CALCIUM-FORTIFIED BEVERAGE SUPPLEMENTATION EFFECTS ON BONE MINERAL DENSITY AND BODY COMPOSITION IN HEALTHY YOUNG WOMEN

by

KIMBERLY SUE PETERSON

B.S., Kansas State University, 2005

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Human Nutrition
College of Human Ecology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2007

Approved by:

Major Professor
Mark D. Haub, Ph.D.

Copyright

Abstract

BACKGROUND: Dietary supplements are increasing in popularity; individuals are looking beyond traditional methods of calorie restriction and exercise to improve health. Calcium is a critical nutrient for bone metabolism that has also been shown to enhance weight loss effects secondary to diet.

PURPOSE: To determine whether eight months of calcium supplementation, in a liquid, shelf-stable form, increases bone mineral density or decreases body weight and/or body fat in free-living young adult women.

METHODS: Volunteer subjects (n=42) were randomly assigned to a supplement group receiving 1,125 mg Ca^{2+}/day (CA-BEV) or to a free-living control group (CON), which did not receive the supplement. At baseline and after the 8-month intervention (POST), dietary intake was assessed using 3-day diet records. Total body composition (body fat percentage, $\%Fat_{TB}$; abdominal percentage fat, $\%Fat_{Ab}$; fat mass, FM; non-bone fat-free mass, FFM) and bone mineral density (lumbar spine and femoral neck; BMD) were measured via dual energy x-ray absorptiometry. Subjects also completed a sub-maximal treadmill exercise test to estimate respiratory fitness at baseline and POST.

RESULTS: At POST, the CA-BEV group's calcium intake (1,868±941 mg/d) was significantly greater than ($p<0.05$) the CON group (867±405 mg/d) and the calcium:protein ratio of the CA-BEV group (29.5±17.1 mg/g) was greater than ($p<0.05$) the CON group (12.9 ±6.2 mg/g). Those differences in calcium did not lead to predicted differences ($p<0.05$) between groups for BMD, body weight, $\%Fat_{TB}$, $\%Fat_{AB}$, FM or FFM.

CONCLUSION: Our findings do not support the hypothesis that increasing calcium intake increases BMD or decreases body weight or body fat in healthy young women over an 8-month period despite a nearly two-fold increase in calcium intake.

Table of Contents

List of Figures

List of Tables

Acknowledgements

I would like express my gratitude to Dr. Mark Haub. I am extremely appreciative for this opportunity to further my education at the graduate level. I have always looked up to him and feel fortunate to have been able to work and learn from him over the years. Without his guidance, I would not be where I am today. Thank you for your patience and for always believing in my success.

I would also like to give special recognition to my committee members, Dr. Katharine K. Grunewald and Dr. Valentina M. Remig, for their encouragement and direction which they have provided throughout my entire college education. Your input has been extremely helpful over the years. Next, I am very appreciative of Chad M. Cook and Dr. Enas K. Al-Tamimi for all of their efforts and contributions throughout the study. I also find it important to acknowledge the Human Metabolism Lab along with the faculty and staff in the Department of Human Nutrition for their time and support through this entire process.

Dedication

I would like to dedicate this work to my dad, whose work ethics and compassionate heart has taught me to always strive towards reaching my dreams. I know that he will always be watching over me with a smile on his face.

I also want to dedicate this to my parents, Mike and Susie, who have been my rock every step of the way. I can't even begin to say what you both mean to me, but thanks for being you!

I love you so much and am very blessed!

CHAPTER 1 - LITERATURE REVIEW

Introduction

Calcium is an essential component of a well balanced diet and also is a key element in the prevention of osteoporosis. Osteoporosis is a disease that affects approximately 25 million Americans (1). Obtaining sufficient amounts of calcium helps increase bone metabolism and improves overall bone health (2). This macronutrient is needed for several purposes: to aid in the growth and maintenance of skeletal bone, muscle and blood vessel contraction, secretion of hormones and enzymes, and sending messages to the central nervous system. The skeleton contains approximately 99% of the total body's calcium stores while the remaining one percent is found throughout the body in blood, muscle, and extra-cellular fluid (3).

The aim of this study was to determine whether a calcium-fortified beverage in the form of calcium citrate malate can facilitate changes in bone mineral density (BMD) and body composition in young adult women. Although previous research has been done using this calcium-fortified supplement beverage in post-menopausal woman, it is important to know if it would be helpful for young adult women. There is little research that focuses on young adult women using a calcium citrate malate supplement beverage in a liquid, shelf stable form. Therefore, the study will provide evidence to support or refute the effectiveness of calcium supplementation in young adult women.

This literature review will focus on the effects of calcium supplementation on bone mineral density and how supplementation effects body composition. First, it will examine the calcium intake of Americans; then the most common forms of calcium supplements on the market will be described with explanation of how calcium is absorbed. Next, the relationship between calcium and weight loss is presented. Finally, the review will highlight select studies whose aim was to evaluate whether calcium supplementation affected weight loss and/or fat loss.

Calcium

Calcium Intake

Increasing numbers of Americans appear to be calcium deficient. Most children older than eight years of age in the United States fall short of meeting their recommended daily intake (RDI) of calcium. But children are not the only ones not meeting their calcium needs. Most females between the ages of 9-18 years of age and those over 30 years of age are not meeting their recommended daily intakes of 1,000 mg/day of calcium (2). Therefore, it becomes even more critical that the foods people consume are nutrient dense. Calcium intake in women over the age of 65 is estimated to be about 650 mg/day or less (4). That is almost half of their recommended daily intake of 1,000-1,200 mg of calcium/day for adults (5).

Calcium is most abundant in dairy products such as yogurt, cheese and milk (Table 1.1). More than 70% of calcium in an American's diet comes from some type of dairy product (6). It is most ideal to consume calcium through the diet because dairy products have higher calcium content, higher calcium bioavailability and a lower monetary cost relative to nutritional value. Foods such as dried beans, broccoli, spinach and kale are sources of calcium, but calcium is not always fully absorbed from plants and it may be difficult to obtain adequate calcium intake from

these foods (7). Therefore, greater quantities of plant-based products need to be consumed to attain an equal amount of calcium as that found in dairy products.

There are several reasons why individuals may not consume adequate amounts of calcium in their diets including: disliking the taste of dairy products, calorie restriction, and/or lactose intolerance which my cause gastrointestinal (GI) distress (8). Individuals who avoid dairy products are at risk of not attaining an adequate amount of calcium and are thus at greater risk of bone loss (9). It is extremely important for children and women of childbearing years to be conscientious about the amount of calcium consumed each day. When achieving a sufficient amount of calcium from the diet is unattainable, one should consider supplementation to meet daily needs.

Calcium Supplementation

Often it is difficult for individuals to make lasting modifications to their diet, which contributes to dietary supplements becoming increasingly popular. Dietary supplement use has been increasing because people take dietary supplements for many reasons including: convenience, weight loss and to improve overall health.

Research shows that the individuals who consume dietary supplements are generally non-smoking, Caucasian women. These women also tend to make an effort to consume fruits and vegetables, are well-educated, and exercise on a regular basis (10), (11). Yet, limited research is reported which describes factors that influence an individual's decision to purchase dietary supplements. Investigators of a cohort study concluded that individuals who took supplements adamantly believed that dietary supplements protected them from illness and disease. Those who took supplements and those that did not believed that the media was a powerful influence affecting their decision (12).

The decision to use or not use dietary supplements is affected by cost. Though dietary supplements are often recommended by health care professionals, the cost may prevent individuals from making this out of pocket expense. Also supplements vary and have different elemental forms in a product ultimately affecting the cost of the supplement. The most common forms of calcium supplement compared by cost are calcium carbonate, calcium citrate, and calcium citrate malate. Also different brand names affect the overall cost (Table 1.2).

Forms of Calcium

Because it is critical to consume adequate amounts of calcium daily, many individuals supplement their diets to prevent decreases in bone mass. Calcium supplementation has been shown to help prevent bone mass from decreasing in postmenopausal women (13). There are different forms of calcium in commercially available supplements that can benefit an individual who is not consuming adequate amounts of calcium.

