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Adolescent Girls:Calcium Intake and Sources

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ADOLESCENT GIRLS: CALCIUM INTAKE AND SOURCES

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An Abstract of the Thesis Presented
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Calcium is an essential nutrient required for normal growth and development of the skeleton and teeth. Adequate calcium intake is critical during early adolescence to optimize peak bone mass and decrease the risk of osteoporosis later in life. Previous researchers, however, have concluded that adolescent girls consume insufficient calcium to meet the demands of rapid skeletal growth. National surveys conducted prior to 1997 showed that the major sources of calcium for adolescent girls were milk and milk products. Shortly after the new Dietary Reference Intakes (DRI's) for calcium were released in 1997, calcium fortified foods became more readily available on the market. Sources of calcium and calcium intake levels of adolescent girls need to be reassessed to determine the role of calcium-fortified foods, which are increasing in prevalence in the food supply.

This research is part of the Seasonal Bone Study, a longitudinal, observational study of bone mineralization in adolescent girls at the University of Maine. The specific hypothesis tested was that the total calcium intake of adolescent girls is significantly greater than in the past, and that calcium-fortified foods are a significant source of dietary calcium. One objective was to compare the mean calcium intake of the subjects in this study to that of the Adequate Intake (AI) and to mean calcium intakes of adolescent

females from previous national and Maine studies. The other objective was to compare the sources of calcium intake of the subjects in this study to those of adolescent females from the USDA 1981-1988 Nationwide Food Consumption Survey.

In January and February of 2001, twenty-four females between the ages of 9.7 to 12.3 years kept 24-hour food records on four discontinuous days consisting of three week days and one weekend day. After the four days of food records were completed, each record was reviewed and the foods and beverages were classified into the following categories: milk and milk products, milk as an ingredient, calcium-fortified non-grains, calcium-fortified grains, grains, and other.

The mean daily calcium intake of adolescent females in this study was 1039 mg, which is significantly higher ($p<0.01$) than in many previous surveys, yet remains significantly lower ($p<0.01$) than the AI of 1300 mg. The most significant sources of calcium in this study were milk and milk products, which is consistent with previous research. Furthermore, calcium fortified foods contributed 7% of the total calcium intake, with 30% of breakfast cereals consumed being calcium-fortified.

The results of this research suggest that calcium intake of adolescent girls may have improved in recent years. It is not possible from this study, however, to determine if the intake for the whole population has increased. Thus, national surveys should be conducted. Although the calcium intake of adolescent girls in this study was higher than in previous studies, calcium intake was still suboptimal. Furthermore, calcium-fortified foods were not a major source of calcium in this study. These results imply that further attention to increasing calcium intakes in adolescent females is warranted.

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INTRODUCTION

Calcium is an essential nutrient, and is a major component of mineralized tissues. Because 99 percent of total body calcium is found in bone, it is required for normal growth and development of the skeleton and teeth. Early adolescence, which is defined as the start of puberty, is a period of rapid bone mineralization. Thus, adequate calcium intake during this period has a major influence on peak bone mass and the risk of osteoporosis later in life. Previous research, however, has concluded that adolescent girls consume insufficient calcium to meet the demands of rapid skeletal growth (1,2). Shortly after the new Dietary Reference Intakes (DRI's) for calcium were released in 1997, calcium fortified foods became more readily available on the market (3-5). Therefore, reassessing calcium intake and sources in adolescent girls after this shift in the food supply is warranted.

LITERATURE REVIEW

Role of Calcium in Health

Adequate dietary calcium is essential during preadolescent and adolescent years to promote optimal accumulation of bone mass. Furthermore, calcium influences the transport functions of cell membranes. It also affects the release of neurotransmitters at synaptic junctions, the function of protein hormones, and the release or activation of intracellular and extracellular enzymes. Calcium is required for nerve transmission, heart muscle function, and blood clotting. The proper balance of calcium, sodium, potassium, and magnesium ions maintains skeletal muscle tone and controls nerve irritability. A significant increase in serum calcium above normal can result in cardiac or respiratory failure, and a decrease causes tetany of skeletal muscles (6).

The skeleton contains 99.5% of total body calcium and serves mechanical and metabolic functions. The mechanical function provides a structural framework that allows support, locomotion, and protection of organs. The metabolic function includes the storage of calcium that can be accessed when required for vital bodily functions. In addition to calcium, phosphate and other ions are stored in bone. The skeleton is a site for hematopoiesis (the formation of blood cells) as well (7).

Bone remodeling is a physiological process of simultaneous bone resorption (breakdown) and formation. Bone remodeling provides a constant calcium source to the body and keeps the skeleton elastic enough to serve structural functions. First, osteoclasts (bone dissolving cells) are activated to carve a cavity on the bony surface. This signals osteoblasts (bone forming cells) to refill the excavation site by forming a layer of cells on bone surfaces. During adolescence, the amount of bone formation is

greater than that of bone resorption, resulting in the acquisition of bone mass. Peak bone mass is defined as the time when bone acquisition is complete and bone mass is at its highest point (7).

Bone Mineralization in Adolescent Girls

The rate of gain in bone mineralization is steady during childhood and accelerates rapidly in early puberty. Bone mass accrual is greatest from the start of puberty at age 9 to 11 until menarche when the rate of bone mineral accrual starts to decline. An estimated 40-50% of peak bone mass is accumulated during the six years that surround puberty (7). Bone mineral accumulates more slowly after menarche, and by 20 years of age, 90-95% of the total body peak bone mass is attained in females, although additional bone mass may accumulate through the third decade of life (8,9). At this time, bone mineral density plateaus until approximately age 40 when it begins to decline as a consequence of reduced physical activity and a decline in the production of estrogen (7).

It is currently recognized that increasing peak bone mass by intervening during early adolescence provides protection against osteoporosis later in life (10,11). Risk of osteoporosis is influenced by the peak bone mass attained in the first two to three decades of life and the rate at which bone is lost after that (8,12). A person's peak bone mass is primarily genetically controlled, but a combination of endogenous factors, such as genetics and hormones, and exogenous factors determine peak bone mass. The exogenous factors include lifestyle factors, such as dietary calcium intake, physical activity, and smoking, and can be controlled through behavior to help prevent

osteoporosis later in life (13). Adequate calcium intake is necessary to achieve optimal peak bone mass.

Calcium Requirements of Adolescent Girls

Calcium requirements in adolescent girls include the amount needed for optimal bone development and the maintenance of bone health. The AI (Adequate Intake) for girls 9-18 years old has been set at 1300 mg/day (14). This amount allows for maximal calcium retention and is higher than the previous 1989 Recommended Daily Allowance (RDA) of 1200 mg (15). The indicators used to set the AI include calcium retention studies, clinical trials measuring bone mineral content, the factorial approach, and epidemiological evidence (14). Calcium requirements are estimated at 1100-1600 mg/day for adolescent girls to attain maximal calcium retention. This requirement was based on information on bone mineral accretion of 228 children age 9 to 19 years followed over 4 years (10). It has been concluded in previous clinical trials that an optimal calcium intake of 1200-1500 mg/day in adolescence is critical to achieve peak bone mass (16-18). The factorial approach takes into account calcium needs for growth, calcium absorption, and calcium losses in urine, feces, and sweat (14). Finally, epidemiological evidence suggests a positive association between calcium consumption in adolescence and greater bone mineral density in adulthood. Requirements vary throughout an individual's life span, as well, with the greatest needs during periods of rapid growth (childhood and adolescence), during pregnancy and lactation, and in later adult life (14).

For females, the calcium accretion rate peaks at about age 13. During the pre-menarchal time period, an estimated 40-60 % of calcium consumed is absorbed. Calcium retention, bone formation, and bone resorption decline rapidly after menarche. The calcium intake required, however, does not decline because fractional absorption of calcium also decreases after menarche (14).

Balance studies have shown that retention of calcium by the body increases with increasing calcium intake up to a threshold. Beyond this point, further calcium intake results in little additional calcium retention. Moreover, excess levels of calcium intake produce several adverse effects. The efficiency of calcium absorption decreases as intake increases, providing a protective mechanism to lessen the risk of calcium intoxication. This adaptive mechanism can be overcome by a calcium intake of greater than four g/day, although even calcium intake levels less than four g/day can cause a healthy person to be more susceptible to developing hypercalcemia or hypercalciuria. Overall, a modest increase in calcium intake above the AI is safe for most people, and no adverse effects of moderate supplementation up to 1500 mg/day have been reported. Because there is a lack of data in this area, the Tolerable Upper Intake Level for calcium has been conservatively set at 2500 mg/day for adolescent girls (14).

Increased calcium intake may interfere with the absorption of other nutrients, such as iron and zinc. Iron absorption can be decreased by as much as 50 percent by many forms of calcium supplements or milk ingestion, but not by calcium forms that contain citrate and ascorbic acid, which enhance iron absorption. However, increased intakes of specific calcium sources might induce iron deficiency in individuals with marginal iron status (14).

Calcium Sources and Absorbability

Optimal calcium intake can be achieved through diet, calcium-fortified foods, calcium supplements, or a combination of these. However, the preferred approach to attain optimal intake is through dietary sources. Although calcium is widely distributed in both plant and animal foods, dairy products have been the major source of dietary calcium for most Americans because of their high calcium content (approximately 250-300 mg/8 oz. milk) and high frequency of consumption. Other food sources of calcium include some green vegetables (broccoli, kale, turnip greens, and Chinese cabbage), calciumset tofu, some legumes, fruits, canned fish, seeds, nuts, calcium-fortified food products, and depending on location, water (1). In The Nationwide Food Consumption Survey, it was found that milk and milk products account for 443 mg or 56% of the total calcium intake (790 mg) for adolescent girls 12-19 years old. Other calcium sources were: milk as an ingredient (163 mg, 21%), eggs and egg products (9 mg, 1%), legumes, nuts, and seeds (8 mg, 1%), grains and grain products (77 mg, 10%), fruits and fruit mixtures (13 mg, 2%), vegetables and vegetable mixtures (23 mg, 3%), sugars, sweets, and beverages (22 mg, 3%), and meat, poultry, fish and their mixtures (32 mg, 3%) (1). According to the National Institute of Health, breads and cereals contribute significantly to calcium intake because of their frequency of consumption. A number of calcium-fortified products are available on the market, including fortified juices, fruit drinks, breads, and cereals. These foods provide multiple nutrients and are frequently consumed, although their contribution and role in the total diet have not been defined (8).

To maximize calcium absorption, food selection decisions should consider bioavailability. In fact, it is possible that the amount of biologically available calcium,

which is the amount absorbed, is actually a more important determinant of bone density than total calcium intake (1). The absorption of calcium from food depends on the food's total calcium content and the presence of components that enhance or inhibit absorption. Lactose may enhance the bioavailability of calcium, while dietary components such as oxalate, phytate, fiber, and phosphorus may reduce the bioavailability by forming insoluble complexes with calcium in the intestine (8). Oxalate, which is present at high levels in some vegetables, such as spinach, depresses absorption of the calcium present in the food, but not of calcium in co-ingested calcium-containing foods. Phytate also depresses calcium absorption, but to a lesser extent. Dietary fiber has not been found to affect calcium absorption significantly, with the exception of large amounts of wheat bran, which depresses calcium absorption from milk (8). The results of a number of studies suggest a negative correlation between bone density and caffeine (19,20) and alcohol (21,22) and a positive association with calcium and vitamin C (23,24), but data are inconclusive. Fat, phosphate, and magnesium have not been found to significantly affect calcium absorption or excretion (8).

