**Consumption of Greek Yogurt During 12 Weeks of High-impact, Loading Exercise Increases Bone Formation in Young, Adult Males – A Secondary Analysis from a Randomized Trial**

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<td>Novelty bullets: points that summarize the key findings in the work:</td>
<td>☐ Greek yogurt, with exercise, increased bone formation in young adult males over 12 weeks., ☐ After 1 week of an osteogenic exercise program, Greek yogurt tended to blunt a rise in bone resorption seen with the placebo, ☐ Greek yogurt is a plausible post-exercise food that supports bone.</td>
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Consumption of Greek Yogurt During 12 Weeks of High-impact, Loading Exercise Increases Bone Formation in Young, Adult Males – A Secondary Analysis from a Randomized Trial.

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Abstract

Exercise combined with protein and calcium has been shown to benefit bone turnover and bone metabolism. Greek yogurt (GY) contains important nutrients that support bone but has yet to be studied with exercise for this purpose. Thirty untrained, university-aged, males were randomized to 2 groups (n=15/group): GY (20g protein, 208mg calcium/dose) or Placebo Pudding (PP; 0g protein, 0g calcium/dose) consumed 3x/d on training days and 2x/d on non-training days. Both groups underwent a resistance/plyometric training program for 12 weeks. Blood was obtained at weeks 0, 1 and 12 to measure procollagen-type-I-N-terminal-propeptide (P1NP) and C-terminal-telopeptide (CTX). After outlier treatment, P1NP increased more over time in GY versus PP (P=0.002; interaction). Both groups decreased CTX over time (P=0.046; time effect). Following one week of training, there was a trend towards a significant increase in CTX in PP with no change in GY (P=0.062; interaction). P1NP changed more in GY than PP (wk12-baseline; P=0.029) as did the P1NP:CTX ratio (P=0.015) indicating a greater increase in formation with GY. Thus, GY added to a high-load, high-impact exercise program positively shifted bone turnover towards increased formation while attenuating resorption. GY could be a plausible post-exercise food to support bone health in young adult males.

Novelty Bullets

✓ Greek yogurt, with exercise, increased bone formation in young adult males over 12 weeks.

✓ After 1 week of an osteogenic exercise program, Greek yogurt tended to blunt a rise in bone resorption seen with the placebo.

✓ Greek yogurt is a plausible post-exercise food that supports bone.

Keywords: Greek yogurt, Resistance training, Bone health, Short-term response, Bone turnover, Loading exercise
Introduction

Exercise has musculoskeletal benefits (Martyn-St. James and Carroll 2006; Behringer et al. 2014; Silk et al. 2015; Specker et al. 2015; Antoniak and Greig 2017). Certain exercise modalities, including those that produce high forces, high impacts, and high loads such as weightlifting/resistance exercise and jumping-based sports and exercises have been associated with greater bone mineral density (BMD) and a reduced fracture risk compared to unloaded sports/exercises (Morel et al. 2001; James and Carroll 2010; Babatunde et al. 2012; Gregov and Salaj 2014; Valente-dos-Santos et al. 2018). The superior osteogenic effects of dynamic loaded exercise stem from the hydrostatic pressure gradients that are created within the fluid-filled lacunar-canalicular network in bone tissue (Turner and Robling 2003) which initiate a signalling cascade eventually leading to increased bone matrix production (Turner and Robling 2003). Muscular contractions (e.g. during resistance training (RT)) also provide a stimulus for bone growth by directing mechanical force to the bone via the tendon (Rubin et al. 2006).

Bone remodelling can take up to 6 months for significant changes to be detected with radiographic methods (i.e. dual-energy x-ray absorptiometry (DXA) or peripheral quantitative computed tomography (pQCT)) (Nikander et al. 2010; Hlaing and Compston 2014) on the other hand, markers of bone turnover are sensitive to small physiological changes and therefore allow for earlier detection of the bone response to an intervention (Banfi et al. 2010). Several longer-term exercise training studies in young adult males have shown benefits of RT (without nutritional manipulation) on bone (Fujimura et al. 1997; Ryan et al. 2004; Evans et al. 2008; Guadalupe-Grau et al. 2009b; Almstedt et al. 2011; Ucan 2014). Previous research from our group and others has also demonstrated that even a single acute bout of high-impact exercise has the ability to transiently alter bone metabolism either alone (Crameri et al. 2004; Whipple et al. 2004; Rantalainen et al. 2009; Kish et al. 2015; Kouvelioti et al. 2019), or with post-exercise protein (Townsend et al. 2017) and/or calcium provision (Guillemant et al. 2004). No study to date has investigated the short-term (e.g. 1 week) effects of impact-exercise plus nutrition on
markers of bone turnover, which would shed light on the cumulative effects of multiple bouts of exercise and nutrient intake within the initial stages of an exercise program, i.e. when the stimulus is most novel. One study in obese adolescents (boys and girls) demonstrated that milk (versus CHO) reduced inflammatory markers following 1 week of daily exercise, but the short-term effect on bone was not measured (Liu et al. 2015).

