# The Effects of Dairy Consumption on Bone, Inflammatory, Antioxidant & Metabolic Biomarker Responses to Exercise or Nutritional Stressors in Humans

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# Abstract

The overall purpose of this dissertation was to examine the impact of dairy product consumption on outcomes of bone, inflammation, antioxidant & metabolic biomarkers both at rest (Chapter 5) and after exposure to different physiological stressors, including exercise (Chapter 3 & 4) and a high-calorie, high-fat meal (Chapter 6). To achieve this, two human intervention studies (divided into 4 papers) that used different stressors in combination with dairy product consumption were conducted. The collective findings indicate that increased dairy product consumption may confer some benefits to post-exercise bone remodeling and basal circulating inflammatory concentrations but does not have a significant effect on postprandial inflammatory, metabolic or glutathione responses. Therefore, including dairy products as part of a balanced diet could have minor beneficial effects if consumed following impact exercise and/or to potentially ameliorate resting low-grade chronic inflammation in people that may be at higher risk of developing metabolic disease.

# Acknowledgements

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First and foremost, I would like to thank my supervisor Dr Andrea Josse for choosing me to be her first PhD student. She has been both an incredible academic mentor, but more importantly, a great human being to have spent the last almost 5 years working with. Throughout my time at York she would answer any question I posed, be there whenever I needed support for early morning data collections and always matched my time and effort when writing grant applications, conference presentations or research paper submissions. I am also grateful for her mentorship on how to be an effective leader and communicator, which I developed after being provided the opportunity to run and manage the DRIVE study. In addition to academics, I am lucky to have had a supervisor that was flexible and understanding about life events, and who was always more than willing to take the time to offer advice and help if needed. I have many fond memories of singing and dancing at conferences as well as from celebrating the achievements of our lab and its members over dinners. The fact that she took the time to drop off preemie baby clothes at the hospital when my daughter was born early is a testament to her caring nature and character. Overall, I couldn't have asked for a better supervisor. I would also like to thank my co-supervisor Dr Chris Perry, and my committee member Dr Nota Klentrou, who I have had the good fortune of meeting with regularly during my doctorate. Having the opportunity to work with Chris and see how his inquisitive mind approached problems was invaluable. His training has improved my ability to both logically think through problems in a step-by-step approach as well as to consider the bigger picture. These are skills that I am very thankful to have developed, and I'm sure will be useful in many areas of my life going forward. He also had the best jokes (and brisket!) and would have participants and our entire research team laughing out loud during data collection, which certainly made the biopsy procedures a lot less scary! Nota's knowledge about bone physiology is unparalleled and I have thoroughly enjoyed learning from her and developing my own expertise and passion for this research area. I really appreciate her ability to get straight to the point of an issue and to noy be afraid to say things how she sees them. It was very refreshing to not have to wonder about her true

thoughts/feelings on a topic! I would also like to thank my dissertation examining committee, Dr. Devin Phillips (chair), Dr. Tarra Penney (internal) and Dr. Lindsay Robinson (external), as well as my comprehensive examination arms-length committee members, Dr. Ali Abdul-Sater and Dr. Michael Riddell for their insightful questions, interesting discussions and helpful suggestions, all of which have helped to improve the work contained within this document.

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support and words of encouragement have allowed me to complete this part of my journey. Even on the hardest of days in the lab, you would be there to make me feel better and pick me back up. I love you, and I'm excited for the next chapter together. Finally, to my daughters Lilia and Faye, thank you for being there to put a smile on my face and to remind me that while my work is important, the true purpose of life is to bring love and happiness to those around you, and to make the world better for those who come next.

As I finish reflecting on my doctoral journey, the following quote from Brandon Sanderson's book, Oathbringer, resonates with me now more than ever:

*"A journey will have pain and failure. It is not only the steps forward that we must accept. It is the stumbles. The trials. The knowledge that we will fail. That we will hurt those around us. But if we stop, if we accept the person we are when we fail, the journey ends. That failure becomes our destination. To love the journey is to accept no such end. I have found, through painful experience, that* ***the most important step a person can take is always the next one****."*

So, while this part of my journey has been hard at times, it has also forged me into a more considerate, resilient and overall better man. The journey continues and I'm excited to continue to keep taking the next step.

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# Chapter 1: General Introduction

Within its complex wholefood matrix (WFM), dairy foods contain a variety of essential macro- and micronutrients, as well as functional and bioactive components that seem to have anti-inflammatory, antioxidant, and musculoskeletal supporting properties [5-7]. At present, limited research has assessed the interaction between wholefood dairy product intake in combination with an acute physiological disturbance or stressor such as exercise or the consumption of a high-calorie, high-fat meal. More specifically, there is little research assessing the effect of acute dairy intake on bone biomarkers following a bout of high-impact, high-load (i.e., highly osteogenic) exercise, as well as on short/medium term mixed dairy intake on biomarkers of inflammation, antioxidants (e.g., glutathione) and blood lipids/glucose following a high-fat meal challenge (especially without a dairy preload meal). The intention of this dissertation was to measure a range of human blood/tissue biomarkers while providing participants with a varied and ecologically valid dairy nutrition protocol in order to better understand how dairy consumption may be modulating acute physiological responses that underly the positive health effects that are consistently observed in the literature within both healthy and chronic disease populations [8-10].

The studies completed herein are distinct from but informed by previous human intervention research carried out by our group and others. For example, our group has previously demonstrated that dairy consumption can change the chronic bone turnover response to exercise training in different populations, including lean adult males [11], adolescent [12] and adult [13] females with overweight/obesity (OW/OB). It has also been observed (outside of our group's work) that in individuals with OW/OB and metabolic syndrome, dairy intake can favourably alter markers of oxidative stress and inflammation after only one week (i.e., short-term), with progressive improvements as the protocol continues over a period of weeks (i.e., long-term) [14,

15]. Research from others has also shown that an acute dairy containing "pre-load" can blunt postprandial inflammation following a high-fat meal challenge [16, 17]. Given these promising findings, this dissertation sought to further explore the effects of consuming dairy products in combination with different physiological disturbances on a range of physiological biomarkers. The ultimate goal of this research was to provide insight into both the acute and chronic responses and interactions of dairy consumption with physiological stressors in different human contexts, which ultimately can be used to inform the optimization of different nutritional strategies for exercise and feeding in both healthy and clinical populations.

## 1.1 Objectives and Hypotheses

The overall objective of this doctoral dissertation was to advance our understanding of the effects of dairy product consumption on bone, inflammation, metabolic and antioxidant outcomes in both healthy adults and those at risk of developing chronic cardiometabolic disease. This was examined through provision of different amounts and durations of dairy product consumption, as well as in the context of an exercise or high-fat meal physiological stressor. To achieve this, two distinct and novel research studies (**Study 1** and **Study 2**) were performed, and four research papers (**Chapter 3-6**) were produced to discuss their findings. Due to the COVID-19 pandemic, **Study 1** was not conducted by me, however I instead completed the data analysis, interpretation and write up (**Chapters 3 and 4**). **Study 2** was a large, multi-disciplinary human crossover trial which I primarily conducted and coordinated. These data were collected from June 2022 to March 2024, and I then conducted the majority of the data analysis, interpretation and write up (**Chapter 5 and 6**). In **Study 2**, vascular measurements and assessments were performed by a separate research group, so while those data appear within, they will not be significantly discussed within this dissertation. The specific objectives for each paper are as follows:

### 1.1.1 Objective 1 (Study 1; Paper 1/Chapter 3)

The objective of this human study was to compare post-exercise consumption of milk vs. carbohydrate on bone biomarker responses to a single bout of combined high-impact, high-load exercise in healthy, young female adults. A randomized crossover design with 2 trial arms was used, and blood was taken pre-exercise, and then 15-minutes, 75-minutes, 24-hours and 48-hours following exercise in order to allow for measurement of the following bone biomarkers from within the serum: cross-linked C-telopeptide of type I collagen (CTX), receptor activator of NF-κB ligand (RANKL), sclerostin (SOST), osteoprotegerin (OPG), osteocalcin (OC). The nutrition intervention was consumed immediately following exercise, and then 1-hour after the completion exercise, and consisted of an isoenergetic and isovolumetric amount of either: 1) skimmed 0% white milk (500 ml); or 2) maltodextrin dissolved in water.

The hypothesis of this study was that, relative to carbohydrate, post-exercise milk consumption would favourably influence acute bone turnover biomarkers by reducing bone resorption markers and increasing bone formation markers.

### 1.1.2 Objective 2 (Study 1; Paper 2/Chapter 4)

Using bone biomarker response data from **Paper 1** in combination with inflammatory biomarker responses assessed from the same samples, we sought to determine whether these biomarkers were related. Thus, the objective of this study was to use linear mixed modelling to investigate the associations between the concentrations of inflammatory and bone biomarkers within the context of a single bout of high-intensity, high-impact exercise and post-exercise nutrition. Dependent variables for the models consisted of the bone biomarkers: serum CTX, OPG, RANKL, SOST, and OC. The fixed effects covariates used were time, supplement condition, and

the following inflammatory cytokines from plasma: interleukin (IL-) 1β, IL-6, IL-10, and tumor necrosis factor-alpha (TNF-α).

We hypothesized that there would be a relationship between bone and inflammatory biomarkers, and that bone biomarkers indicative of breakdown (CTX, RANKL, SOST) would be positively associated with some or all of the proinflammatory cytokines (IL-6, IL-1β, TNF-α) and negatively associated with IL-10 (anti-inflammatory cytokine). We also hypothesized that the bone biomarkers indicative of formation (OPG, OC) would be negatively associated with the proinflammatory cytokines and positively associated with IL-10.

### 1.1.3 Objective 3 (Study 2; Paper 3/Chapter 5)

The objective of this human study was to assess the effect of consuming 3 servings/day of mixed dairy products for 6-weeks on a range of inflammatory, metabolic, vascular and glutathione measures in adults with OW/OB and some cardiometabolic dysfunction. A randomized crossover design was used, consisting of two 6-week dietary intervention periods involving: 1) habitual diet with low dairy product consumption (<1 serving/day) or, 2) additional dietary incorporation of mixed wholefood dairy (3 servings/day of milk, yogurt and cheese). Measurements were taken at the beginning and end of each 6-week period, and circulating inflammatory, antioxidant and metabolic biomarkers were then compared between the two dietary conditions.

We hypothesized that increased dairy product consumption would result in reductions in circulating proinflammatory cytokines and/or increase reduced glutathione (GSH) or reduced-to-oxidised glutathione ratio (GSH:GSSG) and would therefore benefit individuals with chronic disease risk factors who may be in a state of low-grade chronic inflammation (LGCI).

### 1.1.4 Objective 4 (Study 2; Paper 4/Chapter 6)

The objective of this study was to assess the effect of consuming 3 servings/day of mixed dairy products for 6-weeks on a range of acute postprandial inflammatory, metabolic, vascular and glutathione responses to a high fat meal (i.e., a high-fat meal challenge [HFMC]) in adults with OW/OB and some cardiometabolic dysfunction. Data were also obtained during **Study 2** (i.e., the same cohort as **Chapter 5**) which involved a randomized crossover design consisting of two 6-week dietary intervention periods involving: 1) habitual low dairy product consumption (<1 serving/day) or, 2) provision of 3 servings/day of mixed wholefood dairy (milk, yogurt and cheese). A HFMC was implemented after each 6-week intervention period, and postprandial results were compared between dietary conditions. Measurements were taken pre-meal and then repeated 1h, 2h, 4h and 6h postprandially. Vascular measures and muscle tissue samples were taken pre-meal and 4h postprandially.

We hypothesized that increased dairy product consumption would result in improvements in postprandial circulating inflammatory cytokines, metabolic biomarkers (e.g., glucose, lipid, insulin) and blood/muscle antioxidant measures (e.g. GSH or GSH:GSSG).

# Chapter 2: Review of Literature

Consumption of foods and/or isolated nutrients can induce both rapid, acute physiological responses, as well as long-term adaptations that may either support or impair biological functioning and health. It is therefore important to consider and study the effects of both short/acute and chronic consumption of foods and nutrients of interest in order to gain the most comprehensive understanding of how the human body responds to their incorporation within the diet in different contexts. To generate applicable results that best examine cause-effect relationships, this research should utilise human participants and well controlled, randomized control trial (RCT) study designs [18]. There are however a number of unique challenges involved with conducting these nutrition studies, particularly those involving wholefoods. These include appropriate selection of the study population, methods of randomization and blinding, instructions for the prescription and intake of the investigational food, identification of an appropriate "control" food or condition, assessment of compliance, and the statistical analysis and interpretation of study results [19]. It is therefore essential to understand and consider the current body of literature to both effectively identify knowledge gaps, while still following established methodological best practices to allow for results to be appropriately compared and contrasted. By considering these factors, the usefulness of conducting RCTs will be enhanced by providing high-quality evidence to researchers and allowing for stronger policy influence and nutritional messaging for governing bodies and consumers alike. The focus of this dissertation will be on wholefood dairy product consumption, and its ability to interact with circulating physiological components from the inflammatory, skeletal, glutathione and metabolic systems in response to physiological stressors. An overview of dairy foods and those physiological systems are briefly reviewed below, along with research on their interactions with dairy product consumption.

## 2.1 Dairy Food Overview

Dairy foods are produced from the milk of mammals (most commonly cows) and include cheese, yogurt, buttermilk and other closely related products [20, 21]. They have been a component of the human diet for approximately 10,000 years after humans first learned to domesticate ruminants during the Neolithic Revolution in Mesopotamia [22]. Data from the 2015 Canada Community Health Survey (CCHS) indicate that the average daily intake of dairy foods for adult Canadians is 1.36 servings/day, composed on average of ~44% milk, ~30% cheese, ~10% yogurt, ~10% frozen dairy and ~6% "other dairy" [23]. The previous Canada's Food Guide (from 2007-2018) advocated for 2-3 servings/day for adults [24]. The current Food Guide lists dairy foods as part of the protein food grouping (1/4 of the plate) with no specific serving size recommendation [25]. The primary role of milk is to provide mammalian neonates with energy (lipids and lactose) and essential amino acids, fatty acids, vitamins, minerals and water [26, 27]. These nutrients are contained in a complex structure known as the "wholefood matrix" (WFM) which can be defined as the arrangement of food constituents and their interactions at multiple spatial length scales [28]. Differential nutrient-nutrient interactions and physicochemical properties from the WFM can alter digestion and absorption characteristics as well as the resultant short and long-term physiological effects upon consumption of that particular food [29, 30]. The WFM of different dairy products, as it relates to their physiological effects, is of particular interest due to the variability that differentiates them. For example, comparing milk, yogurt and cheese reveals differences in the physical state of the products (liquid vs. semi-solid vs. solid), pH, relative macronutrient and micronutrient composition, amino acid profile, casein to whey protein ratio and absorption kinetics and characteristics (e.g., time taken until blood amino acid concentrations peak) [31-33]. Even the nutritional characteristics of the same type of dairy product can vary due to differences in

processing techniques between manufacturers, the specific breed or diet of the cow, and seasonal/geographic differences at the time of milking [34, 35]. It is therefore important to take these factors (as well the unique challenges generally involved in designing and interpreting studies using wholefood interventions [19]) into account when reviewing and comparing literature in this area.

### 2.1.1 Dairy Composition and Nutrients

Milk, and the dairy products derived from it, typically contain all three macronutrients to support optimal growth and development of newborns, however the percentages of each will vary depending on the specific type of dairy product.

The main form of carbohydrate within milk is lactose (~4.5-5.2g/100g) [27], which is a disaccharide composed of a galactose and glucose subunit [27]. Lactose is a particularly important energy source during the first years of mammalian life, providing almost half of the total energy required by human infants [22]. Human milk contains slightly more lactose than that of the ruminant milk which predominates in adult human nutrition (~7% lactose vs. ~5% lactose) [22]. There is a relatively high prevalence of intolerance to this specific carbohydrate in some adult ethnic groups [36, 37], somewhat limiting the generalizability of blanket dairy intake recommendations to humans as a collective, although lactose free options are becoming more available and generally provide comparable nutrition [38].

Lipids within milk mostly exist as globules in an oil-water emulsion and are typically composed of ~98% triglycerides (TG), ~0.3% diacylglycerols, ~0.8% phospholipids, ~0.3% cholesterols and ~0.1% free fatty acids (FFA) [39]. There are over 400 different types of fatty acids (FA) found within milk, with saturated fatty acids (SFA) making up 64-73% of total FA [40, 41]. The SFA's within dairy have variable chain lengths of 4 to 24 carbons (unique among animal-

derived fats), which can be divided into long-chain (14:0 to 21:0; ~58% of total FA), medium-chain (7:0 to 13:0; ~8% of total FA) and short-chain (4:0 to 6:0; ~5% of total FA) [40, 41]. The most prominent dairy SFA is palmitic acid (16:0; ~35% SFA), followed by myristic acid (14:0; ~12% SFA) and stearic acid (18:0; ~9% SFA) [40]. Dairy also contains significant amounts of odd chain SFA such as pentadecanoic acid (15:0) and heptadecanoic acid (17:0), as well as dairy-specific trans-FA such as 16:1 trans-9 or 18:1 trans-11, and contains low amounts of the n-6 PUFA linoleic acid (18:2n-6) in comparison to vegetable oils and margarines [40, 42].

As for protein, approximately 80% in cow's milk is casein (typical distribution of casein-type is 42% β, 37% αS1, 9% κ-casein and 7% αS2 [43]) whereas the remaining 20% is made up of the major whey proteins β-lactoglobulin and α-lactalbumin as well as other protein constituents (e.g., immunoglobulins, serum proteins, milk fat globule proteins, transferrin, lactoferrin, β2-microglobulin)[26]. Dairy proteins are generally considered to be high-quality because they provide a complete set of amino acids (i.e., both essential and non-essential) and are consistently rated highly when assessed with either the protein digestibility-corrected amino acid score (PDCAAS) or the digestible indispensable amino acid score (DIAAS) [44]. Casein typically contains a higher proportion of histidine, methionine, and phenylalanine whereas whey is rich in branched chain (leucine, isoleucine, and valine) and sulphur containing (cysteine) amino acids [45-47]. Furthermore, some of the components/peptides within dairy's WFM have also been shown to have unique bioactive effects and functional properties that appear to directly influence human physiology upon consumption. These properties include: antibacterial, antiviral, antioxidant, antihypertensive, antithrombotic, and immunomodulatory effects, as well as the ability to modulate digestion/absorption of other nutrients [2, 6, 48-50]. Processing of milk into other dairy products, such as by fermentation with lactic acid, heat treatment and straining into yogurt can also

modify their nutritional characteristics (e.g., changes in whey to casein ratio or lactose concentration), which can have implications for physiological responses and health effects of different dairy foods [51].

Amongst the micronutrients provided by dairy, calcium has been the most extensively studied, with research showing that around 50-75% of human dietary calcium comes from dairy products (~1200mg/L in milk) in western countries [52, 53]. Other components of dairy's WFM can act synergistically to enhance calcium absorption, including milk phosphopeptides which can improve passive absorption by forming soluble chelates with calcium, which augments active transport by increasing gene expression of calcium-binding protein and ultimately increases membrane permeability [20, 53]. Other micronutrients within dairy include magnesium (~120mg/L in milk), selenium (~30µg/L in milk), phosphorous (~950mg/L in milk), zinc (~3-4mg/L in milk), and vitamins A, B2, B12, and K, which are estimated to make up 20-40% of average daily dietary intake in western countries [2, 20]. Dairy products can also be fortified with Vitamin D [54]. In Canada specifically, it is mandatory that Vitamin D be added to milk and margarine (2µg per 100mL as of 2022 regulations [55]) and as of 2024 regulations, is now permitted (but not mandated) to be added to yogurt and kefir [56]. This has positive implications for bone health and other physiological functions that need calcium due to Vitamin D's essential role in promoting calcium intestinal absorption and subsequent homeostasis [57]. A simplified overview of the nutrient composition of dairy is shown in **Figure 1**.

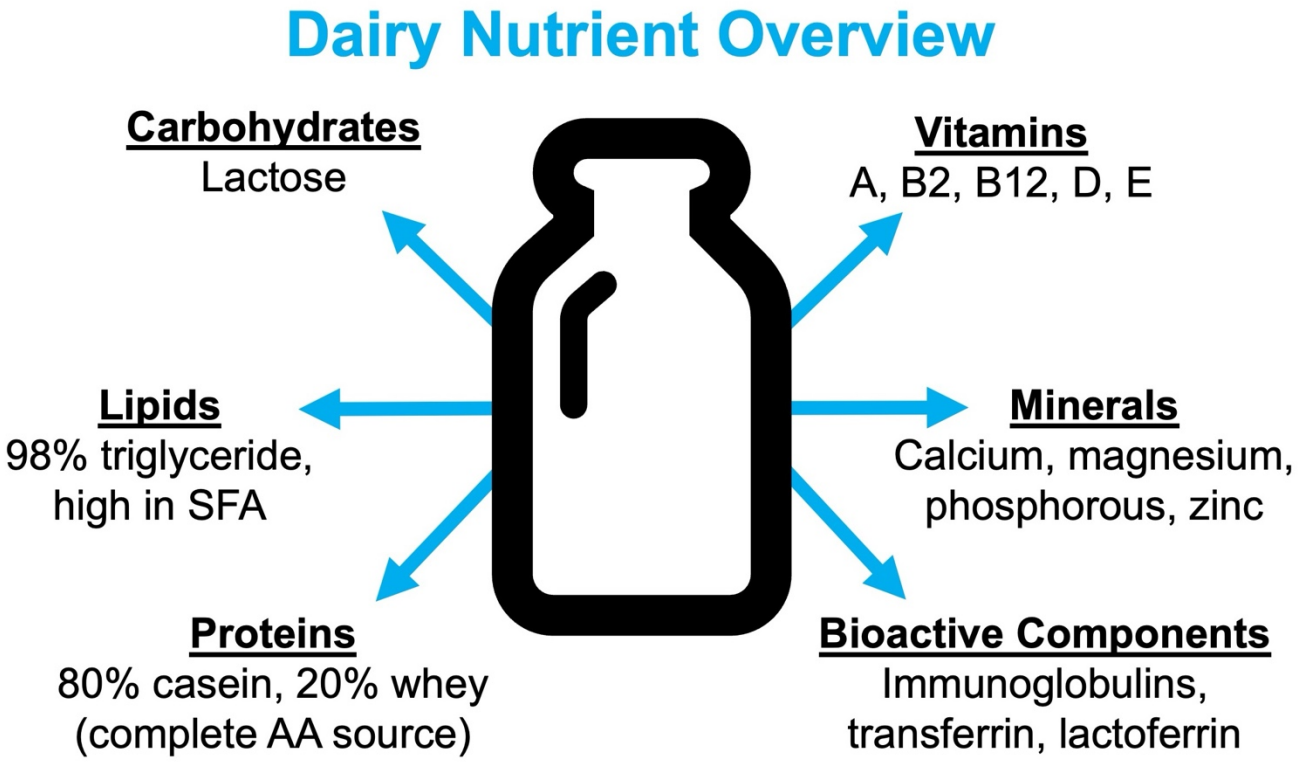


**Figure 1.** Dairy products contain all three macronutrients, as well as vitamins, minerals and unique bioactive components within its wholefood matrix.

## 2.2 Dairy and Bone Health

Considering that milk and dairy products contain many essential nutrients for skeletal maintenance and growth (i.e., protein, calcium, phosphorus, magnesium, manganese, zinc, Vitamin D [7]), a relatively large amount of research has been performed to determine how dairy can influence bone health and osteoporosis risk [58]. Osteoporosis is a disease characterised by deterioration of bone tissue/microarchitecture and reduced bone mass, leading to an increase in the risk of fractures with increased age [59]. Because peak bone mass (i.e., the highest amount of bone mass one will have in their life) is achieved during young adulthood (typically 20-25 years of age [60]), osteoporosis has been referred to by Osteoporosis Canada as a "paediatric disease with geriatric consequences" [61, 62]. Dietary inclusion of dairy products in youth/young adults as a

way to obtain necessary nutrients for skeletal maintenance is thus an important dietary strategy and one focus of this thesis. To date there have been over 70 RCTs (and many other observational studies) investigating the role of dairy products over the lifespan on bone health outcomes, which have recently been comprehensively reviewed by Cheng & Josse (2024) [58].

### 2.2.1 Bone Physiology & Remodelling Overview

The skeletal system is made up of bone which is an essential biomechanical and structural component of the body, affording humans the ability to resist gravity and engage in locomotion. Despite appearing static at the macroscopic level, bone is a dynamic tissue, undergoing continuous regeneration of microdamage and engaging in numerous physiological functions. For example, bone contributes to haematopoiesis, production of mesenchymal stem cells (MSCs) and is the major storage depot of calcium and phosphate in the body [63]. Bone repair or "remodelling" functions by replacing old or damaged bone tissue with new tissue and occurs in both trabecular bone and compact cortical bone. A complete remodelling cycle involves the following five steps: activation, resorption, reversal, formation and mineralization/termination (**Figure 2**) [64].

Remodelling occurs within temporary anatomic structures known as bone remodelling compartments (BRCs), consisting of a canopy of bone lining cells which encapsulates a team of osteoclasts, osteoblasts and osteocytes, known together as the bone (or basic) multicellular unit (BMU) [65, 66]. To maintain homeostasis of bone mass, resorption and formation must be balanced and so several mechanisms have evolved to couple these processes together. First, the BRC serves as an important physical coupling strategy by confining most remodelling factors within a closed compartment (although some can escape into the circulation and be measured) (28). Secondly, cells within the BMU can directly interact with and regulate each other, allowing coupling through autocrine and paracrine signalling. In addition to local regulation, systemic

factors can also regulate bone remodelling, acting through a variety of signalling pathways [67]. Osteocytes, which are dispersed throughout the matrix of the bone, are primarily responsible for orchestrating the remodelling process and make up 90-95% of all bone cells [68]. They originate from osteoblasts that become entombed during bone formation, undergoing both biochemical and morphological transformations [69]. Osteocytes develop dendritic processes which connect them to neighbouring osteocytes and other bone cells, forming a complex intracellular network (lacuno-canalicular network) that facilitates the transport of signalling molecules throughout the bone matrix [70, 71]. Another important attribute of osteocytes is that they can be activated by mechanical stimuli, responding by either converting the mechanical signals into biochemical signals (known as the piezoelectric effect [69]) or by undergoing apoptosis, either of which may initiate formation of the BRC [72]. They respond to loads that are both over (e.g., an exercise stressor) and under the "normal" physiological range, explaining why prolonged bedrest or microgravity reduces bone mineral density (BMD) [73]. The mechanosensitive, interconnected nature of osteocytes makes them ideal for both initiating and regulating bone remodelling [74]. Following osteocyte induced recruitment/activation of the BMU, osteoclasts begin to erode both organic (*via* release of proteases like Tartrate-resistant acid phosphatase type 5b [TRAP-5b] or Cathespin K) and inorganic (using hydrogen ions to create an acidic compartment) bone tissue, forming 40-60μm deep resorption pits known as lacunae [75]. Osteoclasts are large, multinucleated cells that originate from myeloid hematopoietic stem cells (HSCs). These HSCs initially differentiate into monocyte or macrophage progenitors, then to preosteoclasts before finally fusing to become mature osteoclasts within the BRC [64]. Osteoclast formation (i.e., osteoclastogenesis) relies first on the proliferation and survival of preosteoclasts. This is achieved through the binding of a permissive concentration of macrophage colony stimulating factor (M-CSF) to the c-fms

receptor on their cell surface, which stimulates expression of receptor activator of NF-κB (RANK) [76, 77]. The RANK ligand (RANKL) is a member of the tumour necrosis factor (TNF) superfamily and is either expressed as a transmembrane protein or secreted by osteocytes and osteoblasts [77]. This secreted RANKL is able to bind to RANK receptors on the surface of preosteoclasts, leading to the recruitment of adaptor proteins such as the TNF-receptor-associated factor 6 (TRAF-6). Upon binding with TRAF-6, there is an activation of nuclear factor kappa-B (NF-κB) and mitogen-activated kinases (MAPK) pathways, causing gene expression of activator protein-1 (AP1) and nuclear factor of activated T cells and cytoplasmic calcineurindependent 1 (NFATc1), and ultimately resulting in preosteoclast fusion and differentiation into mature osteoclasts [78]. Osteoclastogenesis is also regulated by osteocytes and osteoblasts through the secretion of a paracrine effector molecule called osteoprotegerin (OPG), a soluble decoy receptor that binds to RANKL. OPG reduces osteoclastogenesis by reducing RANK-RANKL binding through competitive inhibition, ultimately leading to reductions in bone resorption [79, 80]. To prevent excessive degradation of surrounding bone, the resorption stage is terminated when osteoclasts either undergo apoptosis or are recycled into osteomorphs *via* fission [64, 81]. During the reversal stage, the lacunae surfaces are prepared for new bone matrix deposition and signals are released to halt resorption and initiate the changeover to formation [64]. It is believed that osteoclasts play a key role in this temporal transition from resorption to formation [80]. Mature osteoclasts express a molecule called ephrinB2 on their membrane which is able to bind with ephrinB4 found on preosteoblasts, and alongside signalling induced by the release of bone matrix breakdown factors (e.g., insulin-like growth factors (IGFs), transforming growth factor β (TGF-β), fibroblast growth factor (FGF)), differentiation of preosteoblasts into mature osteoblasts is initiated and the bone formation phase begins [82, 83]. The bone formation stage predominately

involves osteoblasts, which are large, cuboidal bone cells derived from myeloid MSCs [69]. Mature osteoblast formation (i.e., osteoblastogenesis) is primarily mediated through the canonical Wingless (Wnt)/β-catenin signalling pathway but also involves signalling from bone morphogenetic proteins, hedgehog proteins and notch proteins [64]. Wnt ligands act by binding to a receptor complex consisting of low-density lipoprotein receptor-related protein (LRP) 5 or 6 and one of ten Frizzled molecules [67, 84]. This results in the release and stabilization of cytoplasmic β-catenin, which is then translocated to the nucleus to activate target gene transcription and ultimately stimulates osteoblast differentiation and proliferation [85]. This pathway is negatively regulated by the proteins sclerostin (SOST) and Dickkopf (DKK) 1/2, which bind to co-receptors on LRP 5/6, inhibiting Wnt signalling and thus reducing bone formation [85]. Mature osteoblasts deposit pre-mineralized bone collagen called "osteoid" into the lacunae, which subsequently becomes mineralized and hardened by hydroxyapatite crystals (composed of calcium and phosphate) that get trapped within the tissue [86, 87]. During this mineralization phase, osteoblasts can become imprisoned within the matrix, at which point they undergo three possible fates: 1) transformation into the mechanosensory osteocytes, 2) transformation into bone lining cells, and 3) apoptosis [88, 89]. Osteogenesis is terminated by osteocytes *via* secretion of the Wnt inhibiting proteins SOST and DKK-1, ending the remodelling cycle [64]. Physiological stressors such as exercise are able to influence cellular processes during the bone remodelling cycle, leading to adaptations at the macroscopic level.

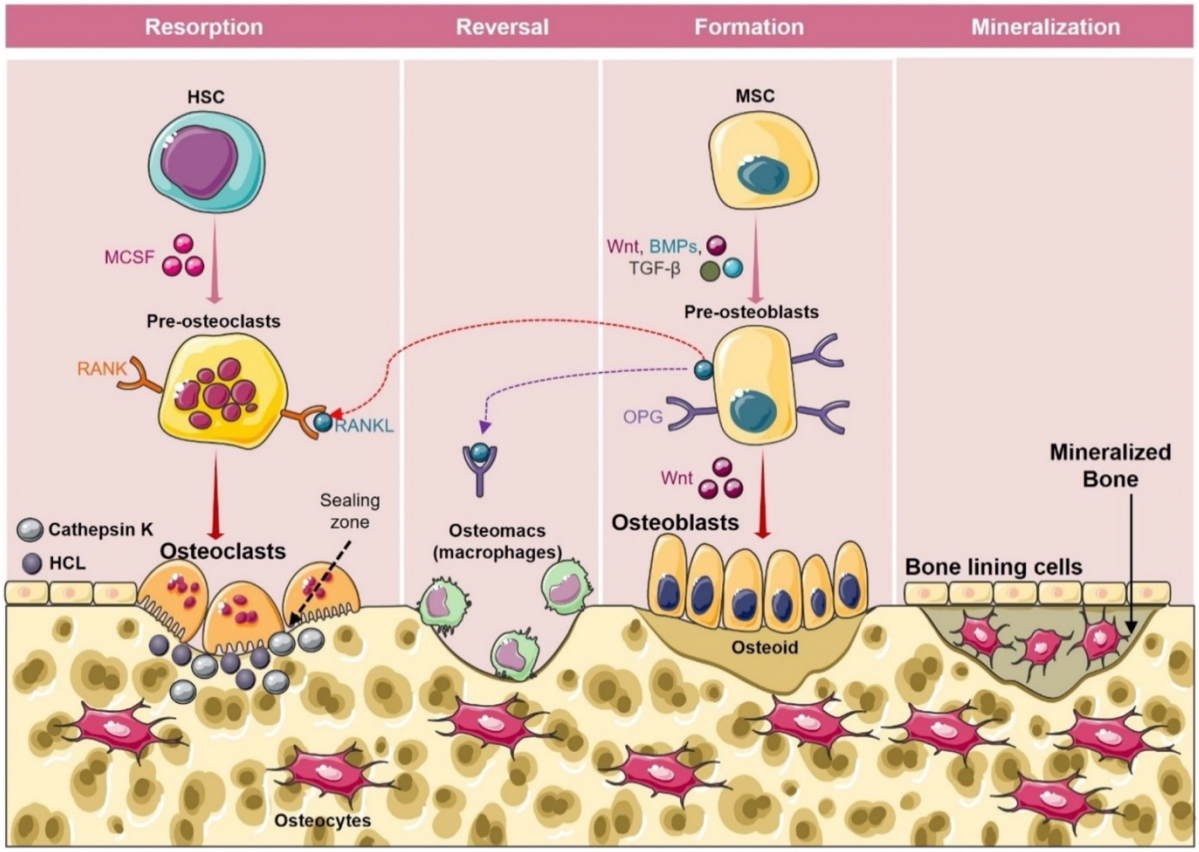


**Figure 2.** Schematic of the bone remodeling cycle which occurs in five phases: 1) Activation; 2) Resorption; 3) Reversal; 4) Formation; 5) Mineralization/termination. Adapted from Bhardwaj et al. (2022) [3].

### 2.2.2 Bone Cellular Responses to Exercise

Bone is responsive to physiological stressors such as exercise, which stimulates remodelling in order to clear old or micro-damaged bone, adapt bone tissue to mechanical loading and maintain the strength and integrity of the skeleton [90]. Bone tissue deformation occurs during exercise, causing mechanosensors (e.g., stretch-activated ion channels, integrins) to change conformation, which subsequently triggers a signalling cascades that induce a biochemical response at the specific site [91, 92]. Osteocytes are a key regulator in the response of bone to exercise due to their specialised ability to detect and respond to mechanical strain [93]. These mechanosensing cells can control bone resorption or formation by modulating the

differentiation of osteoblasts and osteoclasts and by stimulating the expression of OPG [94]. Osteocytes also plays an important role in mediating the anabolic actions of the Wnt/β-catenin pathway, which is activated by mechanical strain and leads to bone formation [95, 96]. These cellular processes are also responsive to systemic circulating changes that occur during exercise. For example, circulating parathyroid hormone (PTH) generated during exercise has been shown to downregulate expression of SOST and upregulate expression of FGF-23 within osteocytes [97]. There is also evidence that exercise can modulate bone remodelling through the OPG/RANKL/RANK signalling pathway, although this has not been consistently observed when measuring these markers in the circulation [98, 99]. In many studies however, it seems that exercise is able to inhibit bone resorption by increasing OPG secretion and/or reducing RANKL secretion [98, 99]. It is also possible that acute inflammatory responses from exercise can play a role in modulating bone remodelling.

### 2.2.3 Bone and Inflammation – Connections & Crosstalk

The emerging field of osteoimmunology has highlighted links and commonalities between the immune and skeletal systems, including shared cellular lineages, transcription factors and receptor targets, that may allow crosstalk and reciprocal regulation [76].

Many cells, but particularly immune cells, express pattern recognition receptors that can recognise molecular motifs from both exogenous pathogens ("pathogen associated molecular patterns" [PAMPs]) and endogenous sources ("damage associated molecular patterns" [DAMPs])[100]. Toll-like receptors (TLRs) are one such receptor, and generally their activation leads to initiation of proinflammatory signalling (e.g., NF-κB pathway) and the release of inflammatory signalling molecules such as cytokines (e.g., TNFs, Interleukins [ILs], and Interferons [IFNs]) which go on to activate and recruit additional leukocytes and cytokines to the

site of injury or infection [100, 101]. The TLR-DAMP/PAMP system is complex, resulting in different outcomes depending upon which PAMP or DAMP is involved, which subtype of TLR they end up binding with, which type of cell that particular TLR is being expressed upon and the maturity of that same cell [100, 101].

In bone, TLR activation within mature osteoclasts and osteoblasts generally results in osteoclastogenesis, which contributes to increased bone resorption [102]. TLR4 has shown to be expressed in cells that directly participate in bone remodelling, and its activation within osteoblasts can lead to the production of inflammatory mediators (e.g. cytokines and chemokines)[103]. A clear negative association between circulating RANKL and RANKL/OPG and BMD has been shown within the literature [104], and accumulating data indicate that immune/inflammatory cells can directly impact bone metabolism by mediating RANK-RANKL signalling [77]. For example, the proinflammatory cytokines IL-1, IL-6, IL-15 and IL-17 have shown to accelerate bone resorption by increasing RANKL secretion from osteoblasts/osteocytes, resulting in greater osteoclastogenesis [77]. In addition, certain immune cell subtypes, including monocytes, neutrophils, dendritic cells (DCs) and B and T cell lymphocytes can produce RANKL themselves and therefore may directly contribute to increased RANK-RANKL signalling [77]. IL-6 has been implicated in the pathogenesis of several bone diseases and may also increase osteoclastic resorption *via* increased osteoclast RANK expression and preosteoclast differentiation [105, 106]. One of the most potent catabolic cytokines in terms of bone is TNF-α, which has potent synergistic effects on the osteoclastogenic potential of RANKL [107]. This occurs due to an increased differentiation of HSCs/macrophages into preosteoclasts (thus increasing the size of the preosteoclast pool) as well as by increasing expression of the RANK receptor on these preosteoclasts [108]. TNF-α can also alter bone formation by suppressing osteoblast

differentiation through various mechanisms [108], such as by increasing production of DKK1 and SOST, which inhibits the Wnt signalling pathway and ultimately leads to a reduction in osteoblastogenesis [108, 109]. Increased ROS can induce inflammation through upstream activation of signalling pathways like the NOD-like receptor containing pyrin domain 3 (NLRP3) inflammasome [110], but they have also been shown to be involved in RANKL-mediated osteoclastogenesis [111]. For example, Ashtar et al. (2020) showed that blunting excessive ROS production (using the xanthine oxidase inhibitor, febuxostat) reduced both RANKL and osteoclast formation, leading to reduced bone resorption [112].

