1   **Nutritional Assessment During a 14-d Saturation Dive: the NASA Extreme Environment**

2   **Mission Operation V Project**

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10 ABSTRACT

11   Ground-based analogs of spaceflight are an important means of studying physiological and

12   nutritional changes associated with space travel, particularly since exploration missions are

13   anticipated, and flight research opportunities are limited. A clinical nutritional assessment of the

14   NASA Extreme Environment Mission Operation V (NEEMO) crew (4 M, 2 F) was conducted

15   before, during, and after the 14-d saturation dive. Blood and urine samples were collected before

16   (D-12 and D-1), during (MD 7 and MD 12), and after (R + 0 and R + 7) the dive. The foods

17   were typical of the spaceflight food system. A number of physiological changes were reported

18   both during the dive and post dive that are also commonly observed during spaceflight. Serum

19   hemoglobin and hematocrit were decreased ($P < 0.05$) post dive. Serum ferritin and

20   ceruloplasmin significantly increased during the dive, while transferrin receptors tended to go

21   down during the dive and were significantly decreased by the last day (R + 0). Along with

22   significant hematological changes, there was also evidence for increased oxidative damage and

23   stress during the dive. 8-hydroxydeoxyguanosine was elevated ($P < 0.05$) during the dive, while

24   glutathione peroxidase and superoxide dismutase activities were decreased ($P < 0.05$) during the

25   dive. Serum C-reactive protein (CRP) concentration also tended to increase during the dive,

26   suggesting the presence of a stress-induced inflammatory response. Decreased leptin during the

27   dive ($P < 0.05$) may also be related to the increased stress. Similar to what is observed during

28   spaceflight, subjects had decreased energy intake and weight loss during the dive. Together,

29   these similarities to spaceflight provide a model to further define the physiological effects of

30   spaceflight and investigate potential countermeasures.

31

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

33   INTRODUCTION

34   Nutrition is essential for the maintenance of crew health before, during, and after spaceflight.

35   Several physiological changes occur during spaceflight, including bone and muscle loss (1),

36   oxidative damage (2), cardiovascular, and hematologic alterations (3). These may involve

37   altered nutritional status to one degree or other. Ground-based models have been used

38   extensively to study human adaptation to spaceflight (4), including disuse (e.g., bed rest) and

39   isolation (e.g., Antarctic, closed chamber studies). Underwater analogs have also been used to

40   simulate the isolation, stress, and constraints of spaceflight. They are used to better understand

41   physiological and psychological effects on humans, to assess training and operational issues, to

42   evaluate hardware and procedures, and to test the effectiveness of potential countermeasures.

43   One underwater-based analog was named the NASA Extreme Environment Mission Operation

44   (NEEMO) project, where subjects live in an underwater habitat for extended periods of time.

45   The unique underwater laboratory provides an environment similar to that aboard the

46   International Space Station (ISS). Not only is the habitat similar in size to modules of the ISS,

47   but the "aquanauts" coordinate operations remotely via a mission control center located onshore

48   (4.5 km away), and also perform extensive science and extravehicular activities during the

49   mission. In some cases, as in the study reported here, the foods provided to the crew are the

50   same as those provided to astronauts on the ISS.

51   The environment in the habitat emulated stress-induced physiological changes commonly

52   observed during spaceflight (5) and in other ground-based analogs (6). One mechanism by

53   which physiological changes occur during spaceflight is the increased stress due to

54   environmental changes such as acceleration during lift-off, weightlessness, confinement, and

55   long-term maintenance of high levels of performance. These types of stress induce hormonal

56 changes and altered immune function (7-9). Furthermore, while these stress-induced changes are

57 known to occur during spaceflight, the confounding effects of altered nutritional status (and the

58 effects on nutritional status) are not well understood and need to be clarified in order to define

59 nutritional requirements for long-term spaceflight.

60   The aim of this study was to evaluate the nutritional status of subjects in a ground-based

61 analog of spaceflight, the fifth NEEMO mission (NEEMO V). A comprehensive nutritional

62 assessment was conducted before, during, and after the mission. We hypothesized that in

63 addition to the effects of stress and confinement, that unique characteristics of the mission and

64 habitat (e.g., increased atmospheric pressure) would also impact nutrition and health.

