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Resistance Training and Milk-Substitution Enhance Body Composition and Bone Health in Adolescent Girls

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ABSTRACT

Background: Increased soft-drink consumption has contributed to poor calcium intake with 90% of adolescent girls consuming less than the RDA for calcium.

Purpose/objectives: The purpose of this investigation was to determine the independent and additive effects of two interventions (milk and resistance training) on nutrient adequacy, body composition, and bone health in adolescent girls.

Methods: The experimental design consisted of four experimental groups of adolescent girls 14–17 years of age: (1) Milk + resistance training [MRT]; $n = 15$; (2) Resistance training only [RT]; $n = 15$; (3) Milk only [M] $n = 20$; (4) Control [C] $n = 16$. A few significant differences were observed at baseline between the groups for subject characteristics. Testing was performed pre and post-12 week training period for all groups. Milk was provided (3, 8 oz servings) for both the MRT and the M groups. The MRT group and the RT groups performed a supervised periodized resistance training program consisting of supervised one-hour exercise sessions 3 d/wk (M, W, F) for 12 wk. Baseline dietary data was collected utilizing the NUT-P-FFQ and/or a 120 item FFQ developed by the Fred Hutchinson Cancer Research Center (Seattle, Washington). Body composition was measured in the morning after an overnight fast using dual-energy X-ray absorptiometry (DXA) with a total body scanner (Prodigy™, Lunar Corporation, Madison, WI). A whole body scan for bone density and lumbar spine scans were performed on all subjects. Maximal strength of the upper and lower body was assessed via a one-repetition maximum (1-RM) squat and bench press exercise protocols. Significance was set at *P* 0.05.

Results: Significant differences in nutrient intakes between groups generally reflected the nutrient composition of milk with greater intakes of protein and improved nutrient adequacy for several B vitamins, vitamin A, vitamin D, calcium, magnesium, phosphorus, potassium, and zinc. Mean calcium intake was 758 and 1581 mg/d, in the non-milk and milk groups, respectively, with 100% of girls in the milk groups consuming > RDA of 1300 mg/d. There were no effects of milk on body composition or muscle performance, but resistance training had a main effect and significantly increased body mass, lean body mass, muscle strength, and muscle endurance. There was a main effect of milk and resistance training on several measures of bone mineral density (BMD). Changes in whole body BMD in the M, RT, MRT, and CON were 0.45, 0.52, 1.32, and 0.19%, respectively ($P < 0.01$).

Conclusions: Over the course of 12 weeks the effects of 1300 mg/d of calcium in the form of fluid milk combined with a heavy resistance training program resulted in the additive effects of greater nutrient adequacy and BMD in adolescent girls. While further studies are needed, combining increased milk consumption with resistance training appears to optimize bone health in adolescent girls.

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Introduction

Lifestyle choices during adolescence, especially for young girls, have a significant effect on health and risk for disease later in life (1–4). While it is recognized that nutrition and exercise habits are essential behaviors to address in adolescents, surprisingly few intervention studies have been conducted in this population, particularly in teenage girls. It is now well known that progressive heavy resistance training can be an effective modality to improve strength and fitness along with other health related outcomes in children with a properly designed and supervised programs (5–7). However, to our knowledge, no investigations examining progressive heavy resistance training in younger girls and the synergistic effects of a dietary intervention have been undertaken. It would appear that diet and exercise do represent modifiable factors that can have a significant impact on the health in this under-studied population (8–14).

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A poor nutrient adequacy and physical inactivity profile have been observed in adolescent girls which along with inadequate intake of calcium and weight-bearing exercise that can compromise the development of peak bone mass (15, 16). It may also contribute to the accumulation of excess body fat (17). According to the US Department of Agriculture (USDA), 90% of teenage girls do not meet the current 1300 mg/d calcium recommendation advised for teenagers (12, 18). It appears that this may well have been mediated by the fact that soft drinks and juices have displaced milk and contributed to poor calcium intakes in addition to inadequate intakes of several other nutrients (19–23). Declining milk and increased consumption of sweetened drinks in adolescent girls and boys has contributed to not only deficient calcium but also folate, phosphorous, magnesium, Vitamin A, iron and riboflavin (24). While still an ongoing line of research is needed, it is possible that deficient intakes of dietary calcium among adolescents' principally young teenage girls may adversely modify their risk of pathologies over the lifespan related to bone and adiposity (2–4, 25). The role of dairy products and its influence on body weight and fat in adolescent girls remains unclear and the synergistic interactions with resistance exercise have not been adequately examined (25).

The importance of regular physical activity during the growing years, especially weight-bearing exercise, in optimizing bone mineralization has also become more apparent (6, 26). Several studies indicate resistance training is effective in improving muscle strength and muscle size in children and during later stages of the teenage years in youth (14, 27). However, there are few prospective heavy resistance training intervention studies examining bone mineral density (BMD) except from our laboratory work in adolescent boys where improvements in bone were seen with such a synergistic interactions with milk as hypothesized for this study in adolescent girls (28). Additionally, prior work from our laboratory studies showed in a cross-sectional study that adolescent boys (14–17 yrs) who were junior Olympic weightlifters had significantly higher BMD compared to age-matched controls (29), providing evidence that heavy resistance training is effective in augmenting bone mineral acquisition during adolescence. One prospective study has examined the effects of a resistance training program on BMD in adolescent girls, which showed a favorable trend on bone after 26 weeks of training (30). This first finding was supported by a meta-analysis and systematic review which include some data on young girls which has indicated that peak bone mass in young girls can be improved with short bouts of school-based high-impact exercise (31). Furthermore, resistance training appears to be an important modality for optimizing preservation and improvement of BMD in women across the lifespan.

The purpose of this investigation was to determine the independent and additive effects of two interventions (milk and resistance training) on nutrient adequacy, body composition, and bone health in adolescent girls. In addition, we sought to characterize the nutrient intakes using two dietary assessment methods and determine the beverage consumption patterns in teenage girls. We hypothesized that providing additional milk, a rich source of calcium and other nutrients, would improve nutrient adequacy. Moreover, we expected that the combination of increased milk and resistance training would have an additive effect on bone health.

Table 1. Subject characteristics at the start of the study.

Variable	MRT ($n=15$)	RT ($n=15$)	CON ($n=16$)	M ($n=20$)
Age (yrs)	15.7 ± 1.3	15.4 ± 1.4	15.4 ± 1.2	15.1 ± 1.5
Height (cm)	166.3 ± 5.9	167.0 ± 4.9	165.7 ± 5.9	162.5 ± 6.4
Weight (kg)	61.6 ± 8.5^{a}	61.0 ± 8.3^{a}	57.3 ± 5.4^{ab}	54.9 ± 8.8^{b}
Body Fat (%)	28.4 ± 6.9	26.8 ± 6.8	27.7 ± 6.6	25.5 ± 5.6
BMD (g/cm^2)	1.140 ± 0.057^{a}	1.159 ± 0.066^{a}	1.073 ± 0.049^{b}	1.123 ± 0.081^{a}
Tanner Stage	4.4 ± 0.5	4.5 ± 0.5	4.2 ± 0.7	4.3 ± 0.7

Abbreviations: MRT: milk+resistance training; RT: resistance training; CON: control; M: milk.
Values are mean ± SD.
Whole Body BMD = bone mineral density.
Values with a different superscript are different (P 0.05).

Methods

Experimental design and approach to the problem

Adolescent girls were placed into one of four groups: 1. Milk + resistance training [MRT]; $n=15$; 2. Resistance training only [RT]; $n=15$; 3. Milk only [M] $n=20$; 4. Control [C] $n=16$. Girls in the milk groups substituted 3 servings of milk for other sugar containing beverages while the RT and control groups maintained their habitual diet for 12 wk. The resistance training groups performed a 12 wk whole body supervised, progressive heavy resistance training program while the M and CON groups were normally active. Testing was performed in all four groups prior to and after the 12 wk intervention. Outcome variables included nutrient adequacy, bone health, body composition (lean body mass and fat mass), and physical performance.

Subjects

All procedures were approved by an Institutional Review Board for use of Human Subjects. Each subject and her parent or guardian were informed of all of the potential risks and procedures to be used prior to the study and subsequently signed an informed consent/assent document to participate. Girls ranging in age from 14 and 17 years were recruited to participate in the study. Groups were matched for general characteristics after screening. The girls in this study were Caucasian, healthy and per medical and dietary screening had no physical, health or eating disorders that would limit their participation in the study or confound the results. The girls were had not participated in any resistance training programs. There were a few significant anthropometric but no developmental differences among the groups. Body mass and whole body BMD in the CON group was slightly lower than MRT and RT (P 0.05). Subject characteristics are shown in Table 1.

Screening procedures

Interested volunteers participated in a two-hour preliminary screening with their parents that included a medical, onset of menarche and menstrual, nutrition and exercise

Table 2. Nutrient composition of the MILK supplement.

1% Milk	Amount
Energy (kcal)	330
Protein (g)	24
Carbohydrate (g)	35
Fat (g)	7.7
Cholesterol (mg)	29.2
Saturated Fat (g)	4.5
Calcium	900
Trans Fatty Acid (g)	0.2
Sodium	365.5
Potassium (mg)	1140.4
Vitamin A (IU)	1500
Vitamin C (mg)	7.0
Iron (mg)	0.369
Vitamin D (IU)	293
Thiamin (mg)	0.29
Riboflavin (mg)	1.22
Niacin (mg)	0.64
Pyridoxine (mg)	0.32
Folate (μg)	37
Cobalamin (μg)	2.69
Biotin (μg)	15
Panothenic acid (mg)	2.36
Phosphorus (mg)	702
Sugar, Total (g)	36
Magnesium (mg)	103
Zinc (mg)	2.8

Values represent daily intakes (i.e., 3 servings or 240 mL).

questionnaires. Maturity status was determined by self-reported Tanner definition methodology with the help of their parent(s) (32). Tanner's methods have been previously validated in adolescents (33). An extensive dietary interview to assess nutrient intake with an emphasis on calcium intake was performed. A detailed dietary food frequency in addition to a separate calcium history interview was conducted in person in order to assess habitual milk and dairy product consumption. Subjects that consumed >3 servings of fluid milk or >1000 mg calcium per day were excluded from participation. Other exclusion criteria included: metabolic and/or endocrine disorders, known history of bone disease (e.g., scoliosis), use of drugs that influence calcium metabolism, smoking, eating disorders and weight change ±10 lbs over the last month. All girls were required to have commenced menarche and have a normal menstrual cycle; additionally girls that took oral contraceptive pills were excluded. Girls were required to habitually engage in moderate aerobic physical activity, defined as participation in organized team sports, school-based physical education classes and recreational activities. No form of resistance training outside the research setting was permitted over the time course of the investigation. Medical clearance by each subject's physician was required for her participation in the study.