The most common and least expensive calcium supplement used is calcium carbonate. However, the disadvantage of this form is that it is not optimally absorbed relative to other forms of calcium (14). Another popular form of calcium supplementation is calcium citrate. Calcium citrate has half the elemental calcium compared to calcium carbonate, thus body absorbs less (15). The form of calcium with the highest bioavailability is calcium citrate malate (CCM). Calcium citrate malate is more bioavailable than either calcium from milk or calcium carbonate (14), (16). CCM is easy to digest, and causes less constipation and gas compared to the other supplements. Also, Studies suggest that the amount of organic acids significantly affect calcium absorption via CCM when it is suspended in a fruit juice (17).

Calcium Absorption

Although there are benefits of using supplements, one of the drawbacks of consumption of a dietary supplement is that the body will not automatically absorb or use it all. Early studies suggested that the solubility of calcium was thought to affect how well it was absorbed (18). More recently a very weak association between solubility and absorbability of calcium has been shown (19), (20). Researchers have identified that absorption depends on several different factors. Absorption depends on bioavailability of the compound in the GI tract. This is determined by factors such as: the type of supplement taken, what it was taken with, the dose and the absorptive response of the individual.

To absorb the maximum amount of calcium from a supplement, it is best to consume no more than 500 mg at one time, (18). It may be challenging to optimally space one's calcium intake throughout the day to match individual is consuming and their individual absorptive responses. Heaney and Recker (21) reported that approximately 40% of middle aged women required more than 1,000 mg of calcium to meet recommended daily intake; yet later work showed that approximately 25% of the middle aged women needed at least 1,500 mg per day to meet recommended intakes because of difficulty with absorption.

Both passive and active mechanisms are involved when calcium is absorbed in the small intestine. Vitamin D plays an important role in the active absorption that occurs in the duodenum for individuals having a low calcium intake (22). It can also be passively absorbed throughout the jejunum and ileum in individuals who normally consume sufficient amounts of calcium. The amount of calcium that the body is able to absorb is also affected by hormones. This is one reason that post-menopausal women who produce less estrogen do not absorb calcium as well as younger women even when they consume their recommended amount (23).

Calcium Supplementation Trials

Several studies have shown that calcium supplementation increases bone mass in pre-menopausal females. In a meta-analysis study completed by Welten et al. (24), calcium supplementation prevented bone loss in pre-menopausal females. For women who were previously consuming between 700-1,000 mg calcium/day, the supplementation of roughly 1,000 mg/day prevented approximately 1% of bone loss per year at all sites except the ulna.

Yet, a meta-analysis study by Shea et al. (13) reviewed 15 trials, which assessed a combined total of 1,806 subjects. The post-menopausal women in these studies were randomized into a calcium supplement group or into a usual calcium intake group. Though investigators could not conclude whether it was the calcium that reduced the incidence of non-vertebral fractures, results did show an overall small positive effect on bone mineral density with calcium supplementation.

It is extremely difficult to determine the effect of calcium alone when studies combine it with vitamin D or vitamin C (25), (26), (27). In studies where calcium was the only intervention, results were dependent upon several factors: 1) whether the women were pre or post-menopausal, 2) length of time since menopause, 3) the type of calcium and 4) the skeletal sites used to determine bone mineral density (28), (29), (30).

Consuming adequate amounts of calcium on a regular basis can help ensure that calcium deficiency is not contributing to decreased bone loss and weakening of the skeleton. Though

dietary supplements can be beneficial, finding a supplement to fit individual needs, expectations, and price range can be a challenge.

Osteoporosis

Osteoporosis is defined by the World Health Organization as being 2.5 standard deviations below the peak bone mass of a 20 year old sex matched individual. It is distinguished by increased bone fragility which causes an increased risk of skeletal fracture. This disease affects both men and women and causes approximately 1.5 million skeletal fractures every year. The National Institute of Health recommends that individuals consume approximately 1,500 mg/day of calcium. Yet, those who have osteoporosis and are at the greatest risk of fractures are reported in the literature to be consuming less than 650 mg/day (31). The amount of calcium that an individual consumes daily has an impact on overall bone health and is one of the more important nutritional factors in osteoporosis (2).

Although this disease which weakens bones can be found in males, approximately 80% of the 44 million Americans who have osteoporosis are women. The natural decrease in the amount of estrogen that the body produces as a result of menopause is a contributor because the amount of estrogen in the body affects calcium absorption (23). With hormone changes that occur during menopause, women tend to lose about three percent of bone mass per year during the first five years following menopause (32). This decrease was at its highest rate during the first five years following menopause.

Bone Mineral Density

Several factors influence an individual's bone mineral density. Experts estimate that up to 60% of an individual's bone mineral density is genetically predetermined. The remaining 40% can be modified through lifestyle (33). Genetics, physical activity, diet, and hormone replacement therapies are all influential factors affecting bone mineral density (34). Although genetics are a non-modifiable risk factor, modifiable risk factors can optimize bone mineral density. There are two other important modifiable lifestyle areas of consideration for preventing osteoporosis. First, exercising on a regular basis is critical. Incorporating weight-bearing activities is highly recommended in the prevention of osteoporosis because it places stress on the bones (35). Second, nutrient density of the diet should be evaluated. Children should not be allowed to replace milk with soft drinks (36).

The skeleton acts as a storage site for calcium. When an individual does not meet daily calcium needs through diet, the body will withdraw calcium from bones over time. The amount of calcium consumed also affects the mineralization of bones throughout life. Low calcium intake also and vitamin D deficiency are key factors involved in bone loss (37). High bone loss means lower bone mineral density and greater risk of osteoporosis over the lifespan of the individual.

Two additional factors affect the risk of skeletal fracture and overall bone health: 1) peak bone mass achieved before the end of the third decade in life and 2) the rate at which bone is lost after menopause occurs (1). Bone mass decreases at a rate of approximately 1% per year once individuals reach skeletal maturity, usually around the age of thirty. This decline generally increases with age, especially through menopause if intake is not adequate.

Peak Bone Mass

Bone mineral density is greatly dependent upon the peak bone mass that is attained before the age of thirty. Once an individual reaches peak bone mass, no additional bone mass is gained (38), (39). It is essential that children have a diet rich in calcium to ensure that peak bone mass is high. Without adequate amounts of calcium, peak bone mass will fall short of its potential and bone development will be (40).

Reaching one's peak bone mass is crucial, yet it is clear that most young women fall short of meeting this goal. Results from the U.S. Department of Agriculture's national food intake survey showed that 14% of adolescent girls between ages 12-19 years fail to consume an adequate amount of calcium. When girls do not reach their peak bone mass, the chance of developing osteoporosis increases, they do not have adequate calcium stored in their bones (41). Obtaining sufficient calcium throughout childhood greatly decreases risk of osteoporosis later in life. Bones need calcium and weight bearing activity to remodel and become stronger (42).

Calcium absorption decreases slowly from as high as 60% in children to 15-20% in adults. Through adolescence, 30% adult bone mass can be reached (38). Once an individual reaches peak mass, the bone mass will either be maintained or it will decrease over time. The rate of decrease is influenced by the amount of calcium that consumed on a daily basis and by the amount of stress placed upon the bone. Engaging in physical activity on a regular basis can help build bone mass (43); weight bearing activities attenuate bone loss (44), (45). The most effective activities include running, gymnastics, and weight lifting, which causes a great amount of stress placed on the bone (42).

Dual-Energy X-Ray Absorptiometry (DXA)

The dual-energy x-ray absorptiometry (DXA) is a way to measure both bone mineral content (BMC) and bone mineral density (BMD). Bone mineral content (BMC) measures the total amount of mineral located within the bone, reported in grams. Bone mineral density (BMD) measures the total amount of mineral found in the bone divided by the area of bone that was measured (g/cm^2). A mathematical calculation has been developed to predict bone mineral apparent density (46).

Bone Mineral Density (BMD) is categorized by reporting the number of standard deviations from the mean of a normal young adult to others same gender and ethnicity. Bone mass within one standard deviation (T score above -1) of a normal young adult, is in the "normal" range for BMD. BMD falls between 1-2.5 standard deviations away from normal is considered to be osteopenia. Osteopenia is a precursor for osteoporosis, with bones slightly to moderately lacking in calcium. Osteoporosis is a condition when 2.5 or more standard deviations below the mean occur. A person in this category, who has had one or more fractures, is considered to have severe osteoporosis.

