

## ORIGINAL ARTICLE

# Effects of prolonged isolation in a confined space on status of oxidative stress and prothrombogenesis: In preparation for possible future manned space expedition to Mars

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### Abstract

**Introduction:** Apart from inflammation and endothelial dysfunction, other key components in the development of atherogenesis include prothrombogenesis and oxidative stress. The effects of long-term confinement and isolation, exposure to radiation and different gravity forces during space travel could potentially increase the long-term risk of atherosclerosis. To the best of our knowledge, this is the first study determining the status of prothrombogenesis and oxidative stress in six cosmonauts subjected to the longest duration of confined isolation period of 520 days in preparation for prospective undetermined manned space travel to Mars. **Materials and Methods:** This collaborative research between the National Space Agency (ANGKASA), Universiti Teknologi MARA, Malaysia and Institute of Biomedical Problems (IBMP), Russia was conducted at the Russian Academy of Sciences IBMP, Moscow, Russia. Six multi-national cosmonauts were assigned to live in a ground-based confined module for 520 days. Standard exercise and diet regime were instituted throughout the isolation phase. Six age, ethnic and gender-matched healthy, free-living ground controls were recruited in parallel. Serial serum and whole blood were analysed for biomarkers of prothrombogenesis [plasminogen activator inhibitor-1 (PAI-1) and homocysteine] and oxidative stress [oxidised low-density lipoprotein (ox-LDL) and malondialdehyde (MDA)]. **Results:** There were significantly lower concentrations of PAI-1 and homocysteine in cosmonauts during confinement compared to the controls. There were no significant differences seen in the concentrations of biomarkers of oxidative stress during confinement but there was a significant percentage change increment for serum MDA in cosmonauts. **Conclusion:** Long-term confinement decreased the risk of prothrombogenesis and this could be attributed to the exercise and diet regime which includes omega-3 fatty acids supplementation given to the crew members during their confinement period. However, oxidative damage could not be excluded and may be attributed to the influence of psychological stress during this prolonged confinement.

**Keywords:** MARS-500, atherogenesis, atherosclerosis, prothrombogenesis, oxidative stress, coronary artery disease

## INTRODUCTION

It has been described that patients with illness are those who live in a normal environment, but have abnormal physiology within them,<sup>1</sup> whilst cosmonauts are humans with normal physiology who are affected by the complex combination of factors during spaceflight, therefore the human body may not necessarily be able to adapt to these changes. These changes have been

observed on whole human organisms and on different physiological systems of cosmonauts following completion of spaceflight that have had a negative impact on their health.<sup>2</sup> With the establishment of the International Space Station (ISS), a permanent space habitat in Low Earth Orbit (LEO) human exploratory missions beyond LEO may be the next step in the human desire to conquer the outer limits of habitability.<sup>3</sup> The

operational necessities of space missions require clinical knowledge which has to be continually updated to ensure proper medical policies and standards are incorporated in a timely manner into space medicine practice.<sup>4</sup>

Mars, the fourth planet from the sun, is less massive than its two neighbours, Jupiter and Earth. The surface of Mars is cold and dry, and any liquid water will very quickly freeze, evaporate or sublime into the atmosphere.<sup>5</sup> Mars is currently considered as a major target in the search for life beyond Earth since it is the only planet located within the habitable zone of our solar system.<sup>6</sup> Besides the human desire to extend the window of habitability, human exploratory missions are driven by several aspects of science, technology, culture and economy. These missions beyond LEO will add a new dimension to human space travel with regards to travel distance, duration of the mission, radiation exposure, gravity levels and the level of isolation and confinement the crew will be exposed to.<sup>7</sup> This has led to the Mars-500 mission, which was an experiment conducted between 2007 and 2011 by the Russian, European and China Space Agencies, in preparation for an unspecified future manned spaceflight to Mars. The experiment's facility is located at the Russian Academy of Sciences Institute of Biomedical Problems (IBMP) in Moscow, Russia.<sup>8</sup>

Malaysia, through the National Space Agency (ANGKASA) has been invited by the IBMP, Russia to participate in their MARS500 scientific programme. The effects of long-term isolation and confinement can be more appropriately addressed via ground-based simulations.<sup>9</sup> A total of 640 experiment days were scheduled which were, divided into three stages of differing duration. During each stage, the crew of volunteers lived and worked in a mock-up spacecraft.<sup>10</sup> The final stage of the experiment, which was intended to simulate a full-length manned mission (520 days) to Mars, began on 3 June 2010 and ended on 4 November 2011. This stage was conducted by an international crew consisting of three Russians, a French, an Italian and a Chinese citizen. During their simulated mission, the crew lived in isolation without fresh food, sunlight or fresh air. Every detail was planned; including diet, daily activities and sleep.<sup>10</sup> This may raise significant health issues especially in the formation of atherosclerosis when the cosmonauts are faced with stressors such as high radiation and various gravity forces as well as psychological stress.

