



**HAL**  
open science

## **Effect of exercise on energy expenditure and body composition in astronauts onboard the International Space Station: Considerations for interplanetary travel**

Pierre Bourdier, Alexandre Zahariev, Dale A Schoeller, Isabelle Chery, Elisa Le Roux, Cécile Thevenot, Alain Maillet, Maël Garnotel, Guillemette Gauquelin-Koch, Audrey Bergouignan, et al.

### ► To cite this version:

Pierre Bourdier, Alexandre Zahariev, Dale A Schoeller, Isabelle Chery, Elisa Le Roux, et al.. Effect of exercise on energy expenditure and body composition in astronauts onboard the International Space Station: Considerations for interplanetary travel: Considerations for interplanetary travel. *Sports Medicine*, 2022, 52 (12), pp.3039-3053. 10.1007/s40279-022-01728-6 . hal-03727261

**HAL Id: hal-03727261**

**<https://hal.science/hal-03727261>**

Submitted on 19 Jul 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Original research article**

2  
3 **Effect of exercise on energy expenditure and body composition in astronauts onboard the International**  
4 **Space Station: Considerations for interplanetary travel**

5  
6 Bourdier Pierre<sup>1</sup>, Zahariev Alexandre<sup>1</sup>, Schoeller Dale A<sup>2</sup>, Chery Isabelle<sup>1</sup>, Le Roux Elisa<sup>1</sup>, Thevenot Cécile<sup>3,4</sup>,  
7 Maillet Alain<sup>3,4</sup>, Garnotel Maël<sup>1</sup>, Gauquelin-Koch Guillemette<sup>5</sup>, Bergouignan Audrey<sup>1,6§\*</sup>, Blanc Stéphane<sup>1§</sup>,  
8 Simon Chantal<sup>7,8§</sup>

9 <sup>§</sup>Co-last authors

10  
11  
12 **Affiliations**

13 <sup>1</sup> CNRS IPHC UMR 7178 Université de Strasbourg, Strasbourg, France

14 <sup>2</sup> Biotechnology and Nutritional Sciences, University of Wisconsin, Madison, WI, USA

15 <sup>3</sup> MEDES, Institut de Médecine et Physiologie Spatiales, Toulouse, France

16 <sup>4</sup> CADMOS-CNES, 18 Av. Edouard Belin, 31401 Toulouse, France

17 <sup>5</sup> Centre National d'Etudes Spatiales, Paris France

18 <sup>6</sup> Division of Endocrinology, Metabolism and Diabetes, Anschutz Health & Wellness Center, University of  
19 Colorado, Anschutz Medical Campus, Aurora, CO, USA

20 <sup>7</sup> CarMen Laboratory, INSERM 1060, INRAE 1397, University of Lyon, F-69600 Oullins, France

21 <sup>8</sup> Human Nutrition Research Centre of Rhône-Alpes, Hospices Civils de Lyon, Lyon, France

22  
23 \*Corresponding author

24 Stéphane Blanc,

25 CNRS, 23 rue Becquerel, 67000 Strasbourg,

26 tel: +33388106900

27 stephane.blanc@iphc.cnrs.fr

28  
29 **ORCID**

30 Bourdier P: 0000-0003-4216-7725

31 Schoeller DA : 0000-0002-5770-3853

32 Le Roux E: 0000-0001-5126-4848

33 Bergouignan A:0000-0002-1266-5144

34

35 **Running heading** (84 characters)

36 Body composition and energy requirements in astronauts onboard the International Space Station

37

38 **Abstract** (277 words)

39 *Objective:* Body mass (BM) loss and body composition (BC) changes threaten astronauts' health and mission  
40 success. However, the energetics contribution of the exercise countermeasure to these changes has never been  
41 investigated during long-term missions. We studied energy balance and BC in astronauts during 6-month missions  
42 onboard the International Space Station.

43

44 *Methods:* Before and after at least 3 months in space, BM, BC, total and activity energy expenditure (TEE and  
45 AEE) were measured using the doubly labeled water method in 11 astronauts (2011- 2017). Physical activity (PA)  
46 was assessed by the SensewearPro® activity-device.

47

48 *Results:* Three-month spaceflight decreased BM (-1.20kg (SE 0.5);  $P=0.04$ ), mainly due to non-significant fat-free  
49 mass loss (FFM; -0.94kg (0.59)). The decrease in walking time (-63.2min/day (11.5);  $P<0.001$ ) from preflight was  
50 compensated by increases in non-ambulatory activities (+64.8min/day (18.8);  $P<0.01$ ). Average TEE was  
51 unaffected but a large interindividual variability was noted. Astronauts were stratified into those who maintained  
52 (stable\_TEE; n=6) and those who decreased (decreased\_TEE n=5) TEE and AEE compared to preflight data.  
53 Although both groups lost similar BM, FFM was maintained and FM reduced in stable\_TEE astronauts, while  
54 FFM decreased and FM increased in decreased\_TEE astronauts (estimated between-group-difference (EGD) in  
55  $\Delta\text{FFMindex}$  [FFMI] 0.87kg/m<sup>2</sup>, 95%CI +0.32 to +1.41;  $P=0.01$ ,  $\Delta\text{FMindex}$  [FMI] -1.09kg/m<sup>2</sup>, 95%CI -2.06 to -  
56 0.11kg/m<sup>2</sup>;  $P=0.03$ ). The stable\_TEE group had higher baseline FFMI, and greater baseline and inflight vigorous  
57 PA than the decreased\_TEE group ( $P<0.05$  for all).  $\Delta\text{FMI}$  and  $\Delta\text{FFMI}$  were respectively negatively and positively  
58 associated with both  $\Delta\text{TEE}$  and  $\Delta\text{AEE}$ .

59

60 *Conclusion:* Both ground fitness and inflight overall PA are associated with spaceflight-induced TEE and BC  
61 changes and thus energy requirements. New instruments are needed to measure real-time individual changes in  
62 inflight energy balance components.

63

64 **Word counts:** 5486

65 **Key points**

66

- Space agencies ranked energy balance control to critical level for human space exploration. Although exercise is a cornerstone of countermeasure programs to prevent microgravity-induced physiological alterations, the energy cost of physical activity during long-term spaceflights has never been studied.
- Six-month missions on the ISS led to large inter-individual variability in body composition changes. Astronauts maintaining pre-flight total and activity energy expenditures (possibly due to both physical training and unexpected non-exercise activity), maintained fat-free mass but lost fat mass. Conversely those who decreased energy expenditures lost fat-free mass but gained fat mass. Fat mass changes reflects unmatched energy intake adaptation to expenditures during flight.
- On average, astronauts who maintained energy expenditures during flight were also fitter on the ground.
- The large between-astronauts variability suggests that energy requirements cannot be derived from general population equations. Methods to track inflight changes in body composition, energy intake and energy expenditure are needed to determine individual energy requirements to ensure astronaut's performance and mission success.

67

68

69 **Supplementary Information** – The online version contains supplementary material available at xxxx.

70

71 **Acknowledgments**

72 The authors are grateful to the astronauts for their dedication to the study. Special thanks also to the Danish  
73 Aerospace Company (former DAMEC, Odense Denmark) and CADMOS Center (Toulouse, France) for the  
74 preparation, operational support during baseline data collection on the different training sites (Houston, TX, USA;  
75 Koln, Germany) but also during the ground support of the inflight sessions. We are thanking Tanya Halliday for  
76 her support in the energy intake assessment.

77

78 **Declarations**

79 *Funding* – This study was funded by the French Space Agency (CNES, French Space Agency) and the European  
80 Space agency which provided financial and operational supports for the Energy experiment.

81 *Conflicts of interest* – PB, AZ, DSA, IC, ELR, CT, AM, MG, GGK, AB, CS and SB declare that they have no  
82 potential conflicts of interest that might be relevant to the contents of this manuscript.

83 *Availability of data and material* – Data are accessible upon reasonable request to the authors after validations by  
84 space agencies.

85 *Ethics approval* – The study was yearly approved by the NASA Institutional Review Board under NASA  
86 7116301606HR. The ESA Medical Board and the JAXA Institutional Review Board for human experiment also  
87 approved the experiment.

88 *Consent to participate* – The study was conducted in conformity with the policy statement regarding the use of  
89 human participants as outlined in the Declaration of Helsinki of 1964. All astronauts received a detailed  
90 presentation of the experiment before enrolling the study and signed a written informed consent.

91 *Consent for publication* – A global authorization from the European Space Agency was obtained to use and publish  
92 the photos (ESA copyright).

