Protein Intake and Physical Performance Following Long-Term Stay on the International Space Station

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INTRODUCTION: Exposure to microgravity reduces muscle mass, volume, and performance. The ingestion of protein, especially combined with carbohydrate intake and exercise after ingestion, improves net muscle protein synthesis and increases muscle mass. However, there are few studies on this relationship during and after a long-term spaceflight. The objective of this study was to investigate the influence of protein and the combined effects of carbohydrate intake on muscle performance following long-term spaceflight.

METHODS: This study is a retrospective cohort study involving secondary analysis of data stored in the NASA Lifetime Surveillance of Astronaut Health Repository. Multivariable analysis was performed to evaluate the impact of protein intake on physical performance by considering covariates potentially associated with each model.

RESULTS: After adjusting for sex, age, flight week, energy intake, and preflight physical performance, protein intake was found to be significantly associated with concentric measurements for knee extension ($\beta = 51.66$), ankle plantar flexion ($\beta = 32.86$), and eccentric measurements for ankle plantar flexion ($\beta = 79.85$) at 5 d after landing. Significant associations remained after controlling for exercise effect. No significant interaction between protein and carbohydrate intake was observed in either model.

DISCUSSION: Protein intake during spaceflight was related to physical performance for knee extension and ankle plantar flexion, even after taking exercise effect into consideration. However, protein and carbohydrate intake provided no synergetic benefit. This suggests that high protein intake, about twice the current average intake, may serve as a countermeasure to offset the negative effects of long-duration spaceflights.

KEYWORDS: astronaut, nutrition, muscle performance, countermeasures.

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Exposure to microgravity reduces muscle mass, volume, and performance, especially in the legs, on both short[1,6,14] and long flights.[4,6,8] The most affected muscle is the triceps surae muscle, wherein 20% fiber atrophy occurs after 6 mo of spaceflight.[6,8] In addition, preferential atrophy of the extensors over the flexors occurs in the first few weeks.[14] One study reported a 10% decrease in strength across different muscle actions, an 8% decrease in the cross-sectional area of the knee extensor and gluteal muscles, and no significant change in the knee flexor muscle cross-sectional area after a 17-d spaceflight.[14] Therefore, for routine mission medical monitoring (MEDB Isokinetic Assessment), standard clinical isokinetic dynamometer measurements on selected muscle groups in the legs have been conducted to evaluate muscle atrophy in astronauts.

Although many types of exercise protocols have been proposed to aid in the maintenance of muscle during flight, an efficient method that combines nutritional and mechanical countermeasures is required. According to various ground studies conducted in the past, it is known that the ingestion of proteins, including branched-chain amino acids, especially

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when combined with carbohydrate intake and exercise after ingestion, improves net muscle protein synthesis and increases muscle mass.[12,15,16] Ferrando et al. reported that increasing protein intake above the recommended daily allowance preserved muscle function in the elderly during 10 d of bed rest.[5] Further, in a 28-d bed rest study, essential amino acid and carbohydrate supplementation provided an anabolic stimulus capable of ameliorating the loss of lean muscle mass and muscle strength.[13] However, there are few studies on the relationship between nutrition and muscle strength during and after long-term spaceflight. The purpose of this study was to evaluate the influence of protein and carbohydrate intake on physical performance following long-term spaceflight using the Longitudinal Study of Astronaut Health (LSAH) database at NASA.

This information can be useful for the development of new space foods for future space exploration. Because the loss of muscle mass and muscle strength in astronauts during spaceflight strongly resembles sarcopenia on Earth, that is, age-related involuntary loss of skeletal muscle mass and strength, this study is expected to be useful for the treatment of sarcopenia as well.

METHODS

Subjects

This is a retrospective cohort study that conducted secondary analysis of data obtained from the NASA LSAH. The LSAH database contains annual preventive medical examination data and other medical data collected on the ground and in orbit from astronauts. The Faculty of Medicine Institutional Review Board of the University of Tsukuba, NASA Institutional Review Board, and JAXA Institutional Review Board for Human Research approved this study. We received approval from the LSAH Advisory Board for use of unattributable medical data, specifically the following: nutrition status assessment data, exercise record, isokinetic assessment data, biochemical testing data, and body composition assessment data of astronauts who met the criteria of the protocol. The inclusion criteria were as follows: 1) subjects whose information on nutrition status assessment, exercise record, and isokinetic assessment was available; and 2) subjects who stayed at the International Space Station (ISS) for a period of 3–9 mo. Subjects with a history of active, uncontrolled gastrointestinal disorders were excluded. A total of 76 records were required for multiple regression tests with three predictors with a medium effect size ($f2 = 0.15$), 80% power, and at a significance level of 5%. Astronauts who were repeat flyers were treated as independent records.

