The Influence of Post-Exercise Feeding on Bone Biomarkers Following an Acute High-Intensity Exercise Bout in Young, Healthy Adults

Nicholas Cheng

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Abstract

Exercise and nutrition, specifically dairy products, are interventions that can influence bone. There is limited evidence on the effects of wholefood dairy consumption following an acute exercise bout on bone biomarkers. The objective of this study was to determine whether the consumption of either milk, Greek yogurt (GY), carbohydrate (CHO) or water, differentially modulated bone biomarkers post-exercise. We recruited twenty participants to partake in this randomized crossover study that provided one of the four trial supplements in a randomized order following an acute multimodal exercise session. The main findings from this study were an attenuation of PTH 4h post-exercise in the MILK trial compared to GY, elevated P1NP in MILK compared to WATER at 24h post-exercise and a suppression of RANKL in MILK compared to all trials at 24h post-exercise. These data suggest that the consumption of milk post-exercise was superior to carbohydrate, GY and water in favourably modulating circulating bone biomarkers.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

25[OH]D3	25-Hydroxyvitamin D3
AUC	Area Under The Curve
ANOVA	Analysis of Variance
BALP	Bone Alkaline Phosphatase
BCAAs	Branched Chain Amino Acids
BIA	Bioelectrial Impedance Analysis
BIONEX	Bone and Inflammatory Outcomes – Nutrition and Exercise
BF%	Body Fat Percentage
BMI	Body Mass Index
BMs	Bone Markers
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMPs	Bone Morphometric Proteins
BMR	Basal Metabolic Rate
BRC	Bone Remodeling Compartment
BMU	Basic Multicellular Unit
c-FMS	Colony-Stimulating Factor-1 Receptor
CHO	Carbohydrate
CV	Coefficient of Variation
e1RM	Estimated 1 Repetition Maximum
ELISA	Enzyme Linked Immunosorbent Assay
JNK	c-Jun N-Terminal Kinase
CTR	Calcitonin Receptor
CtsK	Cathepsin K
CTX	C-Terminal Telopeptide of Type 1 Collagen
DBP	Vitamin D Binding Protein
DIAAS	Digestible Indispensable Amino Acid Score (DIAAS)
Dlx5	Distal-Less Homeobox 5
DXA	Dual X-Ray Absorptiometry
Erk	Extracellular Signal-Related Kinase
FDR	False Discovery Rate
GHR	Growth Hormone Receptor
GI	Gastrointestinal
GSK-3	Glycogen Synthase Kinase 3
GY	Greek Yogurt
H+-ATPase	Hydrogen-Adenosine Triphosphatase
HIIC	High-Intensity Interval Cycling
HIIE	High-Intensity Interval Exercise
HR	Heart Rate
HSCs	Hematopoietic Stem Cells
IGF-1	Insulin-Like Growth Factor 1
LAB	Lactic Acid Bacteria
LCN	Lacunocanalicular Network
Lef/Tcf	Lymphoid Enhancer Factor/T-Cell Factor

LMM	Linear Mixed Model
LRP5/6	Lipoprotein Receptor-Related Proteins 5 or 6
m-CSF	Macrophage Colony-Stimulating Factor
MAPK	Mitogen Activated Protein Kinase
MF	Milk Fat
MMP	Matrix Metalloproteinase
MPS	Muscle Protein Synthesis
NaHep	Sodium Heparin
NCPs	Noncollagenous Proteins
NF-κB	Nuclear Factor Kappa-Light Chain-Enhancer of Activated B Cells
NFATC1	Nuclear Factor-Activated T Cells c1
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OC	Osteocalcin
OPG	Osteoprotegerin
Osx	Osterix
OW/OB	Overweight/Obesity
P1NP	Procollagen Type 1 N-Terminal Propeptide
PAR-Q+	Physical Activity Readiness Questionnaire
PBM	Peak Bone Mass
PDGF	Platelet Derived Growth Factor
PO	Power Output
PTH	Parathyroid Hormone
PTH1R	Parathyroid Hormone 1 Receptor
RANK	Receptor Activator of Nuclear Factor Kappa-B
RANKL	Receptor Activator of Nuclear Factor Kappa-B Ligand
RCT	Randomized Controlled Trial
RPE	Rating of Perceived Exertion
RPM	Revolutions Per Minute
Runx2	Runt-Related Transcription Factors 2
SD	Standard Deviation
SEM	Standard Error Of The Mean
Smad1/5/8	SMA/MAD-Related Protein
SOST	Sclerostin
tAUC	Total Area Under The Curve
TDEE	Total Daily Energy Expenditure
TGF- β	Transforming Growth Factor Beta
TRAF6	Tumor Necrosis Factor Receptor Associated Factor 6
TRAP5b	Tartrate-Resistant Acid Phosphatase 5b
UHT	Ultra-High Temperature
VDR	Vitamin D Receptor
VEGF	Vascular Endothelial Growth Factor
W	Watts

1.0: Introduction

The health of the skeleton should be a priority to prevent the onset of debilitating diseases such as osteoporosis. Osteoporosis is characterized by impaired bone strength and an increased fracture risk (1). Osteoporosis is associated with difficulties such as acute and chronic pain, disability and an increased risk for morbidity and mortality, which can reduce an individual's quality of life (2). In addition, a recent meta-analysis has reported a global prevalence of 18.3% for osteoporosis, with women having almost twice the likelihood of developing this disease compared to men (3). Therefore, with the growing prevalence of osteoporosis worldwide, identifying strategies to help prevent this disease and improve bone health at a younger age is of significant importance.

Prevention of osteoporosis should focus on two key factors. These are: acquiring a greater peak bone mass (PBM) when young and attenuating age-related bone loss (4). PBM refers to the maximum amount of bone mass acquired in one's lifetime (5). This usually occurs during early adulthood (6). Several non-modifiable factors play a role in PBM attainment such as sex, ethnicity and pubertal timing, with ~80% of the variance in PBM resulting from genetics, making it the most important factor for bone development (5). However, there are also modifiable factors that can affect bone health such as exercise and nutrition.

Exercise provides a sufficient stimulus to activate bone cell activity by inducing mechanical stress onto the bone. When bones experience mechanical strain, the adaptive response to this stressor over time results in increased bone formation by osteoblast cells to enhance bone strength (7). Varying types of exercises can elicit mechanical strain on the skeleton. For example, resistance exercises exert a mechanical strain from the contracting muscles pulling on the bone and plyometric exercises apply an external load from the forces that the bone experiences during landing (8,9). These mechanical stressors induce microdamage to

the bone and activate several pathways responsible for bone remodeling, which is the process of replacing and removing damaged bone with newer and healthier bone tissue through the action of bone-resorbing osteoclasts and bone-forming osteoblast cells, respectively (10). Although bone remodeling is beneficial for bone homeostasis, excessive bone remodeling can result in deleterious consequences where bone breakdown exceeds deposition, culminating in decreased bone mass and strength, leading to an elevated risk for fractures (11). Hence, investigating other potential modulators for bone metabolism following exercise may result in improved benefits for bone health compared to exercise alone.

Nutrition may provide additional benefits to bone health as food provides the body with the specific nutrient constituents of bone itself. Bone consists of both calcium in the form of small hydroxyapatite crystals and protein as type 1 collagen and other non-collagenous proteins (12,13). Both calcium and protein are nutrients that can be found in the diet and through supplementation that may aid in the formation of new bone tissue. Meta-analyses on both of these nutrients have shown that their supplementation leads to some significant improvements in bone mineral density (BMD) at certain sites (14–17) and lowered risk for hip fractures (18). Although examining these single nutrients in isolation seems promising, there is a benefit with investigating nutrients contained together in wholefoods as the combination of multiple nutrients within a food (i.e., the unique food matrix) may result in beneficial synergistic effects (19).

The food matrix refers to the microenvironment of a wholefood where nutrients are sequestered and interact with one another (20). The interaction of nutrients may also differ depending on the structure of the matrix, whether these nutrients are held in a liquid, gel, cellular, dense, fibrous or porous matrix (20). Thus, food matrices may exert unique effects depending on its structure and specific composition of nutrients. With respect to bone health, the

dairy matrix seems to be relevant as dairy products are good sources of both protein and calcium, but are found in different matrices including milk, yogurt and cheese. Milk is a nutrient-dense food product as it contributes the most amount of protein and calcium per unit of energy (calorie) than any other foods consumed in a regular diet (21). Furthermore, on top of calcium and protein, the fortification of vitamin D in milk is mandatory in Canada (22), which also plays a vital role in calcium and bone homeostasis. Yogurt is another type of dairy product that is derived from milk. It has a semi-solid food form and is fermented with beneficial bacterial cultures (and sometimes probiotics) (23). Yogurt can go through additional processing to form Greek Yogurt (GY) which has almost double the amount of total protein content compared to regular yogurt (24), which may be more efficacious than regular yogurt for improving bone (and muscle) health. Interestingly, these dairy products may also result in differing bone turnover and metabolic responses due to their unique food matrices, bioavailability of nutrients and nutrient digestion/absorption kinetics (25,26). Despite their matrix and nutrient composition differences, milk and Greek yogurt provide the specific nutritional requirements for optimal bone health and may elicit additive effects in conjunction with exercise (**Figure 1**).

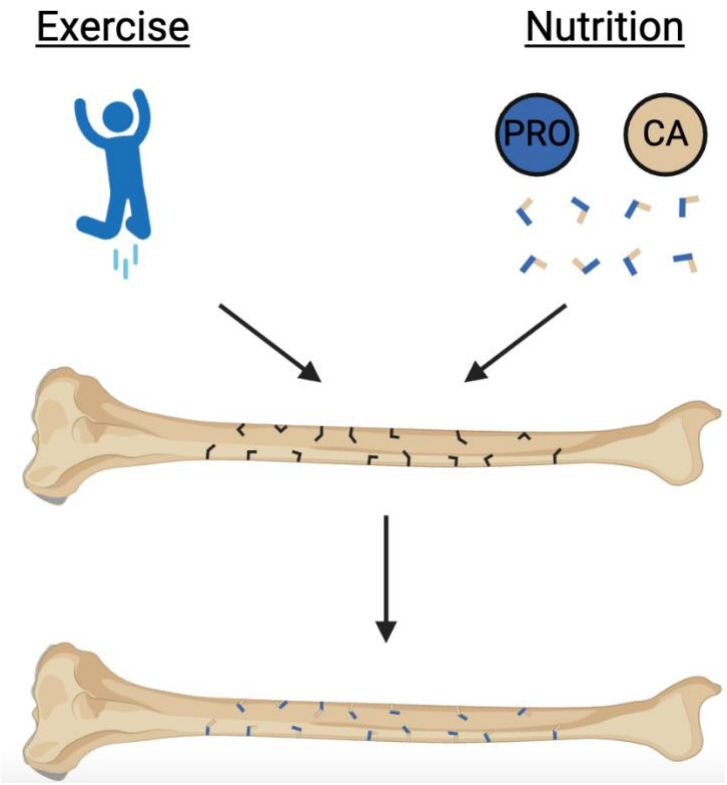

Figure 1: Exercise initiates bone remodeling by inducing microcracks within the bone and nutrition provides the building blocks for bone to repair and renew itself during bone remodeling. Created with BioRender.com

There have been limited studies investigating the effects of exercise training combined with a nutritional intervention such as dairy. Our lab group has conducted four dairy and exercise training studies, demonstrating that a greater number of dairy servings compared to a lower amount resulted in reduced bone resorption in young girls with overweight and obesity (OW/OB) (27) and improved bone formation and decreased bone resorption in adult females with OW/OB (28). The other two studies found that consuming dairy products, either milk or GY compared to an isoenergetic carbohydrate (CHO) supplement, evoked a more advantageous bone response either by lowering bone resorption in women (29) or by increasing bone formation in men (30). Several other studies have been performed with dairy consumption and exercise training across the lifespan in youth (31–37), adulthood (38,39) and older adulthood (40). These studies have reported either increases in BMD or bone mineral content (BMC) or maintenance of BMD with aging following exercise training and dairy consumption. However, there is a lack of

understanding as to how these long-term improvements in bone health arise. Measurements of BMD and/or BMC make it difficult to understand how bones are adapting to long-term interventions as they present static data on bone mass that requires approximately 12 months to see any meaningful results (41). Therefore, when trying to study the mechanisms behind bone adaptations to exercise and nutrition interventions, it is important to design studies using the appropriate methods to assess these outcomes.

1.1: Rationale

Investigating the acute bone response to a single bout of exercise and feeding of bone-supporting nutrients (i.e., through wholefood dairy products) may allow for a greater mechanistic understanding of how these chronic skeletal adaptations are developed physiologically. Utilizing bone markers (BMs), provides information on the dynamic bone metabolic processes that occur following short-term stimuli such as an acute exercise bout and/or feeding (42). BMs reflect both sides of bone remodeling as there are markers of bone resorption and formation which allows for a deeper understanding of what is happening to the bone physiologically following an acute stressor or long-term intervention (43).

Acute exercise has been shown to influence BMs. A recent meta-analysis investigated the BM response to exercise and found that the bone resorption marker, C-terminal telopeptide of type 1 collagen (CTX), was elevated specifically during cycling exercise and was more sensitive to exercise compared to the bone formation marker, procollagen type 1 N-terminal propeptide (P1NP) (44). In addition, nutrition may also have a modulating role as the provision of calcium attenuated the post-exercise CTX response as observed in another meta-analysis examining the impact of nutrition on the BM response to exercise (45). When implementing nutrition around an acute exercise bout, the most optimal feeding window to support exercise-induced osteolytic activity seems to be the post-exercise window due to a longer opportunity for feeding and a

greater amount that can be provided compared to feeding pre-exercise or during exercise (46). This was seen in a study that provided a CHO and protein solution to their participants immediately post-exercise compared to delayed feeding 2-hours post-exercise or to a placebo condition (47). They reported significant decreases in the post-exercise CTX response and an increase in P1NP 4-hours post-exercise compared to the delayed feeding and placebo conditions (47). With respect to dairy wholefoods, only one study (from our laboratory) has investigated the acute BM effects of post-exercise dairy consumption. Prowting and colleagues found that post-exercise milk consumption significantly lowered the CTX area under the curve (AUC) 48-hours post-exercise compared to an isoenergetic CHO beverage in young adult females (48).

Although the study by Prowting and colleagues presents data on the acute interactions between exercise and milk consumption and its effects on bone metabolism, a gap in the literature still exists regarding other dairy products such as GY and their acute regulation of the post-exercise bone response. Even though milk and GY are both dairy wholefoods, their unique dairy matrices and nutrient composition may result in divergent BM responses, warranting a head-to-head investigation within the same controlled, crossover study. The comparison between milk and GY will inform researchers on the different mechanisms of action to improve bone health following their consumption post-exercise. It will also inform health practitioners on the potential 'superior' dairy supplement to consume post-exercise to optimize bone health. If no differences are observed between the two dairy wholefoods, these findings would also provide evidence that there is no "best" dairy product to consume post-exercise and that consumers can choose whichever food they prefer or the one that is the easiest to consume/digest.

2.0: Literature Review

2.1 Bone Physiology

2.1.1: Bone Function and Composition

The biology of bone is highly complex despite our previous understanding of it as a static organ (49). Bone is a crucial organ in the body that has many regulatory functions. It serves as an attachment site for the tendons of muscle for locomotion, provides structure and protection to the many organ systems in the body, produces red blood cells within the red marrow, and houses many minerals such as calcium and phosphate that are essential for muscle contractions, nervous signal propagation and cellular metabolism (50–52). Therefore, healthy bones are vital in ensuring overall physiological health.

Anatomically, when looking specifically at long bones, there are different layers that comprise the bone **(Figure 2)**. The outermost layer of the bone is a membrane known as the periosteum. The periosteum can be subdivided into its outer and inner layers, where the outer layer is mainly made of fibrous connective tissue and the inner layer is made of osteoprogenitor cells with greater osteogenic potential (53). Deeper to the periosteum, is bone tissue known as the cortical bone and deeper to that is the trabecular bone. Cortical and trabecular bone comprise 80% and 20% of overall bone mass, respectively (52). Cortical bone is the dense layer of bone that is solid in structure and can withstand greater external forces than trabecular bone (54). In contrast, trabecular bone has greater porosity, is more elastic, and can tolerate multi-directional forces, as the organization of trabeculae are found in the direction of common stresses that the bone experiences (55). Trabecular bone is also predominantly found at the epiphyses (ends) of long bones, as the inner layer of the diaphysis (middle/shaft) houses the medullary cavity which contains the red and yellow bone marrow, responsible for hematopoiesis and fat storage, respectively (56). The last notable structure of the bone is the inner membrane known as the

endosteum. The endosteum lines the medullary cavity, osteons and trabeculae and contains both osteoprogenitor cells and type 3 collagen fibers (53).

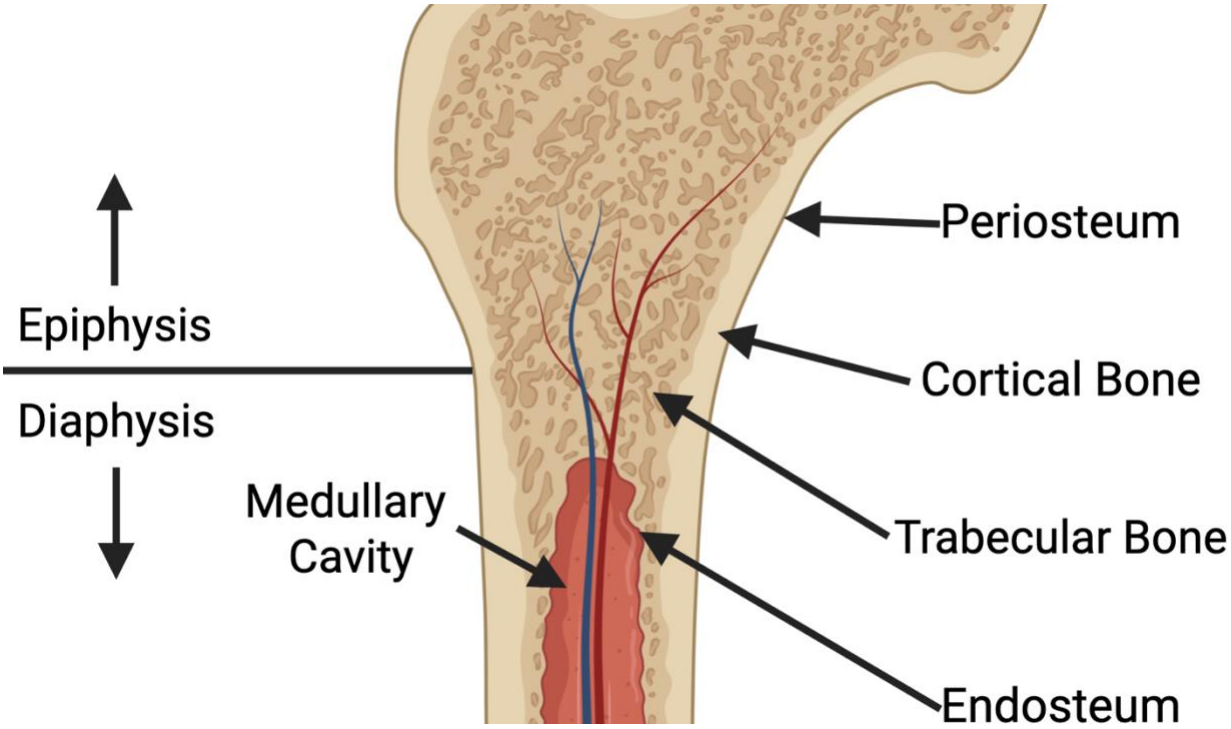

Figure 2: Structures of the bone. Created with BioRender.com

Focusing on the cortical bone, it has structural properties that are responsible for the maintenance of bone health. Within the cortical bone, the main functional unit is the osteon. Osteons are cylindrical structures that have four distinct features; the Haversian canal, lamellae, osteocytes and lacunocanalicular network (LCN) **(Figure 3)** (57). The Haversian canal is the central component of the osteon containing nerves and blood vessels that supply nutrients to the bone and clear waste products (58). These nutrients can be distributed to other osteons through Volkmann canals that run perpendicular between osteons (57). The lamellae are sets of alternating layered bone tissue, composed of hydroxyapatite crystals and collagen fibers that surround the Haversian canal in a circular fashion, making up most of the space within the osteon (59). Within the lamellae are bone cells called osteocytes which are stored within lacunae

(60). Osteocytes are mature bone cells that are trapped within mineralized bone matrix and are responsible for sensing mechanical strain placed on the bone. They signal changes in bone metabolism to counteract stressors (61). Dendrites are extracellular protrusions that aid in cell-cell communication and can be found on osteocytes, connecting them to neighboring osteocytes through tiny canals known as canaliculi (62). These make up the LCN, which facilitates different signals during stress or strain (63). Thus, these four components of the osteon allow for many physiological functions that are required to sustain cortical bone homeostasis.

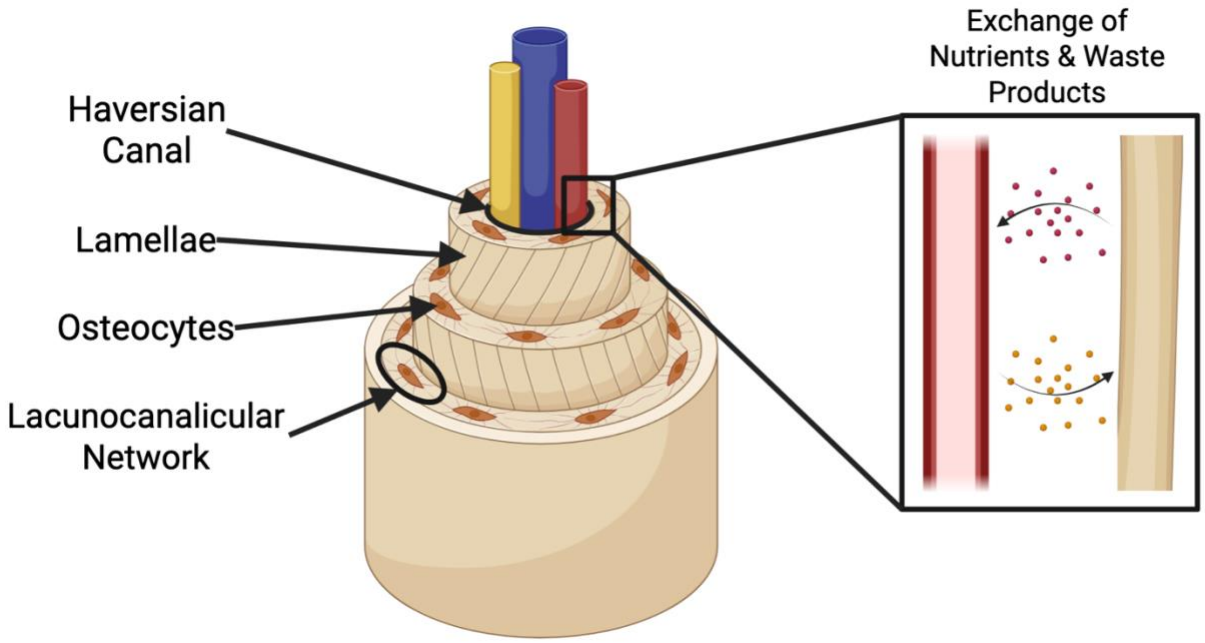

Figure 3: Structure of the osteon. Created with BioRender.com

The bone matrix components consist of water (~10%) and the inorganic (~60%) and organic (~30%) matrices (64). The inorganic matrix is composed mainly of small hydroxyapatite crystals made predominantly from calcium and phosphate that provide stiffness, rigidity, and resistance against compressive forces (55). Conversely, the organic matrix is mostly comprised of type 1 collagen (~90%), with the remaining portion being non-collagenous proteins (~10%) (13). The type 1 collagen proteins within the organic matrix provide elasticity and flexibility to

give bone its tensile strength and to preserve bone against torsion forces (55). The non-collagenous proteins (NCPs) also play an important role as they are responsible for the mineralization of the bone matrix and the regulation of bone cell function and activity (64).

2.1.2: Bone Cells

At the molecular level, there are 4 distinct cell types, osteoblasts, osteocytes, bone-lining cells, and osteoclasts that are found within bone tissue. Osteoblasts are cuboidal shaped bone cells that are responsible for the formation of new bone. Osteoprogenitor cells that derive from multipotent mesenchymal stem cells, proliferate and differentiate into osteoblasts through the canonical Wnt/β-catenin pathway (65), where Wnt binds to the Frizzled receptor and its co-receptor, low density lipoprotein receptor-related proteins 5 or 6 (LRP5/6), to inhibit glycogen synthase kinase 3 (GSK-3), allowing for β-catenin translocation to the nucleus (66). β-catenin binds with lymphoid enhancer factor/T-cell factor (Lef/Tcf) to activate critical transcription factors in osteoblast differentiation such as Runt-related transcription factors 2 (Runx2), Distal-less homeobox 5 (Dlx5) and Osterix (Osx) (67). Additionally, bone morphometric proteins (BMPs) also play a role in osteoblast differentiation by phosphorylating Smad1/5/8 and forming a complex with Smad4, leading to its translocation to the nucleus for the transcription of these osteoblast factors (68,69). When mature osteoblasts are fully differentiated, bone matrix formation occurs in a two-step process, where the osteoid (unmineralized organic matrix), composed of both type 1 collagen and NCPs is first synthesized, followed by the mineralization of the matrix through the formation of hydroxyapatite crystals (70,71). Type 1 collagen is an important component of the bone as it provides elasticity to the bone and also acts as a scaffold for the organization of hydroxyapatite mineralization (72–74). Although type 1 collagen represents a large portion of the osteoid, the NCPs such as osteocalcin (OC), osteopontin and bone sialoprotein play roles in advancing to the mineralization phase. OC specifically, is the

most abundant NCP secreted by osteoblasts, that has the ability to bind calcium due to its Gla residues and support mineralization (75,76). Historically, due to these mechanisms, it was believed that OC was indicative of bone formation, however, conflicting results have been observed in transgenic mice models showing increased bone mass in OC deficient mice (77). Furthermore, OC also gets sequestered into the bone matrix and the breakdown of bone causes its release into the circulation, potentially demonstrating that OC is a marker of overall bone turnover since it can be released into the circulation during the formation and breakdown of bone (71,78). During mineralization, matrix vesicles within the osteoblast will start to form hydroxyapatite crystals from calcium and phosphate derived from the circulation and will release its contents towards areas of type 1 collagen fibrils (79–81). A regulatory enzyme within the osteoblast matrix vesicles which helps with the provision of phosphate is the bone formation marker bone alkaline phosphatase (BALP). It breaks down inorganic pyrophosphate to inorganic phosphate which is required for hydroxyapatite mineralization (82,83). Following bone formation, there are 3 fates for osteoblasts, either they undergo apoptosis (and die), or they convert into osteocytes or bone-lining cells (84).

During this process, a subset of osteoblasts gets encased within the newly synthesized matrix and they become mature bone cells known as osteocytes (70,85). Osteocytes account for ~95% of all bone cells and can live up to ~25 years (86). They are mechanosensitive cells that can detect strain that is placed on the bone. The ability of the osteocytes to detect strain makes them crucial to the maintenance of the bone matrix as they can transmit these stress signals in response to external forces through their dendritic processes that run through the canaliculi (70,71). These dendritic processes are integral as they provide the connection to other neighbouring osteocytes, osteoblasts, and bone-lining cells, aiding in communication of

mechanical stresses placed on the bone and ultimately initiating the necessary physiological response of bone remodeling (55).

