CHANGES IN PROTEIN AND GENE EXPRESSION OF ADHESION MOLECULES AND CYTOKINES OF ENDOTHELIAL CELLS IMMEDIATELY FOLLOWING SHORT-TERM SPACEFLIGHT TRAVEL
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ABSTRACT

Introduction: Spaceflight travel may induce modulation of inflammation and endothelial activation possibly increasing risk of atherosclerosis. However, the mechanisms of changes in protein and gene expression of inflammation and endothelial activation by short-term spaceflight travel are still unclear.

Objectives: To investigate the protein and gene expression of cytokines and adhesion molecules in endothelial cells immediately after a short-term space flight.

Materials and Methods: Human umbilical vein endothelial cells (HUVEC) cultured on microcarriers (Cytodex-3, GE Healthcare Biosciences AB, Uppsala, Sweden) in a fluid processing apparatus (FPA) were flown to the International Space Station by the Soyuz TMA-11 rocket. At the ISS, the HUVEC were incubated at 37°C for 8 days. Following landing, HUVEC supernatant was collected and stored at -80°C until analysis (PF). 2 sets of ground controls were performed using similar spaceflight temperature profile (ATGC) and at 37°C (ITGC). The supernatant from PF and corresponding controls were collected and measured for IL-6, sICAM-1, e-selectin, sVCAM-1 & TNF-α levels using ELISA. For mRNA expressions of IL-6, ICAM-1, e-selectin, VCAM-1, TNF-α, nuclear factor kappa B (NFkB) and endothelial nitric oxide synthase (eNOS) total RNA was extracted from HUVEC on microcarriers and analysed with branched DNA (bDNA) hybridization method using the Quantigene Plex assay kit (Panomics, USA).

Results: Levels of soluble IL-6 and sICAM in PF were higher compared to ATGC (IL-6: 80.9 ± 18.6 vs. 12.6 ± 0.6 pg/ml, p<0.05, sICAM-1: 12994.9 ± 1060.6 vs. 7738.7 ± 225.0 pg/ml, p<0.05). PF had higher soluble IL-6, sICAM-1 and e-selectin compared to ATGC (IL-6: 80.9 ± 37.3 vs. 14.0 ± 3.47 pg/ml, p<0.05, sICAM-1: 12994.9 ± 2121.3 vs. 2601.8 ± 47.1 pg/ml, p<0.05, e-selectin: 2165.0 ± 186.1 vs. 1609.2 ± 41.3 pg/ml, p<0.05). No significant different in levels of IL-6, sICAM-1, sVCAM-1 and TNF-α between ATGC and ITGC were found. No significant different in mRNA expression of IL-6, sICAM-1, e-selectin, sVCAM-1, TNF-α, NFkB and eNOS between ATGC and ITGC could be shown. However there was a significant difference in IL-6 (p<0.01), ICAM-1 (p<0.01), VCAM-1 and NFkB mRNA expression between PF and ATGC. There was a significant difference in IL-6 (p<0.01), ICAM-1 (p<0.01), e-selectin and NFkB mRNA expression between PF and ITGC.

Conclusion: Short-term spaceflight travel enhances soluble endothelial protein expression of IL-6, sICAM-1 and e-selectin suggesting endothelial activation. Short-term spaceflight induces the increment of gene expression of IL-6, ICAM-1 and VCAM-1. A known transcription factor of these markers, NFkB, showed a corresponding increase in expression. These suggest that, short-term spaceflight travel may increase inflammation and endothelial activation in endothelial cells.

Keywords: Microgravity, adhesion molecules, interleukin-6, endothelial cells, inflammation

INTRODUCTION

Endothelial cells are sensitive to oxidative stress and gravity alterations (Buravkova, 2005). During spaceflight the endothelial cells are exposed to microgravity while during launch and landing they are subjected to hypergravity and vibrational forces. Both, micro- and hypergravity can modulate the expression of cytokines and adhesion molecules which may lead to atherosclerosis (Carlsson, 2003). It has been difficult to determine the relative contribution of each of those factors to the development of atherosclerosis. However, studies conducted on astronauts during prolonged space flight have shown an association between the length of the flight and increased risk of arrhythmia susceptibility (D’Aunno, 2003). In orbital flight, astronauts experience an adaptive cardiovascular response known as cardiovascular deconditioning (Baisch, 2000).

