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Effects of Prolonged Confined Isolation on Status of Inflammation, Endothelial Activation and Function: In Preparation for Possible Future Manned Space Expedition to Mars

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ABSTRACT

Inflammation and endothelial dysfunction are key components in atherogenesis. Should the status of these pro-atherogenesis factors be enhanced during prolonged confined space travel, specific countermeasures need to be instituted to prevent these processes to ensure safe outcome for astronauts during space expeditions. Six crew members were exposed to prolonged, confined isolation for 520 days. Standard exercise and diet regime were instituted throughout isolation phase. Age and gender-matched healthy, free living controls were recruited in parallel. Serial serum and whole blood were analysed for biomarkers of inflammation (hsCRP and IL-6) and endothelial activation (sICAM-1, sVCAM-1 and E-selectin). Flow-mediated dilatation (FMD) of the artery was performed following the standard protocols set by the International Brachial Artery Reactivity Task Force by trained personnel. There was decreased sVCAM-1 concentration in crew members compared to baseline. However, there was significant decrease in percentage dilatation from baseline in FMD of the brachial artery in the crew members. Percent change increment was observed in hsCRP while percent change reduction was seen in sVCAM-1. The enhanced inflammation and reduced endothelial function could

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possibly be attributed to the rigorous exercise instituted throughout the confinement period. Furthermore, possible haemoconcentration as a result of psychosocial stress and/or exercise-induced physiological response could further explain elevations in hsCRP, and unlikely pathological. Furthermore, endothelial activation was attenuated during isolation, suggesting that the diet and exercise program instated throughout the period improved endothelial function.

Keywords: Atherogenesis, coronary artery disease, cosmonauts, endothelial activation, endothelial function, inflammation, MARS500

INTRODUCTION

It has been described that patients with illness are those who live in a normal environment but have abnormal physiology. In contrast, cosmonauts are humans with normal physiology who live in an abnormal environment. It is this abnormal environment in space that causes unique and significant alterations to a cosmonaut's physiology that could have a negative impact on their health. The direction of research into space, the final frontier, has shifted from developing ways and means to get there, to currently pushing the boundaries of understanding neighbouring planets and discovering potential resources. The current approach of space programs to stretch space exploration farther away from earth, thus extending durations of expeditions from months to years, will challenge the current capabilities of space medicine.

Mars is the fourth planet from the sun and the second smallest planet in our Solar System. Named after the Roman god of war, it is often described as the 'Red Planet' due to the iron oxide atmosphere giving its surface a reddish appearance (Zubrin & Wagner, 2011; Rees, 2012). Mars is a terrestrial planet with a thin atmosphere, having surface features reminiscent of the craters of the moon and the volcanoes, valleys, deserts and polar ice caps of earth. Over the years, there have been increasing interest to further explore the surface of mars, The Mars-500 mission was a psychosocial isolation experiment conducted between 2007 and 2011 by Russia, the European Space Agency and China, in preparation for an unspecified future manned spaceflight to the planet. The experiment's facility was located at the Russian Academy of Sciences' Institute of Biomedical Problems (IBMP) Moscow, Russia (Basner et al., 2014).

The experiment was designed to allow planning of the methods and means of control and monitoring of the habitat during lengthy crew stays in confined and cramped conditions. During the program, three different crews of volunteers lived and worked in a simulated spacecraft. The final stage of the experiment, which intended to simulate a 520-day manned mission, was conducted by a crew consisting of three Russians, a Frenchman, an Italian and a Chinese citizen. This form of simulation training may have profound impact on the

psychological and physiological aspect of an individual which include decreased physical activity, suboptimal nutrition, sleep deprivation, fatigue and increased stress levels.

Long term confinement and microgravity environment have been suggested to induce inflammatory responses and modulate immune functions that may increase oxidative stress (Arbeille et al., 2014). Inflammation, oxidative stress and endothelial dysfunction are important in the pathogenesis of atherosclerosis and the biomarkers reflecting these processes have been associated with increased risk for developing coronary artery disease (CAD). It has also been established that prolonged isolation can lead to psychological stress which can have an impact on biomarkers of inflammation, endothelial activation and oxidative stress. A meta-analysis of thirty studies determining the effects of psychological stress on inflammatory biomarkers reported increased concentrations of C-reactive protein (CRP), interleukin-1 (IL-1) and interleukin-6 (IL-6) concentrations following psychological stress (Steptoe et al., 2007). Should the status of these pro-atherogenesis factors be enhanced during prolonged confinement and space travel, specific countermeasures need to be instituted in order to prevent these processes to ensure safe outcome for astronauts during their space expeditions.