Aside from becoming osteocytes, osteoblasts are also able to become bone-lining cells. Bone-lining cells are flattened, quiescent osteoblasts that lines areas where bone remodeling does not occur (70,84). They protect bone surfaces from osteoclast activity where resorption is not required and are also able to revert back to active osteoblasts, if necessary (85).

Osteoclasts, another bone cell type, are large multinucleated cells that are responsible for the breakdown of bone (i.e., bone resorption) (13). They are derived from mononuclear cells that originate from hematopoietic stem cells (HSCs) and are tightly regulated by osteokines secreted by the osteoblasts such as macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) (70). M-CSF and RANKL bind to colony-stimulating factor-1 receptor (c-FMS) and receptor activator of nuclear factor kappa-B (RANK) on the surface of osteoclast precursor cells, respectively, in order to regulate the proliferation, survival and differentiation of these cells into mature osteoclasts (87). The binding of RANKL to RANK localizes TNF receptor-associated factor 6 (TRAF6) to the cytoplasmic portion of RANK and activates signaling molecules such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), c-Jun, c-Fos and mitogen activated protein kinases (MAPK) such as extracellular signal-related kinase (Erk), c-Jun N-terminal kinase (JNK) and p38 to promote nuclear factor-activated T cells c1 (NFATc1) activity, the master regulator of osteoclastogenesis (88,89). NFATc1 is then able to transcribe key osteoclastic genes such as tartrate-resistant acid phosphatase 5b (TRAP5b), cathepsin K (CtsK) and calcitonin receptor (CTR) (90). Alternatively, this process of osteoclastogenesis can be inhibited by OPG, as it can act as a decoy receptor for RANKL, preventing its binding to RANK (91,92). Following

osteoclastogenesis, bone resorption can take place by the binding of osteoclasts to the bone matrix. Podosomes on the osteoclasts bind to the bone matrix and create a sealing zone to compartmentalize the bone resorption process to the extracellular environment (84). The process of bone resorption occurs at the ruffled border of the osteoclast and occurs via two processes, acidification of the inorganic matrix and proteolysis of the organic matrix (84,85). First, the release of H+ ions from vacuolar H+-adenosine triphosphatase (H+-ATPase) on the ruffled border, creates an acidic environment with a pH of ~4.5, resulting in the degradation of the inorganic hydroxyapatite matrix (93). Second, the proteolysis of the organic matrix (i.e., type 1 collagen) is facilitated by lysosomal enzymes such as CtsK, TRAP and matrix metalloproteinases (MMPs) (94–97).

2.1.3: Bone Modeling and Remodeling

During childhood and adolescence (i.e., primary somatic growth phases), a process known as bone modeling occurs wherein the shape and structure of bone adapts to external loading (84,98). Bone adapts its shape through longitudinal and radial growth (55). Longitudinal growth primarily occurs at the epiphyseal growth plates located between the epiphysis and metaphysis of long bones (99). At these growth plates, endochondral ossification occurs, where chondrocytes undergo longitudinal proliferation, and differentiation into hypertrophic chondrocytes that become enlarged, subsequently lengthening the growth plate region (100). These chondrocytes are then mineralized and undergo apoptosis, which provides the foundation and framework for osteoblasts to lay down new bone tissue (84,100). On the contrary, radial growth widens the bone by the addition of bone on the outer periosteal surface with concomitant resorption on the inner endocortical surface to maintain cortical thickness (52).

After growth and during adulthood, bone remodeling predominates. This is a tightly coupled process responsible for the resorption of old and damaged bone with the replacement of

new bone (101,102). Two distinct types of bone remodeling are non-targeted (stochastic) and targeted remodeling (71,103). Stochastic remodeling is a systemic form of remodeling regulated by hormones such as parathyroid hormone (PTH) and calcitonin, with the primary goal of ensuring homeostasis of important minerals such as calcium and phosphate in the circulation. Bone breakdown releases these minerals from the matrix into the circulation for systemic use (71,104). Thus, stochastic remodeling regulates calcium homeostasis with the release of calcium from the bone through PTH-induced bone resorption during hypocalcemia or the inhibition of osteoclast activity through calcitonin during hypercalcemia (105–107).

In contrast, targeted remodeling is more specific and site-dependent, as it is initiated by the detection of microdamage to repair these areas (10). The bone remodeling process is governed by the osteocytes, osteoclasts and osteoblasts that are intimately grouped together to form the basic multicellular unit (BMU) which promotes the coupling of their actions (108). These BMUs differ in their structure depending on the type of bone that is being remodeled. In cortical bone, the BMUs form a cutting cone within the cortex, with vasculature and nerves that follow behind (109). In trabecular bone, the BMU occurs at the bone surface and is enclosed within a bone remodeling compartment (BRC), creating a microenvironment for different factors to regulate the coupling of bone remodeling (110). BMUs in both cortical and trabecular bone are linked to the systemic circulation by the Haversian canal or the marrow space, respectively (108).

The process of bone remodeling occurs in 4 distinct stages: activation, resorption, reversal, and formation (52). As mentioned, activation of the bone remodeling cycle can be done systemically by circulating hormones to regulate mineral homeostasis or by mechanical strain placed on the bone causing targeted microdamage (101). During mechanical strain in trabecular

bone, osteocytes are proposed as the fundamental cell responsible for the activation of remodeling. Osteocytes are mechanosensitive and can detect and undergo apoptosis under mechanical loading, triggering the release of RANKL from neighbouring osteocytes and signaling other cells such as osteoblasts and bone-lining cells to release RANKL as well for osteoclastogenesis (111,112). Prior to bone resorption by the osteoclasts, bone-lining cells will prepare the area for osteoclast binding by secreting MMPs to begin the breakdown of the bone matrix (111,113). The bone-lining cells will also form a canopy over the targeted remodeling site to assemble the BRC and are posited as the cells responsible for recruiting capillaries to the BRC from the marrow (114). These capillaries provide HSCs to the BRC and can differentiate into osteoclasts with the release of RANKL from the osteocytes, osteoblasts and bone-lining cells (79). Mature osteoclasts will then resorb the damaged bone surface through acidification and proteolysis to form resorption pits known as Howship's lacunae (84,85).

Once osteoclasts have completed their role in bone resorption, the reversal stage begins, where reversal cells that are proposed to originate from the osteoblast lineage, shift the BRC from a pro-resorptive microenvironment to an osteogenic one by differentiating into osteoblasts (111,115,116). During the reversal stage, a proposed coupling mechanism of resorption and formation is that the resorbed bone releases growth factors that are sequestered within the matrix such as insulin-like growth factor 1 (IGF-1), transforming growth factor-beta (TGF- β), platelet derived growth factor (PDGF) and BMPs into the BRC, that facilitates osteoblast bone formation (113).

Following the reversal stage, osteoblasts will first secrete the osteoid, followed by mineralization, that is characterized by the incorporation of calcium and phosphate (70) and the

removal of H+ ions from the matrix (117), where they nucleate and form hydroxyapatite crystals, resulting in newly synthesized bone tissue where damaged bone used to reside.

Depending on the stage of the remodeling cycle, there are differences in terms of its duration. The activation stage is ~10 days, the resorption stage is ~2-4 weeks, the reversal stage is ~5 days and formation can take ~4-6 months (52,84). With regards to the rate of remodeling, ~25-28% of trabecular bone is remodeled every year whereas in cortical bone, it is only ~3-5% per year (55). In addition, throughout a year, ~5-10% of the entire skeleton is remodeled (108). Thus, bone remodeling is essential for the health and preservation of bone tissue (13), but if bone is excessively remodeled (i.e., resorption is not coupled to formation), this can elicit deleterious outcomes (11). Frequent activation of bone turnover results in the inability for bone formation to catch up with bone resorption, as the duration for resorption is ~2-4 weeks whereas formation requires ~4-6 months (55). Thus, the consequences for excessive bone turnover can lead to a negative net bone balance, where bone breakdown exceeds deposition, culminating in decreased bone mass and a gradual decline in bone strength (55). Therefore, it was proposed by Dr. Robert Heaney in 2003, that bone remodeling should be viewed as a J-shaped curve, where exceedingly lower and higher levels of bone turnover increases the risk for bone weakness and fractures, and that intervention strategies such as nutrition and exercise should target the remodeling response to prevent it from reaching these critical thresholds (11).

2.1.4: Bone Homeostasis in Disease

When bone homeostasis is disrupted, a negative net bone balance can occur where resorption is greater than formation (118). With greater bone breakdown, there is a decrease in bone mass and microarchitectural integrity, leading to osteoporosis (119). Osteoporosis is diagnosed using dual x-ray absorptiometry (DXA), when bone mineral density (BMD) T-scores at different anatomical sites are below 2.5 standard deviations from the mean of young, healthy

adults (120). DXA is the gold standard for measuring bone health but only provides a static measure of BMD. There are other measures of bone health such as bone markers (BMs) that can be used to assess the health of bone by measuring the dynamic process of bone remodeling (121). As BMD typically requires long periods of time to observe measurable changes (noting that the whole turnover process can take several months), the use of BMs provide an alternative to assess the efficacy of interventions to improve bone health, especially shorter-term interventions (122).

2.2: Interventions to Improve Bone Health

2.2.1: Exercise and Bone

Exercise provides the skeletal system with an external stressor that stimulates the bone remodeling response. As previously described, exercise induces mechanical loading on the bone, that can be detected by the osteocytes to activate the remodeling signaling cascade (123). Aside from mechanical loading, declines in serum ionized calcium during exercise also play a significant role in bone remodeling (124). The effects of exercise are most pronounced when performed in a chronic long-term exercise training program. Several systematic reviews and meta-analyses have demonstrated that exercise training has positive influences on BMD (125–128). However, improving our mechanistic understanding by investigating a single bout of exercise can allow for insights into the optimization of an exercise program if each individual exercise session can maximize its benefits on bone. Utilizing BMs would be the optimal method of investigating an acute exercise bout as changes in BMD would not occur following a single exercise session. A broad array of BMs exist that provide insight into the different mechanisms at play during remodelling and can be found in **Figure 4**, along with their functions. A meta-analysis by Dolan and colleagues investigated the effects of acute exercise on BMs, and used their own classification to identify 4 different areas of BMs; bone formation (P1NP, SOST),

resorption (CTX, OPG, RANKL, OPG/RANKL), general remodeling (tOC) and calcium metabolism (PTH) (44). Overall, there were no significant changes in resorption and general remodeling following exercise, with significant but small increases in formation, and a significant and moderate increase in calcium metabolism, post-exercise. For bone resorption, CTX and RANKL did not significantly change, while OPG had a significant but small increase post-exercise. Moderator analyses were conducted on CTX demonstrating that plyometrics and resistance exercises did not change CTX, whereas aerobic exercise, more specifically cycling, resulted in a significant and moderate increase in CTX. For bone formation, both P1NP and SOST had significant changes, with very small and small increases post-exercise, respectively. For general remodeling, tOC had no significant change post-exercise and no change with any of the moderators. For calcium metabolism, PTH had significant and moderate increases post-exercise. Although this meta-analysis provides a comprehensive understanding of the BM response to acute exercise, it combined data from studies over a wide range of populations (older adults, adolescents), training states (trained vs untrained vs sedentary) and exercise modalities (resistance exercises, aerobic exercise, plyometrics) which could have resulted in large amounts of noise, albeit, pooling these studies is necessary because there would not be enough data to conduct an analysis of this caliber. Indeed, looking at training status as a moderator in this meta-analysis revealed that athletes/trained individuals had significant increases in these 4 BM areas while sedentary and recreationally active individuals had no changes. Therefore, this section, where individual BMs are discussed, will focus specifically on young adults who are untrained and performing either plyometrics, resistance exercise and/or cycling, to determine the effect of exercise for each BM of interest in individuals who are in the process of maximizing their PBM or maintaining it.

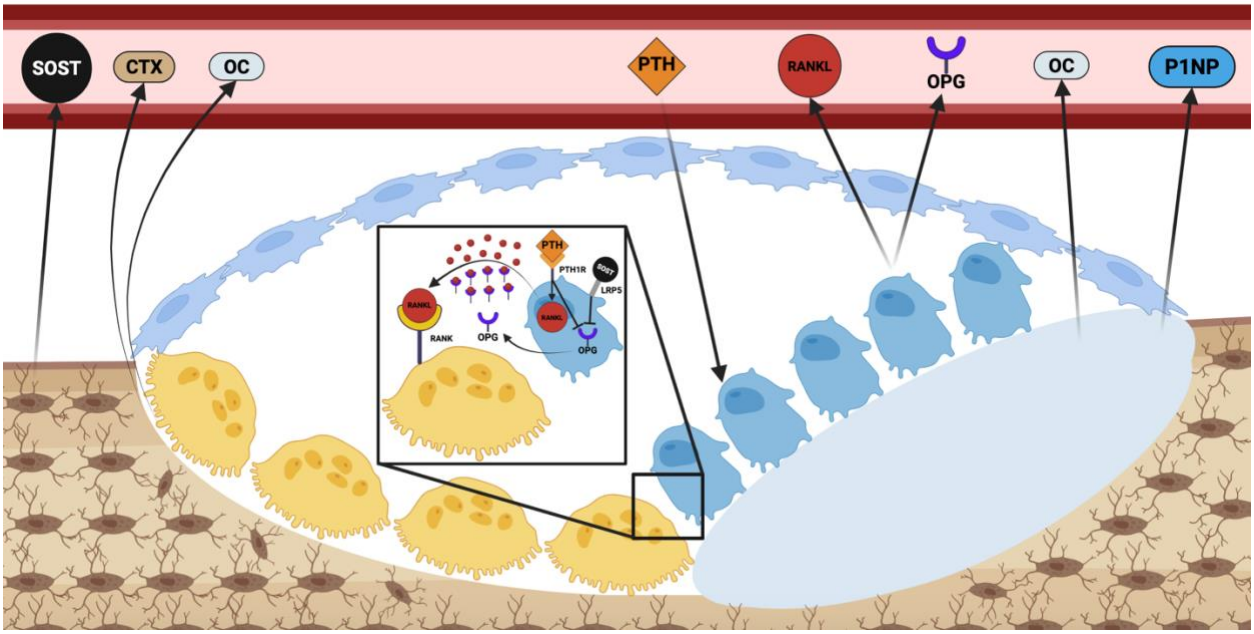

Figure 4: Bone markers and their various functions. During bone resorption, the osteoclasts (yellow cells) will break down bone tissue and release CTX and OC into the circulation. In contrast, osteoblasts (blue cells) are responsible for bone formation and will subsequently release P1NP and OC as well. PTH is released by the parathyroid gland and can bind to PTH1R found on osteoblasts to stimulate the release of RANKL and inhibit OPG production. RANKL can bind to RANK on osteoclast precursor cells to promote osteoclastogenesis. OPG acts as a decoy receptor to RANK to prevent the RANKL-RANK interaction. SOST is released by the osteocytes (brown cells) and can bind to LRP5 to inhibit the Wnt/β-catenin pathway, resulting in the downregulation of osteoblastogenesis and OPG production. Created with BioRender.com

2.2.1.1: Procollagen type 1 N-terminal propeptide (P1NP)

P1NP is a marker of bone formation that reflects the formation of new type 1 collagen in bone. It is recommended to use P1NP as the reference marker for bone formation by the International Osteoporosis Foundation (IOF) (129). P1NP is a byproduct of type 1 collagen formation as osteoblasts synthesize the immature type 1 procollagen, followed by the cleavage of P1NP from the amino terminal end with the aid of procollagen peptidases that form the type 1 collagen in mature bone (130). In the majority of acute exercise studies in untrained adults, P1NP typically had no changes immediately post-exercise (131–135). At 1h (132,133) and 24h post-exercise (131–133,135,136), there was either no change or if there was a transient change, it returned to baseline. Conversely, two studies in recreationally active adult men and inactive adult

women found significant increases in P1NP immediately post-exercise (136,137). These studies included high-impact plyometrics such as 6x25 drop jumps and soccer games, respectively. A study by Nelson and colleagues reported a decrease in P1NP immediately post-exercise and 1h post-exercise, that returned to baseline levels at 24h, in young healthy, untrained women that performed plyometrics (138). Interestingly, a study by Herrmann and colleagues investigated the effects of different cycling intensities (75%, 95% or 110% of anaerobic threshold) in either male or female sedentary adults but only had 3h and 24h timepoints and no immediate post-exercise samples (139). They found that cycling at 75% and 95% of anaerobic threshold decreased P1NP at 3h and remained lower at 24h post-exercise (except females returned to baseline at 24h). When cycling at 110% anaerobic threshold, both groups had no changes in P1NP at 3h and 24h post-exercise except for the males who had an increase at 24h. Overall, aside from the few divergent studies, it seems that in untrained young adults, P1NP does not seem to change much with exercise. This aligns with the mechanisms of bone remodeling as bone resorption typically occurs before bone formation (113).

2.2.1.2: C-terminal telopeptide of type 1 collagen (CTX)

Similar to P1NP, the IOF recommends utilizing CTX as the reference marker for bone resorption (129). CTX is released into the circulation when CtsK cleaves the carboxy-terminal end during type 1 collagen degradation (140). With regards to the CTX response to acute exercise in untrained young adults, most studies found no change in CTX immediately post-exercise (48,131,132,134,135,138,141–143). Similarly, at 1h (132,133,138) and 24h (131–133,135,136,139) post-exercise, there were either no changes or if changes were observed post-exercise, they returned to baseline. Two studies demonstrated that CTX decreased immediately post-exercise in recreationally active men (136,144). These two studies had participants perform either differing intensities of resistance exercise (144) or drop jumps (136) and found that CTX

decreased even further at 1h or remained below baseline at 1.5h, respectively. In contrast, only one study found an increase in CTX post-exercise in recreationally active men performing high-intensity interval cycling (HIIC) (133). Although the literature seems to point towards CTX not changing or increasing post-exercise, most of the studies that have observed the latter are in trained individuals or used different modalities of exercise such as walking/running (145–149). With respect to the trained/competitive athletes, the potential mechanism for an increased CTX response could be that they partake in frequent exercise sessions throughout the week, and this could have potentially greater bone breakdown effects compared to untrained individuals. However, in the context of untrained, young adults, CTX does not appear to change post-exercise.

2.2.1.3: Osteoprotegerin (OPG)

OPG is a negative regulator of osteoclastogenesis. As previously described, OPG is released from osteoblasts and can inhibit osteoclastogenesis as it acts as a decoy receptor for RANKL (150). The binding of RANKL to OPG prevents it from binding to RANK and stimulating osteoclastogenesis. There are very few human studies investigating OPG in the context of acute exercise in untrained young adults, with two studies demonstrating an increase in OPG immediately post-exercise (151,152). The study by Kish and colleagues had participants perform plyometrics such as drop jumps, lung jumps, single leg hops and jumping jacks with ~140 jumps, and found that the increase in OPG remained elevated at 1h and 24h post-exercise (151). The study by Mezil and colleagues had an exercise bout of HIIC with OPG levels increasing post-exercise and returning to baseline at 1h and 24h post-exercise (152). One study by Prowting and colleagues utilized a multi-modal exercise bout of plyometrics and resistance exercises but found no changes in OPG immediately post-exercise (48). Although there are only

a few studies investigating the OPG response in untrained young adults following an acute exercise bout, it seems that OPG may tend to increase post-exercise.

2.2.1.4: Receptor activator of nuclear factor kappa-B (RANKL)

RANKL acts as a positive regulator of bone resorption. Osteoblasts and osteocytes can secrete RANKL and when RANKL binds to RANK found on the surface of osteoclasts, it upregulates signaling pathways that are responsible for osteoclastogenesis (61,64,153). To our knowledge, only three studies have explored the RANKL response to acute exercise in untrained young adults with varying results (48,151,152). The study by Kish and colleagues found no changes in RANKL immediately post-exercise, 1h and 24h post-exercise (151). These data seem to be aligned with the OPG results as OPG was elevated at all timepoints and this may have dampened the RANKL response to exercise. Conversely, Mezil and colleagues reported an increase in RANKL immediately post-exercise that returned to baseline at 1h and remained at baseline levels at 24h post-exercise (152). The exercise bout was HIIC so the concurrent elevations in both RANKL and OPG in this study could be because this exercise bout was able to initiate osteoclastogenesis and the physiological feedback response to this would be to increase OPG. Furthermore, due to the rise in both RANKL and OPG, when expressed as a ratio such as the OPG/RANKL ratio, there was no change across all timepoints. OPG/RANKL ratio is a measure that is used to demonstrate the state of osteoclastogenesis, with an increased ratio meaning a more anti-osteoclastogenic environment and a decreased ratio indicating a shift towards a pro-osteoclastogenic environment (154). Contrasting these studies is the study by Prowting and colleagues that observed a decrease in RANKL immediately post-exercise (48). Due to the decrease in RANKL with no observed changes in OPG, an increase in the OPG/RANKL ratio was observed. With the limited studies in untrained young adults performing

acute exercise that investigated RANKL, it is unclear how RANKL is expected to respond post-exercise.

2.2.1.5: Parathyroid hormone (PTH)

PTH is a circulating hormone released from the parathyroid glands during hypocalcemia that stimulates bone resorption to maintain calcium homeostasis (155). The bone cells that contain PTH1 receptors (PTH1R) are osteoblasts and osteocytes, so the binding of PTH to PTH1R on these cells will secrete RANKL to favour bone resorption (156,157). The literature focusing on untrained young adults has conflicting data with two studies demonstrating a decrease in PTH (158,159), two studies reporting no change (160,161) and one study observing an increase (162). A study by Rogers and colleagues studied both plyometrics and resistance exercises and found that PTH decreased and had no change immediately post-exercise, respectively (163). Another study by Rong and colleagues had two cycling groups and a resistance exercise group and reported that PTH decreased and increased, respectively (164). Hence, the data in untrained individuals seems to be discordant with what was reported in the Dolan meta-analysis, as PTH had a significantly moderate increase post-exercise (44). Similar to CTX, the majority of studies that found heightened PTH concentrations were in athletes/trained individuals and in exercise modalities such as walking/running (47,47,145–149,165–177). Furthermore, from a mechanistic standpoint, an increase in PTH would be expected as exercise utilizes circulating serum calcium for muscular contraction so a rise in PTH would be necessary to sustain circulating calcium homeostasis (124). Therefore, it is unclear what the expected response for PTH would be following an acute exercise bout in untrained young adults and further research is required to tease out these outcomes in this specific population.

2.2.1.6: Sclerostin (SOST)

SOST is a negative regulator of osteoblastogenesis. SOST is secreted by the osteocytes and inhibits the Wnt/β-catenin pathway, subsequently suppressing the ability for preosteoblasts to differentiate into mature osteoblasts (178). Additionally, SOST may also have indirect roles on bone resorption by upregulating RANKL (179). In untrained, young adults, the SOST response to acute exercise seems to reveal an increase immediately post-exercise (132,133,138,162), with a return to baseline or no change at 1h (132,133,138,162) and 24h (132,133,138,162,180) post-exercise. Two studies found no changes in SOST post-exercise and these studies included either a multi-modal exercise bout with plyometrics and resistance exercises or resistance exercise alone (48,159). These studies demonstrate that the post-exercise SOST response may be dependent on many factors such as exercise modality, intensity and/or age. The evidence suggests that resistance exercises may not elicit exercise-induced increases in SOST compared to high-intensity interval exercise (HIIE) (132,133) and plyometrics alone (162). Overall, the limited data in this population of untrained young adults seems to suggest that SOST increases post-exercise.

2.2.1.7: Osteocalcin (OC)

Total OC (tOC) has been historically regarded as a marker of bone formation as it is a NCP released by osteoblasts during bone formation that is subsequently sequestered within the bone matrix (181). Interestingly, OC may also reflect bone resorption as the breakdown of bone can liberate the sequestered OC into the circulation (42). Thus, tOC may be treated as a marker of overall bone remodeling as it can reflect both formation and resorption. The meta-analysis by Dolan and colleagues also characterized OC as a marker of general bone remodeling (44). Therefore, the interpretation of tOC should take into account how the markers of bone formation and resorption are acting. In an acute exercise setting, untrained young adults typically have no

changes in OC immediately post-exercise (48,134,137,160,182,183), 1h (134) and 24h (160,164,183). A single study by Brahm and colleagues had a different finding where following submaximal knee-extension at 60 reps/min for 15 minutes at 61% VO2 peak for one-leg, OC decreased post-exercise and returned to baseline at 1h (161). The study by Rong and colleagues as previously described, observed no changes in OC at 4h and 24h post-exercise with cycling at 55% of their VO2 max, but found that cycling at 85% of their VO2 max and resistance exercises decreased OC at 4h and with a return to baseline at 24h post-exercise (164). Furthermore, the study by Herrmann and colleagues investigated differing intensities of cycling and its effect on OC and found that there were differences in the OC response to varying intensities of cycling and that males and females respond differently at certain intensities (139). Altogether, the data from the majority of these studies suggests that OC does not change post-exercise in untrained, young adults.

2.2.1.8: Summary

In summary, there are limited studies investigating this specific population of untrained, young adults following either plyometrics, cycling or resistance exercise. It seems that the data in untrained individuals are relatively unclear and do not fully align with what was seen in the Dolan meta-analysis that pooled participants and training modalities. **Table 1** summarizes the effects of acute exercise on all the BMs of interest in untrained, young adults.

Table 1: Summary of the immediate post-exercise response for each BM in young, healthy, untrained adults

Bone Marker	Immediate Post-Exercise Response
P1NP (131–135)	↔
CTX (48,131,132,134,135,138,141–143)	↔
OPG (151,152)	↑
RANKL (48,151,152)	?
PTH (158–164)	?
SOST (132,133,138,162)	↑
OC (48,134,137,160,182,183)	↔

Note: ↔ = no change, ↑ = increase, ? = unclear

2.2.2: Nutrition and Bone

Nutrition is another key factor that plays a significant role in bone health. Several fundamental nutrients will be discussed that positively influence bone, including macronutrients like carbohydrates and protein, as well as several micronutrients like calcium, vitamin D and other minerals.

2.2.2.1: Carbohydrates

Many cells within the body require glucose as an energy source to carry out their biological function (184,185). With this in mind, although not inherently thought of as a bone-supporting nutrient, CHO provide glucose to bone cells such as osteoblasts, osteoclasts and osteocytes to fuel cellular activity (186–188). Aside from providing fuel to these cells, CHO ingestion stimulates insulin production, which increases hepatic growth hormone receptor (GHR) expression, thereby improving GH-mediated IGF-synthesis (189). IGF-1 is a growth hormone that promotes osteoblast differentiation and activity, and attenuates osteoblast apoptosis (190,191). CHO is also a commonly consumed nutrient for exercise recovery and several human studies have demonstrated positive BM outcomes with the ingestion of CHO before, during and after exercise (165,167,168,192,193).

2.2.2.2: Protein

The majority of the organic matrix is comprised of type 1 collagen proteins. Thus, protein consumption is necessary to provide amino acids to the skeleton to support type 1 collagen synthesis during bone formation (194,195). Another mechanism by which protein supports bone health is by stimulating an increase in circulating IGF-1 levels to support osteoblast activity and bone formation (196). Furthermore, there has been a growing interest in investigating the role of both muscle and bone health as they are intricately linked both anatomically and metabolically to each other (197). Bone and muscle have crosstalk capacity through paracrine and endocrine

signaling of different factors such as IGF-1, SOST, OC and vascular endothelial growth factor (VEGF) from bone and irisin and myostatin from muscle that can act on their opposing tissues (197). Hence, the provision of protein can also indirectly support bone health by preserving muscle mass and strength (198). Dietary protein can also support bone health by improving intestinal calcium absorption which consequently preserves the liberation of calcium from the skeleton (199). Indeed, several meta-analyses have demonstrated improved BMD and reduced fracture risk with increased protein consumption (14,200–202).