65

66 **METHODS**

67 *Environment*

68   NEEMO V was a 14-d saturation dive, with the crew (n = 6) living in an underwater habitat.

69 The habitat is 14 m long and 4 m in diameter (**Figure 1**). It is located 20 m (47 ft) below the

70 ocean surface, with an atmospheric pressure inside the habitat of 2.5 atm. The NEEMO V

71 mission was completed in June - July of 2003. Supplies were transferred down to the habitat via

72 sealed container, and samples were returned to the surface via the same container. All tubes and

73 hardware were pre-tested to ensure that the pressure change would not alter function. Because of

74 the nature of the dive (i.e., extended-duration saturation dive), a 17-h decompression was

75 required prior to resurfacing.

76

Subjects

78   The crew for NEEMO V consisted of 2 females and 4 males. Three of the six were astronauts

79   (one with previous flight experience), one was a scientist from the Johnson Space Center, and the

80   remaining two were technicians responsible for the maintenance of the habitat. The average age

81   was 35.7 ± 6.6 y (mean $\pm$SD). All subjects were required to pass an Air Force Class III physical

82   and were required to have logged a minimum of 25 dives prior to participation in the study.

83   Before the dive, the average body weight was 69.9 ± 17.3 kg. Body fat mass, bone mineral

84   content, and lean body mass were also recorded for 4 of the crewmembers (15.3 ± 2.25, $2.51 \pm$

85   0.69, and 52.1 ± 14.5 kg, respectively).

86   Subjects were trained on all procedures required for the successful completion of the in dive

87   sample and data collections. Pre-dive dietary data was collected from a standard food frequency

88   questionnaire (REF), while in-dive food intakes were recorded for each meal using a bar code

89   reader. Dietary training was provided by the research dietitian (BLR). Two of the crewmembers

90   were trained in phlebotomy techniques, and they subsequently collected all pre- and in-dive

91   blood samples.

92

93   *Body mass and body composition determinations*

94   Body mass was determined using a calibrated scale on the days when body composition was

95   determined, and using a standard scale on all other days. For the in-dive determinations, a

96   standard scale was tested in the habitat and was found to function reliably in the high pressure

97   atmosphere. This scale was subsequently used for the remainder of the study.

98   Body composition was determined (4 subjects only) before and after the dive using dual

99   energy x-ray absorptiometry (DEXA). Dual-energy x-ray absorptiometry (DEXA) scans were

100 performed using a Hologic QDR 4500W (Hologic, Inc., Waltham, MA) fan beam densitometer.

101 Whole body scans were performed before and after the mission for body composition

102 assessment.

103

104 *Sample Collection and Processing*

105 Blood (25.7 mL) was collected before (dive minus twelve days, D-12 and D-1), during the

106 dive [mission day 7 (MD 7) and MD 12], and post-dive (return plus zero days, designated R + 0,

107 and R + 7). For two of the subjects, the first pre-dive collection was completed at D-5/-4. Blood

108 collections were performed at the same time each day following an 8-h fast.

109 Urine was collected before (D-12, D-11, D-1; except for two subjects where samples were

110 collected on D-5, D-4, and D-1), during (MD 7, MD 12), and after the dive (R + 0, R + 1, R + 7,

111 and R + 8). Pre- and post-dive samples were collected in individual bottles and stored cool until

112 processing (<24 hours). During the dive, the crew collected voids either into a beaker or a

113 graduated cylinder. Volumes were recorded, and a 50 mL aliquot from each void was sent to the

114 surface. All urine and blood samples were kept in a cooler on ice in the habitat before (and

115 during) ascent to the surface. The samples were also kept on ice aboard the boat when returning

116 to shore. 24-h pools were created based on void volumes, and aliquots were prepared and frozen

117 for analysis as soon as possible on shore.

118 For tests where storage would alter the results (e.g., malondialdehyde, hematocrit, and

119 hemoglobin), these were run in the laboratory facilities on shore. Others remained frozen on dry

120 ice until return to the Johnson Space Center in Houston.