This type of ground-based simulation can mimic the effect of long-term confinement and isolation which has been suggested to induce inflammatory responses and endothelial dysfunction primarily from psychological stress, reduced physical activity and diminished nutritional standards.<sup>11,12</sup> This can result in an increased long-term risk of atherosclerosis in cosmonauts. Apart from inflammation and endothelial dysfunction, prothrombogenesis and oxidative stress are also important in the pathogenesis of atherosclerosis.<sup>13</sup> Increases in homocysteine and plasminogen activator inhibitor-1 (PAI-1) concentrations contribute to a more prothrombotic status in the atherosclerotic lesion. PAI-1 represents an extremely promising marker which is overexpressed in the vascular wall adjacent to an arterial thrombus induced by mechanical injuries.<sup>14</sup> Malondialdehyde (MDA) is an end-product of the radical-initiated decomposition of polyunsaturated fatty acids; therefore, it is frequently used as a biomarker of oxidative stress.<sup>15</sup> MDA's reaction with lysine residues generates lysine-lysine cross-links which have been identified in apolipoprotein B (apoB) fractions of oxidised low-density lipoprotein (ox-LDL), and have been postulated to impair the interaction between ox-LDL and macrophages and thereby promote atherosclerosis.<sup>16</sup> Should the status of these pro-atherosclerotic factors be enhanced during prolonged confinement and space travel, specific countermeasures should be instituted to prevent these issues to ensure a safe outcome of cosmonauts during their space expeditions.

To date, there have not been extensive studies examining the effects of long-term isolation under either 1 g facility or actual space travel on *in vivo* prothrombogenesis and oxidative stress. Furthermore, to the best of our knowledge, the longest Earth-based space flight simulation record for continuous confinement was set by Valery Polykov at 437 days.<sup>17</sup> Determining the effects of prolonged living in confined isolation under 1 g environment on these biomarkers will be a pilot and preliminary investigation before a tentative similar study is performed in the actual MARS-500 under microgravity environment. Therefore, the potential changes within the vascular system during extensive prolonged isolation of 520 days remain to be elucidated in order to understand the underlying cellular and metabolic consequence of confinement with regards to these changes in preparation for a possible manned space travel to Mars.

Hence, the objective of this study was to determine the status of prothrombogenesis and oxidative stress status in six cosmonauts subjected to a long duration of ground-based confined isolation period of 520 days in preparation for prospective undetermined manned space travel to Mars.

## MATERIALS AND METHODS

### Subjects recruitment

This study was conducted at the Russian Academy of Sciences IBMP, Moscow, Russia. Six male crew members consisting of three Russians, an Italian, a Frenchman and a Chinese citizen were recruited and subjected to confined space, simulating actual estimated flight travel to Mars.<sup>18</sup> The timeline took into account 250 days for travel to Mars, 30 days sojourn on Mars surface and 240 days for the return flight to earth. The simulation was performed under 1 g (Gravity of Earth).

Six age-, ethnic- and gender-matched and free-living (following a way of life in which they were freely indulged the appetites, desires, etc.), healthy controls is based in Malaysia were recruited which consisted of four Russians and two Chinese males. Inclusion criteria for the control subjects included 1) normotensive (BP <140/90 mmHg), 2) normoglycaemic (fasting plasma glucose <6.0 mmol/L), 3) normal lipid profile [total cholesterol (TC) <5.2 mmol/l, low density lipoprotein cholesterol (LDL-c) <3.4 mmol/L, high density lipoprotein cholesterol (HDL-c) >1.0 mmol/l and triglyceride (TG) <1.7 mmol/L], and 4) non-smoking.

Institutional ethics committee approval was obtained prior to commencement of the study [IBMP Committee on Bioethics (Protocol No. 269 of 06.05.2010, Protocol No. 277 of 12.01.2011, Protocol No. 278 of 14.01.2011) and UiTM Research Ethics Committee (600-RMI (5/1/6)]. The cosmonauts were carefully screened so as to be in fit physical, mental and emotional health, having a healthy lifestyle, normotensive, normoglycaemic, normolipaemic, non-smoker and lean with a body mass index (BMI) below than 25 kg/m<sup>2</sup>.<sup>19</sup> All subjects were given informed consent before prior to commencement of the study.

