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The bone-protective effect and mechanism of soluble corn fiber

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Steven A. Jakeman

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To my mother, who taught me about sacrifice; to my father, who taught me about work; and to my wife, who teaches me about both every day.

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I look back on the last two and a half years and know that my graduate experience was possible because of support from those around me.

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ABBREVIATIONS

AMS	accelerator mass spectrometry
BAP	bone-specific alkaline phosphatase
BCE	bone collagen equivalents
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
DXA	dual energy x-ray absorptiometry
FOS	fructooligosaccharides
GOS	galactooligosaccharides
ICP-MS	inductively coupled plasma mass spectrometry
nCi	nanocuries
NTx	N-terminal telopeptide
OC	osteocalcin
OVX	ovariectomized
PDX	polydextrose
SCF	soluble corn fiber
SCFA	short-chain fatty acid
scFOS	short-chain fructooligosaccharides

SFD	soluble fiber dextrin
SYN1	Synergy1 fiber
U/L	units/liter
vBMD	volumetric bone mineral density

ABSTRACT

Jakeman, Steven A. M.S., Purdue University, May 2015. The Bone-Protective Effect and Mechanism of Soluble Corn Fiber. Major Professor: Connie Weaver.

Postmenopausal women are at greatest risk among healthy individuals of developing osteoporosis and associated fractures. Nondigestible, fermentable dietary carbohydrates have been shown to improve calcium absorption in adolescents and bone-strength parameters in the rat model. Of particular interest is soluble corn fiber (SCF), which improved rat bone strength the most in a survey of novel fibers, and improved calcium absorption by up to 13% in teen girls. Hypotheses about the mechanism behind this effect revolve around the production of short-chain fatty acids (SCFAs) in the large intestine during fermentation. The purpose of this work was to determine if SCF would also benefit postmenopausal women and to assess the effect on calcium absorption of a chronic increase in cecal concentrations of SCFAs in rats.

To study the effect of SCF, a randomized, double-blind, placebo-controlled crossover design was used to assess the effects of daily consumption of 0, 10, and 20 g SCF on bone calcium retention in postmenopausal women. SCF was provided as PROMITOR® 85 SCF, which provides 85% fiber, and was consumed as a constituent of one muffin and one fruit-flavored drink daily for 50 days. To measure net bone calcium

loss, participants' bones were labeled with ^{41}Ca by administration of ≥50 nCi of ^{41}Ca intravenously at least 100 d prior to the start of the study. Every 10 days, 24 h urine was collected, and ^{41}Ca appearance was measured sensitively by Accelerator Mass Spectrometry, while total Ca was measured by ICP-MS. Urinary ^{41}Ca:Ca from nonintervention periods was used to determine an expected rate of bone loss. A decrease in urinary ^{41}Ca:Ca from the expected amount during intervention periods reflected increased bone retention. A dose-response effect was demonstrated, with 10 g/d SCF and 20 g/d SCF improving bone calcium retention by 4.8% (P = 0.013) and 7% (0.007) respectively. Bone formation marker bone-specific alkaline phosphatase (BAP), and bone turnover markers osteocalcin (OC) and N-terminal telopeptide (NTx), were measured at the ends of the baseline period and each washout and intervention period. Of the biomarkers measured, the only statistically significant difference detected was measured in BAP, between placebo and 20 g/d SCF (8%, P = 0.035). Daily SCF consumption significantly increased bone retention in postmenopausal women. It is estimated that 20 g/d SCF would improve bone balance by 50 mg/d, or 2.5% total body BMC per year if the effect persists.

To assess the effectiveness of SCFAs to increase calcium absorption, rats were chronically dosed in a randomized, placebo-controlled trial with SCFAs. Twice daily, the rats received 0, 300, or 600 umol SCFA conjugate base directly to their ceca through a cecal catheter. At the end of 11 days, ^{45}Ca was administered to their ceca, and ^{45}Ca appearance was measured in their plasma. No statistically significant difference was observed between treatments.

These studies indicate that SCF, in achievable dietary intakes, is effective in supporting bone health in postmenopausal women, but also that increasing SCFA concentration in the large intestine may not be the only mechanism causing this benefit. It is possible that the protocol used to assess the effect of chronic SCFA dosing on calcium absorption is inadequate. New protocols should be considered, and other mechanisms, such as a shift in gut pH or microbial populations, should be assessed for their effects on calcium absorption.

1. INTRODUCTION

Osteoporosis is a worldwide problem, resulting in millions of fractures annually (1). Primarily a disease of age, it afflicts an estimated one-in-two women and one-in-five men over the age of 50 (2). Osteoporosis is characterized by low bone-mass, with an exaggerated loss of trabecular bone and structure. The result of this condition is weak, brittle bones that may break due to minor falls or impacts.

The costs of osteoporosis are great. It is estimated that, in the U.S. alone, the annual fiscal burden of osteoporosis is $19 billion annually, relating to two million fractures (2). This is only expected to grow in the coming years. Projections estimate that by 2025, the annual cost will increase to $25.3 billion, related to three million fractures. In addition to the monetary expense and physical pain of fracture, one in five people suffering a hip fracture will die within one year of the event. Many of those who survive are unable to independently complete simple physical tasks, such as walking a block or climbing stairs, 2 years after the fracture (3).

Calcium accounts for 31% of bone mass, and is critical for maintaining bone strength. While 99% of total body calcium is stored in bone, its concentration in blood is tightly regulated. When total absorbed calcium is too low to make up for obligatory losses, bone resorption is triggered to maintain homeostasis (4). Chronic bone resorption,

paired with inadequate bone deposition, can lead to osteoporosis. Thus chronic inadequate dietary calcium can lead to osteoporosis.

Individuals in all age groups, except for young men, are at substantial risk of inadequate dietary calcium (5). Unfortunately, recent changes in dietary patterns and concern over the safety of calcium supplements are likely increasing the risk of calcium inadequacy. Milk consumption, the source of most dietary calcium in the U.S., is declining (6). Likewise, calcium supplement use in the U.S. was down to 18% in 2013 from 22% in 2011 (7). The decline in consumption of relatively bioavailable dietary calcium presents a challenge for professionals striving to reduce incidence of osteoporosis. One dietary strategy is to improve calcium absorption efficiency.

The effect of dietary calcium absorption enhancers, including non-digestible, fermentable carbohydrates has been studied (8). These carbohydrates remain unaffected by human digestion, reaching the large intestine intact. There they are fermented by bacteria into short-chain fatty acids (SCFAs) and other metabolites. The primary hypothesis is that through the production of SCFAs, colonic pH is reduced, increasing calcium solubility and ionization, and ultimately allowing for greater paracellular diffusion. Randomized controlled trials (RCTs) with rats (9-18), adolescents (19-24), and postmenopausal women(25-27) have generally, but not always (20, 28, 29), shown that calcium absorption is improved with dietary supplementation of non-digestible, fermentable carbohydrates.

Of particular interest is soluble corn fiber (SCF), a novel fiber recognized for its tolerability (22, 30), and bone-strengthening effects in the rat model (31). Two studies were recently carried out in adolescents showing 12-13% improved calcium absorption

with 10 – 20 g/d SCF (22, 32). However, no studies have been carried out assessing the bone-protective effects of SCF in postmenopausal women, the population most affected by osteoporosis due to hormone changes, in addition to low calcium status. The purpose of this work was to explore both the benefit of SCF on bone in postmenopausal women, and the mechanism by which this effect occurs.

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2. LITERATURE REVIEW

Calcium, Food and Nutritional Components in Focus, Ed. By Prof. Victor Preedy, ISBN: 978-1-84973-882-8, DOI: 10.1039/978-1-78262-213-0. Forthcoming, 2015

Widely known as the primary mineral constituent of bone, calcium likely encourages other health-promoting effects such as reduced risk for high blood pressure, colon cancer, and cardiovascular disease (1). However, in an evaluation of adults and adolescents in 20 countries, individuals in all groups, except for young men, had a substantial risk of inadequate calcium intake (2). Furthermore, calcium absorption by post-menopausal women, a segment of the population with great risk for low calcium status, is only approximately 30% or less from one serving of dairy or 300g supplements. One strategy to enhance calcium status is to increase the absorption efficiency of this mineral.

Historically, calcium absorption enhancement strategies have primarily focused on identifying the most soluble form of calcium (3). Recently, efforts in several fields, including nutrition, have begun to focus on leveraging microbial cell populations in and on the human body. Such cells account for an estimated 95% of all the cells in the body (4), and some postulate that over the course of millennia, humans have evolved to rely on microbial colonies as a kind of symbiote-dependent super organism. Indeed, human

health parameters modulated by microbes include brain function, cancer development, and nutrient absorption (5). Due to their ability to modulate health through gut bacteria, prebiotics, which are thought to aid the absorption of certain minerals, have become the focus of much calcium-related research.

2.1 What Are Prebiotics?

Prebiotics are dietary components, primarily carbohydrates, that are selectively fermented by beneficial microbes in the large intestine, resulting in positive health outcomes for the human host. These compounds come from a variety of sources, including plants, animals, and synthetic production.

Disaccharides, oligosaccharides, and polysaccharides, carbohydrate members of the prebiotic family vary in both chain length and bond character. Where digestible saccharides primarily contain α(1→4), α(1→6) bonds, and the occasional β(1→4) bond (in the case of lactose) between sugar molecules, prebiotics additionally contain an array of α(1→2), β(1→2), β(1→3), β(1→6), and other bonds that are resistant to human enzymes. It is possible that variation in chain length and bond type could be responsible for differences in prebiotic effectiveness.

The health gains of prebiotics are primarily a result of their consumption and fermentation by beneficial gut microbiota, which result in two distinct benefits: the feeding of good intestinal bacteria and the release of short-chain fatty acids. First, by providing targeted nutrition to good bacteria, such as bifidobacteria, prebiotics encourage these colonies to grow and become metabolically active, providing the human host several favorable outcomes. These include crowding out pathogenic species, relieving

symptoms of lactose intolerance, producing B-vitamins, controlling blood cholesterol levels, relieving gastrointestinal distress, and modulating nutrient absorption (4).

The second distinct benefit is the production and release of short-chain fatty acids (SCFAs) through fermentation. Short-chain fatty acids include acetate, butyrate, and propionate, and are associated with several health gains (6). As the preferred energy source of colonic mucosal cells, butyrate is important to colon health and function. When SCFAs are eaten as part of the diet the majority are absorbed by the small intestine; prebiotics, however, are not broken down in the small intestine, but are instead fermented into SCFAs in the colon. Eating foods that are subsequently fermented into SCFAs is one of the only ways to consistently provide these important nutrients to the large intestine. Thus, prebiotics not only serve as nutrients for gut microbiota, but also for the large intestine itself.

2.2 Calcium Absorption

The body absorbs calcium both actively and passively, potentially at multiple points through the digestive tract. Passive absorption occurs through paracellular diffusion along the length of the intestine. Active absorption occurs through calcium transporters primarily in the small intestine, though recent evidence suggests that the large intestine houses active transport also. Zhao et al. (7) showed through kinetic modeling that when rats are fed a small bolus dose of calcium, the calcium is absorbed monophasically. However, when rats are fed a large bolus dose of calcium they absorb it biphasically. This suggests two relatively efficient sites of calcium absorption, the first, and potentially the second, being saturable. The timing and biphasic nature of calcium

absorption when the dose is high is consistent with absorption in the small and large intestines. This suggests that either passive diffusion is more effective in the large intestine than in the small intestine, or that the large intestine is also capable of active calcium transport.

2.3 Prebiotic Mediated Calcium Absorption

Prebiotics have been linked with increased calcium absorption in the large intestine. For example, ovariectomized (OVX) rats have diminished calcium absorption and are used to model the postmenopausal condition. Mitamura et al. (8) found that a raffinose intervention restored calcium absorption in OVX rats. This benefit was not seen in rats that were cecocolonectomized, suggesting that extracting the cecum and colon removed the site of calcium absorption. Furthermore, prebiotics remain essentially unaffected throughout digestion until they arrive in the large intestine. The exact process by which prebiotics enhance calcium absorption is unclear. However, potential mechanisms that prebiotics may work through include changing the luminal environment of the large intestine, modifying the composition of the large intestine, or by mediating gut microbial population of the large intestine.

2.3.1 Changes in the Lumen Environment

One popular hypothesis is that the short-chain fatty acids generated from carbohydrate fermentation are the driving force behind increased calcium absorption. The theory is that as SCFA concentration increases, the luminal pH of the colon

decreases. This causes greater ionization and solubility of dietary calcium, leading to greater active transport and passive diffusion across the membrane of the large intestine.

While the argument is compelling, a good amount of evidence suggests that decreased luminal pH is not the only player, or even a dominant player, in prebiotic-mediated enhanced calcium absorption. Mineo et al. (9), using the Ussing chamber method to examine rat colon segments, found that treating the cells with SCFAs increased calcium absorption (Figures 4.1 and 4.2), while treating them with HCl did not, indicating that solely decreasing luminal pH does not drive calcium absorption in the large intestine.

Interestingly, Yonezawa et al. (10) found that treating cells from the breast cancer cell line MCF-7 with SCFAs butyrate, propionate, or acetate increased cellular concentrations of calcium. When G-protein GPR43 was silenced, the effect observed due to propionate was inhibited. Thus, it was hypothesized that SCFAs had a signaling effect on the cell, which encouraged calcium uptake.

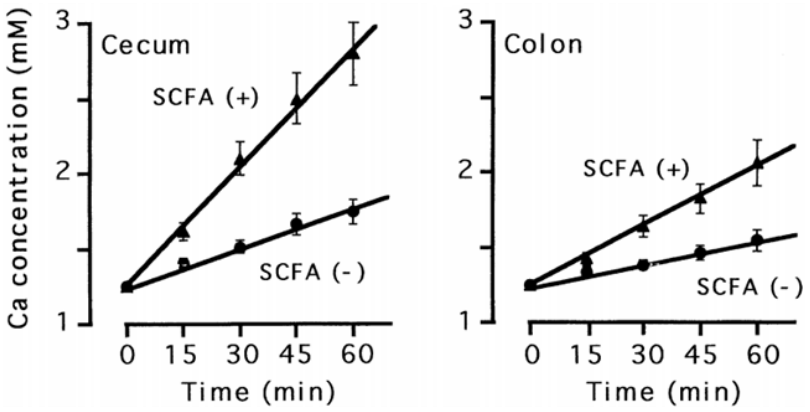

Figure 2.1 SCFAs Increase Calcium Absorption in the Intesine Ex Vivo

Short-chain fatty acids increase calcium absorption in rat cecal and colon segments ex vivo. In this experiment 130 mM SCFA mixture was added to the mucosal side of cecal and colon segments engaged in a using chamber. Calcium concentration was measured on the serosal side of the segments every 15 minutes. Segments treated with SCFA were more efficient at absorbing calcium over time than segments not treated with SCFA ($p<0.001$). Reprinted from Life Sciences, Volume 69, Mineo et al., Short-chain fatty acids enhance diffusional Ca transport in the epithelium of the rat cecum and colon, p 521, 2002, with permission from Elsevier.

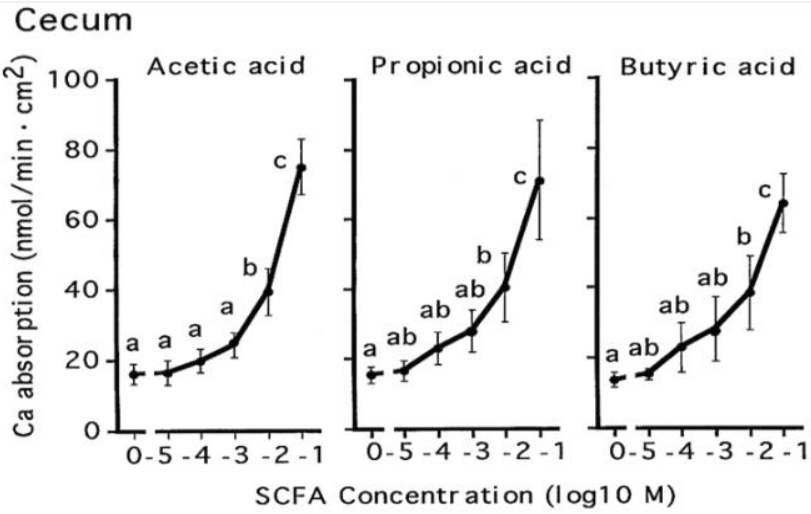

Figure 2.2 Individual SCFAs Increase Cecal Calcium Absorption Ex Vivo in a Dose-Dependent Manner

Various SCFAs increase calcium absorption in cecal segments ex vivo in a concentration-dependent manner. These values were measured 30 minutes after incubation. Values not sharing a common letter are significantly different ($p < 0.05$). Reprinted from Life Sciences, Volume 69, Mineo et al., Short-chain fatty acids enhance diffusional Ca transport in the epithelium of the rat cecum and colon, p 521, 2002, with permission from Elsevier.

Other mechanisms may play a role. For example, in a randomized trial examining the effect of several prebiotic fibers on calcium absorption in rats, Weaver et al. (11) showed that cecal content weight was much more correlated with enhanced calcium absorption than cecal SCFA concentration. Differences in cecal content weight between interventions are likely a function of the water-holding capacity of individual prebiotics. It has been proposed that prebiotics leading to increased cecal content weight may improve calcium absorption due to increased molecular mobility and greater mineral solubilization. Other postulated mechanisms involve changes to the large intestine.

2.3.2 Changes to the Large Intestine

Some investigators have proposed that prebiotics enhance calcium absorption by changing the large intestine itself. Fukushima et al. (12) found that caco-2 cells treated with SCFAs developed significantly more calbindin-D9k, a vitamin-D mediated calcium transporter, than untreated cells. Interestingly, neither vitamin D receptor, nor the cell proliferation marker CDX2 increased in caco-2 cells treated with SCFAs. Similarly, Nzeusseu et al. (13) showed that in rats, inulin and oligofructose consumption increased the expression of calbindin-D9k, though the effect seen by inulin was much stronger.

Prebiotics may also increase calcium absorption by increasing colon cell count and surface area. Nzeusseu et al. (13) saw an increase in cecal wall weight in rats fed inulin or oligofructose. Perez-Conesa et al. (14) observed a similar effect when they provided an intervention to four-week-old Sprague-Dawley rats consisting of diets containing 1.2%, 5%, and 10% GOS for 30 days. At the end of the trial, they analyzed cecal and colon cell density and crypt depth, as well as femur and tibia mineral contents.

They found that under all interventions, the proximal colon experienced a significant increase in crypt depth and cell density compared to the control. Similarly, in the distal colon all prebiotic interventions significantly increased crypt depth, while cell density was only significantly increased in rats consuming 1.2% and 5% diets. As expected, all GOS interventions significantly increased calcium content in both the femur and tibia compared to control groups, implying a connection between increased colon cell count and surface area, and calcium uptake. Interestingly, researchers saw similar results when feeding the rats with probiotics—live exogenous beneficial microbes. Perhaps calcium absorption is increased when prebiotics are consumed because of changes in gut microbial populations, rather than changes to the human cells of the large intestine.

2.3.3 Changes to the Gut Microbial Population

Changing gut microbial populations may also affect calcium absorption. These changes may occur through consuming prebiotics or probiotics. Few studies have been performed to determine the effect of consuming probiotics, live exogenous beneficial microbes, on mineral absorption. Narva et al. (15) showed that post-menopausal women who consumed milk fermented by *Lactobacillus helveticus* had increased calcium absorption above those who consumed milk fermented by other lactic acid bacteria. Kruger et al. (16) showed that true calcium absorption in male rats consuming feed containing *Lactobacillus rhamnosus* for 3 weeks was significantly increased over those consuming a control diet. These rats also experienced a positive, non-significant increase in fractional calcium retention. Importantly, in this study, the intervention group did consume more total calcium than the control group. Kruger et al. (16) also showed that

the bones of OVX rats that consumed *Lactobacillus rhamnosus* were more like the bones of sham-operated rats than the bones of other OVX rats that did not consume the probiotic. Perez-Conesa et al. (17) showed that consuming the probiotics *Bifidobacterium bifidum* and *Bifidobacterium longum* either alone, or in conjunction with prebiotics, increased apparent calcium absorption. In this study, the increase in calcium absorption was also correlated with an increase in crypt depth and cell density in the proximal and distal colon. It is unclear how probiotics increase calcium absorption, but it may occur due to a change in luminal environment, large intestine structure, or through some other metabolic activity unaccounted for by specific bacterial species.

Like probiotics, prebiotics can change the makeup of gut microbial populations. Weaver et al. (18) showed that intestinal microbial populations in rats fed 2%, 4%, 6%, or 8% GOS diets were similar within a dose of GOS, but different between GOS levels. Similarly, Clavijo-Gutierrez et al. (19) found that gut microbial populations of adolescent girls became similar when they moved from a self-selected diet to a controlled diet. When soluble corn fiber (SCF), a prebiotic, was added to the controlled diet, another microbial population shift occurred. Gut microbiota between individuals on the SCF-containing diet were similar; however, gut microbiota between individuals on SCF-containing and non-SCF diets varied. Dietary changes may transform the composition of gut microbial populations by giving an advantage to those organisms that can best utilize new nutrients. This selection for specific microbes may lead to increased calcium absorption.

2.4 Types of Prebiotics

2.4.1 Disaccharides

As one category of the prebiotic family, disaccharides are carbohydrates consisting of two sugar units. Of these prebiotics, perhaps the most studied is lactulose (4-*O*-β-d-galactopyranosyl-d-fructose). This man-made prebiotic is created during heat-sterilization of milk, and was one of the first non-digestible carbohydrates to be linked with enhancing gut microbial populations (20).

Seki et al. (20) investigated the effects of 2g lactulose/day and 4g lactulose/day on 24 young adult men in a double-blind, placebo-controlled, dual-stable isotope study. They found that subjects who consumed 4g lactulose in a test meal experienced significantly higher calcium absorption and urinary excretion than the control group. They did not, however, experience a change in bone resorption markers. It is unclear if calcium balance improved, or if the additionally absorbed calcium was merely excreted.

2.4.2 Oligosaccharides

While experts sometimes disagree on the exact number of polymerized sugar units required to make an oligosaccharide, definitions usually range from 3 to 10 sugar units. Many oligosaccharides exist, but the most common classes of prebiotic oligosaccharides are fructooligosaccharides and galactooligosaccharides.

Fructooligosaccharides (FOS) include a variety of compounds of varying in chain length, primarily composed of fructose molecules connected by β(1→2) linkages and

capped with a terminal glucose. While some FOS are synthetically made, many are found in such plants as bananas, Jerusalem artichoke, chicory, asparagus, and onions.

Fructooligosaccharides (Figure 4.3) have frequently been tied to enhanced calcium absorption. For instance, Morohashi et al. (21) conducted a 3-day metabolic study on rats that revealed that calcium absorption, excretion, and balance were all increased when the rats were fed a diet with 5% FOS. Similarly, van den Heuvel et al. (22) found that adolescent males who consumed 15g FOS/day for 9 days experienced significantly higher fractional calcium absorption. However, Martin et al. (2010) saw no effect on adolescent girls who consumed 9g per day of a FOS/Inulin blend for 3 weeks.