121

122   *Biochemical Analyses*

123   Most analytical determinations were completed using standard, commercial techniques as

124   described previously (6). Hemoglobin, hematocrit (calculated), and mean corpuscular volume

125   were determined using a Coulter T890 instrument (Beckman Coulter, Brea, CA). Serum ferritin

126   and transferrin were analyzed using the Immulite (Diagnostics Products, Los Angeles, CA) and

127   Array 360 instruments, respectively (Beckman Coulter). Transferrin receptors were measured

128   using a commercially available ELISA (Ramco Laboratories, Houston, TX). RBC folate was

129   measured using a commercially available radioreceptor assay (Diagnostic Products, Los Angeles,

130   CA). Ferritin iron content was determined by ICP-MS using a method previously described (6).

131   Whole blood ionized calcium and electrolytes were determined using ion-sensitive electrode

132   techniques with a portable analyzer (i-STAT, Princeton, NJ) (6,10) . Despite attempts to use the

133   portable device *in situ* during the mission, the pressure differential did not allow for proper

134   functioning of the device. These tests were subsequently performed on samples once they were

135   returned to the surface.

136   Urine and serum total calcium was measured by inductively coupled plasma emission mass

137   spectrophotometry techniques (11). Serum intact parathyroid hormone was measured by RIA

138   (Nichols Institute Diagnostics, San Juan Capistrano, CA). Vitamin D metabolites 25-

139   hydroxyvitamin D and 1,25-dihydroxyvitamin D were also determined using commercially

140   available kits (DiaSorin, Stillwater, MN). Bone-specific alkaline phosphatase was measured by

141   ELISA (Quidel Corp, Santa Clara, CA, USA). Serum osteocalcin was measured by commercial

142   radioimmunoassay (Biomedical Technologies).

143   Urine samples were analyzed for collagen cross-links using commercially available kits

144   (METRA PYD and DPD EIA kits, Quidel Corp.; and Osteomark ELISA kit; Ostex International,

145   Inc., Seattle, WA, USA) as previously described (12). Crosslink data were expressed as nmol

146   excretion per day, as we have demonstrated that this reduces within-subject variability (13).

147   RBC superoxide dismutase, glutathione peroxidase, and serum oxygen-radical absorbance

148   capacity were measured spectrophotmetrically using commercially available kits (Randox

149   Laboratories, Crumlin, UK). HPLC techniques (14) were used to determine 8-hydroxy-2'-

150   deoxyguanosine in urine. Plasma MDA was measured using a commercially available kit

151   (Calbiochem Lipid Peroxidation Assay kit, EMD Biosciences, Inc., San Diego, CA).

152   Serum total protein, cholesterol, triglycerides, sodium, potassium, chloride, aspartate

153   aminotransferase, alanine aminotransferase, RBC transaminase, and total alkaline phosphatase

154   were analyzed using a Beckman CX7 automated clinical chemistry system (Beckman Coulter,

155   Brea, CA). Serum albumin and transthyretin were analyzed using the Beckman Appraise and

156   Array 360 instruments, respectively (Beckman Coulter). Urine creatinine was analyzed on the

157   NexCT (Alfa Wassermann, West Caldwell, NJ).

158

159   *Statistical Analysis*

160   Data are reported as means ± SD. Dietary data and biochemical data were analyzed using

161   repeated-measures analysis of variance (ANOVA) with a post-hoc Bonferroni test to determine

162   differences among groups. Statistical analyses were performed using SigmaStat (SPSS, Chicago,

163   IL).

164

165   **RESULTS**

166

167   *Dietary Intake*

168   Pre-dive dietary intakes were determined (mean ± SD) for energy, fat, protein, calcium, and

169   iron (2100 ± 613 kcal, 84.7 ± 24.2 g, 83.1 ± 33.0 g, 774 ± 327 mg, and 19.7 ± 14.4 mg,

170   respectively) using a food frequency questionnaire. Energy intake was significantly lower than

171   the World Health Organization recommendations during the dive on MD 5, MD 6, and MD 11

172   (**Table 1**). Mid-dive means (± SD) were also determined for vitamin D, fat, protein, calcium,

173   and iron (5.46 ± 5.28 μg, 63.3 ± 20 g, 77.5 ± 28 g, 1002 ± 387 mg, and 22.3 ± 9.56 mg,

174   respectively).

