Network Analysis of Skeletal Muscle During Spaceflight in Male Mice

Michael Savaglio

IU School of Medicine,

Department of Orthopaedic Surgery

MARIAN UNIVERSITY
Indianapolis
College of Osteopathic Medicine

Disclaimer

- The views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation.

Background

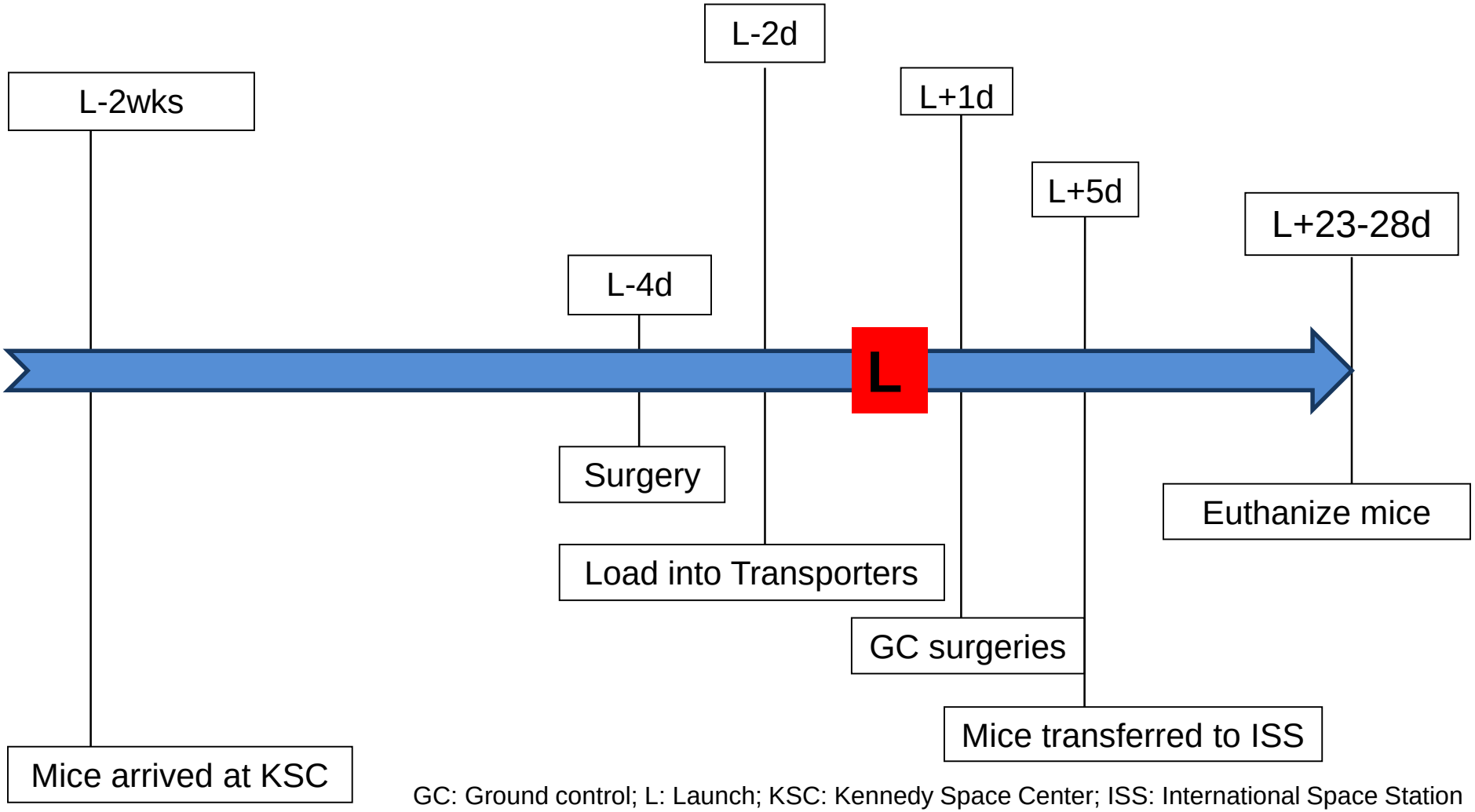
- The unloading associated with spaceflight results in rapid loss of bone and muscle tissue *(Stein, T., European Journal of Applied Physiology, 2012)*
- Loss of bone and muscle tissue presents a challenge for long term occupation of space*(Stein, T., European Journal of Applied Physiology, 2012)*
- In orthopaedics, many patients spend prolonged periods non-weight bearing, especially after traumatic injury*(Kershaw, C., et al., Clinical Orthopedics and Related Research, 2012)*
- The associated atrophy may impair healing and it is important to understand the mechanisms surrounding this *(Androjna, C. et al., Clinical Review Bone Mineral Metabolism, 2012)*
- This is the first time that skeletal muscle changes have been studied in male mice during spaceflight