### Diet regime

The developed diet regimes used in the experiment were based on the content necessary for humans and complied with the accepted

physiological norms for contingents, whose professional activity on energy inputs refers to the category of medium gravity. Food composition of the rations complies with the recommendations of the World Health Organization (WHO),<sup>20</sup> and also agreed with Russian-American norms on the food composition of rations for the crews of ISS.

The subjects were given three diet variations, assigned as Variants 1 to 3. These variants were prepared to cater for the following: 1) Variant 1: during the time of flight from Earth to Mars, 2) Variant 2: during simulation of egress of the three crew members to the surface of the planet, 3) Variant 3: during return to Earth (Table 1). Omega-3 fatty acid supplementation (3 g/day eicosapentaenoic acid + docosahexaenoic acid supplementation compared to placebo groups, double-blind crossover study) was given in the first 250 days of the experiment.

### Exercise programme

The entire 520 days of the experiment was divided into several stages, during which different protocols of physical training were implemented. The chosen training regime provided in conditions of isolation preservation at a high enough level of physical working capacity, that was assessed on the indices of physiological and ergometric loading. The crew was divided into three groups and the training regimes were alternated. During the first stage, different protocols of training which included strength training, expanders and vibration training were executed.

The second stage of training involved the efficiency assessment by locomotor training in active and passive regimes on treadmill and trainings on cyclo-ergometer. The third and final stage of training involved physiological and ergometric loading.

### Blood collection

A week before confinement, fasting blood samples were taken for baseline as an indicator for the pre-isolation period. Subsequent fasting blood samples were taken at day 30 post confinement and two-monthly for the second half of isolation time with a total number of nine samples taken during the intra-isolation period, and one sample at post-isolation period. Blood was taken from the median cubital, basilica or cephalic veins by applying an aseptic technique. Fasting venous blood samples were also collected. Plasma and serum were separated from blood by centrifugation at 3,000 rpm at

**TABLE 1: Diet Variations for Crew Members of Mars-500 Mission**

	<b>VARIANT 1 DIET (g)</b> (Percentage of total diet, %)	<b>VARIANT 2 DIET (g)</b> (Percentage of total diet, %)	<b>VARIANT 3 DIET (g)</b> (Percentage of total diet, %)
	1-250 Days (All crews)	251-269 Days (Crews 5003, 5005, 5006)	I 251-520 Days (Crews 5001, 5002, 5004) II 270-520 Days (Crews 5003, 5005, 5006)
Protein	106.9 (14)	138.2 (17)	112.9 (14.3)
Fat	115.8 (33.2)	126.8 (35)	110.9 (31.9)
Carbohydrate	402.2 (52.8)	370.8 (47)	419.5 (53.7)
Average calorie intake (kCal)	3120	3170	3130

4°C and stored frozen at -80°C until analysis. Samples from Moscow were transported back to Malaysia in a dry-ice box.

**Sample processing**

Blood samples collected in ethylenediaminetetraacetic acid (EDTA), sodium citrate and plain tubes were centrifuged at 4000 rpm for 10 minutes to separate the plasma and serum for biochemical analysis. The plasma and serum were transferred into the appropriate 1.5 mL tubes and labelled accordingly. Plasma obtained from EDTA tubes were added with 950 µL of butylated hydroxytoluene (BHT) solution used primarily as an antioxidant additive before storage.

**Biochemical assay**

The ELISA assay kits for oxidative stress parameter, Ox-LDL (MercoDIA, Germany) and the prothrombogenic markers, PAI-1 and tissue plasminogen activator (tPA) (eBioscience, Austria) were purchased to detect biomarker levels in the serum samples. The kits were used according to the manufacturer’s instruction and achieved optical density (OD) was tabulated and analysed using a data elaboration software. MDA was measured by a method adopted from Ledwozyw, Michalak, Stepien & Kadziolka (1986) and homocysteine by immunoassay method (Immulite, Germany).

**Statistical analysis**

Data were analysed using the SPSS statistical package programme version 22.0. The continuous

variables were expressed as median (95% confidence interval [CI]). Statistical analysis was performed using Kruskal-Wallis (parameter being the effect of confinement) and Chi-squared test.

**RESULTS**

**Demographic data**

Table 2 summarises the demographic data of the cosmonauts and free-living normal controls. Both groups were non-smokers, normotensive with normal lipid profiles, and they were matched for age, gender, ethnicity, smoking status, BMI and blood pressure.

