Protein (and muscle)
**Muscle Protein**

↓ Protein Synthesis  ↑ Protein Breakdown

**Protein Synthesis Breakdown**

Unloading-induced atrophy is a relatively uncomplicated form of muscle loss. 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.

Phelps, et al., J Apil Physiol, 2009

**Amino Acids**

Amino acid supplementation

Issues:

- Protein synthesis vs. breakdown
- Exercise
- Catabolic effectors (e.g., stress/cortisol, hypocaloric diet, T3)
- Intake (and/or supplement) of control group

**Inflammation**

Bed rest  Hindlimb unloading  Spaceflight  Ionizing radiation/UVC

PIF  LPS  RANKL  TNF-α

NF-κB

kB Inhibitor  kB Inhibitor

NF-κB (active)

UB-prot activation  ↓ Muscle proteolysis

**Inflammatory Markers**

Pre  Me  L-1  0  FD  15  FD3  0  FD6  0  FD12  0  FD180  R+0  R+30

0  20  40  5  10  15  20  25

IL-1β (pg/mL)

Pre mean  L-10  FD  15  FD  30  FD  60  FD  120  FD  180  R+0  R+30

0  2000  4000

CXCL5 (pg/mL)

**…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
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$_2$ (ergocalciferol)
- Vitamin D$_3$ (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: Review

Holick, AJCN, 2004

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

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

25 (OH) Vitamin D (nmol/L)

Vitamin D and PTH

Erkal, Osteo Int, 2006
Thomas et al., NEJM, 1998
Chapuy et al., Osteo Int, 1997

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

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

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

Space Food

<table>
<thead>
<tr>
<th>Flight Requirement (per day)</th>
<th>450</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D (IU)</td>
<td>120</td>
</tr>
<tr>
<td>Salmon</td>
<td>396</td>
</tr>
<tr>
<td>Tuna</td>
<td>152</td>
</tr>
<tr>
<td>Breakfast Drink</td>
<td>116</td>
</tr>
<tr>
<td>Tuna Noodle Casserole</td>
<td>94</td>
</tr>
<tr>
<td>Cornflakes</td>
<td>68</td>
</tr>
<tr>
<td>Tuna Sliced Spread</td>
<td>64</td>
</tr>
<tr>
<td>Bran Chex</td>
<td>68</td>
</tr>
<tr>
<td>Scrambled Eggs</td>
<td>64</td>
</tr>
<tr>
<td>Bread Pudding</td>
<td>64</td>
</tr>
<tr>
<td>Granola w/Raisins</td>
<td>44</td>
</tr>
<tr>
<td>Tapioca Pudding</td>
<td>44</td>
</tr>
<tr>
<td>Teriyaki Beef</td>
<td>36</td>
</tr>
<tr>
<td>Pork Chops</td>
<td>32</td>
</tr>
<tr>
<td>Vegetable Quiche</td>
<td>28</td>
</tr>
<tr>
<td>Pudding Soup</td>
<td>28</td>
</tr>
</tbody>
</table>
Upper Limits

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

Sunlight does not result in toxicity
Watch multivitamins (vit A and other nutrients may be in excess)

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

Compliance
84% on average

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

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?
In addition to BMI, the efficacy of vitamin D supplementation is affected by baseline status.

Compliance:
- 2000 IU/d: 91%
- No supplement: 97%

Does a high dose result in a high serum concentration of 25-OH vitamin D (or metabolites) or alter serum or urine calcium?

Residual Questions...

Is a higher, less frequent dose as effective as a daily or weekly dose?
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.

Nutrition SMO

Calcium
**Collagen Crosslinks**

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

Bone resorption is increased during flight

**Calcium Isotopes**

- Higher $\delta^{44}\text{Ca} = $ "heavier"
- Lower $\delta^{44}\text{Ca} = $ "lighter"

$\delta^{44}\text{Ca} = \left(\frac{^{44}\text{Ca}}{^{44}\text{Ca}_{\text{standard}}} - 1\right) \times 1000$

<table>
<thead>
<tr>
<th>Isotope</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>

Smith et al., JCEM, 1998

**Bone Formation/Resorption**

**Calcium Isotopes**

Skula et al., Clin Chem, 2007
URINE PROCESSOR ASSEMBLY

Recycle Filter Tank Assembly

Distilled Assembly

Regenerative ECLSS

UPA

Urine Calcium

Urine Volume

-17%
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?

