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.

Inflammation

Phillis, et al., J. Appl. Physiol, 2009

NF-κB

NF-κB (active)

Muscle proteolysis

Inflammatory Markers

Pierson et al., 2009
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-catabolic conditions associated with proteolysis:
- Cancer cachexia
- Cachexia associated with heart failure
- Sepsis
- Starvation
- Metabolic acidosis
- Stress/trauma associated with excess glucocorticoids
- Space flight

- Eicosapentaenoic acid (EPA)
  - 20-C, omega-3 fatty acid
  - Dietary sources: fish oil, flaxseed, walnuts
  - 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 D₂ (ergocalciferol)
- Vitamin D₃ (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

Other metabolites:
- 24,25(OH)₂D₃
- 25,26(OH)₂D₃
- 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, 2005**

**SN**
**Control**
**Pre flight**
**Post flight**

Vitamin D is associated with:
- 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

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

**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)

**Chapuy et al., Osteo Int, 1997**

**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></th>
<th>Flight Requirement (per day)</th>
<th>VIT D (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menu</td>
<td>450</td>
<td>127 ± 46</td>
</tr>
<tr>
<td>Salmon</td>
<td>396</td>
<td>152</td>
</tr>
<tr>
<td>Tuna</td>
<td>116</td>
<td>84</td>
</tr>
<tr>
<td>Breakfast Drink</td>
<td>64</td>
<td>68</td>
</tr>
<tr>
<td>Tuna Noodle Casserole</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Cornflakes</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Tuna Salad Spread</td>
<td>64</td>
<td>64</td>
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<tr>
<td>Bran Chex</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Scrambled Eggs</td>
<td>64</td>
<td>64</td>
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<tr>
<td>Bread Pudding</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Granola w/Raisins</td>
<td>64</td>
<td>64</td>
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<tr>
<td>Tapioca Pudding</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Teriyaki Beef</td>
<td>64</td>
<td>64</td>
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<tr>
<td>Pork Chops</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Vegetable Quiche</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Pudding</td>
<td>64</td>
<td>64</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?
Polar Vitamin D

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%
- 10,000 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
- 2000 IU/d
- 10,000 IU/wk
- 50,000 IU weekly x4; then 1/mo
- Vit D (and metabolites)
- Ca, etc.
- Diets, etc.

Subjects screened - Fasted blood draw; 24-h urine sample

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

Supervised administration of 2000 IU, 10,000 IU or 50,000 IU vitamin D3 dose
- Time: 48 hr - Blood draw & spot urine
- Time: 24 hr - Blood draw
- Time: 12 hr - Blood draw
- Time: 6 hr - Blood draw

Supervised administration of 50,000 IU vitamin D3 dose 24 h later:
- Fasted blood
- Time: 48 hr - Blood draw & spot urine
- Time: 24 hr - Blood draw
- Time: 12 hr - Blood draw
- Time: 6 hr - Blood draw

Vit D Dosing Study
- 2000 IU/d
- 10,000 IU/wk
- 50,000 IU weekly x4; then 1/mo
- Vit D (and metabolites)
- Ca, etc.
- Diets, etc.

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

Baseline

- 2000 IU/d
- 10000 IU/wk
- 50000 IU/mo

60 Days

- 2000 IU/d
- 10000 IU/wk
- 50000 IU/mo

Placebo

- 6
- 12
- 24
- 48

Placebo

- 0
- 28
- 60
- 90

- 50
- 100
- 150

- 0
- 2
- 5
- 10
- 15

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

- 25(OH) Vitamin D
- 0
- 25
- 50
- 75
- 100
- 125
- 150

Vitamin D mool/L

SN Control

Preflight Postflight

Bone Calcium Pools

Diet Excretion Feces Absorption Secretion

Human Body

Deposition Secretion Bone

Calcium

Human Body

Dist Absorption Excretion Feces

Calcium Pools Excretion Resorption

Bone

+24%
Collagen Crosslinks

Bone Resorption

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

Bone resorption is increased during flight

Smith et al., JCEM, 1998

Bone Formation/Resorption

Calcium Isotopes

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

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

Skulan et al., Clin Chem, 2007
Urine Calcium

+49%

Nutrition SMO
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)

0 1000 2000 3000 4000

Day 1-85

Day 131-179

Day 86-130 (Vitamin K)

Pre

Mission

Post

UOsteocalcin (%)

Fluid Intake

3000

4000

ak

FD 15

FD 20

FD 25

FD 30

FD 35

FD 40

FD 45

FD 50

FD 55

FD 60

FD 65

FD 70

FD 75

FD 80

FD 85

FD 90

FD 95

FD 100

FD 105

FD 110

FD 115

FD 120

FD 125

FD 130

FD 135

FD 140

FD 145

FD 150

Fluid Intake (mL/d)

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Fluid Intake (mL/d)
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.