The effect of nutrients on calcium excretion should be considered as well. For example, the typical American diet contains high amounts of sodium and animal protein, both of which significantly increase urinary calcium excretion and increase the amount of calcium that must be absorbed from the diet, and, hence, the calcium requirement. When aluminum, in the form of an antacid medication, is taken in excess, it may significantly increase calcium excretion in the feces in association with impaired bone mineralization (8).

Vitamin D plays a role in optimal calcium uptake because it is needed for adequate calcium absorption. The active vitamin D metabolite, 1,25-dihydroxyvitamin D, stimulates the active transport of calcium in the small intestine and colon. A deficiency of this metabolite is caused by inadequate dietary vitamin D, inadequate exposure to sunlight, impaired activation of vitamin D, or acquired resistance to vitamin D, and results in reduced calcium absorption. In fact, in the absence of 1,25-dihydroxyvitamin D, less than 10 percent of dietary calcium can be absorbed. As a result, vitamin D deficiency is associated with an increased risk of bone fractures. Sources of vitamin D include supplements, sunlight, vitamin D fortified milk, cod liver oil, and fatty fish. Calcium and vitamin D do not need to be taken together to result in adequate calcium absorption. Furthermore, excessive doses of vitamin D should be avoided because they can introduce risks such as hypercalciuria and hypercalcemia (8).

In 1997, the AI for calcium for adolescent girls increased from 1200 mg/day to 1300 mg/day (14). The AI for calcium for other age groups was increased as well. As a result, fortification of foods with calcium became increasingly more common. A fortified food is one in which vitamins and/or minerals have been added to the product in amounts in excess of at least 10% of the daily value for the nutrient. Calcium-fortified foods are defined as having equal to or greater than 100 mg of added calcium/serving (25). Currently, many products on the market are fortified with calcium, such as breakfast cereals, waffles, juices, margarine, pancake mix, and cereal bars. In fact, a record number of 119 calcium-fortified food products were introduced in the United States in the year 1999, three times the number launched in 1998 (26). It was shown in studies prior to the late 1990's that dairy products were the major source of calcium for

adolescent girls (1). However, since the calcium content of the food supply has shifted, the contribution of fortified foods to calcium intake needs to be assessed.

Calcium Intakes of Adolescent Girls

Research suggests that most adolescent girls consume levels of calcium lower than the optimal amount, which refers to the levels necessary for an individual to maximize calcium retention and to optimize peak adult bone mass. For instance, it was shown in the USDA 1981-1988 Nationwide Food Consumption Survey that 12-19 year old females had a mean calcium intake of 789 mg/day, while females 6-11 years old consumed 808 mg/day. Data was collected from approximately 4,600 households from April 1987 through August 1988 in the 48 contiguous states. Individuals were surveyed in all four seasons and on all days of the week. Dietary intakes were measured over a three-day period using a 24-hour recall followed by a two-day record (1).

In The Nationwide Food Consumption Survey, it was also found that calcium consumption for all age groups varied by region of the country, by ethnic group, and by household income. Calcium intake was highest for individuals living in the west (811 mg), for non-Hispanic whites (765 mg), and for those from households in the highest income group of at least $41,000 per household per year (784 mg). The lowest calcium intake was reported for individuals in the South (685 mg), for non-Hispanic blacks (592 mg), and for individuals from households in the lowest income category of $18,000 per household per year (673 mg) (1).

It was also concluded in The Nationwide Food Consumption Survey that calcium intake decreased with age, and that the only age ranges in which the 1989 RDA was met

by females were under 1 year of age and 6-11 years old. In fact, after age 11, no age group of females achieved even 75% of the RDA for calcium, which was 1200 mg/day for adolescents at the time of this survey. Interestingly, the lowest calcium intake in comparison with the RDA was for females between the ages of 12-19, who consumed less than 60% of the RDA. This finding is troubling, because that is the period in which rapid bone mineralization is occurring (1).

The 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII) was conducted by USDA's Agricultural Research Service. Data were obtained by a 24-hour recall from 969 females ages 6 to 11 years, and from 732 females aged 12 to 19 years. The findings showed that females aged 6 to 11 years consumed a mean intake of 865 mg of calcium daily, while 12 to 19 year old females consumed 773 mg of calcium (27).

According to data collected in the first phase of the National Health and Nutrition Examination Survey (NHANES) III, calcium intakes for non-Hispanic white females were highest between the ages of 6-11 years at 879 (±28) mg/day, and declined during adolescence to 801 (±45) mg/day for 12 to 15 year olds. NHANES III was conducted by the National Center for Health Statistics (NCHS) and the Centers for Disease Control and Prevention (CDC) in two, three-year nationwide phases from 1988-1991 and 1988-1994. A 24-hour recall was used to collect data from 14,801 subjects, 877 of which were females aged 12 to 15 years. Data were collected from 373 females aged 16 to 19 years as well and it was found that the mean calcium intake increased to 866 (±46) mg/day (2).

Several previous studies in Maine have found that the mean calcium intake levels of adolescent girls were below the current AI of 1300 mg. For instance, Cook's research in 1974 (27) concluded that female adolescents aged 11-18 consumed and average of

1124 mg of calcium daily. Data were collected from 41 adolescent females aged 15-18 years using a three-day food record and 24-hour recall. In 1977, Violette et al. (29) used a 24-hour recall to gather data from 41 subjects, and determined that females 15-18 years old consumed an average of 962 (±485) mg of calcium daily, with an intake of 995 (±531) mg for rural females and 941 (±463) mg for urban girls. In Soychak et al.'s (30) research in 1981, data from 33 females 12-13 years old showed the mean daily calcium intake to be 837 (±295) mg. In 1996, Shriver et al. (31) collected data from 40 females 13-17 years old and the mean calcium consumption was 779 (±49) mg/day. Based on these findings, adolescent females in Maine, on average, consumed a level of calcium below the optimal level for bone development.

The previous national and Maine data on calcium consumption in adolescent females was collected prior to 1997, at which time the AI for calcium increased for all age groups. It is possible that because calcium fortified foods have increased in the food supply since these studies were conducted, adolescent females may be consuming a higher level of calcium. The role of calcium fortified foods on calcium intake in adolescent females needs to be determined.

Methods of Assessing Dietary Intake

Methods such as food records, 24-hour recalls, diet histories, and food frequency questionnaires are commonly used to assess dietary intake. Food records involve subjects keeping records of everything consumed in a 24-hour period. One problem with this method is compliance, because writing down everything eaten is tedious. Another problem is a training effect, in which food intake is changed due to participation in a

study. In addition, recording all food consumed is a behavior modification method to reduce intake. For instance, snacks and condiments high in calories, fat, and sugar may not be worth the trouble to eat when keeping a record. Nevertheless, the food record is regarded as a valid method to assess dietary intake (32).

A 24-hour recall is a method in which subjects recall everything consumed in the previous 24-hours, and is used to assess recent nutrient intake. Subjects may use food models to estimate the quantity of food consumed. An advantage to this method is that short-term memory is generally accurate and people can correctly remember what they ate the previous day. It has been found to require less time, money, and subject cooperation than other techniques (32,33). A disadvantage to the 24-hour recall is that a single day's intake does not describe the usual diet, and therefore, multiple recalls must be obtained in order to obtain a more accurate representation of usual nutrient intake (34).

The results of Greger and Etnyre's research (35) showed that with a 24-hour recall, adolescent girls estimated their calcium intakes to within two-thirds to four-thirds of their actual intake. Twenty-four hour recalls were conducted on 32 adolescent girls, 12.5 to 14.5 years of age, who were participating in a 30-day metabolic study. Results showed that the girls more often omitted food items in diet recalls than added or misidentified food items. Unfortunately, the girls commonly misidentified the brand of cereal consumed. This finding is problematic because of the presence of calcium-fortified cereals on the market, and this type of error can affect an estimate of calcium intake. Overall, diet recalls are a valid technique to estimate the dietary calcium intake of adolescent girls, and performing diet recalls more than once improves the accuracy (35).

A diet history is a review of an individual's usual food intake pattern over an extended period of time. In comparison with other methods, the diet history is expensive and time consuming (6).

A food frequency questionnaire obtains the usual frequency of each food from a list of foods for a specific period of time. It is easily standardized and provides an overall picture of intake. The food frequency questionnaire, however, requires literacy skills, does not provide meal pattern data, and requires knowledge of portion sizes. Furthermore, a food frequency questionnaire including all calcium-fortified foods would be cumbersome to use (6).

Subjects show day-to-day variation in dietary intake, and data from several days is required to assess usual dietary intake. Research has shown that data from three days is acceptable to assess energy intake in adolescent girls. Furthermore, energy intakes for each weekday are not significantly different, but intake on weekends is different than intake on weekdays (36). Therefore, data must be obtained from both weekdays and weekend days. A random selection of days is preferable to consecutive days, because it is more reflective of the overall diet, and it gives a better sense of the variety in intake (37).

Overall, 24-hour recalls and food records are valid tools to assess dietary intake. The accuracy of these methods is increased when data are collected on several non-consecutive days, including both weekday and weekend days.

Summary and Rationale for Study

Calcium is an essential nutrient required for normal growth and development of the skeleton and teeth. Adequate calcium intake is critical during early adolescence to optimize peak bone mass and decrease the risk of osteoporosis later in life. Previous studies have concluded that adolescent girls consume insufficient calcium to meet the demands of rapid skeletal growth. National surveys have shown that the major sources of calcium for adolescent girls are milk and milk products. The calcium intake and sources of calcium in adolescent girls need to be reassessed to determine the role of calcium-fortified foods, which are increasing in prevalence in the food supply.

The specific hypothesis to be tested was that the total calcium intake of adolescent girls is significantly greater than in the past, and that calcium fortified foods are a significant source of dietary calcium intake. One objective was to compare the mean calcium intake of the subjects in the Seasonal Bone Study to that of the AI and to mean calcium intakes of adolescent females from previous national and Maine studies. The other objective was to compare the sources of calcium intake of the subjects in the Seasonal Bone Study to those of adolescent females from the USDA 1981-1988 Nationwide Food Consumption Survey.

METHODS AND MATERIALS

Overview

This research is part of the Seasonal Bone Study, a longitudinal, observational study of bone mineralization in adolescent girls at the University of Maine. Subjects in the Seasonal Bone Study will keep four-day food records six times over the course of three years. This research involved the food record data from the first of the six sets of food records and was analyzed for dietary calcium sources and amounts.

Subjects

The subjects were recruited during the summer of 2000 for the Seasonal Bone Study. Inclusion criteria were: age 9-11 years, pre-menarche, non-smokers, no significant medical problems or medications, no vitamin, mineral, or herbal supplements, no eating disorders, and no plans for winter travel to warm climates for greater than one week. The protocol for the study was approved by the Protection of Human Subjects Board at the University of Maine and the Institutional Review Board at St. Joseph's Hospital in Bangor, Maine.