Folate

Radiation

Nutrient Stability

Vitamin E

Zwart et al., J Food Sci, 2009
Vitamin K

Fluid Intake

Fluid Intake (mL/d)

Total Body Water

ECF and Plasma Volumes

Fluid Shift

Renal Stone Risk

Fluid Shift from Pre-flight

Renal Stone Risk

Fluid Shift from Preflight

Fluid Shift from Preflight

Nutrition SMO

UMS

ISU UMS

MISSION DATA INTERFACE CONNECTOR

P/R USB INTERFACE CONNECTOR

MSD INTERFACE CONNECTOR

RS-232 CONNECTOR

FLUSH WATER INLET/OUTLET

MECHANICAL MODULE

*URINE HOSES NOT SHOWN
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:

- Excess sodium intake leads to non-osmotic (i.e., non-fluid retaining) storage of sodium
- The excess sodium is bound to glycosaminoglycans in skin, exchanging with a hydrogen ion.

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

Recap 3

1. The higher the acid load, the slower you went, the more your arterial blood was acid and the higher the body’s acid base balance.
2. Excess dietary sodium may lead to bone loss.
3. Acid-base exchange in skin GAGs.
4. Excess dietary sodium.
5. Acid-base imbalance leads to bone and muscle imbalances.
6. Acid-base imbalances may lead to cardiovascular disease.
7. Acid-base imbalances may lead to renal dysfunction.
8. Acid-base imbalances may lead to osteoporosis.
9. Acid-base imbalances may lead to muscle weakness.
10. Acid-base imbalances may lead to decreased exercise capacity.
11. Acid-base imbalances may lead to decreased exercise performance.
12. Acid-base imbalances may lead to decreased exercise recovery.
13. Acid-base imbalances may lead to decreased exercise efficiency.
14. Acid-base imbalances may lead to decreased exercise endurance.
15. Acid-base imbalances may lead to decreased exercise capacity.
16. Acid-base imbalances may lead to decreased exercise performance.
17. Acid-base imbalances may lead to decreased exercise efficiency.
18. Acid-base imbalances may lead to decreased exercise endurance.
19. Acid-base imbalances may lead to decreased exercise capacity.
20. Acid-base imbalances may lead to decreased exercise performance.
21. Acid-base imbalances may lead to decreased exercise efficiency.
22. Acid-base imbalances may lead to decreased exercise endurance.
23. Acid-base imbalances may lead to decreased exercise capacity.
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63. Acid-base imbalances may lead to decreased exercise capacity.
64. Acid-base imbalances may lead to decreased exercise performance.
65. Acid-base imbalances may lead to decreased exercise efficiency.
66. Acid-base imbalances may lead to decreased exercise endurance.
**Bone Resorption**

- **Week of Bed Rest**
- **% from Baseline**
- **FD**
- **NTX (% change)**

**Potential Countermeasures**

- Nutrition
- Exercise
- Pharmacology
- Gravity

**Artificial Gravity.1**

- **CON**
- **AG**

**Exercise Countermeasures**

- **WISE**

**Bone Resorption**

- **Smith et al., J Appl Physiol, 2009**
- **Shackelford et al., JAP, 2004**
- **Smith et al., Bone 2008**
**Bone Formation**

![Bone Formation Diagram]

**Bisphosphonates**

![Bisphosphonates Diagram]

**Nutrition and Bone**

![Nutrition and Bone Diagram]

**Nutrition and Bone**

![Nutrition and Bone Diagram]

**Vitamin K**

![Vitamin K Diagram]

**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

![Dietary Protein Diagram]