Another common prebiotic type, galactooligosaccharides (GOS) are found as a mixture of oligosaccharides of varying length. Similar to FOS, GOS is comprised of galactose units bound by various β(1→2), β(1→3), β(1→4), and β(1→6) linkages with a terminal glucose (Figure 4.3). Galactooligosaccharides are found naturally in milk and can be created synthetically by the enzymatic action of glycosidic hydrolases on lactose.

Like fructoligosaccharides, galactooligosaccharides (GOS) have been shown to increase calcium absorption. van den Heuvel et al. (23) saw that postmenopausal women who consumed 20g GOS per day experienced increased calcium absorption. The intervention did not affect calcium excretion. Because the storage site for calcium is bone, it is inferred that this excess calcium was being used to create new bone. In another study, dos Santos et al. (24) showed that both gastrectomized and sham-operated rats experienced increased calcium absorption when they were fed 5% GOS diets. Similarly, Weaver et al. (18) showed that rats consuming diets enriched with 2%, 4%, 6%, and 8% GOS had a net increase in calcium balance in a dose-dependent manner. Whisner et al.

(25) also documented a positive effect from GOS intervention. In this study, adolescent girls were fed 0g GOS, 2.5g GOS and 5g GOS twice daily for 3 weeks. Both interventions, low and high dose, were shown to significantly increase calcium absorption, though the benefit was not dose-dependent.

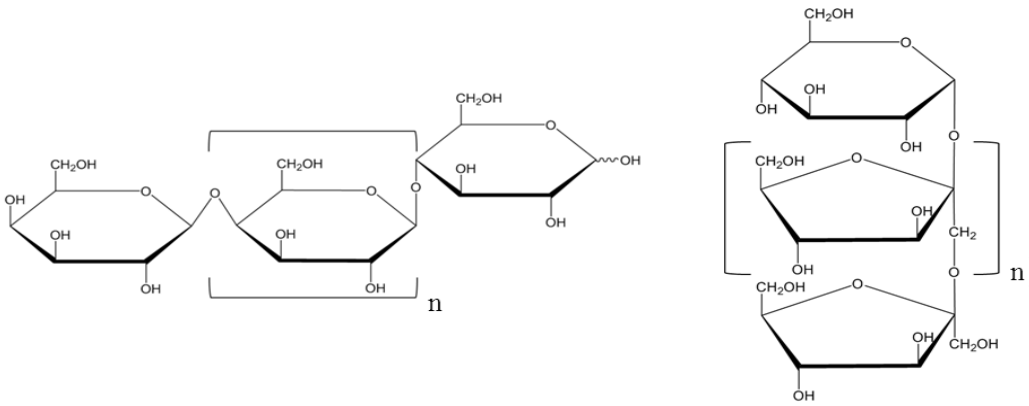

Figure 2.3 General Chemical Structures of GOS and FOS.

2.4.3 Polysaccharides

Polysaccharides are composed of many sugar subunits and include cellulose, hemicellulose, gums, polyglycans, and polyfructans. Some, like cellulose and hemicellulose, are not well fermented and thus cannot be prebiotics. Gums, polyglycans, and polyfructans, on the otherhand, are well fermented which may mean they have prebiotic potential. Thus, calcium absorption research on these compounds may be warranted. For example, Kawase et al. (26) showed that, under ex vivo conditions, intestinal segments from rats fed a diet with 7.5% gum arabic for 10 days had more efficient calcium absorption than intestinal segments from control rats. The most common polyfructan used as a prebiotic is inulin, the parent compound to FOS. Coudray

et al. (27) found that rats on a diet supplemented with 10% inulin had increased calcium absorption over animals on non-supplemented diets.

2.4.4 Synbiotics

Occasionally, prebiotics are eaten in tandem with probiotics, an external source of beneficial bacteria. When these two products, prebiotics and probiotics, are consumed at the same time it is deemed a synbiotic. Examples of synbiotics products include yogurt and kefir. Synbiotics, like prebiotics and probiotics, have been shown to increase calcium absorption. For instance, Perez-Conesa et al. (17) fed weanling rats a diet containing probiotics but no GOS, as well as diets containing 1.2%, 5%, or 10% GOS with and without probiotics. At the end of the study, all interventions showed significantly higher apparent calcium retention than the control group. The synbiotic group did not appear to confer greater benefit to calcium retention than just prebiotics or probiotics alone.

2.4.5 Mixed Prebiotics

It has been hypothesized that the various types of prebiotics are effective at different locations in the large intestine. Some prebiotics are small and ferment quickly. Others take longer to ferment because they are long, highly branched, and include many kinds of bonds. It is possible that rapidly fermenting prebiotics may primarily affect the proximal large intestine, while slowly fermenting prebiotics may primarily affect the transverse and distal colon. However, it is still unknown if the fermentation profile of prebiotics is an important factor in their mineral-absorption enhancing qualities. Therefore, some researchers have studied combinations of prebiotics to gain insight about

fermentation profile and its effect on maximization of calcium absorption. Holloway et al. (28) performed a randomized, double-blind, crossover study using a FOS/Inulin mixture intervention with postmenopausal women. Participants undergoing the intervention consumed 10g of the prebiotic mixture each day for 6 weeks. The study showed a significant increase in calcium absorption after the intervention. This effect was primarily seen in subjects who had low lower-spine bone mineral density.

In a balanced, randomized, crossover design, Griffin et al. (29) fed girls either 8g FOS/day or 8g FOS+inulin mixture/day for 3 weeks. In the mixed prebiotic intervention, calcium absorption was significantly higher than placebo, whereas the intervention with only 8g FOS did not increase calcium absorption. Importantly, there was no inulin-only intervention, and so it is not possible to separate an effect from inulin itself compared to the mixed prebiotic.

Interestingly, Hicks et al. (30) found no difference in absorption between infants consuming formula enriched a with 4g/L GOS+Polydextrin mixture and those consuming non-enriched forumla. Abrams et al. (31) showed that adolescents who received an 8g/d dose of short- and long-chain inulin type fructans experienced an 8% increase in calcium absorption after 8 weeks, and a 6% increase in calcium absorption after 1 year, over the control group.

2.5 Factors that Influence Prebiotic Mediated Calcium Absorption

As described above many, but not all, studies suggest prebiotics can have a promoting effect on calcium absorption. The following sections review distinct factors that may influence the efficacy of prebiotics on calcium absorption.

2.5.1 Prebiotics and Age

One of the many variables that may impact calcium absorption mediated by prebiotics is age. Young people are generally more active than older individuals, inducing more load bearing events on their skeletons, which encourages bone growth. Hormone levels between older and younger individuals also differs drastically. The most critical age stages for bone health are post-menopause, when changes in hormone levels leads to increased bone loss, and adolescence, when the majority of bone is accrued.

Several studies indicate that loss of calcium absorption due to the post-menopausal condition could be alleviated by prebiotics. In one crossover study (23), a daily dose of 20g GOS for 9 days increased true calcium absorption in post-menopausal women compared to a placebo. Similarly, another study (28) showed that fractional calcium absorption in post-menopausal women increased significantly when they consumed 5g FOS/day for 6 weeks. Interestingly, one study (32) showed no effect of 10g FOS/day for 5 weeks on women 2-6 years post menopause. However, they did see a positive trend in women 6 or more years post menopause. Unfortunately, only six participants fell into this age range. In OVX rats, a model intended to mimic the post-menopausal condition, loss of calcium absorption was restored when the rats were fed a diet with 3% raffinose (8).

Data for adolescents are less consistent. Perhaps the best example, one study (29) showed that girls who consumed 8g FOS+inulin mixture/day for 3 weeks experienced significantly higher calcium absorption. Yet a different study (33) showed that adolescent girls who consumed 9g of the same FOS+inulin mixture/day for 3 weeks did not have

increased calcium absorption. The primary difference between these two studies was that the effective intervention was provided with orange juice, while the ineffective intervention was provided in ready-to-eat cereal. Additionally, subjects in the study where no difference was found had high fractional calcium absorption before the study began. Prebiotic supplementation may not have been able to increase it further.

In agreement with these studies, Coudray et al. (34) showed that aged rats, animals suffering from diminished calcium absorption, experienced a greater increase in calcium absorption from inulin intervention than young rats did. However, this study used male rats, and was not comparing OVX rats to young female rats.

2.5.2 Intervention Length and Calcium Status

Both intervention length and calcium status of subjects are thought to be important factors in how well prebiotics enhance calcium absorption. In one study (27), rats were fed a 10% inulin diet for 13 days. Animals on either low- or high-calcium diets experienced increased calcium absorption over animals on normal calcium diets. When the trial was extended to 37 days, the animals on the low calcium diet retained the enhanced calcium absorption, while the animals on a high calcium diet did not. This may be because vitamin D mediated calcium absorption is downregulated when calcium absorption is high. Therefore, because the inulin enhanced calcium absorption, active calcium absorption may have been hormonally downregulated over time in the rats on high-calcium diets, leading to the loss of the benefit seen in the short-term. Supporting this theory, several long-term studies using subjects with adequate calcium intake have

shown significant increases in calcium absorption when they were fed prebiotics (18, 24, 28, 31).

2.5.3 Dose Amount

Intervention dose amount likely determines the strength of the prebiotic response. In one study (17), rat weanlings that consumed a 10% GOS diet absorbed more calcium than those on 1.2% or 5% GOS diets. Similarly, a separate study showed that growing rats consuming 2%, 4%, 6%, and 8% GOS diets experienced increased calcium absorption in a dose-dependent manner. However, Whisner et al. (25) showed that adolescent girls with adequate calcium absorption had increased calcium absorption when consuming either 2.5g or 5g GOS/day. Curiously, in this case, 2.5g GOS/day was more effective than 5g GOS/day.

2.5.4 Genetics and Pre-Existing Conditions

Subject genetics may play a role in the effectiveness of prebiotic intervention. Many studies have been performed in rat populations, which while practical are not the ideal representation of human physiology. For instance, the cecum plays a much more substantial role in fermentation in rats than in humans. Additionally, variation across individuals in a species can greatly change calcium absorption. Replogle et al. (35) showed that, in rats, hormonal response to calcium status varied significantly based on the rat phenotype. Weaver et al. (36) has observed similar effects in human populations with different genetic histories. A similar effect could be seen with prebiotic interventions. In fact, some studies have used the terms *responders* and *non-responders*

to differentiate individuals with different sensitivity to prebiotics. One study showed that postmenopausal women with lower lumbar BMD were more likely to respond to prebiotic intervention (28). Another (32) found that women more than 6 years post menopause were more likely to respond than those closer to menopause.

2.6 Prebiotics on Bone

While increasing calcium absorption is a first step towards improved health outcomes, the primary end outcome of concern is bone strength. This is usually quantified by a proxy measure, such as bone mineral density, or trabecular and cortical thickness. There is evidence that suggests consuming prebiotics improves these parameters. Abrams et al. (31) showed that after 1 year of consuming a FOS+inulin mixture, adolescents had significantly greater whole-body bone mineral content and bone mineral density than the control group. Similarly, Roberfroid et al. (37) found that a diet consisting of 0.5 and 1.0% inulin increased whole-body bone mineral density and whole-body bone mineral content in rats. Another study (13) showed that, in rats, a 5% GOS intervention for 3 months significantly increased trabecular bone area over control. Additionally, a 5% inulin intervention for 3 months increased trabecular bone area more than the GOS intervention. It also increased trabecular bone area in the lower lumbar area, which the GOS intervention did not.

The ultimate test of bone quality is bone strength. Weaver et al. (18) showed that young growing rats had greater volumetric bone mineral density and peak breaking strength when fed a diet with 2%, 4%, 6%, and 8% GOS for 8 weeks. The same research group (11) compared the effect of eight different prebiotic fibers on rat calcium

absorption, bone quality measurements, and peak breaking force. These fibers included two resistant starches, soluble corn fiber (SCF), soluble fiber dextrin (SFD), pullulan, polydextrose, inulin, and a FOS+inulin mixture. Except SCF and pullulan, the prebiotics were effective at increasing calcium content of the rats' bones. While the only fiber to significantly increase femur uptake of calcium was the FOS+inulin mixture, only SFD and SCF significantly increased femoral peak breaking strength. The parameters that both SCF and SFD significantly increased, and the inulin/FOS mixture did not, were total femoral BMD and cortical thickness. These data indicate that some prebiotics are more effective at enhancing bone formation, even when others may increase calcium uptake more efficiently. Scholz-Ahrens et al. (38) found that in OVX rats, tibial bone was spared with an intervention of high calcium, FOS, or a combination of the two. Interestingly, the spared bone had a different trabecular architecture depending on whether the intervention included high calcium, FOS, or both. This indicates that some of the benefit of prebiotics to bone structure and strength may be in addition to the benefit of greater calcium absorption.

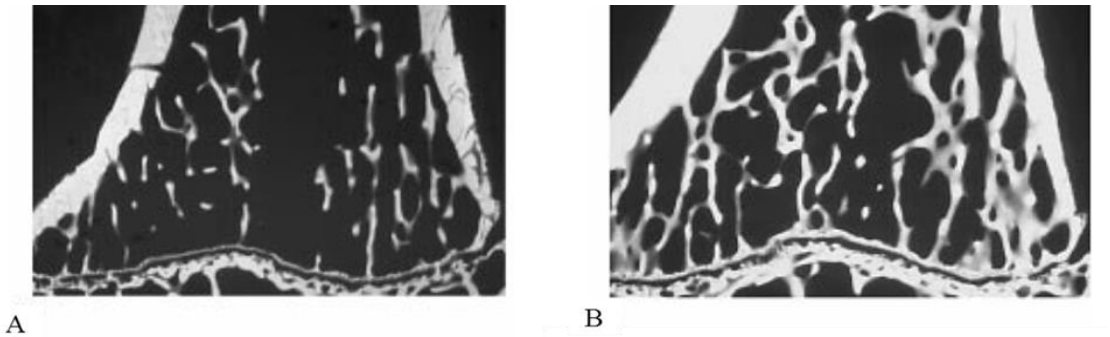

Figure 2.4: FOS/Inulin Supplementation Prevents Boneloss in Rats.

Prebiotics help maintain bone quality. Image (A) is a representative contact microradiograph of the proximal tibia in control ovariectomized rats. Image (B) is a representative contact radiograph of the proximal tibia in ovariectomized rats that were fed a 5% FOS/Inulin mixture. These images show that prebiotic intervention prevented both cortical and trabecular bone loss. Scholz-Ahrens et al., Effect of oligofructose or dietary calcium on repeated calcium and phosporus balances, bone mineralization and trabecular structure in ovariectomized rats. British Journal of Nutrition, 2002, Oct 8(4):368, reproduced with permission.

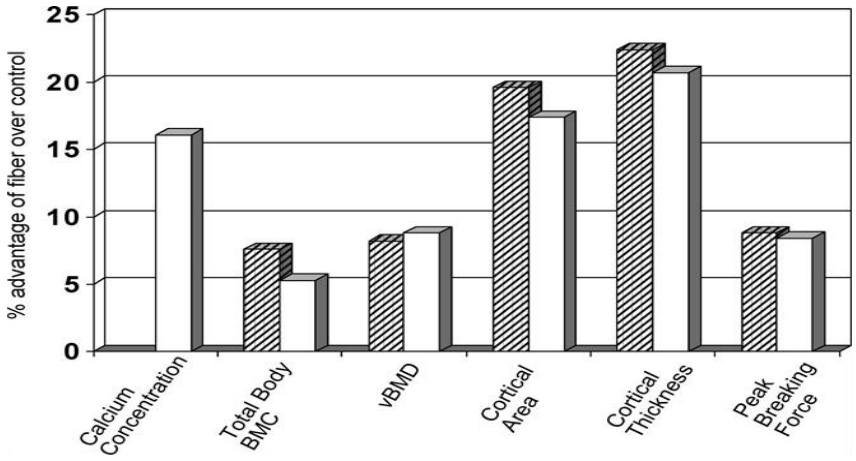

Figure 2.5: SCF and SFD Improve Bone Strength in Rats.

Soluble corn fiber (SCF; slashed bars) and soluble fiber dextrin (SFD; open bars) fed rats had improved femoral bone quality over control fed rats. Reprinted with Permission from Weaver et al. Novel Fibers Increase Bone Calcium Content and Strength beyond Efficiency of Large Intestine Fermentation. J Agr Food Chem. 2010 Aug 25;58(16):8952-7.

2.7 Conclusion and Future Work

The gastrointestinal tract hosts many trillions of microbes, and through prebiotics humans might be able to unlock some of their health-promoting potential. The majority of reports evaluating the effect of prebiotics on calcium absorption indicate that various types of prebiotics enhance calcium absorption and bone deposition. Further research is necessary to determine how calcium absorption is enhanced, and whether bone strength is truly enhanced beyond the effect of increased calcium absorption.

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3. SOLUBLE CORN FIBER INCREASES CALCIUM RETENTION IN POSTMENOPAUSAL WOMEN IN A DOSE-DEPENDENT MANNER

3.1 Abstract

Soluble corn fiber (SCF) significantly improves calcium absorption in adolescents and bone strength and architecture in rodent models. In this study, we aimed to see if PROMITOR® SCF 85, which provides 85% dietary fiber, would also benefit postmenopausal women. We used our novel technology of determining bone calcium retention—by following urinary appearance of ^{41}Ca from prelabeled bone—to rapidly and sensitively evaluate the effectiveness of SCF to reduce bone loss. A randomized-order, crossover, double-blinded trial was performed in 14 healthy postmenopausal women to compare 0 g/d, 10 g/d, and 20 g/d fiber from SCF for 50 days. A dose-response effect was demonstrated with 10 g/d SCF and 20 g/d SCF improving bone retention by 4.8% ($P = 0.013$) and 7% ($P = 0.007$) respectively. Bone turnover biomarkers N-terminal telopeptide and osteocalcin were not influenced by the interventions; however, a significant increase in the bone-formation marker bone-specific alkaline phosphatase was detected between 0 g/d SCF and 20 g/d SCF levels (8%, $P = 0.035$). Daily PROMITOR®SCF consumption significantly increased bone calcium retention in postmenopausal women, improving bone balance by an estimated 50 mg/d.

3.2 Introduction

Osteoporosis is an age-related condition resulting in demineralization of the skeleton which is influenced by genetics as well as various environmental factors. Diet and nutrition, including calcium intake and absorption, are critical for maintaining bone mass and strength at all stages of life, thus reducing the risk for osteoporosis later in life. When women experience menopause and estrogen production ceases, bone resorption far exceeds mineralization, resulting in potential loss of bone mass and strength and increased risk of bone fracture. Additionally, postmenopausal women are at substantial risk of inadequate calcium intake (1). Yet due to recent concern over a link between calcium supplementation and risk of cardiovascular disease and myocardial infarction (2), calcium supplement use has dramatically declined. Therefore, it is particularly important at this stage of life to incorporate alternative dietary routines that will maximize bone health.

Previous studies in our lab have shown that fermentable carbohydrates, specifically soluble corn fiber (SCF), increase calcium absorption in adolescent boys and girls, and improve bone properties in a rodent model. Bone mineral content, bone mineral density, and peak breaking force of the femur were all increased in 4-week-old male rats (3). In teen boys and girls, consuming a chronic dose of 12 g/d SCF over a 3-week period as part of a controlled diet resulted in a 12% increase in calcium absorption compared to a placebo (4). Another study, with teen girls only, showed that interventions of either 10 g/d or 20 g/d SCF over a 4-week period increased calcium absorption by 13% compared

to a placebo (5). However, the effect of SCF on bone health in postmenopausal women has not been explored.

One randomized, double-blinded, crossover study (6) used a single oral calcium isotope (^{44}Ca) to assess the effect of 10 g/d short-chain fructoologisaccharides, a fermentable carbohydrate, on calcium absorption in postmenopausal women. While no effect was detected overall, there was a trend ($P < 0.1$) for increased calcium absorption in women >6 y postmenopausal, despite the small subsample size (6 individuals) and the relatively imprecise method used. Further investigation on bone-protective qualities of fermentable carbohydrates with a larger sample and a very precise method is warranted.

Our laboratory has developed a method to screen efficacy of interventions to improve bone calcium retention using ^{41}Ca, a long-lived radio isotope ($t_{1/2} = \sim 10^5$ y), to label the skeleton (7). Due to the great measurement sensitivity of accelerator mass spectrometry (AMS), urinary ^{41}Ca can be followed in an individual for their lifetime after only a single dose. Changes in urinary ^{41}Ca can be used to estimate the effects of diet or other interventions on bone calcium retention rapidly and sensitively. This study was designed to measure the dose-response effect of chronic SCF intake over a 50-day period on bone resorption in healthy postmenopausal women using the ^{41}Ca methodology.

3.3 Subjects and Methods

3.3.1 Subjects

Fourteen healthy postmenopausal women were enrolled in the study. Each signed a written consent form and completed a screening questionnaire, which included a brief medical history, diet assessment, physical-activity evaluation, and 4-day diet record. All

participants were >6 years post-menopausal due to natural menopause or total hysterectomy and had a blood sample screened for general blood chemistries, verifying normal physiological function. Exclusion criteria included taking medication known to affect calcium metabolism, using illegal drugs, having kidney disease or other diseases known to affect calcium metabolism, or having broken a bone within six months prior to the start date of the study. Participants discontinued vitamin and mineral supplementation, except for a multivitamin (Spectravite® Advanced Formula) provided by the researchers for daily consumption. This multivitamin provided 200 mg/d calcium and 400 IU/d vitamin D.

3.3.2 Study Design

This study was a randomized, placebo-controlled, double-blind crossover trial with three intervention periods beginning after a 100-day equilibration period and a 50-day baseline period. The equilibration period was initiated for individual subjects with the IV administration of 50 nCi ^{41}Ca, a long-lived radio isotope. This isotope was allowed to deposit into bone and to equilibrate with whole-body calcium for at least 100 days. During this equilibration period, subjects collected 24-h urine monthly to monitor the decrease in ^{41}Ca urinary excretion. Two of the subjects were dosed with ^{41}Ca for a previous study and bypassed the equilibration phase. Due to the long half-life of this isotope, urinary ^{41}Ca values were easily measureable in these participants.

Subsequent to the equilibration phase, the baseline level of urinary ^{41}Ca was evaluated over a 50-day baseline period with 24-h urine collections every 10 days. After the baseline phase, participants were randomized to receive each of the three doses of

soluble corn fiber (0, 10, 20 g/d) in one of six sequences. During intervention phases, participants consumed two products daily, including one fruit-flavored drink and one muffin, each containing half of the daily SCF dose. The muffins and drinks containing SCF (Promitor® Soluble Corn Fiber 85) were provided by Tate & Lyle.

3.3.3 Anthropometric Parameters and Bone Measurements

Anthropometric parameters were measured throughout the study. During the baseline clinical visit, waist circumference and hip width and depth were measured. Bone mineral content and bone mineral density were also measured during that first visit by dual-energy X-ray absorptiometry (DXA; GE Lunar iDXA). During morning-time follow-up clinical visits at the beginning of intervention and washout phases, standing height was measured using a wall-mounted stadiometer (Measurement Concepts; QuickMedical®), and fasting weight was measured using a digital scale (Mini Platform Stand-on Scale®; Scale-Trionix).