2.2.2.3: Calcium

Hydroxyapatite is a major component of bone, and it is composed of both calcium and phosphorus. Similar to protein, the provision of calcium is necessary as it is one of the main constituents of bone tissue (203). Aside from bone, the maintenance of serum calcium homeostasis is invaluable and tightly regulated as calcium has many other physiological functions such as aiding in nerve signal transmission and muscular contraction for smooth, cardiac and skeletal muscle (204). As previously mentioned, the dominant hormones responsible for calcium homeostasis are PTH and calcitonin. The main storage site for calcium is the skeleton and calcium extraction from the skeleton is regulated by PTH during periods of hypocalcaemia to maintain circulating calcium levels (205). Additionally, PTH and vitamin D also work synergistically to promote calcium homeostasis as PTH can elevate 1,25[OH2]D3 (also known as calcitriol, the active form of vitamin D) levels to increase intestinal calcium absorption and renal reabsorption to sustain circulating calcium (205,206). In contrast, during periods of hypercalcaemia, the skeleton can act as a reservoir for excess calcium (207). All things considered, there is strong evidence in the literature to support these mechanisms of calcium intake and bone homeostasis as several meta-analyses have reported positive bone

outcomes with calcium ingestion (17,208–210). Considering the physiological importance of calcium, it is without a doubt that adequate calcium intake is a necessity for bone health.

2.2.2.4: Vitamin D

Vitamin D is a micronutrient that supports bone directly through vitamin D receptors (VDR) found on bone cells and indirectly by preserving circulating calcium levels. First, vitamin D is obtained by two mechanisms: 1) Endogenous synthesis by the skin through UVB radiation or 2) consumed from the diet (211). Vitamin D binding protein (DBP) will shuttle vitamin D to the liver so that it can be hydroxylated by 25-hydroxylase to 25-hydroxyvitamin D3 (25[OH]D3), the main circulating form of vitamin D (212,213). 25[OH]D3 will then be transported to the kidney with DBP to be hydroxylated by 1alpha-hydoxylase to become calcitriol (212). Calcitriol is the active form of vitamin D that can act on VDRs of different tissues (214). Specifically, osteoblasts express VDR and the actions of calcitriol-VDR binding can result in elevated RANKL secretion and subsequent osteoclastogenesis and bone resorption to support calcium homeostasis during hypocalcemia, or to promote osteoblast maturation and mineralization of the bone matrix (215). In addition, calcitriol also has indirect methods of preserving bone health by its ability to stabilize serum calcium levels by increasing intestinal calcium absorption and renal calcium reabsorption (216). In light of the various actions that vitamin D has on bone, it is evident that it is an important nutrient to support bone health.

2.2.2.5: Other Important Nutrients

A few other nutrients are worth mentioning that have positive effects on bone health. Although still important for bone, brief descriptions of each nutrient and their effects are summarized in **Table 2**, along with the other nutrients described earlier.

Table 2: Summary of important bone-supporting nutrients and their function on bone

Nutrient	Function
Carbohydrate	Provides energy to bone cells to aid in their cellular function (186–188)
Protein	Provides amino acids to type 1 collagen synthesis, stimulates IGF-1 production and supports muscle protein synthesis (194,196,197)
Calcium	One of the main components of hydroxyapatite crystals in the inorganic matrix (203). As calcium has many physiological roles, its serum levels are tightly controlled through calcium exchange with bone (205,207).
Vitamin D	Supports calcium homeostasis to preserve bone and can directly interact with bone cells expressing VDR (215,216)
Phosphorus	Another component of the inorganic matrix that make up hydroxyapatite along with calcium. Necessary for the mineralization of bone (217,218). Inorganic phosphate may also have direct action on bone cells such as inhibiting osteoclastogenesis (219)
Magnesium	Bone is the primary storage site for magnesium (60% of all magnesium) and magnesium acts as a cofactor for the enzymes 25-hydoxylase and 1ahydroxylase to aid in vitamin D synthesis (220).
Vitamin K	Vitamin K is required for the carboxylation of osteocalcin that is an NCP responsible for bone mineralization (76,221)
Potassium	Helps maintain acid-base balance to reduce bone breakdown as high acid load can result in increased osteoclast activity and ion exchange on bone surfaces (222,223)
Iron	Iron supports bone as a cofactor for enzymes such as prolyl-4-hydroxylase and lysyl-hydroxylases that support collagen maturation (224). Iron is also a cofactor for 25-hydroxylase and 1alpha-hydroxylase which is a regulating enzyme for the vitamin D synthesis pathway (225,226).
Zinc	30% of Zinc can be found in bone and Zinc acts as a cofactor for ALP activity and to stimulate type 1 collagen synthesis. Zinc may also promote IGF-1 and calcitriol activity, as well as activating the Runx2 pathway for osteoblastogenesis (227)

Therefore, it is evident that there are a variety of nutrients that can support bone health. The positive mechanisms of action on bone from these nutrients provide a solid case that the combination of them all could elicit additive effects.

2.3: Dairy and Bone

While individual nutrients are important in their own ways, wholefoods may provide a greater stimulus when all these nutrients are interacting with one another. The field of nutritional sciences has been trying to shift from a reductionist approach of looking at the effects of isolated

nutrients to a more holistic approach by studying the effect of the whole food matrix and its potential synergistic effects on health as humans eat wholefoods in their diet as opposed to individual nutrients (228,229). Wholefoods provide a matrix of nutrients that may elicit a greater response than its individual constituents in isolation (20). For example, a study by van Vliet et al., compared egg whites to whole eggs and found that whole eggs stimulated a greater muscle protein synthesis (MPS) response post-exercise than just egg whites (230) indicating greater skeletal muscle protein turnover. These data were consistent with findings from a 12-week training study demonstrating that whole eggs increased serum testosterone, knee extension and handgrip strength, had a trending increase in lean body mass and reduced body fat percentage compared to egg whites (231). Food matrices are influenced by the nutrients within the food, the food form (liquid vs semi-solid/gels vs solid) and can undergo different types of processing such as fermentation, homogenization, heating etc., that can influence physicochemical properties of the food (232,233). The food matrix influences the bioaccessibility (amount of nutrients liberated from the food matrix upon digestion) and bioavailability (amount of nutrients that are absorbed from the gut into the circulation) of the nutrients that are found in these foods (234).

One of the most studied food matrices is the dairy matrix and is defined as the unique structure of a dairy food, its components (nutrients and non-nutrients) and their interactions (235). There is keen interest in dairy foods because they are nutrient-dense and contain high amounts of protein, calcium, vitamin D (if fortified), magnesium, potassium and phosphorus per calorie compared to other foods (236). The interaction between all these nutrients may elicit a synergistic, more potent effect on physiological health than just their individual nutrients consumed in isolation (20,229). Indeed, there have been several meta-analyses that have demonstrated the positive effects that dairy consumption can have on bone health (237–242).

The most common form of dairy is cow's milk, with different dairy products such as yogurt and cheese being derived from milk following additional processing. These different dairy products have differing dairy matrices that may elicit unique effects on bone health, even when nutrient composition is similar (243).

2.3.1: Milk

The process of making milk into a consumable food item requires many steps. Bovine milk is first collected from cows and undergoes a skimming process that separates the fat from the milk that can be added back later at certain percentages (whole milk [3.25%], 2%, 1% and skim milk [0.1%] (233). The milk is then homogenized to break down the fat globules into smaller ones to prevent creaming and is then pasteurized (heat-treated) to kill any harmful pathogens (244,245). In Canada, it is also mandatory that milk be fortified with vitamin D (22).

Lactose is the main carbohydrate in milk and is a disaccharide that contains both glucose and galactose monosaccharide subunits. Lactose can be broken down and hydrolyzed into glucose and galactose through the lactase enzyme known as β-galactosidase (246). These monosaccharides can then be used to generate energy in different cells such as osteoclasts or osteoblasts (186,188). One of the barriers for some people in consuming dairy products appears to be the inability to digest/break down lactose. The main form of lactose intolerance is due to the regression in lactase gene expression as one ages (247). An increase in lactose concentration (without lactase) increases osmolarity in the intestine that can lead to an excessive buildup of water and subsequent diarrhea (246). To counter the issues of lactose intolerance, a lactase pill that contains the lactase enzyme can be purchased to aid in the digestion of dairy products, or one can purchase lactose-free dairy products as well.

Milk proteins are considered high-quality proteins as they contain all nine essential amino acids (248). When assessed using the digestible indispensable amino acid score (DIAAS), whole

milk, milk protein concentrate and whey protein isolate, all rank as the best source of protein for digestibility and quality (249). The two main types of milk proteins are whey and casein, and they comprise 20% and 80% of the total protein found in milk, respectively. Whey proteins are structured in a globular shape and can be further broken down into β-lactoglobulin, α-lactalbumin and bovine serum albumin (250). Similarly, casein also has different components such as β-casein, κ-casein, αS1-casein, αS2-casein (250). These caseins form a clustered structure known as a casein micelle. κ-casein is typically found on the surface of the casein micelles that can bind to calcium phosphate, which supports the structure of the micelle and aids in the transportation of calcium during digestion (233,251). Comparatively, whey proteins are known as faster digesting proteins as β-lactoglobulin is resistant to pepsin hydrolysis in the stomach so it can move through the gastrointestinal (GI) tract faster, whereas casein tends to coagulate in the stomach and passes through the GI tract more slowly (232). The speed at which these proteins are digested influences their absorption and the timing of amino acid bioavailability in the circulation.

Additionally, milk contains large quantities of many micronutrients such as calcium (316 mg/250 mL), phosphorus (261 mg/250 mL), potassium (404 mg/250 mL), magnesium (28 mg/250 mL) etc. (Canadian Nutrient File [CNF] https://food-nutrition.canada.ca/cnf-fce/ Code: 114). As described earlier, these micronutrients are protective of bone, and the combination of all these nutrients in a nutrient-dense food product such as milk, demonstrate its importance as a wholefood source for bone health. Furthermore, in Canada, it is mandatory for milk to be fortified with Vitamin D (22), which is invaluable as there are few food sources that naturally contain vitamin D and the endogenous synthesis of vitamin D from the sun is limited in countries like Canada that have shorter days in the winter months.

The postprandial effects of milk consumption on BMs were investigated in a study by Kruger and colleagues (252). Participants consumed 200 mL of skimmed milk with either 250 mg, 500 mg or 1000 mg of calcium. They found that serum calcium increased in the first 3 hours for all groups, serum PTH decreased for all groups at each timepoint, and serum CTX progressively decreased in all groups throughout the 5-hour window. Similarly, a study by Hilkens and colleagues looked at the effects of adding either no dairy (NO-D), one dairy product (ONE-D) or two dairy products (TWO-D) to an isocaloric breakfast meal (253). With respect to the plasma amino acid response, there was a dose-response observed for essential and branched-chain amino acids (BCAAs) with total area under the curve (tAUC) (253). For serum calcium, there was a marginal increase in ONE-D and TWO-D with a marginal decrease in the NO-D group (253). Serum PTH decreased at the 1-hour mark and remained below baseline at all timepoints, but ONE-D and TWO-D had a lower tAUC compared to NO-D (253). There was a gradual decrease in serum CTX in all 3 groups until the 3-hour mark, with the ONE-D and TWO-D groups suppressing the rise in CTX seen in the NO-D group at the 4 and 5-hour timepoints (253). Collectively, these data demonstrate the ability for milk to modulate the postprandial BM response.

2.3.2: Greek Yogurt

Yogurt is a dairy product derived from milk that undergoes additional processing during its inception. Lactic acid bacteria (LAB) cultures such as *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* are inoculated into milk to begin fermentation (254). During the fermentation process, these bacteria will ferment lactose to lactic acid which drops the pH, causing micelles to coagulate and form a gel structure (255). Yogurt can undergo ultrafiltration or straining to form Greek yogurt (GY) by removing the liquid portion

of yogurt consisting of lactose and whey leading to a higher protein content than regular yogurt (~10 g/100 g of GY vs ~5 g/100 g of regular yogurt; CNF Code: 6981 & 6948) (24).

There are numerous benefits to GY as a dairy product of choice. Following the straining process, a large amount of lactose is removed from GY, which may ease its digestion and be preferred by consumers who are lactose intolerant (256). GY is also a superior source of dairy protein as it provides more protein per serving than regular yogurt and milk (24). Furthermore, the addition of traditional LAB cultures and/or specific probiotic strains into yogurt can provide unique mechanisms to improve bone health. For example, probiotics are specific live microorganisms that can enhance the health of the host when provided in adequate amounts (257). The consumption of probiotics can modulate the gut microbiota and enhance intestinal calcium absorption, which has downstream effects in supporting bone health (258). The specific mechanisms by which probiotics can elicit beneficial effects are out of the scope of this thesis and have been reviewed in detail by Bashir and colleagues (259).

To date, there is no evidence on the effect of GY consumption on postprandial BMs responses. However, our lab group has published data investigating postprandial GY vs. milk plasma amino acid responses (26) and the combined effects of chronic GY consumption and resistance training on BMs (30) that will be discussed in Section 2.3.3 and Section 2.4, respectively.

2.3.3: Dairy Matrix Differences

Milk and GY are dairy products that can both provide bone-supporting nutrients, however, the mechanisms by which they support bone may be different due to their unique dairy matrices. The most apparent distinction between the two matrices is their physical form as milk is a liquid and GY is a semi-solid food. Liquid matrices are known to result in faster gastric emptying, whereas semi-solid and solid food forms take longer to breakdown and are more

viscous, so they pass through the GI tract at a slower rate (260). Two human studies have measured gastric emptying following either the consumption of milk or yogurt (261,262). Both studies showed consistent results with milk demonstrating faster gastric emptying compared to yogurt.

When comparing their nutrient composition, there is ~10 g of protein per 100 g of GY while there is only ~4 g of protein per 100 mL of milk (CNF Code: 6981 & 114). There are also differences between the protein fractions as milk has more whey protein than GY because the majority of the whey proteins have been removed during the straining process to form GY, resulting in GY predominantly consisting of casein proteins (24). Therefore, it would be plausible to expect that milk would have faster amino acid absorption compared to GY due to semi-solid foods having a longer gastric emptying time compared to liquids, and casein being a slower-digesting protein than whey. However, a study by Brown et al., conducted in our lab investigated the effects GY vs. milk consumption on the postprandial plasma BCAA responses (26). Participants consumed two isonitrogenous doses of 200 g of GY or 555 mL of milk at baseline and at 1-hour with plasma BCAAs (leucine, isoleucine and valine) measured over a 4-hour window (26). These data demonstrated that GY had a greater maximal concentration and AUC for leucine and total BCAAs compared to milk but the time to maximal concentration was not different (26). Thus, it appears that leucine and BCAAs were more readily available in the circulation within the 4-hour window following GY vs. milk. Horstman and colleagues had similar findings as they investigated the postprandial amino acid response to low-fat ultra-high temperature (UHT) milk and yogurt (263). The time to maximal concentration of serum essential amino acids was not different between the groups but yogurt had a greater maximal concentration than the UHT milk (263). The improved amino acid absorption seen with yogurt

compared to milk may be attributed to the proteolytic function of the bacterial cultures (264). An *in vitro* digestion study by Lamothe and colleagues observed greater protein hydrolysis in GY compared to milk and came to a similar conclusion that the bacterial cultures may aid in the proteolytic process contributing to a faster amino acid absorption (260). In summary, yogurt contains more casein protein and differs from milk in both food form and matrix, however, evidence from these studies does not suggest that yogurt has a slower amino acid absorption profile compared to milk.

Between milk and GY, differences also exist in their micronutrient composition. The calcium content in milk is greater than GY as some calcium is lost during the straining process. Per 100 g, milk has 122 g, and GY has 96 g of calcium (CNF Code: 114 & 6981). Two human studies have directly compared the calcium fractional absorption rate between milk and GY (265,266). Both studies had participants consume dairy products that were isocalcemic and labelled with calcium isotopes (265,266). These studies had similar findings and reported no differences in calcium fractional absorption rate between milk and GY (265,266). In addition, a vital nutrient for bone health is vitamin D and although dairy products do not inherently contain this micronutrient, Canada has a mandatory fortification of vitamin D in milk (22). Within 100 mL of milk, there is ~40 IU of vitamin D (CNF Code: 114). Furthermore, GY is not fortified with vitamin D, presenting a potential limitation of GY to support bone compared to milk.

In summary, several dairy matrix differences exist between milk and yogurt. A review of these differences is illustrated in **Figure 5**. There have also been several *in vitro* and *in vivo* studies that have investigated differences between the two dairy products in terms of amino acid and calcium bioavailability which have been reviewed in detail by Fardet et al (243). However, despite all these studies, there are none (to our knowledge) that have compared the effects of

milk vs. GY on bone turnover and metabolism specifically, which is a gap in the literature that this study aimed to fulfill.

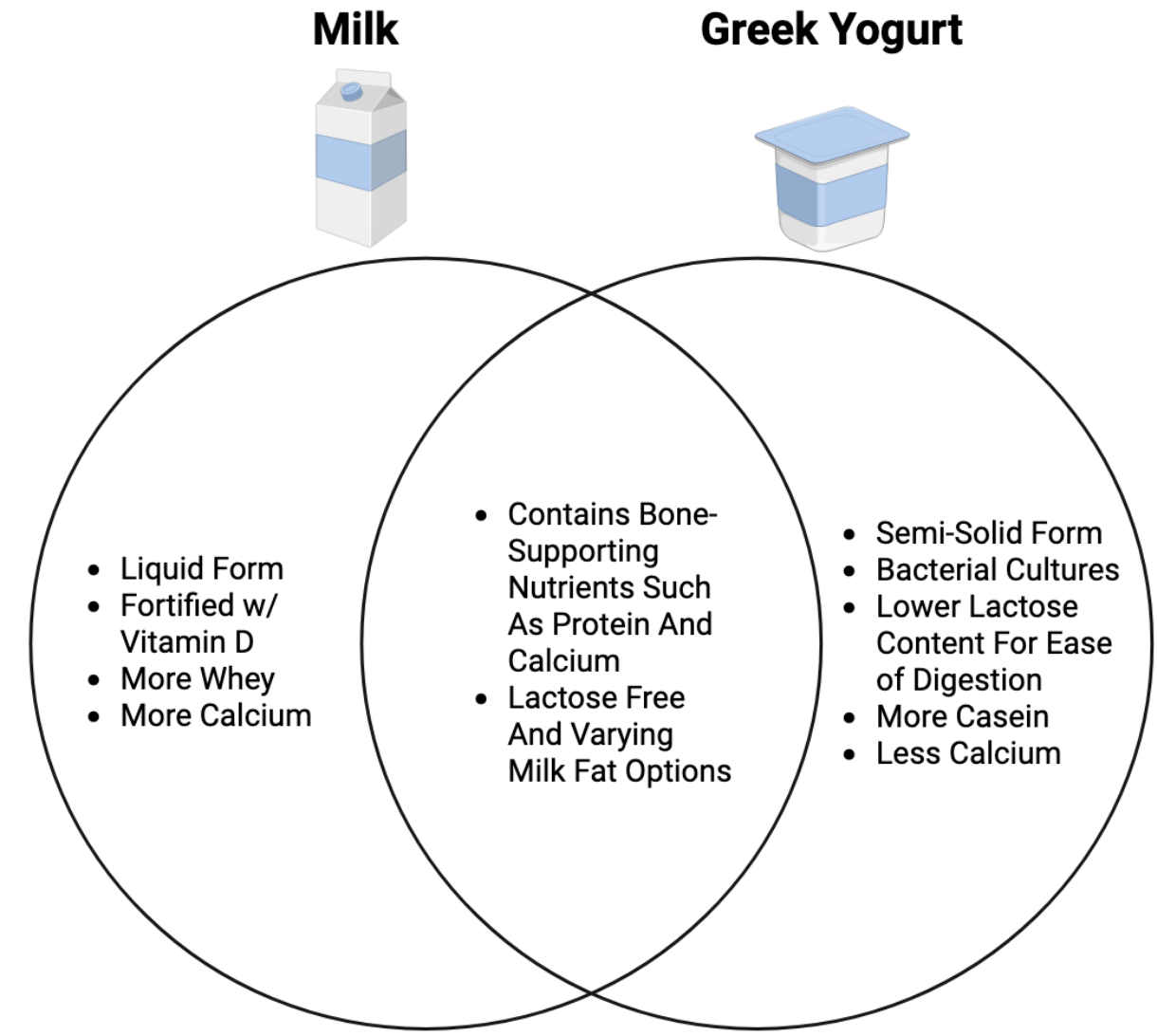

Figure 5: Dairy matrix differences and similarities between milk and Greek yogurt. Created with BioRender.com

2.4: Dairy and Exercise for Bone Health

It is apparent that both exercise and nutrition, specifically dairy products, can support bone. With this in mind, it would be reasonable to suspect that the combination of these two interventions may provide augmented benefits compared to these interventions in isolation. Several studies have been conducted investigating the effects of both chronic exercise training

and dairy product consumption on BMD and/or BMs in adults (28–30,38,39,267). These studies have been summarized and reviewed recently in detail by Cheng and Josse (268). The studies of most relevance to this thesis are two 12-week randomized controlled trials (RCTs) previously performed by our lab comparing exercise training and either milk (29) or GY (30) consumption vs. a carbohydrate control in young, healthy adults. The first RCT by Josse et al., had two groups of female participants (n=10 per group) perform resistance exercise for 5 days/week and consumed either milk or an isoenergetic carbohydrate supplement immediately post-exercise and 1-hour post-exercise (29). The second RCT by Bridge et al., had two groups of male participants (n=15 per group) perform a mix of resistance exercises (2x/week) and plyometric exercises (1x/week) and consumed either GY or a carbohydrate placebo pudding on three occasions during the exercise days (immediately post-exercise, 1-hour post-exercise and before bed) and twice on the non-exercise days (breakfast and before bed) (30). Both studies reported improved bone outcomes with either decreases in PTH (29) or increases in P1NP and P1NP:CTX ratio (30) with chronic dairy consumption, demonstrating the effectiveness of dairy and exercise training on the longer-term BM response to training.

2.4.1: Dairy Consumption and Acute Exercise

These longer-term RCTs (mentioned above) contribute practical evidence for nutritionists, personal trainers and researchers on the promising effects of these combined interventions on skeletal health. However, studying nutrition and acute exercise can provide valuable mechanistic insight into the effects of dairy products and their ability to modulate the acute post-exercise BM response. Similar to the meta-analysis by Dolan and colleagues on the post-exercise BM response (44), the same group performed another complementary meta-analysis assessing the influence of nutrition (45). They did a systematic search of acute exercise studies with carbohydrate, protein, calcium or dairy product supplementation that measured BMs

post-exercise. Only the calcium studies had a meta-analysis performed as the other supplements did not have enough studies for a meta-analysis. There were three studies looking at acute CHO ingestion and running exercise that reported lower CTX values when consuming carbohydrate vs water (165,167) or a low-carbohydrate high-fat meal (192).

Similarly, with acute exercise and whey protein supplementation, three studies have investigated these interventions on BMs. Theocharidis et al., provided either whey protein, a carbohydrate beverage or water to adolescent athletes following a high-intensity swimming bout and found that there were no between-group differences in CTX or P1NP, but only whey protein had a significant decrease in CTX from the 8-hour to 24-hour blood sampling timepoint (269). Aussieker et al., had recreational athletes perform 6 sets of squats at 60% of their 1RM and provided either whey protein, collagen protein or water post-exercise and found that at 2-hours post-exercise, whey protein and collagen protein had significantly lower CTX compared to water (270). The last protein study was conducted by Townsend et al., where trained endurance runners performed exhaustive treadmill running at 75% of their VO2 max and were given either a combined protein/carbohydrate beverage immediately post-exercise (immediate feeding), 2-hours post-exercise (delayed feeding), or water post-exercise (placebo) (47). Their paper reported significantly lower CTX at 1- and 2-hours post-exercise in the immediate feeding group compared to the delayed feeding and placebo group (47). The immediate feeding group also had higher P1NP values at 4-hours post-exercise compared to the other two groups (47). Data from several of these carbohydrate and protein studies demonstrate the ability of these macronutrients to influence the BM response to exercise, specifically reducing CTX levels.

Regarding calcium, Dolan and colleagues were able to perform a meta-analysis of studies combining acute exercise and calcium supplementation on the CTX and PTH response (45).

Eight studies were included for the meta-analysis with a wide range of methodologies for calcium intake such as through the diet, supplementation or through intravenous infusion (45). The results of the meta-analysis revealed that the provision of calcium suppressed the post-exercise CTX and PTH responses (45). Altogether, the findings presented from these studies indicated a beneficial effect of calcium on the post-exercise BM response.

There is limited evidence on the effects of wholefood dairy products and acute exercise on BMs, with only four studies that have been published to date. The first study was a randomized crossover trial conducted by Haakonssen et al., which recruited 32 competitive cyclists and had them perform 80 minutes of stationary cycling at 60% of their max power output (271). The trials differed in their pre-exercise meal as they either consumed a high calcium breakfast containing dairy products such as milk and yogurt that equated to ~1352 mg of calcium, or a control meal with ~46 mg of calcium (271). The high calcium group reported significantly lower PTH values vs. the control group immediately post-exercise and 1-hour post-exercise. Also, the high calcium group had lower CTX values compared to the control group immediately post-exercise, 1-hour post-exercise and at 4-hours post-exercise (271). Similarly, a study by Lundy and colleagues recruited elite male rowers to partake in two acute, high-intensity rowing sessions (3x30 minutes) on the same trial day and provided either a high calcium breakfast with dairy foods such as milk, yogurt and cheese, or a control meal without dairy (272). The high calcium breakfast increased levels of ionized calcium in the circulation compared to the control meal which resulted in lower PTH and CTX post-exercise. The last two acute studies were performed by our group, with one study being done in adolescent girls with overweight and obesity (273), and the other being in young, healthy female adults (48). Kurgan et al., recruited 30 adolescent females with overweight/obesity who underwent a 12-week mixed-

mode exercise training study where participants trained 3x/week and either consumed a higher dairy diet (RDa; 4 servings/day) or a lower dairy diet (LDa; 0-2 servings/day) (273). The participants completed an acute exercise session consisting of plyometrics pre- and post-intervention and collected blood samples pre-exercise, immediately post-exercise and 1-hour post-exercise (273). The main finding from this study revealed that following the intervention, the RDa group significantly increased the relative acute exercise OPG:RANKL ratio response compared to pre-intervention, whereas the LDa group had no change in their relative acute exercise response from pre- to post-intervention (273). This indicates a reduced osteoclastogenic response to acute exercise following an exercise training and dairy intervention. The last study was done by Prowting et al., where 13 young, healthy female participants completed a single mixed-mode exercise bout consisting of plyometrics and resistance exercises and consumed either milk or a carbohydrate supplement post-exercise (48). The results from this study indicate a significantly lower CTX AUC response over 48-hours post-exercise in the milk group compared to the carbohydrate group (48). Collectively, these studies suggest that the provision of dairy products may positively modulate the post-exercise BM response.

