Protein (and muscle)
**Muscle Protein**

![Protein Synthesis](image1)

**Amino Acids**

**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 pro-catabolic hormonal and cytokine environment.

**Inflammation**

![Inflammatory Markers](image2)

**Inflammatory Markers**

![Inflammatory Markers](image3)
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

Hypercatabolism

Hyper-catabolic conditions associated with upregulation of the ubiquitin-proteasome system:
- Cancer cachexia (Lurie et al. 2001; Tisdale et al. 2009)
- Cachexia associated with heart failure (Frippiat et al. 2005, Freeman et al. 1998)
- Sepsis (Vireen et al. 1996; Tiao et al. 1994)
- Starvation (Whitehouse, 2001)
- Metabolic acidosis (Mitch et al. 1994)
- Stress/trauma associated with excess glucocorticoids (Wing et al. 1993, Boku et al. 2000)
- Space flight (Kemote et al. 2001; Rikay et al. 1992)

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$_2$ (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

Other metabolites:
- 24,25(OH)$_2$D$_3$
- 25,26(OH)$_2$D$_3$
- 35 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
- Muscle strength/function
- Cancer
- Cardiovascular health
- Immune function
- Diabetes
- Multiple Sclerosis
- Dementia
- Parkinson’s Disease
- Tuberculosis
- Incidence of C-section
- The common cold

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)

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.

<table>
<thead>
<tr>
<th>Flight Requirement (per day)</th>
<th>VD D (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>100</td>
</tr>
<tr>
<td>172 ± 44</td>
<td>25 (OH) Vitamin D (nmol/L)</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>110</td>
</tr>
<tr>
<td>20</td>
<td>120</td>
</tr>
<tr>
<td>30</td>
<td>130</td>
</tr>
<tr>
<td>40</td>
<td>140</td>
</tr>
<tr>
<td>50</td>
<td>150</td>
</tr>
<tr>
<td>60</td>
<td>160</td>
</tr>
<tr>
<td>70</td>
<td>170</td>
</tr>
<tr>
<td>80</td>
<td>180</td>
</tr>
<tr>
<td>90</td>
<td>190</td>
</tr>
<tr>
<td>100</td>
<td>200</td>
</tr>
</tbody>
</table>

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

Recommendations
- Fortified milk, orange juice
- Fish (salmon, tilapia, tuna)
- Few other sources...

Space Food

<table>
<thead>
<tr>
<th>Food</th>
<th>Vitamin D (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon</td>
<td>296</td>
</tr>
<tr>
<td>Tuna</td>
<td>150</td>
</tr>
<tr>
<td>Breakfast Drink</td>
<td>116</td>
</tr>
<tr>
<td>Tuna Noodle Casserole</td>
<td>86</td>
</tr>
<tr>
<td>Cornflakes</td>
<td>68</td>
</tr>
<tr>
<td>Tuna Salad Spread</td>
<td>64</td>
</tr>
<tr>
<td>Bran Chex</td>
<td>64</td>
</tr>
<tr>
<td>Scrambled Eggs</td>
<td>64</td>
</tr>
<tr>
<td>Bread Pudding</td>
<td>56</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>Potato 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

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

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.

Vitamin D Dosing Study

Nutrition SMO

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

Bone resorption is increased during flight

Bone Formation/Resorption

Calcium Isotopes

40Ca 42Ca 43Ca 44Ca 46Ca 48Ca
97% 0.65% 0.11% 2.09% 0.004% 0.19%

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

Calcium Isotopes

Higher \( \delta^{44}\text{Ca} \) = “heavier”
Lower \( \delta^{44}\text{Ca} \) = “lighter”
Regenerative ECLSS

URINE PROCESSOR
ASSEMBLY

Recycle Filter Tank Assembly
Distilled Assembly

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

Nutrient Stability

Radiation

Vitamin E

Nutrient Stability

Zwart et al., J Food Sci, 2009

Vitamin B6
Vitamin B12
Vitamin C
Thiamin
Niacin
Riboflavin
Folate
Biotin
Pantothenate
Vitamin A (β carotene)
Vitamin D
Vitamin E
Vitamin K
Amino Acids
Fatty acids
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,

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Sodium Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1500 mg/d</td>
</tr>
<tr>
<td>2</td>
<td>2000 mg/d</td>
</tr>
<tr>
<td>3</td>
<td>2500 mg/d</td>
</tr>
</tbody>
</table>

The space food system is very high in sodium.

