

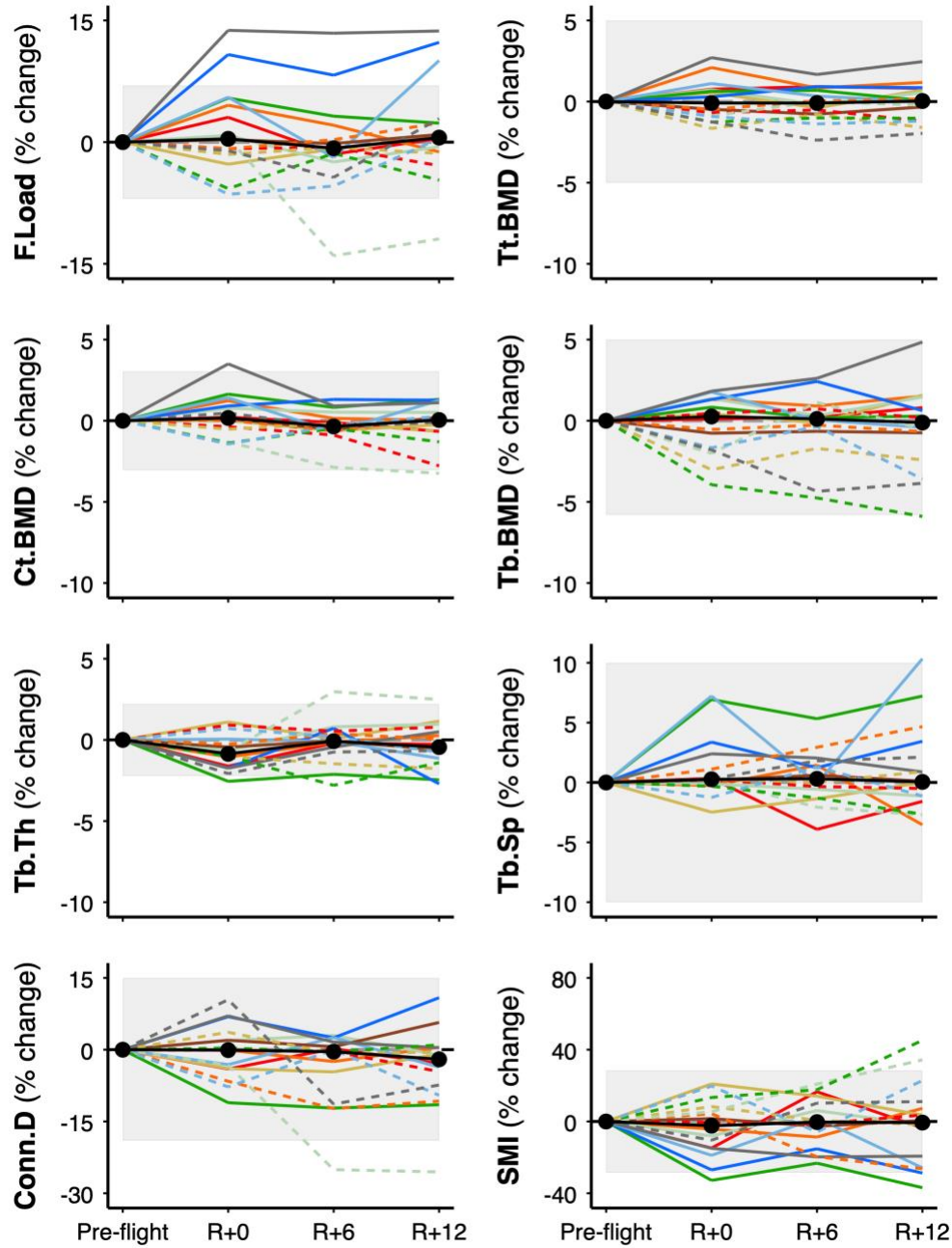
Supplementary Appendix

Incomplete recovery of bone strength and trabecular microarchitecture at the distal tibia one year after return from long duration spaceflight