Procedure

Nutrition status assessment. The crewmembers performed an estimation of weekly food intake using an electronic food frequency questionnaire (FFQ) during flight. The FFQ is designed to provide an estimate of seven key nutrients: fluids ($mL \cdot d^{-1}$), energy ($kcal \cdot d^{-1}$), protein ($g \cdot d^{-1}$), calcium ($mg \cdot d^{-1}$), sodium ($mg \cdot d^{-1}$), iron ($mg \cdot d^{-1}$), and potassium ($mg \cdot d^{-1}$) from a clinical point of view. The FFQ is unique for each space mission, depending on the available food. The foods are categorized so that each line has 1–10 or more food items, based on their content of the seven nutrients. The crew then estimate their intake over the 7 d for each group of foods. If a grouping of foods includes 15 different foods and the crewmember consumed 5 of the same food in that group, he/she will indicate the number consumed as 5. The weekly in-flight summary of the seven nutrients for both raw and supplement intake was calculated from this FFQ. Average daily protein and carbohydrate intake amounts were calculated from the weekly in-flight summary.

Exercise record. At scheduled times during the day, the crewmembers exercised using one or more of the available exercise equipment (Treadmill 2, Cycle Ergometer with Vibration Isolation and Stabilization, and Advanced Resistive Exercise Device). In 1 g, exercise included over-ground running and cycling. The crewmembers recorded the exercise program in real time for the in-flight duration. Exercise data were downloaded minimally once a week and transferred to the Mission Extended Medical Enterprise. The "total time doing aerobic-style exercise (min/week)" and "ARED sessions per week (times/week)" were selected as the covariates for the multiple regression analysis in this study.

1. For all exercise modalities: number of training sessions per week.
2. For Treadmill 2: speed, subject loading, heart rate, exercise duration, and tread resistance (passive mode only); ground reaction forces.
3. For Cycle Ergometer with Vibration Isolation and Stabilization: work rate (Watts), pedaling speed, heart rate, exercise duration, and arm cycle session.
4. For the ARED: exercises performed, prescribed load, dialed in load, number of sets and repetitions, and ground reaction force (GRF) data.

Isokinetic assessment. Muscle performance testing was performed using a standard clinical isokinetic dynamometer on selected muscle groups: concentric knee extension and flexion (seated), concentric ankle plantar flexion and dorsiflexion (prone), eccentric ankle plantar flexion (prone), and dorsiflexion and concentric trunk extension and flexion (separated). A standard procedure for warm-up prior to testing was followed for each muscle group. Testing was performed on the right limb unless previous injury required the left limb to be used for these assessments. Postflight measurements were performed on the same limb as preflight. Pre- and postflight testing consisted of 3 components: 1) 5 reps each of isokinetic concentric measurements at $30° \cdot s^{-1}$ for the ankle and $60° \cdot s^{-1}$ for all other joints to assess muscle strength; 2) 5 reps of isokinetic eccentric measurements at $30° \cdot s^{-1}$ at the ankle only to assess muscle strength; and 3) 21 reps of isokinetic concentric measurements at $180° \cdot s^{-1}$ for the knee only to assess muscle endurance. In the isokinetic measurements, the higher the movement speed, the smaller the peak torque. At the knee, concentric measurements at $60° \cdot s^{-1}$ are used for general muscle strength measurement and endurance is evaluated at $180° \cdot s^{-1}$. For the ankle, $30° \cdot s^{-1}$ is known to be the rate at which the slow type I fiber activity is the largest; therefore, it was selected to assess the effects on them in space. Testing was performed in the United States or Russia before and after the flight. Preflight data were acquired twice (270 and 90 d before launch) and postflight data were acquired twice (5 and 30 d after landing).

