Pediatric RESEARCH

CLINICAL RESEARCH ARTICLE

Dairy product intake decreases bone resorption following a 12-week diet and exercise intervention in overweight and obese adolescent girls

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BACKGROUND: We examined whether increased dairy intake was associated with changes in the levels of bone-related biochemical markers in overweight/obese adolescent girls undergoing a 12-week diet and exercise intervention.
METHODS: Thirty-five girls were assigned to a low dairy group (LDa; 0–2 servings/day; $n = 16$) or a higher dairy group (RDa; 4 servings/day; $n = 19$). Morning, fasted/resting blood samples were collected before and after the intervention and serum concentrations of procollagen-type-1-N-terminal-propeptide (P1NP), β-isomerized-C-terminal-cross-linking-telopeptides (β-CTX), osteocalcin (OC), 25-hydroxyvitamin-D, sclerostin and parathyroid hormone were measured.
RESULTS: At baseline, there were no significant differences between groups in any bone variable. Changes (Δ) over time in β-CTX ($p = 0.035$; interaction) and OC ($p = 0.015$; interaction) were significantly different between groups characterized by decreases in RDa and increases in LDa. P1NP and P1NP:β-CTX ratio decreased in both groups (main time effects: $p = 0.003$, $p = 0.041$, respectively). ΔβCTX ($r = -0.37$; $p = 0.028$) and ΔOC ($r = -0.39$; $p = 0.021$) were correlated with average number of dairy servings consumed during the study and with each other ($r = 0.45$; $p = 0.006$). ΔOC was not correlated with ΔP1NP ($r = 0.19$; $p = 0.27$).
CONCLUSIONS: Our results suggest that the osteogenic response to a diet and exercise program in this population can be improved with increased dairy intake via a decrease in bone resorption.

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IMPACT:

- We demonstrated that bone resorption significantly decreased over the intervention period in the group consuming adequate levels of dairy products compared to the group consuming little to no dairy products. Change in bone resorption was negatively correlated with average number of dairy servings consumed during the study. Our results suggest that the osteogenic response to a diet and exercise program in this population can be improved with increased dairy intake via a decrease in bone resorption.
- This is the first study to date to assess changes in bone marker status following a lifestyle intervention with exercise and different intakes of dairy products in a sample of OW/OB adolescent girls. We provide evidence that increased dairy product intake is associated with beneficial changes in circulating levels of bone-related biochemical markers in these girls undergoing a 12-week lifestyle (nutrition counseling and exercise training) intervention program.
- The main impact of our work relates particularly to the recent changes to Canada's food guide. Using the old recommendations, we demonstrated that the inclusion of 3–4 servings of mixed dairy foods per day improved bone health (primarily as a decrease in resorption) in OW/OB adolescent girls and that this level of dairy product intake appears appropriate and should still be encouraged for this age group. We also demonstrated that adolescent girls, a group that usually does not sufficiently consume dairy products, also improved their BMI percentile and nutrient intake with the inclusion of dairy products in their diets.

INTRODUCTION

Adolescence is a critical time period for the accrual of bone mass.[1] According to the 2016 USA National Osteoporosis Foundation's position statement on lifestyle factors that affect peak bone mass development, the only factors that have strong or moderate level evidence to positively affect bone include the consumption of dairy foods, dietary calcium, vitamin D and exercise.[1] However, dairy consumption among adolescents in the USA[1] and Canada[2] is insufficient, with 94% of girls ages 14–18 years and 83% of girls ages 10–16 years not consuming the recommended minimum intakes of 3 cups of milk/day or 3 servings of dairy/day respectively. In terms of exercise, only 35% of Canadian youth

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ages 5–17 years meet the recommended guidelines for physical activity.[3] Lack of physical activity, as well as an increase in sedentary behavior is linked to overweight and obesity.[4] Low dairy intake, coupled with low levels of physical activity and obesity,[5] likely sets youth on a trajectory into adulthood with compromised bone health and an increased risk of chronic disease.

Children who are OW/OB have excess body fat and may also present with suboptimal bone status and higher fracture incidence.[6] According to previous cross-sectional studies, a negative association exists between fat mass and bone mass in adolescents, once lean mass is accounted for.[7] Our previous work has also shown that a higher body fat content negatively affects markers of bone formation in early and late-pubertal girls.[8] Higher fat content in the bone marrow may negatively affect osteoblast differentiation, increase osteoclastic activity and impair bone mineralization (i.e. bone mineral density; BMD).[9] This is problematic since a lower peak bone mass (defined as the amount of bone accrued by the end of skeletal maturation) and compromised bone strength predicts a greater fragility fracture risk in adulthood.[10,11] A meta-analysis of randomized controlled trials and cohort studies from 2008[11] as well as a systematic review from 2017[12] demonstrated that increased dairy product (and calcium) intake improved bone mineral content in normal weight children and adolescents, particularly those with low baseline calcium intakes,[11] and that this effect was greater when exercise was combined with calcium.[13] No such data exist for OW/OB youth, yet they may be particularly at risk for impaired bone development.