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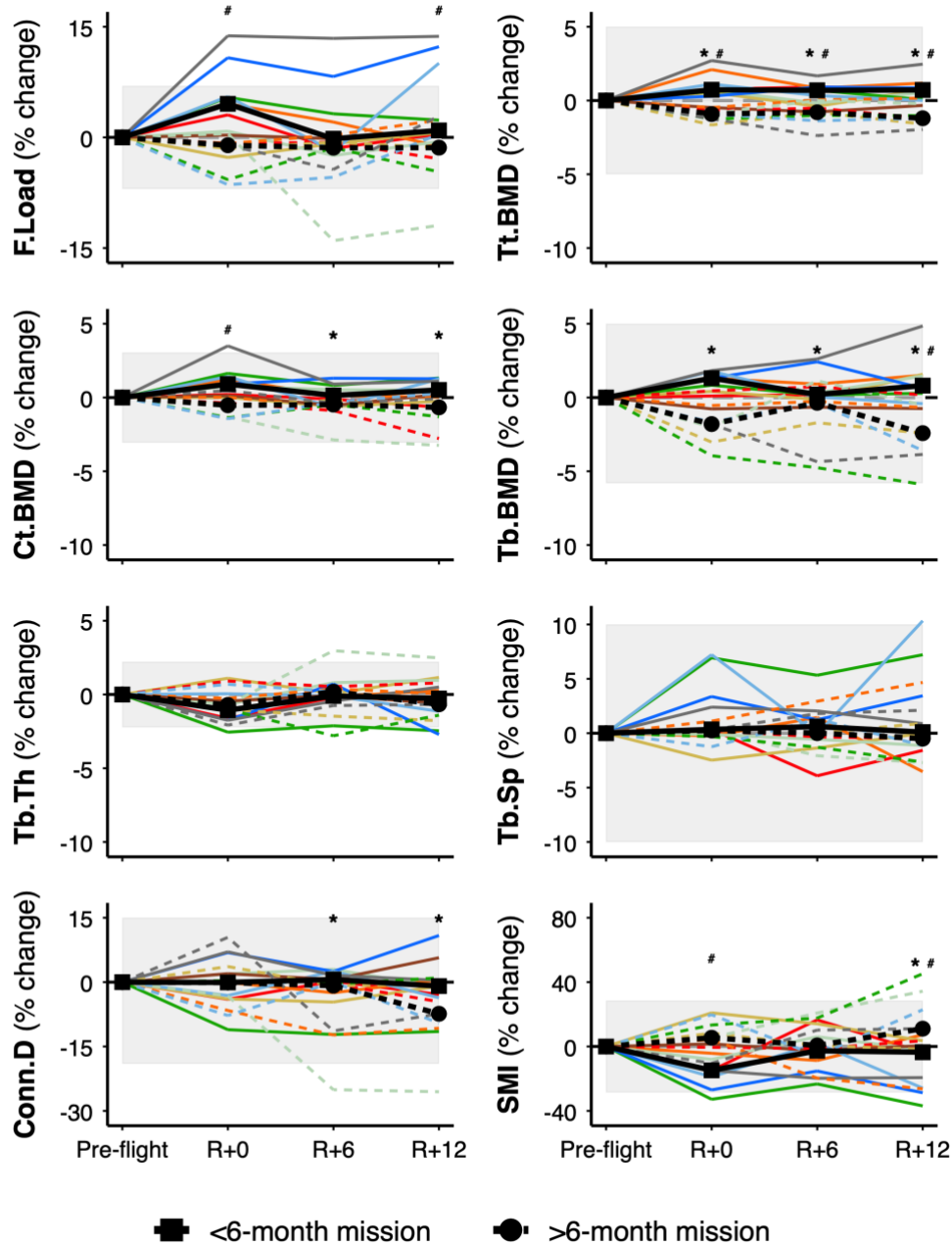
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Radius

