Using Dense Phenotyping and Cluster Analysis to Identify Subgroups Associated with Deconditioning in Bed Rest

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Outline

- CFT70 Bed Rest Study
    - Available Data and Outcomes of Interest
    - Data Cleaning and Preparation
- Statistical Methods
    - Variable Reduction
    - Hierarchical Clustering
    - Phenogrouping Analysis
    - Multivariable Modeling
- Results
    - Dense Chord Diagram
    - Regression Results
- Next Steps

Overview of the CFT 70 Bed Rest Study

CFT 70 Bed Rest Study

- Bed Rest (BR) is used as a spaceflight analog
- Astronaut-like cohort
- 10 Weeks of 6 degree Head Down Tilt (HDT) Bed Rest
- 96 days (14 days pre-BR, 70 days during BR, and 12 days post-BR)
- 4 arms: Control (n = 11), Exercise (n = 10), Exercise + Testosterone (n = 8), Flywheel (n = 8)

A Tightly Controlled Environment

- Monitored 24 hours/day
- Toileting/Showering in HDT
- Standardized wake/sleep schedule
- 3 meals/day controlled and adjusted diet
- Resting metabolic rate/exercise energy expenditure
- All fluid intake/output monitored

Overview of the NASA 70-day Bed Rest Study, Cromwell RL, et. al, Med Sci Sports Exerc. 2018 Sep;50(9):1909-1919.

Bed Rest Measures Collected

Testing	Testing Days[a] Pre–Bed Rest	Bed Rest	Post–Bed Rest
Standard measures			
Bone densitometry	−11	15, 32, 48, 65	+2, +180, +365
QCT	−3		+4, +180, +365
Echocardiography	−6, −2	7, 21, 31, 70	+0, +3, +13
Cardiovascular tilt test	−4	70	+3
Plasma volume	−6, −2	7	+0, +1, +3, +6, +13
Aerobic capacity	−7	4, 25, 46, 68	+0, +11
Vertical jump	−6, −2		+0, +13
Isokinetic testing	−11, −5		+2, +12
Posture and balance	−12, −6, −2		+0, +1, +6, +13
Reflex testing	−10, −4, −1	5, 20, 70	+0, +3, +6
Nutritional assessment	−10, −3	7, 14, 21, 28	+0, +5
Immunologic assessment	−10	28	+0, +5
Investigator measures			
Treadmill locomotion/dynamic visual acuity test	−12, −6, −2		+0, +1, +6, +13
Fine motor control test	−12, −6, −2		+0, +1, +6, +13
FTT	−12, −6, −2		+0, +1, +6, +13
Muscle performance measures	−20, −12, −6, −2		+0, +1, +6, +13
Muscle size—MRI	−5	3, 7, 11, 15, 22, 29, 36, 53, 69	+3, +6, +10
Muscle size—ultrasound scanning	−5	3, 7, 11, 15, 22, 29, 36, 53, 69,	+3, +6, +10
Muscle biopsy		3, 57	
Mood and fatigue questionnaires	−20, −1	Weekly	+0, +13
Glucose tolerance test	−20, −1	37, 66	+12
Nasal patency	−10, −8, −6, −4, −2, −1	21 tests	+0, +1, +2, +4, +7, +10, +12, +180
Odorant test	−10, −8, −6, −4, −2, −1	21 tests	+0, +1, +2, +4, +7, +10, +12, +180
Smell acuity tests	−1	1, 3, 37, 65	+2, +12
Food questionnaires	Daily	Daily	Daily
Vision exam	−13, −5	Weekly	+2, +9
Optical coherence tomography and fundus photography	−13, −5	38	+2, +9
Behavioral assessment	−11, −7	8,50, 65	+5, +11
Brain MRI and fMRI	−12, −8	8, 50, 67	+0, +6, +13
Personality test	−21		
Journal entries	Daily	Daily	Daily
Psychological dimensions survey	Daily	Daily	Daily
Outgoing debrief			+12

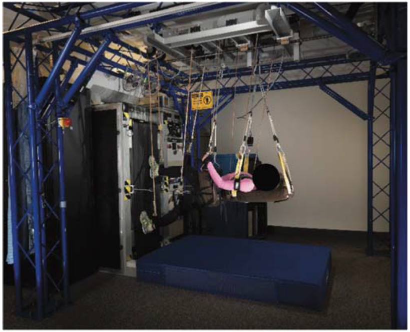
fMRI, functional MRI.

