Nutritional Assessment During a 14-d Saturation Dive: the NASA Extreme Environment Mission Operation V Project

SM Smith, JE Davis-Street, JV Fesperman, MD Smith, BL Rice, SR Zwart
ABSTRACT

Ground-based analogs of spaceflight are an important means of studying physiological and nutritional changes associated with space travel, particularly since exploration missions are anticipated, and flight research opportunities are limited. A clinical nutritional assessment of the NASA Extreme Environment Mission Operation V (NEEMO) crew (4 M, 2 F) was conducted before, during, and after the 14-d saturation dive. Blood and urine samples were collected before (D-12 and D-1), during (MD 7 and MD 12), and after (R + 0 and R + 7) the dive. The foods were typical of the spaceflight food system. A number of physiological changes were reported both during the dive and post dive that are also commonly observed during spaceflight. Serum hemoglobin and hematocrit were decreased (P < 0.05) post dive. Serum ferritin and ceruloplasmin significantly increased during the dive, while transferrin receptors tended to go down during the dive and were significantly decreased by the last day (R + 0). Along with significant hematological changes, there was also evidence for increased oxidative damage and stress during the dive. 8-hydroxydeoxyguanosine was elevated (P < 0.05) during the dive, while glutathione peroxidase and superoxide dismutase activities were decreased (P < 0.05) during the dive. Serum C-reactive protein (CRP) concentration also tended to increase during the dive, suggesting the presence of a stress-induced inflammatory response. Decreased leptin during the dive (P < 0.05) may also be related to the increased stress. Similar to what is observed during spaceflight, subjects had decreased energy intake and weight loss during the dive. Together, these similarities to spaceflight provide a model to further define the physiological effects of spaceflight and investigate potential countermeasures.

Key words: saturation diving, hyperbaric, nutrition, spaceflight analog
INTRODUCTION

Nutrition is essential for the maintenance of crew health before, during, and after spaceflight. Several physiological changes occur during spaceflight, including bone and muscle loss (1), oxidative damage (2), cardiovascular, and hematologic alterations (3). These may involve altered nutritional status to one degree or other. Ground-based models have been used extensively to study human adaptation to spaceflight (4), including disuse (e.g., bed rest) and isolation (e.g., Antarctic, closed chamber studies). Underwater analogs have also been used to simulate the isolation, stress, and constraints of spaceflight. They are used to better understand physiological and psychological effects on humans, to assess training and operational issues, to evaluate hardware and procedures, and to test the effectiveness of potential countermeasures.

One underwater-based analog was named the NASA Extreme Environment Mission Operation (NEEMO) project, where subjects live in an underwater habitat for extended periods of time. The unique underwater laboratory provides an environment similar to that aboard the International Space Station (ISS). Not only is the habitat similar in size to modules of the ISS, but the “aquanauts” coordinate operations remotely via a mission control center located onshore (4.5 km away), and also perform extensive science and extravehicular activities during the mission. In some cases, as in the study reported here, the foods provided to the crew are the same as those provided to astronauts on the ISS.

The environment in the habitat emulated stress-induced physiological changes commonly observed during spaceflight (5) and in other ground-based analogs (6). One mechanism by which physiological changes occur during spaceflight is the increased stress due to environmental changes such as acceleration during lift-off, weightlessness, confinement, and long-term maintenance of high levels of performance. These types of stress induce hormonal
changes and altered immune function (7-9). Furthermore, while these stress-induced changes are
known to occur during spaceflight, the confounding effects of altered nutritional status (and the
effects on nutritional status) are not well understood and need to be clarified in order to define
nutritional requirements for long-term spaceflight.

The aim of this study was to evaluate the nutritional status of subjects in a ground-based
analog of spaceflight, the fifth NEEMO mission (NEEMO V). A comprehensive nutritional
assessment was conducted before, during, and after the mission. We hypothesized that in
addition to the effects of stress and confinement, that unique characteristics of the mission and
habitat (e.g., increased atmospheric pressure) would also impact nutrition and health.

METHODS

Environment

NEEMO V was a 14-d saturation dive, with the crew (n = 6) living in an underwater habitat.
The habitat is 14 m long and 4 m in diameter (Figure 1). It is located 20 m (47 ft) below the
ocean surface, with an atmospheric pressure inside the habitat of 2.5 atm. The NEEMO V
mission was completed in June - July of 2003. Supplies were transferred down to the habitat via
sealed container, and samples were returned to the surface via the same container. All tubes and
hardware were pre-tested to ensure that the pressure change would not alter function. Because of
the nature of the dive (i.e., extended-duration saturation dive), a 17-h decompression was
required prior to resurfacing.
Subjects

The crew for NEEMO V consisted of 2 females and 4 males. Three of the six were astronauts (one with previous flight experience), one was a scientist from the Johnson Space Center, and the remaining two were technicians responsible for the maintenance of the habitat. The average age was $35.7 \pm 6.6$ y (mean ±SD). All subjects were required to pass an Air Force Class III physical and were required to have logged a minimum of 25 dives prior to participation in the study. Before the dive, the average body weight was $69.9 \pm 17.3$ kg. Body fat mass, bone mineral content, and lean body mass were also recorded for 4 of the crewmembers ($15.3 \pm 2.25$, $2.51 \pm 0.69$, and $52.1 \pm 14.5$ kg, respectively).