Per Food Item Na Content

Menu 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).

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

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

Acid/Base and Bone

Excess dietary sodium

Na+/H+ exchange in skin GAGs

CO3\(^{2-}\)

Ca\(^{2+}\)

Excretion

Iron (RBCs, and oxidative damage)

Iron/Hematology

Iron intake

Red Blood Cell Mass (mL/kg body weight)

Mission Duration (days)

Iron and Oxygen

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

Total Body Iron

Males

Females

Total body iron (mg/kg)

Pre Mean

FD1

FD30

FD60

FD120

FD180

R+0

R+30

-5

0

5

10

15

Day 0

5

10

15

20

Mission Duration (days)

Total body iron (mg/kg)
Potential Countermeasures

- Nutrition
- Exercise
- Pharmacology
- Gravity
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 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
**APr:K and Bone**

Zwart et al., AJCN, 2004

Zwart et al., J Appl Physiol, 2005

**EVA Pilot Study**

**Pro K**

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

Blood/Urine markers

**Example Menu**

High APr:K Day 1 Example

- Oatmeal w/ Brown Sugar
- Seasoned Scrambled Eggs
- Grilled Pork Chop
- Smoked Turkey
- Bread Pudding
- Granola Bar
- Cheese
- Grits
- Pasta w/Pesto Sauce
- Butter Cookies
- Fruit Cocktail
- Green Beans & Mushrooms
- Broccoli au Gratin
- Almonds
- Apple Cider
- Cashews
- Peanuts
- Pineapple Drink
- Tea
- Brownies
- Banana Pudding

Low APr:K Day 1 Example

- Oatmeal w/ Raisins & Spice
- Vegetarian Vegetable Soup
- Chicken Noodle Soup
- Waffles
- Celery Sticks
- Corned Beef
- Tuna
- Peanut Butter
- Almonds
- Cheese
- Tortellini
- Curry Sauce w/ Vegetables
- Tortillas
- Cocoa
- Carrot Coins
- Creamed Spinach
- Macadamia Nuts
- Orange Juice
- Tofu w/ Hot Mustard Sauce
- Apples w/ Spice
- Water (250 mL)
- Tea
- Potato Medley
- Candy Coated Almonds
- Candied Yams
- Water (250 mL)
- Japanese Tomato Jelly Drink

**Nutrition and Bone**

**Design**

**Preflight**

- L-180
- L-45
- FD15
- FD30
- FD60
- FD120
- FD180
- FD60

**Inflight**

- FD15
- FD30
- FD60
- FD120
- FD180

**Postflight**

- R+30
- R+180
- R+365

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+6.
Acid/Base and Bone

High protein, low potassium diet

**Acid Load >> Alkali Load**

- **H+ >> Organic anions**
- **Na+/H+ exchange in skin**
- **Na+ exchange in skin GAGs**

- **Ca2+ excretion**

- **Excess dietary sodium**

Inflammation

- Arachidonic acid
- Bed rest
- Hindlimb unloading
- Spaceflight
- Ionizing radiation/UVC

**NF-kB**

- kB Inhibitor
- kB Activator

- **NF-kB (active)**

- **Muscle proteolysis**

**Inflammatory Markers**

- L-10
- R+0
- R+14
- AME

- **NF-κB**
- **κB Inhibitor**

- **NF-κB (active)**

- **Muscle proteolysis**

**Inflammation/Bone**

- **NF-kB**
- **kB Inhibitor**

- **NF-kB (active)**

- **Muscle proteolysis**

- **Bone resorption**

**Inflammatory Markers**

- **CXCL 5 (pg/mL)**
- **TNFα (pg/mL)**

- **NTX (nmol/d)**

- **Pre**
- **Inflight**
- **R+0**
- **R+30**