Supplement intervention

In this study a great deal of time and effort in dietary counseling and record keeping was expended to support efficacy of the intervention. Prior to the battery of testing subjects came to the laboratory for a familiarization session. At this time, an intensive dietary counseling session was conducted. Approximately, one and half hours was spent either individually or in small groups with the subjects and their parents as subjects were provided with specific verbal and written instructions and procedures for reporting foods/beverages consumed. Charts, photographs and other supplemental learning materials were distributed to assist in accurate estimation of serving size portions. Subjects were also given instructions to describe their portion sizes using household measures and weights. Additionally, during the subjects scheduled testing day another brief dietary counseling overview (30 min) was conducted to ensure subjects and their parents fully understood the importance of accurate dietary record keeping and milk intervention compliance.

The MRT and M group were provided with three 8 ounce servings of 1% fluid milk and instructed to consume this in addition to their daily typical milk and dairy consumption. In order to maintain energy balance, subjects in the M group were counseled to substitute fluid milk for a nondairy source of equal energy. Every effort was made to substitute milk for other sugar containing beverages. Subjects in the MRT group were instructed to consume the milk in addition to their normal caloric intake with the assumption this was approximately equal to the energy expenditure associated with the resistance training. Girls in the RT and CON groups did not receive any milk and followed their normal habitual diets and performed all testing.

All groups recorded their daily beverage consumption throughout the entire 12 weeks of the study. Milk was provided for both the MRT and the M groups. Milk was provided to subjects in the MRT group weekly at their exercise training sessions. The MRT subjects consumed two-thirds (2 cups) of their daily supplement after each workout in the presence of a member of the investigative team. Subjects in the MRT group received a weekly supply at their workouts to consume on non-training days. Subjects in the M group received a two-week supply of milk on scheduled visits to the laboratory or arranged drop off sites such as their homes or a local meeting place.

Subjects in all four groups recorded the amount and time they consumed their milk (MRT and M groups) and all other beverages on specific beverage log sheets, which were checked regularly. The MRT and the RT group beverage logs were checked weekly at their workout visits. Logs from the M and C group were checked on scheduled visits to the laboratory, arranged visits off site, or subjects mailed in their beverage logs in frequent increments and follow up phone calls to monitor their entries were made. Each subject continued to receive instructions on how to fill in beverage logs and feedback about their progress in order to maintain contact with the subjects and insure proper dietary intakes. Continuing dietary education also occurred on visits to the laboratory, via phone calls or e-mail communication. All milk was purchased from local grocery stores. The nutrient content of the milk is shown in Table 2.

Progressive and periodized heavy resistance training protocol

The MRT group and the RT groups performed a periodized progressive heavy resistance exercise program consisting of supervised one-hour exercise sessions 3 d/wk (M, W, F) for 12 wk. Make-up workout times were offered and were completed within the same week of the original missed workout slot. The ratio of trainer to subject was no more than 1:3 at each training session allowing for direct supervision of all workouts and their progression. The program consisted of varying training loads within each week of training (i.e., nonlinear periodization) as well as increasing intensity with concomitant decreasing volume over the 12 wk with the goal being to increase maximal strength in order to facilitate changes in body composition and bone mineral density (28, 34). Such variation of training intensity and volume was included to minimize boredom with the weekly training sessions and to optimize strength and power performance gains (34). Both groups exercised in the same facility, at the same time(s) of day, and utilized identical equipment, which consisted of a combination of free weights and Cybex stack plate (CYBEX International, Medway, MA, USA) exercise machines.

Monday workouts involved high-intensity training sessions consisting of 3 sets of 8–10 repetition maximum zones (RMs) during wk 1–5, 6–8 RMs during wk 6–10, and 4–6 RM zone during wk 11–12. Exercises included SMITH squats, bench press, leg press, T-Bar row, dumbbell incline press, narrow latissimus pull-down, machine shoulder press, and sit-ups. Wednesday workouts involved high-volume, moderate-intensity training sessions. Three sets each of back hyperextension, leg extension, leg curl, pec fly, wide latissimus pull-down, triceps push-down, machine arm curl and heel raise were performed using 14 RM, 12 RM, and 10 RM training loads during wk 1–5, 6–10, and 11–12, respectively. Friday workouts involved moderate- to high-intensity training sessions including explosive power exercises. Three sets each of repetitive-body weight squat jumps, barbell squats, explosive hang pulls, bench press, machine row, dumbbell shoulder press and dumbbell curl were performed using 8–10 RM, 6–8 RM, and 4–6 RM training loads during wk 1–5, 6–10, and 11–12, respectively. Abdominal crunches were also performed on Friday (3 sets of 20–30 repetitions). Rest periods ranged from 2 to 3 min between sets and exercises with longer rest periods for heavier loads.

Nutrient intakes testing

As part of the screening process, girls were required to complete a food frequency questionnaire (FFQ)/history assessment utilizing the NUT-P-FFQ and/or a 120 item FFQ developed by the Fred Hutchinson Cancer Research Center (Seattle, Washington). The Fred Hutchinson Cancer Research Center-FHCRC-FFQ has been validated in several studies (e.g., The Women's Health Initiative (WHI) (35–37). The standardized FFQ is the most common dietary assessment tool used in large epidemiologic studies of diet and health. The primary purpose of the FFQ for this study was to screen for calcium intake. During the intervention part of the study, girls completed 7-day diaries (7DD) to determine average dietary energy and macro/micronutrient intakes at weeks 1 and 12. The 7DD were used as the primary tool to assess energy and nutrient intake during the study. The FFQ was also completed during week 12 to capture nutrient intake during the experimental period and compare the results of the FFQ to the 7DD. Analysis of the FHCRC-FFQs was accomplished via optical scanning at FHCRC. Each batch of scanned FHCRC-FFQs yields files containing food consumption data, nutrient intake data, and an error report that specifies questionnaire completion errors (e.g. incomplete erasures, missed questions). The FHCRC-FFQ nutrient database is derived from the University of Minnesota Nutrition Coordinating Center nutrient database (38). After each participant completed the FHCRC-FFQ, a quality assurance check, involving a though review of each FHCRC-FFQ was performed. Food diaries were checked for completeness and accuracy during scheduled visits to the laboratory, during workout sessions, or personal visits to subject's homes. In addition to the 7DD (collected at weeks 1 and 12) all subjects were required to record all beverage consumption (soda, milk, juice, water etc.) consumed everyday during the entire 12-week experimental period.

Dietary information for the entire 12-week experimental study period (6,216 total days) were entered into a data base and analyzed for dietary energy, macronutrient and micronutrient composition using established nutritional software packages with NUTRITIONIST PRO^{TM} (NUT-P), (Version 2.4.1, First Databank Inc., The Hearst Corporation, San Bruno, CA).

Girls were asked to save and return food labels with their completed 7DD and beverage logs to enhance accuracy of the nutrient analysis. When a food/beverage could not be located in the NUT-P database, searches were performed to locate nutrient information. This resulted in an extensively modified database of approximately 900 additional foods. Additionally the program can analyze an unlimited number of recipes that automatically takes into account nutrient losses and gains from cooking. The database contains over 750 quantity and consumer recipes from USDA, Communicating Food for Health newsletter and Good Housekeeping but also has the option to add unlimited recipes. Upon nutrient data entries approximately 100 custom recipes were entered into the data base for analysis.

To classify beverages patterns over the course of the experimental protocol, information from the detailed beverage records were tallied based on the following classification system: water, milk of all types (skim, 1%, 2% whole, soy, and flavored milks), 100% fruit or vegetable juices (distinguishing calcium fortified orange juices), fruit flavored drinks and aides (to include sweetened teas, part juice drinks, kool-aids, etc.), 0 calorie beverages (Crystal Light, diet teas), sports drinks, soft drinks (nondiet and diet) coffee, tea, and coffee drinks, cocoa, and alcohol (including beer, wine, and liquor). The other milk beverage category included shakes and smoothies, and had to contain at least 50% milk (determined by the National Dairy Council) but did not include dairy foods (i.e., yogurt).

Anthropometric measurements

Anthropometric measurements (height, weight, circumferences) were determined using standard procedures at weeks

0, and 12. Body mass was measured on an electronic scale to the nearest 100 grams in the subjects undergarments and height was be determined with a wall-mounted stadiometer to the nearest millimeter without shoes. Circumference measurements (waist and hip) were obtained in duplicate in serial fashion by the same investigator using standard methods (39, 40).

Body composition, abdominal fat, and bone

Body composition was measured in the morning after an overnight fast using dual-energy X-ray absorptiometry (DXA) on a total body scanner (Prodigy^{TM}, Lunar Corporation, Madison, WI). A whole body and second lumbar spine scan was performed in all groups. A urine pregnancy test (Clear Blue®' New Jersey, USA) was administered prior to the scans. For the whole body scan, subjects remained motionless in the supine position for approximately five minutes while the scanning arm of the DXA passed over their body from head to toe in parallel 1-cm strips. Soft tissue mass, which is comprised of fat mass and lean body mass, was measured pixel-by-pixel as a beam of photons penetrate the subjects' body. The lumbar spine scan was performed after the whole body scan. During measurement of the lumbar spine, subjects rested in the supine position and a box was placed underneath their lower legs to facilitate flattening of the spine. The scan duration took about thirty seconds. A phantom spine provided by the company was analyzed over the course of the study to assess quality assurance and daily calibrations were performed prior to all scans using a calibration block provided by the manufacturer.