At an initial meeting during the summer of 2000, a parental informed consent form was reviewed and signed by the parents. The subjects gave verbal assent (see Appendix A for consent and assent forms). At this visit, subjects were trained in keeping food records and in reading food labels for calcium content. The subjects also received a set of plastic food models, including 1 tablespoon, ½ cup, 1 cup, and 3 ounce portions, and were trained in using these to estimate portion sizes. Within the following week, the subjects completed one food record for training purposes.

Food Records Procedure

In early January 2001, the subjects received a letter including instructions and forms for keeping food records (Appendix B). Then, a researcher called each subject to schedule a day to keep the first food record and a time for the subject to be contacted to review the record. The subsequent day of record keeping was scheduled during each phone call.

In January and February of 2001, each subject kept 24-hour food records on four discontinuous days consisting of three week days and one weekend day. A specific form was used by the subjects to record the time the food was eaten and what the food was including: the brand name, the calcium content, and the amount consumed (Appendix C). Within 24-hours of the completion of the food record, each subject was interviewed over the telephone by one of three researchers. The subject read through the record and then the researcher crosschecked for details including portion sizes, preparation methods, brand names, and beverages or other items the subject may have forgotten to mention. The subjects were frequently asked to check food labels to verify the calcium content of food items consumed. The data were then entered into the Nutritionist V (First Data Bank®) computer program by the same researcher. Each day's entries were reviewed. The calcium content of some fortified foods was verified by checking food labels at the grocery story. Entries were edited as needed. Four-day average calcium intakes were calculated.

After the four days of food records were completed, each record was reviewed and the foods and beverages were classified into the following categories: milk and milk products, milk as an ingredient, fortified non-grains, fortified grains, grains, and other.

The milk and milk products category included milk, cheese, cream cheese, and butter. Combination foods, such as pizza, casseroles, and macaroni and cheese, were included in the milk as an ingredient category. Fortified foods were defined as having equal to or greater than 100 mg of added calcium per serving (25). Fortified non-grains included fruit juice, while instant oatmeal, cold cereal, granola bars, english muffins, Poptarts, crackers, and waffles were included in the fortified-grain category. The other category included meat, legumes, eggs, nuts, seeds, fruits, vegetables, sweets, and beverages not fortified with calcium.

In March of 2001, each parent received a letter requesting annual household income data and an envelope in which to return the form to the researcher with the completed information (Appendix D).

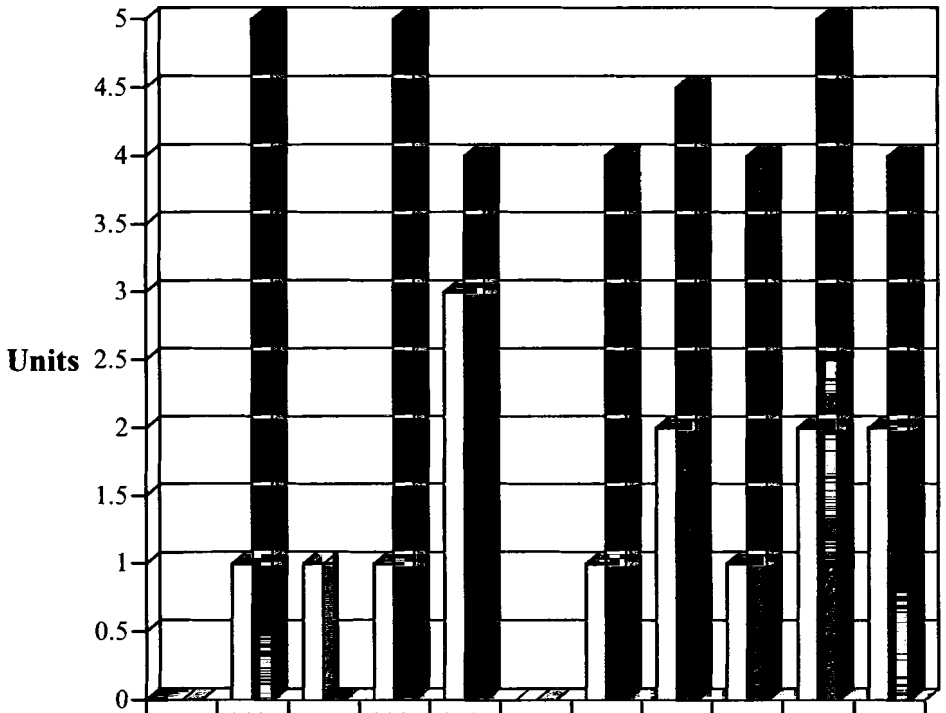
Statistical Analysis

The statistical analysis was performed to compare the mean daily calcium intake from the Seasonal Bone Study to previous studies and to the AI. A one-tailed directional test ($p<0.01$) was used to compare the mean calcium intake to previous studies, while a single sample t-test ($p<0.01$) was used to compare the mean to the AI. Due to unavailable data, estimated standard deviations were used to complete the t-test calculation to compare the mean calcium intake from the Seasonal Bone Study to the intakes from the NFCS (±40 for 6-11 year olds and ±35 for 12-19 year olds), CSFII (±20 for 6-11 year olds and ±30 for 12-19 year olds), and Cook et al. (±400). The standard deviations were estimated by looking at previous data with similar means and number of subjects.

RESULTS

The mean age of the 24 subjects at the time of data collection was 11.25 years, with a range of 9.7 to 12.3 years. A total of 14 of 21 families participating in the study responded to the income survey. The average size of the households submitting income data was 4.4 people. The median year 2000 household income of subjects in the Seasonal Bone Study was between $70,000 and $80,000 (Figure 1). This household income is higher than the median year 2000 household income of all races in the United States ($42,148 ±197) and in Maine ($41,597 ±1152) (38).

Figure 1: Annual Income Levels and Number of People Per Household



	$0-10	$10-20	$20-30	$30-40	$40-50	$50-60	$60-70	$70-80	$80-90	$90-100	> $100
Number of households	0	1	1	1	3	0	1	2	1	2	2
Average number of people in household	0	5	0	5	4	0	4	4.5	4	5	4

Annual household income in $10,000's

□ **Number of households**
■ **Average number of people in household**

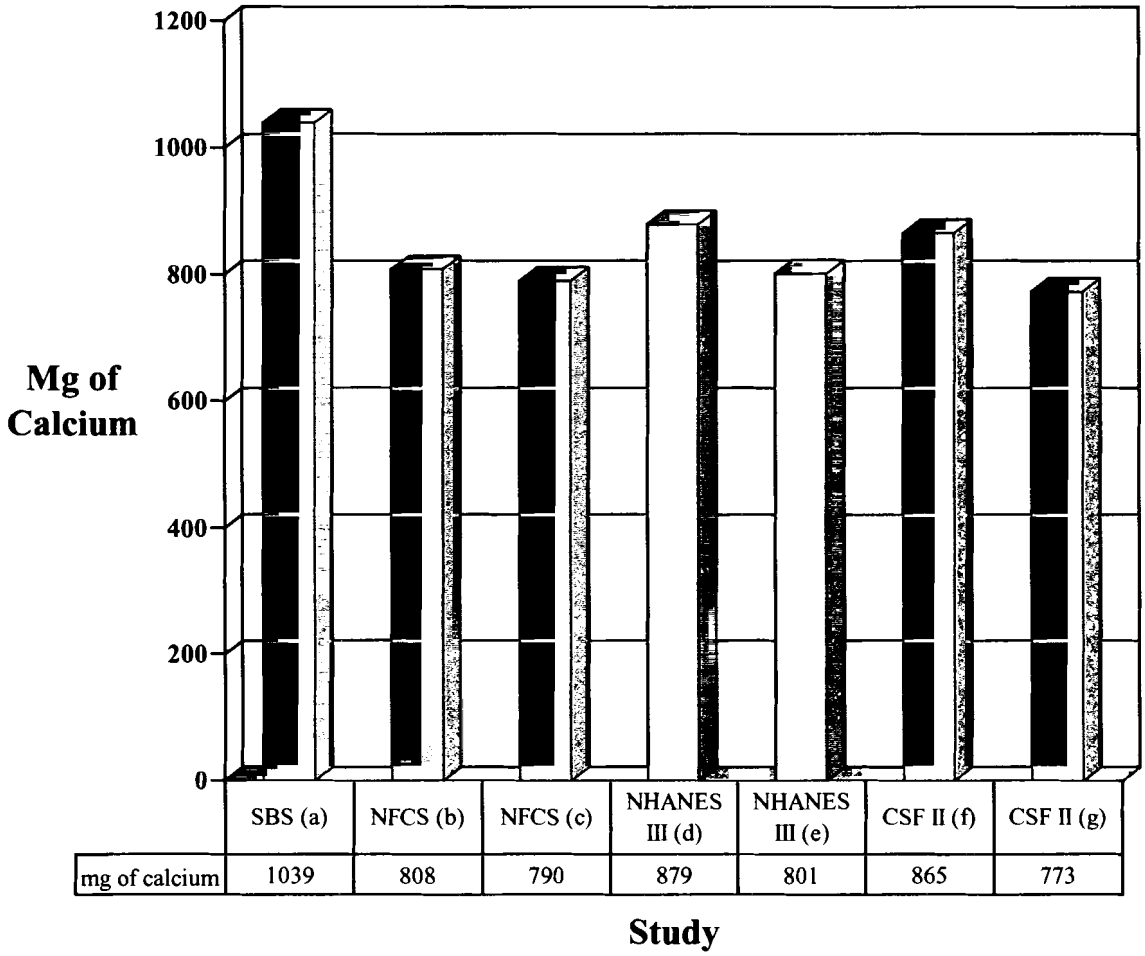
The mean daily calcium intake of participants in the Seasonal Bone Study was 1039 mg (±245) (Figure 2). The average daily calcium intake ranged from 629 mg to 1591 mg, with a median of 960 mg per day. Two (8%) of the subjects met the DRI of 1300 mg for calcium, while eight (33%) of subjects consumed between 1000 mg and 1300 mg of calcium daily. A total of 14 (58%) of subjects consumed less than 1000 mg of calcium daily.

There were no statistically significant differences ($p<0.01$) between the daily mean calcium intake of the Seasonal Bone Study and the mean calcium intakes found by Cook et al. ($t=-0.94$, $df=63$) (28) or Violette et al. ($t=0.72$, $df=63$) (29). However, statistically significant differences ($p<0.01$) were seen between the mean calcium intake of the Seasonal Bone Study and the AI ($t=-5.22$) and previous Maine and national studies, including: NHANES III ($t=16.13$, $df=899$, $t=15.38$, $df=395$) (2), CSFII ($t=19.94$, $df=991$, $t=24.66$, $df=754$) (27), NFCS ($t=17.25$, $df=535$, $t=20.44$, $df=615$) (1), Shriver et al. ($t=6.53$, $df=62$) (31), and Soychak et al. ($t=2.74$, $df=55$) (30) (Table 1, Figures 2 and 3).