175

176   *Body Weights and Composition*

177   Body weights were significantly lower from pre dive weights on MD 7-14 ($P < 0.05$), and

178   higher than pre dive weights on R + 7 (Table 1). Body fat, bone mineral content, and lean body

179   mass were not different (n=4) when these measurements from R + 7 were compared with pre

180   dive measurements (Table 1).

181

182   *Hematology and general chemistry*

183   Hemoglobin and hematocrit were both decreased ($P < 0.05$) at R + 0 compared to pre dive

184   and MD7 (**Table 2**). Serum mean corpuscular volume (MCV) was significantly decreased ($P <$

185   0.05) on the last collection day post flight (R + 7) compared to in-dive (MD 7 and MD 12) and R

186   + 0 (Table 2). There was a significant decrease in serum iron post dive (R + 7) compared to in-

187   dive (MD 7 and MD 12).

188   Serum ferritin was significantly elevated both days in-id dive and R + 0 compared to pre and

189   post (R + 7) dive (Table 2). Similarly, serum ceruloplasmin tended to increase during the dive

190 and was significantly elevated R + 0 (**Table 3**). Transferrin receptors in serum tended to

191 decrease in-dive but the decrease compared to pre dive was only significant on R + 0 (Table 2).

192 Ferritin iron, transferrin, and ferritin saturation were unchanged throughout the study.

193 Triglycerides were elevated post dive (R + 7) compared to pre dive. Serum leptin tended to

194 decrease during the dive and was significantly different from pre dive on R + 0 (Table 3).

195 Electrolyte pools were also altered in response to conditions during NEEMO V. Sodium and

196 chloride excretion were decreased ($P < 0.05$) during the dive (MD 7 and MD 12) compared to

197 pre dive (**Table 4**), while urine excretion volume remained constant during the study. Serum

198 sodium concentration was significantly higher during the dive (MD 7 and MD 12) and after the

199 dive (R + 7) compared to pre dive ($P < 0.05$, Table 3). Whole blood sodium and

200 potassium.....showed the same changes? (data in table, or data not shown...)

201

202 *Calcium and bone metabolism*

203 Urinary calcium was significantly elevated post-dive (R + 8) compared to mid-dive (MD 12)

204 (**Table 5**). Serum total calcium was unaltered during the study; however, serum ionized calcium

205 was increased ($P < 0.05$) on MD 12 and post dive (R + 7) compared to pre dive (Table 5).

206 Urinary collagen crosslinks (NTX, PYD, and DPD) were unaffected during or after the dive.

207 Serum osteocalcin was significantly elevated post dive (R + 7) compared to in-dive (MD 7

208 and MD12), but other markers of bone formation including serum total alkaline phosphatase and

209 bone-specific alkaline phosphatase were unchanged during the study (Table 5). Similarly, there

210 was no effect on serum vitamin D metabolites, 25-hydroxy vitamin D or 1,25-dihydroxy vitamin

211 D (Table 5).

212

213 *Antioxidant status*

214   Urinary 8-hydroxy 2'-deoxyguanosine (8OHdG) excretion was significantly elevated during

215 the dive (MD 7 and MD12) compared to pre dive ($P < 0.05$) **(Table 6)**. Other markers of

216 antioxidant status and function were also altered during and post dive, including whole blood

217 glutathione peroxidase (GPX) activity, superoxide dismutase (SOD) activity, and plasma

218 malondialdehyde (MDA). Whole blood GPX tended to decrease during the dive and R + 0 but it

219 was not significantly decreased until R + 7 (Table 6). Whole blood SOD was decreased MD 7,

220 and this significant decrease ($P < 0.05$) compared to the pre dive continued throughout the

221 remainder of the study (MD 12, R + 0, and R + 7). Plasma MDA was significantly decreased

222 post dive (R + 0 and R + 7) compared to pre dive. Red blood cell glutathione reductase activity

223 was decreased ($P < 0.05$) at the latter part of the dive (MD 12 and R + 0), but was returned to pre

224 dive concentrations 7 d after the dive (Table 3).

225

226 **DISCUSSION**

227   Limitations on resources (e.g., time, power, volume, up/down mass) for spaceflight research

228 necessitate the development of Earth-based analogs. The underwater isolation of the NEEMO

229 missions provides one such analog of spaceflight, with obvious similarities, along with obvious

230 limitations. The study we report here clearly identifies this as a valuable analog environment,

231 with results that resemble many aspects of spaceflight beyond the direct nutritional implications

232 (e.g., dietary intake). In further defining the changes that occurred in crew members, we will be

233 better able to propose, design, and test countermeasures for future missions.