3.0: Objective and Hypotheses

3.1: Objective

The objective of this study was to determine whether two distinct, isonitrogenous dairy wholefood products, milk and Greek yogurt (GY), or an isoenergetic (to milk) carbohydrate (CHO) drink versus a water control consumed following an acute bout of high-intensity exercise differentially modulates circulating systemic markers of bone turnover and metabolism.

3.2: Hypotheses

The consumption of isonitrogenous milk vs. GY post-exercise will elicit differing bone biomarker responses due to their unique food matrices. In addition, consuming milk, GY, CHO and water will result in different bone responses post-exercise. More specifically:

1) Dairy wholefood consumption such as milk and GY post-exercise will favourably affect the acute bone response by enhancing bone formation markers such as P1NP or by reducing bone resorption markers such as CTX, RANKL and SOST compared to CHO and water.
2) CHO consumption post-exercise will result in an intermediary bone response as it will be less effective than dairy wholefoods but will be more beneficial compared to water due to the provision of energy and glucose.

4.0: Methods

4.1: Participants

Twenty healthy adults were recruited for the "Bone and Inflammatory Outcomes – Nutrition and EXercise" (BIONEX) study. Recruitment for this study took place in Toronto (Ontario, Canada) using ethics-approved posters, word of mouth, in-class announcements, and social media posts. Interested participants underwent a virtual screening session that included answering a health and screening questionnaire with a research coordinator to ensure they were eligible for the study. Inclusion criteria for this study included: 1) being between the ages of 18-35 years, 2) body mass index (BMI) of 18.5-24.9 kg/m^2 and 3) lower structured exercise activity (≤2/week). Exclusion criteria included: 1) any medical conditions (inflammatory, autoimmune, musculoskeletal), 2) current smoker, 3) consuming vitamin D and/or calcium supplements, and 4) any allergies to dairy products or lactose intolerance. Once deemed eligible, written informed consent was obtained from participants during the initial lab visit. The BIONEX study has been approved by the York University Research Ethics Board (ORE) File #2019-045 and was registered at www.clinicaltrials.gov (NCT06041087). Data collection spanned from October 2023 to February 2025.

4.2: Experimental Design

This study utilized a randomized, controlled, within-subject, crossover experimental design. Participants underwent 4 trial conditions in a randomized order. The conditions were exercise + milk (MILK), exercise + Greek yogurt (GY), exercise + carbohydrate (CHO), and exercise + water (WATER) (**Figure 6**).

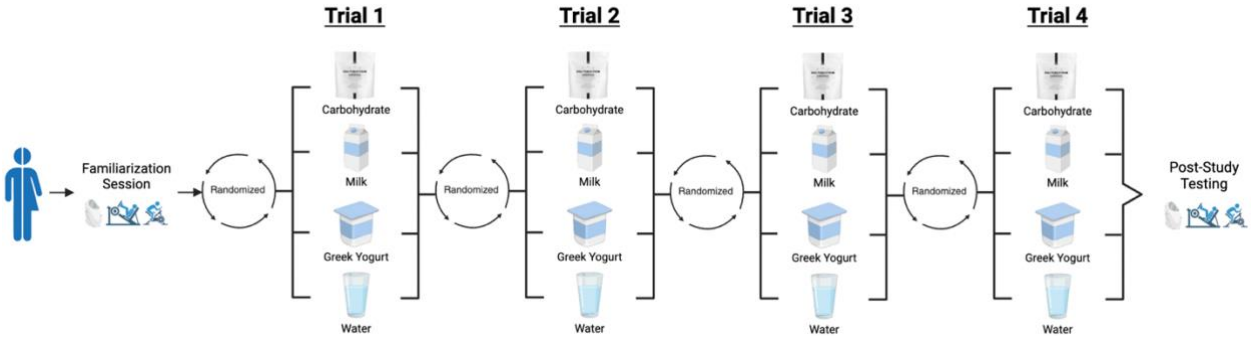

Figure 6: Study timeline of the familiarization session/pre-study testing and post-study testing with the four randomized exercise trials separated by a 2-4 week washout period. Created with BioRender.com

Conditions were coded from 1-4 and were randomized using an online random number generator. Male participants and female participants on hormonal contraceptives had at least a 2-week washout period between trials while naturally cycling female participants had a ~4-week washout period and underwent the trial during the early follicular phase of their menstrual cycle. Preceding each trial, participants arrived at the lab following an 8-hour minimum overnight fast. They were asked to avoid taking non-steroidal anti-inflammatory drugs (NSAIDs), refrain from alcohol consumption and exercise, 12-hours, 24-hours and 48 hours before the trial, respectively. During each trial, participants engaged in an identical acute, high-intensity exercise session to control for the exercise stimulus (described further in the "Exercise Protocol" section). Additionally, nutrition was controlled for by consuming similar foods the day before (Day 0) and the day of the trial (Day 1). To confirm the type and timing of foods consumed, participants used an online food tracking app called "Keenoa" to log each meal. The purpose for controlling their exercise bout and food intake was to ensure that any effects observed in the bone biomarker responses could be attributed as best as possible to the different post-exercise supplements provided and not from any differences in the exercise stimulus or nutritional intake.

Prior to the onset of the study, an initial lab visit was scheduled with participants to provide information and obtain written informed consent. Participants completed the Physical

Activity Readiness Questionnaire (PAR-Q+) and the Godin-Shephard Leisure-Time Exercise Questionnaire to ensure they were cleared for exercise and to confirm their weekly physical activity levels, respectively. Once completed and eligibility was validated, participants began the familiarization session.

4.3: Familiarization Session

Following the attainment of informed consent, participants underwent a familiarization session. During the familiarization session, participants had their height measured in centimeters (cm) and weight measured in kilograms (kg) without shoes using a stadiometer (Health-o-meter Professional, McCook, IL, USA). Body weight and composition (body fat percentage [BF%]) were assessed using 3 different modalities. First, the BodPod (COSMED USA Inc., Concord, CA, USA) was used to measure the participants body density using air displacement plethysmography (274). Before entering, the device was calibrated to determine the volume of air in the empty pod. Participants were then instructed to enter the BodPod wearing only a swim cap and minimal clothing (males wore tight undergarments while females wore tight-fitted shorts and a tank top or sports bra). Body volume was determined by the volume of air displaced which was then used to calculate body density. Using the Siri equation, BF% was estimated from the body density value obtained (275). Second, the Tanita bioelectrical impedance analysis (BIA) device (Tanita BC-418, Tanita Corporation of America Inc., Arlington Heights, IL, USA) was utilized to estimate BF% by sending a weak electrical current through the body with electrodes at the hands and feet and measuring the signal transit time (276). Last, the BodyMetrix (IntelaMetrix, Brentwood, CA, USA) hand-held ultrasonography probe was used to obtain fat thickness at specific sites to estimate BF%. Ultrasound gel was applied to the probe and was placed on the right side of the body on the quadriceps (between the iliac crest and patella), chest (between the axilla and nipple) and waist (2 cm away from the umbilicus) for males and the

quadriceps, triceps (between the acromion and olecranon processes) and hip (just above the iliac crest) for females. The measurements of fat thickness were then inputted into the Jackson & Pollock equation to estimate BF% (277).

Next, participants tested their estimated 1-repetition maximum (e1RM) on the leg press, chest press and seated row selectorized resistance exercise machines. A certified personal trainer demonstrated proper technique for all the exercises. All e1RM tests began with three warm-up sets of 8 repetitions with an increasing load at a rating of perceived exertion (RPE) of 3, 5 and 7, respectively on a 10 RPE Borg scale (278). The load increased by 20-60 lbs depending on their RPE from their previous set. If their RPE was lower than the desired RPE, then the next warm-up set had a larger increase in load. If their RPE was higher than the desired RPE, then the next warm-up set had a smaller increase in load. Once the warm-up sets were completed and participants were familiar with the movement, they performed a set of 5 repetitions with a 20-60 lbs increase in load from their last warm-up set. If 5 repetitions were completed, the load was increased by another 20-40 lbs (following a short rest) until they were no longer able to complete a set of 5 repetitions. When failure to complete a set of 5 repetitions was achieved, the Bryzcki equation was used to estimate their 1RM (279). Following the completion of the three e1RM tests on the resistance exercise machines, participants did a maximum power output (PO) test on a stationary cycle ergometer (Monark 894E, Monark Sports & Medical, Vansbro, Sweden). Their revolutions per minute (RPM) for the test was determined based on their body mass rounded down to the lowest interval of 10 (i.e., a 56 kg female pedalled at 50 RPM). For the maximum PO test, participants warmed up for 4 minutes without any weight loaded onto the cycle basket, resulting in them pedaling between 50-70 watts (W). Following the warm-up, 0.5 kg was loaded

each minute until the participant could no longer maintain their RPM to determine their maximum PO.

The entire familiarization session was repeated at the end of the study during a post-study testing session following their last trial, with a ~2-week washout period, to determine if their body composition and strength changed across the duration of the study.

4.4: Experimental Trial

Participants arrived at the laboratory at ~08:00 am followed by an overnight fast for the experimental trials. Questions regarding their cycle day (if female), recent illnesses, caffeine and medication consumption, timing of last meal and date of last exercise session, were asked to confirm that they met all the requirements of the trial. Their weight was also measured to confirm that they were weight-stable throughout the study. Participants then began the trial with a pre-exercise blood sample. Next, participants entered the exercise laboratory to initiate their high-intensity exercise session that consisted of plyometrics, resistance exercises and high-intensity interval cycling that will be discussed in further detail in the "Exercise Protocol" section. Immediately following the cessation of exercise, a post-exercise (~5-min) blood sample was collected. In a previously determined randomized order, participants consumed their assigned post-exercise supplement of either CHO, MILK, GY or WATER. At 1h following the consumption of the first supplement, there was an additional blood sample and a second consumption of their assigned supplement. A standardized lunch meal was then provided 2h post-exercise. 4h following the consumption of the first supplement, there was one more blood sample for the exercise trial day. Participants were then able to leave the laboratory and were provided with standardized snacks and dinner to take home, as well as their assigned supplement to consume ~1h before bed. The next day (Day 2), ~24h following the end of exercise and the consumption of their first supplement, participants arrived at the laboratory fasted at ~09:30 am

for their last blood sample. The experimental trial was repeated an additional three times with a different post-exercise supplement, followed by a ~2-4 week washout period. **Figure 7** depicts the timeline for the experimental trial day.

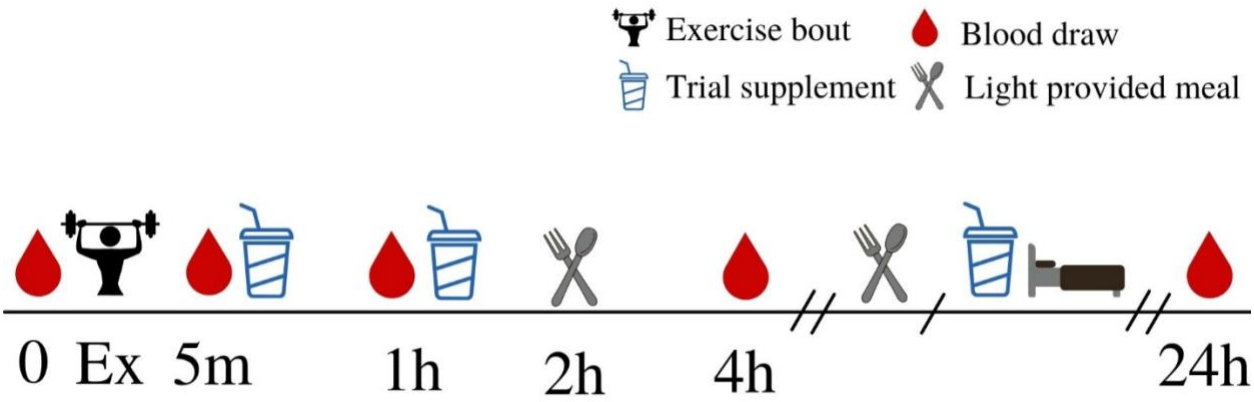

Figure 7: Trial schematic with the various timepoints for blood sampling, supplement and meal consumption.

4.5: Exercise Protocol

The acute exercise bout took place in our exercise research laboratory. Participants began the exercise bout with four plyometric exercises consisting of 3 sets of 10 jumps. The first exercise was *depth jumps* where participants stepped off a box and exploded immediately off the floor with minimal ground contact time. Second, they performed *tuck jumps* where they jumped and brought their knees up to their chest, and similar to the depth jumps, they tried to minimize ground contact time with each jump. Third, they performed *split jumps* where participants had one foot in front of the other and jumped as high as they could while alternating their front foot in mid-air. The last plyometric exercise was the *seated jump*, where participants were in a seated position and swung their arms explosively down and up to jump off the seat as high as they could.

Following the plyometric exercises, participants engaged in three resistance exercises, performing 3 sets of 10 reps at a load of ~70% of their e1RM assessed at the familiarization

session. For the chest press, leg press and seated row, they were instructed to perform the exercises with a full range of motion and verbal encouragement was provided to motivate the participants to complete the sets. The RPE for each set was also recorded to confirm that each exercise session provided a similar, challenging stimulus.

The last exercise that participants performed was high-intensity interval cycling (HIIC). Participants had a heart rate (HR) monitor (Polar H7, Polar Electro, Kempele, Finland) strapped on their chest to measure their inter-set HR response. For the first four minutes, participants warmed up and worked up to 50% of their max PO. The first minute had participants pedal between 50-70 W with no load on the basket. The second minute had an intermediary load before reaching 50% of their max PO on minutes three and four. During the last 10 seconds of the warm-up and for the subsequent one-minute workloads, HR and RPE were recorded. Following the four-minute warm-up, participants went through three one-minute high-intensity interval sprints on the cycle ergometer at 90% of their max PO measured during the familiarization session. Between the sprint intervals, participants had a one-minute active recovery interval of 50% of their max PO. If the HR response during the sprint intervals did not average $\geq$85% of their estimated HR maximum, they performed an additional sprint interval. All exercise sessions were facilitated by a personal trainer and at least one other research volunteer who was unaware of supplement trial allocation.

4.6: Post-Exercise Supplements

Trial supplements were provided to participants on three occasions during each trial: immediately post-exercise, 1h post-exercise and 1h before bed. For the liquid supplements (CHO, MILK, and WATER), participants consumed an isovolumetric amount of 500 mL. The CHO supplement was 48 g of maltodextrin mixed with ~500 mL of water. The MILK supplement was 500 mL of Lactantia 0%MF milk that was isoenergetic to the CHO supplement.

The last supplement was 200 g of 0%MF Oikos high-protein vanilla GY, and it was isonitrogenous to the MILK supplement. Further information on macronutrient and micronutrient contents of each of the trial supplements is in **Table 3**.

Table 3: Nutritional values of the trial supplements

Nutrients	Carbohydrate (48 g)	Milk (500 mL)	Greek Yogurt (200 g)	Water (500 mL)
Calories (kcal)	180	180	149	0
Carbohydrate (g)	45	26	17	0
Protein (g)	0	18	19	0
Fat (g)	0	0.4	0	0
Calcium (mg)	0	600	257	0
Vitamin D (µg)	0	10	0	0

4.7: Blood Sample Collection

Blood samples were collected with venipuncture from the antecubital fossa. Timepoints for the blood samples were pre-exercise, immediately post-exercise (~5-minutes), and 1h, 4h and 24h following the consumption of the first supplement. Approximately 22 mL of blood was collected into five different vacutainers for each timepoint: one 6 mL red top to collect serum, three 4 mL green tops to collect sodium heparin (NaHep) plasma and one 4 mL purple top to collect EDTA plasma. However, for the immediate post-exercise blood sample, only 16 mL of blood was collected into one 6 mL red top, one 6 mL green top and one 4 mL EDTA plasma. When the blood samples were collected, the green and purple top vacutainers were centrifuged (Rotina 38R, Hettich, Tuttlingen, Germany) immediately at 1300 g for 10 minutes at 4°C. The red top vacutainer was kept at room temperature for 30-40 minutes to clot, and was then centrifuged at 1300 g for 10 minutes at 4°C. Once the spin was completed, the plasma and serum components were aliquoted into 1.5 mL Eppendorf tubes and stored at -80°C for bone biomarker analysis at the completion of the study.

4.8: Hematocrit Assessment

Changes in plasma volume were measured for the blood samples on the exercise day (Pre, 5m, 1h and 4h) due to potential fluctuations related to perspiration and water consumption during exercise. A reduction in plasma from exercise can artificially inflate biomarker concentrations (i.e., hemoconcentration) independent of their true response to exercise, warranting its assessment in an acute study with a substantial exercise bout (280). Plasma volume shifts were measured by taking ~300 μL of blood from the EDTA vacutainers prior to centrifugation and aliquoting it into a 1.5 mL Eppendorf tube. Hematocrit was measured in triplicate by collecting the blood into microhematocrit tubes that were sealed and spun in a microhematocrit centrifuge (Haematokrit 210, Hettich, Tuttlingen, Germany) at 10,000 RPM for 5 minutes. Following centrifugation, the plasma and red blood cells were separated, and hematocrit was measured on all three tubes using an evaluation disk. The three values were recorded and averaged for the final hematocrit measure for that specific timepoint. The percent plasma volume change was assessed for each timepoint relative to the pre-exercise value using the Van Beaumont formula (281). If there were significant differences in trial or time, then biomarker concentrations were corrected with the change in plasma volume value.

4.9: Standardized Trial Nutrition

Food diaries were recorded on Day 0 (i.e., the day before exercise) and Day 1 of each trial with the food tracking app Keenoa (Keenoa, Montreal, QC, Canada). These food diaries were used to confirm compliance by ensuring that participants were consuming similar foods on Day 0 of each experimental trial to equate calories and macronutrients prior to their trial. If participants forgot to track their food on Keenoa, verbal dietary recall was used to confirm their compliance. On Day 1, participants were provided standardized meals according to their estimated total daily energy expenditure (TDEE). The Harris-Benedict equation was used to

calculate basal metabolic rate (BMR) and was then multiplied by a "lightly active" (1.375) exercise factor. All lunch meals were the exact same across trials and participants, regardless of their TDEE as seen in **Table 4**. Lunch was consumed in the laboratory.

Table 4: Macronutrients of the standardized foods provided specifically for the MILK trial on Day 1 for an individual requiring ~2000 kcal/day.

Food	Calories (kcal)	Carbohydrates (g)	Protein (g)	Fat (g)
Lunch				
Great Value Vegetable Stir Fry	190	36	8	2
Banana	105	27	1	0
Strawberry Cereal Bar	130	24	1	3
Snacks				
Apple	104	28	1	0
Banana	105	27	1	0
Snack'n Go Hummus & Crackers x2	190 (380)	26 (52)	6 (12)	8 (16)
Strawberry Cereal Bar	130	24	1	3
Dinner				
Healthy Choice Steamers Grilled Balsamic Chicken	270	37	18	6

Following the completion of the 4h blood draw, participants were given snacks and a dinner meal to consume at home. These snacks and meals were modified to meet their energy needs according to their TDEE. The nutritional information for the snacks and dinner meals is in **Table 4**. For the CHO and WATER trials, a protein bar was provided to participants immediately after the 4-hour blood draw so that they could have an even distribution of protein throughout the day, rather than consuming all their protein at dinner as the MILK and GY arms consumed protein already from their supplements. This is because evidence suggests that total daily protein intake may be more effective if distributed evenly throughout the day rather than in less frequent, larger boluses (282). Overall, meals were standardized across trials to match caloric intake, but macro- and micronutrients were different due to the inherent differences in the supplements provided.

4.10: Bone Biomarker Analysis

P1NP and CTX were analyzed in NaHep plasma, while OPG, RANKL, PTH, SOST and OC were analyzed in serum. All samples were measured from pristine aliquots in duplicate for all the assays. Assays were performed as per the manufacturer's instructions, with a few in-house adjustments to optimize assay performance in consultation with the manufacturer. For P1NP and CTX, enzyme linked immunosorbent assays (ELISAs) (Human Procollagen Type 1 N-Terminal ELISA Kit, NBP2-76465, Novus Biologicals, Toronto, ON, Canada; Human CTX-1 ELISA Kit, NBP2-69073, Novus Biologicals, Toronto, ON, Canada) were utilized and optical density (OD) was measured using a BioTek Synergy LX plate reader (Agilent Technologies, Santa Clara, CA, USA). OD was plotted on a four-parameter logistic curve to determine the concentrations in pg/mL and ng/mL in P1NP and CTX, respectively. For RANKL, a microbead single plex assay kit was used (MILLIPLEX Human RANKL Magnetic Bead Single Plex – Metabolism Assay, HRNKLMAG-51K-01, EMD Millipore Corporation, St Louis, MO, USA) and measured with the Luminex 200 (Luminex Corp., Austin, TX, USA). For OPG, PTH, SOST and OC, a microbead multiplex assay kit (MILLIPLEX Human Bone Magnetic Bead Panel – Bone Metabolism Multiplex Assay, HBNMAG-51K-05, EMD Millipore Corporation, St Louis, MO, USA) was used and also measured with the Luminex 200. Belysa was utilized to optimize standard curves for more accurate readings of values (Belysa Immunoassay Curve Fitting Software, 40-122, EMD Millipore Corporation, St Louis, MO, USA). Average intra-assay coefficients of variation (CVs) were 6.52% for P1NP, 11.74% for CTX, 3.01% for OPG, 11.81% for RANKL, 3.44% for PTH, 5.63% for SOST and 6.15% for OC.

4.11: Statistical Analysis

For timepoints that had a CV >20%, these data were visually inspected and assessed in a blinded manner to circumvent any potential bias. P1NP was the exception, and duplicates were

inspected with a CV >10% due to smaller CVs being more influential with larger absolute values. If a replicate appeared to influence the average value away from the mean pattern of change, then it was removed and the single value closest to the mean pattern of change was chosen. This was done for 129/5302 singlets in the entire dataset (2.4% of data).

Following the plasma volume correction of the absolute biomarker concentrations, skewness and kurtosis z-scores were evaluated to assess normality and if any values were ±3, the data were log-transformed to improve normality. The OPG:RANKL ratio was the only marker that was non-normal and log-transformed data were used for statistical analysis; however, the non-transformed data were used when presenting the data graphically. Outliers were also evaluated and classified if values were ± >2SD from the mean. 117/3328 datapoints (3.5% of all datapoints) were deemed outliers and were replaced with the upper/lower 2SD limit.

In total, only 2 blood samples were missed across the entire dataset. However, we were able to draw one vacutainer of serum from one of those two missed samples and had enough to analyze all the markers but P1NP and CTX, since plasma was used for those markers. During biomarker analysis, there were instances where some participants were undetectable and were removed from the final analysis (3 participants for CTX, 1 participant for P1NP, 2 participants for RANKL, and 1 participant for SOST). There were also instances where some values were undetectable. For example, for OPG, OPG:RANKL and SOST, there were three samples that were undetectable from one participant, at 1h, 4h and 24h in their WATER trial. We used a conservative approach and added the participants baseline value as leaving missing values for consecutive missing timepoints may not be as appropriate. For all other missing datapoints such as missed blood samples or undetectable values, these were missing at random and were fitted into the model using restricted maximum likelihood (REML).

Paired t-tests were performed on the familiarization/pre-study vs. post-study e1RM and max PO data. Data were analyzed on GraphPad Prism 10.5.0 (GraphPad Software Inc., San Diego, CA, USA).

One-way ANOVAs (trial [CHO, MILK GY and WATER] as fixed factor) were used to analyze the exercise and dietary data on GraphPad Prism 10.5.0 (GraphPad Software Inc., San Diego, CA, USA) and SPSS (SPSS Inc., Chicago, IL, USA), respectively. The Greenhouse-Geisser correction was utilized if sphericity was violated. For the exercise data, multiple comparisons were corrected with the false discovery rate (FDR).

Two-way repeated-measures linear mixed models (LMMs) were used to analyze hematocrit using R Studio (version 4.5.1). The two fixed effects were time (Pre, 5m, 1h, 4h and 24h) and trial (CHO, MILK, GY and WATER), with participant ID as a random effect. Significant p-values were assessed with post-hoc tests corrected for multiple comparisons using FDR.

Three-way repeated-measures LMMs were used to assess main effects and interactions for the bone biomarkers, using R Studio (version 4.5.1) and the packages lme4, lmerTest and emmeans. The three fixed effects were time (pre-exercise, 5m, 1h, 4h and 24h), trial (CHO, MILK, GY and WATER) and sex (male and female). The three 2-way interactions were time by trial, time by sex, trial by sex and a 3-way interaction of time by trial by sex was also assessed. The effects of sex and their interactions were not displayed unless a significant p-value was observed. Baseline values were added as a covariate to control for baseline variability. Random effects were participant ID and trial by participant ID so that each participant has an individual baseline value for each trial. If significant p-values for main effects and/or interactions were observed, post-hoc analyses were conducted and multiple comparisons were corrected using

FDR. Statistical significance was assumed at an alpha level of 0.05. All graphs were made on GraphPad Prism 10.5.0 (GraphPad Software Inc., San Diego, CA, USA).

5.0: Results

5.1: Participant Enrollment and Characteristics

148 people reached out to the study team to participate in the BIONEX study. Of those, 61 people were excluded due to not meeting the eligibility criteria, 29 were lost to follow-up, and 3 declined to participate after learning more about the study. Thus, 57 people underwent a comprehensive participant eligibility screening in a scheduled meeting. An additional 15 people were excluded due to not meeting eligibility criteria after gathering further information, 3 were lost to follow-up and 2 were waitlisted due to capacity limits. That left 37 participants who attended the initial in-person familiarization session. At this point, 4 more were excluded due to not meeting eligibility criteria (i.e., their actual BMI did not meet criteria after measuring body weight and height in the lab), 2 declined to participate, and 2 were not able to participate due to scheduling. 29 participants were left remaining and were then enrolled in the study but 5 participants dropped out due to scheduling conflicts, 3 had issues with completing the study protocol, and one relocated to another province. Thus, the final sample size for the study was 20 participants, with 18 of these participants completing the post-study testing. The participant characteristics for the 20 participants are in **Table 5**. The full flow of participants in the BIONEX study is illustrated in the CONSORT diagram (**Figure 8)**.

Table 5: Participant Characteristics During Familiarization (Visit 0) (n=20).