Although the DXA is a helpful tool to use when assessing BMD, one of its biggest limitations is it can not determine whether bone turnover is increasing, decreasing or is remaining stable. For this reason, it is important to follow an individual's change in BMD over a long period of time.

Body Composition

Overweight and Obesity

Several factors contribute to an increase in overweight and obesity in adults. Lifestyle promotes the United States to be physically inactive while availability of calorie dense foods increases (47). The number of overweight and obese individuals has been increasing dramatically over time in the United States. Body mass index (BMI) is often used to categorize individuals as being underweight, normal weight, overweight or obese. Results from the 2003-2004 National Health and Nutrition Examination Survey determined that 66.3 % of all adults age 20 years old or older have a BMI of 25 or greater, and 32.2 % of all US adults are considered obese with a BMI of 30 or greater (48).

Overweight and obese individuals without a healthy diet and daily exercise put themselves at higher risk of acquiring heart disease, diabetes, and cancers. This is alarming considering that more children are becoming overweight and obese at an earlier age. Before 1980 approximately 4-6.5 % of all children aged 2-19 were overweight (49). This number has dramatically increased; results of the 2003-2004 NHANES indicate that 17% of children 2-19 years are now overweight (49). Although the prevalence of overweight in children and adolescents is not as high as adults, an increasing number of children are being diagnosed with diseases such as diabetes.

Calcium's Role in Weight Management

Both epidemiological studies and intervention trials have explored the role that calcium may play in weight management. Evidence linking dietary calcium to decreasing weight and fat mass has been accumulating the past two decades. The relationship between calcium and weight was not a focus in the 1980's research. In fact, the relationship between the two variables was almost accidentally discovered. Data from the 1984 NHANES I study primarily focused on hypertension (50). While analyzing the data, researches observed and reported that calcium intake was negatively associated with BMI. A review written by Zemel (51), on a previous study using hypertensive African American males, pointed out the relationship between calcium and body fat. This review noted that the previous study conducted in the 1980's found that men who consumed 2 cups of yogurt per day decreased their body weight by approximately 4.8 kg during the study. Though the results from that study were impressive, the study that was referenced did not have an actual control group. Therefore, outside variables were not controlled and no real conclusions could be made regarding the effects of calcium on body weight.

Many studies now have examined the relationship between calcium and BMI with mixed results. Some studies have found a positive correlation between calcium intake and BMI, while other studies have not.

<u>Studies demonstrating effect</u>

In a study done by Jacqmain et al., a diet low in calcium (< 600 mg) was associated with increased in adiposity (52). Zemel et al. (53) evaluated 32 obese adults and reported a diet high in calcium (~1200 mg/day) increased fat loss. The control group was initially deficient in calcium (~400-500 mg/day) and the subjects decreased the energy intake by 500 kcal/day.

Lloyd et al. (54) observed that increasing calcium intakes from 80% of recommended to 110% via CCM supplementation resulted in significant gains in bone accrual and density in adolescent girls. Their results demonstrate that even modest gains (24 g of bone gain/year) can

lead to substantial benefits (1.3% additional gain/year) and help to achieve great peak bone mass in early adulthood.

In a longitudinal study completed by Rosell et al. (55) approximately 19,000 Swedish women ages of 40-55 years were evaluated during a 9 year period. Women were divided into 4 groups based on calcium intake. Results differed depending on amount and types of dairy product consumed. Women with low intake of whole milk, sour milk, cheese and butter had BMI's statistically higher than women with high intake of those same dairy products. Energy intakes increased with more dairy consumption and the group who consumed low fat milk and sour milk had the least energy increase. A trend that was noted showed a constant relationship between consumption of one or more daily servings of dairy products and decreased risk of gaining one or more kilograms of body weight per year.

A randomized intervention trail by Zemel et al. (56)in 2005 placed obese adults on a calorie restricted diet. Both the control and intervention groups had a 500 kcal deficit per day from usual daily intakes. All subjects were initially not meeting recommended daily intakes for calcium, consuming 500-600 mg of calcium per day. The control group only had the 500 kcal deficit per day, while the intervention group consumed yogurt three times per day bringing their calcium intake to 1100 mg/day. This 12 week trial showed significant weight and body fat loss compared to the control group

A cross sectional survey completed by Kruger et al. compared the calcium intake of how many white women to African American women. Calcium intake and the calcium:fat ratio was significantly higher in the white women. Only the white women's calcium intake was negatively correlated with BMI and percent body fat (57). A noted difference between these two groups was habitual calcium consumption patterns. Mean calcium intake of the white women was approximately 1,000 mg/day, significantly higher than the black women, approximately 500 mg/day.

Studies lacking effect

Shapes et al. (58) conducted a 25 week study investigating whether calcium supplementation affected body fat and/or weight loss. One hundred pre-menopausal and post-menopausal women were divided into two groups, both of which had a 500 kcal deficit/day for six months. One group received 1,000 mg of calcium citrate and the control group a placebo. Difference between groups for changes in body weight and/or body fat were insignificant.

Other similar studies have also demonstrated no changes in body weight or body composition with calcium supplementation (59), (60), (61). The study completed by Phillips et al. (59) looked at the dairy intake and body composition longitudinally over the adolescent period of pre-menarcheal girls. His work suggests no evidence that dairy product consumption affects weight gain or loss.

In a symposium on dairy products and weight regulation, Barr (60) searched MEDLINE to identify randomized trials which used calcium supplementation. Only one study reported that the calcium supplemented group experienced a significant weight loss compared to control group. From the seventeen studies evaluated, none showed significant changes in body weight and/or body composition.

Several factors make it difficult to translate all these studies into reliable information. It is undetermined whether calcium contributed to differences in body weight and/or body fat. Subjects overall diet quality before starting the study is an important aspect. Several studies reported improvements in weight loss or fat loss had individuals who were not initially

consuming adequate calcium. Other factors as exercise habits, total energy intake, macronutrient and micronutrient composition of diet may also contribute to weight and body fat.

Possible Calcium Mechanisms

One possible mechanism found with dairy products may be due to its amino acid composition. It is believed that both leucine and amino acids play a role in metabolism. The importance of this is that leucine plays a role in metabolism and maintaining muscle mass when food is restricted (62). Leucine has also been shown to improve exercise performance with leucine supplementation (63). Leucine and amino acids are most abundant in milk (10% and 21% respectively) compared to other products. Both eggs and muscle proteins have fairly high and comparable amounts of leucine (8.5% and 8 % respectively) and branch chained amino acids (20% and 18% respectively).

Zemel (64) proposed a calcium intake paradox, suggesting that intake of dietary calcium results in lower intracellular levels of calcium. The decreased level of extra-cellular calcium causes an increase in 1, 25-dihydroxyvitamin D. The increase of vitamin D causes an influx of extra-cellular calcium to go into the adipocyte. The increased calcium in the cell stimulates an increase in fatty acid synthase and inhibits lipolysis. Zemel used mice to test this theory and found lipogenesis decreased by 50% with high calcium intake. This model may hold true for a mouse whose environment is completely controlled, does not seem to apply to free-living human subjects.

Another mechanism that has been suggested to alter energy balance is the effect of increased calcium intake to inhibit fat absorption. Welberg et al (65) reported that calcium supplementation increased fecal fat excretion by nearly 1% when subjects increased calcium consumption from 0 g up to 2 g of calcium. Also, Lorenzen et al. (66) observed that calcium from dairy products elicited a decrease in postprandial lipemia and explained this alteration in fat metabolism was due to the calcium being consumed as part of a meal or due to other compounds in the dairy foods.

Summary

Calcium is an essential component to a nutrient dense diet. Therefore, it is important to meet one's RDI via diet or supplementation. Failure to do this may result in decreased BMD causing an increased risk of osteoporosis. It remains unclear whether calcium will facilitate changes regarding body weight and/or body fat.

Purpose

The purpose of this study was to determine whether eight months of calcium-fortified beverage supplementation increased bone mineral density and decreased body weight and fat mass in free-living healthy young women.

Tables for Chapter 1

Table 1.1 List of foods containing calcium.