Atherosclerosis is a progressive disease arising from a combination of endothelial dysfunction and inflammation. Inflammatory mediators appear to play a fundamental role in the initiation, progression and eventual rupture of atherosclerotic plaques (Szmitko, 2003). Markers of inflammation and endothelial activation such as IL-6, TNF-alpha, sICAM-1 and endothelial nitric oxide synthase (eNOS) are useful predictive markers towards cardiovascular disease development and as new targets for treatment (Berg 2003). The transcription factor NFkB is involved in cellular responses to stress, cytokines, free radicals, UV irradiation, oxidized LDL, etc. Incorrect regulation of NFkB has been linked to cancer, inflammatory and autoimmune diseases as well as atherosclerosis. At the cellular level NFkB mediates cell migration and is responsible for an increased expression of many inflammatory cytokines, chemokines and adhesion molecules (Baldwin, 2001).

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Microgravity has been indicated as the cause for the observed changes at cellular level such as gene expressions and metabolic activities (Anderson 2003). Various experiments using simulated microgravity were conducted to investigate the effects of microgravity on endothelial cells. (Villa, 2005, Anderson, 2003, & Buravkova 2005). In simulated microgravity ICAM-1 expression in endothelial cells is increased while the expression of e-selectin and VCAM-1 were undetected (Buravkova 2005). eNOS activity was found to be elevated in microgravity (Versari, 2007). At the cellular level, simulated hypergravity has been reported to affect gene expression of and influences important processes of proliferation, differentiation, cytoskeleton organization, adhesion and motility at the cellular level especially in endothelial cells (Monici, 2006).

Not much has been reported regarding the changes in expression of IL-6, TNFα and adhesion molecules like ICAM, VCAM and e-selectin immediately following a space flight travel due to limited flights and experimental limitations (Sonnenfeld, 2002). Despite experimental limitations endothelial cells were flown to the International Space Station by a Soyuz TMA-11 and the gene and protein expression of IL-6, TNFα, ICAM, VCAM and e-selectin were analysed after a total of 12 days spaceflight travel. Additionally the possible molecular mechanism responsible for the alteration in expressions of these markers was also investigated. Our results demonstrate that space flight travel enhances inflammation and endothelial activation at the protein and molecular level.

**MATERIALS AND METHODS**

**Materials**

Medium 200 and low serum growth supplements (LSGS) were obtained from Cascade Biologics, USA. Accutase was purchased from ICN Biomedical, USA. ELISA test kits for E-selectin, sICAM-1, sVCAM-1, IL6 and TNF-α were purchased from Benda Medsystems, Austria. Cytodex- 3 microcarrier beads were purchased from GE Healthcare Biosciences AB, Uppsala, Sweden. Phosphate buffer saline (PBS) was obtained from MP Biomedicals, France. Quantigene Plex 2 assay kit was purchased from Panomics, USA. Fluid processing apparatus (FPA) were obtained from Bioserve, USA.

**Cell culture**

Human umbilical vein endothelial cell line (HUVEC) was purchased from Cascade Biologics, USA. HUVEC were cultured in medium 200 supplemented with LSGS in a humidified incubator set at 37°C and 5% carbon dioxide (CO₂) until confluent. Split ratio was 1:2. Prior to the addition of HUVEC into FPA, HUVEC were cultured onto microcarrier in Petri dishes and incubated at 37°C in 5% CO₂ incubator for 24 hours. This timeline was sufficient to allow HUVEC to attach to the microcarriers surface. Two days prior to the launch, the cells were loaded into 2 sets of FPA, each in duplicate. Each FPA consists of 3 chambers, A, B and C. For FPA set I, the cells were loaded in chamber A while additional culture medium was loaded in chamber B and C. For FPA set II, the cells were loaded in chamber A, additional culture medium in chamber B and RNAlater fixative in chamber C. Then, the FPAs were incubated at 37°C in 5% CO₂ incubator until being handed over to the engineers at 12 hours before launch, to be placed in Soyuz TMA-11.