3.3.4 Measurement of Bone Biomarkers

At the beginning and end of the baseline period and the end of each intervention and washout phase, fasting blood and urine were collected to measure biochemical markers of bone metabolism. The collected biomarkers included bone-specific alkaline phosphatase (BAP), osteocalcin (OC), and N-terminal telopeptide (NTx). Serum concentration of osteocalcin, a marker of bone turnover, was measured by ELISA (Quidel Corporation). The urinary concentration of bone resorption marker NTx was measured by ELISA (Alere). Serum concentrations of bone formation marker BAP were determined

by ELISA (Quidel Corporation). Creatinine was also measured as a correction value for NTx and was measured by an enzymatic colorimetric assay (COBAS Integra, Roche Diagnostics Ltd.). Interassay percent coefficient of variation was calculated at 4.8, 9.8, and 2.5% for BAP, OC, and NTx, respectively. Intra-assay percent coefficient of variation was calculated at 5.1, 7.6, and 8.4% for BAP, OC, and NTx, respectively.

3.3.5 Compliance and Symptoms

Intervention compliance was measured through participant self-report and product recall. At the end of each intervention period, participants were asked to return any remaining intervention muffins and drinks to the researchers. Participants also kept a log of when they consumed the products.

Abnormal gastrointestinal symptoms were monitored every ten days during intervention phases. Participants were asked to rate gastrointestinal symptoms on scale of 0 (no symptoms) to 5 (severe symptoms). The symptoms on the report included abdominal pain, bloating, flatulence, diarrhea, and stomach noises. Participants also rated their general health.

3.3.6 Measurement and Statistical Analysis of Bone Calcium Retention

After bone deposition and equilibration, ^{41}Ca is released from and reincorporated into bone in proportion to total in-bone and circulating calcium during resorption and bone formation, respectively. Thus, changes in urinary ^{41}Ca:Ca reflects net bone retention. Specifically, a decrease in urinary ^{41}Ca:Ca would indicate improved bone calcium retention. Urinary ^{41}Ca:Ca during all nonintervention (washout and baseline)

periods was used to create a projected rate of net bone calcium loss. Urinary ^{41}Ca:Ca during intervention periods was compared to the projected rate of calcium loss, and the difference was averaged per individual and intervention. These average differences were used to determine statistics. ^{41}Ca was measured by accelerator mass spectrometry (AMS) as previously described (8).

3.3.7 Statistical Analysis of Biochemical Markers and Symptom Severity

Statistical analyses were performed using SAS (version 9.3, SAS Institute, Cary, NC), and statistical significance was set at $p \leq 0.05$. The effects of SCF dose on biochemical markers of bone turnover were examined using a mixed model general linear model. The model included the covariables treatment period and sequence. Comparisons between any dosage level may be modeled, since linear effects are possible.

To further assess significant differences among biochemical markers, two statistical models were constructed using biomarker response variable BAP and an explanatory variable, ID. Model 1 (General Linear Model) used an additional explanatory variable, DOSE, which represented a linear term in the model. Model 2 (ANOVA Model) used an additional explanatory variable, TRT, which represented a categorical term in the model. Tukey multiple comparison procedure was also performed.

Symptom severity between dose levels was compared for each symptom type using the nonparametric Kruskal-Wallis test.

3.4 Results

Of the 18 women that were screened, two were withdrawn from the study due to medical reasons, and two dropped out for personal reasons. A total of 14 women participated in the study. One of those women chose to drop out before entering an intervention period for convenience, and another dropped out after two intervention periods because of diet changes (Figure 5.1). Compliance and gastrointestinal symptoms were calculated based on a sample size of 13 participants, while bone biomarker levels and increased bone retention were calculated based on a sample size of 12 participants. While there were no exclusion criteria regarding race, all participants were white. Participant baseline characteristics are described in Table 5.1.

Four of the women recruited and two of the women who completed the study had undergone hysterectomies, and all were past the stage of rapid menopausal bone loss. While these postmenopausal women were comparable to the white female US population in regards to BMI, total body BMC, total body BMD, intake of calcium, phosphorus, and sodium (9, 10), they had greater femoral neck and lumbar spine BMD (9). They also consumed more fiber but tended to consume lower amounts of potassium and magnesium (Table 5.2).

3.4.1 Compliance

Overall compliance was 92.9 (±1.1%), and there was no significant difference in compliance among phases.

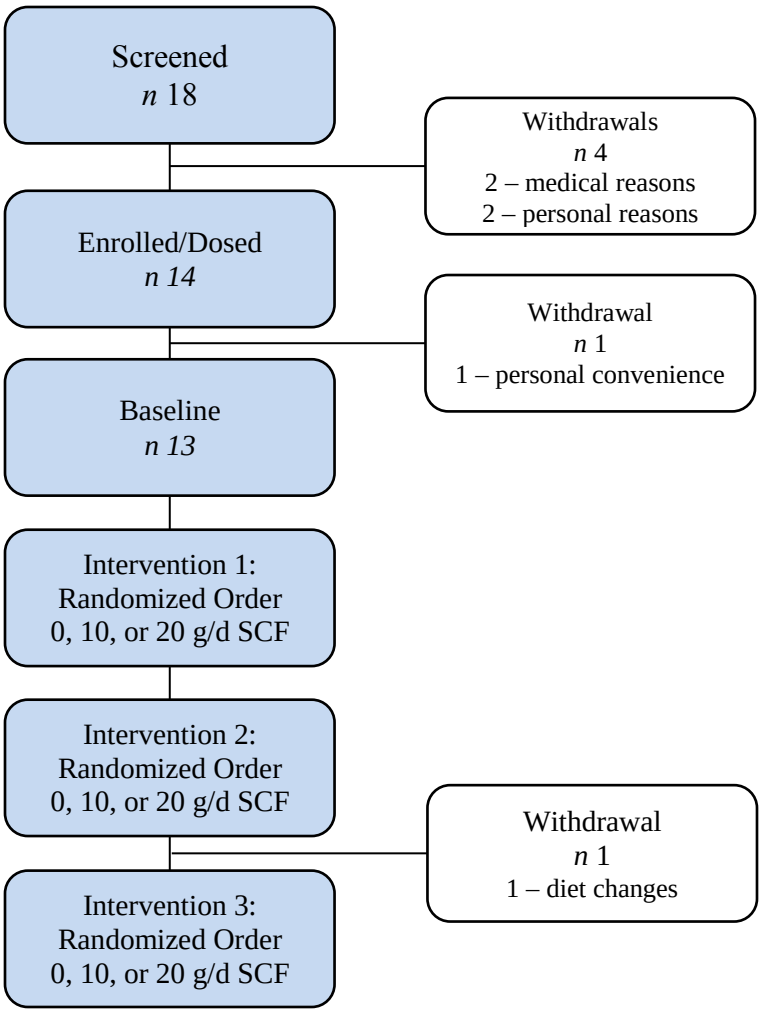

Figure 3.1: Study Design

Table 3.1: Participant Baseline Characteristics

	Mean ± SD
Age (y)	59.8 ± 5.0
Years Postmenopause	12.9 ± 6.8
Pre-study calcium intake (mg)*	862 ± 371
BMI (kg/m2)	28.1 ± 5.2
Total Body BMC (g)	2330 ± 342
Total Body BMD (g/cm2)	1.11 ± 0.10
Total Femoral Neck BMD (g/cm2)*	0.89 ± 0.08
Lumbar Spine BMD (L1-L4) (g/cm2)*	1.12 ± 0.17
Fat Mass (%)	43.1 ± 7.4
Lean Mass (%)	53.8 ± 7.1

(n = 13; * n = 12)
BMD: Bone Mineral Density. BMC: Bone Mineral Content.

Table 3.2 Dietary Intake of Postmenopausal Women throughout the Study.

	Baseline	Washout	0 g/d SCF	10 g/d SCF	20 g/d SCF
Calcium (mg)	964.6 ± 214.9	973.2 ± 320.3	922.0 ± 380.3	731.6 ± 180.7	899.9 ± 240.2
Phosphorus (mg)	1136.0 ± 251.1	1064.7 ±356.7	1006.3 ± 356.0	870.8 ± 259.0	1055.3 ± 355.7
Sodium (mg)	3023.8 ± 546.7	3110.5 ± 686.6	2989.2 ± 939.4	2803.2 ± 561.2	3174.5 ± 477.0
Potassium (mg)	2372.0 ± 721.7	2086.1 ± 630.3	1838.8 ± 726.6	1740.5 ± 514.2	2145.7 ± 645.8
Magnesium (mg)	250.7 ± 69.1	235.4 ± 97.4	214.3 ± 92.4	199.5 ± 81.8	242.5 ± 75.7
Total Fiber (g) (Excluding SCF)	17.8 ± 6.1	18.2 ± 6.6	14.2 ± 6.5	13.7 ± 6.0	15.2 ± 6.8
Soluble Fiber (g) (Excluding SCF)	5.5 ± 1.8	5.6 ± 2.0	4.4 ± 2.2	4.6 ± 1.7	4.7 ± 1.8
Total Fiber (g) (Including SCF)	17.8 ± 6.1	18.2 ± 6.6	14.2 ± 6.5	22.3 ± 6.9	31.5 ± 8.9
Soluble Fiber (g) (Including SCF)	5.5 ± 1.8	5.6 ± 2.0	4.4 ± 2.2	13.2 ± 3.3	21.3 ± 6.3

Mean ± SD

3.4.2 Gastrointestinal Symptoms

Severity of gastrointestinal symptoms was low throughout the study. No significant difference was observed in the severity of gastrointestinal distress between SCF intake levels (0 g/d, 10 g/d, and 20 g/d) (Figure 4.2).

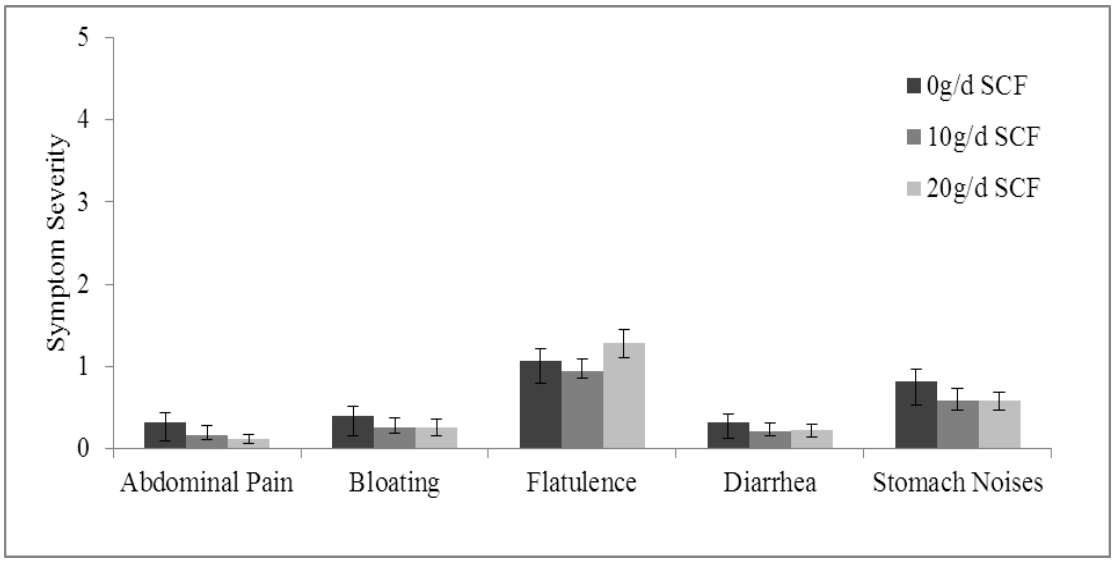

Figure 3.2: Severity of Gastrointestinal Symptoms by Intervention

3.4.3 Bone Calcium Retention

Table 5.3 represents the group mean difference between measured and predicted urinary ^{41}Ca:Ca for each intervention period. Increases in bone calcium retention due to SCF intervention were statistically significant in a dose-dependent manner.

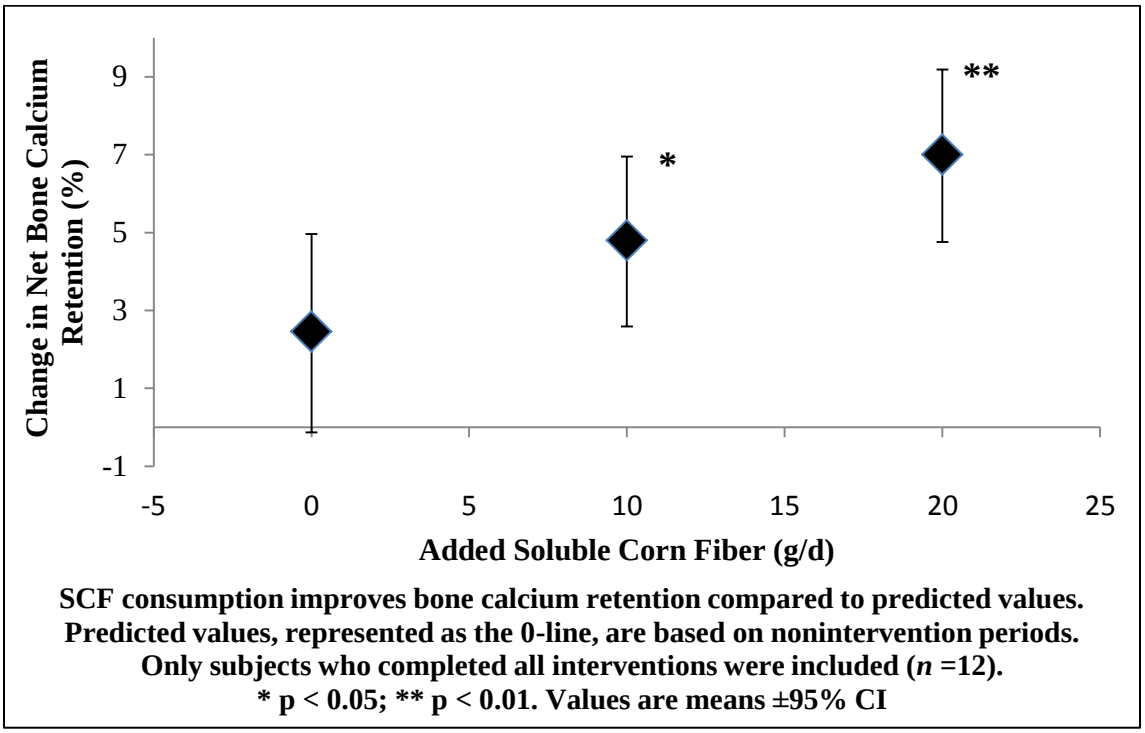

Figure 3.3: Soluble Corn Fiber Increases Net Bone Retention

3.4.4 Bone Biomarkers

Of the bone biomarkers assessed, neither OC nor NTx, both biomarkers of bone resorption, were affected by dietary SCF consumption (OC,p=0.730; NTx, p=0.570). However, a statistically significant effect on serum BAP concentration was observed between placebo and 20 g/d SCF intervention (p=0.034), though the 10 g/d SCF intervention was not significantly different from other levels.

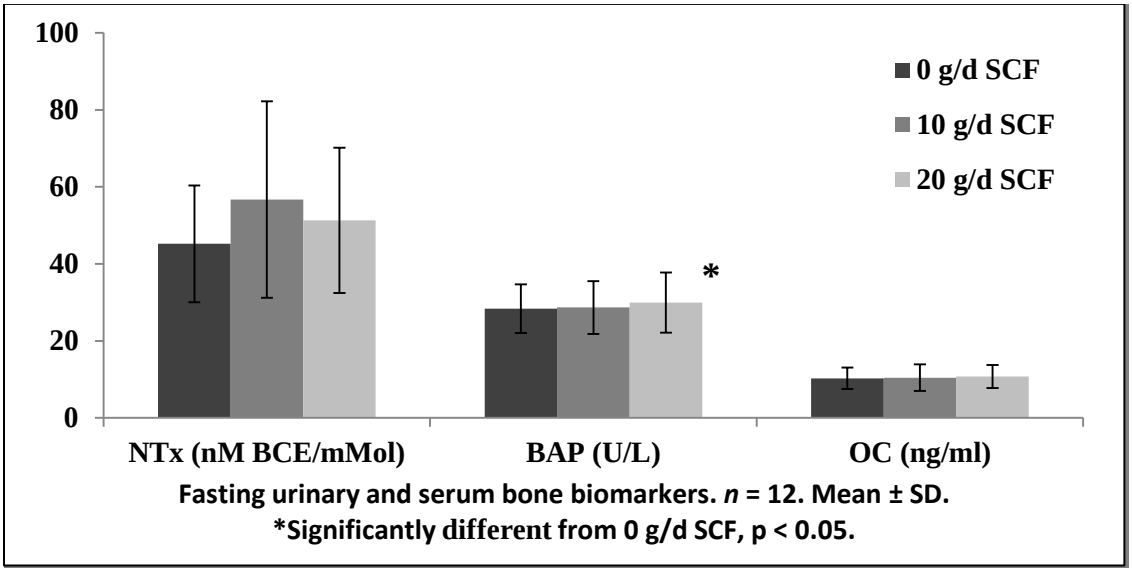

Figure 3.4: Bone Turnover Biomarkers by Intervention

3.5 Discussion

We demonstrated in this randomized order, placebo-controlled crossover trial that SCF significantly increased bone calcium retention in postmenopausal women in a dose-dependent manner. Several other studies suggest fermentable carbohydrates could be used as a dietary intervention to improve bone health in postmenopausal women. Short-term dietary intervention of 10 g/d oligofructose-enriched inulin (SYN1) (11), 20 g/d transgalactooligosaccharides (12), and 5 g/d and 10 g/d lactulose (13) significantly

increased calcium absorption in postmenopausal women. In contrast, Slevin et al. (14) found that 3.6 g/d short-chain fructooligosaccharides (scFOS) did not improve BMD in postmenopausal women over a 24-month period, as measured by DXA. Additionally, Tahiri et al. (6) found that short-term intervention of 10 g/d scFOS did not improve calcium absorption in postmenopausal women. There are several factors that may account for the differences observed. First, the low dose of 3.6 g/d scFOS may not have been large enough to create a measurable effect by DXA (14). However, Tahiri et al. (6) used a much larger dose and also found a negative effect, suggesting that scFOS may not be as effective as other fibers. Second, different fermentable carbohydrates may have distinct fermentation rates and patterns (15), which may play a role in their effectiveness in enhancing calcium absorption. Third, years since menopause may also play a role in the effectiveness of fermentable carbohydrates in influencing bone health. Calcium supplementation was more effective at preventing bone loss in hormone-stable menopause (> 5y) than in early menopause (16, 17). Additionally, hormone-flux-driven bone loss may confound any benefit from fermentable carbohydrates. Further support for this comes from the lack of effect with scFOS supplementation in the overall cohort but a positive trend detected in women >5 y postmenopausal (n = 6) (6).

In adolescents, who are experiencing rapid bone calcium accrual, calcium absorption has been reported to increase with fermentable carbohydrate supplementation. Recently, it was reported that 12 g/d SCF for 3 weeks improved calcium absorption by 12% in 24 adolescent boys and girls on a low-calcium diet (4). Likewise, 10 g/d and 20 g/d SCF for 4 weeks improved calcium absorption by 13% in free-living adolescent girls (5). Whisner et al. (18) showed in an RCT with 31 healthy adolescent girls that modest

amounts of galactooligosaccharides (5 g/d or 10 g/d) for 3 weeks improved calcium absorption by 13%. In a crossover-designed RCT, 15 g/d FOS for 9 days improved calcium absorption in adolescent males (19). Griffin et al. (20) showed in an RCT with 59 adolescent girls consuming 1500 mg/d calcium that 8 g/d FOS for 3 weeks did not improve calcium absorption, but a mixture of FOS and long-chain inulin (SYN1) did. The beneficial effect of SYN1 was confirmed in a follow-up study of the same design, which showed that girls who had low fractional calcium absorption during placebo periods were most likely to have improved fractional calcium absorption due to SYN1 supplementation (21). In contrast, Martin et al (22) found in a similarly designed study that 9 g/d SYN1 supplementation did not improve calcium absorption in adolescent girls 11–13 years old. However, these girls already had high fractional calcium absorption levels, which may have reduced the impact of the intervention. A longer RCT assessing SYN1 was also performed with adolescent boys (n = 50) and girls (n = 50) (23). After 8 weeks or 1 year of 8 g/d SYN1, calcium absorption was increased due to SYN1 supplementation, and after 1 year of supplementation, total body BMC and BMD were improved in the intervention group compared to control.

Studies in adults are limited and inconclusive. Van den Heuvel et al. found that males aged 20–30 y did not experience improved calcium absorption after 21 d with 15 g/d inulin, frutooligosaccharides, or galactooligosaccharides. However, calcium absorption was measured over 24 hours after the dose was received. Since then, it has been determined that calcium absorption is most impacted by fermentable carbohydrates between 24 and 36 hours after dose, implying a lower gut effect. Taking this into account, van den Heuvel et al. assessed the impact of 15 g/d fructooligosaccharides (FOS) for 9

days on adolescent males and found that it increased calcium absorption. It is unclear if the methodological or age discrepancies were responsible for the conflicting results.

Several studies have demonstrated that calcium absorption is enhanced by dietary fermentable carbohydrates in ovariectomized (OVX) rats, the FDA-approved animal model for postmenopausal women. For example, FOS improves calcium absorption and reduces ovariectomy-induced bone loss (24, 25). Additionally, SYN1 improves calcium absorption and reduces ovariectomy-induced bone loss (26, 27). Polydextrose (PDX) has also been shown to improve calcium absorption (26) and promote bone quality in the OVX model (24). Mitamura et al. (28, 29) showed that OVX rats experienced increased calcium absorption with either 3% raffinose or 5% soluble soybean fiber diets for 4 weeks.

Fermentable carbohydrates have also been shown to increase calcium absorption and improve bone status in non-OVX rat models. Inulin was shown to improve short-term calcium absorption in male rats fed low- and high-calcium diets and to improve long-term calcium absorption for rats on low-calcium diets (30, 31). Inulin was also shown to improve total body BMC and BMD in growing male rats on low-, adequate-, and high-calcium diets across all ages, up to 22 weeks old (32). Similarly, a 5% FOS diet improved calcium balance and true calcium absorption after 15 days in 2-month-old male rats (33). One study comparing the effectiveness of inulin and FOS in growing rats over a 3-month period found that both increased total body BMC, tibial BMD, and calcium transporter calbindin D9k, and decreased resorption marker CTx. However, the effect on bone, calbindin D9k, and CTx was greater in the inulin group than in the FOS group (34). In a survey of eight different fibers, including inulin and the inulin/FOS mixture SYN1,

only SCF and soluble fiber dextrin improved peak breaking force of the femur after 12 weeks of feeding (3). A diet including up to 8% galactooligosaccharides was also shown to improve calcium absorption in 4-week-old male rats in a dose-dependent manner (35).