All data analyses from the DXA were performed by the same blinded investigator using commercial software (enCORE version 6.00.270). From the whole body scan, the trunk, arm, and leg regions were identified according to anatomical landmarks manually adjusted by the technician using the region of interest function of the Lunar software (enCORE version 6.00.270). Regional analysis of the abdomen was also assessed by placing a box between the top of L2 and the bottom of L4 of the spinal column. To form the box, the regions of interest tool on the Lunar software was set superiorly in the inter-vertebral space, between the bottom of L1 and the top of L2. The lower part of the box was made with the region of interest tool that was placed posterally in the inter-vertebral space located between the bottom of L4 and the top of L5. This abdominal region of interest has been shown to be a highly reliable and accurate determinant of abdominal obesity compared to multi-slice computed tomography (41). Percent body fat was calculated as soft tissue fat tissue mass divided by the total soft tissue mass plus the estimated bone mineral content. The lumbar spine scan analysis was assessed by making a box between the top of L2 and the bottom of L4 of the spinal column. The region of interest tool on the Lunar software was used to make this box by placing the line superiorly in the inter-vertebral space, between the bottom of L1 and the top of L2. The region of interest tool made the lower line of the box in the inter-vertebral space located between the bottom of L4 and the top of L5. The lumbar spine analysis included the anatomical landmarks of (L2-L4) which included data reported for L2, L3, and L4. Coefficients of variation on repeat scans with repositioning on a group of men and women in our laboratory for BMD, BMC, fat mass and lean body mass were 0.7, 0.6, 1.4, and 0.4, respectively.

Physical performance testing

Physical performance testing was performed on a plyometric power system (PPS) (Norsearch Limited, Lismore, Australia) consisting of a Smith rack and Olympic bar and weights interfaced to an online computer interface. Muscular strength and local muscular endurance were determined at wk 0 and 12. Subjects were familiarized with all testing protocols on a separate day prior to testing. Maximal strength of the upper and lower body was assessed via a one-repetition maximum (1-RM) squat and bench press exercise protocols (42, 43). Each participant attempted a complete single, maximal explosive effort with the required load based on the 1-RM. Maximal strength of the lower body was determined using a squat exercise on a modified Smith machine and maximal strength of the upper body was determined using a bench press exercise. A steady cycling warm up on a bike for 5–7 seven minutes was performed by each subject prior to resistance training warm-up or trials. Several sets of warm-up trials were performed using 30% (8–10 repetitions), 50% (4–6 repetitions), 75% (2–4 repetitions), and 90% (1 repetition) of an estimated 1-RM. The load was increased to a point where the subject had 3–4 maximal efforts to determine the 1-RM. Adequate rest of 3–5 min was allowed between trials. Each subject was asked to lower the bar to a knee angle of ninety degrees (marked by an audible cue and adjustable stoppers) and immediately move the weight upwards in a controlled but forceful fashion to the starting position. The 1-RM bench press test was conducted using the modified Smith machine and the same warm up procedures as for the 1-RM squat test were followed. The 1-RM bench press was performed using a stretch shortening cycle with no pause at the bottom of the movement. The subjects lowered the bar to the chest without bouncing and then lifted the bar to a full elbow extension for a successful lift.

Local muscular endurance was tested using the PPS and consisted of a squat exercise test using 70% of the subject's 1-RM load. Each subject began the exercise in the standing position with the bar in the high squat position. Subjects performed as many repetitions as possible at a specified cadence of 2 s for each repetition. It was explained to each subject that for each beep the subject should be at the top or bottom of the squat. Additionally, the subject had to lower the bar to a knee angle of ninety degrees (marked by an audible cue and adjustable stoppers) and lightly touch the bottom stopper in the bottom position and return to the original standing position. The subject would terminate the test upon volitional fatigue or when the front spotter judged

Table 3. Daily intake of dietary energy and macronutrients.

Nutrient	MRT	RT	CON	M	Group Effect
Energy (kcal)	1970 ± 408	2031 ± 465	1807 ± 360	2119 ± 429	0.172
Protein (g)	77 ± 11^{a}	57 ± 20^{b}	59 ± 16^{b}	80 ± 13^{a}	**0.000**
Protein (%)	16 ± 2^{a}	11 ± 2^{b}	13 ± 2^{c}	15 ± 2^{a}	**0.000**
Carbohydrate (g)	277 ± 69^{ab}	316 ± 56^{a}	256 ± 50^{b}	293 ± 62^{ab}	**0.050**
Carbohydrate (%)	56 ± 3^{a}	63 ± 5^{b}	56 ± 4^{a}	55 ± 3^{a}	**0.000**
Total Fat (g)	63 ± 15	61 ± 21	63 ± 16	72 ± 19	0.282
Total Fat (%)	29 ± 3^{ab}	26 ± 3^{a}	31 ± 3^{b}	30 ± 3^{b}	**0.001**
Saturated Fat (g)	24 ± 6	22 ± 8	22 ± 6	27 ± 8	0.089
Monounsaturated Fat (g)	14 ± 4	13 ± 6	12 ± 6	14 ± 5	0.892
Polyunsaturated Fat (g)	6 ± 2	6 ± 2	7 ± 4	8 ± 5	0.398
Cholesterol (mg)	177 ± 38	157 ± 82	158 ± 66	210 ± 99	0.138
Dietary Fiber (g)	9.9 ± 2.9^{a}	9.3 ± 3.2^{a}	11.6 ± 4.4^{a}	13.7 ± 3.5^{b}	**0.002**

Values are mean ± SD. MRT = Milk + Resistance Training; RT = Resistance Training; CON = Control; M = Milk #Group effect using one-way ANOVA; values with a different superscript are different ($P < 0.05$).

that the subject had not kept the cadence for 3 consecutive repetitions or failed to go through the complete range of motion for 3 consecutive repetitions.

Physical activity

A detailed history of physical activity patterns and sports participation was obtained prior to and throughout this investigation. This included documentation of routine exercise (e.g., mode, duration, intensity, and frequency). Physical activity was defined to include the participation in organized team sports, school-based physical education classes and recreational activities. No form of resistance training outside the research setting was permitted over the course of the investigation.

Statistical analyses

Means and standard deviations were computed for all diet, bone, body composition, performance, and subject anthropometric variables. The mean of the 7DD records were calculated at baseline and week 12. Distributions were examined for approximate normality and transformed (i.e., $\log_{10}$) if appropriate for further analysis. All data analyses were conducted using Statistica 5.5 for windows (StatSoft Inc, Tulsa, OK). Initially, differences among groups in baseline measurements were evaluated using one-way ANOVA with differences further evaluated using Fisher's Least Significant Test. To evaluate the relative impact of Milk and Resistance Exercise treatments over time, a 2 2 2 ANOVA with (Milk x Resistance Exercise) as between factors and the change over time as a within factor was used. When a significant difference at baseline was evident, an ANCOVA was used with the baseline value as a covariate. Nutrient information from 7DD and the delta changes from pre to post among groups were compared using a one-way ANOVA with differences further evaluated using Fisher's Least Significant Test. Nutrient intakes estimated from the 7DD and FFQ were compared using a dependent t-test. Beverage consumption patterns calculated from tallied beverage logs were compared using an independent t-test with alpha level corrections. Relationships among dietary nutrients and body composition and bone measures were assessed using Pearson's product-moment correlation coefficients. Using the nQuery Advisor® software (Statistical Solutions, Saugus, MA) the statistical power for the n size used ranged from 0.78 to 0.93. Significance was set at P 0.05.

Results

Compliance to the resistance training and milk supplementation was excellent. Attendance and completion of workouts in the progressive heavy resistance training groups (MRT and RT) was 100% including make-up sessions. Compliance to the milk supplementation was 97.0% as ascertained primarily via the beverage logs and signed statements by subjects, parents and another household witness. There were a total of 270 missed servings of milk out of 8,820 total servings. The MRT subjects also consumed two-thirds (2 cups) of their daily supplement after each workout in the presence of a member of the investigative team. All girls were menstruating. The average onset of menarche was 12.9 ± 1.4 y. The average years post menarche was 2.4 ± 1.5 y.

Nutrient adequacy

Differences in estimated daily intakes of macronutrients (Table 3), vitamins (Table 4) and minerals (Table 5) from 7DD for the different groups generally reflected the nutrient composition of the additional milk. Energy intake was not significantly different among groups. The mean difference in energy between the M and CON group was 312 kcal/day, similar to the caloric value of the three additional servings of milk, which provided 330 kcal/day. The relative distribution of macronutrients differed between groups. As a percentage of total energy, the RT group consumed significantly less protein (11%), more carbohydrate (63%), and less fat (26%) than other groups. Compared to CON, milk groups consumed more protein (15–16% of total energy). The majority of total fat was derived from saturated fat. There were no significant differences in the relative distribution among saturated fat, polyunsaturated fat, monounsaturated fat, or cholesterol among groups. Dietary fiber was significantly higher in the M group than all other groups. There were significant main groups effects for all vitamins and the majority of minerals and trace elements. As expected based on the nutrient composition of milk, the

Table 4. Daily intake of vitamins.

Nutrient	MRT	RT	CON	M	RDA/AI	Group Effect
Vitamin A (IU)	4608 ± 1189^a	2289 ± 891^b	3702 ± 1176^a	5869 ± 3229^c	4000	**0.000**
Vitamin B1 (mg)	1.2 ± 0.4^a	0.8 ± 0.3^b	0.9 ± 0.4^b	1.4 ± 0.4^a	1.0	**0.000**
Vitamin B2 (mg)	2.5 ± 0.6^a	1.0 ± 0.4^b	1.2 ± 0.5^b	2.3 ± 0.5^a	1.0	**0.000**
Vitamin B3 (mg)	12.7 ± 3.8	10.6 ± 3.5	12.8 ± 5.0	13.9 ± 4.9	14	0.183
Vitamin B6 (mg)	1.2 ± 0.4^a	0.8 ± 0.4^b	1.0 ± 0.4^b	1.3 ± 0.4^a	1.2	**0.001**
Vitamin B12 (μg)	5.5 ± 1.5^a	2.2 ± 1.4^b	2.7 ± 1.5^b	5.5 ± 3.3^a	2.4	**0.000**
Biotin (μg)	23.8 ± 9.1^a	4.9 ± 3.9^b	9.1 ± 8.8^b	25.1 ± 12.8^a	25	**0.000**
Folate (μg)	260 ± 103^a	171 ± 84^b	200 ± 73bc	261 ± 76ac	400	**0.005**
Panothenic Acid (mg)	4.1 ± 0.8^a	1.5 ± 0.8^b	2.0 ± 1.5^b	4.3 ± 1.4^a	5	**0.000**
Vitamin C (mg)	74 ± 52^a	235 ± 64^b	81 ± 37^a	135 ± 265^a	65	**0.018**
Vitamin D (IU)	407 ± 81^a	86 ± 67^b	108 ± 70^b	351 ± 84^a	200	**0.000**
Vitamin E (IU)	4.5 ± 1.3	3.4 ± 1.4	5.6 ± 5.3	6.3 ± 4.2	12	0.101
Vitamin K (μg)	17.0 ± 8.9^a	15.0 ± 10.1^a	40.3 ± 38.7^b	32.8 ± 22.0^b	75	**0.008**

Values are mean ± SD. MRT = Milk + Resistance Training; RT = Resistance Training; CON = Control; M = Milk.
RDA = Recommended Dietary Allowance; AI = Adequate Intake.
#Group effect using one-way ANOVA; values with a different superscript are different ($P < 0.05$).

