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Relationship Between Calcium Consumption from Dairy and Non-Dairy Sources on Bone Mineral Density of College Students

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THE RELATIONSHIP BETWEEN CALCIUM CONSUMPTION FROM DAIRY AND NON-DAIRY SOURCES ON BONE MINERAL DENSITY OF COLLEGE STUDENTS

by

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ABSTRACT

Dairy product consumption has been on the decline for decades in the US and worldwide, and the increase in plant-based substitutes has grown substantially. Osteoporosis and the risk of bone fractures are serious public health issues. To date, little scientific-based studies have compared the relationship between calcium intake from dairy sources vs non-dairy sources and bone mineral density. The purpose of this study is to compare the relationships between calcium consumption from dairy vs. non-dairy sources and bone mineral density among college students. There was a total of 66 participants in the study including 15 (23%) males and 51 (77%) females. The participants' ages ranged from 17 to 43 years with 48 (74%) being white, 16 (25%) African American, and 1 (1%) Hispanic. There was a significant difference found between males (M =.00, SD = .00) and females (M =.62, SD = 1.21) for z-scores, $t(37)$, p <.01. There was a significant difference found between males (M = 126.27, SD = 22.44) and females (M = 110.21, SD = 21.55) for stiffness index scores, $t(60)$, $p < 02$. There was a significant difference found between whites (M = 109.60, SD = 20.67) and non-whites (M = 129.27, SD = 22.86) in stiffness index scores $t(58)$, $p < .01$. There was a significant difference in dairy calcium intake between participants who did not meet the RDA (M = 293.43, SD = 249.36) and participants who did meet the RDA (M = 940.98, SD = 618.89) $t(15.54)$, $p < .01$. This study confirmed that not only were participants who didn't consume dairy calcium not meeting the RDA, but they weren't making up for the difference in non-dairy sources.

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CHAPTER 1

INTRODUCTION

Adequate calcium intake is associated with healthy bone maintenance, not only in childhood but throughout the lifespan. Current dietary recommendations suggest adequate calcium intake must occur throughout the lifespan to help build and maintain strong bones (Bueno & Czepielewski, 2008). The current dietary recommendations from the Institute of Medicine (IOM), the Recommended Dietary Allowances (RDA's) based on adequate bone health, range from 700 to 1300 mg/d for life-stage groups of at least one year of age. These recommendations cover requirements of $\geq$ 97.5% of the healthy population (Institute of Medicine, 2011). Consuming a diet inadequate in calcium can have negative effects on bone health and bone mineral density (Beuno & Czepielewski, 2008; Peacock, 2010; Ross et al., 2011). Current scientific-based evidence shows the importance of calcium intake for achieving peak bone mass and the maintenance of bone health over the lifespan (Beuno & Czepielewski, 2008; Peacock, 2010; Ross et al., 2011).

For many decades, dietary guidelines have recommended dairy products be consumed in order for children, adolescents, and even adults to achieve their daily recommended requirements for calcium intakes; however, a wide range of other foods provide amounts of calcium per serving that are similar to that of dairy products. The growing concern about hormone use in dairy farming has also led to a decrease in dairy

and milk consumption due to the fear that cows treated with the rBGH hormone can increase blood and tissue levels of growth hormone IGF-1 or insulin like growth factor in consumers (Toft, 2016). IGF-1 helps regulate the amount of growth hormone (GH) in the body. Normal IGF-1 and GH functions include bone and tissue growth. An excess of IGF-1 in the body changes the way the body uses glucose as well as causing tissue overgrowth or acromegaly. An excess of GH in the blood can lead to the possibility of pituitary tumors (Toft, 2016).

There is also a concern when animals are given higher than normal amounts of hormones. This can contribute to antibiotic resistant bacteria (American Cancer Society [ACS], 2014). Cows given rBGH tend to develop more udder infections, and in turn are given more antibiotics than cows not given rBGH. This has led to the fear that the antibiotic resistance developing in the cows given more rBGH could be passed on to humans; however, this idea has not been widely examined in humans (American Cancer Society [ACS], 2014).

Consumers are also becoming more aware and concerned about the general welfare of the animals supplying their food (McKendree, Croney, & Widmar, 2014). Public concerns regarding the production methods and treatment of animals in the farm industry have begun to grow (McKendree et al., 2014). Certain production methods, such as factory farming where thousands of animals are grouped together into small, crowded facilities and are fed poor diets, therefore leading to negative health effects, have come under scrutiny. Due to the poor conditions and overcrowded facilities, diseases can become common, which has led consumers to be concerned about possible exposure to

these health effects not only in the meat that they eat, but in the fluid milk products that are on the market as well (Cornish, Raubenheimer, McGreevy, & Phillips, 2016).

Increased detection of cow's milk protein sensitivities and lactose intolerance as well as recent advances in medical technology also have had an impact on the consumption of cow's milk and cow's milk products (Vandenplas et al., 2007). Approximately 2 - 7.5 % of infants are diagnosed with milk protein allergy (Vandenplas et al., 2007). However, not all signs and symptoms of an inability to tolerate cow's milk are related to protein found in cow's milk. Researchers report that between 5 and 15% of individuals exhibit signs and symptoms that resemble an allergic response to the cow's milk protein, but the reactions are more indicative of lactose intolerance (Vandenplas et al., 2007).

Lactose intolerance is defined as the inability to fully digest lactose, or the sugar found in milk or dairy products. Today, approximately 65% of the human population has a reduced ability to digest lactose after infancy. Lactose intolerance is most prevalent in people of East Asian descent. However, it is common in people of West African, Arab, Jewish, Greek and Italian descent. Only about 5% of people of Northern European descent are lactose intolerant (Lactose Intolerance [National Institute of Medicine], 2019).

Statement of the Problem

Osteoporosis and the risk of bone fractures are serious public health issues, and osteoporosis itself is the single most important cause of bone fractures in middle aged and elderly people. Healthy or normal bone mineral density levels have been defined as a T-score of -1.0 or above and a T-score that lies between -1.0 and -2.5 indicates the

individual has low bone density or osteopenia and is at risk for developing osteoporosis, and a T-score of -2.5 or above indicates an individual has osteoporosis. A T-score is the results of an individual's bone density compared with those of a healthy young adult of the same sex as. (Sheu & Diamond, 2016).

Calcium is a major mineral which is mostly stored in the bones and teeth, accounting for approximately 99% of all calcium found in the body (Bueno & Czepielewski, 2008). The bio-availability of calcium from cow's milk products is between 30 and 35%, making it quite easy to provide the body with adequate calcium to support bone health by consuming 2-3, 8-ounce servings of cow's milk per day to provide the body with adequate calcium to support bone health (Dairy Farmers of Canada [DNCA], 2019). Even though non-dairy sources can be high in calcium and potentially high in vitamins and fiber, they have limitations in regards to calcium bioavailability (Amalraj & Pius, 2015).

While the bioavailability of calcium fortified foods is comparable with that of milk, fortified foods do not always provide the same amount of calcium per serving as milk for example calcium fortified soy milk contains only 200 milligrams of calcium per eight ounce serving as compared to dairy milk containing 300 milligrams of calcium per eight ounce serving (Dairy Farmers of Canada [DNCA], 2019).

A study by Gao and colleagues showed diets excluding regular dairy product consumption make it difficult to meet daily calcium needs in adolescents aged 9 to 18 years (Gao, Wilde, Lichtenstein, & Tucker, 2006). The researchers concluded that adequate calcium intake cannot be met with a dairy-free diet. Consumption of adequate

calcium would require large changes in dietary patterns, and the addition of calcium fortified foods would be necessary (Gao et al., 2006).

Despite research that has demonstrated the positive impact of cow's milk and products made from cow's milk has on bone health, the consumption of these products has declined in recent years (Newton, 2019; Thorning et al., 2016). There is cause for concern given that it is estimated that 42% of the population of all ages do not meet their daily calcium requirement (Hoy & Goldman, 2014). This is especially true for college students because the 20's represent the last decade of life to achieve peak bone mass which will serve as protection against the aging process and osteoporosis (Thorning et al., 2016).

The Purpose

The purpose of this study is to compare the relationships between calcium consumption from dairy vs. non-dairy sources and bone mineral density among college students. Current scientific literature indicates calcium supplementation and intake are highly beneficial for bone mineral density levels and overall bone health (Flynn, 2003; Tai et al., 2015; & Winzenberg et al., 2006). However, few studies have compared differences in bone mineral density levels of individuals who obtain the majority of their calcium intake through dairy vs non-dairy sources.