Biochemical test. Blood samples and 48-h void-by-void urine pools were collected for determination of nutritional status and renal stone risk. In this study, total serum protein, serum albumin, serum calcium, γ-glutamyl transferase, creatine kinase, alanine transaminase, aminotransferase, triglyceride, cholesterol, high density lipoprotein, low density lipoprotein (LDL), glucose, blood urea nitrogen, serum creatinine, C-reactive protein (hypersensitive), serum uric acid, plasma vitamin B6-pyridoxal 5'-phosphate, and plasma vitamin C levels were evaluated as markers related to nutritional and muscle status. Testing was performed in the United States or Russia before (90–30 d before launch) and after flight (0 and 14–30 d after landing).

Body composition assessment. The height and weight of the subjects were measured at preflight, in flight, and postflight. Preflight data were acquired twice (21–18 mo and 90–30 d before launch) and postflight data were also acquired twice (0 and 20–30 d after landing). BMI at preflight was calculated as weight (kg) divided by height squared (m^2) at the last measurement before flight.

Statistical Analysis

Variables related to exposure (sex, age, flight duration, weight at preflight, height at preflight, BMI at preflight, physical activity in flight, and dietary factors in flight) and outcome (percentage retention in muscle strength at the return date) were explored to characterize the study subjects. To evaluate the differences between preflight and postflight physical performance measures, the *t*-test was performed. Univariate and bivariate analyses were conducted to find the association between exposure and other covariates related to the outcome. The covariates were selected based on previous literature, including variables significantly associated with physical performance. Multivariable analysis was then performed to test whether protein and carbohydrate intake can modify muscle performance with covariate adjustment. In Model 1, sex, age, flight week, energy intake, and physical performance at preflight were included as covariates. In Model 2, the variables in Model 1, total time doing aerobics-style exercise per week, and ARED sessions per week were included as covariates. Correlations between biochemical testing data and physical performance measures were tested using Pearson's product moment correlation coefficient (r) to support the relationship between physical performance change and muscle-related biochemical parameters. All statistical analyses were performed using SPSS software version 26 for Windows (IBM Corp., Armonk, NY, USA). The result was considered significant if the *P*-value was < 0.05.

RESULTS

There were 62 records (50 men and 12 women) which met the study criteria (some of the 62 records with a study ID were repeat flyers). More than 50% of the subjects were 44–50 yr old. The mean height, weight, and weeks in space were 174.7 ± 6.5 cm, 79.4 ± 11.9 kg, and 21.4 ± 4.5 wk, respectively. Total protein intake was $103.7 \pm 28.7\ g \cdot d^{-1}$ ($1.3 \pm 0.3\ g \cdot kg^{-1} \cdot d^{-1}$) and mean energy intake was $2382.2 \pm 554.0\ kcal \cdot d^{-1}$ (**Table I**).

Isokinetic strength significantly decreased in both the knee flexor and knee extensor and were 87.7 ± 14.1% [$t(57) = 6.673$, $P < 0.001$] and 91.4 ± 13.7% [$t(57) = 5.294$, $P < 0.001$] of preflight levels, respectively. Ankle plantar flexor strength decreased more than dorsiflexor strength in isokinetic concentric measurements and were 86.9 ± 11.9% [$t(57) = 7.675$, $P < 0.001$] and 91.1 ± 15.2% [$t(56) = 4.598$, $P < 0.001$] of preflight levels, respectively (**Table II**).

Total work at knee extension performed during the knee endurance test decreased from before to after the flight and its %Retention from preflight levels was 90.3 ± 10.1% [$t(54) = 6.634$, $P < 0.001$]. There was also a loss in total knee flexion work at postflight and it was 92.3 ± 14.0% [$t(54) = 3.931$, $P < 0.001$] of preflight levels (Table II). Isokinetic concentric measurements of trunk extension and flexion strength postflight were conducted 30 d after landing. Trunk flexion strength had decreased significantly and was 94.4 ± 11.7% [$t(45) = 3.186$, $P = 0.003$] of preflight levels (Table II).

Table I. Characteristics of the Subjects.