There have not been extensive studies examining the effects of long term isolation under either 1g facility, simulated microgravity environment or actual space travel on *in vivo* inflammation, endothelial activation and oxidative stress. Furthermore, the few human experimentations on prolonged isolation have never exceeded a 500 day mark. To the best of our knowledge, the longest reported timeline of prolonged confinement was 438 days (Harvey & Zakutnyaya, 2011). 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 flight to the red planet. This study aimed to determine the effects of prolonged isolation under a 1*g* environment on mechanisms of atherogenesis, namely inflammation, endothelial activation, oxidative stress and thrombogenesis.

METHODS

Subjects Recruitment

The MARS500 mission was conducted between 2007 and 2011 by Russia, the European Space Agency and China, in preparation for an unspecified future manned spaceflight to the planet Mars (Basner et al., 2014). The final stage of the experiment was to isolate a group of crew members in a confined space mimicking the size and structure of a space shuttle for a period of 520 days. Six male crew members consisting of three Russians, an Italian, a Frenchman and a Chinese citizen were recruited and subjected to confinement,

simulating actual estimated flight travel to Mars. The timeline took into account 250 days for the travel to Mars, 30 days sojourn on Mars surface and 240 days for return flight to earth. Simulation was performed under 1*g*. The crew members were carefully screened to be in perfect physical, mental and emotional health, having a healthy lifestyle, non-smoker and lean with a body mass index (BMI) below than 25 kg/m2. As for controls, six free-living, healthy Malaysian-based controls were recruited which consisted of four Russians and two Chinese males. Inclusion criteria for the controls includes normotensive blood pressure (BP<140/90mmHg), normoglycaemic (fasting plasma glucose <6.0 mmol/L), 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 mm0l/L] and non-smoking. This study was reviewed and approved by Universiti Teknologi MARA (UiTM) Research Ethics Committee prior to commencement of the study [ref: 600-RMI (5/1/6)] on 1 September 2010. All subjects were given written informed consent before participating in the study.

The developed diet regimes for the crew members used in the experiment were based on the content necessary for the human 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), and also agreed upon Russian-American norms on the food composition of food rations for the crews of ISS. The subjects were given three diet variations assigned as Variants 1 to 3 (Table 1). These variants were prepared to cater for the following: 1) Variant 1: for the time of flight from Earth to Mars, 2) Variant 2: during simulation of 3 crew members' egress to the surface of the planet, 3) Variant 3: during return to Earth. It is also worth noting that the controls recruited were not standardized for diet or exercise as they were included specifically to compare between a 'free-living' state to compare with the confined living conditions and strict diet and exercise regimes given to the crew members as a preparatory protocol for possible manned mission to mars.

Table 1
The protein, fat, carbohydrate and calorie content of the 3 dietary variants given to the crew members during their isolated confinement period

Variant Diet	Variant 1	Variant 2	Variant 3
Protein [g (%)]	106.9 (14)	138.2 (17)	112.9 (14.3)
Fat [g (%)]	115.8 (33.2)	126.8 (35)	110.9 (31.9)
Carbohydrate [g (%)]	402.2 (52.8)	370.8 (47)	419.5 (53.7)
Average Calorie intake (kCal)	3120	3170	3130

The entire 520 days of the experiment, the crew members were undergoing their routine exercise programme. The programme was divided into several stages, during which different protocols of physical trainings were implemented. The crew was divided into 3

groups and the training regimes alternated. During the first stage, different protocols of trainings which included strength training, expanders and vibro training were executed. The second stage of training was involved efficiency assessment by way of locomotor trainings in active and passive regimes on the treadmill and trainings on cyclo-ergometer. The third and final stage of training was involved physiological and ergometric loading.

Procedure

A week before confinement, blood was taken for baseline as indicator for pre-isolation period. One sample was taken at day 30 after confinement start and two-monthly for second half of isolation time with nine total numbers of samples was taken during intra-isolation period and 1 sample at post-isolation period. Blood were taken from the median cubital, basilica or cephalic veins by applying aseptic technique. Fasting venous blood samples were collected. Plasma and serum were separated from blood by centrifugation at 2000 x *g* in 4^{o}C and was stored frozen at -80^{o}C until analysis. Samples from Moscow were transported back to Malaysia in dry ice box.