Subjects were trained on all procedures required for the successful completion of the in-dive sample and data collections. Pre-dive dietary data was collected from a standard food frequency questionnaire (REF), while in-dive food intakes were recorded for each meal using a bar code reader. Dietary training was provided by the research dietitian (BLR). Two of the crewmembers were trained in phlebotomy techniques, and they subsequently collected all pre- and in-dive blood samples.

Body mass and body composition determinations

Body mass was determined using a calibrated scale on the days when body composition was determined, and using a standard scale on all other days. For the in-dive determinations, a standard scale was tested in the habitat and was found to function reliably in the high pressure atmosphere. This scale was subsequently used for the remainder of the study.

Body composition was determined (4 subjects only) before and after the dive using dual energy x-ray absorptiometry (DEXA). Dual-energy x-ray absorptiometry (DEXA) scans were
performed using a Hologic QDR 4500W (Hologic, Inc., Waltham, MA) fan beam densitometer. Whole body scans were performed before and after the mission for body composition assessment.

Sample Collection and Processing

Blood (25.7 mL) was collected before (dive minus twelve days, D-12 and D-1), during the dive [mission day 7 (MD 7) and MD 12], and post-dive (return plus zero days, designated R + 0, and R + 7). For two of the subjects, the first pre-dive collection was completed at D-5/-4. Blood collections were performed at the same time each day following an 8-h fast.

Urine was collected before (D-12, D-11, D-1; except for two subjects where samples were collected on D-5, D-4, and D-1), during (MD 7, MD 12), and after the dive (R + 0, R + 1, R + 7, and R + 8). Pre- and post-dive samples were collected in individual bottles and stored cool until processing (<24 hours). During the dive, the crew collected voids either into a beaker or a graduated cylinder. Volumes were recorded, and a 50 mL aliquot from each void was sent to the surface. All urine and blood samples were kept in a cooler on ice in the habitat before (and during) ascent to the surface. The samples were also kept on ice aboard the boat when returning to shore. 24-h pools were created based on void volumes, and aliquots were prepared and frozen for analysis as soon as possible on shore.

For tests where storage would alter the results (e.g., malondialdehyde, hematocrit, and hemoglobin), these were run in the laboratory facilities on shore. Others remained frozen on dry ice until return to the Johnson Space Center in Houston.
Biochemical Analyses

Most analytical determinations were completed using standard, commercial techniques as described previously (6). Hemoglobin, hematocrit (calculated), and mean corpuscular volume were determined using a Coulter T890 instrument (Beckman Coulter, Brea, CA). Serum ferritin and transferrin were analyzed using the Immulite (Diagnostics Products, Los Angeles, CA) and Array 360 instruments, respectively (Beckman Coulter). Transferrin receptors were measured using a commercially available ELISA (Ramco Laboratories, Houston, TX). RBC folate was measured using a commercially available radioreceptor assay (Diagnostic Products, Los Angeles, CA). Ferritin iron content was determined by ICP-MS using a method previously described (6).

Whole blood ionized calcium and electrolytes were determined using ion-sensitive electrode techniques with a portable analyzer (i-STAT, Princeton, NJ) (6,10). Despite attempts to use the portable device in situ during the mission, the pressure differential did not allow for proper functioning of the device. These tests were subsequently performed on samples once they were returned to the surface.

Urine and serum total calcium was measured by inductively coupled plasma emission mass spectrophotometry techniques (11). Serum intact parathyroid hormone was measured by RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA). Vitamin D metabolites 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D were also determined using commercially available kits (DiaSorin, Stillwater, MN). Bone-specific alkaline phosphatase was measured by ELISA (Quidel Corp, Santa Clara, CA, USA). Serum osteocalcin was measured by commercial radioimmunoassay (Biomedical Technologies).

Urine samples were analyzed for collagen cross-links using commercially available kits (METRA PYD and DPD EIA kits, Quidel Corp.; and Osteomark ELISA kit; Ostex International,
Inc., Seattle, WA, USA) as previously described (12). Crosslink data were expressed as nmol excretion per day, as we have demonstrated that this reduces within-subject variability (13).

RBC superoxide dismutase, glutathione peroxidase, and serum oxygen-radical absorbance capacity were measured spectrophotometrically using commercially available kits (Randox Laboratories, Crumlin, UK). HPLC techniques (14) were used to determine 8-hydroxy-2'-deoxyguanosine in urine. Plasma MDA was measured using a commercially available kit (Calbiochem Lipid Peroxidation Assay kit, EMD Biosciences, Inc., San Diego, CA).

Serum total protein, cholesterol, triglycerides, sodium, potassium, chloride, aspartate aminotransferase, alanine aminotransferase, RBC transaminase, and total alkaline phosphatase were analyzed using a Beckman CX7 automated clinical chemistry system (Beckman Coulter, Brea, CA). Serum albumin and transthyretin were analyzed using the Beckman Appraise and Array 360 instruments, respectively (Beckman Coulter). Urine creatinine was analyzed on the NexCT (Alfa Wassermann, West Caldwell, NJ).

Statistical Analysis

Data are reported as means ± SD. Dietary data and biochemical data were analyzed using repeated-measures analysis of variance (ANOVA) with a post-hoc Bonferroni test to determine differences among groups. Statistical analyses were performed using SigmaStat (SPSS, Chicago, IL).