Table 5. Daily intake of minerals, trace elements, and electrolytes.

Nutrient	MRT	RT	CON	M	RDA/AI	Group Effect
Calcium (mg)	1612 ± 307^a	674 ± 226^b	842 ± 289^b	1550 ± 261^a	1300	**0.000**
Chromium (μg)	12 ± 7	14 ± 10	21 ± 12	17 ± 12	24	0.071
Copper (mg)	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.1	0.9	0.124
Iron (mg)	11.5 ± 3.5ab	10.9 ± 3.6^a	10.4 ± 3.9^a	13.7 ± 3.6^b	15	**0.046**
Magnesium (mg)	223 ± 47^a	112 ± 42^b	133 ± 62^b	210 ± 42^a	360	**0.000**
Manganese (mg)	1.0 ± 0.6	0.7 ± 0.3	1.1 ± 0.7	1.1 ± 0.4	1.6	0.121
Phosphorus (mg)	1411 ± 271^a	732 ± 289^b	680 ± 261^b	1259 ± 228^a	1250	**0.000**
Potassium (mg)	2563 ± 497^a	2108 ± 633^b	1452 ± 596^c	2412 ± 541ab	4700	**0.000**
Selenium (μg)	58 ± 17	44 ± 16	50 ± 20	57 ± 15	55	0.106
Sodium (mg)	2948 ± 574	2605 ± 782	2714 ± 612	3144 ± 514	1500	0.058
Zinc (mg)	8.8 ± 2.9^a	5.6 ± 2.9^b	5.5 ± 2.6^b	8.5 ± 2.8^a	11	**0.000**

Values are mean ± SD. MRT = Milk + Resistance Training; RT = Resistance Training; CON = Control; M = Milk.
RDA = Recommended Dietary Allowance; AI = Adequate Intake.
#Group effect using one-way ANOVA; values with a different superscript are different ($P < 0.05$).

general pattern was for higher intakes of B vitamins, vitamin A, vitamin D, calcium, magnesium, phosphorus, potassium, and zinc in the Milk groups.

A qualitative analysis of nutrient adequacy was estimated as the percentage of subjects in each group meeting or exceeding two-thirds of the RDA/AI for given vitamins and minerals. Nutrient adequacy was excellent in the majority of subjects consuming milk for most vitamins, with the exception of folate and vitamin E. However, nutrient adequacy in the RT and CON groups was relatively poor with the majority of subjects failing to consume two-thirds of the RDA/AI for biotin, folate, pantothenic acid, vitamin A, vitamin D, and vitamin E. Nutrient adequacy for minerals was also consistently better in the milk groups. As expected 100% of subjects in the milk groups exceeded two-thirds of the RDA/AI for calcium, whereas less than one-third of subjects in the other groups met this criteria. The majority of subjects in the milk group consumed at least two-thirds of the RDA/AI for iron, manganese, phosphorus, selenium, and zinc; however, most were deficient in copper, magnesium, and potassium. Less than 50% of subjects in the non-milk groups consumed two-thirds of the RDA/AI for all the minerals, except for selenium.

Validation of food frequency

Nutrient intakes calculated from 7DD were compared to those obtained using the NUT-P-FFQ (Figure 1). Compared to the 7DD, the NUT-P-FFQ consistently overestimated most nutrients. In particular, the NUT-P-FFQ overestimated the following nutrients by more than 2-fold: monounsaturated fat, polyunsaturated fat, vitamin A, vitamin E, pantothenic acid, magnesium, zinc, copper, and manganese. There were also significant differences between methods for protein, sodium, potassium, vitamin C, iron, vitamin B1, vitamin B2, vitamin B3, vitamin B6, vitamin B12, folate, phosphorus, and fiber. Nutrients not statistically different between methods included total energy, carbohydrate, fat, cholesterol, saturated fat, calcium, vitamin D, biotin, vitamin K, and selenium.

Beverage consumption patterns

Beverage consumption patterns calculated from the beverage logs using a detailed tallying system generally reflect similar patterns among groups except for the supplement intervention. Significant differences for milk, shakes and tea exist. The M group had a greater intake of milk due to the supplement intervention, which is accounted for in the group mean of 70 days and the fluid oz per day. The milk was slightly above 24 fl oz, which was the prescribed amount of the supplement. Tea and shakes in the CON group were significantly greater.

Anthropometric and body composition

There were a few significant differences in some anthropometric or body composition variables at baseline with the

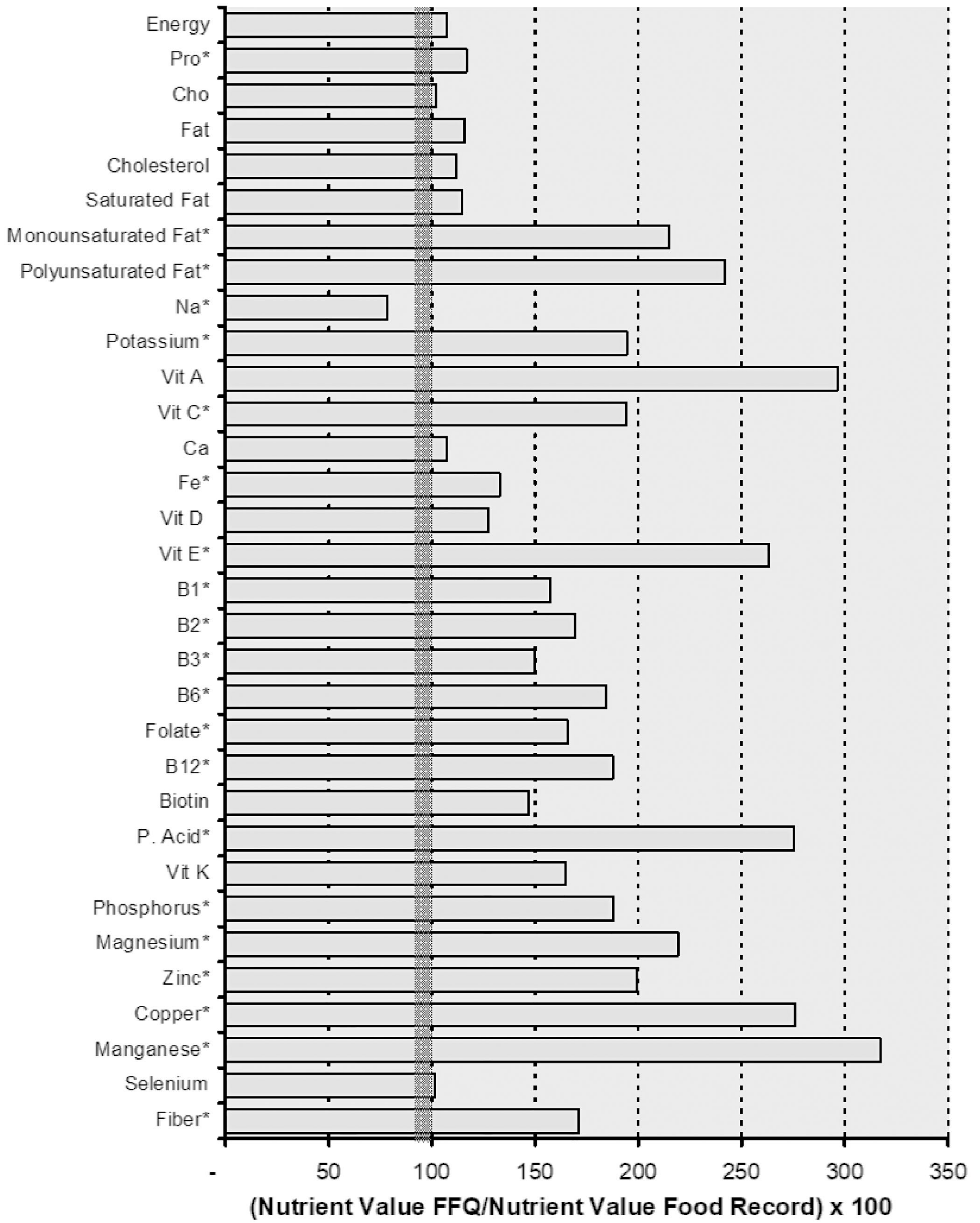

Figure 1. Comparison between nutrients obtained from food records (7DD) and food frequency questionnaire (NUT-P-FFQ) in Control subjects. Food records were obtained for 7 days at Week 1 and 12 and NUT-P-FFQ was performed during Week 12. Nutrient values calculated as: [(food frequency/food record) x 100]. *P* 0.05 between FFQ and Food Record value (dependent *T*-test).

body mass; the M group weighed significantly less than the MRT and RT groups. There were significant increases (main time effects) for height (0.3%), body mass (1.9%), waist circumference (1.5%), hip circumference (1.0%), percent body fat (1.8%), fat mass (3.8%), lean body mass (1.4%), and abdominal fat (3.2%). For the main effect of resistance

Table 6. Whole body and regional bone mineral content responses.