In order to process the blood, 2 EDTA tubes were exception from centrifugation process. This process was required to separate the plasma and serum for biochemical analysis. The remaining tubes were placed in a centrifuge at speed of four 2000 x *g* and spun for 10 minutes. Then, the plasma and serum were transferred into the appropriate 1.5 ml tubes and labelled accordingly. One millilitre (1 ml) of Plasma-EDTA were transferred into 2 purple microcentrifuge tube each and 950 µl were aliquot into 2 black microcentrifuge containing 50 µl butylated hydroxytoluene (BHT) solution that was used as primarily antioxidant additive. One millilitre (1 ml) from two red top plain tubes' plasma were then transferred into 5 reddish orange microcentrifuge tubes each and 0.65 µl plasma-citrate which aliquot 1:3 from the top of plasma were transferred into 2 blue colour microcentrifuge tubes.

Plasma from EDTA tube was tested for fasting serum lipid profiles and high-sensitive C-reactive protein (hsCRP) using automated analyzer, Cobas Integra 400, Roche Diagnostics, USA. Homocysteine levels also tested using the same plasma by automated analyzer, Immulite 1000, Siemens Immunoassay Analyzer, German. Enzyme-linked immunosorbent Assay (ELISA) was carried out using serum from plain tube. This assay was used to detect protein expression in the serum for Interleukin-6 (IL-6), Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Molecule-1 (VCAM-1), E-selectin, and Endothelial Nitric Oxide Synthase (eNOS). All ELISA method used the similar 'sandwich' principle and absorbance reading was done using a microplate reader (TECAN Safire 2, Switzerland)

Flow Mediated Dilatation of the Brachial Artery (FMD) of the brachial artery was measured based on the International Brachial Artery Reactivity Task Force (Corretti et al., 2002). Serial FMD readings were done for the crew members on days -7, 30, 90, 150,

210, 270, 330, 390, 450, +7 of isolation and ground controls on days -14, -7, 30, 150, 280, 390, 520 and +14 of isolation. Subjects were fasted at least 8 hours before the study and FMD measured in a quiet, temperature-controlled room. FMD was divided into two stages as follows: (1) Stage 1 (Endothelium independent FMD). The subject was in a supine position and left arm stretched in a comfortable position, for the imaging of the brachial artery. Brachial artery was imaged above the antecubital fossa in the longitudinal plane. A segment with clear anterior and posterior intimal surfaces between the lumen and vessel wall was selected for continuous 2D imaging and this was recorded as baseline resting image. (2) Stage 2 (Endothelium dependent FMD). A sphygmomanometric cuff was placed either above the antecubital fossa or the forearm to create a flow stimulus. Cuff inflation to suprasystolic pressure (at least 50 mmHg above systolic pressure) occluded the artery for 5 minutes. Cuff deflation was then done to induce brief high flow state (reactive hyperaemia) and the vessel diameter was measured at 60 seconds post deflation. In normal individuals, post occlusion of the brachial artery would dilate to at least 10%. Subjects with endothelial dysfunction such as hyperglycaemia, hypertension, and hyperlipidaemia will cause insult to the endothelial function therefore will have less dilatation post occlusion.

The continuous variables were expressed as median $\pm$ interquartile range. Comparison between 2 groups was determined either by Mann-Whitney U tests. Wilcoxon tests were used to determine the effects of long term confinement of biomarkers between isolation and baseline. The criterion for statistical significance was p value < 0.05. Data was analysed by a statistical package program (SPSS) version 20.0.

RESULTS

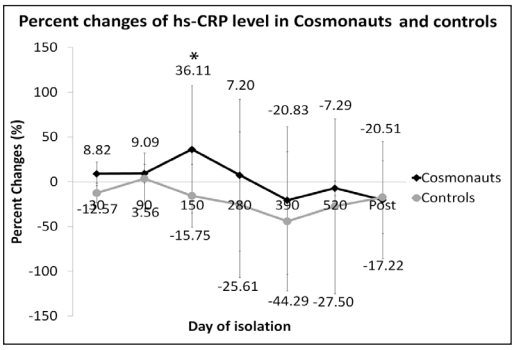
Table 2 summarizes the demographic data of the crew members and free-living normal controls. Both groups were non-smokers, normotensive with normal lipid profile, and they were matched for age, gender, smoking status, BMI and blood pressure. It is also worth noting that there was no significant difference in ethnicity between crew members and controls.