[a] A negative sign indicates the number of days before bed rest; a positive sign indicates the number of days after bed rest; no sign indicates the number of days in bed rest. Day +0 is the day subjects stood up from bed rest.

Overview of the NASA 70-day Bed Rest Study, Cromwell RL, et. al, Med Sci Sports Exerc. 2018 Sep;50(9):1909-1919.

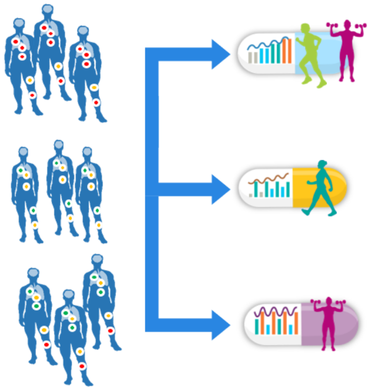
CFT 70 Results

- Exercise mitigated bed-rest induced multisystem deconditioning on average
- There is considerable heterogeneity in spaceflight-induced multisystem deconditioning.
- Methods to identify both physiological systems and individuals at high risk of spaceflight-induced deconditioning are needed.



Our Analysis Objectives

- Apply cluster analysis to identify subgroups associated with deconditioning in bed rest.
- Identifying groups at baseline (phenogroups) that are more likely to have poor outcomes after spaceflight, may help personalize exercise/nutrition/other perscriptions during the mission.



Statistical Methods

Multisystem Variables

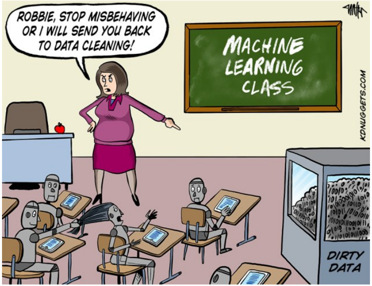
- Over 850 continuous variables assessed at baseline (pre-BR)
- Grouped into the following body systems:
  - Brain Morphology
  - Psychological Questionnaires
  - Body Composition
  - Bone Health
  - Cardiac Function
  - Aerobic Fitness
  - Muscle Strength
  - Blood and Urine Biochemistries
  - Smell
  - Vision
  - Functional Performance
  - Dietary Intake
  - Demographics

Outcomes of Interest

- V02 Max
- Profile of Mood States (POMS) Questionnaire
- Intraocuar Pressure (IOP, Left and Right)
- Fat Free Mass (FFM, grams)
- Total Fat Mass
- Total Bone Mass
- Quadricep Size (cm2)
- Soleus Size (cm2)
- Knee Extensor at 60 degrees/sec (KES60)
- Twist Peak
- Twist Rate
- Heart Rate

Data Cleaning

- Within each system:
  - data were cleaned by removing all variables missing greater than 15% of values
  - variables with 95% or more equivalent values were removed (insufficient variability)
  - single variable imputation was performed using predictive mean matching
  - variable reduction was performed

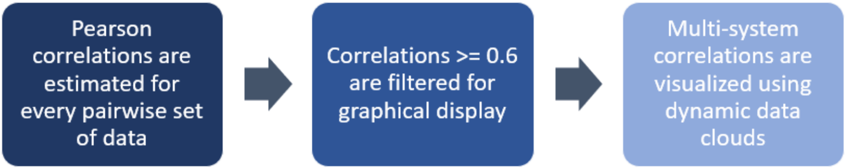


Imputation and Variable Reduction

- Imputation Algorithm:
  - Fits a flexible additive model to predict the target
  - Finds a target variable with an observed value whose predicted value is closest to the predicted value of the missing value
  - Replaces the missing value with observed value
- Variable Reduction using Hierarchical Clustering
  - Variables are merged if there is a decrease in the sum of the squared correlation between the aggregated variables and the cluster center (first principal component)
  - Larger pre-specified tree height thresholds = fewer clusters, smaller thresholds = more clusters
  - One variable is chosen from each cluster based on the variable in that cluster that has the highest squared correlation between the variable and the cluster center