Experimental Design

1) 10 mice with SBD + BMP2 treatment
   - 5 mice: RL fixed for histopathology; remainder frozen for omics
   - 5 mice: carcass frozen for omics
2) 10 mice with SBD + alternative treatment
   - 5 mice: RL fixed for histopathology; remainder frozen for omics
   - 5 mice: carcass frozen for omics
3) 10 mice with SBD + Saline treatment
   - 5 mice: RL fixed for histopathology; remainder frozen for omics
   - 5 mice: carcass frozen for omics
4) 10 mice no SBD **(healthy/sham)**
   - 5 mice: RL fixed for histopathology; remainder frozen for omics
   - 5 mice: carcass frozen for omics

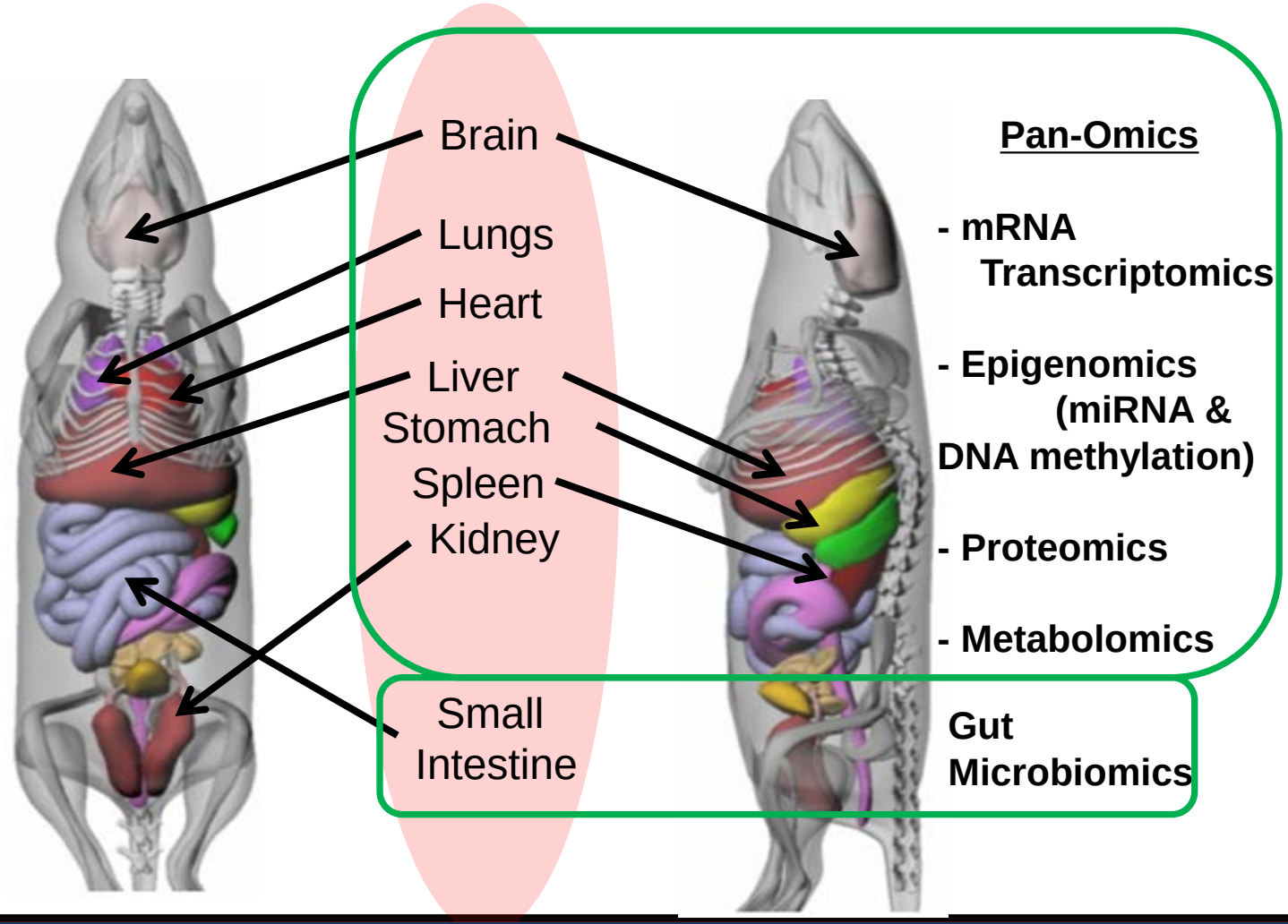
RL: right limb;
SBD induced here

Temporal Work-Breakdown Structure



GC: Ground control; L: Launch; KSC: Kennedy Space Center; ISS: International Space Station

Tissues/Samples to Investigate

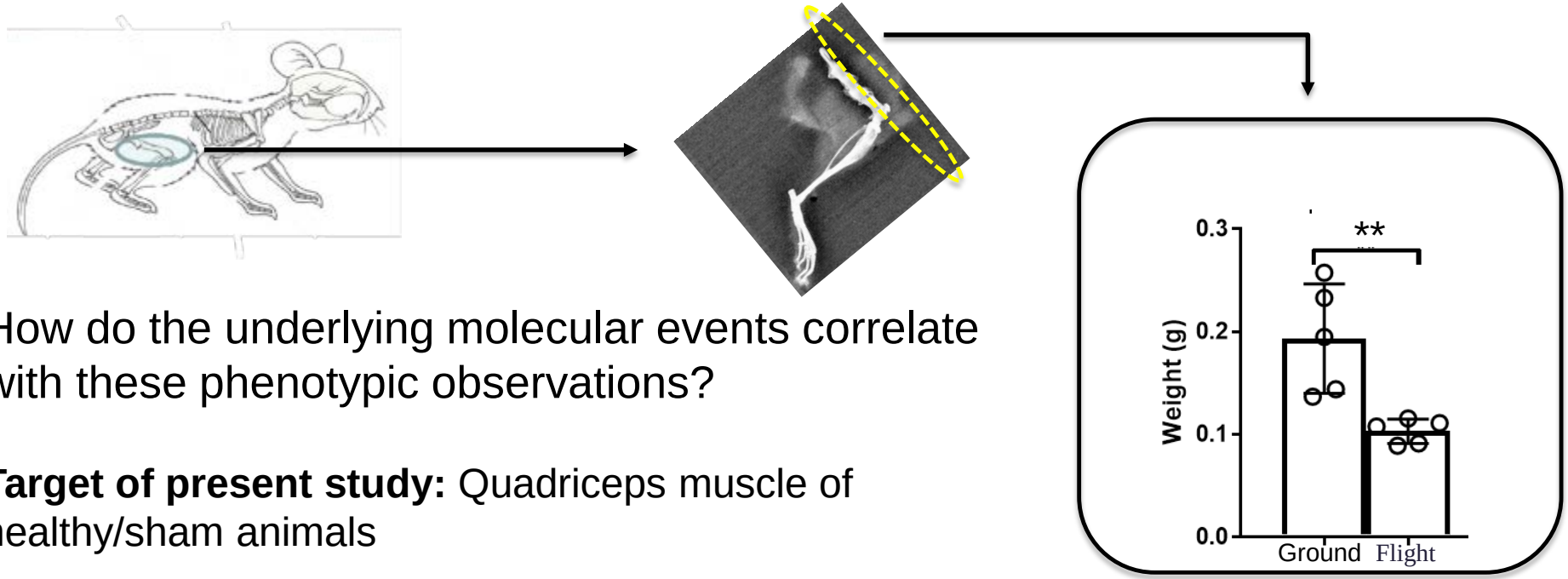


Objective of this presentation

Phenotypic observation so far….

Adverse effects of spaceflight on musculoskeletal health

- Muscle mass was reduced in healthy/sham mice in spaceflight



How do the underlying molecular events correlate with these phenotypic observations?