In contrast, generally anti-inflammatory cytokines such as IL-4, IL-10, IL-13 and IFN-α, -β & -γ can inhibit osteoclastogenesis either directly or indirectly by blocking RANKL signalling [77, 113]. These interactions are complex and not fully elucidated. As an example, while IFN-γ is generally considered to be a proinflammatory cytokine, it can both inhibit and promote osteoclast differentiation depending upon the stage of osteoclast formation [114]. Despite the gaps in our knowledge regarding the complex function of these immune mediators, it is clear that increases in proinflammatory cytokines ultimately tip the balance of the RANKL-RANK-OPG bone metabolic pathway towards greater osteoclastogenesis and enhanced bone breakdown.

Regarding bone, exercise is well established to be osteogenic, seeming to directly or indirectly act on almost every type of bone cell [99, 115]. In addition to the mechanical stimuli of exercise on bone, changes in circulating cytokines from skeletal muscle or systemic immune cells that occur following exercise could also mediate RANK-RANKL signalling. As these cytokines enter bone from systemic circulation (or *via* paracrine/autocrine release of local immune cells), a cytokine-mediated increase in RANK-RANKL binding could stimulate osteoclastogenesis and lead to elevations in bone resorption. However, it is also entirely possible that the mechanically

stimulated osteogenic effects of exercise (e.g., increased OPG and decreased RANKL) provides a more potent signal, ensuring an overall shift towards bone formation. Another thing to consider is that even if post-exercise inflammation may contribute to osteoclastogenesis and bone degradation initially, this may serve a purpose and be ultimately beneficial. For example, the transient elevations in bone resorption may help to ensure that all the damaged bone is actually removed, paving the way for new bone formation and improving long-term tensile strength and/or microarchitecture of the bone by ensuring that there are no underlying vulnerabilities remaining. Blunting acute exercise-induced inflammation (and subsequently not allowing for adequate recovery) has been shown to negatively impact long-term function/regeneration of muscle [116], so it is plausible that a similar outcome could occur within bone. Indeed, it is already known that some degree of inflammation is essential for bone formation, evidenced by the fact that TNF deficient mice suffer from impaired fracture healing [117, 118]. Despite these considerations, our current understanding is that mitigating bone resorption and/or enhancing bone formation are ideal outcomes in order to shift bone into a more anabolic state.

While we know that there are relationships between skeletal and inflammatory systems (at least in cell models), it is unclear how these relate within the context of an exercise bout. Investigating these relationships during/following both acute exercise and prolonged exercise training programs is warranted, as it may provide insight into how to optimise training adaptations or inform recommendations for clinical populations that may want to engage in exercise.

### 2.2.4 Bone Measurement Techniques

Static assessment of bone status can be assessed by measurement of bone mineral density (BMD), bone mineral content (BMC) and/or bone microarchitecture/morphometry, which typically use scanning technologies such as dual-energy x-ray absorptiometry (DXA), quantitative

computed tomography (QCT) or nuclear magnetic resonance (NMR) [119]. Other methods include mechanical testing of bone properties by assessing uniaxial compression or using reference point micro-indentation (RPI), or by evaluating degree of bone mineralization (DBM), turnover rate or organic composition of bone tissue (rarely done experimentally in humans) [119]. However, it is worth noting that there are limitations to these measurement techniques, including the requirement of specialised imaging equipment and radiation training, the inability to readily obtain human bone biopsies in research contexts, as well as the duration required to detect change over time in BMD due to lack of imaging technique sensitivity coupled with relatively slower bone turnover rates (e.g., compared to muscle) [119-121].

Dynamic changes in bone turnover (i.e., resorption and formation) can also be evaluated by measuring various bone biomarkers from blood samples (summarized in **Figure 3**), which can be useful for gaining insight into responses following acute stressors and to measure changes in bone metabolism over shorter time periods versus the months or years required to measure static changes in BMD [122]. Bone resorption markers include C-terminal telopeptide of type I collagen (CTX), N-terminal telopeptide of type I collagen (NTX), cross-linked C-terminal telopeptide of type I collagen (ICTP), hydoxyproline (hyp), hydroxylysine (hyl), pyridinoline (PDL) and deoxypyridinoline (DPD) which are derived from the hydrolysis of type 1 collagen within bone, and bone sialoprotein (BSP) and osteopontin (OP) which are phosphorylated glycoproteins found within the extracellular bone matrix. When bone resorption occurs, these cleaved fragments enter the blood/urine for excretion at which point their concentrations can be measured. The bone formation markers procollagen type I N-propeptide (P1NP) and procollagen type I C-terminal propeptide (P1CP) are collagen precursor components that are released into the circulation following collagen deposition, and so increased concentrations within the blood

suggest increased bone matrix formation [123]. Osteocalcin (OC) is produced by osteoblasts and plays an important role for both bone mineralization and calcium homeostasis as well as metabolism and can be measured within the circulation to provide insight into the magnitude of these formative processes [123]. Enzymes released during resorption (e.g., TRAP-5b and Cathespin K) or formation (e.g., bone-specific alkaline phosphatase [BALP]) can also be measured to provide insight into the response of bone to acute stimuli/stressors. In addition, biomarkers involved with regulating bone remodelling processes can also be measured, and include RANKL, OPG, DKK-1 and SOST. RANKL increases the number of mature osteoclasts, and thus elevated serum concentrations are indicative of increased potential for bone resorption [123]. OPG is a decoy-receptor of RANKL, so increased concentrations of this marker suggest that osteoclast activity and thus bone resorption is being attenuated [124]. To more appropriately infer osteoclast activity, both biomarkers should ideally be measured from the same sample and considered in relation to each other (i.e., OPG:RANKL ratio)[64, 69]. Finally, SOST and DKK-1 inhibit the Wnt signalling pathway, reducing osteoblast differentiation and proliferation, therefore serum concentrations of these biomarkers can be considered to reflect inhibition of bone formation [123]. There are a variety of bone biomarkers that can be chosen, and factors that may determine which to use include participant cohort/characteristics, mechanisms of interest, availability and cost of kits, and convention of the field (to allow for comparison between similar studies).

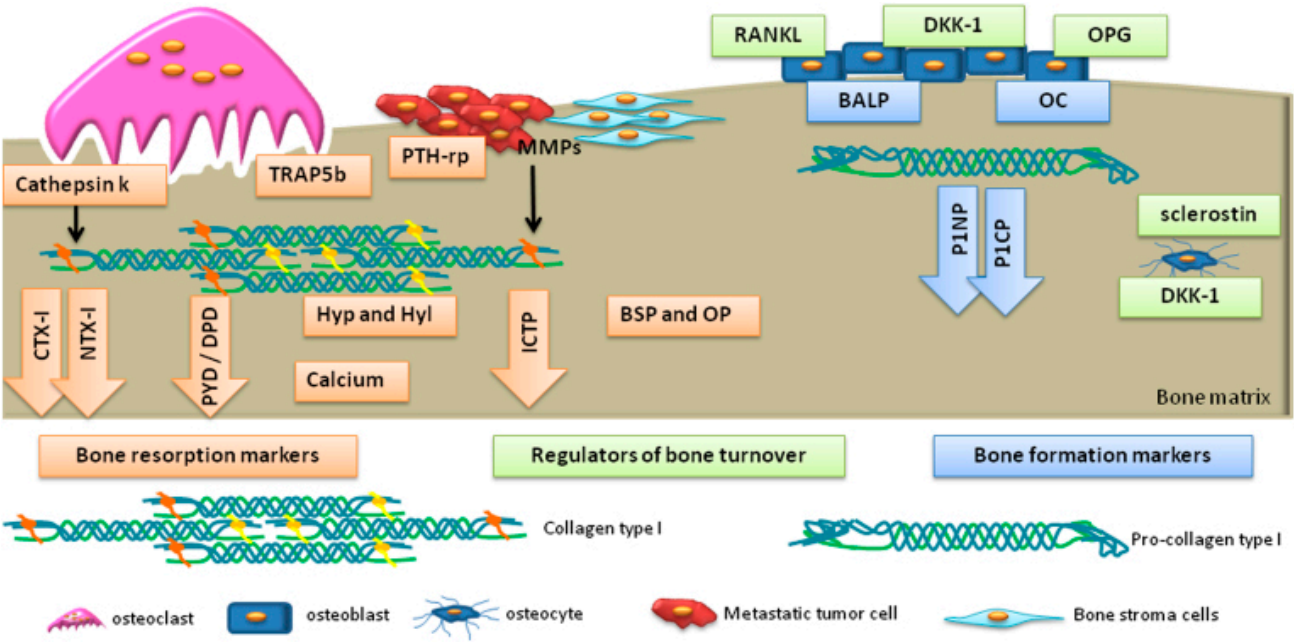


**Figure 3.** Biochemical markers of bone turnover. Blue boxes/arrows represent bone formation markers: bone-specific alkaline phosphatase (BALP); osteocalcin (OC); propeptides of type I procollagen (P1NP and P1CP). Orange boxes/arrows represent bone resorption markers: pyridinoline (PYD); deoxypyridoline (DPD); carboxy-terminal crosslinked telopeptide of collagen type I (CTX-I); amino-terminal crosslinked telopeptide of type I collagen (NTX-I); Hydroxyproline (Hyp); hydroxylysine (Hyl); bone sialoprotein (BSP); osteopontin (OP); tartrate-resistant acid phosphatase 5b (TRAP5b); cathepsin K. Green boxes represent regulators of bone turnover: receptor activator of NF-κB ligand (RANKL), osteoprotegerin (OPG), dickkopf-1 (DDK-1) and sclerostin. Adapted from Ferreria et al. (2015) [4].

While not related to bone turnover directly, there are several other important hormones and serum markers that are often measured in the field of bone research. Parathyroid hormone (PTH) is a peptide hormone secreted in response to low levels of serum calcium or calcitriol (circulating form of Vitamin D), initiating resorption of bone in order to rapidly liberate calcium to raise plasma concentrations [125]. It works by activating the PTH1R receptor on osteoblasts, causing them to release RANKL, which then drives osteoclastogenesis and subsequent bone resorption [126]. While continuous administration of PTH (or hyperparathyroidism) leads to

significant bone loss, when provided intermittently, PTH has the paradoxical ability to stimulate bone formation, and has been used pharmacologically to treat osteoporosis [126, 127]. Despite this finding, the primary function of PTH is to maintain circulating calcium, usually by leeching it from bone [126]. Sex hormones such as estrogens and androgens also play key roles in bone growth and maintenance. During puberty there is an increase in sex steroid production that results in increased bone size in both males and females but occurs to a greater extent in males [128]. When sex hormones decline or are depleted, such as during menopause (or ovariectomy or hypogonadal males), there is a significant acceleration of bones loss [129]. Mechanisms through which sex hormones such as estrogen protect against bone loss include downregulation of cytokine production leading to reduced osteoclast number and activity, decreases in TGF-β resulting in a decreased osteoclast number and activity and increased osteoclast apoptosis, and increased OPG [129]. Finally, Vitamin D is a micronutrient that is important for maintaining bone health. Vitamin $D_2$ or $D_3$ can be derived from exposure to UV rays from sunlight or consumed from foods or supplements. Vitamin D then enters the liver and is converted to 25-hydroxyvitamin D (25(OH)D), the major circulating form of vitamin D (used to determine Vitamin D status) [130]. Vitamin D is essential for calcium uptake and absorption within the small intestine, so deficiencies in this nutrient can lead to reduced BMD and increased fracture risk [130]. This is of particular concern because the prevalence of Vitamin D deficiency (25(OH)D < 20 ng/mL) is high, particularly in places at higher latitudes such as northern Europe where prevalence has been shown to range from 28% to 100% of healthy adults [130, 131]. In Canada, the prevalence of Vitamin D inadequacy was shown to be 19.0% and risk of deficiency was 8.4% [132]. These risk factors are known to be affected by lifestyle, with risk of inadequacy

increased by 2.3x in individuals with BMI >30 kg/m$^2$ (vs. individuals with < 25kg/m$^2$) and 1.41x in individuals that consumed no cow's milk (vs. individuals who consumed 1 serving/day) [132].

### 2.2.5 Bone Health and Dairy Consumption – Clinical Evidence

Dairy products generally contain high amounts of both calcium and protein, which are required for both the organic (collagen protein for bone extracellular matrix) and inorganic (calcium for hydroxyapatite) bone components [133]. The effectiveness of dairy product consumption alone to support/improve bone outcomes are dependent on a variety of factors, with age and baseline calcium intake being the most significant [58]. There have been 6 meta-analyses of RCTs performed to assess outcomes of dairy intake and bone, with most stratified by age. The two meta-analyses assessing outcomes in youth and young adults both showed that dairy intake improved bone mineral mass outcomes such as BMD, as well as biochemical outcomes related to bone health [134, 135]. A meta-analysis in adults indicated that milk supplementation resulted in small increases in BMD at the hip and lumbar spine, as well as reductions in some bone turnover markers (P1NP, CTX, NTX) [136]. The two meta-analyses of RCTs performed in older adults indicated that increased dairy intake may result in small increases in BMD but that they are not significant enough to reduce the risk of bone fractures in this cohort [137, 138]. Finally, the meta-analysis by Ma et al. (2013) analysed results from 11 RCTs (2397 total subjects, not age stratified) and found that milk intake improved BMC as well as concentrations of NTX and OC bone biomarkers [139]. Together, these findings indicate that dairy intake can benefit bone health across the lifespan, although the effects sizes are relatively small.

### 2.2.6 Bone Responses to Combined Dairy Intake & Exercise – Clinical Evidence

Physical activity and exercise are other modifiable lifestyle factors (beyond nutrition) that provide an osteogenic stimulus, with chronic increases generally leading to enhanced bone adaptations [58]. Furthermore, activities that load the bone (e.g., resistance and impact exercise such as landing, jumping, running etc.) may be superior to non-load bearing activities (e.g., swimming, walking, cycling) for stimulating bone adaptations because they can more effectively induce mechanical strain and microcracks to upregulate remodelling [140, 141]. It therefore seems plausible that combining the beneficial effects of exercise to stimulate remodelling, along with the consumption of dairy enabling the provision of key bone supporting nutrients such as calcium and protein may be an optimal strategy in terms of facilitating bone adaptations and long-term outcomes.

To investigate the synergistic effects of these stimuli, there have been 18 RCTs performed in a range of age groups (9 in youth, 6 in young adults and 3 in older adults), a few of which will be briefly summarised [58]. Volek et al. (2003) found that whole body BMD was increased in adolescent males consuming milk compared to fruit juice after 12-weeks of concomitant resistance exercise [142]. Gomez et al. (2021) found superior improvements in whole body, trunk and pelvis BMD with resistance exercise combined with milk intake compared to either alone, or a non-intervention control in adolescent females [143]. Our lab group performed a study in adolescent females with OW/OB in which they completed a combination of exercise types (3x/week, resistance, plyometric, aerobic) alongside either 3-4 servings/day of mixed dairy, or 0-1 servings/day of dairy for 12-weeks [12]. We found significant reductions in the bone resorption marker CTX, which suggested that higher dairy intake with exercise may support bone adaptations by blunting bone catabolism compared to low dairy intake [12]. In adults, we have done three studies showing interactions between dairy intake and resistance exercise on bone outcomes. In

Josse et al. (2010), adult females completed resistance exercise 5x/week with either post-exercise milk or carbohydrate consumption, finding significantly increased serum 25(OH)D and reduced PTH compared to the CHO group, with no differences in OC, BAP or CTX [144]. In Josse et al. (2012), 90 premenopausal females with OW/OB completed exercise training for 16-weeks and were split into 3 groups that differed by dairy and protein intake [13]. The bone resorption markers NTX, CTX and DPD (and bone formation marker OC) increased in the adequate protein, low dairy group whereas in the high-protein, high-dairy group, P1NP and OC increased with no change in resorption marker concentrations [13]. In Bridge et al. (2020), adult males completed 12-weeks of resistance training while supplementing with either Greek yogurt or carbohydrate pudding, and found that CTX (bone resorption marker) was attenuated after 1-week in the Greek yogurt group only, and that P1NP (bone formation marker) was greater in the Greek yogurt group only after 12-weeks [11]. For older male and female adults (>50 years old), Huschtscha et al. (2021) found no change in BMD after 12-weeks of resistance exercise and 2 servings of milk/day [145]. Lee et al. (2017) compared 12-weeks of daily milk consumption and whole-body vibration exercise to a no intervention control within a cohort of older females (>70 years old) and found that BMD decreased in the control group but did not change in the milk group [146]. Overall, these findings suggest that short-to-medium term dairy consumption and exercise are superior to either alone for enhancing bone outcomes (both BMD and bone turnover markers), however less is known about the acute response of bone to combined exercise and dairy consumption.

Dolan and colleagues have compiled and reviewed studies relating to acute bone biomarker responses to both exercise [147] and exercise combined with nutrition [120, 148]. Only five studies to date have assessed the acute bone marker response to exercise and dairy (or dairy components) – three using whey protein and two using wholefood dairy. Townsend and colleagues (2017)

evaluated combined carbohydrate and whey protein consumption on bone biomarker responses to exhaustive treadmill running in trained endurance runners and found a reduction in bone resorption and an increase in bone formation in the protein containing group compared to a non-energy control [149]. Next, Theocharidis et al. (2020) compared post-exercise consumption of whey protein to carbohydrate and water after an intense bout of swimming in adolescent male and female athletes, finding that CTX was significantly reduced 24h later in the whey group alone [150]. Aussieker et al. (2023) compared post-resistance exercise consumption of whey, collagen and a non-energy control in young adult male and females and found that CTX at 2h post-exercise was reduced in both the whey and collagen group, but not the control group [151]. For wholefood dairy, Haakonssen and colleagues (2015) compared bone marker and PTH changes between pre-exercise (90 minute cycling bout) consumption of a dairy containing meal (rolled oats cooked with calcium fortified milk, yogurt and additional milk) to a low calcium control meal (rolled oats cooked in water, fruit and nuts) in well-trained female cyclists [152]. They assessed circulating responses for up to 190 minutes post-exercise and found a reduction in CTX in the group that consumed the dairy containing meal immediately post, 40-minutes post and 190-minutes post-exercise [152]. Finally, our group compared the effect of 12-weeks of habitually higher (4 servings/day) vs lower (0-2 servings/day) dairy intake and resistance exercise on bone biomarker responses to an acute post-intervention bout of plyometric exercise in adolescent females, and found a decrease in the relative RANKL response in the higher dairy group [153]. Not only are data in this area sparse, but it is also a newer area of interest, with all five studies carried out within the last 10 years, and thus significant knowledge gaps remain. Specifically, there has not yet been a well-controlled study evaluating bone responses to a highly osteogenic exercise modality (e.g., high-impact, high-load) in combination with wholefood dairy consumption. Furthermore, the only study thus far that

has assessed acute dairy intake and exercise [152], only measured these bone markers up to 190-minutes post-exercise, however our previous results [153] suggest that important insight may be gained by measuring these markers at later timepoints (24-48h post exercise). Finally, assessment of a range of biomarkers and osteokines (signalling molecules) involved with bone turnover and remodelling (RANKL, OPG, SOST, etc.) may allow us to better understand acute bone responses following the physiological stress of high-impact exercise.

## 2.3 Dairy, Postprandial Responses and Chronic Disease Risk

### 2.3.1 Digestion, Absorption and Postprandial Responses to Dairy

Consumption of foods and/or nutrients results in a cascade of physiological responses throughout the digestive system and beyond, involving various enzymes, transporters and signalling molecules [154]. In addition to the specific nutrients involved, the physicochemical structure of what is consumed can affect digestion kinetics and alter nutrient bioavailability, absorption and downstream physiological responses [155]. To appropriately evaluate the health benefits of wholefoods, the individual components, how they may interact with each other, as well as the physicochemical structure of the food should also be considered. There is evidence that the density and composition of nutrients within dairy products as well as the markedly different WFM properties between different dairy products can significantly impact both acute and chronic physiological responses to their consumption, so it is important to study the physiological effects of consuming multiple forms either together within the diet, or in comparison to one another.

Milk proteins are considered high quality because they contain all essential amino acids (EAAs) in adequate quantities, they are particularly high in branched chain AAs (BCAA) such as leucine, and they also have a high digestibility (~95%) [155]. The main proteins within dairy foods

are whey and casein, and their molecular and physiochemical properties can affect bioavailability of the AAs and other nutrients they contain. Caseins are typically more slowly digested/absorbed because they coagulate more easily within the gastric conditions of low pH, whereas whey proteins are more rapidly digested because they remain relatively soluble [155]. Dairy products with different proportions of these proteins can therefore induce differential responses. For example, a recent study from our group found greater postprandial peak leucine and BCAA responses over 4 hours within the circulation following Greek yogurt compared to milk consumption [156]. This has implications for tissues such as skeletal muscle, as well as other systems throughout the body. A study by Boirie et al. (1997) showed that the faster plasma AA appearance that occurs with whey protein can rapidly increase whole body protein synthesis, but that it did not affect whole body protein breakdown [33]. In contrast, casein consumption resulted in a slower, more prolonged release of AAs that inhibited whole body protein breakdown, as well as increased whole body protein synthesis, but to a lesser extent than whey [33]. Over time, these acute effects of casein and/or whey consumption may result in different adaptations or be suitable for different scenarios. For example, whey protein will stimulate muscle protein synthesis to the greatest degree following an exercise bout so may be superior when rapid recovery is required [157, 158], whereas consumption of the slower digesting casein may be favored when the period until the next feeding opportunity is prolonged (e.g. overnight) [159]. Wholefood dairy provides both these fast and slow digesting proteins within the same bolus and so may be able to provide essential nutrients and thus modulate physiological responses over both the immediate and later postprandial period.

Dairy fat consumption can also differentially alter blood lipid and other metabolic responses (glucose, insulin, lipid etc.) compared to non-dairy foods, or between different dairy products [160]. Masson and colleagues (2011) found that 50g of butter (dairy fat high in SFA)

delayed postprandial plasma TG peak time compared to 50g of sunflower oil (vegetable oil high in n-6 polyunsaturated fatty acids [PUFA]), but that both meals yielded equivalent TG incremental area under the curve (iAUC) and peak concentration over the 8h postprandial period [161]. Bonham et al. (2013) compared two high fat breakfast meals composed of either dairy-based or vegetable oil-based foods, and found that postprandial chylomicron TGs were reduced after the dairy fat-based meal compared to the vegetable oil-based meal, but that there were no differences in total postprandial plasma TGs between test meals [162]. Research has also been performed to compare postprandial lipemia between different dairy products. Clemente and colleagues (2003) compared ingestion of isoenergetic meals including milk, butter or mozzarella cheese in males with T2D, and observed a delay in the TG peak after ingestion of the butter-based meal, possibly due to smaller fat globules in milk and cheese [163]. This delay in TG peak following butter consumption compared to milk has also been observed in healthy participants [164]. Another possible explanation is due to the effect of calcium on dietary fat absorption. Lorenzen et al. (2007) compared test meals containing high, medium and low dairy calcium as well as one test meal with high calcium from a calcium carbonate supplement [165]. They found that the increased calcium intake from dairy products attenuated postprandial lipidemia, and that this was most likely due to reduced fat absorption [165]. Hansson et al. (2019) compared sour cream, whipped cream, cheese and butter and found that sour cream increased TG iAUC compared to the other dairy products [166]. Drouin-Chartier et al. (2017) compared ingestion of 33g of fat from either cheddar, cream cheese or butter that were incorporated into macronutrient matched meals, finding an elevated response of TG at 2h in the cream cheese compared to butter, and lower at 6h compared to cheddar [167]. Tholstrup and colleagues (2004) compared both fasting and postprandial blood responses to isoenergetic amounts of milk, cheese and butter [168]. They found that fasting LDL and TC

concentrations were higher following 3-weeks of dairy fat consumed from butter, and that the postprandial glucose response was elevated in the cheese compared to the milk dietary arm [168]. Despite these observations, contrasting results have also been observed in the literature. Schmid and colleagues (2015) evaluated the postprandial metabolic responses to high fat meals both with and without the inclusion of a mixture of dairy products (full fat milk, cheese and butter) in healthy males and found no differences between the lipid responses to these meals [169]. It is possible that the combination of dairy products that Schmid et al. provided altered the postprandial response compared to an individual dairy food which were used in the other studies. Despite this finding, it seems that in general, both dairy fat composition and dairy WFM structures can influence the postprandial blood lipid response [170].

The inclusion of dairy constituents either prior to or as part of high fat containing meals has also been shown to modulate postprandial responses. A study by Mortensen et al. (2009) compared the effect of consuming different proteins (whey, casein, gluten, cod) prior to a high fat meal challenge (HFMC), and found that the iAUC of TG, FFA and the glucose response were all supressed to the greatest degree by pre-meal whey protein consumption [171]. Akhavan and colleagues (2010) also observed that pre-meal whey consumption was able to reduce postprandial glucose and insulin responses following a HFMC [172]. Furthermore, a study performed by McDonald and colleagues (2019) in prediabetic adults compared postprandial responses between 75g of glucose dissolved in either water, high-fat or low-fat milk [173]. They found that postprandial circulating glucose was lower in the dairy conditions and concluded that dairy milk (regardless of fat content) can attenuate postprandial hyperglycemia [173]. Mariotti and colleagues (2015) compared the addition of whey or casein to a high fat meal on TG responses in overweight men and found that casein reduced postprandial TG and plasma chylomicrons compared to whey

[174]. Demmer et al. (2016) compared postprandial metabolic responses to a HFMC composed of palm oil both with and without an added dairy fraction rich in milk fat globule membrane (MFGM) in individuals with OW/OB, and found that the addition of MFGM reduced postprandial TC, LDL & insulin [17].

When consumed repeatedly/consistently as part of the diet, there can also be differential effects of consuming different dairy products on blood lipids. Feeney et al. (2018) showed that 6-weeks of 40g of dietary fat consumed from cheese compared to butter resulted in significantly lower fasted TC and LDL [175], and Hjerpsted and colleagues (2011) found similar results, in which 6-weeks of dietary fat consumed from cheese compared to butter resulted in lower TC, LDL, and high-density lipoprotein (HDL) concentrations [176]. They also observed that cheese intake did not increase LDL concentrations compared to the habitual diet of participants [176]. Other studies have also shown differences in LDL concentrations between increased dietary fat consumed from either cheese or butter [177, 178]. These findings have implications for longer term health and wellbeing when evaluating the consistent consumption of dairy foods within the diet.

### 2.3.2 Dairy and Chronic Disease

Despite the capacity to support a balanced diet from the multitude of essential nutrients that dairy products contain [179], they have sometimes been perceived negatively and assumed to be associated with an increased risk of developing various non-communicable diseases (e.g., obesity, cardiometabolic disease) [180]. Indeed, considering that some dairy products contain a relatively high level of SFA [168], and that increased dietary SFA intake has been associated with higher circulating LDL levels and a subsequently increased risk of developing cardiovascular disease (CVD) [181, 182], it is understandable how this sentiment developed. The link between

elevated LDL and CVD risk has been contended however and in some parts of the world, a high saturated fat intake is not associated with increased CVD mortality [183]. Furthermore, the associations between health and SFA intake may be food specific. For example, after adjusting for demographics, lifestyle and dietary confounders, de Oliveira Otto et al. (2012) found that a higher SFA from dairy was associated with reductions in CVD risk whereas higher SFA intake from meat was associated with reduced CVD risk [184].

While there have on occasion been individual studies that have found negative health effects associated with consuming dairy [185, 186], when the literature has been collated and reviewed together, increased dairy intake is usually associated with either neutral or beneficial effects on chronic disease incidence and severity. This epidemiological phenomenon is also sometimes referred to as the "French Paradox", and some nutrient and/or combination of nutrients within dairy has been implicated to reduce disease risk [187, 188]. Elwood and colleagues (2010) performed a meta-analysis on prospective cohort studies and found that the relative risk in disease outcomes for subjects with the highest dairy consumption compared to those with the lowest were: 0.87 for all-cause deaths, 0.92 for ischaemic heart disease, 0.79 for stroke and 0.85 for type 2 diabetes (T2D) incidence [189]. Other reviews evaluating the impact of dairy on cardiometabolic disease, including CVD, coronary heart disease, hypertension, stroke and T2D, also found no negative associations [10, 190]. In fact, depending on the specific disease and dairy type, many of the reviewed associations were instead positive, and suggested that dairy consumption was beneficial for health [10, 190]. For example, the research by de Goede and colleagues (2016) identified an inverse association between both milk and cheese on stroke risk, with maximal risk reductions occurring with around 125 g/day for milk and from 25 g/day onwards for cheese [191]. In addition to cardiometabolic disease, evidence suggests that milk and dairy products may protect

against colorectal, bladder, gastric, and breast cancer and that it does not seem to be associated with the risk of developing pancreatic, ovarian, or lung cancer (evidence for prostate cancer risk is inconsistent) [180]. Overall, despite providing increased amounts of SFAs, there does not appear to be any negative effects of dairy consumption (irrespective of fat content) on either cardiometabolic variables (e.g., lipid-related risk factors, blood pressure, insulin resistance, vascular function) or CVD risk [10, 192].

### 2.3.3 Mechanisms of Dairy's Modulation of Lipid Responses and Chronic Disease

Considering the beneficial effects consistently observed between dairy intake, metabolic health and chronic disease, research has been done to attempt to understand the underlying mechanisms. Firstly, calcium (especially dairy calcium) has the ability to bind with FAs in the intestines to form insoluble soaps, which seem to be able to reduce fat absorption into the body [176, 193]. A systematic review and meta-analyse (SRMA) by Christensen et al. (2009) estimated that estimated that increasing dairy calcium intake by 1241mg/day increased the faecal fat excretion by 5.2g day [194]. This reduction in dietary fat absorption is likely contributing to the reductions in circulating lipids. Soerensen and colleagues (2014) compared 2-weeks of either a non-dairy control, a milk containing meal and a cheese containing meal (the dairy conditions having similar calcium content) [195]. They found increased faecal fat excretion in both the milk and cheese condition compared to the control (with no differences between the dairy conditions), and that it correlated with the change in circulating LDL concentrations [195]. Lorenzen et al. (2011) also found a beneficial effect of dairy calcium on circulating lipids, observing that when fat content of the diet increased, increased dairy calcium intake attenuated TC and LDL elevations and did not change HDL concentrations [196]. Other possible explanations may be related to the differences in physical structure of SFA delivery [197]. Butter is a water-in-oil emulsion whereas

cheese is a fermented product in which the fat is contained within milk fat globules, and thus its composition is more comparable to milk (despite the higher SFA content) [197, 198]. Fermented dairy products including yogurt and cheese also contain bacteria, which can produce short-chain FAs (e.g., acetate, propionate, and butyrate) and bioactive peptides within the intestine, the latter of which have shown to inhibit angiotensin-I-converting enzyme (ACE) which regulates blood pressure [197, 199]. Finally, dairy foods and components within them may be able to attenuate oxidative stress and inflammation, which will be discussed further in the next section.

Together, the results from these studies suggest that dairy consumption can have beneficial effects on both postprandial and longer-term lipid/metabolic marker concentrations, which may contribute toward the positive health effects and reduced disease risk that is generally also associated with increased dairy product consumption. At present however, it is not yet entirely clear how dairy is able to influence such a diverse range of conditions, and further work is needed to elucidate the underlying mechanisms responsible. Furthermore, the majority of studies to date have only assessed the effects of consuming a single dairy food, investigating either acute postprandial effects, or outcomes of adding it to the habitual diet. It therefore may be of interest to study the effects of consuming multiple types of dairy foods concomitantly within the diet to more conclusively evaluate physiological responses and long-term health outcomes.

## 2.4 Dairy, Oxidative Stress and Inflammation

In recent decades, research to identify the underlying mechanisms and relevant pathways through which dairy is modulating physiological processes and disease risk factors has begun to accumulate. One hypothesis is that milk and dairy products are able to modulate inflammatory

and/or antioxidant processes throughout the body due to antioxidant and anti-inflammatory properties of nutrients that they contain [2, 6, 200].

### 2.4.1 An Overview of Oxidative Stress and Glutathione

Reactive oxygen species (ROS) are partially reduced oxygen metabolites that possess strong oxidizing capabilities and include molecules such as the superoxide anion ($O2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide ($H_2O_2$)[201]. They are continually produced within the body from biological processes such as cellular respiration (e.g., oxidative metabolism), immune activation (e.g., the NADPH oxidase enzyme found within phagocytes can produce and releases ROS onto phagocytosed objects to destroy them [202]) and cell stress [203]. High levels of ROS are deleterious because they can oxidize protein and lipid components (rendering them non-functional) and may also damage DNA [201, 204]. It is worth noting that ROS at appropriate physiological levels are not necessarily adverse. They also act pleiotropically as signalling molecules that are critically involved in initiating and regulating vital biological processes such as cell proliferation, differentiation, migration and angiogenesis [205]. Essentially, a spectrum exists where at the lower end, physiological ROS concentrations are considered beneficial ("oxidative eustress") and at the higher end, supraphysiological ROS concentrations (termed "oxidative distress" or "oxidative stress") may lead to reversible and irreversible damage to biomolecules and contribute to pathological health states [205]. Indeed, elevated oxidative stress has been linked to cardiometabolic related impairments and diseases such as atherosclerosis, hypertension, T2D and CVD and the development of insulin resistance within adipose and peripheral tissues, primarily by affecting various points in insulin receptor signal transduction [49, 206-208]. Elevations in visceral fat stores have also been shown to increase ROS production by adipocytes, which has shown to also increase expression and secretion of inflammatory adipokines and cytokines [209-211].

Fortunately, the body has developed several defense mechanisms to scavenge and detoxify ROS in order to protect itself against the damaging effects of a highly oxidised microenvironment. The primary intracellular endogenous antioxidant mechanisms include the superoxide dismutase (SOD), catalase and glutathione systems [212]. The glutathione system functions by using glutathione peroxidase (GPx) enzymes to reduce $H_2O_2$ (a prominent ROS) to water by gathering reducing equivalents from glutathione [213]. Glutathione can thus exist in a reduced form (GSH) and upon exposure to ROS, an oxidised form (GSSG). GSH is the most significant contributor to the pool of intracellular thiol (-SH group) and generally over 90% exists as GSH (remainder as GSSG) [214]. The ratio of GSH to GSSG therefore provides an indication of overall oxidative stress within a cell (i.e., lower GSH:GSSG ratio = greater oxidative stress). Glutathione reductase (GRD) in the presence of NADPH can then reduce GSSG back to its GSH form to restore/rebalance the system [212]. In cases where excessive ROS must be neutralised and a rapid production of GSSG occurs (e.g., acute injury/trauma [215]), surplus GSSG can efflux from the tissue into the circulation for degradation, resulting in a net loss of intracellular total glutathione (tGS), and ultimately impairing redox buffering capacity [216]. Furthermore, resting glutathione concentrations can become depleted/reduced with chronic disease [217], leading to impaired functionality of the glutathione system over time, which may also contribute toward further disease progression [218-220]. To counteract the decline in glutathione induced by chronic disease and maintain the ability to adequately neutralize ROS, it may be necessary to engage in strategies to support the synthesis of new glutathione. Indeed, current dogma posits that higher levels of antioxidants and antioxidant enzymes (e.g. GSH and GPx) are generally positive indicators of health status [221, 222].

Glutathione is made up of three precursor amino acids, L-glutamate, cysteine and glycine [223]. For a detailed review on nutritional strategies to enhance glutathione status, please see the paper by Minich and Brown [224]. Briefly, nutritional strategies shown to support/enhance intracellular GSH concentrations in humans include: directly supplementing with GSH [225, 226], supplementing with the prodrug N-acetylcysteine (NAC) [227] and increasing the dietary concentrations of GSH precursor AAs through either increasing dietary protein intake or supplementing with whey protein [228]. Whey proteins are high in cystine (two cysteine AAs linked by a disulfide bond [229]), which is a pepsin and trypsin-resistant molecule that can safely travel through the plasma before being reduced into two sulfur-containing cysteine amino acids upon cell entry [230, 231]. Cysteine is considered to be the rate limiting AA in GSH synthesis [232]. The additional provision of this rate-limiting AAs has been theorized to support and/or enhance glutathione synthesis (and thus antioxidant capacity), which can become depleted with obesity and chronic disease [218, 221, 233]. Supporting GSH synthesis through dietary modulation has been shown to be beneficial in a range of pathological conditions, including those with cystic fibrosis [234], HIV [235, 236], cancer [237] and severe pressure ulcers [238], as well as with aging [239]. Considering that dairy foods are a good source of sulfur containing AAs (such as cysteine) [6], the provision of wholefood dairy may act as a promising vehicle to supply the precursors for glutathione synthesis and support/maintain its concentrations in individuals at increased risk for depleted concentrations.

### 2.4.2 An Overview of Inflammation

Inflammation is an important component of the immune system that primarily involves leukocytes (white blood cells) and cytokines in order to provide a defense mechanism against pathogens or injury [240]. Cytokines are messenger molecules which transiently upregulate and

attract leukocytes to the damaged/infected area so that they can destroy pathogens or remove damaged tissue [241]. In healthy individuals, this proinflammatory response automatically self-regulates and resolves *via* the release of anti-inflammatory cytokines, inhibition of proinflammatory signalling cascades, and activation of regulatory cells [242]. In some situations, such as with obesity or pathological disease states, individuals may be unable to resolve these responses, resulting in a chronic and constant increase in the local and systemic inflammatory milieu throughout the body; a state termed low-grade chronic inflammation (LGCI) [243-245]. The state of LGCI is also associated with the development of more serious disease/dysfunction, including atherosclerosis, osteoporosis, insulin resistance, some cancers, and hypertension [246-248]. It is also worth noting that oxidative stress can be involved with the regulation of inflammation [210], whereby excessive ROS can activate proinflammatory signaling pathways leading to inflammatory responses [201, 249, 250]. Increased oxidant levels can also suppress the adipose tissue expression of adiponectin (an adipokine with anti-inflammatory, anti-apoptotic and antioxidant effects [251]) and have been shown to increase the expression of inflammatory markers such as TNF-α, IL-6 and c reactive protein (CRP), whereas dietary antioxidant intake can exert the opposite effect [15].