Visualizing Body System Relationships

- For every pairwise set of data (e.g., body composition vs. function performance test data), each variable from the first dataset was correlated with every variable from the second dataset using Pearson's correlation coefficient.
- Dynamic data clouds are produced linking all absolute value Pearson correlation coefficients ≥ 0.6, allowing multi-system correlations to be visualized



Identifying Phenogroups

- Outcomes were removed, and filtered variables were standardized
- Hierarchical clustering was performed, dissimilarity matrix given by Euclidean distance and the average linkage score used to join similar clusters
- The optimal number of clusters was selected using the silhouette coefficient
- The silhouette coefficient measures how well data are assigned to its own cluster and how far they are from other clusters
- The resulting number of clusters was validated by visual inspection, insuring a sufficient number of subjects were in each phenogroup

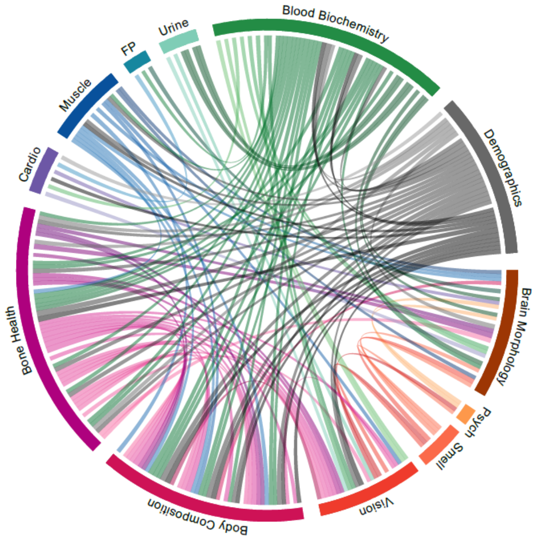
Association of Phenogroups w/ Outcomes

Modeling Outcomes

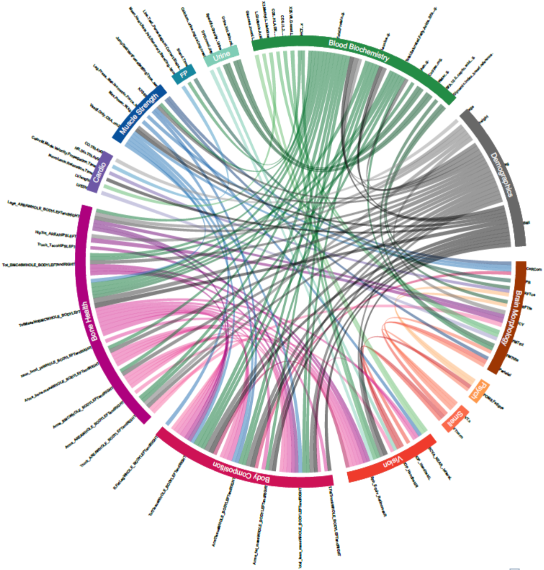
$$
\begin{aligned}
E[OutcomePostBR] = \beta_0 &+ \beta_1\{OutcomeBaseline\} \\
&+ \beta_2\{Cluster2\} \\
&+ \beta_3\{Exercise + Testosterone\} \\
&+ \beta_4\{Exercise\} \\
&+ \beta_5\{Flywheel\}
\end{aligned}
$$