VARIABLES	MEAN	SD	*N*
Sex (Male), *N* (%)	50 (80.6%)		62
Age			
35–43 yr	15 (24.2%)		
44–50 yr	32 (51.6%)		
50+ yr	15 (24.2%)		
Height at preflight (cm)	174.7	6.5	56
Weight at preflight (kg)	79.4	11.9	62
BMI at preflight (kg/m^2)	26.2	3.3	56
Flight duration (wk)	21.4	4.5	62
Physical activity			
Treadmill sessions per week	3.3	1.1	62
Average total amount of time on T2 (min/wk)	96.2	41.2	62
Total time of aerobic exercise (min/wk)	182.5	61.7	62
Total time of aerobic exercise (70% heart rate max) (min/session)	33.4	13.9	44
T2 load (kg)	52.3	8.1	57
ARED Sessions per week	4.8	1.1	61
ARED average squat load (kg)	98.0	20.7	56
ARED deadlift load (kg)	82.4	23.5	56
Dietary factors			
Protein ($g \cdot d^{-1}$)	103.7	28.7	62
Protein ($g \cdot kg^{-1} \cdot d^{-1}$)	1.3	0.3	62
Energy intake ($kcal \cdot d^{-1}$)	2382.2	554.0	62

BMI, body mass index; T2, treadmill 2; SD, standard deviation.

Table II. Changes in Physical Performance Measures Following Spaceflight.

PHYSICAL PERFORMANCE MEASURES	PARAMETERS		N	%RETENTION FROM BASE LINE* MEAN	SD	$P^{\dagger}$
Knee Extension (Total Work: J)	$180° \cdot s^{-1}$	CON	55	90.3	10.1	<0.001
Knee Extension (Peak Torque: Nm)	$180° \cdot s^{-1}$	CON	58	87.1	10.0	<0.001
Knee Extension (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	58	91.4	13.7	<0.001
Knee Flexion (Total Work: J)	$180° \cdot s^{-1}$	CON	55	92.3	14.0	<0.001
Knee Flexion (Peak Torque: Nm)	$180° \cdot s^{-1}$	CON	58	88.5	15.0	<0.001
Knee Flexion (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	58	87.7	14.1	<0.001
Ankle Dorsiflexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	CON	57	91.1	15.2	<0.001
Ankle Dorsiflexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	ECC	51	91.3	7.4	<0.001
Ankle Plantar Flexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	CON	58	86.9	11.9	<0.001
Ankle Plantar Flexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	ECC	54	89.7	17.1	<0.001
Trunk Extension (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	46	99.2	14.7	0.385
Trunk Flexion (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	46	94.4	11.7	0.003

CON, concentric; ECC, eccentric; SD, standard deviation.
*%Retention was calculated as the ratio of physical measures at R-5 to the last measurement before flight. †*t*-test was used to evaluate the differences between physical performance measures at preflight and postflight.

The association between protein intake and energy intake with physical performance measures at 5 d after landing is shown in **Table III**. After adjustment for sex, age, flight week, energy intake, and physical performance at preflight (Model 1), protein intake was positively associated with isokinetic concentric measurements at 60° for knee extension ($\beta = 51.7$, $P = 0.021$), isokinetic concentric measurements at 30° for ankle plantar flexion ($\beta = 32.9$, $P = 0.008$), and isokinetic eccentric measurements at 30° for ankle plantar flexion ($\beta = 79.9$, $P < 0.001$). In addition, significant associations remained after controlling for total time doing aerobic-style exercise and ARED sessions per week (Model 2): protein intake was positively associated with isokinetic concentric measurements at 60° for knee extension ($\beta = 51.4$, $P = 0.017$), isokinetic concentric measurements at 30° for ankle plantar flexion ($\beta = 32.8$, $P = 0.009$), and isokinetic eccentric measurements at 30° for ankle plantar

Table III. Association of Protein Intake ($g \cdot d^{-1} \cdot kg^{-1}$) and Energy Intake ($kcal \cdot d^{-1} \cdot kg^{-1}$) with Physical Performance Measures at Post1 (R-5).