Other dietary interventions for decreasing risk of osteoporotic fracture include flavonoids. Soy Isoflavones, which share structural similarity to estrogen, have been the most prevalently studied flavonoids for bone health. RCTs assessing the effect of isolated soy isoflavones on BMD have been fairly mixed, showing short-term efficacy in reducing postmenopausal bone loss but little effect in interventions at least 2 years long (36). However, BMD is only one predictor of fracture, and flavonoid interventions may be influencing other factors, such as bone composition and architecture. Weaver et al (7), using ^{41}Ca methodology, showed that soy cotyledon and germ effectively increased bone calcium retention in postmenopausal women over a 50-day period by 9% and 5%, respectively; however, this was a short-term study. Plum, high in the flavonoid rutin, has also been assessed as an intervention for improving bone quality in postmenopausal women. One randomized, controlled trial (37) found that postmenopausal women consuming 100 g/d plum for 1 year improved BMD in the ulna and spine. Other flavonoid-containing fruits and vegetables, like blueberry (38), onion (39), and mushroom (40), have been shown to be bone protective in OVX rats, but their efficacy in postmenopausal women has not been confirmed in a long-term randomized, controlled trial.

The ^{41}Ca methodology has several advantages. ^{41}Ca is a virtually stable, long-lived radioisotope ($t_{1/2} = 10^5$ years) that can be measured with great precision and sensitivity via AMS. Intervention effects on bone balance can be detected within 4–8

weeks, whereas studies relying on DXA require about 2 years (41). Lee et al. (42) found that a 14% decrease in ^{41}Ca resulted in a positive bone balance of 100 mg/d Ca. By interpolation, a 7% decrease in Ca^{41}, as observed in the present study, would result in a positive bone balance of approximately 50 mg/d Ca. If the entire effect of SCF is observed in 50 days, then 2.5g bone calcium, or 0.3% TBBMC, would be protected. However, if the effect persisted chronically, it would result in an increased balance of 18.25g bone calcium, or 2.5% TBBMC, per year. This effect would easily be detectable by DXA.

We observed a significant increase in the bone formation marker BAP between placebo and the 20 g/d SCF intervention. The magnitude of this difference was similar to the observed reduction in bone calcium loss. We detected no effect of SCF on bone resorption marker NTx. This contrasts with previous studies predicting that fermentable carbohydrates would reduce bone resorption (12, 14, 27). Zafar et al. (27) showed that SYN1-supplemented OVX rats had a net increase in calcium balance, but experienced both reduced bone formation and resorption, as measured by calcium kinetics. Our results indicate that bone formation may play a greater role in prebiotics-related bone protection than previously thought; however, high intrapersonal variation of bone turnover markers (43) warrant cautious interpretation pending confirmation in future studies.

Our participants' pre-study, baseline, and washout total fiber intakes were about 18 g/d, which is higher than the reported average adult fiber intake of 14.8 g/d in the US (44) but still lower than recommended for adult women (25 g/d) (45). Average total fiber intake during the placebo phase dropped to 14.2 g/d and, without including the fiber

contributed by the intervention, remained at approximately the same level during 10 g/d and 20 g/d SCF phases. The decrease was wholly due to displacement of fiber-rich foods by the muffin intervention. Addition of SCF brought total fiber consumed to 20.4 g/d and 30.9 g/d during the moderate- and high-dose phases, respectively. Soluble fiber followed a similar trend, dropping slightly from about 5.5 g/d in self-selected diet during nonintervention periods, to about 4.5 g/d during intervention periods. SCF supplementation dramatically increased the amount of soluble fiber consumed during the 10 g/d and 20 g/d SCF phases to 13.2 g/d and 21.3 g/d, respectively (Table 5.2).

Even during the high-fiber phase, participants in this study reported low severity and occurrence of gastrointestinal distress. This is not surprising, as adolescents tolerated 12 g/d SCF well (4), and adults tolerated large bolus doses (40 g SCF) and even larger divided doses (65 g SCF) well (46). The tolerability and high compliance rate we observed indicate that SCF supplementation appears to be a reasonable way to increase bone retention while improving fiber intake in most postmenopausal women.

This study has several strengths. The strong randomized-order, crossover design eliminated many confounding variables. The ^{41}Ca methodology measures bone loss in a relatively short time period. Participants were also free living and on self-selected diets, making the results translatable to the public. Limitations exist in this study as well. We used a small, convenient sample, decreasing the generalizability. We did not measure calcium absorption in this study, so we cannot determine the mechanism by which SCF reduced bone loss. Another limitation is the short 50-day intervention length. While ^{41}Ca methodology is rapid and sensitive enough to detect changes in bone retention in this period of time (7, 41), we were not able to assess whether the effect of SCF is persistent

or would diminish with long-term chronic feeding. One complete bone-turnover cycle is about 120 days, well over two times the duration of each intervention.

Chronic doses of 10 g/d and 20 g/d SCF were well tolerated by participants, and increased bone calcium retention in free-living postmenopausal women in a dose-dependent manner. Further research is necessary to determine the mechanism driving bone calcium retention.

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4. EFFECTS OF EXPOSURE TO CHRONIC INCREASE IN CECAL SHORT-CHAIN FATTY ACID SALT CONCENTRATION ON CALCIUM ABSORPTION IN RATS

4.1 Abstract

Several prebiotics have been shown to improve calcium absorption in adolescents, postmenopausal women, and rodent models. In this study, we aimed to assess the effect of a product of prebiotic fermentation, short-chain fatty acids (SCFAs), on calcium absorption. Rats were randomized to receive 0, 300, or 600 uL of a SCFA mixture twice daily for 11 days. Calcium absorption was measured on the 12^{th} day. No significant differences in calcium absorption were detected among the three groups. Cecal weight was significantly increased due to SCFA supplementation (0 vs 300uL SCFA, $p = 0.034$).

4.2 Introduction

Calcium is the major mineral constituent in bone and a critical life-sustaining micronutrient, yet due to scarcity and diet choices, insufficient calcium intake is a common problem worldwide (1). Thus, novel methods to enhance calcium absorption, which is only about 30% from a single serving of dairy or a 300 mg calcium supplement when consumed by postmenopausal women, are being explored. One method for enhancing calcium absorption is the consumption of prebiotics. These nondigestible, fermentable carbohydrates—including fructooligosaccharides (FOS),

galactooligosaccharides (GOS), soluble corn fiber (SCF), and others—have been shown to improve calcium absorption in adolescents (2-7) and postmenopausal women (8-10).

These carbohydrates pass through the majority of the gastrointestinal tract and reach the colon intact, where they are metabolized by gut microbiota. Gut microbiota, which constitute approximately 95% of the cells in the human body, break down these carbohydrates through saccharolytic fermentation, primarily into short-chain fatty acids (SCFAs), especially acetate, propionate, and butyrate (11). Enhanced calcium absorption in humans is observed 24–36h after calcium consumption, which indicates absorption occurs in the large intestine (3, 7).

It has been hypothesized that the production of SCFAs decreases colonic pH, which in turn ionizes and solubilizes additional calcium, enabling higher rates of paracellular calcium diffusion (12, 13). Several rat studies demonstrated that feeding prebiotics decreases cecal or colonic pH in addition to increasing calcium absorption (14-18). However, one study showed *ex vivo* that rat colon and cecal segments had improved calcium absorption due to increased luminal SCFA concentration, but not when HCl was used to depress luminal pH, indicating that SCFAs do not improve calcium absorption solely by reducing luminal pH (19). This study was designed to assess the effect of chronically heightened cecal and colorectal SCFA concentration on calcium absorption *in vivo* without adjusting pH.

4.3 Methods

4.3.1 Study Design

Twenty 3-month-old rats were implanted with cecal catheters (100% silicone Silastic® laboratory tubing; 0.51mm I.D. x 0.94mm O.D.) under anesthesia (isoflurane). On the third day, after two days of recovery, the rats entered the treatment phase of the study. For 10 days, the rats received two treatment doses directly infused into their ceca approximately 12 h apart, at a rate of 100 uL/s. On the ninth day of treatment, at least 2 h after receiving the morning dose, all the rats underwent surgery and had jugular catheters implanted (100% silicone Silastic® laboratory tubing; 0.51 mm I.D. x 0.94 mm O.D.). After a 2-day recovery, following their final treatment dose, the rats were fasted for at least 8 h. The following morning, each rat received a 1 mL cecal dose of calcium ascorbate (316g; 32.4g Ca) and saline. They also received a trace amount of ^{45}Ca either intravenously (10 uCi) or with the cecal calcium infusion (20 uCi). One hundred and seventy uL blood samples were collected at 0, 60, 120, 180, 240, 330, 420, 540, 720, 900, 1260, and 1440 min after dosing. A 100-uL plasma aliquot was collected from each blood sample and counted in a liquid scintillation analyzer (Tri-Carb 2910TR; PerkinElmer). Urine samples were collected as 0–1440 min bulk samples. These were also measured for radioactivity using the liquid scintillation analyzer.

Plasma ^{45}Ca concentration from half of the rats from each group was used to calculate absorption efficiency with the Win-SAAM (Simulation Analysis and Modeling) program as previously described (20). A multicompartmental calcium model was created and plasma ^{45}Ca data was fitted to it. Plasma ^{45}Ca data from IV-dosed rats was used to

establish calcium transfer rates among blood, bone, exchangeable calcium pools, and calcium clearance. Plasma ^{45}Ca data from cecum dosed rats was used to establish calcium absorption, based on calcium transfer rates established by the IV plasma data. Urinary ^{45}Ca provided additional data for constructing the model.

Plasma ^{45}Ca appearance from all of the rats was used to assess calcium absorption via area under the curve.

4.3.2 Treatments

Each of the rats received one of three treatments twice daily: 0 (Placebo; PLC), 300 (Low Dose; LOW), or 600 (High Dose; HIGH) umol SCFA mixture. The amount of SCFA provided in a single HIGH dose is about equal to the total amount found in cecum of a control rat in other studies assessing the effectiveness of fibers to increase calcium absorption (21, 22). Therefore, it was expected that the amount of SCFAs contained in the ceca of HIGH group rats would be doubled at each dosing period. Fiber supplemented rats have increased cecal SCFA concentration up to around 50%, compared to control. The SCFA mixtures were comprised of the molar ratio 5-mol sodium acetate, to 2-mol sodium propionate, to 1-mol sodium butyrate. This is approximately the same ratio as has been observed in rodent ceca when on prebiotic-containing diets.

4.3.3 Cecal Analysis

The animals were euthanized after the calcium kinetics test and their ceca were collected. The cecum was weighed before and after thoroughly rinsing with saline. Cecal content weight and pH (ThermoWorks®; pH Spear) were measured.

4.3.4 Statistical Analysis

ANOVA was performed with Student's t-test using SAS (SAS 9.3; SAS Institute) to determine statistical significance of calcium absorption, cecal pH, and weights among treatment groups. Statistical significance was set at $p \leq 0.05$.

4.4 Results

Significant differences were detected in neither fractional calcium absorption among treatment groups (CON vs LOW: $p = 0.203$; CON vs HIGH: $p = 0.915$; LOW vs HIGH $p = 0.129$), nor area under the curve (CON vs LOW: $p = 0.476$; CON vs HIGH: $p = 0.487$) as determined by plasma ^{45}Ca appearance. Likewise, no significant differences were detected among treatment groups in rodent body weight, cecal pH, or cecal content weight. A statistically significant difference in cecal weight was detected between the CON and LOW groups ($p = 0.034$), while a trend for significance was detected between the CON and HIGH groups ($p = 0.079$).

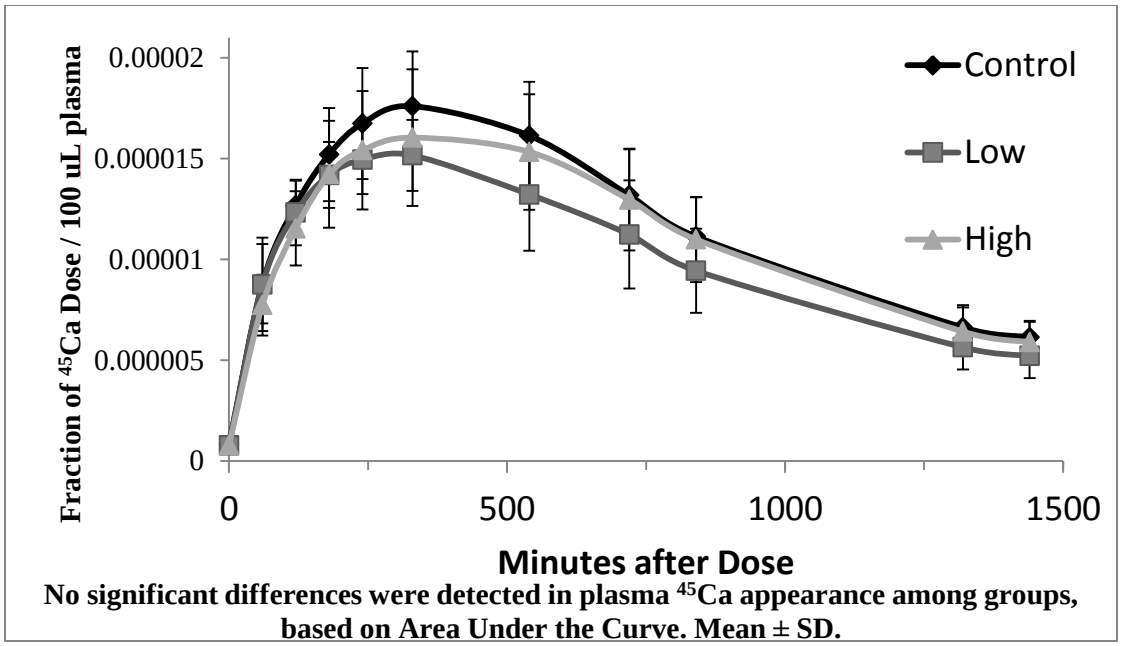

Figure 4.1: Plasma Appearance of ^{45}Ca is Unaffected by Chronic SCFA-Salt Dosing

Table 4.1: Rat Body Weight, Cecal pH, and Cecal Content Weights at Sacrifice (Mean ± SD)

Group	Body Weight (g)	Cecal pH	Cecal Content Weight (g)
CON	384.5 ± 12.0	6.32 ± 0.15	4.23 ± 0.67
LOW	385.7 ± 27.3	6.21 ± 0.13	4.98 ± 1.44
HIGH	391.6 ± 21.2	6.39 ± 0.11	4.18 ± 0.45

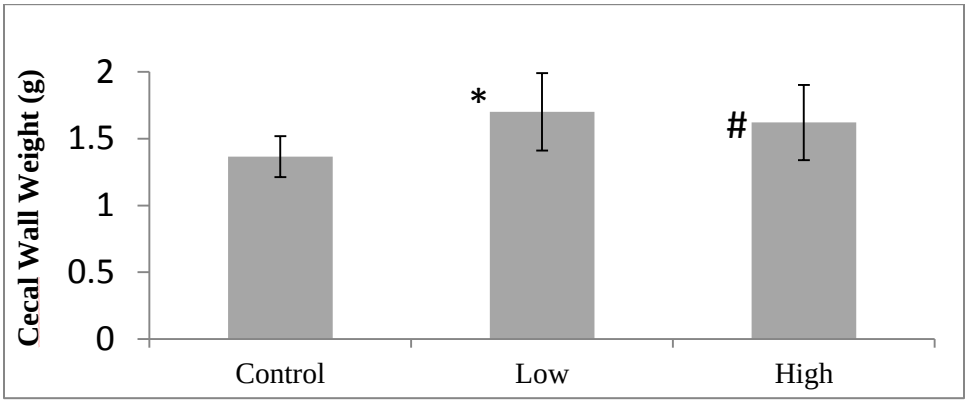

Figure 4.2: Rat Cecal Wall Weight at Sacrifice by Dose

* $p < 0.05$; # $p < 0.10$

4.5 Discussion

Our results failed to show an increase in calcium absorption in the large intestine with a chronic increase in cecal SCFA concentration. This indicates that one or more of the mechanisms proposed with fermentable carbohydrates to increase calcium absorption was not demonstrated in this study. There are several explanations that could be related to a lack of real effect or to a failure in the study design to test the effect of SCFA on calcium absorption.

SCFAs may improve calcium absorption through proton exchange. Protonated SCFAs are absorbed into colonocytes through concentration-dependent non-ionic diffusion. The transported hydrogens may then be exchanged back into the lumen for calcium (23). SCFAs have been shown to improve short-term calcium absorption in the distal colon of humans (23) and rats (24) during an enema or a luminal perfusion, respectively. In the present study, SCFAs were administered chronically in the cecum and were not administered at or near the same time as calcium kinetics were assessed. In agreement with previous work (25), showing a delay compartment between calcium absorption sites in the small and large intestine, our calcium kinetics data indicate that calcium was dosed into a compartment preceding the absorption site. Absorption likely occurs in the colon rather than in the cecum. A mixture of long- and short-chain fructan-type fibers was more effective at improving calcium absorption than only FOS (4), indicating that fermentation along the length of the colon might be more effective at improving calcium absorption than rapid fermentation at the beginning of the colon. Also, the calcium and ^{45}Ca tracer dosing was 12 h after the last SCFA dosing. The

majority of the SCFAs may have already been absorbed and metabolized and could therefore have been unavailable for exchange in any part of the large intestine.

As previously discussed, a decrease in pH due to SCFA production may drive calcium absorption. As cecal and colonic pH decreases, calcium may be further solubilized, leading to increased para- and trans-cellular diffusion. In the present study, neither cecal pH nor calcium absorption were not affected by SCFA-salt dosing.

Decreased pH may play a greater role in calcium absorption if calcium inhibitors are present in the diet. Calcium absorption inhibitors, such as oxalate and phytate, are common in many foods and reduce the bioavailability of minerals, including calcium. Lopez et al. (26) found that rats fed phytase and FOS had less phytate excretion than rats fed phytase alone, suggesting that FOS fermentation may improve phytate degredation in the large intestine. This would release any minerals bound to the phytate at the time of fermentation. Wang et al. (27) showed that calcium absorption was restored in rats fed a phytate-containing diet when they were also fed FOS.

There are several proposed mechanisms in addition to the pH-calcium-solubility hypothesis. Prebiotic fibers have a relatively high water binding capacity, and since they remain intact until the colon, they bring water with them. With an increased solvent pool, a greater proportion of minerals can dissolve and may be more easily absorbed. Incorporation of dietary fermentable fibers leads to greater amounts of cecal soluble calcium and blood flow (13).

Another hypothesis is that fibers enhance calcium absorption through cecal hypertrophy. Dietary fermentable fibers significantly increase cecal wall weight in rats (13, 27, 28). Butyrate is the preferred energy source of colonocytes, likely contributing to

enterocyte proliferation (29). Cecal and colorectal hypertrophy would increase surface area available for both paracellular and transcellular calcium absorption. In one study, GOS supplementation increased wet and dry stool weight in sham and gastrectomized rats (30), indicating an increased excretion of non-water mass, potentially through cell sloughing or intact GOS. In another study, GOS supplementation (31) was not well correlated with an increase in stool moisture content. Weaver et al. (21) found a significant relationship between SCFA production and cecal content weight ($p < 0.05$). It is unclear if this increase was caused from cecal hypertrophy, water-holding capacity of the fermentable carbohydrates, or both. The present study showed that chronic SCFA-salt administration increased cecal wall weight, suggesting increased proliferation. This was not paired with an increase in calcium absorption.

Prebiotic fermentation products may increase calcium transporters in the colon, resulting in increased calcium absorption. FOS supplementation increases rat cecal and colorectal levels of the calcium transporter calcium-binding protein calbindin-D9k, along with increasing calcium absorption (32, 33). Additionally, factors regulating calbindin-D9k transcription, CDX2 and Vitamin D-receptor (VDR), were significantly increased in the colorectal segments of rats on a FOS supplemented diet, potentially increasing transcellular calcium absorption (34). Another study (35) found that Caco-2 cells cultured with sodium propionate and sodium butyrate developed greater levels of calbindin-D9k, a calcium transporter. In MCF-7 cells, SCFA concentration increased intracellular calcium concentration dose dependently, contingent on GPR43 activity (36). GPR43 was also activated by SCFAs in mice colonocytes, though neither calcium absorption nor concentration was not measured (37). GPR43 is expressed in human colonocytes (38),

and these models may be providing some insight into prebiotic-enhanced calcium absorption.

Another way that prebiotics may alter calcium absorption is through the promotion of specific gut microbiota. Several studies have shown shifts in gut microbial populations after adding fermentable carbohydrates to the diet, generally showing an increase in carbohydrate-fermenting organisms (3, 6, 39). However, the mechanism by which these bacteria would enhance calcium absorption is still unclear. It is possible that they work through the mechanisms listed above. Probiotic supplementation (*Bifidobacterium bifidum* and *Bifidobacterium longum*), GOS supplementation, and synbiotic supplementation (probiotic and GOS) in rats for 30 days increased tibia and femur calcium content, proximal-colon crypt depth and cell density, and apparent calcium absorption (17, 31). At the very least, gut microbiota appear to impact calcium absorption efficiency in the colon, since it was shown that antibiotics reduce the calcium absorption benefit provided by GOS (40).

In summary, a chronic increase in cecal SCFA salt concentration alone does not appear to influence calcium absorption using this protocol. Using the acidic form of SCFAs, dosing SCFA salts just prior to dosing Ca, or challenging calcium absorption with inhibitors may be necessary for the mechanism by which prebiotics increase calcium absorption. There are still several potential mechanisms by which fiber could increase calcium absorption that have yet to be examined directly.

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5. SUMMARY

Bone health becomes increasingly important as age progresses and bone demineralization outpaces bone formation. This is particularly important for females, who experience a drastic change in sex hormones at menopause, resulting in increased bone resorption and fracture risk during their postmenopausal years. Yet consumption of calcium, the primary constituent of bone, remains below recommended levels for this demographic (1). Efforts to ameliorate bone loss include the use of fermentable carbohydrates.

Fermentable carbohydrates improve bone health in OVX rats, the approved model of postmenopausal women. In this model, calcium absorption has been shown to be enhanced by fructooligosaccharides (FOS)(2-4), a fructooligosaccharide-inulin mixture (SYN1)(5), polydextrose (PDX)(5), and raffinose (6).

Effectiveness of fermentable nondigestible carbohydrates in enhancing bone metabolism in postmenopausal women has not been as clear. While SYN1 (7), transgalactooligosaccharides(8), and lactulose (9) improved calcium absorption in postmenopausal women, scFOS did not (10, 11). This difference could have been due to the type of fiber, size of dose, length of trial, or sensitivity of method used. The present study showed, using the sensitive novel ^{41}Ca method, that SCF reduces bone loss in

postmenopausal women in a dose-dependent manner. It is estimated that consuming 20 g/d SCF would improve net bone calcium balance by 50 mg/d Ca.

Understanding the mechanism by which these carbohydrates improve calcium metabolism could enhance efforts to reduce fracture risk in all populations and should be investigated. The present study of SCFAs in rodents shows that a chronic increase in cecum concentrations of SCFAs does not increase calcium absorption. Other mechanisms should be explored further, such as depressed pH in and out of the presence of calcium inhibitors, or the selective feeding of gut microbiota.

5.1 Future Work

5.1.1 The Effectiveness of SCF in Postmenopausal Women:

The present study with postmenopausal women showed that SCF improved bone calcium retention over a 50-day period; however, postmenopausal women are at a high risk of fracture for much longer—that is, for the rest of their lives. Therefore, SCF should be assessed for its effectiveness as a long-term intervention for improving bone health in postmenopausal women. Abrams et al. (12) found that chronic dosing of SYN1 improved both calcium absorption and BMD in adolescents after 1 year, and Coudray et al. (13) found that inulin improved calcium absorption after 1 year in male rats with low calcium intake, but further study is necessary. DXA, the recognized gold standard of bone health measurement, and the sensitive ^{41}Ca methodology of bone calcium assessment, should both be employed in assessing the effectiveness of SCF in these long-term studies.

