Protein (and muscle)

Amino Acids

Dietary Protein
Muscle Protein

- Protein Synthesis
- Protein Breakdown

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

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....

Inflammation

- NF-κB
- kB Inhibitor

...procatabolic hormonal and cytokine environment...

Inflammatory Markers
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.

Hyper-catabolism

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

Hyper-catabolism

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 (Fiessman et al. 2005, Filippatos et al. 1998)
- Sepsis (Vietri et al. 1996; Tan et al. 1994)
- Starvation (Whitehouse, 2001)
- Metabolic acidosis (Mitch et al. 1994)
- Stress/trauma associated with excess glucocorticoids (Wing et al. 1993, Babi et al. 2000)
- Space flight (Inamoto et al. 2001, Riley 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

Eicosapentaenoic acid (EPA)

Omega 3 (n3) Fatty Acids

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

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

SN
Control
Pre flight
Post flight

25 (OH) D
(nmol/L)

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

...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>Food</th>
<th>Vitamin D (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flight Requirement (per day)</td>
<td>450</td>
</tr>
<tr>
<td>Menu</td>
<td>172 ± 44</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>64</td>
</tr>
<tr>
<td>Cornflakes</td>
<td>64</td>
</tr>
<tr>
<td>Tuna Salad Spread</td>
<td>64</td>
</tr>
<tr>
<td>Bran Chex</td>
<td>56</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>Pudding</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...

Vitamin D Toxicity

Hypercalcemia, hypercalciuria, soft tissue calcification, kidney stones

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

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

(Excluding subjects with BMI >29 kg/m²)

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

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

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.

Compliance:
- 2000 IU/d: 91%
- 10000 IU/wk: 97%

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?

Vitamin D Dosing Study

- 2,000 IU/d
- 10,000 IU/wk
- 50,000 IU weekly x4; then 1/mo

Vit D (and metabolites)
Ca, etc.
Dist, ur

**Polar I** (2000 IU/d)

** baseline 25D (nmol/L)

<table>
<thead>
<tr>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb</td>
</tr>
<tr>
<td>June</td>
</tr>
<tr>
<td>Sept</td>
</tr>
</tbody>
</table>

Wk 1
Fasted blood & 24-h urine

Wk 2
Fasted blood & 24-h urine

Wk 3
Fasted blood & 24-h urine

Month 1: Time 0
Fasted blood draw/24-h urine sample

Subject screening
Fasted blood draw; 24-h urine sample

Month 2: Time 0
Fasted blood draw/24-h urine sample

Month 3: Time 0
Fasted blood draw/24-h urine sample

Wk 1 Fasted blood & 24-h urine

Wk 2 Fasted blood & 24-h urine

Wk 3 Fasted blood & 24-h urine

Month 4: Time 0
Fasted blood draw/24-h urine sample
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

Bone Resorption

Space Flight:

- Urinary collagen xlinks
- Urinary Ca
- Urinary OH-Proline

Bone resorption is increased during flight

Bone Formation/Resorption

Calcium Isotopes

Calcium Isotopes

Higher δ44Ca = "heavier"
Lower δ44Ca = "lighter"
Regenerative ECLSS

URINE PROCESSOR ASSEMBLY

Recycle Filter Tank Assembly
Distillation Assembly

Urine Calcium

Pre-flight In-flight +24%

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?

**Nutrient Stability**

- Vitamin B6
- Vitamin B12
- Folic acid
- Folate
- Thiamin
- Niacin
- Riboflavin
- Folate
- Biotin
- Pantothenate
- Vitamin D (β-carotene)
- Vitamin D
- Vitamin E
- Vitamin K
- Amino Acids
- Fatty acids

**Radiation**

- RBC folate (% change from preflight)

**Vitamin E**

- Total radiation exposure (µGy)
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. 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.). 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.
Acidosis

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

Mission Duration (days)

-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100

Pre Mean

FD1

FD30

FD60

FD180

R+0

R+30

Radiation and O2 issues have implications for cataracts and other health issues.
Iron and Oxidative Damage

Bed Rest

Total body iron (mg/kg)

8OHdG (ug/day)