**In-flight experiment**

During launch and up to docking, the FPAs containing the HUVEC cells were stowed at ambient temperature (18-22°C) in the Nomex bag. On L+4 (4 days after launch), the Angkasawan (astronaut), Sheikh Muszaphar added the fresh culture medium from chamber B into chamber A of both sets of FPA. The FPAs were placed back in the Kubik Amber. On L+7 (7 days after launch), the Angkasawan took both FPA sets out of the Kubik Amber and transferred to the warmest ambient temperature location in ISS. At ~8 hours before undocking, both sets of FPA were taken out to perform the termination process. For set I, additional culture medium was added into the cells. RNA later was added into the cells for set II. FPAs were then packed into Nomex Bag (CIS kit) and transferred to the Soyuz until landing. Temperature profile was recorded by HOBO which placed in the Nomex bag. Upon landing, the FPA of Set I were placed in a 37°C container to maintain the live cells, while FPA of Set II were kept in a 4°C thermo-container, and transported to the laboratory. The supernatant from set I live cells were collected and stored in a -80°C freezer before further analysis. Cells in RNAlater from set II were collected and kept frozen until analysis. Two sets of asynchronous ground controls were performed using the spaceflight temperature profile according to the HOBO reader (ATGC) and constant 37°C (ITGC). The actual temperature ground controls (ATGC) were performed to control for the effects of temperature fluctuations occurring throughout the space mission. In addition, they were controlled for initial cell density, type of culture medium, growth environment and FPA conditions. However, in this particular experiment, the ground controls were not controlled for vibrational forces and changes in gravity including hyper, micro and 1 g forces throughout the flight. At the end of the 12-day experiment, cells were collected and protein and gene expression analyses were performed together with PF samples. Details of the Soyuz TMA-11 flight code and ISS location were as follow: Soyuz TMA-11 flight code: 2007-045A/32256; ISS location is maintained at an orbit between 278 and 460 km altitude. This particular experiment was conducted in the Russian Segment.
Table 1: Primer sequence for mRNA expression

| i) IL-6     | Forward primer: GCC TTC GGT CCA GTT GCC TT  |
|            | reverse primer: GCA GAA TGA GAT GAG TTG TC |
| ii) ICAM-1 (CD54) | Forward Primer: AGAGGTCTCAGAAGGGACCG       |
|            | Reverse Primer: GGGCCATACAGGACACGAAG       |
| III) VCAM-1 (CD106) | Forward primer: GGTGGGACACAAATAAGGGTTTTGG |
|            | Reverse primer: CTTGCAATTCTTTTACAGCCTGCC   |
| IV) E-selectin | Forward primer: TGAAGCTCCCACCTGAGTCCAA     |
|            | Reverse primer: GGTGCTAATGTCAGGAGGGAGA     |
| V) TNF-α   | Forward primer: CCGGGCGTGGTGGTGAG          |
|            | Reverse primer: TCTGCTTTTGGGTCTTTGTGAATA   |
| VI) eNOS   | Forward primer: ATGGGCAACTTGAGAGCGTGGA     |
|            | Reverse primer: TAGTACTGGTGATGAAGTCC       |

House keeping gene:

| i) GAPDH  | Forward primer: CCACCCATGGCAAATTCATGGCA  |
|          | Reverse primer: TCTAGACGGCAGGTCAGGTCACC |
| iii) HPRT-1 | Forward primer: GGCAAAAACAATGCAAAACCTT |
|          | Reverse primer: CAAGGGCATATCCTACGACAA  |
Expression of cytokines and soluble adhesion molecules

The concentration of inflammation and adhesion molecules in the supernatant of HUVEC cells from Set I FPA were measured by ELISA standard kit (Bender Med System, Vienna, Austria). Tests were performed according to the instructions provided by the manufacturer. Surface levels of IL-6, sICAM-1, sVCAM-1, e-selectin and TNF-α in each of PF and ground controls were performed in triplicates.

mRNA expression

For mRNA expression analysis, HUVEC cells in RNAlater have been collected from set II FPA. In brief, RNA later was removed from the cells through high speed centrifugation. Lysis buffer and proteinase K were added to the HUVEC-onto microcarrier and mixed well by pipetting the solution up and down for about 30 times. Samples were incubated for 30 minutes at 50°C in a thermomixer. Cells were examined under the microscope to ensure complete cell lysis. Cell lysates were mixed with vortex for 15 minutes prior to the high speed centrifugation for 1 minute. Supernatant was collected and followed with a Quantigene Plex assay. The assay was performed according to the protocol provided by the manufacturer. Results were normalized against GAPDH and HPRT-1 to obtain norm ratio. Primer sequence for each measured gene is presented in Table 1.