The immune system and the cytokine responses involved are complex, and their actions may vary depending upon the tissue from which they were produced (immune cells, adipocytes, skeletal muscle, etc.) or act on as well as due to the presence or absence of other cytokines/immune cells in the local area [240]. When attempting to understand the overall state of inflammation or response to an intervention/stimulus, evaluating a range of cytokines may provide a greater insight into the specific mechanisms involved and/or the magnitude of response (i.e., do all proinflammatory markers increase/decrease) [240, 252]. To facilitate a better understanding of

overall function, they can be broadly grouped into those with proinflammatory and those with anti-inflammatory functions. An overview of the cytokines and other inflammatory markers measured as part of the studies within this thesis will now be briefly summarized, and include proinflammatory markers: IL-6, IL-1β, TNF-α, IFN-γ and CRP, and anti-inflammatory markers: IL-4 and IL-10.

IL-6 is a pleiotropic cytokine that is involved in many biological activities, including inflammation and the regulation of metabolic, regenerative, and neurological processes [253]. With regard to inflammation, it has been shown to be involved with both acute (e.g., viral infection [254]) and chronic (e.g. T2D, obesity [255]) inflammation, and is produced by various cell types including leukocytes and adipocytes [256]. It can also be produced by contracting skeletal muscle (as a "myokine"), seeming to have anti-inflammatory properties [257] and beneficial effects on exercise adaptations [258] when produced in this manner. These opposing actions are likely due to the two dichotomous signalling pathways through which IL-6 can stimulate target cells – classical and trans-signalling. In classical signalling, IL-6 binds to a membrane bound IL-6 receptor (mbIL-6R) which is only expressed on relatively few cell types (hepatocytes, monocyte–macrophages, lymphocytes, and skeletal myocytes) [259]. It subsequently binds to membrane bound gp-130 (found ubiquitously on almost all cells), leading to signal transduction and activation of the Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) pathway which goes on to mediate signalling within the cell [253]. In trans-signalling, IL-6 binds to a soluble form of IL-6R (sIL-6R), forming a IL-6/sIL-6R complex which can bind to membrane gp130 without the target cell needing to possess mbIL-6R, which vastly increases the variety of cell types that it can act upon [253]. Trans-signalling leads to stronger activation of the JAK/STAT3 cascade, but unlike classical, also activates the PI3K/Akt and MAPK/ERK pathways [260]. This induces an IL-

6-mediated proinflammatory response, involving release of monocyte chemoattractant protein-1 (MCP-1), promotion of leukocyte infiltration, inhibition of T-cell apoptosis, and impairment of the immunosuppressive actions of regulatory T cells [253, 260, 261]. Concurrent JAK/STAT3, PI3K/Akt, and MAPK/ERK pathway activation, MCP-1 induction, and stronger STAT3 signalling seems to explain why trans-signalling is proinflammatory and classic signalling is anti-inflammatory. The current body of research suggests that the anti-inflammatory and regenerative effects of IL-6 occur primarily through the classical signalling method, whereas trans-signalling leads to proinflammatory outcomes [253]. Research into these signalling pathways within different contexts is still in its infancy, however evidence does exist to indicate that it differs depending on the physiological state/stressor that induces an IL-6 response (e.g., exercise, obesity)[262]. Exercise for example can upregulate mbIL-6Rα expression within skeletal muscle (and also reduce shedding of mbIL-6Rα) [263] and can increase plasma concentrations of soluble gp130 [264, 265] which seems to create a ternary complex with IL-6/sIL-6Rα that leads to inhibition of trans-signalling [266]. These factors shift the IL-6 signalling axis from trans- to classical and may explain the positive effects of IL-6 increases from exercise (rather than it being due to structural/molecular differences in the IL-6 released from muscle) [262]. In contrast, IL-6 trans-signalling and the proinflammatory state it induces seems to dominate within cardiometabolic disease contexts [267]. Obesity induces macrophage recruitment and proliferation into adipose tissue [268, 269], which increases local and systemic inflammation and correlates with clinical measures of insulin resistance and metabolic dysfunction [270]. It has been shown that trans-signalling is a mechanism through which macrophages are recruited to adipose tissue, and that selectively blocking it (*via* Sgp130Fc administration) prevents this accumulation without the adverse effects that can occur with complete blockade of IL-6 [271]. As our understanding of these

mechanisms develops, it may be valuable to develop techniques or find biomarkers related to activation of these pathways in order to determine how nutrition/exercise interventions can alter them. Until then, circulating IL-6 can be used, and considered to reflect proinflammation within cardiometabolic/disease contexts and anti-inflammatory in exercise contexts.

Tumor necrosis factor alpha (TNF-α) is a potent proinflammatory cytokine that is primarily produced from macrophages and T-cells, but has also been shown to be produced by a variety of other cell types (e.g., neutrophils, mast cells, smooth muscle cells, endothelial cells, osteoclasts, osteoblasts and adipocytes) [272]. Production of TNF-α can be triggered through stimuli such as bacterial lipopolysaccharide (LPS), viral antigens, immune complex activation and stimulation by other cytokines such as IL-1, IFN-γ or even itself *via* autocrine mechanisms [272]. It mediates its biological effect on cells through two ubiquitously expressed transmembrane glycoprotein receptors: tumour necrosis factor-alpha receptor 1 and 2 (TNFR1 & TNFR2), however these can also be proteolytically cleaved to form soluble forms (sTNFR) [273]. The TNFR1 also has a death domain which upon binding with TNF-α, plays a crucial role in initiating apoptosis and contributes to activation of signaling pathways like NF-κB [274]. Furthermore, it has also been shown to interact with other cytokines. It enhances the production of other proinflammatory cytokines such as IL-1, IL-6 and IL-8, is upregulated by IFN-γ and itself, and is downregulated by anti-inflammatory cytokines such as IL-4 [274].

There are a number of cytokines within the IL-1 family, with the most commonly studied including IL-1α, IL-1β, IL-1ra and IL-18 [275]. Of these, IL-1β is strongly proinflammatory, and plays a pivotal role in developing both local and systemic inflammation with primary sources of its release including blood monocytes, tissue macrophages, and dendritic cells (DCs) [275]. It is translated into pro-IL-1β which has no biological activity until it becomes activated by caspase-1

and inflammasome complexes such as NLRP3 [276, 277]. Upon IL-1β binding with IL-1R1, signalling cascades are initiated that stimulate activation of NF-κB and MAP kinase kinase (MPKK) pathways [278]. Plasma concentrations of IL-1β are known to increase with obesity and can promote insulin resistance, disrupt storage of FFAs and contribute to atherosclerosis in individuals with obesity [278].

The interferons are a cytokine family involved in inflammation, immunoregulation, and tumor cell recognition [279]. IFN-γ can be produced by cells from both the innate (e.g., natural killer cells, macrophages) and adaptive (e.g., T helper 1 cells, B cells) immune systems, which leads to activation of macrophages, induction of apoptosis of epithelial cells and promotion of cytotoxic activities of other cells [276]. IFN-γ also regulates MHC class I and II expression and antigen presentation (improving recognition in cells such as macrophages and DCs), contributes to macrophage polarization to an M1 (proinflammatory) phenotype and potentiates the release of other proinflammatory cytokines (e.g., TNF-α, IL-1β) [279]. IFN-γ has also been shown to be elevated in obesity [280] and production can be inhibited by nutritional components such as Vitamin A, C and D [281].

Interleukins 4 and 10 are anti-inflammatory cytokines that function mainly by supressing the proinflammatory milieu [282]. IL-4 is produced by TH2 cells, basophils, mast cells, and eosinophils [276]. Functions of IL-4 include being able to cause CD4+ T cell differentiation into TH2 cells while suppressing the development of TH1 cells, acting as a growth factor for B cell, T cell, and mast cells, and being a promoter of immunoglobulin class switching to IgG1 and IgE [283]. It also has potent inhibitory effects on the expression and release of the proinflammatory cytokines from monocytes, including IL-1, TNF-α, IL-6, IL-8, and macrophage inflammatory protein (MIP)-1α [284]. IL-10 is an anti-inflammatory cytokine, produced primarily by monocytes,

type 1 Treg cells, Breg cells), some NK cells, macrophages, and DCs [276]. It is a potent deactivator of monocyte/macrophage proinflammatory cytokine synthesis, inhibiting production of TNF-α, IL-1, IL-6, IL-8, IL-12, granulocyte colony-stimulating factor and MIP-1α & MIP-2α [285, 286]. It has also been shown to inhibit NF-κB nuclear translocation after LPS stimulation, promote degradation of messenger RNA for proinflammatory cytokines and limit the rate of leukocyte infiltration and inflammation in tissue when released from local mast cells [276, 283].

Finally, CRP is an acute phase protein produced by the liver in response to proinflammatory cytokines, with circulating levels rising rapidly in response to inflammation, infection or trauma [287]. Resting circulating CRP can be affected by a variety of factors such as age, sex, smoking status, blood pressure and lipid levels [288]. In healthy Caucasian individuals, serum concentrations are around 0.8mg/L on average, and clinical guidelines suggest that a circulating CRP level of <3mg/L is considered normal, 3-10mg/L is considered high and >10mg/L is considered very high in regard to basal inflammatory status [289, 290].

### 2.4.3 Inflammatory Responses to Exercise and Meal Stressors

Measurement of a series of inflammatory markers can provide insight into both basal/resting inflammatory status (and the presence or absence of LGCI) as well as the response to various stimuli or stressors.

Following a sufficiently strenuous bout of exercise, there is generally an increase in inflammation within skeletal muscle [291]. This is primarily due to mitochondrial uncoupling and the subsequent induction of ROS, as well as tissue microtrauma that results in the release of DAMPs and the activation and recruitment of monocytes/macrophages to the damaged area in order to clear cellular debris/damaged cells [291, 292]. This local inflammatory response leads to an initial transient increase in circulating cytokines, particularly IL-6, which then typically subside

to baseline concentrations within 72h [293, 294]. While the prototypical proinflammatory cytokines TNF-α and IL-1β have been shown to rise post-exercise by us and other groups, this observation is not consistent [291, 295-298], and may be influenced by the type, duration, and intensity of exercise as well as on the training status of an individual [299, 300]. Elevations in IL-6 from exercise do however seem to consistently lead to a later rise in anti-inflammatory cytokines such as IL-10 and IL-1ra [291]. Increases in these cytokines can downregulate production of T cells, proinflammatory cytokines (e.g., IL-1α, IL-1β, TNF-α) and other effector cells in order to initiate the resolution of the inflammatory response [301]. Consistent exercise training alongside adequate rest/recovery intervals seems to result in chronically reduced proinflammatory and increased anti-inflammatory cytokine concentrations [301], and these findings are more consistently found in individuals with LGCI (e.g., OW/OB, aging, T2D) [291, 302].

Other stressors that can induce acute changes in inflammatory profiles include the consumption of a high-energy, high-fat meal [303, 304]. This inflammatory response is typically more severe in individuals with OW/OB [305, 306]. In these individuals, postprandial dysmetabolism, characterized by abnormally high increases in glucose, insulin, and lipids following the consumption of high-calorie and/or high-fat foods, induces acute increases in metabolic oxidative stress and inflammation [306, 307]. One study demonstrated that circulating IL-6, TNF-α and triglycerides were markedly elevated in participants following the consumption of a 760-kcal meal (59% of energy from fat) [308]. Miglio et al. (2012) assessed HFMC responses in OW males, and also found elevations in IL-6 and TNF-α that reached maximal concentrations 8h after meal consumption [309]. This high-fat meal-induced inflammatory response also appears to increase as the total size of the meal increases, with data showing that the IL-6 response is greater following 1500 kcal and 102 g of fat vs. 500 kcals and 34 g of fat [310]. Gregersen and

colleagues (2012) compared 1000kcal meals that were high in either carbohydrate or fat and found that IL-6 expression was increased within both the blood and muscle with both meals, but that only the high carbohydrate meal evoked significant postprandial oxidative stress [311]. There appear to be mixed findings with regard to antioxidant responses following a HFMC, with studies showing both increases and decreases in plasma antioxidant markers [309, 312]. For glutathione specifically, Anderson and colleagues (2009) found that muscle GSH:GSSG of lean human males was reduced 4h after a HFMC as well as after 5 days of a high fat diet [313]. Damage to the endothelial vasculature can also occur due to these postprandial oxidative stress/inflammatory perturbations, which has been implicated as a causal process in the development of CVD and other cardiometabolic disease states [314-316]. Developing strategies that are easily implemented and lower cost to mitigate oxidative stress and inflammation are important in order to combat the development/progression of cardiometabolic diseases [317-321] and the all-cause morbidity and mortality that are associated with them [322]. Dietary strategies including the provision of dairy foods may help in this regard.

## 2.5 The Effect of Dairy Foods on Inflammation and Oxidative Stress

In the last 12 years, five SRMAs have been completed to assess the role of chronic dairy intake on inflammation [5, 200, 323-325]. Labonte and colleagues (2013) reviewed RCTs that evaluated the effect of dairy (milk, yogurt and cheese) on inflammatory biomarkers in OW/OB adults [325]. They concluded that dairy consumption had no adverse effects on inflammation, but methodological factors within the 8 studies assessed left them unable to conclusively determine whether dairy intake had a beneficial or neutral effect. Bordoni et al. (2017) reviewed 52 clinical trials investigating inflammatory marker responses to dairy intake [5]. They determined that dairy

intake (fermented dairy products in particular) can be anti-inflammatory in individuals without a milk allergy, and that these beneficial effects are even more pronounced in individuals with metabolic dysfunction [5]. Ulven and colleagues (2019) reviewed research published between 2012-2018 on inflammatory marker responses to milk and dairy intake [200]. They evaluated outcomes from 16 studies and determined that consumption of milk and dairy did not exert proinflammatory effects, and instead that the majority of studies showed anti-inflammatory effects in healthy and metabolically abnormal individuals [200]. Moosavian et al. (2020) completed a meta-analysis and systematic review (11 RCTs involving 663 participants) evaluating the effect of dairy product consumption on inflammatory biomarkers within adults [323]. They found that dairy intake improved several inflammatory markers, including CRP, TNF-α, IL-6 and MCP-1, was associated with increased adiponectin, but did not alter leptin, intercellular adhesion molecule-1 (ICAM-1) or vascular cell adhesion molecule-1 (VCAM-1) [323]. Finally, Nieman and colleagues (2020) completed a systematic review of studies to evaluate the effect of dairy product (milk, cheese, and yogurt) and dairy protein consumption on LGCI in adults without severe inflammatory disorders [324]. Of the 19 RCTs that evaluated dairy products, 8 reported improvements in at least one biomarker of inflammation while the remainder showed no effect [324]. Furthermore, their analysis showed that beneficial effects were most commonly observed in trials that evaluated individuals with OW/OB and an average age of ~42 years, potentially due to higher baseline systemic inflammation in these individuals [324]. Overall, these SRMAs indicate that dairy consumption is associated with beneficial or neutral effects on inflammatory biomarkers, with more consistently beneficial results observed in those with OW/OB and/or some metabolic dysfunction.

There have been significantly fewer studies evaluating the effect of dairy intake on oxidative stress, and while some RCTs exist, no SRMAs are currently available. A study by Zemel and Xiaocun (2008) found in mice that a dairy containing diet reduced NADPH oxidase mRNA, plasma malondialdehyde (MDA) and adipose tissue ROS compared to a control diet [326]. A follow up study in humans with OW/OB by Zemel and colleagues (2010) showed that 28 days of dairy supplementation reduced oxidative stress (plasma MDA, 8-isoprostane-F2α), inflammatory biomarkers (TNF-α, IL-6, MCP-1) and increased adiponectin compared to soy supplementation [14]. Stancliffe et al. (2011) provided 40 adults (individuals with OW/OB and metabolic syndrome) a low-dairy (<0.5 dairy servings/day) or high-dairy (>3.5 dairy product servings/day of combined milk, yogurt & hard cheese) weight-maintenance diet for 12-weeks [15]. The high dairy condition resulted in significantly attenuated oxidative stress (malondialdehyde: -35%; oxidized LDL: -25%), and inflammation (TNF-α: -35%; IL-6: -21%; MCP-1: -24%), and a 55% increase in adiponectin at the end of the 12-week period [15]. These data together suggest that dairy consumption can positively modulate both oxidative stress and inflammation in humans.

Acute dairy intake may also affect the inflammatory response. Following the consumption of a high-fat meal, there can be a transient metabolic and inflammatory response [303, 304], which is typically greater in severity for individuals with OW/OB [305, 306]. Considering that people in industrialised countries are almost constantly in a postprandial state while awake, assessing postprandial inflammatory responses may be just as important, if not more so than assessing fasted inflammatory concentrations [316, 327]. In consideration of this, several studies have assessed the postprandial inflammatory response to a high-fat meal with dairy product consumption either immediately before (as a pre-load) or as part of the high-fat meal challenge (HFMC) [16, 17]. Pei and colleagues (2018) compared the effects of dairy yogurt vs. soy yogurt consumption on HFMC

responses in premenopausal females both with and without OB [16]. Participants consumed ~330g of either dairy or soy yogurt daily for 9-weeks, and then also consumed it immediately prior to a HFMC breakfast consisting of 2x sausage, egg, and cheese sandwiches and 2 hash browns (~960kcals, ~54% from dietary fat, ~34% from carbohydrates, ~12% from protein). They found that in females with OB, immediate pre-meal consumption of dairy yogurt reduced postprandial IL-6 concentrations compared to the soy yogurt, possibly by mediating hyperglycemia [16, 328]. The pre-meal yogurt was suggested to have acutely altered intestinal lipid absorption *via* formation of calcium soaps [165] which in turn altered the rate of gastric emptying, which affected the magnitude and timing of postprandial glucose and insulin responses [16, 172, 329]. Demmer and colleagues (2016) compared postprandial inflammatory and metabolic responses to a HFMC composed of palm oil with and without an added dairy fraction rich in milk fat globule membrane (MFGM) in individuals with OW/OB [17]. They found the addition of MFGM improved postprandial metabolic responses (reduced TC, LDL & insulin), decreased soluble intracellular adhesion molecule (inflammatory marker) and increased the anti-inflammatory cytokine IL-10 [17]. In contrast, Rogers et al. (2017), found that meals containing MFGM did not elicit different postprandial inflammatory marker responses [330]. Schmid and colleagues (2015) evaluated the postprandial metabolic and inflammatory responses to high-fat meals with and without the inclusion of a mixture of dairy products (full-fat milk, cheese and butter) in healthy males and found no differences between the responses to these meals despite the dairy-containing meal having 30% greater calories [169]. Indeed, this is a response in and of itself indicating that a greater energy/fat load including dairy may have blunted a further increase in response. McDonald and colleagues (2019), in adults with prediabetes, assessed postprandial oxidative stress and vascular responses between 75g of glucose dissolved in either water, high-fat or low-fat dairy milk [173].

They found that, in addition to glucose, markers of oxidative stress (MDA, F2-isoprostanes) and endothelin-1 were also lower in both dairy arms (regardless of fat content), and concluded that this attenuated postprandial hyperglycemia resulted in reduced vascular endothelial function impairments by limiting oxidative stress [173].

At present, no data exist on whether the chronic consumption of dairy products mitigates postprandial inflammation and oxidative stress in the absence of a dairy pre-load. If consuming a habitually higher-dairy diet can attenuate postprandial inflammation, this may have more real-world applicability compared to consuming dairy products immediately prior to a meal, which may not ever become common practice.

## 2.6 Proposed Mechanisms of Dairy's Anti-Inflammatory and Antioxidant Effects

While research relatively consistently shows a benefit of dairy consumption on inflammation, the mechanisms underlying these observations have not been fully elucidated. Factors that have been suggested as contributors to dairy's anti-inflammatory effect include dairy proteins, dairy fats and micronutrients, as well as possible synergy/permissive effects from consuming all these nutrients together in a single food (**Figure 4**) [2].

### 2.6.1 Anti-Inflammatory Mechanisms of Dairy Proteins

Proteins within milk can be divided into casein (~80%) and whey (~20%), which differ by AA profile and other physiochemical properties. Several peptides from these proteins have been identified to have bioactive properties, with examples from casein including: α-, β-, and γ-casein, and from whey including: β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins,

lactoferrin, and proteose-peptone fractions [331]. Caseins (and bioactive tripeptides derived from them) have been shown to have hypotensive effects [332], possibly by modulating endothelial cell function *via* nitric oxide (NO) production [333], through inhibition of angiotensin I converting enzyme (ACE) activity [334], or by moderating monocyte adhesion to vascular endothelium [335]. There are many whey protein derived bioactive peptides which have been reviewed in detail by Ricci-Cabello et al. (2012), and a number of them have been shown to have antioxidant properties (e.g., casein derived peptide: Tyr-Phe-Tyr-Pro-Glu-Leu; β-lactoglobulin A derived peptide: Trp-Tyr-Ser-Leu-Ala-Met-Ala-Ala-Ser-Asp-Ile; fermented milk derived peptide: Ala-Arg-His-Pro-His-Pro-His-Leu-Ser-Phe-Met) [331] and ACE inhibitory activity (e.g., β-casein-derived lactotripeptides: Val-Pro-Pro and Ile-Pro-Pro). Furthermore, α-lactalbumin has been shown to suppress TH1 production of TNF-α and IL-6 [336], and lactoferrin has been shown to downregulate production of TNF-α, IL-1β, IL-6, and IL-8 in monocyte cell lines by disrupting NF-κB activation [337, 338]. In addition to bioactive peptides, intake of specific amino acids found in within dairy products may also play a role in modulating inflammation. For example, cell studies have shown that glycine decreases IL-6 and TNF-α, IL-1β and increases adiponectin and IL-10 gene expression in adipose tissue and monocytes [339, 340]. Cysteine is an amino acid found in relatively high concentrations within whey and, aside from its direct role in promoting glutathione synthesis, has been shown to also inhibit NF-κB activation, leading to decreased IL-6, IL-1β, IL-8, MCP-1 and ICAM expression and production in monocytes and endothelial cells [341, 342].

### 2.6.2 Anti-Inflammatory Mechanisms of Dairy Fats

Dairy fats may confer some modulation of inflammation. Possible effects could be due to short and/or medium chain saturated fatty acids (SFA), dairy-specific trans-FA, or effects of supplying fat with micronutrients like calcium, magnesium, potassium or Vitamins A, E or D [2].

First, milk fat contains a higher proportion of short-chain saturated FA (C4:0 to C8:0, ~3-7% of total FA) which have been shown to reduce IL-6, TNF-α and MCP-1 in a medium containing mouse preadipocytes and macrophages, and to reduce TNF-α stimulated ICAM-1 and VCAM-1 expression in epithelial cells [343, 344]. No studies have demonstrated similar effects in humans so more data regarding the effects of dairy short-chain SFA and inflammation are required. Milk also contains a significant amount of monounsaturated FAs (MUFAs, 24-35% of total FAs), including oleic acid (OA) which has been evaluated in several cell studies [2]. There is some evidence that OA reduces excretion of TNF-α, IL-6 and MCP-1 in human adipocytes [345], and increases adiponectin expression [346], but other studies have shown no effect in adipocytes [347], macrophages [347] or epithelial cells [348]. In addition, dairy fats contain around 2.7% trans-FAs, mainly trans-vaccenic acid (trans-11 18:1), trans-palmitoleic acid (trans-9 16:1) and conjugated linoleic acids (CLAs; PUFAs) [349]. Naturally occurring trans-FAs from dairy products are thought to differentially effect CVD risk compared to other trans-FA sources [350]. There have been mixed findings regarding the effects of these dairy trans-FAs on inflammation, and while anti-inflammatory effects have been observed, they seem to be highly isomer dependent [2]. Overall, while some beneficial effects of dairy fats seem to exist, more research is required to elucidate these effects. Furthermore, few studies have evaluated mixtures of FAs (vs individual FAs alone) on cellular inflammatory responses. Future investigations should consider using mixed dairy FAs in order to better mimic the physiological situation of consuming wholefood dairy [2].

### 2.6.3 Anti-Inflammatory Mechanisms of Dairy Micronutrients

Dairy products are also micronutrient dense, providing a variety of vitamins and minerals together, including calcium, magnesium, potassium, phosphorous, selenium, zinc and Vitamins A, B, D and K [2]. Intake of some of these micronutrients have also been found to effect inflammatory

markers. For example, increased dietary calcium intake has been shown to specifically reduce TNF-α production in mice [351]. In addition, Zemel and colleagues (2008) found that adipose tissue ROS production, TNF-α and IL-6 expression were suppressed in mice after consumption of both a short term, higher calcium supplemented diet and a dairy supplemented diet [326]. Furthermore, ROS and MDA were further decreased by the high-dairy diet in this study, suggesting that while consumption of calcium alone has anti-inflammatory and antioxidant effects, it is further enhanced by other components within dairy [326]. Calcium and certain dairy proteins may also attenuate postprandial dysregulation by delaying gastric emptying and decreasing fat absorption [165, 171, 352], modulating gut microbiome function *via* increasing intestinal mucin and secretory immunoglobulin A and antimicrobial peptide secretion, and/or improving gut permeability by maintaining tight junction functionality [353, 354]. In addition to calcium, human studies have also shown modest inverse associations between magnesium intake and some markers of systemic inflammation and endothelial dysfunction [355]. Finally, dairy products are important sources of Vitamin A and are fortified with Vitamin D (milk only) in countries such as Canada. Mechanistic insights into the effect of Vitamin A from supplements or wholefoods are currently lacking. For Vitamin D however, cell research has shown decreases in the expression of IL-6, MCP-1 and IL-1β in human adipocytes [356]. In humans, it appears that the beneficial effects of higher Vitamin D intake mostly occur within individuals with high basal inflammation and/or who are deficient in Vitamin D [357, 358]. In pediatric populations with OW/OB, Vitamin D supplementation was associated with decreased CRP and a protection of IL-10, suggesting beneficial anti-inflammatory effects in Vitamin D deficient individuals [359].

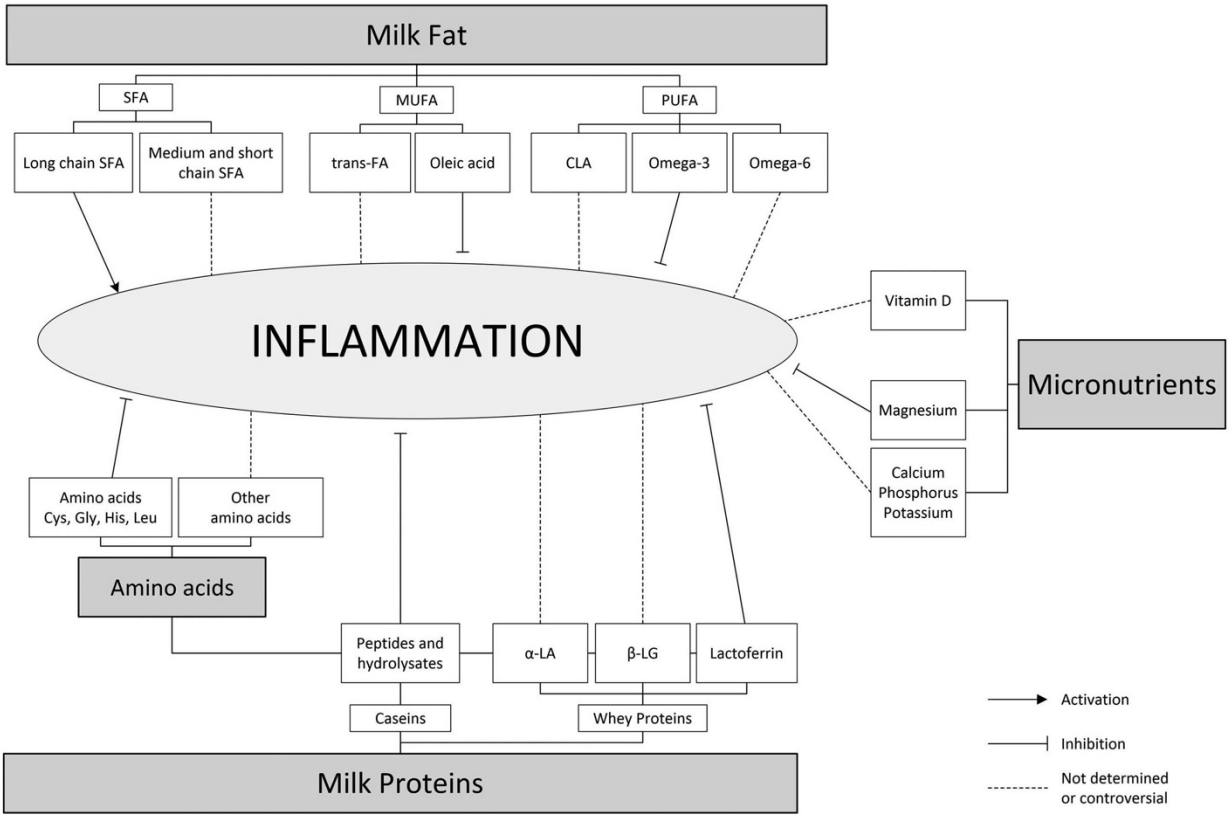


**Figure 4.** Overview of dairy nutrients and their effect on inflammation, adapted from Da Silva et al. (2015) [2]

### 2.6.4 Antioxidant Mechanisms of Dairy

It has been theorised that dairy products and their constituent components can support/enhance antioxidant capacity in a number of ways, which may also contribute to reductions in inflammation [6, 289]. For example, some nutritional components within dairy have the ability to directly scavenge ROS, including Vitamins A and E and carotenoids [6] or support endogenous antioxidant systems by acting as essential enzymatic cofactors (e.g., selenium and GPx, and zinc and SOD)[360]. In addition, bacterial and probiotic organisms (e.g., *Lactobacillus acidophilus*) contained within some dairy products (particularly fermented milks or yogurt and cheese) may enhance the antioxidant capacity of a food by causing the release of "antioxidant

peptides" during the digestion which protects the endothelial cells of the digestive tract against oxidative injury by free radicals [361, 362]. Another possible mechanism is through the provision of GSH precursor components, namely sulfur containing amino acids such as cysteine (the rate limiting precursor amino acid of GSH synthesis [218]) and methionine (dietary restriction in animals has been shown to reduce GSH concentrations [363]). At present, data are relatively sparse regarding the effect of milk/dairy consumption on GSH and GSSG (or related biomarkers); but some studies are discussed below (for the remainder, see the review paper by Fardet et al.[361]). Choi and colleagues [364] measured cerebral GSH concentrations in 60 healthy, older subjects (mean ± SD age: 68.7 ± 6.2 y) whose routine dairy intakes varied. They found significantly greater concentrations of cerebral glutathione in individuals with higher dairy consumption, concluding that dairy foods may serve as a good source of precursor substrates for GSH synthesis. Conventional milk consumption (2 x 250ml/day) has also been shown to increase plasma GSH concentrations in adults, however, milk containing only A2 type β-casein appeared to increase circulating levels to a greater degree than regular milk [365]. Pregnant females that were provided 200g/day of probiotic yogurt (cultures: *L. acidophilus* LA5 & *B. lactis* BB12) for 9 weeks had significantly greater concentrations of plasma GSH, GPx and glutathione reductase (GR) compared to baseline but not compared to a conventional yogurt, which also increased [366]. Lastly, in T2D patients provided with 300g/day of probiotic yogurt for 6-weeks, erythrocyte SOD, GPx activity and total antioxidant status all increased compared to a conventional yogurt [367].

In summary, there are a wide variety of mechanisms through which dairy products may be able to exert an antioxidant and/or anti-inflammatory effect following consumption in humans due to the variety of healthful nutrients contained within their different food matrices, and their potential additive/synergistic effects. It is therefore likely that a combination of these factors may

be contributing to their positive effects on inflammation and/or cardiometabolic disease risk that have been observed within the literature.

## 2.7 Gaps in Literature

While the link between dairy intake and various aspects of physiological function and health have been relatively well studied, gaps remain in our knowledge. As discussed in **Section 2.2.6**, there has not yet been a well-controlled assessment comparing bone responses to wholefood dairy vs. a non-dairy control following a high-impact, high-load acute exercise bout which would sufficiently stress the skeletal system. Furthermore, a study performed under this paradigm should involve a sampling duration of longer than ~3 hours in order to capture later bone remodelling responses, and it should also assess multiple bone turnover and metabolic related markers in order to gain comprehensive insight into the cellular processes happening in the acute post-exercise and nutrition period. In addition, interactions between the immune and skeletal systems are known to exist, primarily through animal and cellular research [76, 77]. At present however, it remains unclear if bone and inflammatory markers are associated with each other in humans and in the context of an exercise and nutrition stressor. Research from this thesis highlighted within **Chapters 3 and 4** will address these gaps.

The recent comprehensive systematic review from Ulven and colleagues (2019) identified a significant lack of well-controlled, transparently reported studies evaluating acute and short-term dairy consumption on a range of inflammatory biomarkers [200]. In addition, most studies thus far have only assessed the effects of consuming a single type of dairy product (usually milk, yogurt, cheese or butter) on lipid responses and inflammation, however considering the possible differential beneficial effects, it may be valuable to study the effects of

mixed dairy product intake [16, 179, 323]. Timon et al. (2020) stated that there has been an over reliance on traditional markers of cardiometabolic health (e.g. lipid responses alone) which may not show subtle physiological changes in response to dairy intake, or elucidate possible causes of beneficial effects, limiting the ability to predict health outcomes [179]. They suggest the use of multiple novel biomarkers that include assessments of different systems as a way to more fully develop our understanding of the interlinked physiology that is affected by food consumption [179]. Finally, the majority of studies to date have assessed fasting inflammatory responses to dairy consumption. Considering that humans in the western world typically spend most of their day in a postprandial state [316, 368], and often consume a diet higher in adverse nutrients [369], it is important to understand and develop strategies to also mitigate the aberrant inflammatory and metabolic stress that can occur following food/drink consumption, which over time compounds to increased chronic disease risk. Research from this thesis highlighted within **Chapters 5 and 6** will address these gaps.

# Chapter 3:

# Acute Effects of Milk vs Carbohydrate on Bone Turnover Biomarkers following Loading Exercise in Young Adult Females

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**Specific Contribution:** I did not conduct/collect data or perform biochemical assays for this study, but did the majority of the data analysis, interpretation, and writing of the manuscript. *ARJ and PK: study design conceptualization.*

***JLP**, LES, NK, ECF, PK, and ARJ: data analysis, interpretation, and drafting and revising the manuscript.*

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# Acute Effects of Milk vs. Carbohydrate on Bone Turnover Biomarkers Following Loading Exercise in Young Adult Females

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Dairy products and impact exercise have previously been identified to be independently beneficial for bone mineral properties, however, it is unknown how the combination of these two osteogenic interventions may alter acute bone turnover. Using a randomized crossover design, we compared the acute effects of consuming milk vs. an isoenergetic carbohydrate control beverage on bone biomarkers following loading exercise. Thirteen healthy female participants (Age = 20.3 ± 2.3y; BMI = 21.0 ± 1.1 kg/m$^2$) consumed either 550 mL of 0% skim white milk (MILK) or 52.7 g of maltodextrin in 550 mL of water (CHO), both 5 min and 1 h following completion of a combined plyometric (198 impacts) and resistance exercise (3–4 sets/exercise, 8–12 reps/set, ∼75% 1-RM) bout. Venous blood samples were obtained pre-exercise, and 15 min, 75 min, 24 h and 48 h post-exercise to assess serum concentrations of bone resorption biomarkers, specifically carboxyl-terminal crosslinking telopeptide of type I collagen (CTX), receptor activator nuclear factor kappa-β ligand (RANKL), and sclerostin (SOST), as well as bone formation biomarkers, specifically osteoprotegerin (OPG) and osteocalcin (OC). When absolute biomarker concentrations were examined, there were no interaction or group effects for any biomarker, however, there were main time effects ($p < 0.05$) for RANKL, SOST, and OC, which were lower, and the OPG: OPG/RANKL ratio, which was higher at 75 min post-exercise compared with baseline in both conditions. In addition to assessing absolute biomarker concentrations at specific timepoints, we also evaluated the relative (% change) cumulative post-exercise response (75 min to 48 h) using an area under the curve (AUC) analysis. This analysis showed that the relative post-exercise CTX response was significantly lower in the MILK compared to the CHO condition ($p = 0.03$), with no differences observed in the other biomarkers. These results show that while milk does not appear to alter absolute concentrations of bone biomarkers compared to CHO, it may attenuate relative post-exercise bone resorption (i.e., blunt the usual catabolic response to exercise).

**Keywords: dairy, milk, carbohydrate, female, acute exercise, bone biomarkers, plyometric exercise, resistance exercise**

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## INTRODUCTION

Osteoporosis is a disease that usually manifests in older adulthood and is characterized by a state of bone fragility and increased fracture risk due to losses in bone mineral density (BMD) and bone strength (1). Peak bone mass (PBM), achieved in early adulthood, is a strong predictor of osteoporosis later in life (2). Early implementation of strategies to increase PBM, particularly during the first 3 decades of life, when bone accrual is the greatest (3), may be one of the most effective preventative strategies for reducing the burden of osteoporosis as we age (2). Several low-cost, non-invasive osteogenic strategies have been identified, including engagement in loading exercise and nutritional modulation.

Regular exercise, particularly exercise that involves skeletal loading (4), is well known to be osteogenic (5). Plyometric and resistance exercise have both been shown to be particularly effective for both increasing PBM in adolescent/young adults and preventing bone loss in the elderly (4). The mechanical and metabolic stress of exercise stimulates the osteocytes embedded throughout the bone matrix to initiate the bone remodeling cycle in which osteoclasts first resorb the damaged bone tissue followed by the osteoblasts depositing new bone collagen (6). These processes are tightly coupled and regulated by several proteins and signaling molecules that may also be modulated by exercise and nutrition (6). For example, receptor activator nuclear factor kappa-β ligand (RANKL) and osteoprotegerin (OPG) oppose each other's function, acting to increase and decrease osteoclast differentiation and function, respectively (7). Other proteins that directly affect bone remodeling include sclerostin (SOST), which is primarily secreted by osteocytes and acts on the Wnt pathway to impair bone formation by inhibiting osteoblast differentiation (8), and osteocalcin (OC) which is secreted by osteoblasts to aid in bone mineralization (9). The acute post-exercise time-course of changes in bone biomarkers is generally consistent with the bone remodeling process, whereby exercise typically elicits an initial transient increase in bone resorption [demonstrated by an increase in carboxyl-terminal crosslinking telopeptide of type I collagen (CTX)] (10–12), whereas bone formation markers are largely unresponsive initially, but can be upregulated later on (13). Although these are the typical responses observed, it is important to note that differences in exercise modality, intensity, duration, and participant training status may cause variations in acute biomarker responses (13). Since bone biopsies cannot be readily obtained in humans, measuring circulating biomarker concentrations to assess both the dynamic cellular processes that govern/modulate different stages of the bone remodeling cycle, as well as the end-products of such processes (e.g., CTX) can be insightful for determining acute responses to exercise and nutrition (7).