Dairy foods contain bone-supporting nutrients including protein, calcium, vitamin D, and phosphorus. These nutrients are crucial to the structural integrity and strength of bone (i.e. the collagen and hydroxyapatite matrices) (Weinsier and Krumdieck 2000; Caroli et al. 2011; Jesudason and Clifton 2011; Bonjour et al. 2013; Rizzoli and Biver 2018). Greek yogurt (GY) has recently become a popular dairy product that is widely accessible and affordable (Darmon and Drewnowski 2015; Drewnowski 2018). It also contains important bone-supporting nutrients (calcium, potassium, phosphorus, and vitamin D, if fortified), and additional structural (e.g. solid vs. liquid) and constitutional features (e.g. increased protein content, lower pH, fermentation/bacterial cultures and higher casein:whey ratio) that contribute to its uniqueness compared to Milk (Van den Heuvel et al. 1999; Scholz-Ahrens and Schrezenmeir 2000; Adolfsson et al. 2004; Hervert et al. 2017; Thorning et al. 2017; Rizzoli and Biver 2018).

There is a paucity of research on the use of loading exercise and whole-food dairy consumption on bone metabolism in young individuals (Josse et al. 2010), and no research in young adult males. Additionally, to our knowledge, no previous research has assessed the effects of GY specifically (plus exercise) on bone health in the short- or longer-term. Only 1 longer-term study assessing bone biomarkers has been undertaken in young adult males that included impact exercise (RT plus running) and a component of dairy (whey protein) (Ballard et al. 2005). Another study in adolescent boys (aged 13-17y) assessed RT plus milk or juice and BMD (Volek et al. 2003). Both showed benefits to bone with nutrient provision and exercise, but neither assessed GY and RT together. Thus, the objective of our
study was to assess the effect of GY versus an isoenergetic, semi-solid, carbohydrate-based placebo with 12 weeks of high-impact, high-load exercise training on two markers of bone turnover (P1NP and CTx) in young, untrained males after 1 and 12 weeks. The analysis of bone markers in this study represent secondary outcomes. The primary outcome from this study (body composition) has been previously published demonstrating that the consumption of GY significantly increased lean mass as well as muscular strength and biceps muscle thickness compared to the carbohydrate placebo (Bridge et al. 2019).

Materials and Methods

This study was a parallel randomized controlled trial and was primarily designed and sufficiently powered to assess the effects of GY on muscular-related outcomes, specifically body composition (lean mass). The present investigation reports on the secondary outcome of bone turnover (type 1 collagen formation and resorption). Participants were randomized using an online-generated randomization scheme (https://numbergenerator.org/) to one of two groups; a Greek yogurt group (GY; n=15) or a placebo pudding group (PP; n=15). Study personnel (AB) enrolled participants and assigned them to the intervention groups based on the randomization scheme. Although participants (and their trainers) could not be blinded to treatment allocation, contents of the PP were concealed, and it was termed the ‘study-designed supplement’. Further study details and results from our strength, muscular thickness, and body composition outcomes have been published elsewhere (Bridge et al. 2019). This study was registered at clinicaltrials.gov (NCT03196856).

Participants

Healthy, university-aged (18-25y) males were recruited for this study from the student population at Brock University between July 2017 and August 2018. Participants underwent screening to
ensure they were healthy (free of medical ailments), had no previous RT experience (RT <0-2x/wk for last 6 months), were of normal body fatness (<25% fat), and did not consume dietary supplements (e.g., vitamins, minerals, protein supplements) in the last 6 months prior to entering the study. Written informed consent was obtained after potential participants met all inclusion criteria. The Brock University Biosciences Research Ethics Board approved our study protocol which conformed to all standards of Canada’s Interagency Panel on Research Ethics for conducting human research (http://www.pre.ethics.gc.ca/eng/index/).

**Study Food Consumption Protocol**

Participants randomized to the GY group consumed 200 g of Oikos of 0% fat, plain GY (~110 Kcals, 20 g protein, 8 g CHO, 208 mg calcium, 283 mg phosphorus, 282 mg potassium; Danone Canada Inc., Boucherville, Quebec) 3 times/day on training days and 150 g, 2 times/day on non-training days. The control group consumed 47 g of a CHO-based placebo pudding (PP), which was mixed with water and made to be isoenergetic and chocolate-flavoured (~110 Kcals, 0 g protein, 28 g CHO, trace minerals from water) at the same time points. Additional details regarding the study foods and timing of intakes have been published elsewhere (Bridge et al. 2019). All participants were provided with nutritional guidance to help them compensate for the added energy consumed from the study foods. During the study, both groups were encouraged to maintain their habitual diets, except for the addition of the study foods.