Statistical analysis

Results are expressed as Mean + SD. ANOVA was performed to assess overall differences between the different samples. Independent T – test was performed to compare the differences between 2 groups of samples. All data was analyzed by a statistical package program, SPSS version 12.1. Significant value was set at p<0.05.

RESULTS

Effects of spaceflight travel on IL-6, ICAM-1, VCAM-1, e-selectin and TNF-α soluble protein expression

Figure 1 shows that levels of IL-6 in the supernatant of PF was significantly higher compared to the ATGC (80.9 + 19.6 vs. 13.1 + 2.0 pg/ml, p<0.05) and ITGC (80.9 + 19.6 vs. 14.0 + 3.4 pg/ml, p<0.05). There was no significance difference in levels of IL-6 between ATGC and ITGC. In Figure 1 it also shows that PF samples have comparable levels of TNF-α with both ground controls. The differences of TNF-α levels between ATGC and ITGC was not seen as illustrated in Figure 1. Figure 2 shows levels of soluble protein of ICAM-1, VCAM-1 and e-selectin in PF, ATGC and ITGC. There was a significant increment in levels of sICAM-1 in PF compared to ATGC (12994.9 + 1060.5 vs. 7738.7 + 225.0 pg/ml, p<0.05) and ITGC (12994.9 + 1060.6 vs. 2601.8 + 33.3 pg/ml, p<0.05). sICAM-1 levels in ATGC were significantly higher compared to ITGC (7738.7 + 225.0 vs. 2601.8 + 33.3 pg/ml, p<0.05). E-
Effects of spaceflight travel on IL-6, ICAM-1, VCAM-1, e-selectin, TNF-α, NFκB and eNOS mRNA gene expression

Gene expression of IL-6 and TNF-α in PF are shown in Figure 3. PF has statistically higher mRNA expression of IL-6 compared to ATGC (p<0.01) and ITGC (p<0.01). No statistically significant different of TNF-α gene expression in PF compared to both ATGC and ITGC as shows in Figure 3. Figure 4 shows ICAM-1, VCAM-1 and e-selectin gene expression in PF, ATGC and ITGC. In that figure, it has been shown that ICAM-1 gene expression was higher in PF compared to both ATGC (p<0.01) and ITGC (p<0.01). There was an increased of e-selectin gene expression in PF compared to ITGC (p<0.05). No significant difference of e-selectin gene expression between PF and ATGC. For VCAM-1, PF shows significantly higher gene expression compared to ATGC (p<0.05) but it was no different compared to ITGC. In overall there was no significance difference in mRNA expressions of IL-6, ICAM-1, VCAM-1, e-selectin and TNF-α between ATGC and ITGC. NFκB and eNOS gene expression in PF, ATGC and ITGC are shown in Figure 5. NFκB gene expression in PF was significantly higher compared to both ATGC (p<0.05) and ITGC (p<0.01). There was no significant difference in gene expression of NFκB between ATGC and ITGC. For eNOS gene expression, PF shows no significant different with both ATGC and ITGC. Between ATGC and ITGC no significant different in gene expression of eNOS were observed.
Figure 4: ICAM-1 (A), VCAM-1 (B) and e-selectin (C) gene expression in PF and ground controls. * p<0.05 compared to ATGC. ** p<0.01 compared to ITGC. Reference gene was GAPDH and HPRT-1. Data are expressed as Mean + SD. PF-Post-flight, ATGC-Actual temperature ground control, ITGC-Ideal temperature ground control.

DISCUSSION

During spaceflight different factors like changes in gravity, temperature, and limitations to the cell culture system need to be taken into account. Reports on protein and gene expression of soluble biomarkers of inflammation and endothelial activation in immediate post-flight samples are scarce. In this experiment, ground controls based on actual and ideal temperature profile were performed to eliminate the temperature changes factor which then leaves only the space flight effects.

The inflammation process occurs as a natural defense mechanism towards toxic compounds such as oxidized proteins and lipids. It has a well recognized role in the development of atherosclerotic lesions. Consequences of inflammation in atherosclerosis are difficult to predict. However it has been postulated that inflammatory processes may be beneficial at early stages (Branen et al., 2004).