Table 1: Comparison of Mean Calcium Intakes of Adolescent Girls in The Seasonal Bone Study with Girls in Previous Surveys

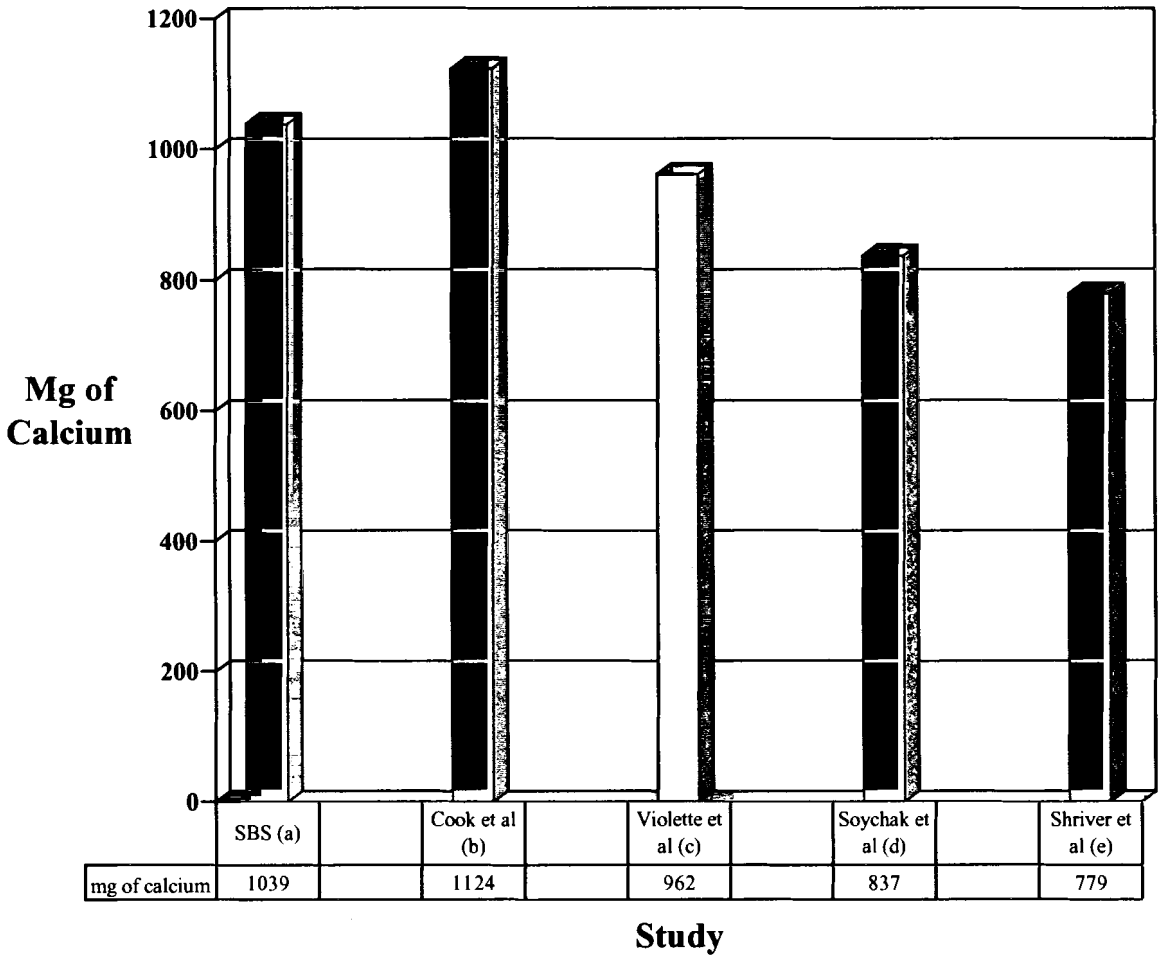
Study	Mean Calcium Intake	Number of Subjects	Standard Deviation	p	t	df
Seasonal Bone Study	1039 mg	24	±245	n/a	n/a	n/a
NFCS (6-11 years old) (1)	808 mg	513	±40 (estimated)	<0.01	17.25	535
NFCS (12-19 years old) (1)	790 mg	593	±35 (estimated)	<0.01	20.44	615
CSFII (6-11 years old) (27)	865 mg	969	±20 (estimated)	<0.01	19.94	991
CSFII (12-19 years old) (27)	773 mg	732	±30 (estimated)	<0.01	24.66	754
NHANES III (6-11 years old) (2)	879 mg	877	±28	<0.01	16.13	899
NHANES III (12-15 years old) (2)	801 mg	373	±45	<0.01	15.38	395
Cook et al (28)	1124 mg	41	±400 (estimated)	<0.01	-0.94	63
Violette et al (29)	962 mg	41	±485	<0.01	0.72	63
Soychak et al (30)	837 mg	33	±295	<0.01	2.74	55
Shriver et al (31)	779 mg	40	±49	<0.01	6.53	62

Figure 2: Comparison of Mean Daily Calcium Intakes of Adolescent Girls in The Seasonal Bone Study with Girls in Previous National Surveys



a. Seasonal Bone Study, 9-12 years old, 2001
b. Nationwide Food Consumption Survey, 6-11 years old, 1987-1988 (1)
c. Nationwide Food Consumption Survey, 12-19 years old, 1987-1988 (1)
d. Third National Health and Nutrition Examination Survey, 6-11 years old, 1988-1991 (2)
e. Third National Health and Nutrition Examination Survey, 12-15 years old, 1988-1991 (2)
f. Continuing Survey of Food Intakes of Individuals, 6-11 years old, 1998 (27)
g. Continuing Survey of Food Intakes of Individuals, 12-19 years old, 1998 (27)

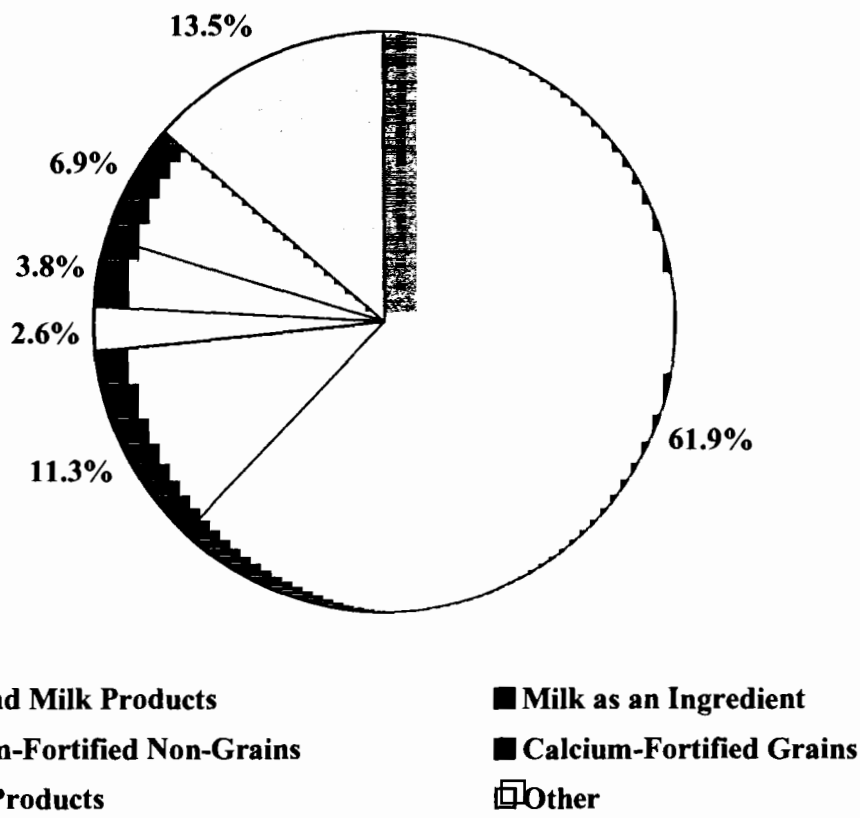
Figure 3: Comparison of Mean Daily Calcium Intakes of Adolescent Girls in The Seasonal Bone Study with Girls in Previous Maine Studies



a. Seasonal Bone Study, 9-12 years old, 2001
b. Cook et al., 11-18 years, 1974 (28)
c. Violette et al., 15-18 years, 1977 (29)
d. Soychak et al., 12-13 years, 1981 (30)
e. Shriver et al., 13-17 years, 1996 (31)

The most significant sources of calcium in the Seasonal Bone Study were milk and milk products (61.9%), followed by other sources (13.5%), milk as an ingredient (11.3%), grain products (6.9%), calcium fortified grain products (3.8%) and calcium fortified non-grain products (2.6%). The other category included meat, legumes, eggs, nuts, seeds, fruits, vegetables, sweets, and beverages not fortified with calcium (Figure 4).

Figure 4: Sources of Calcium Intake of Adolescent Girls in The Seasonal Bone Study



Comparison of the sources of calcium shows little difference between the Seasonal Bone Study and the Nationwide Food Consumption Survey. The most significant sources of calcium continue to be milk, milk products, and milk as an ingredient (Table 1). Overall, calcium-fortified foods contributed 7% of total calcium intake, which is 72 mg of the mean daily calcium intake of 1039 mg. Of the 72.65 cups of breakfast cereal consumed, 30% were calcium-fortified. Furthermore, 8 of the 25 brands of cereal consumed were calcium-fortified (Table 2).

Table 2: Comparison of Calcium Sources From The Seasonal Bone Study and The Nationwide Food Consumption Survey

Calcium Source	Seasonal Bone Study	Nationwide Food Consumption Survey (1)
Milk and Milk Products	62%	56%
Milk as an Ingredient	11%	21%
Grains	Non-fortified: 7% Fortified: 4%	10%
Other	Non-fortified: 13% Fortified: 3%	13%

Table 3: Cereals Consumed by Adolescent Girls in The Seasonal Bone Study

Calcium Fortified Cereals	**Total Amount Consumed**	**Total Amount of Calcium**	**Non-calcium Fortified Cereals**	**Total Amount Consumed**	**Total Amount of Calcium**
Quaker Instant Oatmeal	7 cups	700 mg	Fruit Loops	9 cups	0 mg
Cinnamon Toast Crunch	3.75 cups	500 mg	Raisin Bran	6.25 cups	125 mg
Kix	3.25 cups	366 mg	Honey Bunches of Oats	5 cups	0 mg
Lucky Charms	2.5 cups	250 mg	Frosted Flakes	4.95 cups	0 mg
Cheerios	2.25 cups	225 mg	Frosted Mini Wheats	4.75 cups	0 mg
Honey Nut Cheerios	2 cups	200 mg	Special K	3.5 cups	0 mg
Corn Chex	0.7 cups	75 mg	Corn Pops	3 cups	0 mg
Life	0.5 cups	67 mg	Fruity Pebbles	2.5 cups	0 mg
			Smacks	2 cups	0 mg
			Shredded Wheat	2 cups	40 mg
			Granola	2 cups	60 mg
			Oatmeal	1.5 cups	0 mg
			Cracklin' Oat Bran	1.5 cups	40 mg
			Apple Jacks	1.5 cups	0 mg
			Wheaties	0.5 cups	10 mg
			Cocoa Krispies	0.5 cups	0 mg
			Rice Krispies	0.25 cups	0 mg
TOTAL	**21.95 cups**	**2383 mg**	**TOTAL**	**50.7 cups**	**275 mg**

DISCUSSION

Overview

The specific hypothesis tested was that the total calcium intake of adolescent girls is significantly greater than in the past, and that calcium fortified foods are a significant source of calcium intake. One objective was to compare the mean calcium intake of the subjects in the Seasonal Bone Study to that of the AI and to mean calcium intakes of adolescent females from previous national and Maine studies. The other objective was to compare the sources of calcium intake of the subjects in the Seasonal Bone Study to those of adolescent females from the USDA 1981-1988 Nationwide Food Consumption Survey.

