Effects of Long-Term Space Flight on Erythrocytes and Oxidative Stress of Rodents

Angela Maria Rizzo1*, Paola Antonia Corsetto1, Gigliola Montorfano1, Simona Milani1, Stefania Zava1, Sara Tavella2, Ranieri Cancedda2, Bruno Berra1

1 Dipartimento di Scienze Molecolari Applicate ai Biosistemi, DISMAB, Università degli studi di Milano, Milan, Italy, 2 Università degli Studi di Genova & Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy

Abstract

Erythrocyte and hemoglobin losses have been frequently observed in humans during space missions; these observations have been designated as “space anemia”. Erythrocytes exposed to microgravity have a modified rheology and undergo hemolysis to a greater extent. Cell membrane composition plays an important role in determining erythrocyte resistance to mechanical stress and it is well known that membrane composition might be influenced by external events, such as hypothermia, hypoxia or gravitational strength variations. Moreover, an altered cell membrane composition, in particular in fatty acids, can cause a greater sensitivity to peroxidative stress, with increase in membrane fragility. Solar radiation or low wavelength electromagnetic radiations (such as gamma rays) from the Earth or the space environment can split water to generate the hydroxyl radical, very reactive at the site of its formation, which can initiate chain reactions leading to lipid peroxidation. These reactive free radicals can react with the non-radical molecules, leading to oxidative damage of lipids, proteins and DNA, etiologically associated with various diseases and morbidities such as cancer, cell degeneration, and inflammation. Indeed, radiation constitutes one of the most important hazard for humans during long-term space flights. With this background, we participated to the MDS tissue-sharing program performing analyses on mice erythrocytes flown on the ISS from August to November 2009. Our results indicate that space flight induced modifications in cell membrane composition and increase of lipid peroxidation products, in mouse erythrocytes. Moreover, antioxidant defenses in the flight erythrocytes were induced, with a significant increase of glutathione content as compared to both vivarium and ground control erythrocytes. Nonetheless, this induction was not sufficient to prevent damages caused by oxidative stress. Future experiments should provide information helpful to reduce the effects of oxidative stress exposure and space anemia, possibly by integrating appropriate dietary elements and natural compounds that could act as antioxidants.

Introduction

Over the past 15 years space medicine has become increasingly concerned with the effects of spaceflight on hematological processes; astronauts have consistently returned from space-flight with a decreased red blood cell mass (RBC-M) “spaceflight anemia” and plasma volume (PV) [1,2]. Although PV is known to be labile, current theories for the control of erythropoiesis cannot account for a decrease in RBC-M of 10% in less than 10 days. Erythrocytes exposed to microgravity have a modified rheology and undergo greater hemolysis [3]. We speculate that microgravity changes in the passage of blood cells through the microcirculation. Cell membrane composition plays an important role in determining erythrocyte resistance to mechanical stress and it is well known that cell membrane composition is influenced by external events, such as hypothermia, hypoxia or gravitational strength variations.

The cell membrane is a lipid bilayer essentially formed by phospholipids, cholesterol and glycolipids. Small variations in percentage composition and molar ratio of the different classes of phospholipids and glycolipids, might induce changes in cell membrane’s fluidity and permeability. Moreover this may also influence the activity of intrinsic membrane proteins, such as enzyme’s and channels or ionic pumps. Finally, a different fatty acid composition of membrane components can result in a greater sensitivity to peroxidative stress, with a consequent increase in membrane fragility.

In the human organism, solar radiation or low wavelength electromagnetic radiations (such as gamma rays) from the Earth or space environment can split water to generate the hydroxyl radical, very reactive at the site of its formation, which can initiate chain reactions leading to lipid peroxidation. These reactive oxygen species (ROS) are shown to react with the non-radical molecules, leading to oxidative damage of lipids, proteins and...
DNA, causing various diseases and morbidities, including cancer, cell degeneration, and inflammation [4,5].

In this view, radiation constitutes the most important hazard for humans during long-term space flights. Radiation protection is therefore mandatory to safeguard the well-being of future astronauts or crew members and to prevent the occurrence of future damages [6].