PHYSICAL PERFORMANCE MEASURES AT POST1	PARAMETERS		PROTEIN INTAKE MODEL 1† REGRESSION COEFFICIENT (95% CI)	N	MODEL 2‡ REGRESSION COEFFICIENT (95% CI)	N
Knee Extension (Total Work: J)	$180° \cdot s^{-1}$	CON	295.2 (−171.2, 761.6)	55	288.3 (−188.6, 765.2)	54
Knee Extension (Peak Torque: Nm)	$180° \cdot s^{-1}$	CON	9.0 (−17.8, 35.9)	58	8.3 (−18.5, 35.1)	57
Knee Extension (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	51.7 (8.2, 95.2)*	58	51.4 (9.4, 93.3)*	57
Knee Flexion (Total Work: J)	$180° \cdot s^{-1}$	CON	285.4 (−73.9, 644.7)	55	278.8 (−90.0, 647.6)	54
Knee Flexion (Peak Torque: Nm)	$180° \cdot s^{-1}$	CON	7.3 (−14.4, 29.0)	58	5.2 (−16.1, 26.6)	57
Knee Flexion (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	15.4 (−10.6, 41.3)	58	12.9 (−12.7, 38.6)	57
Ankle Dorsiflexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	CON	−1.2 (−10.5, 8.2)	57	0.0 (−9.1, 9.1)	56
Ankle Dorsiflexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	ECC	−2.2 (−10.3, 5.8)	51	−1.4 (−9.0, 6.2)	50
Ankle Plantar Flexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	CON	32.9 (9.1, 56.6)*	58	32.8 (8.7, 56.9)*	57
Ankle Plantar Flexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	ECC	79.9 (37.7, 122.0)*	54	82.0 (43.4, 120.6)*	53
Trunk Extension (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	n.a.	n.a.	n.a.	n.a.
Trunk Flexion (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	n.a.	n.a.	n.a.	n.a.
			PROTEIN AND ENERGY INTAKE			
Knee Extension (Total Work: J)	$180° \cdot s^{-1}$	CON	−10.1 (−35.8, 15.7)	55	−10.5 (−36.9, 15.8)	54
Knee Extension (Peak Torque: Nm)	$180° \cdot s^{-1}$	CON	0.2 (−1.3, 1.6)	58	0.1 (−1.3, 1.6)	57
Knee Extension (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	0.6 (−1.8, 3.0)	58	0.6 (−1.7, 2.9)	57
Knee Flexion (Total Work: J)	$180° \cdot s^{-1}$	CON	−18.2 (−37.9, 1.5)	55	−19.2 (−39.4, 1.0)	54
Knee Flexion (Peak Torque: Nm)	$180° \cdot s^{-1}$	CON	−0.6 (−1.8, 0.5)	58	−0.7 (−1.9, 0.5)	57
Knee Flexion (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	−0.8 (−2.2, 0.6)	58	−0.9 (−2.2, 0.5)	57
Ankle Dorsiflexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	CON	0.3 (−0.2, 0.8)	57	0.4 (−0.1, 0.9)	56
Ankle Dorsiflexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	ECC	0.2 (−0.3, 0.6)	51	0.2 (−0.2, 0.6)	50
Ankle Plantar Flexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	CON	0.4 (−0.9, 1.7)	58	0.4 (−0.9, 1.7)	57
Ankle Plantar Flexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	ECC	−0.4 (−2.7, 1.9)	54	−0.3 (−2.4, 1.8)	53
Trunk Extension (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	n.a.	n.a.	n.a.	n.a.
Trunk Flexion (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	n.a.	n.a.	n.a.	n.a.

CON, concentric; ECC, eccentric; SD, standard deviation.
*Indicates $P < 0.05$; †Model 1 was adjusted for sex, age, flight week, energy intake, and physical performance at preflight; ‡Model 2 was adjusted for variables in Model 1, total time doing aerobics-style exercise, and ARED session per week.

flexion ($\beta = 82.0$, $P < 0.001$). However, no significant association between protein and carbohydrate intake and physical performance measures at 5 d after landing was observed in either Model 1 or 2.

In the analysis with physical performance measures at 30 d after landing, a significant association between protein intake and isokinetic concentric measurements at 30° for ankle plantar flexion was still observed in Models 1 and 2 ($\beta = 30.9$, $P = 0.026$ and $\beta = 31.5$, $P = 0.027$, respectively) (**Table IV**). Furthermore, the interaction of protein and carbohydrate intake was positively correlated with isokinetic concentric measurements at 30° for ankle dorsiflexion ($\beta = 0.6$, $P = 0.020$) and negatively correlated with isokinetic concentric measurements at 180° for knee flexion ($\beta = -19.6$, $P = 0.037$), and at 60° for trunk flexion ($\beta = -4.5$, $P = 0.014$).

