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Perceived Importance and Actual Intake of Calcium and Vitamin D in Young Female Athletes

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PERCEIVED IMPORTANCE AND ACTUAL INTAKE OF CALCIUM AND VITAMIN D IN YOUNG FEMALE ATHLETES

by

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Calcium needs increase as a child grows. Actual intake often decreases as a child gets older, leaving his/her developing bones at risk for injury during childhood and increasing the risk of developing osteoporosis later in life. The purpose of this study was to investigate relationships between psychosocial factors influencing consumption of calcium and vitamin D-rich foods and actual intakes of these nutrients. We examined the dietary calcium and vitamin D intakes, determined serum vitamin D levels, and assessed nutrition knowledge and perception of a sample of 20 young girls participating in competitive cheerleading in Omaha, Nebraska. From this information we assessed associations that existed among the variables to determine relationships between knowledge and perceived importance of nutrients and actual intakes of these nutrients. Participants completed a 48-item psychosocial survey regarding availability, tolerance, health beliefs, social influence, preference of calcium rich foods; an 18-item nutrition survey for knowledge of calcium rich foods; and a self-assessment Tanner stage questionnaire. A sample of blood was drawn from each participant. Finally, each participant completed one multiple pass 24-hour food recall and a two day food log. Mean daily calcium and vitamin D intakes were determined from the three day intakes.

Pearson correlations were computed among all variables to determine relationships. No significant relationships were found among the variables. There may have been a restriction of range likely due to small sample size as there was not much variance between many of the participants' responses. Assessment of nutrient adequacy indicated that this population consumed inadequate levels of both calcium and vitamin D and had inadequate levels of serum vitamin D. This population may be at risk for bone injuries later in life as they may not reach optimal peak bone mass due to lack of adequate nutrients to meet demands of bone formation in this time of growth.

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Review of Literature

Osteoporosis

Osteoporosis is known as the "pediatric disease with geriatric consequences," (Nicklas, 2003). These consequences include deterioration of the micro-architecture of the bones to the point of increased susceptibility of broken bones, curving of the spine, and often permanent damage later in life. Osteoporosis often limits activities of daily living secondary to decreased mobility and may increase risk of death (Ackerman & Misra, 2011; Nicklas, 2003; Burge et al., 2007). The most frequent areas in which osteoporosis develop are in the femur neck, hip, and wrist. Osteoporosis of the femur neck occurs in 10% of women over 50 and 2% of men over 50 (Looker et al., 2007). Additionally, osteopenia occurs in 49% of women in the same age group and 30% in men. Hip fractures are one of the leading types of bone breaks in the elderly population and are strong predictors of death within the following year of the break.

Health care costs directly associated with osteoporosis treatment in 2005 were $13 to $20 billion a year. This exceeded expectations of $10-15 billion for 2010 set in 2004 (Loud et al., 2005; Burge et al., 2007; Nicklas, 2003). Moreover, it is expected that 60 million people will have osteoporosis by 2020 (Nicklas, 2003) and health care costs to reach $25.3 billion by 2025 (Burge et al., 2007). This condition is costly; however, it may also be preventable if appropriate actions are taken during the childhood years.

A few risk factors of osteoporosis include genetic predisposition, inadequate nutrition, and inactivity. Lifestyle factors play an important role in an individual's risk of

developing osteoporosis. More specifically, osteoporosis is linked to inadequate intakes of calcium and vitamin D in pre-puberty and adolescent years in order to reach peak bone mass as well as a life of physical inactivity (Ackerman & Misra, 2011).

Vitamin D, specifically, is very important and often overlooked by many individuals concerned with their overall health. Intestinal absorption of calcium is enhanced in the presence of vitamin D. In a vitamin D-deficient individual, intestinal absorption of calcium is 10-15% versus 30 to 80% in vitamin D sufficient states (Misra et al., 2008). Calcium is not only important for bone development but also cellular and muscular contractions, such as the heart. This makes regulating circulating calcium levels imperative to maintaining life. To maintain serum levels in dietary calcium deficient state, calcium is pulled from stored sources: bone. Specifically, if circulating serum calcium levels begin to drop, the parathyroid hormone responds by slightly elevating which signals the mobilization of calcium and phosphorus from the bones to bring serum calcium concentrations back to normal. Additionally, this triggers an increase of conversion of inactive vitamin D to active 1-25(OH)D to maintain normal levels, which can further compromise circulating stores of plasma 25(OH)D. If vitamin D levels are low, little calcium will be absorbed from the intestine so again, PTH levels elevate to release calcium from stored sources, and phosphate is spilled into the urine (Misra et al., 2008). Over time, the compensatory action to regulate circulating levels can leave bones with inadequate stores leading to brittle architecture at risk for breaks, or osteoporosis. Therefore, adequate vitamin D levels are imperative throughout the lifespan for prevention of osteoporosis. To maintain bone health, however, adequate vitamin D is

useless for maintaining bone health unless adequate calcium is also present through the diet (Heaney, 2008) and vice versa.

In addition to low vitamin D intake, low intake of foods rich in calcium at critical growth ages can result in deficiencies that can lead to osteoporosis later in life as well (Larson et al., 2006). Calcium is the main building block of the skeletal structure. Therefore, adequate intakes of calcium in the presence of vitamin D are essential for maintaining bone mass and density. If these needs are not met, the bone is not replenished and osteoporosis may begin to develop. If osteoporosis is present, it is difficult to reverse, but interventions may be implemented in order to reduce the progression of the condition especially in younger individuals.

Osteoporosis treatment goals consist of slowing progression of bone loss, and maintaining bone still present. Several sources report a need for 800 to over 2000 international units (IUs) of vitamin D daily and 1000 mg of calcium. Bischoff-Ferrari and colleagues (2006) reviewed several studies that did not show any improvement in bone health with 400IUs of vitamin D. It was not until at least 800IUs of vitamin D were administered that were there improvements on bone health. Heaney suggests that research indicates a need for serum vitamin D levels to reach 30-32ng/mL (Heaney, 2008) which may require 2000IUs daily or more to achieve. Although osteoporosis may have varying etiologies, moderate physical activity, and healthy habits can reduce the risk. Because there is no known cure, prevention of osteoporosis is critical to maintain quality of life and decrease health care costs for the elderly. The appropriate age to begin this prevention process is in the pre-pubertal stage, followed by sustained preventative

lifestyle throughout the lifespan (Runyan, 2003; Loud et al., 2005; Story et al., 2002; Bueno & Czepielewski, 2008; Fiorito et al., 2006; Willis et al., 2008; Larson et al., 2006; Wajszczyk et al., 2006). For example, evidence suggests a significant difference in the bone density of older women who drank milk with every meal during their youth than those who did not (Nicklas, 2003). This proves difficult, however, as this younger age group tends to give more thought and attention to immediate consequences such as social acceptance, rather than focusing on long term goals such as osteoporosis prevention.

Importance of calcium and vitamin D in young girls

Calcium and vitamin D are the key nutritional players in the formation and maintenance of bone structure and density. In fact, researchers agree that adequate calcium intake is a critical factor in achieving, maintaining bone strength, and preventing fractures (Ackerman & Misra, 2011, Wajszczyk et al., 2006). Calcium must be acquired through the diet, however, as it cannot be made endogenously. Daily intake of calcium rich foods with high bioavailability such as milk and dairy products is necessary to meet the body's needs (Bueno & Czepielewski, 2008) (Table 1). According to Bueno & Czepielewski (2008), ideal calcium intake is adequate intake to achieve peak bone mass during child and adolescent, maintain during adulthood, and minimizing bone loss in elderly. Moreover the need for calcium increase in times of growth, such as during childhood and adolescence (Larson et al., 2006; Loud et al., 2007; Hazavehei et al., 2007), although rarely do individuals achieve adequate intakes (Fig 1). For example, Zhu and colleagues (2008) found that consuming calcium and vitamin D has a greater effect

on bone mineral density (BMD) than consuming calcium alone or not at all in Chinese girls. This has not been replicated in the non-Hispanic white population.

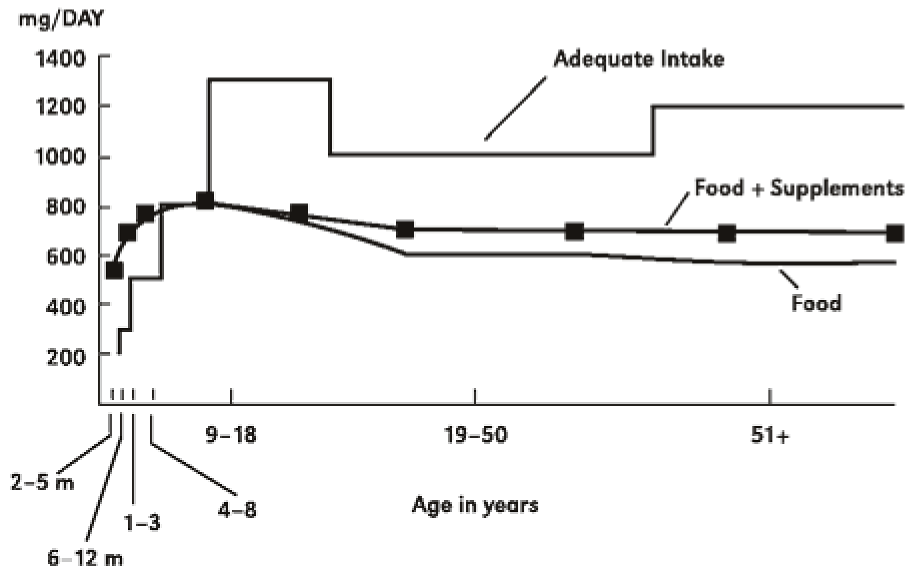
While vitamin D can be absorbed independently of calcium, calcium is dependent on the presence of vitamin D for absorption and excretion. Therefore, vitamin D can be ingested at a different time than calcium and still be available for calcium absorption. In order to aid the public in attaining adequate intakes, many foods are supplemented with calcium. Often these supplements are not absorbed as well as sources that are naturally rich in calcium (Ackerman & Misra, 2011). Dairy sources of calcium contain the highest bioavailability secondary to the lactose and alkaline pH present in the product. These factors increase absorption in the intestine. In order to enhance the absorption further, many are also fortified with vitamin D to ensure vitamin D is present.

Table 1. Calcium content of selected food items

Food item	Amount	Calcium (mg)
Total Raisin Bran	1 cup	1000
Part skim Ricotta cheese	1 cup	669
Plain, skim Yogurt (13g protein)	8 oz	452
Eggnog	1 cup	330
Milk, 1%, fortified	1 cup	305
Collards, boiled	1 cup	266
Spinach, boiled	1 cup	245
Swiss cheese	1 oz	224
Fast food Cheeseburger- single	1 sandwich	195
Salmon, canned with bones and liquid	3 oz	181

Source: adapted from USDA Nutrient Database, accessed 2011

Figure 1. Median Calcium intake of females 1988-1994 Compared to adequate intake recommendations.



Source: Looker 2003.

The recommended intake of calcium for young girls aged 9 to 18 is 1300mg daily. This should be consumed throughout the day as the body is able to absorb 500-600mg at a time (Misra et al., 2008; Ackerman & Misra, 2011). Therefore, many health promotion organizations encourage the intake of calcium though milk at each meal. Eight fluid ounces of milk has 305mg of calcium according to the USDA nutrient database (2010). This means that in order to achieve 1300mg of calcium from milk, one would need to drink 34.6 fluid ounces (4.3 cups) of milk per day (or other combinations of calcium rich foods). In order to ensure proper utilization and increase bioavailability, it is recommended that one should consume dairy products for adequate intakes.

While dairy products are an effective source of natural calcium, adequate intake and absorption proves to be difficult for those with lactose intolerance. In fact, despite similar intakes levels of calcium, individuals with a greater intensity of side effects due to

lactose intolerance have lower BMD (Stefano et al., 2002). Therefore, purportedly those who have higher levels of lactose intolerance may also have a higher predisposition for inadequate bone mineralization and increased risk for osteoporosis (Stefano et al., 2002).

Furthermore, vitamin D aids the absorption of calcium in the stomach. Much of the body's sources come from exposure to sunlight. Due to the increased risk of melanoma, more children are wearing sunscreen that will block the body's absorption of vitamin D from sunlight. In addition, during the winter months many parts of the world do not receive the adequate exposure to sunlight at the correct angle for absorption (Holick, 2007; Misra et al., 2008). The ultra violet B (UVB) rays are absorbed into the atmosphere before reaching the earth's surface or the epidermis. Therefore, in order to increase consumption in the general population many products are fortified with vitamin D (Bueno & Czepielewski, 2008) (Table 2). Since the early 1900s and possibly before, it is known that oral intake of vitamin D via food is often a fraction of what is necessary to show adequate plasma levels of cholecalciferol (Poskitt et al., 1979). Instead, the preferred method is to obtain adequate sunlight exposure for the body's main pathway or supplemental vitamin D. It is critical to examine the role of calcium and vitamin D on the body at a young age and understand environmental factors that influence intake.

Table 2. Vitamin D content of selected food items

	Amount	**IUs of Vitamin D**
Rainbow Trout, farmed, cooked	3 oz (85g)	645
Salmon, Chinook, smoked	3 oz (85g)	583
Swordfish, cooked	3 oz (85g)	566
Salmon, canned	3 oz (85g)	465
Salmon, sockeye, cooked	3 oz (85g)	447
Malted drink mix, chocolate, with added nutrients, powder, prepared with whole milk*	1 cup	326
Tuna, canned in oil	3 oz (85g)	229
Milk, canned, evaporated with added vitamin A and vitamin D*	1 cup	202
Malted drink mix, chocolate, with added nutrients, powder*	3 tsp	200
Halibut, cooked	3 oz (85g)	196
Sardine, canned in oil	3 oz (85g)	164
Rockfish, cooked	3 oz (85g)	156
Tuna, canned in water	3 oz (85g)	154
Milk, chocolate, whole with added vitamin A and vitamin D*	1 cup	128
Eggnog*	1 cup	124
Milk, whole with added vitamin D*	1 cup	124
Milk, chocolate, reduced fat with added vitamin A and D*	1 cup	123
Milk, non fat, with added vitamin A and D*	1 cup	115
Milk shake, thick vanilla*	1 cup	109
Cereal, TOTAL Raisin Bran*	1 cup	104
Egg, whole, raw	1 extra large	48
Shiitake mushrooms, cooked	1 cup	41
Cheese, cheddar	1 oz	7
Yogurt, plain, whole milk	1 cup	5
Ice cream, vanilla, white	½ cup	3
Milk, buttermilk, low fat	1 cup	2

*indicates fortified source of vitamin D Source: Adapted from USDA Nutrient Database, 2010.