Food	Portion	Calcium (mg)	% DV
Plain, fat-free yogurt	1 cup	450	45
American cheese	2 ounces	348	35
Cheddar cheese	1 ½ ounces	305	30
Milk (fat free or low fat)	1 cup	300	30
Orange juice with added calcium	1 cup	300	30
Collards, boiled, frozen	½ cup	179	20
Pudding, made with milk	½ cup	147-160	15
Broccoli, cooked or fresh	1 cup	90	10
Kale, boiled	½ cup	90	10
Ice cream	½ cup	84	8
Cottage cheese, 2% fat	½ cup	78	8
Almonds, dry roasted	1 ounce	71	8
White bread	2 slices	70	8
Oatmeal, instant with added calcium	1 packet	100	11

%DV = % of Daily Value used on food labels. Label values are rounded.
Source from CDC, http://www.cdc.gov/powerfulbones/parents/toolbox/list.html.
Last date accessed, November 2007

Table 1.2 Cost comparison of the different forms of calcium containing products.

Product	Form of Calcium	Amt/day for 1,000 mg	Approx. Annual Cost
Tums Ultra™	Carbonate	3 tablets	$ 36.00
Caltrate 600™	Carbonate	2 tablets	$ 60.00
NatureMade Calcium 500 ™	Carbonate	2 tablets	$ 60.00
Viactive ™	Carbonate	2 chews	$ 100.00
Os-Cal ™	Carbonate	2 tablets	$ 100.00
Citracal ™	Citrate	2 tablets	$ 84.00
Tropicana Pure Premium O.J ™ (calcium + vitamin D)	CCM	24 fl oz	$ 580.00

CCM=calcium citrate malate
Costs are approximations based on current (2007) prices from http://www.drugstore.com and local (Manhattan, KS) grocery stores.

CHAPTER 2 – CALCIUM-FORTIFIED BEVERAGE SUPPLEMENTATION EFFECTS ON BONE MINERAL DENSITY AND BODY COMPOSITION IN YOUNG WOMEN

Abstract

BACKGROUND: Dietary supplements are increasing in popularity; individuals are looking beyond traditional methods of calorie restriction and exercise to improve health. Calcium is a critical nutrient for bone metabolism that has also been shown to enhance weight loss effects secondary to diet.

PURPOSE: To determine whether eight months of calcium supplementation, in a liquid, shelf-stable form, increases bone mineral density or decreases body weight and/or body fat in free-living young adult women.

METHODS: Volunteer subjects (n=42) were randomly assigned to a supplement group receiving 1,125 mg Ca^{2+}/day (CA-BEV) or to a free-living control group (CON), which did not receive the supplement. At baseline and after the 8-month intervention (POST), dietary intake was assessed using 3-day diet records. Total body composition (body fat percentage, $\%Fat_{TB}$; abdominal percentage fat, $\%Fat_{Ab}$; fat mass, FM; non-bone fat-free mass, FFM) and bone mineral density (lumbar spine and femoral neck; BMD) were measured via dual energy x-ray absorptiometry. Subjects also completed a sub-maximal treadmill exercise test to estimate respiratory fitness at baseline and POST.

RESULTS: At POST, the CA-BEV group's calcium intake (1,868±941 mg/d) was significantly greater than ($p<0.05$) the CON group (867±405 mg/d) and the calcium:protein ratio of the CA-BEV group (29.5±17.1 mg/g) was greater than ($p<0.05$) the CON group (12.9 ±6.2 mg/g). Those differences in calcium did not lead to predicted differences ($p<0.05$) between groups for BMD, body weight, $\%Fat_{TB}$, $\%Fat_{AB}$, FM or FFM.

CONCLUSION: Our findings do not support the hypothesis that increasing calcium intake increases BMD or decreases body weight or body fat in healthy young women over an 8-month period despite a nearly two-fold increase in calcium intake.

Introduction

Bone Mineral Density

Adequate calcium intake is an essential component in the prevention of osteoporosis, a condition which affects approximately 25 million Americans (1). Obtaining sufficient amounts of calcium helps increase bone metabolism and improves overall bone health (2). Women who generally consume less than 650 mg/day are at greater risk of developing osteoporosis (31). Two of the many factors that affect the risk of skeletal fracture and overall bone health are: 1) peak bone mass achieved before the end of the third decade in life and 2) the rate at which bone is lost after menopause occurs (1). Bone mass decreases at a rate of approximately1% per year once individuals reach skeletal maturity. This rate increases most during the first 5 years after menopause because calcium absorption decreases due to the loss of estrogen (32).

Since it is critical to acquire adequate calcium on a daily basis, many individuals include a supplement to their diets to help prevent decreases in current bone mass. Calcium supplementation while controversial has been shown to help prevent decreased bone mass in postmenopausal women (13). Moreover, clinical studies indicate that calcium citrate malate (CCM) is the most bioavailable form of calcium (16). Calcium citrate malate is available in a pill, powder and a liquid, shelf-stable form. Additionally, the body absorbs significantly more calcium via CCM when it is in a fortified fruit juice suspension (16), (67), (17).

Body Composition

There are many factors influencing weight management. Both lifestyle and genetic components influence one's body weight, and the prevalence of obesity epidemic. Individuals seeking successful solutions to improve health and achieve weight loss goals have become less enamored with traditional strategies of exercise and calorie restriction. In a recent rat model, calcium was shown to have weight loss effects via a cellular mechanism postulated for regulation of adipocyte metabolism through dietary calcium intake using a rat model (68). A diet low in calcium seems to promote lipogenesis and inhibit lipolysis, while high dietary calcium intake up-regulates lypolysis and down-regulates lipogenesis (69). Zemel and colleagues recommend increasing dietary calcium with calorie restriction to decrease body weight and body fat (53). Zemel also suggests the regulation of intracellular calcium can help alter disease risk (64). There are a limited number of human clinical trials that analyze changes in body composition with subjects who are already consuming the RDI of 1,200 mg for individuals 19-50. Therefore, the purpose of this eight-month randomized clinical trial was to examine the effects of a calcium-fortified beverage supplement on changes in body composition and bone mineral density in young adult women.

METHODS

Study Design

Kansas State University's Institutional Review Board approved this study during the fall of 2002. Subjects were recruited through word of mouth and flyers that were posted on the Kansas State University's campus. Those individuals who showed interest in the study were screened to determine eligibility through a telephone interview. Women who were weight stable six months prior to the study, taking oral contraceptives and between the ages of 18-25 were eligible to participate in this 8-month study. Individuals were excluded from the study if the individual was a smoker, had a recent skeletal fracture (<2 years), or if they were taking any of the following medications known to affect bone metabolism: bisphosphonates, thiazides, corticosteroids, calcitonin, depo provera and tamoxifen.

Sixty women expressed interest in the eight month study (conducted from September 2003-May 2004). Ten individuals were ineligible to participate because they did not meet study requirements, or had scheduling conflicts. Fifty healthy young adult women began the study, and 42 completed it. All 50 subjects enrolled provided a written consent and medical history before beginning the study. Eight subjects dropped out: five had personal reasons unrelated to the study, one disliked the taste of the beverage, one had a change in medication, and one sustained an unrelated injury, leaving her unable to complete testing post intervention. Subjects were randomized to a supplement group which consumed CA-BEV (n=24) or into a free-living control group (CON) that did not receive a supplement (n=26). There were 18 subjects in the CA-BEV group and twenty four in the free-living control group (CON) that completed the study in May 2004 (Figure 2.1).

Supplementation

The 20 fluid ounce calcium-fortified beverage supplement contained skim milk (5-7% depending on the flavor), fruit juice (15%), Vitamin C (50 mg per 10 oz) and calcium (~580-590 mg per 10 oz). CA-BEV provided 1,125 mg of calcium in the form of CCM and approximately 38-54 mg of calcium from the skim milk (depending on the CA-BEV flavor).