The mean calcium intake of adolescent females in the Seasonal Bone Study was 1039 mg per day, which is significantly higher ($p<0.01$) than in many previous surveys, yet remains significantly lower ($p<0.01$) than the AI of 1300 mg. The most significant source of calcium in the Seasonal Bone Study was milk and milk products, which is consistent with previous research. Furthermore, calcium fortified foods contributed 7% of the total calcium intake, with 30% of breakfast cereals consumed being calcium-fortified.

Methodology

An advantage to the methodology used in the Seasonal Bone Study is that food records were kept by the subjects and were reviewed within 24 hours. The food record is an acceptable tool for assessing intake in individuals, and is more accurate than a 24-hour recall. Short-term memory is generally accurate and people can correctly remember what

they ate the previous day (34), and researchers were trained to probe for items that the subjects may have forgotten to record. Research has shown that when a 24-hour recall is used, adolescent girls commonly omit food items such as fruits, vegetables, breads, and cereals, and misidentify the brand of cereal consumed (35). The number of items omitted and misidentified were likely reduced in the Seasonal Bone Study by using food records and reviewing them within 24 hours when memory was more accurate.

The validity of the data obtained in the Seasonal Bone Study was likely improved by subject training and the use of food models. However, because subjects were participating in a bone study and were aware of the importance of an adequate dietary calcium intake, they may have consciously changed food intake patterns on the days records were kept, resulting in an increased total calcium intake being noted. In fact, food records are commonly used as a behavior modification technique to reduce calorie intake (34). Also, keeping a food record is tedious, which may have resulted in underreporting of daily intake in the Seasonal Bone Study. Review of the records over the telephone was done to probe for items that may have been missed. The food record is regarded as a valid method to assess dietary intake.

Obtaining four-day food records likely increased the accuracy of the data collected in the Seasonal Bone Study, because a single day's intake is insufficient to describe the usual diet. Research has shown that data from three days is acceptable to assess energy intake (36). Furthermore, energy intakes for each weekday are not significantly different, but intake on weekends is different than intake on weekdays. Thus, in the Seasonal Bone Study, data were obtained from both weekdays and weekend days. A non-consecutive selection of days was used in the study as well, because this is

more reflective of the overall diet than consecutive days and minimizes the effect of food intake from one day to the next (36). Overall, 24-hour recalls and food records are valid tools to assess dietary intake.

The three national surveys discussed used 24-hour recalls to collect intake data (1,2,27). Interestingly, calcium intake was significantly higher in the Seasonal Bone Study than in the national surveys. This may be partly due to the methodology of data collection, as a food record is a more accurate measure of intake than a 24-hour recall. In Maine, Cook et al. (28) used a food record and a 24-hour recall as tools for data collection, and no significant difference in calcium intake were found between that study and the Seasonal Bone Study. However, Violette et al. (29) used a 24-hour recall, and found calcium intake to be similar to that in the Seasonal Bone Study. The method of data collection for Soychak et al. (30) and Shriver et al. (31) is unknown. It is unclear if the calcium intake results indicate improved consumption, differences in methodology, or differences in the population studied.

Comparison of Results to Previous Studies

The mean calcium intake of participants in the Seasonal Bone Study was significantly higher than that of previous national surveys from the 1980's and 1990's including: NHANES III (2), CSF II (27), and NFCS (1). In Maine, research prior to 1997 had shown that the calcium intake of adolescent girls was declining. However, the mean calcium intake of participants in the Seasonal Bone Study was not significantly different than mean calcium intakes found by Cook et al. (28) or Violette et al (29) in the

1970's, but was significantly higher than the mean calcium intakes found by Soycak (30) and Shriver (31) in the 1980's and 1990's.

The difference in calcium intake between the Seasonal Bone Study and previous national surveys is likely for several reasons. In addition to differences in methodology, an increase in public awareness of calcium and bone health in recent years may have resulted in increased calcium intake among adolescent females. The data from national surveys was collected from 1981-1996, and it might be that the public currently is more knowledgeable regarding calcium and health, especially with some publicity with the release of the 1997 DRI's for calcium.

The subjects in the Seasonal Bone Study differ from the subjects interviewed in national surveys. Although both groups consist of adolescent females, the subjects in the Seasonal Bone Study represent a more homogenous population (same race and geographic location), while subjects in national surveys represent adolescents of all races, incomes, and geographic locations. The subjects in the Seasonal Bone Study were from households of a higher income bracket than the general population, and it has been established that higher incomes are associated with an increased calcium intake (1). Furthermore, unlike subjects from national surveys, subjects in the Seasonal Bone Study were knowingly participating in a bone study, which may influence both consumption and reporting.

The sources of calcium in the diet varied little between the Seasonal Bone Study and the Nationwide Food Consumption Survey. The most significant sources of calcium continued to be milk, milk products, and milk as an ingredient. From the results of the Seasonal Bone Study, it appeared that calcium fortified foods were not a significant

source of calcium for adolescent females. It may be speculated that this is due to the fact that most calcium-fortified foods are not marketed towards adolescents. For instance, the subjects in this study consumed relatively few calcium-fortified breakfast cereals, many of which are marketed towards adult women.

Summary and Implications

The results of the Seasonal Bone Study suggest that calcium intake of adolescent girls may have improved in recent years. If this occurred, the causes are likely for several reasons, with increased public awareness and an increase in the availability of calcium-fortified foods. It is not possible from this study to determine if the intake for the whole population has increased. Thus, more recent national surveys should be conducted to evaluate the effect of calcium-fortified foods on calcium intake in adolescent girls.

Although the calcium intake of adolescent girls in the Seasonal Bone Study is higher than in previous studies, calcium intake is still suboptimal, with only 8% of the subjects meeting the daily calcium requirement. Furthermore, calcium fortified foods accounted for only 7% of total calcium intake, and were not a major source of calcium in the Seasonal Bone Study. These results imply that further attention to increasing calcium intakes in adolescent females is warranted. This may be done through further public awareness of the need for consumption of dairy products during adolescence and by increasing the amount of calcium-fortified foods marketed toward adolescents.

REFERENCES

1. Fleming KH, Heimbach JT. Consumption of calcium in the U.S.: food sources and intake levels. *J Nutr.* 1994;124:1426S-1430S.

2. Alaimo K, McDowell MA, Briefel RR, Bischof AM, Caughman CR, Loria CM, Johnson CL. Dietary intake of vitamins, minerals, and fiber of persons ages 2 months and over in the United States: Third National Health and Nutrition Examination Survey, Phase 1, 1998-91. Advance Data No 258. 1994.

3. Anonymous. *Tufts University Health & Nutrition Letter.* 2000;17:6.

4. Sloan AC. *Food Technology.* 2000;54:20.

5. Whiting S, Wood RJ. *Nutr Rev.* 1997;55 :1 .

6. Anderson JJB. Minerals. In: Mahan LK, Escott-Stump S. Krause's Food, Nutrition, and Diet Therapy. W.B. Saunders Company. Philadelphia, Pennsylvania, 2000. 116.

7. Anderson JJB. The role of calcium, phosphorus, and macronutrients in the maintenance of skeletal health. In: Rosen, CJ. Osteoporosis: Diagnostic and Therapeutic Principles. Humana Press. Totowa, New Jersey, 1996. 18-19.

8. Optimal Calcium Intake. NIH Consensus Statement 1994 June 6-8;12(4):1-31.

9. Cadogan J, Eastell R, Jones N, Baker ME. Milk intake and bone mineral acquisition in adolescent girls: randomized, controlled intervention trial. *BMJ.* 1997;315:1255-1260.

10. Matkovic V, Kostial K, Simonovic I, Buzina R, Bodarec A, Nordin C. Bone status and fracture rates in two regions of Yugoslavia. *Am J Clin Nutr.* 1978;32:540-549.

11. Ott SM. Attainment of peak bone mass. *J Clin Endocrinol Metab.* 1990;71:1082A-1084A.

12. Teegarden D, Weaver CM. Calcium supplementation increases bone density in adolescent girls. *Nutrition Reviews.* 52:171-173.

13. Johnston CC, Selemenda CW. The relative importance of nutrition compared to the genetic factors in the development of bone mass. *Nutritional Aspects of Osteoporosis.* 1991;86:21-26.

14. *Daily Reference Intake for Calcium, Phosphorus, Magnesium, Vitamin D, and Flouride.* National Academy Press, Washington DC. 1997;99-106, 141.

15. *Recommended Dietary Allowances.* National Academy Press, Washington DC. 1989. 5.

16. Johnston CC, Miller JZ, Slemenda CW. Calcium supplementation and increases in bone mineral density in children. *NE J Med.* 1992;327:82-87.

17. Andon MB, Lloyd T, Matkovic V. Supplementation trials with calcium citrate malate: evidence in favor of increasing the calcium RDA during childhood and adolescence. *J Nutr.* 1994;124:1412S-1417S.

18. Chan GM, Hoffman K, McMurry M. Effects of dairy products on bone and body composition in pubertal girls. *J Pediatr.* 1995;126:551-556.

19. Yano K, Heilbryn LK, Wasnich RD. The relationship between diet and bone mineral content of multiple skeletal sites in elderly Japanese-American men and women living in Hawaii. *Am J Clin Nutr.* 1985;42:877-888.

20. Daniell HW. Osteoporosis of the slender smoker. Vertebral compression fractures and loss of metacarpal cortex in relation to postmenopausal cigarette smoking and lack of obesity. *Archives of Internal Medicine.* 1976;136:298-304.

21. Sowers MR, Wallace RB, Lemke JH. Correlates of mid-radius bone density among postmenopausal women: a community study. *Am J Clin Nutr.* 1985;41:1045-1053.

22. Peris P, Peris A, Guanabens N. Determinants of bone density in normal women: risk factors for future osteoporosis? *BMJ.* 1989,298:924-928.

23. Odland LM, Mason RL, Alexeff AI. Bone density and dietary findings of 409 Tennessee subjects. II. Dietary considerations. *Am J Clin Nutr.* 1972;25:908-911.

24. Matkonic V, Kostial K, Simonovic I. Bone status and fracture rates in two regions of Yugoslavia. *Am J Clin Nutr.* 1973;32:540-549.

25. Food and Drug Administration. Calcium-Fortified Foods. http://www.governmentguide.com

26. Springen K. Calcium Overload. *Newsweek.* April 14, 2000:64-65.

27. U.S. Department of Agriculture, Agricultural Research Service. 1999. Food and Nutrient Intakes by Children 1994-1996, 1998. http://www.barc.usda.gob/bhnrc/foodsurvey/home.htm.

28. Cook RA. 1977 & 1978. Nutritional status of non-pregnant and pregnant adolescents. Personal Communication. Dept. of Food Science and Human Nutrition, 5749 Merrill Hall, Room 25, University of Maine, Orono, ME 04469-5749.