Variable (g)		MRT	RT	CON	M	P-M	P-RT
Whole body #	Wk 0	2523 ± 284[a]	2515 ± 291[a]	2236 ± 194[b]	2311 ± 400[b]	0.511	0.339
	Wk 12	2570 ± 305	2564 ± 273	2258 ± 178	2356 ± 414		
Arm #	Wk 0	297 ± 37[a]	301 ± 41[a]	265 ± 27[b]	267 ± 48[b]	**0.020**	0.745
	Wk 12	305 ± 35	304 ± 40	270 ± 28	276 ± 45		
Leg #	Wk 0	976 ± 140[a]	999 ± 153[a]	876 ± 70[b]	907 ± 159[ab]	0.568	**0.039**
	Wk 12	990 ± 145	1013 ± 158	878 ± 70	913 ± 161		
Trunk #	Wk 0	829 ± 128[a]	790 ± 121[ac]	686 ± 103[bc]	709 ± 153[bc]	0.708	0.699
	Wk 12	847 ± 142	810 ± 110	702 ± 85	734 ± 172		
Rib #	Wk 0	284 ± 57[a]	258 ± 46[ab]	228 ± 47[b]	225 ± 65[b]	0.872	0.915
	Wk 12	286 ± 71	267 ± 42	238 ± 39	237 ± 73		
Pelvis	Wk 0	326 ± 54	320 ± 57	276 ± 43	298 ± 65	0.290	0.297
	Wk 12	338 ± 54	328 ± 54	275 ± 40	306 ± 73		
Spine #	Wk 0	219 ± 27[a]	212 ± 29[a]	183 ± 21[b]	186 ± 32[b]	0.517	0.461
	Wk 12	224 ± 25	215 ± 29	187 ± 20	190 ± 34		
L2-L4	Wk 0	—	—	44.0 ± 5.1	45.7 ± 7.5	0.489	
	Wk 12	—	—	44.7 ± 4.9	46.6 ± 7.8		

Values are mean ± SD. MRT = Milk + Resistance Training; RT = Resistance Training; CON = Control; M = Milk.
Significant ($P < 0.05$) main time effect.
#Significant ($P < 0.05$) difference at Wk 0 (one-way ANOVA); values with a different superscript are different ($P < 0.05$).
P-M = Main Effect of Milk; P-RT = Main Effect of Resistance Training.

training, the RT and MRT groups increased body mass and lean body mass by 1.6 and 1.0 kg, respectively; while the M and CON groups increased body mass and lean body mass by 0.6 and 0.1 kg, respectively ($P < 0.05$). Girls in the milk groups gained significantly more abdominal fat than girls in the non-milk group (80 vs. 7 g, respectively). There were no significant main effects of milk for any other anthropometric or body composition variables.

Bone measures

Bone Mineral Content: For the whole body and several regional measures of BMC, the resistance training groups had significantly higher values than the CON and M group (Table 6). There were significant increases (main time effects) for whole body BMC (1.7%), all regional measures of BMC from the whole body scan, and L2-L4 BMC (1.9%) from the lumbar spine scan. For the main effect milk, subjects in the M and MRT groups increased arm BMC by 9 g, while the subjects in the RT and CON groups increased by 4 g ($P < 0.05$). For the main effect of resistance training, the RT and MRT groups increased leg BMC by 15 g, while the M and CON groups increased by 4 g ($P = 0.039$). There were no significant main effects of milk or resistance training for any other measures of BMC.

Bone Mineral Density: Similar to BMC, the resistance training groups had significantly higher BMD values than the CON and M group (see Table 7). There were significant increases (main time effects) for whole body BMD (0.45%) and all regional measures of BMD from the whole body scan except the leg. The increase in L2-L4 BMD (0.9%) from the lumbar spine scan in the CON and M groups was not significant. For the main effect of milk, subjects in the M and MRT groups increased whole body BMD by 0.010 g/cm^2, while subjects in the RT and CON groups increased by 0.002 g/cm^2 ($P < 0.05$). For the main effect of resistance training, subjects in the RT and MRT groups increased whole body BMD by 0.010 g/cm^2, while subjects in the M and CON groups increased by 0.002 g/cm^2 ($P < 0.05$). The significant main effects of Milk and Resistance Training in the absence of a significant interaction indicate that these effects on whole body BMD were independent and additive. A similar pattern of significant main effects of both Milk and Resistance Training were evident for trunk BMD and Pelvis BMD. This is more clearly shown in Figure 2. A main effect of milk only and not resistance training was observed for spine BMD and a trend for arm BMD ($P = 0.082$). There were no significant main effects for leg BMD, rib BMD, or L2-L4 BMD.

Individual responses in whole body BMD and BMC are shown in Figure 3. Mean responses show a clear benefit to milk supplementation and resistance training, however, there is a great deal of variability among subjects with responders and nonresponders present in all groups. All subjects with the exception one had an increase in BMD in the MRT group. The girl who decreased BMD in this group also experienced the largest increase in BMC. All groups but MRT had several people who had decreases in BMD. It is also worth noting that all individual responses in the CON group were below the mean response of the MRT group.

Muscular strength and local muscular endurance performances

There was a significant main effect of resistance training, but not milk, on maximal strength and squat endurance performance (Figure 4). Bench press strength increased by 29, 25, 3, and 5% in the MRT, RT, CON, and M group, respectively. Squat strength increased by 21, 19, 17, and 15% in the MRT, RT, CON, and M group, respectively. Total number of repetitions during the squat endurance protocol improved by 99, 61, 6, and 18% in the MRT, RT, CON, and M group, respectively.

Discussion

The clinical importance of this study resides in the fact that by adding milk and progressive heavy resistance training in young girls, improvement in bone health can be achieved

Table 7. Whole body and regional bone mineral density responses.

Variable (g/cm^2)		MRT	RT	CON	M	P-M	P-RT
Whole body #	Wk 0	1.140 ± 0.057^a	1.159 ± 0.066^a	1.073 ± 0.049^b	1.123 ± 0.081^{ab}	**0.006**	**0.007**
	Wk 12	1.155 ± 0.058	1.165 ± 0.068	1.071 ± 0.049	1.128 ± 0.083		
Arm #	Wk 0	0.849 ± 0.052^a	0.849 ± 0.052^a	0.784 ± 0.043^b	0.802 ± 0.051^b	0.082	0.710
	Wk 12	0.859 ± 0.054	0.850 ± 0.054	0.787 ± 0.041	0.811 ± 0.052		
Leg	Wk 0	1.262 ± 0.101	1.295 ± 0.115	1.208 ± 0.071	1.273 ± 0.110	0.390	0.214
	Wk 12	1.269 ± 0.094	1.294 ± 0.107	1.205 ± 0.068	1.270 ± 0.109		
Trunk #	Wk 0	0.944 ± 0.066^a	0.950 ± 0.072^a	0.863 ± 0.048^b	0.900 ± 0.072^b	**0.001**	**0.033**
	Wk 12	0.966 ± 0.071	0.955 ± 0.070	0.863 ± 0.046	0.909 ± 0.077		
Rib #	Wk 0	0.675 ± 0.056^a	0.664 ± 0.043^a	0.623 ± 0.030^b	0.627 ± 0.064^b	0.237	0.098
	Wk 12	0.683 ± 0.066	0.670 ± 0.039	0.622 ± 0.030	0.635 ± 0.054		
Pelvis #	Wk 0	1.216 ± 0.104^a	1.237 ± 0.118^a	1.096 ± 0.073^b	1.153 ± 0.113^b	**0.004**	**0.007**
	Wk 12	1.254 ± 0.102	1.245 ± 0.103	1.093 ± 0.065	1.166 ± 0.122		
Spine #	Wk 0	1.156 ± 0.105^a	1.151 ± 0.123^a	1.034 ± 0.106^b	1.065 ± 0.131^b	**0.038**	0.575
	Wk 12	1.175 ± 0.115	1.152 ± 0.095	1.051 ± 0.090	1.104 ± 0.136		
L2-L4	Wk 0			1.119 ± 0.081	1.166 ± 0.120	0.511	
	Wk 12			1.125 ± 0.068	1.178 ± 0.119		

Values are mean ± SD. MRT = Milk + Resistance Training; RT = Resistance Training; CON = Control; M = Milk.
Significant ($P < 0.05$) main time effect.
#Significant ($P < 0.05$) difference at Wk 0 (one-way ANOVA); values with a different superscript are different ($P < 0.05$).
P-M = Main Effect of Milk; P-RT = Main Effect of Resistance Training.

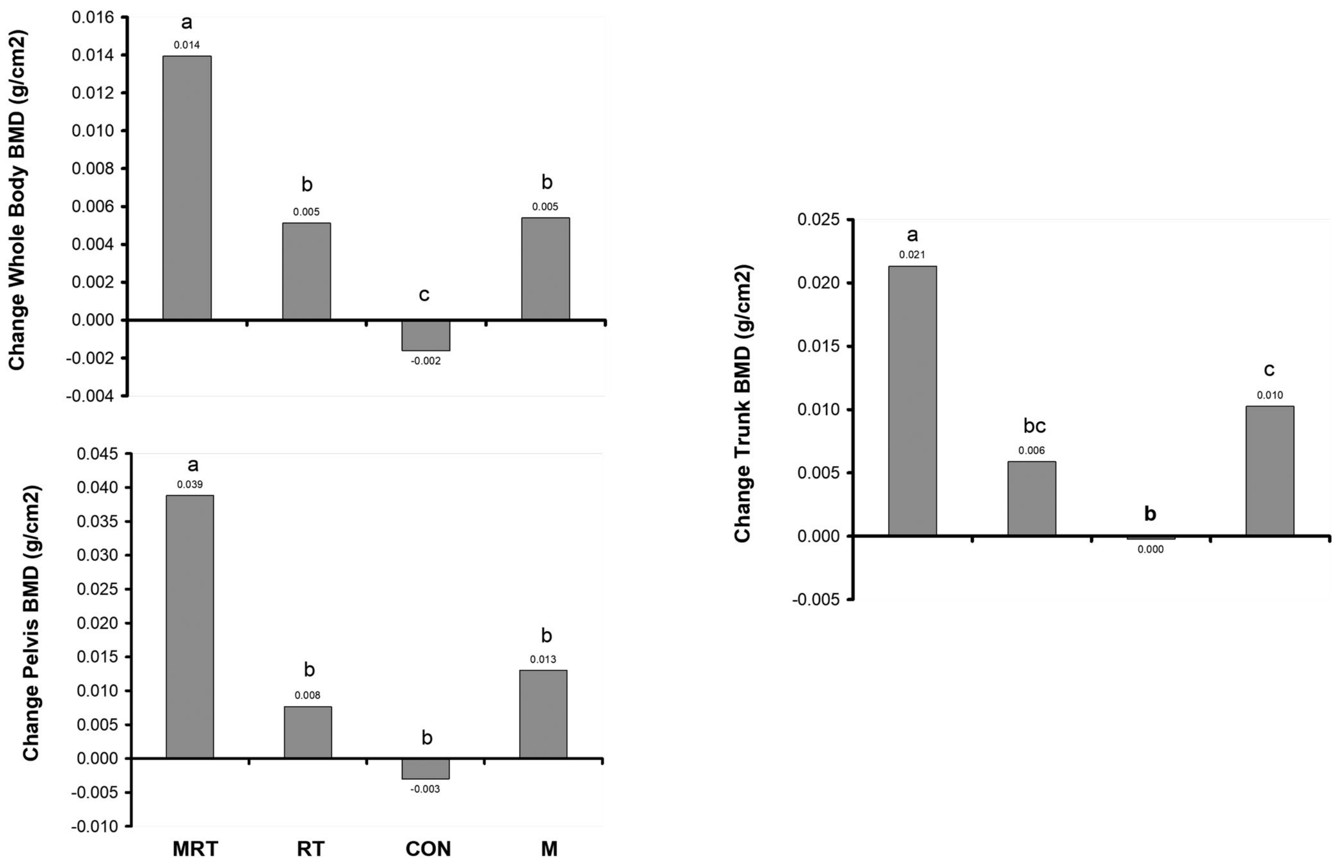

Figure 2. Changes in BMD for whole body, trunk, and pelvis after 12 weeks. MRT = Milk + Resistance Training; RT = Resistance Training; CON = Control; M = Milk. Values with a different letter are different (P 0.05).

