Diet, Gut Microbiome, and Bone Health

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Osteoporosis Prevention

Calcium accretion rates are highest during adolescence\(^1\) 10%↑ in peak bone mass delays osteoporosis by 13 yr.

Calcium intakes remain inadequate especially in girls (9-18 y; mean 918 – 988 mg/d)\(^2\).

Disclosures for Connie Weaver

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<tr>
<th>AFFILIATION/FINANCIAL INTERESTS (past 12 months)</th>
<th>CORPORATE ORGANIZATION</th>
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<td>Grants/Research Support:</td>
<td>NIH, Tate &amp; Lyle, APRE</td>
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Effect of Increasing Dietary Calcium

- **↑ absorption**
- **↓ Bone resorption**

What are Prebiotics?

Basic criteria for selection of prebiotics

- Resistance to digestion
- Hydrolysis and fermentation by colonic microbiota
- Selective stimulation or growth of one or limited number of bacteria
- Beneficial health effects to the host

Greater Intestinal Surface Area

- Crypt depth ↑
- Epithelial cell density ↑
- Cecal vein flow ↑

Net calcium transport 2-fold higher in cecum and distal colon*

Enhanced Mineral Solubility

SCF and SCFA + pH

Blood

SCFA

Greater Intestinal Surface Area

Human studies showing ↑ Ca absorption with inulin-type fructans

- Van den Heuvel et al., 1999
- Griffin et al., 2002, 2003
- Coudray et al., 1997
- Abrams et al., 2005

1-Year Fructan Intervention Trial in 100 boys and girls aged 9-12 y

Objective: Assess the long-term effects of inulin-type fructans on calcium absorption and bone mineral accretion

- 8 g/d mixed short and long inulin-type fructans
- 8 weeks-Ca absorption
- 1 year-Ca absorption and BMD

8 g/d maltodextrin control
- 8 weeks-Ca absorption
- 1 year-Ca absorption and BMD

100 pubertal boys and girls age 9-13

None of these studies evaluated changes in gut microbiota.

Calcium Absorption Increased with Fructan Supplementation

Pubertal adolescents assigned to 8 g/d of mixed short and long chain inulin-type fructans

Whole Body BMD Increased with Fructan Supplementation

* difference from control at 1 yr, p=0.01

* difference from control at 8 wk, p<0.001
** difference from control at 1 yr, p=0.04
**Scientific question**

- Can diet (prebiotic fibers) alter the gut microbiome to improve calcium absorption and utilization?

**Types Prebiotics**

- **Disaccharides**
  - Lactulose
  - Lactitol

- **Oligosaccharides**
  - Short Chain Fructo-oligosaccharides (scFOS)
  - Soybean oligosaccharides
  - Xylo-oligosaccharides
  - Galacto-oligosaccharides (GOS)

- **Polysaccharides**
  - Inulin (long chain FOS)
  - Resistant starches

**Galactooligosaccharide**

- Commercially prepared with β-D-galactosidase
  - Linkages are β(1-4), β(1-2), β(1-6)
  - Similar to human milk oligosaccharides (HMOs)

**GOS Improved Mineral Utilization in Rats\(^1\)**

- 4-week old male Sprague-Dawley Rats, n=75 (15/group)
- 8 week treatment period
- GOS treatments replaced cornstarch by % weight
- Primary Outcomes
  - Mineral Balance (Ca, Mg)
  - Bone mineral density
- Mechanistic Outcomes
  - Cecal Morphology
  - Microbial Community Structure

\(^1\)Weaver et al. J Agric Food Chem 2011; 59: 6501
Mechanistic Outcomes Predicted Calcium Absorption and BMD in Rats

- Cecal pH*: (2.2 to 11.0 % change)
- Cecal Wall Weight*: (13.3 to 141.0 % change)
- Cecal Content Weight*: (45.0 to 342.6 % change)
- Bifidobacteria†: (-25.4 to +265.9 % change)
- Calcium Absorption*: (15.7 – 53.2 % change)
- Distal femur vBMD‡: (4.4 – 7.2 % change)

* p<0.01, † p<0.05, ‡ p<0.0001

GOS

The effects of galactooligosaccharide (GOS) on colonic calcium absorption in pre-menarcheal girls


STUDY DESIGN

Randomized, double-blind crossover design
Healthy, premenarcheal girls, age 9-12 (n=29)
GOS supplemented in two yogurt smoothies/day

- 0g GOS
- 5g GOS
- 10g GOS

- 2% GOS in rat diet = 8g GOS/d in humans (levels proportional to body surface area)