Our previous research utilizing a lifestyle approach to assess changes in bone biomarkers in OW/OB premenopausal adult women (age 18+ years) demonstrated that diets higher in dairy foods, combined with daily exercise, favorably affected bone metabolism.[14] However, it is even more important to examine whether such a lifestyle program can lead to positive bone adaptations in a younger cohort of OW/OB adolescent girls, i.e., precisely during the critical period of peak bone mass accrual and development. Thus, the purpose of this study was to examine whether increased dairy intake (4 servings/day according to the 2007 Canada's Food Guide[15]), during a 12-week diet and exercise intervention program, improves circulating levels of bone-related biochemical markers in OW/OB adolescent girls. Based on the previous results in young OW/OB adult women,[14] we hypothesized that 12 weeks of exercise combined with dairy foods would improve the bone turnover response compared to 12 weeks of exercise without dairy.

METHODS

Participants

The present study includes data from 35 (mean age 14.3 ± 1.5 years; range 12.0–16.9 years) out of 63 adolescent girls (age range 10.0–18.5 years) who took part in a larger lifestyle modification, weight management parallel randomized controlled intervention trial entitled, 'Improving Diet, Exercise And Lifestyle (IDEAL) for Adolescents', which was registered at *ClinicalTrials.gov* (NCT02581813), and was cleared by our institution's Research Ethics Board (BREB file # 14-284). Data and measurements in the present study represent a secondary analysis of the bone turnover response and have not been previously published. Participants were recruited from the Niagara Region, Ontario, Canada via advertisements in local media, social media, university press releases and flyers posted in the community, from pediatric and general practitioner physician clinics, and from elementary and high schools in the District School Board of Niagara. To be eligible for this study, participants had to be menarcheal, between the ages of 12 and 16.9 years, overweight (OW; ≥85–96 percentile BMI) or obese (OB; ≥97 percentile BMI) based on World Health Organization growth charts, low dairy consumers (0–2 servings per day), minimally active (activity 0–2 times per week) and otherwise healthy. All eligible participants and their parents/guardians were invited to Brock University, where they provided informed assent and consent, respectively.

Each participant completed a general health questionnaire to document medical history and medication use. Participants were excluded if they reported an allergy to dairy foods or lactose intolerance, were taking medications related to a chronic condition or that affected bone health, and/or were consuming vitamin or mineral supplements. After all entry criteria were met, participants were randomized. The randomization scheme for the larger/main study was as follows: participants were stratified by BMI percentile (either overweight or obese) and were randomly assigned (using a random number generator) to one of three different groups using an unblocked random allocation ratio of 2:2:1. The three groups were: recommended dairy (RDa), low dairy (LDa) or a no-intervention control group (Con), respectively. Two independent study coordinators enrolled the participants and assigned them to the groups based on the random allocation sequence. The group assignments were concealed from the main investigators. The present secondary analysis includes a subset of participants from the two intervention groups only (RDa and LDa), which were randomized equally (1:1). Supplementary Fig. 1 shows the CONSORT flow diagram pertaining to this study.

Study design and procedures

The *IDEAL for Adolescents Study* was a 12-week, diet and exercise intervention study in OW and OB adolescent girls carried out from June 2016−October 2018. Prior to commencing the intervention, participants visited the laboratory for baseline testing that included anthropometric measurements and a fasting blood sample, and were instructed on how to properly complete a 7-day food record to verify their low dairy intake and to learn about their habitual daily diet. After the initial visit participants came back to the laboratory for an exercise introduction session, where the parent/guardian and participant met their personal trainer, reviewed the exercise program, and outlined the exercise schedule for the next 12 weeks. Pedometers (Fitbit Zip™) were also provided to each participant during this visit for use during the study and after (as part of their compensation for participation). After the exercise introduction session, participants had their first diet consultation with a registered dietitian who reviewed the baseline 7-day food record with them and their parent/guardian and gave detailed instructions for beginning the dietary protocol. If the participant was randomized to the RDa group, dairy products were provided during this visit (and weekly thereafter), and additional instructions were provided regarding their consumption for the rest of the trial.