Maintaining an adequate dietary intake of bone-supporting nutrients is a strategy that has also been shown to support bone health (14). Dairy products provide many of these nutrients, and they contain more protein, calcium, magnesium, potassium, zinc, and phosphorus per calorie than any other food (15). It has been demonstrated through both observational and interventional research that consuming the recommended amount of dairy products can support bone health throughout the lifespan (16). For example, higher dairy intake was associated with elevated bone accrual during childhood (17) and adolescence (18), as well as with greater BMD in older individuals (19–21). In the context of exercise, previous research has indicated that dairy consumption alongside exercise training can push bone turnover status toward formation in young adult males (22), improve BMD and decrease bone resorption markers in female athletes (23), and induce favorable bone biomarker changes in pre-menopausal normal weight and overweight females (24–26). Bone is also acutely responsive to nutrition, as evidenced by changes in biomarkers following the manipulation of energy and carbohydrate intake (27, 28). However, despite positive findings following exercise training with concomitantly increased dairy consumption, little is known about the effect of post-exercise dairy consumption on acute bone cell responses following a single bout of loading exercise. Therefore, the purpose of this study was to examine serum levels of bone biomarkers following a single bout of combined plyometric and resistance (i.e., bone-loading) exercise followed by milk vs. carbohydrate consumption in normal weight, young adult females. Our primary outcome was examination of the absolute bone biomarker concentrations, and our secondary outcome was examination of the relative bone biomarker responses to post-exercise nutrition. We hypothesized that, relative to carbohydrate, post-exercise milk consumption would favorably influence bone turnover biomarkers (i.e., reduced resorption, enhanced/unchanged formation).

## MATERIALS AND METHODS

This study was approved by both the Brock and York University Research Ethics Boards (#17-402 and #2019-045, respectively), met all human research standards of Canada's Interagency Panel on Research Ethics and was registered at clinicaltrials.gov under the following identifier: NCT03615989.

### Participant Characteristics and Study Design

Thirteen young, healthy female adults were recruited from the Brock and York University (Ontario, Canada) student populations. Before informed consent was obtained, participants were initially screened to ensure they met the following inclusion criteria: (1) free of any medical conditions, (2) BMI 18.5–24.9 kg/m$^2$, (3) recreationally active and not participating in a resistance training program, (4) no known allergy to dairy protein or lactose intolerance.

This study utilized a within-subject crossover design where, in a randomized order, participants completed 2 trials consisting of either: (1) exercise bout + carbohydrate (CHO) or (2) exercise bout + milk (MILK). All participants completed an initial lab visit consisting of baseline measurements and an exercise familiarization session before beginning their first trial. Prior to each trial, participants were instructed to arrive at the lab following an overnight fast and to refrain from alcohol and exercise for 48 h before and after the trial. If participants were not on hormonal contraceptives ($n$ = 8), the trials were separated

by a minimum of 4-weeks to allow for both trials to occur during the early follicular phase of the menstrual cycle. If participants were on monophasic hormonal contraceptives ($n = 5$), trials were separated by a minimum 2-week washout, and the trials occurred during hormone delivery.

## Baseline Testing and Familiarization Session

Upon study explanation and obtainment of written informed consent, baseline measurements were taken. These measures included height and weight, measured using a standard scale and stadiometer (with light clothing and shoes off), and body fat percent measured using bioelectrical impedance analysis (BIA; InBody 520 BIA system; Biospace Co., Inc. Los Angeles, CA, United States). To verify baseline physical activity and health status of the participants, the Godin Shephard leisure time physical activity questionnaire (29) and a general health and screening questionnaire were completed. In addition, a Physical Activity Readiness Questionnaire (2018 Par-Q +) (30) was administered to ensure each participant was safe to engage in physical activity. Participants were instructed to keep their diet consistent between trial sessions and to confirm this, were asked to record all their food and drink intake over a 2-day period for each trial (beginning on the day of the exercise bout). Dietary intakes were analyzed using the ESHA Food Processor Program (Food Processor SQL, ESHA Research, Salem, OR).

Participants then completed a familiarization session to learn how to correctly perform the trial exercises and to determine estimated 1 repetition maximum (1 RM) on three machine exercises (Chest Press, Seated Row, Leg Press), thus allowing determination of appropriate loads for the trial exercise bouts. Following a warm up, load was progressively increased in accordance with the American College of Sports Medicine exercise testing recommendations (31) until 5 repetitions or less could no longer be completed. All tests were completed by the same certified personal trainer. Estimated 1 RM was then calculated using the O'Connor calculation {1 RM = weight × [1 + (0.025 × # of reps)]} from the set with the lowest number of completed reps, a method that has been previously validated in females (32).

## Exercise Protocol

All exercise sessions began at approximately 9:00 a.m., were completed in a fasted state, and facilitated by a certified personal trainer. Participants completed the same exercise bout consisting of combined plyometric and resistance exercises for both trials, with each bout typically lasting approximately 70 min. The participants were asked to complete plyometric exercises, including broad jumps, pogo jumps, box drops, and explosive lunges (198 impacts/session), and resistance exercises, including chest press, leg press and seated row (3–4 sets/exercise, 8–12 reps/set, ∼75% 1 RM).

## Supplement Protocol

It was randomly determined (using a random number generator) which of the two isoenergetic and isovolumetric nutrition conditions the participants would complete first, with each being ingested 5–10 min following exercise cessation and again 1 h post consumption of the first trial drink. For the CHO condition, participants consumed 52.7 g of maltodextrin (∼200 kcals) mixed with approximately 550 mL of water plus a calorie-free, fruit flavored sweetener to enhance palatability. For the MILK condition, participants consumed 550 mL of 0% skim milk (Natrel fine filtered skim milk, Agropur, QC, Canada; 200 kcals, 20 g protein, 29 g carbohydrate).

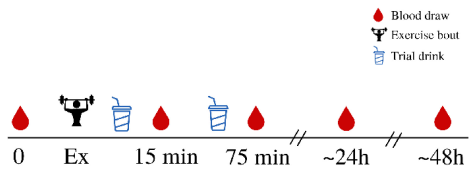


**FIGURE 1 |** An overview of the experimental trial. Participants arrived for a baseline, rested, fasted blood draw before completing the exercise protocol. Immediately following completion of the exercise protocol, participants consumed the first trial drink (either MILK or CHO) which was then immediately followed by a blood draw (approximately 15 min after cessation of exercise). This was repeated 1 h later, with a second, identical, trial drink consumed 1 h after the first drink, and a second blood draw taken after the second drink had been consumed (approximately 75 min after cessation of exercise). Subsequent blood draws were taken 24 h and 48 h following exercise, in the fasted state.

## Blood Sample Collection Protocol

Venous blood samples were collected from the median cubital vein by trained study personnel using a standardized venipuncture technique. Approximately 10 mL of blood was taken at each timepoint, which were pre-exercise (baseline), and 15 min, 75 min, 24 h, and 48 h post-exercise (**Figure 1**). After each draw, blood samples rested for 25 min (serum rested at room temperature, plasma rested at 4°C) before being centrifuged at 1,300 g and 4°C for 15 min. Serum and plasma were then aliquoted into small cryovial tubes and stored at −80°C until required for analysis.

## Bone Biomarker Assessment

Serum β–isomerized carboxy–terminal cross-linking telopeptides (CTX; cat# E-EL-H0960; Elabscience, China) and sclerostin (SOST; cat# DSST00; R&D, Minneapolis, MN) were analyzed in duplicate using enzyme linked immunosorbent assays (ELISA). The average intra-assay coefficients of variation for CTX and SOST were 11.8 and 5.7%, respectively, and the inter-assay coefficients of variation were 9.2 and 4.2%, respectively. Total osteocalcin (OC) and osteoprotegerin (OPG) were analyzed in duplicate using a multiplex human bone magnetic bead assay (cat# HBNMAG-51K; Millipore Corporation, Etobicoke, ON). The average intra-assay coefficients of variation were 8.5 and 6.3%, respectively, and the average inter-assay coefficients of variation were 2.9 and 3.9%, respectively. Serum RANKL was analyzed in duplicate using a single-plex RANKL magnetic bead assay (cat# HRNKLMAG-51K-01; Millipore Corporation, Etobicoke, ON). The average intra-assay coefficient

of variation was 4.8% and the average inter-assay coefficient of variation was 4.6%.

## Statistical Analysis

Data were checked for normality using z-scores for skewness and kurtosis and non-normally distributed data were log transformed (RANKL). Individual missing values were dealt with by either using the same baseline concentration as their CHO trial if baseline values were missing (one value each for CTX, OC, OPG, RANKL in the same participant) or a "last observation carried forward" approach (one value for CTX; two values each for OC and OPG; three for RANKL; one value for SOST out of a total of 130 values per biomarker) (33). For the latter approach, 8 of 9 of these values were to replace missing 48 h values (carried forward from the respective 24 h value). In addition, one participant's serum OC and OPG concentrations were below the assay detection limit at all timepoints and another participant's serum RANKL concentrations were deemed invalid as they were all > 3SDs above the mean. Thus, the sample size was reduced for the analyses conducted on these variables (OC, OPG, RANKL $n = 12$; OPG/RANKL ratio $n = 11$). Average dietary intake data (**Table 1**) were missing for 2 participants, so the data from the 11 remaining participants were reported and analyzed between trial conditions using a paired samples $t$-test (with $p$-values considered significant at $p < 0.05$). Exercise volume load (sets × reps × weight) between the two conditions was also compared using a paired samples $t$-test (with $p$-values considered significant at $p < 0.05$).

The absolute serum levels of all the bone turnover markers during CHO and MILK trials were compared using a two-way repeated measures analysis of variance (2RM-ANOVA), with condition (CHO and MILK) and time (baseline, 15 min, 75 min, 24 h, 48 h) both as within-participant factors. The Huynh-Feldt correction was used when data did not meet the assumption of sphericity. In the event of a significant main effect for time, *post hoc* testing was carried out involving pairwise comparisons using least significant difference (LSD) tests. Statistical analyses were performed using SPSS (Version 27.0 for Windows, Armonk, NY: IBM Corp.) with $p$-values considered significant at $p < 0.05$.

To evaluate our secondary objective, the net area under the curve (AUC) relative to pre-exercise was calculated for all biomarkers from the percent change data to assess short-term response following the post-exercise supplement (i.e., 75 min to 48 h) (34). The 15 min timepoint was excluded from this analysis because it was not affected by the supplement. The AUC for each biomarker was compared between trials using a paired-samples $t$-test for normally distributed data and a Wilcoxon's matched-pairs signed rank test for SOST and OPG/RANKL ratio. The AUC calculations were performed using GraphPad Prism software 9.0 (GraphPad Software, La Jolla, CA, United States).

# RESULTS

Thirteen participants completed both trials, and their baseline characteristics are shown in **Table 2**. Six participants completed the MILK trial before the CHO trial, while the remaining seven participants completed the CHO trial before the MILK trial.

**TABLE 1 |** Average daily dietary intake during the MILK and CHO trials, based on analysis of 2-day food records (day of exercise and day post-exercise) including the provided trial drinks ($n = 11$).

| Dietary variable | CHO | MILK | $p$-value |
|---|---|---|---|
| Energy (kcal) | 1,958 (627) | 1,873 (616) | 0.46 |
| Protein (g) | 67 (27) | 83 (31) | **0.01** |
| Carbohydrate (g) | 274 (91) | 243 (106) | 0.07 |
| Fat (g) | 68 (30) | 68 (24) | 0.98 |
| Vitamin D (IU) | 47 (54) | 408 (50) | **<0.001** |
| Calcium (mg) | 561 (374) | 1,073 (266) | **<0.001** |
| Iron (mg) | 10 (4) | 11 (6) | 0.67 |
| Magnesium (mg) | 194 (145) | 282 (113) | **0.01** |
| Potassium (mg) | 1,768 (1,379) | 2,754 (1,316) | **<0.001** |

*Values are presented as mean (SD). Contribution of two CHO beverages: 401 kcals, 105 g carbohydrate. Contribution of two MILK beverages: 400 kcals, 40 g protein, 58 g carbohydrate, 720IU Vitamin D, 1,200 mg calcium, 1.44 mg iron, 160 mg magnesium, 1,680 mg potassium. Bold p-values denote statistically significant differences ($p < 0.05$).*

**TABLE 2 |** Participant characteristics measured at the start of the study ($n = 13$).

| Participant characteristics | |
|---|---|
| Age (years) | 20.3 (2.3) |
| Height (m) | 1.6 (0.1) |
| Weight (kg) | 56.6 (5.0) |
| BMI (kg/m$^2$) | 21.0 (1.1) |
| Body fat (%) | 23.5 (3.3) |

*Values are presented as mean (SD). BMI, body mass index.*

## Trial Exercise Volume Load

To ensure there were no differences in the exercise stimuli, we compared the exercise load between trial conditions. There was no significant difference in resistance exercise volume load (sets × reps × weight) performed during the exercise bout between trials (CHO trial: 5,487 ± 1,482 kg vs. MILK trial: 5,345 ± 1,485 kg; $p = 0.25$). In addition, for both the CHO and MILK trials, all participants completed 198 impacts/jumps per exercise session.

## Dietary Intake

To ensure nutrient intake during the trials was consistent, we examined 2-day food records with trial drinks included ($n = 11$) between trials, which are reported in **Table 1**. There were no significant differences between the habitual diets of the participants during the trials when analysis was performed with trial drinks removed ($p > 0.05$ for all variables), however, as expected, when the trial drinks were included, some differences in nutrients were noted (**Table 1**).

## Serum Levels of Bone Turnover Biomarkers

Absolute bone biomarker concentrations are shown in **Table 3**. There were no significant main effects for time or condition

and no significant time-by-condition interactions for the absolute concentrations of CTX and OPG ($p > 0.05$). There was a significant main effect for time for OC ($p = 0.01$), SOST ($p < 0.001$), RANKL ($p = 0.004$) and the OPG/RANKL ratio ($p = 0.02$), but no significant main effects for condition or time-by-condition interactions ($p > 0.05$). *Post hoc* analysis of the main time effects indicated that compared to baseline and 15 min post-exercise, the concentrations of OC, SOST, and RANKL were significantly lower at 75 min post-exercise ($p < 0.05$). OC and RANKL returned to baseline levels at 24 h and 48 h post-exercise, however, SOST concentrations were significantly elevated at 24 h and 48 h post-exercise compared to both baseline and 75 min post-exercise ($p < 0.05$). In addition, RANKL concentrations were shown to be significantly below baseline at 15 min post-exercise ($p < 0.05$). The OPG/RANKL ratio was increased 15 min and 75 min post-exercise relative to baseline in both trials ($p < 0.05$), returning to baseline at 24 h.

## Relative Post-exercise Bone Biomarker Response

To address the secondary objective of the study and examine the relative post-exercise and post-nutrition bone marker responses, we determined the net AUC relative to baseline from 75-min to 48 h post-exercise for each bone turnover marker using percent change values (35) (**Figures 2A**, **3A**, **4A**, **5A,C,E**). The AUC analyses demonstrated that the CTX response to exercise was significantly lower in the MILK trial compared to the CHO trial ($p = 0.03$; **Figure 2B**). There was no significant difference in the post-exercise response between trials for OC ($p = 0.39$; **Figure 3B**), SOST ($p = 0.54$; **Figure 4B**), OPG ($p = 0.72$; **Figure 5B**), RANKL ($p = 0.61$; **Figure 5D**) or the OPG/RANKL ratio ($p = 0.70$; **Figure 5F**).

# DISCUSSION

This is the first study to compare the influence of post-exercise milk vs. CHO consumption following plyometric and resistance loading exercise on acute bone biomarker responses in young adult females. We demonstrated that: (1) absolute biomarker concentrations were not differentially altered at any timepoint between the milk and CHO trials, (2) the relative post-exercise CTX response (indicative of bone resorption) was blunted following milk consumption compared to CHO consumption, and (3) a bout of resistance and plyometric loading exercise stimulated an acute bone cell response. Overall, these results suggest that milk (with its important constituent nutrients that support bone) may be a beneficial post-exercise nutritional choice and better than carbohydrates at reducing the typical acute elevations in bone tissue degradation that occur following exercise.

Acute pre-exercise dairy consumption has previously been shown by Haakonssen et al. (36) to attenuate bone resorption biomarkers following a single bout of endurance exercise in well-trained female cyclists. Utilizing a randomized crossover design, their trial consisted of a 90-min bout of intense cycling preceded by either a dairy containing meal (oats with calcium-fortified milk and yogurt) or a control meal (oats with water, tinned fruit, and nuts). The dairy condition displayed significantly reduced CTX concentrations immediately, 40 min and 190 min post-exercise, which is consistent with our findings showing that the relative CTX response was blunted by milk but not by CHO. Furthermore, neither our study nor theirs detected differences in the acute response of bone formation markers [OC, OPG, OPG/RANKL ratio in ours, and procollagen type 1 N-terminal Propeptide (P1NP) in theirs (36)] between the dairy and carbohydrate trials. Other studies have also demonstrated no acute effect of exercise on bone formation biomarkers (35, 37). We recently assessed the effect of Greek yogurt (3 servings/d) vs. carbohydrate consumption on bone biomarkers following 5-days of intense soccer training in adolescent female athletes (38). In this population, neither the intense exercise nor the Greek yogurt appeared to elicit any alterations in absolute bone biomarker concentrations over this period, although methodological differences, such as differences in exercise modality (5-d soccer training vs. single, high-load, high-impact exercise bout), limit the ability to directly compare findings. In addition, the adolescent elite female athletes previously assessed displayed much lower basal CTX concentrations compared to both the participants in the current study as well as to other young adult cohorts previously assessed (39, 40). This may have reduced the ability to detect any potential differences induced by Greek yogurt consumption on bone resorption because of a "floor effect." Despite this, there were differences in the concentration of undercarboxylated OC (but not total OC) in the Greek yogurt condition compared to the carbohydrate condition, indicating that dairy may be uniquely affecting bone cell metabolism in the short-term. Research assessing the effects of acute dairy intake alone (i.e., not in conjunction with exercise) have also observed alterations in bone biomarker responses. For example, Hettiarachchi et al. (41) found that a calcium-fortified dairy supplement significantly reduced CTX and increased P1NP concentrations in the 4 h postprandial period, and Thomas et al. (42) observed significant reductions in CTX in the morning following consumption of 200 mL of skimmed milk fortified with extra calcium. We extend the literature in this area by analyzing the influence of dairy consumption on a variety of biomarkers associated with bone cell metabolism/turnover (specifically CTX, OC, SOST, OPG, and RANKL) and by assessing their responses following a resistance and plyometric exercise protocol. Although CTX was the only biomarker in which we observed significant differences in the relative response between trials, it is considered to be a robust biomarker for evaluating bone resorption [deemed to have higher specificity to bone metabolism and a smaller relative biological variability compared to other biomarkers (43)], and thus these nascent findings that suggest a benefit of post-exercise milk on bone turnover are promising. Collectively, although the data are limited, it currently appears that dairy consumption (either alone or in close proximity to an exercise bout) can acutely attenuate bone resorption in humans.

Despite the paucity of literature specifically evaluating the effect of dairy on post-exercise bone biomarker responses, there is evidence that nutritional status and/or acute feeding of other nutrients can influence bone biomarkers. Sale and

**TABLE 3 |** Absolute serum concentrations of bone biomarkers pre- and post-exercise during the carbohydrate (CHO) and milk (MILK) trials.

| Analyte | CHO | | | | | Milk | | | | | RM-ANOVA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BL | 15 min | 75 min | 24 h | 48 h | BL | 15 min | 75 min | 24 h | 48 h | Cond | Time | Int |
| CTX (pg/ml) | 221 (107) | 212 (103) | 246 (118) | 238 (122) | 208 (90) | 254 (126) | 250 (134) | 224 (95) | 234 (141) | 240 (167) | 0.61 | 0.92 | 0.08 |
| OC (pg/ml) | 16,807 (5,386) | 17,041 (6,074) | 15,557 (5,024) *ab* | 16,006 (5,946) | 16,109 (5,131) | 15,596 (5,228) | 16,810 (5,811) | 13,798 (4,781) *ab* | 16,897 (5,974) | 14,589 (4,774) | 0.20 | **0.01** | 0.31 |
| SOST (pg/ml) | 155 (80) | 178 (84) | 122 (59) *ab* | 176 (84) *ac* | 176 (66) *ac* | 164 (70) | 181 (77) | 140 (56) *ab* | 192 (87) *ac* | 193 (85) *ac* | 0.21 | **<0.001** | 0.85 |
| OPG (pg/ml) | 337 (133) | 351 (145) | 366 (150) | 334 (75) | 340 (78) | 304 (68) | 351 (80) | 355 (65) | 316 (85) | 304 (72) | 0.22 | 0.09 | 0.57 |
| RANKL† (pg/ml) | 40 (21) | 37 (31) *a* | 31 (35) *ab* | 41 (30) *c* | 41 (33) *c* | 47 (37) | 39 (41) *a* | 34 (44) *ab* | 44 (38) *c* | 49 (38) *c* | 0.91 | **0.004** | 0.77 |
| OPG/ RANKL ratio | 11 (8) | 15 (10) *a* | 22 (18) *a* | 11 (7) *bc* | 10 (5) *bc* | 8 (4) | 13 (7) *a* | 20 (16) *a* | 10 (7) *bc* | 9 (7) *bc* | 0.20 | **0.02** | 0.89 |

*Values are mean (SD). CTX, SOST (n = 13); OC, OPG, RANKL (n = 12); OPG/RANKL ratio (n = 11). Post hoc analysis for combined trials (on the main time effects; bold letters): a, significant difference vs. pre-exercise; b, significant difference vs. 15 min post-exercise; c, significant difference vs. 75 min post-exercise using least significant difference tests (p < 0.05). †Log-transformed data used for statistical analysis. BL, baseline; CTX, C-terminal crosslinked telopeptide of type-I collagen; OC, osteocalcin; SOST, sclerostin; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-kβ ligand. Bold p-values denote statistically significant differences (p < 0.05).*

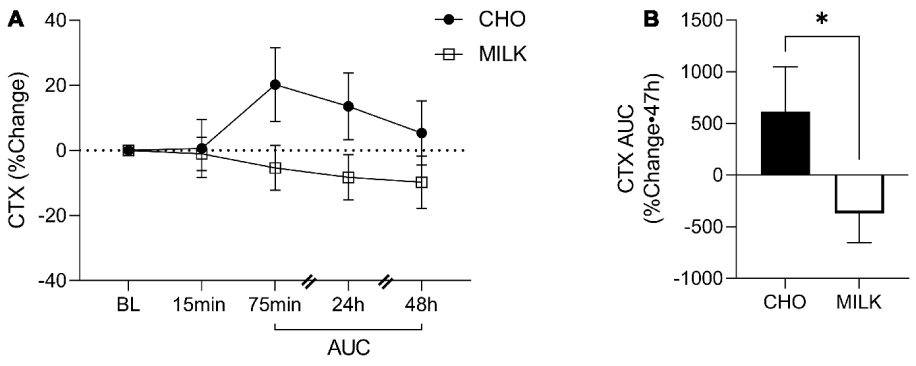


**FIGURE 2 |** Percent change post-exercise **(A)** and area under the curve from 75-min to 48-h **(B)** for C-terminal crosslinked telopeptide of type-I collagen (CTX) in the CHO and MILK trials. Values are means ± SEM. *n* = 13. *Significant difference between conditions based on a paired *t*-test (*p* = 0.03). BL, baseline; AUC, area under the curve.

colleagues (35) found that both CTX and P1NP responses were attenuated for up to 2 h post-exercise with CHO feeding compared to a water control (when assessed using a similar post-exercise AUC analysis to ours), and concluded that CHO consumption during exercise acutely blunts bone turnover in the hours (up to 2 h post) following an exercise bout. The same group, in two additional studies, observed that trained males consuming CHO before, during, and after high-intensity interval running (vs. reduced CHO + high fat, and reduced CHO/energy conditions) (44) and trained adult males consuming CHO + whey protein (a component of dairy protein) immediately post-exercise vs. a water control (45), displayed an attenuated CTX response for up to 3 h post-exercise. When comparing the effect of post-exercise whey protein vs. carbohydrate consumption on bone turnover biomarkers, whey protein has been shown to provide greater reductions in bone resorption (CTX) in athletic samples of swimmers (39) and mountain bikers (37). Furthermore, there are also data showing attenuated CTX with acute calcium supplementation vs. a water or saline control following prolonged strenuous endurance exercise (46, 47). In our study, dietary calcium intake was found to be significantly higher during the MILK vs. the CHO trials, so it is possible that differences in calcium consumption contributed to the blunted relative post-exercise CTX response in the MILK condition. It is worth noting, however, that the previous studies (46, 47) showing a benefit of calcium consumption on bone resorption have done so following an intense endurance exercise bout (1 h in duration) in which there were likely significantly greater calcium losses through sweat than what would have occurred with our exercise protocol. At this

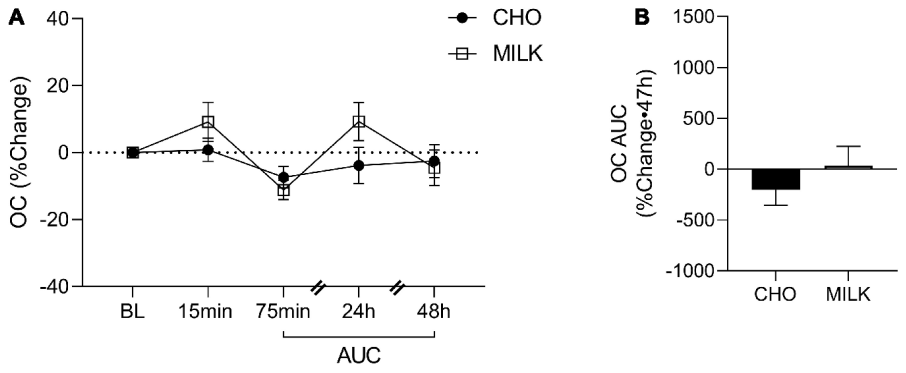


**FIGURE 3 |** Percent change post-exercise **(A)** and area under the curve from 75-min to 48-h **(B)** for osteocalcin (OC) in the CHO and MILK trials. Values are means ± SEM. *N* = 12. BL, baseline; AUC, area under the curve.

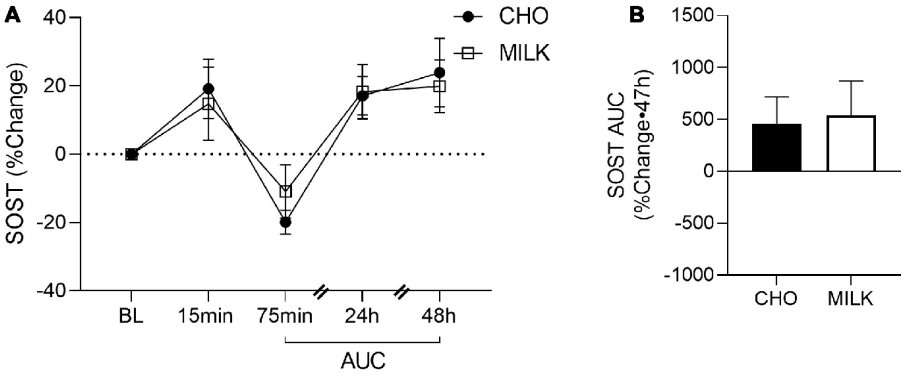


**FIGURE 4 |** Percent change post-exercise **(A)** and area under the curve from 75-min to 48-h **(B)** for sclerostin (SOST) in the CHO and MILK trials. Values are means ± SEM. *n* = 13. BL, baseline; AUC, area under the curve.

time, it remains unclear how isolated calcium supplementation compares to the energy containing, wholefood dairy product we supplied. Therefore, our observation that milk appeared to blunt CTX over the post-exercise period in comparison to an isoenergetic CHO condition and given the previous findings of CHO on the acute bone biomarker responses, it may be prudent to at least consume energy post-exercise, but it is likely more favorable to consume milk (that contains a combination of energy, protein, and bone-supporting nutrients like calcium) post-exercise compared to carbohydrate/energy alone. Although we did not investigate a non-energetic post-exercise control (e.g., water), future studies should consider including this type of trial, and/or a calcium + water trial to compare the relative contribution(s) of energy and other potential functional ingredients within dairy foods on the acute bone response to exercise.

Despite only seeing differences in the relative CTX response between the nutritional interventions, we did observe various time effects elicited by our exercise bout, indicating that a single bout of intense, high load resistance, and plyometric exercise effectively stimulated a response within bone tissue. We have previously demonstrated that plyometric exercise (without post-exercise nutrition) can induce acute bone biomarker changes (including CTX, P1NP, SOST, OC) in both adult and adolescent females (48, 49). Specifically, we have consistently shown an immediate (5 min) post-exercise increase in SOST in studies of both males (50) and females (40, 48, 49, 51). In the present study, SOST tended to increase at 15 min post-exercise ($p$ = 0.11), but the smaller sample size and/or the later sampling time (i.e., 5 min vs. 15 min) may have contributed to the observed non-significant increase. Moreover, we found that SOST, OC, and RANKL concentrations were suppressed 75 min post-exercise, whereas the OPG/RANKL ratio was significantly increased at this timepoint. With acute exercise, we may expect to see an increase (13) or no difference from baseline (52) in bone resorption markers at ∼1 h post-exercise, with bone formation markers unchanged (although bone biomarker responses in previous acute exercise research have been inconsistent) (12, 13, 53). It is likely that our energy containing interventions (both MILK and CHO) influenced the biomarker responses at 75 min post-exercise, resulting in a general shift of bone metabolism away from resorption and toward bone formation. Furthermore, our bone biomarker response is unknown in the later acute post-exercise period (i.e., between 75 min and 24 h). Future research is required to further understand the interactions between loading exercise and energy/nutrition intake on bone biomarker responses, as well as to characterize their responses to loading exercise at different

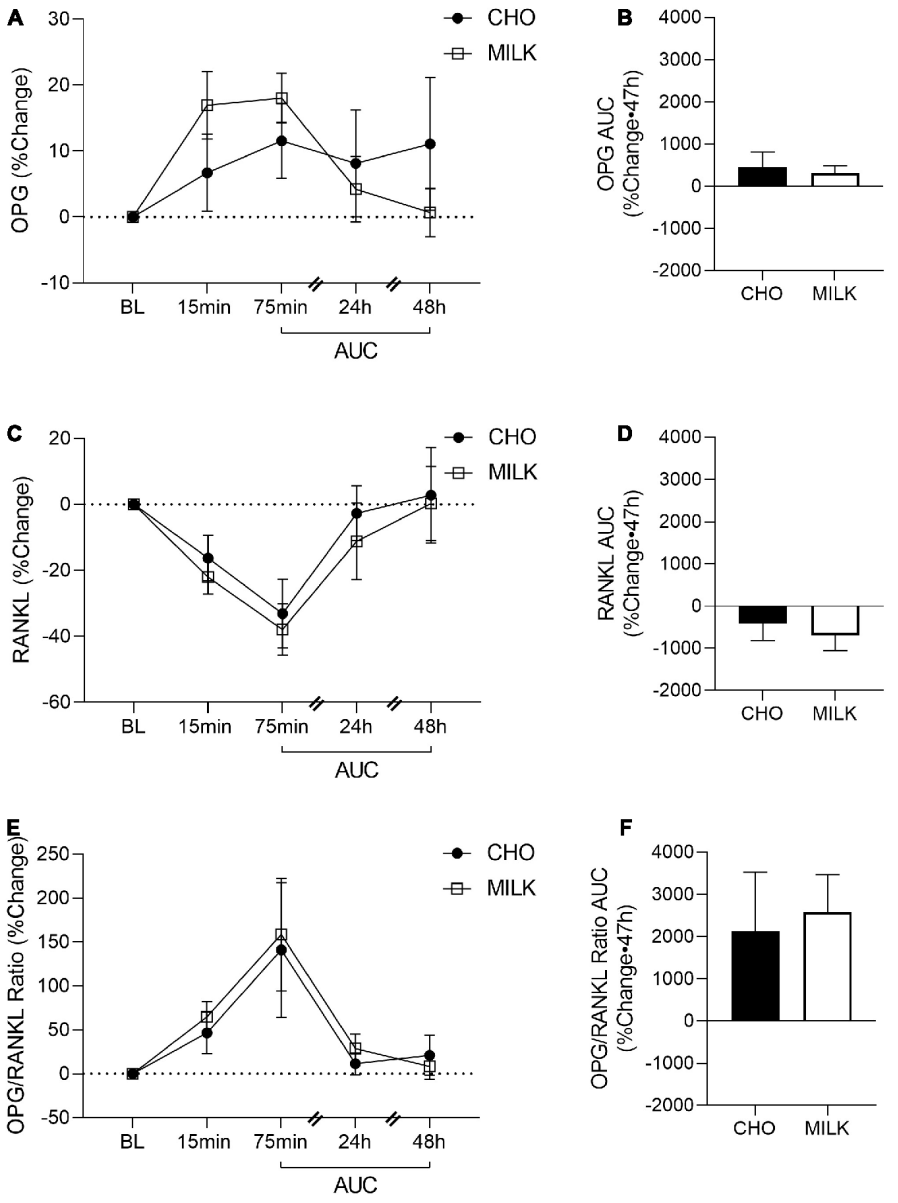


**FIGURE 5 |** Post-exercise percent change in osteoprotegerin (OPG; **A**), receptor activator of nuclear factor-kB ligand (RANKL; **C**) and OPG/RANKL ratio **(E)** and area under the curve from 75-min to 48-h for OPG **(B)**, RANKL **(D)** and OPG/RANKL ratio **(F)** in the CHO and MILK trials. Values are means ± SEM. OPG and RANKL ($n$ = 12); OPG/RANKL ratio ($n$ = 11). BL, baseline; AUC, area under the curve.

sampling timepoints (i.e., the intermediate response between 75 min and 24 h).

Dairy foods may be uniquely suited to enhance bone metabolism because they are a rich source bone-supporting nutrients, including energy, protein, calcium, and other micronutrients. Despite this, the mechanisms through which dairy products appear to alter bone cell metabolism are not well understood. This likely relates to our current inability to directly assess the cellular activity and pathways activated in human bone tissue following an acute stimulus such as exercise. Initial evidence suggests that bioactive peptides/components in dairy foods (e.g., milk-based protein, milk growth factors like IGF-1, lactoferrin, caseinophosphopeptides, glycomacropeptides) can act directly on osteoblastic (Wnt and Bone Morphogenic Protein/Transforming Growth Factor-β) and/or osteoclastic [by controlling production of RANKL and Macrophage-Colony Stimulating Factor (M-CSF)] signaling pathways (54). In this study, we did not observe any significant differences in the acute post-exercise response of circulating RANKL or OPG between nutrition conditions, so it remains unclear through which mechanism milk may have acted to blunt bone resorption. More research is warranted to uncover the mechanistic effects of these dairy-specific bioactives as well as to determine how different dairy products may influence bone metabolism (i.e., fermented dairy products like yogurt have greater concentrations of certain bioactive peptides, and

thus may confer even more potent modulation of bone tissue mechanisms). Furthermore, as discussed in this new and relevant review by Dolan and colleagues (13), the relationship between acute exercise/nutrition related bone biomarker responses and long term positive changes in BMD and bone microarchitecture remains unclear. For example, while it is interesting that milk appeared to blunt the CTX response compared to CHO in our study [and that nutrition/energy provision appears to blunt acute bone resorption in other exercise studies (35, 38, 44, 45)], it may be valuable for future work to extend the observations over a longer duration within the same participants to help determine the link between acute/short-term biomarker responses and longer-term bone outcomes. Chronic studies involving combined exercise and dairy intake have previously demonstrated both improved structural (55) and biomarker (22, 56–58) outcomes. Indeed, Bridge et al. (22) observed a blunted bone resorption (CTX) response following just 1 week (3 bouts) of resistance + plyometric exercise (similar to the exercise bout in this study) with Greek yogurt consumption, which was followed, after 12 weeks, by a significant increase in bone formation (P1NP) vs. those consuming CHO post-exercise (although no BMD/structural outcomes were assessed).

Our study had several strengths, including the use of a crossover design to limit the influence of inter-individual variability, the assessment of multiple biomarkers involved with different aspects of the bone remodeling cycle, and the measurement of biomarkers at multiple timepoints over a 48 h period, allowing for AUC analyses and thus evaluation of the cumulative biomarker response over the entire post-exercise period. Also, we believe performing this research in a population of untrained young adult females is a strength of our study. Not only is this due to the general under-representation of female participants in nutrition and exercise research (59, 60), but also because osteoporosis preferentially affects females compared to males (61).

Our study also had several limitations. First, our sample size was relatively small, although we believe that the crossover design chosen helped to mitigate this concern. Secondly, the measurement and assessment of circulating biomarker concentrations to evaluate acute bone metabolism has its own inherent/established limitations (7, 62), however this currently remains the gold-standard option to assess acute bone responses to exercise and/or nutrition (13). Another potential limitation with systemic biomarker assessment is that there may be site-specific skeletal changes in turnover/metabolism following loading exercise that our systemic measures are not sensitive enough to differentiate. It is therefore possible that the biomarker changes that we are seeing underrepresent meaningful acute changes at specific skeletal sites.

## CONCLUSION

This study advances our understanding of how nutrition can modulate acute bone metabolism following exercise by comparing acute bone biomarker responses to exercise with milk or CHO consumption for the first time. In conclusion, despite no between trial differences in absolute bone biomarker concentrations, our study demonstrated that the consumption of milk following resistance and plyometric exercise, in untrained, healthy adult females, resulted in a greater relative acute suppression of bone resorption in comparison to an isocaloric carbohydrate drink. This novel finding suggests that milk may be a better post-exercise nutritional option (compared to CHO alone) for blunting the initial catabolic effect of exercise on bone. Future research is required to determine how dairy specifically modulates physiological responses within bone, including elucidating which component(s) of dairy are primarily responsible for these observed differences. In addition, RCTs are needed to examine long term effects of these interventions on bone.