The protein and gene expressions of cytokine (IL-6) and ICAM-1 were increased after 12 days of spaceflight. E-selectin and VCAM-1 showed an increment in gene expression, although their protein expression was not affected by spaceflight. TNF-α was not affected by space flight either at the protein or molecular level.

The expression of pro-inflammatory cytokines like TNF-α and IL-6 induce the expression of adhesion molecules. IL-6 is the principal procoagulant cytokine which acts as a messenger cytokine (Willerson et al., 2004). When IL-6 is released into the systemic circulation, the endothelium increases the release of adhesion molecules which amplify inflammatory process. So far, reported data on the effects of spaceflight on IL-6 expression in endothelial cells are scarce. Even in simulated microgravity it has not been much reported. However, IL-6 protein expression in murine microvascular endothelial cells was decreased after 72 hours in simulated microgravity (Cotrupi et al., 2005). The differences in that result with our finding maybe due to the different species of endothelial cells used in that
experiment, the duration of microgravity exposure and that it was conducted on ground microgravity simulation. A study in human fibroblast which were sent into space with Foton-M3 mission reported the increment of soluble protein of IL-6 in the growth medium suggesting there was an increased of inflammatory response (Dieriks et al., 2009).

TNF-α is a key cytokine in the recruitment and in the activation of inflammatory cells. It facilitates influx of inflammatory cells to the vessel wall. Effects of spaceflight in normal WEI38 human fibroblast TNF-α gene expression during STS-93 Space Shuttle mission was also studied by a group of investigators (Semov et al., 2002). In that study, they found that spaceflight upregulated TNF-α related genes which were TNF-α converting enzyme (TACE), TNF-related weak inducer of apoptosis (TWEAK) and TNF superfamily member 15 (TNFSF15) gene expression. TACE may be responsible for the expression of TNF-α protein leading to regulation of proinflammatory process. TWEAK and TNFSF15 are responsible for upregulation of NFkB. At the same time, in that study they found none of the 20 TNF receptors represented in TNF array was up- or down-regulated as a consequence of spaceflight. Similarly, in this study, we found that TNF-α protein and gene expression were not affected by spaceflight travel. However, the TACE, TWEAK and TNFSF15 genes were not measured as they are beyond the scope of this present study.

Simulated microgravity has been reported to increase ICAM-1 protein in HUVEC (Romanov et al., 2001). In another study it was reported that hind-limb unloading in rats stimulated endothelial ICAM-1 expression in rats. Hind-limb unloading which provides microgravity-induced shear stress condition has been used as a model to simulate effects of space-flight on cardiovascular and immune systems in humans (Jung et al., 2005). This model can be used to study changes in immune responses including altered cytokine and adhesion molecule production which may occur during space flight. In this study, it was suggested that presence of ICAM-1 expression on the endothelial cell surface leading to monocyte recruitment to the endothelium is a key event in the pathogenesis of atherosclerosis (Jung et al., 2005). However, an in vivo study has shown decreased circulating soluble ICAM-1 and e-selectin after 5-16 days of spaceflight travel (Mills et al., 2002). This finding was contradictory with our findings.

Table 2: Summary of research findings
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PF – Post-flight
ATGC – Actual temperature ground control
NS - No significant difference

where we found that in-vitro ICAM-1 protein expression was increased after 12 days of space flight travel. The differences between these findings maybe due to the different models of these studies where the present study is
a cell culture in-vitro experiment whilst that of Mills et al. is an in-vivo human experiment. However the results of this present study is in agreement with that of Jung et al. (2005) where it was suggested that this findings may result from vascular site-specific alterations in adhesion molecules expression.

In this present study, VCAM-1 is not affected in the protein level after 12 days spaceflight travel. Reduction of VCAM-1 protein expression was detected in endothelial cells exposed to microgravity for 6-8 hours (Ludmila et al., 2005). In contrast, VCAM-1 protein expression was found to be elevated in endothelial cells taken from 3-weeks hindlimb unweighting simulated microgravity rat when compared to controls group. Data from this study suggested that the upregulation of VCAM-1 protein may contribute to impaired endothelium-dependent relaxation in simulated microgravity rat vasculature (Zhang et al., 2008). Maybe, in this present study, 12 days space flight travel was not sufficient enough to show effects in the protein level. However in the molecular levels, VCAM-1 expression increased in post-flight samples. Despite that, changes in molecular level strongly show that VCAM-1 expression was attenuated by spaceflight travel in this present study. However, a contradictory result was reported in an experiment conducted during a 13-day mission in Space Shuttle Endeavour (STS-118) study. In this particular study, it has been reported that VCAM-1 gene expression in lung samples taken from C57BL/6N mice housed in animal enclosure modules (FLT) was downregulated compared to controls (Tian et al., 2009). The difference of this result can be explained by different cells used in both experiments.