RESULTS
Dietary Intake

Pre-dive dietary intakes were determined (mean ± SD) for energy, fat, protein, calcium, and iron (2100 ± 613 kcal, 84.7 ± 24.2 g, 83.1 ± 33.0 g, 774 ± 327 mg, and 19.7 ± 14.4 mg, respectively) using a food frequency questionnaire. Energy intake was significantly lower than the World Health Organization recommendations during the dive on MD 5, MD 6, and MD 11 (Table 1). Mid-dive means (± SD) were also determined for vitamin D, fat, protein, calcium, and iron (5.46 ± 5.28 μg, 63.3 ± 20 g, 77.5 ± 28 g, 1002 ± 387 mg, and 22.3 ± 9.56 mg, respectively).

Body Weights and Composition

Body weights were significantly lower from pre dive weights on MD 7-14 (P < 0.05), and higher than pre dive weights on R + 7 (Table 1). Body fat, bone mineral content, and lean body mass were not different (n=4) when these measurements from R + 7 were compared with pre dive measurements (Table 1).

Hematology and general chemistry

Hemoglobin and hematocrit were both decreased (P < 0.05) at R + 0 compared to pre dive and MD7 (Table 2). Serum mean corpuscular volume (MCV) was significantly decreased (P < 0.05) on the last collection day post flight (R + 7) compared to in-dive (MD 7 and MD 12) and R + 0 (Table 2). There was a significant decrease in serum iron post dive (R + 7) compared to in-dive (MD 7 and MD 12).

Serum ferritin was significantly elevated both days in-idive and R + 0 compared to pre and post (R + 7) dive (Table 2). Similarly, serum ceruloplasmin tended to increase during the dive...
and was significantly elevated \( R + 0 \) (Table 3). Transferrin receptors in serum tended to
decrease in-dive but the decrease compared to pre dive was only significant on \( R + 0 \) (Table 2).
Ferritin iron, transferrin, and ferritin saturation were unchanged throughout the study.
Triglycerides were elevated post dive (\( R + 7 \)) compared to pre dive. Serum leptin tended to
decrease during the dive and was significantly different from pre dive on \( R + 0 \) (Table 3).
Electrolyte pools were also altered in response to conditions during NEEMO V. Sodium and
chloride excretion were decreased (\( P < 0.05 \)) during the dive (MD 7 and MD 12) compared to
pre dive (Table 4), while urine excretion volume remained constant during the study. Serum
sodium concentration was significantly higher during the dive (MD 7 and MD 12) and after the
dive (\( R + 7 \)) compared to pre dive (\( P < 0.05 \), Table 3). Whole blood sodium and
potassium…..showed the same changes? (data in table, or data not shown…)

**Calcium and bone metabolism**

Urinary calcium was significantly elevated post-dive (\( R + 8 \)) compared to mid-dive (MD 12)
(Table 5). Serum total calcium was unaltered during the study; however, serum ionized calcium
was increased (\( P < 0.05 \)) on MD 12 and post dive (\( R + 7 \)) compared to pre dive (Table 5).
Urinary collagen crosslinks (NTX, PYD, and DPD) were unaffected during or after the dive.
Serum osteocalcin was significantly elevated post dive (\( R + 7 \)) compared to in-dive (MD 7
and MD12), but other markers of bone formation including serum total alkaline phosphatase and
bone-specific alkaline phosphatase were unchanged during the study (Table 5). Similarly, there
was no effect on serum vitamin D metabolites, 25-hydroxy vitamin D or 1,25-dihydroxy vitamin
D (Table 5).
Antioxidant status

Urinary 8-hydroxy 2’-deoxyguanosine (8OHdG) excretion was significantly elevated during the dive (MD 7 and MD12) compared to pre dive (P < 0.05) (Table 6). Other markers of antioxidant status and function were also altered during and post dive, including whole blood glutathione peroxidase (GPX) activity, superoxide dismutase (SOD) activity, and plasma malondialdehyde (MDA). Whole blood GPX tended to decrease during the dive and R + 0 but it was not significantly decreased until R + 7 (Table 6). Whole blood SOD was decreased MD 7, and this significant decrease (P < 0.05) compared to the pre dive continued throughout the remainder of the study (MD 12, R + 0, and R + 7). Plasma MDA was significantly decreased post dive (R + 0 and R + 7) compared to pre dive. Red blood cell glutathione reductase activity was decreased (P < 0.05) at the latter part of the dive (MD 12 and R + 0), but was returned to pre dive concentrations 7 d after the dive (Table 3).

DISCUSSION

Limitations on resources (e.g., time, power, volume, up/down mass) for spaceflight research necessitate the development of Earth-based analogs. The underwater isolation of the NEEMO missions provides one such analog of spaceflight, with obvious similarities, along with obvious limitations. The study we report here clearly identifies this as a valuable analog environment, with results that resemble many aspects of spaceflight beyond the direct nutritional implications (e.g., dietary intake). In further defining the changes that occurred in crew members, we will be better able to propose, design, and test countermeasures for future missions.