Post

Total body iron (% Δ from pre-BR)

Transferrin receptors (% from pre-BR)

8OHdG (ug/day)

Total body iron (mg/kg)

Supplements

Zwart et al., J Nutr, 2009

EVA Pilot Study

Phylloquinone (nmol/L)

25(OH) Vitamin D (nmol/L)

Avg # multivitamins per week

Phylloquinone

25(OH) Vitamin D

Outliers

Avg # multivitamins per week

Pre Mean

FD15

FD30

FD60

FD120

FD180

R+0

R+30

Pre Mean

Supplement

Grape juice

Vitamin E

NAC

Pre Mean

FD15

FD30

FD60

FD120

FD180

R+0

R+30

Pre Mean

FD15

FD30

FD60

FD120

FD180

R+0

R+30

Pre Mean

FD15

FD30

FD60

FD120

FD180

R+0

R+30
Bone Resorption

Week of Bed Rest

% from baseline

NTX (% change)

Smith et al., J Appl Physiol, 2009

Shackelford et al., JAP, 2004

Smith et al., Bone 2008

Potential Countermeasures

- Nutrition
- Exercise
- Pharmacology
- Gravity

Exercise Countermeasures

Bone Resorption

CON
AG

Artificial Gravity.1

Calcium (mg)

Smith et al., JBMR, 2003

Pos

NTX, %

0 5/6 12/1

0 26/2 7

Pos

NTX, %

0 25

Pos

NTX, %

0 25
Bone Formation

Sobel et al., JMRI, 2003
Starr et al., JMRI, 2004
Shafe et al., JBMR, 2003

Bisphosphonates

Sobel et al., JMRI, 2003
Starr et al., JMRI, 2004
Shafe et al., JBMR, 2003

Nutrition and Bone

Smith et al., JBMR, 2003
Shackelford et al., JAP, 2004
Smith et al., Bone 2008

Nutrition and Bone

Smith et al., JBMR, 2003
Shackelford et al., JAP, 2004
Smith et al., Bone 2008

Vitamin K

Vitamin K

Nutrition and Bone

Dawson-Hughes et al. 2002

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

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

Dawson-Hughes et al. 2002
**APro:K and Bone**

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

---

**EVA Pilot Study**

Supplement
Grape juice
Vitamin E
NAC

---

**Pro K**

---

**EXAMPLE Menu**

**High APro/K Day 1 Example**

<table>
<thead>
<tr>
<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oatmeal w/ Brown Sugar</td>
</tr>
<tr>
<td>Grilled Pork Chop</td>
</tr>
<tr>
<td>Smoked Turkey</td>
</tr>
<tr>
<td>Bread Pudding</td>
</tr>
<tr>
<td>Granola Bar</td>
</tr>
<tr>
<td>Cheese Grits</td>
</tr>
<tr>
<td>Pasta</td>
</tr>
<tr>
<td>Fruit Cocktail</td>
</tr>
<tr>
<td>Seasoned Scrambled Eggs</td>
</tr>
<tr>
<td>Grilled Chicken</td>
</tr>
<tr>
<td>Tuna</td>
</tr>
<tr>
<td>Almonds</td>
</tr>
<tr>
<td>Cocoa</td>
</tr>
<tr>
<td>Carrot Coins</td>
</tr>
<tr>
<td>Creamed Spinach</td>
</tr>
<tr>
<td>Macadamia Nuts</td>
</tr>
<tr>
<td>Orange Juice</td>
</tr>
<tr>
<td>Tofu w/ Hot Mustard Sauce</td>
</tr>
<tr>
<td>Apples w/ Spice</td>
</tr>
<tr>
<td>Water (250 mL)</td>
</tr>
<tr>
<td>Brownies</td>
</tr>
<tr>
<td>Banana Pudding</td>
</tr>
<tr>
<td>Tropical Punch</td>
</tr>
</tbody>
</table>