Soluble e-selectin was found to be decreased at post-flight in a human study involving 22 astronauts (Mills et al., 2002). In another study, e-selectin was decreased after 6-8 hours exposed to simulated microgravity in stimulated endothelial cells compared to controls (Ludmila et al., 2005). In this study, e-selectin protein expression in post-flight (PF) was found to be comparable with actual temperature ground control (ATGC). However, post-flight e-selectin protein expression was found to be elevated compared to ideal temperature. At the same time, e-selectin protein expression in ATGC was significantly higher compared to ideal temperature ground control (ITGC). Therefore it is suggested that maybe e-selectin protein expression can be affected by changes in temperature profile. Maybe e-selectin level is attenuated by temperature rather than microgravity and spaceflight.

In this study, it shows that IL-6 and ICAM-1 protein and gene expression of endothelial cells are increased by spaceflight travel. There was a positive correlation between IL-6 and ICAM-1 levels as reported by a group of investigators (Jacques et al., 2006). It explained why in this study, levels of both IL-6 and ICAM-1 are significantly increased.

NFκB is a pleiotropic transcription factor implicated in the regulation of diverse biological phenomena, including apoptosis, cell survival, cell growth, cell division, innate immunity, cellular differentiation, and the cellular responses to stress, hypoxia, stretch and ischemia. In response to physical and biological stresses it will up-regulate the expression of cytokines and adhesion molecules. In this study, we found that short travel of 12 days duration space flight increased the gene expression of NFκB in HUVEC. Similar findings were shown in previous studies where, increased NFκB transcription was observed in mice brain and cardiac muscle cell line exposed to microgravity for 7 days (Wise et al., 2005 & Kwon et al., 2009). In another study, simulated microgravity exposure for 12 days also increased NFκB transcription in mice testis (Sharma et al., 2008). Our present study has also clearly shown an increase in expression of IL-6, ICAM-1, VCAM-1 and e-selectin. Therefore it is suggested that, spaceflight travel upregulates NFκB transcription leading to the increment of IL-6, ICAM-1, VCAM-1 and e-selectin. Since the expression of NFκB gene expression is significantly higher than ground controls, it may be postulated that changes are possibly attributable to gravity. However given that the ground controls were not controlled for vibrational forces in addition of changes in gravity forces, we cannot exclude the possibly of the expression changes be in part attributed to vibrational forces and/or changes in gravity. In addition, the expression changes might be dampened as the cells readjusted to 1g in the first 24 hours upon landing.

eNOS gene expression in PF was comparable with ATGC. In another study, eNOS was found to be increased after 48 hours microgravity simulation in endothelial cells (Versari et al., 2007). However, Jasperse et al. (1999) showed that eNOS expression is decreased by 14 days of hindlimb unloading (HU) in the soleus feed arteries. No significant difference of eNOS protein expression was observed in rat’s aorta exposed to hind limb unloading for 20 days compared to controls (Vaziri et al., 2000). Therefore, it can be postulated that, spaceflight travel might give different effects on eNOS production by HUVEC.

The results of this study suggest that endothelial cells are prone to increased inflammation after short-term space flight travel by secreting cytokines and adhesion molecules which are responsible for the development of atherosclerotic plaques in blood vessel. This may lead to the increased risk of cardiovascular disease among astronauts. The spaceflight experiments reported here indicate the protein and molecular mechanisms of inflammation and endothelial activation which may provide useful information for space travel related diseases.

Results obtained from this study may have a potential to be utilized in the prevention of the atherogenesis leading to cardiovascular disease during space flight travel. Preventive action can be taken in order to slow down the atherosclerotic progression. Preventive anti-inflammatory
supplementation may be beneficial in inhibiting the production of cytokines and adhesion molecules in endothelial activation which in turn may prevent atherosclerosis related complications associated with space travel.

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