The effect of increased dairy consumption during one week of intense training on serum bone markers of adolescent female athletes

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Abstract

While high-impact exercise training typically has a positive effect on bone, intensified training during adolescence, the period of rapid growth and peak bone acquisition, could potentially have an opposite effect. Dairy foods contain bone-supporting nutrients (i.e., calcium) that are crucial to the structural integrity and strength of bone. In this study, 13 female adolescent soccer players (14.3 ± 1.3y) participated in a cross-over, randomized, double-blind trial examining the effects of Greek yogurt (GY) consumption on bone biomarkers during a one-week period of intensified training. The study took place over two intervention weeks, which consisted of a pre-training assessment day, 5-days of consecutive, intense soccer training and a post-training assessment day. Participants completed both the GY condition, and a carbohydrate isocaloric placebo control pudding condition (CHO) condition in random order, 4 weeks apart. Fasted, resting blood samples were collected in the morning at pre- and post-training sessions during each intervention condition. Total osteocalcin (tOC), undercarboxylated osteocalcin (unOC), carboxyl-terminal telopeptide of type 1 collagen (CTX), osteoprotegerin (OPG), and receptor activator nuclear factor kappa-β ligand (RANKL) were measured in serum. Results showed no significant effects for time (from pre- to post-training) and condition, and no interaction in tOC, CTX, OPG, RANKL and OPG/RANKL ratio. There was an interaction (p=0.011) for unOC, which decreased significantly at the end of the intense training period in the GY condition, but not in the CHO condition (-26% vs -3%, respectively). Relative unOC, expressed as a percentage of tOC, also reduced post-training (-16%), but with no differences between intervention conditions. These findings suggest that high-impact intense training had no direct catabolic impact on bone metabolism, at least in the short-term, and thus, GY added no benefit beyond that of the isocaloric CHO control pudding.

Table of Contents

List of Tables

List of Figures

Abbreviations

ANOVA	Analysis of Variance
BMC	Bone Mineral Content
BMD	Bone Mineral Density
CHO	Carbohydrates
CTX	Carboxyl-Terminal Crosslinking Telopeptide of Type I collagen
DXA	Dual Energy X-ray Absorptiometry
GY	Greek Yogurt
OPG	Osteoprotegerin
OC	Osteocalcin
tOC	Total Osteocalcin
unOC	Undercarboxylated Osteocalcin
PBM	Peak Bone Mass
RANK	Receptor Activator of Nuclear Factor kappa-β
RANKL	Receptor Activator Nuclear Factor kappa-β ligand
RDA	Recommended Dietary Allowance
RED-S	Relative Energy Deficiency Syndrome

Chapter 1. Literature Review

1.1 Bone

1.1.1 Bone Physiology

Bones have several important functions in the human body such as providing the structure and framework of the body, protecting the organs, supporting and moving the body, secreting osteokines and growth factors, maintaining homeostasis of minerals such as calcium, and many others (Jenkins, 2007; Kini & Nandeesh, 2012). Bones are living structures made of minerals, matrix, water, cells, and lipids. Bones have two tissue types, compact and trabecular (spongey) tissue. Roughly 75-80% of the skeleton consists of compact tissue, while the other 20-25% is spongey tissue, although the bones' precise consistency can change throughout the body based on its overall function (Clarke, 2008; Kini & Nandeesh, 2012). One of the differences between the two types of bone tissues is their function; compact bone is less metabolically active. Cortical (compact) bone is dense and solid and surrounds the marrow space, whereas trabecular (spongey) bone is composed of a honeycomb-like network of trabecular plates and rods interspersed in the bone marrow compartment (Clarke, 2008). Bone turnover is an important process that involves resorbing or breaking down old bone and the mineralization of the bone matrix to maintain bone strength and mineral homeostasis. The bone matrix includes organic matrix, composed of a collection of type I collagen, and inorganic matrix, which is composed of calcium and phosphate and is associated with bone strength (B. Clarke, 2008; Viguet-Carrin, Garnero, & Delmas, 2006). The organic matrix is primarily made up of collagen, which provides the skeleton with its biochemical structure (Rosen et al., 2000; Viguet-Carrin et al., 2006). Collagen is strong and forms bone, cartilage, skin, and tendons, and has mechanical functions in the connective tissue it is associated with, providing elasticity and structure (Viguet-Carrin et al., 2006). Collagen makes up roughly 80% of the total proteins present in bone. It is a triple helix structure found in bone matrix that has been indicated to play a substantial role in bone's capacity to absorb energy and overall bone toughness (Viguet-Carrin et al., 2006).

Bone tissue including the matrix, surrounds bone cells namely, osteogenic cells, osteocytes, osteoblasts and osteoclasts (Clarke, 2008). Osteogenic cells are non-specialized and derive from mesenchyme tissue (Clarke, 2008; Kini & Nandeesh, 2012; Thomas, 2012). These osteogenic cells eventually differentiate into osteoblasts during bone development and repair. Osteoblasts are bone-building cells, which, when stimulated, coordinate the calcification of the skeleton, regulate osteoclasts, and produce osteoid, the uncalcified organic matrix of bone (Clarke, 2008; Thomas, 2012). These cells become trapped in the osteoid they produce and become osteocytes – mature bone cells. Osteocytes are the longest living bone cell and have an important role in maintaining bone mass and structure because they are the cells that sense mechanical loading and initiate a response (Clarke, 2008; Jenkins, 2007). Osteocytes function as a bone-lining cell to regulate the exchange of mineral in the bone fluid, therefore serving as a bone-blood barrier (B. Clarke, 2008). Lastly, the osteoclasts are the cells involved with bone resorption; these cells release acids and enzymes that break down the protein and mineral aspects of the bone matrix (Clarke, 2008; Jenkins, 2007; Thomas, 2012).

1.1.2 Bone Turnover

Bone growth and modelling is a complex, dynamic process that begins in the womb and continues until humans reach their final skeletal size in adulthood. Bone turnover is a constant process that takes place throughout the lifespan and replaces old damaged bone with new bone (Thomas, 2012; McCullough & Goss., 2012). Bone resorption is the first phase of bone remodelling or bone turnover (Figure 1.1), and it involves the catabolic osteoclasts synthesizing and releasing enzymes that result in the destruction of bone matrix (Clarke, 2008). It is followed by the reversal phase that is characterized by the disappearance of almost all osteoclasts, which makes room for the osteoblasts to move to the site for the bone formation phase, involving osteoblasts synthesizing new bone matrix (Siddiqui & Partridge, 2016). The last phase of the cycle is the Resting Phase, where the environment is maintained until the next wave of remodeling is initiated (Siddiqui & Partridge, 2016). The process of turnover or bone remodelling lasts between 2 and 8 months in adults, and 3 to 4 months in youth (B. Clarke, 2008). In adults, around 5-10% of total body bone mass is involved in the process yearly (Clarke, 2008; Seibel, 2005). Through the remodelling process, new osteons are produced, which act as storage for minerals, such as calcium and phosphorous that are required for various metabolic reactions. Bone strength depends not only on the quantity of bone tissue but also on its quality, which is characterized by a few factors; composition, mass, microstructure and collagen – which forms a scaffold to provide additional strength and structure.

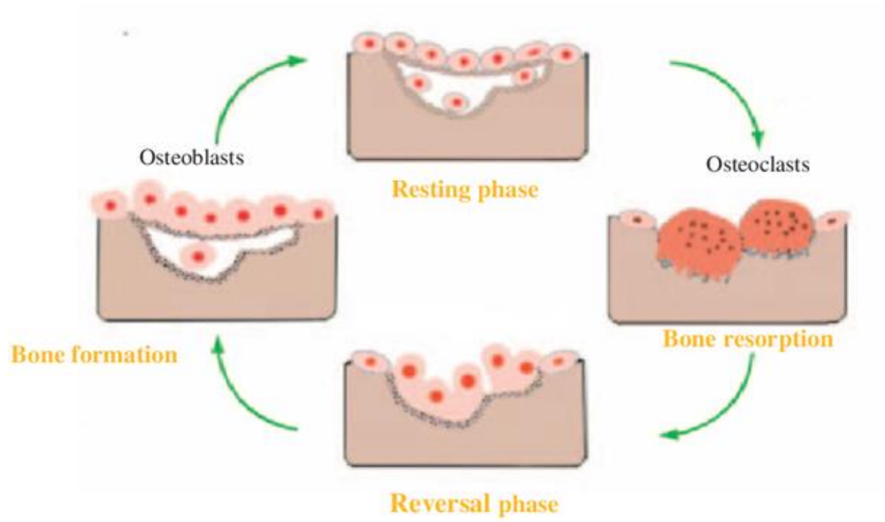


Figure 1.1: The bone turnover cycle (modified from Hlaing & Compston, 2014).

1.1.3 Markers of Bone Turnover

Markers of bone turnover include those that reflect formation and those that reflect resorption. Bone turnover markers are biochemical metabolites measured and used in research and clinical practice, reflecting the activities of bone formation, resorption, or both. In contrast to static measures, which involve imaging techniques such as dual energy x-ray absorptiometry (DXA), levels of bone turnover markers provide dynamic information about the bone status (Calvo, Eyre, & Gundberg, 1996). Bone turnover markers reflect the metabolic activity of the bone during remodelling, as they are quantitative and dynamic indicators of current bone turnover (Thomas, 2012). Osteoblasts secrete bone formation markers, that show the activity of osteoblasts during the different stages of bone formation. Markers of collagen degradation are most commonly used as markers of bone resorption (Vasikaran et al., 2011; Watts, 1999).

According to the International Osteoporosis Foundation (IOF), one of the more common bone resorption markers that should be measured in clinical studies is carboxyl-terminal crosslinking telopeptide of type I collagen (CTX) (Vasikaran et al., 2011). Collagen crosslinks are peptides that are crucial biochemical markers of bone turnover, more specifically bone resorption, that have been created through the process of collagen degradation (Rosen et al., 2000). CTX is a specific type of collagen crosslink that can be measured in both urine and blood (Viguet-Carrin et al., 2006). It is predominantly expressed by osteoclasts and is secreted into the resorption cavity below active osteoclasts resulting in type 1 collagen degradation (Vasikaran et al., 2011). Thus, it is measured as a marker of collagen breakdown as it is the specific product of enzyme mediated bone resorption (Vasikaran et al., 2011). The time of day at which the sample is collected, when using CTX, is critical due to CTX's circadian variation and short half-life (~1 hour) (Shetty, Kapoor, Bondu, Thomas, & Paul, 2016). Therefore, when using CTX, fasting morning samples are recommended for optimal clinical and research use.

Osteocalcin (OC) is a protein that is exclusively synthesized by osteoblasts and can be found in the extracellular bone matrix. OC is a late marker of osteoblastic activity, and is therefore used as a bone formation marker, but it is limited by a short half-life (~5 min) and influence of circadian rhythms (Shetty et al., 2016). Since OC is incorporated into the skeletal matrix and is released during bone resorption it can also be used as a measurement of bone turnover (Figure 1.2). OC is often paired with CTX throughout the literature as markers of bone formation and resorption, respectively (Shetty et al., 2016). However, despite OC being routinely used as a serum marker of osteoblastic bone formation and attributed to regulating mineralization, subsequent work has put forth the notion that osteocalcin functions as an inhibitor of bone mineralization (Zoch, Clemens, Riddle, 2016). This seems counterintuitive since serum osteocalcin levels have been reported to correlate positively with bone formation and have been used clinically to assess bone turnover (Brennan-Speranza & Conigrave, 2015). Thus, OC's role in bone remains unclear, but it seems to support the later stages of bone formation by acting at the bone mineral surface to coordinate matrix mineralization. These effects suggest that osteocalcin plays a small role in the regulation of bone turnover, while it is possible that total circulating OC plays other roles in whole body metabolism (Brennan-Speranza & Conigrave, 2015). Osteocalcin which reflects osteoblast activity may exist in two forms: fully carboxylated osteocalcin and undercarboxylated (unOC) osteocalcin, in which only 0–2 residues are carboxylated. Carboxylated osteocalcin, through binding calcium and consequently hydroxyapatite, plays an active regulatory role in bone formation and mineralization. However, of the total amount of osteocalcin that is released into blood circulation, a significant portion

(40–60%) is undercarboxylated osteocalcin (unOC), the amount of which is sensitive to vitamin K intake (Lin, Brennan-Speranza, Levinger, & Yeap, 2018). Recent advances have indicated that osteocalcin, and in particular its undercarboxylated form, is not only a biomarker indicative of bone health but also an active hormone that mediates glucose metabolism and vitamin K status in experimental studies (Lin et al., 2018). Evidence also suggests that the unOC may have a link, directly or indirectly, to skeletal muscle function (Itamar Levinger et al., 2014). In general, high total OC levels are associated with an increase in bone formation and bone turnover as the carboxylated form has a high affinity for calcium while increased unOC levels, which has a lower affinity for calcium, are associated with lower bone quality (Neve, Corrado, & Cantatore, 2013). Thus, the suggestion is to use the unOC to tOC ratio (unOC/tOC) or the percentage of total OC that is unOC (unOC%) as indicators of bone formation (Lin et al., 2018; Neve et al., 2013). However, most clinical studies have examined total circulating OC. In children, total OC levels are higher than adult levels because there is a higher bone formation rate. It is important to mention that there is a large variability in circulating osteocalcin levels among adolescents, which may be a consequence of the growth spurt period occurring at different ages (Moser & van der Eerden, 2019).

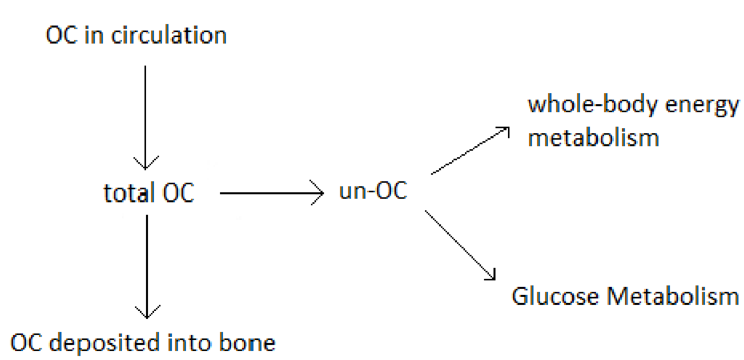


Figure 1.2: Osteocalcin in circulation.

1.1.4 OPG/RANKL Pathway

In healthy bone, the interaction between osteoprotegerin (OPG) and the receptor activator of nuclear factor kappa-β ligand (RANKL) is a key pathway that regulates the outcome of bone remodelling (Boyce & Xing, 2007; Khosla, 2001a; Wasilewska, Rybi-Szuminska, & Zoch-Zwierz, 2010). The OPG-RANKL pathway (Figure 1.3) dictates a large portion of bone turnover, specifically regulation of bone resorption (Boyce & Xing, 2008; Khosla, 2001a; Wasilewska et al., 2010). The binding of receptor activator of nuclear factor kappa-β (RANK) to RANKL, a membrane-bound protein, regulates osteoclast formation, activation, and survival (Figure 1.3). Specifically, RANKL stimulates the fusion of osteoclasts to bone and subsequently promotes their activation and survival, as well as promoting osteocyte apoptosis (Khosla, 2001a).

Osteoprotegerin (OPG) is considered a bone protector, as it acts as a decoy receptor for RANKL. OPG is an anabolic osteokine secreted by osteoblasts that downregulates the catabolic RANKL pathway by inhibiting the binding of RANKL to RANK on the osteoclast precursor cells, thus preventing osteoclastogenesis (Wasilewska et al., 2010). Increases in OPG levels have been associated with lower osteoclast numbers as well as higher bone strength and bone density in animal models (Khosla, 2001a). When OPG increases and RANKL decreases, the OPG/RANKL ratio increases overall, leading to an inhibition of osteoclastogenesis and bone resorption (Khosla, 2001b). Thus, the OPG/RANKL ratio is also used as a key indicator of the rate of osteoclastogenesis.

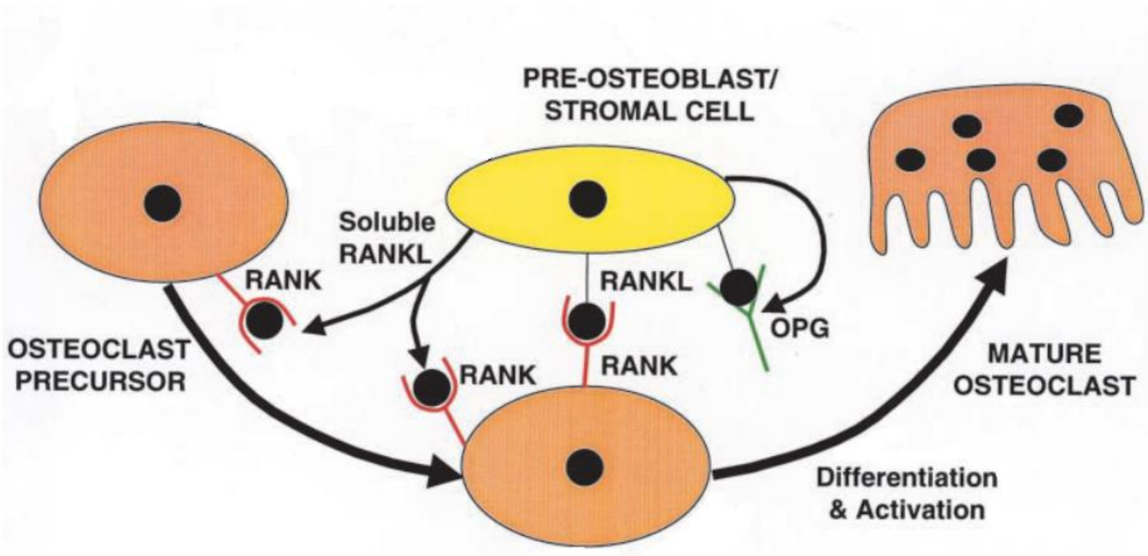


Figure 1.3: OPG and RANKL interaction (modified from Khosla, 2001).