**Training Protocol**

Both intervention groups underwent 12 weeks of supervised exercise training, 3x/week. The exercise protocol featured two weekly RT sessions (at 70% 1-RM) and one weekly plyometric (PLY) session. All training sessions were facilitated by a study trainer. Further details regarding the training protocol have been published elsewhere (Bridge et al. 2019).
Dietary Analysis

Participants were instructed to record their habitual dietary intake 7 days prior to beginning the study, and at the end of the intervention (during the 12th week of training) using 7-day and 3-day food diaries, respectively. Dietary data were inputted and analyzed using the ESHA diet analysis program (Food Processor, ESHA Inc., Salem, OR). Additional information regarding the dietary analysis has been published elsewhere (Bridge et al. 2019).

Bone Turnover Biomarker Analysis

Venous blood samples (from a vein in the antecubital fossa) were collected at weeks 0, 1 and 12 of the intervention using a standard venipuncture technique. Participants arrived at the laboratory for the blood sample in the morning (between 0800 and 1000) after an overnight fast (10+ hours) and with no exercise performed within the previous 48 hours due to the sensitivity of bone markers to diet and exercise (Eastell and Hannon 2008; Chubb 2012; Ferreira et al. 2015; Szulc et al. 2017). Participants were also asked to consume a similar meal to the one consumed prior to the baseline sample before each subsequent blood sample in order to help control for nutrient intake the night before sample acquisition. Blood was collected into SST (with serum separator) vacutainer tubes and was allowed to clot (~10 minutes) before being centrifuged at ≤1300 RCF (g) for 15 minutes. The serum was separated and aliquoted into small polyethylene cryotubes for storage at –80°C until analysis upon study completion. Frozen samples were stored for no more than 14 months and were only thawed once prior to analysis. Total procollagen-type 1 N-terminal-propeptide (P1NP; cat# 03141071 190) was measured from serum at the Mount Sinai Hospital Core Laboratory (Toronto, Ontario) using a Roche Elecsys e411 automated analyzer. Lower and upper detection limits were 5-1200 μg/L (quality control standard CV: 5.2%). β-isomerized Carboxy-terminal cross-linking telopeptides (β-CTX; β-CrossLaps; cat#: 11972308 122) was measured from serum at the Mount Sinai Hospital Core Laboratory (Toronto, Ontario) using a Roche Cobas e602 automated analyzer. Lower and upper detection limits were 0.010-6.00 ng/mL.
(quality control standard CV: 4.8%). Laboratory personnel involved with the serum analyses were blinded to study group allocation. The ratio of these two analytes in ng/L was also calculated (P1NP:CTX) at week 0, week 1 and week 12. P1NP was converted to ng/L before the ratio was calculated. Bone turnover rate and balance were subsequently calculated based on a newer method for graphically assessing the complementary processes of formation and resorption from Bieglmayer and Kudlacek, published elsewhere (Bieglmayer and Kudlacek 2009). According to the initial clinicaltrials.gov registration, osteoprotegerin (OPG) and receptor activator of nuclear factor kappa-B ligand (RANKL) were supposed to be analysed as additional secondary outcomes. Due to unforeseen circumstances and fund-reallocation towards the data-collection phase of the study, these additional bone outcomes were not analysed.

Statistics

Prior to all statistical analyses, data were checked, and normality was verified. Missing data points (because of missing blood samples; week 0=0, week 1=1 [PP], week 12=2 [PP and GY]) were replaced with the mean for that time point (Figure 1). Initial statistical analyses were carried out without outlier treatment, as detailed in Figure 1. Then as a secondary analysis, data points that were more than ±2SD from the mean were categorized as outliers (P1NP: week 0=2, week 1=1, week 12=1; CTX: week 0=0, week 1=0, week 12=1) and were replaced with the mean for that time point. Repeated measures analysis of variance (RMANOVA) were used to analyze time (weeks 0, 1 and 12), group (GY and PP), and interaction effects (group x time) for our outcome variables. The main RMANOVA covered all 3 time points and 2 groups. Separate RMANOVAs covering 2 timepoints each were also carried out to specifically isolate the short- and longer-term effects of the intervention; week 0 to week 1 and week 1 to week 12. Independent t-tests were used to assess baseline differences between groups and the total absolute change from week 0 to week 12. Data were analysed using SPSS (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). P-values were considered significant at α<0.05.
Results

-------- Insert Figure 1 --------

Of the thirty participants randomized, 27 participants completed all 12 weeks of the intervention. The remaining three participants (1 GY, 2 PP) completed at least 6 weeks of exercise, one dropped out due to injury (1 GY; unrelated to the study), and the other two (2 PP) dropped out because they unexpectedly relocated. Blood samples were obtained from two (1 GY and 1 PP) of these three participants at the time of dropout and their data were included in the statistical analyses. Additionally, one blood sample was not obtained from a GY participant who completed the 12 weeks of training due to a scheduling conflict. Thus, blood samples were obtained from all participants at baseline, 29 participants at week 1 (n=15 GY; n=14 PP), and 28 participants at week 12 (n=14 GY; n=14 PP). Baseline characteristics are presented in Table 1.