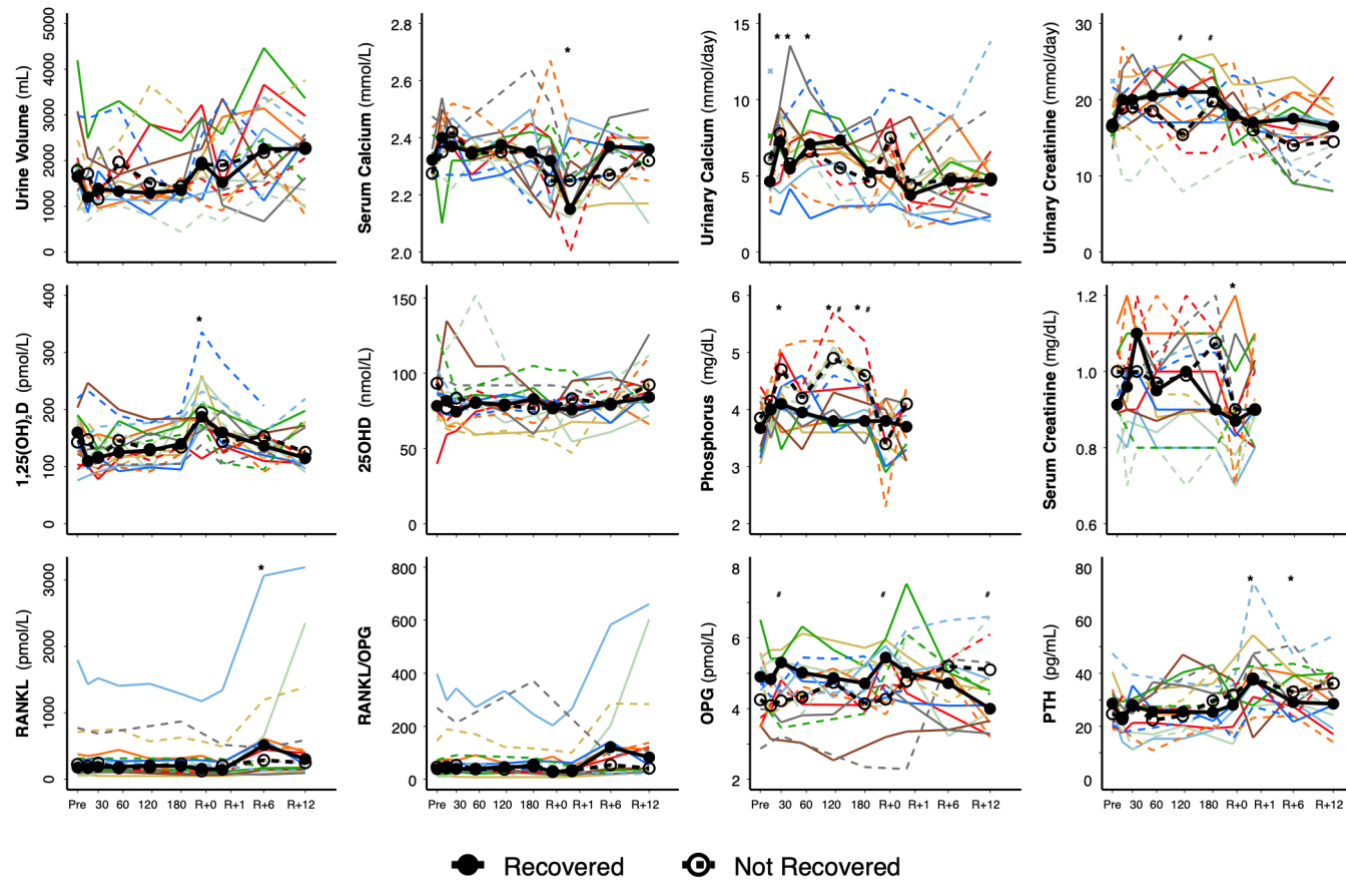


Supplementary Figure S1. Radius bone strength, density, and trabecular microarchitecture. Values are percent change from pre-flight at return (R+0), 6-months (R+6), and 12-months (R+12) recovery. Dashed lines for astronauts on > 6-month missions (n=8) and solid lines for astronauts on < 6-month missions (n=9). Black circles connected by thick solid line indicates median group change. Shaded bars indicate least significant change.⁽¹⁵⁾ F.Load, failure load; Tt.BMD, total bone mineral density; Ct.BMD, cortical bone mineral density; Tb.BMD, trabecular bone mineral density; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; Conn.D, connectivity density; SMI, structure model index.

Radius



Supplementary Figure S2. Radius bone strength, density, and trabecular microarchitecture by mission duration. Values are percent change from pre-flight at return (R+0), 6-months (R+6), and 12-months (R+12) recovery. Dashed lines for astronauts on > 6-month missions (n=8) and solid lines for astronauts on < 6-month missions (n=9). Black circles connected by thick dashed line indicate median change for astronauts on > 6-month missions and black squares connected by thick solid line indicate median change for astronauts on < 6-month missions. Shaded bars indicate least significant change.⁽¹⁵⁾ F.Load, failure load; Tt.BMD, total bone mineral density; Ct.BMD, cortical bone mineral density; Tb.BMD, trabecular bone mineral density; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; Conn.D, connectivity density; SMI, structure model index. * $p < 0.05$ from pre-flight for astronauts on > 6-month missions; # $p < 0.05$ from pre-flight for astronauts on < 6-month missions.



Supplementary Figure S3. Biomarkers of bone turnover relative to bone recovery at pre-flight (Pre), flight days 15, 30, 60, 120, 180, and return (R+0), 1-month (R+1), 6-months (R+6), and 12-months (R+12) recovery. Median group change for astronauts who recovered (n=8) total BMD at R+12 are indicated by black circles connected by a thick solid line, while median group change in astronauts who did not recover (n=9) total BMD at R+12 are indicated by open circles connected by a thick dashed line. Colored dashed lines for astronauts on > 6-month mission and solid lines for astronauts on < 6-month mission. 24-hr urine volume; serum calcium; urinary calcium; urinary creatinine; 1,25(OH)₂D, 1,25 di-hydroxy vitamin D; 25OHD, 25-hydroxyvitamin D; phosphorus; serum creatinine; RANKL, RANKL/OPG, PTH, intact parathyroid hormone; OPG, osteoprotegerin. Note: three astronauts on > 6-month missions did not complete all in-flight measures; thus, for urine volume, calcium, and creatinine these astronauts are indicated by an 'x' at pre-flight and trajectory between R+0 and R+12. Phosphorus and serum creatinine were not measured beyond R+1. **p*<0.05 from pre-flight for entire group. #*p*<0.05 between astronauts whose tibia total BMD recovered and astronauts whose tibia total BMD did not recover, *p*<0.05.

Supplementary Table S1. Pre-flight biochemical markers of bone turnover and bone metabolism and change from pre-flight.