Regarding the correlation between changes in laboratory test analysis levels and changes in physical performance measures during the mission, pre- to postflight changes in serum creatinine were significantly correlated with isokinetic concentric measurements at 180° for knee extension ($r = 0.346$, $P = 0.033$) and at 180° and 60° for knee flexion ($r = 0.420$, $P = 0.009$ and $r = 0.437$, $P = 0.006$, respectively). Changes in C-reactive protein were also positively correlated with isokinetic concentric measurements at 180° for knee extension ($r = 0.485$, $P = 0.009$) and 180° for knee flexion ($r = 0.505$, $P = 0.006$). On the contrary, an inverse correlation was observed between changes in LDL and isokinetic concentric measurements at 180° and 60° for the knee flexion ($r = -0.775$, $P = 0.041$ and $r = -0.991$, $P < 0.001$, respectively).

DISCUSSION

Based on a review of the literature this may be the first study to report the actual impact of protein intake during spaceflight on muscle performance. The findings of this study showed that protein intake during spaceflight was related to some physical performance even after taking the exercise effect into consideration. Protein intake was positively associated with isokinetic concentric measurements for knee extension and isokinetic measurements for ankle plantar flexion at 5 d after landing. Regarding ankle plantar flexion, a significant association was still observed at 30 d after landing. According to the results of the multivariable analysis, 1 $g \cdot kg^{-1} \cdot d^{-1}$ body weight protein intake contributes to a 33 Nm increase in concentric measurements and an 80–82 Nm increase in eccentric measurements for ankle plantar flexion 5 d after landing. The average decrease in plantar flexor strength that occurred following spaceflight was approximately 20 Nm, and high protein intake, approximately twice the current average intake, may be clinically useful for microgravity-induced loss of muscle strength. However, increased consumption of sulfur-containing amino acids is

Table IV. Association of Protein Intake ($g \cdot d^{-1} \cdot kg^{-1}$) and Energy Intake ($kcal \cdot d^{-1} \cdot kg^{-1}$) with Physical Performance Measures at Post2 (R-30).

PHYSICAL PERFORMANCE MEASURES AT POST2	PARAMETERS		MODEL 1† REGRESSION COEFFICIENT (95% CI)	N	MODEL 2‡ REGRESSION COEFFICIENT (95% CI)	N
			PROTEIN INTAKE			
Knee Extension (Total Work: J)	$180° \cdot s^{-1}$	CON	145.4 (−175.8, 466.5)	55	122.9 (−198.5, 444.2)	54
Knee Extension (Peak Torque: Nm)	$180° \cdot s^{-1}$	CON	−1.9 (−22.7, 18.9)	58	−3.7 (−23.7, 16.3)	57
Knee Extension (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	17.6 (24.5, 60.5)*	58	15.5 (−27.5, 58.5)	57
Knee Flexion (Total Work: J)	$180° \cdot s^{-1}$	CON	98.2 (−240.0, 436.5)	55	92.2 (−256.4, 440.7)	54
Knee Flexion (Peak Torque: Nm)	$180° \cdot s^{-1}$	CON	−4.4 (−23.3, 14.5)	58	−6.0 (−25.0, 13.0)	57
Knee Flexion (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	−1.5 (−26.6, 23.6)	58	−2.9 (−28.4, 22.5)	57
Ankle Dorsiflexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	CON	−2.5 (−12.0, 7.1)	57	−2.0 (−11.6, 7.6)	56
Ankle Dorsiflexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	ECC	−3.3 (−12.2, 5.6)	52	−3.0 (−12.1, 6.2)	51
Ankle Plantar Flexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	CON	30.9 (3.9, 57.9)*	58	31.5 (3.8, 59.3)*	57
Ankle Plantar Flexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	ECC	36.1 (10.3, 82.5)*	54	41.7 (−5.1, 88.4)	53
Trunk Extension (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	34.6 (−131.5, 200.7)	46	36.2 (−137.8, 210.2)	45
Trunk Flexion (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	11.5 (−63.4, 86.3)	46	7.9 (−65.1, 80.9)	45
			PROTEIN AND ENERGY INTAKE			
Knee Extension (Total Work: J)	$180° \cdot s^{-1}$	CON	2.1 (−15.7, 20.0)	55	1.1 (−16.8, 18.9)	54
Knee Extension (Peak Torque: Nm)	$180° \cdot s^{-1}$	CON	0.8 (−0.3, 1.9)	58	0.7 (−0.4, 1.8)	57
Knee Extension (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	−0.5 (−2.8, 1.9)	58	−0.6 (−2.9, 1.8)	57
Knee Flexion (Total Work: J)	$180° \cdot s^{-1}$	CON	−19.6 (−38.0, −1.3)*	55	−20.6 (−39.4, −1.7)*	54
Knee Flexion (Peak Torque: Nm)	$180° \cdot s^{-1}$	CON	−0.7 (−1.7, 0.3)	58	−0.8 (−1.8, 0.3)	57
Knee Flexion (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	−0.8 (−2.2, 0.6)	58	−0.8 (−2.2, 0.5)	57
Ankle Dorsiflexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	CON	0.6 (0.1, 1.1)*	57	0.6 (0.1, 1.1)*	56
Ankle Dorsiflexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	ECC	0.1 (−0.4, 0.5)	52	0.1 (−0.4, 0.6)	51
Ankle Plantar Flexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	CON	−1.0 (−2.4, 0.5)	58	−1.0 (−2.4, 0.5)	57
Ankle Plantar Flexion (Peak Torque: Nm)	$30° \cdot s^{-1}$	ECC	−1.7 (−4.2, 0.8)	54	−1.6 (−4.1, 0.9)	53
Trunk Extension (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	1.1 (−7.3, 9.4)	46	0.7 (−7.8, 9.2)	45
Trunk Flexion (Peak Torque: Nm)	$60° \cdot s^{-1}$	CON	−4.5 (−8.0, −1.0)*	46	−4.1 (−7.5, −0.7)*	45