-------- Insert Table 1---------

Adherence to the study foods and exercise program

As previously reported (Bridge et al. 2019), study personnel and trainers ensured that the post-exercise study foods (either GY or PP) were consumed in the laboratory following training. This produced 100% adherence with the post-exercise consumption of the study foods. Bedtime and non-training day study food consumption occurred without direct supervision. Week 12 food diaries indicated 97% and 99% adherence for the intake of the unsupervised study foods by the GY and PP groups, respectively. Attendance at the exercise training sessions was on average; 31.6 and 30.1 out of 36 sessions for GY and PP groups respectively.

Biomarkers of bone turnover

P1NP
In our initial analysis, prior to outlier treatment, the results from the main RMANOVA (between=group; within=time [wk0, wk1, wk12]) revealed a significant main time effect (p=0.041), no effect of group and a trend towards a significant interaction (p=0.054). After outlier treatment, our results revealed a significant main effect of time (p=0.009) and a significant interaction (p=0.002), suggesting that P1NP increased to a greater extent in the GY group compared to the PP group (Figure 2A). Further RMANOVA analyses (between=group; effects=time [wk0 to wk1] or [wk1 to wk12]) revealed a significant interaction between week 1 and week 12 (p=0.004), but not week 0 and week 1.

**CTX**

In our initial analysis, prior to outlier treatment, the results from the main RMANOVA (between=group; within=time [wk0, wk1, wk12]) revealed no significant p-values. After outlier treatment, results showed a significant main effect of time (p=0.046) (Figure 2B). Figure 2B shows that CTX remained relatively constant in the GY group but increased transiently after 1 week of training in the PP group, before returning to near baseline by week 12. Further RMANOVA analyses (between=group; effects=time [wk0 to wk1] or [wk1 to wk12]) revealed a significant main effect of time from week 0 to week 1 (p=0.043) and a trend towards a significant interaction from week 0 to week 1 indicating that the PP group had a greater initial increase in CTX than the GY group (p=0.062). A significant main effect of time was also present from week 1 to week 12 in which both groups decreased CTX concentration (p=0.032).

**P1NP:CTX Ratio**

In our initial analysis, prior to outlier treatment, the results from the main RMANOVA (between=group; within=time [wk0, wk1, wk12]) revealed a trend towards a significant main effect of time (p=0.081), and no effect of group or interaction. After outlier treatment, results showed a
significant main effect of time such that the P1NP:CTX ratio increased in both groups (p=0.014) (Figure 2C). A significant main effect of time was also present from week 1 to week 12 (p=0.005).

-------- Insert Figure 2 --------

Absolute change from baseline

After outlier treatment, an independent t-test indicated a significant difference in absolute change from week 0 to week 12 in which the GY group experienced a greater increase in P1NP (μg/L) than the PP group (Figure 3A, GY: 18.7±18; PP: 5.5±13; p=0.029). No significant changes between groups were present for CTX (ng/L) (Figure 3B, GY: -25±92; PP:5±200; p=0.60). P1NP:CTX ratio increased significantly in the GY group from week 0 to week 12 compared to the PP group (Figure 3C, GY: 30.6±26; PP: -3.8±44; p=0.015). Of note, these data assessing the absolute change from week 0 to week 12, specifically for CTX, fail to demonstrate the initial, transient increase in bone resorption in the PP group. This highlights the importance of assessing the short-term response of these markers, i.e. after 1 week of high-impact exercise training with and without GY.

-------- Insert Figure 3 --------

Bone Turnover Calculations

Bone turnover rate and balance for the two groups are depicted visually in figures 4A and 4B. Each black diamond or grey square on the figure represents a participant in the GY or PP group, respectively. At baseline (4A), the distribution of GY subjects in the quadrants of fast formation, slow formation, fast resorption and slow resorption were 13%, 27%, 27% and 33% respectively. Whereas PP subjects were 27%, 27%, 33% and 13% respectively. Following 12 weeks of the intervention (4B), GY subjects were divided 33%, 20%, 7% and 40% while PP subjects were divided 13%, 33%, 33% and 20%, respectively. The most favourable quadrants to be in are the fast-formation quadrant and slow-resorption quadrant. At week 12, the slope of the line and the ellipse surrounding the GY participants demonstrated this favourable pattern whereas the slope of the line and the ellipse around the PP
participants demonstrated an opposite effect (favouring fast resorption and slow formation). The percentage of GY participants in the fast-resorption and slow-formation quadrants (least favourable) at week 12 were 7% and 20%, respectively, whereas the percentage of PP participants in these quadrants were both 33%. It is important to note that this different way of visually depicting our data is exploratory and may offer another approach to assess the effect of our intervention on bone turnover. Limitations do exist regarding its interpretation that relate to variability in the measures themselves and the use of single-timepoint bone markers to assess temporal changes.

