC form of the SNP, which implies that the difference observed in astronauts was of the magnitude expected with genetic variants. Although tissue concentrations of 5-MTHF and other B vitamins were not measured in astronauts, it is suspected that vascular tissue concentrations of vitamins would be lower in individuals with more SNPs in enzymes of the 1-carbon pathway. Another explanation for the lack of difference in serum homocysteine concentrations is that the SNP effects were localized to a tissue and not systemic and, thus, not reflected in blood biomarkers.

Localized tissue insufficiency of B vitamins is associated with increased tissue homocysteine and increased production of reactive oxygen species and oxidative stress

Altered 1-carbon metabolism, in general, and increased homocysteine and depleted B vitamins, specifically, have many metabolic touchpoints with oxidative stress and antioxidant protection systems (19, 37). Folate itself has antioxidant properties (38), and 5-MTHF—the primary circulating form of folate—has been shown to reduce superoxide production and increase NO synthesis (19, 39, 40). This has been demonstrated at the tissue level with folic acid supplementation in a Wistar rat model in which folic acid pretreatment for 1 wk (10 mg/d) reduced ischemic endothelial dysfunction relative to placebo controls (41). Folic acid and its active metabolite, 5-MTHF, exert their antioxidant functions by preserving the coupling of eNOS with consumption of NADPH (41). Uncoupling eNOS from NADPH consumption leads to decreased synthesis of NO and increased generation of reactive oxygen species (42). Tetrahydrobiopterin (BH_4) is a cofactor of eNOS and is necessary for eNOS coupling

(40). 5-MTHF preserves eNOS coupling by increasing the availability of BH_4 *via* its role in stabilizing BH_4 and facilitating its binding to eNOS (38), as well as through its role in up-regulating dihydrofolate reductase, an enzyme that recycles BH_2–BH_4 (43). This folate-enhanced preservation of eNOS coupling is associated with improved endothelial function (41). Conversely, the decreased availability of folate contributes to oxidative stress and decreased bioavailability of NO *via* a number of mechanisms (19, 39). Decreased NO is a hallmark of endothelial dysfunction. This process is depicted in **Fig. 2**.

Vitamin B_{12} has also been shown *in vitro* to protect against superoxide radicals, as it acts as an efficient intracellular oxygen radical scavenger in cells that are exposed to homocysteine or Cu/Zn superoxide dismutase inhibitors (44). Vitamin B_{12} is inactivated by oxidation and, therefore, vitamin B_{12} status in individuals with more oxidant risk factors is often low or low–normal (45). Unlike a decrease in serum folate, a decrease in circulating vitamin B_{12} is not a generalized phenomenon for affected astronauts, but in some individual cases, circulating B_{12} fluctuates or the metabolic indicator of cobalamin status, methylmalonic acid, is elevated during flight (unpublished results), which reflects vitamin B_{12} insufficiency.