<u>1.2 Bone, Growth and Development</u>

During childhood, bones grow in both length and width, with bone formation exceeding the rate of bone resorption. This increased rate of formation allows bone mass to increase in order to achieve peak bone mass (PBM) (Jenkins, 2007). PBM is defined as the highest level of bone mass achieved as a result of normal growth (Matkovic et al. 1994). Specifically during puberty and during the adolescent growth spurt the increase in bone mass accounts for 25% of the final PBM achieved (Dennison, Harvey, & Cooper, 2013). By the age of sixteen, approximately 90% of total bone mineral content is accrued, making adolescence the critical period for bone accretion (Elgán, Samsioe, & Dykes, 2003; Stager, Harvey, Secic, Camlin-Shingler, & Cromer, 2006). The final PBM is achieved during early adulthood and is typically lower in females than males; the higher the PBM achieved, the less risk of osteoporotic fractures and injuries in elderly years (Dennison et al., 2013). Permanent deficits in bone mass are the result of any process that interferes with normal bone mineral accretion during childhood and adolescence.

From a physiological and hormonal standpoint, males and females are different, especially in their youth, as the magnitude and rate of hormonal changes differ between the sexes (Henry & Eastell, 2000). In females, longitudinal and radial growth of the skeleton occurs prior to menarche under the influence of growth hormone, insulin-like growth factors, and other factors (Clarke & Khosla, 2010). With the onset of menarche at age 11–13 years, estrogen stimulates rapid skeletal mineral acquisition, as well as further longitudinal and radial skeletal growth (Clarke & Khosla, 2010). Indeed, women gain roughly a third of their peak bone mineral density (BMD) within the 4 years around the onset of menarche (Clarke, 2008). Thus, the female reproductive system profoundly stimulates bone growth, modeling, and remodeling throughout the lifespan, and especially during puberty, with modifiable factors, including diet, and physical activity, also playing a significant role (Clarke & Khosla, 2010). Androgens, as well as estrogens, maintain cancellous bone mass and integrity, regardless of age or sex (Vanderschueren et al., 2004). Testosterone in males is an important hormone for both bone gain and maintenance as it can inhibit resorption (De Oliveira, Fighera, Bianchet, Kulak, & Kulak, 2012; Vanderschueren et al., 2004). However, current evidence suggests that estradiol plays a greater role in maintenance of skeletal health than testosterone, but that androgens also have direct beneficial effects on bone (De Oliveira et al., 2012).

1.3 Bone and Exercise Training

1.3.1 Exercise and Bone Turnover

Physical activity is an accessible, low cost, and highly modifiable contributor to bone health. In adults, exercise is known to decrease bone resorption markers, while causing an increase in bone formation markers through an increase in mechanical loading. Specifically, skeletal muscle imposes force on bone, with the largest forces occurring during movement and lifting activities. Muscular contractions are a stimulus for bone growth, through the directing of mechanical forces to the bone via the tendon (Avin, Bloomfield, Gross, & Warden, 2015). In the past two decades, exercise and sports training have been shown to enhance bone mineral density (BMD) in not only adults, but in children and adolescents as well. In general, physical activity has been associated with bone mineral acquisition and maintenance (Valdimarsson, Sigurdsson, Steingrímsdóttir, & Karlsson, 2005) and the prevention of future osteoporosis. Specifically, mechanical strain as a result of physical activity causes bone metabolism to react and initiate bone remodelling in favour of bone formation (Gregov & Šalaj, 2014). The osteogenic effect of exercise in youth is seen mainly following weight-bearing or high-impact exercises, such as running and jumping in sports like soccer (Falk, Galili, Zigel, Constantini, & Eliakim, 2007; López-Calbet, Dorado, Díaz-Herrera, & Rodríguez-Rodríguez, 2001; Söderman, Bergström, Lorentzon, & Alfredson, 2000).

During exercise, the skeleton will typically be subjected to bending, compression, tension and torsion loads (Russo, 2009). During weight-bearing activities, gravity also adds mechanical load to the skeleton. Specifically, in running, there is an increase in muscle force due to higher impact, whereas in walking there is only a modest loading increase on the skeleton above gravity (Russo, 2009). Thus, sports such as soccer or basketball are very effective osteogenic exercises, since they use force and power consistently to maintain high intensity running intervals (Falk et al., 2007; Russo, 2009). Increases in femoral neck BMD have been found in peripubertal girls involved with high-impact weight-bearing recreational and competitive activities (Lehtonen-Veromaa, Möttönen, Nuotio, Heinonen, & Viikari, 2000). Furthermore, resistance training has been shown to have positive effects on femoral BMD in adolescent females when implemented in high school curricula (Nichols, Sanborn, & Love, 2001).

In terms of bone markers, previous studies in adolescent females have shown that a single bout of high impact exercise leads to acute responses in markers of bone turnover, including a decrease in bone resorption markers and a progressive increase in bone formation markers (Dekker, Nelson, Kurgan, Falk, Josse, 2017; Kurgan, McKee, Calleja, Josse, & Klentrou, 2020), This suggests that high impact exercise is beneficial for promoting positive bone turnover responses during this critical period for bone accrual in young females (Rantalainen et al., 2009). However, it is unclear what happens during intense training and whether intense training during adolescence is beneficial or potentially detrimental to bone turnover. In healthy young menstruating women, periods of intense exercise, such as during 5 consecutive days of 70% VO_2 max exercise, have been shown to cause increased bone resorption, as shown by increases in CTX (Ihle & Loucks, 2004). Although not definitive, this suggests that multiple days of intense exercise could lead to an uncoupling or imbalance in bone turnover, favoring increased bone resorption, which may have detrimental effects on bone mass and health (Ihle & Loucks, 2004). Exercise volume and frequency are important factors, which can directly influence bone accrual during adolescence. Indeed, previous studies reported that adolescent female athletes who undertook low frequency, low volume activities had 5-9% higher BMC and BMD when compared to a controlled, less active group (Caputo, Rombaldi, Harmer, & Silva, 2020). On the other hand, a study including a twelve week high-intensity resistance training with university-aged women, found that training did not appear to enhance bone formation or inhibit bone resorption, as assessed by biochemical markers of bone metabolism, specifically OC and bone-specific alkaline phosphatase (BAP) (Mullins & Sinning, 2005). Thus, more research is needed to determine whether, and under what conditions, repeated, intense, high-impact training can have detrimental or beneficial effects on bone.

1.4 Bone and Dairy Food Intake

1.4.1 Dairy Foods

Dairy foods contain bone-supporting nutrients that are crucial to the structural integrity and strength of bone such as, protein, calcium, vitamin D, and phosphorus. Due to the lower pH of yogurt, dairy minerals such as calcium and magnesium are present in their ionic forms, which increases their absorption (Felix Bronner & Pansu, 1999). Yogurt tends to be a more digestible and tolerable source of dairy compared to milk due to naturally occurring enzymes present in the bacterial cultures (Felix Bronner & Pansu, 1999). These enzymes are responsible for the intra-intestinal digestion of lactose, and the bacterial cultures that support healthy gut microflora and provide antipathogenic and anti-inflammatory properties (Markowiak & Ślizewska, 2017; Vatanparast, Bailey, Baxter-Jones, & Whiting, 2010). The acidity of yogurt slows gastric emptying, allowing a prolonged period of nutrient absorption. The probiotic cultures added during yogurt production also offer health benefits, such as a reduced risk of developing type 2 diabetes, reduced allergy symptoms, and several more (Markowiak & Ślizewska, 2017). Bacteria within yogurt also contains proteolytic enzymes and peptidases, which help catabolize the proteins in yogurt making them more easily digested and absorbed.

The main proteins in dairy products are casein and whey. Specifically Greek yogurt, contains primarily casein, which allows for a prolonged influx of amino acids (Moore et al., 2009). Casein exists in a micellar form, which encases the casein molecule making it insoluble in water (Felix Bronner & Pansu, 1999). This causes casein to coagulate in the stomach, resulting in digestion to be slower than other dairy proteins, such as whey, and ultimately slows the absorption of amino acids into the blood. Whey protein is the acid-soluble portion of protein in dairy that is rapidly digested and absorbed into the blood (Felix Bronner & Pansu, 1999). There have been studies in adolescents examining the effects of whole-food dairy consumption on BMD and BMC with the addition of exercise, which have found beneficial results (Volek et al., 2003), however they have not assessed bone turnover or bone biomarkers. Additionally, studies in young adults and adolescents have looked at the impact of whole-food dairy consumption on BMC and BMD long-term and found beneficial results correlated to increased dairy consumption (Ballard, Clapper, Specker, Binkley, & Vukovich, 2006; Cheng et al., 2005; Rizzoli & Biver, 2018; Vannucci et al., 2018).

1.4.2 Effects of Calcium intake on Bone

Calcium is important for the promotion of skeletal health and growth with its influence varying according to age, sex and the bone site studied (Vannucci et al., 2018). Specifically, calcium intake has been shown to be important for the development and maintenance of BMD and bone mineral content (BMC) during the prepubertal years (Vannucci et al., 2018). Importantly, since the early 90s, studies have shown that increased calcium intake is significant to optimizing and enhancing bone mineral acquisition and can favourably modify the attainment of peak bone mass specifically in adolescent girls (Cadogan, Eastell, Jones & Barker, 1997; Valimaki et al. 1994).

Consequently, during periods of rapid growth, it is recommended that girls aged 9-18 years consume 1300 mg of calcium daily (Osteoporosis Society of Canada). However, studies have indicated that in adolescent girls, the average dietary calcium intake is about 50% of the recommended daily allowance (Steelman & Zeitler, 2001). It has also been noted that it is not only young females who are not achieving the RDA of 1300mg/day, but young adults also consume less than 1 serving a day of dairy – well below the recommended levels (Fiorito, Mitchell, Smiciklas-Wright, & Birch, 2006; Health Canada, 2020; Volek et al., 2003).

Furthermore, dairy foods are the main contributor to calcium consumption, making up for 70% of calcium intake (Fiorito et al., 2006). A study in adolescent boys (aged 13–17 years) assessed resistance training during 12 weeks plus milk or juice on BMD (Volek et al., 2003). They showed benefits to bone with nutrient provision and exercise, however the intake of milk provided a 2-fold greater increase in BMD when compared to the juice group (Volek et al., 2003).

There is less evidence, however, in terms of bone turnover markers. In a systematic review on the effects of dairy intake and calcium consumption on bone in children, bone turnover markers were assessed in 6 studies, with 4 showing nonsignificant results and only 2 showing some significant positive changes to bone structure and BMD (Kouvelioti, Josse, & Klentrou, 2017). Moreover, all but one of the studies involved in this systematic review did not include exercise with dairy or calcium consumption, while the one study that did include exercise only looked at BMD and did not measure bone turnover markers (Kouvelioti et al., 2017; Volek et al., 2003). However, this review did demonstrate that the majority of bone turnover markers did not show any significant effects of dairy/calcium consumption by itself, leading researchers to believe the addition of exercise may cause a more prominent effect (Kouvelioti et al., 2017). Finally, a recent study investigated the differences in bone markers after a 12-week exercise and diet intervention paired with calcium intake in young females (age 12-16 years) who were overweight or obese. They found that the group with increased consumption of dairy foods, had beneficial changes in bone turnover compared to the group that had lower consumption of dairy (Josse et al., 2020). Specifically, CTX decreased and was negatively correlated with the average number of dairy servings consumed during the study (Josse et al., 2020). This indicates that a greater intake of dairy foods is associated with lower bone resorption, leading to an idea that whole food dairy products, such as Greek yogurt, milk, or cheese, could have more osteogenic benefits to youth female athletes, than supplements.

1.4.3 Protein Effects on Bone

According to the current USA and Canadian Dietary Reference Intakes, the recommended dietary allowance (RDA) of protein for adolescents 14–18 years of age is 0.85 $g.kg^{-1}$ per day, and for adults over 19 years of age it is 0.80 $g.kg^{-1}$ per day (Canada, 2001). However, these protein RDA's for children are mostly derived from adult studies (Canada, 2001), in an attempt to account for the needs of growth (Volterman & Atkinson, 2016). Importantly, since the RDA for protein refers to the 'average' child, it may still be insufficient for the energy and protein requirements of child athletes, who complete significant amounts of training and competition. In adults, the protein needs of athletes have been reported as higher than non-athletes, at $1.8g.kg^{-1}$ per day (Health Canada, 2020; Phillips & van Loon, 2011). However, the protein requirements for child athletes or highly active children are still unspecified due to the lack of studies examining the effects of protein consumption on bone turnover in young athletes, who may have higher needs for protein than non-athletic youth.

One recent study has demonstrated that protein intake immediately post exercise in adolescents leads to a significantly higher rate of bone turnover at 8 hours and 24 hours post exercise compared to baseline (Theocharidis et al., 2020). Additionally, literature has demonstrated beneficial effects of ingesting protein immediately post-exercise in adult male athletes on bone turnover markers, specifically by seeing a decrease in bone resorption marker CTX (Townsend et al., 2017). However, only one study in male adults (Bridge et al., 2019) has examined the effect of consumption of a whole food protein - Greek yogurt, during intense resistance training 3 times a week for 12 weeks. It was found that the group who consumed Greek yogurt had a greater increase in bone formation post-training compared to the group that consumed an isocaloric control pudding (Bridge et al., 2020). These studies demonstrate the potential importance of protein consumption shortly after intense or resistance exercise in promoting beneficial bone turnover responses in adolescents and adults, respectively. Due to the relatively high protein content of Greek yogurt in comparison to other protein-rich foods or supplements, it may serve as a practical option to increase total protein intake in youth athletes and yield beneficial health adaptations. Greek yogurt is an accessible whole food product that may yield additional benefits to bone when paired with exercise or training (Drewnowski, 2018).

1.4.4 Greek Yogurt Effects on Bone

Greek yogurt (GY) has recently become a popular dairy product that is widely accessible and affordable (Drewnowski, 2018). It also contains important bone-supporting nutrients (calcium, potassium, phosphorus, and vitamin D, if fortified), and additional structural (solid vs. liquid) and constitutional features (increased protein content, lower pH, fermentation/bacterial cultures and higher casein/whey ratio) that contribute to its uniqueness compared with milk. There is a paucity of research on the use of loading exercise and whole-food dairy consumption on bone metabolism in young individuals (Bridge et al., 2020; Josse & Phillips, 2013) and very little research in adolescents. Additionally, to our knowledge, no previous research has assessed the effects of GY specifically (plus exercise) on bone turnover markers in a one-week duration. The combination of the nutritional and unique properties of Greek yogurt makes it an attractive and functional food for the goal of improving important health parameters. However, research has only just begun to investigate the use of Greek yogurt in this way and/or in combination with exercise (Bridge et al., 2019; Bridge et al., 2020). Due to the unique properties of GY, it is plausible that studying individuals who increase their training load while intaking GY may yield similar benefits as to other previously studied protein sources, while also providing additional health benefits due to the bone-supporting and digestive nutrients present specifically within GY.

Chapter 2: Introduction to Research Paper

2.1 Rationale

Adolescence is an essential period of bone accretion that provides a window of opportunity to increase peak bone mass (PBM), and if this opportunity is missed the effects can translate into adulthood – especially with females. In other words, this is the period of life when the highest amounts of bone mass is accrued through an increase in bone formation, without which disease and chronic issues such as osteoporosis and chronic bone mineral deficiencies can occur later in life (Fiorito et al., 2006). Although exercise, especially of high impact, has been previously demonstrated to benefit bone (Deere, Sayers, Rittweger, & Tobias, 2012; Dekker, Nelson, Kurgan, Falk, Josse, & Klentrou, 2017; Mezil et al., 2015; Rantalainen et al., 2009; Weeks, Young, & Beck, 2008), it is unclear what happens in adolescent females during intense training and whether intense training is beneficial or detrimental to bone formation during this crucial period of bone development.

Bone biomarkers reflect formation and resorption rates in the body, and can give a dynamic measure of these processes as they are stimulated by biomechanical forces – such as exercise and training (Kini & Nandeesh, 2012). In our study, we measured tOC, unOC and CTX as markers of bone formation and resorption, respectively, as well as the osteokines OPG and RANKL, which reflect the relative balance of bone turnover and can give insight to which process (resorption or formation) is more favoured (Boyce & Xing, 2008). These five markers can be measured in serum samples to reflect the microenvironment of bones.

Soccer is a sport that demands high amounts of running, through training or gameplay, which is characterized by a high skeletal load. According to previous studies, high-impact activities provoke exercise-induced bone responses in adolescents (Dekker et al., 2017; Kish et al., 2015; Rantalainen et al., 2009), which can have a positive effect by increasing bone formation and turnover. However, longer periods of intense exercise, as seen during 5 consecutive days of exercise at 70% VO_2 max, can cause increased bone resorption (shown by increases in CTX) in young women, who were in an energy restricted state (Ihle & Loucks, 2004). Although not definitive, this suggests that prolonged and intense exercise could potentially lead to an uncoupling or imbalance of bone turnover, favoring increased bone resorption, which may have detrimental effects on bone mass and health (Ihle & Loucks, 2004).