**Low APro/K Day 1 Example**

<table>
<thead>
<tr>
<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oatmeal w/ Raisins &amp; Spice</td>
</tr>
<tr>
<td>Vegetarian Vegetable Soup</td>
</tr>
<tr>
<td>Chicken Noodle Soup</td>
</tr>
<tr>
<td>Waffles</td>
</tr>
<tr>
<td>Soup</td>
</tr>
<tr>
<td>Salad</td>
</tr>
<tr>
<td>Grilled Cheese</td>
</tr>
<tr>
<td>Creamed Spinach</td>
</tr>
<tr>
<td>Carrot Coins</td>
</tr>
<tr>
<td>Savory sauce or Vegetable</td>
</tr>
<tr>
<td>Tofu</td>
</tr>
<tr>
<td>Grilled Chicken</td>
</tr>
<tr>
<td>Tuna</td>
</tr>
<tr>
<td>Almonds</td>
</tr>
<tr>
<td>Cocoa</td>
</tr>
<tr>
<td>Carrot Coins</td>
</tr>
<tr>
<td>Creamed Spinach</td>
</tr>
<tr>
<td>Carrot Coins</td>
</tr>
<tr>
<td>Pasta</td>
</tr>
<tr>
<td>Fruit Cocktail</td>
</tr>
<tr>
<td>Seasoned Scrambled Eggs</td>
</tr>
<tr>
<td>Grilled Pork Chop</td>
</tr>
<tr>
<td>Bread Pudding</td>
</tr>
</tbody>
</table>

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**Nutrition and Bone**
Acid/Base and Bone

High protein, low potassium diet

Acid Load >> Alkali Load

\[ \text{H}^+ \text{ exchange} \]

Na+/H+ exchange in skin GAGs

Excess dietary sodium

High protein, low potassium diet

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

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)

\[ \text{Ub-prot activation} \]

\[ \text{Muscle proteolysis} \]

\[ \text{Muscle proteolysis} \]

\[ \text{Osteoclast differentiation} \]

\[ \text{Bone resorption} \]

\[ \text{Bone resorption} \]

\[ \text{Bone resorption} \]

\[ \text{Bone resorption} \]

Inflammation

Inflammatory Markers

\[ \text{CXCL 5 (pg/mL)} \]

\[ \text{TNFα (pg/mL)} \]

\[ \text{NTX (nmol/d)} \]

\[ \text{Pre mean} \]

\[ \text{L-10} \]

\[ \text{R+0} \]

\[ \text{R+14} \]

\[ \text{AME} \]

\[ \text{FD15} \]

\[ \text{FD30} \]

\[ \text{FD60} \]

\[ \text{FD120} \]

\[ \text{FD180} \]

\[ \text{R+30} \]

\[ \text{0} \]

\[ \text{2000} \]

\[ \text{4000} \]

\[ \text{6000} \]

\[ \text{8000} \]

\[ \text{0} \]

\[ \text{200} \]

\[ \text{400} \]

\[ \text{600} \]

\[ \text{Pre} \]

\[ \text{Inflight} \]

\[ \text{R+0} \]

\[ \text{R+30} \]

\[ \text{Inflammation/Bone} \]

\[ \text{MTX (nmol/d)} \]

\[ \text{TNFα (pg/mL)} \]

\[ \text{Pre} \]

\[ \text{Inflight} \]

\[ \text{R+0} \]

\[ \text{R+30} \]
Omega-3 Fatty Acids (EPA)

Muscle, cancer...

Vanamala et al., Carcinogenesis, 2008

Omega-3 (EPA) and Bone

Muscle, bone, and cancer...

Zwert, et al. 2010

Omega-3 (EPA) and Bone

n-3 FA

Muscle, bone, and cancer...

Zwert, et al. 2010

Nutrient Requirements

Energy
CHO (fiber), Fat, Protein
Fat-Soluble vitamins
Water-sol vitamins
Minerals
Fluid

Countermeasures

Energy
Amino acids
Protein
Sodium
Fatty acids
Antioxidants
Other

Bisphosphonates
KCl
Medications
Exercise
Other

Systems

Bone
Muscle
Cardio
Fluid/Electrolyte
Immunology
Hematology
Neuro, Etho
GI, BHP

Earth

Physiology
Medicine
Technology
Education

Vehicle/Mission

Food System
Duration
Radiation
EVA
Schedule

Zwart, et al. 2010