Antioxidant status reflects the dynamic balance between the antioxidant system of enzymes and molecules and the prooxidants that are constantly being generated. "Oxidative stress" [7], a more pronounced pro-oxidant state, resulting from a serious imbalance favoring oxidation, might be due to an excessive production of ROS, caused by exposure to toxins, radiations or pathological conditions, or from weakening of the antioxidant defense system.

The damage caused by ROS also includes DNA base alteration, which might cause permanent mutations, carbonyl modification of proteins, loss of sulphydryl groups leading to inactivation of enzymes and increased protein oxidation.

A number of defense mechanisms have been developed to protect the non-radical molecules from radical attack, thus limiting the damages. Several antioxidant enzymes can counteract the availability of ROS: superoxide dismutases (SOD), which transforms superoxide anion to hydrogen peroxide; glutathione transferases (GST) and glutathione peroxidases (GPx) [7]. These latter enzymes are associated with the detoxification reactions of xenobiotic compounds that have been activated to electrophilic molecules. The glutathione peroxidase removes hydrogen peroxide generated by the superoxide dismutase. There are also a number of repair enzymes that destroy free-radical-damaged proteins, DNA and oxidized fatty acids from peroxidized molecules. Other molecules contribute to the overall antioxidant defenses of the body against radical damage. They can be found both intra and extracellularly, including tocopherol, reduced glutathione, vitamin C, carotenoids, and urate. Alpha-tocopherol (vitamin E) occurs in membranes and lipoproteins and prevents the chain reaction of lipid peroxidation by scavenging intermediate peroxyl radicals. Vitamin C converts the vitamin E radical back to alpha-tocopherol. Reduced glutathione participates in a number of radical-scavenging reactions, mainly in the respiratory tract and red blood cells. Urate also scavenges free radicals, and beta-carotene functions as a general lipophilic antioxidant [8].

The Mice Drawer System (MDS) is an Italian Space Agency (ASI) facility developed by Thales-Alenia Space to support mice onboard the International Space Station during long-duration missions (from 100 to 150-days) [9]. It was launched with STS-128 on August 28th, 2009 and installed on the ISS. The leading missions (from 100 to 150-days) [9]. It was launched with STS-128 (ASI) facility developed by Thales-Alenia Space to support mice beta-carotene functions as a general lipophilic antioxidant [8].

Experiments have been conducted on MDS-ISS mice during space flight to measure in vivo oxidative stress after a long permanence of the animals on board the ISS.

For these reasons, we determined the Thiobarbituric Acid Reactive Substances (substances formed as byproducts of lipid peroxidation – TBARS) content as an indicator of oxidative stress and the activities of antioxidant enzymes in the mice erythrocytes.

We observed in the PTN MDS-iss mice a statistically significant increase of TBARS in erythrocytes mice compared to both Earth controls (PTN MDS-ground and vivarium), figure 1. The same trend was observed for wild type mice. These data indicated that during space flight mice underwent oxidative stress, which generated lipid peroxidation products such as malondialdehyde.

By analyzing the content of erythrocyte glutathione, the major antioxidant present in these cells, we observed that the total content of this thiol was significantly increased after space flight compared to control in PTN MDS-ISS mice (Fig. 2). In addition, the enzyme involved in glutathione utilization, the glutathione peroxidase, which eliminates hydroperoxides from lipids, was significantly more active in PTN MDS-ISS mice. On the contrary, the activity of glutathione reductase, a very important enzyme that regenerates glutathione after its oxidation was not modified during the space flight.

Several antioxidant enzymes can limit the availability of ROS, including superoxide dismutases (SOD), which transforms superoxide anion to hydrogen peroxide, and Catalase, that removes hydrogen peroxide.

Both enzymes had a significantly higher activity in flown PTN mice erythrocytes (Fig. 3). The same trend was observed for WT mice, even if no statistics may be performed on these data.