Table 3 summarises the serum concentration of biomarkers of prothrombogenesis (PAI-1 and homocystein) and oxidative stress (ox-LDL and MDA). There were significantly lower concentrations of PAI-1 and homocysteine in cosmonauts in the intra compared to pre-isolation period. There was also a significant increment of MDA at 280 days and significant reduction in post-isolation, while no change of ox-LDL concentration was observed in both cosmonauts and control groups when compared to their respective pre-isolation concentrations. None of the biomarkers was significantly different between the cosmonaut and control groups during pre-, intra- and post-isolation.

**Changes of prothrombogenesis and oxidative stress biomarkers**

Table 4 depicts the percentage changes of

TABLE 2. Demographic Data of the Cosmonauts and Free-Living Controls

Parameters	Cosmonauts (n=6)	Controls (n=6)	p-value
<sup>a</sup> Age (years)	31.8 ± 4.8	34.8 ± 7.1	0.419
<sup>b</sup> Gender	6 Males	6 Males	-
<sup>b</sup> Ethnicity (Russian/European/Chinese)	3/2/1	4/0/2	0.174
<sup>b</sup> Current smoker (Yes/No)	0/6	0/6	-
<sup>a</sup> BMI (kg/m <sup>2</sup> )	26.4 ± 2.5	24 ± 2.2	0.109
<sup>a</sup> SBP (mmHg)	118.5 ± 9.7	117.4 ± 8.3	0.212
<sup>a</sup> DBP (mmHg)	75.5 ± 9.8	74.1 ± 6.8	0.462

<sup>a</sup>Data expressed as mean±SD, <sup>b</sup>Data expressed as proportion, *p* <0.05 is statistically significant.

the prothrombogenesis and oxidative stress biomarkers in cosmonauts and normal free-living controls. There was a significant percentage reduction for serum PAI-1 in cosmonauts compared to controls from day 90 of isolation and beyond (when compared to pre-isolation period). There was significant percentage change reduction for serum homocysteine in cosmonauts compared to controls on days 90, 150, 280 and 520 of isolation. There was no significant within-group or between-group differences in percentage change for serum TPA and ox-LDL.

There were percentage reductions for serum ox-LDL in cosmonauts compared to controls from pre-isolation to post-isolation period. However, these changes were not statistically significant. There was a significant percentage change increment of serum MDA in cosmonauts compared to controls on day 280 of isolation. However, there was a slight percentage change reduction post-isolation phase but this did not achieve statistical significance.

## DISCUSSION

The main findings of this study showed significant reductions in the biomarkers of prothrombogenesis particularly PAI-1 and homocysteine among the cosmonauts during long-term confinement. However, mixed results were seen in the biomarkers of oxidative stress where ox-LDL did not show significant change but MDA showed significant percentage increment among the cosmonauts during the confinement period. The increased MDA level could possibly be due to the stress of the confinement which led to increases of lipid peroxidation and altered lipid profile, which may be responsible for the pathophysiology

of cardiovascular, neurological and various diseases.<sup>21,22</sup>

Prolonged confinement and space travel have been suggested to increase the long-term risk of atherosclerosis in cosmonauts by inducing inflammatory responses, endothelial dysfunction, prothrombogenesis and oxidative stress, primarily from exposure to radiation and different gravity levels, psychological stress, reduced physical activity and diminished nutrition intake.<sup>23,24</sup> In particular, increased risk of prothrombogenesis can be shown through the elevation of PAI-1 and homocysteine levels which can increase the risk of atherothrombotic events and promote the progression of vascular disease.<sup>25</sup> PAI-1 is a serine protease inhibitor protein produced by vascular endothelium. It functions as the principal inhibitor of tPA and urokinase (uPA) and hence is an inhibitor of fibrinolysis.<sup>26</sup> Elevated PAI-1 level will thus predispose patients to thrombosis and atherosclerosis.<sup>27</sup>

On the other hand, oxidative damage can occur when there is excessive reactive oxygen species generated *in vivo* causing lipid peroxidation, measured by analysing the oxidative stress biomarkers such as MDA and ox-LDL.<sup>28,29</sup> A significant increase in the activity of glucose-6-phosphate dehydrogenase and superoxide dismutase and a reduction in catalase activity were found in Mars-105 experiment. These indicate the imbalance between the amount of reactive oxygen species generation and antioxidant protection mechanisms in cells observed during the 105-days isolation, due to the body's adaptation to stresses experienced during and after the experiment.<sup>30</sup> MDA is generated *in vivo* via peroxidation of polyunsaturated fatty acids. MDA interacts with proteins and is