Exercise intervention

Participants in both groups (RDa and LDa) completed a structured exercise training program over the 12 weeks (3 times/week). The exercise intervention was individualized but based on the principles of progressive loading whereby the exercise trainers would change exercise variables (i.e. load/reps/duration/speed) to maintain a constant exercise stimulus. Exercise sessions lasted between 60 and 90 min. Each session began with a plyometric-based (jumping) warm-up for 5–10 min, followed by 20 min of aerobic training (on either a treadmill, cycle ergometer, elliptical or rowing ergometer) and either 10–20 min of resistance training using free weights or selectorized resistance exercise machines, or plyometric exercises. Upon completion, participants cooled down by stretching and walking. Participants consumed a drink immediately after each training session. The RDa group drank 1 cup (250 ml) of 1% chocolate milk, and the LDa group had 1 cup of a non-dairy, vitamin D- and calcium-free, carbohydrate-based, electrolyte drink. On days that participants did not receive formal exercise training, they were assigned various forms of physical

activity to achieve a predetermined number of steps during their leisure time.

Dietary intervention

Dietary counseling by a registered dietitian was provided five times during the study (weeks 0, 2, 4, 8, 12) to each participant individually. Energy requirements/expenditures were calculated for each participant using predictive equations from the Academy of Nutrition and Dietetics for OW/OB girls with a sedentary activity factor. This was used to prescribe a diet for weight maintenance (as opposed to weight loss) based on participant's age, height and body mass. Participants were provided with an individualized eating plan outlining their required macronutrient intakes in food group servings from Canada's 2007 Food Guide[15] corresponding to their calculated daily energy requirements to maintain energy balance. This was the current food guide throughout the duration of the intervention. All participants were counseled on consuming a healthy diet of fruit, vegetables, high fiber foods, whole grains, lean meats and meat alternatives. Participants were also asked to avoid processed foods, foods high in "bad" fats (trans and some saturated fat (SFA)), sugar-sweetened beverages, pastries and confection, and they were instructed not to take any vitamin or mineral supplements during the study.

The study was designed such that the RDa and LDa groups differed primarily in the source of protein they consumed (and associated nutrients), as the RDa group consumed half of their daily protein (~20% of total energy intake) from dairy sources. The RDa group was provided with (for the entire duration of the intervention) and instructed to consume 4 servings/day of mixed dairy products as recommended by the 2007 Canada's Food Guide.[15] These included: 2 cups of milk (white and chocolate 1%), 2 × 100 g cartons of 0% or 2% MF Greek yogurt (any flavor) and 42 g of full-fat cheddar or marble cheese. The LDa group maintained their low dairy intake (as per this study entry criterion) of 0–2 servings/day and continued to consume protein from other sources including: meat, egg, fish, chicken, legumes and grains. They were also asked to continue to refrain from consuming calcium-fortified beverages/foods.

Measurements

Anthropometrics and maturity. Height (cm), sitting height (cm), and body mass (kg) were assessed for each participant at weeks 0 and 12 by the same investigator. Standing and sitting height were measured using a stadiometer (Seca 213 Portable Stadiometer, CME Corp., Warwick, RI) to the nearest 0.1 cm with light clothing and no shoes. An additional measure of leg length (cm) was derived by subtracting sitting height from standing height. Body mass was assessed using a standard scale (Digital Physician Scale, Rice Lake Weighing Systems, Rice Lake, WI).

The somatic maturity offset (years from peak height velocity) was estimated using a sex-specific regression equation.[16] This is a simple, noninvasive method of assessing somatic maturity in children using known differential growth measures of height, seated height and leg length.

Food records. All participants provided 7-day food records at weeks 0 and 12 and 3-day food records at weeks 2, 4 and 8 before each dietetic counseling session to assess dietary intake, track compliance with the nutrition protocol and to provide guidance moving forward. The same investigator analyzed all food records using the Food Processor Diet analysis software program (ESHA Research, Inc. Salem, OR).