93

94 **Author contribution** – SB, DAS, GGK and AM designed the study. PB, AB, CS and SB drafted the manuscript.  
95 AZ, CT, AM, GGK, CS and SB collected data. PB, ELR, AZ, DAS, IC, MG, AB, CS and SB analyzed the data.  
96 CS and PB realized the statistical analysis. All the authors read and approved the final version of the manuscript.

97

98 **1 INTRODUCTION**

99 The human spaceflight program has entered a new phase of space exploration directed towards the Moon and  
100 Mars. Space Agencies have developed roadmaps that define research priorities enabling planetary exploration.  
101 Among those, understanding inflight energy balance regulation has been placed as a top priority, as energy  
102 homeostasis is vital for both medical and operational reasons. Any energy deficit will not only impact fat mass  
103 (FM), but it can also increase loss of bone and of muscle mass and strength, favor cardiovascular deconditioning  
104 and impair numerous other physiological functions. Overall it conditions astronaut's performance and health, and  
105 ultimately may affect mission success [1].

106 A loss of body mass (BM) is a hallmark of spaceflights. On Mir (4 months)[2, 3], Shuttle (4 to 19 days)[4, 5] and  
107 early International Space Station (ISS) missions (128 to 195 days)[6, 7], astronauts lost more than 5% of their  
108 preflight BM despite sufficient food onboard with no clear relationship with mission duration [8]. In some cases,  
109 this loss even exceeded 10% of preflight BM, which is clinically significant. Conversely, BM was successfully  
110 maintained in few missions, such as SpaceLab Life Sciences Space Shuttle missions SLS1 and SLS2 in the 1990s  
111 [9] or, more recently, on the ISS [7, 10]. Yet, recent reports from the ISS show that astronauts still lose from 2 to  
112 5% of their initial BM with a large between-subject variability [6, 11]. We recently hypothesized that an energy  
113 balance dysregulation, i.e. an uncoupling between energy intake (EI) and expenditure, occurs in space [8]. Very  
114 little data, however, exist to support this hypothesis.

115 The regulation of energy balance was measured with objective doubly labeled water (DLW) methods in three  
116 studies during short term spaceflights only [4, 9, 12]. The results of two of these studies indicate that energy  
117 balance is not optimal in space when exercise is performed, i.e. compensatory changes in EI are insufficient to  
118 match increases in energy expenditure [13]. During the 17-day Life and Microgravity Science (LMS) Space Shuttle  
119 mission, Stein et al. [4] observed in four astronauts an energy deficit as large as 5.7 MJ/day that was associated  
120 with the loss of up to 2 kg of fat mass (FM). Concomitantly, even though heavy exercise training was specifically  
121 prescribed during this mission to prevent loss of muscle mass and bone, astronauts presented a negative nitrogen  
122 balance indicating protein loss related to a maladjustment of energy and protein intakes to high requirements. By  
123 contrast, during the SLS1 and SLS2 missions, during which no exercise was prescribed, the astronauts maintained  
124 stable energy balance with only a moderate negative nitrogen balance likely thanks to the maintenance of protein

125 intakes despite an adapted reduction in energy intakes. [9]. Because exercise is the cornerstone of the  
126 countermeasure program to prevent unloading-induced loss of skeletal muscle mass and strength, bone, aerobic  
127 capacity and other health-related outcomes [14, 15], these observations suggest that understanding its role in  
128 energy balance regulation is critical, notably for long-duration missions.

129 This exploratory study was therefore designed to measure total energy expenditure (TEE) and activity energy  
130 expenditure (AEE), using the gold standard DLW method, in relation to body composition in 11 astronauts after  
131 at least 3 months onboard the ISS. Inferences to evaluate energy requirements in space were derived. We  
132 hypothesized that astronauts' individual BM and body composition changes are explained by inflight AEE.

133

## 134 **2 MATERIALS AND METHODS**

135 Protocols, methodological validation and related assumptions of the calculations used are fully described in **online**  
136 **supplemental data**.

137

### 138 **2.1 Participants**

139 Eighteen astronauts (16 men and 2 women) voluntarily took part in the study between 2011 and 2017. All were  
140 subjected to extensive physical and medical examination prior to the flight. None had history of chronic disease,  
141 and all of them were healthy throughout the mission. Out of the eighteen astronauts, three (2 men and 1 woman)  
142 performed preflight measurements only, due to rescheduling priorities during the flight, and four (3 men and 1  
143 woman) served as controls to correct for background isotopic changes on the ISS during an experimental session  
144 (see **supplemental methods** and **supplemental Figs 1 to 3**). Data presented here were therefore collected in 11  
145 men.

146 The study was yearly approved by NASA Institutional Review Board (IRB) under NASA 7116301606HR.  
147 European Space Agency (ESA) Medical Board and Japanese Space Agency (JAXA) IRB for human experiments  
148 also approved the protocol. Written informed consent was obtained from all astronauts.

149

## 150 2.2 Protocol

151 For the 11 remaining astronauts, each completed two research sessions of 10 days, one on Earth (ground) and one  
152 onboard the ISS (flight). The ground session was conducted within the year before flying, while astronauts were  
153 at the European Astronaut Center (EAC) in Koln, Germany. On average, the mean delay between ground  
154 measurements and the flight was 99 days (SD 78). The astronauts were deemed in energy balance the year before  
155 launch (see **Fig 1A** and **supplemental methods**). The flight session was conducted after at least three months in  
156 space and before the last month onboard the ISS. This three-to-five-month window was selected to provide data  
157 for long-term spaceflights while avoiding stress associated with the preparation for return to Earth. On average,  
158 flight sessions were conducted after 108 days (19) in space.

159 Ground and flight experiments, strictly similar, were realized under the supervision of ESA and French Space  
160 Agency (CNES) science officers in charge of the experiment and the investigators from Toulouse Space Center  
161 (CADMOS, France), and were preceded by dry runs of the experiments conducted at EAC. BM and body  
162 composition, TEE and its components (resting metabolic rate (RMR) and AEE) and physical activity (PA) were  
163 measured. Additionally, post-flight BM and composition were measured on the ground after landing.

164

## 165 2.3 Total energy expenditure and its components

166 TEE was measured over 10 days using the doubly-labeled water (DLW) method as previously described [16]. The  
167 DLW is the gold standard to assess TEE in free living conditions. The technique is fully described in **supplemental**  
168 **methods**. Briefly, it is based on the exponential elimination of the stable isotopes  $^2\text{H}$  and  $^{18}\text{O}$  after a bolus dose of  
169 water labeled with both isotopes. The  $^2\text{H}$  are lost as water, whereas the  $^{18}\text{O}$  are lost both as water and as  $\text{CO}_2$ . Thus,  
170 the excess disappearance rate of  $^{18}\text{O}$  relative to  $^2\text{H}$  is a measure of the  $\text{CO}_2$  production rate. This latter is converted  
171 to TEE using the food quotient or the respiratory quotient and the classic indirect calorimetry equations. On the  
172 DLW dosing day, RMR was measured in fasting state, at rest for 45 min using the Pulmonary Function System  
173 (PFS, manufactured by the Danish Aerospace Company, former DAMEC)[17]. AEE was calculated from TEE  
174 and RMR assuming a diet-induced thermogenesis of 10% of TEE. PA level (PAL) was calculated as the ratio  
175 between TEE and RMR.

176

177 **2.4 Body composition**

178 Pre- and post-flight ground BM measures were obtained during NASA medical operations with a calibrated scale  
179 at EAC, and fat-free mass (FFM) and FM by dual X-ray energy absorptiometry (DXA, Hologic, Marlborough,  
180 MA, USA; software version 12.7.3.1 in 2011 to 15.5.3 in 2016). Inflight BM was measured using the  
181 SLAMMD (Space Linear Acceleration Mass Measurement Device) within one day of the DLW dosing day, and  
182 FFM and FM were obtained from the DLW method. BM, FFM and FM indexes (BMI, FFMI, FMI), were  
183 calculated by dividing BM, FFM and FM (in kg) by squared height (in squared m).

184

185 **2.5 Energy intake**

186 Inflight mean daily EI, expressed in MJ/day, was calculated from the changes in body composition and TEE  
187 between the preflight and inflight DLW sessions deeming astronauts were in stable energy balance on ground  
188 measurements [18], and compared with theoretical EI calculated from the 2001 Dietary Reference Intakes (DRI)  
189 [19]. Details are provided in **supplemental methods**.