Subjects were asked to consume one bottle of CA-BEV per day; 10 ounces (295 ml) of CA-BEV in the morning and the remaining 10 ounces (295 ml) in the afternoon or evening. Subjects came into the Human Metabolism Lab (HML) once per week to pick up the beverage supplements and turned in a weekly supplement intake form at the HML. Women who were traveling during the semester break (2-4 weeks) had an option to carry the CA-BEV with them or to take two 600 mg calcium citrate malate (CCM) pills per day (one pill in the morning and one in the evening). The weekly supplement intake form was used to document missed dosages. Reported reasons for missed dosages included: gastrointestinal complications, flu, family emergency, and forgetfulness. The self-reported weekly supplement intake forms indicated 90% compliance from subjects.

Measurements

Subjects came into the HML for baseline measurement of height, weight, body composition, and sub-maximal VO_2 test. This information, along with a 3-day diet record was also collected at baseline and at completion of the eight month intervention (POST). Both height and weight were measured with shoes and outdoor clothing removed. Height was measured by

using a wall-mounted stadiometer (SECA™, Hanover, MD) and weight was measured using a digital scale (OHAUS™, Pine Brook, NJ).

Body Composition: Dual-energy x-ray absorptiometry (DXA) software version 5.6 (GE Lunar Corp.™, Milwaukee, WI) was used to measure the following body composition variables: total body fat percentage (%Fat), abdominal fat percentage (%FatAb), total fat mass (FM), non-bone fat free mass (FFM), and bone mineral density (BMD). Bone mineral density was measured at the lumbar spine (LS; L1-L4), and femoral neck (FN). Body composition measurements were assessed by the same trained technician using the DXA.

Dietary Intake: Subjects received both written and verbal instructions on completing the 3-day diet record. A trained individual instructed the subjects about portion sizes while showing them common measuring utensils. Subjects were instructed to record everything that they put into their mouths including food, water, gum, mints, vitamins, and medications. The 3-day diet record included two "typical" weekdays and one "typical" weekend day. This was the same nutritional assessment tool used in a similar study done by Haub et al. which evaluated body composition in post-menopausal women (61). The 3-day diet record was completed before subjects began the study and again post intervention. Diet records were analyzed using Nutritionist Pro™ software program, version 2.0 (First DataBank, San Bruno, CA).

Physical Fitness Assessment: Subjects were asked to continue their usual daily activities throughout the duration of the study. To determine whether the subjects' physical fitness levels changed during the study, a sub-maximal treadmill exercise test was conducted. The treadmill incline was set at a 5% grade and subjects were instructed to walk at a constant speed between 2.0-4.5 mph. Subjects' heart rate was measured during the 4 minute treadmill test. This procedure was completed prior to the subjects starting the study and post intervention. The following model was used (70):

VO_2 max = 15.1 + 21.8*SPEED (mph) -0.327*HEART RATE (bpm) - 0.263*SPEED*AGE (yr) + 0.00504*HEART RATE*AGE + 5.98*GENDER.

Statistical Analyses

Independent *t*-tests were used to compare means between the supplement group and the free-living control group. Paired *t*-tests were used to compare means with in groups. Pearson correlations were used to assess the relationships between dietary intake and bone mineral density (BMD). All analyses were computed using the Statistical Product and Service Solution (SPSS) software for Windows software (version 11.5; SPSS Inc., Chicago, IL). Statistical significance was determined by two-tailed p-values ($p<0.05$). All values are presented as mean ± standard deviation unless otherwise noted.

RESULTS

Dietary Intake

There were no differences between or within groups from baseline to post intervention (POST) for the following dietary components: energy intake, carbohydrate, fat, carbohydrate:protein ratio, vitamin C, and vitamin D . The CA-BEV group significantly increased the amount of dietary calcium ($p<0.001$) along with their calcium:protein ratio ($p<0.001$) intake during the 8-month intervention. The CON group's intake of dietary calcium and calcium:protein ratio decreased slightly during the eight month intervention, but these results were not significant. Finally, there was a significant difference ($p<0.001$) in the amount of calcium consumed along with the calcium:protein ratio by the CA-BEV group compared to the CON group, (Table 2.2).

Bone Mineral Density, Body Composition & Fitness

Both the CA-BEV and CON groups significantly increased ($p=0.033$, $p=0.015$, respectively) their total body BMD from baseline to POST. However, the CA-BEV group's femoral BMD remained indifferent ($p=0.063$). Whereas, the CON group's femoral BMD significantly ($p=0.003$) decreased from baseline to POST. No significant changes were observed between or within either group's spine BMD. Finally, there were no significant differences between or within groups from baseline to POST for body weight, total body fat, abdominal fat, fat mass, non-bone fat free mass and aerobic fitness variables, (Table 2.3).

DISCUSSION

Based on the study design and methods used, our findings suggest that additional calcium above the recommend daily intake (RDI) for eight months, compared with habitual intake, does not significantly affect bone mineral density (BMD) or body composition in healthy young women. Even though the CA-BEV group consumed nearly twice the amount of calcium consumed by the control group, the increased calcium did not elicit any significant benefits.

Bone Mineral Density and Body Composition Trends

The results from this study do not support that increasing calcium intake elicits increased BMD in young women. The present results support the work of Barger-Lux et al. (71) that demonstrated calcium supplementation in young women, of similar age to those in the present study, and who ate a low calcium diet did not experience improved BMD after 36 months relative to a placebo-controlled group. Moreover, in a longitudinal study, Mein et al. (72) observed a negative correlation between calcium intake and BMD in young women (age = 18-27 yr) over a nine year period. While CCM was not effective in this sample, it might be effective in even younger individuals as bone accrual was significantly increased in a clinical trial using CCM supplementation in adolescent females (73). That said, the CA-BEV intervention seemed to prevent a decrease in BMD at the femur, whereas the CON group experienced a significant decrease in femoral BMD.

Body Composition

The body composition findings were similar to a 25 week randomized, double blind, placebo-controlled study completed by Shapes et al. (2004), where 100 pre-menopausal and

post-menopausal women were divided into two groups, with each group having a 500 kcal deficit/day for six months. One group received 1,000 mg of calcium citrate and the control group was given a placebo tablet. The difference between groups for changes in body weight and body fat were not significant.

Other similar studies also failed to show changes in body weight or body composition as a result of calcium or dairy supplementation (60), (61). In a paper reviewing the effects of dairy products on weight regulation, Barr (60) searched MEDLINE to find seventeen randomized trials using calcium supplements. Only one study out of 17 found that the calcium supplemented group experienced significant weight loss compared to the control group. None of the other studies showed significant changes in body weight or body composition. The study by Haub et al (61) also used CCM, but in older women. They reported no difference for body composition between the calcium supplemented group or the free-living reference group of women.

However, according to a study by Jacqmain et. al. (52), a diet low in calcium (< 600 mg) was associated with an increased adiposity. Moreover, Zemel et al. (53) completed a randomized clinical trial demonstrating that diets high in calcium (~1,200 mg/day) or dairy food (~1200 mg/day from yogurt) increased fat loss compared to an energy deficit alone. One possible explanation may the control group was initially deficient in calcium (~400-500 mg/day), while the volunteers in the present study were consuming about twice that amount.

Based on the work by Davies (74), which was devised to estimate annual changes in body weight based on the calcium:protein intake ratio, we expected the CA-BEV group to lose weight. However, the CA-BEV group gained 0.7±2.0 kg and the CON group gained 0.1±2.0 kg over the 8-month period. Our results suggest that the weight effects are likely secondary to one's calcium intake. Based on that equation (74), the CA-BEV group should have lost 0.32 kg. However, the same equation predicted that the CON group would increase their weight 0.20 kg. Thus, the purposeful increase in the calcium:protein ratio induced by supplementing with CCM did not affect body weight. Studies have shown that obtaining calcium from food may have a more profound effect on changes in body weight and body composition compared to calcium taken in the supplement form (53), (75). But this was not likely to be an issue for this study because this study's calcium was in the form of CCM, which has very high bioavailability (67), (17).

As for limitations, the duration of this study may not have been sufficient to detect significant changes in BMD. Our study sample primarily contained females who had BMI's in the "normal" range and were not calcium deficient. Their energy intakes and exercise routines were not modified from their usual daily lifestyle. Thus, according to energy balance principles, a change in body composition would not be expected even if calcium intake were increased. Furthermore, since they were not calcium deficient, calcium supplementation is less likely to have an effect (76). Finally, there could have been a seasonal effect. Individuals are generally less active during the winter months. Therefore, there may have been less stress placed on the bone which would attenuate bone resorption, thus partially explaining part of the reason for the decrease in femoral BMD. That said, young women, even if they are "normal" weight and apparently healthy, still seek means to decrease body weight (77).