## DATA AVAILABILITY STATEMENT

The data sets presented in this article are not readily available. Requests to access the data sets should be directed to AJ, ajosse@yorku.ca.

## ETHICS STATEMENT

This study involving human participants was reviewed and approved by the Brock University Research Ethics Board—reb@brocku.ca and the York University Office of Research Ethics—ore@yorku.ca. The participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

AJ and PK: study design conceptualization. JP, LS, NK, EF, PK, and AJ: data analysis, interpretation, and drafting and revising the manuscript. All authors approved of the final version of this manuscript.

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## REFERENCES


1. Osterhoff G, Morgan EF, Shefelbine SJ, Karim L, McNamara LM, Augat P. Bone mechanical properties and changes with osteoporosis. *Injury.* (2016) 47:S11–20. doi: 10.1016/S0020-1383(16)47003-8
2. Kralick AE, Zemel BS. Evolutionary perspectives on the developing skeleton and implications for lifelong health. *Front Endocrinol (Lausanne).* (2020) 11:99. doi: 10.3389/fendo.2020.00099
3. Baxter-Jones AD, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA. Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. *J Bone Miner Res.* (2011) 26:1729–39. doi: 10.1002/jbmr.412
4. Xu J, Lombardi G, Jiao W, Banfi G. Effects of exercise on bone status in female subjects, from young girls to postmenopausal women: an overview of systematic reviews and meta-analyses. *Sports Med.* (2016) 46:1165–82. doi: 10.1007/s40279-016-0494-0
5. Yuan Y, Chen X, Zhang L, Wu J, Guo J, Zou D, et al. The roles of exercise in bone remodeling and in prevention and treatment of osteoporosis. *Prog Biophys Mol Biol.* (2016) 122:122–30. doi: 10.1016/j.pbiomolbio.2015.11.005
6. Kenkre J, Bassett J. The bone remodelling cycle. *Ann Clin Biochem.* (2018) 55:308–27. doi: 10.1177/0004563218759371
7. Kuo TR, Chen CH. Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. *Biomark Res.* (2017) 5:1–9. doi: 10.1186/s40364-017-0097-4
8. Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, et al. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. *EMBO J.* (2003) 22:6267–76. doi: 10.1093/emboj/cdg599
9. Wang JS, Mazur CM, Wein MN. Sclerostin and osteocalcin: candidate bone-produced hormones. *Front Endocrinol (Lausanne).* (2021) 12:584147. doi: 10.3389/fendo.2021.584147
10. Scott JP, Sale C, Greeves JP, Casey A, Dutton J, Fraser WD. Effect of fasting versus feeding on the bone metabolic response to running. *Bone.* (2012) 51:990–9. doi: 10.1016/j.bone.2012.08.128
11. Barry DW, Hansen KC, Van Pelt RE, Witten M, Wolfe P, Kohrt WM. Acute calcium ingestion attenuates exercise-induced disruption of calcium homeostasis. *Med Sci Sports Exerc.* (2011) 43:617. doi: 10.1249/MSS.0b013e3181f79fa8
12. Rantalainen T, Heinonen A, Linnamo V, Komi PV, Takala TE, Kainulainen H. Short-term bone biochemical response to a single bout of high-impact exercise. *J Sports Sci Med.* (2009) 8:553.
13. Dolan E, Varley I, Ackerman KE, Pereira RMR, Elliott-Sale KJ, Sale C. The bone metabolic response to exercise and nutrition. *Exerc Sport Sci Rev.* (2020) 48:49–58. doi: 10.1249/JES.0000000000000215
14. Rizzoli R. Dairy products, yogurts, and bone health. *Am J Clin Nutr.* (2014) 99:1256S–62S.
15. Caroli A, Poli A, Ricotta D, Banfi G, Cocchi D. Invited review: dairy intake and bone health: a viewpoint from the state of the art. *J Dairy Sci.* (2011) 94:5249–62. doi: 10.3168/jds.2011-4578
16. Rizzoli R. Dairy products and bone health. *Aging Clin Exp Res.* (2021) 34:9–24. doi: 10.1007/s40520-021-01970-4
17. Xueqin D, Zhu K, Trube A, Zhang Q, Ma G, Hu X, et al. School-milk intervention trial enhances growth and bone mineral accretion in Chinese girls aged 10–12 years in Beijing. *Br J Nutr.* (2004) 92:159–68. doi: 10.1079/BJN20041118
18. Cadogan J, Eastell R, Jones N, Barker ME. Milk intake and bone mineral acquisition in adolescent girls: randomised, controlled intervention trial. *BMJ.* (1997) 315:1255–60. doi: 10.1136/bmj.315.7118.1255
19. Sahni S, Tucker KL, Kiel DP, Quach L, Casey VA, Hannan MT. Milk and yogurt consumption are linked with higher bone mineral density but not with hip fracture: the Framingham offspring study. *Arch Osteoporos.* (2013) 8:119. doi: 10.1007/s11657-013-0119-2
20. Dawson-Hughes B, Harris SS. Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. *Am J Clin Nutr.* (2002) 75:773–9. doi: 10.1093/ajcn/75.4.773
21. Rizzoli R, Biver E, Bonjour JP, Coxam V, Goltzman D, Kanis J, et al. Benefits and safety of dietary protein for bone health—an expert consensus paper endorsed by the European society for clinical and economical aspects of osteopororosis, osteoarthritis, and musculoskeletal diseases and by the international osteoporosis foundation. *Osteoporos Int.* (2018) 29:1933–48. doi: 10.1007/s00198-018-4534-5
22. Bridge AD, Brown J, Snider H, Ward WE, Roy BD, Josse AR. Consumption of Greek yogurt during 12 weeks of high-impact loading exercise increases bone formation in young, adult males–a secondary analysis from a randomized trial. *Appl Physiol Nutr Metab.* (2020) 45:91–100. doi: 10.1139/apnm-2019-0396
23. Josse AR, Phillips SM. Impact of milk consumption and resistance training on body composition of female athletes. *Med Sport Sci.* (2012) 59:94–103. doi: 10.1159/000341968
24. Josse AR, Tang JE, Tarnopolsky MA, Phillips SM. Body composition and strength changes in women with milk and resistance exercise. *Med Sci Sports Exerc.* (2010) 42:1122–30. doi: 10.1249/MSS.0b013e3181c854f6
25. Woo J, Lau W, Xu L, Lam CWK, Zhao X, Yu W, et al. Milk supplementation and bone health in young adult Chinese women. *J Womens Health.* (2007) 16:692–702. doi: 10.1089/jwh.2006.0222
26. Josse AR, Atkinson SA, Tarnopolsky MA, Phillips SM. Increased consumption of dairy foods and protein during diet– and exercise-induced weight loss promotes fat mass loss and lean mass gain in overweight and obese premenopausal women. *J Nutr.* (2011) 141:1626–34. doi: 10.3945/jn.111.141028
27. Clowes J, Hannon R, Yap T, Hoyle N, Blumsohn A, Eastell R. Effect of feeding on bone turnover markers and its impact on biological variability of measurements. *Bone.* (2002) 30:886–90. doi: 10.1016/s8756-3282(02)00728-7
28. Papageorgiou M, Dolan E, Elliott-Sale KJ, Sale C. Reduced energy availability: implications for bone health in physically active populations. *Eur J Nutr.* (2018) 57:847–59. doi: 10.1007/s00394-017-1498-8
29. Godin G. The Godin-Shephard leisure-time physical activity questionnaire. *Health Fit J Can.* (2011) 4:18–22.
30. Warburton DE, Bredin SS, Jamnik VK, Gledhill N. Validation of the PAR-Q+ and ePARmed-X+. *Health Fit J Can.* (2011) 4:38–46. doi: 10.1139/h11-044
31. Pescatello LS, Riebe D, Thompson PD. *ACSM's Guidelines for Exercise Testing and Prescription.* Philadelphia, PA: Lippincott Williams & Wilkins (2014).
32. Mayhew JL, Johnson BD, LaMonte MJ, Lauber D, Kemmler W. Accuracy of prediction equations for determining one repetition maximum bench press in women before and after resistance training. *J Strength Cond Res.* (2008) 22:1570–7. doi: 10.1519/JSC.0b013e31817b02ad
33. Dziura JD, Post LA, Zhao Q, Fu Z, Peduzzi P. Strategies for dealing with missing data in clinical trials: from design to analysis. *Yale J Biol Med.* (2013) 86:343.
34. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem.* (1993) 39:561–77. doi: 10.1093/clinchem/39.4.561
35. Sale C, Varley I, Jones TW, James RM, Tang JC, Fraser WD, et al. Effect of carbohydrate feeding on the bone metabolic response to running. *J Appl Physiol.* (2015) 119:824–30. doi: 10.1152/japplphysiol.00241.2015
36. Haakonssen EC, Ross ML, Knight EJ, Cato LE, Nana A, Wluka AE, et al. The effects of a calcium-rich pre-exercise meal on biomarkers of calcium homeostasis in competitive female cyclists: a randomised crossover trial. *PLoS One.* (2015) 10:e0123302. doi: 10.1371/journal.pone.0123302
37. Oosthuyse T, Bosch AN, Kariem N, Millen AM. Mountain bike racing stimulates osteogenic bone signaling and ingesting carbohydrate-protein compared with carbohydrate-only prevents acute recovery bone resorption dominance. *J Strength Cond Res.* (2021) 35:292–9. doi: 10.1519/JSC.0000000000003928
38. Klentrou P, McKee K, McKinlay BJ, Kurgan N, Roy BD, Falk B. Circulating levels of bone markers after short-term intense training with increased dairy consumption in adolescent female athletes. *Children.* (2021) 8:961. doi: 10.3390/children8110961
39. Theocharidis A, McKinlay BJ, Vlachopoulos D, Josse AR, Falk B, Klentrou P. Effects of post exercise protein supplementation on markers of bone turnover in adolescent swimmers. *J Int Soc Sports Nutr.* (2020) 17:1–11. doi: 10.1186/s12970-020-00350-z
40. Kouvelioti R, Kurgan N, Falk B, Ward W, Josse A, Klentrou P. Response of sclerostin and bone turnover markers to high intensity interval exercise in young women: does impact matter? *Biomed Res Int.* (2018) 2018:4864952. doi: 10.1155/2018/4864952
41. Hettiarachchi M, Cooke R, Norton C, Jakeman P. Temporal change in biomarkers of bone turnover following late evening ingestion of a

calcium-fortified, milk-based protein matrix in postmenopausal women with osteopenia. *Nutrients.* (2019) 11:1413. doi: 10.3390/nu11061413
42. Thomas SD, Morris HA, Nordin B. Acute effect of a supplemented milk drink on bone metabolism in healthy postmenopausal women is influenced by the metabolic syndrome. *Nutr J.* (2015) 14:1–6. doi: 10.1186/s12937-015-0092-2
43. Bauer D, Krege J, Lane N, Leary E, Libanati C, Miller P, et al. National bone health alliance bone turnover marker project: current practices and the need for US harmonization, standardization, and common reference ranges. *Osteoporos Int.* (2012) 23:2425–33. doi: 10.1007/s00198-012-2049-z
44. Hammond KM, Sale C, Fraser W, Tang J, Shepherd SO, Strauss JA, et al. Post-exercise carbohydrate and energy availability induce independent effects on skeletal muscle cell signalling and bone turnover: implications for training adaptation. *J Physiol.* (2019) 597:4779–96. doi: 10.1113/JP278209
45. Townsend R, Elliott-Sale KJ, Currell K, Tang J, Fraser WD, Sale C. The effect of postexercise carbohydrate and protein ingestion on bone metabolism. *Transl J Am Coll Sports Med.* (2017) 2:129–37. doi: 10.1249/MSS.0000000000001211
46. Guillemant J, Accarie C, Peres G, Guillemant S. Acute effects of an oral calcium load on markers of bone metabolism during endurance cycling exercise in male athletes. *Calcif Tissue Int.* (2004) 74:407–14. doi: 10.1007/s00223-003-0070-0
47. Kohrt WM, Wherry SJ, Wolfe P, Sherk VD, Wellington T, Swanson CM, et al. Maintenance of serum ionized calcium during exercise attenuates parathyroid hormone and bone resorption responses. *J Bone Miner Res.* (2018) 33:1326–34. doi: 10.1002/jbmr.3428
48. Nelson K, Kouvelioti R, Theocharidis A, Falk B, Tiidus P, Klentrou P. Osteokines and bone markers at rest and following plyometric exercise in pre-and postmenopausal women. *Biomed Res Int.* (2020) 2020:7917309. doi: 10.1155/2020/7917309
49. Kurgan N, McKee K, Calleja M, Josse AR, Klentrou P. Cytokines, adipokines and bone markers at rest and in response to plyometric exercise in obese vs normal weight adolescent females. *Front Endocrinol (Lausanne).* (2020) 11:531926. doi: 10.3389/fendo.2020.531926
50. Kouvelioti R, LeBlanc P, Falk B, Ward W, Josse A, Klentrou P. Effects of high-intensity interval running versus cycling on sclerostin, and markers of bone turnover and oxidative stress in young men. *Calcif Tissue Int.* (2019) 104:582–90. doi: 10.1007/s00223-019-00524-1
51. Kouvelioti R, Kurgan N, Falk B, Ward WE, Josse AR, Klentrou P. Cytokine and sclerostin response to high-intensity interval running versus cycling. *Med Sci Sports Exerc.* (2019) 51:2458–64. doi: 10.1249/MSS.0000000000002076
52. Mezil YA, Allison D, Kish K, Ditor D, Ward WE, Tsiani E, et al. Response of bone turnover markers and cytokines to high-intensity low-impact exercise. *Med Sci Sports Exerc.* (2015) 47:1495–502. doi: 10.1249/MSS.0000000000000555
53. Maimoun L, Manetta J, Couret I, Dupuy A, Mariano-Goulart D, Micallef J, et al. The intensity level of physical exercise and the bone metabolism response. *Int J Sports Med.* (2006) 27:105–11. doi: 10.1055/s-2005-837621
54. Bu T, Zheng J, Liu L, Li S, Wu J. Milk proteins and their derived peptides on bone health: biological functions, mechanisms, and prospects. *Compr Rev Food Sci Food Saf.* (2021) 20:2234–62. doi: 10.1111/1541-4337.12707
55. Gómez AL, Kraemer WJ, Maresh CM, Lee EC, Szivak TK, Caldwell LK, et al. Resistance training and milk-substitution enhance body composition and bone health in adolescent girls. *J Am Coll Nutr.* (2021) 40:193–210. doi: 10.1080/07315724.2020.1770636
56. Dolan E, Sale C. Protein and bone health across the lifespan. *Proc Nutr Soc.* (2019) 78:45–55. doi: 10.1017/s0029665118001180
57. Josse AR, Ludwa IA, Kouvelioti R, Calleja M, Falk B, Ward WE, et al. Dairy product intake decreases bone resorption following a 12-week diet and exercise intervention in overweight and obese adolescent girls. *Pediatr Res.* (2020) 88:910–6. doi: 10.1038/s41390-020-0834-5
58. Josse AR, Atkinson SA, Tarnopolsky MA, Phillips SM. Diets higher in dairy foods and dietary protein support bone health during diet- and exercise-induced weight loss in overweight and obese premenopausal women. *J Clin Endocrinol Metab.* (2012) 97:251–60. doi: 10.1210/jc.2011-2165
59. Costello JT, Bieuzen F, Bleakley CM. Where are all the female participants in sports and exercise medicine research? *Eur J Sport Sci.* (2014) 14:847–51. doi: 10.1080/17461391.2014.911354
60. Devries MC, Jakobi JM. *Importance of Considering Sex and Gender in Exercise and Nutrition Research*. Ottawa, ON: NRC Research Press (2021).
61. Alswat KA. Gender disparities in osteoporosis. *J Clin Med Res.* (2017) 9:382. doi: 10.14740/jocmr2970w
62. Shetty S, Kapoor N, Bondu JD, Thomas N, Paul TV. Bone turnover markers: emerging tool in the management of osteoporosis. *Indian J Endocrinol Metab.* (2016) 20:846. doi: 10.4103/2230-8210.192914

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

# Chapter 4:

# Inflammatory Biomarkers are Associated with Bone Biomarkers in the Context of Acute High-impact Exercise in Young Female Adults

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**Specific Contribution:** I did not conduct/collect data or perform biochemical assays for this secondary analysis, but did the majority of the data analysis, interpretation, and writing of the manuscript.

***JLP**, ECF, PK & ARJ conceived the study. **JLP** prepared and wrote the paper. AE, ECF and **JLP** were responsible for statistical analyses.*

## 4.1 Abstract

Following high-intensity exercise there are well-characterized increases in bone resorption biomarkers and inflammatory cytokines, but it is unclear if these biomarkers are related. The purpose of this secondary analysis was to investigate the association between concentrations of inflammatory biomarkers and bone biomarkers following a single bout of high-intensity/impact exercise and post-exercise nutrition. We performed a secondary analysis on data collected from a repeated measures crossover study, in which 13 healthy, young adult females (age = 20.3 ± 2.3y) performed combined high-intensity resistance and high-impact plyometric exercise followed by nutrient consumption (consumed 5-min and 75-min post-exercise; skim milk [MILK] vs. isoenergetic carbohydrate beverage [CHO]). Venous blood was obtained pre-exercise, 15-min, 75-min, 24-h, and 48-h post-exercise. Serum bone biomarkers (cross-linked C-telopeptide of type I collagen [CTX], receptor activator of NF-κB ligand [RANKL], sclerostin [SOST], osteoprotegerin [OPG], osteocalcin [OC]) and cytokines (interleukin [IL]-1β, IL-6, IL-10, and tumor necrosis factor-alpha [TNF-α]) were measured. To examine the relationships between the inflammatory and bone biomarker responses, we applied a separate linear mixed regression model for each bone biomarker. There were significant associations between bone and inflammatory biomarkers, including: CTX with TNF-α (positive association) and IL-10 (negative association), OPG with TNF-α (negative association) and IL-10 (positive association), RANKL with IL-6 (positive association) & SOST with TNF-α (negative association) and IL-10 (positive association). Overall, our findings suggest that relationships exist between inflammatory and bone biomarkers that relate to the physiology of the bone remodeling cycle in the context of an exercise stressor.

**Key Words:** Bone, Inflammation, Biomarker, Relationship, Post-exercise, Regression

**Abbreviations:**
BMD: bone mineral density
BMI: body mass index
CHO: carbohydrate
CI: confidence intervals
CTX: C-telopeptide of type I collagen
DAMP: damage associated molecular patterns
NF-κB: nuclear factor-kappa B
IL : interleukin
OC: osteocalcin
OPG: osteoprotegerin
RANKL: receptor activator of NF-κB ligand
SOST: sclerostin
TLR: toll-like receptors
TNF-α: tumor necrosis factor-alpha

## 4.2 Introduction

The emerging field of osteoimmunology explores links between the immune system and the skeleton [77]. Several commonalities exist between these systems, including shared cellular lineages [370], transcription factors and receptor targets [371], as well as the ability for these tissues/cells to "cross-talk" and reciprocally regulate one another [372, 373]. While innate factors such as genetics, age and sex [374, 375] play a significant role in determining the health/function of these systems, they can also be modulated by lifestyle factors, including nutrition and exercise [376].

Loading the skeleton through exercise [377] stimulates mechanosensing osteocyte cells within bone tissue, which then initiate the sequence of bone remodelling [64]. The process begins catabolically, with osteoclasts breaking down and resorbing damaged (i.e., micro-fractured) or old bone, which is followed by the anabolic process of osteoblasts depositing new bone collagen [64, 67]. This new bone collagen gets mineralized into resilient, functional mature bone tissue, which

entombs and transforms osteoblasts into osteocytes, establishing mechanosensing capabilities within this newly formed tissue [69]. In addition to mechanical loading [378], acute exercise can stimulate bone tissue through various other mechanisms, including metabolic signalling, calcium kinetics [379], redox balance [380] and *via* crosstalk with other systems/tissues [381], including muscle and adipose tissue, and immune cells [147]. Repeated exercise (i.e., multiple bone cell stimulations over time) is adaptively osteogenic, consistently resulting in enhancements of bone mineral density (BMD) and reductions in fracture risk across the life span [58, 382-384]. Human bone can be a challenging tissue to study both *in vivo* and *in vitro* due to our inability to obtain bone biopsies, as well as the prolonged time course (months to years) over which bone mass changes occur [67]. The latter factor renders static indicators of bone health, such as BMD measured by dual energy X-ray absorptiometry or bone quality (i.e., microarchitecture) by computed tomography, ill-suited to assess acute responses to stimuli [147]. Instead, analysing bone biomarkers can be used to evaluate both the dynamic cellular processes that govern/modulate the bone remodeling cycle (e.g. OPG, RANKL, SOST, OC), as well as biological fragments released during these processes (e.g., CTX) [123]. Measurement of circulating biomarkers is therefore commonly used in both clinical/diagnostic settings [123], as well as for research involving acute/short term experiments and interventions that influence bone (such as nutrition or exercise) [1, 120, 147].

Acute bouts of exercise can also initiate a complex cascade of immune/inflammatory responses, differing based on the type, duration, and intensity of the exercise (in addition to other factors like training status, age, and health of the individual) [385]. Following high-intensity exercise there are generally increases in peripheral blood immune cell numbers, granulocyte activity, lymphocyte proliferation and cytokines, with an initial release of pro-inflammatory

cytokines (e.g. TNF-α, IL-1β), followed by the release of anti-inflammatory mediators (IL-4, IL-10) in order to resolve/attenuate the inflammatory response and initiate recovery [385, 386]. Interestingly, this somewhat mirrors the pattern of the bone biomarker responses, whereby an initial transient increase in bone resorption (i.e., increases in CTX) [387, 388], is sometimes followed by a later (~60 minute) upregulation of bone formation markers [148]. Considering the close relationship between the immune and skeletal systems, it is plausible that one of the mechanisms mediating bone remodelling following exercise is inflammation, communicated *via* circulating cytokines.

Nutrition can also influence the circulating levels of bone and inflammatory biomarkers at rest and post-exercise [1, 120, 298]. To ensure bones are sufficiently able to recover, they must be supplied with sufficient calories, and specific macro- and micronutrients post-exercise to replenish nutrient losses and repair microdamage [389]. A recent review by Dolan & colleagues [120] indicated that nutritional interventions may attenuate post-exercise bone resorption, with wholefood dairy products being of interest considering that they supply energy, carbohydrates, protein and essential bone supporting micronutrients within the same bolus. Meta-analyses, as well as data from our lab, have also indicated that dairy products may play a role in reducing inflammation [5, 298, 323]. At present, despite knowing that both bone and inflammatory markers both change post-exercise, and that their responses can be modulated by post-exercise nutrition, very little is known regarding how these biomarkers (and the systems they control) interact with each other.

Therefore, the purpose of this study was to investigate the association between concentrations of inflammatory biomarkers and bone biomarkers in the context of a single bout of high-intensity/impact exercise and post-exercise nutrition. We hypothesize that there will be a

relationship between bone and inflammatory markers in this acute post-exercise context, and that pro-inflammatory cytokine concentrations will be positively associated with catabolic bone marker concentrations (CTX, RANKL, SOST), and negatively associated with anabolic bone marker concentrations (OPG, OC), and vice versa with anti-inflammatory cytokines.

## 4.3 Methods

The bone biomarker and inflammatory cytokine response data from our acute exercise study (clinical trial registration: NCT03615989) have been previously and separately published [1, 298]. This paper will investigate the relationship between these biomarkers as a novel secondary analysis. Thirteen young, healthy female adults were recruited after initial screening ensured they met the following inclusion criteria: 1) free of any metabolic or musculoskeletal medical conditions, 2) body mass index (BMI) 18.5–24.9 $kg/m^2$, 3) recreationally active and not participating in a resistance training program, 4) no known allergy to dairy protein or lactose intolerance.

In a randomized within-subject crossover design, participants completed 2 trials consisting of either: 1) exercise bout + post-exercise carbohydrate consumption (CHO) or 2) exercise bout + post-exercise milk consumption (MILK). Prior to their first trial, all participants had baseline measurements taken and completed an exercise familiarization session. These measures included height, weight, and body fat percentage measured using bioelectrical impedance analysis (BIA; InBody 520 BIA system; Biospace Co., Inc. Los Angeles, CA, United States). They also completed a Godin Shephard leisure time physical activity questionnaire, a general medical/health questionnaire and a physical activity readiness questionnaire (2018 Par-Q +) [1]. Participants were instructed to keep their diet consistent between trial sessions and were asked to record all their

food and drink intake over a 2-day period for each trial (beginning on the day of the exercise bout). Dietary intakes were analyzed using the ESHA Food Processor Program (Food Processor SQL, ESHA Research, Salem, OR). Dietary intakes throughout the two trials have been previously reported [1, 298]. On trial days, participants were instructed to arrive at the lab following an overnight fast and to refrain from alcohol and exercise for 48h before and after the trial session. A minimum of 4-weeks separated the trials in those not on hormonal contraceptives (n = 8), and all testing was completed during the early follicular phase of the menstrual cycle to help ensure consistency between trials. Participants on monophasic hormonal contraceptives (n = 5) completed trials with a minimum 2-week washout between, during the hormone delivery phase.

**Exercise Protocol**

Participants completed a familiarization session to learn how to correctly perform the trial exercises and to determine their estimated 1 repetition maximum (1 RM) on three resistance exercise machines (Chest Press, Seated Row, Leg Press) in accordance with the American College of Sports Medicine exercise testing recommendations. This was then used to determine appropriate loading for the trial exercise bouts, totalling 3–4 sets/exercise, 8–12 reps/set at ∼75% 1 RM. Exercise bouts were performed from approximately 09:00am - 10:00am and participants completed the same exercise bout in both trials, consisting of combined plyometric (broad jumps, pogo jumps, box drops, explosive lunges; 198 impacts/session), and resistance exercises (chest press, leg press, seated row). Additional details regarding the exercise protocol have been previously published [1, 298].

**Nutrition Protocol**

Two isoenergetic and isovolumetric nutritional supplements were given in a randomized trial order, with the two beverages being consumed 5–10 min following exercise cessation, and 1h

following completion of the first trial drink. For the CHO condition, each drink consisted of 52.7 g of maltodextrin (each drink: 200 kcals, 0 g protein, 50g carbohydrate, 0 mg calcium) mixed with approximately 550 mL of water plus a calorie-free, fruit flavored sweetener. For the MILK condition, each drink consisted of 550 mL of 0% skim milk (Natrel fine filtered skim milk, Agropur, QC, Canada; each drink: 200 kcals, 20 g protein, 29 g carbohydrate, 660 mg calcium).

**Blood Sample Collection Protocol**

Venous blood samples were collected from the median cubital vein pre-exercise as well as 15-min, 75-min, 24-h, and 48-h post-exercise (**Figure 10**). After each draw, blood samples were centrifuged (serum samples were rested for 25 minutes, plasma samples were spun immediately) at 1300 g and 4°C for 15 min, with serum and plasma then aliquoted and stored at –80°C until analysis.

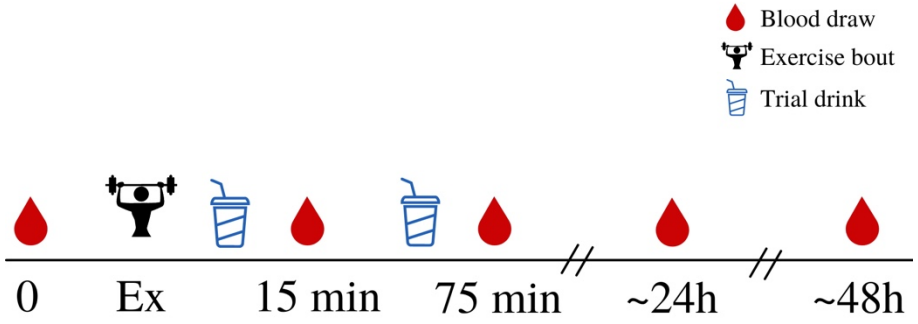



**Figure 10.** Overview of the experimental trial. Originally published in Prowting et al. (2022) [1].

**Biomarker Analysis**

Serum concentrations CTX (cat# E-EL-H0960; Elabscience, China) and SOST (cat# DSST00; R&D, Minneapolis, MN)) were analyzed in duplicate using enzyme linked immunosorbent assays (ELISA), RANKL (cat# HRNKLMAG-51K-01; Millipore Corporation, Etobicoke, ON) using a single-plex magnetic bead assay, and total OC and OPG using a multiplex

human bone magnetic bead assay (cat# HBNMAG-51K; Millipore Corporation, Etobicoke, ON). Plasma concentrations of the inflammatory markers IL-6, TNF-α, IL-1β, and IL-10 were analyzed in duplicate using microbead multiplex assay kits (Human high sensitivity T cell panel HSTCMAG-28SK, Millipore Corp, Burlington, MA, USA).

**Statistical Analysis**

Detailed descriptions for how data were selected and processed have been previously published [1, 298]. Dietary intake data and exercise volume load (sets × reps × weight) were compared between trial conditions using a paired samples *t*-test (with *p*-values considered significant at $p \leq 0.05$).

To examine the relationships between the inflammatory and bone biomarkers, we analyzed the data using linear mixed models, creating a separate regression model for each bone biomarker. Analyses were conducted using the software environment R-studio version 4.3.1 (2023) and utilised the following packages: lme4, lmerTest, performance, ggeffects, and MuMin.

Dependent variables for the models consisted of the bone biomarkers: serum CTX, OPG, RANKL, SOST, and OC. The fixed effects covariates used were time, supplement condition (milk or carbohydrate, with carbohydrate being the baseline), plasma IL-6, IL-10, IL-1ß and TNF-α. Participant ID was used as the random effects covariate. Significant fixed effects coefficients along with their corresponding p-values and 95% confidence intervals (CI) were reported. Marginal (fixed effects only) and conditional (fixed and random effects) $R^2$ values were calculated and reported as the percentage of variance explained by each model. A significance level of $\alpha \leq 0.05$ was set and both unadjusted and adjusted (using Benjamini & Hochberg's False Discovery Rate correction [390]) p-values are reported for each model.

## 4.4 Results

### Participant Characteristics, Nutrient Intake, Training Load

Participant characteristics at baseline for the 13 participants that completed both intervention arms are shown in **Table 4**.

**Table 4.** Participant characteristics measured at the start of the study (n=13). BMI = body mass index. Originally published in Prowting et al [1].

| Participant Characteristics | |
|---|---|
| Age (years) | 20.3 ± 2.3 |
| Height (m) | 1.6 ± 0.1 |
| Weight (kg) | 56.6 ± 5.0 |
| BMI (kg/m$^2$) | 21.0 ± 1.1 |
| Body Fat (%) | 23.5 ± 3.3 |

Values are presented as mean ± standard deviation (SD). Abbreviations: BMI = body mass index.

As reported previously [1], comparison of nutrient intake showed no significant differences between the two intervention arms when trial drinks were removed, but some expected differences when they were included (**Table 5**). There were also no significant differences in training load of the trials between the two intervention arms as reported previously [1].

**Table 5.** Average daily dietary intake during the MILK and CHO trials, based on analysis of 2-day food records (day of exercise and day post-exercise) including the provided trial drinks (n=11). Originally published in Prowting et al [1].

| Dietary Variable | CHO | MILK | p-value |
|---|---|---|---|
| **Energy (kcal)** | 1958 ± 627 | 1873 ± 616 | 0.46 |
| **Protein (g)** | **67 ± 27** | **83 ± 31** | **0.01** |
| **Carbohydrate (g)** | 274 ± 91 | 243 ± 106 | 0.07 |
| **Fat (g)** | 68 ± 30 | 68 ± 24 | 0.98 |
| **Vitamin D (IU)** | **47 ± 54** | **408 ± 50** | **<0.001** |
| **Calcium (mg)** | **561 ± 374** | **1073 ± 266** | **<0.001** |
| **Iron (mg)** | 10 ± 4 | 11 ± 6 | 0.67 |
| **Magnesium (mg)** | 194 ± 145 | 282 ± 113 | **0.01** |
| **Potassium (mg)** | **1768 ± 1379** | **2754 ± 1316** | **<0.001** |

Values are presented as mean ± (SD). Significant differences ($p<0.05$) denoted by bolded text.

## CTX

Using both fixed and random effects, our model explains 86.7 % of the variation in CTX, with 17.0 % of the variation coming from fixed effects only. Both TNF-α and IL-10 concentrations were found to have small, but significant associations with CTX concentrations. While holding all other variables constant, an increase of 1 pg/mL of TNF-α was associated with a 0.03 pg/ml increase in CTX (**Figure 11A**; $p<0.001$; adjusted $p<0.001$ 95% CI [0.013, 0.039]), whereas an

increase of 1 pg/mL of IL-10 was associated with a 0.01 pg/ml decrease in CTX (**Figure 11B**; p=0.034, adjusted p=0.089; 95% CI [-0.019, -0.0002]).

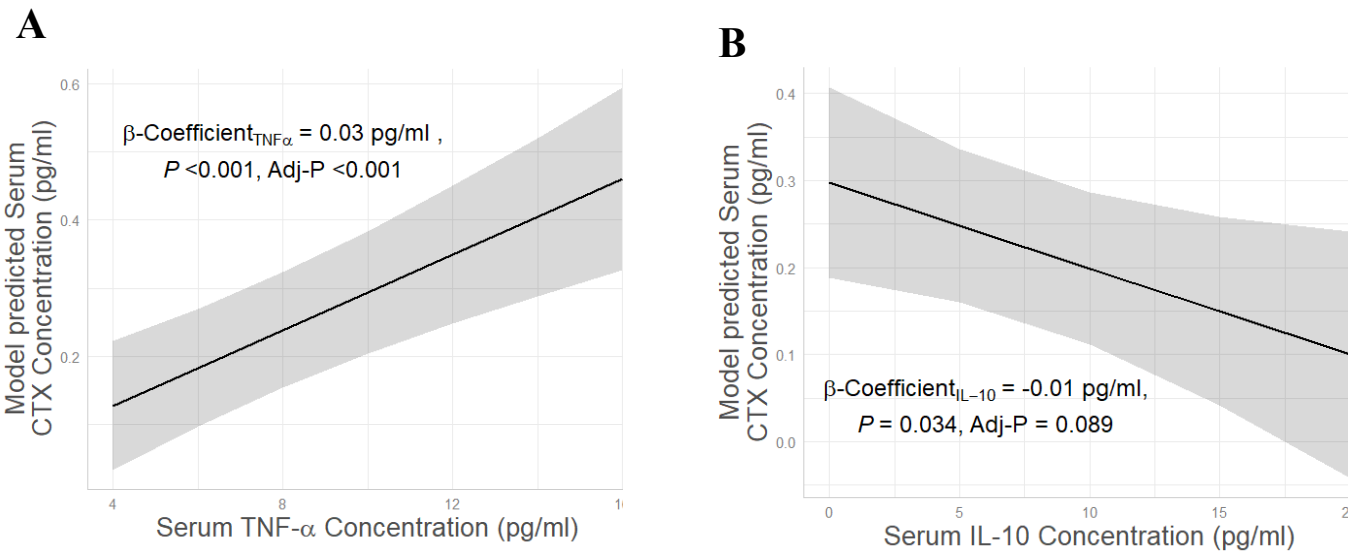



**Figure 11.** Graphical representation of the significant associations between: **A**) CTX and TNF-α, **B**) CTX and IL-10. Shading represents 95% confidence intervals. Abbreviations: CTX = C-telopeptide of type I collagen; TNF-α = tumor necrosis factor-alpha; IL-10 = Interleukin-10.

**OPG**

Using both fixed and random effects, our model explains 83.1% of the variation in OPG, with 10.8% of the variation coming from fixed effects. Variables found to have significant association with circulating OPG concentrations include TNF-α and IL-10. While holding all other variables constant, an increase of 1 pg/mL of TNF-α was associated with a decrease of 11.3 pg/mL of OPG (**Figure 12A**; p=0.035; adjusted p=0.087; 95% CI [-21.73, 2.72]), an increase of 1 pg/mL of IL-10 was associated with an increase of 11.2 pg/mL of OPG (**Figure 12B**; p=0.007; adjusted p=0.034; 95% CI [2.49, 18.79]).

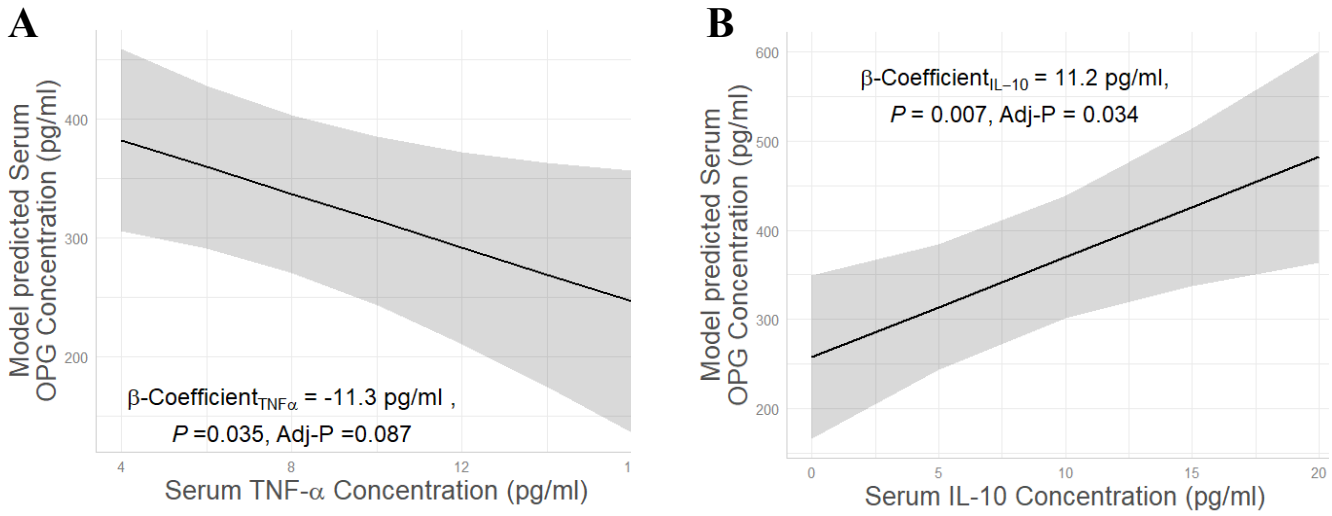



**Figure 12.** Graphical representation of the significant associations detected between: **A**) OPG and TNF-α and **B**) OPG and IL-10. Shading represents 95% confidence intervals. Abbreviations: OPG = osteoprotegerin; TNF-α = tumor necrosis factor-alpha; IL-10 = Interleukin-10.