	Pre-flight	Δ FD15	Δ FD30	Δ FD60	Δ FD120	Δ FD180	Δ R+0m	Δ R+1m	Δ R+6m	Δ R+12m
N [#]	17	14	13	14	13	15	17	17	17	16
Bone resorption										
CTx (ug/d)	1513 (1068, 1935)	1309 (709, 2191)^b	1478 (1155, 2359)^b	2267 (1327, 2831)^b	1296 (798, 2788)^b	1625 (754, 1970)^b	1325 (904, 1848)^b	369 (-60, 662)	-2 (-332, 150)	-231 (-400, 150)
CTx (ug/mmol Cr)	91 (63, 135)	44 (31, 75)^b	80 (38, 111)^b	101 (64, 143)^b	92 (64, 124)^b	82 (49, 115)^b	57 (44, 113)^b	20 (-2, 44)	-3 (-24, 11)	-10 (-30, 20)
NTx (nmol/d)	390 (323, 439)	209 (163, 393)^b	277 (126, 338)^b	357 (174, 482)^b	196 (131, 406)^b	216 (96, 389)^b	251 (136, 309)^b	86 (54, 158)	-131 (-141, -32)	-105 (-176, -7)
NTx (nmol/mmol Cr)	23 (20, 28)	8 (4, 21)^b	9 (5, 14)^b	12 (8, 21)^b	10 (6, 18)^b	12 (5, 22)^b	12 (8, 20)^b	4 (2, 10)	-3 (-8, 6)	-3 (-6, 5)
Bone formation										
BSAP (U/L)	21.2 (18.0, 22.5)	-1.1 (-2.3, 0.9)	0.7 (-0.2, 1.0)	1.9 (1.3, 3.4)	3.7 (1.9, 6.6)^b	4.7 (4.0, 11.4)^b	7.3 (3.5, 10.1)^b	8.6 (4.1, 12.2)^b	-0.9 (-1.6, 0.5)	-2.5 (-3.9, -0.6)^b
OC (ng/mL)	20.9 (18.3, 23.6)	1.1 (-0.5, 2.8)	2.0 (0.0, 3.9)	5.5 (3.8, 9.1)^b	10.7 (7.7, 11.9)^b	14.3 (7.4, 16.4)^b	4.2 (1.1, 7.9)^b	11.7 (7.3, 17.9)^b	4.2 (1.0, 8.3)^a	0.6 (-3.8, 2.7)
P1NP (ug/L)	48.8 (42.3, 57.1)	-4.5 (-9.0, -0.6)	10.6 (3.8, 18.9)	26.8 (14.0, 37.5)^b	41.0 (22.5, 52.9)^b	51.3 (38.4, 66.2)^b	40.4 (13.8, 49.4)^b	57.5 (45.5, 71.1)^b	7.8 (0.5, 14.1)	-1.4 (-11.3, 9.6)
Osteocyte activity										
Sclerostin (pmol/L)	26.2 (21.3, 31.3)	2.5 (-0.9, 4.2)	2.9 (1.2, 9.1)	1.9 (-1.0, 5.6)	2.0 (0.3, 5.1)	1.3 (-1.8, 3.5)	-0.2 (-3.4, 1.1)	-0.3 (-2.2, 1.6)	2.7 (-2.1, 7.3)	2.2 (-0.5, 5.2)
Regulators of bone metabolism										
