Unloading-induced atrophy is a relatively uncomplicated form of muscle loss, where most of the loss of muscle mass during disuse atrophy can be accounted for by a depression in the rate of protein synthesis.

whereas in disease states associated with inflammation (cancer cachexia, AIDS, burns, sepsis, and uremia), there is a procatabolic hormonal and cytokine environment.

...procatabolic hormonal and cytokine environment...
It is imperative that these studies include examination of dynamic measures of muscle protein turnover and putative metabolic controllers... unless we have a clear idea of the basic responses to immobilization per se, the effects of such factors will not be easily teased out and therapeutic goals will remain largely unattainable.

Hypercatabolism

Hyper-catabolic conditions associated with proteolysis:
- Cancer cachexia
- Cachexia associated with heart failure
- Sepsis
- Starvation
- Metabolic acidosis
- Stress/trauma associated with excess glucocorticoids
- Space flight

Omega 3 (n3) Fatty Acids

- Eicosapentaenoic acid (EPA)
  - 20-C, omega-3 fatty acid
  - Dietary sources: fish oil, flaxseed, walnuts

- Beneficial effects on cholesterol, lipid metabolism, and cardiovascular health

n3/EPA and Muscle

Whitehouse et al. 2001
Omega-3 and Cancer

Proportion of tumor bearing rats

Vanamala et al., Carcinogenesis, 2008

Vitamin D

Sources
- UVB radiation
- Food
  - Seafood, mushrooms, egg yolk, fortified foods

Nomenclature
- Vitamin D$_3$ (ergocalciferol)
- Vitamin D$_2$ (cholecalciferol)
- 25-OH vitamin D
- 1,25 (OH)$_2$ vitamin D

Vitamin D Intake Guidelines

RDA (1997 IOM)
- 19-50 y: 200 IU/d
- 50-70 y: 400 IU/d

The 2005 Dietary Guidelines for Americans recommendation advised older adults, people with dark skin, and people exposed to insufficient sunlight to consume 1000 IU/d.

Vitamin D Status

Other metabolites:
- 24,25(OH)$_2$D$_3$
- 25,26(OH)$_2$D$_3$
- 3 others

Contributing Factors to Vitamin D Status

Age
Ethnicity
Salt-sensitive hypertension
Increased protein excretion in salt-sensitive individuals and Dahl rats with salt loading
Adiposity/obesity
Vitamin D is associated with:
- Calcium metabolism
- Fracture Risk/BMD

Smith et al., J Nutr, 2006
Smith et al., J Nutr, 2005

Control
Pre flight
Post flight

25 (OH) Vitamin D (nmol/L)

25 (OH) Vitamin D (nmol/L)

Vitamin D is associated with:
- Calcium metabolism
- Fracture Risk/BMD
- Muscle strength/function
- Cancer (prostate, breast, colon)
- Multiple sclerosis
- Dementia
- Parkinson’s disease
- Tuberculosis
- Incidence of C-section
- The common cold

Smith et al., J Nutr, 2006
Smith et al., J Nutr, 2005

SN
Con
Control
Pre flight
Post flight

25 (OH) Vitamin D (nmol/L)

25 (OH) Vitamin D (nmol/L)

Cardiovascular health
Immune function
Diabetes
Multiple Sclerosis
Dementia
Parkinson’s Disease
Tuberculosis
Incidence of C-section

Smith et al., J Nutr, 2006
Smith et al., J Nutr, 2005

SN
Co
Control
Pre flight
Post flight

25 (OH) Vitamin D (nmol/L)

25 (OH) Vitamin D (nmol/L)

Vitamin D status has been related to:
- Fractures, fracture risk, BMD
- Muscle strength/function, falls
- Cancer (prostate, breast, colon)
- Multiple sclerosis
- Blood pressure/heart disease
- Diabetes (type 1)

Bischoff-Ferrari, Am J Clin Nutr, 2006

Recommendations
Encourage adequate vitamin D:
- Intake
  - Fortified milk, orange juice
  - Fish (salmon, tilapia, tuna)
  - Few other sources...
- Sunlight
- Supplements

Encourage adequate vitamin D:
- Intake
  - Fortified milk, orange juice
  - Fish (salmon, tilapia, tuna)
  - Few other sources...
- Sunlight
- Supplements

...the criterion for broad-based supplementation in the general population is not fulfilled, except for in high risk groups, such as the elderly...all others persons with negligible exposure to sunshine.
Upper Limits

2000 IU/day is currently defined IOM no observed adverse events limit (NOAEL)
Studies of higher levels have proven safe...

Vitamin D Toxicity

Hypercalcemia, hypercalciuria, soft tissue calcification, kidney stones

Vitamin D

Vitamin D status goes down after long-duration spaceflight.