Results

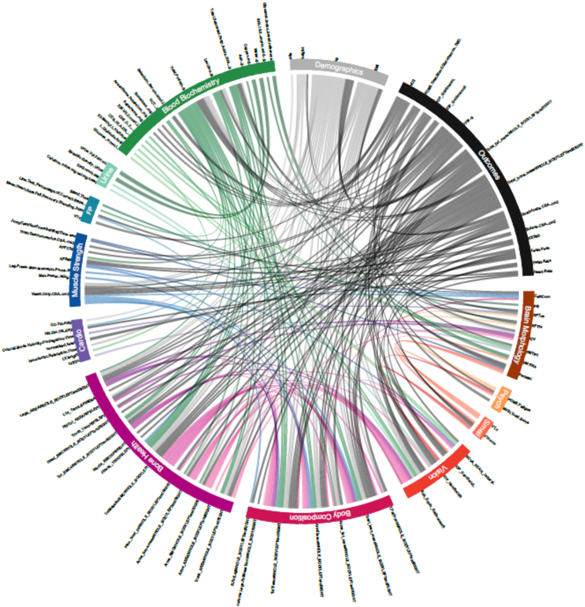
Dynamic Data Cloud



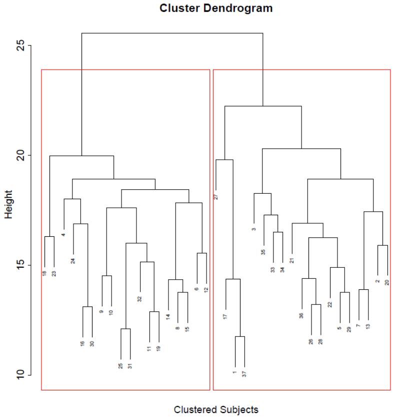
Detailed Dynamic Data Cloud

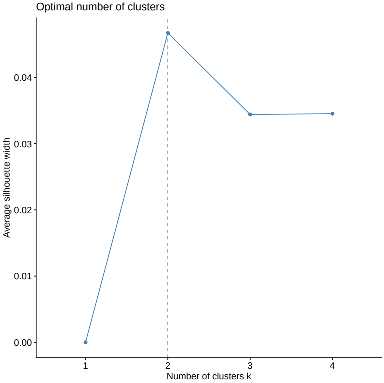


Data Cloud with Outcomes



Identification of Phenogroups





Characterization of Phenogroups

	N	Cluster 1	Cluster 2	Test Statistic
		$N = 19$	$N = 18$	
Bodycomp				
T.FatTrunk	33	24.54 28.47 30.77	13.51 17.35 26.23	$F_{1,31}$=13.87, P<0.001
Total_lean	33	57839.87 60351.53 64615.24	51865.88 55949.89 59426.63	$F_{1,31}$=8.33, P=0.007
Arms_fat_m	33	1658.44 2008.04 2255.16	920.07 1149.89 1450.98	$F_{1,31}$=26.3, P<0.001
ArmTissueW	33	9289.63 10033.21 10304.40	7365.40 8201.57 9063.72	$F_{1,31}$=19.35, P<0.001
TotTissueW	33	75103.69 78528.26 82310.08	59135.69 65470.23 69146.24	$F_{1,31}$=32.58, P<0.001
FattMassAr	33	0.70 0.84 0.93	0.80 1.02 1.11	$F_{1,31}$=7.22, P=0.011
R.FatLegWH	33	22.60 24.14 25.36	15.37 19.36 23.02	$F_{1,31}$=7.76, P=0.009
Bone				
Trunk_AREA	33	864.03 891.98 903.23	788.34 837.10 873.28	$F_{1,31}$=6.23, P=0.018
Arms_AREAW	33	450.43 467.94 514.30	426.99 453.16 487.31	$F_{1,31}$=2.17, P=0.151
Arms_BMCWH	33	461.02 485.44 507.95	391.40 420.59 442.14	$F_{1,31}$=15.33, P<0.001
Arms_bone.	33	461.02 485.44 507.95	391.40 420.59 442.14	$F_{1,31}$=15.33, P<0.001
zsco_bmd_y	33	0.79 1.01 2.45	-0.10 0.38 1.02	$F_{1,31}$=8.04, P=0.008
TotMassNoB	33	39719.07 41495.40 43655.35	31319.59 34443.14 36708.43	$F_{1,31}$=36.43, P<0.001
HipTot_.AM	32	96.76 99.37 110.07	90.90 93.84 103.47	$F_{1,30}$=2.07, P=0.16
FNeck_.YNH	33	98.19 107.66 114.74	88.60 92.13 100.36	$F_{1,31}$=7.22, P=0.011
Wards_AREA	33	3.06 3.44 3.90	2.93 3.14 3.38	$F_{1,31}$=2.29, P=0.141
Wards_BMCH	33	2.82 3.33 4.00	2.34 2.73 2.99	$F_{1,31}$=7.76, P=0.009
Tot_BMC46W	33	3226.49 3343.81 3597.44	2782.60 2906.89 3138.99	$F_{1,31}$=11.35, P=0.002
Head_BMCWH	33	556.12 587.78 631.84	482.03 516.09 560.50	$F_{1,31}$=4.74, P=0.037
Troch_Tsco	33	-0.83 0.03 0.72	-1.24 -0.86 -0.07	$F_{1,31}$=3.69, P=0.064
HipTot_ARE	33	35.89 36.53 39.84	34.63 36.06 38.25	$F_{1,31}$=2.17, P=0.151
L14_TscoLS	33	-0.61 0.17 1.12	-1.12 -0.01 0.64	$F_{1,31}$=0.57, P=0.456
Legs_AREAW	33	863.64 898.48 941.40	818.91 868.94 904.92	$F_{1,31}$=2.66, P=0.113
Cort.TotHi	28	0.50 0.52 0.55	0.49 0.51 0.54	$F_{1,26}$=0.66, P=0.425
Trab.TotHi	28	0.11 0.13 0.16	0.12 0.14 0.16	$F_{1,26}$=0.27, P=0.607
L1.Centr.3	28	0.18 0.21 0.25	0.21 0.23 0.25	$F_{1,26}$=0.58, P=0.453
Demographics				
age	36	28.20 36.24 38.90	27.72 31.83 33.44	$F_{1,34}$=2.62, P=0.115
height	36	174.67 179.50 182.70	170.88 178.10 182.80	$F_{1,34}$=0.41, P=0.524
kg	36	75.20 84.75 88.98	65.17 73.15 76.30	$F_{1,34}$=16.57, P<0.001
BMI	36	24.57 26.16 27.13	21.30 22.69 24.47	$F_{1,34}$=23.14, P<0.001