Furthermore, studies have indicated that only about 67-70% of the adolescent population is consuming the recommended daily allowance (RDA) of dietary calcium intake (1300mg), particularly in adolescent females (Fiorito et al., 2006; Health Canada, 2020; Vatanparast et al., 2010). Calcium requirements increase during periods of rapid growth, therefore calcium is an important nutrient when it comes to bone development and accretion, especially during the prepubertal years (F. Bronner, 2001; Vannucci et al., 2018). However, since our bodies do not produce calcium, the only source of calcium is from nutrition and/or supplementation. Low dietary calcium can result in a low bone mineral density (BMD) and content (BMC), leading to increased risk of breaks or fractures (Felix Bronner & Pansu, 1999; Cormick & Belizán, 2019). Thus, calcium consumption, whether lower or higher than the recommended intake, could respectively exacerbate, or counterbalance any potentially negative effects of intense exercise on bone (Cormick & Belizán, 2019). Additionally, protein supplementation may also play an instrumental role in bone tissues that undergo stress during exercise and attenuate some of the negative effects of over training (Bonjour, Ammann, Chevalley, & Rizzoli, 2001). For example, according to a study in adult male endurance athletes, a protein beverage combined with carbohydrates consumed immediately post-exercise resulted in increased circulating levels of bone formation markers, and decreased levels of bone resorption markers (Townsend et al., 2017). However, the protein requirements for child athletes or highly active children are still unspecified due to the lack of studies examining the effects of protein consumption on bone turnover in young athletes, who may have higher needs for protein than non-athletic youth.

Greek yogurt (GY) has both a higher protein and calcium content, nearly triple and double that of regular yogurt, respectively (Caroli, Poli, Ricotta, Banfi, & Cocchi, 2011; Huncharek, Muscat, & Kupelnick, 2008; Rizzoli & Biver, 2018; Skotidakis Inc, 2021; Weinsier & Krumdieck, 2000). Due to these relatively higher concentrations, GY may serve as a practical option to increase total calcium and protein intake, yielding beneficial bone adaptations. Providing effective nutrition to the adolescent athlete population, specifically females, may be enough to counter any potential negative effects of intense exercise on bone. Therefore, this study was designed to examine the effects of GY consumption on markers of bone turnover during short-term intense training in adolescent female soccer athletes.

2.2 Objectives and Hypotheses

The purpose of this study was to examine whether consumption of three daily doses (at breakfast, immediately following each training workout, and before bedtime) of GY in comparison to an isocaloric carbohydrate control pudding (CHO, designed for the study) would affect markers of bone turnover during a period of short-term intense training (one week of high volume, high intensity) in female soccer players. It was hypothesized that during the period of short-term intense training, the consumption of GY would help maintain the systemic levels of tOC, unOC, CTX, OPG, RANKL and OPG/RANKL ratio near their pre-training levels, compared with the isocaloric carbohydrate control pudding, where we expected to see a catabolic effect of intense training, mainly reflected by an increase in unOC, CTX and/or RANKL, with unchanged or decreased tOC and OPG.

Chapter 3: Methods

3.1 Participants

A total of 21 competitive, female soccer players (12-16yrs) were recruited from local soccer clubs in Southern Ontario, Canada to participate in a clinical trial primarily designed to examine the effects of GY consumption on indices of inflammation and recovery. However only 13 participants completed all parts of this study while the other 7 participants dropped out for reasons not pertaining to the study. All participants had a minimum 2 years of elite competitive experience, training ≥ 3 sessions/week, were free of any musculoskeletal injury or medical condition that would prevent them from participating in maximal exercise, had no hypersensitivity or allergy to dairy products and did not take any medication or supplements. The age of the participants was 14.3 ± 1.3y, and their age from peak height velocity was 0.8 ± 0.8y, with no differences in the participants' anthropometric characteristics between the intervention weeks (Table 3.1).

Table 3.1 Participants' physical characteristics at pre-training during each nutritional intervention (n=13).

	Greek Yogurt	Carbohydrate
Height (cm)	165.9 ± 5.2	166.0 ± 5.3
Body mass (kg)	59.1 ± 7.5	59.3 ± 7.4
Body fat (%)	22.2 ± 6.5	22.2 ± 5.4

Values are mean ± standard deviation.

All participants and their parents/guardians received a thorough explanation of the study's purpose, procedures, benefits, and potential risks, and consent was obtained from both the participants and their parents/guardians prior to study commencement. The study was cleared by the Research Ethics Board of Brock University (REB# 18-289) and was registered at Clinicaltrials.gov, registration #NCT03947801.

3.2 Study Design

The study was carried out using a cross-over, double-blind, placebo-controlled design. All participants participated in an information session, and two intervention weeks, each consisting of a pre-training testing day, one week of consecutive soccer training and one post-training testing day (see procedures and measurements below). Intervention weeks were scheduled 4 weeks apart to correspond with the same phase of menstrual cycle for each player and to allow for an adequate wash-out period.

Following the first week of intervention, participants resumed regular soccer activities in their respective teams for four weeks (wash-out period). Upon completion of the wash-out period participants were again reminded to not partake in any physical activity 24h prior to baseline testing. The second week of intervention was identical to the first, with the exception that supplementation was crossed over (i.e., each participant received the opposite supplement from the first week of training). An overview of the cross-over design can be found in Figure 3.1.

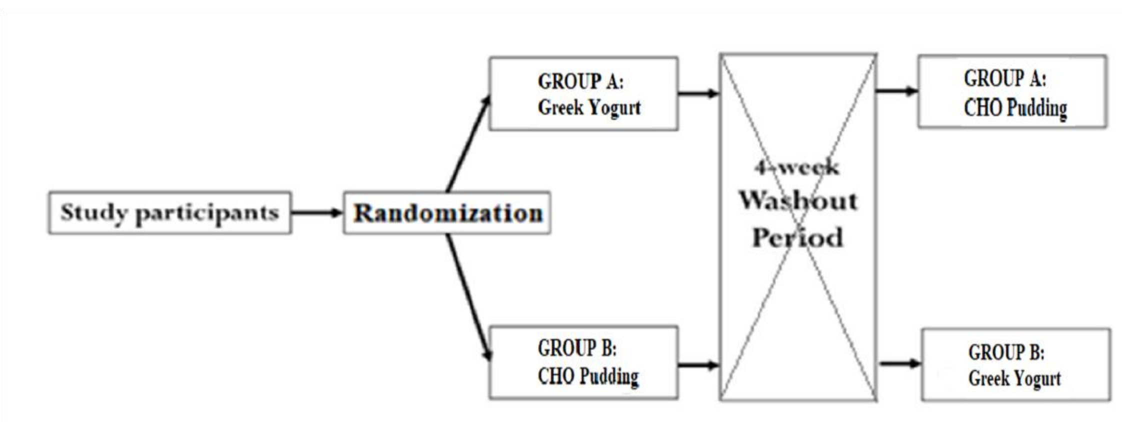


Figure 3.1: Study, crossover design.

3.3 Experimental Procedures and Measurements

The information session occurred ~3 weeks prior to the study's commencement. During this visit, participants and their parents/guardians were provided with information regarding the study's purpose, procedures, and benefits and risks associated with participation. Informed consent and assent were then obtained, followed by the completion of a medical screening questionnaire which included the reporting of any injuries, allergies, and/or health-related conditions, along with information regarding menstruation. Participants also completed a training history questionnaire. Parents were then instructed to have participants refrain from exercise 24h prior to the initial baseline data collection visit.

Standing and seated height were measured using a portable stadiometer (SECA - 217, Canada), and recorded to the nearest 0.5 cm. These measurements were used to calculate somatic maturity offset (years from the age of peak height velocity), as previously described (Mirwald, Baxter-Jones, Bailey, & Beunen, 2002). Body mass and percent body fat (%BF) were measured by bioelectrical impedance analysis (Biospace.228, Los Angeles, CA, USA), and were recorded to the nearest 0.1 kg and 0.1%, respectively. A fasted saliva and venous blood sample was then taken from an antecubital vein, followed by a standardized breakfast, which included one granola bar, one muffin, fruit (banana, apple, strawberries) and a juice box or water (~400-500 kcal total).

Following this visit, participants were instructed to limit dairy consumption (i.e 1-serv/d) during the 5-day training period and were then provided with a food frequency questionnaire (Block 2014.1_6Mo, Nutrition Quest, USA), to be completed with the assistance of a parent by the end of the study. During the period between the first blood draw and the first training session, participants were randomized to one of two experimental conditions: Greek yogurt (GY) or an isocaloric study-designed carbohydrate pudding (CHO), by an independent research assistant (described below).

3.4 Training Sessions

Training sessions began the day following baseline blood draw and consisted of 5 consecutive days of soccer-specific training, structured to mimic a heavy-volume, high-intensity training week (microcycle). Training sessions occurred at 18:00-20:00h each day and were administered by a certified technical soccer coach and knowledgeable training staff. The coach-to-participant ratio during all training sessions was 1:3.

Each session began with a 15min dynamic warm-up followed by 90-min of soccer-specific training, ending with a 15-min cool-down. The 90-min of soccer-specific drills were performed at maximal effort and consisted of agility, sprinting and plyometric drills as well as ball-handling, small-sided games (rondo) and shooting. Specifically, on Monday, Wednesday and Friday, work-to-rest ratios during activities and drills were 1:1 and 1:2 and on Tuesday and Thursday rest intervals were increased (i.e., 1:3), due to more explosive drills (i.e., plyometric exercise). Following the completion of each training session participants were asked to individually rate how hard the practice was, using a standard rating of perceived exertion scale. Indeed, the mean rating was similar ($p = 0.16$) between the two intervention conditions (GY: 8 [8 – 9], CHO: 8 [8 – 9]).

3.5 Nutritional Intervention

Each participant received three daily servings of their respective supplement during the five consecutive days of training. The study-designed pudding was created daily in the laboratory using a combination of fat-free vanilla Jell-O instant pudding (6g) and maltodextrin (24g) mixed in water. Both supplements were served in clear containers by an independent research assistant, separate from the coaching staff (double-blind). While the palatability and taste of GY was noticeable to participants, the true contents of the study designed pudding was concealed. Participants were informed that this supplement was created to maximize their performance. Participants consumed three servings of 160g of GY (~115 Kcals, 17g protein, ~11.5 carbs) (Skotidakis Inc., St. Eugene, Ontario, Canada), or 30g of isocaloric CHO pudding (~115Kcal, 0.04g protein, ~28.6g carbs) immediately following the training session, 1h prior to bedtime, as well as one serving between breakfast and lunch on the subsequent day. Following the last training session, participants consumed only two servings (immediately post-session and prior to bedtime).

Although the literature reports a weak to moderate effect on timing of protein consumption and bone metabolism immediately post-exercise (Townsend et al., 2017), we chose to give participants protein immediately post-exercise to partly control supplement consumption adherence and to mimic the design of similar studies using milk for comparison purposes. Due to the specific digestion and absorption kinetics of casein protein (which comprises the majority of protein in GY), we gave participants 160g of GY prior to sleep as research shows that protein, specifically casein, prior to sleep may maintain an elevated net protein balance throughout sleep (Schoenfeld, Aragon, & Krieger, 2013). In addition to protein timing, we also wanted to ensure that our supplementation of GY allowed participants to meet the overall protein intake of 1.6 g/kg/day in efforts to maximize bone adaptations.

During both training portions of the study, participants were asked to self-report all food consumed in a 24h period for 5 consecutive days. The 24h food recall form was provided to each participant in a folder with a portion sheet stapled to the inner flap. Each day participants would complete and return the food record and be provided with a blank one for the following 24h period. Photocopies of the food records over the course of the first training week were made and provided back to participants prior to the second week of training. Participants were instructed to follow the same diet as best they could. All food records were analyzed using a diet analysis program (Food Processor, ESHA Inc., Salem, OR), and inputted and analyzed by the same examiner for consistency. Habitual dietary intake was assessed using the Block Food Frequency Questionnaire (FFQ), designed to assess dietary habits through a recall of foods eaten in the last 6 months (Berkly, CA. USA).

3.6 Blood Collection and Analysis

A total of 10ml of blood was collected from an antecubital vein by a certified phlebotomist using a standard venipuncture technique. Blood samples were obtained on four occasions (pre- and post-training during training week 1 and 2) 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 for 20 minutes at room temperature (23°C) before being centrifuged at 4°C for 15 min at 1,405 RCF (g). All Ethylenediaminetetraacetic acid (EDTA) plasma tubes were centrifuged immediately at room temperature (23°C) for 15 min at 1,405xg. Serum was separated and aliquoted into 0.5ml polyethylene cryotubes that were stored at -80°C until analysis upon study completion.

All analytes were measured in duplicate, with the average coefficients of variations (CV) estimated in-house. Serum concentrations of tOC and OPG were measured 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 tOC were 2.7% and 8.4%, and for OPG were 0.1% and 1.5%, respectively. Serum concentration of unOC was measured using an ELISA kit (cat. # MK118, Takara Bio, USA) with an inter-assay CV of 4.7% and an intra-assay CV of 7.0%. Serum CTX was measured using an ELISA kit (cat. # E-EL-H0835, Elabscience, China) with an inter-assay CV of 17.0% and an intra-assay CV of 5.8%. RANKL was also measured in serum using a microbead multiplex kit (Human RANKL MAG Bead Single Plex Kit, cat.# HRNKLMAG-51K-01, EMD Millipore, Darmstadt, Germany) with an average intra-assay CV of 5.2%, and an inter-assay CV of 5.7%. The OPG:RANKL ratio was calculated for each participant at each time point by dividing the OPG concentration in pg/ml with the RANKL concentration in pg/ml.

In addition, although each participant was in the same phase of their menstrual cycle for the two training weeks, participants were of different gynecological age and were not tested in the same phase of the cycle. Given the potential for estrogen concentrations to affect bone metabolism, morning, fasting circulating estradiol concentrations were measured during the two baseline visits only to confirm that each participant had similar estradiol levels in both intervention conditions. Specifically, estradiol was measured in serum using an ELISA assay (Human Estradiol E2 kit, Abcam, Toronto, Ontario, Canada). The estradiol inter-assay CV was 5.2%, intra-assay CV was 8.0%. The sensitivity of this assay was 10-1000 pg/ml with 101.3% recovery in serum.

3.7 Statistical Analysis

Prior to analysis, data were screened for normality using the Shapiro-Wilk test, z-scores for skewness and kurtosis of ±3 and visual screening of histograms for symmetry. The screening showed that unOC, tOC, CTX, RANKL and OPG/RANKL ratio were not normally distributed and were log-transformed for the analysis. Three participants with missing values for unOC, CTX and RANKL due to undetectable concentration (i.e., below the detection limit of the biochemical assay) were excluded from the analysis of these markers.

A one-way analysis of variance (ANOVA) was used to examine differences between the two baseline trial visits in terms of physical characteristics, training history, estradiol concentration, and bone markers. Changes in each of the biomarkers from pre- to post-training were examined using a two-way repeated measures analysis of variance, with two within-subject main-effects (time and condition). An alpha value of $p<0.05$ was used to determine statistical significance. Statistical analyses were performed using SPSS version 25.0 for Windows.

Chapter 4: Results

Our nutritional intervention was successful in creating two distinct conditions, with no differences in daily energy or fat intake between GY and CHO (Table 4.1). In contrast, participants consumed significantly ($p \leq 0.001$) more protein in the GY condition compared to the CHO condition, and significantly ($p \leq 0.001$) more carbohydrates in the CHO condition compared to the GY condition (Table 4.1). Likewise, when training diet (via food record) was compared to participants' habitual diets (via FFQ), no significant differences were observed with respect to the daily energy intake ($p = 0.22$) and relative fat consumption ($p = 0.82$), but relative habitual protein and carbohydrate consumption were significantly higher compared to the intervention weeks, where CHO ($p = 0.003$) and GY ($p \leq 0.001$) were consumed, respectively (Table 4.1).

Table 4.1 Habitual nutrition intake, and intervention nutritional intake.

Condition	Energy intake (kcal)	Fat intake ($g \cdot kg^{-1} \cdot d^{-1}$)	Carbohydrate intake ($g \cdot kg^{-1} \cdot d^{-1}$)	Protein intake ($g \cdot kg^{-1} \cdot d^{-1}$)
Habitual	1622 ± 502	1.1 ± 0.4	3.3 ± 0.7	1.1 ± 0.3
Greek Yogurt	1892 ± 287	1.0 ± 0.2	4.0 ± 1.0	1.9 ± 0.3*
Control CHO Pudding	1959 ± 441	1.0 ± 0.4	5.2 ± 1.2†	1.0 ± 0.3

Values are mean ± standard deviation; habitual diet was assessed by food frequency questionnaire; energy and macronutrient consumption (including the supplements) during the Greek yogurt (GY) and isocaloric control (CHO) conditions were assessed using diet record. * indicates GY significantly greater ($p < 0.05$) than CHO and habitual. † indicates CHO significantly greater ($p < 0.05$) than GY and habitual.

Basal estradiol concentrations were similar at the beginning of the GY and CHO intervention conditions (13.1 ± 11.2 $pg \cdot ml^{-1}$ versus 13.4 ± 12.1 $pg \cdot ml^{-1}$, respectively). Resting and post-training biomarker levels were not significantly different between conditions (Table 4.2). The high variability is evident from the %CV ranging from 19% in OPG to 88% in the

OPG/RANKL ratio (Table 4.2). This variability is also evident in Figures 4.1 to 4.5 presenting the individual and group pre- to post-training changes in all markers. As shown in Table 4.2, there were no significant differences in the resting, pre-training concentrations of bone turnover markers and osteokines between intervention conditions.

Table 4.2 Resting, morning, pre-training concentrations of bone turnover markers and osteokines during each nutritional intervention condition.