5.1.2 Calcium-Absorption Enhancement Mechanism

Many studies show that calcium absorption is enhanced with dietary supplementation with fermentable fibers, but few assess the mechanism driving this effect. The present investigation on chronic SCFA dosing in rats provides a framework for assessing this mechanism by singling out one of the effects of dietary fibers. Other effects of fibers should be assessed directly for their abilities to affect calcium absorption.

The fermentation of nondigestible fibers into SCFAs decreases culture pH *in vitro* (14) , and may decrease the pH of the large intestine *in vivo*, leading to increased calcium solubility. Increased concentration of solubilized calcium may increase passive diffusion of calcium across the wall of the large intestine. Therefore, the effect of luminal pH on calcium absorption should be directly assessed. *Ex vivo,* calcium absorption in a segment of large intestine was enhanced by SCFA concentration, but not by a decrease in pH by HCl (15), however, the present study showed that chronically increasing the concentration of cecal SCFA-conjugate bases does not improve calcium absorption *in vivo* when there is an exposure gap between SCFA and ^{45}Ca exposure. The observed difference in the effectiveness of SCFAs to improve calcium absorption could be attributed to the exposure gap, model (i.e. ex vivo vs in vivo), intestinal segment, study duration, or the acidity of the SCFAs used. Therefore, future studies should assess these differences.

Similarly, increasing the acidity of the large intestine could free calcium bound to common calcium absorption inhibitors, such as phytate and oxalate, and thus increase calcium bioavailability. Future research should explore this relationship.

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APPENDICES

A. Postmenopausal Women SCF & ^{41}Ca Study: Recruitment and Data Collection Materials

A study to determine the effect of soluble corn fiber on bone resorption in post-menopausal women

Post-menopausal women are needed for a calcium study in the Department of Nutrition Science. It is an 18 month study which involves consuming soluble corn fiber snack products for three phases, each 50 days long. Researchers will study how fiber may influence the amount of calcium absorbed in bones.

Maximum total compensation for completing the entire study is $825.

Eligibility requirements include: healthy, post-menopausal women who are at least 4 years past the onset of menopause due to natural menopause or total hysterectomy, not currently taking estrogen replacement therapy or treatment for osteoporosis.

For more information, contact Berdine Martin at martinb1@purdue.edu 49-46559 or 49-40385.

The principal investigator is Connie Weaver, distinguished professor and head of the Department of Nutrition Science.

Corn Fiber and Bone Health

Osteoporosis is a disease affecting 25 million Americans which causes bone loss and fractures. One in three women over 50 years of age will suffer an osteoporotic fracture.

We are **SEEKING VOLUNTEERS** to take part in an 18 month study, which will involve consuming snacks which contain soluble corn fiber that may influence the amount of calcium that is retained in your bones.

Your are eligible if you are:

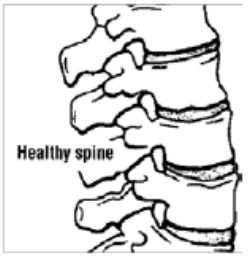
- Female
- At Least 4 years post Menopause
- Not currently taking estrogen replacement therapy
- or treatment for osteoporosis

You will be paid for your participating in the study

Please contact:

bmartin1@purdue.edu

494-6559



Research Project Number_____

For IRB Office Use Only
APPROVED
MAR 20 2012
EXPIRES 3/19/13
PURDUE UNIVERSITY
INSTITUTIONAL REVIEW BOARD

RESEARCH PARTICIPANT CONSENT FORM

The Effect of Soluble Corn Fiber (SCF) on Bone Resorption in Post-Menopausal Women using ^{41}Ca Technology

Dr. Connie M. Weaver
Purdue University
Nutrition Science

Purpose of Research

This study is designed to compare the effect of a soluble fiber found in corn kernels on calcium retention and bone loss. The study will compare two different doses of the fiber to no daily supplemental fiber.

Specific Procedures to be Used

In order to determine if you are eligible for the study we will ask you to complete the following procedures:

1. a) Verify that you are at least 4 years post-menopausal
   b) Fill out questionnaires concerning your health habits, medical history, physical activity and food patterns and usual diet.
   c) Come to the clinical facilities in Smith Hall where we will draw a small amount of blood (1 Tablespoon) to ensure that you are healthy and to verify your hormonal status.

2. If you meet the eligibility requirements and the enrollment goals have not been met you will be enrolled into the study for participation in the following procedures. If you are currently taking a vitamin mineral supplement we will ask you to quit taking the supplement for at least 30 days before the beginning of the baseline period and throughout the study. We will ask you to take the supplement that we provide.

3. If you have not already received a dose of special calcium because of your participation in a previous study then we will ask you to go to the Indiana University School of Medicine where the medical personnel will administer a form of calcium, Ca-41, that is very slightly radioactive (loses energy very slowly) by inserting a needle into one of your veins and transferring about a teaspoon of solution (intravenous infusion). An equilibration period of 100 days will be necessary following this administration so that the calcium can become deposited in your bones. We will ask you to collect urine every month during this phase. Then a 50 day baseline period will begin.

4. If you have already been dosed with Ca-41 from a previous study, the equilibration period is eliminated and you will begin a baseline period immediately.

5. Baseline Period:
   Every 10 days during the baseline period, you will collect urine for 24 h. Arrangements will be made for you to deliver it to the lab or to advise lab personnel where collections can be picked up. During the phase you will receive a bone scan of your total body, both hips, and spine. This involves lying on a table for about 45 minutes while a low energy X-ray beam from the instrument scans your body. We will also complete several body measurements including height, weight, waist circumference, hip circumference, and hip width. We will ask you to record everything that you eat for 4 days during the 50 day period.

6. Treatment Phases:
   During the treatment phases you will consume two snack products per day which contain soluble corn fiber (5 or 10 g) or no fiber for 50 days. The products may include a breakfast bar, cookie, gummie, or powder to add to a beverage. You will record your product consumption on a calendar that will be provided to you. You will collect 24 h urine every 10 days in containers that will be provided to you. We will make arrangements to receive your collections in the lab or to pick them up at your residence. We will also ask you to record everything you eat for 4 days.

Initials_____Date_____

Research Project Number_____

7. Recovery Phases:
   After each intervention phase we will ask you to eat your normal diet for 50 days and continue collecting urine every 10 days.

8. Clinical Visits:
   At the end of each 50 day period you will come to Smith Hall in a fasted state. You be asked to give a small urine sample and a small blood sample (~ 1 Tablespoon). We will repeat body measurements and receive your diet records and compliance calendars.

Duration of Participation

The maximum number of days for the study if you have already been dosed is 350. If you have not been dosed then the total study will last 450 days. There will be a total of 9 clinical visits which will take no longer than 30-60 minutes each time.

Benefits to the Individual

You will receive no direct benefits from this study. However, we will share the results from your bone scan and the analysis of your diet records.

Risks to the Individual

The health risks involved in this study include drawing of blood which can lead to pain, fainting, bruising and infection. Precautions will be taken to minimize these risks by using sterile technique and applying pressure to the site after the needle is withdrawn.

The average absorbed dose of radiation from the bone measurement is 1.424 mRem. For comparison, absorption from a single dental x-ray is 1 mRem and from a chest x-ray is 6 mRem.

The lifelong radiation exposure associated with receiving Ca-41 is less than 1-100,000th of a set of dental x-rays.

The fiber that will be in the snack products is no different than the fiber found in normal corn that you eat. As with all dietary studies we will contact you at least once during each intervention phase to ask you about any adverse events such as abdominal pain, nausea, headache, diarrhea, constipation, or allergic like reactions. We will also ask you to report any events when you come for your clinical visits. The study physician will evaluate your reports.

There is always a chance that confidentiality will be breached. However, precaution will be taken to avoid this possibility.

Compensation

You will be paid according to the following schedule:
Screening: $25
Equilibration: $50
Baseline: $50
Phase 1-3: $150
Recovery 1-3 (washout): $50
Total compensation for completing the entire study if you have not been dosed previously: $825
Total compensation if you have been previously dosed: $750

Initials_____Date_____

Research Project Number_____

You will receive a check which will be sent to your home after completing each phase. Your name, social security number and address will be provided to the business office at Purdue University for the purpose of facilitating payment for participating in this study.

There will be no extra costs to you other than the time and cost of travel for the clinical visits. If you choose to drive your own car to Indianapolis for the dosing procedure you will be reimbursed for your mileage.

Injury and Illness

You understand that Purdue University will not provide medical treatment or financial compensation if you are injured or become ill as a result of participating in this research project. This does not waive any of your legal rights nor release any claim you might have based on negligence.

Confidentiality

If you do not meet the eligibility requirements, any data that has been collected during the screening process will be destroyed immediately. If you do participate in the study your identity will remain confidential. Health questionnaires and forms will be stored in a secure file in Dr. Weaver's lab. All blood and urine samples will be labeled only with your study ID. The code which connects your ID with your name will be stored in a separate file. The key code and any remaining specimens or files will be destroyed after 5 years. Data that is shared with the sponsor or that is published will have no reference or connection with your name. However, the project's research records may be reviewed by the sponsor, Tate and Lyle Ingredients Americas, LLC or by departments at Purdue University responsible for regulatory and research oversight.

Voluntary Nature of Participation

If you do not meet eligibility requirements you will be withdrawn from the study. However, if you do meet Eligibility requirements, you still do not have to participate in this study. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. You may also discontinue participation at any time without penalty or loss of benefits.

Contact Information

If you have any questions about this research project, you can contact Dr. Connie M. Weaver at 494-8231(weavercm@purdue.edu) or Dr. Berdine Martin at 494-6559 (bmartin1@purdue.edu).
If there are concerns about the treatment of research participants, contact the Institutional Review Board at Purdue University, Ernest C. Young Hall, 10th Floor, Room 1032, 155 S. Grant St., West Lafayette, IN 47907-2114. The phone number for the Board is (765) 494-5942. The email address is irb@purdue.edu .

Documentation of Informed Consent

I have had the opportunity to read this consent form and have the research study explained. I have had the opportunity to ask questions about the research project and my questions have been answered. I am prepared to participate in the research project described above. I will receive a copy of this consent form after I sign it.

_____ _____

Participant's Signature Date

_____

Participant's Name

_____ _____

Researcher's Signature Date

FREQUENTLY ASKED QUESTIONS ABOUT
Soluble Corn Fiber Study
Department Nutrition Science
Connie Weaver, Principal Investigator
2013

Who is eligible for the study?
Only women will be studied. At least 4 years post menopause. Not currently taking estrogen replacement therapy or treatment for osteoporosis.
If you have had a total hysterectomy you would be eligible but not if you have had only a partial hysterectomy.

When will the study begin?
The study will begin immediately and continue until we have enrolled ~12-15 participants.. The screening procedure includes: 1) Signing consent form, 2) A small blood sample will be taken to verify that you are healthy. 3) Completing a screening questionnaire and diet record.

How much time does it involve and what do we have to do?
If you have never participated in one of our studies before:
The first step after you are enrolled involves a trip to University Hospital in Indianapolis. You will receive a very small dose of a special form of Ca intravenously. This calcium is very slightly radioactive equivalent to 1/100,000 of a dental x-ray. After receiving the calcium you must wait at least 100 days before the baseline and treatment phases can begin. During this 100 day equilibration phase, you will collect urine once per month.

If you have participated in one of our studies before and you have already been dosed with special calcium you can begin immediately with the baseline phase of the study.

What do the treatment phases involve?
Each treatment phase lasts for 50 days. During each of the treatment phases, you will be asked to consume 2 products per day that contain soluble corn fiber (0, 5 or 10 g fiber per product). These products will include a muffin and a fruit flavored drink. Every 10 days you will be asked to collect urine for 24 hours. At the end of each treatment and washout phase, we will ask you to come to our clinical facility where we will draw a small sample of blood and collect a small amount of urine.

How many treatment phases are there?
There is a baseline phase when you will only have to collect urine every 10 days. Then there are 3 treatment phases, each separated by a 50 day washout period.

Do I have to alter my diet in any way?
We will not ask you to alter your diet in any way. However, we will ask you to record what you eat for four days during each treatment phase.

Can I continue to take vitamin/mineral supplements during the study?
We will ask you to quit taking any vitamin mineral supplements for one month before you start the study until the end of the study. This excludes prescription drugs.
We will provide you with a supplement that we will ask you to take daily.

What if we miss a urine collection?
Obviously we would hope that you would not miss any collections.
However, if that happens we ask you to keep a log and note any missed collections. We can also be somewhat flexible about the days that you must collect urine.

How much will I be compensated for participation in this study?
You will be paid $25 for screening. During the equilibration phase you will be paid $50. For baseline and each treatment phase -$150. You will be paid $50 for each recovery period. If you complete all phases of the study that totals $825. If you discontinue the study, you will be paid for each phase completed.

How do we store urine samples?
We will deliver urine collection containers to your home or office. We will also give you a small cooler that you could use to carry containers with you. (It will look like a lunch cooler) There will also be designated refrigerators in Stone Hall that you can leave your containers in during the day. Any collections at home can be stored in the cooler provided. We will ask you to bring the urines to your office and we will pick them up promptly from your office. If day 10 falls on a weekend or if it is not possible to bring the cooler to campus we will pick the urine coolers up from your home.

Will there be any side effects from eating the products?
It is not expected to notice any side effects from the products. Dr. Munro Peacock, Endocrinologist at IU Med Center in Indianapolis is one of the collaborating investigators and is available for consultation about any side effects which may occur.

PURDUE UNIVERSITY

Department of Nutrition Science

Calcium and Soluble Corn Fiber
Postmenopausal 2013

SCREENING RECORD

Subject Number/Initials	_____/_____
Date/ Time	_____/_____/_____ _____:_____AM/PM
Name	_____, _____, _____ Last First MI

Script

____ Objectives of Study
____ Screening Process
____ Drug Restrictions
____ Supplement Restrictions (Hormone/Calcium/Herbal)
____ Intervention: Muffins, drink/diet record
____ Clinical Visit-blood/urine/poop/bone scans
____ Confidentiality and payment

Conditions

____ Is the subject currently using any vitamin/mineral supplements?__(Y/N)

- If yes, are they willing to quit taking them at least 30 days before baseline period starts and throughout the study? __(Y/N)
- Are they willing to take the supplement that we provide? __(Y/N)

____ The subject has been informed of the urine collection schedule (24-hour urine once every 10 days) __(Y/N)

____ The subject has been briefed about the use of Ca-41, the slightly radioactive calcium isotope used in this study. __(Y/N)

Ca-41 Status

Has the subject been dosed with Ca-41 previous to this study? __(Y/N)

Consent/Assent

____ The subject was given ample time to read the consent form. All questions were answered. The subject signed and dated the informed consent form prior to participation in the study. A signed copy of the informed consent forms were given to the subject.

Examination

Blood Drawn By:_____

____ Blood draw time: _____
____ Blood draw volume: _____ml
____ 4 ml lavender
____ 8.5 ml SST
____ 9 ml redtop

_ _

____ Subject height: _____cm
____ Subject weight: _____kg

Blood Sendout	___Chem Panel ___CBC ___Lipid ___FSH
Forms	Screening Instrument Given_____ Rec'd _____ 4-Day Food Record Given_____ Rec'd _____ Diet Assessment Tool Given_____ Rec'd _____ Diet Record Instructions Given_____
Status	___Accept ___Deny ___Notified __/___/__ Date
NOTES	
Staff	_____

First Name_____ ID_____ Date_____

Diet Assessment Tool

Please answer all questions !
This will take only 5 minutes to complete.

The following food prompts refer to what you ate **over the past month**.

For example, if you drank one cup of hot cocoa every week this past month, then your answer would look like this:

HOW OFTEN DID YOU EAT OR DRINK THESE FOODS	NEVER OR <1 PER MONTH	1-3 TIMES PER MONTH	ONCE PER WEEK	2-6 TIMES PER WEEK	ONCE PER DAY	2 OR MORE TIMES PER DAY
Cocoa (hot chocolate) made with milk (1 cup)	○	○	●	○	○	○

Fill in the bubble with the best response for <u>each</u> item. Please answer all of the items.

HOW OFTEN DID YOU DRINK THESE FOODS	NEVER OR <1 PER MONTH	1-3 TIMES PER MONTH	ONCE PER WEEK	2-6 TIMES PER WEEK	ONCE PER DAY	2-3 TIMES PER DAY	4 OR MORE TIMES PER DAY
Milk to drink, white or chocolate (1 cup or 1 carton)	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Instant breakfast drink such as Carnation Instant breakfast(r) (1 packet or 1 glass)	⬭	⬭	⬭	⬭	⬭	⬭	⬭

HOW OFTEN DID YOU EAT OR DRINK THESE FOODS	NEVER OR <1 PER MONTH	1-3 TIMES PER MONTH	ONCE PER WEEK	2-6 TIMES PER WEEK	ONCE PER DAY	2 OR MORE TIMES PER DAY
Café Latte, Café Mocha, Cappuccino, or Café Au Lait (1 tall or 1 large))	⬭	⬭	⬭	⬭	⬭	⬭
Cocoa (hot chocolate) made with milk (1 cup	⬭	⬭	⬭	⬭	⬭	⬭
Yogurt, not frozen (1 container)	⬭	⬭	⬭	⬭	⬭	⬭
Frozen yogurt or ice cream (1/2 cup or 1 scoop or 1 bar)	⬭	⬭	⬭	⬭	⬭	⬭
Milk shake, malt, or frappe (1 shake, 1 malt or 1 frappe)	⬭	⬭	⬭	⬭	⬭	⬭
Cheese (1 slice, 1 stick, or a 1 inch cube)	⬭	⬭	⬭	⬭	⬭	⬭
Cold cereal (1 cup or 1 bowl)	⬭	⬭	⬭	⬭	⬭	⬭
Chocolate candy bar (1 regular size bar, ½ king size bar)	⬭	⬭	⬭	⬭	⬭	⬭

HOW OFTEN DID YOU EAT THESE FOODS	NEVER OR <1 PER MONTH	1-3 TIMES PER MONTH	ONCE PER WEEK	2-4 TIMES PER WEEK	5 OR MORE TIMES PER WEEK
Macaroni and cheese (1 cup)	⬭	⬭	⬭	⬭	⬭
Hamburger or hot dog with cheese on a bun (1 hamburger or 1 hot dog)	⬭	⬭	⬭	⬭	⬭
Enchilada: cheese (1 enchilada)	⬭	⬭	⬭	⬭	⬭
Chile relleno (1 chile)	⬭	⬭	⬭	⬭	⬭
Tofu (1/2 cup)	⬭	⬭	⬭	⬭	⬭

Your calcium score can be estimated by adding the points assigned to your responses. Circle the score of your response and place that number under the "My Score" column.

HOW OFTEN DID YOU DRINK THESE FOODS	NEVER OR <1 PER MONTH	1-3 TIMES PER MONTH	ONCE PER WEEK	2-6 TIMES PER WEEK	ONCE PER DAY	2-3 TIMES PER DAY	4 OR MORE TIMES PER DAY	My Score
Milk to drink, white or chocolate (1 cup or 1 carton)	0	1	2	8	15	37	60	
Instant breakfast drink such as Carnation Instant breakfast(r) (1 packet or 1 glass)	0	1	2	8	15	37	60	

HOW OFTEN DID YOU EAT OR DRINK THESE FOODS	NEVER OR <1 PER MONTH	1-3 TIMES PER MONTH	ONCE PER WEEK	2-6 TIMES PER WEEK	ONCE PER DAY	2 OR MORE TIMES PER DAY	My Score
Café Latte, Café Mocha, Cappuccino, or Café Au Lait (1 tall or 1 large)	0	1	2	8	15	30	
Cocoa (hot chocolate) made with milk (1 cup)	0	1	2	8	15	30	
Yogurt, not frozen (1 container)	0	1	2	8	15	30	
Frozen yogurt or ice cream (1/2 cup or 1 scoop or 1 bar)	0	1	2	8	15	30	
Milk shake, malt, or frappe (1 shake, 1 malt or 1 frappe)	0	1	2	8	15	30	
Cheese (1 slice, 1 stick, or a 1 inch cube)	0	1	2	8	15	30	
Cold cereal (1 cup or 1 bowl)	0	1	2	8	15	30	
Chocolate candy bar (1 regular size bar, ½ king size bar)	0	1	2	8	15	30	

HOW OFTEN DID YOU EAT THESE FOODS	NEVER OR <1 PER MONTH	1-3 TIMES PER MONTH	ONCE PER WEEK	2-4 TIMES PER WEEK	5 OR MORE TIMES PER WEEK	My Score
Macaroni and cheese (1 cup)	0	1	2	6	10	
Hamburger or hot dog with cheese on a bun (1 hamburger or 1 hot dog)	0	1	2	6	10	
Enchilada: cheese (1 enchilada)	0	1	2	6	10	
Chile relleno (1 chile)	0	1	2	6	10	
Tofu (1/2 cup)	0	1	2	6	10	
Add up the numbers in "My Score" column here **BCAT Score :**						

Things to know about your Baseline Visit:

- Baseline visits will be held in Stone Hall. If you don't have a campus parking pass, we will be able to provide you with an A-pass. Please let us know ahead of time so you will have it prior to your visit.
    - There are A parking spots behind Smith Hall, on Russell St, parking garage, etc.
- We will meet you in Stone Hall, Room 124, at the time of your scheduled appointment. Please enter and have a seat in the waiting area. There have been a few minor changes to the protocol. We will review those and have you sign the new consent form.
- At your visit, we will need you to be prepared to collect fasting urine and fasting blood samples. This means that we ask you not to eat or drink anything but water for at least 8 hours before your visit.
- We will also complete a DXA scan to measure bone mineral density. Since this is an X-ray, you cannot wear any metal during the scan (clothing with zippers, eyelets, hooks or jewelry, etc). We advise you to wear comfortable clothing to make the scan faster and easier. We will have scrubs available for you to change to if you forget.
- Bring with your schedule/calendar for us to go over and verify urine collection dates with you. If you have any special dates when you will be on vacation or unavailable bring them with so we can plan around them.
- This visit will probably take approximately 45 minutes.

Please let us know if you have any other questions or concerns about your visit.

Text for instructions after Visit 0
Send as e-mail

Thank you for coming for your baseline visit this morning. It is good to get this study started and we really appreciate your help. I just want to review the details that we covered this morning so you have them in writing and you can transfer them to your calendar.

First of all you will be collecting urine every 10 days from now until your next visit. The dates which we decided would fit your calendar are the following. Remember to collect after the first void of the morning and including the first void of the following morning. Place the bottles in the cooler that we provided and please set it on your porch. I will come by sometime before noon to pick it up and I will leave you another cooler. Unless you contact me I will assume that you will be true to your schedule so you don't need to contact me every time you collect urine. If however, you forget please let me know that you are collecting on the following day so I can rearrange my collection schedule. The last collection you will bring with you when you come the following day for your clinical visit.