Moreover it seems that flown PTN mice reached an higher level of TBARS, GSH and GSH peroxidase after space flight; these may be related to an higher sensitivity of PTN mice to oxidative stress. In fact in primary culture of human osteoblasts from osteoporotic tissue it was recently demonstrated that a down regulation of PTN is paralleled by an up regulation of genes involved in response to reactive oxygen species probably due to
| REF. | PTN-1 | PTN-2 | WT-1 | PTN-1 | PTN-2 | WT-1 | PTN-1 | PTN-2 | WT-1 | PTN-1 | PTN-2 | PTN-3 | WT-1 | WT-2 | WT-3 |
|------|-------|-------|------|-------|-------|------|-------|-------|------|-------|-------|-------|------|------|------|
| MDS | 106/f | 966    | MDS  | 113   | 110   | MDS  | 106   | 105   | MDS  | 106   | 105   | MDS  | 106   | 105   | MDS  | 106   | 105 |
| ISS | 10.6  | 10.6   | ISS  | 10.6  | 10.6  | ISS  | 10.6  | 10.6  | ISS  | 10.6  | 10.6  | ISS  | 10.6  | 10.6  | ISS  | 10.6  | 10.6 |
| MDC | 4.5   | 4.5    | MDC  | 4.5   | 4.5   | MDC  | 4.5   | 4.5   | MDC  | 4.5   | 4.5   | MDC  | 4.5   | 4.5   | MDC  | 4.5   | 4.5 |
| MCH | 13    | 13     | MCH  | 13    | 13    | MCH  | 13    | 13    | MCH  | 13    | 13    | MCH  | 13    | 13    | MCH  | 13    | 13 |
| MCHC | 30   | 30      | MCHC | 30    | 30    | MCHC | 30    | 30    | MCHC | 30    | 30    | MCHC | 30    | 30    | MCHC | 30    | 30 |
| RDW | 0.2   | 0.2     | RDW  | 0.2   | 0.2   | RDW  | 0.2   | 0.2   | RDW  | 0.2   | 0.2   | RDW  | 0.2   | 0.2   | RDW  | 0.2   | 0.2 |
| WBC  | 7.1  | 7.1     | WBC  | 7.1  | 7.1   | WBC  | 7.1  | 7.1   | WBC  | 7.1  | 7.1   | WBC  | 7.1  | 7.1   | WBC  | 7.1  | 7.1 |
| Neutrophils | 20-50 | Neutrophils | 20-50 | Neutrophils | 20-50 | Neutrophils | 20-50 | Neutrophils | 20-50 | Neutrophils | 20-50 | Neutrophils | 20-50 | Neutrophils | 20-50 | Neutrophils | 20-50 |
| Eosinophils | 0-3 | Eosinophils | 0-3 | Eosinophils | 0-3 | Eosinophils | 0-3 | Eosinophils | 0-3 | Eosinophils | 0-3 | Eosinophils | 0-3 | Eosinophils | 0-3 | Eosinophils | 0-3 |
| Basophils | 0-3 | Basophils | 0-3 | Basophils | 0-3 | Basophils | 0-3 | Basophils | 0-3 | Basophils | 0-3 | Basophils | 0-3 | Basophils | 0-3 | Basophils | 0-3 |
| Lymphocytes | 50-50 | Lymphocytes | 50-50 | Lymphocytes | 50-50 | Lymphocytes | 50-50 | Lymphocytes | 50-50 | Lymphocytes | 50-50 | Lymphocytes | 50-50 | Lymphocytes | 50-50 | Lymphocytes | 50-50 |
| Monocytes | 2-8 | Monocytes | 2-8 | Monocytes | 2-8 | Monocytes | 2-8 | Monocytes | 2-8 | Monocytes | 2-8 | Monocytes | 2-8 | Monocytes | 2-8 | Monocytes | 2-8 |
| Platelets | 10^11/ul | Platelets | 10^11/ul | Platelets | 10^11/ul | Platelets | 10^11/ul | Platelets | 10^11/ul | Platelets | 10^11/ul | Platelets | 10^11/ul | Platelets | 10^11/ul | Platelets | 10^11/ul |
| MPV | 5.6 | 5.6     | MPV  | 5.6 | 5.6   | MPV  | 5.6 | 5.6   | MPV  | 5.6 | 5.6   | MPV  | 5.6 | 5.6   | MPV  | 5.6 | 5.6 |
| PDW | 0.285 | PDW | 0.285 | PDW | 0.285 | PDW | 0.285 | PDW | 0.285 | PDW | 0.285 | PDW | 0.285 | PDW | 0.285 | PDW | 0.285 |

REF. reference intervals are obtained from common normal inbred strains of mice.

doi:10.1371/journal.pone.0032361.t001
increased exposure of osteoblasts to oxidative stress in osteoporotic tissue [14].