Current intake of calcium and vitamin D in young girls

As girls age and grow, the intake of calcium and milk decreases (CDC, 2010). In the last twelve years much information has been developed about the influence of calcium and vitamin D consumption in the younger generation. In fact, while following dietary intakes of young girls over a four year period, Fisher and colleagues (2004) found that at age five, over 50% of girls met the 800mg requirement for their age, but by age nine, only 10% reached the 1300mg requirement. Similarly, Fiorito and colleagues (2006) followed five year old girls for six years (until age 11) to study dietary intake of calcium. They found that at age five, 69% met calcium recommendations for their age. By age 11, only 39% of the same sample met recommendations for their age. According to Ervin and colleagues (2004), girls aged 12-19 averaged an intake of 793mg of Calcium compared to their male counterparts at 1081mg Calcium. In 2009 only 8.7% of high school girls drank 3 servings of milk or more per day. According to the Department of Health and Human Services, this is a "significant linear decline" in intake since 1999.

One reason for this decline is that calcium is consumed typically through milk as a beverage for most young children (Fiorito et al., 2006; Loud et al., 2005). Evidence indicates that as a girl ages, her consumption of sweetened beverages increases and her consumption of milk as a beverage decreases (Fiorito et al., 2006; Loud et al., 2005). Therefore, it can easily be ascertained why most girls do not reach their daily requirement of calcium. Although there is an increase in the consumption of cheese via commercially prepared and manufactured foods (Fiorito et al., 2006) as an alternative source of calcium for children, these products are often foods that have more fat and sodium than milk

increasing risk of other health issues such as hypercholesterolemia and hypertension. Moreover, studies have found that there seems to be no change in vitamin D status with consumption of these sources of calcium (Gordon et al., 2004).

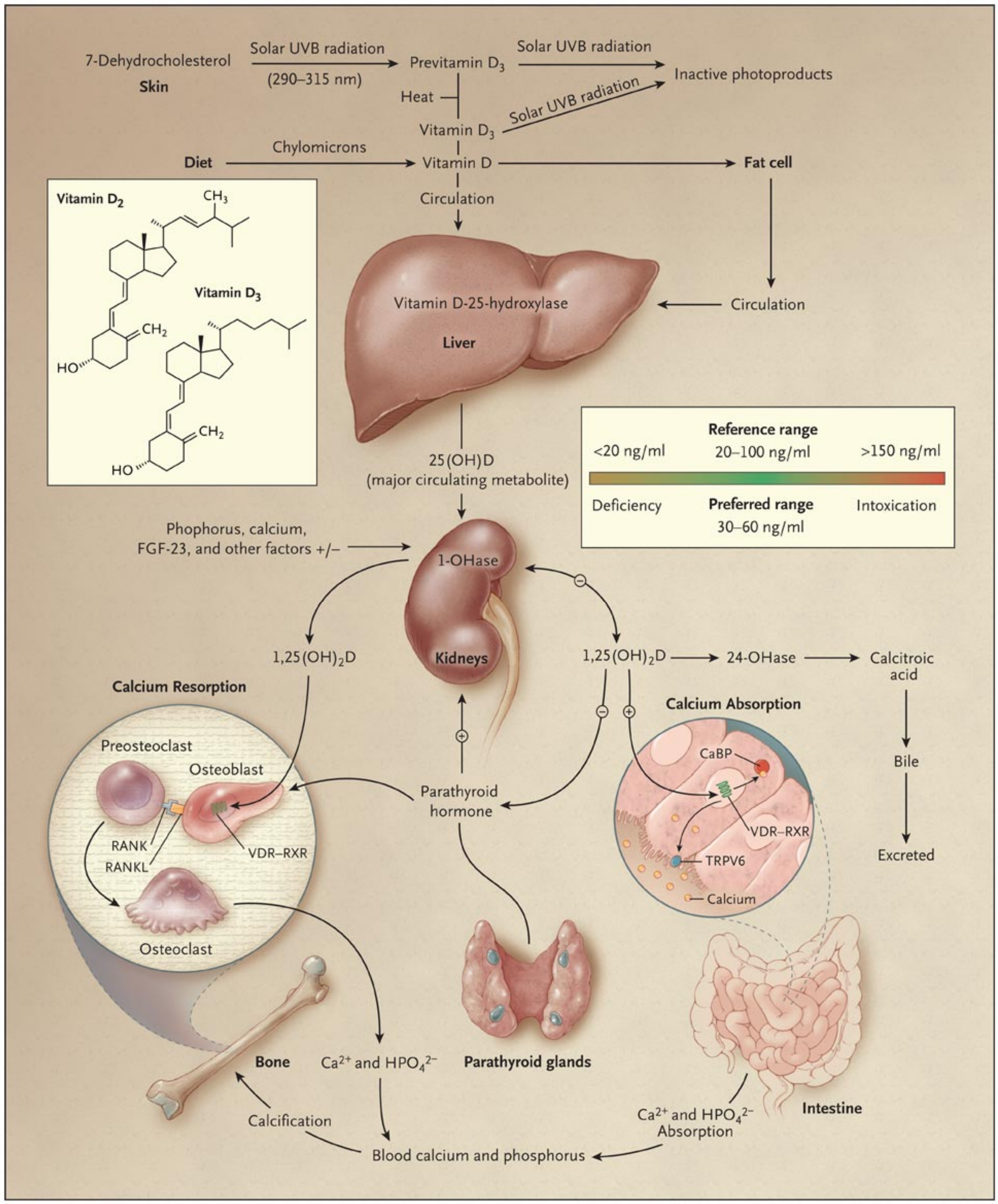
The American Academy of Pediatrics recently increased recommends for supplemental vitamin D from 200 to 400IUs for any infant during the first 2 months of life and recommends continuing supplementation into adolescence (Misra et al., 2008, Wagner et al., 2008). On the other hand, the Canadian Paediatric Society guidelines recommend supplementing 800IUs per day for infants born in winter months to breastfeeding mothers. Vitamin D intakes are inadequate even after infancy according to much of what the current literature suggests as adequate. One study found that only 50% of girls 9-13 had an oral intake of vitamin D greater than 200 IUs, and only 32% of girls in the next age bracket were able to reach the same levels (cited in Ackerman & Misra, 2011). Even by the Institute of Medicine's (IOM) standards this is insufficient to maintain "good" bone health. Bueno and colleagues (2008) outlines multiple studies which have similar findings that both boys and girls across all age groups have inadequate intakes of both calcium and vitamin D.

Sun exposure and coetaneous generation of vitamin D

It has been well known since the early 1900s that exposure to sunlight is the best source of vitamin D (Armas et al., 2007). In fact oral intake of vitamin D is not necessary to reach optimal circulating levels of inactive vitamin D during summer months in North America if a person has adequate exposure to the sun's rays. When the skin is exposed to

UVB from the sun, 7-dehydrocholesterol, located in the plasma throughout the epidermis, is converted to pre-cholecalciferol (previtamin D). At that point it is sent to the liver for hydroxylation to circulating 25-hydroxyvitamin D (25(OH)D and is circulated throughout the body by vitamin D-binding proteins. This is the inactive form of vitamin D. In the kidneys and other cellular structures 25(OH)D is converted to its active form, 1-25-hydroxyvitamin D as needed. The kidneys highly regulate this concentration in the body and 1-25(OH)D level in the blood is normally not disturbed. Once the kidneys are able to activate vitamin D, it is available to assist the absorption of Calcium into the bone. (Fig. 2).

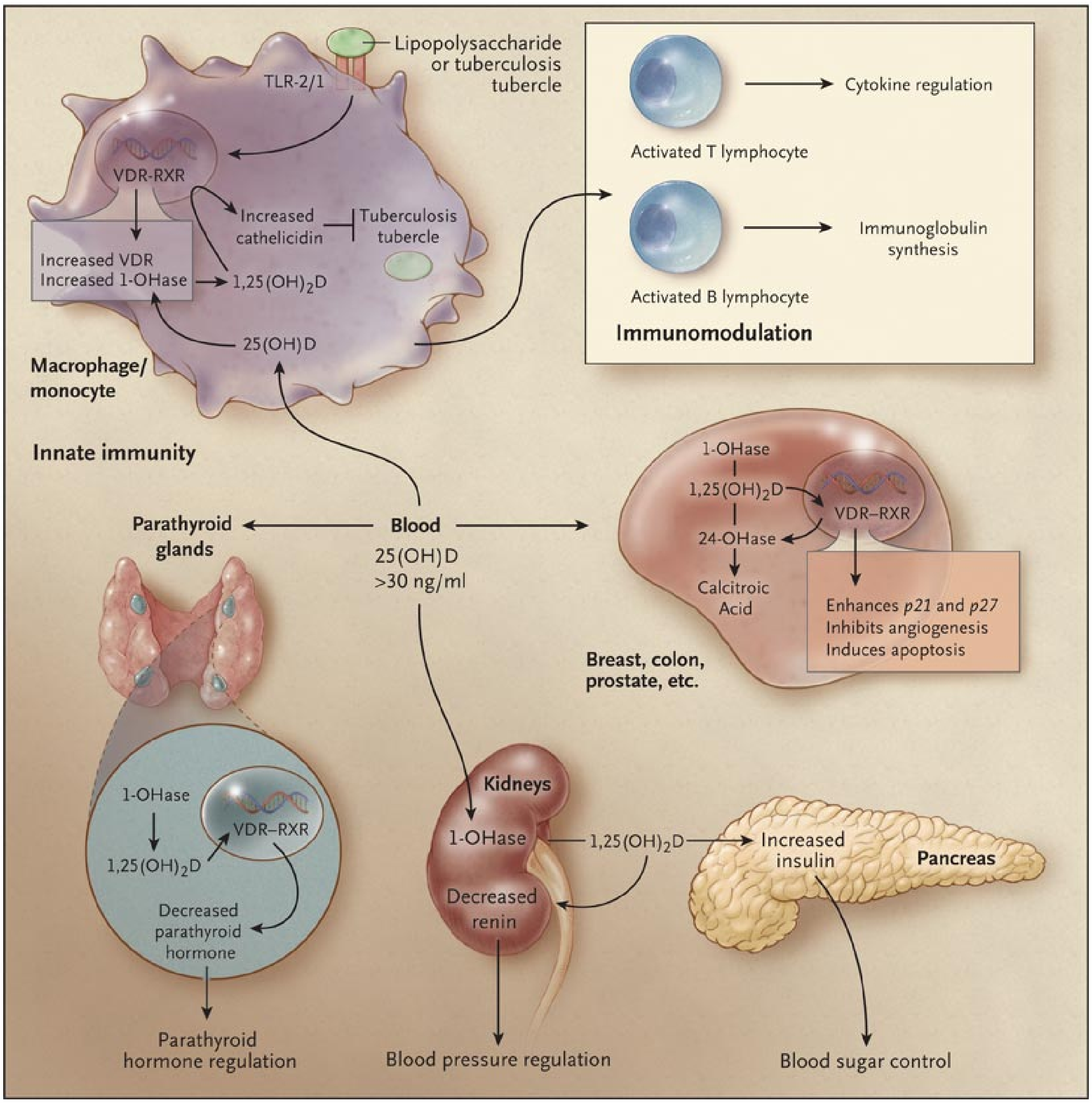
Figure 2. Vitamin D pathways



Holick 2007

Evidence is growing that the kidneys are not the only organ with the capability of converting 25(OH)D to 1-25(OH)D. In fact Holick (2007) outlines multiple extra-renal receptor sites for inactive vitamin D which stimulates conversion to active 1-25(OH)D. These are found in the heart, brain, intestine, and multiple cells, such as macrophages, responsible for immune function, and antimicrobial peptides (Willis et al., 2008; Holick, 2007) (*Fig 3).* With this in mind, it may be even more important to monitor vitamin D status in athletes for more than just bone function but also for rate of illness and infection.

Figure 3. Non-skeletal pathways of circulating vitamin D.



Holick 2007

Circulating vitamin D as it remains inactive, represents the body's total vitamin D exposure (Tylavsky et al., 2005). Researchers agree that the average white skinned adult can obtain adequate vitamin D levels by exposing hands and face to 15 minutes of sunlight between the hours of 10am and 3pm during summer months. This will result in adequate conversion of inactive 25(OH)D to maintain both bone and non-bone health (Holick, 2007; Armas et al., 2007; Heaney et al., 2009). The adequacy of this exposure can depend on age, skin pigmentation, obesity, time of year (Holick, 2007). Moreover, regular sunscreen use, clothing, cloud coverage, and latitude during winter months as discussed previously can also interfere with adequate vitamin D absorption form UVB radiation (Misra et al., 2008). Moreover, as mentioned, the absorption of vitamin D from sunlight is dependent on the angle of the sun compared to the earth's surface. During winter months (October to March) above the 35th degree latitude, UVB radiation is absorbed into the atmosphere and does not reach the ground (Kimlin et al., 2007; Holick, 2007). Therefore, the endothelial exposure to sunshine does not stimulate conversion of vitamin D in certain regions during winter months.

To demonstrate this, Halliday and colleagues (2010) found that young college athletes (n=41) involved in outdoor sports had significantly higher 25(OH)D levels in the fall than those involved in indoor sports. During winter months both groups had significant drops in their vitamin D levels from normal to borderline insufficient (30.5+/-9.4ng/mL). This study used 40ng/mL as the cutoff for optimal. Moreover, 25(OH)D levels were correlated with multivitamin use in the winter, and tanning and frequency of illness in the spring. Other lifestyle factors did not seem to impact vitamin D status in

these participants. Gordon and colleagues (2004) found that in their population 42% of healthy adolescents had insufficient vitamin D levels (<20ng/mL). Compoundingly, this study also found that vitamin D levels were 24% lower in the winter compared to summer. Others report 5-14% increase in deficiency in the winter (Tylavsky et al., 2005). Moreover, oral intake of dietary vitamin D is correlated to vitamin D status in the winter but not the summer in a study of Finnish girls (Lehtonen-Veromaa et al., 1999). This demonstrates that oral intake has a greater effect on serum levels during the winter months whereas in the summer months, it has less effect on total levels as natural conversion occurs.

Without exposure to solar UVB radiation or ingestion of foods or supplements containing vitamin D, plasma levels of vitamin D slowly decline over time. Because strictly restricting environmental and nutritional factors is not ethical for the human population, a sample of pigs was used to study how vitamin D impacts circulating vitamin D in the blood. Heaney and colleagues (2009) found that 2000IUs of vitamin D supplied the body with 7 days worth of circulating vitamin D in this pig sample. Therefore, a person not exposed to sufficient UVB radiation during the winter months and inadequate oral intake of vitamin D may lose their stores from the fall within several weeks as the half life of 25(OH)D is reported as 2-8weeks (Veith, 1999, Misra et al., 2008). This may explain much of the evidence for insufficient vitamin D levels in many populations in northern America during winter months.

On the other hand, Willis and colleagues (2008) assessed athletes exposed to adequate coetaneous UVB exposure at latitudes where vitamin D conversion is possible

year round. They found that 40% of a small group of runners had insufficient levels. It is not noted, however, if sunscreen usage or skin color was considered in this population. Individuals with darker skin pigmentation often have insufficient levels as well as those who use sunscreen before being exposed to UVB.

Some literature suggests that lower levels of serum 25(OH)D may be expected during puberty. Tylavsky and colleagues (2005) discuss that 2 years prior to the onset of puberty 25(OH)D levels may be higher than at onset of puberty. This needs more examination as the third national health and nutrition examination survey (NHANES III) data does not reflect these findings (Tylavsky et al., 2005). Even with regular 400IU supplementation, hypovitaminosis D was prevalent in a group of Finnish girls. This group was not categorized by stage of puberty, however. Another study reported by Wagner and colleges (2008) found a 14.3% and 17.2% increase in bone density in 11-12 year olds with 200IU and 400IU of vitamin D supplementation compared to controls.