Another 1-carbon pathway metabolite—asymmetric dimethylarginine (ADMA)—is formed upon the conversion of methionine to homocysteine and is an inhibitor of eNOS. It pushes eNOS toward the uncoupled pathway and subsequently generates reactive oxygen species. Of interest, in our study that investigated the effects of 1-carbon pathway genetics and B vitamin status on ocular changes after spaceflight, we did observe a small but significant correlation between ADMA and the change in diopters that individuals experienced after their long-duration spaceflight (**Fig. 3**). These ADMA data are from a blood sample that was collected for genetic analyses and not with any specific timing relative to spaceflight (*i.e.*, some were collected before flight, some were collected long after flight). It is worthwhile to note that the mean homocysteine concentration in these same blood samples
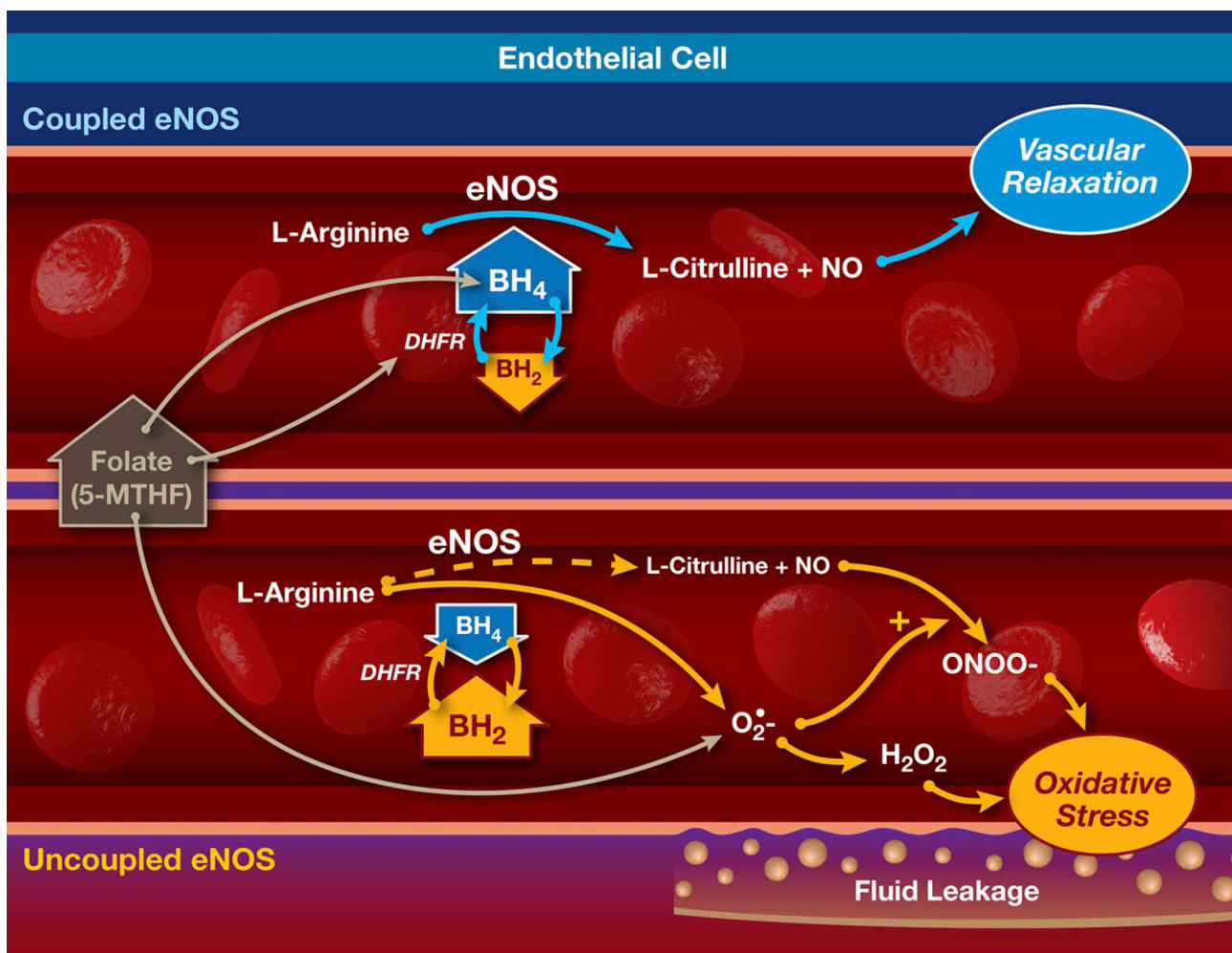

Figure 2. A simplified diagram of eNOS coupling with consumption of NADPH and uncoupling. eNOS is present as a dimer and when its action is coupled with NADPH consumption, arginine is converted to citrulline and NO is produced (top). BH_4 is a cofactor for eNOS and preserves eNOS dimerization. When BH_4 is limited, either by decreased synthesis or increased oxidation, eNOS becomes uncoupled and reactive oxygen species are formed instead of NO (bottom). 5-MTHF can act favorably on endothelial function by preserving eNOS coupling by stabilizing BH_4 and facilitating its binding to eNOS. 5-MTHF also acts as a superoxide radical scavenger and can also upregulate dihydrofolate reductase (DHFR) to recycle oxidized tetrahydrobiopterin to BH_4. Figure adapted from refs. 43–45.

was higher in affected astronauts, which matched the pattern found in pre-, in-, and postflight samples. These data are not conclusive, by any means, but they do suggest that individuals who experienced ophthalmic issues generally had higher concentrations of 1-carbon pathway intermediates that are known to contribute to endothelial dysfunction.