If at any time you need more bottles let us know and we can bring you some more.

Collection 1:
Collection 2:
Collection 3:
Collection 4:
Collection 5:

Now for the fecal collections!
The timing for fecal collections during the baseline period is as follows:
You will collect the first specimen sometime during the first week of the baseline phase ( )
During the last week of the baseline phase you will collect 3 specimens ( )

Each time you collect a specimen please send me an e-mail or call me (see info below) and leave me a message. Place the sample in the small cooler with the ice pack and set it out so that I can pick it up.

If you have any questions please feel free to contact me.

Again we really appreciate your cooperation with this study. You should be receiving the $50 payment for the Equilibration period within a couple of weeks. I submitted the invoice today.

Instructions for Consumption of Muffins and Drinks

1. **Each muffin and drink bottle contains equal amounts of fiber. (0-10 g)**
2. **Please consume 1 muffin each day. Please keep all muffins frozen as they are very moist and can accumulate mold very easily at room temperature. Follow the instructions and take the muffin out of the freezer the night before and put it in the refrigerator to thaw for the next day.**
3. **Fill the bottle with normal tap water and drink one each day. You may choose to vary the amount of water that you add due to variation in flavor. However, please add enough so that all contents are dissolved and no "sludge" is left in the bottom.**
4. **Please record each event on your compliance record.**
5. **Do not consume both products at the same time as high amounts of fiber consumed at the same time could result in GI symptoms.**
6. **Please double check us and make sure that the same number and color is on each of the products that we give you.**

THANKS FOR BEING SUCH COMPLIANT SUBJECTS!!!

DXA Results

1. **We cannot diagnose osteoporosis or osteopenia**
2. **Explain total body chart first.**

   Definitions:

   BMD : Bone Mineral Density (g mineral per cm^2 bone area)

   BMC:- g mineral in that bone

   YA T-score: a T score of 0 means your BMD is equal to the norm for a healthy young adult . A T-score between +1 and -1 is considered normal. A T-score between -1 and -2.5 indicates that you have low bone mass.

   Age matched Z score: your measurement compared to that of women your age and weight.

   Lines on colored chart are mean ± 1 SD of age matched Z score

Ask subjects to share these results with their physician

Effect of Soluble Corn Fiber (SCF) on Calcium Absorption in Post Menopausal Women

Subject ID_____ **Date** _____/_____/_____
MM DD Year

Mark an 'X' on the line next to each symptom to indicate the degree to which you experienced each of these symptoms for each specified time period. The more discomfort you feel, the larger the number. Your mark may fall anywhere on the line. It does not have to fall directly on one of the numbers. Please rate your experience of abdominal pain, bloating, flatulence, and diarrhea/loose stools at the end of **EACH 10 day period, i.e the day you collect urine**. Indicate any medicines you have taken and answer the remaining questions about bowel movements and how you felt during the week.

Abdominal Pain

0	1	2	3	4	5
None	Slight	Mild	Moderate	Moderately Severe	Severe

Bloating

0	1	2	3	4	5
None	Slight	Mild	Moderate	Moderately Severe	Severe

Flatulence (Gas)

0	1	2	3	4	5
None	Slight	Mild	Moderate	Moderately Severe	Severe

Diarrhea

0	1	2	3	4	5
None	Slight	Mild	Moderate	Moderately Severe	Severe

Stomach Noises

0	1	2	3	4	5
None	Slight	Mild	Moderate	Moderately Severe	Severe

1. Did you take any medication for pain or discomfort during the week? Yes No

If yes, circle the type.

Antacids Ibruprofen Acetaminophen Aspirin Antibiotic Other(specify)_____

2. How frequently did you go poop this week? _____

3. On average, what type (hard, soft, runny) of poop did you have this week? _____

4. How did you feel overall physically for the week? (Check the appropriate box)

Excellent	Very Good	Same as usual	Poor	Very Poor

TL41 Visit 1 Check sheet

1. **_____ Verify Fasting Status**
2. **_____ Verify Medical Status, Review Screening Form**
3. **_____ Receive Diet Record**
4. **_____ Receive Compliance Calendar**
5. **_____ Explain DXA results (see Explanation of DXA)**
6. **_____ Explain muffin and drink protocol (see Instructions for Muffins and Drinks)**
7. **_____ Explain symptom sheet (deliver with 1st and 3rd urine pick up)**
8. **_____ Collect Fasting urine**
9. **_____ Collect Fasting Blood (Fill out Request form for Doug)**
10. **_____ Deliver Muffins and record quantity**
11. **_____ Deliver Drinks and record quantity**
12. **_____ Make plan for delivery of additional muffins and drinks (preferable to deliver the rest of the products at the time we pick up the second urine collection but depends on how many muffins they can store)**
13. **_____ Review dates for urine collection and next appointment (note any changes from original planned dates)**
14. **_____ Review Compliance Record Sheet**
15. **_____ Deliver additional urine bottles if necessary**
16. **_____ Complete Subject Invoice**

Hints to Make Diet Records More Accurate

- Portions
  - Peanut butter, dressings, other condiments:
    - Typical servings are 2 Tablespoons = size of a ping pong ball
  - Pizza: Slices or squares?
    - Large= 14"
    - Medium = 12"
    - Small = 10"
  - Popcorn: Personal 100 calorie bag or regular large bag?
  - Chips: Potato chips or pretzels?
    - 1 small handful = ½ cup.
    - 1 personal bag = 1 oz
  - Cheese slices: Cracker sized, pre-sliced sandwich sized, hand cut size?
    - 1 stick of cheese = 1 oz
  - Cereal and Oatmeal: was it "1.75 cups" dry or prepared?
  - Frozen meals: Please write down weight of package
  - Meat:
    - Thickness and size of your palm = 3 oz
  - Candy (eg Reese's) can come in all different sizes- please write down weight if possible so there is no miscommunication
  - Soup:
    - If from a can: how many cups?

- Preparation
  - Veggies: Raw, boiled, grilled, steamed, sautéed?
    - If sautéed—did you use butter or oil? Did you add salt?
  - Meat: Fried, grilled, baked
    - Marinated? Coated?
    - Butter, salt?

- What kind?
  - Cookie: Oreo, homemade chocolate chip, bakery snicker doodle?
  - Creamer: liquid or powder? Did you use a packet, a tub, a spoon?
  - Tea and Iced tea: sweetened?
  - Milk: Whole, 2%, skim?
  - Yogurt: Made with what kind of milk? Give brand name if possible
  - Bread: Whole wheat, white, with oats?
  - Ice cream: Plain vanilla, double chocolate with fudge, containing candy pieces?
  - Wine: Red or White?
  - Juice: Orange juice, apple juice, cranberry juice?

- Pizza:
  - From a restaurant? Brand name?
  - Thin crust, thick crust, hand tossed
  - Toppings?

- Extra Detail
  - Salads:
    - Do your best to portion each ingredient added, our program doesn't have all the restaurant ones readily available
  - Restaurant Entrées:
    - Did it come with sides?
  - Sandwiches and Burgers: Be specific about toppings!
    - Meats, ketchup, mustard, lettuce, cheese, tomatoes, onions, etc
  - Baked Potatoes:
    - Butter, sour cream, onions, cheese, bacon?
  - Cakes and cupcakes:
    - Does it have icing? What kind?
  - Fruit: Fresh or canned?
    - Canned: Syrup pack or water pack?
  - Fries, potato wedges, chips, etc:
    - Ketchup, mustard, BBQ sauce, ranch?
  - Almonds:
    - Dry roasted or honey roasted?
    - Salted? Cocoa dusted?
  - Drinks:
    - With ice?

4-Day Food Record

Name of Participant:

_____

Day (e.g., Friday)	Date

First Day

Date: _____

Vitamins and Mineral Supplements

Did you take vitamins or minerals today? Y N

If yes, check the type you used:

☐ One-a-day type multivitamins with minerals, such as Centrum A-Z. State Brand: *(Example: Flintstones Gummies)*

_____

☐ Herbal supplement, such as ginseng. State Brand: _____

Did you take any vitamins or minerals as a separate pill? Y N

If yes, check the type you used:

		Dose per tablet or spoon	# of tablets/spoons per day	Brand Name
__	Vitamin C	_____mg	_____	_____
__	Vitamin B	_____mg	_____	_____
__	Vitamin E	_____IU	_____	_____
__	Vitamin A	_____IU	_____	_____
__	β-carotene	_____IU	_____	_____
__	Calcium	_____mg	_____	_____
__	Iron	_____mg	_____	_____
__	Folic Acid	_____ug	_____	_____
__	Other	_____ mg/ug	_____	_____

Don't forget to fill out the recipe pages at the end of each day's entry if you ate something like a casserole, etc. that had a recipe.

Meal (and time) B=Breakfast L=Lunch D=Dinner **S**=Snacks		**Place Prepared** **H**=Home **R**=Restaurant O=Other	**Foods and Beverages**	**Amount**
1				
2				
3				
4				
5				
6				

7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				

Recipe 1: _____

Number of servings recipe made: _____
Number of servings you ate: _____

Ingredients: *Amount:*

Recipe 2: _____

Number of servings recipe made: _____
Number of servings you ate: _____

Ingredients: *Amount:*

Notes:

Instructions for 4-day Diet Record

Please record all foods you eat in the **Food Record** booklet. Record foods you eat for a total of **four days**, one of which should be a weekend day. The days do not have to be consecutive days. Please return the completed form when you come for your next clinical visit.

Diet Record Information

Please use the following pages to record your food and beverage intake over a FOUR DAY PERIOD. It is important that you be as accurate as possible in completing the food record. Eat what you would normally eat. Follow the instructions given below, and don't hesitate to contact Berdine (494-6559) if you have any questions. Thank you and have fun!

1. Your record for each day should start from 12:01am and end 11:59pm.

2. Record everything you eat, drink or swallow in each 24-hour period, in the order in which you eat them. Record each food when taken or immediately after so that you don't forget to mark them down.

3. There are two blank boxes on the diet record sheet. In the first box, please indicate which meal you are eating (B-breakfast, L-lunch, D-dinner, S-snacks). In the second box, please indicate where the meal is prepared (H-home, R-restaurant, O-other). Describe each food and indicate whether it is raw or cooked. If it is cooked, write how it is prepared (boiled, steamed, fried, broiled, grilled, baked etc), and how it is served (for example: with butter, cream sauce, dressing etc).

4. Record the amount of everything you eat, drink or swallow. Please estimate portion sizes of everything, in cups, spoons, ounces, grams, or by comparison with a common object (for example, "a piece of steak is the size of a deck of cards"). See the attached sheet for samples of portion sizes.

5. Record the place where you consumed the item.

6. Please record any supplements you take, such as vitamins, minerals and herbs, in addition to all your food and drinks.

7. If you eat a mixed food such as a casserole, please at least specify the major ingredients. If you have the recipe please include that also.

Consider the following tips as you record the beverages and foods you eat.

B.E. C.O.M.P.L.E.T.E.

- **B**rand: When possible, indicate the brand of processed foods, such as Dannon yogurt, Meijer waffles, etc. For frozen dinners, indicate the brand and name of the meal.

- **E**xtra: Remember to record salad dressing, butter/sour cream, sugar or cream in tea or coffee, mayonnaise, syrup, etc.

- **C**ut: For meats, specify the cut if possible. When using ground beef, list the type: regular, ground round, ground chuck, ground sirloin, etc. For chicken, indicate which piece(s) of chicken you ate (leg, breast, etc.)

- **O**rder-in foods: indicate restaurant name and how it was cooked.

- **M**odification: It will always be assumed that you used the regular version of a food unless you indicate that the food was low-fat, low-sugar, low-sodium, calcium-fortified, etc.

- **P**reparation: Specify the way your food was cooked – such as fried, baked, boiled, etc. Be sure to list any added fat.

- **L**ive the way you were. Do not alter your dietary habits.

- **E**ating outside: If you eat your food away from home, be sure to list the restaurant name and how it was cooked.

- **T**horoughness: Think "detail". Tell us whether you ate white or whole wheat bread; butter vs. margarine; skin on your potato or chicken; whole, 2%, or skim milk; regular or flavored milk; fresh, frozen, or canned juices or fruit; sweetened or unsweetened tea; hot chocolate made with water or milk.

- **E**ating portions: When possible, measure portions using cups, teaspoons, etc. until you are familiar with portion sizes.

Picturing Portions

A "portion" is the amount of food you actually eat. As much as possible, measure your food when you keep your diet records. When that is not possible, you can compare the size or amount to common objects, such as:

= A medium potato is the size of a computer mouse

= A medium size fruit or vegetable is the size of your clenched fist

= ½ cup of rice, pasta, cereal, or chopped vegetables or fruit is a rounded handful

= ¼ cup of dried fruit or raisins in the size of a golf ball

= An average bagel is the size of a hockey puck

= A pancake or a slice of bread is the size of a compact disk

= A cup of fruit is the size of a baseball

= A cup of lettuce is four leaves

= Three ounces of cooked meat or poultry is the size of a cassette tape or a deck of cards

= Three ounces of grilled fish is the size of a checkbook

= One ounce of cheese is the size of four dice or two dominoes

= One teaspoon of salad dressing is the size of a thumb tip

= Two tablespoons of peanut butter is the size of a ping-pong (table tennis) ball

= One cup of cooked dry beans is the size of a tennis ball

= An ounce of nuts or small candies is one small handful

= An ounce of chips or pretzels is a large handful

COMPLIANCE RECORD		August	2014					
ID			PHASE					
DAY	DATE	Muffin	Drink	Supplement	Urine	Feces	Diet Record	Visit
Fri	1							
Sat	2							
Sun	3							
Mon	4							
Tues	5							
Wed	6							
Th	7							
Fri	8							
Sat	9							
Sun	10							
Mon	11							
Tues	12							
Wed	13							
Th	14							
Fri	15							
Sat	16							
Sun	17							
Mon	18							
Tues	19							
Wed	20							
Th	21							
Fri	22							
Sat	23							
Sun	24							
Mon	25							
Tues	26							
Wed	27							
Thurs	28							
Fri	29							
Sat	30							
Sun	31							

24 hour urine collection instructions

On the day of your collection, void, but **do not** collect, your first void of the morning.

Write the **date** and **time** of your first void of the day on the urine container. This starts the timing of your collection.

Collect ALL urine for the next 24 hours.

The first void of the next morning will be the last collection of the 24 hour specimen.

Write the date and time of the first void of the day on each urine container used.

Example:

Wake up at 7:00am and void. **Discard** urine. Beginning of collection is at 7:00am. Write the date and 7:00am on the label. Collect all urine from morning, afternoon and evening. If you get up during your sleeping hours, collect this urine also. Next morning, wake up at 7:00am. Collect this specimen as your last void of the 24 hour collection. Write the date and 7:00am on the label.

Please use the lunch bag/cooler to hold urine bottles while you are collecting away from home. The collection does NOT have to be refrigerated.

When you are finished collecting, you may bring the bottles in the lunch bag to Stone G36, email Berdine Martin at bmartin1@purdue.edu, or call us at 494-6559 or 494-0385 for a staff member to pick up the collection.

Please keep the white urine collection container that fits in the toilet. This will be used for future collections.

B. Postmenopausal Women SCF and ^{41}Ca Study: Data

Table A.1 Bone Biomarker Concentrations

ID	Phase	BAP (U/L)	OC (ng/ml)	NTx (nM BCE/mMol Cr)
200	1	27.89	6.17	28.76
200	2	25.20	6.64	36.47
200	3	26.78	7.07	28.01
200	4	21.97	8.46	25.51
200	5	23.28	7.03	41.70
200	6	23.70	6.31	36.71
200	7	22.27	7.59	33.35
201	1	37.28	9.31	80.81
201	2	43.75	8.15	86.20
201	3	51.70	12.36	n/a
201	4	44.41	15.63	76.69
201	5	40.49	13.86	41.06
201	6	39.00	10.60	53.49
201	7	41.03	11.13	25.77
202	1	22.52	7.92	22.48
202	2	31.79	7.63	29.41
202	3	27.03	4.94	35.81
202	4	37.37	7.31	31.24
202	5	25.82	9.06	32.11
202	6	27.22	8.80	32.53
202	7	n/a	n/a	n/a
204	1	24.28	12.57	68.25
204	2	23.16	11.65	88.26
204	3	23.40	14.20	92.72
204	4	30.11	15.10	64.22
204	5	36.81	13.79	98.55
204	6	24.91	13.48	37.43
204	7	25.93	7.38	76.40
205	1	34.36	11.15	54.26
205	2	28.18	12.10	62.06
205	3	34.16	13.42	80.77
205	4	30.77	11.26	36.41
205	5	28.31	11.76	n/a
205	6	25.94	12.52	45.09
205	7	26.17	9.68	57.14
206	1	28.08	4.26	16.30
206	2	30.96	4.19	29.08
206	3	28.81	5.57	18.54

ID	Phase	BAP (U/L)	OC (ng/ml)	NTx (nM BCE/mMol Cr)
206	4	31.94	5.54	24.46
206	5	28.90	5.40	46.43
206	6	33.69	5.67	35.08
206	7	33.17	5.88	32.13
208	1	35.72	11.54	40.91
208	2	40.30	12.08	48.38
208	3	36.77	9.46	44.49
208	4	34.20	10.86	51.11
208	5	35.80	9.77	39.39
208	6	34.41	n/a	98.95
208	7	33.98	n/a	47.65
209	1	21.16	10.01	54.68
209	2	23.72	10.93	54.87
209	3	21.51	10.62	59.44
209	4	22.45	11.80	61.57
209	5	21.60	10.66	55.80
209	6	22.89	9.64	40.69
209	7	22.98	10.30	59.30
210	1	22.05	7.07	33.29
210	2	21.33	7.45	27.58
210	3	20.94	8.62	28.14
210	4	23.94	7.46	29.46
210	5	21.51	6.01	31.26
210	6	21.11	8.55	23.02
210	7	23.59	8.13	37.84
211	1	30.83	13.35	100.32
211	2	29.11	12.34	70.13
211	3	45.63	19.38	73.33
211	4	29.37	12.64	69.48
211	5	27.52	12.98	95.54
211	6	28.71	9.47	73.17
211	7	23.90	10.17	63.23
213	1	36.38	15.34	93.99
213	2	29.43	12.79	n/a
213	3	37.72	15.38	135.18
213	4	35.96	14.33	92.43
213	5	33.65	15.65	64.89
213	6	36.61	16.24	64.57
213	7	33.26	14.41	48.78
214	1	26.97	13.00	47.67

ID	Phase	BAP (U/L)	OC (ng/ml)	NTx (nM BCE/mMol Cr)
214	2	25.83	13.06	56.18
214	3	18.70	12.13	48.58
214	4	30.11	10.35	56.33
214	5	27.25	14.36	41.73
214	6	29.79	12.93	40.88
214	7	26.57	10.41	35.14
218	1	18.49	8.83	64.84
218	2	17.71	12.99	43.10
218	3	19.46	8.11	57.56
218	4	18.49	9.56	50.98
218	5	17.93	9.04	46.09
218	6	18.83	9.86	44.81
218	7	18.23	7.61	45.04

Table A.2: Urinary ^{41}Ca

Subject ID	Urine ID	Treatment	Days Post Dose	^{41}Ca:Ca (10^{-10})	^{41}Ca:Ca (10^{-10}) 1σ	Urine Volume (ml)	Pellet Weight (g)
200	U0.1	Baseline	108.00	4.042	0.176	1783.0	0.784
200	U0.2	Baseline	118	3.411	0.083	2021.2	0.4987
200	U0.3	Baseline	129	3.012	0.173	1383.9	1.194
200	U0.4	Baseline	137	2.766	0.079	1830.4	0.7999
200	U0.5	Baseline	147	2.499	0.087	1959.1	0.8401
200	U1.1	843	158	2.325	0.106	2135.0	0.8075
200	U1.2	843	168	2.418	0.069	2580.3	0.5274
200	U1.3	843	178	1.990	0.063	1765.8	0.6464
200	U1.4	843	187	1.937	0.095	2613.0	0.5264
200	U1.5	843	198	1.733	0.079	1648.9	0.8097
200	U2.1	R1	209	1.713	0.056	1172.4	1.7263
200	U2.2	R1	222	1.809	0.107	1936.1	0.5947
200	U2.3	R1	229	1.793	0.063	1348.8	1.0529
200	U2.4	R1	239	1.560	0.049	1655.7	0.7218
200	U2.5	R1	248	1.460	0.045	1364.8	1.1531
200	U3.1	709	260	1.369	0.051	2024.7	0.5355
200	U3.2	709	269	low current	low current	938.0	1.9424
200	U3.3	709	278	1.312	0.042	1326.3	1.1859
200	U3.4	709	289	1.300	0.042	2366.8	0.5519
200	U3.4	709	298	1.388	0.052	2018.2	0.6477
200	U4.1	R2	309	1.398	0.053	1959.4	0.7827
200	U4.2	R2	319	1.534	0.062	1170.9	1.1728
200	U4.3	R2	328	1.290	0.060	1468.4	1.1309
200	U4.4	R2	338	1.342	0.053	1510.1	0.8999
200	U4.5	R2	353	1.349	0.052	1670.5	0.766
200	U5.1	576	362	1.370	0.057	2142.7	0.5471
200	U5.2	576	372	1.336	0.089	2473.2	0.41
200	U5.3	576	381	1.314	0.056	1298.6	1.1624
200	U5.4	576	392	1.251	0.059	1454.2	1.2169
200	U5.5	576	401	1.300	0.052	1756.7	0.6389
200	U6.1	R3	413	1.562	0.039	1687.5	0.5625
200	U6.2	R3	423	1.319	0.052	1576.7	0.7077
200	U6.3	R3	431	1.444	0.049	1455.8	1.3742
200	U6.4	R3	441	1.512	0.063	1523.8	1.1524
200	U6.5	R3	451	1.533	0.072	2065.9	0.7363

Subject ID	Urine ID	Treatment	Days Post Dose	$^{41}Ca:Ca$ (10^{-10})	$^{41}Ca:Ca$ (10^{-10}) 1σ	Urine Volume (ml)	Pellet Weight (g)
201	U0.1	Baseline	111	4.781	0.198	587.3	0.9829
201	U0.2	Baseline	120	4.088	0.175	709.1	0.5951
201	U0.3	Baseline	129	3.667	0.135	680.9	0.2783
201	U0.4	Baseline	139	3.117	0.129	647.0	0.7892
201	U0.5	Baseline	149	3.104	0.079	243.2	0.5962
201	U1.1	709	160	2.389	0.097	884.8	0.1
201	U1.2	709	170	2.803	0.135	364.9	0.1953
201	U1.3	709	179	2.134	0.063	583.6	1.0441
201	U1.4	709	189	1.990	0.067	976.4	0.0
201	U1.5	709	203	2.257	0.067	190.3	0.5837
201	U2.1	R1	214	2.246	0.121	1030.8	0.1
201	U2.2	R1	225	1.816	0.051	644.3	0.7625
201	U2.3	R1	231	1.870	0.057	414.5	0.5226
201	U2.4	R1	244	1.503	0.060	710.0	0.1
201	U2.5	R1	255	1.750	0.055	1275.9	0.1711
201	U3.1	843	273	1.510	0.044	592.5	0.3028
201	U3.2	843	280	1.515	0.034	1646.1	0.2927
201	U3.3	843	293	1.626	0.080	565.1	0.5297
201	U3.4	843	303	1.619	0.045	323.8	0.5954
201	U3.5	843	310	1.676	0.073	324.2	0.6058
201	U4.1	R2	324	1.562	0.061	1011.6	0.2281
201	U4.2	R2	332	1.423	0.064	751.0	0.6484
201	U4.3	R2	341	1.486	0.050	1172.4	0.3439
201	U4.4	R2	354	1.485	0.096	519.5	0.5
201	U4.5	R2	364	1.452	0.239	572.4	0.3203
201	U5.1	576	379	1.521	0.053	416.4	0.9302
201	U5.2	576	389	1.486	0.060	936.9	0.4507
201	U5.3	576	401	1.596	0.054	272.0	0.3243
201	U5.4	576	409	1.691	0.034	291.0	0.1541
201	U5.5	576	419	1.636	0.036	944.4	0.3365
201	U6.1	R3	430	n/a	n/a	n/a	n/a
201	U6.2	R3	442	1.461	0.041	687.1	0.6086
201	U6.3	R3	449	1.408	0.066	1482.4	0.5954
201	U6.4	R3	465	1.664	0.054	357.8	0.5839
201	U6.5	R3	470	1.481	0.065	1804.8	0.3737