The question remains if the demands for bone accrual naturally cause a decrease serum vitamin D levels during puberty. Wajszczyk and colleagues (2006) found that there was a significant difference between BMD in pre-menstrual girls and menstruating girls. They found that serum vitamin D levels did not seem to influence BMD in menstruating girls, but those who were pre-menstrual had higher BMD with serum vitamin D levels of greater than 20ng/mL. Therefore it is important to note stage of puberty when assessing impact of vitamin D on bone health.

Oral intake of vitamin D

According to the USDA nutrient database, few foods naturally contain significant amounts of vitamin D (*Table 2)*. When listed by content, only 37 foods have 100IUs or more per serving. Moreover, only 16 of those items lists are not fortified and all of those happen to be sources of fish. Misra and colleagues (2008) report that less than 10% of vitamin D is obtained orally.

When examining the diets of young adolescents and their vitamin D status, there is a correlation with vitamin D deficiency and intake of soft drinks, fruit juice, and tea (Gordon et al., 2004). Furthermore, there was no effect for vitamin D status compared to yogurt, cheese or ice cream but there is an increase in vitamin D status with milk and cereal consumption (Gordon et al., 2004).

The DRI for vitamin D by the IOM for young girls is 600 IUs, up from 200IUs in 1989. The IOM determined this to be an adequate amount with minimal sunlight exposure to maintain plasma levels of 20 nanograms per milliliter. They determined that this is the adequate level in order to maintain "good" bone health (IOM report brief). Others, however, report this is the amount to merely prevent rickets (Veith, 1999) which has been proven to be an unrealistic goal to meet through food alone. For example, Lehtonene-Veromaa and colleagues (1999) reported their subjects, 9-15 year old girls, had an average dietary intake of vitamin D of 116 IUs at baseline. With 3 months supplementation of 400IUs of vitamin D, deficiency was found in 63.4% of their subjects. It was not until that dose was doubled to 800IUs did they find an increase in circulating vitamin D status, which was still lower than that of the summer months

(Lehtonene-Veromaa et al., 1999). This insinuates that 600-800IUs daily is not sufficient to maintain vitamin D levels for total health and to fulfill BMD potential during winter months. To meet 800IUs through food, one would need to eat 1/2 fillet of a whole salmon or drink 8 cups of milk per day.

Milk, however, may or may not have the amount of vitamin D indicated on the label. In 1936, it was discovered that the amount of vitamin D in the milk was dependent on the cow's exposure to sunlight and the time of year (Bechtel & Hoppert, 1936). In the winter months and/or with poor exposure to sunlight, the vitamin D content in the fat of the milk was lower than in summer months and/or increased exposure to sunlight. This variation in vitamin D content was as much as a 900% difference. Furthermore, in 1992 Holick and colleagues found that the actual vitamin D content of cow's milk sold to customers varied greatly across date of purchase to fat content in the milk. They found that only 29% of the samples tested contained between 80-120% of the 100IUs reported on the food label per cup. Furthermore, they found several samples of skim milk with no detectable vitamin D despite 25% of the daily value (100IUs) indicated on the label. In 2007, a similar study was conducted finding that 49% of samples contained between 100-125% of stated vitamin D content on label (Patterson et al., 2010). Whole milk averaged about 150% and several samples had no detectable levels of vitamin D. Actual content levels ranged from 0 IUs to 800 IUs per eight fluid ounces. Therefore, although improved, it is greatly important to avoid depending solely on milk for sources of vitamin D as it is highly variable from sample to sample.

Supplementation of 100IUs of vitamin D increases serum levels by 1ng/ml (Cranney et al., 2007). To raise an individual from a serum level of 28ng/mL to 33ng/mL, for example, this individual would need a daily dose of 600IUs in addition to normal sun exposure, dietary patterns and current supplementation. To raise that same individual from 28 to 50ng/mL, the level many researchers suggest for optimal health, this individual would now need a daily dose of 2200IUs to reach 50ng/mL and maintain that level. This is much higher than many national recommendations and should be noted during treatment of vitamin D deficiency.

Assessing vitamin D status

The most accurate measure of assessing vitamin D status is to test the inactive vitamin D3 (cholecalciferol) in the blood. Cholecalciferol is the best indicator of sufficient vitamin D because many parts of the body require vitamin D for proper health (Holick, 2007) and is reflective of absorption, whereas 1-25(OH)D levels are tightly regulated by the kidneys. When plasma levels of cholecalciferol are inadequate, PTH levels increase to assist to maintain calcium in the bone. By assessing plasma levels and obtaining adequate vitamin D, PTH levels in healthy individuals will remain normal. Additionally, if this level is adequate, bone health is likely to be satisfactory. Just as measuring plasma calcium is not a good indicator of calcium status secondary to regulation by the kidneys, assessing active vitamin D is not as accurate in predicting bone health because it is also regulated by the kidneys, and so stays constant regardless of

deficiency. Therefore, it is necessary to assess inactive circulating cholecalciferol to obtain an accurate indication of levels.

Many suggested reference points and terminology are expressed in the literature. Although there are no current standardized reference ranges for vitamin D, current research suggests below 20ng/mL is deficient, 20-30ng/mL is insufficient, 30-100ng/mL is normal for adults. The IOM, however, describes 20ng/mL as adequate. Children, however, have fewer sources and references for adequate levels. Misra and colleagues (2008) note that there are lower ranges suggested for children, however, they also suggest that these may not be adequate for several populations. No literature reviewed for this research reflects the recommendation set forth by the IOM when studying intake and exposure verses plasma levels. Circulating vitamin D levels are dependent on several factors including the amount of inactive vitamin D needed to prevent hyperparathyroidism (Holick, 2007; Hollis, 2005).

Interpreting literature for circulating 25(OH)D should be done with a cautious eye. There are several ways of testing vitamin D status that have not been standardized. Differences in results are noted between testing methods (Phinney, 2008; Misra et al., 2008). NHANES researchers caution that the data collection between the dates of 2003-2004 and 2005-2006 may have been shifted due to drifts in the reformulated DiaSorin assay and many articles referenced in this review are from that time period. It has since been updated, but the limits of interpretation of literature from that time frame must be noted. Furthermore, NHANES researchers acknowledge a need for adjustment between the 1988-1994 data and the 2000-2006 data as methods used for testing vitamin D were

different: the former using the older DiaSorin radioimmunoassay (RIA) kit and the latter using an adjusted version. The Center for disease Control and Prevention (CDC) used this information to account for differences between dates for vitamin D status. After adjustment, the mean vitamin D level shifted from 25.3 to 24.3 in the 2003-2004 data and from 22.2 to 23.9 in the 2005-2006 data. In the future the CDC plans to use isotope dilution tandem mass spectrometry (LC-MS/MS) and then further adjust the previous data to account for methodology differences. This method was tested and compared to six other analysis methods using a cohort of 300 blood samples. Most other methods demonstrated variation from results of the preferred method.

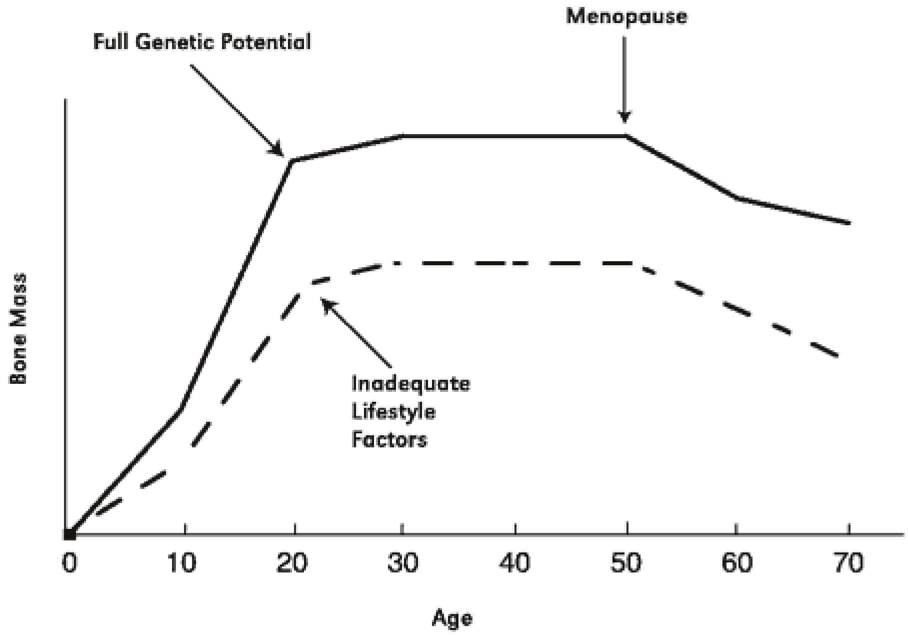
LC-MS/MS and high pressure liquid chromatography (HPLC) seems to be the preferred way to analyze serum vitamin D levels and is encouraged for researchers to use this method to begin to have a standardized method for comparing results. At this time, there is still a wide variance in methodology between laboratories as many of the preferred methods are labor-some and often available (Misra et al., 2008).

Carter et al., 2009, however, concludes that the LC-MS/MS assay is not “sufficiently robust” and instead suggests the gas chromatography-mass spectrometry method of analyzing serum vitamin D. Therefore, it can be concluded that although the CDC recommends the LC-MS/MS method, other methods continue to be preferred choices in other laboratories. Hence, inter-laboratory comparisons are limited due to discrepant methods.

Normal growth and bone mineralization in young girls

Skeletal maturity and normal bone density development are crucial for maintenance throughout the lifespan. Under certain favorable conditions all individuals contain a genetic potential for optimal and normal growth and development (Bueno & Czepielewski, 2008; Willis et al., 2008). The maximum bone density reached during growth is known as peak bone mass (PBM). Although 60 to 80% of a person's PBM is attributed to genetics (Ackerman & Misra, 2011), the limiting factors to reaching optimal growth are environmental factors such as physical activity, economic factors, disease, psychosocial factors, hormone regulation, and nutrition (Ackerman & Misra, 2011; Bueno & Czepielewski, 2008). Without the availably and acquisition of these most favorable conditions, growth and development occur but not to full capacity allowing these limiting factors to interrupt maintenance later in life *(Fig. 4)*. A window of opportunity exists for acquiring the lifestyle factors for optimal bone density. This window extends from the onset of puberty to the third decade of life (Ackerman & Misra, 2011) and even throughout the 30s (Zanker et al., 2004) in both men and women, at which time the individual reaches PBM.

Figure 4. Bone Mass versus Age with Optimal and Suboptimal Bone



Acquisition

Beyond nutrition and physical activity, hormone regulation is connected to the utilization of nutrients. Hormone regulation is stimulated by the bioavailability of many of these factors. The production of growth hormone (GH), Insulin-like Growth Factor (IGF)-1,2, and binding proteins for bone mineral increases at the onset of puberty. GH often surges during puberty which stimulates bone generation during the growth spurt and then levels off after the growth spurt. Through a variety of mechanisms, osteoblast differentiation, anabolism, and proliferation are all tightly regulated (Ackerman & Misra, 2011). IGF enhances the conversion of 25(OH)D to 1-25(OH)D in the kidney. This stimulates calcium and phosphorus absorption in the intestine further enhancing bone generation. Sex hormones regulate the production of growth hormone and IGF. Glucocorticoids inhibit calcium absorption through a mechanism dependent on vitamin

D, and interfere with the survival of the osteoblast. Also, hormones from the thyroid can increase bone turnover (Ackerman & Misra, 2011).

Upon the onset of puberty, hormones related to energy balance seem to have an effect on bone metabolism although this is inconclusive (Ackerman & Misra, 2011). Notably, however, is the increase in cortisol during energy deficiency that has a negative impact on PBM. Energy deficiency is noted in disordered eating and is found to be higher in female runners, gymnasts, and dancers. Therefore, during the puberty, it is essential to have adequate intakes of nutrients to meet needs of optimal growth.

Menstruation and bone health

The female menstrual cycle is largely influential in the accrual of bone mineral density (Ackerman & Misra, 2011). When assessing bone health in children and adolescent girls, it is crucial to understand their pubertal stage and age of menarche. The onset of menarche in young girls marks the onset of puberty and acceleration of growth. The average age of menarche is 12.54 years (Anderson et al., 2003). The conversion of 25(OH)D to 1-25(OH)D is accelerated during puberty to support growth in the skeletal structure (Tylavsky et al., 2005; Ackerman & Misra, 2011). An increase in 1-25(OH)D levels has been reported during the transition between female Tanner stage 1 and stage 2 (Aksnas & Aarskog, 1982). This increase in 1-25(OH)D has been correlated with a decrease in circulating 25(OH)D which emphasizes the need for adequate inactive reserves from oral or coetaneous intake (Lehtonene-Veromaa et al., 1999) in order to

satisfy needs of active vitamin D. These changes are stimulated by the sex hormone release by the gonadal system introduced at this time period.

Chalfant and colleagues (2011) analyzed the age of onset of puberty related to acquisition of bone density in adolescent boys and girls and found that as the age of onset of puberty is younger, the skeletal maturity is higher. It can be interpreted that skeletal maturation begins with puberty. Those who start puberty later can expect to develop bone density later in life also. This reflects the changes in the hormones during puberty. In females, Loud and colleagues (2005) reinforced that menarche occurs at the end of the main growth spurt in girls which correlates with rapid increases in bone mass and density. Sex hormones and steroids released during the onset of puberty are necessary for utilization of the rest of the system for development. Therefore, environmental factors delaying the onset of puberty may interrupt the system. Nonetheless, pubertal changes require higher amounts of calcium and vitamin D than pre-pubertal needs to build strength in the bones during growth and to be biologically available during times of hyper up-regulation by the hormonal changes. This can be further reinforced by adequate physical activity (Markou et al., 2010). If a young girl is not supplying her body with the sources it needs, her bones are at higher risk of fragility and she becomes a more likely candidate for stress fractures or other bone problems as she grows (Loud et al., 2005).

Regular menstrual cycles are show to be correlated to fewer stress fractures in athletes (Loud et al., 2007). Conversely, the absence of a menstrual period, or amenorrhea, largely increases the likelihood of developing stress fractures. Lappe and colleagues (2008) found a 91% increase in risk of stress fractures in young women with

amenorrhea. Due to the high intensity and long duration of physical activity required of most gymnasts and cheerleaders, amenorrhea can be common if caloric intake is inadequate to maintain needs.

Onset of menarche may be delayed in girls who participate in intense physical activity, which has often been interpreted as normal. Given that as the intensity of physical activity increases, so does the likely hood negative calorie balance and of amenorrhea, delayed onset of menarche should not be considered normal because it poses a risk to the onset of maturation of the skeletal structure, leading to higher risk of injury. In young athletes, this may result in the delay of onset of menarche through the age of 16, which is considered primary amenorrhea. Secondary amenorrhea refers to individuals who have started menstruation but then have three consecutive missed periods (Sherman & Thompson, 2004).