The increase of these enzymatic activities and of glutathione intracellular content may indicate an induction of these proteins and may be also correlated to the age of newly synthesized erythrocytes; in fact it is demonstrated that during RBC aging the level and the activity of these enzymes significantly decrease [15].

An induction of antioxidant enzymes has been reported in the liver of mice flown for 13 days in previous space missions [16]. Moreover, simulated microgravity and chronic stresses induced antioxidant responses in lymphocytes, brain and muscles [17–20].

Finally, we investigated the lipid composition of RBC membrane phospholipids. The membrane phospholipid content and composition of mice erythrocytes is reported in table II.

As the lipid membrane composition may be greatly influenced by food quality and consumption [21] we first compared the fat composition of food bars used in the MDS facility during flight and ground control (produced by Mucedola, Italy) with the one of the standard food utilized for vivarium mice (Global Diet 2018, Harlan, Italy). As the fat content was different between the two diets, we considered for statistical comparison only the membrane lipid composition of MDS-ISS mice and MDS-ground control mice that had the same diet.

In the RBC membrane of MDS-ISS mice there was an increase of phospholipids, statistically significant for the PTN mice, with changes in their relative percentage composition. In particular, the content of phosphatidylcholine (PC) was significantly reduced, whereas the phosphatidylserine content was increased in MDS-ISS PTN mice. Moreover, we purified the single phospholipid classes and analyzed the fatty acid composition of each phospholipid. We noticed a significant decrease of the total polyunsaturated fatty acids of the omega-3 series in PTN mice (from 13.39% in MDS-ground mice to 12.89% in MDS-ISS mice).

In particular, in the purified PC the amount of docosahexaenoic acid (DHA) was significantly reduced from 2.43 to 0.62% (Fig. 4).

These data are in agreement with the increase of TBARS, which could have been generated from oxidative damage of membrane polyunsaturated lipids and may be related to changes in membrane fluidity of erythrocyte or may be the results of microgravity adaptation [3].

It is noteworthy that, in addition to structural roles, omega 3 fatty acids, especially DHA, play major roles in signal transduction and are increasingly being recognized as reservoirs of lipid messengers. Specific precursors are cleaved from membrane phospholipids, in particular from PC, upon stimulation by neurotransmitters, neurotrophic factors, cytokines, membrane depolarization, ion channel activation, etc.

Impaired polyunsaturated fatty acid (PUFA) status is observed, and measured in erythrocyte, in numerous non physiological states and chronic diseases, like heart disease, metabolic syndrome, rheumatoid arthritis, and other inflammatory conditions, pulmonary disorders, and some psychiatric disorders [22,23].

There are several potential mechanisms that mediate the effects of PUFA on human health including antithrombotic and anti-arrhythmic effects, decreased heart rate variability and resting blood pressure, decreased serum low density lipoproteins (LDL) cholesterol and triglyceride content and increased insulin sensitivity. Other possible mechanisms of omega-3 PUFA are favorable effects on endothelial function, anti-inflammatory effects as well as neuroendocrine influences including modulation of the hypothalamic-pituitary-adrenocortical axis activity [24].

The operation of the space station and the human exploration will require long duration missions. However, mission duration will be limited by the degree of knowledge on the level of the physiological adaptation as well as on the acceptable limits of exposure to specific conditions during the permanence in space environment; our observations may be of particular importance for future manned long term space flight to prepare and implement a nutritional and nutraceutical program to maintain crew health.