The hematological findings are striking, and extend those from earlier dive studies, as well as apply to hematological changes seen during spaceflight. Reductions in hemoglobin


concentration and increases in serum ferritin concentration are well established in deep saturation
dives (depths up to 660 m, 31 – 67 atm) (15-17). These same effects were observed after the 14-
d shallow saturation dive described here (14.3 m, 2.5 atm). Reduced hemoglobin concentrations
suggest a reduction in red blood cell mass, which could be due to decreased production of new
red blood cells, as seen in spaceflight (18), or destruction of existing red blood cells by oxidative
damage (15,19).

Increased serum ferritin in-dive and decreased transferrin receptors R + 0 were also observed
and would be expected when iron stores and intracellular iron availability are high. It is likely
that the increased oxygen availability, induced by the increased atmospheric pressure,
contributed to a decreased need for red blood cells, and iron pools were consequently shifted
from hemoglobin to a storage form. This process, termed neocytolysis, has been documented in
spaceflight (20,21), as well as in subjects traveling from high to low altitude (22).

While ferritin iron content did not increase along with the increased serum ferritin, ferritin
iron and serum iron both tended to go up during the dive. One possibility for lack of significance
is the small sample size (n = 6 for pre dive, MD 7, R + 0, and R + 7; n = 5 for MD 12). Another
possibility is that the increase in total serum ferritin is indicative of recruitment of ferritin from
preexisting stores, and that the time course is too short for enrichment of ferritin with excess iron
to alter the reflection in the serum. There is also a possibility that the changes of serum ferritin
during the dive were due to an acute inflammatory response since there were other indications
that such a response might have occurred. Other acute phase proteins tended to go up during the
dive. While not significant, serum C-reactive protein tended to be elevated during the dive
compared to pre and post dive. The large variances prevented these findings from being
significant. Again, we are limited with the very small sample size in this study. Furthermore,
other studies suggest that oxidative stress is increased during the acute inflammatory phase of
many illnesses (24,25), which was also observed in one subject prior to the dive.

Alterations in antioxidant markers were hypothesized due to the hyperbaric environment.
Along with the increased 8OHdG excretion observed during the dive, decreased activities of
GPX and SOD post (GPX and SOD) and in-dive (SOD) imply increased oxidative stress. A
number of other parameters (besides the environment) could have contributed to this, including
changes in nutrient intake or changes in stress hormones. The significant decrease in MDA
suggests that lipid peroxidation is decreased in and post dive compared to pre dive, which does
not support the theory of increased oxidative damage. This decrease is not easily explained since
we would have expected to see similar changes during the dive for 8OHdG and MDA.

Pre dive measurements for all parameters are averages of measurements recorded twice before the dive,
and there appeared to be differences between the two collection points (pre dive day 12 for MDA
was high compared to pre dive day 1; specifically 1.25 ± 0.96 and 0.26 ± 0.18 µmol/L for pre
dive days 12 and 1, respectively). If only pre dive day 1 was used for comparison (instead of the
average of the two), MDA then tended to increase during the dive compared to pre and post dive.
Mean body weights were significantly lower than pre dive weights during the latter part of the
dive (MD 7-14). During the dive, energy intakes were lower than World Health Organization
recommendations (Table 1). This is a similar phenomenon that consistently occurs during
spaceflight (6,26,27), and explains why body weights were concurrently decreased. Serum leptin
was measured in these individuals and we found that these concentrations tended to go down
during the dive and were significantly decreased by the last day (R + 0). Leptin is normally
involved in the regulation of food intake and in the maintenance of energy balance, but its role in
the decreased energy intake in this study is unknown and warrants further investigation. Other
studies have linked decreased leptin concentration with periods of intense exercise, possibly indicative of increased stress or inflammation (28,29). Again, consistent with other findings outlined above, the decreased leptin observed here may support the presence of an acute inflammatory response during the dive.

Despite increased pressure in the habitat, there was no evidence for alterations in bone formation/resorption during the dive. While osteocalcin was significantly higher post dive (R + 7) compared to in-dive (MD 7 and MD 12), other bone formation markers in the serum, including alkaline phosphatase and bone-specific alkaline phosphatase, were unchanged during the study. Bone resorption markers were unchanged during the dive. Parathyroid hormone and vitamin D concentrations tended to decrease, but not significantly. Both of these indices might have reached statistical significance with a longer mission (due to lack of ultraviolet light exposure) or with additional subjects. Furthermore, these findings enhance our recent observations that lower body negative pressure (LBNP) can mitigate disuse-induced bone resorption (30). The current study, one of whole body positive pressure, suggests that the findings with LBNP may be more related to circulatory changes than to pressure itself. Such suggestions that circulatory influences may impact weightlessness-induced bone loss are not new (31,32).

It is evident that there are indeed many physiological and nutritional changes that occurred during NEEMO V that are also commonly observed during spaceflight. Changes in nutritional status during spaceflight are of critical concern for future long duration space travel, and spaceflight analogs such as NEEMO V may be increasingly important to further investigate potential countermeasures.
Acknowledgements

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REFERENCES


Table 1. Body weight and dietary intake data from NEEMO V.