Target of present study: Quadriceps muscle of healthy/sham animals

Molecular Assay

Muscle tissues

Cryo-mill

Homogenized Samples

Precellys

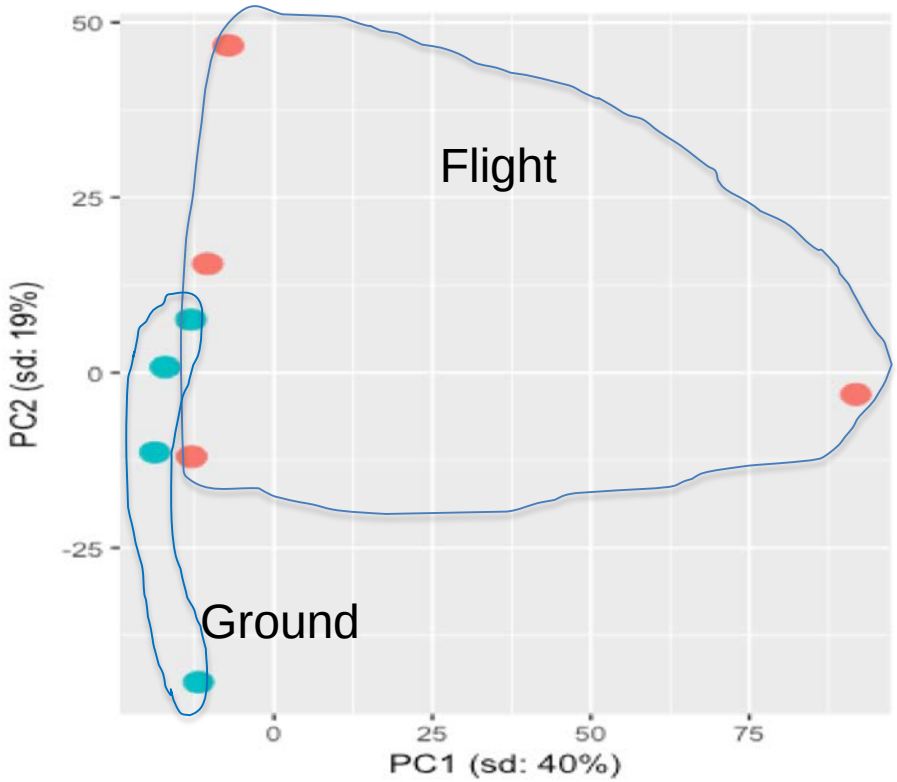
RNA extraction (Trizol method)

mRNA seq: Illumina HiSeq

The quality of the filtered 14,228 genes and their performance metrics in mapping to mouse genome

	Raw read counts/ gene			Map to Genome: Stats		
	Min	Max (M)	Mean (M)	Min	Max	Avg
Ground Sham	12	1.3	0.02	65.5%	79.5%	74.1%
Flight Sham	16	1.4	0.02	77.5%	81.5%	79.5%

Principal Component Analysis



- 840 differentially expressed genes (DEG) met t-test $p<0.05$
- 19 genes met False Discovery Rate (FDR) 0.1
  14 genes met FDR 0.05

Genes meeting FDR 0.1

Symbol	Log(FC)	FDR	Gene Name	Location	Type
TNNT1	-3.6	1.8 E-4	troponin T1, slow skeletal type	Cytoplasm	other
MYH7	-5.2	6.8 E-4	myosin heavy chain 7	Cytoplasm	enzyme

5 genes (all down regulated) are related to Myosin proteins

Myosin:

- **General Molecular motors**
- **Interact with actin filaments: Utilize energy to generate mechanical force**

GOLGA7B	1.4	0.03	golgin A7 family member B	Other	other

3 genes (all down regulated) are related to Troponin proteins

Troponin:

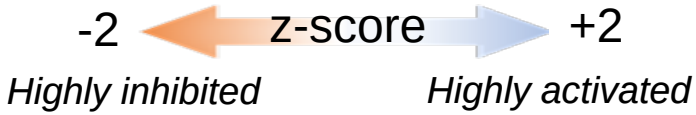
- **Regulate the myofibril contractile apparatus of striated muscles**

PFN2	-0.8	0.08	profilin 2	Cytoplasm	enzyme
DCAF4	0.9	0.09	DDB1 and CUL4 associated factor 4	Nucleus	other

Functional Analysis and Significantly Regulated Networks

Selection criteria of biological functions/networks of interest:

- Significantly enriched by differentially expressed genes (840 genes, $p<0.05$) -log(p value) < 1.3
- Degree of regulation (z score)