Marker	**Group**	**Pre-training**	**Post-training**
tOC (ng·ml^{-1})	GY	74.0 ± 29.9 [40%]	74.0 ± 29.1 [39%]
	CHO	73.2 ± 30.2 [41%]	78.0 ± 33.5 [43%]
unOC (ng·ml^{-1})*#	GY	8.9 ± 4.5 [50%]	6.6 ± 3.5 [54%]
	CHO	8.6 ± 4.5 [52%]	8.4 ± 4.6 [54%]
unOC/tOC (%)*	GY	12.4 ± 6.1 [49%]	9.4 ± 5.0 [53%]
	CHO	11.6 ± 4.6 [40%].	10.5 ± 4.4 [42%]
CTX (pg·ml^{-1})	GY	0.17 ± 0.11 [65%]	0.16 ± 0.10 [62%]
	CHO	0.16 ± 0.11 [68%]	0.16 ± 0.11 [68%]
OPG (pg·ml^{-1})	GY	1388.2 ± 475.9 [34%]	1223.8 ± 233.0 [19%]
	CHO	1206.8 ± 363.4 [30%]	1273.1 ± 344.9 [27%]
RANKL (pg·ml^{-1})	GY	34.3 ± 22.1 [64%]	29.8 ± 21.4 [72%]
	CHO	30.3 ± 21.4 [71%]	35.0 ± 17.9 [51%]
OPG/RANKL (ratio)	GY	57.4 ± 48.5 [84%]	69.5 ± 57.1 [82%]
	CHO	57.1 ± 48.2 [84%]	50.6 ± 44.7 [88%]

Values are mean ± standard deviation [% coefficient of variation]; tOC= Total Osteocalcin; unOC = Undercarboxylated Osteocalcin; CTX = Carboxyl-Terminal Crosslinking Telopeptide of Type I Collagen; OPG Oosteoprotegerin; RANKL = Receptor Activator Nuclear Factor kappa-β ligand; * denotes significant main effect for time; # denotes significant time by condition interaction

Total OC (Figure 4.1) showed no main effect for condition (F=0.021; p=0.886, partial η^2=0.001) or time (F=0.285; p=0.589, partial η^2=0.012), and no interaction (F=0.280; p=0.602, partial η^2 =0.012). Circulating unOC showed a significant main effect for time (F=16.0; p=0.001, partial η^2=0.471) and a significant time by condition interaction (F=7.919; p=0.011, partial η^2=0.306) with no condition effect (F=0.15; p=0.707, partial η^2=0.008). Specifically, unOC (Figure 4.2) decreased post-training in the GY condition by -26% on average (p=0.002; 95% CI = -14% to -38%), but in the CHO condition this decrease was only -3% (p=0.638; 95% CI = -17% to +10%). The relative unOC to tOC (%unOC/tOC) ratio showed a significant main effect for time (F=12.2; p=0.003, partial η^2=0.405), reflecting an overall decrease (-16%, p=0.011; 95% CI = -28% to -4%) from pre- to post-training (Figure 4.3), but with no significant time by condition interaction (F=2.80; p=0.0112, partial η^2=0.135) and no condition effect (F=0.002; p=0.961, partial η^2=0.000).

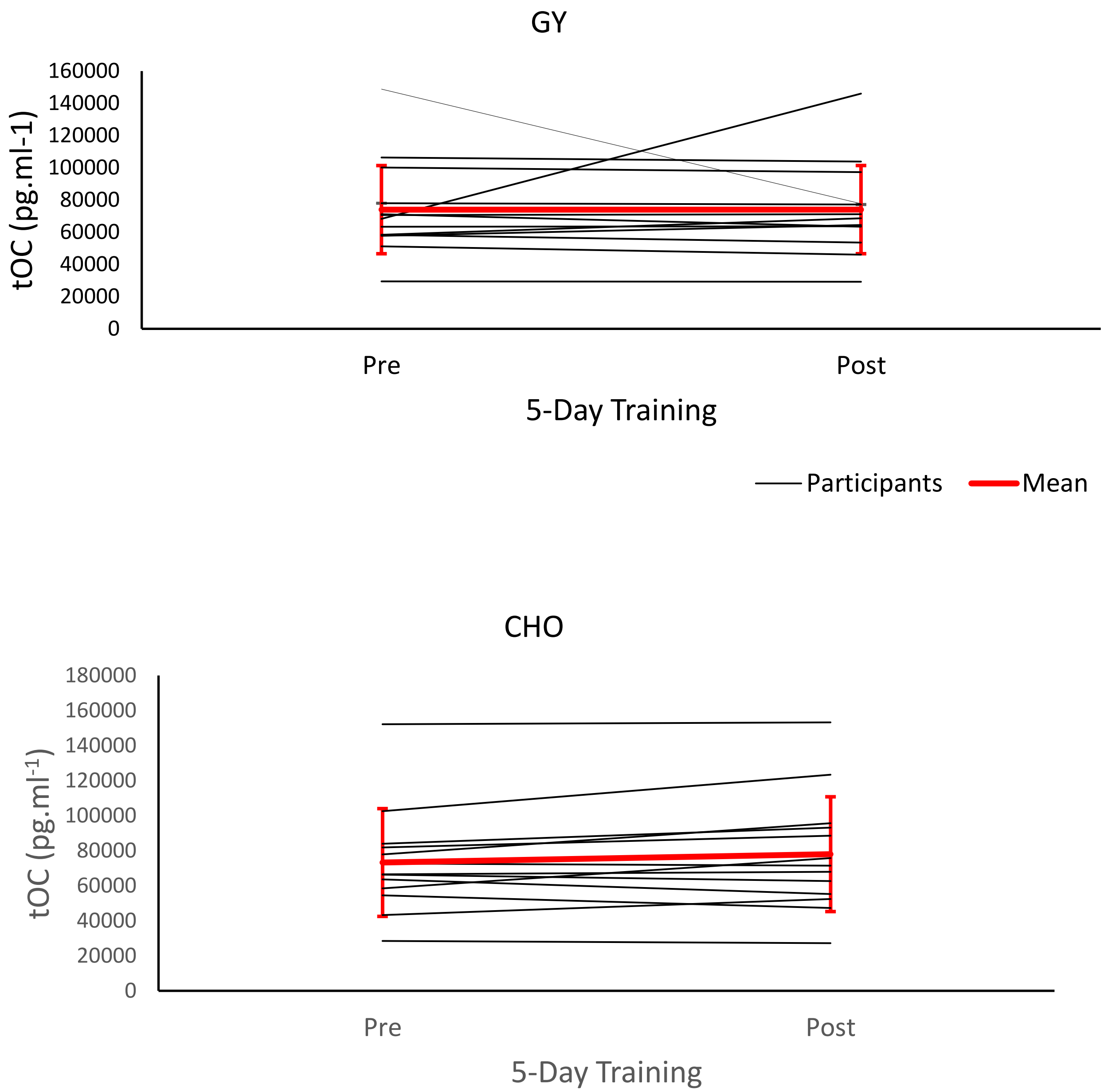


Figure 4.1: Pre- and post-training serum concentrations (mean ± standard deviation, n=13) of total osteocalcin (tOC), during the Greek Yogurt (GY) and the Carbohydrate (CHO) intervention conditions. Values are absolute concentrations, not log-transformed.

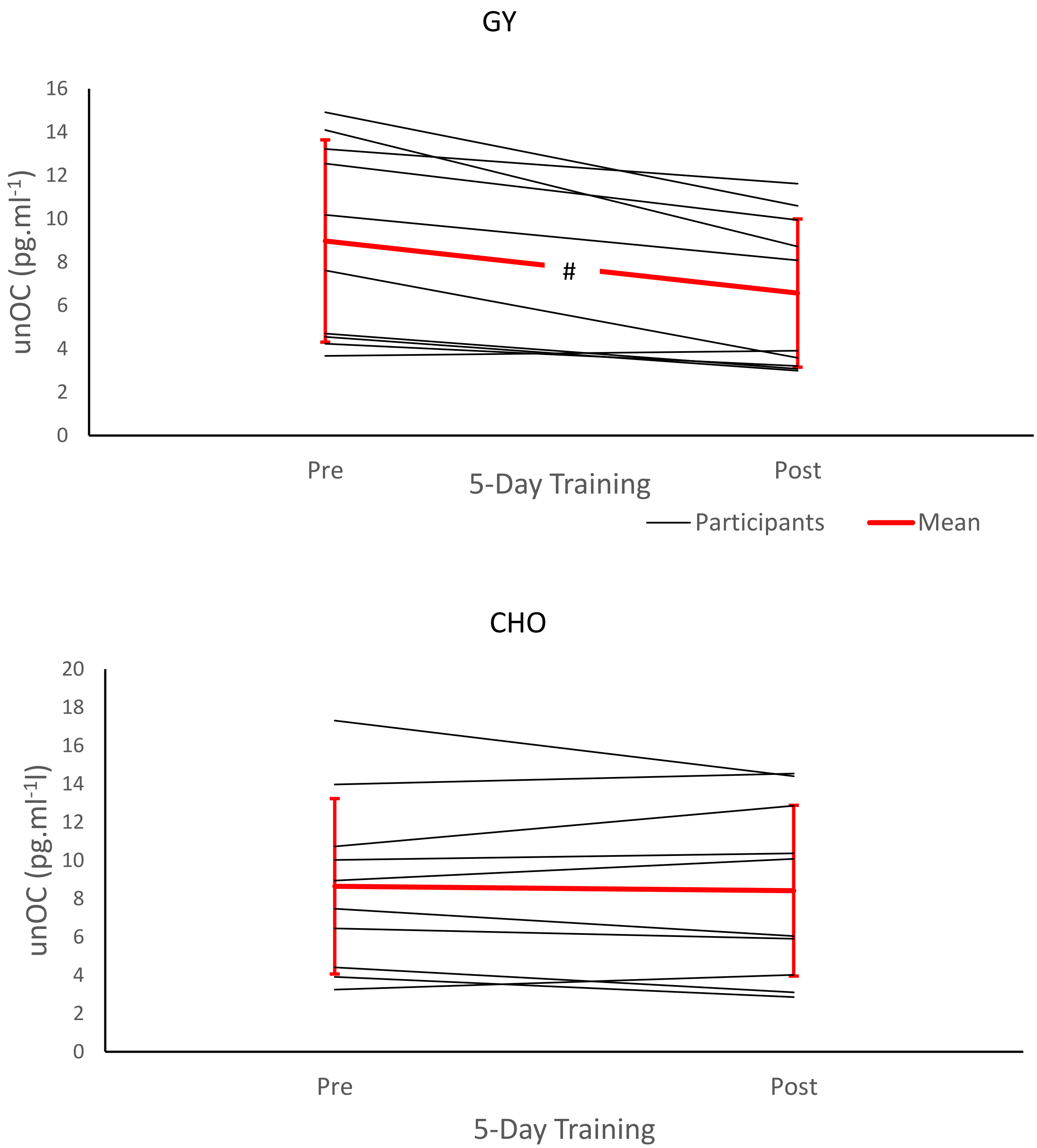
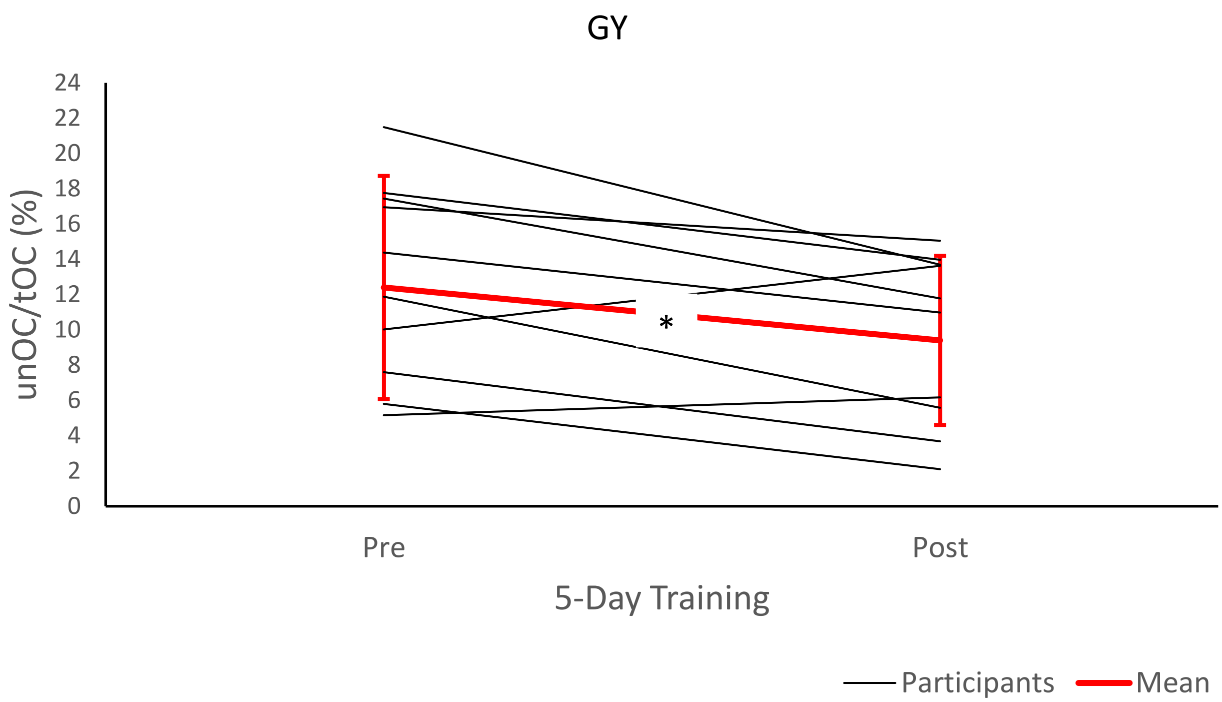


Figure 4.2: Pre- and post-training serum concentrations (mean ± standard deviation, n=10) of undercarboxylated osteocalcin (unOC), during the Greek Yogurt (GY) and the Carbohydrate (CHO) intervention conditions. # denotes significant time by condition interaction reflecting a decrease in GY condition only (p<0.05). Values are absolute concentrations, not log-transformed.



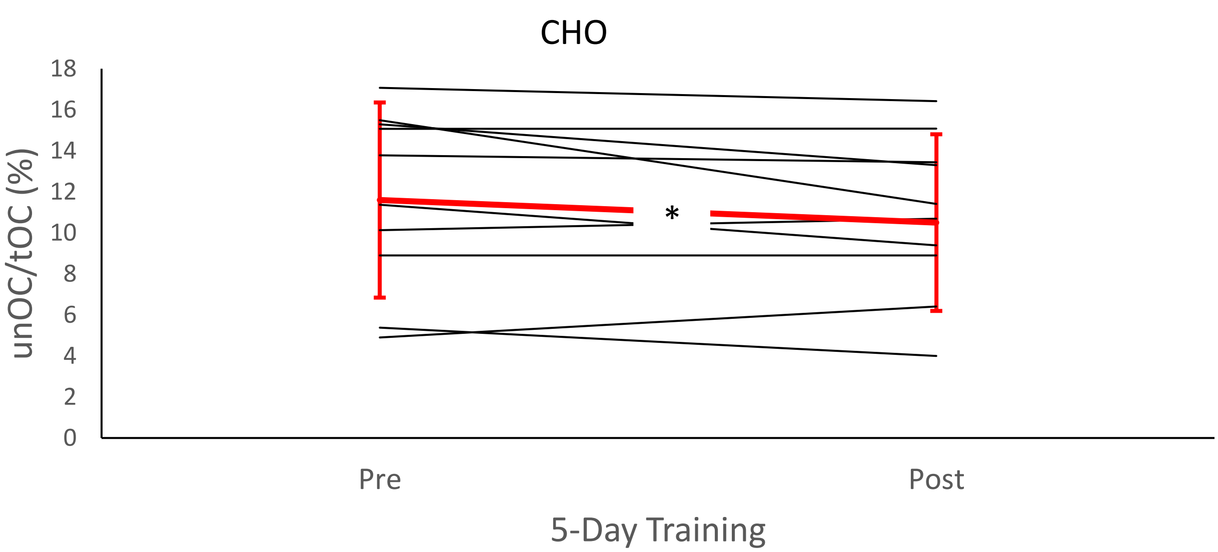
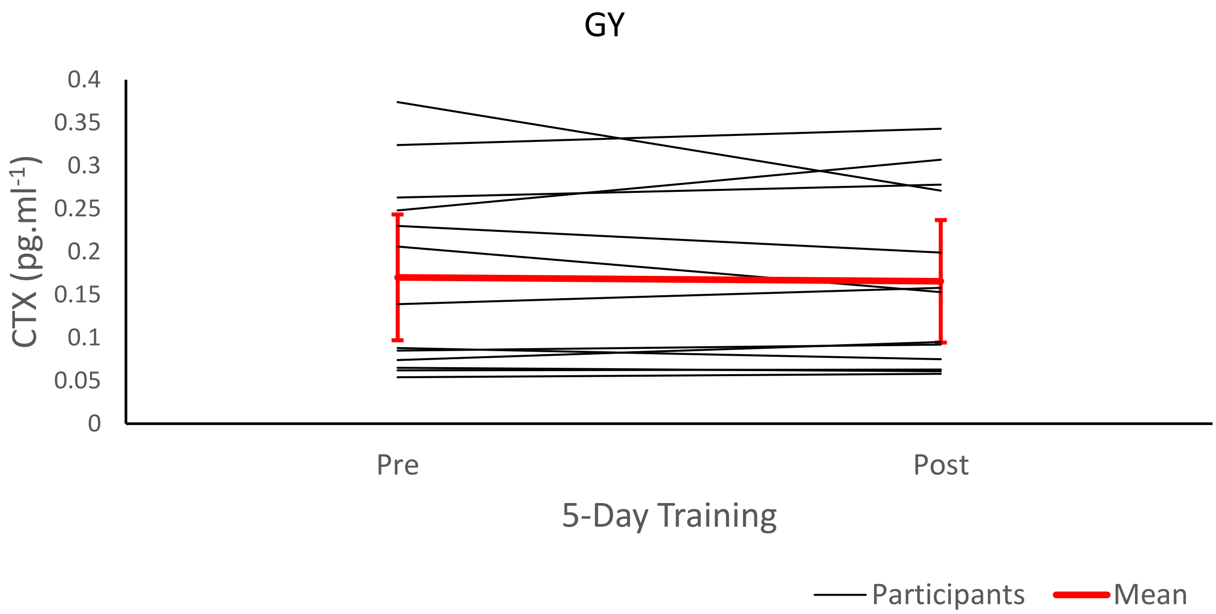


Figure 4.3: Pre- and post-training of relative undercarboxylated osteocalcin to total osteocalcin (%unOC/tOC) ratios (mean ± standard deviation, n=10) during the Greek yogurt (GY) and Carbohydrate (CHO) intervention conditions. * denotes significant main effect for time ($p<0.05$). Values are absolute concentrations, not log-transformed.