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<tr>
<th></th>
<th>PRE</th>
<th>MD2</th>
<th>MD3</th>
<th>MD4</th>
<th>MD5</th>
<th>MD6</th>
<th>MD7</th>
<th>MD8</th>
<th>MD9</th>
<th>MD10</th>
<th>MD11</th>
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<tr>
<td>Energy Intake, kcal</td>
<td>2590 ± 613</td>
<td>2690 ± 925</td>
<td>2900 ± 651</td>
<td>2610 ± 689</td>
<td>1790 ± 654</td>
<td>2394 ± 555</td>
<td>2390 ± 510</td>
<td>2550 ± 471</td>
<td>2490 ± 685</td>
<td>2110 ± 392</td>
<td></td>
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<tr>
<td>Energy Intake (% WHO)</td>
<td>90.0 ± 7.2</td>
<td>92.2 ± 21.1</td>
<td>99.5 ± 16.2</td>
<td>71.4 ± 27.9</td>
<td>10.0 ± 12.9</td>
<td>83.1 ± 17.3</td>
<td>83.3 ± 16.9</td>
<td>91.0 ± 17.4</td>
<td>82.5 ± 9.5</td>
<td>75.1 ± 12.4</td>
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<td>Water Intake, mL</td>
<td>2560 ± 989</td>
<td>2520 ± 912</td>
<td>2510 ± 791</td>
<td>1960 ± 689</td>
<td>1930 ± 1140</td>
<td>2410 ± 377</td>
<td>2750 ± 810</td>
<td>3100 ± 1950</td>
<td>2880 ± 1240</td>
<td>2200 ± 544</td>
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<tr>
<td>BW (kg)</td>
<td>76.0 ± 16.1</td>
<td>75.2 ± 16.6</td>
<td>75.4 ± 15.9</td>
<td>75.1 ± 16.2</td>
<td>75.0 ± 15.5</td>
<td>74.0 ± 15.3</td>
<td>74.3 ± 15.5</td>
<td>74.5 ± 15.6</td>
<td>74.8 ± 15.6</td>
<td>74.2 ± 15.8</td>
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<td>Body Mass (DEXA) (kg)</td>
<td>99.9 ± 17.3</td>
<td>-</td>
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<tr>
<td>Body Fat (kg)</td>
<td>15.3 ± 2.3</td>
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<tr>
<td>BMC</td>
<td>2.5 ± 0.7</td>
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<tr>
<td>LBM (kg)</td>
<td>102.1 ± 14.5</td>
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</table>

BW, body weight; BMC, bone mineral content; LBM, lean body mass. Values are means ± SD, n = 6. **Energy intake is significantly different from WHO recommendations (P < 0.05).

*Significantly different from pre dive (P < 0.05).
Table 2. Hemotologic, iron, and folate status indicators before, during, and after NEEMO V.\textsuperscript{1}

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>MD 7</th>
<th>MD 12</th>
<th>R + 0</th>
<th>R + 7</th>
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<tbody>
<tr>
<td><strong>Serum Hgb, g/L</strong></td>
<td>136 ± 15\textsuperscript{ac}</td>
<td>138 ± 10\textsuperscript{ac}</td>
<td>137 ± 14\textsuperscript{ad}</td>
<td>128 ± 13\textsuperscript{bd}</td>
<td>131 ± 13\textsuperscript{ad}</td>
</tr>
<tr>
<td><strong>Serum HCT</strong></td>
<td>0.41 ± 0.04\textsuperscript{ac}</td>
<td>0.41 ± 0.03\textsuperscript{ac}</td>
<td>0.40 ± 0.04\textsuperscript{ad}</td>
<td>0.38 ± 0.04\textsuperscript{bd}</td>
<td>0.40 ± 0.04\textsuperscript{cd}</td>
</tr>
<tr>
<td><strong>Serum MCV, fL</strong></td>
<td>91 ± 3\textsuperscript{ab}</td>
<td>92 ± 3\textsuperscript{a}</td>
<td>92 ± 4\textsuperscript{a}</td>
<td>92 ± 3\textsuperscript{a}</td>
<td>90 ± 2\textsuperscript{bc}</td>
</tr>
<tr>
<td><strong>Serum Iron, umol/L</strong></td>
<td>19 ± 7\textsuperscript{ac}</td>
<td>26 ± 10\textsuperscript{a}</td>
<td>27 ± 10\textsuperscript{a}</td>
<td>22 ± 5\textsuperscript{bc}</td>
<td>12 ± 4\textsuperscript{bc}</td>
</tr>
<tr>
<td><strong>Ferritin Iron, umol/L</strong></td>
<td>0.34 ± 0.15</td>
<td>0.48 ± 0.19</td>
<td>0.44 ± 0.17</td>
<td>0.47 ± 0.17</td>
<td>0.32 ± 0.12</td>
</tr>
<tr>
<td><strong>Serum Ferritin, ug/L</strong></td>
<td>102 ± 63\textsuperscript{a}</td>
<td>168 ± 82\textsuperscript{b}</td>
<td>219 ± 98\textsuperscript{b}</td>
<td>196 ± 92\textsuperscript{b}</td>
<td>117 ± 70\textsuperscript{a}</td>
</tr>
<tr>
<td><strong>Ferritin Saturation, %</strong></td>
<td>22.8 ± 9.2</td>
<td>18.2 ± 8.7</td>
<td>12.0 ± 3.2</td>
<td>14.6 ± 3.8</td>
<td>18.6 ± 8.5</td>
</tr>
<tr>
<td><strong>Transferrin Receptors, ug/mL</strong></td>
<td>4.8 ± 1.3\textsuperscript{ac}</td>
<td>4.6 ± 1.1\textsuperscript{cd}</td>
<td>4.3 ± 0.8\textsuperscript{cd}</td>
<td>3.9 ± 0.9\textsuperscript{bd}</td>
<td>4.1 ± 1.0\textsuperscript{cd}</td>
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<tr>
<td><strong>Transferrin, g/L</strong></td>
<td>2.63 ± 0.18</td>
<td>2.66 ± 0.29</td>
<td>2.85 ± 0.14</td>
<td>2.53 ± 0.24</td>
<td>2.62 ± 0.18</td>
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<tr>
<td><strong>RBC Folate, nmol/L</strong></td>
<td>1705 ± 486</td>
<td>1598 ± 575</td>
<td>1683 ± 365</td>
<td>1445 ± 486</td>
<td>1554 ± 429</td>
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</tbody>
</table>