In patients with coronary artery disease, supplementation with folic acid leads to decreased superoxide production—secondary to improved eNOS coupling—and improved endothelial function, specifically, flow-mediated dilation (46). Studies of spontaneously hypertensive rats show that folate deficiency leads to symptoms of metabolic syndrome, including oxidative stress, insulin insensitivity, and increased systolic blood pressure (47). Whether these effects are a result of homocysteine itself, a reduction of antioxidant enzyme systems, or the inherent antioxidant effects of folate is not well understood, but the level of folic acid supplementation required to improve endothelial function far exceeds the amount required for simply lowering homocysteine; therefore, it is likely that the mechanism of action of folate is related to its effects on preservation of eNOS coupling as described above and shown in Fig. 2. Regardless of the mechanism, clear evidence links inefficient aberrations in 1-carbon pathway metabolism, homocysteine, and endothelial oxidative stress (48).

Thus, the combination of a lower B vitamin status and/or slightly elevated homocysteine and 1-carbon pathway genetic variations among astronauts with ocular changes after flight may contribute to increased oxidative stress at the tissue level. We did not find an association between circulating markers of oxidative stress and the development of ophthalmic changes during spaceflight (unpublished results), but several limitations to the subject, sample, analyte stability, and available data collection sets may have obscured the identification of a relationship.

In addition to the oxidative stress caused by variations in 1-carbon pathway genetics, other spaceflight sources of physiologic and/or environmental stress that can contribute to endothelial dysfunction or vascular changes are fluid shifts, radiation exposure, ambient CO_2, and hormonal or metabolic influences

The occurrence of a fluid shift toward the head during exposure to microgravity is well documented (49–51).

Approximately 2 L of fluid from the legs is shifted upward toward the head, and simultaneously cardiac output increases approximately 20% (52, 53). Plasma volume decreases 10–15% (54), but total body water is unchanged (55), which suggests that the excess fluid moves into the interstitial and intracellular spaces (56). It has been postulated, though not documented, that the fluid shift toward the head diminishes NO bioavailability in the cerebral vasculature, and thus decreases cerebral perfusion (57).

Studies of fluid shifts in relation to cardiovascular and ocular examination during long-duration spaceflight are currently underway with ISS astronauts. Ophthalmic issues have been assessed in head-down tilt (HDT) bed rest, an often used analog of weightlessness, with some subclinical findings but none as overt as those observed after actual spaceflight (3, 58–60). Diuresis occurs in HDT bed rest but not during spaceflight, and total body water decreases during HDT bed rest but is unchanged during spaceflight (56). Differences in the nature and regulation of fluid shifts during spaceflight and bed rest studies (61, 62) could contribute to an understanding of why the ocular changes observed after flight do not occur in bed rest, but caution is urged in comparing the findings obtained under these two conditions.

Radiation exposure is inevitable during spaceflight. Sources of radiation include solar particle events and highly charged galactic cosmic rays (63), and the dose each astronaut receives depends on the shielding provided by the spacecraft, the location of the vehicle during extravehicular activities, and solar activity during the mission. Ionizing radiation can cause senescence and apoptosis of endothelial cells, which decrease NO production and can contribute to endothelial dysfunction (64). It has also been suggested that, in the brain and spinal cord, with free flow of CSF, the potential inflammatory effects of radiation are diluted relative to their state in the more stagnant ONS CSF in which the effects may be more pronounced (9).