In yet a secondary factor, the type of physical activity may influence density acquisition, regardless of amenorrhea (Robinson et al., 1995). Robinson and colleagues found that amenorrhea and ogliomenorrhea did not seem to impact bone density equally between college-aged gymnasts and runners. Despite the similar incidence of amenorrhea and ogliomenorrhea, in each athletic group, the gymnasts had higher bone density. The repetitive high impact of jumping seems to effect bone mineralization differently than distance running. This study found similar intakes of calcium between groups, although both groups consumed less calcium than the DRI. So, calcium intake in this sample may not have been a strong enough factor to be taken into consideration to draw conclusions.

Physical activity and bone health

As mentioned in previous sections, physical activity is important in maintaining bone health in most populations (Ackerman & Misra, 2001; Loud et al., 2005). Weight-bearing activity in particular stimulates bone mineralization and is indicated to promote bone health during adolescent years (Loud et al., 2007). For example, the 5 to 30% increase in BMD attained in healthy athletes may reduce the risk of fracture by 50 to 80%. A meta-analysis performed by Burrows (2007) supports the effect of exercise on bone mineralization in prepubescent subjects. Pre-pubertal girls seem to benefit from *moderate* physical activity for bone accrual. Moreover, the most gain seems to be from jumping activities around the age of 10.2 and continue into the puberty years (Burrows, 2007). Gymnasts and elite dancers have higher BMD than other sports. Regardless, it seems that activity in the pre-pubertal years, if sustained, may decrease the risk of developing osteoporosis later in life. It is unclear if an active child with an inactive adult life will have a decreased risk.

On the other hand, there may be a threshold at which excessive physical activity can have detrimental effects on bone health (Robinson et al., 1995; Ackerman & Misra, 2011). During intense training bone formation increases which in turn increases the demand for adequate calcium intake (Lappe et al., 2008; Loud et al., 2007). Additionally, physical activity promotes sweating. According to Lappe and colleagues (2008), coetaneous calcium loss in sweat can be as much as 433mg per two hours of physical activity. Sources agree that greater than 16 hours per week of moderate to vigorous activity places a person at high risk for stress fractures (Loud et al., 2007). Even on a

smaller scale each additional hour per week of high impact activity increases risk of stress fracture (Loud et al., 2007).

Girls who participate in more than 16 hours of physical activity per week have been found to be 1.88 times more likely to have a history of stress fractures than those who participated in less than four hours per week. Specifically those participating in running, cheerleading, and gymnastics have been found to be at an increased risk of stress fracture than those in other sports (Loud, 2005). These researchers also found that girls who participated in long distance running or cheerleading/gymnastics were more likely to have disordered eating habits and more incidences of stress fractures. They also found no correlation between BMI, calcium intake, or vitamin D intake after controlling for age (Loud et al., 2005). Average intakes of both calcium and vitamin D for this population, however, were under the current daily recommended intakes. It is possible that calcium and vitamin D could have had a positive impact on bone health if participants had adequate intakes.

Though the literature surrounding long distance running and increased risk of stress fractures is consistent, others have not found these results for gymnastics. Information in the literature agrees that distance runners are at a higher risk of bone injuries than any other athlete including gymnasts. The repetitive load from running seems to diminish bone health at a great rate than the remodeling of the bone, resulting in bone mineral deficiency and stress fractures (Robinson et al., 2005; Ackerman & Misra, 2011). Runners also tend to have more instances of energy deficiency and amenorrhea, both of which have been linked to elevated risk for osteoporosis. As mentioned, gymnasts

tend to have higher bone mineral density despite instances of amenorrhea. The relative force at which gymnasts' bodies are impacted is two to four times as high as a runners force, resulting in elevated stimulation of osteoblasts to maintain peak bone mass in gymnasts (Robinson et al., 1995).

A study that supports the theory that energy and nutrition adequacy can decrease risk of injury the bone was conducted at Creighton University. Lappe and colleagues (2008) found that supplementing 2000mg of calcium and 800IUs of vitamin D for eight weeks sufficiently reduced the incidence of stress fractures by 20% versus a control sample in female Navy recruits during basic training. This population was approximately 19 years old and, most women engaged in little previous physical activity. The recruits in the control sample who had a history of regular physical activity of greater than three times per week had a lower incidence of stress fractures. It is important to note that while other studies did not find these results, many doses of vitamin D and calcium used in such studies were considerably less. Most research that finds positive results of supplementing calcium and vitamin D use amounts similar to that used by Lappe and colleagues.

In addition to nutritional factors, the age of initiation of regular physical activity may have a larger influence on developing osteoporosis (Karlsson 2007). Bass and colleagues (1998) examined the effect of pre-puberty exercise on long term bone density in adulthood in female gymnast. This article studied 45 active pre-pubertal female gymnasts and 35 control participants (Tanner breast stage 1). Notably, this study was conducted in Melbourne, Australia (37 degrees latitude). This geographical setting may

have access to adequate vitamin D exposure from the sunlight during more months out of the year than other areas which influences the likelihood of adequate serum vitamin D. This study did not indicate time of year, however. Although dietary intake was evaluated, it was not included in the evaluation of bone density. They did find that the pre-pubertal gymnasts consumed equal amounts of calcium to the controls despite having lower total energy intakes. Next, these participants were compared to elite gymnasts retired for 8 years and matched controls. Both the retired gymnasts and the controls participated in similar amounts of physical activity at the time of the study. Bass and colleagues (1998) found that compared to controls, the retired gymnast had a 1-1.5SD higher bone mass. Long term outcomes of exercise-induced bone accrual as reduction of risk of osteoporosis is still under investigation (Burrows, 2007).

Familial resemblance in BMD may give some insight. Runyan and colleagues (2003) compared bone density between early adolescent girls, their pre-menopausal mothers, and their postmenopausal maternal grandmothers. Calcium intake and physical activity, but not vitamin D intake/exposure, were measured and compared as well. They found that bone mineral density (BMD) was highly correlated between the girls and their mothers. Calcium intake was weakly inversely correlated with mother's BMD but not the daughters or the grandmothers. Physical activity, however, was strongly correlated with BMD in both mothers and adolescent girls. This was also independent of calcium intake which, according to adequate intakes (AI), was inadequate in all groups. And again, because calcium intake was below recommended levels, the findings in this study may not accurately represent calcium's role in BMD. In the daughters, height, weight, and

onset of puberty were all strongly associated with measured BMD (Runyan et al., 2003). Although many studies discuss heredity as the main factor in BMD, results from Runyan and colleagues (2003) suggest that lifestyle factors (physical activity) may be as influential as heredity, namely between adolescent girls and their pre-menopausal mothers. Furthermore, this study suggests that moderate physical activity may be able to compensate for lack of calcium intake for BMD. Moreover, they suggest that even as calcium intake approaches normal, physical activity may still have a stronger influence in achieving optimal bone density than calcium intake. It is important to note that vitamin D was not factored into this prediction. Because vitamin D exposure was not accounted for and is essential for absorption of calcium, it may weaken the evidence for the effect of calcium on BMD compared to physical activity especially during the years of menarche.

Research does suggest a positive correlation with BMD and circulating 25(OH)D. The greatest BMD has been observed at serum concentrations of 40ng/mL (Bischoff-Ferrari et al., 2006). In fact, serum levels of 33 are necessary to reflect adequate BMD in the older population (Heaney, 2004).

Exercise impacts bone growth during puberty, as well. During puberty higher bone mass and bone growth is related to the stimulation of growth hormone secretion by exercise and release of insulin-like growth factor I (Bass et al., 1998). Pre-puberty growth, however, is independent of these hormones as the regulation of these hormones changes when the sex hormones are activated (Ackerman & Misra, 2011).

One study, however, explored the factor of “kids being kids”. They suggested that their data do not support the concept that endocrine changes during puberty increase the

risk of bone development and risk of fracture. When comparing multiple factors including calcium intake, vitamin D intake, and outdoor activity in the winter and summer months, total physical activity, BMD, results indicated that increased physical activity in children is associated with higher risk of bone injury, regardless of BMD. This is important to consider while examining children's risk of bone injury. How many injuries are due to low BMD and how many are due to purely the increased exposure to risk of impact great enough to result in bone related injuries (Clark et al., 2008)? These researchers propose that physical activity in children is a means for increased bone density and decreased risk of osteoporosis later in life, although it is important to consider all factors included in bone injuries in children when comparing risk of bone injuries in late adulthood.

After reviewing the literature related to physical activity and its effect on bone health, it can be suggested that there is a threshold for girls' activity at which point it becomes detrimental to bone health, rather than promoting bone health. This effect occurs when intakes of energy, calcium, and vitamin D are inadequate. Because of the variety of variables involved in the role of physical activity on bone mineralization and many studies failing to control for calcium and vitamin D at adequate intakes to see results, it is difficult to make solid conclusions about physical activity other than that moderate physical activity throughout the life in otherwise healthy individuals proves to reduce the risk of fractures throughout the lifespan. It seems that, more than anything, excessive exercise coupled with a negative energy balance increases the risk of developing bone related injuries (Ackerman & Misra, 2011; Burrows, 2007). Moreover, moderate physical

activity coupled with balanced energy and adequate micronutrient profiles throughout the lifespan act as a preventative measure for developing bone related injuries later in life. It remains unclear if an exercise-induced increase in BMD acquired during growth alone is sufficient for reducing risk of developing osteoporosis later in life.

Influential social factors of calcium and vitamin D intakes

The environment in which a person makes food decisions is highly influential on the outcome of the dietary profile. Junior high students tend to consume more milk and calcium than senior high students and students in a lower economic status consume less milk than those in a higher economic status (Larson et al., 2006). Factors that are highly correlated with intake of calcium and milk include familial support, consumption, modeling, and availability (Larson et al., 2006; Fisher et al., 2004). It makes sense that if milk is not available, one cannot drink it. Therefore, it is important that milk be available to young girls and that it is observed as a regular beverage by other family members. When girls observe their family members drinking milk, they also have increased consumptions compared to others (Fisher et al., 2004). Moreover, when the habit of drinking milk regularly is in place by pre-school age, it is more likely that this same habit will be in place by age nine (Fisher et al., 2004). Even until age 14, family influence seems to influence dietary patterns as this age group continues to eat most meals and snacks at home (Story et al., 2002).

Another factor that is thought to contribute to young girls' dietary intake of nutrient rich foods is peer acceptance. Several studies report that up to the age of 11, peer

influence does not affect dietary intake like once believed as it does with influence of participation in physical activity, however (Coppinger et al., 2010;). There is a stronger *familial* influence on food decisions than peer influence including added fat intake (Story et al., 2002). Moreover, among 419 adolescents, peer influence rated the lowest factor in making decisions regarding snack food choices (cited in Story et al., 2002). Therefore, parents, not peers, should be targeted as potential catalysts for adequate calcium intake in young girls.

Female Athlete Triad

The Female Athlete Triad is an interrelated syndrome many female athletes and non-athletes (Raymond-Barker et al., 2007). It is diagnosed as the co-existence of disordered eating, amenorrhea, and osteoporosis (Sherman & Thompson, 2004; Raymond-Barker et al., 2007). It can occur by excessive exercise or disordered eating which in turn diminishes essential nutrients in the body. This can lead to disruption of endocrine function including amenorrhea which in turn can lead to osteoporosis. When all three occur, and other conditions are ruled out, the Female Athlete Triad can be diagnosed.

Athletes in sports that require a thin or lean frame seem to be more at risk than those in other sports. Furthermore, athletes in sports that require judging and/or reveling attire such as gymnastics, figure skating, diving (Sherman & Thompson., 2004) as well as competitive gymnastics may have additional pressure and are at increased risk for developing this syndrome. Many assume that this condition is more prevalent in athletes

than non athletes. When comparing non-athletes to elite athletes, a study in Norway found that 6 out of 10 athletes and 7 out of 10 non-athletes were at risk for the Female Athlete Triad (Torstveit & Sundgot-Borgen, 2005).

As discussed, energy deficiency has been shown to increase cortisol levels in the body and this further exacerbates calcium release from bone stores (Ackerman & Misra, 2011) leaving bones with a decrease in skeletal architecture and higher risk of injury. Energy deficiency also signals the hypothalamus to “turn off” the reproductive system (Sherman & Thompson, 2004). As previously discussed, girls who have started their menstrual cycle have higher BMDs (Loud et al., 2005, 2007; Chalfant et al., 2011; Ackerman & Misra, 2011) and so those who prolong reaching puberty decrease chance of reaching PBM.

Excessive physical activity’s influence on bone health, however, continues to be under debate. On one hand, sweating promotes calcium release from the body, and stress fractures seem to increase with intensity and duration of activity. On the other hand, Loud and colleagues (2005) found no association between age at menarche, BMI, calcium intake, or vitamin D intake and stress fractures. Only duration of physical activity greater than 16 hours a week had an effect on prediction of stress fractures. Alternatively, the type of physical activity may have more to do with bone density than intensity. For example, distance runners seem to have lower BMD and higher rates of stress fractures than sports that require jumping or vertical motions like gymnastics. Other evidence identified distance running and cheerleading/gymnasts were the only groups that were associated with greater incidences of stress fractures, which are contrary to previous

findings (Loud et al., 2005). This multifaceted disorder, therefore, can have several etiologies and different outcomes.

The effects of disordered eating and/or excessive exercise on bone health are seen in early indications of osteoporosis in young female athletes. If a girl is showing signs of the other two components of the Female Athlete Triad, she may lose 1-5% of her total bone mass each year that may also be irreversible (Sherman & Thompson, 2004). The deficiency of energy and nutrients results in a decrease in estrogen. This can leave fewer osteoblasts (bone building cells) than osteoclasts (bone resorption cells) causing more resorption of bone than formation of bone and subsequently early signs of osteoporosis.

Although osteoporosis is established at a young age and is difficult to reverse, early identification and treatment of any disorder improves the outcome of the individual facing the disorder (Sherman & Thompson, 2004). In a case study of the Female Athlete Triad, a female athlete diagnosed with amenorrhea for 12 years was monitored. The athlete's BMD at 2 locations, weight, and BMI were monitored. The researchers noted that as her weight and BMI declined so did her bone density. At the initiation of treatment of transdermal estradiol (an estrogen based medication used in menopausal women for osteoporosis prevention) despite no weight gain, the loss of bone stopped. At age 33 when the athlete voluntarily began to gain weight, her BMD in her proximal femur increased but her lumbar spine did not. Therefore, earlier prevention and treatment of the Female Athlete Triad is warranted in order to avoid permanent damage (Zanber et al., 2004). In the case of bone health, this is usually done by those adults in the lives of the young girls such as parents, teachers, and coaches.

While coaches play an important role in the prevention of and intervention on the Female Athlete Triad, this condition is not always visible to coaches and can even be mistaken as normative behavior. First, excessive exercising may be encouraged or seen as a “strong will” rather than a disorder. Many coaches do not understand that athletes can perform at high levels while having a disorder. Also, amenorrhea is very common in female athletes to the point of being considered “normal”. Finally, osteoporosis usually does not demonstrate many side effects or symptoms at a young age (Sherman & Thompson, 2004). With these factors combined it may be easy to overlook the Female Athlete Triad. Moreover, there seems to be little relationship between knowledge of nutrition and positive nutrition behaviors in the younger population of female athletes under 7th grade (Raymond-Barker et al., 2007). Therefore, attention to this disorder by coaches and other adults is needed.