There was no main effect for condition (F=0.137; p=0.715, partial η^2=0.006) or time (F=0.060; p=0.809, partial η^2=0.002), and no interaction (F=0.064; p=0.802, partial η^2=0.003) for CTX (Figure 4.4).



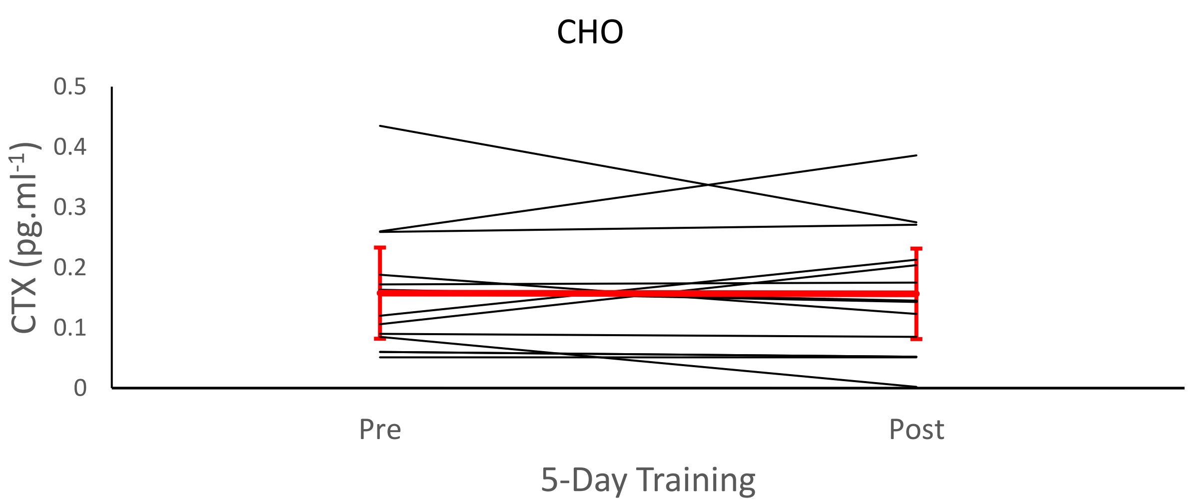


Figure 4.4: Pre- and post-training serum concentrations (mean ± standard deviation, n=10) of carboxyl-terminal crosslinking telopeptide of type I collagen (CTX), during the Greek Yogurt (GY) and the Carbohydrate (CHO) intervention conditions. Values are absolute concentrations, not log-transformed.

OPG (Figure 4.5) showed no significant main effect for condition (F=0.35; *p*=0.56, partial η^2=0.014) or time (F=0.30; *p*=0.59, partial η^2=0.012), and no significant interaction (F=1.65; *p*=0.21, partial η^2=0.064). RANKL (Figure 4.6) also showed no significant main effect for condition (F=0.11; *p*=0.74, partial η^2=0.005) or time (F=0.48; *p*=0.49, partial η^2=0.019), and no significant interaction (F=2.73; *p*=0.11, partial η^2=0.102).

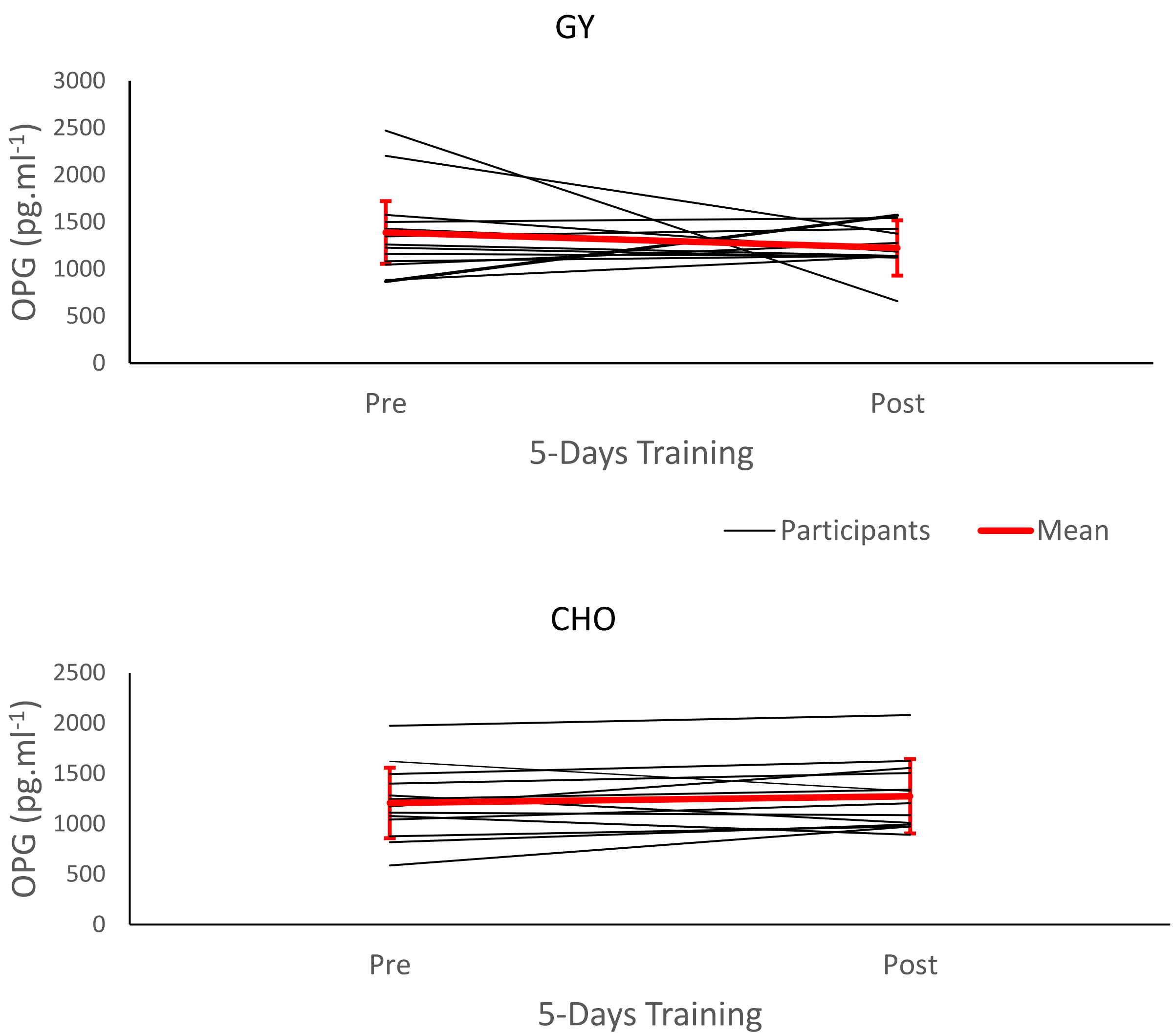


Figure 4.5: Pre- and post-training serum concentrations (mean ± standard deviation, n=13) of osteoprotegerin (OPG), during the Greek Yogurt (GY) and the Carbohydrate (CHO) intervention conditions.

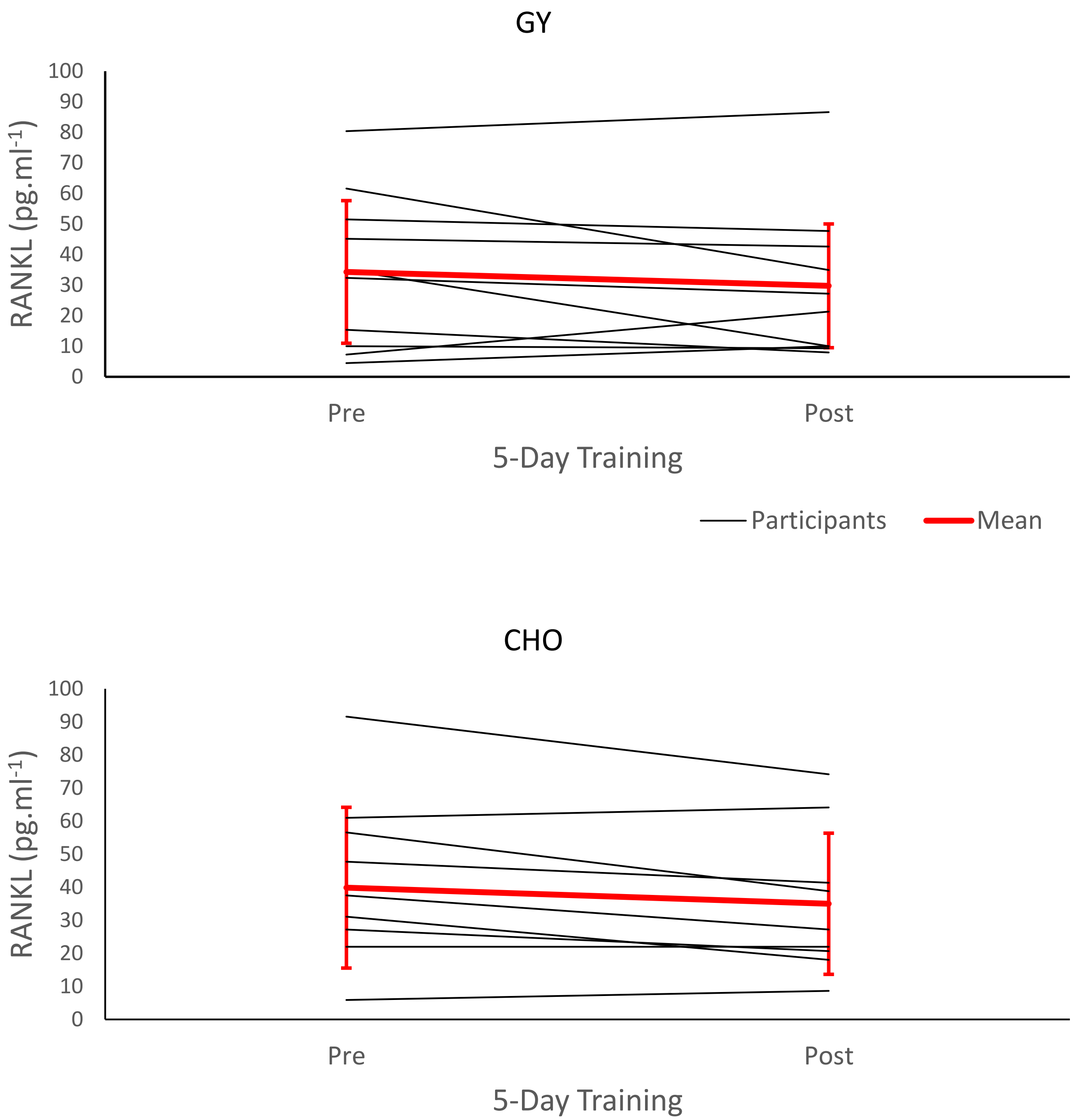


Figure 4.6: Pre- and post-training serum concentrations (mean ± standard deviation, n=10) of receptor activator of nuclear factor kappa-B ligand (RANKL), during the Greek Yogurt (GY) and the Carbohydrate (CHO) intervention conditions. Values are absolute concentrations, not log-transformed.

Finally, for the OPG/RANKL ratio (Figure 4.7), there was no significant main effect for condition (F=0.16; p=0.69, partial η^2=0.007) or time (F=0.13; p=0.73, partial η^2=0.005), and no significant interaction (F=1.22; p=0.28, partial η^2 =0.049).

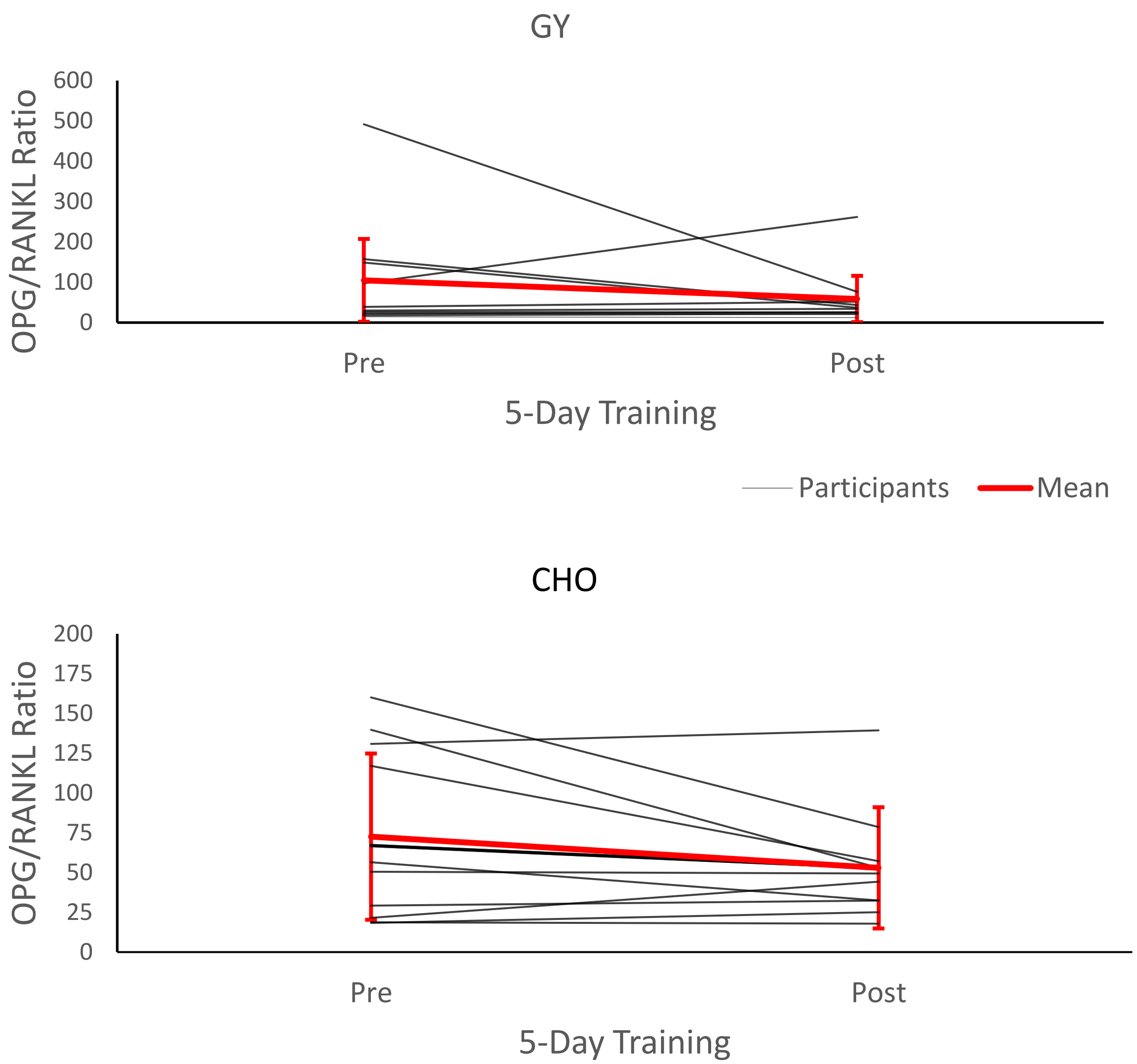


Figure 4.7: Pre- and post-training OPG/RANKL ratios (mean ± standard deviation, n=10) during the Greek Yogurt (GY) and Carbohydrate (CHO) intervention conditions. Values are absolute concentrations, not log-transformed.

Chapter 5. Discussion

5.1 General Discussion

This is the first study to our knowledge examining the effects of consuming Greek Yogurt compared with an isocaloric carbohydrate control pudding, on bone biomarkers following one week of intense training in adolescent girls. Our hypothesis was that the consumption of GY would help to maintain the systemic levels of bone formation and resorption markers near their pre-training levels, compared with the isocaloric carbohydrate control pudding condition, in which we expected mainly an increase in unOC, CTX and/or RANKL, with unchanged or decreased tOC and OPG. This hypothesis was not confirmed. There were no effects of GY supplementation compared to an isocaloric CHO supplement and no adverse effects from intense soccer training on CTX as well as OPG, RANKL and their ratio following intense training. Although there were no effects of training or condition on total osteocalcin, unOC decreased significantly at the end of the intense training period in the GY condition, but not in the CHO condition. Interestingly, however, relative unOC, expressed as a percentage of tOC was reduced post-training with no differences between the GY and CHO conditions. As previously suggested, a decrease in the %unOC/tOC reflect a lower risk of bone fracture, or potentially an increase in BMD. Adolescence is a crucial period of high bone turnover, growth, and development, and although this study did put the adolescent girls through intense training, there is a possibility that this period of high turnover in bone protects them from the deficits of over training or chronic exercise. In other words, it might be possible that their growing processes are able to counter any negative effects of high-impact intense training for multiple days, especially when combined with good energy and nutritional intake.