The ISS cabin CO_2 concentration is approximately 10 times higher than concentrations found on Earth. NASA's long-duration Spacecraft Maximum Allowable Concentration for CO_2 is 0.7% (a CO_2 partial pressure of 5.3 mmHg) (65). Carbon dioxide affects cerebral blood flow (66) and, therefore, has been considered a key issue related to ICP and ophthalmic issues in astronauts. In the years since ocular issues were first reported in ISS astronauts, cabin CO_2 concentrations have been reduced. Despite this reduction, some crewmembers continue to develop ocular
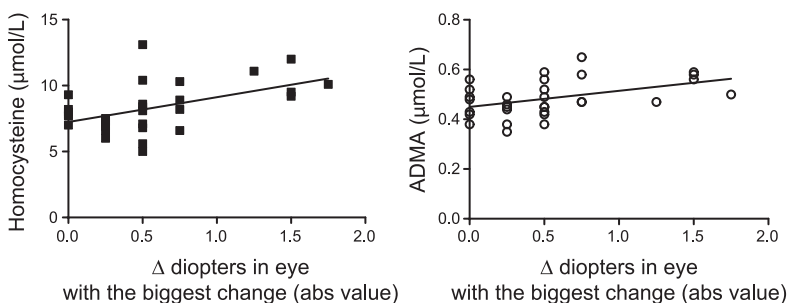

Figure 3. Correlation of ADMA or plasma homocysteine with the change in diopters that individuals experienced after their long-duration spaceflight. Correlation of the change in diopters (from cycloplegic refraction data) in the eye with the biggest change after 4–6 mo of spaceflight with plasma homocysteine ($P < 0.01$) or ADMA concentration ($P = 0.01$) measured in a blood sample collected before or up to 5 yr after flight (unpublished results). Abs, absolute.

signs and symptoms. In our initial assessment of homocysteine in astronauts, we found that the cabin CO_2 concentration on the day of blood collection was higher in astronauts who experienced ophthalmic issues. Whereas that provides a snapshot of the atmospheric composition for the mission, we believe this is a potential contributing factor. Indeed, if the response, such as changes in cerebral blood flow, to CO_2 exposure is different in individuals who have 1-carbon pathway SNPs, this could provide a plausible link between individual genetics and spaceflight effects on cerebrovascular function.

When considering exposure to increased atmospheric CO_2, formate is a 1-carbon pathway metabolite that must be included (67). The properties of CO_2 in aqueous solution are such that 90% is converted to carbonic acid, and 10% remains as dissolved CO_2 (68, 69). Of that 10% of dissolved CO_2, 25% is converted to oxalate *via* a head-to-head dimerization, and 75% is converted to formate *via* a head-to-tail dimerization (68). ISS cabin air also contains sources of formaldehyde that could contribute to formate production (70). Decreased status of folate or vitamin B_{12} can increase plasma and urinary formate, as well. Formate is an intermediate of the 1-carbon pathway, and if B vitamin cofactors are not present in sufficient quantities to convert formate to 10-formyl tetrahydrofolate, then formate can build up in the plasma (71). Excess formate can affect the eye by damaging Müller cells, which are glial cells that span the entire thickness of the retina (72). Müller cells constitute a link between retinal neurons and vasculature, by which they exchange nutrients. It is not known whether Müller cells in astronauts are affected by long-duration spaceflight.

Hormones, including testosterone and insulin, can contribute to endothelial dysregulation. Although the mechanism is not known, plasma testosterone concentration is associated with arterial elasticity and microvascular function in middle-aged men (73). Serum total and free testosterone were also associated with flow-mediated dilation in men age 25–85 yr (74). Testosterone represents a unique and potentially overarching aspect of the hypothesis presented herein. It has been associated in the medical literature with many other elements of this hypothesis: increased homocysteine, altered carbohydrate metabolism, thrombosis, and endothelial dysfunction. With respect to 1-carbon pathway genetics, the *MTHFR* C677T polymorphism in healthy women has been associated with elevated testosterone (75), and testosterone has been implicated in the regulation of cystathionine β-synthase enzyme activity and the sex difference in circulating homocysteine concentrations (76). Whereas testosterone concentrations do not typically change during spaceflight or bed rest (77), we demonstrated that the response of serum testosterone to spaceflight (*i.e.*, the area under the curve for testosterone concentration during spaceflight) was greater in astronauts who experienced ophthalmic issues (14), as was that group's preflight concentration of dehydroepiandrosterone (14).