Urinary calcium (mmol/d)	4.86 (4.20, 6.14)	2.39 (0.41, 3.79)^b	1.36 (0.61, 1.86)^a	1.83 (1.08, 3.62)^b	1.44 (-0.18, 2.13)	0.33 (-0.38, 1.71)	0.58 (-0.14, 2.00)	-0.72 (-2.04, 0.19)	-0.68 (-1.28, 0.88)	-0.18 (-1.63, 1.50)
Serum calcium (mmol/L)	2.30 (2.28, 2.35)	0.08 (0.04, 0.12)	0.05 (-0.03, 0.14)	0.04 (-0.03, 0.10)	0.06 (0.01, 0.09)	0.07 (-0.06, 0.12)	-0.01 (-0.07, 0.05)	-0.10 (-0.17, -0.01)^a	0.02 (-0.06, 0.07)	0.01 (-0.06, 0.06)
PTH (pg/mL)	25.2 (22.1, 31.5)	-2.6 (-5.5, 1.4)	-3.2 (-8.0, 1.4)	-0.4 (-7.9, 2.6)	1.3 (-5.6, 4.9)	-0.1 (-4.6, 5.1)	0.3 (-1.3, 5.9)	12.9 (6.6, 14.7)^b	5.6 (-0.4, 7.6)^a	5.9 (-3.9, 10.2)
1,25(OH) ₂ D (pmol/L)	145 (128, 187)	-8 (-49, 13)	-26 (-43, 1)	-25 (-43, 6)	-36 (-45, -2)	-6 (-25, 30)	36 (17, 85)^b	-4 (-20, 63)	-25 (-40, 5)	3 (-50, 21)
25OHD (nmol/L)	86 (76, 96)	-4 (-9, 4)	-7 (-17, 1)	1 (-13, 13)	-7 (-11, 6)	-14 (-21, 4)	-10 (-18, 0)	-10 (-20, 2)	-7 (-14, 4)	3 (-15, 20)
Phosphorus (mg/dL)	3.8 (3.4, 4.0)	0.4 (0.0, 0.7)	0.3 (-0.1, 1.1)^b	0.1 (-0.1, 0.6)	0.7 (0.3, 1.2)^b	0.5 (0.1, 0.7)^a	-0.2 (-0.6, 0.3)	0.0 (-0.1, 0.2)	NA	NA
OPG (pmol/L)	4.7 (3.7, 5.2)	0.1 (-0.8, 0.2)	-0.1 (-0.6, 0.6)	0.1 (-0.5, 0.7)	-0.2 (-0.4, 0.3)	0.2 (-0.7, 0.6)	0.2 (-0.4, 0.7)	0.2 (-0.2, 0.9)	-0.4 (-0.7, 1.3)	-0.1 (-0.8, 0.7)
RANKL (pmol/L)	217 (124, 312)	2 (-3, 27)	4 (-14, 11)	5 (-18, 41)	3 (-42, 19)	-6 (-60, 17)	-20 (-46, -6)	-19 (-59, -5)	113 (-2, 337)^a	42 (-14, 230)
RANKL/OPG	46 (31, 68)	3 (0, 9)	4 (-4, 6)	4 (-10, 14)	1 (-9, 5)	-2 (-10, 7)	-6 (-17, 13)	-11 (-23, -3)	12 (-2, 89)	14 (-3, 76)
Chemistry										
24-hr Urine volume (mL)	1741 (1475, 2062)	-588 (-789, 7)	-405 (-674, 128)	-207 (-498, 172)	-490 (-841, 254)	-232 (-832, 191)	-149 (-438, 694)	-15 (-388, 306)	572 (-147, 904)	437 (-334, 830)