CON, concentric; ECC, eccentric; SD, standard deviation

*Indicates $P < 0.05$; †Model 1 was adjusted for sex, age, flight week, energy intake, and physical performance at preflight; ‡Model 2 was adjusted for variables in Model 1, total time doing aerobics-style exercise, and ARED session per week.

associated with increased bone resorption.[11] Zwart et al. have described the potential for low-grade metabolic acidosis during 30 d of bed rest and suggest that the ratio of animal protein to potassium intake predicts the rate of bone resorption.[19] This means that future unloading studies should evaluate increased protein intake via nonsulfur containing essential amino acids to protect both muscle and bone health since optimizing skeletal muscle parameters via increasing protein intake to 1.5–2.0 $g \cdot kg^{-1} \cdot d^{-1}$ may worsen bone outcomes.

The results of this study are in accordance with those of a previous study that reported that the extensor muscles are the first to be affected by microgravity and the calf muscle is one of the most highly affected limb muscles.[6] Moreover, the changes in strength and volume are different among individual muscle groups.[10] Gopalakrishnan et al. reported that the average ratio of %isokinetic strength change/%volume change in the knee extensors was about 1:1.2, while the ratio in the knee flexor was 2.9:1.[10] With the limitation that volume is a gross measure subject to error because of water content and other factors, the force-generating capacity of muscle is dependent not only on cross-sectional area but also on a number of other factors such as motor neuron control,[3] muscle length,[9] and muscle fiber recruitment.[2] In this study, the same level changes from preflight for isokinetic strength as in previous studies was observed at the knee and ankle: the strength changes in concentric action at the knee extension (91.4%), knee flexion (87.7%), ankle dorsiflexion (91.1%), and ankle plantar flexion (86.9%). These findings suggest that the same level of fiber atrophy may occur in this study population as reported in previous long flight studies, and a strong effect of protein intake is observed in the muscle group, where protein intake significantly contributes to muscle strength.