3 week consumption of GOS separated by 2-week washouts
2 day clinical visit at end of each 3-week treatment

GOS Improved Calcium Absorption

p=0.0149
Summary of GOS Effects in Girls

- GOS treatment had a differential response on levels of bifidobacteria
- 5 and 10 g/d GOS supplementation increased calcium absorption by 10% relative to control
  - Increase in absorption would account for 5.4% of total skeletal calcium after a year
- Increased absorption occurs between 24-36h post-treatment, suggesting lower gut mechanisms
- GOS may help reduce fracture risk by maximizing bone mass accrual during growth


Observation on Mechanisms

- All fibers except resistant starches led to increased total SCFA, acetate, butyrate
- But total SCFA largely unrelated to bone parameters
- Cecal content weight better predictor of bone mineral density and strength

Weaver CM et al. JAgri Food Chem. 2010.
TWO RANDOMIZED, DOUBLE BLIND, CROSSOVER SCF STUDIES WITH TEENS

Study 1: Efficacy

- Subject Randomization
- N = 28 (G)
- 0, 10, or 20 g/d of fiber as SCF
- 3 week intervention
- 1 week washout
- 3 week intervention

Study 2: Effectiveness

- Subject Randomization
- N = 24 (B/G)
- 0, 10, or 20 g/d of fiber as SCF
- 4 week intervention
- 3 week washout
- 4 week intervention

Period-End Clinical Visit: Dual Tracer Calcium Absorption


Teen Study 1 Efficacy Study

- Adolescent girls (n=9; 12-14 y) and boys (n=15; 13-15 y)
- Controlled diets with 600 mg/d calcium and 15 g/d fiber (not including SCF)
- PROMITOR® SCF in fruit snacks

Primary objective:

- Determine the effect of SCF on calcium absorption and retention in adolescent boys and girls

Secondary objective:

- Assess the effect of SCF on fecal microbiota in relation to calcium absorption

SCF INCREASES CALCIUM ABSORPTION IN THE LOWER GUT BY 12%

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Soluble Corn Fiber’s Effect on Bone Health in Postmenopausal women

Hypothesis: SCF will increase bone calcium retention in a dose-dependent manner in postmenopausal women.

Bone turns over at a rate of 26%/year for trabecular bone and 3%/year for cortical bone.

FDA approved approach to study interventions:
- 4 year RCT of BMD
- We developed a 50 day screening method.

Traditional DXA measures are time consuming in humans

Study design

- Measures atom level quantities $^{14}$C, $^{41}$Ca, $^{129}$I, $^{26}$Al, $^{10}$Be
- $^{41}$Ca hat $t_{1/2}$ ~ 100,000 years
- 99% of Ca in the body resides in the skeleton
- 24-hr Urine collection every ~10 days
- $^{41}$Ca/Ca in urine
- Baseline, Treatment 1, Recovery 1

Purdue University Cooperative Extension Service is an equal access/equal opportunity institution.

Rapid Screening Method Accelerator Mass Spectrometry for Tracer Quantification
Effect of Added Soluble Corn Fiber on Net Bone Calcium Retention in Postmenopausal Women (Mean ± 95% CI)

- Different from predicted values determined at baseline and recovery periods

Symptom Severity by Dose (Mean ± SEM)

No significant difference in severity of GI distress between SCF intake levels

**BIOMARKERS OF BONE TURNOVER**

- BAP – Bone-Specific Alkaline Phosphatase (Bone Formation)
- NTx – N-Terminal Telopeptide (Bone Resorption)
- OC – Osteocalcin (Bone Turnover)
Dietary prebiotic results in significant differences in Microbiome of subjects

**Effectiveness Study**

All Female White Subjects

SCF treatments

- B-0 g control
- B-10 g
- B-20 g
- E-0 g control
- E-10 g
- E-20 g

B=Beginning E=End

Principal Coordinate Analysis (PCoA) of Euclidean Distances

**SCF treatment**

- B-0 g control
- B-10 g
- B-20 g
- E-0 g control
- E-10 g
- E-20 g

Effectiveness Study

All Female White Subjects

Whisner CM et al. (submitted)

Dietary prebiotic results in significant differences in Microbiome of subjects.

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SCF increased bone calcium retention in a dose response manner in postmenopausal women.

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**Thank you!**

- **Funders:**
  - FrieslandCampina
  - Tate & Lyle
  - NICHD

- **Collaborators/Lab:**
  - Cindy Nakatsu
  - George McCabe
  - Munro Peacock
  - Meryl Wastney
  - Linda McCabe
  - Berdine Martin
  - Corrie Whisner
  - Pam Lachcik
  - Steven Jakeman