234   The hematological findings are striking, and extend those from earlier dive studies, as well as

235 apply to hematological changes seen during spaceflight. Reductions in hemoglobin

236   concentration and increases in serum ferritin concentration are well established in deep saturation

237   dives (depths up to 660 m, 31 – 67 atm) (15-17). These same effects were observed after the 14-

238   d shallow saturation dive described here (14.3 m, 2.5 atm). Reduced hemoglobin concentrations

239   suggest a reduction in red blood cell mass, which could be due to decreased production of new

240   red blood cells, as seen in spaceflight (18), or destruction of existing red blood cells by oxidative

241   damage (15,19).

242   Increased serum ferritin in-dive and decreased transferrin receptors R + 0 were also observed

243   and would be expected when iron stores and intracellular iron availability are high. It is likely

244   that the increased oxygen availability, induced by the increased atmospheric pressure,

245   contributed to a decreased need for red blood cells, and iron pools were consequently shifted

246   from hemoglobin to a storage form. This process, termed neocytolysis, has been documented in

247   spaceflight (20,21), as well as in subjects traveling from high to low altitude (22).

248   While ferritin iron content did not increase along with the increased serum ferritin, ferritin

249   iron and serum iron both tended to go up during the dive. One possibility for lack of significance

250   is the small sample size (n = 6 for pre dive, MD 7, R + 0, and R + 7; n = 5 for MD 12). Another

251   possibility is that the increase in total serum ferritin is indicative of recruitment of ferritin from

252   preexisting stores, and that the time course is too short for enrichment of ferritin with excess iron

253   to alter the reflection in the serum. There is also a possibility that the changes of serum ferritin

254   during the dive were due to an acute inflammatory response since there were other indications

255   that such a response might have occurred. Other acute phase proteins tended to go up during the

256   dive. While not significant, serum C-reactive protein tended to be elevated during the dive

257   compared to pre and post dive. The large variances prevented these findings from being

258   significant. Again, we are limited with the very small sample size in this study. Furthermore,

259   other studies suggest that oxidative stress is increased during the acute inflammatory phase of

260   many illnesses (24,25), which was also observed in one subject prior to the dive.

261   Alterations in antioxidant markers were hypothesized due to the hyperbaric environment.

262   Along with the increased 8OHdG excretion observed during the dive, decreased activities of

263   GPX and SOD post (GPX and SOD) and in-dive (SOD) imply increased oxidative stress. A

264   number of other parameters (besides the environment) could have contributed to this, including

265   changes in nutrient intake or changes in stress hormones. The significant decrease in MDA

266   suggests that lipid peroxidation is decreased in and post dive compared to pre dive, which does

267   not support the theory of increased oxidative damage. This decrease is not easily explained since

268   we would have expected to see similar changes during the dive for 8OHdG and MDA. Pre dive

269   measurements for all parameters are averages of measurements recorded twice before the dive,

270   and there appeared to be differences between the two collection points (pre dive day 12 for MDA

271   was high compared to pre dive day 1; specifically 1.25 ± 0.96 and 0.26 ± 0.18 μmol/L for pre

272   dive days 12 and 1, respectively). If only pre dive day 1 was used for comparison (instead of the

273   average of the two), MDA then tended to increase during the dive compared to pre and post dive.

274   Mean body weights were significantly lower than pre dive weights during the latter part of the

275   dive (MD 7-14). During the dive, energy intakes were lower than World Health Organization

276   recommendations (Table 1). This is a similar phenomenon that consistently occurs during

277   spaceflight (6,26,27), and explains why body weights were concurrently decreased. Serum leptin

278   was measured in these individuals and we found that these concentrations tended to go down

279   during the dive and were significantly decreased by the last day (R + 0). Leptin is normally

280   involved in the regulation of food intake and in the maintenance of energy balance, but its role in

281   the decreased energy intake in this study is unknown and warrants further investigation. Other

282　studies have linked decreased leptin concentration with periods of intense exercise, possibly

283　indicative of increased stress or inflammation (28,29). Again, consistent with other findings

284　outlined above, the decreased leptin observed here may support the presence of an acute

285　inflammatory response during the dive.