Table 2
Demographic data of the crew members and controls

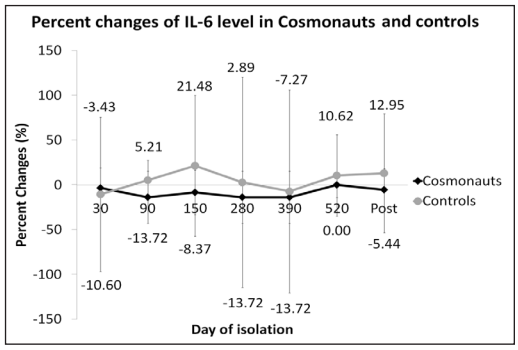
Parameters	Crew Members (n=6)	Controls (n=6)	p value
[a]Age (years)	31.8 $\pm$ 4.8	34.8 ± 7.1	NS
[b]Gender	6 Males	6 Males	NS
[b]Ethnicity (Russian/European/Chinese)	3/2/1	4/0/2	NS
[b]Current smoker (Yes/No)	0/6	0/6	NS
[a] BMI (kg/m^2)	26.4 $\pm$ 2.5	24.0 ± 2.2	NS
[a] SBP (mmHg)	118.5 $\pm$ 9.7	117.4 ± 8.3	NS
[a] DBP (mmHg)	75.5 $\pm$ 9.8	74.1 ± 6.8	NS

Note. [a]data expressed as mean$\pm$SD; [b]data expressed as proportion; NS not significant

Figures 1(a), 1(b), 2(a), 2(b), 2(c) and 3 depict the percent change of the biomarkers in crew members and controls. There was a higher percent change increment for serum hsCRP concentration in crew members compared to controls on day 150 isolation. There was significant percent change reduction in serum Soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1) concentration on days 90 and 150 and E-selectin concentration on days 150

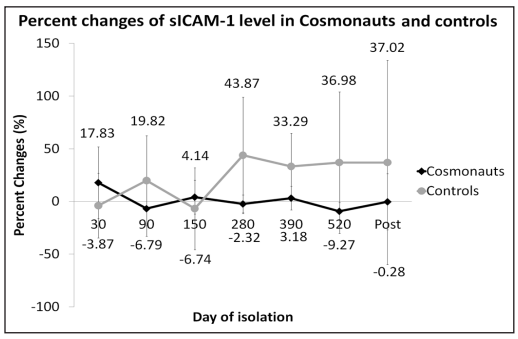


(a)



(b)

Figure 1. Percent change of inflammation biomarkers concentrations in crew members and controls: (a) hsCRP; and (b) IL-6.



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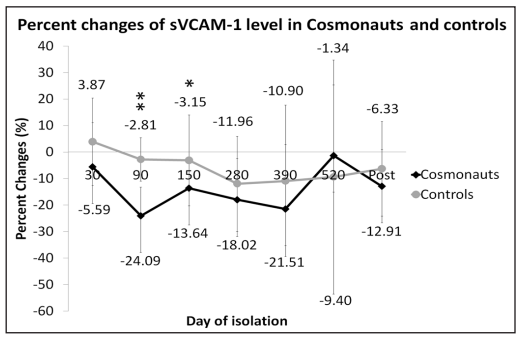

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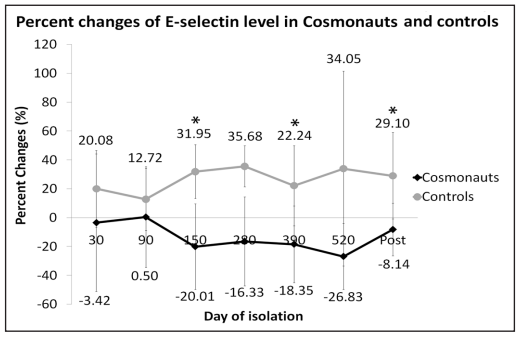

(c)

Figure 2. Percent change of endothelial activation biomarkers concentrations in crew members and controls: (a) sICAM-1; (b) sVCAM-1; and (c) E-selectin.