Participant Characteristics	Mean ± SD
Sex (M/F)	8/12
Age (years)	22.7 ± 4.0
Height (cm)	165.5 ± 8.8
Weight (kg)	60.3 ± 8.3
BMI (kg/m^2)	22.0 ± 2.3
Body Fat (%)	23.4 ± 9.6
Peak Power Output (W)	166 ± 41

Abbreviations: BMI: body mass index

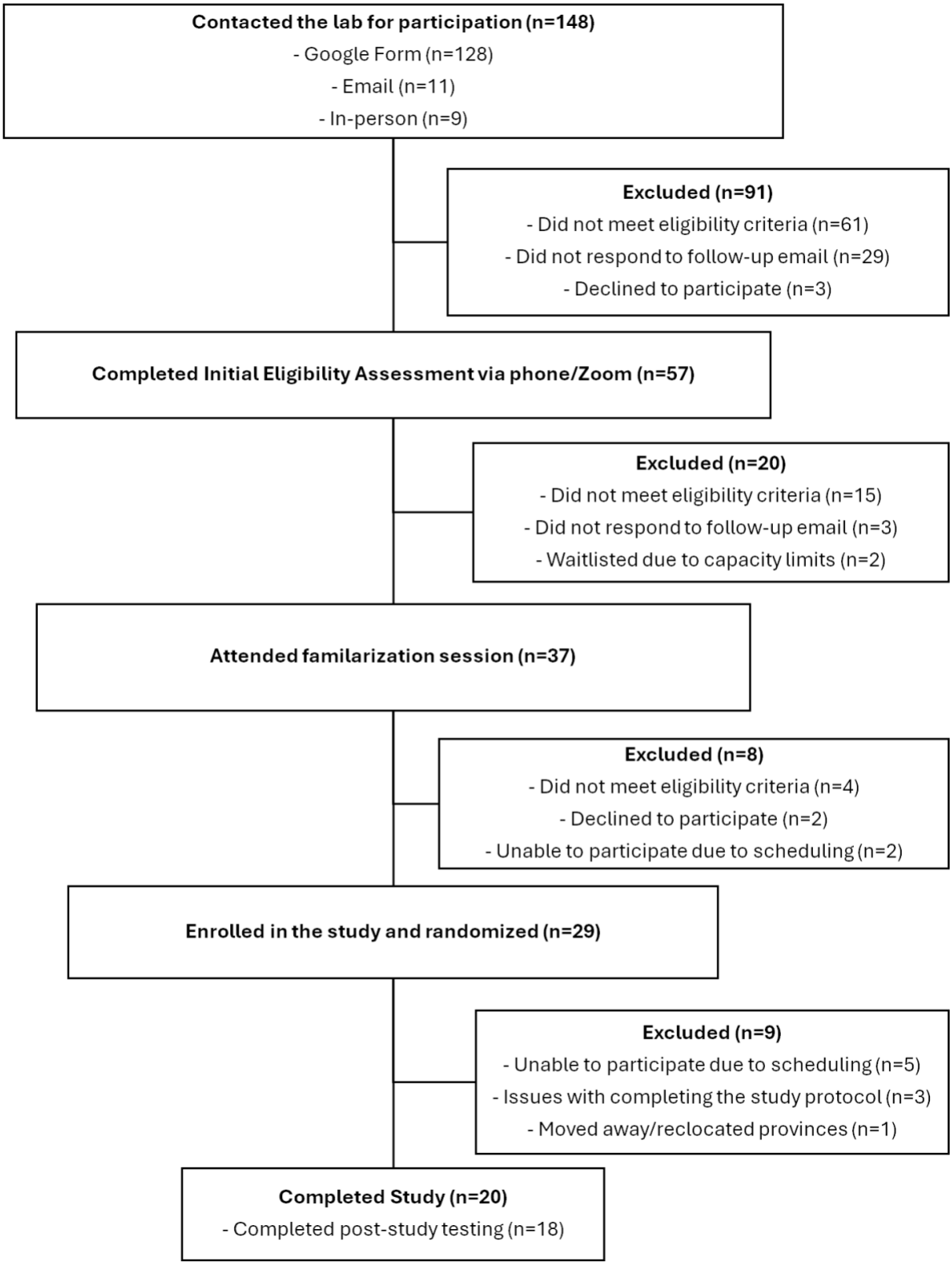

Figure 8: CONSORT diagram outlining participant recruitment, eligibility and final sample size.

5.2: Exercise Data

In all trials, participants completed 120 impacts per session for the plyometric exercises. For the resistance exercises, there were no significant differences between exercise intensity expressed as % e1RM, volume load or RPE between trials. Similarly, for the HIIC, there were no significant differences in HRmax%, volume load and RPE **(Table 6)**.

Table 6: Comparison of number of impacts, resistance exercise volume load and cycling volume load for carbohydrate (CHO), MILK, Greek yogurt (GY) and WATER trials (n=20).

Exercises	CHO	MILK	GY	WATER	p-value
Impacts/Session	120	120	120	120	1.000
Chest Press					
e1RM (%)	69.12% ± 4.57%	68.38% ± 4.92%	69.12% ± 4.57%	69.71% ± 3.96%	0.272
Volume Load (kg)	1075.05 ± 460.19	1065.90 ± 457.84	1075.05 ± 460.19	1079.60 ± 454.14	0.485
RPE	4.74 ± 1.46	4.99 ± 1.23	5.28 ± 1.25	4.57 ± 1.54	0.109
Leg Press					
e1RM (%)	68.31% ± 8.69%	68.31% ± 8.69%	68.27% ± 8.68%	68.31% ± 8.69%	0.330
Volume Load (kg)	2189.85 ± 1097.02	2189.85 ± 1097.02	2188.50 ± 1096.25	2189.85 ± 1097.02	0.330
RPE	5.29 ± 1.81	5.47 ± 1.32	5.51 ± 1.65	5.05 ± 1.83	0.580
Seated Row					
e1RM (%)	67.60% ± 5.87%	67.60% ± 5.87%	67.60% ± 5.87%	67.60% ± 5.87%	1.000
Volume Load (kg)	939.20 ± 319.74	939.20 ± 319.74	939.20 ± 319.74	939.20 ± 319.74	1.000
RPE	5.41 ± 1.66	5.58 ± 1.73	5.80 ± 1.44	5.47 ± 1.80	0.448
Cycling					
HRmax (%)	89.50% ± 8.03%	92.25% ± 5.25%	92.85% ± 3.90%	92.45% ± 6.45%	0.078
Volume Load (kJ)	28.43 ± 7.12	28.16 ± 6.98	27.59 ± 6.89	28.08 ± 7.33	0.277
RPE	7.50 ± 2.08	7.80 ± 1.91	8.10 ± 1.68	7.63 ± 1.71	0.292

Note: Volume load was determined by sets*repetitions*weight for resistance exercises and sets*time*Watts for cycling. RPE was measured using Borg's 0-10 Critical Rating scale (278). %HRmax and cycling RPE was taken from the last working interval (third or fourth). HRmax was estimated using 220-age (283). Abbreviations: CHO: carbohydrate; GY: Greek yogurt; %e1RM: Percentage of estimated one repetition maximum; RPE: ratings of perceived exertion; %HRmax: percentage of heart rate maximum.

Additionally, when the familiarization (pre-study) e1RM and Max PO cycling bout were compared to the post-study testing e1RM and Max PO cycling, all exercises significantly increased strength (leg press [p<0.001], chest press [p=0.002], seated row [p=0.002]) and power output (p=0.023) **(Figure 9)**. In addition, there was a significant increase in body mass from pre- to post-study (Δ1.39 ± 1.54; p=0.001), with no differences observed in body fat percentage (p=0.432) **(Figure 10)**.

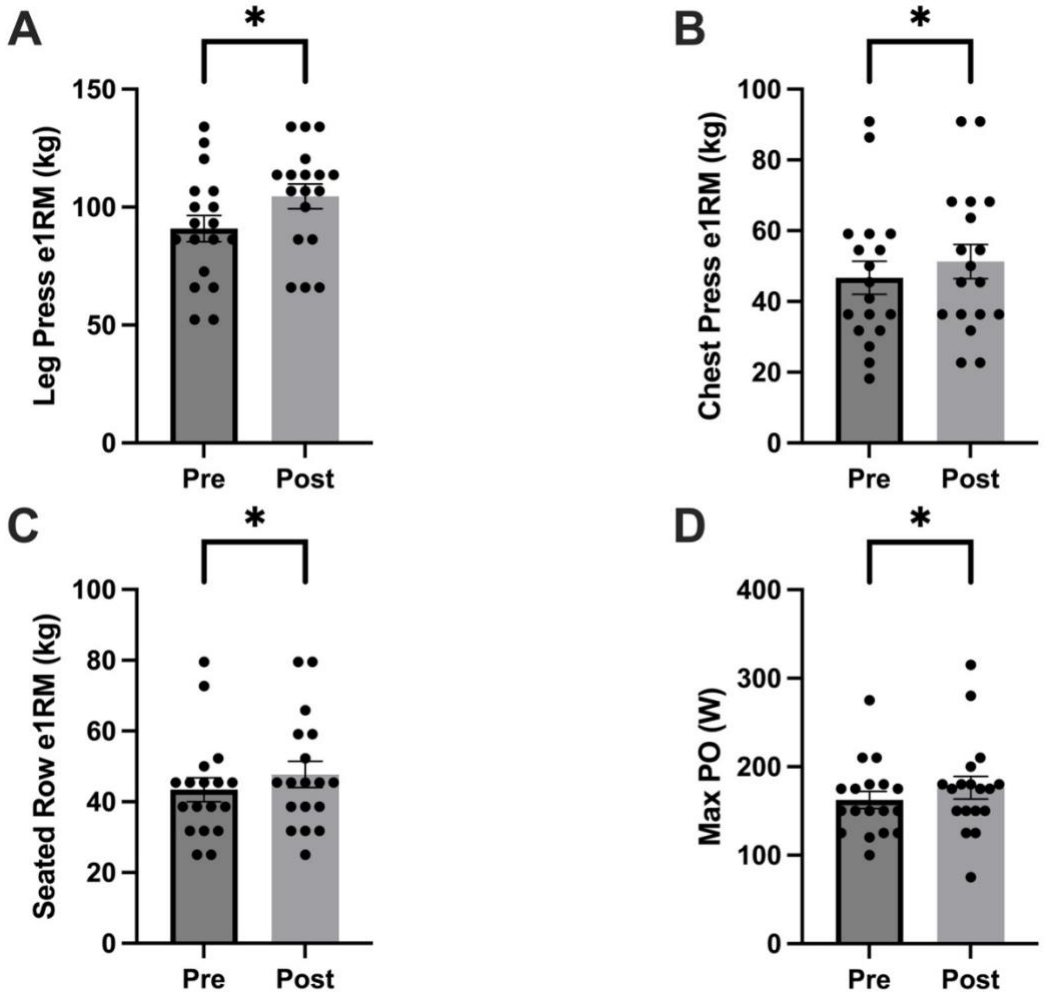

Figure 9: Pre- vs. post-study comparison of strength and power measures (n=18). e1RMs of leg press (A), chest press (B) and seated row (C) are displayed, along with max PO on the cycle ergometer (D). * = significant difference between pre- and post-study. Statistical significance was p≤0.05. Values are mean ± SEM. Abbreviations: e1RM: estimated 1 repetition maximum, PO: power output.

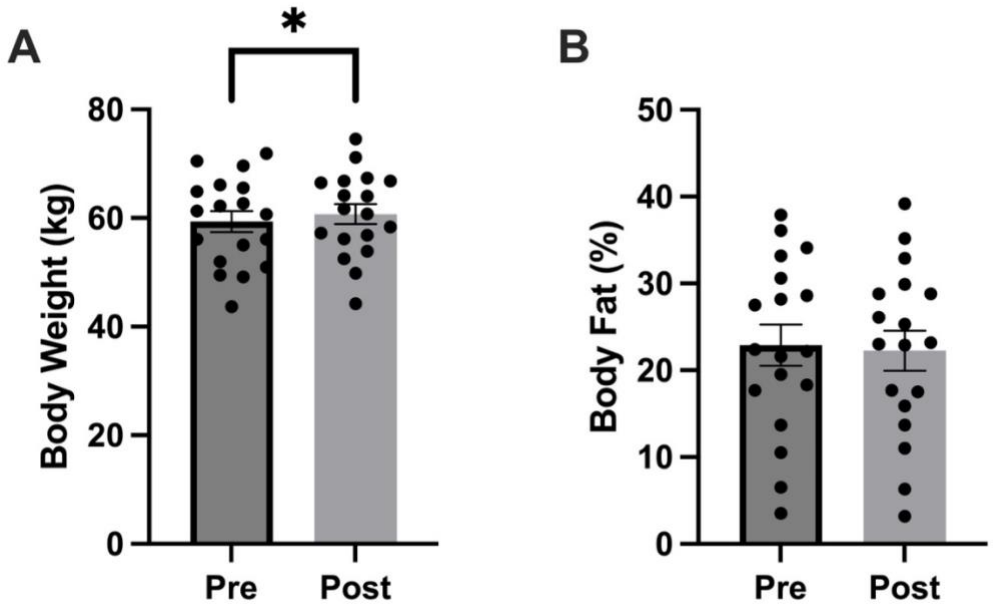

Figure 10: Pre- vs. post-study comparison of body composition measures (n=18). Body weight (A) and body fat percentage (B) are displayed. * = significant difference between pre- and post-study. Statistical significance was $p \leq 0.05$. Values are mean ± SEM.

5.3: Hematocrit Analysis

There were no main effects of trial ($p=0.409$) or a trial by time interaction ($p=0.307$) but a significant effect of time for hematocrit was present ($p=0.001$), revealing an increase in hematocrit at 5m vs. Pre, followed by a reduction in hematocrit at 1h and 4h vs. Pre and 5m ($p<0.001$ for all) **(Figure 11)**.

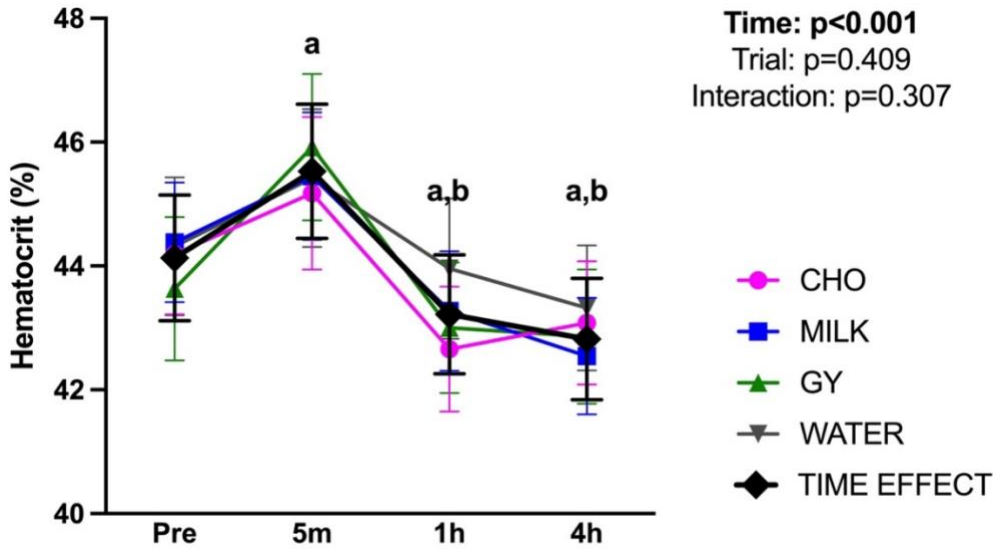

Figure 11: Hematocrit percentage across the four timepoints on the exercise trial day (n=20). a = difference from Pre, b = difference from 5m. Statistical significance was $p \leq 0.05$. Values are mean ± SEM.

5.4: Dietary Data

Dietary data from Day 0 (pre-trial day) found no significant differences in energy intake or any of the macro- and micronutrients except for sodium (p=0.036) **(Table 7)**. Post-hoc analyses revealed that the GY trial consumed significantly greater sodium than the CHO and WATER trials and that the MILK trial also consumed significantly greater sodium than the CHO trial on Day 0. On the trial day, energy intake was not significantly different across trials (p=0.231) as was expected with our study design **(Table 8)**. However, all other macro- and micronutrients (aside from trans-fat) were significantly different across trials primarily due to the differences in nutrient composition between the trial supplements.

Table 7: Dietary intake data from the pre-trial day (Day 0; n=20)

Dietary Variable	CHO	MILK	GY	WATER	p-value
Energy intake (kcal)	1533 ± 544	1620 ± 633	1498 ± 519	1443 ± 543	0.369
Protein (g)	64 ± 29	68 ± 30	65 ± 31	58 ± 24	0.460
Fat (g)	57 ± 28	61 ± 34	59 ± 30	55 ± 28	0.803
Saturated Fat (g)	17 ± 9	20 ± 12	21 ± 12	18 ± 12	0.387
Trans Fat (g)	0 ± 0	1 ± 1	1 ± 1	1 ± 0	0.697
Carbohydrate (g)	194 ± 67	203 ± 77	182 ± 59	182 ± 68	0.243
Sugar (g)	66 ± 35	75 ± 49	62 ± 30	72 ± 35	0.281
Fibre (g)	11 ± 6	12 ± 6	12 ± 5	11 ± 5	0.701
Calcium (mg)	437 ± 275	536 ± 348	536 ± 400	452 ± 220	0.349
Iron (mg)	9 ± 5	9 ± 5	9 ± 5	9 ± 5	0.873
Sodium (mg)	2049 ± 884	2659 ± 1453	2697 ± 1479	2190 ± 1181	**0.036**
Potassium (mg)	1569 ± 711	1697 ± 731	1586 ± 789	1694 ± 743	0.678
Vitamin D (IU)	98 ± 130	129 ± 146	119 ± 134	134 ± 147	0.331
Magnesium (mg)	166 ± 92	172 ± 100	168 ± 99	177 ± 86	0.895
Phosphorus (mg)	699 ± 381	738 ± 418	735 ± 479	673 ± 308	0.746

Note: Values are mean ± SD. Abbreviations: CHO: carbohydrate, GY: Greek yogurt.

Table 8: Dietary intake data from the trial day including the trial supplements (Day 1; n=20)

Dietary Variable	CHO	MILK	GY	WATER	p-value
Energy intake (kcal)	2017 ± 237	1969 ± 262	1979 ± 214	1975 ± 242	0.231
Protein (g)	58 ± 10	101 ± 14	107 ± 9	78 ± 12	**<0.001**
Fat (g)	35 ± 7	34 ± 7	33 ± 7	46 ± 7	**<0.001**
Saturated Fat (g)	7 ± 1	5 ± 1	5 ± 1	8 ± 1	**<0.001**
Trans Fat (g)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.109
Carbohydrate (g)	385 ± 38	332 ± 39	333 ± 33	334 ± 39	**<0.001**
Sugar (g)	88 ± 14	161 ± 17	149 ± 13	111 ± 18	**<0.001**
Fibre (g)	27 ± 4	28 ± 5	31 ± 5	35 ± 6	**<0.001**
Calcium (mg)	242 ± 27	1937 ± 241	988 ± 30	319 ± 36	**<0.001**
Iron (mg)	12 ± 2	11 ± 2	11 ± 2	15 ± 2	**<0.001**
Sodium (mg)	1839 ± 315	2379 ± 355	2045 ± 291	2343 ± 313	**<0.001**
Potassium (mg)	1981 ± 365	4511 ± 548	3280 ± 280	2622 ± 426	**<0.001**
Vitamin D (IU)	0 ± 0	1032 ± 267	0 ± 0	0 ± 0	**<0.001**
Magnesium (mg)	64 ± 15	234 ± 25	128 ± 4	67 ± 16	**<0.001**
Phosphorus (mg)	63 ± 14	1537 ± 210	939 ± 9	66 ± 14	**<0.001**

Note: Values are mean ± SD. Abbreviations: CHO: carbohydrate, GY: Greek yogurt.

5.5: Absolute Concentrations of Bone Biomarkers

5.5.1: Markers of Bone Turnover

P1NP had a significant main effect of trial ($p=0.039$), time ($p<0.001$) and a trial-by-time interaction ($p<0.001$) **(Figure 12A)**. For the between-trial differences, GY had the lowest P1NP at 5m compared to all trials (GY vs. CHO [$p<0.001$], MILK [$p=0.002$], WATER [$p<0.001$]). At 1h, CHO was greater than MILK and GY (CHO vs. MILK [$p<0.001$], GY [$p<0.001$]) and WATER was also greater than MILK ($p=0.046$). No differences were observed at the 4h timepoint but at the 24h timepoint, MILK had greater P1NP than water ($p=0.018$). Regarding the within-trial differences across time **(Supplementary Table 1)**, P1NP peaked at 1h (1h vs. Pre [$p=0.039$], 5m [$p=0.016$], 4h [$p<0.001$], 24h [$p=0.002$]), dipped below baseline at 4h ($p=0.045$) and returned to baseline at 24h ($p=0.329$) for the CHO trial. For the MILK trial, P1NP significantly decreased at 5m ($p=0.049$) and remained lower than baseline at 1h ($p=0.047$). At 4h ($p=0.899$) and 24h ($p=0.559$), P1NP returned to baseline in the MILK trial. For the GY trial, P1NP was significantly lower at 5m compared to all other timepoints ($p<0.001$ for all timepoints), returned to baseline at 1h ($p=0.058$) and remained at baseline for the remaining timepoints (4h: $p=0.070$, 24h: $p=0.210$). For the WATER trial, P1NP significantly decreased at 24h compared to Pre ($p=0.042$), 1h ($p=0.013$) and 4h ($p=0.013$).

CTX had a significant effect of time ($p<0.001$) and a trial by time interaction ($p<0.001$), but no effect of trial ($p=0.266$) **(Figure 12B)**. With the between-group differences, CHO was greater than GY at 5m ($p=0.015$) and was greater than WATER at 1h ($p=0.001$). There were no trial differences for CTX observed at the 4h and 24h timepoints. For the within-trial differences across time **(Supplementary Table 1)**, CTX peaked at 5m (5m vs. Pre [$p<0.001$], 4h [$p=0.006$], 24h [$p<0.001$]) and remained elevated above baseline at 1h ($p<0.001$) and 4h ($p=0.009$) before returning to baseline at 24h ($p=0.798$) for the CHO trial. In the MILK trial, CTX gradually

increased until the 4h timepoint (Pre vs. 5m [p=0.015], 1h [p=0.031], 4h [p<0.001]) and returned to baseline at 24h (p=0.176). For the GY trial, CTX significantly increased at 1h (p<0.002) and peaked at 4h (4h vs. Pre [p<0.001], 5m [p=0.002], 24h [p<0.001]), with a return to baseline at 24h (p=0.535). For the WATER trial, CTX significantly increased at 5m (p=0.026), returned to baseline at 1h (p=0.507), followed by a trending increase at 4h (p=0.056), and a return to baseline at 24h (p=0.694).

For the P1NP:CTX ratio, there was a significant effect of time (p<0.001), with no effect of trial (p=0.499) or trial by time interaction (p=0.470) **(Figure 12C)**. P1NP:CTX ratio was significantly lowered at the 5m (p<0.001), 1h (p=0.002) and 4h (p<0.001) timepoints relative to baseline, but returned to baseline at 24h (p=0.247).

OC had a significant effect of time (p<0.001), with no trial (p=0.157) or trial by time interaction (p=0.745) **(Figure 13A)**. OC significantly increased and peaked at 5m (p<0.001 for all timepoints), and returned to baseline at 1h (p=0.571). There was then a significant decrease at 4h compared to all other timepoints (p<0.05 for all timepoints), with OC returning to baseline at 24h (p=0.614). There was also a significant effect of sex (p=0.005) **(Figure 13B)**, with males having significantly greater OC compared to females.

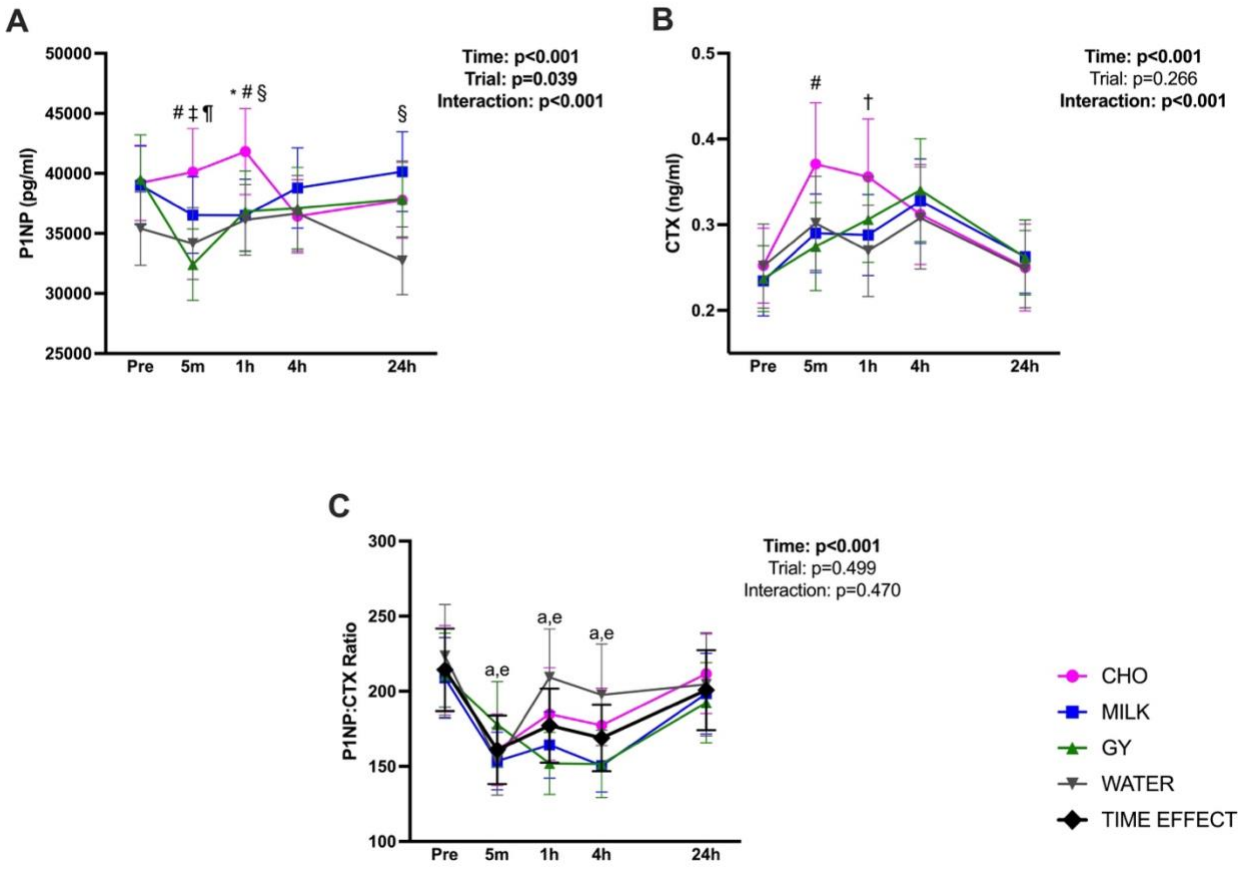

Figure 12: Absolute concentrations of markers of bone turnover. The bone formation marker P1NP (n=19) (A), resorption marker CTX (n=17) (B), and their ratio (n=16) are displayed. When a significant interaction was present, only significant differences between trials were shown. * = CHO vs. MILK, # = CHO vs. GY, † = CHO vs. WATER, ‡ = MILK vs. GY, § = MILK vs. WATER, ¶ = GY vs. WATER. When a significant effect of time was present in the absence of a significant interaction, significant differences between timepoints were shown. a = difference from Pre, e = difference from 24h. Statistical significance was $p \leq 0.05$. Values are mean ± SEM. Abbreviations: P1NP: procollagen type 1 N-terminal propeptide, CTX: C-terminal telopeptide of type 1 collagen.