Urinary Creatinine (mmol/d)	17 (16, 18)	2 (1, 4)	3 (1, 4)	3 (1, 5)	2 (-3, 5)	3 (-1, 6)	1 (-2, 3)	0 (-1, 0)	-1 (-5, 2)	-1 (-3, 0)
Serum Creatinine (mg/dL)	1.00 (0.90, 1.00)	0.02 (-0.04, 0.08)	0.05 (0.00, 0.10)	-0.03 (-0.05, 0.06)	0.03 (-0.05, 0.10)	0.00 (-0.10, 0.10)	-0.07 (-0.14, -0.03)^a	-0.04 (-0.10, 0.00)	NA	NA

Data are median (interquartile range) for pre-flight and median change from pre-flight.

^a***p*<0.05**; ^b***p*<0.01** compared with pre-flight (mean of L-180 and L-45) based on linear mixed effects model with small sample adjustment and Bonferroni correction. BSAP, OC, P1NP, sclerostin, 25OHD, RANKL, and RANKL/OPG were log-transformed for analysis. [#]maximum sample size for data collection period. Three participants did not have CTx analyzed at any time point, phosphorus was not analyzed at R+6, nor was serum creatinine at R+6 or R+12.

CTx, type I collagen C-terminal cross-linked telopeptide; NTx, type I collagen N-terminal cross-linked telopeptide; BSAP, bone-specific alkaline phosphatase; OC, osteocalcin; P1NP, procollagen type 1 amino-terminal propeptide; PTH, intact parathyroid hormone; 1,25(OH)₂D, 1,25 di-hydroxy vitamin D; 25OHD, 25-hydroxyvitamin D; OPG, osteoprotegerin.