190

191 **2.6 Physical exercise and activities**

192 Physical training sessions were composed of both aerobic and resistive exercises and prescribed 6 days/week, as  
193 detailed in **supplemental methods**. Briefly, 30-45min of aerobic exercise per session were prescribed on the cycle  
194 ergometer (CEVIS) or on a treadmill (T2) with vibration isolation system (**Supplemental Fig 4**) [20, 21]. During  
195 treadmill exercise, an external vertical load (60-100% of BM) was applied to the astronauts to partially compensate  
196 for the absence of body weight in microgravity environment. The resistance exercise program consisted of 2 to 3  
197 workouts per session that were performed on the advanced resistive exercise device (ARED) (**Supplemental Fig**  
198 **4**). Each workout was composed of 5 to 7 exercises primarily focused on the lower body using 8 to 15 repetitions  
199 for 3 to 4 sets. Loads were individually adapted by the personal exercise trainers throughout the flight. Parameters  
200 of the exercise training actually performed during the spaceflight were obtained from the exercise dairy logs.  
201 Overall PA was further evaluated from a Sensewear Pro (SWP) activity monitor (Body media Inc®, Pittsburgh,  
202 PA USA).

203 Self-reported exercise logs were provided by NASA. These logs included date and duration of each aerobic  
204 exercise session, the average speed and external load for T2 and average power and revolution per minute for  
205 CEVIS. For ARED, they included date and detailed exercise workouts of each resistive exercise session, with  
206 number of sets and repetitions, and loads. Mean daily and weekly exercise parameters were calculated over a  
207 period beginning 2 months before the experimental flight session (**details in supplemental files**). An estimation  
208 of T2 power was derived from speed and external load from the American College of Sports Medicine (ACSM)  
209 formulas [22], and aerobic exercise workloads were calculated as power\*duration. Resistive exercise workloads  
210 were calculated as sets\*repetitions\*loads [23]. Because the duration for the resistive exercise sessions was not  
211 provided, muscular time under tension was estimated from the number of repetitions, assuming a 2-to-5 s count  
212 for each repetition (i.e. including both concentric and eccentric phases), according to movement complexity from  
213 online video featuring this kind of exercise [24]. An additional 1-min resting time by set was added to approximate  
214 time involved with exercise on ARED. The goal of this calculation was to allow a comparison with the SWP-  
215 derived data.

216 The SWP, an activity multi-sensor armband that includes a bi-axial accelerometer, was worn on the non-dominant  
217 arm throughout the two 10-day DLW sessions. Its companion software (professional version 8.0) incorporates a  
218 proprietary machine-learning activity classification algorithm based on heat flux, galvanic skin response, skin and  
219 near-body ambient temperature and accelerometry measures patterns [25]. It was used to identify non-wear periods  
220 and, after exclusion of non-valid days, to obtain steps and categorize each minute in 4 classes: inactivity, walking,  
221 running and all other detected activities groups as non-ambulatory PA. Inflight valid data were obtained for 9  
222 astronauts only. The norm of the 1-min acceleration signal mean amplitude deviation (MAD) was used as an  
223 additional proxy of PA workload and intensity [26]. MAD removes the static component due to gravity from the  
224 acceleration signal to keep only the dynamic component due to body movements and changes in velocity. It is  
225 therefore poorly influenced by microgravity. Aggregation of 1-min MAD during activities, a composite variable  
226 that corresponds to the product of duration by the intensity of a given effort/exercise bout or of overall daily PA,  
227 are presented in milli-g (i.e., 0.001 g). Vigorous PA (VPA) was estimated using a MAD-cutpoint of 16 milli-g/min.  
228 Details are provided in **supplemental methods**.

229

## 230 2.7 Statistical analyses

231 Data were first analyzed for the entire group of astronauts (n=11). To understand the inter-individual variability,  
232 astronauts were then stratified according to inflight TEE, i.e. either maintained close to preflight values  
233 (stable\_TEE; n=6) or decreased (decreased\_TEE n=5).

234 Differences between the two groups stratified for inflight TEE changes at baseline and for inflight physical training  
235 were examined using unpaired Student's t-tests. Linear mixed-effects models accounting for repeated  
236 measurements, with subjects as random effect and indicator for flight as fixed factor, were used to test the overall  
237 effect of spaceflight on the anthropometric, energetic and PA outcomes. The group effect (stable\_TEE vs  
238 decreased\_TEE) on the flight-induced changes was tested by adding a group x flight interaction term. Our  
239 statistical inference was respectively on the overall outcome net changes and their between-group differences  
240 estimated (EGD) with their 95% Confidence Interval (CI). Effect size was estimated using Hedges'g, calculated  
241 as the EGD divided by the estimation of the groups' weighted pooled standard deviation, with a Hedges'g greater  
242 than 0.2, 0.5, and 0.8 indicating small, medium, and large effect size, respectively [27]. TEE, AEE, EI and EB  
243 were further adjusted for mean BM, and RMR for both mean FM and mean FFM (whole group mean baseline  
244 values were used as reference values).

245 General linear models were used to examine the associations of 1) FFMI and FMI changes (inflight-preflight) with  
246 TEE and AEE changes (inflight-preflight), and 2) inflight FFMI and FMI with different inflight PA variables.

247 Baseline values and inflight physical training variables are presented as means (standard deviation, SD). Unless  
248 otherwise noted, results of the linear mixed-models are presented as least square means (standard error, SE).

249 Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, North Carolina) with a significance level  
250 at 5%. Figures were realized with Prism 9 (GraphPad, San Diego, California).

251

## 252 3 RESULTS

### 253 3.1 Few spaceflight changes for the whole group of astronauts

254 At baseline (**Table 1**), astronauts had a mean age of 45.7 years (SD 7.7), a BMI of 24.3 kg/m<sup>2</sup> (2.1) with a normal-  
255 to-high FFMI of 19.6 kg/m<sup>2</sup> (1.9). They were quite active, as indicated by a TEE of 13.2 MJ/day (1.9), an AEE of

256 4.9 MJ/day (1.1) and a PAL of 1.90 (0.20). SWP-derived daily overall PA time was 161.8 min/day (56.3). This  
257 included 66.8 min/day (34.1) walking and 8.1 min/day (10.5) running, corresponding to a total of 10077 steps/day  
258 (2834) and 7.4 min/day (9.2) of SWP-derived VPA.

259 - Please insert Table 1 near here -

260 **Fig 1a** presents the changes in BM and composition throughout the whole mission and **Table 2** details the effect  
261 of at least 3 months in space on the main outcomes. Astronauts were in energy balance the year before launch and  
262 spaceflight had modest impact on BM, FM and FFM. Compared to preflight values, BM decreased by 1.20 kg (SE  
263 0.50;  $P=0.04$ ) mainly due to a non-significant reduction in FFM of 0.94 kg (0.59;  $P=0.14$ ).

264 - Please insert Figure 1 near here -

265 No significant overall changes in TEE, RMR and AEE were noted between preflight and flight (**Table 2**).  
266 However, inflight SWP-derived ambulatory activities dramatically dropped with daily steps decreasing by 6583  
267 steps/day (SE 730;  $P<0.001$ ) and walking time by 63.2 min/day (SE 11.5;  $P<0.001$ ). While SWP-derived running  
268 time remained stable at 14.4 min/day (SD 6.4), in agreement with the 13.3 min/day (SD 4.6) of self-reported time  
269 spent exercising on the T2, this reduction in ambulatory activities was almost fully compensated by an increase in  
270 SWP-derived non-ambulatory activities of 64.8 min/day (SE 18.8;  $P<0.01$ ). This explained the absence of  
271 significant net changes in SWP-derived overall active time and accelerometry MAD, a proxy of overall PA  
272 workload. Daily SWP-derived-VPA was not significantly different from ground values. The one-hour increase in  
273 non-ambulatory activities, leading to a total of 151.8 min/day (SE 18.9; range 119.7-194.5), was only partially  
274 explained by the 10.7 min/day (SD 4.4) spent on the CEVIS and the about 29.0 min/day (SD 14.3) on the ARED  
275 as reported by the astronauts (**Supplemental Table 1**). This suggested an increase in PA not related to physical  
276 training. Overall, the astronauts reported 6.2 aerobic exercise sessions/week (SD 1.4; range 3.6-8.6) corresponding  
277 to a total duration of 167.8 min/week (SD 43.9; range 86.9-257.8). They reported 4.5 resistance training  
278 sessions/week (SD 1.8; range 1.8-6.6), consisting of 5.9-10.2 exercises using 8.8 to 15.1 repetitions for 2.2-3.2  
279 sets. This led to a total of 1481 repetitions/week (SD 834; range 468-3044) and a muscular time under tension of  
280 81.8 min/week (SD 43.5; range 28.8-161.2). These numbers exhibit a great inter-individual variability in the  
281 adherence to the exercise prescriptions. They are to be compared to the 180-270 min/week aerobic, and 6-resistive  
282 exercise sessions/week (corresponding to about 1500 repetitions/week) recommendations [21].