29. Violette CA. 1977. Nutritional status assessment of adolescent females with a factor analysis of the evaluation of variables. Masters Thesis, University of Maine.

30. Soychak JM. 1981. Development of a nutritional surveillance tool among an adolescent population. Masters Thesis, University of Maine.

31. Shriver W. 1996. Vitamin/mineral intakes and factors related to nutritional status of central Maine adolescents. Masters Thesis, University of Maine.

32. Christakis, G. Nutritional assessment in health programs. *AJPH* 63:Supplement, November 1973.

33. Burk MC, Pao EM. Methodology for Large Scale Surveys of Household and Individual Diets. Home Economics Research Report #40, 1976.

34. Barrett-Connor E. Nutrition epidemiology: how do we know what they ate? *Am J Clin Nutr.* 1991;54:182S-187S.

35. Greger JL, Etnyre GM. Validity of 24-hour dietary recalls by adolescent females. *AJPH.* 1978;68:70-72.

36. Todd KS, Hudes M, Calloway DH. Food intake measurement: problems and approaches. *Am J Clin Nutr.* 1983;37:139-146.

37. Larkin FA, Metzner HL, Guire KE. Comparison of three consecutive-day and three random-day records of dietary intake. *J Am Diet Assoc.* 1991;91:1538-1542.

38. US Census Bureau. Year 2000 Median Household Incomes. http://www.census.gov/

APPENDICES

APPENDIX A

Consent and Assent Forms

MAINE CENTER FOR OSTEOPOROSIS RESEARCH AND EDUCATION
PATIENT INFORMATION AND INFORMED CONSENT STATEMENT- PARENTS

TITLE: **Calcium, Vitamin D, and Seasonal Bone Turnover in Adolescent Girls**

DATE: **June 5, 2000**

My daughter has been asked to take part in a research study being conducted at the Maine Center for Osteoporosis Research and Education, an affiliate of St. Joseph Hospital, in conjunction with the University of Maine. The study has been explained to me by Clifford J. Rosen, M.D. or his representative.

PURPOSE OF THE STUDY:

My daughter has being asked to take part in a study of bone mineral density and dietary factors in 25 adolescent girls. She is being asked because she is between the ages of 9 and 11 years of age and is healthy.

Calcium is the main mineral in bones and lots of calcium in the bones makes them strong and less likely to fracture. Osteoporosis and bone fractures are a common problem for post-menopausal women. Adolescence is a time when large amounts of calcium are being added to the bones. The calcium added to a girl's bones between the ages of 10 and 14 years (puberty) is very important in determining how strong her bones are and what her risk of bone fracture will be throughout her life. It is important to gather as much information as possible about the process of adding calcium to bones during adolescence to learn how to prevent weak bones later in life. The purpose of the study is to determine if more calcium is added to bones during the summer than in the winter in adolescent girls. This might be true if vitamin D levels are higher in the summer because of more time spent in the sun.

PROCEDURES TO BE FOLLOWED:

If I agree and my daughter meets the screening criteria, she and a parent or guardian will return to the Maine Center for Osteoporosis Research and Education seven times over the next three years for bone mineral density measurements and other testing.

My daughter will return for her first visit during the month of September 2000. Measurements taken at this visit will include:

- height and weight,
- blood tests for serum calcium, phosphorus, parathyroid hormone, vitamin D, bone-specific alkaline phosphatase (measure of bone formation), and insulin-like growth factor-I (IGF-I),
- bone mineral density via dual-energy X-ray absorptiometry (DXA) of the spine.

Bone mineral testing is a quick, comfortable method of measuring the density, or thickness, of my daughter's bones. The amount of radiation in this exam is very low. During the exam my daughter will lie on a padded table and the arm of the machine will pass over her body. I understand she will not be enclosed in anyway during this exam.

_____ Sub. initials 1

My daughter will be expected to bring with her a urine sample to measure urinary N-telopeptide (a measure of bone breakdown).

While she is at the Center, my daughter will be asked several questions about her health over the past six months. She will also be asked to rate her sexual maturity using a questionnaire that contains pictures of female breasts and pubic hair for her to select which one best rates her stage of development in puberty.

Each visit is expected to take one to two hours.

My daughter and her parent or guardian will return for six more visits over the next three years. The months for these visits will be March 2001, September 2001, March 2002, September 2002, March 2003, and September 2003. At each of these visits, the testing and questions will be the same as described above.

In between each of these visits, my daughter will be contacted four separate times by mail and asked to complete a record of her physical activity and food intake for one day. The day after she keeps this record, the investigator will call her to find out her physical activity and food and beverage intake for the previous day. The information collected over the phone for four days in the summer and four days in the winter will be used to assess her dietary intake, level of physical activity, and the amount of time she spends out in the sun.

POSSIBLE BENEFITS OF THE STUDY:

My daughter and her doctor may benefit from knowing the results of her bone mineral density tests and how they change over time. Decisions can be made to help decrease her future risk of osteoporosis based on bone density information. Since bone mineral density has a genetic component, my daughter's biological siblings and mother may also benefit from identification of abnormal bone density in my daughter. A summary of my daughter's data will be given to us at the end of the study with education on the prevention of osteoporosis.

Information from the diet analysis can help my daughter understand how to achieve healthier eating habits. In general, information from this study will help researchers further understand the process of adding calcium to bones that takes place in adolescence and the factors involved. They can use this information to help girls achieve maximal bone mineral density and decrease risk of fractures.

POSSIBLE RISKS OR DISCOMFORTS OF THE STUDY:

The tests that will be done at her visits may have some small risks. The bone density (DXA) scan has radiation exposure less than one tenth of a dental x-ray.

My daughter will have blood drawn on seven different occasions. At each visit, total blood volume drawn will be less than 30 mls or 2 tablespoons. There may be possible pain or a black and blue spot at the site of the venipuncture for the blood tests.

To fill out the sexual maturity index questionnaire at each visit, my daughter will be shown photographs of

breasts and pubic hair of females and asked to rate her own development. These photographs are standard pictures used by the medical profession to assess sexual maturity. Some children may become uncomfortable with answering these questions. Any questions that my child does not want to answer, she is free to skip.

PAYMENTS:

For my daughter's time and effort she will be paid a stipend for each visit. There will be no cost to me for any of the tests listed above.

CONFIDENTIALITY:

The results of all testing will be recorded on special forms. These forms will have no name on them, but will use a code number to protect my daughter's privacy. I have been assured my daughter's name will not be used in any publication describing this research without my permission.

VOLUNTARY PARTICIPATION:

My daughter's taking part in this study is voluntary. She is free to leave the study at any time or Dr. Rosen can remove her from the study without my consent. This will not affect our medical care or other benefits she or I may be entitled to. I understand there will be no charge to me or to my insurance company for tests or services that are required by this study. St. Joseph Hospital does not provide financial payment for any injury resulting from this study.

ADDITIONAL INFORMATION:

My daughter and I have had time to consider if she wants to take part in this study. I have read or had read to me this consent form and had it explained to me in language that I am able to understand. I have had a chance to ask questions of Dr. Rosen or his representative, and I have received answers that fully satisfy my questions. I understand the information in this consent form. I willingly agree to let my daughter take part in this study. I understand the study has been reviewed and approved by an ethical research review committee to protect our legal rights. I have been given a copy of this informed consent. If I have any further questions about this study, I may contact Susan Sullivan at (207)-581-3130 or Dr. Clifford J. Rosen at (207)-262-1176 or 1-800-839-8311. If I have any questions about my daughter's rights as a research subject, I may contact Sister Mary Norberta at (207)-262-1000.

Subject Signature/Date	Investigator or Representative Signature/Date
Parent or Guardian Signature/Date	Witness Signature/Date

_____ Sub. initials

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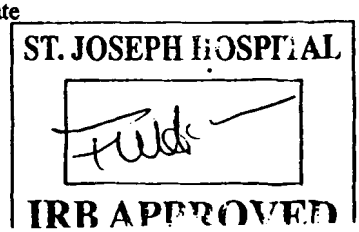

MAINE CENTER FOR OSTEOPOROSIS RESEARCH AND EDUCATION
PATIENT INFORMATION AND ASSENT STATEMENT- SUBJECTS

TITLE: Calcium, Vitamin D, and Seasonal Bone Turnover in Adolescent Girls

I have been asked to be a subject in a research study at the Maine Center for Osteoporosis Research and Education. The purpose of the research is to study how calcium is added to my bones as I grow. Calcium is needed by bones to make them strong and less likely to fracture (break). Girls my age add calcium to their bones at a fast rate. These researchers will study my bones in winter and summer to see if calcium is added faster or slower at different times of the year. They will collect information on my eating habits, my exercise pattern, how often I go out in the sun, and my stage of puberty (or development). They will measure my bone density with a weak X-ray test twice per year. They will also draw a small amount of my blood for tests twice per year. I will go to the Center twice per year for 3 years to have these tests, and I will bring a record of my eating and a sample of my urine (pee) for them to test. Each time I go to the Center, it will take about 2 hours to have the tests and answer the questions.

The questions will be something like this:

- "How often do you drink milk?" and "How often do you eat cheese?"
- "How many hours do you spend playing outdoors each day?" and "How often do you use sunscreen lotion?"
- "How many times per week do you participate in activities that make you breath hard for at least 15 minutes at a time?" and "Do you play on any sports teams?"

At each visit, I will be shown photographs of breasts and pubic hair of females and asked to rate my own physical development. These photographs are standard pictures used by the medical profession to assess sexual maturity.

There are no right or wrong answers to the questions. I can skip questions if I do not want to answer them. The researchers will not tell anyone my name or my test results or my answers to the questions.

Information on my food intake and bone density can help me understand how to have healthier eating habits and stronger bones. Researchers will use information from this study to help other girls have strong bones and less risk of fractures as they grow up.

I will be free to quit the study any time I want to. I have had time to think about it and want to be a subject in this research study.

_____ _____
Subject Signature Date

_____ _____
Parent or Guardian Signature Date

APPENDIX B

Letter Including Instructions for Keeping Food Records

The Seasonal Bone Study

University of Maine
5749 Merrill Hall • Orono, ME 04469

January 8, 2001

Parent name
Parent address

Dear Parent:

Happy New Year! I hope you enjoyed the holidays. Here at the Seasonal Bone Study, we are gearing up for our next data collection point. We will measure bone density and blood tests on the girls again in March. You should already have the appointment for the March visit to the Maine Center for Osteoporosis Research and Education. The March visit will take a bit longer than the last one. Please plan for a half hour.

Before March we would like to have (daughter's name) complete four days of food and activity records. I have enclosed instructions and the forms for her to use. The procedure will be the same as for the practice run last summer. We will call (daughter's name) to arrange the date for the first day of record keeping, and will set a time to call the next day and collect the information from her. All the girls did a great job with the practice run. This time it is for real and we hope they will continue to keep the records as carefully as possible. We would like to record three typical winter weekdays (school days) and one typical weekend day. The days should be separate from each other (not in a row).

To start the process off, Jen Cobb, my research assistant, will call (daughter's name) to arrange the first day of record keeping and to schedule a follow-up phone call for the next day. One of three people will call to collect the information, Jen Cobb, myself, or Laura Agard who is another graduate student helping with this project. During the first phone call, the date for the second day of record keeping will be arranged and the second phone call scheduled. During the second phone call, the third date will be set and so on. We will try to make the dates of record keeping as convenient as possible for the girls.