Hypotheses

Four hypotheses will be tested:

1. There will be no significant difference in total average calcium consumed (milligrams/day) based on gender, school classification, and race.

2. There will be no significant difference in the average calcium (mg/day) consumed from dairy products based on gender, classification, and race.
3. There will be no significant difference in the average calcium (mg/day) consumed from non-dairy sources based on gender, classification, and race.
4. There will be no difference in student's fracture risk based on average total calcium intake; dairy calcium intake; or non-dairy calcium intake.

Justification

Milk and dairy product consumption have been on the decline for decades in the US and worldwide, and the increase in plant-based substitutes has grown substantially in line with this decline (Yu, 2017). Peak bone mass is reached by age 30, and maintenance of healthy bone mineral density levels has a profound effect on bone health as individuals age and become elderly (Bueno & Czepielewski, 2008). Therefore, it is not surprising that randomized controlled trials have found lower than normal bone density levels across all ages and genders (Black et al., 2002; Cullers, King, Van Loan, Gildengorin & Fung, 2019; Feshkanich, Meyer, Fung, Bischoff-Ferrari & Willett, 2018). Several studies have demonstrated improvement in bone mineral density when calcium supplementation is provided (Cullers et al., 2019; Flynn, 2003). To date, little scientific-based studies have compared the relationship between calcium intake from dairy sources vs non-dairy sources and bone mineral density.

CHAPTER 2

REVIEW OF LITERATURE

Calcium is one of the key elements in the human body that is available only through dietary sources (Peacock, 2010). There are a wide range of biological functions in which calcium plays an important role; however, the most important role of dietary calcium in biological functions is in regards to bone or skeletal mineralization. Calcium provides strength and rigidity to the human skeleton, and also serves as a dynamic store that can help maintain intra- and extracellular levels of this essential mineral (Peacock, 2010).

Calcium Metabolism

Calcium homeostasis is regulated through an integrated hormonal system that controls calcium transport in the gut, kidneys, and bone (Peacock, 2010). The two main calcium-regulating hormones are parathyroid hormone or PTH and its receptor PTHR and vitamin D and the vitamin D receptor or VDR. Serum ionized calcium and the calcium sensing receptor (CaR) also play a role as well in maintaining calcium homeostasis. The way these hormones work together to maintain serum calcium homeostasis not only maintains extracellular ionized calcium levels, but also allows the flow of calcium to and from essential stores, including the bone (Peacock, 2010).

The mechanisms the body uses to regulate serum calcium levels is an interplay of the rate at which calcium is absorbed in the intestines, the movement of calcium into and out of the bones, and the kidney's reclamation and excretion of calcium into the urine (Peacock, 2010). If serum calcium levels fall, this triggers the release of parathyroid hormone (PTH) from the parathyroid glands into the blood. PTH signals cells in bone called osteoclasts to release calcium from the surface of bones. PTH also signals the kidneys to reclaim more calcium before excretion into the urine (Peacock, 2010).

Decreases in serum calcium inactivate the calcium sensing receptor (CaR) in the parathyroid glands, which increase PTH secretion that acts on the parathyroid hormone receptor (PTHR) in the kidneys to increase secretion of vitamin D (Peacock, 2010). Vitamin D functions in calcium metabolism by stimulating intestinal calcium and phosphorus absorption, stimulating bone calcium mobilization, and by increasing renal reabsorption of calcium in the distal tubule. Vitamin D is particularly important in regards to low calcium intakes. Passive diffusion is the absorptive mechanism that dominates at high calcium intakes. At normal calcium intakes, a vitamin D dependent transport system is responsible for the majority of calcium absorption. This in turn activates the vitamin D receptor (VDR) in the gut to increase calcium absorption. This signaling will also decrease PTH secretion in the parathyroid glands and in bone to increase calcium resorption. This integrated hormonal response restores serum calcium to normal levels and closes this negative feedback loop. If there is a rise in serum calcium levels, this process is reversed in order to maintain calcium homeostasis (Peacock, 2010).

Almost all dietary calcium is absorbed in the upper intestine (Peacock, 2010). As a result, frequent meals or oral calcium supplements promote a larger net calcium

absorption. The bioavailability of calcium can be enhanced by phosphate binders, lactose and other sugars, as well as citric and other acids, and can be reduced by calcium binding agents such as cellulose, phosphate, and oxalate. During periods of rapid growth and development, children and adolescents are in what is referred to as positive bone balance, which means there is more calcium absorption creating more bone rather than bone loss. As adolescents' transition to early adulthood they remain in positive bone balance as long as they maintain and active lifestyle and healthy diet. As they transition into adulthood, they transition into a neutral bone balance or a state of equilibrium where bone formation equals absorption and bone loss. Around the age of 50 years, negative bone balance appears and is reflected by some bone loss due to less development and less absorption (Peacock, 2010).

The average adult human body contains about 1200 grams of calcium, which equates to between 1 and 2 percent of total body weight (Cashman, 2002). About 99 percent of calcium is found in bones and teeth. Calcium is required to achieve normal growth and development of the human skeleton, which continue until skeletal maturation, which occurs around the early twenties in humans (Cashman, 2002). Over the past few decades, several studies have reported positive effects of increased calcium during childhood and adolescence on bone mineral density in early adulthood (Cashman, 2002; Bueno & Czepielewski, 2008; Tai, Leung, Grey, Reid, & Bolland, 2015).

Bone Mineral Density

Bone mineral density, also referred to as bone mineral content, is defined as a measurement of long-term calcium balance (Peacock, 2010). Increases in bone mineral content are largest during childhood, reach a peak during adolescence, stay relatively

constant or at maintenance during early to late adulthood, and begin to decline in old age (Peacock, 2010).

Osteoporosis is a disease characterized by low bone mass and deterioration of bone structure that causes bone fragility and increases the risk of fracture (Office of the Surgeon General, 2004). Osteopenia or bone loss is a condition that can occur when the body's rate of bone formation or ossification is exceeded by the rate of bone reabsorption. This makes the bones weaker than normal but not as weak as would be associated with osteoporosis (Office of the Surgeon General, 2004). Rickets is softening or weakening of the bones in children usually caused by inadequate vitamin D intake. The condition results from a delay in depositing calcium into growing bones. In adults the condition is called osteomalacia (Office of the Surgeon General, 2004).

Bone loss is more rapid in women and usually begins after menopause due to a decrease in the hormone estrogen. Male bone loss typically occurs when the production of testosterone begins to decline around age 45 to 50 (Calhoun et al., 2018). Women are far more likely to develop osteoporosis than men due to having smaller and less dense bones. While female sex, family history, and age are major risk factors for developing osteoporosis, there are other modifiable, and non-modifiable risk factors (Calhoun et al., 2018).

Individuals of European or Asian decent are at a higher risk for developing osteoporosis when compared to other ethnicities. This is largely due to differences in bone mineral density (Cauley, 2011). A study of 359 adult Chinese women found significantly lower bone mineral density at the lumbar spine, total hip, and femoral neck when compared with white women. African-Caribbean women have the highest mean

bone mineral density reported for women (Leslie, 2012). The largest multi-ethnic study to date is the National Osteoporosis Risk Assessment study or NORA (Leslie, 2012). The study has a cohort of 197,848 women undergoing a variety of peripheral bone mineral density measurements. When ethnicities were compared, African-American women had a lower risk for T-score in the osteoporotic range and a similarly low risk for fracture whereas Hispanic and Native American women had similar risk to white women. Among Asian women, the unadjusted risk for osteoporosis was higher than that of white women (Leslie, 2012).

Certain medications, specifically corticosteroids taken for an extended period of time can cause bone thinning (Calhoun et al., 2018). Even certain surgeries such as having the ovaries removed prior to menopause can place an individual at greater risk for developing osteoporosis. People who have a long history of dieting or eating disorders such as anorexia nervosa and female athletes who have infrequent menstrual cycles due to low body fat percentages are at higher risk as well (Calhoun et al., 2018).

Hormonal changes can have a considerable impact on bone health specifically in postmenopausal women (Rizzoli, Ferrari, Hughes, & Weaver, 2014). Menopause is the natural decline in reproductive hormones that usually occurs between the fourth and fifth decades of life. Osteoporosis affects one out of three postmenopausal women (Rizzoli et al., 2014). Menopause causes a rapid drop in estrogen production which leads to an increase in bone turnover and accelerated bone loss that is accompanied by microstructural alterations. Menopause, the largest risk factor for women developing osteoporosis, may promote an average bone loss of 2-3% in the first few years after menopause with the rate decreasing by .5-1% in subsequent years (Rizzoli et al., 2014).