Materials and Methods

The Mice Drawer System (MDS) is an Italian Space Agency (ASI) facility, which is able to support mice onboard the
Space Flight Induce Oxidative Stress in Rodents

**TOTAL GLUTATHIONE**

|            | Transgenic | Wild Type |
|------------|------------|-----------|
| **nmol/g Hb** |            |           |
|            |            |           |

**GLUTATHIONE PEROXIDASE**

|            | Transgenic | Wild Type |
|------------|------------|-----------|
| **Ug Hb**  |            |           |
|            |            |           |

**GLUTATHIONE REDUCTASE**

|            | Transgenic | Wild Type |
|------------|------------|-----------|
| **Ug Hb**  |            |           |
|            |            |           |
International Space Station during long-duration exploration missions (from 100 to 150-days) by providing living space, food, water, ventilation and lighting.

Mice can be accommodated either individually (maximum 6) or in groups (4 pairs). MDS is integrated in the Space Shuttle middeck during transportation (ascent and descent) to the ISS and in an EXPRESS Rack in Destiny, US Laboratory during experiment execution.

MDS was launched on board of ISS with 3 wild type mice (C57BL/10) and 3 transgenic mice with the Pleiotrophin (PTN), a growth and differentiation factor. The major aim was to study the genetic mechanisms underlying the bone mass pathophysiology and test the hypothesis that mice with an increased bone density are likely to be more protected from osteoporosis, when the increased bone mass is a direct effect of a gene involved in skeletogenesis (skeleton formation).

Figure 2. Antioxidant enzymes and glutathione content in PTN and wild type mice erythrocytes after space flight. A: Total glutathione (GSH) content (nmol/g Hb, mean±sd) B: glutathione peroxidase (GPx) (U/g Hb, mean± SD) C: Glutathione reductase (GR) (U/g Hb, mean± SD). MDS-ISS (black bars), Ground MDS controls (line bars) and vivarium (white bars). * p<0.05 MDS-ISS vs Ground MDS control. Statistical analysis performed only on PTN mice.

doi:10.1371/journal.pone.0032361.g002

Figure 3. Catalase (Panel A, U/mg Hb±SD) and Superoxide dismutase (Panel B U/g HB±SD) activities in PTN and wild type mice erythrocytes after space flight. MDS-ISS (black bars), Ground MDS controls (line bars) and vivarium (white bars). * p<0.05 MDS-ISS vs Ground MDS control. Statistical analysis performed only on PTN mice.

doi:10.1371/journal.pone.0032361.g003
For flight details refer to the paper of Cancedda et al. After flight, only 3 mice survived: 2 PTN transgenic mice and one wild type mouse.

Controls were run asynchronously in Genoa laboratories. In particular, 2 types of control were utilized. MDS ground control consisted in mice housed in a MDS payload replica on ground with the same profile of food, temperature, humidity and oxygen level. Vivarium mice, housed in normal conditions, constituted the second set of control mice. The three groups of mice will be hereafter referred as MDS-ISS, MDS-Ground and Vivarium. For additional information refer to the paper of Cancedda et al. (published in the same collection).

In all phases of the experiment (pre-flight, during the flight and post-flight) handling of animals was in accordance with the principles expressed in the “Guide for the care and the use of laboratory animals” (Office of Science and Health Reports of the USA National Institute of Health, Bethesda, USA). The approval of the MDS experiment was requested and obtained by the American Institutional Animal Care and Use Committee (IACUC protocol n° FLT-09-070 – Kennedy Space Centre) as well as by the Ethics Committee of the Animal Facility of the National Institute for Cancer Research (Genoa, Italy) and by the Public Veterinary Health Department of the Italian Ministry of Health (Ministero del Lavoro, della Salute e delle Politiche Sociali prot n° 4347-09/03/2009 DGSA.P.).

The main authors of this article were not directly involved in/responsible for designing and/or executing the animal maintenance part of the experiment. Instead they were allowed access to the mice at the end of the flight mission and of the ground control experiments and participated in the specific tissue collection. Additional