In conclusion, the results from this study suggest that calcium supplementation does not significantly affect total body fat percentage, abdominal fat percentage, fat mass, non-bone fat free mass, or bone mineral density in healthy young women already consuming close to the RDI of calcium. Had the women been calcium deficient we might have observed significant differences between groups. Although previous studies reported that increased calcium intake affected weight loss and fat loss, our results do not concur with those observations.

Tables and Figure for Chapter 2

Table 2.1 Baseline characteristics for subjects who drank the supplement beverage (CA-BEV) and those in the free-living control group (CON).

	CA-BEV	CON
Subjects (n)	24	26
Age (yr)	20.9±1.6	20.4±1.2
Weight (kg)	62.3±8.0	63.9±6.2
BMI (kg/m^2)	22.6±3.4	22.8±2.3
Body Fat (%)	31.6±7.0	31.7±6.7
VO_2 max (ml/kg/min)	32.8±3.5	32.3±4.2

Mean ± SD
There were no significant differences ($p<0.05$) between groups.

Table 2.2 Dietary intake data at baseline and after completion of the 8-month intervention for subjects who drank the supplement beverage (CA-BEV) and those in the free-living control group (CON).

	Baseline	POST	P-Value [1]	Δ [2]	P-Value [3]
Energy Intake (MJ)					
CA-BEV	7.7±2.1	7.8±2.1	0.264	0.1±1.9	0.260
CON	7.7±1.6	8.5±2.0		0.8±1.9	
Carbohydrate (g)					
CA-BEV	259±79	264±80	0.404	5±75	0.232
CON	251±65	286±84		35±80	
Protein (g/kg)					
CA-BEV	68±20	66±17	0.808	-2.0±22	0.802
CON	67±18	68±19		1.0±25	
Fat (g)					
CA-BEV	61±25	61±24	0.223	0.0±18	0.534
CON	66±20	71±23		5±25	
Carbohydrate:Protein (g/g)					
CA-BEV	4.0±1.3	4.1±1.3	0.509	0.1±0.3	0.312
CON	3.9±1.3	4.3±1.2		0.4±0.6	
Vitamin C (mg)					
CA-BEV	102±68	173±121 * †	0.058	71±129	0.101
CON	95±64	113±78		18±76	
Vitamin D (µg)					
CA-BEV	4.8±3.6	3.9±4.1	0.376	-0.9±157	0.610
CON	3.3±3.7	3.0±2.7		-0.3±134	
Calcium (mg)					
CA-BEV	988±408	1,868±941 ** ††	0.000	880±873 ** ††	0.000
CON	902±532	867±405		-35±476	
Calcium:Protein (mg/g)					
CA-BEV	15±5.9	29.5±17.1 ** ††	0.000	-14.5±.75 ** ††	0.000
CON	13.1±5.5	12.9±6.2		-0.2±0.50	

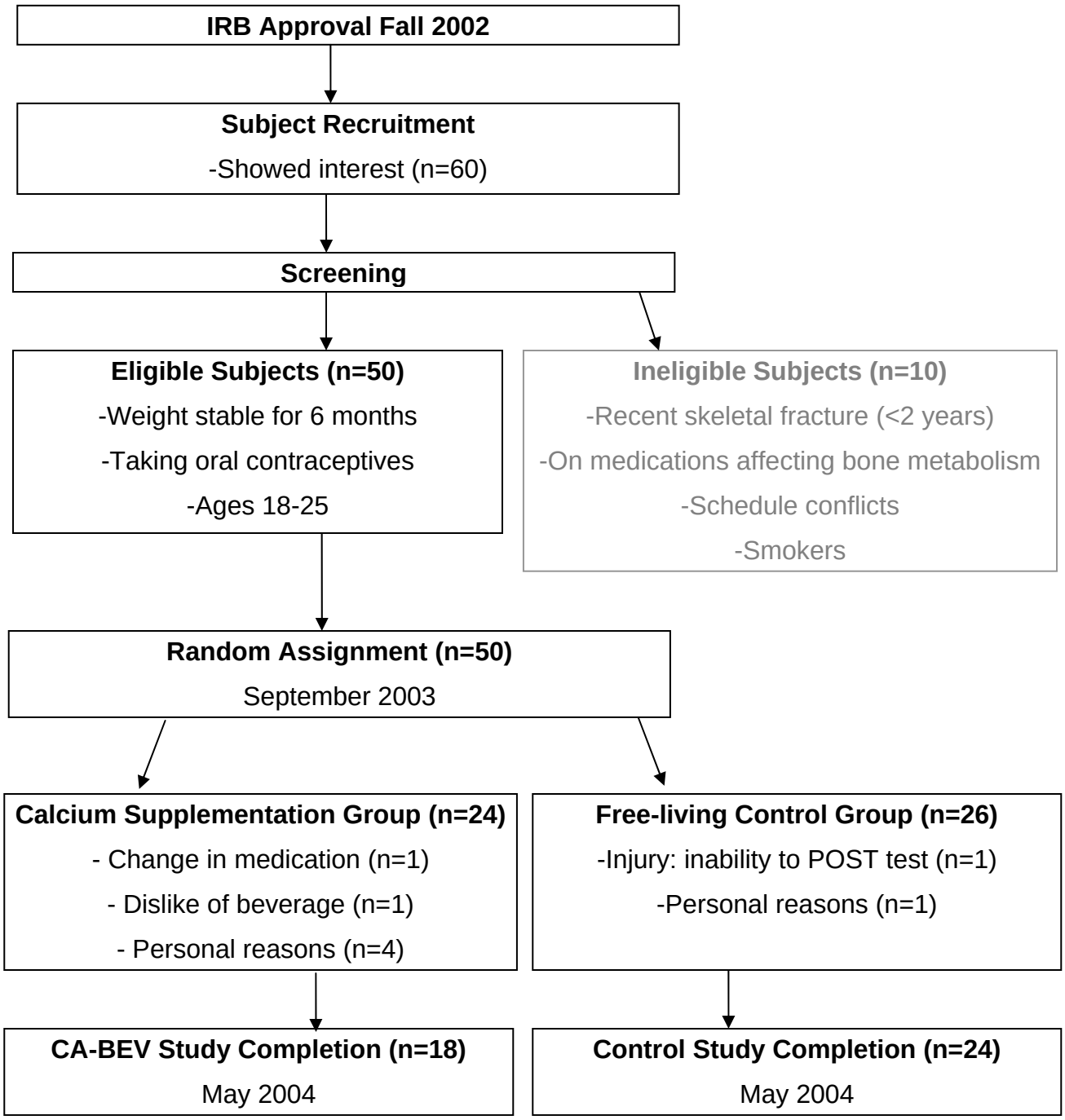
Mean ± SD; P-Value[1] = p-value for the difference between groups at POST; P-Value[2] = Absolute difference between groups at POST; P-Value[3] = p-value for the difference between the change in groups at POST; * = $p<0.050$, ** = $p<0.010$ difference within group from baseline to POST; † = $p<0.050$, †† = $p<0.010$ difference between groups.

Table 2.3 Body Composition, bone density and aerobic fitness data at baseline and after completion of the 8-month intervention for subjects who drank the supplement beverage (CA-BEV) and those in the free-living control group (CON).

