Protein (and muscle)
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. .....
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 upregulation of the ubiquitin-proteasome system:
- Cancer cachexia (Lorke et al. 2001; Tisdale et al. 2009)
- Cachexia associated with heart failure (Filippatos et al. 2005, Freeman et al. 2005)
- Sepsis (Viret 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, Borko et al. 2000)
- Space flight (Kennedy et al. 2001, Rikky et al. 1992)

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_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.

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) 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

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.

Space Food

<table>
<thead>
<tr>
<th>Food</th>
<th>Vitamin D (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flight Requirement (per day)</td>
<td>400</td>
</tr>
<tr>
<td>Tuna</td>
<td>152</td>
</tr>
<tr>
<td>Salmon</td>
<td>116</td>
</tr>
<tr>
<td>Breakfast Drink</td>
<td>72</td>
</tr>
<tr>
<td>Cornflakes</td>
<td>64</td>
</tr>
<tr>
<td>Tuna Noodle Casserole</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 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

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%
- 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?
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:

N-Telopeptide

Bone resorption is increased during flight

Bone Formation/Resorption

Calcium Isotopes

\[ \delta^{44}\text{Ca} = \left( \frac{^{44}\text{Ca}}{^{40}\text{Ca}} \right)_{\text{sample}} / \left( \frac{^{44}\text{Ca}}{^{40}\text{Ca}} \right)_{\text{standard}} - 1 \] x 1000

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

Skulan et al., Clin Chem, 2007
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 (mL/d)

Day 1-85

Day 131-179

Day 86-130 (Vitamin K)

Pre                Mission               Post

UOsteocalcin (%)

Fluid Intake

3000

4000

a ke

FD 15

FD 20

FD 25

FD 30

FD 35

FD 40

FD 50

FD 55

FD 60

FD 70

FD 80

FD 90

FD 95

FD 100

FD 110

FD 115

FD 12

FD 130

FD 135

FD 140

FD 145

FD 150

FD 160

FD 170

FD 175

FD 180

FD 185

FD 190

FD 195

FD 200

FD 205

FD 210

FD 215

FD 220

FD 225

Fluid Intake (mL/d)

Total Body Water

ECF and Plasma Volumes

SLS-1 and SLS-2

Leach et al. 1996

Fluid Shift

Renal Stone Risk

Nutrition SMO

UMS

ISS UMS

AVIONICS MODULE

MAIN POWER & AM/MM INTERFACE CONNECTOR

RS-232 CONNECTOR

(UORP ON-ORBIT DATA EXTRACTION METHOD)

ISS CONFIG ONLY

PRIME DATA EXTRACTION METHOD – ISS CONFIG ONLY

KEYPAD

LCD

UMS MECHANICAL MODULE

*URINE/AIR INLET

*URINE/AIR OUTLET

*URINE HOSES NOT SHOWN

INTERFACE CONNECTOR

SAMPLE PORT

MAIN POWER CONNECTOR, POWER SWITCH, CB

FLUSH WATER INLET/OUTLET

Renal Stone Risk

Nutrition SMO
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.).

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

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

H+ release contributes to acid load.
Acidosis

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

Acid/Base and Bone

Excess dietary sodium

Excess GAG 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
Bone Resorption

Week of Bed Rest
% from baseline

Pre Measure
FD15
FD30
FD60
FD12
FD18
R+0
R+30

Nutrition
Exercise
Pharmacology
Gravity

Potential Countermeasures

Artificial Gravity.1

CON
AG

Bone Resorption

Exercise Countermeasures

NTX (% of pre-BR)

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

Nutrition
Exercise
Pharmacology
Gravity

Artificial Gravity.1

CON
AG

Bone Resorption

Exercise Countermeasures

NTX (% of pre-BR)

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

Nutrition and Bone

Dietary Protein

Animal vs. Vegetable

Dietary protein increases urinary calcium
Oxidation of excess protein yields acid (H\(^+\), H\(_2\)SO\(_4\))
- 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 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
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>Ingredient</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oatmeal w/ Brown Sugar</td>
<td></td>
<td>Hot and Sour Soup</td>
</tr>
<tr>
<td>Granola Bar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seasoned Scrambled Eggs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoked Turkey</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bread Pudding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit Cocktail</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tropical Punch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brownies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana Pudding</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Low APro/K Day 1 Example

<table>
<thead>
<tr>
<th>Item</th>
<th>Ingredient</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oatmeal w/ Raisins &amp; Spice</td>
<td></td>
<td>Vegetarian Vegetable Soup</td>
</tr>
<tr>
<td>Waffles</td>
<td></td>
<td>Cheese Tortellini</td>
</tr>
<tr>
<td>Carrot Coins</td>
<td></td>
<td>Creamed Spinach</td>
</tr>
<tr>
<td>Cocoa</td>
<td></td>
<td>Tofu w/ Hot Mustard</td>
</tr>
<tr>
<td>Almonds</td>
<td></td>
<td>Candy Coated Almonds</td>
</tr>
<tr>
<td>Grilled Pork Chop</td>
<td></td>
<td>Potato Medley</td>
</tr>
<tr>
<td>Candy</td>
<td></td>
<td>Japanese Tomato Jelly</td>
</tr>
<tr>
<td>Smoked Turkey</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waffles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled Chicken Tuna Peanut</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Almonds</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Waffles</td>
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</tr>
</tbody>
</table>

Nutrition and Bone
Acid/Base and Bone

Acid Load >> Alkali Load

High protein, low potassium diet

H+ >> Organic anions
Na+/H+ exchange in skin
Excess dietary sodium

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

\[ \text{Na}^+ \]

\[ \text{Ca}^{2+} \]

\[ \text{GAG}^- \]

\[ \text{NF-\kappa B} \]

Inflammation

Bed rest
Hindlimb unloading
Spaceflight
Ionizing radiation/UVC

Arachidonic acid
PIF
LPS
RANKL
TNF-\alpha

NF-\kappa B

\( \kappa B \) Inhibitor

NF-\kappa B (active)

Ultraprot activation

Muscle proteolysis

Inflammatory Markers

Inflammation/Bone

Arachidonic acid
Bed rest
Hindlimb unloading
Spaceflight
Ionizing radiation/UVC

NTX

TNF-\alpha

Pre
Inflight
R+0
R+30

NF-\kappa B (active)

Ultraprot activation

Muscle proteolysis

Bone resorption

Pre mean

Inflight

R+0

R+30