| WILD TYPE | MDS-ISS | MDS-GROUND | VIVARIUM | PTN | MDS-ISS | MDS-GROUND | VIVARIUM |
|-----------|---------|------------|----------|-----|---------|------------|----------|
| PL μg/mg HB | 27.65 | 23.45 | 36.02±9.40 | 36.09±0.21* | 28.33±4.57 | 44.97±16.43 |
| % PE | 30.83 | 30.71 | 36.65±0.76 | 38.14±4.19 | 36.16±2.21 | 31.92±6.77 |
| % PI | 1.93 | 6.48 | 4.75±0.61 | 3.41±1.61 | 6.82±4.35 | 4.74±1.56 |
| % PS | 27.72 | 9.70 | 12.01±1.85 | 19.74±2.71* | 12.54±1.39 | 9.63±0.82 |
| % PC | 13.95 | 27.41 | 26.94±2.54 | 22.15±1.50* | 28.16±1.83 | 27.42±6.08 |
| % SM | 25.57 | 25.71 | 19.64±0.88 | 16.56±1.37 | 16.32±2.57 | 26.28±10.68 |

(PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, Phosphatidylcholine; SM, sphingomyelin).

* = p<0.05 MDS-ISS vs MDS-Ground control.
doi:10.1371/journal.pone.0032361.t002

![Figure 4. Total omega-3 fatty acid content (black bars) and phosphatidylcholine DHA content (white bars) in PTN mice erythrocytes after space flight (MDS-ISS, Ground MDS controls). * p<0.05 MDS-ISS vs Ground MDS control.](https://plosone.org/)
information about the MDS hardware adopted for housing the animal in space, the animal behavior during the flight and the efforts made to reduce mice pain and suffering during the whole experiment are reported in the companion article by Cancedda et al.

Blood Collection
Collection of blood of flight and ground mice was performed by retroorbital sinus puncture. Blood from each animal was divided into 2 microtubes containing EDTA, for hematocrit determination, and L-heparin for erythrocyte separation from plasma respectively.

The heparin tubes after gentle agitation were centrifuged at 850 g at 4°C for 5 min. After centrifugation, plasma was immediately separated and frozen in dry ice.

The erythrocyte pellet was washed twice with 10 vol of cold PBS, subdivided into Eppendorf microcentrifuge tubes, and immediately frozen on dry ice. An aliquot of 15 µl of erythrocytes was freshly used to prepare glutathione extract. For this purpose, cells were homogenized on ice in 10% metaphosphoric acid, centrifuged at 13,000 rpm for 10 min and the supernatant was frozen on dry ice.

Samples were transported from Kennedy Space Center (flight samples) and Genoa laboratories (ground control samples) to our laboratory in Milan as frozen samples in dry ice using a specialized courier.

Laboratory of analysis for hematochemistry
Blood collected into the EDTA tubes was utilized for hematocrit count and standard laboratory analysis. Flight samples were analyzed by USA Miller laboratory, School of Medicine University of Miami, whereas ground control samples were analyzed in Genoa by a private veterinarian laboratory.

RBC separation and analysis
Erythrocyte unsealed ghosts were prepared by hypotonic lysis (30 min, 4°C) in 40 vol of 5 mM NaHPO4 buffer pH 8.0. Membranes were washed in 20 vol of the same buffer and this process was continued until the ghosts were free of residual hemoglobin.

Cell hemolyzates were obtained from washed cells by two cycles of freezing, unfreezing and centrifuging. Ghosts were used to assess cell membrane lipid composition (fatty acids, cholesterol and phospholipids) and lipid peroxidation as thiobarbituric acid-reactive substances (TBARS). The homolysed fraction was tested for catalase, peroxidase, reductase and SOD enzyme activities.

Lipid extraction and analysis
Ghosts were extracted with three different chloroform/methanol mixtures (1:2, 2:1, 1:1 v/v), dried and partitioned with 1 vol of water and 2 vol of chloroform/methanol/water 3/48/47 (v/v/v) to obtain an organic and an aqueous phase which were both dried and resuspended in chloroform/methanol 2/1. Lipids were quantified as previously described [25].

Enzyme assay
Hemoglobin was determined on cell lysate as previously described [26] and used to normalize enzyme determinations.

Superoxide Dismutase (SOD) activity assay: the enzyme activity was assayed on aliquots of the hemolysate fraction using the method based on inhibition of NAD(P)H oxidation, according to Paolletti and Mocail [27]. One unit of SOD activity is defined as the amount of enzyme required to inhibit the rate of NADPH oxidation by 50%.