286　　Despite increased pressure in the habitat, there was no evidence for alterations in bone

287　formation/resorption during the dive. While osteocalcin was significantly higher post dive (R +

288　7) compared to in-dive (MD 7 and MD 12), other bone formation markers in the serum,

289　including alkaline phosphatase and bone-specific alkaline phosphatase, were unchanged during

290　the study. Bone resorption markers were unchanged during the dive. Parathyroid hormone and

291　vitamin D concentrations tended to decrease, but not significantly. Both of these indices might

292　have reached statistical significance with a longer mission (due to lack of ultraviolet light

293　exposure) or with additional subjects. Furthermore, these findings enhance our recent

294　observations that lower body negative pressure (LBNP) can mitigate disuse-induced bone

295　resorption (30). The current study, one of whole body positive pressure, suggests that the

296　findings with LBNP may be more related to circulatory changes than to pressure itself. Such

297　suggestions that circulatory influences may impact weightlessness-induced bone loss are not new

298　(31,32).

299　　It is evident that there are indeed many physiological and nutritional changes that occurred

300　during NEEMO V that are also commonly observed during spaceflight. Changes in nutritional

301　status during spaceflight are of critical concern for future long duration space travel, and

302　spaceflight analogs such as NEEMO V may be increasingly important to further investigate

303　potential countermeasures.

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396

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

	PRE	MD2	MD3	MD4	MD5	MD6	MD7	MD8	MD9	MD10	MD11
Energy Intake, kcal	-	2590 ± 613	2660 ± 925	2600 ± 851	2010 ± 886**	1790 ± 654**	2384 ± 658	2380 ± 510	2550 ± 471	2400 ± 688	2110 ± 362**
Energy Intake (% WHO)	-	90.0 ± 7.2	92.2 ± 21.1	89.6 ± 16.2	71.4 ± 27.9	60.9 ± 12.9	83.1 ± 17.8	83.3 ± 10.6	91.0 ± 17.4	82.5 ± 9.5	75.1 ± 12.4
Water Intake, mL	-	3000 ± 999	3260 ± 912	2510 ± 751	1960 ± 689	1910 ± 1140	2410 ± 877	2790 ± 810	3810 ± 1960	2680 ± 1210	2200 ± 634
BW (kg)	76.0 ± 16.1	75.2 ± 16.6	75.4 ± 15.9	75.1 ± 16.2	75.0 ± 16.0	75.0 ± 15.5	74.6 ± 15.7*	74.3 ± 15.8*	74.5 ± 15.9*	74.6 ± 15.6*	74.2 ± 15.8*
Body Mass (DEXA) (kg)	69.9 ± 17.3	-	-	-	-	-	-	-	-	-	-
Body Fat (kg)	15.3 ± 2.3	-	-	-	-	-	-	-	-	-	-
BMC	2.5 ± 0.7	-	-	-	-	-	-	-	-	-	-
LBM (kg)	52.1 ± 14.5	-	-	-	-	-	-	-	-	-	-

[1]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).

402   Table 2. Hemotologic, iron, and folate status indicators before, during, and after NEEMO V[1].

	Pre	MD 7	MD 12	R + 0	R + 7
Serum Hgb, g/L	136 ± 15^{ac}	138 ± 10^{ac}	137 ± 14^{cd}	128 ± 13^{bd}	131 ± 13^{cd}
Serum HCT	0.41 ± 0.04^{ac}	0.41 ± 0.03^{ac}	0.40 ± 0.04^{cd}	0.38 ± 0.04^{bd}	0.40 ± 0.04^{cd}
Serum MCV, fL	91 ± 3^{ab}	92 ± 3^{a}	92 ± 4^{a}	92 ± 3^{a}	90 ± 2^{bc}
Serum Iron, umol/L	19 ± 7^{ac}	26 ± 10^{a}	27 ± 10^{a}	22 ± 5^{ac}	12 ± 4^{bc}
Ferritin Iron, umol/L	0.34 ± 0.15	0.48 ± 0.19	0.44 ± 0.17	0.47 ± 0.17	0.32 ± 0.12
Serum Ferritin, ug/L	102 ± 63^{a}	168 ± 82^{b}	219 ± 98^{b}	196 ± 92^{b}	117 ± 70^{a}
Ferritin Saturation, %	22.8 ± 9.2	18.2 ± 8.7	12.0 ± 3.2	14.6 ± 3.8	18.6 ± 8.5
Transferrin Receptors, ug/mL	4.8 ± 1.3^{ac}	4.6 ± 1.1^{cd}	4.3 ± 0.8^{cd}	3.9 ± 0.9^{bd}	4.1 ± 1.0^{cd}
Transferrin, g/L	2.63 ± 0.18	2.66 ± 0.29	2.65 ± 0.14	2.53 ± 0.24	2.62 ± 0.18
RBC Folate, nmol/L	1705 ± 486	1598 ± 575	1683 ± 365	1445 ± 486	1554 ± 429