Thank you for coming in for the September 2000 visit. We have reviewed the bone density results, but cannot draw any conclusions until we see how your daughter's bone density changes over time. Most of the blood samples have been frozen and will be analyzed as a batch later. We will be able to discuss the results of the food records at the visit in March. I look forward to seeing you and (daughter's name) then. Please feel free to call with any questions.

Sincerely,

Susan Sullivan
581-3130

The Seasonal Bone Study

Instructions for Keeping Your Food Record:

- Please keep a record of your food intake for one day. The day after you keep your record, we will call you on the phone to talk about your record.

Food Intake Worksheet

- On the day you are assigned to keep your record, use your "Food Intake Worksheet" to keep track of everything you eat. Make sure to record all food that you eat or drink throughout the entire day. Include the brand name of the food (for example, Kellogg's Corn Flakes; Mott's Apple Juice) when appropriate. As you record each food, remember to write down the amount of food you eat. Estimate the amount to the nearest ounce, cup, tablespoon or other common measure. Compare to food models or use measuring cups to measure actual amount eaten. The orange 3-oz rubber food model is only used to estimate the weight of meat or cheese eaten. Here are a few hints for recording the foods that you eat.

  - ✓ Meats: When estimating the size of meat portions (like chicken, fish, steak), it helps to know that a piece the size of a deck of cards is about 3 ounces (or use orange food model for 3 ounces).

  - ✓ Beverages: Measure the amount of fluid in your drinking glasses. Record as cups or ounces. Make sure to list all kinds of beverages, such as: whole, skim, 1%, 2%, or chocolate milk; carbonated soda; fruit-flavored drink or juice. Also, note if the beverage is calcium-fortified and how much calcium is listed on the food label.

  - ✓ Snack foods: Look at the label on your snack food package. Snack foods are often recorded in ounces or individual pieces. Record the number of chips or crackers eaten. Save labels to refer to during the phone call.

  - ✓ Vegetables, Starches and Canned Fruits: Record as cups.

- ✓ Pizza: Record the number of slices as a fraction of the whole pizza (for example, 2 of 8 = 2/8) and record the brand name or restaurant that the pizza came from.

- ✓ Bread: List the type of bread or rolls you eat as whole wheat, rye, white, multigrain, oatmeal, Italian, etc.

- ✓ Cereal: Record portions in cups.

- ✓ Desserts: Measure size of cake or cookie in inches.

- ✓ Mixed Foods, Casseroles and Salads: Record all the ingredients. For example: a ham-and-cheese sandwich can be listed as 2 slices of bread, 1 tablespoon of mayonnaise, 2 slices of ham, and 1 slice of cheese. If you don't know all the ingredients, estimate the amounts of only the major ones.

Sample Food Intake Worksheet

Time	Food (include brand name)	Amount Eaten
8:00 am	Kellogg's Rice Krispies	1 cup
	2% Milk	1 cup
	Tropicana Orange Juice - Calcium fortified	¾ cup
10:00 am	Kellogg's Strawberry Nutrigrain Bar - Calcium fortified	1 bar
12:00 pm	McDonald's cheeseburger	1
	French fries - McDonald's	1 small
	Pepsi - Regular - McDonald's	1 small
3:00 pm	Peach	1 medium
	Rold Gold Pretzels	1 ounce package
5:30 pm	Baked chicken	3 oz
	Green Beans - fresh	1 cup
	Kraft Macaroni & Cheese (made with 2% milk & regular Promise margarine)	1 cup
	2% milk	1 cup

Figure B1: Subject Food Intake Worksheet

The Seasonal Bone Study

Your Food Intake Worksheet

Time	Food (include brand name)	Amount Eaten
EXAMPLE 8:00 am	EXAMPLE Colombo Lowfat Blueberry Yogurt	EXAMPLE 1 container/8 oz

APPENDIX C

Interviewer Recording Form

Figure C1: Interviewer Recording Form

Subject: _____ **Date:** _____

Recorder: _____ **Day:** _____

24-hour Recall of Food Intake

Time	Food (include brand name)	Amount Eaten	Ca Content

APPENDIX D

Letter Requesting Annual Household Income Data

The Seasonal Bone Study

University of Maine
5749 Merrill Hall • Orono, ME 04469

Dear Parent:

Thank you for helping your child participate in our research study. I am so pleased with how well the girls are doing in the study. The purpose of this letter is to request some information about your household income. I will collect this information anonymously so that your privacy is not violated. My goal is to compare the household income of our sample with the data in other research studies. If you agree to supply this information, please fill in the blanks below and return this form in the envelope provided. Do not put your name on the letter or envelope. These letters and envelopes are not marked, so we will not know whom they are coming from. As always, I am grateful for your assistance.

Sincerely,

Sue

Susan Sullivan

Please choose the range that best approximates the total income in your household (include wages, interest, dividends, self-employment income etc...).

Annual Income Level	**Choose One**
$0-10,000 per year	
$10,001-20,000 per year	
$20,001-30,000 per year	
$30,001-40,000 per year	
$40,001-50,000 per year	
$50,001-60,000 per year	
$60,001-70,000 per year	
$70,001-80,000 per year	
$80,001-90,000 per year	
$90,001-100,000 per year	
> $100,000 per year	

Total number of people living in the household that are supported by this income: _____

*Note- if the girls live in two different households, information on either one of the households is adequate.

APPENDIX E

Calcium Intake Data for Each Subject

Table E1: Calcium Intake Data for Each Subject

	Milk/ Milk Products	Milk as an Ingredient	Foritified Non-grains	Fortifed Grains	Grain Products	Other	Total
Subject 1							
Day 1	840.3	152.9	100.0	0.0	0.0	0.0	0.0
Day 2	755.8	27.2	100.0	0.0	54.0	311.6	1,248.6
Day 3	453.4	396.7	0.0	0.0	43.5	46.2	939.8
Day 4	302.3	0.0	100.0	0.0	54.0	273.0	729.3
Total	2,351.9	576.8	300.0	0.0	151.5	630.8	4,010.9
Daily average	588.0	144.2	75.0	0.0	40.4	180.4	1,027.9
% of Calcium	*57.2%*	*14.0%*	*7.3%*	*0.0%*	*3.9%*	*17.5%*	
Subject 2							
Day 1	439.9	165.7	0.0	200.0	30.0	54.1	889.7
Day 2	628.3	259.7	0.0	0.0	36.0	145.7	1,069.7
Day 3	943.3	0.0	0.0	0.0	133.8	190.7	1,267.8
Day 4	299.1	299.5	0.0	0.0	54.0	20.8	673.5
Total	2,310.6	725.0	0.0	200.0	253.8	411.3	3,900.7
Daily average	577.6	181.2	0.0	50.0	63.5	102.8	975.2
% of Calcium	*59.2%*	*18.6%*	*0.0%*	*5.1%*	*6.5%*	*10.5%*	
Subject 3							
Day 1	839.3	279.6	0.0	0.0	83.5	151.6	1,353.9
Day 2	857.2	54.3	0.0	20.0	60.0	60.3	1,051.8
Day 3	964.3	162.5	0.0	20.0	75.9	54.0	1,276.7
Day 4	601.3	116.8	0.0	0.0	74.6	32.5	825.2
Total	3,262.1	613.2	0.0	40.0	294.0	298.4	4,507.7
Daily average	815.5	153.3	0.0	10.0	73.5	74.6	1,126.9
% of Calcium	*72.4%*	*13.6%*	*0.0%*	*0.9%*	*6.5%*	*6.6%*	
Subject 4							
Day 1	523.5	76.6	0.0	0.0	87.0	58.6	745.6
Day 2	1,079.2	582.8	0.0	0.0	91.6	54.4	1,808.0
Day 3	800.8	1,079.8	100.0	0.0		58.2	2,038.7
Day 4	654.7	79.4	0.0	0.0	82.7	492.0	1,308.7
Total	3,058.1	1,818.5	100.0	0.0	261.3	663.2	5,901.1
Daily average	764.5	454.6	25.0	0.0	65.3	165.8	1,475.3
% of Calcium	*51.8%*	*30.8%*	*2.0%*	*0.0%*	*4.4%*	*11.2%*	
Subject 5							
Day 1	1,010.6	400.0	100.0	0.0	45.9	396.5	1,953.0
Day 2	389.7	195.0	0.0	0.0	79.8	647.6	1,312.1
Day 3	874.0	233.5	0.0	0.0	60.6	402.8	1,570.9
Day 4	688.8	281.2	0.0	0.0	43.4	514.7	1,528.1
Total	2,963.1	1,109.8	100.0	0.0	229.7	1,961.6	6,364.1
Daily average	740.8	277.5	25.0	0.0	57.4	490.4	1,591.0
% of Calcium	*46.7%*	*17.4%*	*2.0%*	*0.0%*	*3.6%*	*30.8%*	

	Milk/ Milk Products	Milk As an Ingredient	Foritified Non- grains	Fortifed Grains	Grain Products	Other	Total
Subject 6							
Day 1	534.2	0.0	0.0	66.7	62.4	26.8	690.0
Day 2	1,141.9	0.0	25.0	0.0	64.7	66.0	1,297.6
Day 3	432.2	0.0	0.0	200.0	85.5	89.0	806.8
Day 4	444.1	46.4	0.0	112.5	317.7	62.7	983.3
Total	2,552.3	46.4	25.0	379.2	530.3	244.5	3,777.7
Daily average	638.1	11.6	6.3	94.8	132.6	61.1	944.4
% of Calcium	*67.6%*	*1.2%*	*0.7%*	*10.0%*	*14.0%*	*6.5%*	
Subject 7							
Day 1	300.1	0.0	0.0	250.0	230.4	216.5	997.0
Day 2	882.8	206.4	0.0	320.0	44.5	61.6	1,515.3
Day 3	450.2	96.9	262.5	0.0	107.5	97.0	1,014.1
Day 4	624.7	255.4	400.0	50.0	112.1	66.0	1,508.1
Total	2,257.8	558.7	662.5	620.0	494.4	441.1	5,034.4
Daily average	564.4	139.7	165.6	155.0	123.6	110.3	1,258.6
% of Calcium	*44.9%*	*11.1%*	*13.0%*	*12.3%*	*9.8%*	*8.8%*	
Subject 8							
Day 1	520.8	233.1	0.0	66.7	0.0	126.9	947.5
Day 2	1,281.4	0.0	0.0	0.0	74.9	122.4	1,478.7
Day 3	140.1	0.0	350.0	0.0	0.0	157.4	647.5
Day 4	959.1	0.0	350.0	0.0	56.2	34.2	1,399.4
Total	2,901.5	233.1	700.0	66.7	131.1	440.9	4,473.2
Daily average	725.4	58.3	175.0	16.7	32.8	110.2	1,118.3
% of Calcium	*64.9%*	*5.2%*	*16.0%*	*1.5%*	*2.9%*	*9.9%*	
Subject 9							
Day 1	596.8	180.0	0.0	0.0	150.0	210.4	1,137.2
Day 2	296.7	465.3	0.0	0.0	174.0	66.3	1,002.3
Day 3	1,572.6	0.0	0.0	0.0	120.2	131.8	1,824.6
Day 4	296.7	383.7	0.0	0.0	52.5	84.9	817.8
Total	2,762.8	1,029.0	0.0	0.0	496.7	493.4	4,781.9
Daily average	690.7	257.2	0.0	0.0	124.2	123.4	1,195.5
% of Calcium	*57.8%*	*21.5%*	*0.0%*	*0.0%*	*10.4%*	*10.3%*	
Subject 10							
Day 1	600.2	175.2	0.0	0.0	54.0	187.0	1,016.4
Day 2	1,050.2	54.3	0.0	0.0	35.6	147.2	1,287.4
Day 3	601.4	81.5	0.0	0.0	59.8	88.7	831.4
Day 4	250.2	0.0	0.0	0.0	40.0	149.9	440.1
Total	2,502.2	311.0	0.0	0.0	189.4	572.8	3,575.3
Daily average	625.5	77.7	0.0	0.0	47.3	143.2	893.8
% of Calcium	*70.0%*	*8.7%*	*0.0%*	*0.0%*	*5.3%*	*16.0%*	