Subject ID	Urine ID	Treatment	Days Post Dose	$^{41}Ca:Ca$ (10^{-10})	$^{41}Ca:Ca$ (10^{-10}) 1σ	Urine Volume (ml)	Pellet Weight (g)
202	U0.1	Baseline	112	3.185	0.165	1306.7	0.7804
202	U0.2	Baseline	120	2.629	0.102	1450.3	0.609
202	U0.3	Baseline	132	2.183	0.085	1243.4	2.0846
202	U0.4	Baseline	141	1.878	0.203	869.6	2.1672
202	U0.5	Baseline	149	1.866	0.063	969.1	1.0015
202	U1.1	843	159	1.840	0.076	2005.2	0.7676
202	U1.2	843	168	1.700	0.061	1361.0	0.7928
202	U1.3	843	179	1.827	0.105	1826.0	0.2377
202	U1.4	843	187	1.513	0.071	1450.7	0.7931
202	U1.5	843	203	1.335	0.054	1239.8	1.2535
202	U2.1	R1	215	1.484	0.100	792.6	1.1379
202	U2.2	R1	223	1.187	0.033	1612.6	1.7376
202	U2.3	R1	230	1.052	0.054	1127.6	1.0001
202	U2.4	R1	243	1.200	0.044	807.5	0.9178
202	U2.5	R1	256	1.235	0.068	1036.6	1.1566
202	U3.1	576	269	0.924	0.051	729.7	1.1581
202	U3.2	576	277	1.003	0.031	1440.5	0.9293
202	U3.3	576	291	1.054	0.042	1129.5	0.8565
202	U3.4	576	297	0.984	0.030	1295.1	0.6641
202	U3.5	576	310	1.052	0.068	1027.3	1.0796
202	U4.1	R2	323	1.020	0.048	1089.5	1.3132
202	U4.2	R2	332	0.987	0.039	1974.1	0.2215
202	U4.3	R2	n/a	n/a	n/a	n/a	n/a
202	U4.4	R2	354	0.937	0.039	1718.0	0.5503
202	U4.5	R2	364	0.880	0.033	1623.0	0.4267
202	U5.1	709	375	0.866	0.028	2504.9	0.3083
202	U0.1	Baseline	112	3.185	0.165	1306.7	0.7804
202	U0.2	Baseline	120	2.629	0.102	1450.3	0.609
202	U0.3	Baseline	132	2.183	0.085	1243.4	2.0846
202	U0.4	Baseline	141	1.878	0.203	869.6	2.1672
202	U0.5	Baseline	149	1.866	0.063	969.1	1.0015
202	U1.1	843	159	1.840	0.076	2005.2	0.7676
202	U1.2	843	168	1.700	0.061	1361.0	0.7928
202	U1.3	843	179	1.827	0.105	1826.0	0.2377

Subject ID	Urine ID	Treatment	Days Post Dose	^{41}Ca:Ca (10^{-10})	^{41}Ca:Ca (10^{-10}) 1σ	Urine Volume (ml)	Pellet Weight (g)
204	U0.1	Baseline	5267	8.053	0.175	1946.1	0.4798
204	U0.2	Baseline	5277	9.779	0.296	1433.4	0.6263
204	U0.3	Baseline	5287	10.755	0.568	1886.1	0.5172
204	U0.4	Baseline	5297	9.355	0.227	1596.5	0.5607
204	U0.5	Baseline	5306	9.845	0.365	1598.2	0.513
204	U1.1	843	5317	9.643	0.372	2015.5	0.4991
204	U1.2	843	5327	9.561	0.286	1650.4	0.5339
204	U1.3	843	5336	9.673	0.354	1667.1	0.5639
204	U1.4	843	5346	9.956	0.409	1754.3	0.5656
204	U1.5	843	5357	9.822	0.372	2255.8	0.4358
204	U2.1	R1	5368	10.369	0.245	1500.2	0.6019
204	U2.2	R1	5378	9.642	0.159	1648.3	0.3774
204	U2.3	R1	5388	9.620	0.188	1805.2	0.4205
204	U2.4	R1	5398	9.274	0.183	1709.4	1.0524
204	U2.5	R1	5407	9.333	0.236	1607.2	0.6353
204	U3.1	709	5421	9.339	0.232	1581.6	0.621
204	U3.2	709	5428	9.557	0.296	1813.2	0.4147
204	U3.3	709	5437	9.997	0.212	1628.6	0.5609
204	U3.4	709	5448	9.939	0.255	1867.7	0.4282
204	U3.5	709	5457	10.044	0.245	2424.7	0.4481
204	U4.1	R2	5468	10.396	0.346	1315.8	0.5815
204	U4.2	R2	5478	9.939	0.231	1653.7	0.353
204	U4.3	R2	5487	9.668	0.212	1959.9	0.3699
204	U4.4	R2	5497	9.061	0.340	2108.0	0.4857
204	U4.5	R2	5512	9.972	0.241	1655.5	0.5043
204	U5.1	576	5521	10.152	0.284	1905.2	0.4581
204	U5.2	576	5528	10.414	0.291	1729.0	0.5035
204	U5.3	576	5540	10.459	0.216	2177.5	0.4696
204	U5.4	576	5551	10.045	0.280	2273.5	0.4015
204	U5.5	576	5560	9.253	0.269	2168.3	0.3807
204	U6.1	R3	5572	9.492	0.199	2070.3	0.3367
204	U6.2	R3	5581	9.218	0.177	2120.1	0.2987
204	U6.3	R3	5590	8.709	0.274	2633.7	0.3631
204	U6.4	R3	5600	9.570	0.372	2248.9	0.3168
204	U6.5	R3	5610	9.551	0.237	2381.0	0.4153

Subject ID	Urine ID	Treatment	Days Post Dose	^{41}Ca:Ca (10^{-10})	^{41}Ca:Ca (10^{-10}) 1σ	Urine Volume (ml)	Pellet Weight (g)
205	U0.1	Baseline	117	3.072	0.127	1816.9	0.2497
205	U0.2	Baseline	118	2.807	0.089	1686.8	0.4403
205	U0.3	Baseline	128	2.452	0.086	1875.2	0.4519
205	U0.4	Baseline	138	2.213	0.054	1652.5	0.1666
205	U0.5	Baseline	147	2.095	0.051	2384.8	0.3105
205	U1.1	576	159	2.071	0.084	2098.0	0.2857
205	U1.2	576	166	1.814	0.064	2345.4	0.1551
205	U1.3	576	179	1.734	0.037	2003.6	0.2405
205	U1.4	576	188	1.776	0.064	2067.6	0.185
205	U1.5	576	197	1.770	0.048	2119.3	0.1881
205	U2.1	R1	207	1.699	0.076	1962.6	0.1655
205	U2.2	R1	217	1.512	0.042	1928.0	0.4328
205	U2.3	R1	229	1.503	0.058	1758.7	0.1666
205	U2.4	R1	236	1.419	0.038	1392.9	0.2352
205	U2.5	R1	248	1.395	0.041	2164.3	0.7107
205	U3.1	709	260	1.257	0.049	2067.6	0.5131
205	U3.2	709	269	1.275	0.054	2848.0	0.36
205	U3.3	709	280	1.302	0.038	2678.9	0.2316
205	U3.4	709	291	1.295	0.046	2254.2	0.5205
205	U3.5	709	298	1.279	0.035	2158.9	0.5244
205	U4.1	R2	310	1.173	0.048	1789.5	0.2394
205	U4.2	R2	319	1.106	0.036	2409.5	0.1914
205	U4.3	R2	327	1.106	0.061	2007.9	0.1
205	U4.4	R2	338	1.043	0.057	2436.1	0.2026
205	U4.5	R2	371	1.070	0.036	1790.5	0.4308
205	U5.1	843	384	1.020	0.036	2154.1	0.2028
205	U5.2	843	396	0.966	0.030	2526.3	0.1851
205	U5.3	843	403	0.994	0.031	2007.3	0.2624
205	U5.4	843	413	0.913	0.026	1672.0	0.3522
205	U5.5	843	422	1.262	0.034	1755.3	0.1388
205	U6.1	R3	433	0.950	0.044	2817.0	0.2565
205	U6.2	R3	442	0.877	0.028	2667.7	0.222
205	U6.3	R3	452	0.893	0.037	2331.4	0.3252
205	U6.4	R3	463	0.917	0.034	2392.4	0.2155
205	U6.5	R3	472	0.932	0.035	2567.1	0.276

Subject ID	Urine ID	Treatment	Days Post Dose	$^{41}Ca:Ca$ (10^{-10})	$^{41}Ca:Ca$ (10^{-10}) 1σ	Urine Volume (ml)	Pellet Weight (g)
206	U0.1	Baseline	109	6.035	0.225	1660.7	0.3132
206	U0.2	Baseline	120	4.933	0.173	1621.6	0.4491
206	U0.3	Baseline	130	3.917	0.156	1941.2	0.4915
206	U0.4	Baseline	139	3.537	0.103	977.4	1
206	U0.5	Baseline	148	3.854	0.113	1001.1	0.5444
206	U1.1	843	160	3.468	0.144	1000.9	0.3884
206	U1.2	843	170	3.154	0.093	1013.6	0.8476
206	U1.3	843	182	3.142	0.108	998.1	0.622
206	U1.4	843	189	3.249	0.177	872.2	0.4687
206	U1.5	843	197	2.689	0.095	951.4	0.9602
206	U2.1	R1	209	2.605	0.069	1002.6	1.6388
206	U2.2	R1	218	2.635	0.086	998.4	0.7867
206	U2.3	R1	229	2.320	0.067	947.6	0.8933
206	U2.4	R1	237	2.276	0.069	1241.0	0.8849
206	U2.5	R1	250	2.167	0.056	975.1	0.8578
206	U3.1	576	260	2.128	0.064	941.9	0.2615
206	U3.2	576	270	1.746	0.065	600.0	1.5182
206	U3.3	576	281	1.914	0.049	976.7	0.5814
206	U3.4	576	297	1.774	0.039	1390.2	0.421
206	U3.5	576	301	1.795	0.072	1034.4	0.5774
206	U4.1	R2	312	1.716	0.058	1000.8	0.3819
206	U4.2	R2	321	1.628	0.046	1186.9	0.4838
206	U4.3	R2	332	1.615	0.051	999.9	0.7112
206	U4.4	R2	341	1.519	0.058	1250.5	0.5584
206	U4.5	R2	355	1.443	0.069	689.5	0.8734
206	U5.1	709	365	1.559	0.060	999.5	0.8412
206	U5.2	709	376	1.490	0.058	1044.3	0.8778
206	U5.3	709	384	1.427	0.055	977.7	0.7466
206	U5.4	709	396	1.328	0.060	730.0	1.4762
206	U5.5	709	406	1.309	0.028	1005.9	0.605
206	U6.1	R3	412	1.442	0.034	931.8	0.616
206	U6.2	R3	427	1.473	0.053	1190.6	1.0491
206	U6.3	R3	436	1.286	0.049	1157.5	1.0418
206	U6.4	R3	446	1.242	0.056	1455.2	1.2501
206	U6.5	R3	457	1.266	0.048	1739.3	0.8298

Subject ID	Urine ID	Treatment	Days Post Dose	$^{41}Ca:Ca$ (10^{-10})	$^{41}Ca:Ca$ (10^{-10}) 1σ	Urine Volume (ml)	Pellet Weight (g)
208	U0.1	Baseline	109	3.824	0.114	2596.4	0.3552
208	U0.2	Baseline	119	3.778	0.101	2444.0	0.4962
208	U0.3	Baseline	130	3.507	0.092	2063.6	0.1692
208	U0.4	Baseline	139	2.850	0.093	1804.0	0.9736
208	U0.5	Baseline	150	2.813	0.108	1911.9	0.538
208	U1.1	709	162	low current	low current	1772.5	0.1605
208	U1.2	709	171	2.360	0.079	2165.4	0.3263
208	U1.3	709	182	2.011	0.070	2089.3	0.3473
208	U1.4	709	191	2.258	0.096	2426.6	0.1
208	U1.5	709	199	2.478	0.115	1254.5	0.1807
208	U2.1	R1	209	2.226	0.058	2440.2	0.5552
208	U2.2	R1	220	2.072	0.057	2930.6	0.5023
208	U2.3	R1	229	2.122	0.063	1690.4	0.7449
208	U2.4	R1	239	2.172	0.096	2686.1	0.1938
208	U2.5	R1	249	1.934	0.059	1776.9	0.798
208	U3.1	576	263	1.854	0.124	1793.4	0.9359
208	U3.2	576	269	1.795	0.078	1811.5	0.3546
208	U3.3	576	278	1.713	0.054	1758.6	0.7377
208	U3.4	576	289	1.882	0.055	2191.9	0.4176
208	U3.5	576	298	1.907	0.059	1747.7	0.4465
208	U4.1	R2	309	1.992	0.064	2441.4	0.3478
208	U4.2	R2	320	1.894	0.087	1798.8	0.4722
208	U4.3	R2	330	1.891	0.081	1667.3	0.4952
208	U4.4	R2	339	1.852	0.079	1793.5	0.3861
208	U4.5	R2	353	1.719	0.081	1916.2	0.4661
208	U5.1	843	361	1.700	0.073	1661.7	0.5005
208	U5.2	843	372	1.748	0.073	2358.7	0.4249
208	U5.3	843	382	1.736	0.066	1905.0	0.3946
208	U5.4	843	392	1.780	0.046	2146.9	0.4483
208	U5.5	843	402	1.706	0.066	1914.8	0.3455
208	U6.1	R3	413	1.688	0.040	2134.0	0.2066
208	U6.2	R3	423	low current	low current	1683.0	0.3155
208	U6.3	R3	431	1.603	0.050	2133.1	0.4154
208	U6.4	R3	441	1.599	0.042	2059.7	0.3632
208	U6.5	R3	454	1.484	0.058	2227.0	0.1249

Subject ID	Urine ID	Treatment	Days Post Dose	^{41}Ca:Ca (10^{-10})	^{41}Ca:Ca (10^{-10}) 1σ	Urine Volume (ml)	Pellet Weight (g)
209	U0.1	Baseline	127	2.454	0.126	901.0	1.2181
209	U0.2	Baseline	136	2.234	0.094	944.9	0.8076
209	U0.3	Baseline	148	2.169	0.062	745.9	0.6658
209	U0.4	Baseline	156	1.640	0.077	814.8	2.7162
209	U0.5	Baseline	166	2.218	0.070	647.5	0.3035
209	U1.1	709	181	2.139	0.062	854.4	0.3848
209	U1.2	709	191	2.039	0.077	862.7	0.637
209	U1.3	709	202	2.168	0.048	544.0	0.2039
209	U1.4	709	210	1.886	0.049	1003.4	0.3985
209	U1.5	709	221	1.701	0.068	857.3	0.8844
209	U2.1	R1	235	1.601	0.037	1266.8	0.705
209	U2.2	R1	242	1.638	0.082	751.6	1.3194
209	U2.3	R1	252	1.472	0.056	969.7	1.6456
209	U2.4	R1	264	1.584	0.068	581.5	1.2091
209	U2.5	R1	273	1.597	0.036	540.2	0.963
209	U3.1	576	287	1.571	0.054	842.1	0.3365
209	U3.2	576	296	1.423	0.080	899.2	0.907
209	U3.3	576	306	1.545	0.059	495.1	0.7302
209	U3.4	576	318	1.349	0.052	638.6	1.0896
209	U3.5	576	328	1.423	0.049	1118.7	0.3295
209	U4.1	R2	343	0.817	0.094	648.0	1.4602
209	U4.2	R2	349	1.271	0.043	856.3	0.4248
209	U4.3	R2	360	1.064	0.054	577.0	1.8086
209	U4.4	R2	368	1.183	0.034	471.9	0.681
209	U4.5	R2	378	1.098	0.063	691.4	1.1438
209	U5.1	843	390	1.174	0.025	813.4	0.458
209	U5.2	843	399	1.116	0.033	939.7	0.9373
209	U5.3	843	410	1.123	0.030	722.6	1.3238
209	U5.4	843	419	1.118	0.042	518.2	1.6781
209	U5.5	843	430	0.952	0.040	687.0	0.3761
209	U6.1	R3	440	1.152	0.056	787.7	1.0645
209	U6.2	R3	451	1.077	0.055	736.6	0.9405
209	U6.3	R3	462	1.129	0.111	703.0	1.7891
209	U6.4	R3	471	0.918	0.028	816.8	1.5009
209	U6.5	R3	482	0.984	0.059	532.6	1.0537

Subject ID	Urine ID	Treatment	Days Post Dose	$^{41}Ca:Ca$ (10^{-10})	$^{41}Ca:Ca$ (10^{-10}) 1σ	Urine Volume (ml)	Pellet Weight (g)
210	U0.1	Baseline	112	4.208	0.224	618.5	1.5997
210	U0.2	Baseline	120	3.834	0.126	584.0	1.2173
210	U0.3	Baseline	129	3.964	0.128	974.8	0.7234
210	U0.4	Baseline	139	3.260	0.126	521.5	1.0821
210	U0.5	Baseline	149	3.335	0.101	1186.2	0.5916
210	U1.1	576	159	2.644	0.091	415.9	1.1883
210	U1.2	576	170	3.169	0.106	652.2	1.0977
210	U1.3	576	179	2.992	0.158	782.0	0.59
210	U1.4	576	190	2.784	0.131	775.2	1.2872
210	U1.5	576	201	2.548	0.080	840.8	0.7722
210	U2.1	R1	210	2.114	0.061	740.5	1.3622
210	U2.2	R1	221	2.236	0.107	819.4	0.9791
210	U2.3	R1	230	1.958	0.049	7.8	1.0093
210	U2.4	R1	242	2.064	0.085	505.3	1.0689
210	U2.5	R1	250	1.889	0.094	620.4	2.0028
210	U3.1	843	259	1.752	0.063	798.2	1.3818
210	U3.2	843	270	1.927	0.060	649.0	1.3233
210	U3.3	843	282	1.842	0.046	1210.5	0.5575
210	U3.4	843	291	1.872	0.054	889.8	0.5057
210	U3.5	843	300	1.702	0.082	538.9	1.583
210	U4.1	R2	311	1.698	0.078	676.3	1.0416
210	U4.2	R2	320	1.550	0.061	732.5	1.2039
210	U4.3	R2	332	1.561	0.053	1162.2	0.4985
210	U4.4	R2	342	1.611	0.119	596.3	0.9433
210	U4.5	R2	360	1.575	0.062	735.8	1.4549
210	U5.1	709	371	1.652	0.050	806.6	0.856
210	U5.2	709	382	1.224	0.055	821.2	0.9849
210	U5.3	709	391	1.440	0.038	1008.9	0.3946
210	U5.4	709	402	1.341	0.040	716.5	1.1235
210	U5.5	709	413	1.421	0.056	820.9	1.8697
210	U6.1	R3	422	1.458	0.051	536.8	1.1758
210	U6.2	R3	432	1.452	0.064	991.9	0.6382
210	U6.3	R3	444	1.567	0.074	590.3	1.0864
210	U6.4	R3	453	1.582	0.076	553.3	0.6386
210	U6.5	R3	463	1.715	0.138	654.3	1.053

Subject ID	Urine ID	Treatment	Days Post Dose	$^{41}Ca:Ca$ (10^{-10})	$^{41}Ca:Ca$ (10^{-10}) 1σ	Urine Volume (ml)	Pellet Weight (g)
211	U0.1	Baseline	100	2.837	0.123	1240.3	1.9044
211	U0.2	Baseline	113	2.900	0.113	1857.3	0.5961
211	U0.3	Baseline	120	2.658	0.110	893.2	1.1252
211	U0.4	Baseline	132	2.443	0.100	1303.8	0.7567
211	U0.5	Baseline	142	2.192	0.082	1036.4	1.8579
211	U1.1	709	153	2.293	0.091	1369.4	1.1437
211	U1.2	709	162	1.975	0.061	862.3	1.4494
211	U1.3	709	173	2.170	0.106	1209.2	0.8178
211	U1.4	709	183	2.013	0.064	981.0	1.0703
211	U1.5	709	193	1.932	0.114	1180.8	0.8794
211	U2.1	R1	204	1.734	0.046	866.1	1.438
211	U2.2	R1	214	1.722	0.048	816.1	0.9064
211	U2.3	R1	221	1.528	0.051	1115.5	0.7783
211	U2.4	R1	235	1.553	0.051	1065.6	0.9378
211	U2.5	R1	244	1.502	0.088	769.5	1.5987
211	U3.1	843	256	1.595	0.051	1225.3	2.0109
211	U3.2	843	266	1.627	0.053	1354.5	0.9323
211	U3.3	843	277	1.775	0.064	1466.2	0.8025
211	U3.4	843	283	1.557	0.065	670.7	1.4932
211	U3.5	843	293	1.485	0.058	1076.9	1.4473
211	U4.1	R2	303	1.492	0.053	1231.2	0.861
211	U4.2	R2	313	1.493	0.062	958.4	0.9232
211	U4.3	R2	324	1.311	0.060	1100.3	0.934
211	U4.4	R2	335	1.472	0.076	1014.4	1.0748
211	U4.5	R2	347	1.541	0.082	1062.9	1.1948
211	U5.1	576	357	1.504	0.053	947.4	1.1524
211	U5.2	576	367	1.533	0.054	1093.0	0.9771
211	U5.3	576	377	1.354	0.068	1011.6	1.0036
211	U5.4	576	388	1.332	0.054	978.3	1.3222
211	U5.5	576	n/a	n/a	n/a	n/a	n/a
211	U6.1	R3	409	1.346	0.046	888.6	2.2772
211	U6.2	R3	419	1.322	0.046	1449.6	0.9159
211	U6.3	R3	429	1.401	0.055	1072.3	0.9807
211	U6.4	R3	443	1.443	0.047	1177.3	0.8909
211	U6.5	R3	450	1.329	0.065	1765.0	0.79