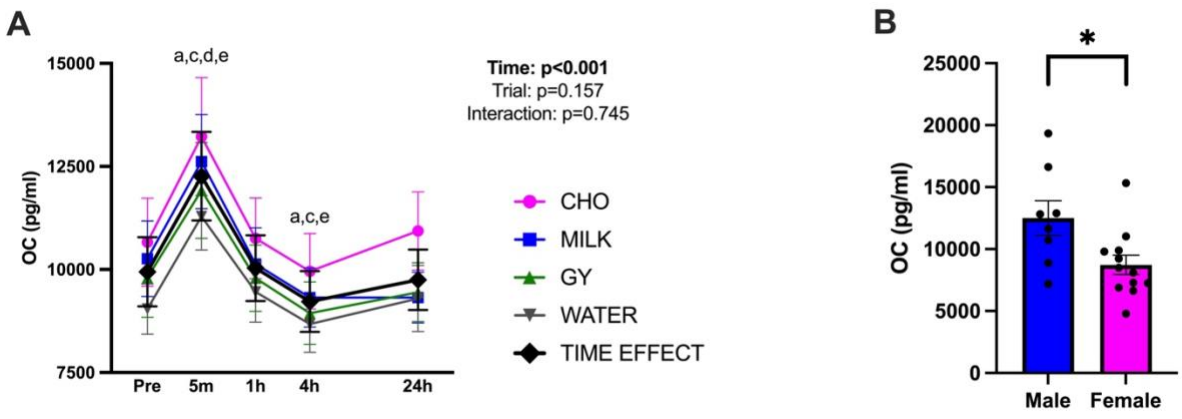

Figure 13: Absolute concentrations of OC (n=20) (A) and the comparison between males and females (B) are displayed. a = difference from Pre, c = difference from 1h, d = difference from 4h, e = difference from 24h, * = difference between sex. Statistical significance was $p \leq 0.05$. Values are mean ± SEM. Abbreviations: OC: osteocalcin.

5.5.2: Markers of Bone Resorption

For PTH, there was a significant effect of time ($p<0.001$) and trial by time interaction ($p=0.003$), but no effect of trial ($p=0.340$) **(Figure 14A)**. At 5m, CHO was significantly greater than GY ($p=0.001$) and WATER ($p=0.014$) and at 4h, MILK was significantly lower than GY ($p=0.047$). There were no trial differences observed at 1h and 24h. For the time effects within each trial **(Supplementary Table 1)**, only CHO significantly increased at 5m ($p=0.001$), followed by all groups having significantly lower PTH at 1h relative to baseline ($p<0.001$). At 4h, GY and WATER returned to baseline ($p=0.894$ and $p=0.531$, respectively), with CHO and MILK attenuating the rise in PTH at 4h ($p=0.035$ and $p=0.004$, respectively). All trials returned to baseline at 24h (CHO: $p=0.120$, MILK: $p=0.134$, GY: $p=0.729$, WATER: $p=0.181$).

With SOST, there was a significant effect of time ($p<0.001$), but no trial ($p=0.371$) or trial by time interaction ($p=0.147$) **(Figure 14B)**. SOST significantly increased and peaked at 5m ($p<0.001$ for all timepoints) but returned to baseline at 1h and remained unchanged for the rest of the timepoints.

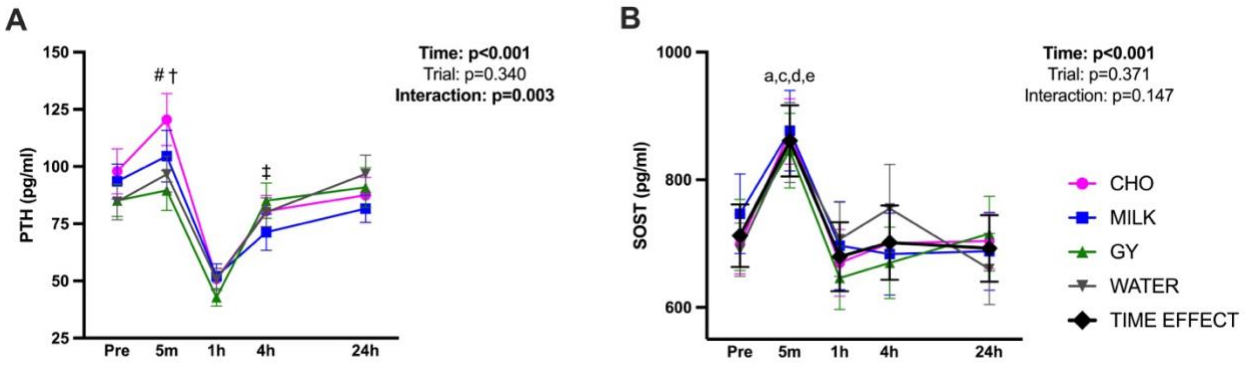

Figure 14: Absolute concentrations of markers of bone resorption. The bone resorption marker PTH (n=20) (A), and SOST (n=19) (B) are displayed. When a significant interaction was present, only significant differences between trials were shown. # = CHO vs. GY, † = CHO vs. WATER, ‡ = MILK vs. GY. When a significant effect of time was present in the absence of a significant interaction, significant differences between timepoints were shown. a = difference from Pre, c = difference from 1h, d = difference from 4h, e = difference from 24h. Statistical significance was $p \leq 0.05$. Values are mean ± SEM. Abbreviations: PTH: parathyroid hormone, SOST: sclerostin.

5.5.3: Markers of Osteoclastogenesis

A significant time effect ($p<0.001$) was observed for OPG, but no trial ($p=0.878$) and trial by time interaction ($p=0.138$) **(Figure 15A)**. OPG significantly increased at 5m ($p<0.001$), 1h ($p<0.001$) and 4h ($p<0.001$) relative to baseline, with a peak in OPG at 1h. At 24h, OPG returned to baseline.

With RANKL, there was a significant effect of time ($p<0.001$) and a trial by time interaction ($p=0.009$), but no effect of trial ($p=0.700$) **(Figure 15B)**. There were no differences at the 5m, 1h and 4h timepoints, but at 24h, RANKL was significantly lower in the MILK trial compared to all other trials (MILK vs. CHO [$p=0.004$], GY [$p=0.014$], WATER [$p=0.004$]). For the within-trial differences across time **(Supplementary Table 1)**, GY and WATER responded similarly with a gradual decrease at 5m (GY: $p=0.021$, WATER: $p=0.012$) until the 4h timepoint ($p<0.001$ for both GY and WATER at the 1h and 4h timepoints). Both trials returned to baseline at 24h (GY: $p=0.514$, WATER: $p=0.665$). Likewise, CHO and MILK responded similarly with no significant changes at 5m (CHO: $p=0.435$, MILK: $p=0.053$), and a gradual decrease at 1h and

4h ($p<0.001$ for both CHO and MILK at 1h and 4h timepoints). However, CHO returned to baseline at 24h ($p=0.928$), but MILK remained below baseline ($p=0.001$).

When OPG and RANKL are expressed as a ratio, there was a significant effect of time ($p<0.001$) and a trial by time interaction ($p=0.047$), but no trial effect ($p=0.855$) **(Figure 15C)**. Similar to RANKL, no between-trial differences were observed at 5m, 1h and 4h, but at 24h, MILK was significantly greater than WATER ($p=0.011$). For the within-trial differences across time **(Supplementary Table 1)**, all groups gradually increased from Pre to 4h ($p<0.05$ for all trials at all timepoints), with a return to baseline at 24h for all trials (CHO: $p=0.460$, GY: $p=0.367$, WATER: $p=0.731$) except for the MILK trial as it was still significantly elevated at 24h ($p=0.008$).

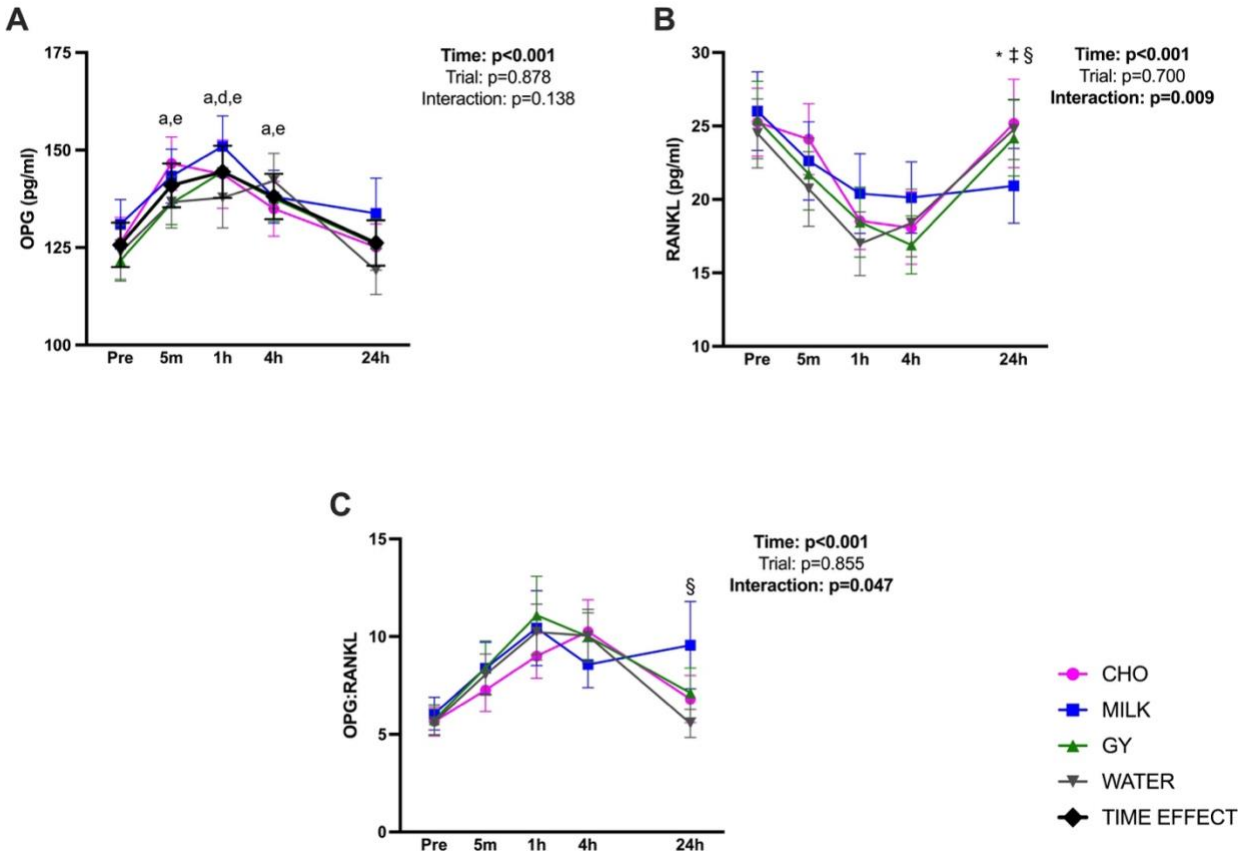

Figure 15: Absolute concentrations of markers of osteoclastogenesis. OPG (n=20) (A), RANKL (n=18) (B) and their ratio (n=18) (C) are displayed. When a significant interaction was present, only significant differences between trials were shown. * = CHO vs. MILK, ‡ = MILK vs. GY, § = MILK vs. WATER. When a significant effect of time was present in the absence of a significant interaction, significant differences between timepoints were shown. a = difference from Pre, d = difference from 4h, e = difference from 24h. Statistical significance was $p \leq 0.05$. Values are mean ± SEM. Abbreviations: OPG: osteoprotegerin, RANKL: receptor activator of nuclear factor kappa-B ligand

6.0: Discussion

The use of a four-arm, randomized, controlled, crossover study comparing post-exercise milk, GY, carbohydrate and water consumption provides novel data on the influence of nutrition on bone biomarker responses following a high-intensity, multimodal acute exercise bout. We also provide unique insight into the influence of the dairy matrix by comparing the consumption of post-exercise isonitrogenous milk vs. GY head-to-head in the same crossover trial. The main findings from this study demonstrate that the provision of MILK post-exercise significantly

increased P1NP vs. WATER **(Figure 12A)** and attenuated the rise in RANKL vs. all trials **(Figure 15B)** at 24h post-exercise. The reduction in RANKL at 24h in the MILK trial was reflected in the OPG:RANKL ratio as MILK had the highest OPG:RANKL ratio at 24h and was significantly greater than the WATER trial **(Figure 15C)**. There was also a difference between MILK and GY for PTH at 4h, where milk suppressed the PTH response compared to GY **(Figure 14A)**. These findings suggest that the ingestion of milk following an acute high-intensity exercise bout was better able to positively modulate circulating bone biomarker responses compared to GY, carbohydrate and water **(Figure 16)**.

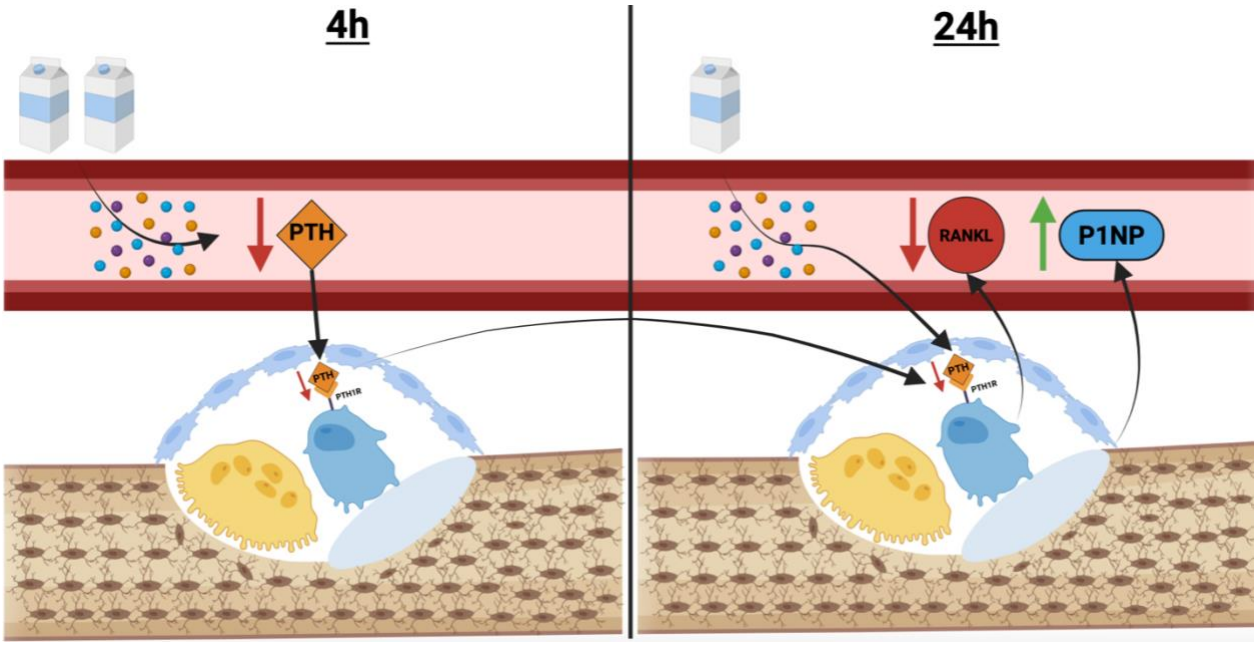

Figure 16: Summary diagram demonstrating the positive effects of milk consumption post-exercise on bone markers. At the 4h timepoint, 2 boluses of milk have been consumed post-exercise that provides bone-supporting nutrients such as protein (blue circles), calcium (orange circles) and vitamin D (purple circles). These nutrients may aid in reducing circulating PTH and subsequent PTH1R activation. The reduction in PTH may have implications at the 24h timepoint, in combination with the additional bolus of milk consumption before bed, in reducing RANKL production and increasing P1NP into the circulation. Created with BioRender.com

6.1: Modulating Effects of Post-Exercise Nutrition

To our knowledge, the acute high-intensity exercise bout utilized in the present study was a novel design that has not been used previously in this context. Participants performed

plyometrics, resistance exercises and high-intensity interval cycling in the same exercise session lasting for 1 hour. Our aim was to trigger an elevated post-exercise bone response (as well as an inflammatory response) by combining different modalities of bone-stimulating exercise into a single exercise session. To evaluate the effect that nutrition may have on modulating the post-exercise bone response, we assessed trial by time interactions, specifically at the 1h, 4h and 24h timepoints, to determine how specific supplements differentially modulated the bone biomarker response post-exercise. For P1NP and CTX, there was an interaction and at the 1h timepoint, the CHO trial had the greatest P1NP and CTX vs. the dairy trials and the WATER trial, respectively **(Figure 12A & B)**. However, this appeared to be largely influenced by the variability observed at the 5m post-exercise timepoint. Nonetheless, at the 24h timepoint the MILK trial had the highest P1NP compared to all groups and was significantly greater than the WATER trial **(Figure 12A)**. To our knowledge, our study provides novel evidence demonstrating a between-trial difference in P1NP following the acute consumption of milk vs. water post-exercise. There are only two other studies that have investigated the P1NP response at 24h post-exercise, following the provision of either a protein supplement (269) or a combined carbohydrate and protein supplement (47). These studies found no difference between protein or a combined carbohydrate and protein supplement vs. water at 24h post-exercise, suggesting that the provision of a dairy wholefood as seen in the current study, may be superior than just consuming bone-supporting nutrients in isolation. The greater P1NP response observed in the MILK trial at 24h in our study may be explained by the differences in nutrient composition. The MILK trial provided the greatest amount of calcium (1800 mg), protein (54 g, except for GY) and vitamin D (30 µg) compared to the other trials **(Table 3)**, and these nutrients may have contributed to a more osteogenic post-exercise environment that supported the anabolic formation process observed

24h after exercise. Furthermore, when assessing how the individual trials change differently over time, only the WATER trial significantly decreased P1NP relative to baseline at 24h **(Supplementary Table 1)**, indicating the importance of post-exercise nutrition to adequately refuel and provide essential bone-supporting nutrients following an intense bout of exercise.

Another interaction was observed with PTH, as the MILK trial had significantly lower PTH than the GY trial at the 4h timepoint **(Figure 14A)**. The differences observed may be due to the influence of the distinct dairy matrices between MILK and GY. Milk is a liquid food form and there is evidence to suggest that liquid matrices result in faster gastric emptying relative to semi-solid food matrices like GY (261,262). Furthermore, milk has greater calcium and vitamin D vs. GY **(Table 3)** and these differences in micronutrients may explain the ability of milk to attenuate the rise in PTH. PTH is released when circulating calcium is lowered to restore calcium homeostasis, so the greater amount of exogenous calcium provided in the MILK vs. GY trial may have contributed to the suppression of PTH (205). Several lines of evidence have demonstrated that the provision of calcium pre-exercise can attenuate post-exercise increases in PTH (146,271,272,284), however, more work is needed to investigate the effects of PTH following the consumption of calcium or calcium-rich wholefoods post-exercise. Additionally, vitamin D also improves intestinal calcium absorption and renal calcium reabsorption, which may be another potential beneficial mechanism for milk compared to GY (216). Taken together, the MILK trial may have had an improved ability to increase systemic circulating calcium through dietary calcium and vitamin D provision which is reflected in the lower PTH concentrations observed in the MILK vs. GY trial at the 4h timepoint **(Figure 14A)**.

The final interactions we observed were in RANKL and the OPG:RANKL ratio. At 24h, we saw that RANKL was significantly lower in the MILK trial compared to all other trials

(Figure 15B). Similarly, in the OPG:RANKL ratio, MILK had the greatest ratio and was significantly greater than the WATER trial at 24h **(Figure 15C)**. There was no interaction observed with OPG **(Figure 15A)** so it appears that the OPG:RANKL ratio response at 24h is mainly driven by the difference in RANKL. Our current findings differ from our previous work in young, healthy females as we did not see any differences in RANKL following milk vs. CHO consumption post-exercise (48). However, the differences seen between our studies may be due to the fact that the participants in Prowting et al., only consumed two boluses of the supplement (immediately post-exercise and 1h post-exercise), with no extra bolus consumed before bed (48). The extra bolus provided before bed in this current study may have had a greater influence on the BM response at the 24h timepoint. From a mechanistic perspective, the ability for milk to attenuate the rise in RANKL at 24h may be driven by the suppression of PTH at the 4h timepoint **(Figure 14A)**. A rise in PTH is typically upstream of any osteolytic activity as PTH binding to the PTH1R can stimulate the release of RANKL and subsequent osteoclastogenesis, triggering bone resorption (156,157). Therefore, the decreased PTH in the MILK trial observed at the 4h timepoint **(Figure 14A)** may have diminished the subsequent release of RANKL at the 24h timepoint **(Figure 15B)**.

6.2: Post-Exercise Bone Response

This section mainly addresses the immediate post-exercise bone response at the 5m timepoint by evaluating any markers with a main effect of time or a trial by time interaction. In general, the effect of exercise on the BM response is not as clear in the literature, specifically in this population of young, healthy, untrained adults. This is related to training status, but it may also be partially due to the different exercise modalities used in these trials (e.g., prolonged endurance exercise vs. high-intensity exercise). For the markers of bone turnover (P1NP, CTX and OC), the literature points towards no change immediately following exercise (without

nutritional provision) in young, healthy, untrained adults (**Table 1**, (48,131–135,137,138,141–143,160,182,183). We report slightly different outcomes with no change in P1NP in the non-dairy groups but a significant decrease in the dairy groups immediately post-exercise **(Supplementary Table 1)**. It is unclear as to why the dairy groups decreased P1NP immediately post-exercise while the non-dairy groups had no change. We expected to see similar responses across all groups immediately post-exercise (before the influence of nutrition) since the exercises and relative loads were standardized across trials within participants. Similarly, our CTX data do not seem to be in agreement with the literature in young, untrained individuals **(Table 1)**, as we found that the CHO, MILK and WATER trials significantly increased post-exercise, but the GY trial did not ($p=0.114$; **Supplementary Table 1)**. Regardless of these differences, our data appear to mostly align with the Dolan et al., acute exercise meta-analysis as they found that when stratified specifically by exercise modality, cycling significantly increased CTX post-exercise (44). Thus, the immediate CTX response may be more dependent on exercise modality as 6 of the 9 studies (48,131,135,138,141,143) that found no change in CTX post-exercise in young, healthy, untrained adults, did not have cycling as an exercise modality.

When P1NP and CTX were expressed as a ratio, there was a significant decrease post-exercise, indicating an increase in bone resorption **(Figure 12C)**. These data also align with our OC results displaying a significant effect of time, where all groups significantly increased OC post-exercise **(Figure 13A)**. Historically, OC has been viewed as a marker of bone formation as osteoblasts secrete OC during mineralization, however, there has been a recent shift in the literature and OC is now viewed more as a marker of bone remodeling, as OC can be released from the bone matrix into the circulation during bone resorption (42). Hence, OC may indicate an increase in bone turnover as the turnover process begins with bone resorption, which we

observed with an increase in CTX **(Supplementary Table 1)** and a reduction in the P1NP:CTX ratio **(Figure 12C)**. Although our OC results are not in agreement with the literature in young, healthy, untrained adults as there is typically no change in OC immediately post-exercise **(Table 1,** (48,134,137,160,182,183)**),** in the context of the present study, an increase in OC may be expected since there was a reduction in the P1NP:CTX ratio, indicating an increase in bone resorption and subsequent release of OC into the circulation. In addition, we also saw a main effect of sex for OC, with males demonstrating overall higher OC concentrations compared to females **(Figure 13B)**. Our OC data reflect what is commonly seen as males typically demonstrate greater OC levels compared to females (285,286). Altogether, our P1NP:CTX ratio and OC data suggest that bone turnover was initiated following our acute, high-intensity, high-load exercise bout.

Previous research in both cell and murine models demonstrate a reduction in SOST following mechanical loading (287–289). As a negative regulator of osteoblastogenesis/bone formation (i.e., a bone resorption marker), SOST works through the inhibition of the Wnt/β-catenin pathway and can promote RANKL-induced osteoclastogenesis through the suppression of OPG (290). At face value, the cell and murine data would suggest that the downregulation of SOST following mechanical loading would be an adaptive response to prevent further bone breakdown and to promote bone formation, however, findings from human studies in young, healthy, untrained adults seem to report opposing responses with SOST increasing immediately following acute exercise in HIIE and plyometrics (132,133,138,162). From a bone remodeling perspective, this response would be reasonable as bone resorption precedes formation to clear any damaged areas or to restore calcium homeostasis (291). Our SOST data align with the literature as we have also observed a significant increase immediately post-exercise, with a

return to baseline levels thereafter **(Figure 14B)**. Another resorption marker, PTH, appears to have an unclear immediate post-exercise response in young, healthy, untrained individuals as there have been studies showing either no change (160,161), increases (162) or decreases (158,159) in PTH following exercise. Although, mechanistically, there is reason to expect an increase in PTH following exercise due to the need to systemically replenish calcium in the circulation from bone tissue stores, only the CHO trial increased post-exercise PTH with all other groups remaining unchanged **(Supplementary Table 1)**. The meta-analysis by Dolan et al., on the effects of acute exercise on bone markers (without nutrition), found that with training status as a moderator, only trained individuals/athletes had an increase in PTH post-exercise compared to individuals who were sedentary or recreationally active (44). Therefore, our findings demonstrated a similar response to that seen in the Dolan et al., meta-analysis as our participants were untrained individuals. Furthermore, another marker that we could have assessed would be serum ionized calcium to determine if our exercise bout was stimulating enough to transiently lower circulating calcium levels or not, which may help explain the immediate null changes in PTH.

The OPG, RANKL and OPG:RANKL axis are markers of osteoclastogenesis. Our data align with what is seen in the literature (151,152) as we found an increase in OPG post-exercise **(Figure 15A)**. With regards to the post-exercise RANKL response, the evidence seems to be unclear (48,151,152), however, our findings are in agreement with the mechanisms of OPG inhibiting RANKL binding to RANK (150) as we found that all groups (trending decrease for MILK) had a significant decrease in circulating RANKL, except for the CHO trial **(Supplementary Table 1)**. When expressed as the OPG:RANKL ratio, we found a significant

increase post-exercise, suggesting that there is a blunted osteoclastogenic response acutely following high-intensity exercise in this population **(Supplementary Table 1)**.

The collective results of all these biomarkers paint an interesting perspective on the bone response following high-intensity exercise (**Figure 17)**. From a systemic perspective, it does not appear that there was an endocrine role as PTH was not significantly elevated post-exercise in all groups except for the CHO trial **(Supplementary Table 1)**. Therefore, it is possible that the bone response may be a targeted/local response at the cellular level. SOST is the next upstream marker and can inhibit osteoblastogenesis and stimulate RANKL-induced osteoclastogenesis by attenuating OPG production (290). The actions of SOST appear to be more influential regarding osteoblastogenesis, as we did not see an increase in the bone formation marker P1NP. Instead, we saw either no changes or decreases in P1NP **(Supplementary Table 1)**. When considering the bone remodeling cycle, the absence of bone formation in the earlier stages would be expected as bone formation occurs following resorption (292). However, regarding the actions of SOST on resorption, SOST can impede OPG production by inhibiting the Wnt/β-catenin pathway, subsequently increasing RANKL (290). However, we still saw an increase in OPG immediately post-exercise that persisted longer than the quick rise and fall in SOST **(Figure 15A)**. These findings suggest that there may be multiple signaling pathways that are acting upon OPG that are SOST-independent such as estradiol or inflammatory cytokines (293,294). Nonetheless, the influence of OPG was evident as we observed a decrease in RANKL in the GY, WATER and MILK trial (trending) at the 5m timepoint **(Supplementary Table 1)**, suggesting an anti-osteoclastogenic environment facilitated through OPG. Interestingly, our RANKL data does not seem to align with our CTX data as there was an increase in CTX in all trials, except for the GY trial **(Supplementary Table 1)**. We speculate that perhaps immediately post-exercise, it is not

the osteoclasts but the bone-lining cells that are initially breaking down bone collagen tissue. Early work in the 80's by Chambers and Fuller demonstrate that bone-lining cells are able to digest areas of unmineralized collagen surfaces to aid in osteoclastic bone resorption (295). Thus, it has been suggested that bone-lining cells assist in the initiation of bone remodeling by exposing the mineralized bone surface for osteoclasts to bind (111). As collagen breakdown by bone-lining cells precedes osteoclastic bone resorption, this could be a potential mechanism as to why we see an increase in CTX post-exercise despite RANKL being downregulated.