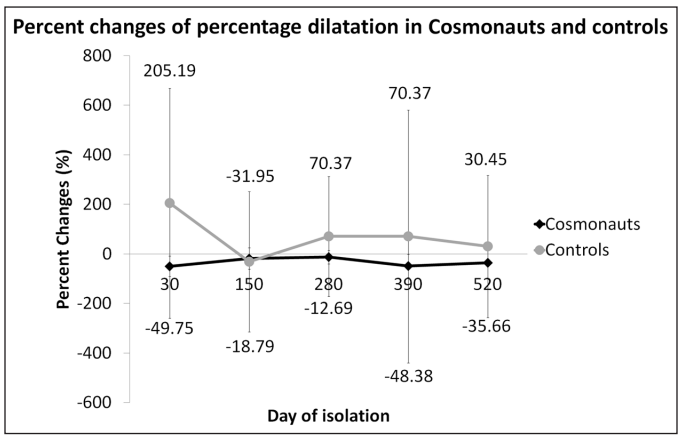

Figure 3. Mean diameter of brachial artery of crew members and controls at pre, 30D, 150D, 280D, 520D and post confined isolation.

and 390. However, there was a slight percent change increment post-isolation phase but this did not achieve statistical significance. There were no significant within-group or between-group differences in percent change for serum IL-6 and Soluble intercellular adhesion molecule-1 (sICAM-1) concentrations.

Table 3 summarizes the serum concentration of biomarkers of inflammation (hsCRP and IL-6) and endothelial activation (sICAM-1, sVCAM-1 and E-selectin). There was significantly lower concentration of sVCAM-1 in cosmonauts compared to pre-isolation period. There were no significant differences in hsCRP, IL-6, sICAM-1 and E-selectin in the crew and control groups compared to pre-isolation. None of the biomarkers were significantly different between groups.

Table 3 refers to the absolute percentage in arterial dilatation and Figure 3 illustrates the flow mediated dilatation (FMD) of the cosmonauts' pre-isolation and during confinement. There were significantly lower FMD readings on days 390 and 520 in the cosmonauts' group ($p<0.01$). There were no between group differences in FMD ($p>0.05$). There were no significant percent change differences in FMD between the crew members and normal controls ($p>0.05$) and neither was there significant difference between timelines ($p>0.05$).

DISCUSSION AND CONCLUSION

This is the first study to report the effects of prolonged confinement that extended well beyond 500 continuous days on the biomarkers of coronary risks such as hsCRP, IL-6, sICAM-1, sVCAM-1 and E-selectin. This study reported percent change increment in hsCRP amongst the crew members compared to free-living controls. The increment seen for hsCRP could be attributed to changes in the plasma volume where it has been established that acute psychological stress stimulates reductions in plasma volume leading

Table 3
Concentration of Serum hsCRP, IL-6, sVCAM-1, sICAM-1, E-Selectin and flow mediated dilatation (FMD) of the brachial artery