**TABLE 3: Serum Concentration of Biomarkers of Prothrombogenesis and Oxidative Stress**

Variables	Pre	30	90	150	280	390	520	Post
<b>Cosmonauts</b>								
PAI-1 (pg/mL)	338 (16)	<b>145 (76)*</b>	<b>140 (36)**</b>	<b>145 (13)**</b>	<b>138 (26)**</b>	<b>129 (28)**</b>	<b>112 (48)**</b>	<b>113 (15)**</b>
tPA (pg/mL)	3830 (633)	4145 (1697)	3074 (1812)	4082 (669)	4904 (2357)	3330 (2946)	3090 (1159)	2816 (2241)
Homocysteine (g/L)	10.68 (3.25)	9.26 (1.01)	8.44 (2.88)	<b>7.58 (0.92)*</b>	7.99 (1.9)	8.34 (2.86)	7.91 (0.58)	7.92 (2.02)
Ox-LDL (mU/L)	72538 (23892)	55423 (26137)	66227 (7662)	55751 (12287)	64454 (6965)	58141 (10186)	65575 (10276)	57257 (16038)
MDA (nmol/mL)	1.38 (0.18)	1.17 (0.23)	1.29 (0.18)	1.18 (0.18)	<b>1.51(0.06)*</b>	1.49 (0.58)	1.28 (0.36)	<b>1.07 (0.21)*</b>
<b>Controls</b>								
PAI-1 (pg/mL)	58 (49)	107 (53)	104 (27)	<b>98 (28)*</b>	92 (12)	<b>111 (50)*</b>	<b>112 (94)*</b>	99 (39)
tPA (pg/mL)	1724 (670)	1904 (459)	2045 (813)	2109 (1236)	2479 (1324)	2151 (1099)	2356 (2224)	2677 (1510)
Homocysteine (g/L)	9.93 (2.84)	10.3 (2.52)	10.25 (3.51)	10.85 (2.3)	11.40 (2.41)	9.37 (1.80)	11 (1.73)	9.86 (2.76)
Ox-LDL (mU/L)	43439 (4848)	42914 (1476)	45602 (14123)	43908 (9420)	42435 (13860)	44579 (12839)	50794 (13141)	47602 (8949)
MDA (nmol/mL)	0.95 (0.24)	0.84 (0.25)	0.87 (0.1)	0.78 (0.02)	0.78 (0.02)	0.83 (0.17)	0.82 (0.1)	0.93 (0.13)

Data are presented as median [interquartile range, (IQR)]. \**p*<0.05 compared to baseline.

Table 4. Percentage Changes of Biomarkers of Prothrombogenesis and Oxidative Stress

Variables	30	90	150	280	390	520	Post
Cosmonauts							
PAI-1 (pg/mL)	-3.43 (0.02)	<b>-13.72 (0.03)*</b>	<b>-8.37 (0.02)**</b>	<b>-13.72 (0.03)**</b>	<b>-13.72 (0.03)**</b>	<b>0 (0.01)**</b>	<b>-5.44 (0.02)**</b>
tPA (pg/mL)	-2.65 (37.14)	-24.15 (42.76)	-2.88 (42.97)	10.5 (58.09)	1.82 (81.06)	-18.35 (64.49)	-12.72 (65.07)
Homocystein (g/L)	-5.37 (28.36)	<b>-11.51 (11.03)*</b>	<b>-29.64 (21.45)**</b>	<b>-20.73 (9)**</b>	-27.12 (14.63)	<b>-27.98 (24.8)*</b>	-25.63 (36.47)
Ox-LDL (mU/L)	-3.68 (71)	-1.40 (25.15)	-21.57 (9.39)	-4.39 (10.25)	-13.91 (21.98)	-8.11 (36.33)	-1.32 (29.72)
MDA (nmol/mL)	-2.33 (19.28)	0.67 (21.95)	-16.19 (13.42)	<b>18.43 (36.08)*</b>	19.33 (38.95)	5.42 (30.10)	-25.77 (11.82)
Controls							
PAI-1 (pg/mL)	-10.6 (86.28)	5.21 (22.45)	21.48 (78.72)	2.89 (117.48)	-7.27 (113.46)	10.62 (45.47)	12.95 (66.49)
tPA (pg/mL)	14.6 (16.73)	15.7 (13.16)	13.35 (36.32)	20.53 (34.75)	20.82 (63.05)	32.10 (166.09)	24.59 (71.34)
Homocystein (g/L)	4.85 (7.76)	9.91 (23.49)	14.86 (14.61)	20.53 (15.90)	-2.99 (2.66)	21.16 (22.66)	10.48 (28.60)
Ox-LDL (mU/L)	3.42 (15.22)	9.43 (13.29)	4.80 (22)	2.24 (11.2)	8.03 (18.43)	35.30 (51.65)	8.43 (45.57)
MDA (nmol/ml)	-6.12 (5.46)	-8.98 (20.97)	-8.64 (6)	-17.86 (12.42)	-13.47 (10.53)	-16.62 (14.85)	3.87 (35.53)