The risk of osteoporosis can be reduced through healthy lifestyle changes which include adequate intakes of dietary calcium as well as adequate vitamin D intake, weight bearing exercise, reduction in alcohol consumption, and smoking cessation (Rizzoli et al., 2014).

Dietary Calcium

The largest sources of dietary calcium for the majority of people residing in developed countries is cow's milk, dairy products made from cow's milk, and calcium supplements (Omidvar, 2015). Dairy products contain the largest amount of calcium per serving but other sources of calcium have been shown to contribute to adequate daily calcium intakes of 1200 to 1500 milligrams per day (Rozenberg et al., 2016). In recent years, numerous non-dairy products and dairy substitutes have been introduced to the market. These products may be foods fortified with calcium, some of which are identified as milk substitutes (Newton, 2019). In fact, most of these products, including ones such as orange juice, have been fortified with enough calcium to reach levels that are similar to that of one, eight-ounce serving of cow's milk (Thorning et al., 2016). Alternatives, such as lactose free and low lactose milks also exist for individuals with lactose intolerance. Calcium supplementation, using products such as calcium citrate, is a viable option for individuals who cannot or will not consume adequate amounts of calcium from appropriate dietary sources (Thorning et al., 2016).

One cup of milk, either 2%, whole, or skim, and one cup of yogurt contains approximately 300 mg of calcium. A one ounce serving of cheese contains 200 mg of calcium. Vegetarian sources of calcium include kale, spinach, soybeans, carrots and potatoes and contain between 26 and 300 milligrams of calcium per one cup serving (Yang, Punshon, Guerinot, & Hirschi, 2012). One disadvantage of vegetarian sources is

calcium density and bioavailability are lower when compared with cow's milk and products made with cow's milk (Yang et al., 2012). Due to this aspect, larger servings are needed in order to equal the total calcium absorption that can be obtained from dairy products (Gao et al., 2006).

A study done for the United States Department of Agriculture by Stewart, Diangsheng, and Carlson (2013) explored why Americans are consuming less fluid milk. Specific focus was on generational differences. The researchers pointed out that younger generations are consuming less fluid milk than older generations, and suggested this could be attributed to a change in the overall way of life, such as increased fast food availability as well as a wider selection of different beverage choices at supermarkets and convenience stores. Soft drinks, sports drinks, bottled water, and other beverages compete with fluid milk for the consumers' appetite. Differences in generational intake of fluid milk continue to make it difficult to reverse current consumption trends. As newer generations replace older ones, the population's average fluid milk consumption likely will continue to decline (Stewart, Diangsheng, & Carlson, 2013).

Recommended Dietary Allowances for Calcium

The Institute of Medicine used available evidence to establish the estimated average requirements (EARs) and RDAs for calcium and vitamin D for all life-stages except for infants (Ross et al., 2011). Prior to the 2011 update, there was insufficient evidence for the estimation of EARs and DRIs. The 2011 RDA committee aimed to address three main questions: (1) What health outcomes were influenced by vitamin D and/or calcium intake? (2) How much calcium and vitamin D are needed to achieve desirable health outcomes? and (3) How much is too much?

The 2011 dietary reference intakes (DRIs) for calcium are based on the calcium content of human breast milk for infants, calcium balance studies for ages 1-50 years, and observational and clinical trial evidence for those over the age of 50, using bone health as the main indicator (Ross et al., 2011). The tolerable upper limit of calcium intake is 2500 mg/d from ages 1-8, 3000 mg/d for ages 9-18, 2500 mg/d for ages 19-50, and 2000 mg/d from ages 51-71+ for both males and females. The RDAs, or intake that covers approximately 97.5% of the population is 700 mg/d for ages 1-3, 1000 mg/d for ages 4-8, 1300 mg/d for ages 9-18, 1000 mg/d for ages 19-50 for both males and females, 1000 mg/d for ages 51-70 for males, 1200 mg/d for ages 51-70 for females, and 1200 mg/d for ages 71+ for both males and females (Ross et al., 2011).

Non-Dairy Sources of Calcium

Recent evidence shows fluid-milk substitutes such as almond milk and coconut milk-based beverages have experienced drastic increases in sales. Compound growth rates have been 66% for almond-based milk substitutes and 111% for coconut-based milk substitutes (Newton, 2019). A 2014 report published for the United States Department of Agriculture examined the calcium intake of the US population and in particular, the specific sources of calcium and the percentage of calcium those sources contribute to the American diet. The researchers determined non-dairy sources of calcium such as grains contributed only 12% of overall calcium intake to diets and non-dairy beverage substitutes contributed only 8% of total calcium intake. Other fortified beverages such as fortified orange juice contributed 4% of total calcium intake, and fruits and vegetables containing calcium contributed 4% (Hoy & Goldman, 2014).

More and more consumers are replacing their dairy sources with products that are plant-based (Thorning et al., 2016). In fact, the market research firm called Mintel™ released a report forecasting total US dairy sales during the period from 2015 to 2020 will decrease by 15.9 billion dollars, which equates to an 11% drop from previous reporting periods. Mintel™ reported a three-billion-dollar growth in non-dairy beverage sales from 2015 to 2020, stating US consumption of fluid milk and dairy products has been on the decline for decades (Yu, 2017).

The demand for non-dairy beverages is expected to continue to rise, and companies will need to invest research and development activities in this direction. The non-dairy beverage and product market will continue to evolve as the demand continues to increase and current estimates and trends are predicting a continued increase in sales and consumption in the future (Deora & Deswal, 2018).

Currently, the scientific literature is limited in regards to investigating the effects of non-dairy food sources and bone health. Most studies exploring the effects of non-dairy calcium on bone health do so from the perspective of calcium supplementation. In addition, these studies often use young girls and pre or post-menopausal women to conduct their investigations. A 2006 meta-analysis showed that calcium supplementation had no effect on bone mineral density at the femoral neck or lumbar spine and that there was a small effect on total body mineral content. The researchers concluded the small positive effect of calcium supplementation on bone mineral density in the upper limb is unlikely to reduce the risk of fracture either in childhood or later in life to a degree that matters for public health (Winzenberg, Shaw, Fryer & Jones, 2006).

A more recent study performed on a younger population and published in 2019 analyzed the effect of prenatal calcium supplementation on bone health in 64 women during pregnancy and for 1 year postpartum. The calcium supplementation group had significantly greater increases in total bone mineral density levels at one year postpartum than the placebo group. Researchers concluded that supplemental calcium provided during pregnancy may improve bone recovery postpartum in women consuming a typical US diet (Cullers et al., 2019).

Non-dairy foods high in calcium that are plant based may contain compounds that interfere with calcium absorption (Amalraj & Pius, 2015). These compounds, which include oxalates and phytates, inhibit calcium absorption by either binding to calcium in the gut and rendering it indigestible, or by being present in food in the form of an indigestible calcium salt that cannot be broken down or absorbed. Commonly consumed foods that contain calcium and inhibitors such as phytates and oxalates, include leafy greens such as spinach and beet greens, and to some extent kale. Commonly eaten foods that contain phytates as well as calcium are whole grains, nuts, seeds, and legumes (Amalraj & Pius, 2015).

Food manufacturers have responded to the decline in cow's milk sales by fortifying their products with calcium to enhance their sales revenue and meet the growing consumer demand for non-dairy sources of calcium (Newton, 2019). Fortification is the manufacturing process of adding micronutrients to food and is carried out to reduce the number of people with dietary deficiencies in the population (Gharibzahedi & Jafari, 2017). Calcium can be found in orange juice, margarine, and ready-to-eat cereals. One study examining the calcium content of fortified beverages

showed calcium salts tend to settle at the bottom of the carton, and vigorous shaking may not be enough to resuspend the calcium salts (Rafferty, Walters, & Heany, 2007).

Dairy Sources of Calcium

Significant increases in the proliferation of anti-dairy media stories, and internet posts are also contributing to the decline in milk and dairy product sales (Thorning et al., 2016). Certain people and organizations are claiming milk and dairy products can increase risks of developing chronic diseases including obesity, type 2 diabetes, cardiovascular disease, osteoporosis, and even cancer (Thorning et al., 2016).

Milk price volatility, the proliferation of imitation milk products and bottled water products, reduced consumption of ready-to-eat cereals, as well as legislation limiting school milk options have caused a sharp decline in milk consumption over the last several decades (Newton, 2019). Prior to the 1980's, more than 50% of the milk regulated by the United States Department of Agriculture's (USDA) Federal Milk Program was beverage milk. By 2015, only 33% of milk in the Federal Order program was fluid milk (Newton, 2019). It was during this time that per-capita consumption of beverage milk declined by 25% to approximately 18 gallons per person (Newton, 2019). USDA agricultural data indicate that from 2012 to 2016, annual conventional milk sales declined by more than four billion pounds or approximately 8% (Newton, 2019).