Groups	Variables	Days of isolation							
		Pre	30	90	150	280	390	520	Post
Crew Members	hsCRP (mg/l)	0.35 (0.20)	0.46 (0.19)	0.32 (0.12)	0.37 (0.14)	0.32 (0.28)	0.38 (0.16)	0.31 (0.12)	0.28 (0.11)
	IL-6 (pg/ml)	1.86 (0.64)	1.56 (0.00)	1.56 (0.00)	1.56 (0.00)	1.56 (0.00)	1.56 (0.00)	1.56 (1.20)	1.56 (0.61)
	ICAM-1 (ng/ml)	262.72 (42.65)	275.57 (96.35)	231.75 (98.96)	257.33 (65.73)	244.16 (46.93)	252.50 (36.93)	219.44 (34.86)	268.28 (105.94)
	VCAM-1 (ng/ml)	718.56 (105.47)	654.06 (52.87)	541.31** (52.22)	586.06* (21.38)	579.50* (53.34)	535.00* (124.94)	717.13 (126.88)	646.44 (93.59)
	E-selectin (ng/ml)	73.14 (28.72)	92.19 (30.47)	84.68 (11.20)	79.66 (20.97)	74.20 (50.49)	71.01 (9.05)	64.46 (26.39)	70.31 (8.82)
	FMD (%)	12.25 (2.54)	6.80 (7.47)		10.49 (7.67)	12.53 (6.68)	7.72* (5.00)	8.34** (2.55)	
Controls	hsCRP (mg/l)	0.37 (0.14)	0.31 (0.11)	0.41 (0.15)	0.28 (0.11)	0.22 (0.21)	0.17 (0.11)	0.21 (0.25)	0.23 (0.19)
	IL-6 (pg/ml)	2.97 (1.28)	2.89 (1.19)	3.14 (0.89)	3.35 (2.09)	3.25 (2.18)	3.50 (1.95)	3.23 (0.73)	2.86 (0.87)
	ICAM-1 (ng/ml)	275.64 (95.67)	260.24 (31.79)	278.99 (22.41)	303.29 (39.05)	359.54 (87.04)	335.12 (115.67)	307.82 (37.75)	324.05 (183.43)
	VCAM-1 (ng/ml)	708.71 (155.72)	722.81 (113.57)	706.88 (128.16)	729.92 (95.06)	616.10 (128.78)	693.31 (265.88)	648.40 (187.16)	679.46 (98.50)
	E-selectin (ng/ml)	57.88 (20.38)	69.65 (43.37)	66.67 (18.48)	71.74 (18.40)	70.16 (22.92)	69.11 (31.11)	72.77 (19.59)	77.23 (22.79)
	FMD (%)	5.53 (9.82)	10.99 (7.53)		8.00 (5.13)	10.42 (4.86)	13.04 (2.22)	9.65 (12.84)	

Note. Data is expressed as Median (IQR); * indicates $p<0.05$ compared to baseline; ** indicates $p<0.01$ compared to baseline.

to haemoconcentration (Allen & Patterson, 1995; Mischler et al., 2005). This results in the inability for large molecules such as cytokines and CRP to migrate passively through the vascular endothelium resulting in its rising concentration.

It should be highlighted that hsCRP utilizes sensitive measurements that allows very reliably low detection limit of CRP where slight increases will be detectable among healthy subjects. There have been numerous epidemiological studies which have shown an association between elevated hsCRP and vascular events (Cao et al., 2003; Kuller et al., 1996; Liuzzo et al., 1994; Mischler et al., 2005; Ridker et al., 1997; Ridker et al., 1998a; Ridker et al., 1998b; Ridker et al., 2001; Thompson et al., 1995) and from these significant findings, the American Heart Association (AHA) and the Centers for Disease Control and Prevention (CDC) have released a clinical practice guideline which utilizes hsCRP in coronary risk stratification (Pearson et al., 2003). The specific cut-offs that are useful in evaluating a patient's risk for future cardiovascular events have been determined as <1mg/dl for low risk, 1-3mg/dl for moderate risk and >3mg/dl for high risk categories. In our study, among the crew members isolated in a confined space, their mean serum hsCRP concentration was mean$\pm$SD: 0.37$\pm$0.17mg/L which is well below the cut-off for low cardiovascular risk despite observations of percent change increase in the concentration during isolation period. These increments could be stress-related from the prolonged confinement as reported by Wang et al. (2014), where his team highlighted the salient finding of positive bias on valence rating of novel unpleasant stimulus over the prolonged confinement among the cosmonauts. It was observed that the positive bias evaluation on negative stimuli was influenced by psychological stress over time, which was consistent with the fluctuation of hormone levels such as cortisol, dopamine and serotonin. The serum hsCRP concentration reduced post isolation well below that of pre-isolation readings although it did not reach statistical significance. Therefore, these changes observed could possibly be due to physiological adaptation rather than a pathological process.

Various studies have shown that exercise can influence acute phase reaction (APR) (Istvan et al., 2006). Therefore, it can be postulated that the series of rigorous exercise regimes implemented throughout the confinement period could have led to the elevations in serum hsCRP concentration in this study. A report by Mouridsen et al. (2014), had shown that hsCRP was not independently associated with CAD, and that increases in hsCRP by 0.13mg/L (IQR 0.05-0.24mg/L) were associated with peak exercise. Another study reported no change in serum hsCRP concentration but reduction in serum IL-6 following exercise (Kasapis & Thompson, 2005). A review on 5 prospective studies determining effects of exercise on inflammatory response reported exercise inducing transient increase in CRP with enhancement of APR being proportional to the amount of activity and muscle injury (Kasapis & Thompson, 2005).