283 The astronauts were stratified into two groups, according to their inflight TEE changes, i.e. no change in TEE or  
284 decrease in TEE compared to preflight values. Fortuitously, the two groups ended up having almost equal number  
285 of subjects.

286 - Please insert Table 2 near here -

### 287 **3.2 Baseline Characteristics of inflight-TEE-changes groups**

288 At baseline (**Table 1**), both groups were of the same age and had similar FMI but, compared to the decreased\_TEE  
289 group, the stable\_TEE astronauts had higher FFMI (20.6 kg/m<sup>2</sup> [SD 1.5] vs 18.3 kg/m<sup>2</sup> [1.6];  $P=0.03$ ) and  
290 presented a more active profile. TEE, AEE and daily SWP-derived overall active time did not differ between the  
291 two groups. However, the stable\_TEE astronauts spent more time on ground in SWP-derived VPA (12.4 min/day  
292 [9.8] vs 1.3 min/day [2.6];  $P=0.04$ ) and running ( $P=0.04$ ), and had higher running-related MAD ( $P=0.04$ ) resulting  
293 in greater SWP-derived mean activity intensity (4.9 milli-g/min [0.9] vs 3.5 milli-g/min [0.9];  $P=0.03$ ) than the  
294 decreased\_TEE astronauts.

295

### 296 **3.3 Anthropometric changes by inflight-TEE-changes groups**

297 Compared to preflight, both groups lost equivalent BM, but contrasted changes in body composition were noted  
298 (**Table 2** and **Fig 1a and 1b**). While the stable\_TEE group maintained FFMI and displayed a slight FMI loss, their  
299 counterparts lost FFMI and gained FMI. The EGD for FFMI changes (stable\_TEE compared to decreased\_TEE  
300 group) was +0.87 kg/m<sup>2</sup> (95%CI 0.32 to 1.41; effect size=1.7;  $P<0.01$ ); and the EGD for FMI changes -1.09 kg/m<sup>2</sup>  
301 (95%CI -2.06 to -0.11; effect size=1.2;  $P=0.03$ ).

302

### 303 **3.4 Energy expenditures changes by inflight-TEE-changes groups**

304 The EGD for TEE changes adjusted for BM was +2.98 MJ/day (95%CI 1.75 to 4.22; effect size=2.6;  $P<0.001$ ),  
305 mainly related to differences in AEE changes (BM-adjusted EGD +3.02 MJ/day, 95%CI 1.81 to 4.23; effect  
306 size=2.7;  $P<0.001$ ) while RMR remained stable in both groups (**Table 2** and **Fig 2a**). As illustrated in **Fig 2b**, the  
307 individual FMI net changes were negatively associated with net changes in TEE ( $R^2=0.58$ ;  $P<0.01$ ) and AEE  
308 ( $R^2=0.54$ ;  $P=0.01$ ) while net changes in FFMI were positively associated with net changes in TEE ( $R^2=0.64$ ;  
309  $P<0.01$ ) and, to a lesser degree, net changes in AEE ( $R^2=0.47$ ;  $P=0.02$ ).

310

- Please insert Figure 2 near here -

311

### 312 **3.5 Physical activity changes by inflight-TEE-changes groups**

313 Net changes in SWP and accelerometry-derived activity parameters (**Table 2** and **Fig 3a**) were globally similar  
314 across groups. However, like what was observed on the ground, the stable\_TEE astronauts still spent more time  
315 inflight in SWP-derived VPA (16.7 min/day [SE 3.1] vs 4.6 min/day [3.4];  $P=0.02$ ), had higher overall-activity  
316 accelerometry MAD (884 milli-g/day [125] vs 505 milli-g/day [140];  $P=0.06$ ), and SWP-derived mean activity  
317 intensity (4.60 milli-g/min [0.41] vs 3.35 milli-g/min [0.45];  $P=0.06$ ) than the decreased\_TEE group. Reported  
318 inflight resistance training characteristics and time spent performing CEVIS were not significantly different  
319 between groups (**Supplemental Table 1**). The stable\_TEE group reported non-significant higher inflight aerobic  
320 workloads than the decreased\_TEE group ( $P=0.10$ ), due to more time spent on T2 (15.7 min/day [SD 3.0] vs 10.4  
321 min/day [4.6];  $P=0.05$ ) but also to higher average speeds (11.2 [0.9] vs 8.5 [2.1] km/h;  $P=0.02$ ). This was in good  
322 agreement with higher SWP-derived running-times (18.0 min/day [SE 3.3] vs 9.9 min/day [3.7];  $P=0.12$ ) and  
323 running-related accelerometry MAD (461 milli-g/day [SE 87] vs 154 milli-g/day [98];  $P=0.03$ )

324 As illustrated in **Fig 3b**, after at least 3 months on the ISS individuals' inflight FMI were inversely associated with  
325 SWP-derived overall activity-related accelerometry MAD ( $R^2=0.73$ ;  $P<0.01$ ), time spent in SWP-derived VPA  
326 ( $R^2=0.56$ ;  $P=0.02$ ) and self-reported T2 relative workload ( $R^2=0.71$ ;  $P=0.001$ ). Conversely FFMI values were  
327 positively associated with SWP-derived overall activity-related accelerometry MAD ( $R^2=0.48$ ;  $P=0.04$ ), time  
328 spent in SWP-derived VPA ( $R^2=0.62$ ;  $P=0.01$ ) and self-reported T2 relative workload ( $R^2=0.40$ ;  $P=0.04$ ). Practice  
329 of ARED and CEVIS was not associated with any body composition parameter.

330

- Please insert Figure 3 near here -

331

### 332 **3.6 Energy intakes by inflight-TEE-changes groups**

333 EI (BM-adjusted) calculated from changes in body composition from the time of beginning of space flight to the  
334 time of starting the inflight TEE measurement was 13.6 MJ/day (SE 0.5) in the stable\_TEE group and 11.3 MJ/day  
335 (0.6) in the decreased\_TEE group, representing 93% and 75% of DRIs, respectively (**Fig 4**).

336

- Please insert Figure 4 near here -

337

## 338 **4 DISCUSSION**

339 The aim of the ENERGY study was to assess the regulation of energy balance and body composition during 6-  
340 month missions onboard the ISS by specifically considering the energy cost of PA using the gold standard DLW  
341 method. Despite no significant overall changes in body composition and energy expenditures, a slight decrease in  
342 BM (-1.5%) was observed in the whole group of astronauts. A large inter-individual variability was however  
343 noticed. Astronauts who maintained pre-flight total and activity energy expenditures kept fat-free mass at baseline  
344 levels but lost fat mass. Conversely, those who expended less energy during the flight than on the ground lost fat-  
345 free mass and gained fat mass. Astronauts who maintained stable TEE during the flight spent more time inflight  
346 running and engaged in VPA, and were also the ones with the best fitness on the ground. These results suggest  
347 that 1) inflight AEE, possibly due to both physical training and non-exercise PA, drives inflight body composition  
348 regulation, 2) inflight energy requirements should be individually evaluated during the whole duration of the  
349 spaceflight, 3) baseline participants' characteristics need to be considered for the prescription of both preflight and  
350 inflight exercise training; 4) fat mass changes result from unmatched spontaneous EI adaptation to changes in AEE  
351 in space that require further investigation.

352

### 353 **4.1 Ground fitness and PA performance influence changes in body composition during spaceflight**

354 After at least 3 months onboard the ISS, the 11 astronauts presented a slight BM loss, mainly due to a reduction in  
355 FFM, despite no significant changes in TEE. The modest effect of spaceflight on BM and body composition in the  
356 whole group masked a large interindividual variability with half of the group of astronauts who maintained a stable  
357 FFM favoring a slight BM loss (stable\_TEE group), while the other half displayed a FFM loss but an increase in  
358 FM (decreased\_TEE group). This between-subject variability seemed to be associated with heterogeneity in  
359 astronauts' ground body composition and engagement in exercise of high intensity [15]. Although all the astronauts  
360 were quite fit and active on the ground, those who maintained their FFM during the flight had higher FFMI and  
361 spent more time running or in VPA on Earth. These results are in line with the study of Matsumoto et al. [6] that  
362 showed habitual PA on Earth was a better predictor of inflight BM loss than PA performed during spaceflight.