Blood and biochemical markers. Fasting venous blood samples were collected by phlebotomists from the median cubital vein in the antecubital fossa of each participant's arm using a standard venipuncture technique. Blood samples were obtained on two occasions (pre- and post intervention) between the hours of 0800 and 1000 after an overnight fast of 10–12 h. Blood was collected into SST vacutainer tubes and was allowed to clot (~10 min) before being centrifuged at ≤1300 RCF (*g*) for 15 min. Serum was separated and aliquoted into 0.5 ml polyethylene cryotubes that were stored at −80 °C until analysis upon study completion. Total Procollagen-type-1-N-terminal-propeptide (P1NP; cat# 03141071 190), β-isomerized-C-terminal-cross-linking-telopeptides (β-CTX; β-CrossLaps; cat#: 11972308 122), and total 25-hydroxyvitamin D (Vitamin D; # 05894913 190) were measured from serum at the Mount Sinai Hospital Core Laboratory (Toronto, Ontario) using a Roche e411 Elecsys automated analyzer for P1NP, and a Roche Cobas e602 automated analyzer for β-CTX and total 25-hydroxyvitamin D. Lower and upper detection limits were 5-1200 µg/l (quality control standard CV: 5.2%), 0.010–6.00 ng/ml (quality control standard CV: 4.8%) and 3.00–70.0 ng/ml (or 7.5–175 nmol/l; quality control standard CV: 6.2%), for P1NP, β-CTX and 25-hydroxyvitamin D, respectively. Sclerostin, osteocalcin (OC) and parathyroid hormone (PTH) were measured in duplicate using a microbead multiplex kit (Human bone magnetic bead panel, cat.# HBNMAG-51K-08, EMD Millipore, Darmstadt, Germany). The average inter- and intra-assay coefficients of variation (CV) for OC were 5.7% and 5.5%, for sclerostin were 13.5% and 6.4%, and for PTH were 4.3% and 5%, respectively. The ratio of bone turnover markers was calculated by dividing each participant's P1NP (ng/l) value with their corresponding β-CTX (ng/l) values at pre- and post intervention, as previously reported.[14]

Compliance. Compliance was calculated separately for the exercise and dairy components of the study. For the structured exercise program, compliance was determined as the percentage of exercise sessions attended relative to the number of scheduled exercise sessions over the 12 weeks. Compliance with the consumption of the dairy products for the RDa group was calculated as the average daily servings of dairy products consumed by the participants, as reported in their food records at weeks 4, 8 and 12, compared to those prescribed/provided. RDa participants were considered compliant if they consumed ≥3 of the 4 prescribed servings/day. LDa participants were considered compliant if dairy intakes were kept ≤2 servings/day.

Statistical analysis

Results are presented as mean ± standard deviation (SD) in the tables and mean ± standard error (SE) in the figures. Missing values (two values each for P1NP, β-CTX and vitamin D; one value each for OC, sclerostin, and PTH) were replaced with either the corresponding measured pre- or post-intervention value for that participant. Data were then assessed for normality using visual screening of histograms, *z*-scores for skewness and kurtosis and the Kolmogorov−Smirnov test. All variables except for P1NP, OC, PTH and the P1NP:β-CTX ratio were normally distributed. These became normal after log transformation. Two-way repeated measures ANOVAs (RMANOVAs) were conducted on all variables to examine the change over time (pre- to post intervention, time effect), the differences between groups (RDa vs. LDa, group effect), and the difference between groups in the changes over time (group-by-time interaction). If a significant interaction was present, post-hoc pairwise comparisons (paired *t* tests) were conducted for each group separately. Independent *t* tests were also conducted to examine differences between groups at baseline. Following this, a sensitivity analysis was conducted, after outlier identification and treatment. Outlying values (with *z*-scores >2) were identified and replaced with the group mean value ±2 SD. This method of treating outliers retains their extremeness within the range limits of the dataset and helps to normalize our data.[17] From the 70 data points per marker (35 total participants, and 2 timepoints: pre- and post intervention), there were two outliers for β-CTX, six for P1NP, five for OC, four for sclerostin, one for 25-hydroxyvitamin D, and none for PTH. After outlier

Table 1. Anthropometrics and nutritional intake pre- and post intervention in the recommended dairy (RDa) and low dairy (LDa) groups of overweight and obese adolescent girls (mean ± SD).

Variable	RDa $N = 19$		LDa $N = 16$		RMANOVA (p values)		
	Pre	Post	Pre	Post	Group	Time	Group × time
Years from peak height velocity	2.0 ± 0.9	2.1 ± 0.9	2.5 ± 0.9	2.6 ± 0.9	0.12	<0.001	0.23
Height (cm)	163.4 ± 8.4	163.8 ± 8.4	163.5 ± 5.7	163.8 ± 5.5	0.98	0.002	0.53
Body weight (kg)	79.7 ± 15.5	79.7 ± 14.4	78.9 ± 13.9	77.6 ± 13.4	0.76	0.26	0.28
BMI percentile (%)	96.5 ± 4.6	95.6 ± 6.4	94.4 ± 6.1	93.3 ± 6.9	0.27	0.027	0.75
Energy (kcal/day)	1749.2 ± 369.7	1756 ± 364.7	1591.6 ± 468.2	1510.9 ± 180.5	0.065	0.56	0.49
Protein (g/day)	67.0 ± 18.8	86.7 ± 12.3*	64.71 ± 17.6	74.2 ± 11.0*	0.12	<0.001	0.044
Carbohydrates (g/day)	216.2 ± 50.9	207.5 ± 47.2	194.3 ± 57.0	177.3 ± 31.1	0.076	0.10	0.54
Fat (g/day)	70.9 ± 15.7	66.6 ± 22.1	64.8 ± 23.3	59.7 ± 10.8	0.15	0.31	0.93
Vitamin D (mcg/day)	2.2 ± 1.5	5.7 ± 1.0*	1.4 ± 0.9	1.7 ± 1.0	<0.001	<0.001	<0.001
Calcium (mg/day)	699.3 ± 269.3	1295.7 ± 185.2*	489.3 ± 179.7	504.2 ± 222.0	<0.001	<0.001	<0.001
Phosphorous (mg/day)	828.6 ± 332.2	1335 ± 204.8*	722.5 ± 205.8	877.3 ± 166.5*	<0.001	<0.001	0.001
Potassium (mg/day)	1555.8 ± 623	2359.4 ± 489.7*	1508.5 ± 494.6	1881.7 ± 437.6*	0.086	<0.001	0.031
Magnesium (mg/day)	167.3 ± 72.6	224.6 ± 54.6	158.5 ± 51.4	236.0 ± 71.0	0.94	<0.001	0.42