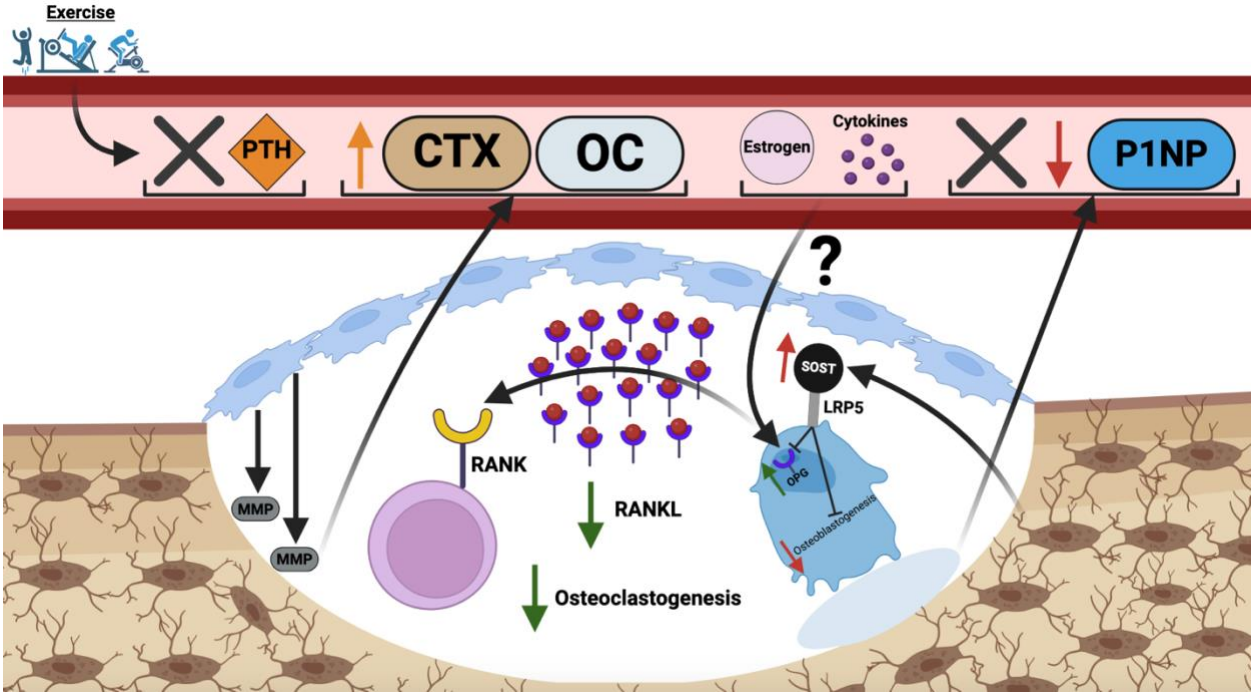

Figure 17: Summary of the post-exercise bone marker response. There were no changes seen in circulating PTH following exercise but there was a local upregulation of SOST, which may have contributed to the lack of an increase in P1NP due to decreased osteoblastogenesis. SOST can also inhibit OPG secretion from the osteoblast, however, an increase in OPG was observed. Other potential pathways may be at play that are more potent than SOST in elevating OPG secretion such as circulating estrogen or cytokines. The release of OPG may be an influencing factor in the decrease in RANKL post-exercise, which would suggest a lowered osteoclastogenic response. Additionally, there was an increase in CTX and OC post-exercise which would indicate an increase in collagen breakdown but not by osteoclasts as RANKL was downregulated. A potential explanation could be that the bone-lining cells are preparing the BMU for remodeling by secreting MMPs that digest collagen to prepare the surface for subsequent osteoclastic bone resorption. Created with BioRender.com

6.3: Strengths and Limitations

Our study had several strengths that are worth highlighting. First, it should be emphasized that in human research, there is high individual variability, so we utilized a randomized crossover design in an attempt to minimize variability between trials. In crossover trials, carryover effects may exist so to limit this, we ensured that there was at least a 2-week washout period in males and females on hormonal contraceptives between trials. For females who were naturally cycling, we standardized their trials by having them complete trials during the early follicular phase of their menstrual cycle, to reduce the potential effects of any fluctuating sex hormones on the bone biomarker response. We also implemented a few improvements from our previous acute intervention study (48) as we tried to ensure that the nutrition between each trial was as similar as possible except for the post-exercise supplement provided, by encouraging the participants to eat similar foods before each trial and by standardizing their meals on the trial day. We also added a control group with no post-exercise nutrition (i.e., the WATER trial) to tease out the effects of exercise alone. Furthermore, the timing of the trials (i.e., earlier morning) and blood samples were also standardized to the best of our ability to reduce diurnal variation in the circulating biomarkers. Lastly, due to any potential differences in perspiration or *ad libitum* water consumption, we corrected our biomarkers for plasma volume shifts that may have occurred during exercise.

Our study also had some limitations. We observed small but significant differences in strength and body composition from pre- to post-study testing **(Figure 9 & 10)**. Ideally, we would have wanted the participants to remain weight stable and not see any gains in strength throughout the investigation. The increased weight and strength could have influenced the relative intensity of the exercise bout throughout the study as we standardized only the absolute load for each exercise modality. Importantly, while significant, the increase in body weight was

1.39±1.54 kg **(Figure 10)**, which was small and unlikely to meaningfully affect our results. Despite small, uniform increases in strength and body weight, our trials were randomized, meaning that any slight increases in these parameters would have affected our trials similarly. In addition, we observed no significant differences in exercise intensity for all of the exercises between trials **(Table 6)**. The absence of any differences in exercise intensity across the trials could be attributed to the randomization of the trials. Another potential limitation was our large age range of 18-35 years. Bone turnover occurs at different rates across the lifespan and the participants on the younger side may have elevated bone markers and/or turnover rates as they are still in the process of achieving PBM compared to the older participants who have more mature bones and stable turnover rates (6,296). To account for this, we ran a statistical model controlling for age and found that age did not significantly influence any of the bone marker responses (data not shown) so it was not retained as a covariate in our model. It was also highly colinear with sex so it was inappropriate to keep in the model alongside the covariate of sex.

6.4: Future Directions

There are many interesting future directions that can extend this area of research. First, there are some other markers of interest that could be measured if a similar study like this were to be replicated. To further tease out these mechanisms, especially with PTH, ionized calcium could be measured to determine whether the null changes we saw with PTH were due to the exercise bout not depleting circulating calcium enough to elicit increases in PTH. Markers such as bone-specific alkaline phosphatase (BALP; bone formation) and tartrate-resistant acid phosphatase 5b (TRAP5b; bone resorption) may be interesting to measure in conjunction with P1NP and CTX. Evidence suggests that following acute exercise, changes in BALP can occur over a 24h period as demonstrated by Kish and colleagues following plyometric exercises in young men (151). The combination of P1NP as a measure of collagen formation and BALP as a

measure of mineralization through inorganic phosphate, provides a more comprehensive outlook on the effects of exercise and nutrition on bone formation (82,83). The measurement of TRAP5b and matrix metalloproteinase (MMPs) would also provide more insight on the bone resorption process occurring post-exercise. TRAP5b and MMPs are released by osteoclasts to breakdown bone tissue (94), however, only MMPs are expressed in bone-lining cells and not TRAP5b (111). Therefore, if we were to observe no change in TRAP5b but an increase in MMPs, this could provide evidence to our hypothesis that the increased CTX response we see post-exercise, may be a result of the bone-lining cells initiating bone resorption, rather than the osteoclasts.

Another future direction could be to investigate the effects of a dairy wholefood such as milk or yogurt vs. their individual nutrient constituents, to further explore the idea that the combination of nutrients in a wholefood is superior to these nutrients in isolation. For example, in the MILK trial, milk was consumed in 3 boluses on the exercise day, providing 1800 mg of calcium, 54 g of protein and 30 µg (1200 IU) of vitamin D **(Table 3)**. Another trial arm could provide the same amount of calcium, protein and vitamin D, but as individual supplements and not as a wholefood to help tease out their individual effects. Thus, this potential study would be able to elucidate the effects of the wholefood matrix vs. individual nutrients as their nutrient composition would be equated.

Another important future direction could be to determine whether the effects observed in this acute study could translate to bone health adaptations in a longer-term exercise training and dietary intervention study. In the present acute study, we observed some beneficial effects of milk seen as a reduction in PTH compared to GY at the 4h timepoint and a reduction in RANKL **(Figure 14A)** concentrations compared to all trials at the 24h timepoint **(Figure 15B)**. We also saw that the MILK trial had the greatest P1NP at the 24h timepoint and was significantly greater

than the WATER trial **(Figure 12A)**. Therefore, it would be interesting to see if the positive effects of consuming milk post-exercise can translate to longer-term bone adaptations with exercise training such as an increase in BMD. We have seen increases in bone formation (i.e., P1NP) with dairy consumption over the longer term (12-16 weeks) in exercise training trials, but BMD was not measured (28,30).

6.5: Implications

The present study provides novel data on the acute effects of post-exercise dairy consumption in the form of either milk or GY on various bone turnover biomarkers and markers of bone metabolism. We have shown that the provision of milk can better support bone health following an acute exercise bout compared to GY, carbohydrate or water. Data from this study are in agreement with our previously published work demonstrating that post-exercise milk consumption was superior (to carbohydrate consumption) for reducing bone breakdown, albeit through different markers of bone resorption (48). Collectively, these studies provide evidence to health practitioners such as personal trainers, exercise physiologists, dietitians and physicians on a beneficial post-exercise nutritional supplement to consume to optimize bone health. The utility of investigating the effects of nutrition following a single exercise bout can help elucidate the mechanisms of how different dairy wholefoods can improve bone health such as through reductions in bone resorption (RANKL and PTH) and potentially enhancing bone formation (P1NP). These new insights on post-exercise nutrition can help practitioners maximize individual exercise sessions within a training and dietary intervention program to foster the greatest bone health benefits. Furthermore, these mechanistic insights allow researchers and the broader scientific community to develop a deeper understanding of the interplay between nutrition, exercise and bone physiology.

6.6: Conclusion

In conclusion, our study investigated bone biomarker responses to a novel high-intensity exercise bout that consisted of plyometrics, resistance exercises and HIIC and we demonstrated that the consumption of milk post-exercise can favourably modulate bone biomarkers and markers of bone metabolism compared to carbohydrate, GY and water. The provision of milk was able to augment the bone formation marker P1NP, at 24h post-exercise, and attenuate the differentiation of bone-resorbing osteoclasts as seen with the decrease in RANKL. A potential mechanism for the suppression of RANKL could be through PTH as the MILK trial had the lowest PTH at 4h post-exercise and was significantly different than GY, which may have downstream effects by reducing RANKL secretion. These findings have implications for health practitioners looking to integrate wholefood consumption following exercise and provide some mechanistic evidence for researchers as to how exercise and nutrition beneficially influence the skeleton. Future work should investigate additional biomarkers of interest, comparing wholefoods to their nutrient constituents in isolation, and extend this work into longer-term nutrition and exercise intervention studies measuring BMD. Overall, these findings suggest that the provision of milk post-exercise may be the most optimal wholefood to consume to maximize the acute bone biomarker response compared to GY, carbohydrate and water in young, healthy, untrained adults.

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8.0: Appendices

Appendix A: Supplementary Table

Supplementary Table 1: Absolute concentrations of the bone biomarkers with between-trial differences and within-trial differences across time.

Bone Biomarkers	CHO					MILK					GY					WATER					Trial	Time	Trial* Time
	Pre	5m	1h	4h	24h	Pre	5m	1h	4h	24h	Pre	5m	1h	4h	24h	Pre	5m	1h	4h	24h			
P1NP (ng/ml) (n=19)	40.08 (13.51)	40.11 (14.98) c #	41.82 (15.58) ade *#	36.44 (13.31) a	37.77 (13.74)	39.01 (14.31)	36.53 (13.8) a ‡	36.52 (13.05) a *§	38.80 (14.55) c	40.14 (14.47) bc §	39.51 (16.12)	32.40 (12.98) acde #‡¶	36.84 (14.57) #¶	37.10 (14.80)	37.88 (13.80)	36.05 (13.39)	34.16 (12.67) ¶	36.13 (12.79) §¶	36.67 (13.35)	32.72 (12.32) acd §	**0.039**	**<0.001**	**<0.001**
CTX (ng/ml) (n=17)	0.252 (0.180)	0.371 (0.286) ade #	0.356 (0.279) ade †	0.312 (0.241) ae	0.250 (0.203)	0.234 (0.168)	0.290 (0.189) a	0.288 (0.188) a	0.328 (0.197) ae	0.262 (0.176)	0.237 (0.158)	0.275 (0.212) d #	0.306 (0.206) ae	0.341 (0.247) ae	0.262 (0.181)	0.251 (0.202)	0.302 (0.226) ae	0.269 (0.219) †	0.308 (0.238) e	0.248 (0.186)	0.266	**<0.001**	**<0.001**
P1NP:CTX (n=16)	220.73 (120.50)	158.45 (97.20) ae	184.86 (123.45) ae	177.25 (99.34) ae	209.19 (109.55)	208.93 (109.55)	153.61 (76.73) ae	163.71 (91.2\1) ae	145.01 (69.53) ae	198.41 (107.49)	210.67 (112.03)	177.95 (114.23) ae	151.97 (82.75) ae	151.63 (89.72) ae	192.46 (106.83)	231.75 (137.63)	157.85 (90.82) ae	209.28 (128.78) ae	195.81 (139.78) ae	204.61 (137.18)	0.499	**<0.001**	0.470
OPG (pg/ml) (n=20)	126.34 (28.71)	146.56 (29.81) ae	143.84 (39.05) ade	134.96 (31.47) ae	125.15 (26.64)	131.01 (28.30)	143.32 (30.96) ae	150.96 (34.96) ade	138.02 (30.28) ae	133.77 (40.31)	121.68 (23.62)	136.38 (24.51) ae	144.41 (30.29) ade	137.57 (26.14) ae	125.76 (29.35)	123.80 (31.24)	136.65 (29.43) ae	137.77 (33.53) ade	142.10 (30.79) ae	119.14 (26.71)	0.878	**<0.001**	0.138
RANKL (pg/ml) (n=18)	25.25 (9.79)	24.11 (9.94) cd	18.55 (8.26) ae	18.07 (10.49) ae	25.18 (12.74) *	26.02 (11.34)	22.63 (11.27)	20.41 (11.52) a	20.13 (10.32) a	20.93 (10.78) a *‡§	25.42 (11.19)	21.74 (10.39) acd	18.46 (10.10) ae	16.91 (8.39) ae	24.18 (10.94) ‡	24.50 (9.97)	20.72 (10.77) ace	16.99 (9.18) ae	18.40 (9.76) ae	24.77 (8.73) §	0.700	**<0.001**	**0.009**
OPG:RANKL (n=18)	5.67 (3.17)	7.26 (4.49) acd	9.01 (4.88) a	10.25 (6.88) ae	6.80 (5.10)	6.06 (3.56)	8.38 (5.62) a	10.44 (8.14) a	8.57 (4.99) a	9.56 (9.49) a §	5.74 (3.29)	8.41 (5.81) acde	11.09 (8.47) ae	10.00 (5.18) ae	7.12 (5.43)	5.63 (2.74)	8.05 (4.48) acde	10.23 (5.90) ae	10.04 (5.59) ae	5.56 (2.95) §	0.855	**<0.001**	**0.047**
PTH (pg/ml) (n=20)	97.91 (43.84)	120.51 (49.50) acde #†	50.92 (19.72) ade	80.53 (30.14) a	87.44 (35.23)	93.51 (33.78)	104.58 (50.58) cde	52.02 (24.13) ade	71.31 (35.53) a ‡	81.59 (26.71)	85.15 (31.40)	89.54 (38.97) #	42.82 (17.20) abde	85.05 (34.49) ‡	90.89 (38.87)	84.58 (34.99)	96.63 (34.99) †	50.67 (21.87) abde	80.02 (28.11)	96.79 (36.69)	0.340	**<0.001**	**0.003**
SOST (pg/ml) (n=19)	699.15 (202.22)	875.80 (217.26) acde	669.79 (227.91)	700.46 (251.19)	704.19 (190.29)	746.79 (271.49)	876.89 (274.25) acde	696.69 (300.11)	683.63 (278.96)	688.03 (265.58)	713.38 (243.38)	845.51 (254.18) acde	646.11 (215.18)	669.82 (243.32)	715.35 (255.58)	690.37 (181.52)	858.24 (272.65) acde	706.79 (245.36)	754.95 (293.23)	660.09 (236.79)	0.371	**<0.001**	0.147
OC (ng/ml) (n=20)	10.66 (4.78)	13.55 (6.42) acde	10.76 (4.38)	9.96 (4.09) ace	10.94 (4.25)	10.26 (4.09)	12.62 (5.11) acde	10.13 (3.94)	9.32 (3.20) ace	9.32 (2.74)	9.80 (4.29)	11.92 (5.20) acde	9.79 (3.60)	8.94 (3.37) ae	9.45 (3.20)	9.04 (2.73)	11.29 (3.65) acde	9.46 (3.28)	8.68 (3.07) ae	9.30 (3.58)	0.157	**<0.001**	0.745

Note: Absolute concentrations of bone biomarkers. Between-trial differences at specific timepoints are denoted by the following symbols: * = CHO vs. MILK, # = CHO vs. GY, † = CHO vs. WATER, ‡ = MILK vs. GY, § = MILK vs. WATER, ¶ = GY vs. WATER. Within-trial differences between timepoints are denoted by the following letters: a = difference from Pre, b = difference from 5m, c = difference from 1h, d = difference from 4h, e = difference from 24h. Statistical significance was $p \leq 0.05$. Values are mean ± SD. Abbreviations: P1NP: procollagen type 1 N-terminal propeptide, CTX: C-terminal telopeptide of type 1 collagen, OPG: osteoprotegerin, RANKL: receptor activator of nuclear factor kappa-B, PTH: parathyroid hormone, SOST: sclerostin, OC: osteocalcin

Appendix B: Ethics Approval

YORK
UNIVERSITÉ
UNIVERSITY

Certificate #:	**2019-045**
Initial Approval:	**01/31/19-01/31/20**
Amendments:	**6th Amendment approved: 08/11/23; 7th Amendment approved: 02/09/24; 8th Amendment approved: 07/15/24**
Renewals:	**02/04/22-02/04/23; 02/03/23-02/03/24 02/09/24-02/09/25; 02/10/25-01/10/26**
Current Approval Period:	**02/10/25-01/10/26 *Final**

OFFICE OF RESEARCH ETHICS (ORE)

4700 Keele St.
Toronto ON
Canada M3J 1P3
ore@yorku.ca
www.research.yorku.ca

ETHICS RENEWAL

To: **Professor Andrea Josse**
Department of Kinesiology
Faculty of Health
ajosse@yorku.ca

From: Alison M. Collins-Mrakas, Director, Research Ethics
(on behalf of Gillian Parekh, Chair, Human Participants Review Committee)

Date: Friday, February 28, 2025

Title: **Cre-Ex Study: Does Creatine Augment the Acute Effect of Exercise and Dairy on Bone Turnover?**

Risk Level: ☒ Minimal Risk ☐ More than Minimal Risk

Level of Review: ☒ Delegated Review ☐ Full Committee Review

I am writing to inform you that this research project, **"Cre-Ex Study: Does Creatine Augment the Acute Effect of Exercise and Dairy on Bone Turnover?"** has received ethics review and renewal by the Human Participants Review Sub-Committee, York University's Ethics Review Board and conforms to the standards of the Canadian Tri-Council Research Ethics guidelines.

Note that renewal is granted for one year. Ongoing research – research that extends beyond one year – must be renewed prior to the expiry date.

Any changes to the approved protocol must be reviewed and approved through the amendment process by submission of an amendment application to the HPRC prior to its implementation.

Any adverse or unanticipated events in the research should be reported to the Office of Research ethics (ore@yorku.ca) as soon as possible.

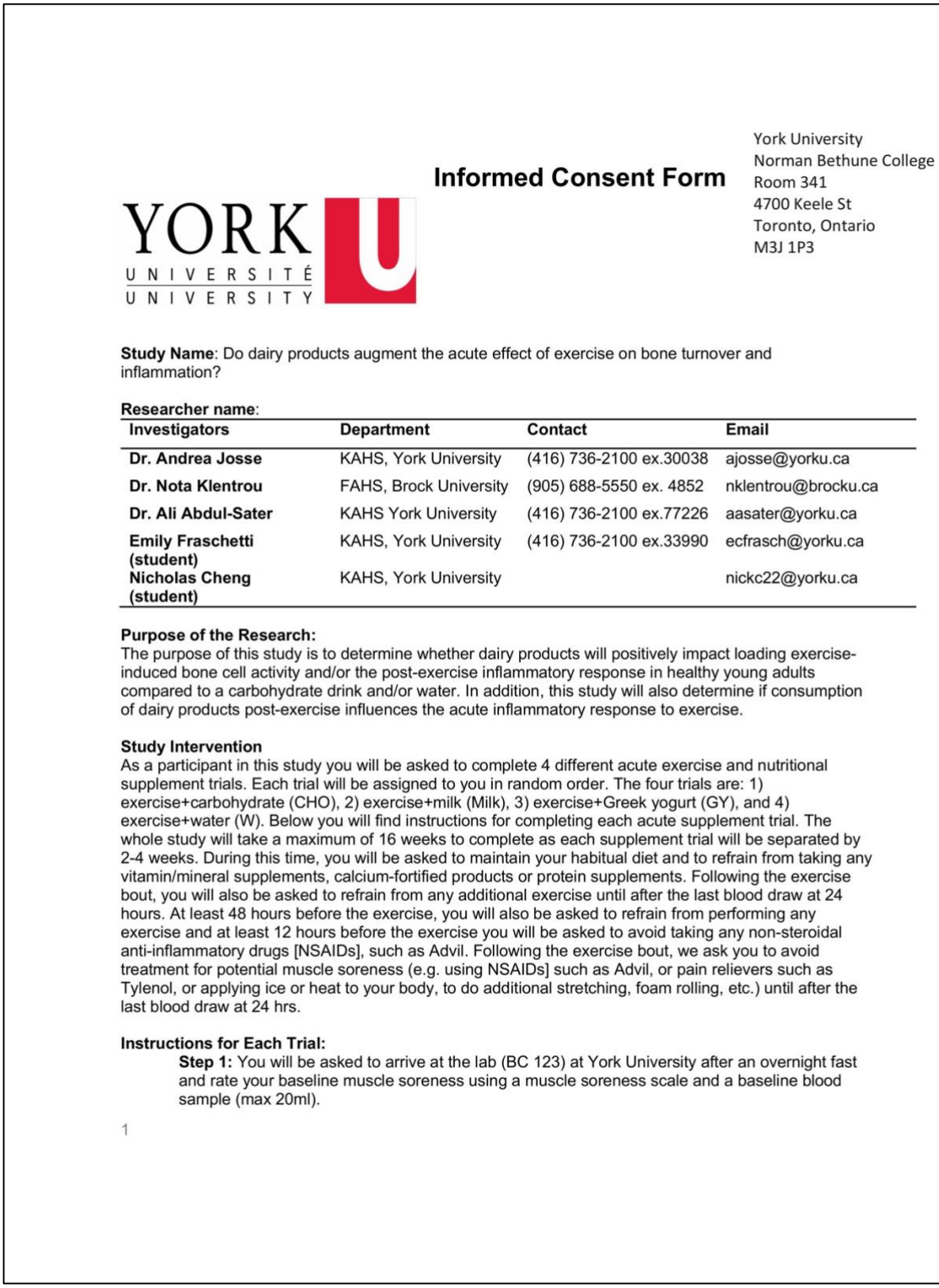
For further information on researcher responsibilities as it pertains to this approved research ethics protocol, please refer to the attached document, **"RESEARCH ETHICS: PROCEDURES to ENSURE ONGOING COMPLIANCE"**.

Should you have any questions, please feel free to contact me at: ore@yorku.ca

Yours sincerely,

Alison M. Collins-Mrakas M.Sc., LLM
Director, Office of Research Ethics

Appendix C: Informed Consent Form



Informed Consent Form

York University
Norman Bethune College
Room 341
4700 Keele St
Toronto, Ontario
M3J 1P3

Study Name: Do dairy products augment the acute effect of exercise on bone turnover and inflammation?

Researcher name:

Investigators	Department	Contact	Email
Dr. Andrea Josse	KAHS, York University	(416) 736-2100 ex.30038	ajosse@yorku.ca
Dr. Nota Klentrou	FAHS, Brock University	(905) 688-5550 ex. 4852	nklentrou@brocku.ca
Dr. Ali Abdul-Sater	KAHS York University	(416) 736-2100 ex.77226	aasater@yorku.ca
Emily Fraschetti (student)	KAHS, York University	(416) 736-2100 ex.33990	ecfrasch@yorku.ca
Nicholas Cheng (student)	KAHS, York University		nickc22@yorku.ca

Purpose of the Research:
The purpose of this study is to determine whether dairy products will positively impact loading exercise-induced bone cell activity and/or the post-exercise inflammatory response in healthy young adults compared to a carbohydrate drink and/or water. In addition, this study will also determine if consumption of dairy products post-exercise influences the acute inflammatory response to exercise.

Study Intervention
As a participant in this study you will be asked to complete 4 different acute exercise and nutritional supplement trials. Each trial will be assigned to you in random order. The four trials are: 1) exercise+carbohydrate (CHO), 2) exercise+milk (Milk), 3) exercise+Greek yogurt (GY), and 4) exercise+water (W). Below you will find instructions for completing each acute supplement trial. The whole study will take a maximum of 16 weeks to complete as each supplement trial will be separated by 2-4 weeks. During this time, you will be asked to maintain your habitual diet and to refrain from taking any vitamin/mineral supplements, calcium-fortified products or protein supplements. Following the exercise bout, you will also be asked to refrain from any additional exercise until after the last blood draw at 24 hours. At least 48 hours before the exercise, you will also be asked to refrain from performing any exercise and at least 12 hours before the exercise you will be asked to avoid taking any non-steroidal anti-inflammatory drugs [NSAIDs], such as Advil. Following the exercise bout, we ask you to avoid treatment for potential muscle soreness (e.g. using NSAIDs] such as Advil, or pain relievers such as Tylenol, or applying ice or heat to your body, to do additional stretching, foam rolling, etc.) until after the last blood draw at 24 hrs.

Instructions for Each Trial:

Step 1: You will be asked to arrive at the lab (BC 123) at York University after an overnight fast and rate your baseline muscle soreness using a muscle soreness scale and a baseline blood sample (max 20ml).

Step 2: You will be asked to perform a jump height test.
Step 3: You will then be asked complete a supervised high-intensity exercise bout consisting of a mix of resistance, plyometric (jumping) and/or aerobic exercise.
Step 4: Immediately after exercise you will be asked to rate your muscle soreness and perform the jump height test again.
Step 5: You will then be asked to consume the trial supplement (either a carbohydrate drink, milk, Greek yogurt or water) and a blood sample will be taken 5 minutes after exercise (max 20ml).
Step 6: Another blood sample will be taken 1 hour after exercise (max 20ml), and you will consume an additional trial supplement.
Step 7: You will be provided with a light lunch meal 2 hours after the exercise bout.
Step 8: Another blood sample will be taken at 4 hours after the exercise bout (max 20ml). You will then rate your muscle soreness again and perform another jump height test.
Step 9: You will be provided with a dinner meal to take home and an additional trial supplement to consume before bed.
Step 10: You will be asked return to the lab (BC 123) at York University after an overnight fast (8h minimum), 24 hours later to rate your muscle soreness once more, perform the jump height tests, and complete a final blood sample~~s~~ (max 20ml).