Although much research exists stating that girls with the Female Athlete Triad may have a higher risk of osteoporosis, the case study by Frederickson & Kent (2005) demonstrated that although the bone mineralization process may be disrupted, athletes may be able to build bone into their third decade if intervention and regain is initiated. Therefore, the risk for osteoporosis should be addressed at a young age.

Health Belief Model (HBM) and adolescent girls

The HBM of cognition addresses a person’s belief about their health and draws connections between the person’s behavior and their health belief (Becker, 1974). Additionally, it identifies one’s perceived ability to make changes or perceived risk of

acquiring health impairment. The theory states that motivation to change stems from perceived risk of disease. For example, Hazavehei and colleagues (2007) assessed the impact of interventions targeting perceived risk of osteoporosis in adolescent Iranian girls. They compared an HBM-based intervention, a basic education about osteoporosis, and a control group with no education and found that the HBM-based intervention resulted in significant changes in health belief and action as compared to traditional education and controls (Hazavehei et al., 2007). This model is relevant for young female gymnasts because it may be that their perception of risk of disease also may be correlated to their health action.

Nutrition knowledge and associated behavior

Researchers seem to suggest that athletes are exposed to and utilize more nutrition information than their non-athletic counter parts (Torstveit & Sundog-Borgen, 2005). Raymond-Barker and colleagues (2007), however, found there was no difference in the nutrition knowledge between athletes at risk of the Female Athlete Triad versus controls. They concluded that nutrition knowledge, or lack thereof, was not associated with restricted eating found in the Female Athlete Triad. On the other hand, Sharma and colleagues (2010) found that 66.1% of the responses to knowledge of calcium rich sources were answered correctly. They also found that knowledge about sources of calcium, self-efficacy, and outcome expectations were all correlated with higher calcium intakes. Milk available at home was found to be correlated with adequate calcium intake (Sharma et al., 2010; Larson et al., 2006).

Age differences have been associated with behavior regarding using acquired nutrition knowledge. In fact, Pirouznia in 2001 found that there was no association between sixth graders (11 year olds) knowledge and subsequent nutrition behavior but there was for seventh and eighth graders (12 and 13 year olds). Thus, 12 and 13 year olds were more likely to make nutrition choices that reflected their nutrition knowledge.

Summary

Young girls generally do not consume adequate intakes of calcium or vitamin D. Serum levels of 25(OH)D also are usually insufficient in this group. Both adequate intake of calcium and vitamin D have been shown to increase BMD in this population, and therefore, it is concerning that neither is adequate in this population. Girls aged 11-14 are going through multiple biological changes affecting their bone density. Their behaviors can influence their bone density outcomes in a positive way which starts at home. Girls will more likely chose nutrient rich sources of calcium if it is available and if their family's promote and demonstrate this behavior.

The standard serum vitamin D level in pubertal young girls is equivocal. Seasonal differences of vitamin D status are demonstrated across the research spectrum. Moreover, there seems to be a decrease in serum vitamin D on onset of puberty when adequate vitamin D is necessary for adequate bone development. It also remains unclear if children and adults should have the same reference points or if different reference points should be in place for serum vitamin D levels. This makes consistent diagnosis for hypovitaminosis D of young girls difficult between studies.

Physical activity can promote bone accrual in this age group. In fact, the more weight baring activity before menarche the more likely adequate bone density will be reached. This seems to be extraordinarily beneficial before menarche and then has little added effect after onset of menarche on accrual although seems to improve maintenance of bone health.

Onset of menarche is the main indication that a girl's bone density is building and therefore is even more essential to eat nutritiously and to practice weight baring physical activity. The later the onset of menarche in the absence of adequate physical activity, the more likely that that young girl will be at risk for bone injury. Factors that can influence onset of menarche include genetics, race, and adequate energy intake to meet expenditure.

As the age of onset of menarche is different, it is important to collect this information from participants in this age range to predict bone maturity and relative risk of bone injury and determine the critical time period of optimal bone development. Assessing Tanner stage is important for determining a girls' pubertal status. This can help the researcher assess when the girl will require higher amounts of calcium and vitamin D to promote bone anabolism (Clark et al., 2008; Marshall & Tanner, 1969).

Research is lacking in the area of knowledge and perceived importance of nutrition and health behaviors associated with bone health. Of the studies that have inquired about perceived importance, behavior often does not reflect perception of importance until later in adolescents. Even then, environmental factors such as time spent away from home can promote behavior such as inadequate nutrient rich calcium intake,

and decrease physical activity. Moreover, athletes seem to demonstrate adequate nutrition knowledge, but also may not demonstrate the behaviors that reflect that knowledge. Better understanding the connection between knowledge and bone health behaviors will assist researchers in developing programs for promoting bone health and osteoporosis prevention for this age group. To this writer's knowledge, there appears to be no current research related to the attitudes and beliefs regarding calcium and vitamin D in young female competitive cheerleaders in the United States.

Methodology

This study examined the perception of young, female competitive cheerleaders about the importance of dietary calcium and vitamin D. We hypothesized that there would be a positive correlation between the girls' perceived importance of calcium and vitamin D and their actual intake of calcium and to plasma values of vitamin D. This population was selected because there is very little research on perceived importance and the adequacy of diets for these girls.

Specific study objectives: (1) To determine calcium intake and plasma vitamin D levels of young female athletes, (2) Analyze the participants' perception of the nutrients' importance to their health, (3) Determine correlations that exist among knowledge and perception of importance of nutrients, calcium and vitamin D intake, and serum vitamin D levels.

Before data collection began, approval was received from the Institutional Review Board at the University of Nebraska-Lincoln.

Data collection

Participants were recruited from Elite Cheer in Omaha, Nebraska. All 11-14 year old girls involved in competitive cheerleading at Elite Cheer were invited to participate (n = 33). Twenty-eight girls showed interest in participating. Twenty-three girls completed consent forms, two did not meet eligibility criteria, one did not complete the blood draw, and 20 girls completed the study. The coaches at Elite Cheer received parental informed

consents and informed assent letters and forwarded them to the participants and their parents. The letters also included information to ensure the participants met eligibility criteria. These criteria included the absence of severe food allergies or chronic medical problems that would affect food intake, and the absence of dietary restrictions involving dairy products. The participants returned their signed informed consents and assents back to their coach who returned all forms to the investigator at the start of the study. Those that did not complete the informed consents and assents prior to the beginning of the study were given the opportunity to do so if they wished to participate by signing assent and consent forms on site prior to the first date of the data collection. The researcher collected all data on site at Elite Cheer and we hired phlebotomists were hired to collect a blood sample from each of the participants and assigned an identification number to all participants protect their privacy.

The study took place over a series of five weeks. At the first meeting, the participants were given privacy to complete two questionnaires:

1a. Demographics/Anthropometrics: (Appendix A) Questionnaire including demographics, anthropometrics and self-reported height and weight, UVB exposure via tanning beds, supplementation usage, estimated sunlight exposure, menstruation status, and previous bone injuries.

1b. Psychosocial construct survey: (Appendix A) A nutrition survey based on the psychosocial constructs for adolescent girls' calcium intake survey developed by Glanz and colleagues in 2008. This survey inquired in more detail about assessed participants' view of foods containing calcium, their perception of other individuals' consumption of

calcium rich foods, and their preference for foods rich in calcium. This study was validated and had high internal consistency reliability (a>.75) and good test–retest reliability (0.73 to 0.78). Questions were included to assess the participants' knowledge of food sources of vitamin D. The participants rated their answers on a 5 point scale with 1 being "never" or "strongly disagree" 3 being "sometimes" and 5 being "always" or "strongly agree". Glanz granted this researcher permission to use the items from this construct. Within the questionnaire, categories of questions include those pertaining to availability of calcium rich foods, social influence (including coaches, parents, and peers), perceived health benefits, preference for milk, perception of taste of milk, perceived negative influence of milk on weight, and knowledge of calcium rich and vitamin D rich foods.

2. Self-assessment Tanner staging questionnaire: (Appendix B). The participants completed a self-assessment Tanner stage questionnaire adapted from Jenner and colleagues (2009) and Azevedo and colleagues (2009). The participants answered 5 questions concerning their pubertal development. They assessed their pubertal development by circling the photograph that best described their body. The photographs included sets of 5 different photographs of breast development or pubic hair grown in girls at similar stages of prepubescent development. Finally, the participants determined at what time they would be available during the next week to complete their blood work.

Blood collection

Each participant gave a blood sample in a private area separated from the rest of the participants. Physicians Lab of Omaha, NE came to the site on two separate occasions with one or two licensed phlebotomists at each session. Participants completed consent forms ahead of time and presented them at the time of the blood draw. Physician's Lab used Enzyme Immunoassay (EIA) IDS to analyze the blood to determine vitamin D levels. EIA can be used with serum or plasma to quantify 25(OH)D. Samples are diluted with biotin and then incubated in wells coated with sheep 25(OH)D antibodies for 2 hours. Next the samples are aspirated and washed. An enzyme, avidin, is added which binds to biotin. After being washed one more time, color develops using chromogenic substrate. Interpretation of this indicates that the deeper the color intensity, the lower the concentration of 25(OH)D (Immunodiagnostic Systems Inc, Fountain Hills, Arizona, 2003). Results were returned to the investigator via US mail. All participants received their vitamin D results.

Nutrient intake

The subjects participated in a 24-hour recall using the multiple pass method along with 3-dimensional food models and common household measures to determine portion sizes (Johnson et al., 1996; Burrows et al., 2010). Participants received verbal as well as written instructions on how to document a two day food record. Each participant was

given a food log to collect this information for one week day and one weekend day and instructed to bring the completed record back the following week (Appendix C).

On the last day, each participant returned their completed food record and received a gift card as a thank you for completing the study. The participants were advised that they would receive one phone call from the investigator at a later date to clarify any of the food records if needed.

Analysis of data

Total caloric intake, calcium intake, vitamin D intake, servings of milk, and servings of dairy were assessed using Nutrition Data Systems for Research (NDS-R, Nutrition Coordination Center, University of Minnesota). All data was coded and analyzed with Statistical Analysis System (SAS Inc., Cary, North Carolina) and Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois) to compare anthropometric information on this population. Measures were gathered for average calcium, vitamin D, and total average calories consumed; knowledge of calcium rich foods; availability of calcium and vitamin D rich foods; social influence of intake; food preferences; weight influence; tolerance of calcium rich foods; convenience of calcium rich foods; supplement use; source knowledge; and taste preferences.

Frequencies, means, standard deviation, and range were determined for all variables. Correlations were used to identify significant relationships between variables with a significance level of $P = 0.05$. Percentages of participants' scores were calculated by adding the individual scores from each item in each group of measurements for a

measurement group total score and comparing individual totals to available score totals. For example, in the “availability” measure, there were 10 items each with 5 points possible (strongly disagree =1, strongly agree = 5) for a total of 50 points. These totals were considered ideal scores. Any inverse questions were reversed for consistency.

Results

A total of 23 girls ages 11-14 volunteered for participated in this study. Of those girls, all completed the questionnaires, 21 completed the blood draw and the two day food records. Of the girls who completed the study, one was ineligible secondary to both anticonvulsive medications and a milk allergy.

Of the 20 participants in this sample, 95% were of white ethnicity. The average Tanner stage was 3.5(+/- 0.77). The mean age was 12.5 (+/-1.05) years at the time of the study. The mean daily calcium, vitamin D, and total energy intake were 878.7 (+/- 75.8 mg), 255.5 (+/- 42.8IUs), and 1647 (+/-371) kcals per day, respectively. The mean height, weight, and BMI were 157.4 (+/-11.24) cm, 47.2 (+/-9.7)kg, and 19.6 (+/- 3.0), respectively. Forty-five percent of the girls had reached menses with an average age of 11.3 (+/-0.5) years at onset. Fifteen percent of the girls reported use tanning beds regularly (19 minutes per week average). Four girls reported a history of non-digit bone injuries. Five girls used dietary supplements on a regular basis, two of which had both calcium and vitamin D included (Table 3).

Table 3. Anthropometric and dietary information of sample.

	Frequency (n)	Mean	Std Dev. (+/-)	Minimum	Maximum
Age (yrs)	20	13	1.1	11	14
Height (cm)	19	154.5	11.2	129.5	167.6
Weight (kg)	19	47.2	9.7	34.1	65.9
BMI	20	19.6	3.0	14.7	25.7
BMI%	20	58%	29%	7%	92%
Tanner Stage	20	3.5	0.77	2	5
Age of Menarche (yrs)	9	11.3	0.5	11	12
Estimated calcium (mg/day)	20	878.7	75.8	311.3	1790
Estimated vitamin D (IUs/day)	20	255.5	42.8	12.6	631.5
Serum vitamin D (ng/mL)	20	27	11	9	59
Calories	20	1647	371	986.3	2128.3
Minutes in Tanning bed per week	3	19	7.2	12	30

Dietary calcium intake

The goal for calcium intake is 1300mg per day for 11-14year old girls. The mean calcium intake of the participants was 878.7mg (std dev +/- 339.2). In the current sample, we divided calcium intake into categories of 300mg (average amount of calcium in one cup of milk). Of the 20 participants, two girls consumed on average calcium intake of 1201-1500mg per day, eight girls consumed an average between 901-1200mg per day, six girls consumed 601-900mg per day, three girls consumed 301-600mg per day, and one girl consumed more than 1501mg of calcium on average per day (Fig 5).