There is a larger body of scientific evidence regarding the effect of calcium from dairy sources on bone health. Two studies conducted in 2017 examined the association of dairy products with bone mineral density as well as reduced risk for hip fracture in older adults over the age of 50. Both studies showed higher intakes of milk, fluid dairy, and yogurt and cheese were associated with higher lumbar spine bone mineral density, and a

higher intake of milk, fluid dairy, and yogurt and cheese were protective against trochanter bone mineral density loss and lowered risk of hip fracture among vitamin D supplement users but not among nonusers (Feskanich et al., 2018; Sahni et al., 2017). A recent meta-analysis showed that milk consumption at higher levels was associated with lower risk of hip fractures versus consumption at the lowest levels in case control studies, and that the consumption of yogurt and cheese was associated with lower risk of hip fracture in cohort studies (Bian et al., 2018).

Non-Dietary Factors

Factors other than calcium intake also can have effects on bone mineral density and bone health (Bueno & Czepielewski, 2008. While genetics have been shown to be a large determinant of an individual's peak bone mass, factors such as exercise, smoking, and coffee consumption have been implicated in affecting bone health (Bueno & Czepielewski, 2008).

A 2001 review analyzed calcium, physical activity, and bone health and their relation to one another. The review summarized the role that calcium, other vitamins and minerals, and physical activity play in achieving peak bone mass. The researchers concluded that the "functional demand that exercise imposes on bone is a large determinant of its structure. The researchers conclude that the full genetic potential for peak bone mass can be achieved by combining adequate intake of nutrients that are associated with bone health and weight bearing physical activity (Branca & Vatuena, 2001).

A 2017 systematic review analyzed the effects of exercise on areal bone mineral density in perimenopausal and postmenopausal women in an effort to provide

information on the most suitable bone-loading exercise regimens for improving bone health in these populations. The review confirmed the positive effects of impact exercises combined with other forms of training such as strength training on increasing bone mineral density in the lumbar and femoral neck as well as the preservation of bone mineral density in perimenopausal and postmenopausal women (Sanudo et al., 2017).

A meta-analysis published in 2001 showed that smokers had significantly lower bone mass when compared with non-smokers at all bone sites. The researchers estimated that smoking increases the lifetime risk of vertebral fracture by 13% in women and 32% in men. They concluded smoking has an independent, dose-dependent effect on bone loss, which increases fracture risk and may be partially reversed by smoking cessation (Ward & Klesges, 2001). Wong, Christie and Wark (2007) used data from twin studies and the three main published meta-analyses and concluded that smoking cessation should be a major component of any bone therapeutic program (Wong, Christie & Wark, 2007).

Coffee consumption has been thought to be associated with bone health because of caffeine metabolism. However, it is currently unknown coffee/caffeine consumption leads to sustained bone mineral loss or if it is an individual, dose-dependent response (Hallstrom et al., 2010).

A study published in 2010 examined the relationship between consumption of coffee and bone mineral density at the proximal femur in men and women. Researchers concluded a high consumption of coffee seems to contribute to a reduction in BMD of the proximal femur in elderly men but not in women. BMD was lower in high caffeine consumers suggesting that rapid metabolizers of caffeine may constitute a risk group for bone loss induced by coffee (Hallstrom et al., 2010). A more recent study published in

2014 did not support the idea that coffee is a risk factor for impaired bone health in Korean premenopausal women (Choi et al., 2014).

Summary

Several studies in children and adolescents have demonstrated that dietary calcium intake from dairy foods or calcium supplements positively contributes to increases in bone mineral density (Demmer, Cifelli, Houchins & Fulgoni, 2016; Gao, Wilde, Lichtenstein & Tucker, 2006; Winzenberg, Shaw, Fryer & Jones, 2006). Maintaining adequate calcium intake during childhood and adolescence is essential for achieving peak bone mass and maintaining adequate calcium intakes during adulthood has been shown to be necessary for bone mineral maintenance and diminishing bone loss while reducing the risk for fractures during the aging process (Bueno & Czepielewski, 2008; Demmer, Cifelli, Houchins & Fulgoni, 2016; Miller, Jarvis & McBean, 2001). Optimal calcium intake is especially relevant during adolescence when most mineral accretion occurs (Greer & Krebs, 2006). Further research is needed to determine if college students are achieving adequate daily calcium intakes despite a decline in dairy and milk consumption across the United States, and to determine the dietary sources of calcium they are consuming and what impact, if any this has on bone mineral density.

CHAPTER 3

METHODS

The purpose of this study is to compare the relationships between calcium consumption from dairy vs. non-dairy sources and bone mineral density among college students. A descriptive, cross-sectional study design will be employed.

Subjects

A convenience sample of college students enrolled in undergraduate nutrition courses offered during the fall of 2019 at Louisiana Tech University. The target sample size was 100 participants who meet the inclusion criteria. Inclusion criteria was individuals between the ages of 18 and 45 years of age. The participants who agreed to participate have provided informed consent (Appendix A).

Data Collection Instruments

Three separate collection instruments were used for this study. The first assessment tool was a 24-hour dietary recall designed to obtain students' dietary intake over the previous 24 hours. The tool reflects, time, food or beverage consumed, serving size, and supplements taken. The second assessment tool was a food frequency calcium scoring sheet developed by Dr. Terri Vanderlinde and available at https.//bestgyn.com.

The tool reflects a 24-hour time frame, the inclusion of foods known to be high in calcium, and correct portion sizes to reflect accurate reporting of those foods. The tool also records students' gender, race, and school classification. The tool was modified to reflect a wider variety of calcium sources that college students commonly consume, such as whey protein, caffeinated beverages made with dairy or non-dairy products, and a more detailed section on calcium supplementation including a daily multi-vitamin. A copy of this instrument is provided in (Appendix B).

The final assessment tool was a bone mineral density measurement tool, the Achilles EXP™ from General Electric Healthcare. The Achilles EXP™ is an ultrasound bone densitometer that measures bone mineral density by using quantitative ultrasound (QUS) without the use of ionizing radiation to accurately predict fracture risk in study participants. QUS uses ultrasound waves that pass through fluid and human tissues and undergo attenuation based on the density of the calcaneus bone. The analysis of this attenuation will be used to generate empirical measurement that is expressed as a numeric value. This numeric value is then compared to the QUS stiffness index which lists ranges for QUS values that correlate to particular T-scores (General Electric Co., 2017). The clinical standard for normal bone mineral density is defined as a T-score of greater than -1.0 or above. A T-score between -1.0 and -2.5 is considered osteopenic, and T-scores less than -2.5 are considered osteporotic (Hammad, 2013). A T-score is the results of an individual's bone density compared with those of a healthy young adult of the same sex as. (Sheu & Diamond, 2016).

Data Collection Procedure

Approval was obtained from the Human Use Committee at Louisiana Tech University will be obtained (Appendix A). Subject selection began in the fall of 2019, and data was collected after the participants had received instruction on estimating portion sizes from the instructors of both courses, Dr. Catherine Fontenot and Mrs. Amy Hogan. Students practiced completing a 24-hour dietary recall during the first week of classes. Dr. Catherine Fontenot and Mrs. Amy Hogan provided students with instructions on the first Monday of class, and had students complete a sample 24-hour dietary recall. During the next class period, students participated in a discussion to ask questions. The Friday of the first week of class, students took home a 24-hour dietary recall food to complete over the weekend and submit in class the following Monday. Students were then assigned a date and time for bone mineral density testing. At the appropriate date and time, students reported to the nutrition assessment lab on Louisiana Tech University's campus for bone mineral density testing using the Achilles EXP™ ultrasound bone densitometer. Bone mineral density measurements were taken by Dr. Catherine Fontenot and/or Dr. Vicky Green. All participant data was stored in a locked filing cabinet in Dr. Catherine Fontenot's office and only the researchers had access to this data. All data was shredded upon completion of the study.

Data Analysis

The 24-hour recalls were scored by the researcher. The 24-hour food recall calcium scoring sheet used to convert food frequency to milligrams of calcium is provided in Appendix B. Daily intake of total calcium, dairy calcium, and non-dairy calcium was extrapolated from the 24-hour food recalls, which were then used for data

analysis. Once the students completed both the 24-hour recall and BMD test and the data have been matched, data was identified and analyzed by the researcher.

The statistical software used to analyze the data for this study was Statistical Package for the Social Sciences (SPSS) BASE for Students, version 25. Descriptive statistics include frequencies and percentages for gender, school classification, and race; and means and standard deviations for average 3-day calcium intake and bone mineral density scores.