Questions:
- Is the stability of vitamin D in the food system and supplement different during spaceflight?
- Is the daily dose not high enough to maintain status?
- Does vitamin D metabolism change during spaceflight?

Stability Study

Stability of vitamin D in food/supplement is not altered during spaceflight

Question:
- Is the daily dose simply not high enough to maintain status in an environment with no sun exposure?
3 levels of vitamin D supplementation:
- 400 IU/d (n = 18)
- 1000 IU/d (n = 19)
- 2000 IU/d (n = 18)

3 blood collections and diet logs
- 25D, 1,25D, PTH, Ca, VDBP, NTX

Double blinded supplementation

Double blinded supplementation (Smith et al., 2009)

Vitamin D status is related to body weight…
- what if we exclude subjects with BMI >29 kg/m²?

1000 or 2000 IU/d was enough to reach 80 nmol/L and maintain vitamin D status

Compliance 84% on average

Residual Questions…
- Could compliance be improved with a weekly dose instead of a daily dose?
- Is vitamin D status related to observed changes in immune function during polar winters?
**Residual Questions…**

Is a higher, less frequent dose as effective as a daily or weekly dose?  
Does a high dose result in a high serum concentration of 25-OH vitamin D (or metabolites) or alter serum or urine calcium?
1 subject in 2000 IU/d group had 2 values >150 nmol/L.
2 subjects in 50000 IU group had 3-5 values > 150 nmol/L.
**Collagen Crosslinks**

Space Flight:
- Urinary collagen xlinks
- Urinary Ca
- Urinary OH-Proline

Bone resorption is increased during flight

**Bone Resorption**

Space Flight:
- Urinary collagen xlinks
- Urinary Ca
- Urinary OH-Proline

Bone resorption is increased during flight

**Bone Formation/Resorption**

**Calcium Isotopes**

<table>
<thead>
<tr>
<th>Calcium Isotopes</th>
<th>40Ca</th>
<th>42Ca</th>
<th>43Ca</th>
<th>44Ca</th>
<th>46Ca</th>
<th>48Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>97%</td>
<td>0.65%</td>
<td>0.11%</td>
<td>2.09%</td>
<td>0.004%</td>
<td>0.19%</td>
</tr>
</tbody>
</table>

\[ \delta^{44}\text{Ca} = \left( \frac{^{44}\text{Ca}}{^{40}\text{Ca}} \right)_{\text{sample}} \times 1000 \]

Higher \( \delta^{44}\text{Ca} \) = "heavier"
Lower \( \delta^{44}\text{Ca} \) = "lighter"

**Calcium Isotopes**

Weeks of Bed Rest

\[ \delta^{44}\text{Ca} = \left( \frac{^{44}\text{Ca}}{^{40}\text{Ca}} \right)_{\text{sample}} \times 1000 \]

Higher \( \delta^{44}\text{Ca} \) = "heavier"
Lower \( \delta^{44}\text{Ca} \) = "lighter"
Current folate intakes do not maintain folate status

How much folate is in the food? If enough – then:
Is folate stable on orbit? If it is – then:
What is changing?
Vitamin K

Fluid Intake

Fluid Intake

Fluid Shift

Renal Stone Risk

Nutrition SMO

UMS
Excess sodium intake (and related effects on acid/base physiology) is associated with a number of health issues:

- Bone loss
- Increased renal stone risk
- Impaired muscle performance/protein catabolism
- Altered glucose metabolism
- Altered vitamin D metabolism
- Hypertension

With the exception of hypertension, all of these other factors have been raised as concerns for space travelers.

The space food system is very high in sodium.

Sodium intake during flight is very high:

- ISS Sodium Intake
- US Dietary Reference Intake Tolerable Upper Intake Level
- NASA exploration requirement

High sodium has been shown in bed rest (and ambulatory) studies to exacerbate bone breakdown (Heer, et al.).

The excess sodium is bound to glycosaminoglycans in skin, exchanging with a hydrogen ion.

NOTE: This is the basis for the ESA sponsored SOLO experiment on ISS.

Mechanism: Excess sodium intake leads to non-osmotic (i.e., non-fluid retaining) storage of sodium.

**NOTE:** only a few JAXA food items are on the standard menu at this point (and no ESA or CSA). These are included in the bonus foods per crew request (along with other non-standard foods).
Acidosis

Recap 3

From Dr. L. Frassetto (UCSF) 10/6/09 JSC presentation

Acid/Base and Bone

Excess dietary sodium

Na+/H+ exchange in skin GAGs

Iron (RBCs, and oxidative damage)

Iron and Oxygen

Radiation/oxygen issues have implications for cataracts and other health issues.

Total Body Iron

Males

Females
1. **Bone Resorption**

- Chart showing percentage change from baseline over weeks of bed rest.