Figure 5. Frequency of calcium consumption

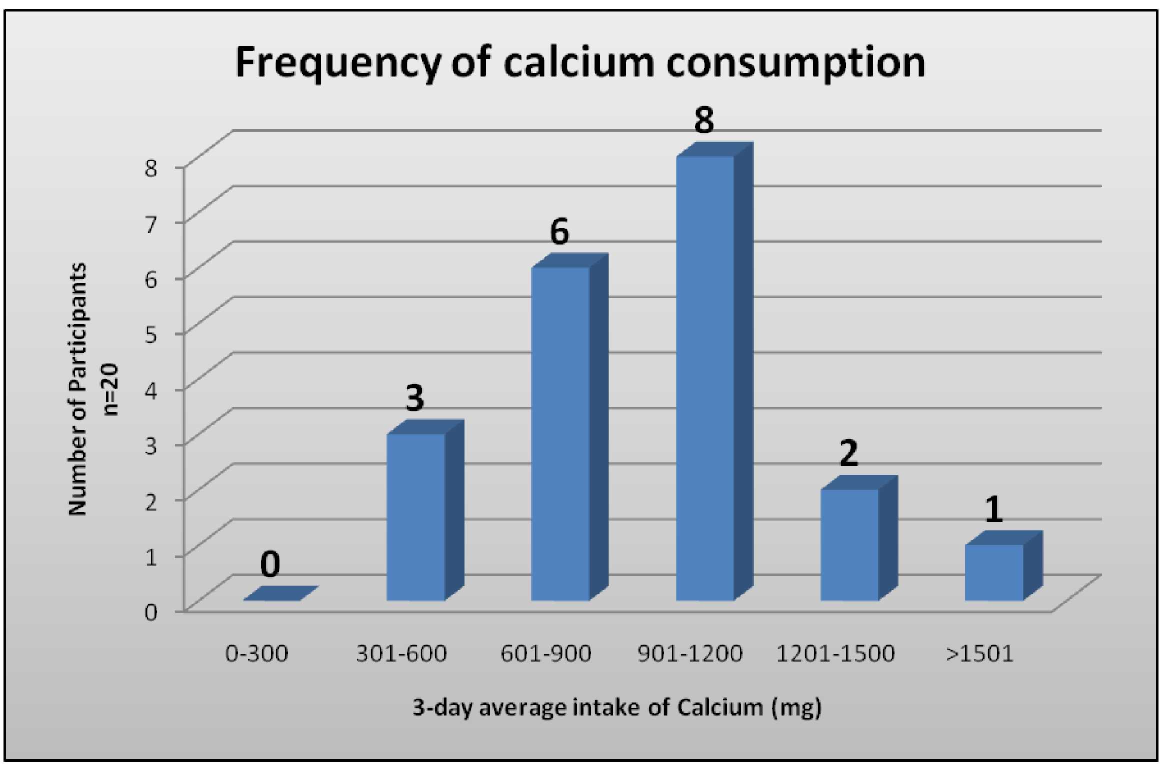
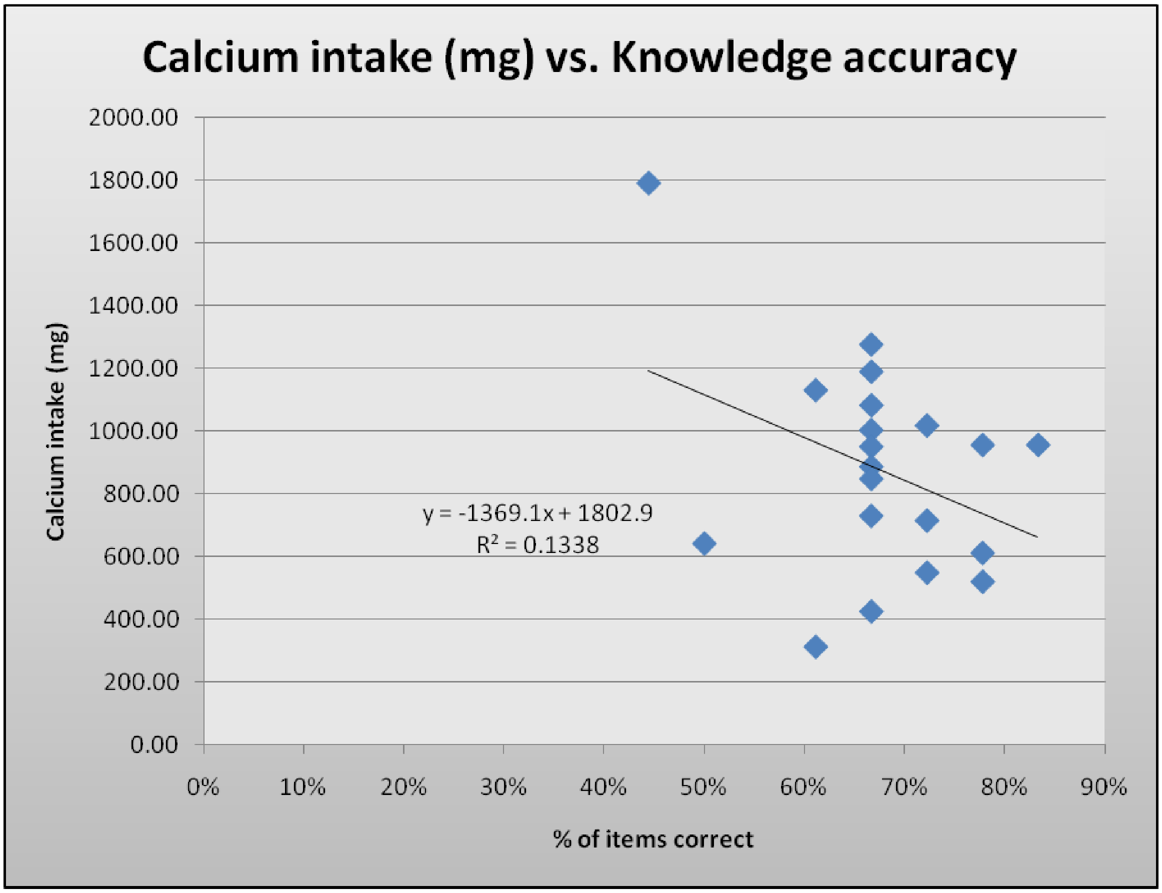
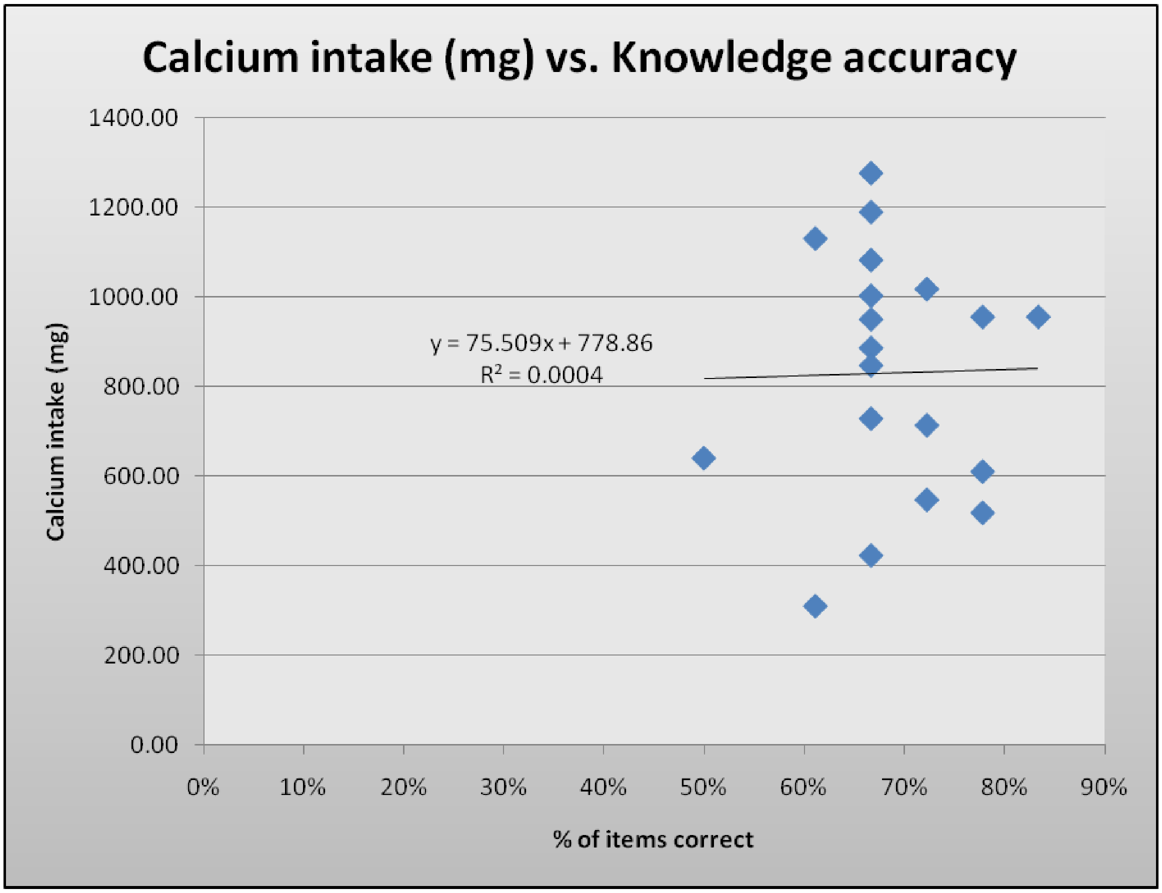


Figure 6. Calcium intake (mg) vs. Knowledge accuracy



The results of the Pearson correlation indicated that there was no relationship between calcium intake and knowledge of calcium sources (r = -.366) (Fig 6). With the removal of the one outlier the line changes to a very slight positive relationship but continues to be insignificant (r = .02) (Fig 7).

Figure 7. Calcium intake (mg) vs. Knowledge accuracy- no outlier

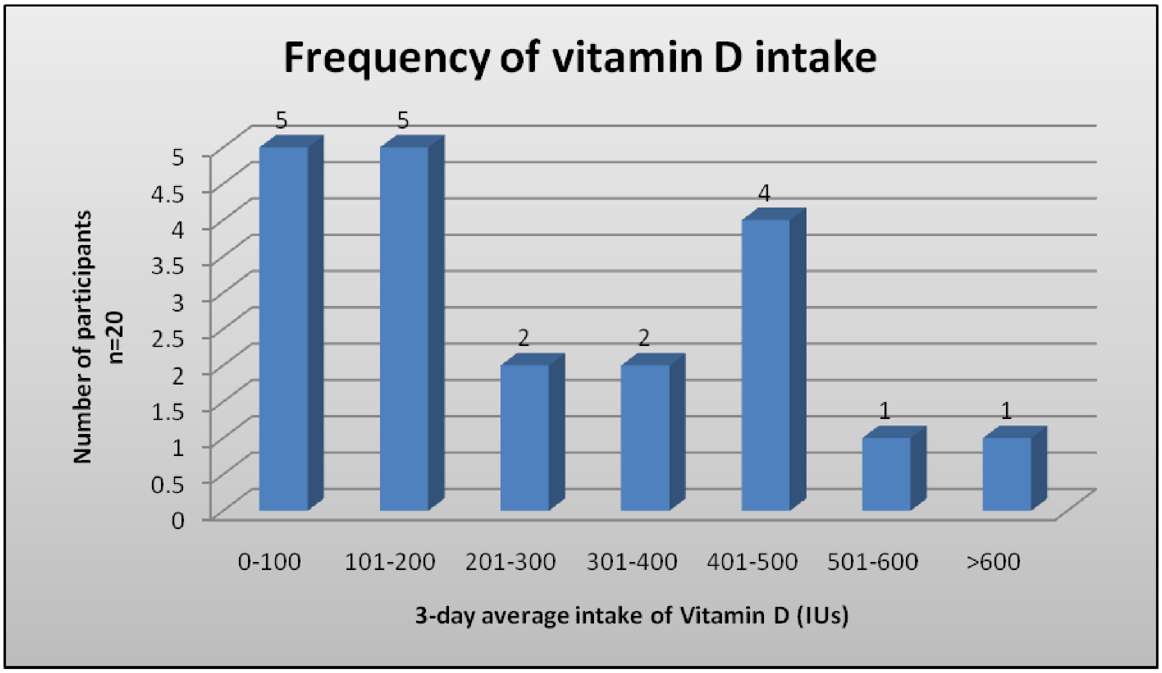


Dietary vitamin D intake

According to the IOM, the recommended intake of vitamin D is 600IUs per day. The mean estimated dietary intake of the participants was 225.4IUs (std dev +/- 191.6). Vitamin D was divided into increments of 100IUs, the average IU per one cup of milk. One participant consumed between 501-600IUs, four participants consumed 401-500IUs, and ten participants consumed an average of less than 201 IUs of vitamin D (Fig 8).

These results are similar to results quoted from Bueno & Czepielewski (2008) which reported an average vitamin D intake of 240IUs per day in children.

Figure 8. Frequency of vitamin D (IU) intake

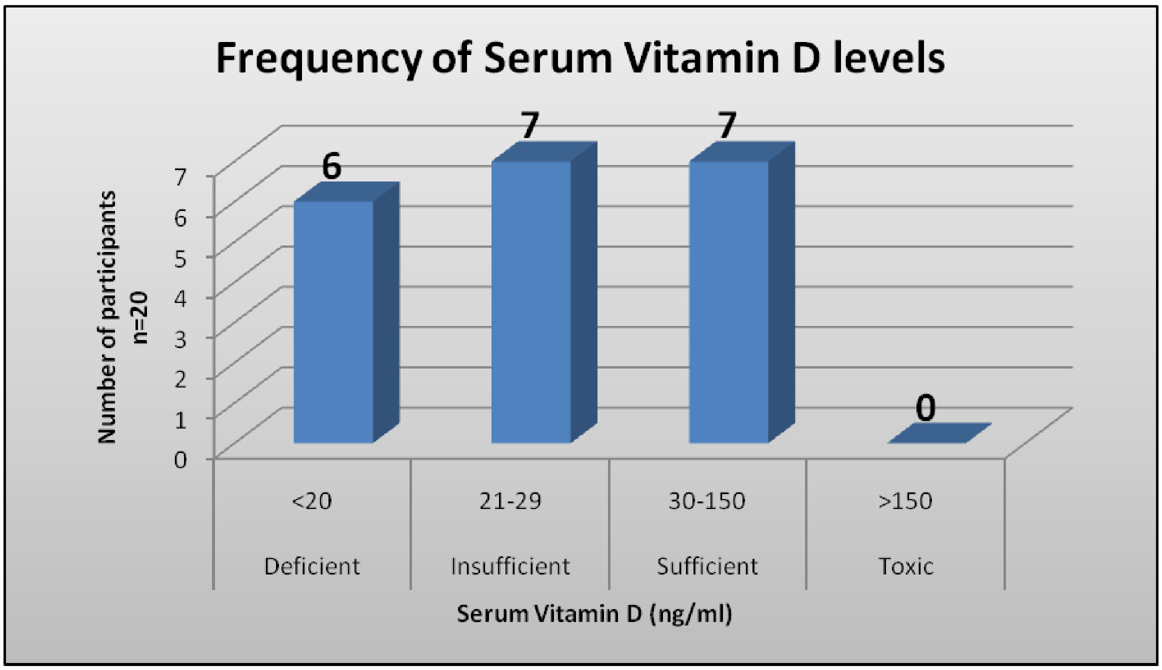


Serum vitamin D levels

Holick (2007) describes adequate serum vitamin D levels to be above 30ng/mL. Others use 20ng/mL for children (Bueno & Czepielewski, 2008) but it is suggested that his is not adequate to meet needs of bone health. Adequate levels describe by Holick will be used for this study. The mean serum vitamin D for this sample was 27.1 (+/- 10.9) which is considered insufficient. The distribution for serum vitamin D demonstrates 30% of participants were deficient, 35% were insufficient, and 35% were in the sufficient range (Fig 9). Of the girls who were in the sufficient ranges, three of the seven girls used

tanning beds on a regular basis. There were no tanning bed users in the insufficient or deficient categories. Of the girls who were sufficient, five of the seven girls had serum vitamin D levels less than 40ng/ml.

Figure 9. Frequency of serum vitamin D (ng/mL) levels



Association between knowledge, perception, and nutrient status

No significant correlations were found among independent and dependent variables. (Table 4). There was no association between average calcium, vitamin D, caloric intake and any other item measured. Because of the small sample sizes, frequencies and distributions of responses may give better insight.

