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

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Running heading (84 characters)

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

Abstract (277 words)

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

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

Results: Three-month spaceflight decreased BM (-1.20kg (SE 0.5); P=0.04), mainly due to non-significant fat-free mass loss (FFM; -0.94kg (0.59)). The decrease in walking time (-63.2min/day (11.5); P<0.001) from preflight was compensated by increases in non-ambulatory activities (+64.8min/day (18.8); P<0.01). Average TEE was unaffected but a large interindividual variability was noted. Astronauts were stratified into those who maintained (stable_TEE; n=6) and those who decreased (decreased_TEE n=5) TEE and AEE compared to preflight data. Although both groups lost similar BM, FFM was maintained and FM reduced in stable_TEE astronauts, while FFM decreased and FM increased in decreased_TEE astronauts (estimated between-group-difference (EGD) in ΔFFMindex [FFMI] 0.87kg/m^2, 95%CI +0.32 to +1.41; P=0.01, ΔFMindex [FMI] -1.09kg/m^2, 95%CI -2.06 to -0.11kg/m^2; P=0.03). The stable_TEE group had higher baseline FFMI, and greater baseline and inflight vigorous PA than the decreased_TEE group (P<0.05 for all). ΔFMI and ΔFFMI were respectively negatively and positively associated with both ΔTEE and ΔAEE.

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

Word counts: 5486

Key points

- 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.

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

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Declarations

Funding **–** This study was funded by the French Space Agency (CNES, French Space Agency) and the European Space agency which provided financial and operational supports for the Energy experiment.
Conflicts of interest **–** PB, AZ, DSA, IC, ELR, CT, AM, MG, GGK, AB, CS and SB declare that they have no potential conflicts of interest that might be relevant to the contents of this manuscript.
Availability of data and material **–** Data are accessible upon reasonable request to the authors after validations by space agencies.
Ethics approval **–** The study was yearly approved by the NASA Institutional Review Board under NASA 7116301606HR. The ESA Medical Board and the JAXA Institutional Review Board for human experiment also approved the experiment.
Consent to participate **–** The study was conducted in conformity with the policy statement regarding the use of human participants as outlined in the Declaration of Helsinki of 1964. All astronauts received a detailed presentation of the experiment before enrolling the study and signed a written informed consent.
Consent for publication – A global authorization from the European Space Agency was obtained to use and publish the photos (ESA copyright).

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

1 INTRODUCTION

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

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

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

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

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

2 MATERIALS AND METHODS

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

2.1 Participants

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

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

2.2 Protocol

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

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

2.3 Total energy expenditure and its components

TEE was measured over 10 days using the doubly-labeled water (DLW) method as previously described [16]. The DLW is the gold standard to assess TEE in free living conditions. The technique is fully described **in supplemental methods**. Briefly, it is based on the exponential elimination of the stable isotopes ^{2}H and ^{18}O after a bolus dose of water labeled with both isotopes. The ^{2}H are lost as water, whereas the ^{18}O are lost both as water and as CO_2. Thus, the excess disappearance rate of ^{18}O relative to ^{2}H is a measure of the CO_2 production rate. This latter is converted to TEE using the food quotient or the respiratory quotient and the classic indirect calorimetry equations. On the DLW dosing day, RMR was measured in fasting state, at rest for 45 min using the Pulmonary Function System (PFS, manufactured by the Danish Aerospace Company, former DAMEC)[17]. AEE was calculated from TEE and RMR assuming a diet-induced thermogenesis of 10% of TEE. PA level (PAL) was calculated as the ratio between TEE and RMR.

2.4 Body composition

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

2.5 Energy intake

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

2.6 Physical exercise and activities

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

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

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

2.7 Statistical analyses

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

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

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

Baseline values and inflight physical training variables are presented as means (standard deviation, SD). Unless otherwise noted, results of the linear mixed-models are presented as least square means (standard error, SE). Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, North Carolina) with a significance level at 5%. Figures were realized with Prism 9 (GraphPad, San Diego, California).

3 RESULTS

3.1 Few spaceflight changes for the whole group of astronauts

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

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 included 66.8 min/day (34.1) walking and 8.1 min/day (10.5) running, corresponding to a total of 10077 steps/day (2834) and 7.4 min/day (9.2) of SWP-derived VPA.

- Please insert Table 1 near here –

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

- Please insert Figure 1 near here -

No significant overall changes in TEE, RMR and AEE were noted between preflight and flight (**Table 2**). However, inflight SWP-derived ambulatory activities dramatically dropped with daily steps decreasing by 6583 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 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 spent exercising on the T2, this reduction in ambulatory activities was almost fully compensated by an increase in SWP-derived non-ambulatory activities of 64.8 min/day (SE 18.8; P<0.01). This explained the absence of significant net changes in SWP-derived overall active time and accelerometry MAD, a proxy of overall PA workload. Daily SWP-derived-VPA was not significantly different from ground values. The one-hour increase in non-ambulatory activities, leading to a total of 151.8 min/day (SE 18.9; range 119.7-194.5), was only partially 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 as reported by the astronauts (**Supplemental Table 1**). This suggested an increase in PA not related to physical training. Overall, the astronauts reported 6.2 aerobic exercise sessions/week (SD 1.4; range 3.6-8.6) corresponding to a total duration of 167.8 min/week (SD 43.9; range 86.9-257.8). They reported 4.5 resistance training 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 sets. This led to a total of 1481 repetitions/week (SD 834; range 468-3044) and a muscular time under tension of 81.8 min/week (SD 43.5; range 28.8-161.2). These numbers exhibit a great inter-individual variability in the adherence to the exercise prescriptions. They are to be compared to the 180-270 min/week aerobic, and 6-resistive exercise sessions/week (corresponding to about 1500 repetitions/week) recommendations [21].