Acute Exercise Bout
As part of each acute supplement trial, you will be asked to perform one bout of exercise. Exercise will take place in the morning hours between 8 and 11am at York University in Dr. Josse's Exercise Research Lab in the Life Science Building (Rm 002). The bout will consist of a mix of resistance exercise (e.g. bench press, lat pulldown, squats, leg press, free weights) combined with plyometric (jumping) exercise (e.g. box jumps, drop-box jumps, frog jumps) and/or a high-intensity aerobic exercise session (e.g. cycling or running). Each session will last for approximately 60 minutes. Each exercise session will be facilitated by a personal trainer and/or a trained kinesiology student who will ensure safety and correct technique. Please ensure you are properly dressed for the exercise sessions. This includes running shoes, shorts/jogging pants and a t-shirt/tank-top. You may also bring a water bottle. We ask that you maintain your current pre-study physical activity patterns during the study and refrain from starting any new forms of physical activity until the study is complete. Due to the nature of the exercise bout, muscle soreness is likely to occur the evening and day or two following. Researchers will contact you the first and/or second day after each exercise bout to ensure there are no issues or concerns. Please do not treat your muscle soreness as described on the first page of the consent form.

Description of Testing Procedures
1. Questionnaires
You will be asked to complete three short questionnaires at the beginning of the study to see if you meet our inclusion criteria: i) Health and Screening questionnaire to gain insight about your health and lifestyle (this asks questions about your general health and lifestyle including questions on alcohol consumption and smoking). ii) A PAR-Q to determine readiness to participate in physical activity. iii) A physical activity questionnaire to determine your current levels of physical activity. We ask that you complete these questionnaires as truthful as possible and ask us any questions you may have. You may choose to leave questions blank with no penalty if you do not wish to provide an answer. You will also be asked to answer a standard single question about muscle soreness several times throughout the trials to assess the degree of soreness you experience after the exercise and up to 48 hours later.

2. Familiarization Session
You will be asked to come to the lab an additional time prior to beginning the study for an exercise familiarization session to acclimatize yourself to the exercises that will be completed. During this session, we will also determine the workrate for the interval cycling/running component of the exercise and how much weight you can lift/push/pull on certain machines (1 repetition maximums) such as the Leg Press, Leg Curl, Seated Row and Machine Chest Press. This will enable us to determine the appropriate weights that you will use for the following exercise bouts.

3. Food diaries
You will be asked to complete a 2-day food diary for each trial. The 2 days include the day before the exercise bout, and the day of the exercise bout. We ask that you try to consume a similar diet/eating pattern on the day before the exercise bout for all supplement trials. On the day of exercise, we will be providing you with a lunch and dinner meal as well ~~at~~ as the supplements to consume in the lab and at home. This will allow us to accurately assess your current nutrient intake and control the foods you eat on the day of the trial. We ask that these diaries be filled out truthfully and with as much detail as possible. We encourage any questions you may have about these diaries and we will go over how to fill them out with you prior to you filling it out on your own.

4. Blood collection
We would like to collect blood from your arm (front part of the elbow using a standard technique, and taken by a trained professional) 5 times per supplement trial. During each blood sample, a maximum of 20ml of blood will be taken: upon arrival to the lab, 5 minutes post-exercise, 1 hour post-exercise, 4 hours post-exercise and 24 hours post-exercise. This is a total of 100ml of blood (over 2 days) for each supplement trial. For context, those that donate blood provide ~450-500 ml at one time. We ask that you arrive to our laboratory (BC 123) for testing after an overnight fast (minimum 8 hours), but you may/should drink water. We also ask that you refrain from exercise for at least 48 hours prior to your first blood sample and until after the last blood draw at 24 hours. We will use your blood to measure different bone markers that reflect activity inside the bone and/or inflammatory markers that are related to muscle soreness. Once blood is collected, it will be transferred to special tubes. These tubes will then be stored in a freezer for later analysis. **Note**: You may also leave the lab to use the washroom or grab water briefly between the 5 minutes post-exercise and 4 hour post-exercise blood draws.

5. Anthropometry and Body Composition
We would like to measure your body weight and body composition. Body composition will be measured through Bio Electrical Impedance (BIA) or through air displacement plythsmography (Bodpod). Please wear athletic clothing (shorts, t-shirt) for this component. This measurement is routinely done in laboratory and is very safe. This will take a few minutes to complete. If requested, a female researcher can complete these measurements for you.

6. Jump Height Test
We would like you to perform a jump height test at each blood draw (baseline, post-exercise, 4hr, and 24hr post-exercise). We ask that you perform this test to the best of your ability. During the familiarization session we will discuss proper technique for performing the jump and show you how to perform the jump height test.

What You Will Be Asked to Do in the Research:
Maximal Time Commitment

Treatment Number	Acute trial 1	Acute Trial 2	Acute Trial 3	Acute Trial 4
Initial meet, signing of consent form, questionnaires Familiarization Session (Introduce exercises, body composition, 1RM testing)	(1.5 hours)			
Day 1 – Pre-exercise testing (15min), exercise (60 mins), Blood Sample (240 mins)	(5 hours, 15 mins)	(5 hours, 15 mins	(5 hours, 15 mins)	(5 hours, 15 mins)
Day 2 - 24 Hour Blood Sample	(15 mins)	(15 mins)	(15 min)	(15 mins)
Food Diary	(30 mins)	(30 mins)	(30 mins)	(30 mins)

Post-study testing session (body composition, 1RM testing)				(1 hour)
Total Per acute trial:	**7.5 hours**	**6 hours**	**6 hours**	**7 hours**

Estimate of total time commitment in hours to complete initial familiarization session and all 4 acute trials: ~ 26.5 hours.

Upon completion of all trials or withdrawal, you will be compensated $20 for each completed trial and an additional $20 for completing the post-study testing.

Risks and Discomforts:
There is little direct risk to you, you may experience some uneasiness or anxiety due to the personal nature of the questions asked on the questionnaires or due to the measurements taken (i.e., anxiety around the blood sample or body composition testing or exercise). We assure you that this is nothing to be anxious about and we will do our best to minimize these feelings. The only foreseeable physical risks involved in participation include a) potential muscle soreness/discomfort after completing the exercise bout which may take 2-3 days to subside. This is completely normal; b) you may experience slight pain, and/or tingling, and/or bruising in the area after the blood samples. In some instances, you may unexpectedly feel faint or lose consciousness if looking at the needle or blood. It is also possible that a hematoma (bruise) could form under the skin beside the puncture site. Applying adequate pressure following the blood draw will mimimize this. It is also important to know that there is always a risk of infection whenever the skin is punctured. Please inform the researchers if you experience any of the above-mentioned events or you are concerned about them.

Benefits of the Research and Benefits to You:
By participating in this research study, you will: (1) become exposed to a research study, (2) contribute to the advancement of science; (3) gain knowledge about your own body, health and nutrition. Your own individual results or group results provided to you upon request.

Voluntary Participation and Withdrawal: Your participation in the study is completely voluntary and you may choose to stop participating at any time. Your decision not to volunteer, to stop participating, or to refuse to answer particular questions will not influence the nature of the ongoing relationship you may have with the researchers or study staff, or the nature of your relationship with York University either now, or in the future. If you decide to stop participating, you may withdraw without penalty, financial or otherwise, and you will still receive the promised inducement. In the event you withdraw from the study, all associated data collected will be immediately destroyed wherever possible. Should you wish to withdraw after the study, you will have the option to also withdraw your data up until the analysis is complete.

Confidentiality:
Once you begin the study, you will be given a personal participant identification number. All research related documents will only use only this ID number to identify you and your data, not your name. This way, those who may be analyzing your online/computer data will not be able to link it to you. You maintain your right to withdraw from the study at any time, including research data (if applicable). However, your personal information will not be linked to your data. The identification key and all information collected about you during this study will remain confidential and will be stored in locked offices and on secured computers to which only the principal investigator, co-investigator and student investigator will have access to. Unless you choose otherwise, all information you supply during the research will be held in confidence and unless you specifically indicate your consent, your name will not appear in any report or publication of the research. Results of this study will be made available to scientists, through publication in scientific journals, but your name and personal data will not appear on its own. We will only report group information. Electronic and paper information will be kept safely for 5 years after publication (~2030), at which time, all information will be destroyed. In addition, you will have access to your own results, as well as results from the group when our analysis is completed and available. Please let us

know if you would be interested in receiving the results. Confidentiality will be provided to the fullest extent possible by law. The data collected in this research project may be used – in an anonymized form – by members of the research team or approved colleagues in subsequent research investigations exploring similar lines of inquiry. Such projects will still undergo ethics review by the HPRC, our institutional REB. Any secondary use of anonymized data by the research team or approved colleagues will be treated with the same degree of confidentiality and anonymity as in the original research project.

Questions About the Research? If you have questions about the research in general or about your role in the study, please feel free to contact Emily Fraschetti ecfrasch@yorku.ca (student investigator) or the main supervisor of this research, Andrea Josse at ajosse@yorku.ca and/or (416) 736-2100 (30038). You may also contact the Graduate Program in Kinesiology and Health Science at kahs@yorku.ca and/or (416) 736-5728.

This research has received ethics review and approval by the Delegated Ethics Review Committee, which is delegated authority to review research ethics protocols by the Human Participants Review Sub-Committee, York University's Ethics Review Board, and conforms to the standards of the Canadian Tri-Council Research Ethics guidelines. If you have any questions about this process, or about your rights as a participant in the study, please contact the Sr. Manager & Policy Advisor for the Office of Research Ethics, 5th Floor, Kaneff Tower, York University (telephone 416-736-5914 or e-mail ore@yorku.ca).

Legal Rights and Signatures:

I_____consent to participate in this study conducted by Dr. Andrea Josse. I have understood the nature of this project and wish to participate. I am not waiving any of my legal rights by signing this form. My signature below indicates my consent.

Signature_____ **Date**_____
Participant

Signature_____ **Date**_____
Principal Investigator

Appendix D: Recruitment Poster

YORK UNIVERSITÉ UNIVERSITY

PARTICIPANTS NEEDED FOR THE BIONEX STUDY

SCAN FOR MORE INFORMATION!

ARE YOU BETWEEN THE AGES OF 18-35 YEARS WITH LIMITED EXERCISE EXPERIENCE?

HELP US DETERMINE IF POST-EXERCISE NUTRITION CAN INFLUENCE BONE & INFLAMMATION!

STUDY INFORMATION

- 4 TRIALS: 2 VISITS/TRIAL 27 TOTAL HOURS
- 4 POST-EXERCISE SUPPLEMENTS (MILK, GREEK YOGURT, CARBOHYDRATE, WATER)
- 5 BLOOD SAMPLES/TRIAL

BENEFITS OF PARTICIPATING

- 4 EXERCISE SESSIONS WITH A PERSONAL TRAINER
- LUNCH & DINNER MEALS PROVIDED ON STUDY DAYS
- UP TO $100 COMPENSATION

Contact: york.nutrition.research@gmail.com or by phone (416) 736-2100 x33990

Dr. Andrea R. Josse (Principal Investigator): ajosse@yorku.ca or by phone (416) 736-2100(30038)

*should prospective participants choose to interact with this post on Facebook, their privacy and confidentiality may be compromised

Approved by the York University Research Ethics Board (ORE) File# (2019-045)

Appendix E: Health and Screening Questionnaire

Participant ID#: BIONEX Trials

Researcher:
Date & Time:

1. Participant Details			
Name:			
Telephone #:		Email:	
Preferred Contact method?	| | Telephone | | Zoom | | Email		
Age:		| | **18 - 30 years old?**	
Date of Birth:			
Sex:			
Self-Identified Ethnicity:			
Height:			
Weight:			
Calculated BMI:		| | **18.5 - 25 kg/m^2?**	
2. Exercise/Activity Level			
How many times per week do you perform structured exercise (excluding walking)?			
		| | **≤ 2 structured weekly sessions?**	
Type of activity and duration (per day or week):			
How many times per week do you engage in any weight-lifting or jumping exercises (plyometrics)?			
		| | **≤ 2 structured weekly sessions?**	
Type of activity and duration (per day or week):			
3. Supplement consumption			

Do you consume nutrition supplements (e.g., Multivitamin, Calcium, Vitamin D, Iron, B12, protein supplements, etc.)	| | Yes*	| | No
If YES, please specify the:		
Types:		
Amount:		
Frequency (per day or average per week):		
Have you been instructed by a physician to take these supplements?		
If yes, would you be willing to not take these supplements during the duration of the study (maximum 18 weeks)?	| | Yes*	| | No

4. Health & Lifestyle Questions		
Do you regularly or occasionally smoke/vape tobacco and/or use cannabis products?	| | Yes	| | No
If yes, please explain (specify if using tobacco or cannabis, smoking/vaping or consuming edibles and frequency of use):		
Do you consume alcohol on a regular basis (at least every couple of days)?	| | Yes	| | No
Are you lactose intolerant (diagnosed by doctor)?	| | Yes	| | No
Do you believe you may be lactose-intolerant or lactose-sensitive?	| | Yes	| | No
Do you have an allergy to dairy/milk protein?	| | Yes	| | No
Do you have any other food allergies, or	| | Yes	| | No

dietary restrictions (gluten free, vegetarian) If yes, explain:		
Do you regularly take any pain management medication, such as Tylenol or Advil? (Please select yes if you usually take pain management medication for menstrual cramps?)	| | Yes	| | No
Do you have Celiac Disease?	| | Yes	| | No
Do you have any other gastrointestinal diseases or condition?	| | Yes	| | No
Have you ever had any bone, joint or muscle injury? If yes, when and how did this injury occur? How was it treated and does it affect your physical ability?	| | Yes	| | No
Have you ever had any major joint instability or ongoing chronic pain such as in the knee, back or elbow? If yes, when and how did this occur? How was this treated and does this affect your physical ability?	| | Yes	| | No
5. Dairy Intake Questionnaire		
Do you regularly consume dairy products (milk, yogurt, cheese)?	| | Yes	| | No
Do you regularly consume dairy-containing products (ice cream, sour cream, whipped cream etc.,)	| | Yes	| | No
What dairy products do you consume?		
What dairy-containing products do you consume?		
On average, how many dairy products do you consume per week?		

On average, how many dairy-containing products do you consume?		
6. Medical History Questions		
Heart disease/condition	| | Yes	| | No
Kidney disease/condition	| | Yes	| | No
Liver disease/condition	| | Yes	| | No
Pancreatic disease/condition	| | Yes	| | No
Hepatitis B	| | Yes	| | No
Hepatitis C	| | Yes	| | No
HIV/AIDS	| | Yes	| | No
Other (please specify):		
Do you take any prescription medication?	| | Yes	| | No
If YES, please specify (anything prescribed by a doctor, including supplements):		
7. Menstrual Cycle and Hormonal Questions		
Do you experience a menstrual cycle?	| | Yes	| | No
If yes, do you experience a regular menstrual cycle?	| | Yes	| | No
Have you ever been diagnosed with any menstrual imbalances or disorders (such as PCOS, endometriosis, etc.)? If yes, please specify, including when you were diagnosed:		

Are you currently taking any type of hormonal contraception? If yes, what kind of contraceptive and how long have you been taking it?	
What was the approximate date of your last period (mm/dd/yy)?	
What day of your cycle are you currently at (with day 0 being the day menstrual bleeding starts then day 1 is the next full 24 hours of bleeding)?	
How many days in duration is your monthly cycle?	
What is the average or most consistent length & if it varies, what is the range?	

Eligible: | | Yes | | No/consult with coordinator

Appendix F: PAR-Q+

2018 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	**YES**	**NO**
1) Has your doctor ever said that you have a heart condition ☐ OR high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** _____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it ***does not limit your current ability*** to be physically active. **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.
Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

PARTICIPANT DECLARATION

If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness centre may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at **www.eparmedx.com** before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

01-11-2017

2018 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?

If the above condition(s) is/are present, answer questions 1a-1c — If **NO** ☐ go to question 2

1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES ☐ NO ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES ☐ NO ☐

2. Do you currently have Cancer of any kind?

If the above condition(s) is/are present, answer questions 2a-2b — If **NO** ☐ go to question 3

2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES ☐ NO ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES ☐ NO ☐

3. Do you have a Heart or Cardiovascular Condition? *This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm*

If the above condition(s) is/are present, answer questions 3a-3d — If **NO** ☐ go to question 4

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES ☐ NO ☐
3c.	Do you have chronic heart failure?	YES ☐ NO ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES ☐ NO ☐

4. Do you have High Blood Pressure?

If the above condition(s) is/are present, answer questions 4a-4b — If **NO** ☐ go to question 5

4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES ☐ NO ☐

5. Do you have any Metabolic Conditions? *This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes*

If the above condition(s) is/are present, answer questions 5a-5e — If **NO** ☐ go to question 6

5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES ☐ NO ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES ☐ NO ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES ☐ NO ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES ☐ NO ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES ☐ NO ☐

01-11-2017

2018 PAR-Q+

6. Do you have any Mental Health Problems or Learning Difficulties? *This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome*

If the above condition(s) is/are present, answer questions 6a-6b — If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. Do you have a Respiratory Disease? *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*

If the above condition(s) is/are present, answer questions 7a-7d — If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. Do you have a Spinal Cord Injury? *This includes Tetraplegia and Paraplegia*

If the above condition(s) is/are present, answer questions 8a-8c — If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. Have you had a Stroke? *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*

If the above condition(s) is/are present, answer questions 9a-9c — If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. Do you have any other medical condition not listed above or do you have two or more medical conditions?

If you have other medical conditions, answer questions 10a-10c — If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: _____

_____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

01-11-2017

2018 PAR-Q+

If you answered NO to all of the FOLLOW-UP questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact

www.eparmedx.com
Email: eparmedx@gmail.com

Citation for PAR-Q+
Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration.
The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada 4(2):3-23, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

Key References

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01-11-2017

Appendix G: Godin-Shephard Leisure-Time Exercise Questionnaire

BIONEX Trials
Bone and Inflammatory Outcomes, the Nutrition and Exercise Trials

ID :_____

GODIN-SHEPHARD LEISURE-TIME EXERCISE QUESTIONNAIRE

1. Considering a **7-day period** (a week), how many times on average do you do the following kinds of exercise for **more than 15 minutes** during your **free-time** (write on each line the appropriate number)?

	Times Per Week
(a) STRENUOUS EXERCISE **(HEART BEATS RAPIDLY)** (i.e. running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)	_____
Please specify the types of exercise:	
(b) MODERATE EXERCISE **(NOT EXHAUSTING)** (i.e. fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)	_____
Please specify the types of exercise:	
(c) MILD EXERCISE **(MINIMAL EFFORT)** (i.e. yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)	_____
Please specify the types of exercise:	

2. Considering a **7-day period** (a week), during your leisure-time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

1. OFTEN (≥4x/week)	**2. SOMETIMES (2-3x/wk)**	**3. NEVER/RARELY (0-1x/wk)**
☐	☐	☐

Appendix H: Familiarization Tracking Sheet

BIONEX: Bone and Inflammatory Outcomes, the Nutrition and Exercise Trials
Familiarization Session Tracking Sheet

Participant ID: _____ Date: _____
Age: _____ Time: _____

Trainer: _____
Researchers Present: _____

Body Composition

Height (cm):	Mean Weight:	BMI (kg/m^2):
BodyMetrix	Male Quads (mm): Chest (mm): Waist (mm): Body Fat %:	Female Quads (mm): Triceps (mm): Hip (mm): Body Fat %:
	Weight (kg)	Body Fat %
BIA		
BodPod		

Notes: (For BodPod, please note the clothing worn and any jewelry that remained on during the test. For the BIA, please note an estimate of water consumption.)

Jump Height Test:

Standing Reach: _____ feet _____ inches _____ total inches _____ total cm

Vertical Jump Attempt 1: _____ feet _____ inches _____ total inches _____ total cm

Vertical Jump Attempt 2: _____ feet _____ inches _____ total inches _____ total cm

Vertical Jump Attempt 3: _____ feet _____ inches _____ total inches _____ total cm

Best Vertical Jump – Standing Reach = _____ total inches _____ total cm

BIONEX: Bone and Inflammatory Outcomes, the Nutrition and Exercise Trials

Familiarization Session Tracking Sheet

Leg Press

Seat Height:

Intensity	**Weight**	**Reps**	**RPE**
Warm-Up			
RPE 3 (Easy)		8	
RPE 5 (Somewhat Difficult)		8	
RPE 7 (Hard)		8	
Trial Sets Until Failure			
Sets	**Weight**	**Reps**	**RPE**
Set 1			
Set 2			
Set 3			
Set 4			
e1RM: weight/[(1.0278) – (0.0278 x repetitions completed)] e1RM =			

Chest Press

Seat Height:

Intensity	**Weight**	**Reps**	**RPE**
Warm-Up			
RPE 3 (Easy)		8	
RPE 5 (Somewhat Difficult)		8	
RPE 7 (Hard)		8	
Trial Sets Until Failure			
Sets	**Weight**	**Reps**	**RPE**
Set 1			
Set 2			
Set 3			
Set 4			
e1RM: weight/[(1.0278) – (0.0278 x repetitions completed)] e1RM =			

BIONEX: Bone and Inflammatory Outcomes, the Nutrition and Exercise Trials

Familiarization Session Tracking Sheet

Seated Row

Intensity	**Weight**	**Reps**	**RPE**
Warm-Up			
RPE 3 (Easy)		8	
RPE 5 (Somewhat Difficult)		8	
RPE 7 (Hard)		8	
Trial Sets Until Failure			
Sets	**Weight**	**Reps**	
Set 1			
Set 2			
Set 3			
Set 4			
e1RM: weight/[(1.0278) – (0.0278 x repetitions completed)] e1RM =			

Cycling

Resting HR: Estimated Max HR (220-Age): 90% of Max HR:

Seat Height:

Workload	Time	HR	RPE
	4 min		
	1 min		
	1 min		
	1 min		
	1 min		
	1 min		
	1 min		
	1 min		
	1 min		

Warm-up Watts will be roughly their weight in kg, rounded to the lowest 10kg.
Increase by 0.5kg each min.
Watts = RPM*resistance (kg)

Notes: (Any notes about the veins of participants, food preferences and allergies.)

Appendix I: Exercise Trial Tracking Sheet

Exercise Tracking Sheet

Participant ID: _____ Date:_____

Trial: Milk GY CHO W Trainer:_____

Participant Maxes & Seat Height

Exercise	e1RM	60%	Actual Weight	70%	Actual Weight	80%	Actual Weight	Seat Height
Chest Press								
Leg Press								
Seated Row								

Exercise	Sets	Reps	Intensity	Load	Notes (RPE)		
Depth Jumps	3	10		BW			
Tuck Jumps	3	10		BW			
Split Jumps	3	10		BW			
Seated Box Jumps	3	10		BW			
Chest Press	3	10	60-80% 1RM				
Leg Press	3	10	60-80% 1RM				
Seated Row	3	10	60-80% 1RM				

*Take note of how many steppers used for depth jump

*Add RPE for each set for resistance exercises

Completed all 3 sets for the same reps and weight? Yes ☐ No ☐

If no, record the exercise, reps, weight and RPE for the incomplete exercise.

Exercise Tracking Sheet

Age:
Estimated Max HR (220-age):
90% of Max HR:
Cycling Max PO: 50%: 90%: Seat Height:
Weights Required
50%: 90%:

Cycling			RPM:	
Intensity	Time	Workload	HR	RPE
Warm-up Ramping Protocol				
50-70 W	1 min			
	1 min			
50% of max	1 min			
50% of max	1 min			
High Intensity Interval Bouts				
90% of max	1 min			
50% of max	1 min			
90% of max	1 min			
50% of max	1 min			
90% of max	1 min			
50% of max	1 min			
90% of max	1 min			
50% of max	1 min			
50% of max	2 min			

If the HR average of the first 3 intervals is greater than 85% of HR max, they are done.
[(HR1 + HR2 + HR3)/3]/HR max
[(____ + ____ + ____)/3]/____ =
If it is below 85%, do a 4th interval.

Appendix J: BIONEX Trial Data Collection Sheet

BIONEX: Bone and Inflammatory Outcomes, the Nutrition and Exercise Trials
Trial Data Collection Sheet

ID: _____ Date: _____

Keenoa ID: _____ Time: _____

Trial Number:

Supplement: GY / Milk / CHO / W

Estimated Energy Expenditure:

Meal Type: Regular / Vegetarian

Meal/Snack modifications:

Cycle day (naturally cycling):

Any illness in the last week: Details:		☐ YES	☐ **NO**
Feeling well today:		☐ **YES**	☐ NO
Time of last food/caloric drink:	Fasted 8+ hours?	☐ **YES**	☐ NO
Last exercise session:	Rested state?	☐ **YES**	☐ NO
Caffeine consumed:		☐ YES	☐ **NO**
Any medication taken today: Details:		☐ YES	☐ NO

BIONEX: Bone and Inflammatory Outcomes, the Nutrition and Exercise Trials
Trial Data Collection Sheet

Standing Reach (inches):

Timeline	Time of Day	Time post-exercise (h/min)	Mean Hematocrit	Muscle Soreness Rating (0-5)	Jump Height (cm)	Serum and Plasma Aliquots (# and Volume)
Baseline Draw						Serum: Plasma:
Exercise Session	Start: End:					
5min Post Exercise						Serum: Plasma:
Supplement 1						
1h Post Supplement						Serum: Plasma:
Supplement 2						
Lunch (2h post-exercise)						
4h Post Supplement						Serum: Plasma:
Provide Participants with Snacks and Meals, and final supplement for before bed. Remind participant to log food data on Keenoa and note the time of consumption. Remind participants to not treat their muscle soreness in any way (no stretching, Advil/Tylenol, exercise, icing, applying heat, massage etc.,). Remind them to come fasted in the morning, and not eat after ~10pm.						

BIONEX: Bone and Inflammatory Outcomes, the Nutrition and Exercise Trials
Trial Data Collection Sheet

Trial Day 2:

Feeling well today:		☐ **YES**	☐ NO
Time of last meal/supplement:	Fasted 8+ hours?	☐ **YES**	☐ NO
Caffeine consumed:		☐ YES	☐ **NO**
Any medication taken today: Details:		☐ YES	☐ NO
Muscle Soreness Treatment: (i.e., stretching, heat, ice etc.) Details:		☐ YES	☐ **NO**

Timeline	**Time of Day**	**Time post-exercise (h/min)**	**Mean Hematocrit**	**Muscle Soreness Rating (0-5)**	**Jump Height (cm)**	**Serum and Plasma Aliquots (# and Volume)**
24h Post Exercise			[redacted]			Serum: Plasma:

BIONEX: Bone and Inflammatory Outcomes, the Nutrition and Exercise Trials
Trial Data Collection Sheet

Raw Hematocrit values:

Draw	Measure 1	Measure 2	Measure 3	Mean
Pre-exercise				
5 min post-exercise				
1h post-exercise				
4h post-exercise				
24h post-exercise				

Notes (e.g., issues with blood sampling, changes in protocol):