----- insert Figure 4 -----  

Nutrition

As expected, the GY group increased their protein and calcium intakes and decreased their CHO intake compared to the PP group (p<0.05 for interactions). Fat intake remained the same between groups and over time. Further details regarding these nutrients (actual intakes and differences between groups) have been published elsewhere (Bridge et al. 2019). In terms of other dairy-related nutrients, phosphorus and potassium also significantly increased in the GY group compared to the PP group (Phosphorus: GY= +548±335 mg, PP= +9±86 mg, p<0.001 for interaction; Potassium: GY= +509±560 mg, PP= -51±616 mg, p=0.026 for interaction). Dietary vitamin D was also measured but there were no differences between the groups or over time (Vitamin D: GY= -0.18±1.36 mcg, PP= 0.43±1.56 mcg). The latter was not surprising since the GY that we used was not fortified with vitamin D.

Discussion

Our study demonstrated that habitual consumption of fat-free, plain GY during a 12-week RT and PLY training program in young males resulted in a significantly greater increase in bone formation (Figure 2; after outlier treatment) compared to an isoenergetic CHO-based placebo, devoid of protein and calcium. We also demonstrate a greater change from baseline in P1NP concentration (Figure 3) and
P1NP:CTX ratio (Figure 3) in the GY group (also after outlier treatment). Furthermore, GY was also able to attenuate the initial and transient rise in CTX concentration that was observed in the PP group following the first week of exercise training.

Our study is one of a few studies to investigate bone health outcomes in young, adult males, and the only one to assess bone outcomes with GY consumption in combination with high-impact, high-load exercise (RT and PLY) both in the short- and longer-term. Although males may not be at the greatest risk for developing osteoporosis (Alswat 2017), and the absolute prevalence of osteoporosis in males is less than in females (although 1 in 5 males will suffer from an osteoporotic fracture in their lifetime according to Osteoporosis Canada (Melton et al. 1998; Kanis et al. 2000)), cases in males can be more complex (due to multiple/different factors) and are more often associated with mortality compared to females (Briot et al. 2009). Thus, maximizing the accrual of bone mass and increasing bone strength and density in young adulthood is still an important and strategic approach in males to prevent bone-related disease and fractures later in life (Van Langendonck et al. 2003; Nordström et al. 2004; Tveit et al. 2010; Rizzoli et al. 2010).

Only a few studies have investigated dairy (or a component of dairy) plus exercise on bone health outcomes in young, untrained males. Ballard et al., (2005) demonstrated that whey protein supplementation (42 g, 2x/d) during 6 months of mixed exercise training (RT and running, 5d/wk) elicited greater increases in BAP and IGF-1 compared to a CHO placebo in both males and females (Ballard et al. 2005). In this study, additional protein supplementation increased habitual protein consumption from 1.1 g/kg/d to 2.2 g/kg/d which may explain the greater increase in bone formation markers compared to the placebo group, as protein is important for bone. Our study also demonstrated a beneficial response to bone formation when protein was increased to 1.74 g/kg/d in the GY group compared to 1.22 g/kg/d in the PP group. A study by Hartman et al., (2007) found that milk consumption (500ml, 26g protein) and RT lead to a greater but non-significant percent change in bone mineral
content (BMC) in young males following 12 weeks of RT compared to the provision of isonitrogenous soy protein and isoenergetic CHO (milk= 1.7%, soy= 0.8%, CHO= 0.6%) (Hartman et al. 2007). Other longer-term studies in young males, without an accompanying nutritional intervention have demonstrated the positive effects of RT on BMD (Ryan et al. 2004; Almstedt et al. 2011; Ucan 2014), and bone formation biomarkers (Fujimura et al. 1997; Woitge et al. 1998; Evans et al. 2008; Guadalupe-Grau et al. 2009). The results from our study corroborate these results, as our PP group was also able to increase bone formation with training. However, this is not entirely comparable since the PP group was also consuming energy as CHO post-exercise and were habitually consuming a relatively high (above the RDA) amount of protein at 1.22 g/kg/d. This prevented them from acting as a true, exercise-only, control group (as seen in the studies mentioned above without a nutritional intervention.