Table 4. Correlations among variables					
Correlation P value	Calcium intake	Servings of dairy	Servings of milk	Vitamin D intake	Serum vitamin D
Health Behavior	0.05 0.85	0.02 0.92	-0.04 0.88	0.25 0.3	-0.42 0.89
Availability	-0.24 0.31	-0.2 0.4	-0.16 0.49	0.14 0.57	0.35 0.13
Social influence	-0.16 0.5	0.08 0.74	0.014 0.95	0.16 0.49	0.22 0.35
Preference	0.05 0.84	-0.12 0.60	-0.29 0.21	-0.20 0.39	-0.19 0.42
Weight	-0.1 0.68	0.15 0.52	0.15 0.53	-0.16 0.49	0.13 0.33
Tolerance	0.34 0.15	0.24 0.31	0.26 0.26	0.31 0.18	-0.29 0.21
Knowledge	-0.37 0.11	-0.24 0.32	-0.27 0.25	-0.1 0.68	0.23 0.34
Taste	-0.002 0.99	0.5 0.53	0.23 0.32	0.14 0.55	0.11 0.64
Serum Vitamin D	-0.24 0.31	-0.003 0.99	-0.11 0.66	-0.08 0.75	1.00

Perception of importance

According the response from the health belief section of the questionnaire, of the girls who participated 70% indicated that they *strongly agree* or *agree* with the statement “I drink milk so I can have strong bones now.” Seventy percent indicated they *strongly agree* or *agree* with the statement “If I drink milk now, my bones will be stronger when

I'm older." All of the participants *strongly disagreed* or *disagreed* with the statement "Milk is not healthy", 85% *strongly agreed* or *agreed* with the statement "It is important for people my age to drink milk.", 95% *strongly disagreed* or *disagreed* with the statement "only little kids need milk." And 80% *strongly agreed* or *agreed* with the statement "I drink milk because it is good for me." (Table 5).

Table 5. Distribution and frequency of response based on Healthy Behavior section

	Strongly Disagree 1	**2**	**Sometimes 3**	**4**	**Strongly Agree 5**
I drink milk so I can have strong bones now.	0	4 20%	2 10%	6 30%	8 40%
If I drink milk now, my bones will be strong when I'm older.	1 5%	1 5%	4 20%	5 25%	9 45%
Milk is not healthy.	18 90%	2 10%	0	0	0
It is important for people my age to drink milk.	0	0	3 15%	3 15%	14 70%
Only little kids need milk (reversed).	17 85%	2 10%	1 5%	0	0
I drink milk because it is good for me.	1 5%	0	3 15%	4 20%	12 60%

Other variables assessed

The means of the total points for each subcategory were compared to the total possible favorable points for the corresponding subcategory (Table 6). As a group, the girls scored availability of calcium rich foods (69.1%), social influence (61.7%), health benefits (88.2%), preference for milk (56%), taste 67.8%, negative influence on weight (8.3%), knowledge of calcium rich and vitamin D rich foods 67.5%.

Table 6. Variable statistics

Label	Points available	%	Mean Sum	Std Dev	Minimum	Maximum
Serum vitamin D	30		27.1	10.95	9.0	59.0
Availability of calcium rich foods	50	69.1	34.55	3.05	30.0	39.0
Social influence	45	61.7	27.80	5.88	17.0	38.0
Perceived health benefits	30	88.2	26.45	3.24	20.0	30.0
Preference	40	56	22.40	5.62	14.0	32.0
Perceived taste	30	67.8	20.35	2.62	16.0	24.0
Perceived effect on weight	15	91.7	13.75	0.97	12.0	15.0
Tolerance	10		10.0	3.0	2.11	8.95
Knowledge of calcium rich sources	18	67.5	12.15	1.63	8.0	15.0
Average calcium	1300	67.6	878.7	339.18	311.3	1790.0
Average vitamin D IU	600	37.6	225.46	191.60	12.6	631.5
Average servings of dairy*	4	49.3	1.97	1.25	0.5	4.9
Average servings of milk/yogurt**	4	31	1.24	0.97	0.1	3.0

*Dairy includes all foods made from milk: milk, yogurt, cheese, etc.

**Milk includes cow's milk and yogurt

Discussion

The purpose of this study was to investigate correlations between intake of calcium, vitamin D, and serum vitamin D with perceived importance of these nutrients. Although no correlations were found, it is apparent through frequency of responses that this population agreed with statements of importance to calcium. These attitudes are associated with higher calcium intakes in other studies. The actual intakes of vitamin D and calcium were inadequate compared to the Dietary Reference Intake (DRI) for this population. It is interesting that their intake of calcium and vitamin D did not reflect their attitudes towards milk and other nutrient-rich sources. There may be many explanations for this discrepancy.

The results from the current study demonstrated that this sample of girls did not consume adequate amounts of dietary calcium or dietary vitamin D to meet the standard recommended daily allowance (RDA) for this age group, on average. Inadequate intake of calcium and vitamin D is observed nationally by the CDC as well. Girls seems to be increasing their intake of other beverages and decreasing intake of milk as a beverage leaving fewer opportunities to meet RDAs for calcium (Fisher et al., 2004; Fiorito et al., 2006; Ervin et al., 2004, Nicklas, 2003; Loud et al., 2005; Gordon et al., 2004). Although this sample's intake of calcium and vitamin D is lower than RDA, it is similar to suboptimal findings by other studies of this age group (Fiorito et al., 2006; Ervin et al 2004), and the 200 IUs of vitamin D noted by several other studies (Cited in Ackerman & Misra, 2011). This may indicate that our sample is representative of this age group for consumption of dietary calcium and vitamin D.

These results further support prior research that hypovitaminosis D is common among this age group (Lehtonen-Veromaa et al., 1999) in winter months. It is common for samples to have lower levels in the winter than in the summer months (Halliday et al., 2010). Some literature indicates that the goal for normal may be in the 50s ng/mL for overall health. For bone health, however, a low-normal range (30-39 ng/mL) may be sufficient. Of our sample, 35% of the girls had serum vitamin D levels within normal limits. Furthermore, 100% of the girls who had UVB exposure via tanning beds fell in this group, which comprised almost half of the total number of girls in the group. This can be expected because exposure to UVB radiation increases likelihood of optimal serum vitamin D levels (Armas et al., 2007). Additionally, of the girls whose serum vitamin D levels were within the normal range, only one fell above the low-normal range. This is also consistent with literature from all populations for this latitude in winter months. This study was conducted in January at latitude of 41 degrees. Due to the angle of the sun's rays hitting the earth, UVB rays do not reach ground which eliminates opportunity for conversion of vitamin D in the skin (Holick, 2007). At this latitude, UVB does not reach the earth from the months of October to March. Therefore, as the half-life of vitamin D is 2-8 weeks, this sample did not have any exposure to UVB from the sun at that time of the study. Without education about the need for supplementation during winter months, it makes sense that this sample had low levels of serum vitamin D as they are unable to consume adequate amounts in fortified foods to reach needs.

Results from the questionnaire assessing perceived importance indicated that this population is able to acknowledge the health benefits of calcium rich foods. Of the six

items of the questionnaire that focus on health belief, at least 70% of the girls indicate they are in strong agreement with the health benefit statements. In fact, 100% of the girls indicate that they strongly disagree or disagree with the statement “Milk is not healthy”. It seems as though the perceived importance of milk is not correlated to actual intake of milk until later in life (Pirouznia, 2001). When girls are younger (below seventh grade) their behavior is not as highly influenced by their health belief. This can be seen in our results because there were no significant correlations between indications of perceived importance and actual intake of calcium, vitamin D, or serum vitamin D. This is consistent with the results found by Sharma and colleagues (2010), Larson and collegues (2006), or Pirouznia (2001) for this age group. In addition, as girls get older, their knowledge about calcium rich foods is positively correlated to calcium intake (Nicklas, 2003). This could be due to young children’s difficulty grasping long term consequences for current behaviors. As children develop into adults insight can improve with education.

This does not necessarily mean that they consume more calcium as they get older. In fact, most studies find that as girls get older they consume less calcium (Bueno & Czepielewski, 2008). Not only are they consuming less than what they were consuming at a younger age, it is magnified by the fact that their needs increase at this new stage of development. The RDA is 800mg per day for girls until the age of nine years. Due to growth needs that number increases to 1300mg per day, which is at least one more serving of milk. If girls become accustomed to a dietary pattern, it may be difficult to alter that pattern by including another serving of milk into their day as their interest in milk as a beverage deceases.

Although no significant correlations were found between psychosocial constructs and dietary patterns, several relationships approached significance, such as knowledge of calcium-rich sources. Although the relationship between calcium intake and knowledge of calcium rich food sources was approaching significance ($p = 0.11$), it was inversely related in our sample. Therefore, in this population, knowledge of calcium-rich sources actually may have been related to lower intakes of calcium. This is not consistent with findings in larger samples. In fact all other studies mentioned that have measured this have found a relationship between knowledge of calcium rich sources and calcium intake (Sharma et al., 2010; Larson et al., 2006). This may have been skewed by an outlier in our study; once the outlier is removed, the relationship become positive. Calcium intake and tolerance to milk was approaching significant ($p = 0.15$) as well as availability of calcium rich foods and serum vitamin D levels ($p = 0.13$). This further extends prior research findings that having sources of calcium available will increase consumption (Larson et al., 2006).

There is evidence to suggest that dietary recalls and food logs may be insufficient for gathering accurate intake for this population. We used one multiple pass 24-hour recall and a two-day food log in our study. Although Johnson and colleagues (1996) found multiple pass 24-hour recalls to be accurate measures for 4-7 year olds, a review by Burrows and colleagues (2010) found children tend to under report their intake in food records and over report their intake in 24-hour recalls. This review indicates that diet histories proved the best estimates of children's diets for ages up to 16 years. Because we were investigating serum vitamin D levels as well, which can be highly variable

depending on UVB exposure and can change in a few weeks, a diet history would not be an accurate way of comparing dietary intake of vitamin D to serum levels of vitamin D at the time of the blood draw. It may be likely that inaccurate food logs contributed to the insignificant findings and the low intake of calcium and vitamin D.

This age group, 11-14 years, is the optimal time for developing peak bone mass (Looker, 2003). Calcium intake, vitamin D intake, and physical activity are all proponents for osteoblast formation and bone development. Although gymnasts are less susceptible to bone injuries because of the benefit of the high impact for stimulation of osteoblast formation, it is possible that this group may be at risk for the Female Athlete Triad and developing weak bones. Perhaps this sample surpassed the excessive physical activity threshold of 16 hours per week. Every additional hour of physical activity after a moderate level increases risk of bone injury. Inadequate intake of calcium and inadequate serum vitamin D levels further perpetuate this risk. Furthermore, a few participants consumed an average of less than 1200kcals over three days which increases risk of amenorrhea, and consequently weakens bone mass.

On the other hand, if this population does not practice more than 16 hours a week, they may be at less of a risk than we predicted. Gymnasts tend to have stronger bones than other athletes at risk of the Female Athlete Triad. This group seems to have a positive attitude towards milk in general, and a good knowledge of which foods contain calcium. Moreover, this group generally has optimal responses to questions regarding attitude towards dieting. In fact most girls indicated they were not concerned about their weight in the questionnaire. Additionally, because in general this sample has optimal

responses to health belief items, their behavior may start to reflect their attitudes as they grow older as research suggests. What is more, this population seems to have a healthy menstruation for the most part. This group is going through puberty and of the girls who indicated that they have started menstruating all started at either 11 or 12 years. There were two participants who were 13 years at the time of the study and indicated that they had not started their menstrual cycle at the time of the study, both of which reported inadequate calories (less than 1200kcals per day on average). Athletes who have regular menstrual cycles tend to have stronger bones. We cannot draw any strong conclusions about this population's bone health as we do not have results of bone mass.

One sign of inadequate bone mass is not only a break in the bone but the site of a break. Many participants reported a bone injury, but many of these are digit injuries which may be a result of using their hands for tumbling drills rather than low bone mass. Probability of sustaining a bone injury increases with exposure to physical activity (Clark et al., 2008) without increasing risk of osteoporosis. Breaks and fractures in the lower back and growth plates are two major indicators of weak bones, however. One of the participants reported a hairline fracture in her lower back. This particular individual also had the lowest serum vitamin D level (9ng/mL), reported the lowest total score for positive social influence on calcium intake (17 out of 45 possible points), and second lowest score for availability (31 out of 50 possible points). Both of these environmental factors are correlated to calcium intake in larger studies as noted previously. While she reported adequate caloric intake, she reported an inadequate average intake of vitamin D of about 100IUs per day. Vitamin D is necessary for calcium absorption. This is one

example of an individual who may be at risk for more bone injuries and osteoporosis later in life.

In order to more precisely estimate a girl's risk of osteoporosis or reaching peak bone mass, it is ideal to evaluate her done density. The best way to assess an individual's BMD is to use dual-energy x-ray absorptiometry (DEXA). If bone loss is identified, a treatment can be put into place in an attempt to reverse the loss. This must be done before a woman reaches her mid-to-late 20s because bone mass may be difficult to reverse at later stages in life.

Future research utilizing a larger sample size in order to better determine correlates between nutrient intake and psychosocial factors is warranted. This information will be helpful in determining possible predictors for behavior change in young female athletes. Athletes are susceptible to bone injuries both from insufficient nutrient intake to account for the demands of physical activity and from repeated exposure to more forceful impact on the bones.

Limitations

One possible explanation for the discrepant findings in the current study may be a result of limited power to detect an effect due to the small sample size. Because sample size was small and many of the participants responded homogeneously, a restriction in range is likely. All participants had relatively high knowledge of calcium rich foods and so there was no way of identifying a pattern of how level of knowledge is related to intake. There were no low-knowledge participants for comparison. Restriction of range in

data can obscure correlation results because variability is necessary in order to identify relationships among variables.

A limitation to finding a relationship between behavior and insight to nutrient-rich foods is that this study did not investigate the level of self-efficacy in these participants. Believing that one can achieve a certain behavior increases likelihood of that behavior developing. In larger samples, intake of calcium was correlated to self-efficacy (Sharma et al., 2010).

Moreover, secondary amenorrhea is an indicator for the Female Athlete Triad. Although this study investigated onset of menarche, it did not ask about menstrual regularity. This can help determine nutritional adequacy, risk of the Female Athlete Triad, and possible risk of osteoporosis later in life.

Insight into average hours of physical activity per week may have helped determine adequacy of caloric intake. There may be individuals who practice greater than 16 hours per week, which has been found to increase risk for bone injuries. This study also did not analyze bone accrual in the sample, which would help identify the adequacy of nutrients compared to level of physical activity. All of these questions (self efficacy, bone density, physical activity, menstrual regularity) could be potential opportunities to gain further understanding of this population's relative risk of developing bone injuries later in life.

Future directions

To our knowledge this is the first study that examines the impact of perceived importance of calcium and vitamin D on actual intake in gymnasts. The paucity of research on this topic leaves potential for further investigation into the influence of sense of importance of nutrition on behavior. To improve this study, a larger sample size would be beneficial to gain power. Additionally, it would be interesting to interview the next age group older than this sample to compare relationships of perceived importance and actual intakes and compare responses to the younger girls.