Sodium intake during flight is very high.

- ISS Sodium Intake: 3500 mg/d
- US Dietary Reference Intake (RDA): 2300 mg/d
- NASA exploration requirement (JSC-63555)

In 2005-2006, the average US intake of Na was estimated at 3,436 mg Na/d*
In 1990-1999, the average US intake of Na was estimated at:
- 3,377 mg for 31-50 yo M
- 3,539 mg for 31-50 yo F

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

**SOLO, etc.**

Mechanism

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.

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Na+-Balance</th>
<th>Low Na</th>
<th>High Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>0</td>
<td>500</td>
</tr>
<tr>
<td>10</td>
<td>1000</td>
<td>0</td>
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<tr>
<td>15</td>
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</tr>
<tr>
<td>20</td>
<td>2000</td>
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<td>2000</td>
</tr>
<tr>
<td>25</td>
<td>2500</td>
<td>0</td>
<td>2500</td>
</tr>
</tbody>
</table>

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

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

Acid/Base and Bone

Excess dietary sodium

Iron (RBCs, and oxidative damage)

Iron and Oxygen

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

Total Body Iron

Mission Duration (days)

Red Blood Cell Mass (mL/kg body weight)

Iron intake
Bone Resorption

Week of Bed Rest

% from baseline

Bone Resorption

Potential Countermeasures

- Nutrition
- Exercise
- Pharmacology
- Gravity

Artificial Gravity.1

Smith et al., J Appl Physiol, 2009

Exercise Countermeasures

Brain Resorption

Smith et al., J Appl Physiol, 2009

Shackelford et al., JAP, 2004

Smith et al., Bone 2008
**Bone Formation**

- **Exercise** vs. **Control**
- **Week of Bed Rest**
- **Percentage from baseline**

**Bisphosphonates**

- **Control** vs. **Alendronate**
- **Week of Bed Rest**
- **Percentage from baseline**

**Nutrition and Bone**

- **Vitamin K**
- **SOLO**
- **Study Day**
- **CTX (μg/d)**

**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
Controlled dietary intake
High or Low APro:K
Monitored dietary intake
Blood/Urine markers

EXAMPLE Menu

Nutrition and Bone

High APro:K Day 1 Example
Oatmeal w/ Brown Sugar
Oatmeal w/ Raisins & Spice
Granola Bar
Fruit Cocktail
Green Beans & Mushrooms
Tropical Punch
Chicken Noodle Soup
Canned Spinach
Creamed Spinach

Low APro:K Day 1 Example
Oatmeal w/ Brown Sugar
Oatmeal w/ Brown Sugar
Granola Bar
Fruit Cocktail
Green Beans & Mushrooms
Tropical Punch
Chicken Noodle Soup
Canned Spinach
Creamed Spinach

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

H+ >> Organic anions

Na+/H+ exchange in skin GAGs

Excess dietary sodium

CO3^2- Ca^2+ CO3^2- Ca^2+

H+ >> Organic anions

Arachidonic acid
Bed rest
Hindlimb unloading
Spaceflight
Ionizing radiation/UVC

PIF
RANKL
TNF-α
α-LPS

Inflammation

NF-kB
kB Inhibitor

kB Inhibitor

NF-kB (active)

Muscle proteolysis

Osteoclast differentiation

Bone resorption

Inflammation/Bone

Inflammatory Markers

Inflammatory Markers

NF-kB
kB Inhibitor

NF-kB (active)

Muscle proteolysis

Osteoclast differentiation

Bone resorption

Inflammation

NF-kB
kB Inhibitor

NF-kB (active)

Muscle proteolysis

Osteoclast differentiation

Bone resorption

Inflammation/Bone

Inflammatory Markers

NF-kB
kB Inhibitor

NF-kB (active)

Muscle proteolysis

Osteoclast differentiation

Bone resorption

Inflammation/Bone

Inflammatory Markers

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Bone resorption