## RANKL

Using both fixed and random effects, our model explains 88.1% of the variation in RANKL, with 60.1 % of the variation coming from fixed effects only. IL-6 was found to have a significant association with RANKL. While holding all other variables constant, an increase of 1 pg/mL of IL-6 was associated with an increase of 1.8 pg/mL of RANKL (**Figure 13**; p<0.001; adjusted p=0.004; 95% CI [0.98, 2.55]).

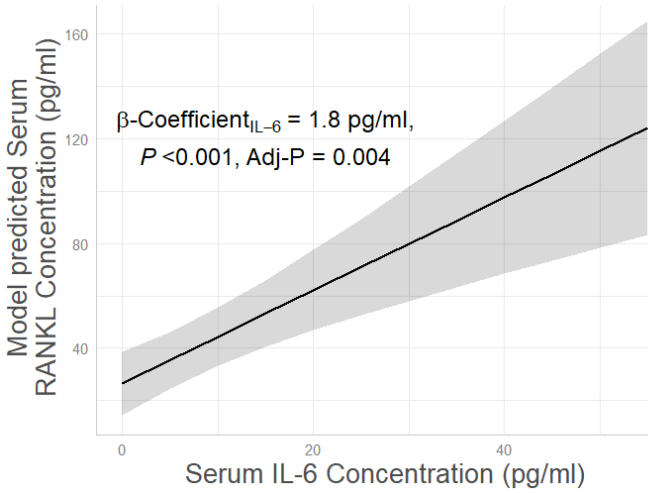
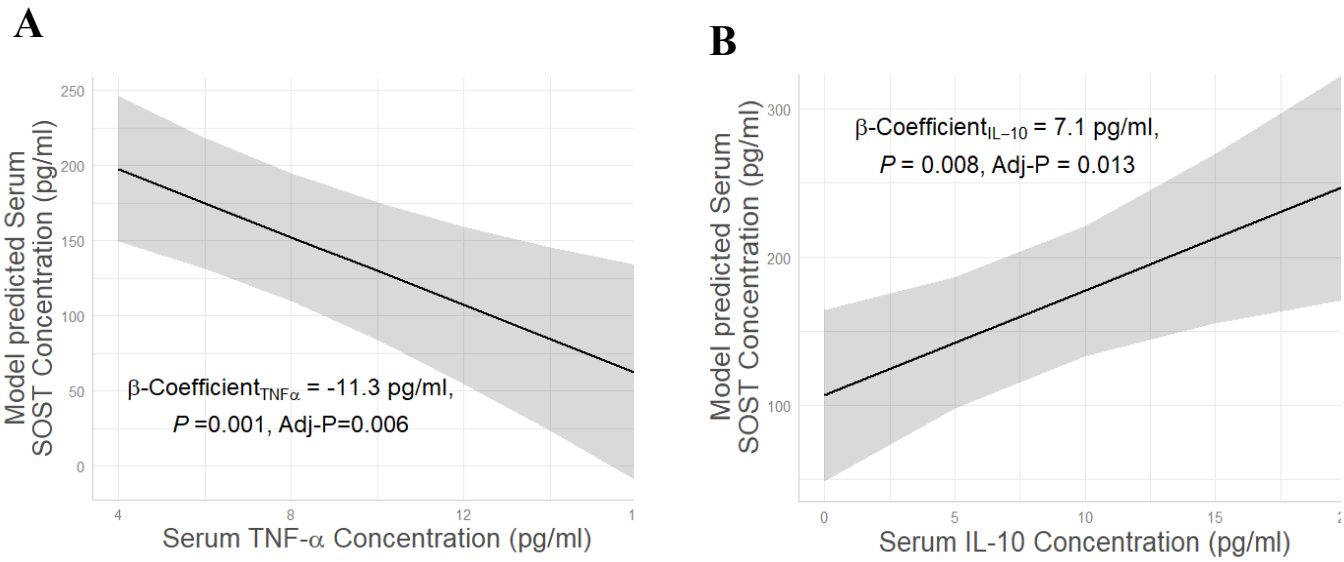


**Figure 13.** Graphical representation of the significant associations detected between RANKL and IL-6. Shading represents 95% confidence intervals. Abbreviations: RANKL = receptor activator of NF-κB ligand; IL-6 = Interleukin-6.

**SOST**

Using both fixed and random effects, our model explains 83.8% of the variation in SOST, with 18.7% of the variation coming from fixed effects only. The variables significantly associated with SOST include TNF-α, IL-10 and dietary condition,. While holding all other variables constant, an increase of 1 pg/mL of TNF-α was associated with a 11.3 pg/ml decrease in SOST (**Figure 14A**; p=0.001, adjusted p=0.006; 95% CI [-17.79, -4.12]), and an increase of 1 pg/mL of IL-10 was associated with a 7.1 pg/ml increase in SOST (**Figure 14B**; p=0.008, adjusted p=0.013; 95% CI [1.97, 12.08]). On average, while holding the other variables constant, SOST levels were higher by 19.5 pg/ml (**Figure 14C**; p=0.007; adjusted p=0.013; CI [5.59, 33.24]) in MILK compared to CHO (post-hoc analysis determined that this was due to higher baseline levels).



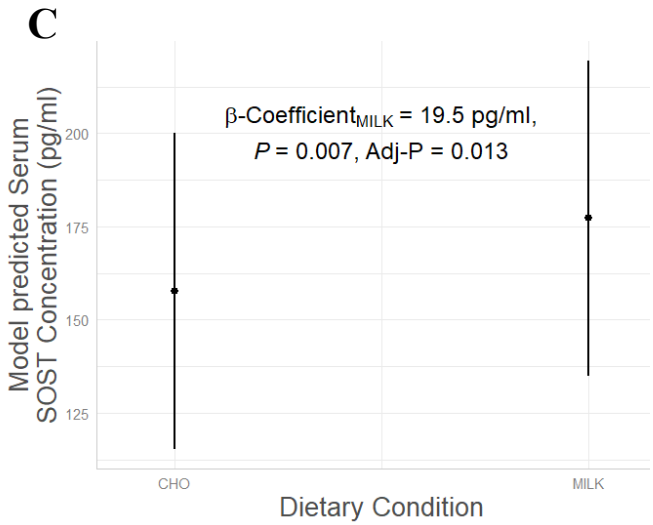



**Figure 14.** Graphical representation of the significant associations detected between: **A**) SOST and TNF-α, **B**) SOST and IL-10, and **C**) SOST and dietary condition. Shading represents 95% confidence intervals. Abbreviations: SOST = sclerostin; TNF-α = tumor necrosis factor-alpha; IL-10 = Interleukin-10.

## OC

Using both fixed and random effects, our model explains 81.0% of the variation in OC, with 3.6% of the variation coming from fixed effects only. There were no significant associations between OC and inflammatory markers when assessed with our model.

## 4.5 Discussion

Our findings show that a relationship exists between inflammatory and bone turnover marker responses following a bout of high-intensity exercise combined with nutrient consumption. We observed that CTX, a bone marker related to catabolism, was positively associated with TNF-α (pro-inflammatory) and negatively associated with IL-10 (anti-inflammatory) in the context of high intensity impact and resistance exercise. For the bone formation marker OPG, we observed a negative association with pro-inflammatory TNF-α, and a positive association with anti-inflammatory IL-10. We also observed that the resorptive RANKL biomarker was positively associated with IL-6 concentrations. We also observed a negative association between SOST and the pro-inflammatory TNF-α cytokine, and a positive association with the anti-inflammatory IL-10 cytokine. Finally, we observed that SOST concentrations were greater in the MILK compared to CHO, however, post-hoc testing indicated that differences in baseline concentrations of SOST were the main driver of this effect.

Pathological chronic inflammation is well established as a promoter of bone resorption [75, 391], and both systemic and local mechanisms involving cytokines have been implicated in this catabolic process [118]. Although post-exercise inflammatory responses have been posited to influence acute post-exercise bone turnover responses [120, 392], this relationship has not yet been well studied. We have previously found correlations between bone and inflammatory responses to exercise [295, 297], but sought for the first time herein to further investigate these relationships using more sophisticated modelling techniques that appropriately control for the other covariates.

Assessing circulating bone turnover markers provides insight into acute or short-term bone responses, given that months or years may be required to see meaningful alterations in static measures (e.g. BMD) [120]. In this secondary analysis of data from our two previously published

studies [1, 298], we found a significant association between the bone breakdown marker CTX and two of the inflammatory biomarkers that were concomitantly measured. Specifically, increases in pro-inflammatory TNF-α were associated with increases in CTX, and increases in anti-inflammatory IL-10 were associated with decreases in CTX. Thus, our data provide supporting evidence that these systems are interlinked such that pro-inflammation predicted increased bone resorption and anti-inflammation predicted decreased bone resorption. Mechanisms for the link between the systems may relate to exercise acting as a systemic stressor and inducing damage associated molecular patterns (DAMPs). When toll-like receptors (TLRs; TLR-4 in particular [103]) are stimulated by DAMPs, pro-inflammatory pathways such as the NOD-like receptor protein 3 (NLRP3) inflammasome and nuclear factor kappa B (NF-κB) are activated, leading to recruitment of cytokines and leukocytes to the damage site [103, 393]. These TLRs are also expressed on bone cells [102], and their stimulation combined with the localised (between endosteum and periosteum) macrophage-induced release of TNF-α and IL-1 [75, 394] can increase the activity of osteoclasts, which leads to elevated bone resorption [102]. Other prominent DAMPs generated during exercise are reactive oxygen species (ROS) which may be involved in RANKL-mediated osteoclastogenesis [111]. Our findings suggest that inflammatory signalling processes relate to bone metabolism/remodelling, and that pro- and anti-inflammatory biomarkers are positively and negatively related to bone catabolism, respectively.

The OPG/RANKL system is an important mechanism through which bone homeostasis is maintained [98], and involves either RANKL-RANK binding (increasing osteoclast activity) or OPG-RANK binding (competitively inhibiting RANK-RANKL binding, reducing osteoclast activity). Our analysis showed that as the anti-inflammatory cytokine IL-10 increased, so did OPG, but that as the pro-inflammatory cytokine TNF-α increased, OPG decreased. It is therefore

possible that the balance between pro- and anti-inflammatory signalling may affect bone resorption by altering OPG release. If the anti-inflammatory IL-10 is driving increased OPG production/release, then this would block RANKL binding, reduce osteoclast activity and shift bone turnover to a more formative state. In osteoblastic cell models, the effects of cytokines on OPG production have been mixed, with some research showing that IL-1β and TNF-α increase expression of OPG [395], whereas others have shown that cytokines such as IL-7 (pro-inflammatory) reduce OPG production from osteoblasts [396]. It should be noted that while *in vitro* studies have traditionally ascribed OPG production to be almost exclusively from osteoblasts, in recent decades it has been shown that lymphocytes (both T and B cells) play an important role in both producing (B cells may be responsible for 45% of OPG production in mice [397]) and stabilising OPG [398]. Although the B cell response to exercise is inconsistent [399], it is plausible that the relationship we found is between circulating cytokines and B cell derived OPG, and may not be related to bone directly. A recent study in mice suggests that OPG primarily regulates bone turnover in a paracrine manner, and that circulating OPG concentrations may not reflect what is happening locally within this tissue [400]. More research is needed to better understand the local and systemic responses of bone in relation to OPG, but to our knowledge, our study is the first to demonstrate a relationship between OPG and inflammatory cytokines within an exercise context in humans.

As for RANKL, we observed a significant positive association between its response and the response of IL-6. This cytokine is particularly interesting because its function can vary depending on the tissue in which it is released from. For example, it can be released from various immune cells (monocytes, T cells, B cells etc.) and when overproduced can induce a proinflammatory response [401, 402]. In recent decades though, it has been identified to be

released by muscle (referred to as a myokine) following exercise, with some data indicating that muscle derived IL-6 may have positive effects on health [403] as well as an opposing anti-inflammatory function compared to immune cell derived IL-6 [404]. These opposing actions also appear to hold true in regard to bone remodelling, with research showing that IL-6 can have both positive and negative effects on osteoblast and osteoclast differentiation [405]. In cell models, the effects of osteoclastogenesis were found to be due to the induction of RANKL in osteoblasts and fibroblast-like synoviocytes by IL-6/IL-6R signals [406, 407]. Other animal and cell model research has demonstrated that TNF-α can have potent synergistic effects on the osteoclastogenic potential of RANKL *via* increased differentiation of hemopoietic stem cells and macrophages into preosteoclasts (increasing the size of the osteoclast pool) as well as through increasing expression of RANK on these preosteoclasts (increasing their sensitivity to RANKL) [75, 108]. Research within pathological cohorts that display elevated chronic inflammation (e.g. chronic obstructive pulmonary disease, rheumatoid arthritis) have observed inverse relationships between circulating RANKL (or OPG/RANKL ratio) and BMD [408, 409], but it is not clear if this relationship exists in healthy individuals. A cross sectional study in healthy post-menopausal females observed a negative association between circulating RANKL and BMD but also found that C-reactive protein (a commonly assessed clinical marker of inflammation) was not associated with BMD [104]. Therefore the positive relationship we observed between CTX (bone breakdown byproduct) and TNF-α may be the result of OPG/RANKL system modulation by cytokines, or an alternate mechanism such as by increasing RANK expression on osteoclast precursors which would increase their sensitivity to RANKL. Another hypothesis is that some cytokines (such as TNF-α) may result in an uncoupling of bone formation from bone resorption, perhaps by disrupting the differentiation of osteoblasts [410] or by directly interrupting signaling that couples these two

processes [75]. Alternatively, it may be that changes to local but not circulating RANKL concentrations (paracrine signalling) lead to increased bone resorption (i.e., increased CTX), but that we were unable to detect this in the venous blood samples measured.

In addition to osteoclast activity and bone resorption, net bone turnover is also determined by osteoblast activity and bone deposition. Mature osteoblast formation (i.e., osteoblastogenesis) is primarily mediated through the canonical Wingless (Wnt) signalling pathway, in which Wnt ligands bind to a receptor complex consisting of low-density lipoprotein receptor-related protein (LRP) 5 or 6 and one of ten Frizzled molecules [67]. This results in the release and stabilization of cytoplasmic b-catenin, which is then translocated to the nucleus to activate target gene transcription and ultimately stimulate osteoblast differentiation and proliferation [67]. This pathway is negatively regulated by the proteins SOST and Dickkopf (DKK) 1/2, which bind to co-receptors on LRP 5/6, inhibiting Wnt signalling and thus reducing bone formation [85]. Our results indicated a significant negative relationship between pro-inflammatory TNF-α with SOST. In contrast, an increase in the anti-inflammatory IL-10 cytokine was associated with an increase in circulating SOST. These findings are unexpected considering that TNF-α has previously been shown in mice to upregulate the expression of SOST through the NF-κB signaling pathway, by inducing the production of DKK1, and by contributing toward apoptosis of osteoblasts [108, 411]. In the original study [1], we observed a significant main time effect in which SOST initially increased 15-min post-exercise, dropped at 75-min post-exercise and then remained elevated at 24-h and 48-h post-exercise. In previous exercise studies performed by our group [295, 412], we also observed immediate elevations in SOST following exercise, but have not consistently observed the 24-h and 48-h elevations. We did however find a significant positive correlation between post-exercise SOST and TNF-α in one of these prior studies [295], which is the opposite

of what was found during this analysis. It is currently unclear why our results are divergent from previous observations, but it may be related to differences in study design, such as the difference in exercise modality used (high impact jumping and resistance exercise vs. running and cycling) or the post-exercise provision of energy that was involved with this study vs. no post-exercise provisions in the other study.

Through our statistical modelling we observed several significant relationships between bone and inflammatory markers in the context of an exercise stressor, however there are some limitations which must be considered. First, although relationships seem to be present between bone and inflammatory markers in an exercise context, we cannot be certain on the direction of causality (i.e., are inflammatory cytokines responsible for bone biomarker concentrations, or is it the other way around). That said, physiologically it is most feasible that inflammatory signalling is modulating bone biomarkers rather than the opposite. Secondly, despite using a crossover design, our sample size was relatively small, which may have reduced power. Finally, while our models generally were able to explain a good amount of the variance in bone markers using our fixed effects predictors, they were able to much better explain that variance using both the fixed and random effects. This means that differences between participants made up the majority of the model fit, potentially suggesting missing relevant predictors which are controlled for but can not be commented on in our models. Further research should continue to explore how these interconnected systems modulate one another, perhaps with more timepoints, and the assessment of how different exercise types, durations and intensities, as well as different post-exercise nutritional provisions can change these relationships.

## 4.6 Conclusion

Findings from this secondary analysis suggest that inflammatory and bone biomarkers are physiologically related in the context of an exercise and nutrition stimulus. Our models generally found that proinflammatory and anti-inflammatory cytokines, were positively and negatively associated with catabolic bone biomarkers, respectively. Improving our understanding of the underlying mechanisms and relationships between these interconnected systems could help to optimise acute exercise recovery strategies (i.e., resolving inflammation and supporting bone turnover/formation) as well as therapeutic techniques for those with chronic disease and increased inflammation to ensure exercise is not catabolic for bone. Future research should investigate the temporal relationship between post-exercise inflammation and bone responses in humans and how post-exercise nutrition may influence the bone-immune axis.

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**Ethics Approval:** This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committees of Brock University (REB: 17-402, 3 August 2018) and York University (REB: 2019-045, 31 January 2019).

# Chapter 5:

## Six Weeks of Increased Dairy Intake Imparts Minor Benefits to Inflammatory Cytokine Profiles in Individuals with Overweight and Obesity – A Randomized Crossover Trial

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**Specific Contribution:** I was the lead investigator and coordinator for the 2-year long data collection period of this study. I also completed biochemical assays, and did the majority of the data analysis, interpretation, and writing of the manuscript. Vascular measures, analysis and interpretation were performed by Dr Heather Edgell's group.

***JLP**, CGRP, DW, HE, PK and ARJ designed research. **JLP**, ECF, TJP, JALT, SG, NC and ARJ conducted research. **JLP**, TJP and ARJ analyzed data; **JLP** wrote the paper.*

## 5.1 Abstract

**Background**: Dairy products are an important source of macro/micronutrients and contain bioactive components with anti-inflammatory, antioxidant, and musculoskeletal supporting properties. Few studies have assessed the short-term effect of consuming a variety of wholefood dairy products (as opposed to single foods/nutrients) on a range of interrelated inflammatory, metabolic, antioxidant and vascular outcomes. **Objective:** The objective of this study was to compare responses of these outcomes between 6-weeks of a high mixed-dairy diet to 6-weeks of a habitually low dairy diet. **Methods:** Using a randomized crossover design, 30 adult human participants (18/12 female/male) with overweight/obesity (OW/OB) (BMI: 32.0±4.5kg/$m^2$) and pre-existing cardiometabolic disease risk factors were randomized to consume either a high-dairy (HD) diet (participants were provided with 3 servings/d of a mix of low-fat milk, Skyr yogurt, full-fat cheddar cheese) or to continue their habitual low-dairy (LD) diet (≤1 serving/d) for a 6-week period (separated by a ≥4-week washout). At baseline and then 6-weeks later, fasted blood samples were collected, body composition was assessed, and vascular measurements were taken. **Results:** There were no significant interactions or main effects for IL-6 (primary endpoint), however, there were significant interactions for tumor necrosis factor-alpha (TNF-α; p=0.038), and interleukin-4 (IL-4; p=0.022) such that the HD diet decreased and increased, respectively, vs. the LD diet. Main trial effects were present for both IL-1β (p=0.047; HD>LD) and carotid intima-media thickness (IMT; p=0.031; HD<LD), and a main time effect was found for reduced:oxidized glutathione ratio (GSH:GSSG; p=0.009; Baseline>6-weeks). There were no significant effects for other inflammatory, metabolic, glutathione, or vascular measures. **Conclusions:** Our study demonstrated that increasing mixed dairy product intake for 6-weeks favourably modulated some cytokines in the absence of metabolic marker, vascular, body weight or fat changes, and therefore, may help ameliorate low-grade chronic inflammation (LGCI) in individuals with OW/OB and cardiometabolic dysfunction.

**Abbreviations:**

CRP: c reactive protein
FMD: flow mediated dilation
GSH: reduced glutathione
GSSG: oxidized glutathione
HD: high dairy

HDL: high density lipoproteins
IL-1β: interleukin 1 beta
IL-4: interleukin 4
IL-6: interleukin 6
IL-10: interleukin 10
IFN-γ: interferon gamma
IMT: intima-media thickness
LD: low dairy
LDL: low density lipoproteins
LGCI: low grade chronic inflammation
OW/OB: overweight and obesity
ROS: reactive oxygen species
TC: total cholesterol
TG: triglycerides
tGS: total glutathione
TNF-α: tumor necrosis factor alpha

**Clinical Trial Registry:** NCT04902417
https://clinicaltrials.gov/study/NCT04902417?term=NCT04902417&rank=1
**Keywords**: inflammation; Dairy foods; cardiometabolic risk; blood biomarkers; glutathione

## 5.2 Introduction

Obesity is a highly prevalent, non-communicable disease characterized by excess adiposity and is considered a major public health concern due to its deleterious effects on human physiological function as well as its direct role in the development of chronic metabolic disease [413, 414]. The immune system of individuals with overweight/obesity (OW/OB; BMI ≥25 kg/m$^2$) can become dysregulated due to increased production of pro-inflammatory molecules (e.g., tumor necrosis factor-alpha [TNF-α], interleukin-6 [IL-6], IL-1, c-reactive protein [CRP]) from adipose or other tissues [242, 381]. Inflammation is an important defense mechanism that typically involves a transient upregulation of immune mediators (e.g., cytokines) to deal with threats like infection or injury [241]. In healthy individuals, this pro-inflammatory response automatically self-regulates and resolves *via* the release of anti-inflammatory cytokines, inhibition of pro-

inflammatory signalling cascades, and activation of regulatory cells [242]. However, individuals with OW/OB may experience an inability to resolve these responses, resulting in a chronic and constant increase in the local and systemic inflammatory milieu throughout their body (i.e., low-grade chronic inflammation [LGCI]) [244, 245]. This state of LGCI is also associated with the development of more serious disease/dysfunction, including atherosclerosis, osteoporosis, insulin resistance, some cancers, and hypertension [246, 247]. Research on effective lifestyle modification strategies to counteract OW/OB as well as the concomitant state of LGCI is a priority. Dietary modulation is a key strategy that may fulfill this purpose and improve the health and well-being of individuals with OW/OB, even in the absence of weight loss [415]. In recent decades, there is compelling evidence suggesting that the types of foods consumed can play a role as a suppressor or promoter of LGCI both within OW/OB and normal weight individuals and that specific dietary patterns, food groups, or nutrients can modulate these inflammatory effects [416].

Dairy products, such as milk and yogurt, are a food type that is associated with beneficial (or neutral) effects on systemic inflammation [416], cardiometabolic disease risk and mortality [10, 200]. Several review studies have indicated that increased dairy consumption may reduce circulating inflammatory biomarkers (e.g. TNF-α, IL-6, CRP) particularly in individuals with metabolic dysfunction [5, 200]. These wholefoods/beverages are relatively inexpensive compared to dietary supplements, are available within most grocery stores, and provide an important source of dietary macro/micronutrients, and thus significantly improve diet quality when habitually consumed [44, 180]. They also provide important bioactive components that have been shown to reduce the risk of developing cardiometabolic disease [9, 417], which may relate to the ability of dairy to modulate inflammation and oxidative stress [2, 6, 29, 200]. Components of dairy that have been identified as contributors to its anti-inflammatory effect include peptides and hydrolysates in

casein, lactoferrin (a whey protein peptide), oleic acid, calcium and magnesium [2]. Other dairy constituents may contribute to the maintenance of antioxidant defense systems by mitigating oxidative stress that can activate pro-inflammatory signaling pathways [250] *via* directly scavenging reactive oxygen species (ROS) (e.g., vitamin A, zinc, phosphorus) or by supporting the function of intracellular antioxidant systems (e.g., the glutathione system) [6]. The glutathione system uses glutathione peroxidase (GPx) enzymes to reduce ROS, such as hydrogen peroxide, to water by gathering reducing equivalents from glutathione, which is a tripeptide synthesized *de novo* from the amino acids glycine, cysteine, and glutamic acid [218]. Glutathione exists in both a reduced ("GSH"; ~90% in this form) and an oxidized form ("GSSG"; remaining 10% exists in this form) with the proportion of GSSG increasing upon exposure to ROS [213]. Therefore, the ratio between these glutathione forms indicates oxidative stress within cells (i.e., lower GSH:GSSG = greater oxidative stress). It has been theorized that dairy products can support/enhance glutathione synthesis, and thus antioxidant capacity, by providing additional precursor components, namely sulfur-containing amino acids such as cysteine (the rate-limiting precursor amino acid of GSH synthesis [218]). Therefore, if increased dairy consumption can support the glutathione system more effectively, this may decrease oxidative stress and provide a possible mechanistic explanation for the reductions in systemic inflammation previously observed [5, 200, 323].

Despite these hypotheses, questions remain regarding the mechanisms of action associated with changes in inflammatory biomarkers, as well as the time course over which consumption of wholefood dairy may provide benefit. Furthermore, a recent comprehensive systematic review from Ulven and colleagues (2019) identified a significant lack of well-controlled, transparently reported studies evaluating acute and short-term dairy consumption responses on a range of inflammatory biomarkers [200]. Finally, the majority of studies thus far have only assessed the

effects on inflammation of consuming a single type of non-fat or low-fat dairy product (usually milk or yogurt), which may not be representative of how dairy foods are typically consumed within the diet according to national nutrition surveys (e.g., cheese is estimated to account for 30% of dairy intake within Canada) [16, 23, 323]. Therefore, the purpose of this study was to address these gaps in the literature by completing a short-term (6-week), crossover study to assess the effect of consuming 3 servings per day of mixed wholefood dairy products (milk, yogurt and cheese) on a broad range of inflammatory and other interrelated physiological outcome measures in individuals with OW/OB. We hypothesized that increased dairy product consumption would result in reductions in circulating pro-inflammatory cytokines (specifically IL-6) and would therefore benefit those individuals in a state of LGCI. Considering the relationship between oxidative stress, inflammation and cardiovascular health, the current study also investigated the impact of 6-weeks of dairy supplementation on glutathione, metabolic biomarkers, vascular structure and endothelial function, which we hypothesized would improve alongside an improved inflammatory biomarker profile.

## 5.3 Methods

This study reports data from a larger randomized crossover clinical trial. It was approved by the Office of Research Ethics at York University, Toronto, Canada (certificate#: 2021-177), and conformed to the most recent Canadian government Tri-council funding policy statement (TCPS2) on the use of human participants in research. The full trial was registered at clinicaltrials.gov (identifier: NCT04902417). The trial was conducted from June 2022 to March 2024, and participants were recruited from York University and the greater Toronto area. A total of 493 people contacted the lab for assessment: 211 completed the initial phone screening, 106 completed the secondary in-person screening visit, 36 were enrolled, and 30 participants

completed the study (**Figure 15**). Our sample size calculation for the larger trial was based on Pei et al. (2018) [16], and deemed 29 participants per group sufficient to see differences in postprandial IL-6 (a main outcome not reported herein). For fasted IL-6 (main outcome of this study), power calculations using data from Stancliffe et al [15] also deemed 29 participants as sufficient. The initial phone screening process consisted of obtaining informed consent and completing questionnaires pertaining to health, activity levels, medical history, and habitual dairy consumption. Potential participants who passed the phone screening process were invited for an in-person screening where they attended the laboratory in a fasted state and had the following measurements taken: height, weight, waist circumference, blood pressure, and a finger prick blood sample, which was analysed using the Cholestech LDX Analyzer (Abbott; Chicago, IL, USA) to obtain a full lipid profile (total cholesterol [TC], triglycerides [TG], low density lipoprotein [LDL], high-density lipoprotein [HDL] and glucose measurement). Those who continued to meet inclusion criteria (detailed below) were then required to complete a 7-day food diary to assess their habitual dietary intake (and corroborate their habitually low dairy (LD) intake indicated from the phone screening questionnaire). Participants were instructed by a registered dietitian (RD) on how to accurately record their dietary intake using the mobile phone dietary intake app Keenoa (Keenoa, Montreal, QC, Canada, version 1.03), which was chosen to allow for consistent monitoring and reduced participant burden [418].

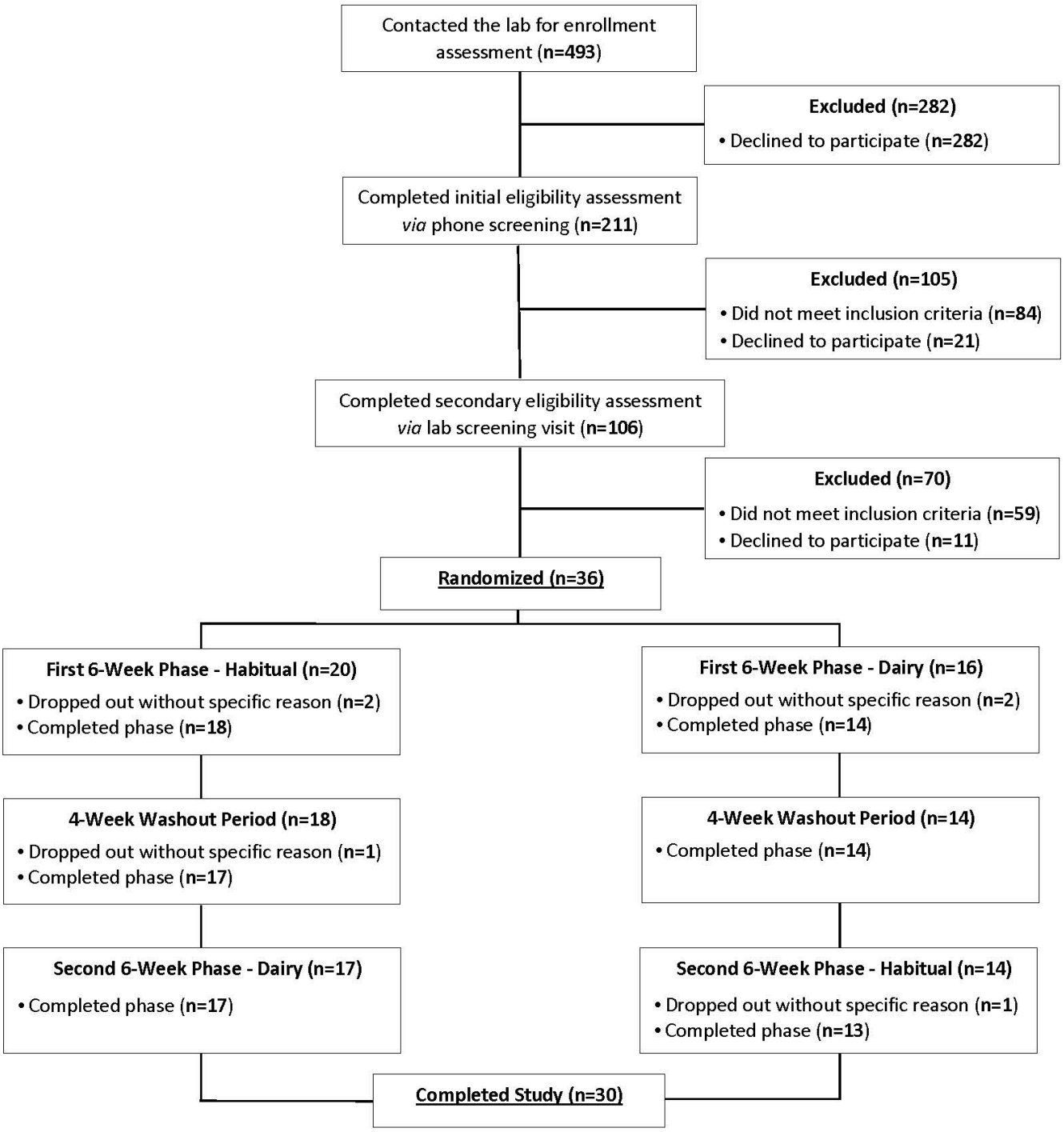


**Figure 15.** CONSORT diagram showing participant flow through the trial, including recruitment, randomization, and completion.

**Participant Criteria and Characteristics**

Inclusion criteria for the study included: aged 18 - 70 years old, BMI ≥25 kg/m$^2$, low physical activity levels (<2 structured exercise sessions per week as per the Canadian 24-h movement guidelines for adults [419]), and low habitual dairy intake (≤1 serving per day). In addition to the aforementioned criteria, participants also had to display at least two other cardiometabolic risk factors (proxy for LGCI) from the following list (based on clinical guidelines [420]): elevated waist circumference (males ≥102cm; females ≥88cm), elevated resting blood pressure (BP; ≥130/85), impaired fasting glucose (≥5.6 mmol/L), borderline high TC (≥5.2 mmol/L) or fasting LDL (≥3.5 mmol/L), impaired fasting HDL (males <1.03 mmol/L; females <1.3 mmol/L), and impaired fasting TG (≥1.7 mmol/L). Exclusion criteria included: any allergy to dairy foods or diagnosed lactose intolerance, previous history of diabetes and/or related cardiovascular disease, any inflammatory/immune disease, use of multiple medications for managing lipids, glucose, and/or blood pressure, and current smoker. In total, thirty participants with OW/OB and two or more pre-existing cardiometabolic disease risk factors completed the study, and their characteristics at study onset are shown in **Table 6**. Participants were randomized to 1 of 2 diet sequences (AB/BA design, 1:1 allocation ratio) – high dairy (HD)/low dairy (LD) or LD/HD using a computer-generated randomizer by a research team member without any prior knowledge of participants' status or screening results. The sequence for each participant was then passed on to the study coordinator before being told to participants during their first baseline testing visit.

**Table 6.** Participant characteristics and screening criteria for all 30 participants who completed the study. Study eligibility required having some combination of 2 or more of the cardiometabolic risk factors listed below.

| CHARACTERISTICS | MEAN ± SD | N THAT MET INCLUSION CRITERIA |
|---|---|---|
| Age (years) | 31.8 ± 12.3 | 30 |
| BMI (kg/m$^2$) | 32.0 ± 4.5 | 30 |
| Height (cm) | 164.0 ± 8.0 | |
| Body Mass (kg) | 85.0 ± 14.4 | |
| BIA Body Fat (%) | 37.5 ± 7.3 | |
| BodyMetrix Body Fat (%) | 34.1 ± 4.9 | |
| Sex | Male n = 12<br>Female n = 18 | |
| Cardiometabolic risk factors: | | |
| Waist Circumference (cm) | 99.4 ± 10.1 | 25/30 |
| Systolic BP (mmHg) | 125 ± 14 | 12/30 |
| Diastolic BP (mmHg) | 76 ± 11 | 5/30 |
| Blood Glucose (mmol/L) | 5.5 ± 0.7 | 11/30 |
| Total Cholesterol (mmol/L) | 4.8 ± 0.8 | 11/30 |
| LDL Cholesterol (mmol/L) | 3.0 ± 0.6 | 7/30 |
| HDL Cholesterol (mmol/L) | 1.2 ± 0.3 | 19/30 |
| Triglycerides (mmol/L) | 1.6 ± 0.7 | 9/30 |

Data are displayed as mean ± SD, n=30. Abbreviations: BIA = bioelectrical impedance analysis; BMI = body mass index; BP = blood pressure; LDL = low density lipoprotein; HDL = high density lipoprotein.

## Study Design and Procedures

This study used a within-subject cross-over design where all participants completed two 6-week dietary intervention arms in random order. Initially, after completing the screening process, eligible participants were invited to the laboratory at York University to complete baseline testing. They were required to complete an overnight fast, avoid strenuous exercise 48h prior, avoid alcohol consumption 24h prior, and avoid caffeine or pain medication 12h prior to the visit. All

visits were performed in the morning between 7am – 9am, and efforts were made to complete follow-up visits at the same time as their initial baseline visit. To begin with, participants rested for 5-minutes in a supine position before having measurements of vascular function assessed (detailed below under *Vascular Measures*). Following this, body composition measurements were taken, which included body mass (BC-418 TANITA scale, Tanita Corp, Tokyo, Japan) and body fat percentage measured using two techniques: 1) bioelectrical impedance analysis (BIA) using a BC-418 TANITA scale (Tanita Corp, Tokyo, Japan), 2) ultrasound assessment of subcutaneous tissue thickness at 3 skinfold sites using the BodyMetrix™ BX2000 device (IntelaMetrix, Brentwood, CA, USA) [421]. A registered phlebotomist then obtained approximately 20mL of blood from the median cubital vein for future analysis. To avoid blood clotting and auto-oxidation of glutathione, 500µL of whole blood was immediately mixed with 11.5µL of tripotassium ethylenediaminetetraacetic acid (k3-EDTA) and 50µL of 20 µmol l−1 N-ethylmaleimide (NEM) before being transferred and stored at –80˚C until analysis. Vacutainers coated with EDTA and sodium heparin (for plasma) were immediately centrifuged at 1300 g and 4˚C for 10 minutes, and serum separator tubes (for serum) rested for 30 minutes at room temperature before also being centrifuged at 1300 g and 4˚C for 10 minutes. Plasma and serum were then aliquoted into small cryovials and stored at –80˚C until analysis. The primary outcome measure of this study was IL-6 followed by the other measured inflammatory biomarkers. Secondary outcomes also included the metabolic biomarkers, glutathione, and vascular measures. Exploratory subgroup analyses stratifying by sex were also performed on inflammatory markers.

Participants then began the first of the two 6-week dietary intervention arms. The intervention arms consisted of: 1) high-dairy (HD) consumption arm, where 3 servings/d of a mix of milk, cheese and yogurt were provided to participants (250mL of 2% white milk [Lactantia],

200g of plain, vanilla, strawberry or black cherry flavoured (based on participant preference) 2% milk fat Icelandic skyr yogurt [Siggis], and 42g of 35% fat cheddar cheese [Cracker Barrel]); or 2) their usual/habitual low-dairy (LD) diet (≤1 serving of dairy/d, no dairy foods provided). Upon completion of the first 6-week intervention arm, participants returned to the laboratory at the same time of day to complete post-testing, in which all the measurements taken at baseline were repeated. They then completed a ≥4-week washout before commencing the second 6-week dietary intervention arm, following the same procedures as during the first intervention arm.