Since there is limited comparable research available in the pediatric population, acceptable baseline ranges for bone turnover markers are currently unknown. However, the tOC concentrations in our adolescent soccer players were very similar to those previously reported in adolescent female athletes (ages 14-18), i.e., within a 6000 pg·ml-1 to 8,000 pg·ml^{-1} range (Jürimäe et al., 2021). In the circulation, osteocalcin is considered a marker of bone formation, yet its exact role in the control of bone matrix formation, mineralization or maintenance is not fully understood (Harada & Rodan, 2003; Lin et al., 2018). It is generally accepted, however, that circulating tOC levels, are indicative of osteoblast activity, and were found to increase in adults during exercise (Moser & van der Eerden, 2019). In children, a study comparing the response to plyometric exercise in normal weight and overweight adolescent females found that tOC levels increased significantly 1h post-exercise (Kurgan, McKee, Calleja, Josse, & Klentrou, 2020). Their results suggest that using a higher impact modality of exercise may be beneficial for promoting positive bone turnover responses during this critical period for bone accrual (i.e., adolescence). Thus, our results of no change in bone markers over 5 days of intense high impact exercise training may also suggest that the increased mechanical stimulus of the activity could counterbalance the potential negative effect of the high intensity, repetitive load, at least in the short term (one week).

Circulating osteocalcin comprises both undercarboxylated and carboxylated form (Lin et al., 2018), of the total amount of osteocalcin that is released into the circulation, a substantial proportion (40–60%) is unOC (Lin et al., 2018). A higher percentage of unOC has been associated with an increased risk of bone fracture in older adults, particularly women. Therefore, the detection of circulating unOC has long been recognized as having clinical predictive value as a biomarker and indicator of fracture risk (Lin et al., 2018). In humans, however, most bone studies have measured total osteocalcin instead of the undercarboxylated form suggesting a measurement bias. Interestingly, we found a training-induced decrease in unOC in the GY condition, but not in the CHO condition, which could be interpreted as GY preserved bone turnover after training better than CHO. However, this can be misleading because unOC is implicated in glucose metabolism, so this decrease could reflect differences in the overall metabolic use/allocation between conditions. The results of the few studies that have measured unOC in response to exercise have been equivocal (Booth, Centi, Smith, & Gundberg, 2013). One study in obese men reported that exercise increases circulating levels of unOC and that increases in unOC correlated with the post-exercise reductions in serum glucose levels (Levinger et al., 2011). Since unOC has been reported to regulate glucose metabolism, which provides energy to muscles during exercise (Ferron, McKee, Levine, Ducy, & Karsenty, 2012; Lee et al., 2007; Zoch, Clemens, & Riddle, 2016), it has been determined that skeletal muscle and adipose tissue respond to osteocalcin by increasing their sensitivity to insulin (Ferron et al., 2012; Lee et al., 2007; Zoch et al., 2016). It is possible that unOC concentrations were positively impacted in both our nutritional conditions by the added 345 kcal·d-1 (3 servings x 115 kcals per serving). This could counterbalance any training-induced catabolic effect on bone formation as energy availability does play a role in bone metabolism (Moser & van der Eerden, 2019). Indeed, relative unOC, which is a better reflection of bone turnover status, decreased following the intense training in our adolescent athletes with no differences between GY and CHO. This finding supports the suggestion that there was no negative effect on bone formation following intense training in our adolescent athletes.

The resting CTX levels seen in this study were lower than previously measured in young adult women (Kouvelioti et al., 2018), as well as in adolescent swimmers (Theocharidis et al., 2020). These low levels of CTX could be due to either the younger age of our adolescent female participants or their involvement in a high impact sport. Since this is the time of peak bone formation in their lives, resorption levels may be lower in adolescent females, and even lower in these participants as they are high-impact athletes. In a recent study looking at adolescents during low impact, high intensity swimming, it was found that 8h after exercise there was an increase in CTX before returning to baseline 24hours after exercise (Theocharidis et al., 2020). Interestingly, however, our CTX levels were only slightly lower than what has been seen in young adult males during high-impact exercise (0.41-0.67 $pg \cdot ml^{-1}$) in a previous study by Rantalainen et al., 2009. In addition, we found no significant changes in CTX the high-intensity, high-impact training, and no differences between the GY and CHO conditions. Previously, protein combined with carbohydrate consumption has shown to acutely decrease CTX concentrations 2-4 hours after exhaustive running in endurance-trained male adults (Townsend et al., 2017). Additionally, a study by Bridge et al. (2020), examining the effect of GY supplementation over a 12-week period of training found that CTX increased acutely after exercise, but returned to baseline over time in the group that consumed GY. However, the results of both these studies were in adult males and cannot be directly compared with our findings in adolescent females.

Our group recently published an article on the impact of 12 weeks of dairy consumption and exercise on bone-biomarkers in overweight adolescents, there were 2 groups consisting of a dairy consumption at the recommended amount and a lower dairy consumption group (Josse et al., 2020). In that study, we found decreases in both OC and CTX after 12-weeks of moderate intensity intervention (Josse et al., 2020), while we saw no change in these two markers with the 1-week, high intensity intervention (Josse et al., 2020). Though our present study was shorter in duration than this previous study, we add to the literature by showing that bone markers remained constant during 5 consecutive days of intense high-impact training, with consumption of both dairy and CHO (3 servings/day), in a unique cohort of highly active, elite adolescent female athletes. The maintenance of bone turnover may contribute to an enhanced bone accretion during this period of growth of our participants' lives. The beforementioned study by Kurgan et al. (2020) noted a significant decrease in CTX following plyometric exercise in both normal weight and overweight adolescent females. The decrease in CTX was seen immediately post-exercise as well as one hour post-exercise (Kurgan, McKee, et al., 2020), suggesting a shift towards a supressed bone resorption, i.e., an anabolic response to high-impact exercise in female adolescents. However, the present study demonstrated that CTX levels were maintained (no increase or decrease) after 5 consecutive days of high-impact exercise, suggesting that the acute decreases in CTX following one intense session are transient in this young population.

We found no significant changes over time and no condition by time interaction in OPG, so we can note that the OPG levels did not change during the training weeks, and that GY provided no added benefit compared with CHO. OPG is secreted by osteoblasts and osteogenic stromal stem cells to protect the skeleton from excessive bone resorption by binding to RANKL and preventing the interaction with RANK (Boyce & Xing, 2008). In our study, both RANKL and the OPG/RANKL ratio were not affected by the intense training, suggesting no increase in catabolism because of training. A study in college females (non-regular exercisers) also found no significant changes in the OPG and RANKL levels after a 12-week combined endurance and resistance training program (Kim, Kim, & Kim, 2019). As RANKL is an indicator of osteoclast activity, our research potentially demonstrates that intense training does not increase osteoclast activity in young female athletes. Since soccer is a high-impact activity, these athletes already have a positive bone formation to resorption ratio. As mentioned earlier, OPG protects the skeleton from excessive bone resorption, therefore making the OPG/RANKL ratio an important determinant of bone mass and skeletal integrity (Boyce & Xing, 2007). It has been confirmed that osteoclast activity is dependent on the relative balance of OPG and RANKL, which is why it has been suggested that the OPG/RANKL ratio is a critical factor for determining the osteoclastic activation at the bone level (Wasilewska et al., 2010). The study by Kim et al., (2019) in college-aged females also explored the OPG/RANKL ratio and found no significant differences after their exercise program. However, they did have much lower ratio results at baseline and post-exercise when compared to our study. The OPG/RANKL balance is critical for bone remodeling and the preservation of bone mass.

5.2 Strengths and Limitations

One of the strengths of this study is its cross-over design, making this study very compelling since each participant acted as their own control. Another strength of the study is that all measurements were done in the morning and before breakfast, thus eliminating diurnal fluctuations and nutritional effects. This was important since most bone turnover markers are stable and do not vary much throughout the day or when food is consumed, but CTX is known to be at its peak during morning hours and is influenced by food intake (Seibel, 2005).

Another strength of this study is its ecological validity. First, we opted to have participants complete a full week of training sessions that were sport-specific, as increased training frequency is common in this population. Each training session included drills, conditioning, tactical and technical ball work, as well as plyometric training. The training was periodized and training variables were manipulated throughout the intervention to ensure continuous progressions and efforts from the participants. We included plyometric training in addition to soccer-specific work, primarily because of its effects on bone health, as well as the combination of ball work and plyometric training potentially being more similar to the typical physical activity patterns in our specific population. Second, we chose 1 week for our training intervention as this is a typical duration for high level soccer players when leading into a game or tournament, so we tried to mimic that training.

The study also has its limitations, first being the small sample size. Although it is not less than previously used in cross-over studies, the study was underpowered (observed power 1-b = 0.10 to 0.60). It is also important to mention that bone turnover markers reflect overall bone homeostasis, i.e., the activity of osteoblasts and osteoclasts in physiological conditions, and do not reflect bone cellular activity at a specific site (Bhattoa, 2018).

5.4 Conclusions and Future Directions

When compared to an isocaloric carbohydrate control pudding, three daily dosages of GY during high-intensity, high-impact, short-term training, led to no differences in bone formation or resorption markers, either at rest or in response to training. We also found no markable adverse effects on markers of bone metabolism because of the intense soccer training. This brings us to the conclusion that, overall, when female adolescent athletes are well nourished, they are protected from the potential negative impact on bone metabolism, and eventually bone accrual, due to high volume training, at least in the short-term. It is important to note that soccer is a high-impact sport, however this finding of no major effect of intense training may not be the same for athletes of low-impact activities, in which case GY supplementation may play a different role. Promoting bone turnover in favour of bone formation during adolescence is beneficial because it can promote bone accretion, leading to higher peak bone mass and improved bone mineral density in the long term.

Future research should also focus on the long-term benefits of calcium and protein consumption on bone turnover markers in combination with a chronic or prolonged period of exercise training in children and adolescents. Specifically, future research should examine the effects of different doses of protein on bone turnover in pediatric populations. This research may help shed light on the impact that calcium and protein could have on this population and athletic cohort. An additional avenue could be to study the common issue of female athletes dealing with poor nutrition and over exercising habits. These habits can lead to individuals developing the female athlete triad or RED-S. Relative energy deficiency syndrome (RED-s), is the clinical phenomenon commonly known for the three tiers including; energy availability, menstrual function and bone health (Mountjoy et al., 2014). However, it is not a clinical phenomenon, rather a syndrome that has the potential to affect many aspects of health, athletic performance, and physiological function in females of all ages (Mountjoy et al., 2014).

With this up-and-coming research domain on adolescents and specifically athletic female adolescents, there may benefit to female athletes and provide the appropriate nutrition for maintaining bone health and optimal athletic performance. Adolescence is a crucial period for bone accrual, therefore, studying of the combined effects of intense training and protein consumption on bone metabolism could offer future valuable inputs to adolescent health and overall bone knowledge.

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Appendices

Appendix 1: Ethics Approval

Clinicaltrials.gov registration: NCT03947801

Brock University
Office of Research Ethics
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE:	5/16/2019		
PRINCIPAL INVESTIGATOR:	KLENTROU, Nota - Kinesiology		
FILE:	18-289 - KLENTROU		
TYPE:	Ph. D.	STUDENT: SUPERVISOR:	Brandon McKinlay Nota Klentrou

TITLE: Dairy consumption during a high-intensity, high-volume training week in young athletes

ETHICS CLEARANCE GRANTED

Type of Clearance: NEW Expiry Date: 5/1/2020

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from to **5/1/2020**.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before 5/1/2020. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Office of Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved:

Craig Tokuno, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

Appendix 2: Screening and Medical History Questionnaire

APPLIED PHYSIOLOGY RESEARCH GROUP
DEPARTMENT OF KINESIOLOGY, BROCK UNIVERSITY

Your responses to this questionnaire are confidential and you are asked to complete it for your own health and safety. If you answer "YES" to any of the following questions, please give additional details in the space provided and discuss the matter with one of the investigators. You may refuse to answer any of the following questions.

Name: ______________________________ Date: ______________________________

1. Have you ever been told that you have a heart problem?
   YES NO

2. Have you ever been told that you have a breathing problem such as asthma?
   YES NO

3. Have you ever been told that you sometimes experience seizures?
   YES NO

4. Have you ever had any major joint instability or ongoing chronic pain such as in the knee, back or elbow?
   YES NO

5. Have you ever been told that you have kidney problems?
   YES NO

6. Have you had any allergies to medication?
   YES NO

7. Have you had any allergies to food or environmental factors?
   YES NO

   If so, please provide details: ____

8. Have you had any stomach problems such as ulcers?
   YES NO

9. When you experience a cut do you take a long time to stop bleeding?
   YES NO

10. When you receive a blow to a muscle do you develop bruises easily?
    YES NO

11. Are you currently taking any medication (including aspirin) or have you taken any medication in the last two days?
    YES NO

12. Is there any medical condition with which you have been diagnosed and are under the care of a physician (e.g. diabetes, high blood pressure)?
    YES NO

Girls only:

Please answer the following questions:

1. Have you had your period?

YES NO

2. If yes, what date was your last period? ____________________

3. How often do you get periods? (e.g. every 28 days) _____________________________

4. Approximately how many days does your period last? ________________________________

Appendix 3: Training History Questionnaire

TRAINING HISTORY QUESTIONNAIRE FOR ATHLETES

Please fill in the table below to the best of your knowledge.

If you have any difficulties, discuss the matter with one of the investigators.

Activity/Sport	Level of Competition	# of years	Sessions/week	Min/session	Intensity (light, moderate, intense, very intense)	Seasonal length
Swimming						
Soccer						
Hockey						
Gymnastics						
Running						
Resistance						
Other						

Appendix 4: 24-Hour Food Record for Nutrition and Compliance

Participant ID:________________ Date: __________________

COMPLIANCE:

I consumed my yogurt or study designed supplement immediately after the training session ☐

I consumed my yogurt or study designed supplement at least 1h prior to bed ☐

I consumed my yogurt or study designed supplement at breakfast prior to training ☐

Please list all food/beverages/water – ***use the nutrition questionnaire portion size as your guide for the amount section***

TIME	AMOUNT	DESCRIPTION – What did you eat?

Appendix 5: Food Frequency Questionnaire

FOOD AND ACTIVITY QUESTIONNAIRE

RESPONDENT ID #

TODAY'S DATE

	DAY	YEAR
○ Jan		
○ Feb		
○ Mar	0 0	2014 ○
○ Apr	1 1	2015 ○
○ May	2 2	2016 ○
○ Jun	3 3	2017 ○
○ Jul	4	2018 ○
○ Aug	5	2019 ○
○ Sep	6	2020 ○
○ Oct	7	2021 ○
○ Nov	8	2022 ○
○ Dec	9	2023 ○

ABOUT THIS SURVEY

Please answer each question as best you can. Estimate if you aren't sure.

- **DETACH THE LAST PAGE OF THIS BOOKLET.** These are your portion pictures.
- **USE ONLY A NUMBER 2 PENCIL.**
- **FILL IN THE CIRCLES COMPLETELY** and erase completely if you make any changes.

ABOUT YOU

SEX
○ Male
○ Female

If female, are you pregnant or breast feeding?
○ No
○ Yes
○ Not female

AGE

WEIGHT pounds

HEIGHT feet inches

PROOF

INSTRUCTIONS

This form is about the foods you usually eat. Think about your intake over the last 6 months. This includes all meals or snacks, at home, in a restaurant, or carry-out.

Please tell us...

1. **HOW OFTEN,** on average, did you eat the food?
   DO NOT SKIP any foods. Mark "Never" if you didn't eat any of the food.
2. **HOW MUCH** of the food did you usually eat on the days you ate it?
   Sometimes we ask "how much" as A, B, C or D. **LOOK AT THE PORTION PICTURES.**
   Pick the picture that looks the most like the serving size you usually eat.
   (If you don't have pictures: A=1/4 cup, B=1/2 cup, C=1 cup, D=2 cups.)
3. **WHAT TYPE?** For some foods we ask the type (low-fat, low-sugar...) near the end of the survey.

EXAMPLE: This person drank orange juice twice a week, and had one glass each time.
Once a week this person ate a "C"-sized serving of cold cereal (about 1 cup).