For the first three hypotheses,

1. There will be no significant difference in total average calcium consumed (milligrams/day) based on gender, school classification, and race.
2. There will be no significant difference in the average calcium (mg/day) consumed from dairy products based on gender, classification, and race.
3. There will be no significant difference in the average calcium (mg/day) consumed from non-dairy sources based on gender, classification, and race.

The dependent variable is milligrams of calcium consumed (total, dairy, and non-dairy). The independent variables are gender, race, and school classification. Hypotheses will be analyzed with t-tests, correlations, and chi-squared tests depending on number of groups in independent variables.

For the fourth hypothesis,

4. There will be no difference in student's fracture risk based on average total calcium intake; dairy calcium intake; or non-dairy calcium intake.

The dependent variable is fracture risk and the independent variables are total, dairy, and non-dairy calcium intake. This hypothesis was tested using t-tests, correlations, and chi-squared tests. The bone mineral density scores T-scores were provided on the printout for each students' test with the GE Achilles EXP bone density machine assigned each student a fracture risk based on T-scores provided by the machine's printout for each test (low risk=T score of -1.0 or above, medium risk=T score between -1.0 and -2.5, high=T score less than -2.5). The supporting tables and figures are presented in Appendix B.

Plans to Share Results

Results will be shared by submitting a manuscript to the *Journal of Clinical Nutrition*. Findings will also be shared with Louisiana Tech University in Ruston, Louisiana.

CHAPTER 4

RESULTS

There was a total of 66 participants in the study including 15 (23%) males and 51 (77%) females. The participants' ages ranged from 17 to 43 with 48 (74%) being white, 16 (25%) African American, and 1 (1%) Hispanic. The percentage of Hispanic students was too small to statistically analyze therefore race was categorized as whites, and non-whites. Eight of the participants were freshman, twenty-one sophomores, thirteen were juniors, and twenty-two were seniors at the university. Nineteen (31%) of the participants weighed less than 127 pounds and 43 (69%) weighed more than 127 pounds. The body weight categories of under 127 pounds or over 127 pounds were the only weight-related data reported by the Achilles EXP bone density scanner report printed for each participant. We did not collect measured heights and weights of the participants and therefore did not calculate BMI. Fifteen participants reported they currently smoke cigarettes while 47 were non-smokers. Weight, and smoking status was missing for four participants, two participants did not report academic classification, and one participant did not report race. See Table 1.

Table 1

Participant Characteristics (N=66)

Characteristic	Number	Percent
Gender		
Male	15	23%
Female	51	77%
Age		
17-20	32	48%
21-25	29	44%
26-43	5	8%
Race		
White	48	74%
African American	16	25%
Hispanic	1	1%
Class Classification		
Freshman	8	13%
Sophomore	21	33%
Junior	13	20%
Senior	22	34%
Weight		
Under 127 lbs.	19	31%
Over 127 lbs.	43	69%
Smoking		
Yes	15	24%
No	47	76%

Stiffness index scores use the theory of quantitative ultrasound and is based on the principle that bone as a porous material will absorb, scatter and transmit sound wave dependent on stiffness, density and volume of the material. Both sound attenuation and the sound velocity are combined to form the stiffness index used in commercial units. The Achilles EXP II from GE Healthcare calculates stiffness index as Stiffness

Index = (0.67 * BUA + 0.28 * SOS) – 420. Normal stiffness index scores are > 100 for ages 20-59, > for ages 60 and above, and stiffness index scores of 60 and below are considered osteoporotic (Sheu & Diamond, 2016).

Bivariate correlations were used to explore the relationships between the variables. There was a strong significant positive correlation between total calcium intake and dairy calcium intake, $r = 0.93$, $p < 0.05$. There was a strong significant positive correlation between T-score, and Z-scores, $r = 0.94$, $p < 0.05$. There was a strong significant positive correlation between T-score and stiffness index, $r = 0.98$, $p < 0.05$, as well as a strong significant positive correlation between Z-scores and stiffness index, $r = 0.91$, $p < 0.05$. Table 2 shows the study correlations.

Table 2

Correlations between the Variables

Variable	*n*	*M*(*SD*)	1	2	3	4	5	6	7
Age	66	21.35 (3.72)							
Daily Ca	61	822.06 (674.41)	-0.16 [0.23]						
Dairy Ca	66	472.97 (628.51)	-0.21 [0.10]	0.93** [0.00]					
Non-Dairy Ca	61	274.60 (240.48)	0.07 [0.55]	0.32* [0.01]	0.08 [0.55]				
T-Score	48	0.76 (1.24)	-0.08 [0.58]	-0.05 [0.75]	-0.11 [0.46]	0.18 [0.22]			
Z-Score	42	0.56 (1.16)	-0.07 [0.68]	-0.05 [0.76]	-0.28 [0.08]	0.22 [0.17]	0.94** [0.00]		
Stiffness	62	114.10 (22.67)	-0.15 [0.24]	0.18 [0.17]	0.14 [0.29]	0.16 [0.22]	0.98** [00]	0.91** [00]	

*$p < .05$, **$p < .01$. *P* values are in brackets.

Independent samples t-tests were conducted to determine whether variables differed between the genders, white and non-white races, smoking behavior, and calcium intake. Tables 3, 4, 5 and 6 display the results of the sampling. There was a significant difference found between males (M =0.00, SD = 0.00) and females (M = 0.62, SD = 1.21) for z-scores, $t(37)$, p <.01. There was a significant difference found between males (M = 126.27, SD = 22.44) and females (M = 110.21, SD = 21.55) for stiffness index scores, $t(60)$, p < 02.

Table 3

T-Test Comparisons of Variables by Gender

Independent Samples T-Test by Gender								
Influencing Factor	Male		Female					
	n	*M*(*SD*)	*n*	*M*(*SD*)	*t*	*df*	*Sig* (*2-tailed*)	*95% CI*
Daily Ca	15	1070.26 (1109.76)	46	741.13 (442.79)	1.66	59	0.10	[-66.31, 724.58]
Dairy Ca	15	786.43 (1032.63)	51	380.78 (420.49)	2.27	64	0.03	[48.06, 763.26]
Non-Dairy Ca	15	283.83 (216.49)	46	318.97 (268.45)	-0.46	59	0.65	[-188.08, 117.81]
T-Score	10	1.41 (1.15)	38	0.59 (1.21)	1.92	46	0.06	[-0.04, 1.68]
Z-Score	4	0.00 (0.00)	38	0.62 (1.21)	-3.17**	37	0.00	[-1.02, -0.22]
Stiffness	15	126.27 (22.44)	47	110.21 (21.55)	2.49*	60	0.02	[3.15, 28.96]

*$p < .05$, **$p < .01$

Table 4

T-Test Comparisons of Variables by Race

Independent Samples T-Test by Race								
Influencing Factor	White		Non-White					
	n	*M*(*SD*)	*n*	*M*(*SD*)	*t*	*df*	*Sig* (*2-tailed*)	*95% CI*
Daily Ca	44	825.61 (685.28)	15	813.21 (708.30)	0.06	57	0.95	[-401.31, 426.12]
Dairy Ca	48	436.65 (633.09)	16	566.46 (663.35)	-0.70	62	0.49	[-499.43, 239.83]
Non-Dairy Ca	44	349.26 (274.32)	15	208.99 (168.60)	2.33**	40	0.03	[18.92, 261.62]
T-Score	40	0.71 (1.15)	8	1.01 (1.68)	-0.62	46	0.54	[-1.27, 0.67]
Z-Score	35	0.53 (1.09)	7	0.70 (1.58)	0-.34	40	0.74	[-1.15, 0.82]
Stiffness	45	109.60 (20.67)	15	129.27 (22.86)	-3.11**	58	0.00	[-32.33, -7.00]

*$p < .05$, **$p < .01$

Table 5

T-Test Comparisons of Variables by Smoking

Independent Samples T-Test by Smoking								
Influencing Factor	No		Yes					
	n	*M*(*SD*)	*n*	*M*(*SD*)	*t*	*df*	*Sig* (*2-tailed*)	*95% CI*
Daily Ca	43	807.38 (737.00)	15	752.42 (407.18)	0.27	56	0.79	[-347.48, 457.41]
Dairy Ca	47	470.24 (672.18)	15	351.26 (365.66)	0.65	60	0.52	[-245.53, 483.49]
T-Score	32	0.98 (1.28)	13	0.65 (0.87)	0.87	43	0.39	[-0.45, 1.12]
Z-Score	29	0.83 (1.22)	10	0.27 (0.53)	1.39	37	0.17	[-0.26, 1.37]
Stiffness	44	116.82 (22.99)	15	107.73 (16.15)	1.41	57	0.16	[-3.80, 21.97]