Subject ID	Urine ID	Treatment	Days Post Dose	^{41}Ca:Ca (10^{-10})	^{41}Ca:Ca (10^{-10}) 1σ	Urine Volume (ml)	Pellet Weight (g)
213	U0.1	Baseline	110	5.302	0.140	1686.3	0.3392
213	U0.2	Baseline	123	5.664	0.228	1806.2	0.1773
213	U0.3	Baseline	131	5.143	0.167	1536.7	0.4487
213	U0.4	Baseline	140	4.548	0.124	1962.9	0.3694
213	U0.5	Baseline	150	4.262	0.117	1797.4	0.175
213	U1.1	576	164	3.854	0.145	1243.7	0.937
213	U1.2	576	173	3.800	0.085	1915.0	0.1775
213	U1.3	576	184	3.403	0.114	1899.8	0.4181
213	U1.4	576	193	3.165	0.100	1718.7	0.1
213	U1.5	576	200	3.243	0.061	1892.7	0.2204
213	U2.1	R1	212	3.657	0.207	1198.7	0.3789
213	U2.2	R1	223	3.671	0.306	1473.7	0.3335
213	U2.3	R1	230	3.435	0.084	1816.8	0.3979
213	U2.4	R1	240	3.372	0.071	1671.6	0.406
213	U2.5	R1	250	3.348	0.105	1241.9	0.4726
213	U3.1	843	264	3.050	0.120	1058.5	0.4643
213	U3.2	843	274	2.939	0.124	1164.9	0.1961
213	U3.3	843	282	3.099	0.111	1348.4	0.3028
213	U3.4	843	291	2.916	0.066	1184.4	0.6035
213	U3.5	843	295	2.825	0.055	1415.5	0.2721
213	U4.1	R2	316	2.841	0.098	1236.7	0.5207
213	U4.2	R2	324	2.893	0.088	1461.4	0.3794
213	U4.3	R2	335	2.679	0.070	1335.6	0.3189
213	U4.4	R2	352	2.412	0.088	1493.2	0.3772
213	U4.5	R2	358	2.212	0.105	1493.3	0.3328
213	U5.1	709	369	2.139	0.074	1511.6	0.6373
213	U5.2	709	380	2.063	0.056	1836.0	0.3341
213	U5.3	709	388	2.359	0.061	1831.9	0.1718
213	U5.4	709	399	2.248	0.108	1366.1	0.4886
213	U5.5	709	410	2.010	0.049	1032.0	0.2103
213	U6.1	R3	421	1.876	0.076	979.4	0.448
213	U6.2	R3	431	1.971	0.052	1182.4	0.6332
213	U6.3	R3	440	1.777	0.059	1541.9	0.4835
213	U6.4	R3	454	1.749	0.056	1037.9	0.5776
213	U6.5	R3	461	1.901	0.060	1592.0	0.4949

Subject ID	Urine ID	Treatment	Days Post Dose	$^{41}Ca:Ca$ (10^{-10})	$^{41}Ca:Ca$ (10^{-10}) 1σ	Urine Volume (ml)	Pellet Weight (g)
214	U0.1	Baseline	106	6.068	0.172	1429.8	0.6571
214	U0.2	Baseline	120	5.337	0.297	1114.1	0.4015
214	U0.3	Baseline	129	4.733	0.107	1455.5	0.3297
214	U0.4	Baseline	138	4.462	0.088	1454.9	0.2615
214	U0.5	Baseline	148	4.117	0.123	1215.4	0.6878
214	U0.6	Baseline	161	3.544	0.144	1403.1	0.2414
214	U1.1	843	173	3.300	0.075	1717.4	0.1836
214	U1.2	843	171	3.087	0.143	1314.9	0.4356
214	U1.3	843	190	3.104	0.067	1343.7	0.2865
214	U1.4	843	201	3.061	0.095	-304.2	0.3135
214	U1.5	843	211	2.865	0.108	849.7	0.6185
214	U2.1	R1	223	2.413	0.060	1539.9	0.2589
214	U2.2	R1	231	2.524	0.063	1103.2	0.3648
214	U2.3	R1	242	2.575	0.111	1417.4	0.2139
214	U2.4	R1	256	2.115	0.059	1740.0	0.2457
214	U2.5	R1	265	2.466	0.063	1128.6	0.2719
214	U3.1	709	272	2.154	0.060	1287.4	1.3149
214	U3.2	709	284	2.101	0.041	1828.8	0.2756
214	U3.3	709	293	2.099	0.054	1489.1	0.8031
214	U3.4	709	304	2.127	0.045	1298.9	0.4377
214	U3.5	709	314	2.196	0.055	321.8	0.2916
214	U4.1	R2	324	1.873	0.074	1193.0	0.4717
214	U4.2	R2	334	1.872	0.053	1702.1	0.3951
214	U4.3	R2	348	1.743	0.064	1189.1	0.5281
214	U4.4	R2	356	1.927	0.077	1697.7	0.4029
214	U4.5	R2	368	1.741	0.043	1464.2	0.3507
214	U5.1	576	380	1.637	0.038	1342.2	0.1533
214	U5.2	576	388	1.669	0.047	1757.0	0.2352
214	U5.3	576	402	1.667	0.036	1121.6	0.5539
214	U5.4	576	409	1.687	0.037	1298.0	0.445
214	U5.5	576	419	2.464	0.061	1676.9	0.1307
214	U6.1	R3	431	1.623	0.041	1758.5	0.2551
214	U6.2	R3	440	1.603	0.065	1709.2	0.4533
214	U6.3	R3	450	1.353	0.043	1401.4	0.482
214	U6.4	R3	460	1.438	0.062	1273.1	0.6016

Subject ID	Urine ID	Treatment	Days Post Dose	^{41}Ca:Ca (10^{-10})	^{41}Ca:Ca (10^{-10}) 1σ	Urine Volume (ml)	Pellet Weight (g)
218	U0.1	Baseline	2011	0.664	0.032	1169.5	0.7813
218	U0.2	Baseline	2019	0.631	0.031	1354.7	0.4013
218	U0.3	Baseline	2030	0.705	0.036	973.4	0.6235
218	U0.4	Baseline	2040	0.726	0.042	1256.0	0.5031
218	U0.5	Baseline	2051	0.856	0.393	1675.1	0.1782
218	U1.1	576	2064	no data	no data	1541.7	0.2697
218	U1.2	576	2073	0.722	0.024	2574.0	0.1
218	U1.3	576	2080	0.718	0.032	1576.1	0.9042
218	U1.4	576	2093	0.718	0.060	1122.6	0.7184
218	U1.5	576	2106	0.649	0.042	2173.6	0.1
218	U2.1	R1	2116	0.737	0.037	1488.6	1.0897
218	U2.2	R1	2127	0.662	0.045	1674.4	0.8594
218	U2.3	R1	2136	0.729	0.031	1999.5	0.8222
218	U2.4	R1	2147	0.708	0.039	1167.6	1.0162
218	U2.5	R1	2155	0.867	0.123	2024.0	0.2024
218	U3.1	709	2167	0.696	0.051	1823.5	0.4185
218	U3.2	709	2177	0.714	0.031	1416.5	0.4681
218	U3.3	709	2184	0.648	0.050	1680.8	0.334
218	U3.4	709	2197	0.679	0.034	1447.6	0.3375
218	U3.5	709	2207	0.804	0.062	1186.8	0.6348
218	U4.1	R2	2215	low current	low current	1222.7	0.3293
218	U4.2	R2	2227	low current	low current	1295.9	0.358
218	U4.3	R2	2238	0.792	0.070	1013.9	0.74
218	U4.4	R2	2247	0.751	0.024	1096.4	0.6103
218	U4.5	R2	2259	0.820	0.022	1634.6	0.3221
218	U5.1	843	2269	low current	low current	1675.4	0.2611
218	U5.2	843	2280	0.778	0.043	1241.1	0.6264
218	U5.3	843	2289	0.723	0.036	1037.0	0.7744
218	U5.4	843	2300	0.794	0.046	1424.6	0.4843
218	U5.5	843	2310	0.804	0.051	1575.5	0.6918
218	u6.1	R3	2320	0.776	0.082	826.7	1.1868
218	u6.2	R3	2331	0.771	0.082	850.8	1.0106
218	u6.3	R3	2342	0.894	0.056	1046.1	0.1659
218	u6.4	R3	2351	0.772	0.056	1374.4	0.4644
218	u6.5	R3	2362	0.792	0.070	1680.7	0.4002

C. Cecal SCFA and Calcium Absorption Study: Data

Table A.3: ^{45}Ca Kinetics Pool Size and Compartment Transfer Rates

		Control Rats		
Unit	301	301	303	303
(/min)	L(2,1)	0.076361	L(2,1)	0.076361
(/min)	L(1,2)	0.004851	L(1,2)	0.004851
(/min)	L(2,3)	0.002574	L(2,3)	0.002574
(/min)	L(3,2)	0.007893	L(3,2)	0.007893
(/min)	L(0,3)	0.001328	L(0,3)	0.001328
(/min)	L(6,1)	5.58E-05	L(6,1)	5.58E-05
(/min)	L(10,9)	0.002314	L(10,9)	0.00241
(/min)	L(1,10)	0.000636	L(1,10)	0.000481
(/min)	L(5,10)	0.005362	L(5,10)	0.005898
(/min)	L(12,11)	0.076361	L(12,11)	0.076361
(/min)	L(11,12)	0.004851	L(11,12)	0.004851
(/min)	L(12,13)	0.002574	L(12,13)	0.002574
(/min)	L(13,12)	0.007893	L(13,12)	0.007893
(/min)	L(0,13)	0.001328	L(0,13)	0.001328
(/min)	L(16,11)	5.58E-05	L(16,11)	5.58E-05
(/min)	L(20,19)	0.002314	L(20,19)	0.00241
(/min)	L(11,20)	0.000636	L(11,20)	0.000481
(/min)	L(15,20)	0.005362	L(15,20)	0.005898
(mL)	P(1)	15.9432	P(1)	15.9432
(mL)	P(11)	15.9432	P(11)	15.9432
(fract. Of dose)	P(20)	0.029523	P(20)	0.272897
(fract. Of dose)	P(16)	0.001059	P(16)	0.000206

LOW Dose Rats					
305	305	306	306	307	307
L(2,1)	0.097809	L(2,1)	0.097809	L(2,1)	0.097809
L(1,2)	0.003818	L(1,2)	0.003818	L(1,2)	0.003818
L(2,3)	0.001588	L(2,3)	0.001588	L(2,3)	0.001588
L(3,2)	0.00408	L(3,2)	0.00408	L(3,2)	0.00408
L(0,3)	0.001208	L(0,3)	0.001208	L(0,3)	0.001208
L(6,1)	0.000199	L(6,1)	0.000199	L(6,1)	0.000199
L(10,9)	0.004305	L(10,9)	0.004688	L(10,9)	0.006076
L(1,10)	0.000362	L(1,10)	0.000359	L(1,10)	0.000346
L(5,10)	0.003136	L(5,10)	0.002858	L(5,10)	0.0025
L(12,11)	0.097809	L(12,11)	0.097809	L(12,11)	0.097809
L(11,12)	0.003818	L(11,12)	0.003818	L(11,12)	0.003818
L(12,13)	0.001588	L(12,13)	0.001588	L(12,13)	0.001588
L(13,12)	0.00408	L(13,12)	0.00408	L(13,12)	0.00408
L(0,13)	0.001208	L(0,13)	0.001208	L(0,13)	0.001208
L(16,11)	0.000199	L(16,11)	0.000199	L(16,11)	0.000199
L(20,19)	0.004305	L(20,19)	0.004688	L(20,19)	0.006076
L(11,20)	0.000362	L(11,20)	0.000359	L(11,20)	0.000346
L(15,20)	0.003136	L(15,20)	0.002858	L(15,20)	0.0025
P(1)	15.9432	P(1)	15.9432	P(1)	15.9432
P(11)	15.9432	P(11)	15.9432	P(11)	15.9432
P(20)	0.202137	P(20)	0.003498	P(20)	0.189972
P(16)	0.000109	P(16)	0	P(16)	0

HIGH Dose Rats							
308	308	309	309	310	310	311	311
L(2,1)	0.073019	L(2,1)	0.073019	L(2,1)	0.073019	L(2,1)	0.07301
L(1,2)	0.004379	L(1,2)	0.004379	L(1,2)	0.004379	L(1,2)	0.00437
L(2,3)	0.00226	L(2,3)	0.00226	L(2,3)	0.00226	L(2,3)	0.0022
L(3,2)	0.007511	L(3,2)	0.007511	L(3,2)	0.007511	L(3,2)	0.00751
L(0,3)	0.001215	L(0,3)	0.001215	L(0,3)	0.001215	L(0,3)	0.00121
L(6,1)	4.59E-05	L(6,1)	4.59E-05	L(6,1)	4.59E-05	L(6,1)	4.6E-05
L(10,9)	0.002085	L(10,9)	0.002153	L(10,9)	0.002278	L(10,9)	0.00231
L(1,10)	0.000622	L(1,10)	0.000346	L(1,10)	0.001147	L(1,10)	0.00048
L(5,10)	0.004743	L(5,10)	0.003357	L(5,10)	0.012805	L(5,10)	0.00572
L(12,11)	0.073019	L(12,11)	0.073019	L(12,11)	0.073019	L(12,11)	0.07301
L(11,12)	0.004379	L(11,12)	0.004379	L(11,12)	0.004379	L(11,12)	0.00437
L(12,13)	0.00226	L(12,13)	0.00226	L(12,13)	0.00226	L(12,13)	0.00226
L(13,12)	0.007511	L(13,12)	0.007511	L(13,12)	0.007511	L(13,12)	0.00751
L(0,13)	0.001215	L(0,13)	0.001215	L(0,13)	0.001215	L(0,13)	0.00121
L(16,11)	4.59E-05	L(16,11)	4.59E-05	L(16,11)	4.59E-05	L(16,11)	4.6E-05
L(20,19)	0.002085	L(20,19)	0.002153	L(20,19)	0.002278	L(20,19)	0.00231
L(11,20)	0.000622	L(11,20)	0.000346	L(11,20)	0.001147	L(11,20)	0.00048
L(15,20)	0.004743	L(15,20)	0.003357	L(15,20)	0.012805	L(15,20)	0.00572
P(1)	15.9432	P(1)	15.9432	P(1)	15.9432	P(1)	15.943
P(11)	15.9432	P(11)	15.9432	P(11)	15.9432	P(11)	15.943
P(20)	0.115949	P(20)	0.130263	P(20)	0	P(20)	0.07781
P(16)	0.000516	P(16)	0.000269	P(16)	0.001277	P(16)	0.00046

D. Additional Rodent SCFA Pilot Experiments

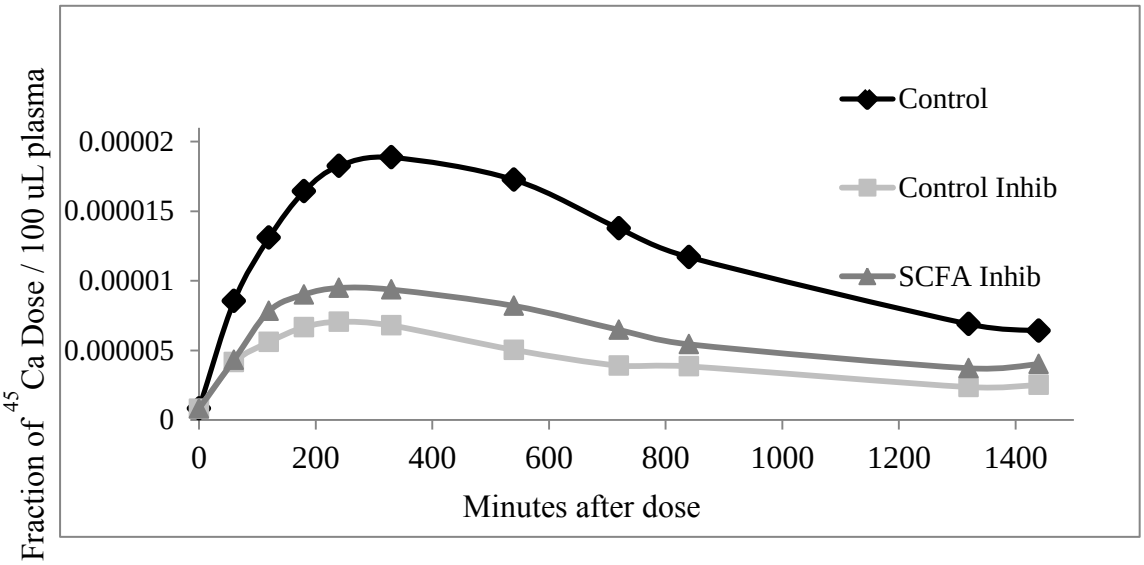


Figure D.1: SCFAs May Protect Against Absorption Inhibition

Calcium absorption inhibitor Ammonium Oxalate immediately prior to ^{45}Ca cecal dose at a molar ratio of 85% of the amount of Calcium Ascorbate infused.

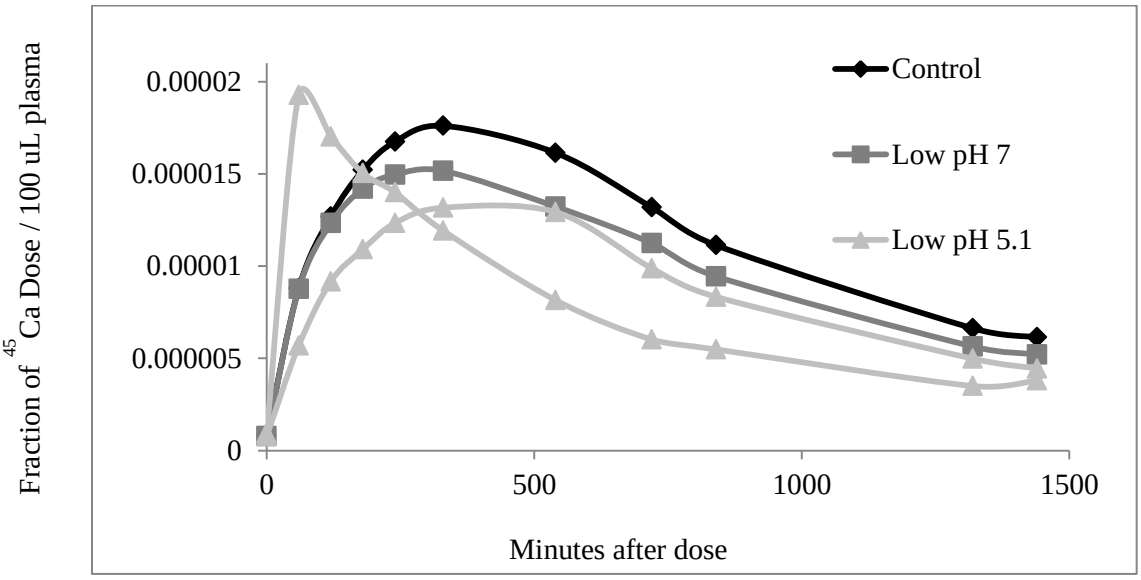


Figure D.2: The Effect of Reduced pH SCFAs on Ca Absorption

Control was saline only. HCl was used to reduce the pH of the Low dose to 5.1.

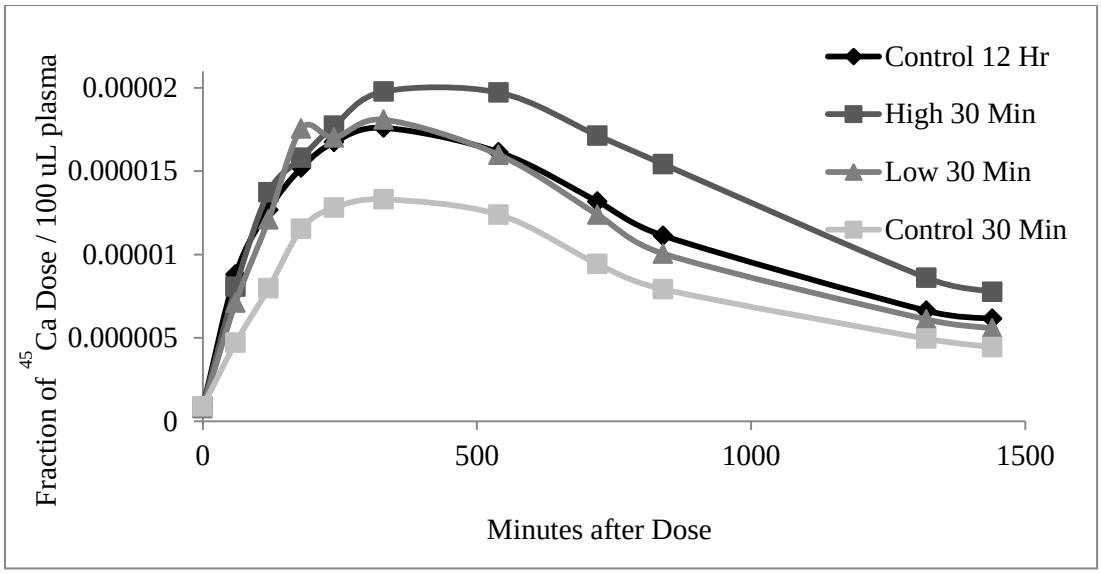


Figure D.3: SCFA Dose Timing May Effect Calcium Absorption

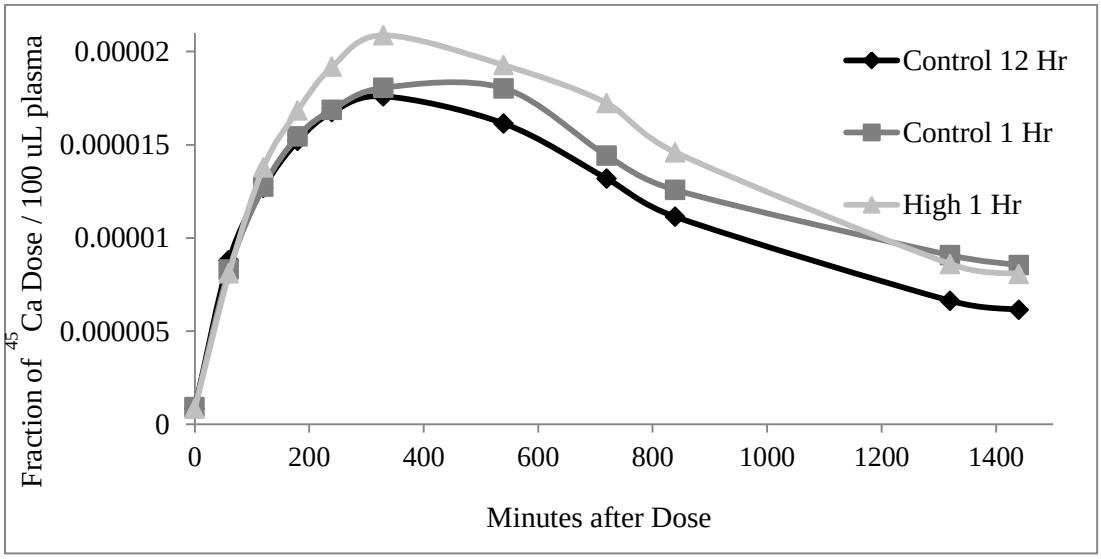


Figure D.4: Dosing of Saline Prior to Calcium Dosing