363 Whether habitual ground activity levels affect inflight body mass and composition directly or are due to greater  
364 engagement of fitter astronauts in exercise during the mission requires further investigation. Of interest, ground  
365 differences in SWP-derived VPA and running persisted during the flight with a significant reduction in inflight  
366 energy expenditures in the astronauts who were the least active on Earth, but not in the others. We also observed  
367 significant associations of inflight self-reported running or SWP and accelerometry proxies of PA workload and  
368 intensity with body composition. While improvement of exercise hardware capabilities and prescription have led  
369 to better post-flight performances, predicting appropriate inflight exercise loads remains difficult because of the  
370 difference in training environment (gravity vs microgravity) and hardware [20, 21]. Beyond their energetics  
371 implications, our findings indicate that gaining a better understanding of the associations between ground and  
372 inflight exercise characteristics may help designing preflight and inflight prescriptions that favor adherence and  
373 enhance inflight performance including for the occupation-related astronaut tasks. Altogether these data suggest  
374 that pre-flight astronauts' individual anthropometric and fitness characteristics require more attention for onboard  
375 exercise prescriptions and EI recommendations.

376

#### 377 **4.2 Does the exercise countermeasure play a role in the regulation of body composition?**

378 The strong associations of TEE and AEE changes with FFMI and FMI changes suggest that activity-related energy  
379 expenditure, in interaction with microgravity exposure, plays a role in inflight body composition changes. In the  
380 absence of gravity, TEE is driven by the exercise countermeasure and intra- and extra-vehicular activities while  
381 energy expenditure related to standing posture and weight-bearing muscle activity is suppressed. For the first time,  
382 SWP and accelerometry used in this study gave a more detailed insight into onboard overall PA patterns. It clearly  
383 highlighted the expected decrease in ambulatory activities, except for running activity performed on the T2  
384 treadmill. Contrary to our expectations, averaged overall PA time and workload, as estimated by SWP and  
385 accelerometry MAD, were not reduced on the ISS; the decrease in ambulatory activities was compensated by non-  
386 ambulatory activities. Of interest, accelerometry MAD removes from accelerometry signal any static gravitation  
387 component [26] and SWP utilizes the signature of several physiological systems (temperature, sweat, and heat flux  
388 and dissipation) in addition to accelerometry, which allows for the disambiguation of activities and contexts (e.g.  
389 microgravity) that may confuse a single sensor [25]. Inflight non-ambulatory activities (almost 2.5 hours/day) can  
390 only be partially explained by the CEVIS and ARED exercises, which represent about 40 min/day altogether. This

391 suggests that routine and off-nominal mission tasks may be another important component of overall PA.  
392 Unfortunately, the identification of specific onboard activities based on the SWP other than walking and running,  
393 was not possible due to the arm-placement of the device and the absence of activity learning studies in  
394 microgravitational conditions.

395 Mean self-reported training exercises were slightly lower than the recommendations. This was in agreement with  
396 previous observations made on the ISS [28]. The lower levels of aerobic exercise reflect a recent tendency to favor  
397 resistive exercise for its effect on muscle and bone mass and strength [29, 30]. More importantly a large inter-  
398 individual variability in exercise adherence was observed with some astronauts reporting no more than 87  
399 min/week of aerobic exercise and less than 500 repetitions/week in resistive exercise with a muscular time under  
400 tension lower than 30 min/week.

401 Even if efficiency is lower in space than on the ground, AEE does not seem to be fully explained by exercise  
402 training inflight. Treadmill exercise, characterized by higher weekly duration and speed in the astronauts who  
403 maintained their TEE, was undoubtedly a contributor of higher AEE and was inversely associated with astronauts'  
404 FM changes. Conversely both inflight SWP-derived VPA and T2 workload were positively associated with FFM  
405 confirming the protective role of exercise intensity on muscle mass [30]. Of note, time spent on the treadmill alone  
406 did not seem to explain the between-group differences in SWP-derived VPA. This suggests that VPA may also  
407 reflect an engagement in energy-demanding mission-related activities. However, any extrapolation from SWP  
408 sensors about the exact energy cost of both non-training and specific training activities in the context of  
409 spaceflights was not possible. Although resistive exercise is considered important to prevent muscle mass and  
410 bone loss, we surprisingly did not find any relationship between ARED practice and FFM.

411

### 412 **4.3 Energy intakes during long-term spaceflight**

413 Even if, by reviewing data from past space missions, no association was found between inflight unadjusted EI and  
414 BM loss [6], insufficient EI has often been reported during long-term missions [7, 13]. Astronauts in this study  
415 consumed about 85% of dietary prescriptions [19], which is similar to self-reported values observed by Smith et  
416 al.[7] during past missions onboard the ISS. Hyporexia due to lower food attractivity, altered smell or taste,  
417 sickness, or other microgravity-induced disorders [8] are expected to impair the spontaneous adjustment of EI to

418 the changes in energy needs [7, 31]. This may explain why the astronauts who maintained their TEE to preflight  
419 values lost FM despite EI close to their theoretical energy requirements. Conversely, the reduction in EI observed  
420 in the less active astronauts was expected as their TEE was reduced by about 20%, due to a decrease in AEE.  
421 However, despite EI only representing 75% of the dietary recommendations, the astronauts who displayed a  
422 decrease in TEE during spaceflight were still in positive energy and fat balance.

423

#### 424 **4.4 How to maintain energy balance during long-term spaceflight?**

425 Exercise countermeasure is the cornerstone of countermeasure programs during human spaceflights. Exercise  
426 training is known to benefit several physiological systems, including muscle strength, bone density and aerobic  
427 capacity, but effects of the exercise on these health outcomes were beyond the scope of this study. Here we showed  
428 that FFM was maintained in the stable\_TEE astronauts despite some of them completing less than the prescribed  
429 exercise sessions. Our results further suggest that the non-ambulatory non-training activities play a role in the  
430 regulation of energy balance. The extent to which these activities, likely related to the occupational tasks on the  
431 ISS, influence energy expenditures and body composition remains to be addressed. For example, it is not known  
432 how it contributes to the preservation of muscle mass or other critical key performance outcomes. This finding has  
433 potential implications for future spaceflights as it calls for considering non-training activities and their associated  
434 energy cost when developing the exercise countermeasure [15], especially for long term space missions.

435 This does not preclude the need for improving strategies to help astronauts to comply with the exercise  
436 prescriptions. Space agencies must find the best combination between modalities, i.e. duration and intensity for  
437 the aerobic exercise, and load and repetitions for the resistive exercise, to benefit most of the physiological systems  
438 without affecting others. For example, using high intermittent interval training (HIIT) that efficiently stimulates  
439 musculoskeletal and cardiovascular systems with a relatively low impact on energy requirements may be a  
440 promising alternative [8, 15, 23, 32]. Other countermeasure such as nutrition or artificial gravity may also be used  
441 to optimize the benefits of exercise and PA. In parallel, changes in the agenda of the astronauts could be considered  
442 to improve time dedicated to meals for the whole crew together, and food could be further improved to favor a  
443 better adjustment to actual energy needs [33, 34].

444 Overall, the results of this study show that both the measurement and regulation of energy balance inflight is  
445 challenging. One of the key findings is the large between-subject variability in changes in TEE, AEE and body  
446 composition. This variability was previously reported for EI. This has challenging consequences for both exercise  
447 and EI prescriptions as a complex tradeoff needs to be assessed. Such a tradeoff would need to consider both the  
448 positive impact on health and various physiological functions of high AEE along with adequate EI; but also the  
449 negative impact of too low AEE and excessive FM gain. This is why research on both exercise countermeasures  
450 and the development of loggers accurately assessing on real-time exercise, energy expenditure and body  
451 composition at the individual level is a top priority. Clearly current available devices (along with their algorithms)  
452 do not allow to do so and further interdisciplinary studies are needed.