a b c represent the lower quartile a, the median b, and the upper quartile c for continuous variables.
N
is the number of non–missing values.
Test used:
Wilcoxon test

Association of Phenogroups w/ Outcomes

Models	N	aCoefficient 95% CI	P-value
Vo2 Model			
Intercept	33	0.17 (-0.5,0.85)	0.62
VO2 Baseline		0.69 (0.48,0.89)	<0.001
Cluster 2 vs. 1		0.09 (-0.13,0.31)	0.43
Ex. + T. vs. Cont.		0.72 (0.42,1.02)	<0.001
Ex. vs. Cont.		0.68 (0.39,0.97)	<0.001
Flywheel vs. Cont.		0.58 (0.28,0.88)	<0.001
POMS Total Model			
Intercept	24	7.82 (-0.74,16.37)	0.09
POMS Baseline		0.47 (0.13,0.81)	0.01
Cluster 2 vs. 1		-0.87 (-9.14,7.39)	0.84
Ex. + T. vs. Cont.		-5.96 (-15.89,3.97)	0.25
Ex. vs. Cont.		-8.12 (-17.98,1.75)	0.12
IOP Goldmann L Model			
Intercept	14	-0.88 (-13.38,11.62)	0.89
IOP Goldman L Baseline		1.01 (0.18,1.83)	0.04
Cluster 2 vs. 1		-1.02 (-4.41,2.37)	0.57
Ex. + T. vs. Cont.		4.07 (-0.08,8.22)	0.09
Ex. vs. Cont.		0.9 (-2.4,4.2)	0.61
IOP Goldmann R Model			
Intercept	14	5.68 (-0.98,12.34)	0.13
IOP Goldman R Baseline		0.64 (0.21,1.07)	0.02
Cluster 2 vs. 1		-0.62 (-3.38,2.14)	0.67
Ex. + T. vs. Cont.		1.4 (-1.89,4.69)	0.43
Ex. vs. Cont.		0.21 (-2.48,2.9)	0.88
FFM Model			
Intercept	25	-800.37 (-10801.81,9201.07)	0.88
FFM Baseline		0.96 (0.8,1.13)	<0.001
Cluster 2 vs. 1		1426.97 (-633.71,3487.65)	0.19
Ex. + T. vs. Cont.		4372.14 (1979.49,6764.78)	<0.001
Ex. vs. Cont.		1458.64 (-852.12,3769.4)	0.23
Quad Size Model			
Intercept	34	-9.16 (-18.33,0.01)	0.06
Quad Size Baseline		1.05 (0.94,1.15)	<0.001
Cluster 2 vs. 1		1.51 (-1.25,4.27)	0.29
Ex. + T. vs. Cont.		8.41 (4.71,12.12)	<0.001
Ex. vs. Cont.		6.55 (3.1,10.01)	<0.001
Flywheel vs. Cont.		2.01 (-1.84,5.86)	0.31