When endothelial cells of the blood vessels undergo inflammatory activation, the increased expression of selectins, VCAM-1 and ICAM-1 promotes monocytes adherence. Adhesion molecule expression is induced by pro-inflammatory cytokines such as IL-1β and tumor necrosis factor-α (TNF-α), by the acute-phase protein CRP that is produced by the liver in response to IL-6, by protease-activated receptor signaling, by oxLDL uptake via oxLDL receptor-1 (LOX-1), and by CD40/CD40 ligand (CD40L and CD154) interactions (Chen et al., 2002; Collins & Cybulsky, 2001; Kaplanski et al., 1998; Schönbeck & Libby, 2001; Verma et al., 2002). This study interestingly denoted percent change reductions in endothelial activation biomarkers, sVCAM-1 and E-selectin, in the crew members when compared with controls. This would suggest that the environment they were in, coupled with aggressive lifestyle modification during the 520 days of experimentation, have somewhat attenuated release of adhesion molecules which would influence monocyte binding to endothelial cells along the vessel wall during atherogenesis. This is in keeping with previous studies which showed similar findings following exercise and healthy diet (Hamdy et al., 2003; Ziccardi et al., 2002). Furthermore, although confined to isolation for a prolonged period, they were not completely deprived from communication with 'earth'. Communication methods with 'ground control' and family members, although restricted, were still maintained through mimickery of communication accessibility as that of space missions. This could have potentially attenuated forms of stress that may occur during confinement that would elevate these biomarkers.

Endothelial function, measured by FMD, reduced during isolation among the cosmonauts when compared to pre-isolation period, as depicted by the lower percent dilatation of brachial artery among them during isolation compared to pre-isolation readings. This is contrary to the mitigation of endothelial activation biomarkers observed among this group. The possible reason for this discrepancy is that the endothelium has several functions, one of which is to maintain patency of the vessel lumen. The presence of atherosclerotic plaques which starts with increased adhesion molecules and selectins secretion which in turn will increase tunica intimal wall thickness eventually reducing lumen size. However, in the setting of healthy vasculature, the mechanism by which arterial vasodilation reduced is not in relation to the formation of lumen-narrowing atherosclerotic plaques. Previous studies have shown paradoxical decline in FMD following exercise (Birk et al., 2013; Dawson et al., 2013) which has been attributed to oxidative stress which occurs during workouts. There is evidence to support a biphasic response in FMD following exercise of which the strength and direction of this relationship is influenced by several factors which include duration, mode, and intensity of exercise, changes in artery diameter during the exercise and fitness level. Factors that are likely to lead to a decrease in FMD include increased oxidative stress, exercise-induced increase in baseline diameter, shear-induced substrate depletion, elevated retrograde shear, and decreased sensitivity or reduced shear stimulus during the FMD test as a result of in-exercise increases in shear.

The following limitations to the study are worth highlighting. Firstly, this study only looked at prolonged isolation which was estimated as the time taken to travel to mars, dedicating 10 days there for exploratory work and travel back to earth. However, the study design was unable to assess these biomarkers during high g-force rocket launch and subsequent microgravity environments once in space and on mars as we were unable to replicate these scenarios. Secondly, this experimental design only recruited six crew members to best simulate the actual number of cosmonauts planned for a mission to mars. Therefore, the small sample size in this study may have led to the some of the results not being able to achieve statistical significance.

Long term confinement in ground isolation facility up to 520 days attenuated endothelial activation which could be due to the exercise and diet regime given to the crew members during their confinement period. However, their inflammatory status was enhanced possibly attributed to the influences of exercise and possibly psychological stress on the inflammatory response. Nevertheless, despite there being increases in hsCRP concentrations among the crew members, the mean hsCRP concentration was well below the cut-off for low risk of cardiovascular disease. The reduction in endothelial function as depicted by decreased FMD possibly denotes a more physiological response to exercise given throughout the isolation period, rather than a pathological process as the endothelial activation status appears to be attenuated during the experimentation. These findings suggest that instituting appropriate and effective diet and exercise regime during prolong confinement helps reduce stress-related increases in inflammatory biomarkers and adhesion molecules that are involved with atherogenesis. Future research to identify changes in these biomarkers and endothelial function at zero gravity during long confinement will answer the question of whether or not similar findings would be observed in a more identical setting to a manned mission to Mars.

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