403

404   [1]Hgb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; RBC, red blood cell.

405   Values are means ± SD, n = 6 (n = 5 on MD 12 for all parameters, except transferrin receptors

406   where n = 6 for all days). Significant differences in rows are represented by different letters (P <

407   0.05).

408 Table 3. General blood chemistry before, during, and after NEEMO V[1].

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

409

410 [1]RBP, retinol binding protein; RBC, red blood cell; GSH, glutathione reductase; ALT, alanine

411 aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein. Values are means

412 $\pm$ SD, n = 6 (n = 5 on MD 12 for all parameters except for serum RBP, where n = 6 for all days).

413 Significant differences in rows are represented by different letters (P < 0.05).

414   Table 4. General urine chemistry before, during, and after NEEMO V[1].

	Pre	MD 7	MD 12	R + 0	R + 1	R + 7	R + 8
Urine pH	6.0 ± 0.16	6.22 ± 0.41	6.25 ± 0.33	6.07 ± 0.47	6.46 ± 0.45	6.21 ± 0.55	6.04 ± 0.31
Urine excretion volume, mL	2160 ± 676	1970 ± 1260	2410 ± 944	2670 ± 1390	1670 ± 647	2110 ± 1040	2620 ± 1940
Urine Sodium, mmol/d	182 ± 21^a	120 ± 71^b	106 ± 44^b	98.7 ± 32.1	124 ± 42	163 ± 31	191 ± 58
Urine Potassium, mmol/d	62.2 ± 15.7^{ab}	47.3 ± 15.0^a	53.0 ± 18.5^{ab}	46.8 ± 18.2	53.8 ± 24.1	72.5 ± 25.2	64.8 ± 20.7
Urine Chloride, mmol/d	176 ± 16^a	98.5 ± 60.4^b	90.2 ± 31.9^b	87.7 ± 30.6	107 ± 24	159 ± 25	193 ± 69
Urine 3MH, umol/d	245 ± 122	242 ± 138	222 ± 116	230 ± 93	270 ± 104	271 ± 112	269 ± 114
Urine Iodine, mmol/d	2.46 ± 0.91	2.12 ± 0.84	1.77 ± 0.71	2.68 ± 1.13	1.80 ± 0.29	2.18 ± 0.96	2.60 ± 1.39
Urine Mg, mmol/d	3.89 ± 1.05	4.34 ± 1.39	3.78 ± 1.44	4.67 ± 1.67	3.41 ± 1.96	4.43 ± 0.94	5.06 ± 1.60
Urine Phos, mmol/d	30 ± 7	34 ± 11	33 ± 12	27 ± 10	23 ± 8	29 ± 11	29 ± 6
Urine Creatinine, mmol/d	16.3 ± 3.7	15.2 ± 5.2	17.5 ± 5.2	16.2 ± 4.0	13.5 ± 7.4	16.3 ± 4.0	16.1 ± 3.5

415

416   [1]3MH, 3-methylhistidine. Values are means ± SD, n = 6 (n = 5 on R + 1). Significant

417   differences in rows are represented by different letters (P < 0.05).