Other metabolic influences could contribute to endothelial dysfunction during flight, including altered carbohydrate metabolism or insulin sensitivity. Hughson *et al.* (78) demonstrated higher homeostatic model assessment of insulin resistance—the insulin resistance index—during ISS missions in 9 male and female astronauts. Earlier studies revealed altered glucose tolerance curves (79) and increased C-peptide excretion during flight (80, 81). Ground analog (*i.e.*, bed rest) studies have also shown altered carbohydrate and insulin metabolism in simulated spaceflight (81–84). Whereas none of these studies examined the relationship of insulin or carbohydrate metabolism to ophthalmic issues in astronauts, our previous study did document metabolomic profiles that suggested greater insulin resistance in astronauts with ophthalmic issues (14). Insulin resistance and endothelial dysfunction have been suggested to coexist (37, 85), and obesity and insulin resistance are also associated with endothelial dysfunction (85).

Endothelial dysfunction leads to vascular leakage and edema

Many sources of oxidative stress can deplete endothelium NO, which leads to endothelial dysfunction (86, 87). Oxidative stress increases vascular endothelial permeability and the widening of the interendothelial junctions, which results in microvascular protein and fluid leakage into the interstitial space (88, 89). Mullick *et al.* (90) documented increased arterial stiffness and permeability in hyperhomocysteinemic mice that was associated with increased oxidative stress, reduced NO availability, and increased arterial permeability (90). This effect was reversed by the provision of a NO donor and was found to occur with hyperhomocysteinemia that was induced by folate or excess methionine (90–93).

With respect to spaceflight, Hughson's group at the University of Waterloo recently showed increased blood vessel stiffness after ISS missions, which was estimated to represent 10–20 yr of aging after only 6 mo of spaceflight (78). This study was completed in a small group of astronauts, which is typical of space research, but of more importance, without reference to their experience of ophthalmic issues. This strengthens our hypothesis that something unique about the spacecraft environment alters endothelial function, and in some individuals, this is exacerbated to the point of presenting with clinical findings. We posit that these individuals are those with 1-carbon pathway SNPs.

Edema can impair CSF drainage, which leads to a buildup of pressure on the optic nerve and eye; variations in optic nerve structure within the orbit may also contribute to decreased CSF drainage and buildup of pressure on the optic nerve

An imbalance between reactive oxygen species and NO production can elicit a vast array of endothelial responses, including endothelial barrier dysfunction, thrombogenesis, and changes in vascular permeability (89). An increase in interstitial fluid as a result of fluid shifts during spaceflight, and subclinical endothelial dysfunction from a number of contributing factors, including 1-carbon

pathway genetics, radiation, CO_2, and hormonal and metabolic changes during flight, could lead to decreased drainage of the CSF and, subsequently, increased pressure on the optic nerve. It has been hypothesized that astronaut ophthalmic issues might arise from altered CSF flow in the SAS secondary to microgravity-induced fluid shifts (4), but not to endothelial dysfunction that is associated with the 1-carbon pathway and other factors as proposed herein.

Structural variants in the region of the optic canal could contribute to a compartmentalized increase in pressure around the optic nerve, but this idea has not been investigated. Roughly 30–60% of individuals (94) have an anatomic variation, superior to the sphenoid sinus, described as Onodi cells. These are typically considered benign structural variations. Onodi cells are ethmoid bony air cells that may be of particular relevance because of their close spatial relationship with the optic nerve, and that are commonly associated with an identifiable optic canal bulge (95). The influence of Onodi cells might also help explain why many astronauts are only affected unilaterally (7, 9).