*$p < .05$, **$p < .01$

Table 6

T-Test Comparisons of Variables by RDA

Independent Samples T-Test by RDA								
Influencing Factor	<1000 mg		>3000 mg					
	n	*M*(*SD*)	*n*	*M*(*SD*)	*t*	*df*	*Sig* (*2-tailed*)	*95% CI*
Daily Ca	45	542.52 (269.89)	15	1419.53 (390.02)	-8.09 **	18.68	0.00	[-1104.25, 649.76]
Dairy Ca	45	293.43 (249.36)	15	940.98 (618.89)	-3.95 **	15.54	0.00	[-996.18, -298.92]
T-Score	33	0.85 (1.23)	9	0.47 (1.38)	0.81	40	0.42	[-0.58, 1.35]
Z-Score	29	0.60 (1.15)	8	0.26 (1.23)	0.73	35	0.47	[-0.61, 1.28]
Stiffness	45	113.33 (22.44)	15	118.53 (24.21)	-0.76	58	0.45	[-18.86, 8.46]

*$p < .05$, **$p < .01$

There was a significant difference found between whites (M = 109.60, SD = 20.67) and non-whites (M = 129.27, SD = 22.86) in stiffness index scores $t(58)$, $p < 0.01$. Also, the non-dairy calcium intake of whites (M = 349.25, SD = 274.32) was significantly higher than non-whites (M = 208.99, SD = 168.60) $t(40.05)$, $p < 0.05$. A regression analysis was run using stiffness as the dependent variable and age, daily calcium intake, dairy calcium intake, and smoking as the independent variables. The analysis showed that smoking did not significantly predict stiffness index score ($\beta = 0.34$, $t(4)$, $p < 0.05$. There were no significant differences found between variables in the T-Test for smoking.

Participants were categorized as those who did or did not consume adequate calcium according to the National Academy of Sciences Recommended Dietary

Allowance (RDA) (Ross et al., 2011). There was a significant difference found in calcium intake for those who did not meet the RDA (≤ 1000 mg) (M = 542.52, SD = 269.89) and those who did meet the RDA (≥1000 mg) (M = 1419.53, SD = 390.02) for daily calcium intake t(18.68), $p < .01$. There was a significant difference in dairy calcium intake between those participants who did not meet the RDA (M = 293.43, SD = 249.36) and participants who did meet the RDA (M = 940.98, SD = 618.89) t(15.54), $p < .01$. We attempted to run Chi-Squared tests to determine the differences between RDA and gender, RDA and race, RDA and age, and RDA and smoking however, these tests could not be run due to small cell size counts of less than five.

CHAPTER 5

DISCUSSION

Hypothesis one stated that there would no significant difference found in total average calcium consumed (milligrams/day) based on gender, school classification, and race. Our study found no significant difference in daily calcium consumption between genders, classification, or race.

Hypothesis two stated that there would be no significant difference in the average calcium (mg/day) consumed from dairy products based on gender, classification, and race. We found no significant difference in dairy calcium consumption based on gender, classification, or race however, from our findings, there was a strong correlation between total daily calcium consumption and dairy calcium consumption. As the participants' dairy calcium consumption increased, so did total calcium consumption. Participants, who did not consume calcium from dairy products, did not meet the RDA for daily calcium consumption. Participants who consumed at least 1000 mg of calcium per day had statistically significant higher dairy calcium intakes. The strong positive correlations between total daily calcium intake and dairy calcium underscores the importance of dairy calcium sources to overall intake. Participants who did not consume a minimum of 1000 mg of daily calcium likely needed dairy products to meet the RDA because vegetarian sources of calcium provide smaller amounts per serving. This is important as participants

who were not consuming the RDA for calcium intake were not only consuming inadequate dairy sources of calcium, but they were not making up the difference in vegetarian or plant-based sources.

Hypothesis three stated that there would be no significant difference in the average calcium (mg/day) consumed from non-dairy sources based on gender, classification, and race. There was a significant difference found between non-dairy calcium intake between whites and non-whites, with whites having a higher intake. The significant difference found suggests that there is a higher intake possibly related to plant based sources of calcium specifically, vegetables. This could possibly suggest that access to non-dairy calcium sources were limited or financial resources for those food sources might be more limited in the non-white participants than in the white participants.

Hypothesis four stated that there would be no difference in student's fracture risk based on average total calcium intake; dairy calcium intake; or non-dairy calcium intake. T-Scores are a comparison of a person's bone density with that of a healthy thirty-year-old of the same sex and Z-Scores are a comparison of a person's bone density with that of an average person of the same age and sex. Z-scores are considered useful in healthy, premenopausal women, men under the age of 50, and children. A z-score of -2.0 or less is considered below the expected range for the age and a z-score above -2.0 is within the expected range for age (Sheu & Diamond, 2016). There is no difference in fracture risk as measured by the stiffness index. T and Z scores are indicative of risk for osteoporosis which increases the risk for fracture. Stiffness index fracture in indicative of fracture risk.

There were no significant correlations found between stiffness index and daily calcium, dairy calcium, and non-dairy calcium consumption. The strong correlations

found between t-scores and z-scores as well as the correlation between z-scores and stiffness index scores show that participants who had higher t-scores also had higher z-scores and participants who had higher z-scores also ranked higher on the stiffness index. Individuals who have high t-scores, as well as z-scores rank higher on the stiffness index and therefore are at a lower risk for osteoporosis.

The significant differences found between males and females z-scores as well as the correlation between z-scores and stiffness index are congruent with current literature regarding gender as a risk factor for osteoporosis (Calhoun et al., 2018). The significant differences found between whites and non-whites and their stiffness index scores is also consistent in line with current literature (Leslie, 2012).

Certain ethnicities are also at increased risk for developing osteoporosis. Specifically, individuals of European descent are at higher risk than other ethnicities (Cauley, 2011). The findings in this study supported this risk in this age group as a significant difference was found between whites and non-whites stiffness index scores. This is consistent with current literature indicating African-Americans have higher stiffness index scores than that of individuals of European descent resulting in lower risk for developing osteoporosis (Leslie, 2012).

Gender, specifically female gender, is a risk factor for developing osteoporosis. There was a significant difference found between males and females z-scores as well as male and female stiffness index scores. These findings are in line with the current literature regarding females being higher risk than males for osteoporosis (Calhoun et al., 2018). These findings are also important because these differences were evident in these young adults.

While the effects of smoking on bone health are reported in the literature (Ward & Klesges, 2001) & (Wong, Christie & Wark, 2007), we did not see significant differences in our T-Tests between smokers and non-smokers. This may be in part due to the limited length of time smokers may have smoked due to their age. It is worth noting that the mean differences were higher for participants who do not smoke versus the participants who did smoke and the results of the T-Tests were approaching statistical significance. The mean differences between the groups may be an early indicator of the effect of smoking on bone health, likely beginning to appear in our participants, however have not quite reached statistical significance.

There were several limitations of this study. We did not have measured heights and body weights for the participants, only a report of being below or above 127 pounds. Some participants did not report weight category nor smoking status. Having exact heights and body weights would have allowed calculation of body mass index. Missing data in this relatively small group might have impacted the results of some statistical tests. The positive effect of exercise on bone density is well documented in current literature (Sanudo et al., 2017) and we did not control for exercise. This may have also assisted with better characterization of results and should be included in future studies.

CHAPTER 6

CONCLUSION

While non-dairy calcium and its consumption can be useful in helping individuals meet the RDA for daily calcium consumption, the results of this study confirmed that not only were participants who didn't consume dairy calcium not meeting the RDA, but they weren't making up for the difference in non-dairy sources. This study also confirmed that previous research regarding osteoporosis risk in gender and ethnicity begin to show in young adults. While smoking status has yet to be shown in research to affect young adults bone mineral density, the results of our study almost reach statistical significance regarding smoking status.

Based on the findings in this study, there are several implications for future research for this age group. Having a larger sample size and focusing on smoking status and bone mineral density might begin to show how early on smoking can begin to effect bone mineral density and might further our understanding of the importance or smoking abstinence or cessation in young adults. Having a larger sample size and more data such as accurate heights and weights to calculate BMI could also affect statistical analysis in this age group.

One area with large implications for practice in this age group is a lack of intake of calcium in participants who do not consume dairy calcium. Based on the findings from

our study, the participants who do not consume dairy calcium are not making up for the difference in non-dairy sources. An emphasis should be placed on the education and importance of ensuring adequate calcium intake from individuals who choose not to consume dairy calcium.