Soleus Size Model			
Intercept	34	0.12 (-2.15,2.4)	0.92
Soleus Size Baseline		0.82 (0.74,0.91)	<0.001
Cluster 2 vs. 1		0.39 (-0.24,1.01)	0.24
Ex. + T. vs. Cont.		2.31 (1.41,3.21)	<0.001
Ex. vs. Cont.		2.42 (1.58,3.25)	<0.001
Flywheel vs. Cont.		2.01 (1.1,2.91)	<0.001
KES60 Model			
Intercept	33	-30.46 (-64.97,4.04)	0.09
KES60 Baseline		0.92 (0.77,1.07)	<0.001
Cluster 2 vs. 1		2.51 (-10.02,15.04)	0.7
Ex. + T. vs. Cont.		28.88 (11.33,46.42)	<0.001
Ex. vs. Cont.		37.14 (20.18,54.1)	<0.001
Flywheel vs. Cont.		18.83 (1.21,36.46)	0.05
Twist Peak Model			
Intercept	24	25.56 (17.63,33.49)	<0.001
Twist Peak Baseline		0 (-0.46,0.45)	0.98
Cluster 2 vs. 1		-2.33 (-5.87,1.21)	0.21
Ex. + T. vs. Cont.		-6.14 (-10.45,-1.83)	0.01
Ex. vs. Cont.		-7.53 (-11.86,-3.19)	<0.001
Twist Rate Model			
Intercept	24	99.56 (57.46,141.66)	<0.001
Twist Rate Baseline		0 (-0.64,0.63)	0.99
Cluster 2 vs. 1		-14.51 (-38.59,9.56)	0.25
Ex. + T. vs. Cont.		-17.22 (-49.25,14.8)	0.3
Ex. vs. Cont.		-20.75 (-49.94,8.44)	0.18
Heart Rate Model			
Intercept	25	55.91 (31.5,80.31)	<0.001
Heart Rate Baseline		0.6 (0.19,1)	0.01
Cluster 2 vs. 1		-3.54 (-10.63,3.55)	0.34
Ex. + T. vs. Cont.		-27 (-35.15,-18.85)	<0.001
Ex. vs. Cont.		-20.65 (-28.72,-12.59)	<0.001

Conclusions

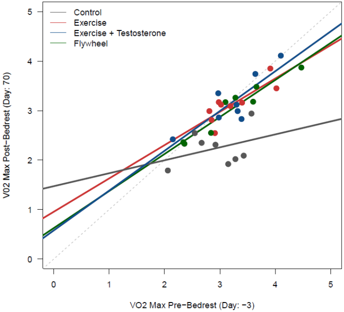
- Preliminary findings demonstrate the feasibility of using a novel unsupervised phenotypic clustering strategy to:

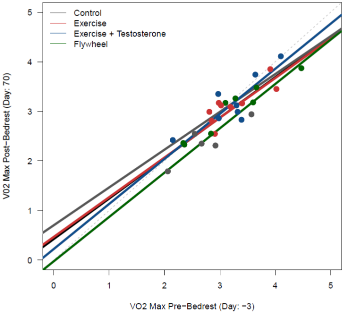
    1. identify a subset of relevant variables for acquisition in future trials

    2. identify mutually exclusive phenogroups of individuals according to baseline characteristics.

Limitations

- This is an exploratory analysis!
- Small sample size and homogeneous population carefully selected
- Study is very tightly controlled - the countermeasures do seem to be working!
- The flywheel group is significantly younger than other groups, and was added to the study later





Next Steps

- Account for variability in correlations and clustering using bootstrapping methods
- Remove the flywheel group from analysis
  - Ideally we would have a large enough sample size to perform entire analysis within the control group
- Choose variables scientifically not analytically
  - With a limited sample size it may pragmatic to use the most robust measures from each system

Questions?

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