2. **Potential Countermeasures**

- Nutrition
- Exercise
- Pharmacology
- Gravity

3. **Exercise Countermeasures**

4. **Bone Resorption**

5. **Artificial Gravity.1**

- Graph comparing CON and AG treatments over weeks.
Bone Formation

- Graphs showing bone formation over time for Control and Exercise groups.
- Smith et al., JBMR, 2003
- Shackelford et al., JAP, 2004
- Smith et al., Bone 2008

Bisphosphonates

- Graphs showing percentage change from baseline for Control and Alendronate groups.
- LeBlanc et al., JMNI, 2002

Nutrition and Bone

- Images of food items and nutritional facts.

Vitamin K

- SOLO study showing vitamin K levels.
- Stacked bar chart showing Pre, Middle, and Post vitamin K levels.
- Stacked column chart showing Pre, Middle, and Post vitamin K levels.

Dietary Protein

- Dietary protein increases urinary calcium.
- Oxidation of excess protein yields acid (H+, H2SO4).
- Renal buffering.
- Bone: reservoir of base.
- Osteoclasts are more active at lower pH.
- Excess protein: beneficial or harmful to bone?
- Many factors influence the net effect.

Animal vs. Vegetable

- Animal protein: Diets rich in animal protein tend to have greater overall acid potential.
- Renal net acid excretion.
- Vegetables/fruits: Also contain substantial amounts of base precursors (and K).
- APro/K provides an estimation of acid/alkali load.

Studied by Dawson-Hughes et al. 2002.
Pro K

Controlled dietary intake
High or Low APro:K
Monitored dietary intake

Blood/Urine markers

EXAMPLE Menu

High APro:K Day 1 Example

<table>
<thead>
<tr>
<th>Item</th>
<th>Preflight</th>
<th>L-45</th>
<th>FD15</th>
<th>FD30</th>
<th>FD60</th>
<th>FD120</th>
<th>FD180</th>
<th>FD60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oatmeal w/ Brown Sugar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seasoned Scrambled Eggs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled Pork Chop</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoked Turkey</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bread Pudding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granola Bar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple Cider</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cashews</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pineapple</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drink</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brownies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana Pudding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Low APro:K Day 1 Example

<table>
<thead>
<tr>
<th>Item</th>
<th>Preflight</th>
<th>L-45</th>
<th>FD15</th>
<th>FD30</th>
<th>FD60</th>
<th>FD120</th>
<th>FD180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oatmeal w/ Raisins &amp; Spice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetarian Vegetable Soup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken Noodle Soup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waffles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled Chicken</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creamed Spinach</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocoa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cereal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled Ground</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cabbage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled Tuna</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mashed Potato</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meatballs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meatballs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled Tuna</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meatballs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled Tuna</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meatballs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled Tuna</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meatballs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled Tuna</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meatballs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled Tuna</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meatballs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled Tuna</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meatballs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: the low ratio diet is NOT low protein, and NOT vegetarian

NOTE: the pattern above (red or blue) is an example, your pattern may vary

Blood/Urine (24 h F; 48 h G) will be collected at the end of each session, and on L-10 and R+0.
Acid/Base and Bone

High protein, low potassium diet

Acid Load >> Alkali Load

$\text{H}^+\text{ >> }\text{Organic anions}$

$\text{Na}^+\text{}/\text{H}^+\text{ exchange}$ in skin GAGs

Excess dietary sodium

$\text{Ca}^{2+}\text{ excretion}$

$\text{CO}_3^{2-}\text{ Ca}^{2+}\text{ CO}_3^{2-}\text{ GAG}$

Arachidonic acid

Bed rest

Hindlimb unloading

Spaceflight

Ionizing radiation/UVC

PIF

RANKL

TNF-$\alpha$

$\alpha$

LPS

Inflammation

NF-$\kappa$B

kB Inhibitor

kB Inhibitor (active)

$\downarrow$ Muscle proteolysis

$\uparrow$ Osteoclast differentiation

Muscle proteolysis

Bone resorption

Inflammatory Markers

Inflammation/Bone

NF-$\kappa$B

kB Inhibitor

kB Inhibitor (active)

$\downarrow$ Muscle proteolysis

$\uparrow$ Osteoclast differentiation

Bone resorption

Pre mean

L-10

FD15

FD30

FD60

FD120

FD180

R+0

R+30

0

2000

4000

6000

8000

L-10 0 2000

R+0 0 2000

R+30 0 2000

CXCL 5 (pg/mL)

Pre

Inflight

R+0

R+30

0

2000

4000

6000

8000

1000

1500

Pre 0

Inflight

R+0

R+30

0

500

1000

1500

3-MH ($\mu$mol/g creat)

TNF-$\alpha$ (pg/mL)

NTX (nmol/d)

Activa

Infl for

Bone

MTX (nmol/d)

TNF-$\alpha$ (pg/mL)