APPENDIX A

APPROVAL DOCUMENTS

A.1 Human Use Committee Project Information Form

A-1 STUDY/PROJECT INFORMATION FOR HUMAN SUBJECTS COMMITTEE

Do you plan to publish this study?	X **YES**	□ NO
Will this study be published by a national organization?	X **YES**	□ NO
Are copyrighted materials involved?	X **YES**	□ NO
Do you have written permission to use copyrighted materials?	X **YES**	□ NO
Researchers must comply with all training requirements from their funding agency. Are all Researchers Up to Date on Human Subjects Training? (attach certificates) Training is on www.citiprogram.org	X **YES**	□ NO
Do any Special Permissions Need to be attached? (School district, data holder, Agency)	X **YES**	□ NO

Describe your study/project in detail for the Human Subjects Committee. Please include the following information:

TITLE: The relationship between calcium consumption from dairy and non-dairy sources and bone mineral density of college students.

PROJECT DIRECTOR(S): Andrew Pescovitz, Nutrition and Dietetics Graduate Student; Dr. Catherine Fontenot, Professor of Nutrition and Dietetics

EMAIL: pescova@miamioh.edu**,** maryf@latech.edu

PHONE: (Andrew Pescovitz) 513-225-0581, (Dr. Mary Fontenot) 225-803-2684

DEPARTMENT(S): School of Human Ecology

PURPOSE OF STUDY/PROJECT: The purpose of this study is to examine the relationship between calcium consumption from dairy vs. non-dairy sources and bone mineral density of college students.

SUBJECTS: The participants for this study will be selected from two undergraduate nutrition courses during the fall of 2019 at Louisiana Tech University. The classes are FNU 203 Introduction to Human Nutrition and FNU 103 Weight Management. The goal of the study is to recruit a total of 100 participants who meet the inclusion criteria. The initial inclusion criteria will be students of Louisiana Tech University who are enrolled in either of the fall 2019 courses, FNU 103 Weight Management or FNU 203 Introduction to Nutrition, must be between the ages of 18 to 22 years old, and healthy. The participants who agree to participate will complete the human use form and will be asked to complete a food frequency questionnaire designed to assess their current dietary calcium intake and have their bone mineral density assessed. Any participant who does not give consent or who does not meet the set inclusion criteria will be excluded from the study.

DETAILED PROCEDURE: Three separate assessment tools will be used for this study. The first assessment tool will be a 24-hour dietary recall that has been designed to analyze the

participant's daily food intake. The second tool is a calcium intake scoring sheet designed to extrapolate the students' daily calcium intake by using portion sizes and well as calcium source reporting over a one-day time frame. The other assessment tool will be a bone mineral density measurement tool called the Achilles EXP ™ from General Electric Healthcare.

INSTRUMENTS AND MEASURES TO INSURE PROTECTION OF CONFIDENTIALITY, ANONYMITY: The Achilles EXP™ ultrasound bone densitometer is a device that uses quantitative ultrasound or QUS without the use of ionizing radiation to accurately predict fracture risk in the study participants. QUS uses ultrasound waves that pass through fluid and human tissues and undergo attenuation based on the density of the calcaneus bone. The analysis of this attenuation will be used to generate empirical measurement. This test will be administered to participants on campus at Louisiana Tech University in the nutrition assessment lab. A food frequency questionnaire will be administered to each of the qualifying participants at the beginning of the study. All collected information will be held confidential and only viewed by the researchers.

RISKS/ALTERNATIVE TREATMENTS: Bone Mineral Density levels will be determined using the Achilles EXP™ ultrasound bone densitometer. There is no relative risk involved with using this machine.

BENEFITS/COMPENSATION: 10 bonus points for class enrolled in fall 2019 quarter.

SAFEGUARDS OF PHYSICAL AND EMOTIONAL WELL-BEING: This study does use physical contact to analyze bone mineral density of the participants using the Achilles EXP™ ultrasound bone mineral densitometer. A certification is not required to operate the machine nor to analyze the data the machine provides. No person will be given access to the data collected other than the researchers.

The following is a brief summary of the project in which you are asked to participate. Please read this information before signing the statement below. You must be of legal age or must be cosigned by parent or guardian to participate in this study.

TITLE OF PROJECT: The relationship between calcium consumption from dairy and non-dairy sources on bone mineral density of college students.

PURPOSE OF STUDY/PROJECT: To examine the relationship between calcium intake, calcium sources, and bone mineral density.

SUBJECTS: The participants for this study will be selected from two undergraduate nutrition courses during the fall of 2019 at Louisiana Tech University. The classes are FNU 203 Introduction to Human Nutrition and FNU 103 Weight Management.

PROCEDURE: If you agree to participate in this study, you will be asked to fill out a 24-hour dietary recalls to obtain your daily food intake. This recall will be used to obtain a daily calcium intake by the researcher. You will also be asked to report to the nutrition assessment lab on Louisiana Tech's Campus to obtain a bone mineral density measurement of your heel. Bone mineral density measurements will be taken using the Achilles EXP ™ from General Electric Healthcare.

BENEFITS/COMPENSATION: 10 bonus points for class enrolled in fall 2019 quarter.

RISKS, DISCOMFORTS, ALTERNATIVE TREATMENTS: Bone Mineral Density levels will be determined using the Achilles EXP™ ultrasound bone densitometer. There is no relative risk involved with using this machine.

The participant understands that Louisiana Tech is not able to offer financial compensation nor to absorb the costs of medical treatment should you be injured as a result of participating in this research.

The following disclosure applies to all participants using online survey tools: This server may collect information and your IP address indirectly and automatically via "cookies".

I, , attest with my signature that I have read and understood the following description of the study, "( )", and its purposes and methods. I understand that my (Or my Child's) participation in this research is strictly voluntary and my (or my child's) participation or refusal to participate in this study will not affect my relationship with Louisiana Tech University or my grades in any way. Further, I understand that I may withdraw (my child) at any time or refuse to answer any questions without penalty. Upon completion of the study, I understand that the results will be freely available to me upon request. I understand that the results of the material will be confidential, accessible only to the principal investigators, myself, or a legally appointed representative. I have not been requested to waive nor do I waive any of my rights related to participating in this study.

_____ _____

Signature of Participant or Guardian Date

_____

Name of child if Applicable

CONTACT INFORMATION: The principal experimenters listed below may be reached to Answer questions about the research, subjects' rights, or related matters.

PRINCIPAL INVESTIGATOR: _____

CO-INVESTIGATOR: _____

Members of the Human Use Committee of Louisiana Tech University may also be contacted if a problem cannot be discussed with the experimenters:

Dr. Richard Kordal, Director, Office of Intellectual Property & Commercialization Ph: (318) 257-2484, Email: rkordal@latech.edu

- Provide your age, race, school classification, and gender in the spaces provided

Age: _____ Race: _____ Classification: _____
Gender:_____

A.2 Human Use Approval Letter

LOUISIANA TECH
UNIVERSITY

MEMORANDUM

OFFICE OF SPONSORED PROJECTS

TO: Mr. Andrew Pescovitz and Dr. Catherine Fontenot

FROM: Dr. Richard Kordal, Director of Intellectual Property & Commercialization (OIPC)
rkordal@latech.edu

SUBJECT: HUMAN USE COMMITTEE REVIEW

DATE: September 11, 2019

In order to facilitate your project, an EXPEDITED REVIEW has been done for your proposed study entitled:

"The Relationship between Calcium Consumption from Dairy and Non-dairy Sources and Bone Mineral Density of College Students"

HUC 20-014

The proposed study's revised procedures were found to provide reasonable and adequate safeguards against possible risks involving human subjects. The information to be collected may be personal in nature or implication. Therefore, diligent care needs to be taken to protect the privacy of the participants and to assure that the data are kept confidential. Informed consent is a critical part of the research process. The subjects must be informed that their participation is voluntary. It is important that consent materials be presented in a language understandable to every participant. If you have participants in your study whose first language is not English, be sure that informed consent materials are adequately explained or translated. Since your reviewed project appears to do no damage to the participants, the Human Use Committee grants approval of the involvement of human subjects as outlined.

Projects should be renewed annually. ***This approval was finalized on September 11, 2019 and this project will need to receive a continuation review by the IRB if the project continues beyond September 11, 2020.*** **ANY CHANGES** to your protocol procedures, including minor changes, should be reported immediately to the IRB for approval before implementation. Projects involving NIH funds require annual education training to be documented. For more information regarding this, contact the Office of Sponsored Projects.