418   Table 5. Calcium and bone markers before, during, and after NEEMO V[1].

	Pre	MD 7	MD 12	R + 0	R + 1	R + 7	R + 8
Serum iPTH, pg/mL	31.1 ± 7.82	25.8 ± 9.72	24.3 ± 6.87	28.8 ± 10.2	-	29.6 ± 9.81	-
Serum Osteocalcin, ng/mL	9.30 ± 2.10^{ac}	7.43 ± 1.02^{a}	7.75 ± 1.46^{a}	8.35 ± 1.73^{ac}	-	10.7 ± 2.90^{bc}	-
Alk P'ase							
Serum total Alk Phosphata	57.7 ± 22.0	61.0 ± 17.7	66.6 ± 20.0	61.7 ± 22.3	-	61.3 ± 21.5	-
Serum BSAP, U/L	21.7 ± 7.80	20.7 ± 7.20	21.4 ± 8.60	21.7 ± 8.06	-	19.7 ± 7.45	-
Serum 25 OH Vit D, nmol/L	92 ± 23	86 ± 30	81 ± 18	86 ± 18	-	81 ± 24	-
Serum 1,25 OH Vit D, pmol/L	211 ± 52.4	212 ± 105	221 ± 80.6	198 ± 108	-	201 ± 64.1	-
Calcium							
Serum TCa, mmol/L	2.56 ± 0.11	2.70 ± 0.18	2.57 ± 0.15	2.57 ± 0.10	-	2.67 ± 0.22	-
Serum ICa, mmol/L	1.18 ± 0.03	1.23 ± 0.05	1.26 ± 0.02*	1.21 ± 0.03	-	1.21 ± 0.03*	-
Urine Ca, mmol/L	5.49 ± 0.73^{ac}	4.83 ± 1.85^{ac}	3.98 ± 0.91^{a}	5.13 ± 1.51^{ac}	4.92 ± 1.21^{ac}	6.12 ± 2.33^{ac}	6.82 ± 2.18^{bc}
DPD, nmol/d	63.9 ± 7.4	73.7 ± 24.9	70.2 ± 20.2	61.6 ± 15.5	60.3 ± 8.6	66.8 ± 17.3	57.0 ± 19.8
NTX, nmol/d	464 ± 235	690 ± 384	480 ± 287	430 ± 208	499 ± 271	539 ± 363	556 ± 240
PYD, nmol/d	256 ± 51	307 ± 85	292 ± 78	297 ± 98	224 ± 68	285 ± 87	260 ± 89

419

420

421   [1]iPTH, intact parathyroid hormone; Alk Phosphatase, alkaline phosphatase; BSAP, bone-specific

422   alkaline phosphatase; DPD, deoxypyridinoline; NTX, n-telopeptide; PYD, pyridinium crosslinks.

423   Values are means ± SD, n = 6 (n = 5 on MD 12 fortotal alkaline phosphatase and n = 5 on R + 1

424   for DPD, NTX, and PYD). Significant differences in rows are represented by different letters (P

425   < 0.05). *Significantly different from pre dive (P < 0.05).

426   Table 6. Antioxidant/oxidative damage indices from NEEMO V crew members[1].

	Pre	MD 7	MD 12	R + 0	R + 7
8(OH)dG, ug/d	5.71 ± 1.28^a	7.25 ± 2.26^{bce}	7.41 ± 2.12^{bd}	6.83 ± 2.44	5.79 ± 1.52
GPX, U/g Hgb	63.8 ± 6.4^a	54.1 ± 9.1^{ab}	58.6 ± 9.7^{ab}	53.1 ± 9.2^{ab}	48.1 ± 7.2^b
MDA, umol/L	0.75 ± 0.46^a	0.61 ± 0.22^{ab}	0.60 ± 0.25^{ab}	0.26 ± 0.13^b	0.20 ± 0.05^b
SOD, U/g Hgb	1240 ± 185^a	877 ± 173^b	912 ± 185^b	1030 ± 170^b	997 ± 101^b
TAC, mmol/L	1.12 ± 0.03	1.15 ± 0.15	1.39 ± 0.47	1.06 ± 0.09	1.12 ± 0.06

427

428   [1]8(OH)dG, 8-hydroxy-2'deoxyguanosine; GPX, glutathione peroxidase; MDA,

429   malondialdehyde; SOD, superoxide dismutase; TAC, total antioxidant capacity. Values are

430   means $\pm$ SD, n = 6 (n = 5 for GPX, MDA, and SOD for MD 12 and n = 5 for 8(OH)dG on R +

431   1). Significant differences in rows are represented by different letters ($P < 0.05$).

432