Dairy products were provided to participants biweekly and were kindly donated by Lactalis, Canada. Due to the nature of the two interventions, it was impossible to blind participants to the dietary arm that they were completing, however members of the research team were blinded to dietary condition during analysis. Participants received dietary counselling from the RD on how to incorporate the dairy foods into their diet by exchanging them with other foods of similar caloric value (of their choosing) to avoid participants increasing their total daily energy intake and gaining body mass over the course of the HD diet arm. Dietary intake was recorded using the Keenoa mobile phone application for a 7-day period during weeks 1 and 6, and a 3-day period during weeks 2 and 4, and was routinely checked/monitored by the RD and study personnel. Participants also attended the laboratory biweekly to have their body weight recorded and to verbally confirm dairy consumption compliance with the research team. The combination of dietary intake data and body mass measurements allowed concurrent feedback to be provided to participants, ensuring that they remained weight stable during the intervention period and were effectively complying with the consumption of all 3 daily servings of dairy provided. Despite consistent prompting, four participants regularly omitted meals and thus under-reported their food intake (defined as having

a ratio of energy intake:estimated energy requirement of <0.7 (34)). Diaries from these 4 participants were removed from the dietary analysis.

**Inflammatory Biomarkers**

The primary outcome measure of this study was circulating IL-6, however a range of inflammatory markers were also assessed including: IL-1β, IL-4, IL-10, TNF-α and interferon gamma (IFN-γ). Analysis was performed by Eve Technologies Corporation (Calgary, Alberta, Canada) using the Luminex® 200™ system (Luminex Corporation/DiaSorin, Saluggia, Italy) with Bio-Plex Manager™ software (Bio-Rad Laboratories Inc., Hercules, California, USA). Six markers were measured together in the samples using a Human High Sensitivity T-Cell 6-Plex Custom Multiplex Assay (MILLIPLEX® Human High Sensitivity T-Cell Magnetic Bead Panel Cat#: HSTCMAG-28SK, MilliporeSigma, Burlington, Massachusetts, USA) as per the manufacturer's instructions for use. Assay sensitivities (pg/mL) and coefficients of variation (CV%) of these markers were as follows: IL-6 = 0.11 and 8.4%, IFN-γ = 0.48 and 8.7%, IL-1β = 0.14 and 8.8%, IL-4 = 1.12 and 8.9%, IL-10 = 0.56 and 10.1%, TNF-α = 0.16 and 6.4%.

**Metabolic Biomarkers**

The following 7 metabolic biomarkers were measured: CRP (Cat#: 07876424-190), glucose (Cat#: 05168791-190), insulin (Cat#: 12017547122), TC (Cat#: 03039773-190), HDL cholesterol (Cat#: 07528566-190), TG (Cat#: 20767107) and Apolipoprotein-B (Cat#: 03032574). These analyses were performed by Mt Sinai Services (Mount Sinai Hospital, Toronto, ON, Canada) on frozen serum samples. Biomarkers were measured using the Cobas c503 & e801 modules (Roche Diagnostics, GmbH, Mannheim, Germany). LDL cholesterol concentrations were derived from TC, HDL and TG results using the Friedewald formula [422]..

**Glutathione Analysis**

Both reduced (GSH) and oxidized (GSSG) glutathione were measured from the same whole blood sample (frozen with pre-added EDTA and NEM) using a high-performance liquid chromatography (HPLC) method adapted from Giustarini et al. [423] that has previously been used by our lab [424-428]. All samples were analyzed using an Agilent HPLC 1100 (Agilent Technologies, Mississauga, ON, Canada) with a Zorbax C18 (4.6mm x 150mm, 5µm) column (Agilent Technologies). Concentrations for both GSH and GSSG were calculated using standard concentration curves, and total glutathione (tGS) was calculated as GSH + 2*GSSG. Results were expressed in relation to hemoglobin concentration and reported in µmol/g Hb.

For GSH, 100µL of blood was combined with 100µL of 15% trichloroacetic acid before being shaken thoroughly and then centrifuged at 14 000g for 2 minutes. The deproteinized supernatant was transferred to HPLC autosampler vials for column separation and then pumped under isocratic conditions using a 0.25% glacial acetic acid mobile phase with 6% acetonitrile at a flow rate of 1.05 $mL/min^{-1}$. The GSH-NEM conjugate peak was detected using a modular variable wavelength detector (UV monitoring) at 265nm wavelength.

For GSSG, 100µL of blood was combined with 100µL of 15% perchloric acid before being shaken thoroughly and then centrifuged at 14 000g for 2 minutes. Then 100µL of the deproteinized supernatant was combined with 500µL of 0.5M NaOH, mixed thoroughly, and finally combined with 37.5µL 0.1% o-Pthalymide (OPA; Sigma-Aldrich) in methanol. This was incubated on a rocker in complete darkness for 15 minutes to create a GS-OPA conjugate. Following incubation, samples were transferred to HPLC autosampler vials for column separation and pumped under isocratic conditions using a mobile phase of 25mM $Na_2HPO_4$ in HPLC-grade water with 15% methanol at 0.5 mL/min flow rate. The oxidized glutathione peak was detected by fluorescence-HPLC monitoring, involving excitation at 350 nm and emission detection at 420 nm.

## Vascular Measures

Vascular measurements were performed in accordance with best practices [429]. Intima media thickness (IMT) of the carotid artery was assessed as an index of vascular health, and was analyzed using automated analysis in EchoPAC software (GE Healthcare Systems, Canada). Blood pressure was taken immediately prior to carotid insonation using an automated cuff (BP Tru, BPM 200, Coquitlam, CA). Subsequently, flow-mediated dilation (FMD) of the brachial artery was used to assess endothelial function with a 10MHz linear probe (Vivid i, GE Healthcare systems, Mississauga, Canada). Brachial artery diameter and blood flow velocity were acquired in duplex mode. Assessment of endothelial function included a 2-minute baseline followed by 5-minutes of supra-systolic (+50 mmHg) forearm occlusion and a 3-minute recovery phase that included the rapid deflation of the blood pressure cuff causing reactive hyperemia. Continuous video was captured using video grabber (AV.io HD, Epiphan Video), which was subsequently analyzed using edge detection software (Cardiovascular Suite, Quipu, Italy). FMD percent (FMD%) was automatically calculated as the difference in peak artery diameter during reactive hyperemia compared to baseline diameter.

## Statistical Analysis

Data analyses were performed while blinded to condition using GraphPad Prism (version 10.4.0; GraphPad Software Inc., San Diego, CA, USA), with a p of $\leq 0.05$ considered statistically significant. Replicate data from biochemical assays were assessed in a blinded fashion, and those with a CV of >15% were visually inspected, and if one of the replicates was considered to be erroneous, a single data point was used instead of the average. Thirty participants completed the study however of the 120 blood sampling points, 2 are missing for each blood biomarker due to unsuccessful blood draws (1 HD baseline timepoint, 1 LD baseline timepoint for two participants).

Additionally, 8 out 120 FMD assessments were lost due to software (n=3) or equipment (n=1) issues and movement artifacts (n=4) (3 HD baseline timepoints, 2 post-HD timepoints, 1 LD baseline timepoint, 2 post-LD timepoints). Data were checked for normality using skewness and kurtosis (±3 deemed acceptable), and non-normally distributed data were log-transformed (IL-6, glucose, insulin, triglycerides) for statistical analysis. A 2x2 mixed-effects model (within-within repeated measures) using a Greenhouse-Geisser correction was used to assess interactions and main effects of time (baseline vs. week 6) and diet (HD vs. LD) of the absolute circulating biomarker concentrations and vascular measurements. Posthoc testing was performed when significant interactions or main effects were found, and a false discovery rate (FDR) correction (two-stage step-up method by Benjamini, Krieger and Yekutieli) for multiple comparisons was used, as recommended for health studies [430, 431]. Both corrected and uncorrected p-values are reported. To account for inter-individual variability, percentage change scores were also calculated and then compared using a paired samples t-test. Although it was not the primary purpose of the study, exploratory analyses were also conducted to evaluate the independent effects of the intervention on inflammation in both sexes. Data were stratified by sex and analysed using a separate 2x2 mixed-effects model for males and females. Effect sizes were calculated for t-test and post-hoc results that displayed significant differences using Cohen's d to determine the magnitude of an effect independent of sample size. Small effect sizes are considered as $d < 0.2$, moderate effect sizes as $d = 0.2–0.8$, and large effect sizes as $d > 0.8$ [432].

## 5.4 Results

### Body Mass & Body Fat Percentage

Both body mass (Baseline HD: 84.7 ± 15.4kg, 6-Week HD: 85.1 ± 15.8kg, Baseline LD: 85.5 ± 15.1kg, 6-Week LD: 85.7 ± 15.6kg) and body fat percentage were measured using BIA (Baseline HD: 37.2 ± 7.0%, 6-Week HD: 37.7 ± 7.3%, Baseline LD: 38.0 ± 7.4%, 6-Week LD: 37.2 ± 7.6%) and ultrasound (Baseline HD: 34.6 ± 5.2%, 6-Week HD: 34.1 ± 5.2%, Baseline LD: 34.1 ± 4.9%, 6-Week LD: 34.1 ± 4.7%) were unchanged during the trial with no significant main effects or interactions observed for any measurement ($p>0.05$).

### Dietary Intake Assessment

Food diaries were collected from all 30 participants; however, due to habitual under-reporting by four participants, data from n=26 were analyzed and reported (**Table 7**). Dietary intake was assessed at multiple timepoints throughout the study. Participant's 7-day food diaries recorded during weeks 1 and 6 of each intervention arm were combined to provide a representative sample of their nutritional intake during each intervention arm. Table 7 also shows the nutrients obtained from the 3 servings/d of dairy products provided. Dairy nutrients were calculated from a representative daily intake of 2% white milk (250mL), 2% skyr plain yogurt (200g), full fat cheddar cheese (42g). Paired sample t-tests indicated protein ($d=0.489$), saturated fat ($d=0.899$), calcium ($d=3.021$), Vitamin A ($d=1.098$) and Vitamin D ($d=1.163$) were significantly higher during the HD diet compared to the LD diet (all $p<0.05$).

**Table 7.** Average daily dietary intake during the HD and LD intervention arms, based on analysis of 7-day food records completed by participants during weeks 1 and 6 of each dietary intervention period. Nutrients from a representative 3 servings of dairy provided is also shown.

| Nutrients | Dairy Nutrients | HD | LD | P-value | Cohens d |
|---|---|---|---|---|---|
| Energy (kcals) | 424 | 2091 ± 371 | 2050 ± 404 | 0.353 | |
| Protein (g) | 37.4 | **108.4 ± 25.2** | **95.0 ± 29.4** | **0.015** | **0.489** |
| Fat (g) | 22.7 | 86.2 ± 16.5 | 89.3 ± 19.2 | 0.287 | |
| Saturated Fat (g) | 13.9 | **33.5 ± 6.9** | **27.2 ± 7.2** | **<0.001** | **0.899** |
| Carbohydrate (g) | 18.4 | 225.1 ± 54.1 | 225.4 ± 53.2 | 0.975 | |
| Sugar (g) | 14.7 | 91.3 ± 31.6 | 83.2 ± 31.4 | 0.073 | |
| Calcium (mg) | 842 | **1216 ± 252** | **567 ± 170** | **<0.001** | **3.021** |
| Magnesium (mg) | 36.3 | 238 ± 60 | 261 ± 66 | 0.052 | |
| Vitamin A (ug) | 291.6 | **445 ± 158** | **287 ± 128** | **<0.001** | **1.098** |
| Vitamin E (mg) | 0.4 | 7.1 ± 1.6 | 7.7 ± 2.2 | 0.060 | |
| Vitamin D (IU) | 180 | **199 ± 101** | **100 ± 66** | **0.0001** | **1.163** |
| Vitamin B12 (mg) | 1.5 | 4.3 ± 2.1 | 4.0 ± 2.4 | 0.484 | |

Data are displayed as mean ± SD, n=26. Significant differences ($p \leq 0.05$) bolded. Abbreviations: HD = high dairy; LD = low dairy. Dairy nutrients provided were calculated from: 2% white milk (250mL), 2% skyr plain yogurt (200g), full fat cheddar cheese (42g).

**Inflammatory Markers**

Absolute concentrations for all inflammatory biomarkers assessed in each diet are displayed in **Table 8**. There were no significant interactions or main effects for our primary endpoint IL-6. A significant interaction effect was found for the pro-inflammatory cytokine TNF-α ($p=0.036$) where dairy consumption decreased TNF-α compared to no dairy consumption. Posthoc changes within the HD diet were significant prior to FDR correction (Δ-0.52pg/mL; corrected $p=0.208$; uncorrected $p=0.040$; $d=0.166$) but not within the LD diet (Δ0.48pg/mL; corrected $p=0.525$; uncorrected $p=0.308$; $d=0.202$). There was also a significant interaction for the anti-inflammatory cytokine IL-4 ($p=0.022$); however, posthoc changes from pre to post were not statistically significant for the HD diet (Δ2.74pg/mL; corrected $p=0.277$; uncorrected $p=0.132$;

d=0.065) or the LD diet (Δ-3.67pg/mL; corrected p=0.277; uncorrected p=0.080; d=0.093). There was a significant main effect of trial for IL-1β (p=0.047), such that IL-1β concentration was higher in HD vs. LD trial over the intervention period. There were no significant interactions or main effects for CRP, IFN-γ, IL-6 or IL-10.

**Table 8.** Absolute concentrations of inflammatory markers measured during each baseline visit and at the end of each 6-week dietary intervention in both the HD and LD arms.

| Inflammatory Markers | | Baseline | 6-Weeks Post | Time | Trial | Interaction |
|---|---|---|---|---|---|---|
| CRP (mg/L) | HD | 2.98 ± 1.54 | 3.25 ± 1.81 | 0.219 | 0.469 | 0.608 |
| | LD | 3.14 ± 2.30 | 3.57 ± 1.92 | | | |
| IFN-γ (pg/mL) | HD | 10.46 ± 5.01 | 9.71 ± 4.63 | 0.245 | 0.863 | 0.122 |
| | LD | 9.75 ± 4.19 | 10.01 ± 4.78 | | | |
| IL-1β (pg/mL) | HD | 6.17 ± 2.59 | 5.90 ± 2.61 | 0.357 | **0.047** | 0.392 |
| | LD | 5.58 ± 2.30 | 5.72 ± 2.47 | | | |
| IL-4 (pg/mL) | HD | 51.89 ± 39.00 | 54.63 ± 44.06 | 0.879 | 0.726 | **0.022** |
| | LD | 53.89 ± 42.05 | 50.22 ± 36.08 | | | |
| IL-6 (pg/mL) | HD | 5.55 ± 8.17 | 5.31 ± 7.27 | 0.359 | 0.217 | 0.976 |
| | LD | 5.11 ± 7.24 | 4.94 ± 7.03 | | | |
| IL-10 (pg/mL) | HD | 21.30 ± 26.97 | 20.25 ± 24.52 | 0.171 | 0.408 | 0.787 |
| | LD | 19.57 ± 23.13 | 18.54 ± 21.80 | | | |
| TNF-α (pg/mL) | HD | 12.83 ± 3.22 | 12.31 ± 3.03 | 0.625 | 0.377 | **0.036** |
| | LD | 11.97 ± 2.28 | 12.44 ± 2.43 | | | |

Data are displayed as mean ± SD, n=30. Significant differences (p≤0.05) bolded. Abbreviations: HD = high dairy; LD = low dairy; CRP = c reactive protein; IFN-γ = interferon gamma; Il-1β = interleukin 1 beta; IL-4 = interleukin 4; IL-6 = interleukin 6; IL-10 = interleukin 10; TNF-α = tumor necrosis factor alpha.

The percentage change scores were also analysed for inflammatory markers and are shown in **Figure 16A – G**. Only IL-4 was significantly different between the two diets (Δ11.54 ± 26.35 %; p=0.028; d=0.564), displaying a moderate effect size. There were no significant differences for percentage change of CRP, IFN-γ, IL-1β, IL-6, IL-10 or TNF-α (all p>0.05).

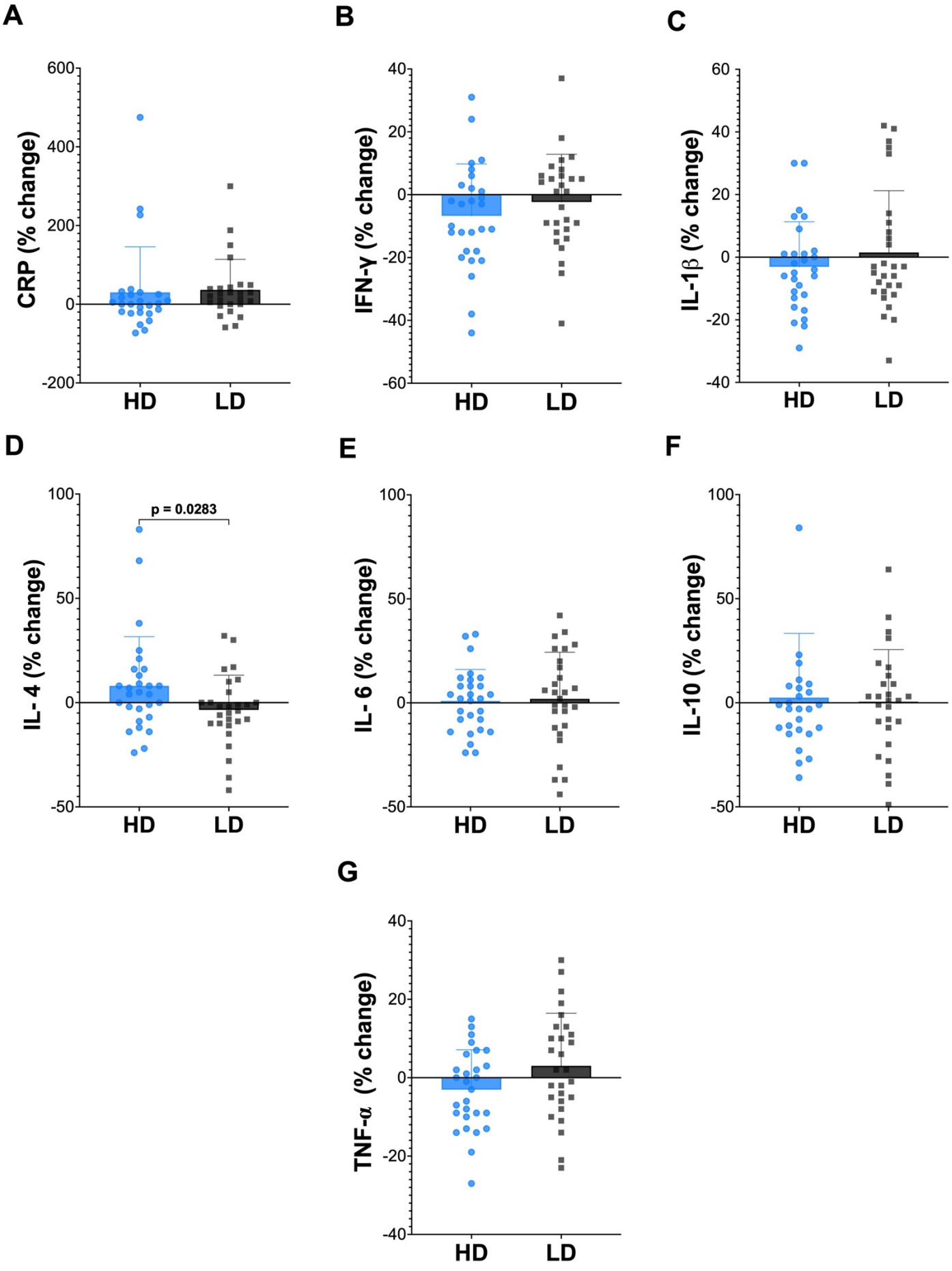
A
CRP (% change)
600
400
200
0
-200
HD
LD
B
IFN-γ (% change)
40
20
0
-20
-40
-60
HD
LD
C
IL-1β (% change)
60
40
20
0
-20
-40
HD
LD
D
IL- 4 (% change)
p = 0.0283
100
50
0
-50
HD
LD
E
IL- 6 (% change)
100
50
0
-50
HD
LD
F
IL-10 (% change)
100
50
0
-50
HD
LD
G
TNF-α (% change)
40
20
0
-20
-40
HD
LD

**Figure 16.** Percentage change (between baseline and 6-week post) of inflammatory markers measured during each 6-week dietary intervention in both the HD and LD arms (n=28 for all). **A**: CRP = c-reactive protein; **B**: IFN-γ = interferon gamma; **C**: IL-1β = interleukin 1 beta; **D**: IL-4 = interleukin 4; **E:** IL-6 = interleukin 6; **F**: IL-10 = interleukin 10; **G**: TNF-α = tumor necrosis factor alpha. Error bars = SD. Statistically significant at $p \leq 0.05$.

To evaluate dietary responses by sex, absolute inflammatory marker concentrations were stratified by sex and then analyzed. Results for both analyses are displayed in the same figure, and the main effects/interactions are detailed below (**Figure 17A-D**). For females only, there was a significant interaction effect for TNF-α ($p=0.026$) with post-hoc analysis indicating that concentrations were significantly lower in the HD diet at 6-weeks compared to the LD diet ($\Delta 1.09$pg/mL; $p=0.011$; $d=0.42$). For males, there were significant main effects of trial for TNF-α ($p=0.009$) and IL-1β ($p=0.033$) showing that concentrations were higher in the HD arm, and a significant main effect of time for IFN-γ ($p=0.009$) showing a decrease at 6-weeks compared to baseline. There were no significant main effects or interactions for CRP, IL-10 or IL-6 when stratified by sex (all $p>0.05$, data not shown).

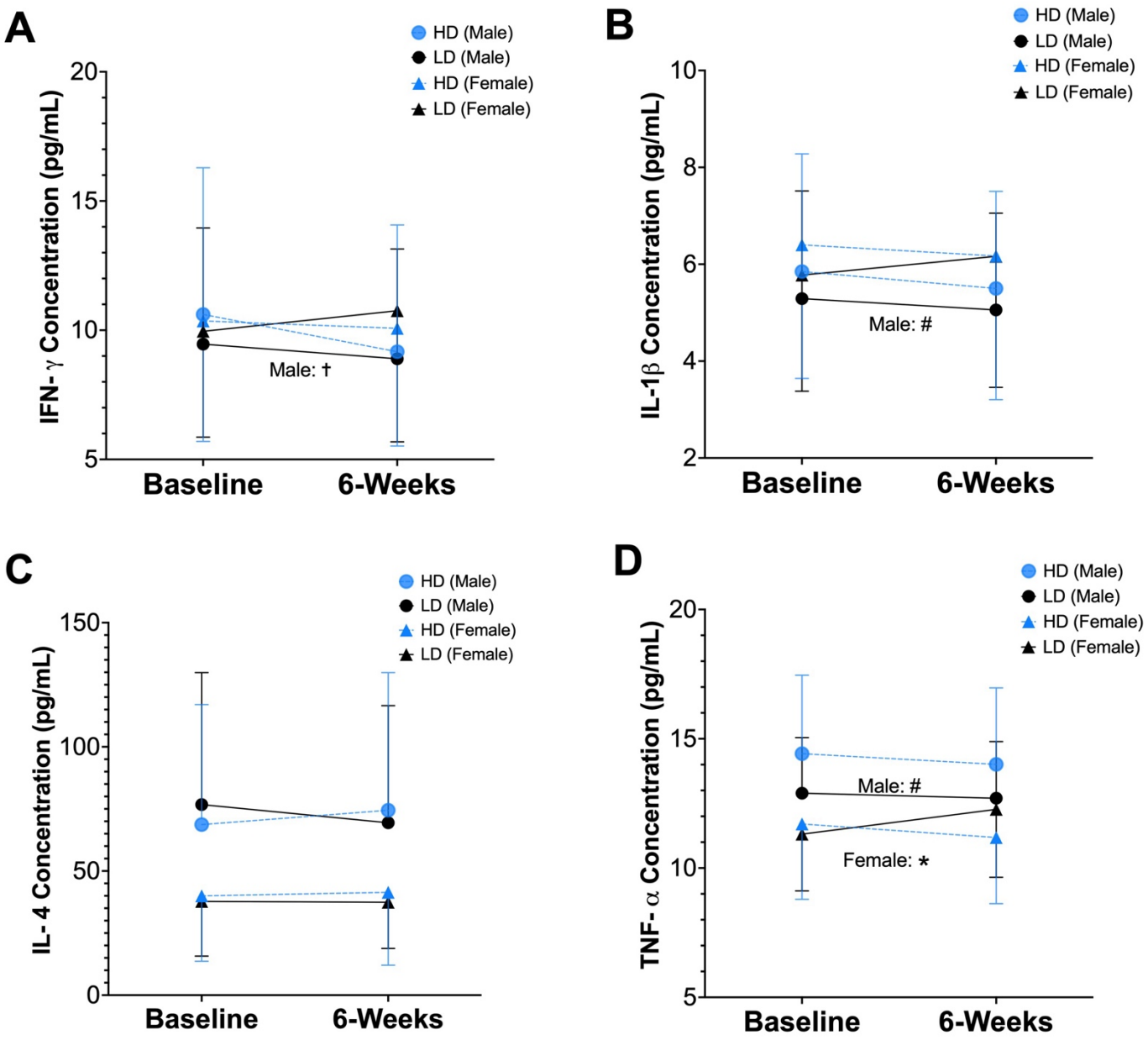


**Figure 17.** Analysis of absolute concentrations of inflammatory markers stratified by sex (males n=12; females n=18) in both the HD and LD diets. **A**: IFN-γ = interferon gamma; **B:** IL-1β = interleukin 1 beta; **C**: IL-4 = interleukin 4; **D**: TNF-α = tumor necrosis factor alpha. Error bars = SD. Statistically significant at $p \leq 0.05$. Significant interaction = *, trial = #, time = †.

**Metabolic Markers**

Absolute concentrations for all metabolic biomarkers assessed in each diet are displayed in **Table 9**. There were no significant main effects or interactions for blood glucose, insulin, TG, HDL, LDL, TC or Apo-B (all $p>0.05$).

**Table 9.** Absolute concentrations of metabolic markers measured during the initial baseline visit and at the end of the 6-week dietary intervention for both the HD and LD diets.

| Metabolic Markers | | Baseline | 6-Weeks Post | Time | Trial | Interaction |
|---|---|---|---|---|---|---|
| Apo-B (g/L) | HD | 0.87 ± 0.20 | 0.91 ± 0.22 | 0.708 | 0.157 | 0.979 |
| | LD | 0.90 ± 0.21 | 0.93 ± 0.23 | | | |
| Glucose (mmol/L) | HD | 5.09 ± 0.89 | 5.15 ± 1.15 | 0.240 | 0.729 | 0.471 |
| | LD | 5.01 ± 0.95 | 5.16 ± 0.91 | | | |
| HDL (mmol/L) | HD | 1.24 ± 0.26 | 1.24 ± 0.25 | 0.941 | 0.333 | 0.699 |
| | LD | 1.26 ± 0.27 | 1.26 ± 0.26 | | | |
| Insulin (pmol/L) | HD | 146.1 ± 159.9 | 139.4 ± 87.7 | 0.108 | 0.236 | 0.616 |
| | LD | 110.2 ± 91.5 | 139.3 ± 102.7 | | | |
| LDL (mmol/L) | HD | 2.81 ± 0.62 | 2.91 ± 0.64 | 0.210 | 0.144 | 0.644 |
| | LD | 2.95 ± 0.71 | 2.97 ± 0.81 | | | |
| TC (mmol/l) | HD | 4.69 ± 0.70 | 4.77 ± 0.79 | 0.252 | 0.208 | 0.881 |
| | LD | 4.79 ± 0.75 | 4.86 ± 0.89 | | | |
| TG (mmol/L) | HD | 1.44 ± 1.04 | 1.38 ± 0.81 | 0.882 | 0.623 | 0.308 |
| | LD | 1.29 ± 0.60 | 1.39 ± 0.79 | | | |

Data are displayed as mean ± SD, n=30. Statistically significant at $p\leq0.05$. Abbreviations: HD = high dairy; LD = low dairy; Apo-B = apolipoprotein B; HDL = high density lipoprotein; LDL = low density lipoprotein; TC = total cholesterol; TG = triglycerides.

**Glutathione Measures**

Absolute blood concentrations for reduced glutathione (GSH) and oxidized glutathione (GSSG), along with the total glutathione (tGS) and GSH:GSSG are displayed in **Table 10**. There was a significant main effect for time for GSH:GSSG (p=0.009). There were no significant interactions or main effects for GSH, GSSG or tGS (all p>0.05).

**Table 10.** Absolute whole blood concentrations of glutathione measurements during the initial baseline visit and at the end of the 6-week dietary intervention for both the HD and LD diets.

| Glutathione Measures | | Baseline | 6-Weeks Post | Time | Trial | Interaction |
|---|---|---|---|---|---|---|
| GSH (µmol/g Hb) | HD | 3.15 ± 0.60 | 3.12 ± 0.68 | 0.068 | 0.961 | 0.074 |
| | LD | 3.20 ± 0.53 | 3.04 ± 0.51 | | | |
| GSSG (µmol/g Hb) | HD | 0.330 ± 0.074 | 0.333 ± 0.063 | 0.201 | 0.928 | 0.509 |
| | LD | 0.331 ± 0.071 | 0.337 ± 0.074 | | | |
| GSH:GSSG Ratio | HD | 9.92 ± 2.30 | 9.42 ± 1.47 | **0.009** | 0.912 | 0.427 |
| | LD | 9.97 ± 2.35 | 9.24 ± 1.65 | | | |
| tGS (µmol/g Hb) | HD | 3.83 ± 0.68 | 3.78 ± 0.78 | 0.209 | 0.971 | 0.184 |
| | LD | 3.86 ± 0.62 | 3.71 ± 0.63 | | | |

Data are displayed as mean ± SD, n=30. Significant differences (p≤0.05) bolded. Abbreviations: HD = high dairy; LD = low dairy; GSH = reduced glutathione; GSSG = oxidized glutathione; Hb = hemoglobin; tGS = total glutathione.

The percentage change scores for glutathione measurements were also calculated and compared using a paired samples t-test (**Figure 18A – D**). The percentage change over the 6-week period for reduced GSH was significantly different between the two diets (Δ5.2 ± 13.7%; p= 0.050; d=0.498), displaying a moderate effect size. There were no significant differences for percentage change of GSSG, GSH:GSSG or tGS (all p>0.05).

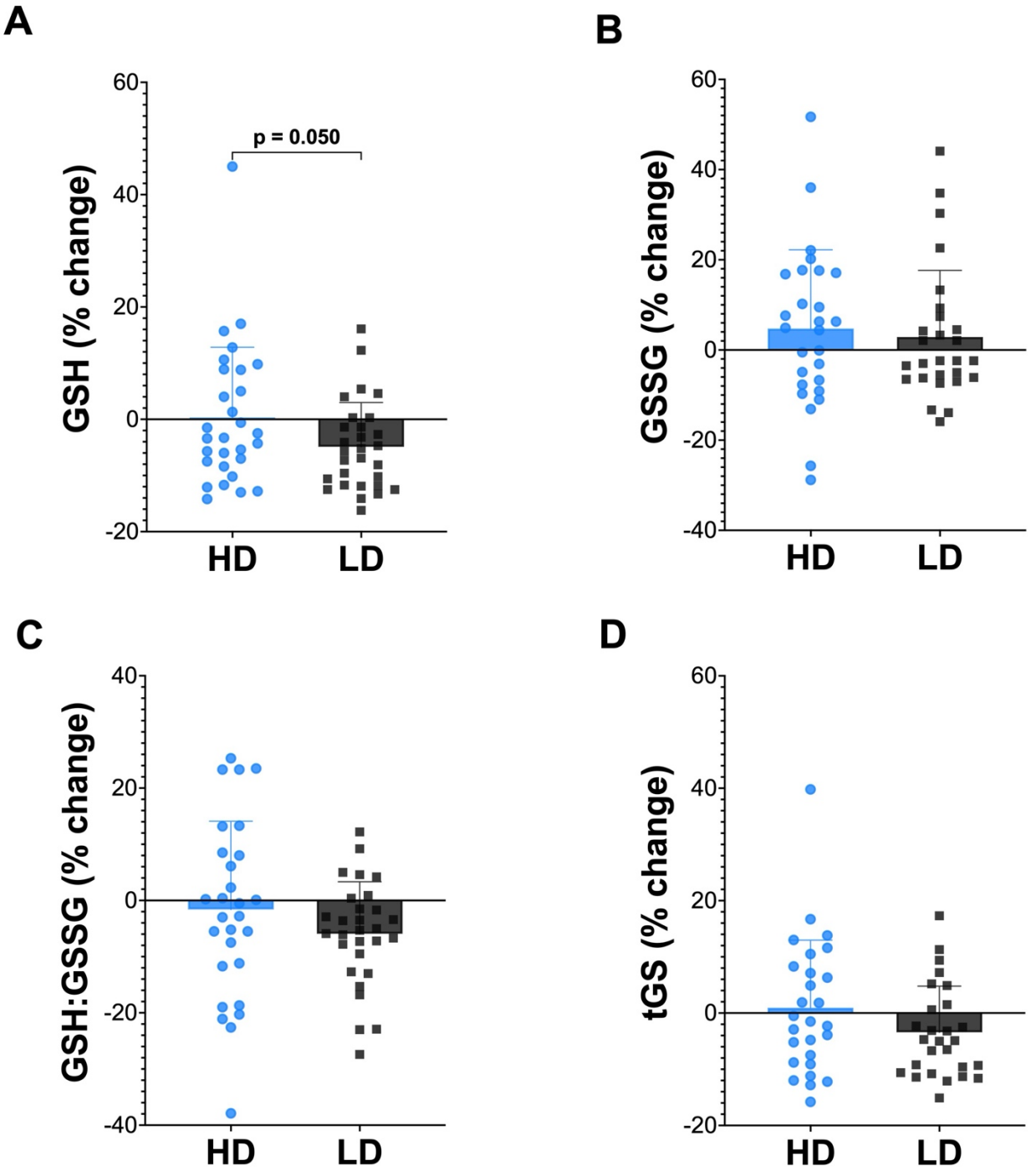


**Figure 18.** Percentage change (between baseline and 6-week post) of circulating glutathione measured during each 6-week dietary intervention in both the HD and LD arms (n=28 for all). **18A**: GSH = reduced glutathione; **18B**: GSSG = oxidized glutathione, **18C**: GSH:GSSG = reduced to oxidized glutathione ratio, **18D**: tGS = total glutathione. Error bars = SD. Statistically significant at $p \leq 0.05$.

**Vascular Measurements**

Results for the vascular measures assessed during each dietary trial are displayed in Table 6. A significant main effect of trial was found for carotid IMT, which was smaller throughout the HD arm of the study (p=0.031; **Table 11**). There were no interaction effects or main effects of diet or trial for systolic blood pressure, diastolic blood pressure, FMD% or Max Shear (all p>0.1; **Table 11**).

**Table 11.** Vascular measurements taken during the initial baseline visit and at the end of the 6-week dietary intervention for both the HD and LD diets.

| Vascular Measures | | Baseline | 6-Weeks Post | Time | Trial | Interaction |
|---|---|---|---|---|---|---|
| Carotid IMT (cm) | HD | 0.049 ± 0.009 | 0.050 ± 0.006 | 0.801 | **0.031** | 0.113 |
| | LD | 0.053 ± 0.012 | 0.052 ± 0.011 | | | |
| Diastolic BP (mmHg) | HD | 77 ± 11 | 75 ± 9 | 0.127 | 0.848 | 0.519 |
| | LD | 76 ± 11 | 75 ± 10 | | | |
| FMD (%) | HD | 7.24 ± 4.86 | 6.55 ± 4.37 | 0.660 | 0.103 | 0.136 |
| | LD | 5.92 ± 3.72 | 6.26 ± 3.83 | | | |
| Max Shear | HD | 1337 ± 482 | 1301 ± 423 | 0.652 | 0.815 | 0.295 |
| | LD | 1304 ± 404 | 1402 ± 480 | | | |
| Systolic BP (mmHg) | HD | 120 ± 12 | 119 ± 10 | 0.238 | 0.691 | 0.923 |
| | LD | 121 ± 13 | 119 ± 12 | | | |

Data are displayed as mean ± SD, n=30. Significant differences (p≤0.05) bolded. Abbreviations: HD = high dairy; LD = low dairy; BP = blood pressure; IMT = intima-media thickness; FMD = flow mediated dilation.

## 5.5 Discussion

In this randomized, controlled crossover intervention study, we observed small but significant interactions between some (TNF-α and IL-4) but not all inflammatory biomarkers (including our primary outcome of IL-6), as well as within the percentage change of GSH following 6-weeks of a high mixed dairy diet compared to a low dairy diet in individuals with OW/OB. Furthermore, when stratified by sex, the improvement in TNF-α concentrations during the HD condition was only present in females, suggesting that differences in responses to dairy intake may exist between sexes. Overall, these findings suggest that for individuals with increased metabolic disease risk factors, incorporating 3 servings of mixed dairy products (low-fat milk, skyr yogurt, and full-fat cheddar cheese) into the diet may have minor beneficial effects on inflammatory and antioxidant status over a relatively short period of time, without causing changes in body weight, body fat, vascular measures or the lipid/metabolic profile.