453

#### 454 **4.5 Limitations and strengths of the study**

455 Spaceflight imposes some limitations and strengths that need to be acknowledged. The sample size was relatively  
456 low, due to the limited number of inflight experiments. This study was conducted in male astronauts only, and  
457 results may not apply to women. Because space flights are limited, this study is one of the only three studies that  
458 determined energy requirements and body composition changes inflight using gold standard objective methods  
459 such as DLW and activity monitors, but it is the first one to focus on long-duration missions. Utilization of a unique  
460 combination of sensors for continuous physiological monitoring related to PA is a strength of the SWP device. As  
461 already underlined, the activity classification algorithms were however developed for detecting activities on Earth,  
462 limiting their current recognition capabilities and energy extrapolations in the context of spaceflights.  
463 Unfortunately, the R&D service of the SenseWearPro company no longer exists and the SWP software is no longer  
464 updated. The current version of the software only provides one-min aggregate measures rather than raw signals,  
465 which precludes any additional data treatment and validation. Also, heart rate monitoring, an additional valuable  
466 physiological signal in the context of exercise and energy evaluation, was available for few astronauts and during  
467 the training sessions only. On the other side, making assumptions on exercise performance based on crewmember  
468 exercise logs was challenging and not possible for non-training activities. Many of the findings are correlations, which  
469 suggests the need for future studies to prove causation.

470

471 **5 CONCLUSION**

472 The between-astronauts variability in body composition and energy expenditure changes observed during  
473 spaceflight was related to ground fitness, and inflight practice of activities of high intensity. Importantly, a high  
474 inter-individual variability in inflight training was noted with some crewmembers reporting values far below the  
475 recommendations, but there was an unexpected engagement in non-ambulatory activities related to the nature of  
476 the missions onboard the ISS. These results suggest that energy requirements in space must be individually derived  
477 based on real-time measurements of AEE and changes in body composition rather than on current general  
478 recommendations and exercise prescriptions. This requires validating in space the use of tri-accelerometry on  
479 different body parts, along with other sensors including heart rate monitors, and the development of specific  
480 algorithms to detect and quantify all physical activities and derive activity-specific energy expenditures during the  
481 space missions. Despite some spontaneous adjustments, we further observed an uncoupling between energy  
482 intakes and expenditures that led to energy imbalance. Methodological developments are therefore vital for the  
483 control of both sides of the energy balance, i.e., energy intakes and expenditures, or at least body composition  
484 evolution during long-duration space missions, which are considered a top priority for exploration by the  
485 international space agencies.

486

487 **REFERENCES**

- 488 1. Bergouignan A, Stein TP, Hahold C, Coxam V, O' Gorman D, Blanc S. Towards human  
 489 exploration of space: The THESEUS review series on nutrition and metabolism research priorities. *NPJ*  
 490 *Microgravity*. 2016;2:16029.
- 491 2. Smith SM, Davis-Street JE, Rice BL, Nillen JL, Gillman PL, Block G. Nutritional status  
 492 assessment in semiclosed environments: ground-based and space flight studies in humans. *J Nutr*.  
 493 2001;131(7):2053-61.
- 494 3. Smith SM, Wastney ME, Morukov BV, Larina IM, Nyquist LE, Abrams SA, et al. Calcium  
 495 metabolism before, during, and after a 3-mo spaceflight: kinetic and biochemical changes. *Am J Physiol*.  
 496 1999;277(1 Pt 2):R1-10.
- 497 4. Stein TP, Leskiw MJ, Schluter MD, Hoyt RW, Lane HW, Gretebeck RE, et al. Energy  
 498 expenditure and balance during spaceflight on the space shuttle. *Am J Physiol*. 1999;276(6 Pt 2):R1739-  
 499 48.
- 500 5. Wade CE, Miller MM, Baer LA, Moran MM, Steele MK, Stein TP. Body mass, energy intake,  
 501 and water consumption of rats and humans during space flight. *Nutrition*. 2002;18(10):829-36.
- 502 6. Matsumoto A, Storch KJ, Stolfi A, Mohler SR, Frey MA, Stein TP. Weight loss in humans in  
 503 space. *Aviat Space Environ Med*. 2011;82(6):615-21.
- 504 7. Smith SM, Zwart SR, Block G, Rice BL, Davis-Street JE. The nutritional status of astronauts is  
 505 altered after long-term space flight aboard the International Space Station. *J Nutr*. 2005;135(3):437-43.
- 506 8. Laurens C, Simon C, Vernikos J, Gauquelin-Koch G, Blanc S, Bergouignan A. Revisiting the  
 507 Role of Exercise Countermeasure on the Regulation of Energy Balance During Space Flight. *Front*  
 508 *Physiol*. 2019;10:321.
- 509 9. Stein TP, Leskiw MJ, Schluter MD. Diet and nitrogen metabolism during spaceflight on the  
 510 shuttle. *J Appl Physiol* (1985). 1996;81(1):82-97.
- 511 10. Smith SM, Heer MA, Shackelford LC, Sibonga JD, Ploutz-Snyder L, Zwart SR. Benefits for  
 512 bone from resistance exercise and nutrition in long-duration spaceflight: Evidence from biochemistry  
 513 and densitometry. *J Bone Miner Res*. 2012;27(9):1896-906.
- 514 11. Zwart SR, Launius RD, Coen GK, Morgan JL, Charles JB, Smith SM. Body mass changes  
 515 during long-duration spaceflight. *Aviat Space Environ Med*. 2014;85(9):897-904.
- 516 12. Lane HW, Gretebeck RJ, Schoeller DA, Davis-Street J, Socki RA, Gibson EK. Comparison of  
 517 ground-based and space flight energy expenditure and water turnover in middle-aged healthy male US  
 518 astronauts. *Am J Clin Nutr*. 1997;65(1):4-12.
- 519 13. Stein TP. Nutrition in the space station era. *Nutr Res Rev*. 2001;14(1):87-118.
- 520 14. Scott JPR, Weber T, Green DA. Introduction to the Frontiers Research Topic: Optimization of  
 521 Exercise Countermeasures for Human Space Flight - Lessons From Terrestrial Physiology and  
 522 Operational Considerations. *Front Physiol*. 2019;10(173):173.
- 523 15. Comfort P, McMahon JJ, Jones PA, Cuthbert M, Kendall K, Lake JP, et al. Effects of  
 524 Spaceflight on Musculoskeletal Health: A Systematic Review and Meta-analysis, Considerations for  
 525 Interplanetary Travel. *Sports Med*. 2021;51(10):2097-114.
- 526 16. Schoeller DA. Measurement of energy expenditure in free-living humans by using doubly  
 527 labeled water. *J Nutr*. 1988;118(11):1278-89.
- 528 17. Moore AD, Jr., Downs ME, Lee SM, Feiveson AH, Knudsen P, Ploutz-Snyder L. Peak exercise  
 529 oxygen uptake during and following long-duration spaceflight. *J Appl Physiol* (1985). 2014;117(3):231-  
 530 8.
- 531 18. Votruba SB, Blanc S, Schoeller DA. Pattern and cost of weight gain in previously obese women.  
 532 *Am J Physiol Endocrinol Metab*. 2002;282(4):E923-30.
- 533 19. Institute of Medicine. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty  
 534 Acids, Cholesterol, Protein, and Amino Acids. Washington, DC: The National Academies Press; 2005.  
 535 p. 184-5.