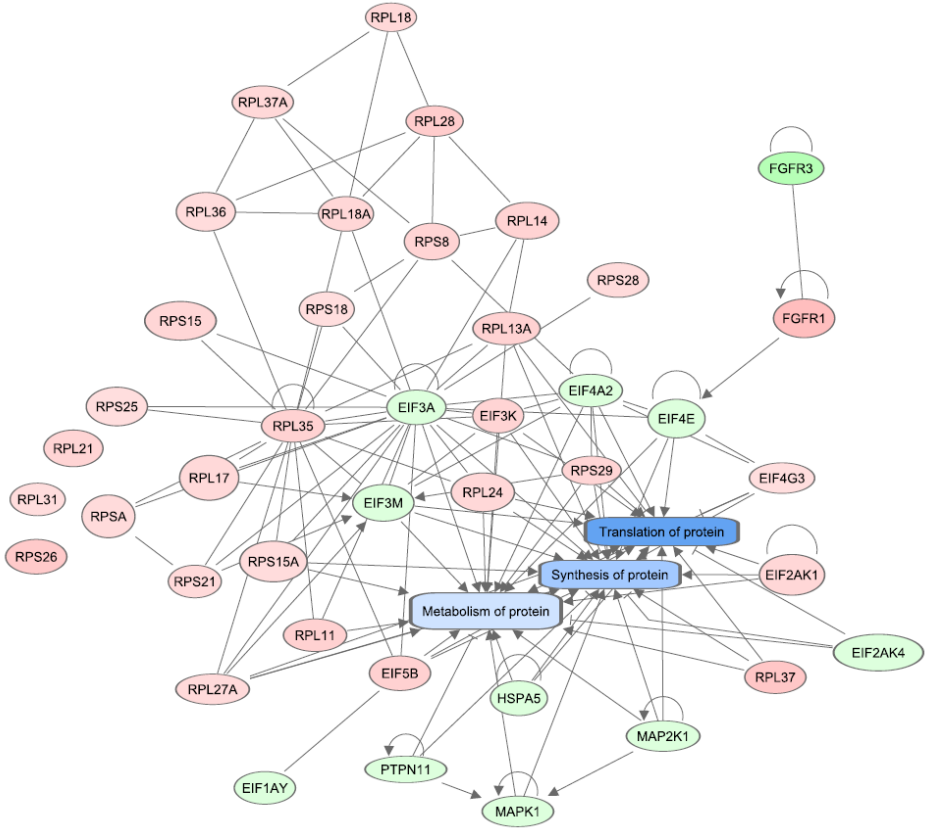


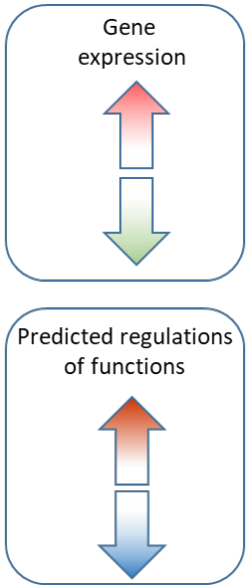
- Involved with protein metabolism

Canonical Networks	-log(p-value)	z-score
EIF2 Signaling	18.5	2.8
GPCR-Mediated Nutrient Sensing	0.283	2.0
Cell Cycle: G1/S Checkpoint Regulation	1.15	1.3
p53 Signaling	0.771	1.3
Type I Diabetes Mellitus Signaling	1.1	-2.0
STAT3 Pathway	4.47	-2.1
Ephrin Receptor Signaling	1.15	-2.3
Integrin Signaling	3.8	-2.3

- Involved with myogenesis
- Integral factor of muscle development

Eukaryotic Initiation Factor (eIF2) Signaling





Activated eIF2 signal induced inhibition of protein synthesis, translation and metabolism