**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

![Animal vs. Vegetable Diagram]
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>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oatmeal w/ Brown Sugar</td>
<td>Hot and Sour Soup</td>
</tr>
<tr>
<td>Granola Bar</td>
<td>Cheese</td>
</tr>
<tr>
<td>Seasoned Scrambled Eggs</td>
<td>Grilled Pork Chop</td>
</tr>
<tr>
<td>Smoked Turkey</td>
<td>Bread Pudding</td>
</tr>
<tr>
<td>Fruit Cocktail</td>
<td>Pudding</td>
</tr>
<tr>
<td>Apple Cider</td>
<td>Brownies</td>
</tr>
<tr>
<td>Tropical Punch</td>
<td>Brownies</td>
</tr>
</tbody>
</table>

**Low APro/K Day 1 Example**

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oatmeal w/ Raisins &amp; Spice</td>
<td>Vegetable Soup</td>
</tr>
<tr>
<td>Green Beans &amp; Mushrooms</td>
<td>Chicken Noodle</td>
</tr>
<tr>
<td>Waffles</td>
<td>Curry Sauce</td>
</tr>
<tr>
<td>Grilled Chicken</td>
<td>Tuna</td>
</tr>
<tr>
<td>Almonds</td>
<td>Peanut Butter</td>
</tr>
<tr>
<td>Carrot Coins</td>
<td>Cocoa</td>
</tr>
<tr>
<td>Creamed Spinach</td>
<td>Carrot Coins</td>
</tr>
<tr>
<td>Macadamia Nuts</td>
<td>Pineapple Drink</td>
</tr>
<tr>
<td>Orange Juice</td>
<td>Japanese Tomato</td>
</tr>
<tr>
<td>Candy Coated Almonds</td>
<td>Water (250 mL)</td>
</tr>
</tbody>
</table>

---

**Nutrition and Bone**

**Pro K**

**EVA Pilot Study**

**Supplement**
- Grape juice
- Vitamin E
- NAC

**Max Voluntary Contraction**

**Endurance - Reps**

**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
Acid/Base and Bone

High protein, low potassium diet
Acid Load >> Alkali Load

\[ H^+ \gg \text{Organic anions} \]

Na+/H+ exchange in skin GAGs

\[ \text{CO}_3^{2-} \rightarrow \text{Ca}^{2+} \text{CO}_3^{2-} \rightarrow \text{Ca}^{2+} \text{excretion} \]

Excess dietary sodium

Inflammation

Bed rest
Hindlimb unloading
Spaceflight
Ionizing radiation/UVC

Arachidonic acid
PIF
LPS
RANKL
TNF-α

NF-κB
kB Inhibitor

NF-κB (active)

kB Inhibitor

Inflammatory Markers

NF-κB

Inflammation/Bone

Inflammation

Bed rest
Hindlimb unloading
Spaceflight
Ionizing radiation/UVC

Arachidonic acid
PIF
LPS
RANKL
TNF-α

NF-κB
kB Inhibitor

NF-κB (active)

kB Inhibitor

Bone resorption

Muscle proteolysis

\[ 0 \rightarrow 1000 \rightarrow 2000 \rightarrow 3000 \rightarrow 4000 \rightarrow 5000 \rightarrow 6000 \]

Inflammatory Markers

L-10
R+0
R+14
AME

Pre mean

FD15
FD30
FD60
FD120
FD180
R+30

NFκB

Pre
Inflight
R+0

NFκB

Pre
Inflight
R+0

3-MH

(CXCL 5 (pg/mL))

Pre
Inflight
R+0

1000
1500

Pre
Inflight
R+0

TNFα (pg/mL)

Pre
Inflight
R+0

NTX (nmol/d)

Pre
Inflight
R+0

Muscle proteolysis

Bone resorption

Osteoclast differentiation

kB Inhibitor

NFκB

NFκB

Usenet activation

Usenet activation

3-MH

(CXCL 5 (pg/mL))

Pre
Inflight
R+0

1000
1500

Pre
Inflight
R+0

TNFα (pg/mL)

Pre
Inflight
R+0

MTX (nmol/d)

Pre
Inflight
R+0

TNFα (pg/mL)