- 536 20. Hackney KJ, Scott JM, Hanson AM, English KL, Downs ME, Ploutz-Snyder LL. The  
537 Astronaut-Athlete: Optimizing Human Performance in Space. *J Strength Cond Res.* 2015;29(12):3531-  
538 45.
- 539 21. Loehr JA, Williams ME, Petersen N, Hirsch N, Kawashima S, Ohshima H. Physical Training  
540 for Long-Duration Spaceflight. *Aerosp Med Hum Perform.* 2015;86(12 Suppl):A14-A23.
- 541 22. American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and*  
542 *Prescriptions.* 6th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2000. p. 163-76.
- 543 23. English KL, Downs M, Goetchius E, Buxton R, Ryder JW, Ploutz-Snyder R, et al. High intensity  
544 training during spaceflight: results from the NASA Sprint Study. *NPJ Microgravity.* 2020;6:21.
- 545 24. Trappe S, Costill D, Gallagher P, Creer A, Peters JR, Evans H, et al. Exercise in space: human  
546 skeletal muscle after 6 months aboard the International Space Station. *J Appl Physiol (1985).*  
547 2009;106(4):1159-68.
- 548 25. Andre D, Pelletier R, Farringdon J, Safier S, Talbott S, Stone R, et al. The Development of the  
549 SenseWear® Armband, a Revolutionary Energy Assessment Device to Assess Physical Activity and  
550 Lifestyle: BodyMedia, Inc.; 2006.
- 551 26. Vähä-Ypyä H, Vasankari T, Husu P, Mänttari A, Vuorimaa T, Suni J, et al. Validation of Cut-  
552 Points for Evaluating the Intensity of Physical Activity with Accelerometry-Based Mean Amplitude  
553 Deviation (MAD). *PLoS One.* 2015;10(8):e0134813.
- 554 27. Cohen J. A power primer. *Psychol Bull.* 1992;112(1):155-9.
- 555 28. Hughson RL, Robertson AD, Arbeille P, Shoemaker JK, Rush JW, Fraser KS, et al. Increased  
556 postflight carotid artery stiffness and in-flight insulin resistance resulting from 6-mo spaceflight in male  
557 and female astronauts. *Am J Physiol Heart Circ Physiol.* 2016;310(5):H628-38.
- 558 29. Jones TW, Petersen N, Howatson G. Optimization of Exercise Countermeasures for Human  
559 Space Flight: Operational Considerations for Concurrent Strength and Aerobic Training. *Front Physiol.*  
560 2019;10:584.
- 561 30. Petersen N, Jaekel P, Rosenberger A, Weber T, Scott J, Castrucci F, et al. Exercise in space: the  
562 European Space Agency approach to in-flight exercise countermeasures for long-duration missions on  
563 ISS. *Extrem Physiol Med.* 2016;5:9.
- 564 31. Heer M, Boerger A, Kamps N, Mika C, Korr C, Drummer C. Nutrient supply during recent  
565 European missions. *Pflugers Arch.* 2000;441(2-3 Suppl):R8-14.
- 566 32. Matsuo T, Ohkawara K, Seino S, Shimojo N, Yamada S, Ohshima H, et al. An exercise protocol  
567 designed to control energy expenditure for long-term space missions. *Aviat Space Environ Med.*  
568 2012;83(8):783-9.
- 569 33. Landon LB, Douglas GL, Downs ME, Greene MR, Whitmire AM, Zwart SR, et al. The  
570 Behavioral Biology of Teams: Multidisciplinary Contributions to Social Dynamics in Isolated,  
571 Confined, and Extreme Environments. *Front Psychol.* 2019;10:2571.
- 572 34. Douglas GL, Zwart SR, Smith SM. Space Food for Thought: Challenges and Considerations for  
573 Food and Nutrition on Exploration Missions. *J Nutr.* 2020;150(9):2242-4.

574

575 **Table1:** Astronauts' baseline characteristics for the whole group and by inflight-TEE-changes groups

	All Astronauts (n=11)	Astronauts with stable TEE (n=6)	Astronauts with decreased TEE (n=5)	Group difference P Value <sup>b</sup>
<b>Age &amp; anthropometry</b>				
Age (yr)	45.7 (7.7)	43.5 (8.7)	48.4 (6.0)	0.32
Height (cm)	180.3 (6.7)	182.0 (5.7)	178.3 (7.9)	0.39
BM (kg)	79.4 (10.6)	83.9 (8.0)	73.9 (11.6)	0.13
BMI (kg/m <sup>2</sup> )	24.3 (2.1)	25.3 (1.4)	23.2 (2.4)	0.10
FFM (kg)	63.8 (8.8)	68.3 (6.0)	58.3 (8.9)	0.05
FFMI (kg/m <sup>2</sup> )	19.6 (1.9)	20.6 (1.5)	18.3 (1.6)	0.03
FM (kg)	15.6 (3.9)	15.57 (4.5)	15.6 (3.5)	0.99
FMI (kg/m <sup>2</sup> )	4.8 (1.0)	4.7 (1.2)	4.9 (1.0)	0.71
<b>Energetics</b>				
TEE (MJ/day)	13.2 (1.9)	14.0 (1.4)	12.2 (2.1)	0.13
TEE <sub>BM</sub> (MJ/day) <sup>a</sup>	13.2 (1.0)	13.3 (1.2)	13.0 (1.3)	0.70
RMR (MJ/day)	7.0 (1.2)	7.4 (1.4)	6.6 (0.8)	0.32
RMR <sub>FFM &amp; FM</sub> (MJ/day) <sup>a</sup>	7.0 (1.0)	7.0 (1.3)	7.0 (1.4)	0.93
AEE (MJ/day)	4.9 (1.1)	5.2 (1.0)	4.4 (1.1)	0.24
AEE <sub>BM</sub> (MJ/day) <sup>a</sup>	4.9 (0.9)	5.0 (1.1)	4.7 (1.1)	0.71
PAL	1.90 (0.20)	1.94 (0.26)	1.85 (0.09)	0.50
Energy intake (MJ/day)	13.2 (1.9)	14.0 (1.4)	12.2 (2.1)	0.13
Energy intake <sub>BM</sub> (MJ/day) <sup>a</sup>	13.2 (1.1)	13.3 (1.2)	13.0 (1.3)	0.70
<b>SWP-derived physical activities</b>				
Steps (number/day)	10077 (2834)	1046 (2816)	9614 (3111)	0.65
Overall activity & exercise (min/day)	161.8 (56.3)	164.8 (67.5)	158.3 (47.0)	0.86
Walking (min/day)	66.8 (34.1)	60.2 (27.1)	74.6 (43.1)	0.53
Running (min/day)	8.1 (10.5)	13.7 (11.5)	1.5 (2.8)	0.04
Non-ambulatory activity & exercise (min/day)	87.9 (63.4)	90.9 (61.7)	82.3 (72.4)	0.84
Vigorous physical activity (MAD>16 milli-g; min/day)	7.4 (9.2)	12.4 (9.8)	1.3 (2.6)	0.04
<b>Accelerometry MAD</b>				
Daily total (milli-g/day)	1353 (278)	1487 (309)	1193 (124)	0.08
Overall activity & exercise (milli-g/day)	692 (313)	814 (368)	546 (164)	0.17
Walking (milli-g/day)	286 (171)	250 (104)	329 (235)	0.47
Running (milli-g/day)	188 (242)	322 (259)	27 (54)	0.04
Non-ambulatory activity & exercise (milli-g/day)	218 (141)	241 (151)	190 (140)	0.58

Overall activity & exercise mean intensity (milli-g/min)	4.3 (1.1)	4.9 (0.9)	3.5 (0.9)	0.03
--	-----------	-----------	-----------	------

576 BMI=body mass index; FM=fat mass; FFM=fat-free mass; FMI=fat mass index; FFMI=fat-free mass index;  
577 TEE=total energy expenditure, RMR=resting metabolism rate, AEE=activity energy expenditure; EI=energy  
578 intake MAD=acceleration mean absolute deviation. Values are means (SD). SWP =Sensewear Pro activity-device.  
579 Physical activities were derived from a combination of different physiological signals with an in-built machine-  
580 learning activity classification algorithm.  
581 <sup>a</sup> BM (TEE, AEE, EI) or FFM and FM (RMR) adjusted lsmeans (SD) (whole group mean baseline values were  
582 used as reference values). All astronauts were male.  
583 <sup>b</sup> Statistical analyses used Student's t-tests.  
584

585 **Table2:** Astronauts' anthropometric, energetic and physical activity changes between preflight and flight for the whole group and by inflight-TEE-changes groups