over a relatively short time of training. This has important implications for health and fitness programs for young women and may potentially impact peak bone density acquisition during adolescence. This was the first study to our knowledge to investigate the combined effects of milk supplementation and resistance training on bone except for our prior work in young boys (28). Both milk supplementation and resistance training had independent effects on BMD. Statistically, significant main effects of these interventions in the absence of a significant interaction effect indicate additive effects of the treatments. One other study has examined the independent and additive effects of calcium supplementation (1000 mg/d) and exercise on bone in adolescent girls, but used a cardiovascular exercise intervention for 15.5 mo (44), as opposed to heavy resistance training in this study. The girls were very close in age to the girls in the present study. An independent effect of calcium supplementation, but not exercise, was found. However, the study had very

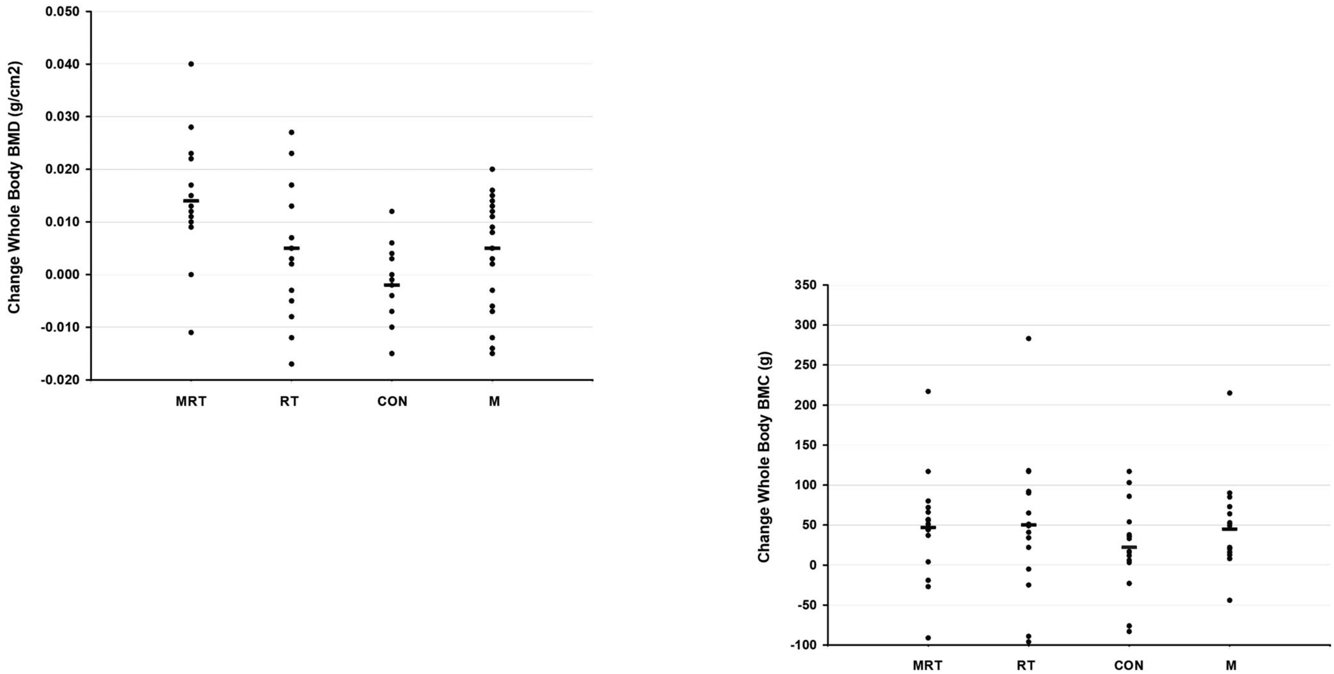

Figure 3. Individual changes in whole body BMD and BMC. Mean for the group is shown by a dashed line. MRT = Milk + Resistance Training; RT = Resistance Training; CON = Control; M = Milk.

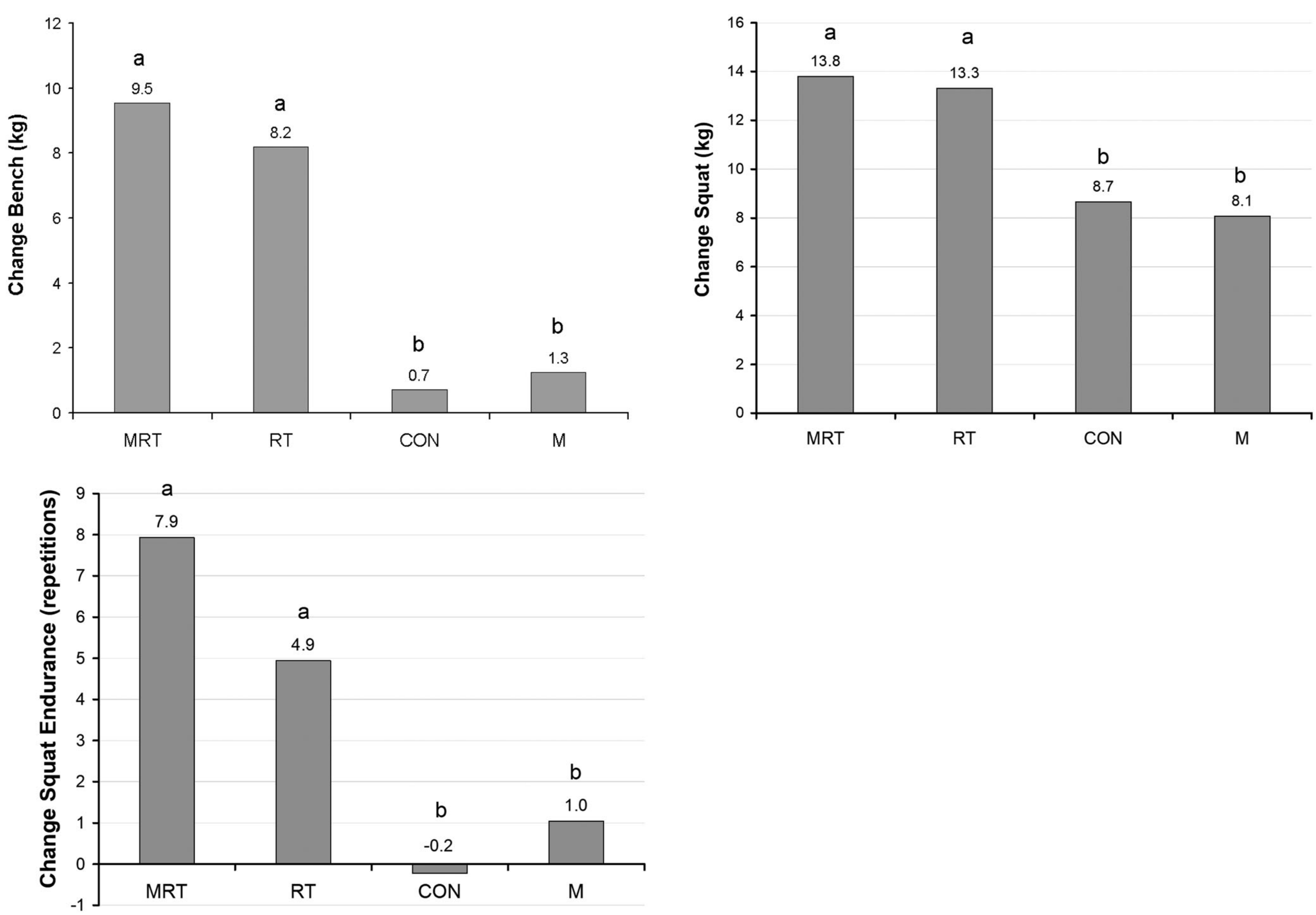

Figure 4. Changes in maximal bench press strength, squat strength, and repetitions during a squat endurance test after 12 weeks. MRT = Milk + Resistance Training; RT = Resistance Training; CON = Control; M = Milk. Values with a different letter are different (P 0.05).

poor compliance with the exercise intervention again making interpretation of the potential additive effects impossible to evaluate accurately.

The finding of an additive effect of milk supplementation and resistance training in this study was novel and illustrates that prior work in this area has not established an optimal intervention to impact BMD in adolescent girls. An additive effect was hypothesized because both interventions work through different mechanisms. Milk supplementation provided additional building blocks in the form of calcium to support an improved BMD, whereas resistance training provided an overload stimulus to increase strength and therefore density of bone. These findings highlight the plasticity of bone to lifestyle interventions in adolescent girls. Combination approaches to improve bone measures should be investigated to validate and refine these findings.

Although this investigation was short-term, the results in regards to the bone health and muscular strength, and composition provide powerful data. This indicates that the combined use of both resistance training and milk substitution provides a powerful tool for young girls bone health. What remains to be demonstrated is whether these changes will continue to increase over a longer intervention period (e.g., 6–12 mo). Additionally it is uncertain if changes will persist into adulthood, both interventions were highly supervised and it is unknown if subjects will continue to consume milk and/or train after the study is over without supervision in a free living environment. However there is data to support that fluid milk consumption has lasting effects on bone (up to 1.5–3.5 years) over calcium supplementation after the supplementation has ceased (45, 46). Measurement of nutrient intakes by self-report of free living populations is semi-quantitative and despite the fact that there was a great deal of time and effort in dietary counseling and record keeping to support efficacy of the milk intervention there is always some degree of error associated with nutrient intakes. Thus the reason a 7DD was used was because it is preferred over a 24 h recall, FFQ and short duration diet record collections (47, 48). Every effort outside of a feeding study was employed to minimize error from the 7DD and FFQ, which are typically self-administered. We stayed with participants and parents while they filled out the questionnaires.