Significant at $p < 0.05$ (RMANOVA).
*Significant at $p < 0.05$ (post-hoc paired/within-group t test).

treatment, P1NP and PTH still required log transformation but OC and the P1NP:β-CTX ratio did not. Pearson correlations were conducted on the outlier-treated data to assess the relationships between nutrition, dairy servings and change in bone turnover markers as well as the relationships between maturity offset (years from age at PHV) and bone markers at the pre-intervention timepoint (baseline). For all statistical tests, significance was assumed at an alpha level of <0.05. Statistical analyses were performed using SPSS version 25.0 for Windows (SPSS, Chicago, Illinois, USA).

RESULTS

At baseline (pre-intervention), there were no significant differences in the anthropometric measures, years from peak height velocity, and dietary intakes for energy, protein, carbohydrates, fat, phosphorous, potassium and magnesium between groups. Intakes of vitamin D and calcium were significantly different between groups at baseline ($p = 0.040$ and $p = 0.012$, respectively). Both groups made improvements to their diet by demonstrating increased intakes of several nutrients over time (Table 1; protein, phosphorus, potassium and magnesium). BMI percentile also decreased in both groups (Table 1) without an accompanied increase in body weight and with a small increase in height. Daily intakes of dairy-related nutrients including protein, vitamin D, calcium, phosphorous and potassium showed significant group-by-time interactions indicating that the RDa group had increased intakes compared to the LDa group (by virtue of the study design) (Table 1; interactions).

At baseline, there were no significant differences between the groups in any of the bone biochemical markers or the bone turnover ratio. Two-way RMANOVAs showed significant group-by-time interactions for β-CTX (ng/l; RDa −97 ± 223 vs. LDa +68 ± 216) and OC (pg/ml, RDa −945 ± 1954 vs. LDa +696 ± 1665; log-transformed) such that both decreased from pre- to post intervention in the RDa group and increased in the LDa group (Table 2, Figs. 1, 2). Post-hoc pairwise comparisons indicated that OC significantly decreased ($p = 0.031$) and CTX showed a trend towards a decrease ($p = 0.077$) from pre- to post intervention in RDa with no change in LDa ($p = 0.18$ and $p = 0.23$, respectively). P1NP and the ratio of P1NP:β-CTX (both log-transformed) showed significant main effects of time indicating that P1NP and P1NP:β-CTX decreased overall. Following outlier treatment, the interactions remained for β-CTX ($p = 0.020$) and OC (not log-transformed; $p = 0.009$), but the main time effects for P1NP (log-transformed) and the P1NP:β-CTX ratio (not log-transformed) were no longer statistically significant ($p = 0.058$ and $p = 0.69$, respectively). Post-hoc pairwise comparisons following the interactions on the outlier-treated data indicated that both OC ($p = 0.029$) and CTX ($p = 0.038$) significantly decreased from baseline in RDa with no change in LDa ($p = 0.23$ and $p = 0.14$, respectively).

Pearson correlations were conducted on the outlier-treated data across the whole sample ($n = 35$). Change in β-CTX ($r = -0.37$; $p = 0.028$) and change in OC ($r = -0.39$; $p = 0.021$) were negatively correlated with average number of dairy servings consumed during the study. Of note, change in β-CTX was also correlated with change in OC ($r = 0.45$; $p = 0.006$) and change in sclerostin was correlated with change in (log-transformed) PTH ($r = 0.49$; $p = 0.003$). No other bone variables were correlated with each other.