\textsuperscript{1}Hgb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; RBC, red blood cell.

Values are means ± SD, n = 6 (n = 5 on MD 12 for all parameters, except transferrin receptors where n = 6 for all days). Significant differences in rows are represented by different letters (P < 0.05).
Table 3. General blood chemistry before, during, and after NEEMO V.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>MD 7</th>
<th>MD 12</th>
<th>R + 0</th>
<th>R + 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Sodium, mmol/L</td>
<td>138 ± 2a</td>
<td>141 ± 2b</td>
<td>141 ± 2b</td>
<td>139 ± 1a</td>
<td>141 ± 2b</td>
</tr>
<tr>
<td>Serum Potassium, mmol/L</td>
<td>4.2 ± 0.3</td>
<td>4.1 ± 0.2</td>
<td>4.2 ± 0.7</td>
<td>3.9 ± 0.3</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Serum Chloride, mmol/L</td>
<td>106 ± 2</td>
<td>106 ± 3</td>
<td>105 ± 3</td>
<td>105 ± 2</td>
<td>107 ± 3</td>
</tr>
<tr>
<td>Serum Creatinine, umol/L</td>
<td>94 ± 13</td>
<td>94 ± 13</td>
<td>77 ± 40</td>
<td>93 ± 17</td>
<td>94 ± 16</td>
</tr>
<tr>
<td>Serum Triglyceride, mmol/L</td>
<td>1.03 ± 0.34</td>
<td>1.07 ± 0.38</td>
<td>1.13 ± 0.62</td>
<td>1.54 ± 0.55</td>
<td>1.72 ± 1.00</td>
</tr>
<tr>
<td>Serum RBP, mg/L</td>
<td>67.4 ± 7.21</td>
<td>58.8 ± 17.9</td>
<td>59.3 ± 11.4</td>
<td>55.0 ± 11.2</td>
<td>65.3 ± 13.4</td>
</tr>
<tr>
<td>RBC GSH Activity, % act</td>
<td>13.9 ± 9.95</td>
<td>7.2 ± 10.5</td>
<td>5.7 ± 5.0</td>
<td>6.0 ± 7.6</td>
<td>10.4 ± 4.9</td>
</tr>
<tr>
<td>RBC Transaminase act, % act</td>
<td>81.6 ± 13.4</td>
<td>86.0 ± 15.4</td>
<td>79.5 ± 16.6</td>
<td>86.7 ± 15.4</td>
<td>82.1 ± 13.8</td>
</tr>
<tr>
<td>Serum ATL, U/L</td>
<td>18.4 ± 7.0</td>
<td>13.8 ± 6.2</td>
<td>16.2 ± 4.8</td>
<td>14.2 ± 3.5</td>
<td>18.8 ± 6.9</td>
</tr>
<tr>
<td>Serum AST, U/L</td>
<td>25.9 ± 2.6</td>
<td>29.7 ± 4.6</td>
<td>29.6 ± 10.6</td>
<td>26.2 ± 3.9</td>
<td>25.3 ± 5.8</td>
</tr>
<tr>
<td>Serum Ceruloplasmin, mg/L</td>
<td>430 ± 130a</td>
<td>470 ± 150ab</td>
<td>480 ± 120ab</td>
<td>520 ± 120b</td>
<td>500 ± 140ab</td>
</tr>
<tr>
<td>Serum Transthyretin, mg/L</td>
<td>305 ± 58</td>
<td>285 ± 24</td>
<td>312 ± 22</td>
<td>305 ± 38</td>
<td>327 ± 59</td>
</tr>
<tr>
<td>Serum Cholesterol, mmol/L</td>
<td>5.15 ± 0.67</td>
<td>5.14 ± 1.10</td>
<td>4.26 ± 2.26</td>
<td>4.78 ± 0.86</td>
<td>5.31 ± 0.91</td>
</tr>
<tr>
<td>Serum pH</td>
<td>7.4 ± 0.02</td>
<td>7.4 ± 0.02</td>
<td>7.4 ± 0.05</td>
<td>7.4 ± 0.02</td>
<td>7.4 ± 0.04</td>
</tr>
<tr>
<td>Total protein, g/L</td>
<td>68 ± 4</td>
<td>70 ± 3</td>
<td>70 ± 7</td>
<td>70 ± 3</td>
<td>69 ± 4</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>43 ± 3</td>
<td>46 ± 2</td>
<td>45 ± 3</td>
<td>45 ± 3</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>5.8 ± 3.5ab</td>
<td>4.2 ± 2.6b</td>
<td>2.7 ± 1.2b</td>
<td>2.3 ± 1.1c</td>
<td>5.6 ± 2.4bd</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>1.8 ± 1.9</td>
<td>11.9 ± 18.1</td>
<td>12.4 ± 23.3</td>
<td>4.7 ± 7.4</td>
<td>2.1 ± 2.4</td>
</tr>
</tbody>
</table>