PROPOSED FUTURE WORK

There is much work to be done to test the proposed hypothesis so that the means to prevent or treat AOS may be developed and validated in affected individuals. Initial studies are underway to better characterize both 1-carbon metabolic function and associated genetic polymorphisms, as well as studies of fluid shifts and ICP during spaceflight (independent of genetics).

Studies of endothelial function in individuals who have risk alleles of SNPs in the 1-carbon pathway would be one logical step, and studies of the effects of CO_2 and HDT in individuals with these same SNPs would also be valuable. Another step is to examine BBB function in light of radiation exposure during spaceflight. If indeed the BBB is compromised, crewmembers are at risk for cerebral folate and other vitamin deficiencies. We have proposed—and initiated—pilot studies to characterize these phenomena in women with polycystic ovary syndrome (PCOS), given that patients with PCOS have many of the characteristics of AOS: higher homocysteine, higher incidence of 1-carbon pathway SNPs, possible intracranial hypertension, thicker retinal nerve fiber layers, and altered testosterone and insulin metabolism. If patients with PCOS indeed have a significant number of AOS symptoms, additional testing of countermeasures in these patients could be beneficial for the treatment of both PCOS and AOS.

Ultimately, we seek countermeasures to prevent AOS or to treat it in affected individuals. If the hypothesis presented herein is supported in ongoing studies, B vitamin supplementation could be a plausible, low-risk countermeasure to AOS. This would be a simple, and again, low-risk approach to correct endogenous antioxidant decrements and even to improve BBB function, which has been shown to occur in patients with hyperhomocysteinemia that is treated with B vitamin supplementation (96). Riboflavin supplementation has been shown to reduce blood pressure in hypertensive patients with the minor form of the MTHFR 677 polymorphism (TT), and may play a role in preventing endothelial dysfunction (97, 98). Attention to details, including the dose and form of vitamins, would need to be considered because folic acid can inhibit 5-MTHF transport across the BBB and potentially exacerbate a cerebral folate deficiency (22, 99, 100); therefore, use of reduced folate (5-MTHF or THF) should be considered. Although the temptation to provide antioxidants is often an immediate reaction, providing the precursors to mitigate these effects in the first place seems a more likely solution than trying to provide the downstream intermediates. Vitamins and antioxidants were not effective in mitigating the acute effects of methionine-induced hyperhomocysteinemia on endothelial dysfunction (101); however, they may have been ineffective simply because providing them exogenously may not—or may not rapidly—increase intracellular reservoirs of antioxidants.

SUMMARY

We maintain that AOS is genetically predisposed, is associated with endothelial dysfunction induced by oxidative stress, and leads to elevated ICP or locally increased SAS pressures within the orbit as a result of these same factors—with or without increased ICP—in affected individuals. Preventing this syndrome will mitigate an important human health risk for NASA and will provide valuable advances for terrestrial medicine. Extensive literature of the past 15 yr has attempted to characterize the interrelationships of 1-carbon pathway genetics, B vitamins, homocysteine, oxidative stress, endothelial dysfunction, and cardiovascular disease. Aspects of these interrelationships have long been argued and debated and, too often, dismissed. The results from studies of generally healthy astronauts before, during, and after space missions provide a unique perspective that may help crystallize the understanding of these relationships and that may have significant implications for both aerospace and terrestrial medicine. [FJ]

AUTHOR CONTRIBUTIONS

S. R. Zwart and S. M. Smith conceived and designed the original hypothesis; S. R. Zwart, C. R. Gibson, J. F. Gregory, T. H. Mader, S. H. Zeisel, and S. M. Smith conducted the research, which was discussed and further developed among all authors; S. R. Zwart and S. M. Smith wrote the initial draft of the paper, with input and revision by all authors; P. J. Stover participated in discussion and

development of the research, and provided input to revision of the initial draft of the paper; S. R. Zwart and S. M. Smith have primary responsibility for final content; and all authors have read and approved the final manuscript.

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