You are requested to maintain written records of your procedures, data collected, and subjects involved. These records will need to be available upon request during the conduct of the study and retained by the university for three years after the conclusion of the study. If changes occur in recruiting of subjects, informed consent process or in your research protocol, or if unanticipated problems should arise it is the Researchers responsibility to notify the Office of Sponsored Projects or IRB in writing. The project should be discontinued until modifications can be reviewed and approved.

Please be aware that you are responsible for reporting any adverse events or unanticipated problems.

APPENDIX B

DATA ANALYSIS TOOLS

B.1 Research Design Matrix

Purpose Statement	Hypothesis	Variables	Type of Data	Statistics
The purpose of this study is to examine the relationship between calcium consumption from dairy vs. non-dairy sources and bone mineral density of college students.	1. There will be no significant difference in total average calcium consumed (milligrams/day) based on gender, classification, and race.	Independent Variables- Gender, classification, and race. Dependent Variables- Milligrams of calcium consumed (total, dairy, and non-dairy)	Nominal, ordinal, and interval ratio	T-Test Correlations
	2. There will be no significant difference in the average calcium (mg/day) consumed from dairy products based on gender, classification, and race.	Independent Variables- Gender, classification, and race. Dependent variable- Milligrams of calcium consumed (total, dairy, and non-dairy)	Nominal, ordinal, and interval ratio	T-Test Correlations
	3. There will be no significant difference in the average calcium (mg/day) consumed from non-dairy, sources based on gender, classification, and race.	Independent Variables- Gender, classification, and race. Dependent variable- Milligrams of calcium consumed (total, dairy, and non-dairy)	Nominal, ordinal, and interval ratio	T-Test Correlations
	4. There will be no difference in students' fracture risk based on average total calcium intake; dairy calcium intake; or non-dairy calcium intake.	Independent Variable- calcium intake (total, dairy, and non-dairy) Dependent Variable- fracture risk	Nominal, ordinal, and interval ratio	Chi-Squared Tests

B.2 Data Collection Tools

Data Collection Tool 1: 24-Hour Recall

24-Hour Dietary Recall Form

1. Complete the 24 Diet Recall as accurately as possible.
2. Record all food and beverages consumed yesterday, starting with the 1st food and/or beverage consumed after waking up and the last food and/or beverage consumed just before going to bed.
3. Example for recording information has been provided below.

(Example) Time	**Food Consumed**	**Serving Size**	**Beverage Consumed**	**Serving Size**	**Supplement(s) Taken** ***Provide name & Mg Calcium provided/tablet***
8:00 am	Cinnamon-Raisin Toasted Bagel	1, mini	1% Milk	8 oz.	Viactive, 650 mg/chew
	Cream Cheese	1 tablespoon	Coffee	8 oz.	
	Banana	6" long			
	Sugar	1 tsp.			
	Half-n-Half	1 tablespoon			
Time	**Food Consumed**	**Serving Size**	**Beverage Consumed**	**Serving Size**	**Supplement(s) Taken** ***Provide name & Mg Calcium provided/tablet***

Data Collection Tool 2: Researcher Data Collection Calcium Frequency Scoring Sheet

FOOD	SERVING SIZE	#SERVINGS	Mg Ca/ Svg	TOTAL
Dairy Products Hard Cheese - Chedd, Mozz • Cubes (7 cubes) • Shredded (1/4 cup) • Slices (1 slice)	1oz			
	TOTAL SERVINGS		x200 mg =	
Parmesan Cheese Homemade Mac & Cheese Boxed Mac & Cheese Milk Shake Milk – skim, 1%, 2%, whole buttermilk, flavored milk	1oz ½ cup ½ cup 8oz 8oz			
	TOTAL SERVINGS		x350 mg =	
Yogurt – Plain Yogurt- Flavored	8oz			
	TOTAL SERVINGS		x450 mg =	
Processed Cheese Slices Soft Cheeses – Feta, Camembert	2 slices 2 oz			
	TOTAL SERVINGS		x250 mg =	
Whey Protein Powder	1oz scoop			
	TOTAL SERVINGS		x200 mg =	
Pudding – made with milk Tofu – made with calcium Yogurt, frozen	½ cup 3 oz ½ cup			
	TOTAL SERVINGS		x150 mg =	
Cottage Cheese	1 cup			
	TOTAL SERVINGS		x100 mg =	
Ice Cream	½ cup			
	TOTAL SERVINGS		x75 mg =	
Non-Dairy Substitutes/Fortified Beverages				
Iced/Hot coffee beverage-soy milk Iced/Hot coffee beverage-non-fat milk Cappuccino-soy/non-fat milk Iced/Hot Cafe Latte-soy/non-fat milk	12 oz 12 oz 12 oz 12 oz			
	TOTAL SERVINGS		X500 mg =	
Almond Milk-enriched with calcium	8oz			
	TOTAL SERVINGS		x450 mg =	
Calcium Fortified Orange Juice Rice Milk-enriched with calcium Soy Milk-enriched with calcium	8oz 8oz 8oz			
	TOTAL SERVINGS		X350 mg =	
Oat Milk	8oz			
	TOTAL SERVINGS		x121 mg =	

FOOD	SERVING SIZE	#SERVINGS	Mg Ca/ Svg	TOTAL
Vegetables/Fruit/Legumes/Nuts				
Almonds Beans – Soy, Baked, White Kidney Beans, Lima Beans, Lentils, Chick Peas Kale, Bok Choy, Spinach, Collard Greens Broccoli, cooked	23 almond 1 cup 1 cup 1 cup 1 cup 1 cup 1 cup			
	TOTAL SERVINGS		x150 mg =	
Orange –fruit itself	1 medium size			
	TOTAL SERVINGS		x50 mg =	
Fish Salmon – with bones Sardines – with bones	 3oz 11 small			
	TOTAL SERVINGS		x250 mg =	
Grains Calcium Enriched Cereals (Cheerios™, Raisin Bran™, Corn Flakes™)	 1cup			
	TOTAL SERVINGS		x100 mg =	
Pancakes/Waffles -made with milk Cheese Pizza	3 medium 1 slice-4”			
	TOTAL SERVINGS		x150 mg =	
Bread	2 slices			
	TOTAL SERVINGS		x50 mg =	
Supplements				
Multivitamin-with calcium	1 serving			
	TOTAL SERVINGS		x100 mg =	
Calcium Supplements Calcium Citrate Calcium Carbonate	1 serving 1 serving 1 serving			
	TOTAL SERVINGS		x600 mg =	
Calcium Gluconate	1 serving			
	TOTAL SERVINGS		x500 mg =	
Calcium Lactate	1 serving			
	TOTAL SERVINGS		x255 mg =	

For Researcher Use Only:

Total Daily Estimated Milligrams of Calcium Consumed = _____ mg/day

Adapted from: https://bestgyn.com

Data Collection Tool 3: Bone Heel Measurement Scheduler

Date: Participant Name: Time (20 mins):

Participant Number:

Participant Signature: _____

Data Collection Tool 4: Measurement Record

Participant Name: Date:

Participant Number:

Measurement:

Z-Score:

Fracture Risk:

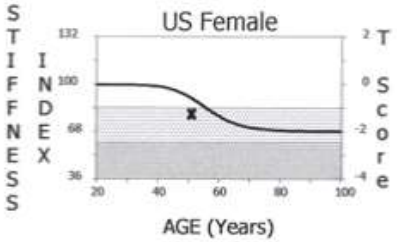
Participant Signature: _____

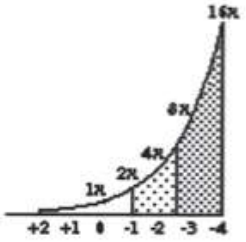
B.3 Sample Achilles Exp™ Report

General Electric Company GE Medical System(China)

No.19,Changjiang Road
Wuxi,Jiangsu,P.R.C 214000

Name:	Sucre, Femando	ID:	110503125455
Age/Birthday:	51 years. 1960.08.08	Doctor:	David Rice
Sex:	Female	Test Date:	2011.05.03
HEEL:	Left		





Relative Fracture Risk versus T-score

Stiffness Index: 81	T Score: -1.2	BUA: 71.1
% Young Adult: 81	Z Score: -0.6	SOS: 1620.8
% Age Matched: 89		

Clinical Risk Factors:
Ever HRT,Ever Smoke,Failed Chair Test,

Comments: Medium Risk of Osteoporotic Fracture

Follow Up: ☐None ☐6 Months ☐12 Months ☐18 Months ☐24 Months

GE Healthcare Achilles Serial Number 60019 Software Version1.10A

imagination at work

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