Increasing the consumption of yogurt/dairy foods provides increased intakes of both calcium and protein, both of which are crucial to the growth and maintenance of the skeleton (Heaney 2009). Participants in our study consumed 200g/dose of GY which contains 20g of protein. This amount has been demonstrated to be a sufficient single dose of protein to support positive muscle-related outcomes (Moore et al. 2009, 2015; Witard et al. 2014). More importantly to our study, this amount represents a practical dose of GY which is likely to be consumed in a real-life setting (i.e. GY purchased as 2x100g cartons from the grocery store). Interventions providing dairy foods in this way assess the incorporation of the whole food into the diet and are generally unable to tease out the potential individual effects of protein and/or calcium on bone, but may be more informative from a practical standpoint. Nonetheless, the components of dairy, namely protein and calcium have been extensively studied in relation to bone health. Meta-analyses indicate that higher protein (above the current recommended dietary allowance of 0.8 g/kg/d) and calcium intakes are associated with increased BMD and favourable changes in bone turnover markers across the lifespan (Darling et al. 2009; Behringer et al. 2014; Silk et al. 2015; Tai et al. 2015; Antoniak and Greig 2017; Wu and Sun 2017; Shams-White et al. 2018).
Only one meta-analysis in 2015 was conducted exclusively in healthy males (n=867 from 6 studies, age range 16-84 years) and determined that calcium supplementation (with and without vitamin D) improved BMD in this population (Silk et al. 2015). This review also called for high quality RCTs in younger males to further explore the effects of calcium (and calcium-containing foods) on bone accrual in youth as only one study included within their analysis (Prentice et al. 2005) was undertaken in adolescent boys (Silk et al. 2015). Despite limited research existing in young adult males, our study corroborates these findings, demonstrating that GY consumption, which provides protein and calcium, increased bone formation greater than the PP group, which was still consuming protein in excess (~150%) of the current RDA. In contrast, both groups were consuming calcium well below the RDA at baseline (GY: 699 mg; PP; 668 mg). Daily GY consumption increased calcium intake to the RDA, whereas PP participants continued to under consume calcium at week 12 (GY: 1069 mg; PP; 585 mg). Specifically, by week 12, 64% (range: 753 mg to 1769 mg) of participants in the GY group met the calcium RDA (with 85% being above 850 mg), whereas 13% (2 participants) in the PP group met the RDA (range: 303 mg to 1064 mg). Again, our study cannot pinpoint which nutrients within GY were responsible for the greater increase in bone formation. While this provides a different and important avenue for research, by design, this was not the intention of our study. Indeed, in certain circumstances, it may be more beneficial to consume whole, multi-nutrient foods, such as GY because they offer additional health benefits beyond their nutrient contents relating to the food matrix, food consistency, nutrient bioavailability, food accessibility (Thorning et al. 2017). For example, calcium obtained from dairy sources is absorbed more efficiently and increases bone mineral mass greater than supplementation with calcium tablets (Cheng et al. 2005).

Our study measured P1NP and CTX. P1NP is enzymatically cleaved off before type 1 collagen is deposited in bone making it a blood/serum indicator of bone formation (Hlaing and Compston 2014). When type 1 collagen is degraded during bone resorption, CTX and NTX are released (Ferreira et al.
Serum CTX and P1NP are considered the reference markers of bone turnover (Ferreira et al. 2015). They are also the most widely used clinically, and their responses correlate with DXA-measured changes in BMD (Vasikaran et al. 2011; Baldini et al. 2014; Szulc et al. 2017). Our P1NP and CTX values were slightly higher than other reported values in adult males from Germany (25-30 years; P1NP=31.1–95.9 ug/L; CTX=120–830 ng/L), and Australia (CTX=170-600 ng/L, 25-40 years; P1NP=15-80 ug/L; 25-70 years) (Vasikaran et al. 2014). Our values are plausible since participants in our study were younger (between 18-22 years) than those in both aforementioned studies. Bone turnover declines with age, thus it is to be expected that our values are slightly higher than these values in males that are older.

A newer method of assessing bone turnover balance and rate has been purposed (Bieglmayer and Kudlacek 2009). Using P1NP and CTX at a given time, a composite measure of individual turnover rates and balances can be calculated and plotted on a cartesian plane. The quadrants represent different combinations of rates (fast/slow) and balances (resorption/formation). This exploratory analysis allows for a visual assessment of bone turnover data, making differences between the groups noticeable. Additionally, the linear relationship between rate and balance for a given group can be plotted. Visually interpreting these figures corroborates our analyses showing that bone formation was greater in the GY group.