	HOW OFTEN in the past 6 months?									HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D				
	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY					
Orange juice	○	○	○	○	○	●	○	○	○	How many glasses	● 1	○ 2	○ 3	○ 4
Cold cereal	○	○	○	○	●	○	○	○	○	Which bowl	○ A	○ B	● C	○ D

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

Block 2014.1_6Mo ©www.NutritionQuest.com Phone 510-704-8514

EGGS and DAIRY FOODS	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D				
Breakfast sandwiches or breakfast burritos with eggs or meat	○	○	○	○	○	○	○	○	○	How many sandwiches in a day	○ 1	○ 2		
Other eggs like scrambled or boiled, or quiche (not egg substitutes)	○	○	○	○	○	○	○	○	○	How many eggs a day	○ 1	○ 2	○ 3	○ 4
Yogurt (not frozen yogurt)	○	○	○	○	○	○	○	○	○	Which bowl or glass		○ B	○ C	○ D
Cottage cheese, ricotta cheese	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Cream cheese, sour cream, dips	○	○	○	○	○	○	○	○	○	How many tablespoons	○ 1	○ 2	○ 3	○ 4
Cheese, sliced cheese, cheese spread, including in sandwiches and quesadillas	○	○	○	○	○	○	○	○	○	How many slices	○ 1	○ 2	○ 3	○ 4
CEREALS, GRAINS, BREADS														
Cold cereals, ANY KIND, like corn flakes, fiber cereals, sweetened cereals	○	○	○	○	○	○	○	○	○	Which bowl		○ B	○ C	○ D
Oatmeal, or whole grain cereal like Wheatena or Ralston	○	○	○	○	○	○	○	○	○	Which bowl	○ A	○ B	○ C	○ D
Grits, cream of wheat, cornmeal mush	○	○	○	○	○	○	○	○	○	Which bowl	○ A	○ B	○ C	○ D
Milk or milk substitutes on cereal	○	○	○	○	○	○	○	○	○					
Brown rice, or dishes made with brown rice	○	○	○	○	○	○	○	○	○	How much in a day		○ B	○ C	○ D
White rice, or dishes made with rice, like rice and beans	○	○	○	○	○	○	○	○	○	How much in a day		○ B	○ C	○ D
Pancakes, waffles, French toast, crepes	○	○	○	○	○	○	○	○	○	How many	○ 1	○ 2	○ 3	○ 4
Breakfast pastries, like muffins, scones, sweet rolls, Danish, Pop Tarts, pan dulce	○	○	○	○	○	○	○	○	○	How many pieces	○ 1 sm	○ 1 med	○ 2	○ 3
Biscuits, not counting breakfast sandwiches	○	○	○	○	○	○	○	○	○	How many	○ 1 sm	○ 1 med	○ 2	○ 3
Corn bread, corn muffins, hush puppies	○	○	○	○	○	○	○	○	○	How many pieces in a day	○ 1/2	○ 1	○ 2	○ 3
Hamburger buns, hotdog buns, submarine or hoagie buns	○	○	○	○	○	○	○	○	○	How many buns in a day	○ 1/2	○ 1	○ 2	○ 3
Bagels or English muffins, dinner rolls, pita, naan	○	○	○	○	○	○	○	○	○	How many	○ 1/2	○ 1	○ 2	○ 3
Tortillas (not counting in tacos or burritos)	○	○	○	○	○	○	○	○	○	How many in a day	○ 1	○ 2	○ 3	○ 4
Any other bread or toast, including white, dark, whole wheat, and what you have in sandwiches	○	○	○	○	○	○	○	○	○	How many slices in a day	○ 1	○ 2	○ 3	○ 4
VEGETABLES														
Broccoli, Chinese broccoli, or Brussels sprouts	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Carrots and mixed vegetables containing carrots	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Corn	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Green beans, string beans, green peas	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Cooked greens like spinach, collards, turnip greens, kale, mustard greens	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D

	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D				
Cabbage, cole slaw, Chinese cabbage	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Green salad with lettuce or raw spinach	○	○	○	○	○	○	○	○	○	How much	○ 1/2 cup	○ 1 cup	○ 2 cups	○ 3+ cups
Raw tomatoes	○	○	○	○	○	○	○	○	○	How much	○ 1/4	○ 1/2	○ 1	○ 2
Salad dressing	○	○	○	○	○	○	○	○	○	How many tablespoons	○ 1	○ 2	○ 3	○ 4
Avocado, guacamole	○	○	○	○	○	○	○	○	○	How many tablespoons	○ 1	○ 2	○ 3	○ 4
Sweet potatoes, yams	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
French fries, home fries, hash browns, tater tots	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Potatoes <u>not</u> fried, like baked, boiled, mashed, or in stew or potato salad	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Any other vegetable, like squash, cauliflower, peppers, okra, nopales	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
FRUITS														
How often do you eat the following fruits? Include fresh or frozen fruits. Only include canned or dried fruit when mentioned.														
Watermelon, cantaloupe, honeydew, other melons	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Strawberries or other berries	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Bananas	○	○	○	○	○	○	○	○	○	How many in a day	○ 1/2	○ 1	○ 2	
Apples or pears	○	○	○	○	○	○	○	○	○	How many in a day	○ 1/2	○ 1	○ 2	
Oranges, tangerines, grapefruit	○	○	○	○	○	○	○	○	○	How many	○ 1/2	○ 1	○ 2	
Peaches and nectarines	○	○	○	○	○	○	○	○	○	How many	○ 1/2	○ 1	○ 2	
Any other fresh fruit, like grapes, plums, mango, fruit salad	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Raisins, dates, other dried fruit	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	
<u>Canned</u> fruit, like applesauce, fruit cocktail, canned peaches or pineapple	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
BEANS, TOFU, and MEAT SUBSTITUTES Include those eaten alone, or in mixed dishes like burritos, chili, stir-fry, salad														
Refried beans, bean burritos, or hummus	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Pinto beans, black beans, kidney beans, baked beans, lentils	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Tofu or tempeh	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Meat substitutes, like veggie burgers, veggie chicken, vegetarian hot dogs or vegetarian lunch meats	○	○	○	○	○	○	○	○	○	How much	○ A	○ B 1 patty or dog	○ C	○ D

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

SOUPS, MIXED DISHES, and NOODLES	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D				
Split pea, bean, or lentil soup	○	○	○	○	○	○	○	○	○	Which bowl		B	C	D
Vegetable soup, vegetable beef soup, or tomato soup	○	○	○	○	○	○	○	○	○	Which bowl		B	C	D
Any other soup, including chicken noodle, cream soups, Cup-A-Soup, ramen	○	○	○	○	○	○	○	○	○	Which bowl		B	C	D
Pizza or pizza pockets	○	○	○	○	○	○	○	○	○	How many slices	1	2	3	4
Macaroni and cheese	○	○	○	○	○	○	○	○	○	How much		B	C	D
Spaghetti, lasagna, other pasta <u>with tomato sauce</u>	○	○	○	○	○	○	○	○	○	How much		B	C	D
Other noodles like plain pasta, pasta salad, sopa seca	○	○	○	○	○	○	○	○	○	How much		B	C	D
Egg rolls, won tons, samosas, empanadas	○	○	○	○	○	○	○	○	○	How many pieces	1	2	3	4
MEAT and CHICKEN														
Hamburgers, cheeseburgers, turkey burger, at home or from a restaurant	○	○	○	○	○	○	○	○	○	How many	1 sm	1 lrg	2	3
Hot dogs or dinner sausage like Polish, Italian, chicken apple	○	○	○	○	○	○	○	○	○	How many	1	2	3	4
Bacon or breakfast sausage	○	○	○	○	○	○	○	○	○	How many pieces	1	2	3	4
Lunch meats like bologna, sliced ham, sliced turkey, salami	○	○	○	○	○	○	○	○	○	How many slices	1	2	3	4
Meat loaf, meat balls	○	○	○	○	○	○	○	○	○	How much		B	C	D
Steak, roast beef, pot roast, including in frozen dinners or sandwiches	○	○	○	○	○	○	○	○	○	How much	A	B	C	D
Tacos, burritos, enchiladas, tamales, tostadas, with meat or chicken	○	○	○	○	○	○	○	○	○	How much	A	B	C	D
Ribs, spareribs	○	○	○	○	○	○	○	○	○	How much	A	B	C	D
Pork chops, pork roast, cooked ham (including for breakfast)	○	○	○	○	○	○	○	○	○	How much	A	B	C	D
Any other <u>beef or pork</u> dish like stew, pot pie, corned beef hash, chili, Hamburger Helper, curry	○	○	○	○	○	○	○	○	○	How much		B	C	D
Liver, including chicken livers or liverwurst	○	○	○	○	○	○	○	○	○	How much	A	B	C	
Pigs feet, neck bones, oxtails, tongue, chitlins	○	○	○	○	○	○	○	○	○	How much	A	B	C	
Veal, lamb, goat, deer meat, other game	○	○	○	○	○	○	○	○	○	How much	A	B	C	
<u>Fried</u> chicken, including chicken fingers, chicken nuggets, wings, chicken patty	○	○	○	○	○	○	○	○	○	How many medium pieces	1	2 pcs/ 6 nuggets	3	4
Roasted or broiled chicken or turkey	○	○	○	○	○	○	○	○	○	How much	A	B medium piece	C	D half chicken
Any other <u>chicken or turkey</u> dish, like chicken stew or curry, chicken salad, stir-fry, Chinese chicken dishes	○	○	○	○	○	○	○	○	○	How much		B	C	D

FISH, SEAFOOD	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D				
Oysters	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	
Shellfish like shrimp, scallops, crab	○	○	○	○	○	○	○	○	○	How much fish	○ A	○ B	○ C	○ D
Tuna, tuna salad, tuna casserole	○	○	○	○	○	○	○	○	○	How much of the tuna	○ A	○ B	○ C	
Salmon, mackerel, sea bass, trout, sardines, herring, <u>without breading</u>	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Fried fish, fish sticks, fish sandwich, <u>breaded</u> fillets	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Any other fish	○	○	○	○	○	○	○	○	○	How much fish	○ A	○ B	○ C	○ D
NUTS, SEEDS, SNACKS														
Peanut butter or other nut butters	○	○	○	○	○	○	○	○	○	How many tablespoons	○ 1/2	○ 1	○ 2	○ 3
Walnuts or flax seeds (don't count flaxseed oil)	○	○	○	○	○	○	○	○	○	How much	○ 1 Tbsp	○ 2 Tbsp	○ 1/4 cup	○ 1/2 cup
Peanuts, sunflower seeds, other nuts or seeds	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Energy or protein bars, like Power Bar, Clif, Balance, Luna, South Beach, Atkins	○	○	○	○	○	○	○	○	○	How much	○ Small	○ Medium	○ Large	
Breakfast bars, cereal bars, granola bars (<u>not</u> energy or protein bars)	○	○	○	○	○	○	○	○	○	How many	○ 1	○ 2	○ 3	
Popcorn	○	○	○	○	○	○	○	○	○	How many cups	○ 1-2	○ 3-6	○ 7-9	○ 10-12
<u>Whole grain</u> crackers, like Wheat Thins, RyeKrisp, Ryvita, Wasa	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Any other crackers, like saltines, Ritz, Cheez-Its, cheese-filled pretzels	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Tortilla chips or corn chips, like Fritos, Doritos, corn nuts	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Any other snack chips, like potato chips, Cheetos, Chex mix	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
SWEETS AND DESSERTS														
Donuts	○	○	○	○	○	○	○	○	○	How many	○ 1 mini	○ 1 med	○ 2	○ 3
Cake or snack cakes like cupcakes, Twinkies, pound cake, banana bread	○	○	○	○	○	○	○	○	○	How many pieces	○ 1 sm	○ 1 med	○ 2	○ 3
Cookies, brownies	○	○	○	○	○	○	○	○	○	How many	○ 1-2	○ 3-4	○ 5-6	○ 7+
Pumpkin pie, sweet potato pie	○	○	○	○	○	○	○	○	○	How many pieces	○ 1/2	○ 1	○ 2	○ 3
Any other pie or cobbler, including fast food pies, snack pies	○	○	○	○	○	○	○	○	○	How many pieces	○ 1/2	○ 1	○ 2	○ 3
Ice cream, ice cream bars, frozen yogurt, fast food milkshakes	○	○	○	○	○	○	○	○	○	How much		○ B	○ C	○ D
Pudding, custard, rice pudding, flan	○	○	○	○	○	○	○	○	○	How much		○ B	○ C	○ D
Chocolate or other flavored sauces or syrup, on ice cream	○	○	○	○	○	○	○	○	○	How much	○ 1-2 Tbspn	○ 3-4 Tbspn	○ 1/2 cup	

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D
Popsicles, jello, frozen fruit bars, slushies, sherbet (don't count sugar-free)	○	○	○	○	○	○	○	○	○	How much: ○ A ○ B ○ C ○ D
Chocolate candy, candy bars like Snickers, Hershey's, M&Ms	○	○	○	○	○	○	○	○	○	How much in a day: ○ 1 mini ○ 1 med ○ 1 lrg ○ 1 king
Any other candy, not chocolate, like hard candy, Lifesavers, Skittles, Starburst, breath mints, chewing gum (NOT sugar free)	○	○	○	○	○	○	○	○	○	How much in a day: ○ 1-2 pcs ○ 1/2 pkg ○ 1 pkg ○ 2 pkgs
SPREADS, SAUCES, OTHER FOODS										
Margarine (not butter) on bread, rice, vegetables, or other foods	○	○	○	○	○	○	○	○	○	How many pats (tsps): ○ 1 ○ 2 ○ 3 ○ 4
Butter (not margarine) on bread, rice, vegetables, or other foods	○	○	○	○	○	○	○	○	○	How many pats (tsps): ○ 1 ○ 2 ○ 3 ○ 4
Mayonnaise, sandwich spreads	○	○	○	○	○	○	○	○	○	How many tablespoons: ○ 1/2 ○ 1 ○ 2 ○ 3
Ketchup, salsa, chili sauce, chili peppers	○	○	○	○	○	○	○	○	○	How many tablespoons: ○ 1/2 ○ 1 ○ 2 ○ 3
Mustard, barbecue sauce, soy sauce	○	○	○	○	○	○	○	○	○	How many tablespoons: ○ 1/2 ○ 1 ○ 2 ○ 3
Gravy, or other rich sauces like Alfredo, white sauce, mole, peanut sauce	○	○	○	○	○	○	○	○	○	How many cups: ○ 1/4 ○ 1/2 ○ 1
Jam, jelly, marmalade	○	○	○	○	○	○	○	○	○	How many tablespoons: ○ 1/2 ○ 1 ○ 2 ○ 3
Pickles, pickled vegetables, sauerkraut, kimchi	○	○	○	○	○	○	○	○	○	How much: ○ A ○ B ○ C ○ D
Salt, added to your food at the table	○	○	○	○	○	○	○	○	○	How many shakes in a day: ○ 1-3 ○ 4-5 ○ 6-7 ○ 8+
BEVERAGES										
Chocolate milk, cocoa, hot chocolate	○	○	○	○	○	○	○	○	○	How many 12 ounce servings: ○ 1/2 ○ 1 ○ 2 ○ 3
Glasses of milk or soy milk, not counting on cereal, in coffee, or chocolate milk	○	○	○	○	○	○	○	○	○	How many 8 ounce servings: ○ 1 ○ 2 ○ 3 ○ 4
Meal replacement drinks like Slim Fast, Ensure, or high protein drinks or powders	○	○	○	○	○	○	○	○	○	How many cans or glasses: ○ 1 ○ 2 ○ 3 ○ 4
Tomato juice, V-8, other vegetable juice	○	○	○	○	○	○	○	○	○	How many 8 ounce servings: ○ 1/2 ○ 1 ○ 2 ○ 3
Real 100% orange juice or grapefruit juice. Don't count orange soda or Sunny Delight.	○	○	○	○	○	○	○	○	○	How many 8 ounce servings: ○ 1/2 ○ 1 ○ 2 ○ 3
Other 100% juices, like apple, grape, 100% fruit blends, or fruit smoothies	○	○	○	○	○	○	○	○	○	How many 8 ounce servings: ○ 1/2 ○ 1 ○ 2 ○ 3
Hi-C, cranberry juice cocktail, Hawaiian Punch, Tang	○	○	○	○	○	○	○	○	○	How many 12 ounce servings: ○ 1/2 ○ 1 ○ 2 ○ 3
Drinks with some juice like Sunny Delight, Knudsen	○	○	○	○	○	○	○	○	○	How many 12 ounce servings: ○ 1/2 ○ 1 ○ 2 ○ 3
Iced tea, homemade, instant or bottled, like Nestea, Lipton, Snapple, Tazo	○	○	○	○	○	○	○	○	○	How many 16-oz. glasses or bottles: ○ 1/2 ○ 1 ○ 2 ○ 3
Gatorade, Powerade, or other sports drinks	○	○	○	○	○	○	○	○	○	How much in a day: ○ 1 16-ounce bottle ○ 1 20-ounce bottle ○ 2 16-ounce bottles ○ 2 20-ounce bottles

	NEVER	A FEW TIMES PER 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D
Energy drinks like Red Bull, Rockstar, Monster	○	○	○	○	○	○	○	○	○	How much in a day: ○ 1 8-ounce can ○ 1 12-16 ounce can ○ 1 20-ounce can ○ 24 ounces or more
Kool-Aid, lemonade, fruit flavored drinks, like Crystal light, atole, horchata (<u>not</u> iced tea)	○	○	○	○	○	○	○	○	○	How much in a day: ○ 1 8-ounce glass ○ 1 12-16-ounce glass or bottle ○ 1 20-ounce bottle ○ 30 ounces or more
Soft drinks, soda, pop, like cola, 7-Up, orange soda, regular or diet	○	○	○	○	○	○	○	○	○	How many in a day: ○ 1 can ○ 1 20-ounce bottle ○ 2 cans ○ Big Gulp or 3 cans
Beer or non-alcoholic beer	○	○	○	○	○	○	○	○	○	How much in a day: ○ 1 can ○ 2 cans ○ 3-4 cans or small pitcher ○ 5+ cans or large pitcher
Wine or wine coolers	○	○	○	○	○	○	○	○	○	How many glasses in a day: ○ 1/2 glass ○ 1 glass ○ 2 glasses, 1/2 bottle ○ 4+ glasses
Liquor or mixed drinks, cocktails	○	○	○	○	○	○	○	○	○	How many drinks: ○ 1 ○ 2 ○ 3 ○ 4
Water, bottled or tap	○	○	○	○	○	○	○	○	○	How many glasses: ○ 1 ○ 2 ○ 3-4 ○ 5+
Milky coffee drinks like latte, mocha, cappuccino, Frappuccino	○	○	○	○	○	○	○	○	○	How much in a day: ○ 12 oz ○ 16 oz ○ 20 oz ○ 24+ oz
Coffee (brewed or instant), regular or decaf	○	○	○	○	○	○	○	○	○	How many in a day: ○ 1 ○ 2 ○ 3 ○ 4+
Hot tea (<u>not</u> including herbal tea)	○	○	○	○	○	○	○	○	○	How many cups in a day: ○ 1 ○ 2 ○ 3 ○ 4+

<u>MILKY COFFEE DRINKS:</u> What kind do you usually drink? MARK ONLY ONE
○ Frappuccino ○ Mocha ○ Latte or cappuccino ○ Café con leche ○ Some of each ○ Don't drink them

What are your milky coffee drinks usually made with? **MARK ONLY ONE**
○ Whole milk ○ Skim milk or non-fat ○ Something else
○ 1 or 2% milk (reduced fat) ○ Soy milk ○ Don't drink

<u>COFFEE:</u> Is your coffee usually regular or decaf? ○ Decaf ○ Regular ○ Both kinds ○ Don't drink coffee