RBP, retinol binding protein; RBC, red blood cell; GSH, glutathione reductase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein. Values are means ± SD, n = 6 (n = 5 on MD 12 for all parameters except for serum RBP, where n = 6 for all days). Significant differences in rows are represented by different letters (P < 0.05).
Table 4. General urine chemistry before, during, and after NEEMO V.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>MD 7</th>
<th>MD 12</th>
<th>R + 0</th>
<th>R + 1</th>
<th>R + 7</th>
<th>R + 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine pH</td>
<td>6.0 ± 0.16</td>
<td>6.22 ± 0.41</td>
<td>6.25 ± 0.33</td>
<td>6.07 ± 0.47</td>
<td>6.46 ± 0.45</td>
<td>6.21 ± 0.55</td>
<td>6.04 ± 0.31</td>
</tr>
<tr>
<td>Urine excretion volume, mL</td>
<td>2160 ± 676</td>
<td>1970 ± 1260</td>
<td>2410 ± 944</td>
<td>2670 ± 1390</td>
<td>1670 ± 647</td>
<td>2110 ± 1040</td>
<td>2620 ± 1940</td>
</tr>
<tr>
<td>Urine Sodium, mmol/d</td>
<td>182 ± 21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>120 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>106 ± 44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.7 ± 32.1</td>
<td>124 ± 42</td>
<td>163 ± 31</td>
<td>191 ± 58</td>
</tr>
<tr>
<td>Urine Potassium, mmol/d</td>
<td>62.2 ± 15.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>47.3 ± 15.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>53.0 ± 18.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>46.8 ± 18.2</td>
<td>53.8 ± 24.1</td>
<td>72.5 ± 25.2</td>
<td>64.8 ± 20.7</td>
</tr>
<tr>
<td>Urine Chloride, mmol/d</td>
<td>176 ± 16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.5 ± 60.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.2 ± 31.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.7 ± 30.6</td>
<td>107 ± 24</td>
<td>159 ± 25</td>
<td>193 ± 69</td>
</tr>
<tr>
<td>Urine 3MH, umol/d</td>
<td>245 ± 122</td>
<td>242 ± 138</td>
<td>222 ± 116</td>
<td>230 ± 93</td>
<td>270 ± 104</td>
<td>271 ± 112</td>
<td>269 ± 114</td>
</tr>
<tr>
<td>Urine Iodine, mmol/d</td>
<td>2.46 ± 0.91</td>
<td>2.12 ± 0.84</td>
<td>1.77 ± 0.71</td>
<td>2.68 ± 1.13</td>
<td>1.80 ± 0.29</td>
<td>2.18 ± 0.96</td>
<td>2.60 ± 1.39</td>
</tr>
<tr>
<td>Urine Mg, mmol/d</td>
<td>3.89 ± 1.05</td>
<td>4.34 ± 1.30</td>
<td>3.78 ± 1.44</td>
<td>4.87 ± 1.67</td>
<td>3.41 ± 1.98</td>
<td>4.43 ± 0.94</td>
<td>5.08 ± 1.80</td>
</tr>
<tr>
<td>Urine Phos, mmol/d</td>
<td>30 ± 7</td>
<td>34 ± 11</td>
<td>33 ± 12</td>
<td>27 ± 10</td>
<td>23 ± 6</td>
<td>29 ± 11</td>
<td>29 ± 6</td>
</tr>
<tr>
<td>Urine Creatinine, mmol/d</td>
<td>18.3 ± 3.7</td>
<td>15.2 ± 5.2</td>
<td>17.5 ± 5.2</td>
<td>18.2 ± 4.0</td>
<td>13.5 ± 7.4</td>
<td>16.3 ± 4.0</td>
<td>16.1 ± 3.5</td>
</tr>
</tbody>
</table>

<sup>1</sup>3MH, 3-methylhistidine. Values are means ± SD, n = 6 (n = 5 on R + 1). Significant differences in rows are represented by different letters (P < 0.05).
Table 5. Calcium and bone markers before, during, and after NEEMO V. 