In an attempt to relate the bone changes to the body composition changes reported elsewhere (Bridge et al. 2019), only the change in CTX was negatively correlated with the change in total strength \( (r = -0.58; p = 0.024) \) in the PP group but not the GY group. This may relate to the fact that the variability around the changes in CTX were larger for the PP group compared to the GY group (GY: -25 ± 92 ng/L; PP: 5 ± 200 ng/L). However, the changes in strength (GY: 98±37 kg; PP: 57±15 kg) were not. No other correlations with individual or combined intervention groups existed.
To our knowledge, this is the first study to assess the short-term (1 week) effect of combined dairy and RT/PLY on bone turnover. This aspect of our study was novel and hypothesis-generating as previous studies have either assessed bone markers <48 hours following exercise (Crameri et al. 2004; Whipple et al. 2004; Rantalainen et al. 2009; Falk et al. 2016; Kouvelioti et al. 2019), or weeks/months following exercise training (Ballard et al. 2005; Mullins and Sinning 2005; Josse et al. 2010, 2012). For CTX, we demonstrated a trend towards a significant interaction within the first week of the intervention indicating that bone resorption was higher in the PP group compared to the GY group. The lack of change in CTX during this time in the GY group may be due to the sufficient provision of calcium and protein (via GY) to support the onset of a new, intense, osteogenic exercise program. This short-term change may also be congruent with the time course of bone remodelling noting that the cycle begins with resorption followed by the deposition of new bone matrix facilitating formation and improved microarchitecture (Banfi et al. 2010). The latter part of the cycle is dependent on the provision and delivery of key nutrients (i.e. protein and calcium) to the site facilitating new bone accrual over time (Shams-White et al. 2017; Wallace and Frankenfeld 2017). We posit, while recognizing that it is speculative, that GY with RT/PLY, in previously untrained males with initial low dairy and calcium consumption (but not low total protein intakes), facilitated this role which lead to a significant increase in bone formation compared to the PP over 12 weeks. Indeed, these effects were observed despite baseline habitual protein intakes being well above the RDA in both groups. Similarly, the early rise in CTX in the PP group was likely related to the initiation of this cycle with ostensibly adequate protein but without adequate micronutrient support. Thus, our data likely show that adequate levels of bone-supporting macro- and micronutrients are needed (and can be supplied by dairy) to achieve the greatest bone benefit in both the short- and longer term.

Our study had several strengths. First, in regards to the bone turnover markers analyzed, P1NP and CTX are both markers of type 1 collagen turnover (Ferreira et al. 2015). Including the beneficial...
reasons mentioned above, they are also regarded by the International Osteoporosis Foundation as the most precise and sensitive markers of bone turnover (Vasikaran et al. 2011; Szulc et al. 2017). In terms of the exercise and nutritional program, additional strengths were described elsewhere (Bridge et al. 2019). There were also limitations. We did not measure whole-body or site-specific BMD or bone mineral content. Indeed, due to the relatively short duration of our intervention, these measures may not have been useful (Nikander et al. 2010; Banfi et al. 2010). We also did not measure or control for vitamin D, calcium or PTH status (or confirm they were within normal ranges), and the GY used was not fortified with Vitamin D (as most Canadian GY is not). However, there is no reason to believe our participants were insufficient or deficient in vitamin D during the intervention. Seasonal variations in vitamin D were also minimized as an equal number of participants from each group began the study at different times throughout the year. It is possible that participants had higher levels of PTH due to their lower calcium intakes at baseline.

In summary, daily consumption of plain, fat-free GY versus an isoenergetic CHO placebo pudding was able to increase bone formation (P1NP) following 12 weeks of RT/PLY training in untrained, university-aged males. GY was also able to attenuate the initial trending rise in CTX observed in the placebo group following the first week of training. GY as a dairy product contains bone-supporting nutrients and its unique characteristics beyond that of milk may increase the bioavailability and absorption of these nutrients. Further research regarding these differences is warranted. Our results suggest that GY can be consumed following exercise to promote bone adaptations, especially during the initial phase of an intense, osteogenic exercise program.

**Conflict of Interest statement:** The authors have no conflicts of interest to report.
References


Table 1: Baseline data for the two intervention groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Greek Yogurt (GY, n=15)</th>
<th>Placebo Pudding (PP, n=15)</th>
<th>Independent T-Test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>1.8 ± 0.0</td>
<td>1.8 ± 0.1</td>
<td>0.37</td>
</tr>
<tr>
<td>Structured exercise sessions/week</td>
<td>0.27 ± 0.5</td>
<td>0.17 ± 0.4</td>
<td>0.51</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>69.9 ± 9.6</td>
<td>69.7 ± 10.4</td>
<td>0.96</td>
</tr>
<tr>
<td>Energy (kcal/d)</td>
<td>2146 ± 407</td>
<td>1989 ± 398</td>
<td>0.27</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>90.6 ± 15.2</td>
<td>85.7 ± 14.6</td>
<td>0.45</td>
</tr>
<tr>
<td>Protein (g/kg/d)</td>
<td>1.31 ± 0.3</td>
<td>1.25 ± 0.3</td>
<td>0.56</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>246.1 ± 52.2</td>
<td>225.0 ± 54.9</td>
<td>0.28</td>
</tr>
<tr>
<td>Carbohydrate (g/kg/d)</td>
<td>3.46 ± 0.9</td>
<td>3.31 ± 0.9</td>
<td>0.48</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>79.2 ± 18.0</td>
<td>79.9 ± 27.5</td>
<td>0.93</td>
</tr>
<tr>
<td>Fat (g/kg/d)</td>
<td>1.18 ± 0.3</td>
<td>1.15 ± 0.4</td>
<td>0.81</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>699 ± 267</td>
<td>668 ± 332</td>
<td>0.88</td>
</tr>
<tr>
<td>Phosphorus (mg/d)</td>
<td>764 ± 299</td>
<td>661 ± 344</td>
<td>0.41</td>
</tr>
<tr>
<td>Potassium (mg/d)</td>
<td>1565 ± 693</td>
<td>1401 ± 453</td>
<td>0.45</td>
</tr>
<tr>
<td>Vitamin D (mcg/d)</td>
<td>2.19 ± 1.05</td>
<td>1.47 ± 1.21</td>
<td>0.11</td>
</tr>
<tr>
<td>P1NP (ug/L)</td>
<td>98.3 ± 31</td>
<td>110.0 ± 39.6</td>
<td>0.79</td>
</tr>
<tr>
<td>CTX (ng/L)</td>
<td>820.4 ± 218.6</td>
<td>797.4 ± 248</td>
<td>0.39</td>
</tr>
<tr>
<td>P1NP:CTX Ratio (ng/L)</td>
<td>115.9 ± 25</td>
<td>142.4 ± 44</td>
<td>0.053</td>
</tr>
</tbody>
</table>