Pearson correlations were also conducted on the outlier-treated data across the whole sample between variables at the pre-intervention timepoint (baseline). Maturity offset (years from age at PHV) was negatively correlated with β-CTX ($r = -0.68$; $p < 0.001$), P1NP (log-transformed: $r = -0.71$; $p < 0.001$) and OC ($r = -0.44$; $p = 0.008$). These relationships indicate that as maturity increases, bone turnover decreases.

For exercise compliance, the RDa group attended 86% (range: 71–97%) of their scheduled exercise sessions, whereas the LDa group attended 80% (range: 56–94%) of their scheduled exercise sessions (independent t test between groups; $p = 0.065$). On average, both groups complied with the dairy protocol, according to the combined food record data from the weeks 4, 8 and 12. The RDa group consumed 3.9 ± 0.4 servings/day and the LDa consumed 0.2 ± 0.2 servings/day. Overall, 19/19 participants in the RDa group complied with consuming ≥3 servings/day of dairy products, and 16/16 participants in the LDa group maintained their habitually low intakes of ≤2 servings/day.

Table 2. Bone biochemical markers and bone turnover ratio in two groups pre- and post intervention in the recommended dairy (RDa) and low dairy (LDa) groups of overweight and obese adolescent girls (mean ± SD).

Variable	RDa $N = 19$		LDa $N = 16$		RMANOVA (p values)		
	Pre	Post	Pre	Post	Group	Time	Group × Time
P1NP (μg/l)[‡]	376.9 ± 308.7	300.1 ± 224.8	258.3 ± 200.0	251.4 ± 205.3	0.20	0.003[€]	0.066
β-CTX (ng/l)	1015.4 ± 331.3	918.9 ± 364.6[†]	831.6 ± 290.7	899.5 ± 317.2	0.34	0.71	0.035
Osteocalcin (pg/ml)[‡]	15380.1 ± 6081.0	14434.9 ± 4992.1[†, *]	12611.3 ± 5475.3	13307.3 ± 6335.3	0.22	0.82	0.015
Sclerostin (pg/ml)	2667.6 ± 1057.1	2576.8 ± 1200.6	2175.5 ± 1011.6	2036.8 ± 1082.3	0.17	0.12	0.74
25-hydroxyvitamin D (nmol/l)	81.7 ± 20.9	83.7 ± 22.3	77.2 ± 25.3	78.1 ± 27.6	0.51	0.59	0.85
Parathyroid hormone (pg/ml)[‡]	113.2 ± 68.2	108.0 ± 70.2	81.9 ± 37.2	78.8 ± 35.3	0.14	0.38	0.66
Bone turnover ratio (P1NP:β-CTX)[‡]	337.8 ± 164.4	304.8 ± 121.9	280.9 ± 127.7	252.9 ± 123.6	0.20	0.041[€]	0.57

No outliers have been treated/adjusted.
*Significant within-group pre−post difference, $p < 0.05$; post-hoc paired t test.
[‡]Statistics were performed on log-transformed data.
[€]Following outlier treatment, nonsignificant ($p > 0.05$) main time effect (P1NP $p = 0.058$; P1NP:CTX $p = 0.69$).
[†]Following outlier treatment, significant within-group pre−post difference $p < 0.05$; post-hoc paired t test (RDa: β-CTX $p = 0.038$ and OC $p = 0.029$).

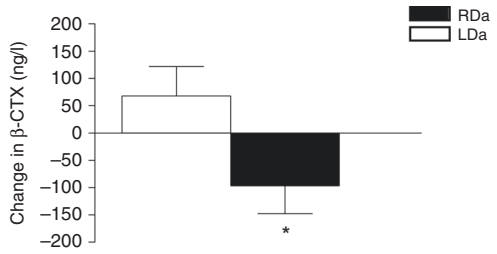

Fig. 1 Change in β-CTX concentration in the RDa and LDa groups (mean ± SE). *denotes significant difference between the two groups (RMANOVA interaction, $p = 0.035$).

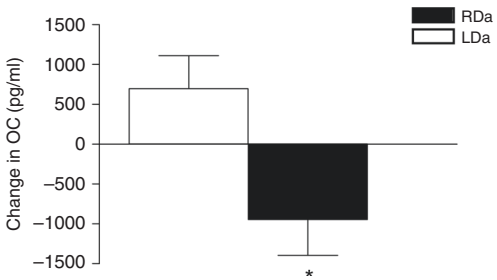

Fig. 2 Change in osteocalcin (OC) concentration in RDa and LDa groups (mean ± SE). *denotes significant difference between the two groups (RMANOVA interaction, $p = 0.015$).