	Baseline	POST	P-Value [1]	Δ	P-Value [2]
Body Weight (kg)					
CA-BEV	62.3±8.0	63.0±8.8	0.184	0.7±2.0	0.388
CON	63.9±6.2	64.0±6.6	0.799	0.1±2.0	
Total-body fat %					
CA-BEV	31.6±7.0	32.3±8.1	0.146	0.7±2.0	0.333
CON	31.7±6.7	31.9±6.3	0.777	0.1±2.0	
Abdominal fat %					
CA-BEV	35.0±10.	36.0±11.7	0.086	1.1±2.5	0.150
CON	36.6±8.7	36.4±8.2	0.805	-0.1±2.7	
Fat mass (g)					
CA-BEV	19.2±6.5	19.8±7.7	0.178	0.7±2.0	0.342
CON	19.5±5.6	19.7±5.6	0.723	0.1±1.7	
Non-bone fat free mass (g)					
CA-BEV	40.2±3.3	40.0±3.5	0.278	-0.2±0.7	0.591
CON	41.3±3.5	41.2±3.1	0.958	0.0±1.2	
BMD (Total Body)					
CA-BEV	1.19±0.0	1.20±0.08	0.033	0.01±0.02	0.803
CON	1.18±0.6	1.19±0.07	0.015	0.01±0.02	
BMD (Spine)					
CA-BEV	1.22±0.1	1.23±0.12	0.297	0.01±0.02	0.735
CON	1.25±0.1	1.25±0.12	0.643	0.00±0.03	
BMD (Femur)					
CA-BEV	1.12±0.1	1.10±0.11	0.063	-0.04±0.06	0.397
CON	1.14±0.1	1.10±0.13	0.003	-0.03±0.05	
VO_2 max (ml/kg/min)					
CA-BEV	32.3±4.2	31.6±3.7	0.307	-0.7±2.8	0.645
CON	32.8±3.5	32.5±4.3	0.713	-0.3±3.3	

Mean ± SD; BMD = bone mineral density; P-Value[1] = p-value within-group differences; P-Value[2] = absolute difference from baseline to POST. There were no differences between or within groups ($p>0.050$).

Figure 2.1 Subject Recruitment flow chart



Literature Cited

1. Lane JM, Nydick M: Osteoporosis: current modes of prevention and treatment. *J Am Acad Orthop Surg* 7:19-31, 1999
2. Cashman KD: Calcium intake, calcium bioavailability and bone health. *Br J Nutr* 87 Suppl 2:S169-177, 2002
3. Anonymous: Dietary Supplement Fact Sheet: Calcium, Office of Dietary Supplements. NIH Clinical Center. National Institute of Health. In http://ods.od.nih.gov/factsheets/calcium.asp. *Last accessed September 2007*
4. Alaimo K, McDowell MA, Briefel RR, Bischof AM, Caughman CR, Loria CM, Johnson CL: Dietary intake of vitamins, minerals, and fiber of persons ages 2 months and over in the United States: Third National Health and Nutrition Examination Survey, Phase 1, 1988-91. *Adv Data*:1-28, 1994
5. Bryant RJ, Cadogan J, Weaver CM: The new dietary reference intakes for calcium: implications for osteoporosis. *J Am Coll Nutr* 18:406S-412S, 1999
6. Huth PJ, DiRienzo DB, Miller GD: Major scientific advances with dairy foods in nutrition and health. *J Dairy Sci* 89:1207-1221, 2006
7. Weaver CM: Calcium bioavailability and its relation to osteoporosis. *Proc Soc Exp Biol Med* 200:157-160, 1992
8. Goulding A, Taylor RW, Keil D, Gold E, Lewis-Barned NJ, Williams SM: Lactose malabsorption and rate of bone loss in older women. *Age Ageing* 28:175-180, 1999
9. Lee MF, Krasinski SD: Human adult-onset lactase decline: an update. *Nutr Rev* 56:1-8, 1998
10. Lyle BJ, Mares-Perlman JA, Klein BE, Klein R, Greger JL: Supplement users differ from nonusers in demographic, lifestyle, dietary and health characteristics. *J Nutr* 128:2355-2362, 1998
11. Kirk SF, Cade JE, Barrett JH, Conner M: Diet and lifestyle characteristics associated with dietary supplement use in women. *Public Health Nutr* 2:69-73, 1999
12. Conner M, Kirk SF, Cade JE, Barrett JH: Why do women use dietary supplements? The use of the theory of planned behaviour to explore beliefs about their use. *Soc Sci Med* 52:621-633, 2001
13. Shea B, Wells G, Cranney A, Zytaruk N, Robinson V, Griffith L, Ortiz Z, Peterson J, Adachi J, Tugwell P, Guyatt G: Meta-analyses of therapies for postmenopausal osteoporosis. VII. Meta-analysis of calcium supplementation for the prevention of postmenopausal osteoporosis. *Endocr Rev* 23:552-559, 2002
14. Sakhaee K, Bhuket T, Adams-Huet B, Rao DS: Meta-analysis of calcium bioavailability: a comparison of calcium citrate with calcium carbonate. *Am J Ther* 6:313-321, 1999
15. Heller HJ, Greer LG, Haynes SD, Poindexter JR, Pak CY: Pharmacokinetic and pharmacodynamic comparison of two calcium supplements in postmenopausal women. *J Clin Pharmacol* 40:1237-1244, 2000
16. Smith KT, Heaney RP, Flora L, Hinders SM: Calcium absorption from a new calcium delivery system (CCM). *Calcif Tissue Int* 41:351-352, 1987
17. Andon MB, Peacock M, Kanerva RL, De Castro JA: Calcium absorption from apple and orange juice fortified with calcium citrate malate (CCM). *J Am Coll Nutr* 15:313-316, 1996

18. Harvey JA, Zobitz MM, Pak CY: Dose dependency of calcium absorption: a comparison of calcium carbonate and calcium citrate. *J Bone Miner Res* 3:253-258, 1988
19. Sheikh MS, Santa Ana CA, Nicar MJ, Schiller LR, Fordtran JS: Gastrointestinal absorption of calcium from milk and calcium salts. *N Engl J Med* 317:532-536, 1987
20. Heaney RP, Recker RR, Weaver CM: Absorbability of calcium sources: the limited role of solubility. *Calcif Tissue Int* 46:300-304, 1990
21. Heaney RP, Recker RR: Estimating true fractional calcium absorption. *Ann Intern Med* 108:905-906, 1988
22. Bronner F: Mechanisms and functional aspects of intestinal calcium absorption. *J Exp Zoolog A Comp Exp Biol* 300:47-52, 2003
23. Heaney RP, Recker RR, Stegman MR, Moy AJ: Calcium absorption in women: relationships to calcium intake, estrogen status, and age. *J Bone Miner Res* 4:469-475, 1989
24. Welten DC, Kemper HC, Post GB, van Staveren WA: A meta-analysis of the effect of calcium intake on bone mass in young and middle aged females and males. *J Nutr* 125:2802-2813, 1995
25. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE: Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *N Engl J Med* 337:670-676, 1997
26. Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD, Meunier PJ: Vitamin D3 and calcium to prevent hip fractures in the elderly women. *N Engl J Med* 327:1637-1642, 1992
27. Morton DJ, Barrett-Connor EL, Schneider DL: Vitamin C supplement use and bone mineral density in postmenopausal women. *J Bone Miner Res* 16:135-140, 2001
28. Dawson-Hughes B, Dallal GE, Krall EA, Sadowski L, Sahyoun N, Tannenbaum S: A controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. *N Engl J Med* 323:878-883, 1990
29. Lau EM, Woo J, Lam V, Hong A: Milk supplementation of the diet of postmenopausal Chinese women on a low calcium intake retards bone loss. *J Bone Miner Res* 16:1704-1709, 2001
30. Storm D, Eslin R, Porter ES, Musgrave K, Vereault D, Patton C, Kessenich C, Mohan S, Chen T, Holick MF, Rosen CJ: Calcium supplementation prevents seasonal bone loss and changes in biochemical markers of bone turnover in elderly New England women: a randomized placebo-controlled trial. *J Clin Endocrinol Metab* 83:3817-3825, 1998
31. Optimal calcium intake. *NIH Consens Statement* 12:1-31, 1994
32. Gallagher JC, Goldgar D, Moy A: Total bone calcium in normal women: effect of age and menopause status. *J Bone Miner Res* 2:491-496, 1987
33. Krall EA, Dawson-Hughes B: Heritable and life-style determinants of bone mineral density. *J Bone Miner Res* 8:1-9, 1993
34. Lewis RD, Modlesky CM: Nutrition, physical activity, and bone health in women. *Int J Sport Nutr* 8:250-284, 1998
35. Sherman S: Preventing and treating osteoporosis: strategies at the millennium. *Ann N Y Acad Sci* 949:188-197, 2001
36. Harnack L, Stang J, Story M: Soft drink consumption among US children and adolescents: nutritional consequences. *J Am Diet Assoc* 99:436-441, 1999
37. Alevizaki CC, Ikkos DG, Singhelakis P: Progressive decrease of true intestinal calcium absorption with age in normal man. *J Nucl Med* 14:760-762, 1973