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>MD 7</th>
<th>MD 12</th>
<th>R + 0</th>
<th>R + 1</th>
<th>R + 7</th>
<th>R + 8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum iPTH, pg/mL</strong></td>
<td>31.1 ± 7.62</td>
<td>25.8 ± 9.72</td>
<td>24.3 ± 6.87</td>
<td>28.8 ± 10.2</td>
<td>-</td>
<td>29.6 ± 9.81</td>
<td>-</td>
</tr>
<tr>
<td><strong>Serum Osteocalcin, ng/mL</strong></td>
<td>9.30 ± 2.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.43 ± 1.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.75 ± 1.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.35 ± 1.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>10.7 ± 2.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td><strong>Alk Pase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum total Alk Phosphatase</td>
<td>57.7 ± 22.0</td>
<td>61.0 ± 17.7</td>
<td>66.6 ± 20.0</td>
<td>61.7 ± 22.3</td>
<td>-</td>
<td>61.3 ± 21.5</td>
<td>-</td>
</tr>
<tr>
<td>Serum BSAP, UIL</td>
<td>21.7 ± 7.60</td>
<td>20.7 ± 7.20</td>
<td>21.4 ± 6.60</td>
<td>21.7 ± 8.06</td>
<td>-</td>
<td>19.7 ± 7.45</td>
<td>-</td>
</tr>
<tr>
<td>Serum 25 OH Vit D, nmol/L</td>
<td>92 ± 23</td>
<td>88 ± 30</td>
<td>81 ± 18</td>
<td>86 ± 18</td>
<td>-</td>
<td>81 ± 24</td>
<td>-</td>
</tr>
<tr>
<td>Serum 1,25 OH Vit D, pmol/L</td>
<td>211 ± 52.4</td>
<td>212 ± 105</td>
<td>221 ± 80.6</td>
<td>196 ± 108</td>
<td>-</td>
<td>201 ± 64.1</td>
<td>-</td>
</tr>
<tr>
<td><strong>Calcium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum TCa, mmol/L</td>
<td>2.56 ± 0.11</td>
<td>2.70 ± 0.18</td>
<td>2.57 ± 0.15</td>
<td>2.57 ± 0.10</td>
<td>-</td>
<td>2.67 ± 0.22</td>
<td>-</td>
</tr>
<tr>
<td>Serum iCa, mmol/L</td>
<td>1.18 ± 0.03</td>
<td>1.23 ± 0.05</td>
<td>1.28 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.21 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>1.21 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>Urine Ca, mmol/L</td>
<td>5.49 ± 0.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.83 ± 1.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.98 ± 0.91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.13 ± 1.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.92 ± 1.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.12 ± 2.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.82 ± 2.16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DPD, nmol/d</td>
<td>63.9 ± 7.4</td>
<td>73.7 ± 24.9</td>
<td>70.2 ± 20.2</td>
<td>61.6 ± 15.5</td>
<td>60.3 ± 6.6</td>
<td>66.8 ± 17.3</td>
<td>57.0 ± 19.8</td>
</tr>
<tr>
<td>NTX, nmol/d</td>
<td>484 ± 235</td>
<td>690 ± 384</td>
<td>480 ± 287</td>
<td>430 ± 208</td>
<td>499 ± 271</td>
<td>539 ± 383</td>
<td>558 ± 240</td>
</tr>
<tr>
<td>PYD, nmol/d</td>
<td>256 ± 51</td>
<td>307 ± 85</td>
<td>262 ± 78</td>
<td>297 ± 98</td>
<td>224 ± 68</td>
<td>265 ± 67</td>
<td>260 ± 89</td>
</tr>
</tbody>
</table>

<sup>1</sup>iPTH, intact parathyroid hormone; Alk Phosphatase, alkaline phosphatase; BSAP, bone-specific alkaline phosphatase; DPD, deoxypyridinoline; NTX, n-telopeptide; PYD, pyridinium crosslinks.

Values are means ± SD, n = 6 (n = 5 on MD 12 for total alkaline phosphatase and n = 5 on R + 1 for DPD, NTX, and PYD). Significant differences in rows are represented by different letters (P < 0.05). *Significantly different from pre dive (P < 0.05).
Table 6. Antioxidant/oxidative damage indices from NEEMO V crew members.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>MD 7</th>
<th>MD 12</th>
<th>R + 0</th>
<th>R + 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>8(OH)dG, ug/d</td>
<td>5.71 ± 1.28³</td>
<td>7.25 ± 2.26³</td>
<td>7.41 ± 2.12³</td>
<td>6.83 ± 2.44</td>
<td>5.79 ± 1.52</td>
</tr>
<tr>
<td>GPX, U/g Hgb</td>
<td>63.8 ± 6.4³</td>
<td>54.1 ± 9.1³</td>
<td>58.6 ± 9.7³</td>
<td>53.1 ± 9.2³</td>
<td>48.1 ± 7.2³</td>
</tr>
<tr>
<td>MDA, umol/L</td>
<td>0.75 ± 0.46³</td>
<td>0.61 ± 0.22³</td>
<td>0.60 ± 0.25³</td>
<td>0.26 ± 0.13³</td>
<td>0.20 ± 0.05³</td>
</tr>
<tr>
<td>SOD, U/g Hgb</td>
<td>1240 ± 185³</td>
<td>877 ± 173³</td>
<td>912 ± 185³</td>
<td>1030 ± 170³</td>
<td>997 ± 101³</td>
</tr>
<tr>
<td>TAC, mmol/L</td>
<td>1.12 ± 0.03</td>
<td>1.15 ± 0.15</td>
<td>1.39 ± 0.47</td>
<td>1.06 ± 0.09</td>
<td>1.12 ± 0.06</td>
</tr>
</tbody>
</table>

³8(OH)dG, 8-hydroxy-2’deoxyguanosine; GPX, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase; TAC, total antioxidant capacity. Values are means ± SD, n = 6 (n = 5 for GPX, MDA, and SOD for MD 12 and n = 5 for 8(OH)dG on R + 1). Significant differences in rows are represented by different letters (P < 0.05).