Because this population's serum vitamin D levels were low in general, more education should be proved to influencing members of these athletes lives. Coaches, parents, and teachers should be involved to assist young female athletes in making healthful choices for reaching optimal bone health. As evidence suggests, this age group may begin to perceive calcium and vitamin D important and still have a high influence from parents. Therefore, education with parents to demonstrate nutrient intake, to encourage nutrient rich sources of calcium and vitamin D available at home, and to create an environment where their daughters are more likely to eat and drink foods rich in these nutrients is important to assist girls in reaching peak bone mass. This may assist with implementing positive health behaviors throughout a lifetime, which can reduce risk of osteoporosis later in life.

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Appendices

Appendix A

Participants Name: ____

Last First MI (DOB)

Parent or Guardian's Name:

____

Phone number: _____-_____-_______ Other contact information: ________________

Best time to call: ____

Age: _________ Height: ___ft ___ in Weight: ____ lbs

Race:

Age of first menstrual cycle: ________ (N/A If have not started menstruation)

Known food allergies, intolerances, or medical conditions:

____

(use back of page for more room)

Please list any dietary supplements, vitamins, minerals, herbals, and medications you take. Please indicate how frequently you take these. (ex. Centrum Kids 1x/day, Vitamin D3 1000IU 1x/day)

____

(use back of page for more room)

On average, how many minutes a week to you use UVA/UVB tanning beds: _____________ minutes

In the last month, how many days have you spent outdoors in the sun between the hours of 10am and 3pm? ______ days _______ to _______ range of minutes each day

Please list any bone related injuries such as breaks, stress fractures, shin splints, etc:

back of page for more room)

For Office Purposes Only:
ID No: _________ Date:__________ Interviewer: ____

Appendix A, continued

Please indicate your response to each question by circling the appropriate number that corresponds to your response. 1 = Never, 3 = Sometimes, 5 = Everyday

There is milk in my home.				
1 Never	2	3 Sometimes	4	5 Everyday
There is yogurt in my home.				
1 Never	2	3 Sometimes	4	5 Everyday
There is frozen yogurt in my home.				
1 Never	2	3 Sometimes	4	5 Everyday
There is ice cream in my home.				
1 Never	2	3 Sometimes	4	5 Everyday
There is cheese in my home.				
1 Never	2	3 Sometimes	4	5 Everyday
There is calcium-fortified orange juice in my home.				
1 Never	2	3 Sometimes	4	5 Everyday
There is cereal at home.				
1 Never	2	3 Sometimes	4	5 Everyday
There are vegetables at home.				
1 Never	2	3 Sometimes	4	5 Everyday
There are corn tortillas at home.				
1 Never	2	3 Sometimes	4	5 Everyday
There is salmon at home.				
1 Never	2	3 Sometimes	4	5 Everyday
My mom makes me drink milk.				
1 Never	2	3 Sometimes	4	5 Everyday
My dad makes me drink milk.				
1 Never	2	3 Sometimes	4	5 Everyday
Everyone in my family drinks milk.				
1 **Never**	2	3 Sometimes	4	5 Everyday
My mom drinks milk.				

Appendix A, continued

1	2	3	4	5
Never		Sometimes		Everyday
My dad drinks milk.				
1	2	3	4	5
Never		Sometimes		Everyday
I drink milk because my coach says to.				
1	2	3	4	5
Strongly Disagree		Sometimes		Strongly Agree
My family makes me take calcium supplements.				
1	2	3	4	5
Strongly Disagree		Sometimes		Strongly Agree
My mom or female guardian regularly drinks milk or eats calcium-rich foods.				
1	2	3	4	5
Strongly Disagree		Sometimes		Strongly Agree
My dad or male guardian regularly drinks milk or eats calcium-rich foods.				
1	2	3	4	5
Strongly Disagree		Sometimes		Strongly Agree
What do most of your friends drink at lunch?				
Milk	Soda	Juice	Punch	Water
Other:				
What do your parents give you to drink at dinner/supper most nights?				
Milk	Soda	Juice	Punch	Water
Other:				
I drink milk so I can have strong bones now.				
1	2	3	4	5
Strongly Disagree		Sometimes		Strongly Agree
If I drink milk now, my bones will be strong when I am older.				
1	2	3	4	5
Strongly Disagree		Sometimes		Strongly Agree
Milk is not healthy.				
1	2	3	4	5
Strongly Disagree		Sometimes		Strongly Agree

Appendix A, continued

It is important for people my age to drink milk.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
Only little kids need milk.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
I drink milk because it is good for me.				
1 Strongly Disagree	2	3	4	5 Strongly Agree
I like tofu.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
I like salmon.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
I like to eat seaweed.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
I like to eat pudding.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
I like to eat green vegetables.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
I like to eat stir-fry dinners.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
When it is cold outside, I like to drink cocoa.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
Now that I'm older, I'd rather drink soda pop or coffee instead of milk.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
I like the taste of soda.				

Appendix A, continued

1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
Milk tastes good.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
Whole milk is too thick.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
Skim milk tastes gross.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
Milk at school tastes bad.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
I love chocolate milk.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
I don't drink milk because it is fattening.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
I am trying to lose weight, so I don't drink milk.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
Milk is high in calories.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
Milk makes me sick/I am allergic to it.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
After I drink milk, my stomach hurts.				
1 Strongly Disagree	2	3 Sometimes	4	5 Strongly Agree
Soda pop and other drinks are easier to take with you than milk.				
1	2	3	4	5

Appendix A, continued

Strongly Disagree	Sometimes	Strongly Agree

Please place an 'X' to indicate which foods are good sources of calcium:
_____ Broccoli

_____ Chicken

_____ Bananas

_____ Fish

_____ Strawberries

_____ Milk

_____ Yogurt

_____ Seaweed

_____ Soda

_____ Cheese

Please place an 'X' to indicate which foods are good sources of Vitamin D:
_____ Milk

_____ Corn

_____ Beef

_____ Certain Mushrooms

_____ Salmon

_____ Blueberries

_____ Yogurt

_____ Fortified Orange Juice

Appendix B

Physical changes during puberty are important events. We are interested in knowing about your physical growth and whether you have begun to notice any changes in your body. If so, we would like to know which ones and when they happened. Puberty usually starts at different times for each person. It is normal for some people to begin puberty as early as 8 years while others do not start until 15 years.

The following questions will help us understand your state of physical development. Please answer on this paper by circling the answer that is true to you. For each question you answer yes, you will be asked to tell how old you were when this happened.

1. Have you started to grow taller?
   a. Yes If yes, how old were you when this first begun? ______________
   b. No

2. Have your breasts started to grow at all?
   a. Yes If yes, how old were you when this first begun? ______________
   b. No

3. Do you have any body hair around your sex organs?
   a. Yes If yes, how old were you when this first begun? ______________
   b. No

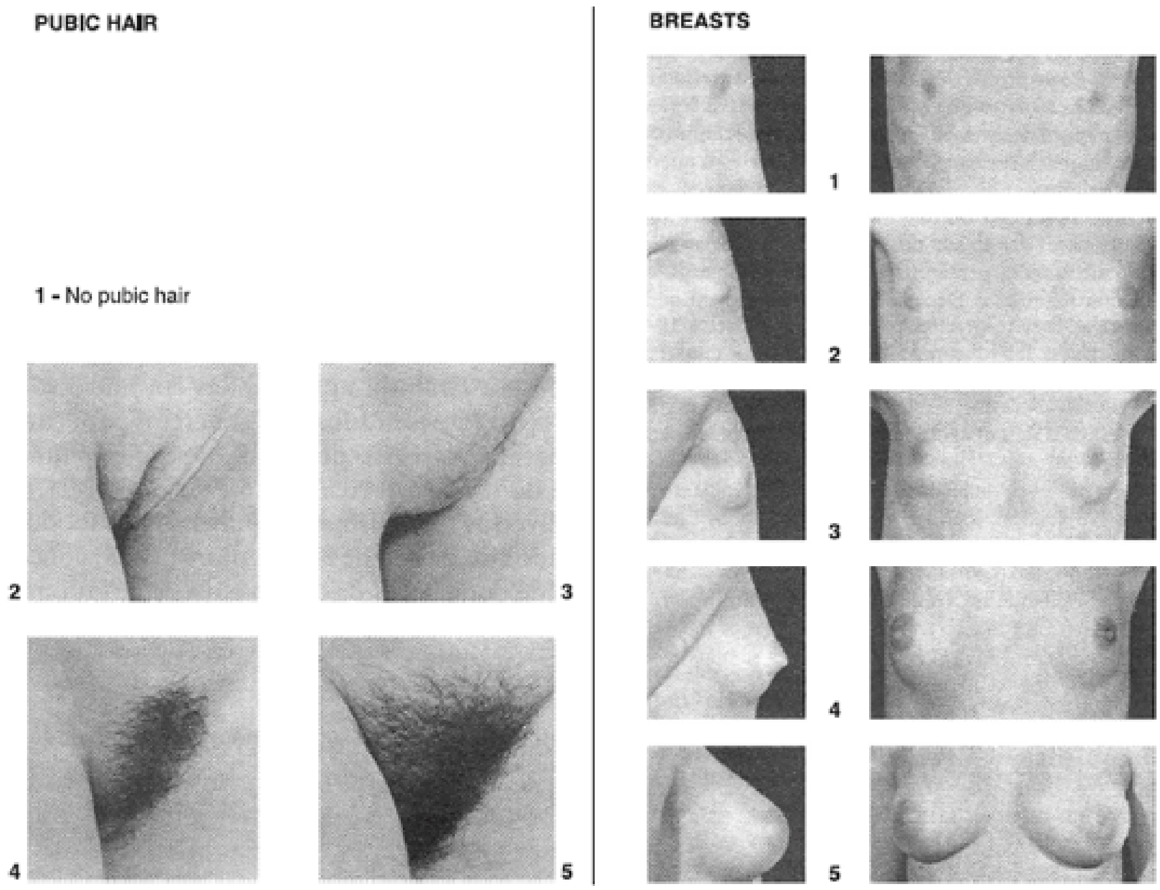
4. Have you started your period?
   a. Yes If yes, how old were you when this first begun? ______________
   b. No

5. Do you have any pimples (acne)?
   a. Yes If yes, how old were you when this first begun? ______________
   b. No

Appendix B, continued

Self-assessment of Tanner Stage (female)

Please indicate the picture for both pubic hair and breasts that most closely resembles you.



Appendix C

Please complete the following food record and return to Miriam Flack by December ___, 2010. Please choose 1 weekend day and 1 week days for a total of **2 days**. Please choose days that are typical (avoid days with parties, holidays, etc). Once you have completed your record, please return it to **John Yenny or Kevin Hooker** or mail it to the following address: Thank you for your participation

Dr. Kaye Stanek-Krogstrand
202J Ruth Leverton Hall
University of Nebraska
Lincoln, NE 68583-0806

Here is an example:

Day 1	Date: 01/25/2010	ID # 9412				
Time/type (circle)	**Item/Additions/Details**	**Brand**	**How much?** (circle)	**Fat?**	**Salt?**	**Other info:**
07:30 a p / B L D S	Honey Nut Cheerios		__1__ floz oz C T ts ea.	Reg		
	1% milk		_3/4_ floz oz C T ts ea.	1%		
	Orange juice	Tropicana	__6__ floz oz C T ts ea.			no pulp Calc/VitD
	water		__4__ floz oz C T ts ea.			Tap
			_____ floz oz C T ts ea.			
10:30 a p / B L D S	Fruit snacks	Our family	__1__ floz oz C T ts ea.	Reg		Sugar free
	apple		__1__ floz oz C T ts ea.			Tennis ball size
	water	Auquafina	__20_ floz oz C T ts ea.			Bottle
			_____ floz oz C T ts ea.			
			_____ floz oz C T ts ea.			
12:30 a p / B L D S	Sandwich w/		_____ floz oz C T ts ea.			
	Bread	Sara Lee	_2___ floz oz C T ts ea.	Reg	Reg	100% whole wheat
	Cheese	Kraft	_1___ floz oz C T ts ea.	Low	Reg	2% American single
	Ham	HyVee	__2__ floz oz C T ts ea.	Reg	Reg	Deli style thin slice
	Mayonnaise	Kraft	_2___ floz oz C T ts ea.	Reg	Reg	
	Lettuce		__1__ floz oz C T ts ea.			Iceberg
~~__:__ a p / B L D S~~	Mountain Dew		_12__ floz oz C T ts ea.			Regular can
6:30pm Dinner	Burger King Whopper Jr.		_1/2_ floz oz C T ts ea.			
	French fries		__1__ floz oz C T ts ea.			2 packets of ketchup

A = am, p = pm, B= breakfast, L= lunch, D = dinner, S = snack, floz = fluid ounce, oz = ounce weight, C = cup, T = tablespoon, ts= teaspoon, ea. = each or package or item

Appendix C, continued

2 Day Food Record

Day 1	Date: __ /__ /____	ID #				
Time/type (circle)	**Item/Additions/Details**	**Brand**	**How much?** (circle)	**Fat?**	**Salt?**	**Other info:**
__:__ a p / B L D S			_____ floz oz C T ts ea.			
			_____ floz oz C T ts ea.			
			_____ floz oz C T ts ea.			
			_____ floz oz C T ts ea.			
			_____ floz oz C T ts ea.			
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__:__ a p / B L D S			_____ floz oz C T ts ea.			
			_____ floz oz C T ts ea.			
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			_____ floz oz C T ts ea.			
__:__ a p / B L D S			_____ floz oz C T ts ea.			
			_____ floz oz C T ts ea.			
			_____ floz oz C T ts ea.			
			_____ floz oz C T ts ea.			
			_____ floz oz C T ts ea.			
			_____ floz oz C T ts ea.			

A = am, p = pm, B= breakfast, L= lunch, D = dinner, S = snack, floz = fluid ounce, oz = ounce weight, C = cup, T = tablespoon, ts= teaspoon, ea. = each or package or item

Appendix C, continued

Time/type (circle)	**Item/Additions/Details**	**Brand**	**How much?** (circle)	**Fat?**	**Salt?**	**Other info:**
__:__ a p / B L D S			______ floz oz C T ts ea.			
			______ floz oz C T ts ea.			
			______ floz oz C T ts ea.			
			______ floz oz C T ts ea.			
			______ floz oz C T ts ea.			
			______ floz oz C T ts ea.			
			______ floz oz C T ts ea.			
__:__ a p / B L D S			______ floz oz C T ts ea.			
			______ floz oz C T ts ea.			
			______ floz oz C T ts ea.			
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			______ floz oz C T ts ea.			
__:__ a p / B L D S			______ floz oz C T ts ea.			
			______ floz oz C T ts ea.			
			______ floz oz C T ts ea.			
			______ floz oz C T ts ea.			
			______ floz oz C T ts ea.			
			______ floz oz C T ts ea.			
			______ floz oz C T ts ea.			

A = am, p = pm, B= breakfast, L= lunch, D = dinner, S = snack, floz = fluid ounce, oz = ounce weight, C = cup, T = tablespoon, ts= teaspoon, ea. = each or package or item

Fat? = indicate % fat, added oil/butter/margarine/lard/reduced fat/fat free/

Salt? = indicate salt added/reduced sodium/low sodium/no added/