38. Chevalley T, Rizzoli R, Nydegger V, Slosman D, Rapin CH, Michel JP, Vasey H, Bonjour JP: Effects of calcium supplements on femoral bone mineral density and vertebral fracture rate in vitamin-D-replete elderly patients. *Osteoporos Int* 4:245-252, 1994
39. Frost HM: The mechanostat: a proposed pathogenic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents. *Bone Miner* 2:73-85, 1987
40. Tortolani PJ, McCarthy EF, Sponseller PD: Bone mineral density deficiency in children. *J Am Acad Orthop Surg* 10:57-66, 2002
41. Riggs BL: Overview of osteoporosis. *West J Med* 154:63-77, 1991
42. Taaffe DR, Snow-Harter C, Connolly DA, Robinson TL, Brown MD, Marcus R: Differential effects of swimming versus weight-bearing activity on bone mineral status of eumenorrheic athletes. *J Bone Miner Res* 10:586-593, 1995
43. Ulrich CM, Georgiou CC, Gillis DE, Snow CM: Lifetime physical activity is associated with bone mineral density in premenopausal women. *J Womens Health* 8:365-375, 1999
44. Brooke-Wavell K, Jones PR, Hardman AE: Brisk walking reduces calcaneal bone loss in post-menopausal women. *Clin Sci (Lond)* 92:75-80, 1997
45. Welsh L, Rutherford OM: Hip bone mineral density is improved by high-impact aerobic exercise in postmenopausal women and men over 50 years. *Eur J Appl Physiol Occup Physiol* 74:511-517, 1996
46. Carter DR, Bouxsein ML, Marcus R: New approaches for interpreting projected bone densitometry data. *J Bone Miner Res* 7:137-145, 1992
47. French SA, Story M, Jeffery RW: Environmental influences on eating and physical activity. *Annu Rev Public Health* 22:309-335, 2001
48. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM: Prevalence of overweight and obesity in the United States, 1999-2004. *Jama* 295:1549-1555, 2006
49. Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM: Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. *Jama* 291:2847-2850, 2004
50. McCarron DA, Morris CD, Henry HJ, Stanton JL: Blood pressure and nutrient intake in the United States. *Science* 224:1392-1398, 1984
51. Zemel MB: Regulation of adiposity and obesity risk by dietary calcium: mechanisms and implications. *J Am Coll Nutr* 21:146S-151S, 2002
52. Jacqmain M, Doucet E, Despres JP, Bouchard C, Tremblay A: Calcium intake, body composition, and lipoprotein-lipid concentrations in adults. *Am J Clin Nutr* 77:1448-1452, 2003
53. Zemel MB, Thompson W, Milstead A, Morris K, Campbell P: Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults. *Obes Res* 12:582-590, 2004
54. Lloyd T, Andon MB, Rollings N, Martel JK, Landis JR, Demers LM, Eggli DF, Kieselhorst K, Kulin HE: Calcium supplementation and bone mineral density in adolescent girls. *Jama* 270:841-844, 1993
55. Rosell M, Hakansson NN, Wolk A: Association between dairy food consumption and weight change over 9 y in 19,352 perimenopausal women. *Am J Clin Nutr* 84:1481-1488, 2006
56. Zemel MB, Richards J, Milstead A, Campbell P: Effects of calcium and dairy on body composition and weight loss in African-American adults. *Obes Res* 13:1218-1225, 2005

57. Kruger HS, Rautenbach PH, Venter CS, Wright HH, Schwarz PE: An inverse association between calcium and adiposity in women with high fat and calcium intakes. *Ethn Dis* 17:6-13, 2007
58. Shapses SA, Heshka S, Heymsfield SB: Effect of calcium supplementation on weight and fat loss in women. *J Clin Endocrinol Metab* 89:632-637, 2004
59. Phillips SM, Bandini LG, Cyr H, Colclough-Douglas S, Naumova E, Must A: Dairy food consumption and body weight and fatness studied longitudinally over the adolescent period. *Int J Obes Relat Metab Disord* 27:1106-1113, 2003
60. Barr SI: Increased dairy product or calcium intake: is body weight or composition affected in humans? *J Nutr* 133:245S-248S, 2003
61. Haub MD, Simons TR, Cook CM, Remig VM, Al-Tamimi EK, Holcomb CA: Calcium-fortified beverage supplementation on body composition in postmenopausal women. *Nutr J* 4:21, 2005
62. Layman DK: Protein quantity and quality at levels above the RDA improves adult weight loss. *J Am Coll Nutr* 23:631S-636S, 2004
63. Crowe MJ, Weatherson JN, Bowden BF: Effects of dietary leucine supplementation on exercise performance. *Eur J Appl Physiol* 97:664-672, 2006
64. Zemel MB: Calcium modulation of hypertension and obesity: mechanisms and implications. *J Am Coll Nutr* 20:428S-435S; discussion 440S-442S, 2001
65. Welberg JW, Monkelbaan JF, de Vries EG, Muskiet FA, Cats A, Oremus ET, Boersma-van Ek W, van Rijsbergen H, van der Meer R, Mulder NH, et al.: Effects of supplemental dietary calcium on quantitative and qualitative fecal fat excretion in man. *Ann Nutr Metab* 38:185-191, 1994
66. Lorenzen JK, Nielsen S, Holst JJ, Tetens I, Rehfeld JF, Astrup A: Effect of dairy calcium or supplementary calcium intake on postprandial fat metabolism, appetite, and subsequent energy intake. *Am J Clin Nutr* 85:678-687, 2007
67. Miller JZ, Smith DL, Flora L, Slemenda C, Jiang XY, Johnston CC, Jr.: Calcium absorption from calcium carbonate and a new form of calcium (CCM) in healthy male and female adolescents. *Am J Clin Nutr* 48:1291-1294, 1988
68. Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC: Regulation of adiposity by dietary calcium. *Faseb J* 14:1132-1138, 2000
69. Xue B, Greenberg AG, Kraemer FB, Zemel MB: Mechanism of intracellular calcium ([Ca2+]i) inhibition of lipolysis in human adipocytes. *Faseb J* 15:2527-2529, 2001
70. Ebbeling CB, Ward A, Puleo EM, Widrick J, Rippe JM: Development of a single-stage submaximal treadmill walking test. *Med Sci Sports Exerc* 23:966-973, 1991
71. Barger-Lux MJ, Davies KM, Heaney RP: Calcium supplementation does not augment bone gain in young women consuming diets moderately low in calcium. *J Nutr* 135:2362-2366, 2005
72. Mein AL, Briffa NK, Dhaliwal SS, Price RI: Lifestyle influences on 9-year changes in BMD in young women. *J Bone Miner Res* 19:1092-1098, 2004
73. Lloyd T, Martel JK, Rollings N, Andon MB, Kulin H, Demers LM, Eggli DF, Kieselhorst K, Chinchilli VM: The effect of calcium supplementation and Tanner stage on bone density, content and area in teenage women. *Osteoporos Int* 6:276-283, 1996
74. Davies KM, Heaney RP, Recker RR, Lappe JM, Barger-Lux MJ, Rafferty K, Hinders S: Calcium intake and body weight. *J Clin Endocrinol Metab* 85:4635-4638, 2000

75. Zemel MB, Miller SL: Dietary calcium and dairy modulation of adiposity and obesity risk. *Nutr Rev* 62:125-131, 2004
76. Iuliano-Burns S, Wang XF, Evans A, Bonjour JP, Seeman E: Skeletal benefits from calcium supplementation are limited in children with calcium intakes near 800 mg daily. *Osteoporos Int* 17:1794-1800, 2006
77. Taylor CB, Bryson S, Luce KH, Cunning D, Doyle AC, Abascal LB, Rockwell R, Dev P, Winzelberg AJ, Wilfley DE: Prevention of eating disorders in at-risk college-age women. *Arch Gen Psychiatry* 63:881-888, 2006