Data are presented as median [interquartile range, (IQR)]. \* $p < 0.05$  and \*\* $p < 0.01$  compared to controls.

itself potentially atherogenic. MDA's reaction with lysine residues generates lysine-lysine cross-links, which have been identified in apoB fractions of ox-LDL and have been postulated to impair the interaction between ox-LDL and macrophages, thereby promoting atherosclerosis.<sup>31</sup> Ox-LDL formation occurs primarily within vascular walls where it is taken up by macrophages via scavenger receptor pathways to form foam cells. Accumulation of ox-LDL within the vascular walls also stimulates the overlying endothelial cells to produce proinflammatory cytokines including adhesion molecules such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and endothelial selectin.<sup>32</sup> Thus, increasing ox-LDL levels correlate with increasing severity of CVD disease.

Unexpectedly, our study has shown improvement in the prothrombogenesis markers in cosmonauts compared to normal free-living controls during prolonged confinement. The possible explanation for the reduction of PAI-1 and homocysteine levels could be due to strict diet and exercise programmes instituted during the confinement period. Exercise and diet-induced weight loss have been shown to improve endothelial function associated with reduction in blood markers of vasoconstriction or inflammation. The exercise component of our intervention was therefore, at least in part, likely responsible for the improved endothelial function we observed.<sup>32</sup> The reduction seen for PAI-1 could be attributed to the moderate intensity exercise which induces favourable changes in lowering lipid profile and thus reducing the risk of cardiovascular diseases.<sup>33</sup> In fact, the reduction in PAI-1 could also be linked with omega-3 polyunsaturated fatty acid (PUFA) intake. A study has shown that omega-3 PUFA intake reduces PAI-1 levels without changing the TPA antigen. These observations may be related to a decrease in thrombotic activity upon consumption of large amounts of fish or fish-derived products.<sup>34</sup> Moreover, omega-3 has been shown to significantly decrease the inflammatory [C-reactive protein (CRP), interleukin-6 (IL-6)], oxidative stress [MDA and glutathione peroxidase (GPx)] markers.<sup>35</sup> A healthy diet consisting of protein and vegetables, moderate consumption of alcohol and low consumption of dairy products and meat has been shown to reduce endothelial dysfunction and low-grade inflammation in adults at risk of cardiovascular disease,<sup>36</sup> but the underlying mechanisms remain unclear.

Despite the reduction of prothrombogenesis biomarkers which may be attributed to regular exercise and diet-induced weight loss, our study has shown that the oxidative stress biomarkers, in particular ox-LDL did not show significant change and MDA showed significant percentage increment at 280 days but no significant increment in cosmonauts in pre- and intra-isolation period. In addition, MDA also showed significantly increased the MDA level at 280 days and post-isolation period. This can possibly be explained by the fact that physical exercise has been known to cause free radical-mediated tissue damage.<sup>37</sup> Although regular physical exercise has many health benefits on the long run including reducing the risk of cardiovascular disease, diabetes and many others,<sup>38</sup> paradoxically, prolonged and intense exercise during that period of intra isolation can result in oxidative damage to cellular constituents as contracting skeletal muscles are able to generate free radicals.<sup>39</sup> This could possibly explain the transient significant elevation of serum MDA at that time point.

## CONCLUSION

Long-term confinement in ground isolation facility up to 520 days decreases the risk of prothrombogenesis and this could be attributed to exercise and diet regime which includes omega-3 fatty acids supplementation given to the crew members during their confinement period. However, oxidative damage could not be excluded and may be attributed to the influences of psychological stress during prolonged confinement.

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## Declaration of Conflicting Interests

The authors declare that there is no conflict of interest or direct financial relation with respect to the commercial identities mentioned in this paper.

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