What do you usually add to your regular or decaf coffee? **MARK ONLY ONE**
○ Cream or half-n-half ○ Condensed milk ○ None of these
○ CoffeeMate, non-dairy creamer ○ Any other milk

Do you usually add sugar (or honey) to coffee? ○ No ○ Yes IF YES, how many teaspoons <u>each cup</u>? ○ 1 ○ 2 ○ 3 ○ 4

<u>HOT TEA:</u> Is your hot tea usually regular or decaf? ○ Decaf ○ Regular ○ I drink both kinds ○ Don't drink tea

What do you usually add to your hot tea? **MARK ONLY ONE**
○ Cream or half-n-half ○ Condensed milk ○ None of these
○ CoffeeMate, non-dairy creamer ○ Any other milk

Do you usually add sugar (or honey) to hot tea? ○ No ○ Yes IF YES, how many teaspoons <u>each cup</u>? ○ 1 ○ 2 ○ 3 ○ 4

If you eat the following foods, what type do you usually eat? MARK ONLY ONE ANSWER FOR EACH QUESTION

Milk	○ Whole milk	○ 2% milk	○ 1% milk (low-fat)	○ Skim milk, non-fat
	○ Soy milk	○ Rice milk	○ Almond milk, other	○ Don't drink
Slimfast, Ensure, or high protein drinks	○ Slimfast, Ensure, regular	○ Slimfast, Ensure, light or low-carb		
	○ High protein drinks, regular	○ High protein drinks, light or low-carb	○ Don't know/Don't drink	
Real 100% orange or grapefruit juice	○ Calcium-fortified	○ Not calcium fortified	○ Don't know	○ Don't drink
Iced tea	○ Home-made, no sugar	○ Bottled, no-sugar	○ Don't drink	
	○ Home-made, with sugar	○ Bottled, pre-sweetened		
Drinks like Kool-Aid, lemonade, Crystal Light	○ Low-calorie, sugar-free	○ Regular		○ Don't drink
Energy drinks like Red Bull, Monster	○ Sugar-free	○ Regular		○ Don't drink
Soft drinks, soda, pop	○ Diet, low-calorie	○ Regular	○ Don't drink	
Do they usually have caffeine?	○ Has caffeine	○ No caffeine	○ Don't drink	
Beer	○ Regular	○ Light	○ Non-alcoholic	○ Don't drink
Wine or wine cooler	○ Red wine	○ White wine	○ Both red and white wine	○ Don't drink
Cheese	○ Low-fat	○ Regular-fat	○ Don't eat	
Yogurt	○ Plain (no sugar or fruit)	○ With fruit or other flavors		
Yogurt	○ Low-fat	○ Non-fat	○ Regular (whole milk)	○ Don't eat
Salad dressing	○ Low-fat, lite	○ Fat free	○ Regular	○ Oil & vinegar	○ Don't use
Spaghetti or lasagna	○ Meatless	○ With meat sauce or meatballs		○ Don't eat
Noodles, pasta	○ Rarely whole grain	○ Sometimes whole grain	○ Usually whole grain	○ Don't know/don't eat
Burgers	○ Hamburger	○ Cheeseburger	○ Turkey burger	○ Don't eat
Beef or pork	○ Avoid eating the fat	○ Sometimes eat the fat	○ Often eat the fat	○ Don't eat
Chicken or turkey	○ Avoid eating the skin	○ Sometimes eat the skin	○ Often eat the skin	○ Don't eat
Hot dogs, dinner sausage	○ Beef or pork	○ Chicken or turkey, low-fat		○ Don't eat
Lunch meats	○ Beef or pork	○ Chicken or turkey, low-fat		○ Don't eat
Cakes, snack cakes, cupcakes	○ Low-sugar, low-carb	○ Low-fat	○ Regular-fat	○ Don't eat
Cookies, brownies	○ Low-sugar, low-carb	○ Low-fat	○ Regular-fat	○ Don't eat
Ice cream, frozen yogurt	○ Low-sugar, low-carb	○ Low-fat or frozen yogurt	○ Regular	○ Don't eat
Energy or protein bars	○ High energy	○ High protein	○ Some of each	○ Don't know	○ Don't eat
Bagels, English muffins, rolls	○ White	○ Multi-grain	○ 100% whole wheat	○ Eat all kinds	○ Don't eat
Burger, hot dog, submarine buns	○ White	○ Multi-grain	○ 100% whole wheat	○ Eat all kinds	○ Don't eat
Bread	○ White (not whole grain)	○ 100% whole wheat		○ Don't eat
	○ Multi-grain, rye, or other brown bread	○ Eat some of each		
Tortillas	○ Corn tortillas	○ Flour tortillas (wheat)	○ Eat all kinds or don't know	○ Don't eat
Popcorn	○ Air popped, fat-free	○ Low-fat or Light	○ Regular	○ Caramel corn	○ Don't know	○ Don't eat
Crackers, pretzels	○ Low-fat, including RyeKrisp, rice cakes, or plain pretzels		○ Don't know	
	○ Regular-fat crackers or filled pretzels		○ Don't eat	
Mayonnaise or sandwich spreads	○ Low-fat, light	○ Regular	○ Don't eat	

If you eat **cold cereals,** what do you usually eat? Choose **ONE or TWO** that you eat most often. If you usually eat just one kind, only choose one.

○ All Bran Original	○ Cinnamon Toast Crunch	○ Grape Nuts	○ Special K, plain
○ All-Bran Complete, Complete	○ Cocoa Krispies, Pebbles, Puffs	○ Honey Bunches of Oats	○ Special K, flavors
○ Apple Jacks, Cookie Crisp	○ Corn Flakes, Corn Puffs	○ Kashi GOLEAN, Heart to Heart	○ Total
○ Bran Flakes	○ Corn Pops	○ Life	○ Wheaties
○ Cap'n Crunch	○ Fiber-One, Bran Buds	○ Lucky Charms, Fruity Pebbles	○ *Other sweet cereal*
○ Cheerios, plain or Multi-Grain	○ Froot Loops	○ Oatmeal Squares, Oat Bran	○ *Other unsweetened cereal*
○ Cheerios, Honey Nut, flavors	○ Frosted Flakes	○ Raisin Bran	○ *Other whole grain cereal*
○ Chex, Wheat	○ Frosted Mini-Wheats	○ Rice Krispies, puffed rice	○ *Other bran or fiber cereal*
○ Chex, other	○ Granola	○ Shredded Wheat	○ *Don't eat cereal*

Which **fats or oils** are used most often for **cooking or frying** (not baking) in your home? **MARK ONLY ONE OR TWO**

○ Non-stick spray or none	○ Soft tub margarine	○ Corn oil, vegetable oil and blends	○ Other oil
○ Butter or ghee	○ Low-fat margarine	○ Peanut oil	○ Don't know
○ Butter/margarine blend	○ Olive oil	○ Lard, fatback, or bacon fat	
○ Stick margarine	○ Canola oil, safflower oil	○ Vegetable shortening, Crisco	

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

What vitamin supplements do you take fairly regularly?

Multiple Vitamins. Do you take...	HOW OFTEN: DIDN'T TAKE	A FEW DAYS per MONTH	1 DAY per WEEK	2 DAYS per WEEK	3-4 DAYS per WEEK	5-6 DAYS per WEEK	EVERY DAY	FOR HOW MANY YEARS?: LESS THAN 1 YEAR	1-4 YEARS	5-9 YEARS	10+ YEARS
Prenatal vitamins	○	○	○	○	○	○	○	○	○	○	○
Regular One-A-Day, Centrum, "senior" vitamins or house brands of multiple vitamins	○	○	○	○	○	○	○	○	○	○	○
Stress-tabs or B-Complex type	○	○	○	○	○	○	○	○	○	○	○
Antioxidant combination, eye formula	○	○	○	○	○	○	○	○	○	○	○
Single Vitamins or Minerals, taken alone or in combination. Do not count what is in your multiple vitamins above.											
Vitamin A (not beta-carotene)	○	○	○	○	○	○	○	○	○	○	○
Vitamin B-6	○	○	○	○	○	○	○	○	○	○	○
Vitamin B-12	○	○	○	○	○	○	○	○	○	○	○
Vitamin C	○	○	○	○	○	○	○	○	○	○	○
Vitamin D	○	○	○	○	○	○	○	○	○	○	○
Vitamin E	○	○	○	○	○	○	○	○	○	○	○
Folic acid, folate	○	○	○	○	○	○	○	○	○	○	○
Calcium or antacids with calcium, like Tums	○	○	○	○	○	○	○	○	○	○	○
Iron	○	○	○	○	○	○	○	○	○	○	○
Zinc	○	○	○	○	○	○	○	○	○	○	○
Cod liver oil, other fish oils, omega-3, flax seed oil, algae	○	○	○	○	○	○	○	○	○	○	○
Fiber supplements like Benefiber, Metamucil	○	○	○	○	○	○	○	○	○	○	○

If you take One-A-Day, Centrum or other types of multiple vitamins, do you usually take types that

○ Contain minerals, iron, zinc, etc. ○ Do not contain minerals ○ Don't know

If you take vitamin C, how many milligrams of **vitamin C** do you usually take, on the days you take it? (Select the closest amount)

○ 100 ○ 250 ○ 500 ○ 750 ○ 1000 ○ 1500 ○ 2000 ○ 3000+ ○ Don't know

If you take vitamin E, how many IUs of **vitamin E** do you usually take, on the days you take it? (Select the closest amount)

○ 100 ○ 200 ○ 300 ○ 400 ○ 600 ○ 800 ○ 1000 ○ 2000+ ○ Don't know

If you take calcium, how many milligrams of **calcium** do you usually take, on the days you take it? (Select the closest amount)

○ 100 ○ 350 ○ 650 ○ 1250+ ○ Don't know

If you take vitamin D, how many IUs of **vitamin D** do you usually take, on the days you take it? (Select the closest amount)

○ 400 ○ 600 ○ 800 ○ 1000 ○ 2000 ○ 3000 ○ 4000 ○ 5000+ ○ Don't know

If you take omega-3 supplements, what type do you usually take? **MARK ALL THAT APPLY**

○ Fish oil ○ Flax oil, hemp oil, other seed oil ○ Krill oil ○ Algae ○ Don't know

SOME LAST QUESTIONS ABOUT YOU

	RARELY	1-2 per WEEK	3-4 per WEEK	5-6 per WEEK	1 per DAY	1 1/2 per DAY	2 per DAY	3 per DAY	4+ per DAY
About how many servings of vegetables do you eat, not counting salad or potatoes? 1 serving = 1/2 cup.	○	○	○	○	○	○	○	○	○
About how many servings of fruit do you eat, not counting juices? 1 serving = 1/2 cup or 1 medium fruit.	○	○	○	○	○	○	○	○	○
How often do you eat foods prepared at home that are cooked or fried in **fat or oil?**	○	○	○	○	○	○	○	○	○

During a regular day, how many **meals and snacks** do you usually eat?

Meals per day	○ 1	○ 2	○ 3	○ 4	○ 5+
Snacks per day	○ 1	○ 2	○ 3	○ 4	○ 5+

PHYSICAL ACTIVITY SURVEY

Think about the last 6 months. How often did you do the activities listed below?

	HOW OFTEN IN THE PAST 6 MONTHS						HOW MUCH TIME ON THOSE DAYS			
	RARELY OR NEVER	A FEW TIMES A MONTH	ONCE OR TWICE A WEEK	3-4 TIMES A WEEK	5-6 TIMES A WEEK	ALMOST EVERY DAY	LESS THAN 30 MINUTES	30-60 MINUTES	1-2 HOURS	3 OR MORE HOURS
Cooking, shopping, light cleaning like doing laundry or dusting, or running errands	○	○	○	○	○	○	○	○	○	○
Slow walking like walking the dog, or light work around the house like watering	○	○	○	○	○	○	○	○	○	○
Work on the job involving standing, like store clerk, or work involving driving (like truck driver)	○	○	○	○	○	○	○	○	○	○
Taking care of children (feeding, dressing), or moderate housework like sweeping, mopping, cleaning the tub	○	○	○	○	○	○	○	○	○	○
Weeding, raking, mowing the lawn, or light house repairs	○	○	○	○	○	○	○	○	○	○
Brisk walking, dancing, hunting or fishing, golf (NOT using a golf cart), or 'friendly' outdoor games like softball	○	○	○	○	○	○	○	○	○	○
Factory work, mechanic, restaurant work, or work involving walking, like mail carrier	○	○	○	○	○	○	○	○	○	○
Construction, painting, feeding livestock, or homecare like caring for an adult family member	○	○	○	○	○	○	○	○	○	○
Heavy work like moving boxes, heavy digging or shoveling snow, farm chores like baling hay, or other HARD labor	○	○	○	○	○	○	○	○	○	○
Exercising at the gym or at home, aerobics, weight training, jogging, or vigorous sports like basketball, soccer, tennis	○	○	○	○	○	○	○	○	○	○
Bicycling or swimming for exercise	○	○	○	○	○	○	○	○	○	○

Are you ○ Hispanic or Latino ○ Not Hispanic or Latino ○ Do not wish to provide this information

What race do you consider yourself to be? **MARK ALL THAT APPLY**

○ White
○ Black or African American
○ Asian
○ American Indian or Alaska Native
○ Native Hawaiian or Other Pacific Islander
○ Do not wish to provide this information

Thank you very much for filling out this questionnaire.
Please take a minute to go back and fill in anything you may have skipped.

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

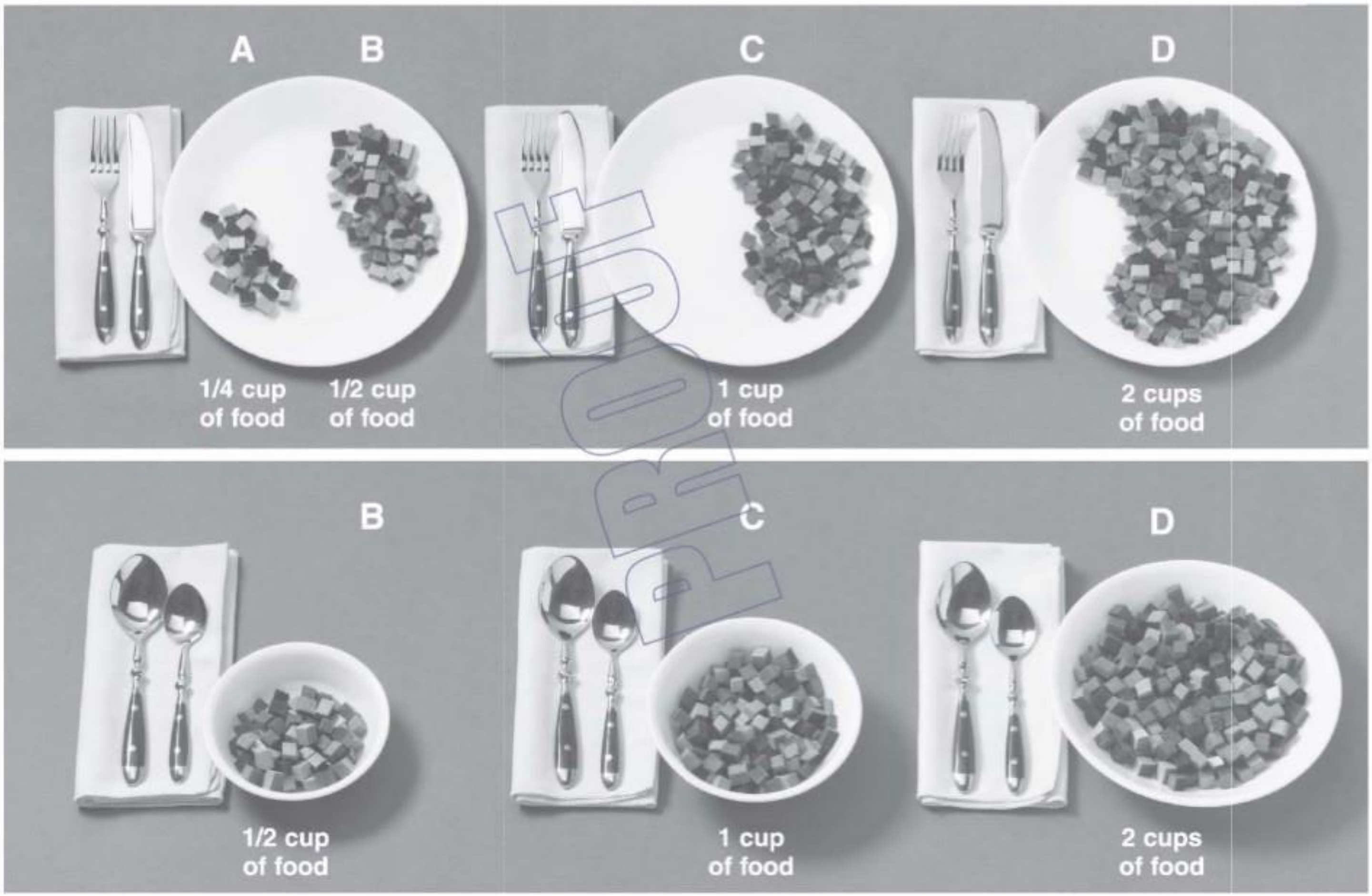
1/4" SPINE PERF

Carefully remove this page and use the portion size pictures on the back.

Portion Size Choices

Keep this in front of you while you are filling out The Food Questionnaire. You may use either the plates or the bowls to help you choose your usual portion size.

Choose A, B, C or D: **A** = 1/4 Cup of Food **B** = 1/2 Cup of Food **C** = 1 Cup of Food **D** = 2 Cups of Food



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Biochemical markers of bone turnover – uses and limitations

Author: Thinn Thinn Hlaing, Juliet E Compston
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