	Milk/ Milk Products	Milk as an Ingredient	Foritified Non-grains	Fortifed Grains	Grain Products	Other	Total
Subject 11							
Day 1	967.704	116.8	0.0	0.0	79.1	200.7	1,364.3
Day 2	1117.704	142.7	0.0	169.2	17.9	123.8	1,571.2
Day 3	967.888	431.5	0.0	0.0	54.7	83.4	1,537.5
Day 4	417.131	0.0	0.0	0.0	63.6	60.0	540.7
Total	3470.427	690.9	0.0	169.2	215.4	467.8	5,013.7
Daily average	867.607	172.7	0.0	42.3	53.8	117.0	1,253.4
% of Calcium	*69.2%*	*13.8%*	*0.0%*	*3.4%*	*4.3%*	*9.3%*	
Subject 12							
Day 1	619.68	0.0	0.0	0.0	34.1	208.5	862.3
Day 2	601.44	51.2	0.0	133.3	75.2	163.2	1,024.4
Day 3	230.148	0.0	0.0	400.0	135.0	126.8	892.0
Day 4	737.124	0.0	0.0	0.0	73.8	70.4	881.3
Total	2188.392	51.2	0.0	533.3	318.1	568.9	3,659.9
Daily average	547.1	12.8	0.0	133.3	79.5	142.2	915.0
% of Calcium	*0.6%*	*1.4%*	*0.0%*	*15.0%*	*8.7%*	*15.5%*	
Subject 13							
Day 1	411.336	0.0	0.0	100.0	0.0	101.3	612.6
Day 2	639.554	0.0	0.0	0.0	59.8	68.3	767.6
Day 3	445.056	60.0	0.0	0.0	38.2	80.3	623.6
Day 4	342.528	0.0	40.5	0.0	0.0	129.3	512.3
Total	1838.474	60.0	40.5	100.0	98.0	379.1	2,516.1
Daily average	459.619	15.0	10.1	25.0	24.5	94.8	629.0
% of Calcium	*0.7%*	*2.4%*	*1.6%*	*4.0%*	*3.9%*	*15.1%*	
Subject 14							
Day 1	302.33	0.0	175.0	20.0	62.1	271.3	830.7
Day 2	334.186	0.0	175.0	0.0	112.3	136.6	758.1
Day 3	154.765	291.4	175.0	20.0	105.0	40.0	786.1
Day 4	0	116.6	87.5	0.0	124.8	103.6	432.5
Total	791.281	407.9	612.5	40.0	404.3	551.4	2,807.4
Daily average	197.82	102.0	153.1	10.0	101.1	137.9	701.9
% of Calcium	*0.3%*	*14.5%*	*21.8%*	*1.0%*	*14.4%*	*19.6%*	
Subject 15							
Day 1	622.1	30.2	0.0	0.0	314.9	145.9	1,113.1
Day 2	570.9	233.1	0.0	0.0	347.2	72.2	1,223.4
Day 3	760.7	382.7	0.0	160.0	9.8	10.6	1,323.8
Day 4	819.5	551.6	0.0	0.0	67.0	4.4	1,442.5
Total	2,773.2	1,197.6	0.0	160.0	738.9	233.1	5,102.8
Daily average	693.3	299.4	0.0	40.0	184.7	58.3	1,275.7
% of Calcium	*54.4%*	*23.5%*	*0.0%*	*3.1%*	*14.5%*	*4.6%*	

	Milk/ Milk Products	Milk as an Ingredient	Foritified Non-grains	Fortifed Grains	Grain Products	Other	Total
Subject 16							
Day 1	147.0	241.3	0.0	0.0	0.0	68.7	457.0
Day 2	634.7	447.0	0.0	0.0	54.0	170.0	1,305.8
Day 3	500.5	0.0	0.0	0.0	10.5	383.5	894.5
Day 4	691.1	0.0	0.0	0.0	27.4	377.6	1,096.1
Total	1,973.3	688.3	0.0	0.0	91.9	999.9	3,753.5
Daily average	493.3	172.1	0.0	0.0	23.0	250.0	938.4
% of Calcium	*52.6%*	*18.3%*	*0.0%*	*0.0%*	*2.5%*	*26.6%*	
Subject 17							
Day 1	951.9	0.0	0.0	0.0	50.7	268.5	1,271.0
Day 2	725.7	75.0	0.0	0.0	60.6	126.1	987.3
Day 3	440.6	0.0	0.0	0.0	118.4	185.8	744.9
Day 4	301.2	0.0	0.0	0.0	173.3	179.5	654.0
Total	2,419.3	75.0	0.0	0.0	403.0	759.9	3,657.2
Daily average	604.8	18.8	0.0	0.0	100.8	190.0	914.3
% of Calcium	*66.2%*	*2.1%*	*0.0%*	*0.0%*	*11.0%*	*20.8%*	
Subject 18							
Day 1	670.4	0.0	0.0	84.6	81.8	113.1	949.8
Day 2	1,032.6	0.0	0.0	133.3	17.5	73.1	1,256.5
Day 3	60.0	0.0	0.0	0.0	0.0	98.6	158.6
Day 4	580.4	64.6	0.0	112.8	106.2	100.2	964.1
Total	2,343.3	64.6	0.0	330.7	205.4	385.0	3,329.0
Daily average	585.8	16.2	0.0	82.7	51.4	96.2	832.3
% of Calcium	*70.4%*	*1.9%*	*0.0%*	*9.9%*	*6.2%*	*11.6%*	
Subject 19							
Day 1	593.4	0.0	0.0	0.0	35.6	73.1	702.2
Day 2	498.4	64.6	0.0	0.0	57.8	181.5	802.3
Day 3	666.2	0.0	0.0	0.0	38.7	114.6	819.5
Day 4	148.4	116.6	0.0	0.0	35.6	202.3	502.9
Total	1,906.3	181.2	0.0	0.0	167.8	571.5	2,826.8
Daily average	476.6	45.3	0.0	0.0	42.0	142.9	706.7
% of Calcium	*67.4%*	*6.4%*	*0.0%*	*0.0%*	*5.9%*	*20.2%*	
Subject 20							
Day 1	425.9	129.2	0.0	150.0	16.5	73.9	795.5
Day 2	693.8	189.2	0.0	150.0	0.0	150.9	1,184.0
Day 3	578.1	0.0	0.0	0.0	0.0	66.3	644.4
Day 4	145.7	129.2	0.0	0.0	16.5	80.8	372.2
Total	1,843.5	447.7	0.0	300.0	33.0	372.0	2,996.2
Daily average	460.9	111.9	0.0	75.0	8.3	93.0	749.0
% of Calcium	*61.5%*	*14.9%*	*0.0%*	*10.0%*	*1.1%*	*12.4%*	

	Milk/ Milk Products	Milk as an Ingredient	Foritified Non-grains	Fortifed Grains	Grain Products	Other	Total
Subject 21							
Day 1	604.7	233.1	0.0	225.0	23.5	27.3	1,113.6
Day 2	1,554.5	0.0	0.0	0.0	198.0	18.2	1,770.8
Day 3	604.7	233.1	0.0	200.0	0.0	64.8	1,102.6
Day 4	1,012.2	0.0	0.0	0.0	103.5	54.8	1,170.5
Total	3,776.0	466.2	0.0	425.0	325.1	165.1	5,157.4
Daily average	944.0	116.6	0.0	106.3	81.3	41.3	1,289.4
% of Calcium	*73.2%*	*9.0%*	*0.0%*	*8.2%*	*6.3%*	*3.2%*	
Subject 22							
Day 1	495.8	0.0	0.0	0.0	99.0	48.8	643.6
Day 2	300.3	0.0	0.0	0.0	78.8	203.0	582.1
Day 3	1,308.2	0.0	0.0	0.0	58.8	29.6	1,396.6
Day 4	602.7	0.0	0.0	0.0	112.3	45.1	760.1
Total	2,707.0	0.0	0.0	0.0	348.8	326.6	3,382.3
Daily average	676.7	0.0	0.0	0.0	87.2	81.6	845.6
% of Calcium	*80.0%*	*0.0%*	*0.0%*	*0.0%*	*10.3%*	*9.7%*	
Subject 23							
Day 1	614.2	0.0	0.0	0.0	0.0	149.2	763.4
Day 2	600.2	0.0	0.0	0.0	0.0	100.8	701.1
Day 3	746.1	120.0	0.0	125.0	0.0	173.4	1,164.5
Day 4	900.4	0.0	0.0	0.0	113.7	285.4	1,299.5
Total	2,860.9	120.0	0.0	125.0	113.7	708.9	3,928.6
Daily average	715.2	30.0	0.0	31.3	28.4	177.2	982.1
% of Calcium	*72.8%*	*3.1%*	*0.0%*	*3.2%*	*2.9%*	*18.1%*	
Subject 24							
Day 1	446.7	150.0	0.0	0.0	23.0	188.0	807.7
Day 2	604.5	200.0	0.0	200.0	119.1	95.8	1,219.5
Day 3	1,531.4	459.0	0.0	0.0	72.7	104.6	2,167.7
Day 4	756.9	65.0	0.0	0.0	80.9	65.3	968.1
Total	3,339.5	874.0	0.0	200.0	295.8	453.7	5,163.0
Daily average	834.9	218.5	0.0	50.0	73.9	113.4	1,290.7
% of Calcium	*64.7%*	*16.9%*	*0.0%*	*3.9%*	*5.7%*	*8.8%*	

BIOGRAPHY OF THE AUTHOR

Laura Agard was born in Milwaukee, Wisconsin on July 1, 1978. She was raised in Milton, Wisconsin and graduated from Milton High School in 1996. She attended the University of Wisconsin-Madison and graduated in 2000 with a Bachelor of Science Degree in Dietetics. She began her graduate studies at the University of Maine in September 2000, and worked as a teaching assistant in the Department of Food Science and Human Nutrition from September 2000 to May 2001. Laura completed her dietetic internship at Maine Medical Center in Portland, Maine in December 2001. She is a candidate for the Master of Science degree in Food Science and Human Nutrition from The University of Maine in May, 2002.