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

- Please insert Table 2 near here -

3.2 Baseline Characteristics of inflight-TEE-changes groups

At baseline (**Table 1**), both groups were of the same age and had similar FMI but, compared to the decreased_TEE group, the stable_TEE astronauts had higher FFMI (20.6 kg/m² [SD 1.5] vs 18.3 kg/m² [1.6]; *P*=0.03) and presented a more active profile. TEE, AEE and daily SWP-derived overall active time did not differ between the two groups. However, the stable_TEE astronauts spent more time on ground in SWP-derived VPA (12.4 min/day [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 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 decreased_TEE astronauts.

3.3 Anthropometric changes by inflight-TEE-changes groups

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

3.4 Energy expenditures changes by inflight-TEE-changes groups

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

- Please insert Figure 2 near here -

3.5 Physical activity changes by inflight-TEE-changes groups

Net changes in SWP and accelerometry-derived activity parameters (**Table 2** and **Fig 3a**) were globally similar across groups. However, like what was observed on the ground, the stable_TEE astronauts still spent more time 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 accelerometry MAD (884 milli-g/day [125] vs 505 milli-g/day [140]; *P*=0.06), and SWP-derived mean activity 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 inflight resistance training characteristics and time spent performing CEVIS were not significantly different between groups **(Supplemental Table 1)**. The stable_TEE group reported non-significant higher inflight aerobic 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 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 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 running-related accelerometry MAD (461 milli-g/day [SE 87] vs 154 milli-g/day [98]; *P*=0.03)

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

- Please insert Figure 3 near here -

3.6 Energy intakes by inflight-TEE-changes groups

EI (BM-adjusted) calculated from changes in body composition from the time of beginning of space flight to the 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 (0.6) in the decreased_TEE group, representing 93% and 75% of DRIs, respectively (**Fig 4**).

- Please insert Figure 4 near here -

4 DISCUSSION

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

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

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

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

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

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

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

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

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

4.3 Energy intakes during long-term spaceflight

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

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

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

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

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

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

4.5 Limitations and strengths of the study

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

5 CONCLUSION

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

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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 [b]
Age & anthropometry				
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^2)	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^2)	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^2)	4.8 (1.0)	4.7 (1.2)	4.9 (1.0)	0.71
Energetics				
TEE (MJ/day)	13.2 (1.9)	14.0 (1.4)	12.2 (2.1)	0.13
TEE_{BM} (MJ/day) [a]	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
$RMR_{FFM\ \&\ FM}$ (MJ/day) [a]	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_{BM} (MJ/day) [a]	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 $intake_{BM}$ (MJ/day) [a]	13.2 (1.1)	13.3 (1.2)	13.0 (1.3)	0.70
SWP-derived physical activities				
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
Accelerometry MAD				
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

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, AEE=activity energy expenditure; EI=energy intake MAD=acceleration mean absolute deviation. Values are means (SD). SWP =Sensewear Pro activity-device. Physical activities were derived from a combination of different physiological signals with an in-built machine-learning activity classification algorithm.

[a] BM (TEE, AEE, EI) or FFM and FM (RMR) adjusted lsmeans (SD) (whole group mean baseline values were used as reference values). All astronauts were male.

[b] Statistical analyses used Student's t-tests.

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**
Age & anthropometry							
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^2)	-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^2)	-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^2)	-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
Energetics							
TEE_{BM} (MJ/day) [a]	-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
$RMR_{FFM\ \&\ FM}$ (MJ/day) [a]	-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_{BM} (MJ/day) [a]	-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 $intake_{BM}$ (MJ/day) [a]	-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 $balance_{BM}$ (MJ/day) [a]	-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
SWP-derived physical activities							
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
Accelerometry MAD							
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
Activity NASA logs [c]							
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)[d]	29.0 (4.3)	.	30.9 (6.1)	26.8 (6.7)	-4.1 (9.0)	-24.5 to 16.4	0.66

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, AEE=activity energy expenditure; EI=energy intake; EB=energy balance; MAD=Acceleration mean absolute deviation; T2= treadmill device onboard the ISS; CEVIS= Cycle Ergometer with Vibration Isolation and Stabilization system; ARED= Advanced Resistive Exercise Device. SWP =Sensewear Pro device.

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

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

[a] BM (TEE, AEE, EI, EB) or FFM and FM (RMR) adjusted Models (whole group mean baseline values were used as reference values).

[c] Inflight Values

[d] 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 set)

Legends

Fig 1: Body mass and composition changes during flight

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

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

Fig 2: Energy expenditure changes during flight

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

TEE_{BM}= total energy expenditure adjusted for body mass; AEE_{BM}= activity-related energy expenditure adjusted for body mass; $RMR_{FFM\ \&\ FM}$= resting metabolic rate adjusted for fat-free mass and fat mass; FFMI= fat-free mass index; FMI= fat mass index.

Fig 3: Physical activity changes during flight

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

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

Fig 4: Estimated energy intakes during flight

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