DISCUSSION

This is the first study to date to assess changes in bone markers following a lifestyle intervention of dietary advice, exercise and different intakes of dairy products in a sample of OW/OB adolescent girls. We provide evidence that increased dairy intake is associated with healthful changes in circulating levels of bone-related biochemical markers in these girls undergoing a 12-week lifestyle (nutrition counseling and exercise training) intervention program for weight maintenance. Specifically, we found divergent changes in β-CTX and OC such that both decreased in the RDa group compared to the LDa group. Additionally, change in β-CTX ($r = -0.37$; $p = 0.028$) and change in OC ($r = -0.39$; $p = 0.021$) were negatively correlated with the average number of dairy servings consumed during the study, indicating that a greater intake of dairy foods is associated with lower bone resorption and, thus, lower bone turnover. The RDa group also showed greater increases (compared to LDa) in the intakes of several bone-supporting nutrients, including protein, calcium, vitamin D, phosphorous and potassium, likely related to the provision of dairy products throughout the 12-week intervention. The LDa group also increased their intakes of some of these nutrients from other food sources but not to the same extent as the RDa group. Nonetheless, positive changes in some nutrient intakes across both groups demonstrate that our dietary intervention (healthy eating advice to both and dairy product provision to the RDa group only) improved dietary intakes in both groups.

The decision to use dairy foods in this study was based on previous evidence in OW/OB premenopausal women demonstrating that the consumption of three or more servings/day of mixed dairy foods with exercise had beneficial effects on biochemical markers of bone metabolism.[14] These results are now extended by our study to younger girls. As P1NP decreased in both groups (but no significant change following outlier treatment), the changes experienced by the LDa group in comparison to the RDa group were more compromising to bone health. Specifically, the LDa group experienced an increase in collagen resorption, whereas the RDa group experienced a decrease in collagen resorption, as reflected in the β-CTX interaction and the post-hoc test in the RDa group, respectively. The decrease in collagen resorption is concomitant with the decrease in OC in the RDa group. Importantly, OC has a role in mediating overall bone matrix turnover, not just bone formation.[18] Change in OC was correlated with change in β-CTX ($r = 0.45$; $p = 0.006$) but not with change in P1NP ($r = 0.19$; $p = 0.27$), suggesting that increases in bone turnover are more strongly associated with and may be driven by increases in β-CTX. Thus, our data demonstrate that the benefits of dairy consumption for bone turnover seem to be more apparent in the process of bone resorption, which is less dominant during growth compared to bone formation.

Concentrations of some of the measured bone markers in our OW/OB girls were consistent with those of normal weight girls, while others were not. For example, the P1NP values observed in our study were within the normal range of reference values reported in the literature for girls of this age group.[19–21] OC levels are at the lower end of the normal range reported for girls of this age[19] while β-CTX is higher than the reference values previously reported.[21] These differences may relate to the adverse influence of excess fat tissue (particularly in the visceral region[22,23]) on BMD and bone metabolism in obese youth.[24] Obese adolescent girls tend to have higher circulating concentrations of leptin, estrogen, insulin and IGF-1, and these changes are not only associated with

accelerated pubertal development and bone maturation, but also with the increased infiltration of adipose tissue into the bone marrow which may negatively affect these processes.[25]

In the context of somatic growth, for which our participants were still undergoing, bone markers are naturally changing.[8] Our study demonstrated that at pre-intervention, somatic maturity (years from age of peak height velocity) was negatively correlated with β-CTX ($r = -0.68$; $p < 0.001$), P1NP (log-transformed: $r = -0.71$; $p < 0.001$) and OC ($r = -0.44$; $p = 0.008$). These relationships reflect that as maturity increases, bone turnover decreases. During the intervention, we observed that P1NP decreased over time in both groups (but no significant change following outlier treatment), which is to be expected with increased age[21,26]), but β-CTX behaved differently in both groups; increasing in LDa and decreasing in RDa. Also, there were no associations between P1NP and dairy intake. This is in contrast to what we have previously observed in young adult males where P1NP increased and β-CTX did not change following 12 weeks of Greek yogurt consumption and resistance training,[27] and in young adult females following 16 weeks of a high protein/high dairy diet with exercise in the context of weight loss.[14] It is possible that P1NP did not increase in this adolescent age group because it was already quite high, whereas concentrations were much lower in adult females[14] and males[27] allowing for greater increases in response to an osteogenic stimulus. There may be an age-related ceiling effect of formation since adolescents already have higher rates of bone formation than adults[28] and therefore, theoretically less room to improve.