Catalase (CAT) activity assay: the enzyme activity was assayed on aliquots of the hemolysate fraction measuring the consumption of H2O2 according to Aebi [28]. One unit of CAT activity is defined as the amount of enzyme required to catalyze the decomposition of 1 µmole H2O2 min-1.

Glutathione Reductase (GR) activity assay: the enzyme activity was assayed on aliquots of the haemolysate fraction following the oxidation of NADPH according to Pinto et al. [29].

Glutathione Peroxidase (GPx) activity assay: the enzyme activity of the selenium-dependent GPs was assayed on aliquots of the hemolysate fraction according to Prohaska and Ganthier [30].

One unit of GR or GPx is defined as the amount of enzyme required to catalyze the oxidation of 1 µmole NADPH min-1.

Total Glutathione was assayed with an enzymatic method according to Griffith [31].

TBARS (fluorimetric assay)
Samples of ghost extracts, standards (from 2.5 to 100 pmol) (1,1,3,3-tetraethoxypropane) and blank were assayed according to TBARS method [32] with and without stimulation with FeSO4 and ascorbic acid.

TBARS Fluorescence was quantified comparing samples with standard curve (Varian Cary Eclipse; excitation wavelength: 517 nm, emission wavelength: 550 nm).

Statistics
Enzymatic assays were repeated at least 4 times. Data report mean ± S.D. values. t test was performed to compare flight (MDS-ISS) with ground controls (MDS-ground) and vivarium, only for PTN mice.

Acknowledgments
AMR, PAC, GM, SM, and SZ want to acknowledge the support and the continuous advice received by Prof. Bruno Berra who unfortunately passed away in April 2010. He started in the laboratory the space life sciences program with particular interest in cell membrane biochemistry and was a real mentor for all of them.

All authors acknowledge Dr. Y. Liu for the perfect organization of the tissue sharing protocol and Dr. A. Ruggiu for her help.

Author Contributions
Conceived and designed the experiments: AMR BB RC. Performed the experiments: AMR GM PC SM SZ ST. Analyzed the data: AMR GM PC. Wrote the paper: AMR.