	All Astronauts (n=11)		Astronauts with stable TEE (n=6)	Astronauts with decreased TEE (n=5)	Linear Mixed Model Analyses		
	Changes from preflight	P value	Changes from preflight	Changes from preflight	EGD in changes from preflight	95%CI	P value
<b>Age &amp; anthropometry</b>							
BM (kg)	-1.20 (0.50)	0.04	-1.59 (0.69)	-0.74 (0.75)	-0.86 (1.02)	-3.17 to 1.46	0.42
BMI (kg/m <sup>2</sup> )	-0.39 (0.17)	0.04	-0.49 (0.24)	-0.28 (0.26)	-0.22 (0.35)	-1.01 to 0.57	0.55
FFM (kg)	-0.94 (0.59)	0.14	0.34 (0.56)	-2.48 (0.61)	2.82 (0.83)	0.94 to 4.69	<0.01
FFM index (kg/m <sup>2</sup> )	-0.29 (0.18)	0.13	0.10 (0.16)	-0.76 (0.18)	0.87 (0.24)	0.32 to 1.41	<0.01
FM (kg)	-0.26 (0.87)	0.77	-1.93 (0.93)	1.74 (1.02)	-3.67 (1.38)	-6.78 to -0.56	0.03
FM index (kg/m <sup>2</sup> )	-0.11 (0.27)	0.70	-0.60 (0.29)	0.49 (0.32)	-1.09 (0.43)	-2.06 to -0.11	0.03
<b>Energetics</b>							
TEE <sub>BM</sub> (MJ/day) <sup>a</sup>	-0.39 (0.73)	0.60	0.90 (0.37)	-2.08 (0.40)	2.98 (0.54)	1.75 to 4.22	<0.001
RMR <sub>FFM &amp; FM</sub> (MJ/day) <sup>a</sup>	-0.15 (0.19)	0.43	-0.35 (0.28)	0.11 (0.33)	-0.46 (0.48)	-1.51 to 0.59	0.36
AEE <sub>BM</sub> (MJ/day) <sup>a</sup>	-0.19 (0.70)	0.79	1.12 (0.36)	-1.90 (0.39)	3.02 (0.53)	1.81 to 4.23	<0.001
PAL	-0.02 (0.10)	0.86	0.23 (0.08)	-0.31 (0.09)	0.54 (0.12)	0.28 to 0.80	0.001
Energy intake (MJ/day)	-0.82 (0.38)	0.06	0.06 (0.34)	-1.86 (0.37)	1.92 (0.50)	0.78 to 3.05	<0.01
Energy intake <sub>BM</sub> (MJ/day) <sup>a</sup>	-0.61 (0.40)	0.16	0.29 (0.33)	-1.75 (0.36)	2.04 (0.48)	0.96 to 3.13	<0.01
Energy balance (MJ/day)	-0.15 (0.29)	0.61	-0.68 (0.33)	0.48 (0.36)	-1.15 (0.49)	-2.19 to -0.12	0.03
Energy balance <sub>BM</sub> (MJ/day) <sup>a</sup>	-0.14 (0.30)	0.65	-0.63 (0.30)	0.50 (0.33)	-1.13 (0.44)	-2.06 to -0.20	0.02
<b>SWP-derived physical activities</b>							
Steps (number/day)	-6583 (730)	<0.001	-6277 (1402)	-7231 (1554)	954 (2093)	-3483 to 5391	0.66

Overall activity & exercise (min/day)	13.2 (23.8)	0.59	24.4 (33.2)	-0.9 (36.8)	25.2 (49.6)	-79.9 to 130.3	0.62
Walking (min/day)	-63.2 (11.5)	<0.001	-57.3 (16.0)	-70.1 (17.7)	12.8 (23.9)	-37.8 to 63.4	0.60
Running (min/day)	6.3 (4.0)	0.13	4.4 (4.5)	8.5 (5.0)	-4.1 (6.7)	-18.3 to 10.1	0.55
Non-ambulatory activity & exercise (min/day)	64.8 (18.8)	<0.01	77.3 (36.3)	60.8 (40.2)	16.5 (54.2)	-98.3 to 131.4	0.76
Vigorous physical activity (MAD>16 milli-g; min/day)	2.8 (2.1)	0.23	4.3 (4.2)	3.3 (4.6)	1.0 (6.2)	-12.2 to 14.1	0.88
<b>Accelerometry MAD</b>							
Daily total (milli-g/day)	-40.8 (74.7)	0.60	12.6 (147.3)	-67.9 (163.1)	80.5 (219.8)	-385.4 to 546.4	0.72
Overall activity & exercise (milli-g/day)	11.1 (93.2)	0.91	70.2 (169.0)	-40.4 (187.2)	110.7 (252.2)	-424.0 to 645.4	0.67
Walking (milli-g/day)	-264.6 (57.3)	<0.001	-235.0 (79.4)	-299.9 (87.9)	64.9 (118.5)	-186.2 to 316.1	0.59
Running (milli-g/day)	113.2 (73.8)	0.16	138.4 (118.3)	127.5 (131.1)	10.9 (176.6)	-363.4 to 385.3	0.95
Non-ambulatory activity & exercise (milli-g/day)	151.8 (60.9)	0.02	166.8 (83.8)	132.0 (92.9)	34.8 (125.1)	-230.4 to 300.0	0.78
Overall activity & exercise mean intensity (milli-g/min)	-0.23 (0.35)	0.53	-0.32 (0.55)	-0.16 (0.61)	-0.16 (0.82)	-1.90 to 1.58	0.84
<b>Activity NASA logs <sup>c</sup></b>							
T2 (min/day)	13.3 (1.4)	.	15.7 (1.6)	10.4 (1.7)	-5.3 (2.3)	-10.5 to -0.1	0.04
CEVIS (min/day)	10.7 (1.3)	.	10.0 (1.9)	11.4 (2.1)	1.4 (2.8)	-4.9 to 7.7	0.63
ARED (min/day) <sup>d</sup>	29.0 (4.3)	.	30.9 (6.1)	26.8 (6.7)	-4.1 (9.0)	-24.5 to 16.4	0.66

586 BMI=body mass index; FM=fat mass; FFM=fat-free mass; FMI=fat mass index; FFMI=fat-free mass index; TEE=total energy expenditure, RMR=resting metabolism rate,  
587 AEE=activity energy expenditure; EI=energy intake; EB=energy balance; MAD=Acceleration mean absolute deviation; T2= treadmill device onboard the ISS; CEVIS= Cycle  
588 Ergometer with Vibration Isolation and Stabilization system; ARED= Advanced Resistive Exercise Device. SWP =Sensewear Pro device.

589 Physical activities were derived from a combination of different physiological signals with an in-built machine-learning activity classification algorithm.

590 Values are estimated Lsmeans (SE) from a mixed-effects models for repeated measurements.

591 <sup>a</sup> BM (TEE, AEE, EI, EB) or FFM and FM (RMR) adjusted Models (whole group mean baseline values were used as reference values).

592 <sup>c</sup> Inflight Values

593 <sup>d</sup> ARED duration was approximate from number of repetitions and sets (2-to5 sec/repetition, according to movement complexity, with addition of 1-min recuperation time by  
594 set)

595

596 **Legends**

597 **Fig 1:** Body mass and composition changes during flight

598 Changes in body mass and composition throughout the whole mission (a) and during the experimental sessions  
599 (b). Values are means (SE) (a), and lsmeans (SE) (b) from mixed-effects models for repeated measurements with  
600 estimated group differences (EGD) presented with their 95% confidence interval; \* $P < 0.05$ ; \*\* $P < 0.01$  (b).

601 BM= body mass; FFM= fat-free mass; FM= fat mass; DXA= dual X-ray absorptiometry; SLAMMD= Space  
602 Linear Acceleration Mass Measurement Device; DLW= doubly labeled water

603

604 **Fig 2:** Energy expenditure changes during flight

605 Changes in total energy expenditure and its components during the experimental sessions (a) and scatterplots for  
606 the relationship between body composition changes and energy expenditure components changes (b). Values are  
607 lsmeans (SE) from mixed-effects models for repeated measurements with estimated group differences (EGD)  
608 presented with their 95% confidence interval; \*\*\* $P < 0.001$  (a). Least square regression lines are plotted with their  
609 95% confidence interval in shaded areas (b).

610 TEE<sub>BM</sub>= total energy expenditure adjusted for body mass; AEE<sub>BM</sub>= activity-related energy expenditure adjusted  
611 for body mass; RMR<sub>FFM & FM</sub>= resting metabolic rate adjusted for fat-free mass and fat mass; FFMI= fat-free mass  
612 index; FMI= fat mass index.

613

614 **Fig 3:** Physical activity changes during flight

615 Changes in SWP-derived activity duration and acceleration-MAD (as a proxy of activity workload) during the  
616 experimental sessions (a) and scatterplots for the relationship between inflight body composition and inflight SWP-  
617 derived or reported physical activity (b). Values are lsmeans (SE) from mixed-effects models for repeated  
618 measurements (a). Least square regression lines are plotted with their 95% confidence interval in shaded areas;  
619 individual values are available for 9 astronauts only (b).

620 FFMI= fat free mass index; FMI= fat mass index; T2= treadmill device onboard the ISS; SWP= Sensewear Pro  
621 activity monitor; MAD= acceleration mean amplitude deviation; VPA= vigorous physical activity (MAD>16  
622 milli-g)

623

624 **Fig 4:** Estimated energy intakes during flight

625 Percentage of energy intake (EI) estimated from the changes in body composition and total energy expenditure  
626 between the preflight and inflight experimental sessions from the 2001 Dietary Reference Intakes (DRIs)<sup>[19]</sup> for  
627 each groups stratified for inflight TEE changes. Values are individual data and mean (SE)