Our correlations between dairy product intakes and bone markers as well as our main result of decreased bone resorption with increased dairy intake and exercise highlight the importance of the incorporation of dairy foods/nutrients into the diets of growing girls for improved skeletal health. This finding is particularly important now given the deemphasis of dairy foods in the new Canada's Food Guide,[29,30] and the very low documented consumption of these foods in adolescent girls.[1,2] Indeed, it has recently been reported that the dietary pattern recommended by the new Canada's Food Guide, while adequate in many micronutrients, is inadequate for meeting the recommended dietary allowances (RDAs) for calcium and vitamin D.[30] Our use of (3 to) 4 servings per day of dairy foods in the RDa group represented the previous recommendation, from the 2007 Canada's Food Guide.[15] Even though this is technically not the current recommendation in terms of servings (in fact, there are no current serving recommendations, only suggested relative proportions of three food groupings (i.e. 50% of the plate as vegetables and fruits, 25% as protein foods and 25% as whole grains)[29,30]), in our study, this level of dairy intake increased calcium, protein and most other dairy nutrients to near or above their respective RDAs.[31] Therefore, this level of dairy intake appears appropriate and should still be encouraged for this age group.

Despite the beneficial effects of dairy on bone resorption seen in our study, a recent randomized controlled trial by Vogel et al.[26] reported no effect of three daily servings of dairy (providing 900 mg/day of calcium) for 18 months on bone mineral accrual (assessed by DXA and pQCT) in OW or normal weight boys and girls. However, this was an effectiveness-focused intervention that did not include a structured exercise component or specific dietary advice/nutritional counseling. It also included less study visits and was undertaken across a wider age range (8–16 years; spanning puberty). Relatedly, 67% of the variability in total-body bone mineral content was attributed to growth variables (change in height, weight, maturity). Indeed, we also demonstrated strong correlations between bone variables and growth variables. Other reasons for the disparate results between our study and Vogel et al. may lie in the number of dairy products consumed as we provided 4 servings/day vs. their 3 servings/day of dairy. They also alluded to the inherent difficulty in repeatedly measuring these variables in growing children, hence why we narrowed our age range for the present analyses. Of note, another study carried out in healthy prepubertal Chinese girls who habitually consumed a low calcium/vitamin D diet reported that one milk drink fortified with calcium (560 mg/drink) and vitamin D (5–8 µg/drink) per school-day for 2 years increased bone mineralization (BMC and BMD), height, sitting height and body weight relative to the control group (habitual diet, no supplementation).[32] The divergent results between Vogel et al.[26] and Du et al.[32] are likely attributable to the different youth populations studied, their varying levels of maturity and growth (and age ranges), and to the background dietary habits/intakes of these different populations. Two other 2-year supplementation studies, one in mostly Caucasian older adolescent girls (aged 15–18 years)[33] and the other in Caucasian (Finnish) peri-pubertal girls,[34] demonstrated positive effects of long-term dairy food intake on BMD/BMC.

Our group recently published a systematic review assessing the studies of dairy consumption and bone properties in youth.[12] Several other RCTs,[35–38] but not all[39,40] demonstrated positive effects of dairy consumption on bone-related variables (mineralization and/or bone-turnover markers) in youth populations (although none exclusively in OW/OB youth), and only one study reported an increase in BMD and BMC with 3 servings of milk/day plus resistance exercise for 12 weeks in normal weight boys (13 −17 years).[37] While our study was shorter in duration than most of the above-cited studies, included an exercise intervention and did not measure bone health radiographically, we uniquely add to the literature by demonstrating a benefit of mixed dairy product consumption (4 servings/day) specifically on bone resorption in menarcheal, OW/OB adolescent girls undergoing a structured diet and exercise program. The reduced bone resorption may have contributed to enhanced bone accretion during this period.

In conclusion, our study provides new evidence that a healthy diet with increased dairy product intake favorably affected circulating markers of bone metabolism in OW/OB adolescent girls participating in a 12-week weight management intervention. In the RDa group, bone markers changed in a manner which suggests that increased provision of dairy foods combined with exercise reduces bone resorption. Additionally, our lifestyle intervention, which included structured dietary advice, succeeded in improving nutrient intakes across both groups regardless of the inclusion of dairy or not, although greater positive changes were seen with dairy product provision. More research is needed to further advance our understanding of the role of whole foods, including dairy foods, in weight management interventions through lifestyle modification for bone health in pediatric populations.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.R.J., B.F., W.E.W. and P.K.; methodology, M.C., I.A.L., A.R.J., B.F., W. E.W. and P.K.; formal analysis, R.K., I.A.L., A.R.J., P.K.; investigation, I.A.L., M.C., A.R.J.; data collection, I.A.L., M.C., A.R.J.; writing—original draft preparation, A.R.J., P.K., R.K.; writing—review and editing, A.R.J., I.A.L., M.C., B.F., W.E.W. and P.K.; project administration, M.C., I.A.L., A.R.J.; funding acquisition, A.R.J. [PI], B.F., W.E.W. and P.K.

ADDITIONAL INFORMATION

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