References
1. Smith SM (2002) Red blood cell and iron metabolism during space flight. Nutrition 18: 864–866.
2. Allfrey VP, Udden MM, Leach-Huntoon CS, Driscoll T, Pickett MH (1996) Control of red blood cell mass in spaceflight. J App Physiol 81: 98–104.
3. Grigoriev AI, Maksimov GV, Morukov BV, Ivanova SM, Yarlikova YV, et al. (2004) Investigation of erythrocyte shape, plasma membrane fluidity and conformation of haemoglobin haemoporphyrin under the influence of long-term space flight. J Gravit Physiol 11: 79–80.
4. Voulgaridou GP, Anestopoulos I, Franco R, Panayioudis MI, Pappa A (2001) DNA damage induced by endogenous aldehydes: current state of knowledge. Mutat Res 711: 13–27.
5. Ghosh N, Ghosh R, Mandal SC (2011) Antioxidant protection: A promising therapeutic intervention in neurodegenerative disease. Free Radic Res 45: 888–905.
6. Schimmerling W (2010) Accepting space radiation risks. Radiat Environ Biophys 49: 325–329.
7. Yu BP (1994) Cellular defences against damage from reactive oxygen species. Physiol Rev 74: 139–162.
8. Papas AM (1996) Determinants of antioxidant status in humans. Lipids 31: S77–S82.
9. Liu Y, Biticchi R, Gilli M, Piccardi F, Ruggiu A, et al. (2009) Mouse Drawer System (MDS): An automated payload for supporting rodent research on the international space station. Basic Applied Myology 19: 87–95.
10. NASA website. Available: http://www.nasa.gov/mission_pages/station/research/experiments/MDS.html Accessed 2012 February 10.
11. Ballas SK, Marcolina MJ (2006) Hyperhemolysis during the evolution of uncomplicated acute painful episodes in patients with sickle cell anemia. Transfusion 46: 105–10.
12. Gridley DS, Nelson GA, Kostenuik PJ, Bateman TA, et al. (2003) Genetic models in applied physiology: selected contribution: effects of spaceflight on immunity in the C57BL/6 mouse. II. Activation, cytokines, erythrocytes, and platelets. J Appl Physiol 94: 2095–2103.
13. Stein TP (2002) Space flight and oxidative stress. Nutrition 18: 367–371.
14. Trost Z, Trebse R, Prezelj J, Komadina R, Logar DB, et al. (2010) A microarray based identification of osteoporosis-related genes in primary culture of human osteoblasts. Bone 46: 72–80.
15. Glass GA, Gershon D (1984) Decreased enzymatic protection and increased sensitivity to oxidative damage in erythrocytes as a function of cell and donor aging. Biochem J 218: 531–537.
16. Baqai FP, Gridley DS, Slater JM, Luo-Owen X, Stodieck LS, Ferguson V, et al. (2009) Effects of spaceflight on innate immune function and antioxidant gene expression. J Appl Physiol 106: 1933–1942.
17. Fleming SD, Edelman LS, Chapes SK (1991) Effects of corticosterone and microgravity on inflammatory cell production of superoxide. J Leukoc Biol 50: 69–76.
18. Guillot C, Steinberg JG, Dellaux S, Kipson N, Jammes Y, et al. (2008) Physiological, histological and biochemical properties of rat skeletal muscles in response to hindlimb suspension. J Electromyogr Kinesiol 18: 276–283.
19. Stojilkovic V, Todorovic A, Kasapovic J, Pejić S, Pajovic SB (2005) Antioxidant enzyme activity in rat hippocampus after chronic and acute stress exposure. Ann N Y Acad Sci 1048: 373–376.
20. Rizzo AM, Montorfano G, Negroni M, Corsetto P, Berselli P, et al. (2009) Simulated microgravity induce glutathione antioxidant pathway in Xenopus laevis embryos. Cell Biol Int 33: 893–898.
21. Hodson L, Skeaff CM, Fielding RA (2008) Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Prog Lipid Res 47: 348–80.
22. Rondanelli M, Giacosa A, Opizzi A, Pelacchi C, La Vecchia C, et al. (2011) Long chain omega 3 polyunsaturated fatty acids supplementation in the treatment of elderly depression: effects on depressive symptoms, on phospholipids fatty acids profile and on health-related quality of life. J Nutr Health Aging 15: 37–44.
23. Sinopoulos AP (2011) Importance of the omega-6/omega-3 balance in health and disease: evolutionary aspects of diet. World Rev Nutr Diet 102: 10–21.
24. Calder PC, Yaqoob P (2009) Understanding omega-3 polyunsaturated fatty acids. Postgrad Med 121(4): 140–57.
25. Rizzo AM, Montorfano G, Negroni M, Adorni L, Berselli P, et al. (2010) A rapid method for determining arachidonic: eicosapentaenoic acid ratios in whole blood lipids: correlation with erythrocyte membrane ratios and validation in a large Italian population of various ages and pathologies. Lipids Health Dis 2010 9: 7.
26. Beutler E (1984) Red Cell Metabolism: A Manual of Biochemical Methods, (third ed.), Grune and Stratton, Orlando.
27. Paletti F, Mocari A (1990) Determination of superoxide dismutase activity by purely chemical system based on NADPH oxidation. In: Meth Enzymol 106: 209–220.
28. Aebi H (1984) Catalase in Vitro. In: Meth Enzymol 105: 121–126.
29. Pinto C, Mata A, Lopez Barca J (1984) Reversible inactivation of Saccharomyces Cerevisiae glutathione reductase under reducing conditions. Arch Biochem Biophys 229: 1–12.
30. Prohaska JR, Gauthier HE (1976) Selenium and glutathione peroxidase in developing rat. J Neurochem 27: 1379–1387.
31. Griffith OW (1993) Glutathione and glutathione disulphide. In: Bergmeyer HU, ed. Methods of Enzymatic analysis, vol VIII. New York: Academic press. pp 521–529.
32. Wey HE, Pyron L, Woolery M (1993) Essential fatty acid deficiency in cultured human keratinocytes attenuates toxicity due to lipid peroxidation. Toxicol Appl Pharmacol 120: 72–79.