The findings of a 28-d bed rest study by Paddon-Jones et al. involving healthy men showed that essential amino acid and carbohydrate supplements could preserve lean leg mass and ameliorate the loss of single-leg one-repetition maximum leg extension strength compared to those in the bed rest group who did not consume the supplements.[13] They also showed that the protective effects of the supplement were associated with high mixed-muscle protein synthesis rates and not mediated by changes in plasma cortisol, which were unaltered by the supplement. Furthermore, Fitts et al. found that bed rest plus essential amino acid and carbohydrate supplements protected against the bed rest-induced loss of peak force in the slow type I fibers of the soleus, but not the slow fibers of the vastus lateralis.[7] In their study, fiber velocity of the vastus lateralis type II fibers was higher in the supplement group than in the prebed rest and postbed rest control groups. This increase in type II fiber velocity seems to be responsible for the increased peak power in our study group. Since the microgravity-induced loss of fiber function is primarily caused by inhibition of muscle protein synthesis and loss of myofibrillar protein, fiber atrophy, and loss of fiber force and power,[8,18] it seems reasonable to speculate that intake of proteins containing essential amino acids would reduce the deleterious changes associated with spaceflight and bed rest.

The concept underlying the nutrition countermeasure of proteins, including essential amino acid and carbohydrate supplementation, is well established in many ground studies. However, the physical performance data in this study suggest that synergistic benefits of increasing muscle strength in the ground studies may not translate into the benefit of microgravity-induced loss of muscle strength. Furthermore, this study showed that protein and carbohydrate intake was negatively associated with the total work of isokinetic measurements for knee flexion and the peak torque of isokinetic measurements for trunk flexion at 30 d after landing. Trappe et al. reported that a leucine-enriched high protein diet during a 60-d bed rest led to an additional loss of thigh muscle volume and a decrease in dynamic muscle strength and total work measured by the supine squat, whereas the calf muscle volume was maintained.[17] They reported that it was not clear why this effect was only seen in the thigh muscle, but the regulation of atrophy and response to additional nutrition during bed rest may be muscle- or fiber-type specific. Thus, as we indicated, protein and carbohydrate intake can induce a delayed decline in the total work of knee flexion muscle by promoting the loss of related muscles and fiber types in some way. Regarding the performance decline of knee flexion and trunk flexion, the negative effect of BMI was also reported, but a significant association was not detected. The relationship between nutrition and the regulation of muscle performance of trunk flexion may be the same as that of flexor muscles.

We evaluated the correlation between changes in biochemical test values and in muscle strength. The blood creatinine concentration, which is known to reflect muscle mass, has a weak correlation with endurance test results of the knee flexor and extensor muscles, suggesting that this kind of physical performance is strongly related to muscle mass. By contrast, ARED sessions per week are significantly related to knee flexion measures, and knee flexion has a strong negative correlation with LDL, which is known to be related to exercise volume. This suggests that exercise may have a significant effect on the volume of the muscle involved in knee flexion.

There were some limitations to this study which may affect the interpretation of the data. The primary limitation is the small number of subjects, which reduces the reliability of the result, but the power for each model was $>$ 90% and the sample size was appropriate for the analyses. Moreover, not all required data were available for all subjects or all variables. The LSAH is a valuable database for understanding the effects of spaceflight on humans. However, some records and measurements were designed as clinical tools. The FFQ was only intended to provide a ballpark figure of intake and the reported intake of the seven key nutrients was considered an estimate; therefore, an analysis according to protein types including amino acid composition was not possible in this study. Although the FFQ was based on the weekly recall of the subjects, familiarization and training, including an overview of all nutritional assessment procedures, were conducted before the flight for all astronauts to keep the data as reliable as possible. Regarding the outcomes related to muscle status, direct information such as lean body mass was not included in the database, and only isokinetic measurements were available. This means that the effect of protein intake on direct muscle volume during spaceflight was still unclear in this study. Although the dataset included repeated measurements on astronauts who flew multiple missions, all data were analyzed as independent records since astronaut identities were not linked to missions in the analyses. In addition, there were few female subjects, reflecting the astronauts' gender ratio; thus, caution should be taken when generalizing the findings to women. Finally, although we considered several previously identified confounders, the associations might have been influenced by residual or unmeasured confounders, such as the impact of daily workload for each astronaut on the muscle performance or the impact of the time-shift activity on the metabolism.

In conclusion, protein intake during spaceflight was related to some physical performance, such as isokinetic measurements for ankle plantar flexion, even after taking the exercise effect into consideration. However, the nutrition countermeasure of protein and carbohydrate intake provided no synergetic benefit for muscle strength. Although further research is required on the muscle group-specific mechanism, this result supports that protein intake can serve as a major candidate for countermeasures to offset the negative effects of long-duration spaceflight on the performance of the most affected muscle group.

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