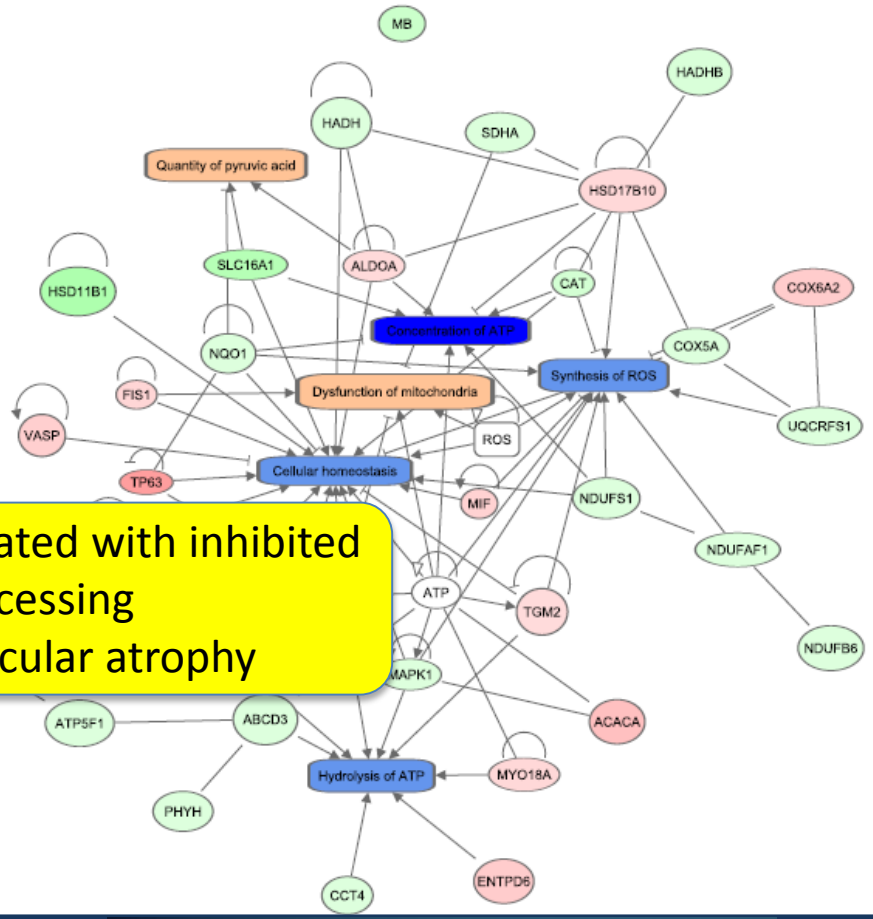
Functions Related to Muscle and Proteins

Biological Function	Status in Spaceflight
166 genes linked to Protein synthesis and degradation	
Protein synthesis	Inhibited
Protein catabolism	Inhibited
Protein metabolism	Inhibited
Protein localization	Inhibited
118 genes linked to muscle related functions	
Formation of muscle	Inhibited
Muscular contraction (GEO term)	Activated
Contractility of muscle/ Muscular inotropy	Inhibited
Development of striated muscle	Inhibited
Apoptosis of muscle cells	Inhibited
Proliferation of muscle cells	Activated
Proliferation of smooth muscle cells	Activated
Morphology of muscles	No change

Additional Functions of Interest

Biological Function	Status in Spaceflight
22 gene linked to Ca+2 signal	
Ca+2 burden	Inhibited
Muscular contraction (GEO term)	Activated
Contractility of muscle/ Muscular inotropy	Inhibited
Contraction of striated muscle	Inhibited
Formation of muscle	No change
Morphology of muscles	No change
differentiation of muscle	Activated
44 genes linked to energy production and mitochondrial dysfunction	
cellular homeostasis	Inhibited
ATP hydrolysis	Inhibited
Concentration of ATP	Inhibited
Hydrogen peroxide	Inhibited
Synthesis of ROS	Inhibited
Quantity of pyruvic acid	Activated
Dysfunction of mitochondria	Activated

Energy Network- Inhibited in Space



Lack of energy is correlated with inhibited protein processing and elevated muscular atrophy

Gene expression

Predicted regulations of functions

Conclusions

- Spaceflight-induced stress including prolonged weightlessness potentially coordinated with reduced muscle synthesis and contractibility, and activated proliferation.
- The reduced mass of the quadriceps is possibly linked to changes in networks such as eIF2 signaling, integrin, and calcium signaling, as well as down regulation of genes related with troponin and myosin.
- A comprehensive deprivation of energy is suggested. Protein synthesis and metabolism, lipid synthesis and metabolism, and ATP hydrolysis and concentration were reduced. In parallel, mitochondrial dysfunction was activated. The energy deprivation is correlated with reduced mass of quadriceps.
- In the near future, we hope metabolomic analysis will increase our confidence in our current findings, and give deeper insight into the processes taking place.

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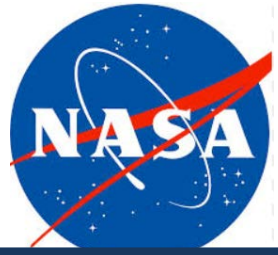

Questions?

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