Exercise has a beneficial effect on bone but only a few studies have specifically examined the impact of resistance training on bone in adolescent girls. In this study, the effects of resistance training were most evident on BMD assessed in the whole body, trunk, and pelvis. There was also a trend for greater BMC in the legs. Morris et al. (49) studied premenarcheal girls aged 9-10 y involved in a weight training program involving exercises for all major muscle groups performed at the same frequency (3x/wk) as this investigation, but for a longer duration (10 mo). In the Morris et al. (49) study the resistance training group demonstrated significantly greater increases in BMC measured at whole body, lumbar spine, and femoral neck and BMD measured at whole body, lumbar spine, femoral neck, pelvis, leg, and arm compared to control. Although the authors acknowledged a large proportion of bone mineral accrual was related to growth, the resistance training group had a positive effect on bone and lean body mass compared to normally active controls. The greater increase in BMC by Morris et al. (49) would be expected because the girls were younger and therefore accruing bone mass at a greater rate compared to the girls in this study. Also, the intervention was over a longer period of time compared to this study.

Blimkie et al. (30) and Nichols et al. (50) reported no significant effects of a 6.5 mo and 15 mo (respectively) whole body resistance training program (3x/wk) on whole body and lumbar spine BMD in adolescent girls more similar to the age of the girls in this present study (aged 14–17 y). In this investigation the training program was a periodized progressive varying training load program designed to increase in intensity with a concomitant decrease in volume over the 3 mo to maximize strength and power and thus facilitate changes in body composition and bone mineral density. In the Blimkie et al. (30) study, the resistance training program involved a circuit of 4 sets of 13 exercises performed on hydraulic machines that provided an isoinertial resistance. The resistance training did result in consistently higher BMC and BMD responses, especially in the lumbar spine, compared to controls, but failed to reach significance. Potential reasons for the lack of significant effects could be the lack of appropriate intensity, progression, and periodization of the program, provision of a weight belt that in some cases may have unloaded muscle forces in the lift, and/or the relatively low calcium intake assessed via 3-day diet records (<1000 mg/d). The program used by Nichols et al. (50), consisted of 15 exercises incorporating a combination of machines and free weights similar in design to this investigation. However the results must be interpreted cautiously because of the large attrition (i.e., only 5 of 46 girls completed the 15 mo training program).

One possible reason for the lack of significant effects of resistance training on bone reported by Blimkie et al. (30) and Nichols et al. (50) could be the low calcium intake. The mean calcium intake assessed via 3-day diet records in the exercise group was less than 50% of the RDA at 545 mg/day (50). In the Morris et al. (51) study that showed a benefit of resistance training on bone, calcium intakes were assessed via FFQ twice during the 10 mo protocol and were at least closer to the RDA at 1000 mg/d). The lower calcium is probably not the sole reason because the RT group in this study experienced benefits on BMD despite a mean calcium intake of 703 mg/d. However, increasing calcium to levels greater than the RDA are probably beneficial because in this study the effects of milk supplementation and resistance training were additive. Thus, the background calcium intake appears to have some impact on the influence of resistance training on bone mass.

Resistance training is a potent stimulus for muscle hypertrophy and thus it was hypothesized that lean body mass would be increased. Resistance training also targets primarily the lean components of body composition. Indeed, heavy resistance training resulted in significant increases in lean body mass compared to controls. For the main effect of resistance training, the RT and MRT groups increased body mass and lean body mass by 1.6 and 1.0 kg, respectively; while the M and CON groups increased body mass and lean body mass by 0.6 and 0.1 kg, respectively ($P < 0.05$). In the Morris et al. (51) study the preadolescent girls exercise group gained significantly more lean mass, less body fat content, however no significant changes were noted in anthropometric measures. Conversely, Blimkie et al. (30) and Nichols et al. (50) reported no effects of training on any anthropometric or body composition variables. This again could be due to the lack of an effective training program design or adherence to the needed heavy intensity of lifting in adolescent girls.

Weight training stimulates protein synthesis and muscle hypertrophy as well as the acute hormonal responses of important anabolic hormones and growth factors that are closely related to growth and development of muscle (i.e., GH, IGF-1). especially over adolescence (14). Although, specific training program variables can be configured differently the training induced gains as reported in this investigation and the Morris et al. (51) study are associated from a more effective stimulus for protein synthesis and muscle hypertrophy. The stimulus in the Blimkie et al. (30) and Nichols et al. (50) investigations were probably not effective due to the lack of appropriate intensity, progression, and periodization of the program, and possibly the provision of a weight belt in the Blimkie et al. (30) study. Whereas, in the Nichols et al. (50) investigation the power to evaluate the results must be interpreted carefully because due to the large attrition with only 5 of 46 girls completed the training program. This study indicates that adolescent girls can safely benefit from periodized heavy resistance training and have favorable results in terms of strength and body composition gains. Fat mass was not affected probably because energy intake was not reduced.

Few data exist regarding the effects of a periodized resistance training program in adolescent girls. It was hypothesized that resistance training would have a greater impact on muscular performance compared to matched controls. As expected, muscular performance was significantly greater in the resistance training groups RT and MRT compared to M and CON. While not a particularly surprising finding it serves to further demonstrates that resistance training, when performed and implemented properly, provides many benefits for adolescent girls especially for strength gains (14, 52, 53). The 1-RM squat and bench in the RT and MRT groups significantly increased compared to the M and C groups. The M and CON groups did have a slight improvement from pre to post testing 1-RM's but these changes were not significant and were likely due to a minor learning effects despite familiarization sessions. Squat endurance repetitions were also significantly greater in the MRT and RT groups compared to the CON and M groups. Expected hierarchy of exercise types were observed in that squat 1-RM's compared to bench press gains were greater. No data exists in adolescent girls to make any age appropriate comparisons. The use of a heavy periodized resistance training protocol in adolescents girls used in this investigation differs greatly with the three previously presented resistance training investigations in adolescent girls (30, 50) and preadolescent girls (51). However, because these three training programs would not be considered heavy resistance training protocols that provide progressive overload using near-maximum efforts the interpretations gathered from the strength gains in their respective studies would differ from this investigation (34).

The girls in this study were all low milk drinkers and in late puberty (mean Tanner stage 4.3 ± 0.6) and represent a population that has been relatively understudied in regards to nutrition and exercise interventions, especially resistance exercise training. Overall, the findings indicate that both milk supplementation and resistance training each had independent effects on bone health. Further, resistance training had benefits on lean body mass and muscle strength. Again, a major finding in this study was that the effects of milk supplementation and resistance training on BMD were additive. The findings provide strong evidence that life style modifications (i.e., changing nutrient adequacy through milk supplementation and overloading the musculo-skeletal system through progressive heavy resistance training) are effective to impact bone health in girls during the late adolescent period.

As expected, milk supplementation resulted in improved overall nutrient adequacy. Compliance to the milk supplementation protocol in this study was excellent as verified by 7DD, beverage records and signed statements by subjects, parents and another household witness. Energy intake and the distribution of macronutrients were similar to national trends from 1994 to 1996 in adolescents (54). Total fat accounted for approximately 30% of total energy, slightly below the 1995 average of 33%. The type of fat was mainly saturated, with moderate amounts of monounsaturated, and very little polyunsaturated fat. This balance of fatty acids is reflective of some of the more common food choices made by the girls in this study (e.g., fast food burgers, French fries, and pizza). Protein was higher in the milk groups (about 15% of total energy) compared to the non-milk groups (about 12% of total energy).

Milk is a rich source of several vitamins and thus contributed to a more favorable nutrient intake for the majority of water-soluble and fat-soluble vitamins (see Table 5). Nutritionally, the majority of subjects in the MRT and M groups had exceptional intakes for most vitamins, with the exception of folate and vitamin E, which were also poor in the non-milk groups. Nutrient adequacy in the RT and CON groups was relatively poor with the majority of subjects failing to consume two-thirds of the RDA/AI for several B vitamins and fat-soluble vitamins (e.g., vitamins A, D, and E). It is worth noting that none of subjects in the non-milk groups reached two-thirds of the RDA for vitamin D, whereas 100% reached this level in the milk groups. All subjects in the milk groups reached two-thirds of the RDA for vitamin B1, vitamin B2, vitamin B12, and vitamin A.

Milk is also a rich source of several minerals. Non-milk groups were deficient in many minerals, with less than half of subjects meeting two-thirds of the RDA for calcium, copper, iron, magnesium, manganese, phosphorus, potassium, and zinc. Milk groups had better nutrient adequacy for calcium, iron, magnesium, phosphorus, potassium, and zinc; however they still had poor intakes of copper, magnesium, and potassium.

Energy intake overall has been increasing over the years and the source has been from carbohydrates, specifically simple sugars, derived from soft drinks, juices, and candy. These also represented some of the most common food consumed by the majority of girls in this study. A surprising observation from the detailed beverage logs kept daily for the entire 12 weeks was regular consumption of flavored coffee drinks, which contain primarily empty calories and contribute to poor nutrient adequacy. To our knowledge this beverage pattern has not been documented in any of the published reports of dietary behaviors in adolescents and may reflect the increased availability of numerous combinations of coffee drinks and a trend of socializing in coffee houses. The majority of these coffee drinks had to be manually entered into the nutrient analysis software.

Calcium intake in the non-milk group was 758 mg/d or 58% of the RDA for adolescent girls. This poor calcium status is similar to other studies reporting nutrient intakes in adolescent girls (55, 56). Calcium intake in the milk groups was 1581 mg/d or 122% of the RDA. Noteworthy is the fact that each girl in both the M and the MRT group had an individual calcium intake greater than the RDA (1300 mg/d). Many calcium intervention studies had poor compliance and used an intent-to-treat analysis where data from drop-outs and noncompliant subjects are used. The physiological relevance of this approach is questionable. A major advantage of this study was that compliance was high and drop-outs low, which resulted in groups that were dichotomous in calcium intakes (i.e., all girls in the milk group had significantly highly calcium intakes than the non-milk groups). From a practical point of view, it is important to note that despite being low dairy consumers at the start of the study, girls were very amendable to drinking an additional three servings of milk as evidenced by the high compliance and further verified by personal communication with subjects. Additionally, taste did not seem to be a reason most girls were low milk drinkers. Increasing availability and educating girls on the health aspects of milk would seem to be effective measures to increase nutrient adequacy of adolescent girls.