Baseline values displayed as mean ± SD. Statistical analysis was by independent t-test between groups (GY and PP). Significance was set at p<0.05.
Figure Captions

FIGURE 1 — Consort diagram depicting the participant flow through the study.

FIGURE 2 — Concentration of P1NP (ug/L) (2A), CTX (ng/L) (2B), and P1NP:CTX ratio (ng/L) (2C) at week 0, week 1 and week 12 in GY (closed triangles) and PP (open circles) groups. † denotes a significant time effect (p<0.05), * denotes a significant interaction effect (p<0.05). Effect symbols are shown for the main RMANOVAs (all timepoints), and the other smaller RMANOVAs (0 versus 1 and 1 versus 12). The horizontal line indicates which specific RMANOVA (inclusive time points) the effect is based on. P-values to the right of the figure reference the main RMANOVA (2 groups x 3 times) with outlier treatment. Data are displayed as means ± SEM.

FIGURE 3 — The absolute change for P1NP (3A), CTX (3B), and CTX:P1NP ratio (3C) from week 0 to week 12 in GY (n=15) and PP (n=15) groups assessed by Independent T-test with outlier treatment. * indicates a significant difference between groups (p<0.05). Data are displayed as means ± SEM.

FIGURE 4 — Bone marker plots and linear trendline with 95% confidence ellipses for the Greek yogurt (black diamonds) and Placebo (grey squares) groups at baseline (4A) and at 12 weeks (4B). Turnover rate is depicted on the y-axis, with fast above the x-axis and slow below the x-axis. Balance is depicted on the x-axis, with resorption to the left of 0 and formation to the right of 0. Individual data points predict the rate and phase (balance) of bone metabolism for each participant.
Assessed for eligibility (n=72)

Excluded (n=42)
- Not meeting inclusion criteria (n=33)
- Declined to participate (n=9)

Allocated to Greek Yogurt group (n=15)
Received allocated intervention (n=15)

Lost to follow-up (n=0)
Week 1 blood samples obtained (n=15)
Week 12 blood samples obtained (n=14)
Discontinued intervention (injured: n=1. Blood sample obtained: n=1)

Analysed (n=15)
- Initial analysis with outliers in
- Missing values replaced with series mean for all analyses
  - Week 12 (P1NP and CTX; n=1)
- Outliers replaced with series mean for secondary analysis
  - Week 0 (P1NP n=1)
  - Week 1 (P1NP n=1)

Allocated to Placebo Pudding group (n=15)
Received allocated intervention (n=15)

Lost to follow-up (n=0)
Week 1 blood samples obtained (n=14)
Week 12 blood samples obtained (n=14)
Discontinued intervention (moved: n=2. Blood sample obtained: n=1)

Analysed (n=15)
- Initial analysis with outliers in
- Missing values replaced with series mean for all analyses
  - Week 1 (P1NP and CTX; n=1)
  - Week 12 (P1NP and CTX; n=1)
- Outliers replaced with series mean for secondary analysis
  - Week 0 (P1NP n=1)
  - Week 12 (P1NP n=1, CTX n=1)
FIGURE 2— Concentration of P1NP (ug/L) (2A), CTX (ng/L) (2B), and P1NP:CTX ratio (ng/L) (2C) at week 0, week 1 and week 12 in GY (closed triangles) and PP (open circles) groups. † denotes a significant time effect (p<0.05), * denotes a significant interaction effect (p<0.05). Effect symbols are shown for the main RMANOVAs (all timepoints), and the other smaller RMANOVAs (0 versus 1 and 1 versus 12). The horizontal line indicates which specific RMANOVA (inclusive time points) the effect is based on. P-values to the right of the figure reference the main RMANOVA (2 groups x 3 times) with outlier treatment. Data are displayed as means ± SEM.
FIGURE 3 — The absolute change for P1NP (3A), CTX (3B), and CTX:P1NP ratio (3C) from week 0 to week 12 in GY (n=15) and PP (n=15) groups assessed by Independent T-test with outlier treatment. * indicates a significant difference between groups (p<0.05). Data are displayed as means ± SEM.