The role of increased dairy consumption in adolescent girls has been brought into question as to not having any effect (25) albeit other findings suggest that increased dairy consumption is associated with decreased adiposity (10, 11, 57). Other work has focused on the role of calcium/dairy in promoting or preventing unhealthy weight gain in children and adolescents (10, 11, 57). In this study there were significant main time effects for height, body mass, fat mass and lean body mass indicating these girls were still growing, but milk did not affect the rate of change in any variables with the exception of body mass and abdominal fat. For example, the weight gain in the M group was 1.2 kg versus 0.0 kg in the CON group. This is in contrast to the findings of other studies that reported supplementation with calcium-rich foods does not increase weight gain in preadolescent and adolescent girls and another that showed no effect in girls who were overweight (10, 11, 25, 57). Despite similar calcium levels in the present investigation compared to these studies, the finding in this study was that weight gain was greater in the M group compared to CON. The M group consumed an average 312 additional kcal/d in this project, which would account for weight gain. The reason these other studies failed to find a weight gain despite greater caloric intake remains unclear.

Cross-sectional studies conducted in adolescent girls have concluded that changes in body composition were related to the mix of nutrients found in dairy foods, specifically calcium and protein may allow the body to break down and burn fat more effectively. Protein is 3–4-fold more thermogenic than carbohydrates or fat (58), but the higher protein intake in this study did not fully compensate for the greater energy intake resulting in greater weight gain. It could be argued that the predicted increase in body weight based on an increase of 330 kcal/d should have been more than the observed weight gain. The thermogenic effect of protein and the possible loss of some energy in the feces could have blunted the increase in body weight. For example, one study showed that one week of a high calcium (1800 mg/d) moderate protein (15% of total energy) diet resulted in an increase fecal energy loss of 84 kcal/d. This mechanism may explain many of the studies showing greater weight loss with calcium-rich diets. Lastly, calcium is not the "magic bullet" (59) and effectively maintaining energy balance in individuals, weather through diet, exercise or both continues to be the primary battle in the global epidemic of obesity.

The use of nutrient intakes calculated from 7DD were compared to those obtained using the NUT-P-FFQ. Compared to the 7DD, the NUT-P-FFQ consistently overestimated most nutrients. While, several nutrients were within a reasonable range reported by the NUT-P-FFQ, diet records provide greater accuracy and reproducibility than a FFQ or a 24 h recall (not used in this investigation) (47, 48). The number of days needed for food records to accurately estimate mean daily calcium intakes within 10% of its true value has been shown to range from 7 to 12 days for young females (60, 61). A strength of the current investigation was the use of two 7DD resulting in a total of 14 days of dietary information. All of the exercise interventions, and the majority of the dietary interventions, except for one that used 7DD (10), used FFQ and 3DD or did not clearly specify how nutrient intakes were calculated. Although FFQ are a validated measure of nutrient intakes (62), diet records are a superior measure (63) and thus caution must be used when interpreting the results of FFQs. In this investigation calculated nutrient intakes via the FFQ resulted in significantly greater intakes for many nutrients compared to 7DD, although for calcium it was not significantly different. It should be pointed that the time spent providing instructions and ensuring accuracy was probably above that performed in other studies that use FFQ to quantify nutrient intakes and thus the differences between these two assessment techniques may be underestimated.

In order to better understand the baseline effects of the milk intervention supplementation on the beverage consumption patterns during weeks 2–11 beverage consumption patterns were tallied via the beverage logs for the two control groups. A total of 70 days of consumption patterns were ascertained and revealed that adolescent girls have similar patterns between groups with the exception of the milk intervention, shakes and teas. Teas, shakes, and soda were significantly greater in the CON group versus M group. The milk supplement as reflected by the 1% milk was greater in the M group compared to CON. The amount of milk intake reported by the girls in the CON group is lower 5 fl oz/d compared to a national survey for milk 8 fl oz/d (32). In contrast, the amount of non diet soda the girls in this investigation drank was 4.5 fl oz/d compared to the national survey of 14 and 16 fl oz/d (19, 64). The prevalence of soft drink consumption among youth, particularly the ages of 6–18 years has increased 48% (64). The mean intakes of soft drinks have more than doubled from 5 fl oz to 12 fl oz/d. Although not reflected in the beverage consumption patterns in the girls in this investigation the prevalence of obesity among youth has increased dramatically, suggesting that energy intake exceeds needs. Increased carbonated soft drinks, fruit drinks, fruit drinks, could increase adiposity in youth (64). These adolescent girls in this investigation were normally active and were not overweight. Questions exist as to the effects of declining activity levels while the beverage patterns observed in this study persist with higher non-diet soda intakes than milk.

As hypothesized, milk supplementation resulted in significant improvements in several bone measures. The intake of milk had a significant effect on arm BMC and whole body, arm, trunk, and pelvis BMD. There was a trend for improved BMD at the spine from the whole body scan ($P = 0.082$), but this was not confirmed by the specific spine scan from L2-L4 ($P = 0.511$). The general lack of an effect of milk on BMC was expected. Peak bone mass is generally reached during mid to late adolescence (18 y) (65–67). However, certain sites such as the arm may continue to accrue bone mass into adulthood (66). The significant effect of milk on arm BMC in this study indicates that this region may be sensitive to the effects of increased dairy/calcium during the mid to late adolescent period.

The favorable effects of milk on BMD in this study were consistent with several other intervention studies in adolescent girls involving either calcium supplements or milk/dairy supplementation. Two notable exceptions were 12 mo calcium supplement interventions, which both failed to show significant effects on BMC and BMD. Calcium supplementation was adequate (1000 mg/d) in the Rozen et al. (68) study, but compliance was poor with only 23% of the calcium supplemented group having a calcium intake >1300 mg/d. In contrast, compliance was relatively high in the Molgaard et al. (69) study, but calcium supplementation may have been inadequate (500 mg/d) resulting in suboptimal effects on bone.

Studies showing a positive effect of calcium supplementation (70–73) or dairy interventions (10, 11, 74, 75) on BMD in adolescent girls have ranged in duration from 6 months to 7 years. This is the first study to our knowledge to show significant effects after 3 mo. Despite the short duration, the magnitude of increase in this study after 3 mo was similar or greater to longer duration interventions. Several methodological aspects of this study may have contributed to the significant increases in such a short time period. First, compliance was extremely high in this study, whereas it was poor to moderate in other investigations and tended to decline as a function of time. Also, the fact that milk was used in this study may have contributed to better results compared to studies that used calcium supplements because of the potential benefit of other nutrients (i.e., vitamin D, protein, CLA, and other micronutrients). Finally, calcium intake was well above the RDA (>1500 mg/d) and this was consistently achieved by all subjects.

The studies with dairy interventions are more directly comparable to the current study because of the similarity in the supplement. Most used milk and or a combination of milk and other dairy products. Chan et al. (11) investigated the effect of a 12 mo daily, milk and dairy product supplement of 1200 mg/d compared to 900 mg/d in this investigation and the dairy group had a significantly greater increase in lumbar spine BMD and whole body BMC than controls. Similarly, our BMD in the whole, arm, trunk and pelvis significantly increased. The spine skeletal site was almost significant and had the study been a longer duration investigation the lumbar spine BMD likely would have been significantly increased as well due to lumbar bone being primarily comprised of trabecular bone. Cadogan et al. (10), Merrilees et al. (75), and Matkovic et al. (74) all reported improvements in BMD and some reported BMC with in an increase milk and or dairy supplementation versus control. The sites that were significantly improved included the lumbar spine, femoral neck and the greater trochanter. Although this investigation did not measure the greater trochanter or the hip the type of bone at this site is primarily trabecular.

Other studies have demonstrated that the beneficial effects of calcium supplementation are most evident during the early adolescence period (70, 71). This study is unique because positive effects of milk supplementation were observed in girls that were in mid to late adolescence and Tanner stage 4 and 5. Thus, girls may still benefit from additional calcium/dairy even if they have gone through their primary growth spurt. It is unknown whether the increases in BMD in this study will be maintained into adulthood. Supplementing with milk has been shown to have lasting effects on bone mass for 1.5 to 3.5 years after discontinuation (45, 46). Therefore, one could predict that that increased BMD in the current study would be maintained for a similar time period.

In summary, inadequate calcium consumption among adolescent girls is a serious health problem that has been identified since at least 1970 (76). Campaigns to increase milk and dairy consumption in all populations but particularity adolescent girls and young women have essentially failed. However, efforts to educate and promote milk consumption should continue because milk and dairy products are an excellent source of calcium and other nutrients and constitute the major source of calcium in the diet. Milk may also be obtained at a very low cost relative to its nutritional value and offers high calcium bioavailability. Additionally, calcium supplementation and the continuation of calcium fortification should be explored to help adolescent girls achieve their calcium recommendation of 1300 mg/day of calcium. With the incidence of individuals with osteoporosis increasing in the United States and the rate of fracture increasing in youths due to increased soda consumption, more attention should be paid to dietary and exercise habits over the lifespan (77–80). Improved optimal dietary intakes and exercise interventions may be keys to reversing these negative trends. In the face of a host of misinformation on resistance training for adolescents, especially girls (e.g. fears of getting big), and trends of alternatives to milk consumption, our findings indicate that both can provide important synergism for optimizing health and fitness benefits.

Conclusion

The use of resistance training with optimal diets containing more dairy products and/or calcium supplementation may help to impact peak bone mineral density and content. Based on the favorable effects of increasing fluid milk when combined with 12 wks of resistance training in this investigation a longer duration study of milk supplementation and resistance training is needed. Therefore, over the course of 3 mo the effects of 1300 mg/d or more of fluid milk combined with a heavy resistance training program resulted in the additive effects of greater nutrient adequacy and BMD in adolescent girls.

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Disclosure statement

Dr. Kraemer is a compensated consultant for the American College of Nutrition and a compensated member of the AdvoCare's scientific and medical advisory board. Dr. Jeff Volek has received royalties for books on ketogenic diets, he has served on scientific advisor boards for Virta Health, UCAN, Advancing Ketogenic Therapies, Axcess Global and Atkins Nutritionals, and is a founder and holds stock in Virta Health. None of the other authors have any conflict of interests to claim for this study.

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