We did not observe any significant effects of increased dairy consumption on IL-6. This finding differs from the investigation by Labonté et al. (2014), in which 4 weeks of similar dairy consumption vs an energy-matched non-dairy control resulted in a significant decrease in IL-6 on both arms [433]. Despite reductions in IL-6, Labonte et al., also reported no changes in CRP, adiponectin or in the expression of inflammatory gene transcription factors, suggesting, similarly to our study, that the magnitude of an anti-inflammatory effect was relatively small [433]. Pei and colleagues (2017) compared 9-weeks of dairy yogurt to soy yogurt in obese and non-obese premenopausal females [434]. They also found no difference in IL-6 concentrations between the two dietary conditions in either participants with normal weight or obesity, but did observe significantly lower TNF-α after 9-weeks in the dairy yogurt groups only. We also observed a significant interaction effect for TNF- α between the HD and LD diets, which suggested that dairy

consumption decreased concentrations of this inflammatory cytokine compared to the LD diet. TNF-α is a potent proinflammatory cytokine that can be synthesized and released from white adipose tissue and thus is commonly elevated in individuals with OB [242, 435]. TNF receptors are almost ubiquitously found throughout the body, and while the responses are dependent on the specific tissue/cell that it binds to [436], common effects include activation of lipolysis/inhibition of gluconeogenesis, increases in vascular permeability, changes in ROS production, and facilitation of leukocyte adhesion [272, 436]. Our findings are in line with those of van Meiji et al. (2010), who observed that 8-weeks of low-fat dairy intake (2 servings/d; 1.5% milk and yogurt) reduced plasma TNF-α concentrations by 0.16pg/mL in OW/OB, but otherwise healthy participants [437]. They also found that not all inflammatory markers assessed during their study (e.g., IL-6, monocyte chemoattractant protein-1 [MCP-1], vascular cell adhesion molecule-1 [VCAM-1]) changed with increased dairy intake either, suggesting that the ability for dairy to reduce inflammation is either minor or only acting through some specific mechanisms, potentially involving TNF-α. Similarly, a randomized parallel design study by Stancliffe et al. (2011) in individuals with OW/OB compared 12-weeks of higher dairy (3.5 servings/d) to low dairy (<0.5 servings/d) intake and observed a reduction in both inflammatory (TNF-α, IL-6, MCP-1) and oxidative stress (malondialdehyde, oxidized LDL) in the higher dairy group [15].

We also observed a significant interaction effect in absolute IL-4 concentrations and significant differences in IL-4 percent change between the HD and LD diets, which indicated that dairy consumption increased concentrations of this anti-inflammatory cytokine compared to the LD diet. This cytokine is highly pleiotropic and has been shown to have significant inhibitory effects on the expression and release of proinflammatory cytokines, including IL-1, TNF-α, IL-6, IL-8, and macrophage inflammatory protein-1a, and has also been shown to suppress macrophage

cytotoxic activity and macrophage-derived nitric oxide production [438, 439]. Few studies have shown that increasing dairy intake can influence IL-4 [200]; however, research has shown that other anti-inflammatory markers may also be altered with dairy consumption. In healthy young adults, 20g/d of butter consumption for 8 weeks increased circulating IL-10 [440], and in participants with inflammatory bowel disease, probiotic yogurt intake increased the proportion of putative CD4+ CD25++ Treg cells in the peripheral blood, which is indicative of a more favourable anti-inflammatory environment [441].

Contrary to our hypotheses, 6-weeks of the HD diet did not significantly impact vascular structure, blood pressure, or endothelial function. In a newly published systematic review, most studies reported no effect of dairy on FMD [442]. Of the studies that found a positive effect, most supplemented with isolated milk proteins [443-445] or monounsaturated fatty acid-enriched dairy products [446, 447]. This likely suggests that specific dairy products may be better at improving cardiovascular health than others and that the lack of observed differences in the current study may be due to the heterogeneity of dairy products consumed rather than a specific product. Indeed, higher yogurt consumption has been associated with lower subclinical atherosclerosis (characterized by the presence of plaque or an IMT diameter $\geq$ 0.8 mm) in a cohort of females without CVD or cancer [448]. Alternatively, differences may have been due to the different populations assessed. Machin and colleagues (2015) observed that 4-weeks of a HD diet significantly increased FMD in middle-aged adults with OW/OB and hypertension [449]. In contrast, our participants were younger and had healthier vasculature (i.e. lower IMT and higher FMD%), which would reduce the potential therapeutic effectiveness of dairy consumption due to a floor effect [8].

Milk (and the products derived from it) is a nutrient-rich food containing high-quality proteins, carbohydrates, fatty acids, and essential micronutrients [2]. Specific milk proteins such as caseins and whey may have immunomodulatory effects (e.g., by suppressing lymphocyte proliferation [450, 451] or interferon secretion from mitogen-activated lymphocytes [452]) and have been shown previously to improve both inflammatory and oxidative stress markers in animals and humans, although *in vivo* studies are still lacking [453, 454]. A study by Zemel & Xiaocun (2008) in mice found that dairy consumption was superior to calcium carbonate supplementation alone for inhibiting ROS production within adipose tissue [326]. The same group followed up on this finding in a human cohort, comparing 28 days of dairy vs. soy-supplemented diets in individuals with OW/OB. In the dairy-supplemented group, they observed significant reductions in markers of oxidative stress (plasma malondialdehyde and 8-isoprostane-F2α) and reductions in inflammatory biomarkers (TNF-α, IL-6, MCP-1) [14]. In addition to the effects of whole proteins (primarily consumed as wholefoods) on these outcomes, specific dairy peptides or amino acids may also play a role [6]. Whey proteins are high in cystine (two cysteine amino acids linked by a disulfide bond [229]), which is a pepsin- and trypsin-resistant molecule that can safely travel through the plasma before being reduced into two sulfur-containing cysteine amino acids upon cell entry [230]. The provision of these rate-limiting amino acids has been theorized to support and/or enhance glutathione synthesis (and thus antioxidant capacity), which can become depleted with obesity and chronic disease [218, 221]. We observed a moderately sized significant difference between the groups for percent change in reduced glutathione (GSH; antioxidant marker), which was higher during the HD compared to the LD diet. Studies evaluating the effect of milk/dairy consumption on GSH and GSSG (or related biomarkers) are sparse, but some data suggest that a potential link exists [361]. A study performed in pregnant females found that consuming an

additional 200g of probiotic yogurt daily for 9 weeks significantly increased plasma GSH, GPx and glutathione reductase compared to baseline [366]. In a cross-sectional study, Choi and colleagues (2021) measured cerebral GSH concentrations in older adults using MRI and found significantly greater concentrations of cerebral GSH in those with higher habitual dairy consumption [364]. Considering that we observed both improvements in some inflammatory markers and in circulating GSH, the provision of key amino acids that support the glutathione system may be a possible mechanism through which dairy consumption can attenuate oxidative stress and inflammation. However, further investigations are needed considering the small magnitudes of change we observed.

Micronutrients such as calcium, magnesium, potassium, vitamin D, and vitamin A may also directly or synergistically affect inflammatory signalling [2]. For example, increased dietary calcium intake has been shown to specifically reduce TNF-α production in mice [351]. Indeed, during the HD diet, participants in our study consumed more than double the calcium (1216 mg/d) and significantly more protein, saturated fat, and vitamins A and D compared to the LD diet. The ability for dairy products to provide these nutrients simultaneously through its wholefood matrix means that a combination of mechanisms may have played a role. It seems likely that dairy foods provided a steady intake of various anti-inflammatory nutrients [2], which may have helped to slightly shift the internal environment toward a less inflammatory state over 6 weeks compared to a diet containing no/few dairy products.

Another factor that should be considered is whether sex differences exist regarding the immunomodulatory effects of dairy consumption. We stratified our results by sex to explore this possibility and found that TNF-α responded differently between the diets for female participants only. Dugan and colleagues (2016) performed a crossover study in which participants with

metabolic syndrome were given 3 servings/d of mixed dairy or carbohydrate control for 6-weeks with a 4-week washout between diets [455]. They found that while liver function improved for all participants in the dairy diet, TNF-α and MCP-1 concentrations were reduced for female participants only [455]. In contrast, Wennersburg et al. (2009) performed a 6-month parallel design study involving males with OW/OB in which they were instructed to consume 3-5 dairy products of their choice per day (suggested foods: milk containing 0.5–3% fat, yogurt, sour milk, cream, crème fraiche, cheese, butter, butter containing spreads, cottage cheese) for 6-months [456]. While they found no significant differences in any marker of inflammation, body weight/composition, or oxidative stress (measured via 8-iso-prostaglandin F2α), they did observe an increase in leptin (appetite-regulating hormone) and VCAM-1 (a marker of inflammatory-induced vascular activation) in female participants only [456]. Their findings of unchanged body weight and composition with increased dairy intake align with our results and suggest that individuals with OW/OB can maintain *ad libitum* energy intake and weight balance when instructed to incorporate a variety of energy-dense dairy foods into their diet. The role of the menstrual cycle on these observations is also unclear. While we recorded the menstrual cycle phase of our female participants, we could not ensure that testing happened at the same point in the cycle due to logistical constraints involved with a 6-week dietary intervention period. Although it is not conclusive that circulating TNF-α concentrations are affected by menstrual cycle phase [457], there is evidence to suggest that it can change (alongside other inflammatory markers) due to estrogen and progesterone fluctuations across the cycle [458, 459]. This means that our findings could have been influenced by the timing of the testing visit, in addition to the effect of the dairy consumption. Overall however, the results from our exploratory sex differences analyses, in combination with findings from the broader literature suggest that sex-based metabolic and

inflammatory responses to dairy consumption may exist. Neither our study nor those in the literature assessed sex hormones, so future studies should consider measuring these outcome measures to further explore this observation and determine if they play a direct role in metabolic or inflammatory differences either between sexes, or across the menstrual cycle.

Taken together, our findings showing that TNF-α decreased and IL-4 increased (along with GSH-sparing) in the HD diet compared to the LD diet suggests an overall minor benefit of short-term dairy consumption on LGCI. The improvements we observed within some biomarkers may however require a longer period of time to manifest as improvements within other tissues, organs or systems (i.e., vascular system). Our results are in line with the broader literature (including epidemiological data), as several large meta-analyses in different populations (including those with metabolic risk factors) have consistently shown that dairy consumption is associated with either neutral or minor anti-inflammatory benefits [5, 200, 323]. Our study had some strengths. We implemented a crossover design in free-living conditions, provided a mixture of commonly consumed dairy products, and utilized a population with OW/OB (who are more at risk of LGCI) which increased ecological validity and improved the generalizability of our findings. Our study also had some limitations. First, while we did utilize a randomized, crossover (AB/BA) design, the variability in our biological measures was high, which may have obfuscated our ability to detect statistical differences with a smaller sample size. We did not control visits by menstrual cycle stage which could have confounded some of our biomarker measurements (e.g., inflammatory cytokines). Furthermore, two participants had significantly higher concentrations of some inflammatory markers throughout (IL-6 in particular), which may have unequally weighted our mean concentrations. However, we did evaluate percentage change in an attempt to account for these inter-individual differences. Next, our goal was to recruit individuals with both OW/OB and

cardiometabolic dysfunction to avoid a floor effect of studying inflammation in healthy individuals. However, while all our participants met at least two inclusion criteria related to cardiometabolic dysfunction, many were only borderline above the threshold for LGCI (CRP > 3mg/L), so the magnitude of dysfunction may have been lower than expected. That said, while we did not observe any changes in CRP between diets, and the mean concentration across all timepoints was 3.24 mg/L, indicating that our participants were experiencing some degree of LGCI, albeit on the lower end of the 'high' criteria (clinical guidelines suggest that a circulating CRP level of < 3mg/L is considered normal, 3-10 mg/L is considered high and >10 mg/L is considered very high [289]). Another limitation is that compliance within the HD diet condition was not quantitatively assessed. Despite this, we are confident that compliance was high considering both the verbal confirmation with participants (during check-ins with the research team) and regular viewing of their food records through the online app (which included photographs taken by participants of the dairy products immediately prior to being consumed). Lastly, due to our deliberate decision to use participants' habitual control diets as the comparator, we were unable to nutrient match the two dietary arms, so we are unable to specifically comment on individual nutrient or food effects.

## 5.6 Conclusion

In conclusion, increased mixed dairy intake, vs. a low dairy diet favourably modulated some circulating plasma cytokines over a 6-week intervention period in the absence of any body weight or percent fat changes, however the differences were relatively small in magnitude. These changes may have been mediated, in part, by improved maintenance of GSH. Chronically elevated circulating inflammation has the potential to negatively interfere with a range of

physiological processes and tissues throughout the body [321], and promote chronic metabolic disease [460]. Therefore, reducing resting, fasted concentrations of pro-inflammatory cytokines and/or increasing levels of anti-inflammatory cytokines while maintaining antioxidant defenses through dietary modulation may improve the functioning of these systems over time and reduce disease risk. Our results suggest that by increasing habitual dairy consumption, resting systemic inflammation may be attenuated in individuals with OW/OB and some cardiometabolic dysfunction and thus may support improved long-term health and well-being if incorporated into a balanced diet.

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# Chapter 6:

# Six Weeks of Increased Dairy Intake Does Not Affect the Postprandial Inflammatory Response following a High-Fat Meal in Individuals with Overweight and Obesity

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**Specific Contribution:** I was the lead investigator and coordinator for the 2-year long data collection period of this study. I also completed biochemical assays, and did the majority of the data analysis, interpretation, and writing of the manuscript. Vascular measures, analysis and interpretation were performed by Dr Heather Edgell's group.

***JLP**, CGRP, DW, HE, PK and ARJ designed research. **JLP**, ECF, TJP, JALT, SG, NC and ARJ conducted research. **JLP**, TJP and ARJ analyzed data; **JLP** wrote the paper.*

## 6.1 Abstract

There is evidence that consuming dairy products either prior to, or as part of a meal can attenuate postprandial inflammation, but it is unclear if increasing dairy consumption within the habitual diet imparts similar benefits. The purpose of this study was to compare 6 weeks of consuming a diet either high or low in dairy on postprandial inflammatory, antioxidant, metabolic and vascular measures following consumption of a high-fat meal. Using a randomized crossover design, 30 participants with overweight/obesity (OW/OB) (18/12 female/male; age: 31.8 ± 12.3years; body mass: 85.0 ± 14.4kg; %body fat: 37.6 ± 7.2%; BMI: 32.0 ± 4.5kg/$m^2$) and pre-existing cardiometabolic disease risk factors were randomized to consume either 6-weeks of a high-dairy (HD) diet (3 servings/d [2% milk, Skyr yogurt, cheddar cheese]) or 6-weeks of their habitually low-dairy (LD) diet (≤1 serving/d) before returning to complete a high-fat meal challenge (HFMC). This was followed by a ≥4-week washout period and then completion of the other dietary condition. Inflammatory cytokines were measured within plasma, metabolic biomarkers within serum, and glutathione within whole blood and quadriceps muscle tissue. The HFMC resulted in changes in inflammatory, metabolic, glutathione and vascular measurements over the 6-h postprandial period, but increased dairy consumption for 6-weeks prior did not significantly affect these responses. Therefore, while a HFMC induced postprandial physiological changes, increased mixed dairy product consumption for a short/moderate time period before the HFMC did not attenuate the potentially negative postprandial inflammatory responses that can occur.

## 6.2 Introduction

Inflammation is an essential immune system response that can restore homeostasis following internal damage or pathogenic invasion [460]. If the resolution of this inflammatory response is dysregulated (such as with overweight/obesity [OW/OB] [243-245, 413, 414]; defined as BMI ≥25 kg/m$^2$), then, over time, a state of low-grade chronic inflammation (LGCI) can ensue. Consistent elevations in circulating immune markers (interleukins [IL], tumor necrosis factors [TNF], interferons [IFN], C-Reactive Protein [CRP], etc.) can disrupt normal physiological functioning/signalling and may also contribute towards the development of chronic cardiovascular and digestive diseases and/or certain cancers [246-248, 460]. In addition to experiencing LGCI while fasted, individuals with OW/OB may also experience postprandial dysmetabolism, characterized by abnormally high increases in glucose (even in the absence of diabetes), insulin, and lipids following the consumption of food (particularly foods high in fat and sugar) [306, 307]. This postprandial dysmetabolism can be followed by acute increases in inflammation and oxidative stress [307], and these postprandial metabolic insults have also been associated with the development of cardiometabolic disease [314, 315]. Considering that people in the western world typically spend the majority of their day in a postprandial state [316, 368], it is important to understand and develop strategies to mitigate the aberrant inflammatory and metabolic stress that can occur following food/drink consumption. Specific foods and nutrients may be able to modulate inflammation [416, 461]; however, relatively few studies have evaluated the effect of these potentially anti-inflammatory nutrient sources on postprandial inflammatory responses.

Dairy products provide an important source of macro/micronutrients, are relatively inexpensive and significantly improve diet quality when incorporated habitually [44, 180, 462]. They also contain important bioactive components, with meta-analyses indicating that their

increased habitual consumption is associated with minor reductions in circulating inflammatory cytokines and CRP, particularly in those with OW/OB and cardiometabolic dysfunction [5, 200, 416]. Furthermore, higher levels of dairy consumption have also been associated with reduced cardiometabolic disease risk and improved mortality, which may be related to the observed amelioration of inflammation and oxidative stress by the incorporation of these foods into the diet [2, 6, 10, 29, 200]. These anti-inflammatory effects have been hypothesized to be related to interactions between dairy constituents and internal inflammatory pathways/antioxidant systems [201, 249, 250]. For example, bioactive peptides and hydrolysates in casein or whey (e.g., lactoferrin) may be able to attenuate inflammation by interfering with Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation or downregulating cytokine production from monocytes [2]. Similarly, micronutrients such as zinc can support reactive oxygen species (ROS) scavenging as enzymatic cofactors [6], and key amino acids in dairy are required to maintain intracellular antioxidant systems (e.g. cysteine to support glutathione synthesis) [6, 218]). Calcium and certain dairy proteins may also attenuate postprandial dysregulation by delaying gastric emptying and decreasing fat absorption [165, 171, 352], modulating gut microbiome function *via* increasing intestinal mucin and secretory immunoglobulin A and antimicrobial peptide secretion, and/or improving gut permeability by maintaining tight junction functionality [353, 354].

Few studies have evaluated the effects of dairy consumption on postprandial metabolic, and inflammatory responses, but there is some evidence that it may help mitigate adverse postprandial spikes in inflammation seen in individuals with OW/OB. Pei and colleagues (2017) found that acute pre-meal dairy yogurt consumption blunted the rise in IL-6 in females with OW/OB following a high-fat meal challenge (HFMC) compared to a soy yogurt [16]. However, it

may be impractical to consume yogurt immediately prior to a meal, limiting the real-world applicability of such a strategy. Instead, the purpose of our study was to evaluate the effects of increasing habitual dairy consumption (daily yogurt, as well as milk and cheese consumption) for a short-term period (6-weeks) on the postprandial response to a HFMC in individuals with OW/OB. We measured a wide range of inflammatory, antioxidant (glutathione), vascular and metabolic biomarkers to understand how these postprandial responses may be modulated by increased habitual dairy consumption.

## 6.3 Methods

### Participant Screening & Recruitment

Our randomized crossover clinical trial was registered at clinicaltrials.gov (identifier: NCT04902417), conformed to Canadian Tri-council funding policy statement (TCPS2) on the use of human participants in research and was approved by the Office of Research Ethics at York University, Toronto, Canada (certificate#: 2021-177). Participants were screened and recruited from the greater Toronto area and data collection was conducted from June 2022 to March 2024. In total, 30 participants completed the entire study, after 493 people contacted the lab for assessment, 211 completed the initial phone screening, 106 completed the secondary in-person screening visit and 36 were enrolled (**Figure 19; CONSORT diagram**). Phone screening was initially completed to obtain informed consent and information relating to health, activity levels, medical history, and habitual dairy consumption. Individuals who were eligible following phone screening attended an in-person screening visit at York University. They arrived fasted and had the following measurements performed: height, weight, waist circumference, blood pressure (BP), and a finger prick blood sample, which was analysed using the Cholestech LDX Analyzer (Abbott;

Chicago, IL, USA) to obtain a full lipid profile (total cholesterol [TC], triglycerides [TG], low density lipoprotein [LDL], high-density lipoprotein [HDL]) and blood glucose measurement. They were then instructed to complete a 7-day food diary to assess their habitual diet and average level of dairy intake. A registered dietitian (RD) instructed participants on how to accurately record their dietary intake using the mobile phone dietary intake app Keenoa (Keenoa, Montreal, QC, Canada, version 1.03) [418, 463].

**Participant Criteria and Characteristics**

The study inclusion criteria were as follows: BMI ≥25 kg/m$^2$, aged 18 - 70 years old, sedentary (<2 structured exercise sessions per week as per the adult Canadian 24-h movement guidelines [419]), and low habitual dairy intake (≤1 serving per day). Participants also had to exhibit at least two other cardiometabolic risk factors [420] from the following list: increased waist circumference (males ≥102cm; females ≥88cm), increased resting BP (≥130/85), impaired fasting glucose (≥5.6 mmol/L), borderline high TC (≥5.2 mmol/L) or fasting LDL (≥3.5 mmol/L), impaired fasting HDL (males <1.03 mmol/L; females <1.3 mmol/L), and impaired fasting TG (≥1.7 mmol/L). Individuals were excluded for the following reasons: allergy to dairy foods or diagnosed lactose intolerance, previous history of diabetes and/or related cardiovascular disease, any inflammatory/immune disease, use of multiple medications for managing lipids, glucose, and/or blood pressure, and current smoker. Participant characteristics at study onset for the 30 participants that completed the study are shown in **Table 12**. They were then randomized to 1 of 2 diet sequences – high dairy (HD)/low dairy (LD) or LD/HD using a computer-generated randomizer by a research team member without any prior knowledge of participants' status or screening results. The sequence for each participant was then passed on to the study coordinator before being told to participants upon study enrollment.

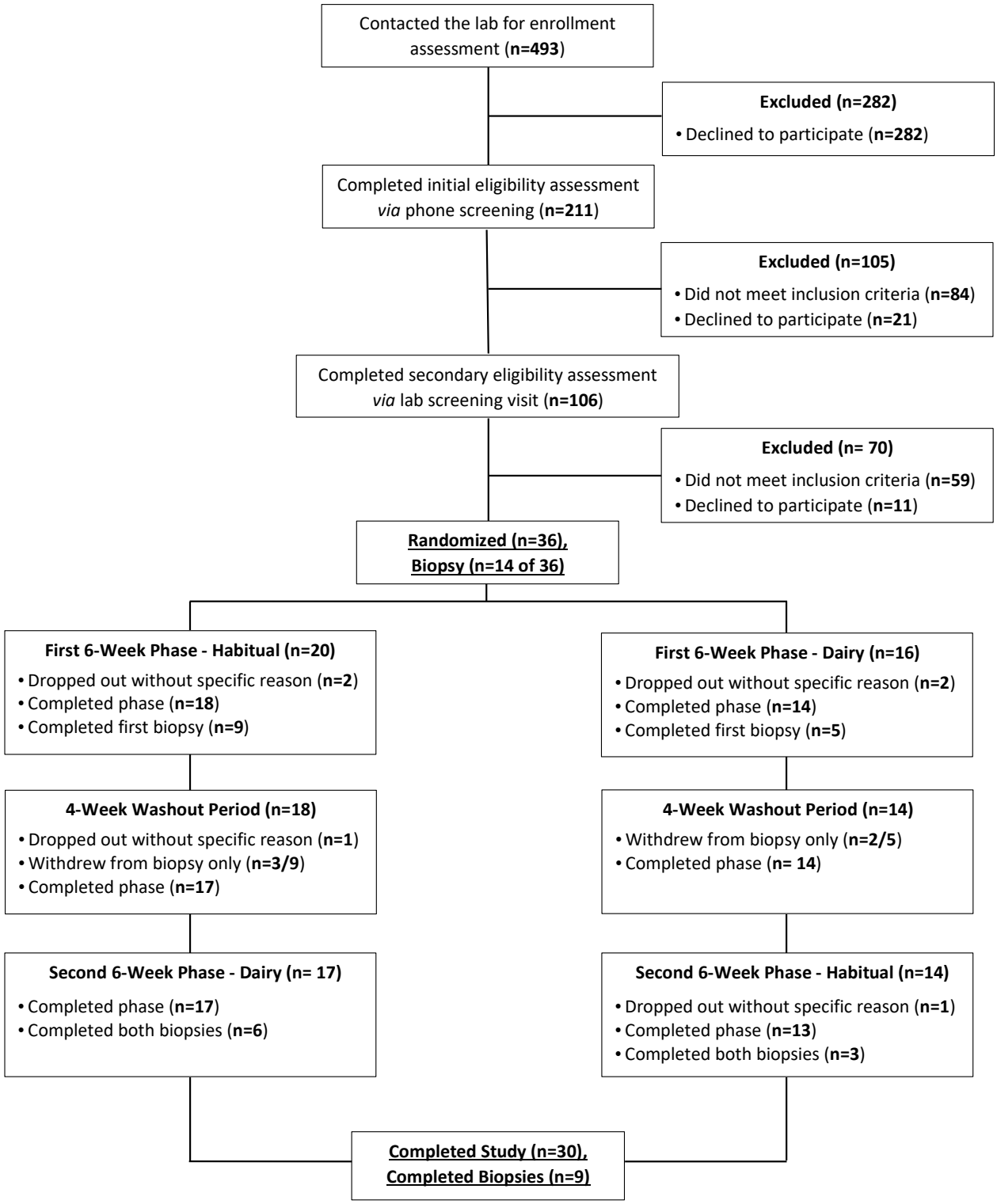



**Figure 19.** CONSORT diagram showing participant flow through the trial, including recruitment, randomization, and completion

**Table 12.** Participant characteristics and screening criteria for all participants who completed the study. Study eligibility required having some combination of 2 or more of the cardiometabolic risk factors listed below.

| Characteristics | Mean ± SD | Meeting inclusion criteria (n) |
|---|---|---|
| Age (years) | 31.8 ± 12.3 | 30 |
| BMI (kg/m$^2$) | 32.0 ± 4.5 | 30 |
| Height (cm) | 164.0 ± 8.0 | |
| Body Mass (kg) | 85.0 ± 14.4 | |
| BIA Body Fat (%) | 37.5 ± 7.3 | |
| BodyMetrix Body Fat (%) | 34.1 ± 4.9 | |
| Sex | Male n = 12<br>Female n = 18 | |
| Cardiometabolic risk factors: | | |
| Waist Circumference (cm) | 99.4 ± 10.1 | 25/30 |
| Systolic BP (mmHg) | 125 ± 14 | 12/30 |
| Diastolic BP (mmHg) | 76 ± 11 | 5/30 |
| Blood Glucose (mmol/L) | 5.5 ± 0.7 | 11/30 |
| Total Cholesterol (mmol/L) | 4.8 ± 0.8 | 11/30 |
| LDL Cholesterol (mmol/L) | 3.0 ± 0.6 | 7/30 |
| HDL Cholesterol (mmol/L) | 1.2 ± 0.3 | 19/30 |
| Triglycerides (mmol/L) | 1.6 ± 0.7 | 9/30 |

Data are displayed as mean ± SD, n=30. Abbreviations: BIA = bioelectrical impedance analysis; BMI = body mass index; BP = blood pressure; HDL = high density lipoprotein; LDL = low density lipoprotein.

**Study Design Overview**

This study utilised a within-subject cross-over design where all participants completed two 6-week dietary intervention arms in a random order. It was part of a larger trial in which fasting measurements were also compared both before (baseline) and after 6-weeks of HD or LD consumption, details and results of which are reported elsewhere (Prowting et al., under review).

After completing the screening process, participants then began the first of the two 6-week dietary intervention arms. The intervention arms consisted of: 1) HD consumption, 3 servings per day of a mix of milk, cheese and yogurt (250mL of 2% white milk [Lactantia], 200g of vanilla,

strawberry or black cherry flavoured 2% milk fat Icelandic skyr yogurt [Siggis], and 42g of 35% fat cheddar cheese [Cracker Barrel]); or 2) their usual/habitual LD diet (≤1 serving of dairy per day). After each 6-week intervention arm, participants completed the high fat meal challenge (HFMC). Measurements were taken while fasted, and then for up to 6 hours following the consumption of a high fat meal. They then completed a ≥4-week washout before commencing the second 6-week dietary intervention arm and subsequent meal challenge. The primary outcome measures of this study were inflammatory biomarkers, and the secondary outcome measures were metabolic biomarkers, glutathione, and vascular measures.

**Intervention Period Monitoring**

Two weeks supply of dairy products were provided to participants every other week and were kindly donated by Lactalis, Canada. It was impossible to blind participants to the dietary arm that they were completing, however, the research team were blinded to dietary condition during analysis. The RD provided individualised counselling on how to appropriately incorporate the 3 additional servings of dairy foods into their diet, including information on how to exchange them with other foods of similar caloric value (of their choosing) to avoid body mass increases over the course of the 6-week HD intervention period. The Keenoa mobile phone application was again used to record and monitor food and drink intake for two 7-day periods (Week 1 and 6), as well as for two 3-day periods (Week 2 and 4). During Weeks 2 and 4, participants also had their body weight recorded and completed an activity questionnaire to allow for ongoing monitoring of weight status.

**Testing Procedures and High Fat Meal Challenge (HFMC) Overview**

After 6-weeks, participants returned to the laboratory at York University for post-intervention arm testing. They were required to complete an overnight fast, avoid strenuous

exercise for 48h prior, avoid alcohol consumption for 24h prior, and avoid caffeine or pain medication for 12h prior to the testing visit. All visits began in the morning between 7am – 9am, and efforts were made to complete follow-up visits at the same time as their initial visit. To begin with, participants rested for 5-minutes in a supine position before having measurements of vascular function assessed (detailed below under *Vascular Measures*). Following this, body composition measurements were taken, which included body mass (BC-418 TANITA scale, Tanita Corp, Tokyo, Japan) and body fat percentage measured using two techniques: 1) bioelectrical impedance analysis (BIA) using a BC-418 TANITA scale (Tanita Corp, Tokyo, Japan), and 2) ultrasound assessment of subcutaneous tissue thickness at 3 skinfold sites using the BodyMetrix™ BX2000 device (IntelaMetrix, Brentwood, CA, USA) [421]. A peripheral intravenous (IV) catheter was then inserted into a vein within the antecubital fossa to facilitate multiple blood draws of approximately 20mL of blood per timepoint throughout the visit. To avoid blood clotting and auto-oxidation of glutathione, 500µL of whole blood was immediately mixed with 11.5µL of tripotassium ethylenediaminetetraacetic acid (k3-EDTA) and 50µL of 20 µmol l−1 N-ethylmaleimide (NEM) before being transferred and stored at –80˚C until analysis. Vacutainers coated with EDTA and sodium heparin (for plasma) were immediately centrifuged at 1300g and 4˚C for 10 minutes, and serum separator tubes (for serum) rested for 30 minutes at room temperature before also being centrifuged at 1300g and 4˚C for 10 minutes. Plasma and serum were then aliquoted into small cryovials and stored at –80˚C until analysis. A subset of participants (n=9) underwent two muscle biopsies (one on each leg) during each HFMC. One was taken fasted pre-meal and the other 4h post-meal consumption (procedure detailed below).

Participants were then provided with a fast-food meal that consisted of approximately 50% of their total daily energy expenditure (estimated using the Mifflin-St Jeor equation [464]),

resulting in meals typically between 800-1400kcals, and 50% of those calories were from dietary fat. This meal did not contain any dairy and was typically made up of a sausage and egg breakfast sandwich (no cheese), hash browns and donut holes (Tim Horton's, Toronto, ON, Canada). Ten participants had dietary restrictions (e.g., vegetarian or religious) and had the sausage breakfast sandwich replaced with a vegetarian patty instead (Beyond Meat, California, USA) which closely matched the energy and macronutrient content of the meat containing meal. Participants were encouraged to complete the entire meal within 15 minutes and the postprandial period began upon meal completion. During this postprandial period, participants remained at rest within the lab (leaving only for bathroom breaks) and were allowed to consume water *ad libitum* but could not consume any other food or drink.

At the sampling timepoints of pre-meal and then 1h, 2h, 4h and 6h postprandially, approximately 20mL of venous blood was collected from the IV catheter each time (~120mL total during the 7h laboratory visit). The catheter was flushed with saline every 30 min to maintain patency. At the 4h timepoint, the vascular measurements and muscle biopsy (if applicable) were repeated. After completion of the 6h blood draw, the IV catheter was removed and participants were asked to complete a ≥4-week washout period before beginning the second dietary intervention arm. Six weeks after that, they returned to our laboratory to repeat the HFMC and testing procedures (**Figure 20**).

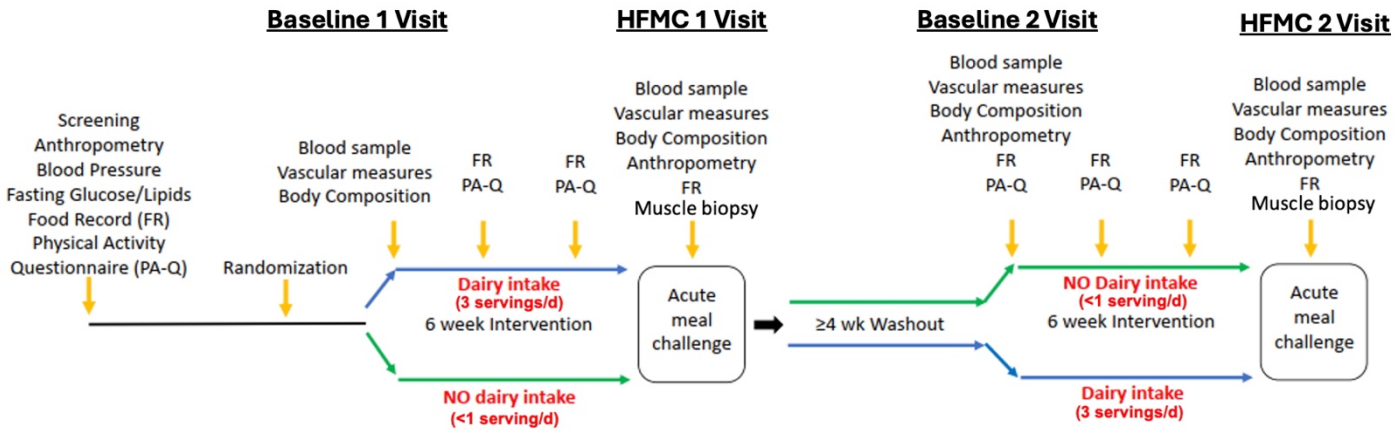



**Figure 20.** Schematic of 16-week study timeline, indicating when different measurements were taken and during which visit. Abbreviations: FR = food record; HFMC = high-fat meal challenge; PA-Q = physical activity questionnaire.

## Muscle Biopsy

This part of the trial was optional. Immediately prior to completion of the muscle biopsy, procedure details and risks involved were repeated to the participants, and a brief medical questionnaire was completed to confirm eligibility to proceed (i.e., ensure no blood thinners were recently taken, inquire about recovery from previous biopsies, etc.). Skeletal muscle biopsies were performed using the Bergström needle biopsy technique [465] modified with manual suction [466] as used previously by our group [467, 468]. With the participant lying supine on a bed, the skin over the vastus lateralis was first sterilized using Stanhexadine (Omega Laboratories, Montreal, QC, Canada) before local subcutaneous anesthesia of ~2 ml of 2% xylocaine without norepinephrine (Mckesson Canada Corporation, Saint-Laurent, QC, Canada) was injected. After numbing had taken effect (usually 5-10 minutes), a ~2cm long incision was made in the skin, underlying layers and deep fascia with a scalpel, prior to vertical insertion (~90° angle) of the Bergström needle (~4 mm diameter). The cutting window of the needle was positioned at a depth of ~1 cm below the surface of the fascia. Three to four cuts (80–150 mg total) were sampled over

a period of ~5-7s with ~30-degree rotations separating each cut prior to removing the needle from the vastus lateralis. Muscle was then immediately flushed out of the needle with sterile saline, placed in a cryotube and flash frozen in liquid nitrogen, before being frozen at -80˚C for future analysis. The incision site was manually compressed for 5-7 minutes before being closed using Steri-Strips (3M Canada Inc, London, ON, Canada), and covered and sealed with a 10cm x 12cm Tegaderm Transparent Dressing (3M Canada Inc, London, ON, Canada). The leg was then elevated and ice was applied using a compression (tensor) bandage for approximately 15-minutes. Participants were provided with extra alcohol swabs, Steri-Strips and Tegaderm transparent dressings and instructed on how to clean and care for the wound over the following days. Research personnel followed up with each participant in the evening of, and days following the biopsy procedure to provide oversight on the recovery process and answer questions, if necessary.

**Vascular Measures**

At pre-meal and 4h post-meal, intima media thickness (IMT) of the carotid artery was assessed as an index of vascular health and was analyzed using automated analysis in EchoPAC software (GE Healthcare Systems, Canada). Blood pressure was taken immediately prior to carotid insonation using an automated cuff (BP Tru, BPM 200, Coquitlam, CA). Subsequently, flow-mediated dilation (FMD) of the brachial artery was used to assess endothelial function with a 10MHz linear probe (Vivid i, GE Healthcare systems, Mississauga, Canada) [469]. Brachial artery diameter and blood flow velocity were acquired in duplex mode. Assessment of endothelial function included a 2-minute initial measurement followed by 5-minutes of supra-systolic BP (+50 mmHg) forearm occlusion and a 3-minute recovery phase that included the rapid deflation of the BP cuff causing reactive hyperemia. Continuous video was captured using video grabber (AV.io

HD, Epiphan Video), which was subsequently analyzed using edge detection software (Cardiovascular Suite, Quipu, Italy) to determine changes in shear stress and arterial diameter. FMD percent (FMD%) was automatically calculated as the difference in peak artery diameter during reactive hyperemia was compared to initial diameter.

**Inflammatory Biomarkers**

Assessment of inflammation was primarily achieved by measuring the circulating concentrations of six inflammatory biomarkers, which included: IL-1β, IL-4, IL-6, IL-10, TNF-α and interferon gamma (IFN-γ). Analysis was performed by Eve Technologies Corporation (Calgary, Alberta, Canada) using the Luminex® 200™ system (Luminex Corporation/DiaSorin, Saluggia, Italy) with Bio-Plex Manager™ software (Bio-Rad Laboratories Inc., Hercules, California, USA). The markers were measured in duplicate together in the samples using a Human High Sensitivity T-Cell 6-Plex Custom Multiplex Assay (MILLIPLEX® Human High Sensitivity T-Cell Magnetic Bead Panel Cat#: HSTCMAG-28SK, MilliporeSigma, Burlington, Massachusetts, USA) as per the manufacturer's instructions for use. Assay sensitivities (pg/mL) and coefficients of variation (CV%) of these markers were as follows: IL-6 = 0.11 and 8.1%, IFN-γ = 0.48 and 8.7%, IL-1β = 0.14 and 8.8%, IL-4 = 1.12 and 8.7%, IL-10 = 0.56 and 10.1%, TNF-α = 0.16 and 6.4%.

**Metabolic Biomarkers**

The following 7 biomarkers were measured using the Cobas c503 & e801 modules (Roche Diagnostics, GmbH, Mannheim, Germany): CRP (Cat#: 07876424-190), glucose (Cat#: 05168791-190), insulin (Cat#: 12017547122), TC (Cat#: 03039773-190), HDL cholesterol (Cat#:

07528566-190), TG (Cat#: 20767107) and Apolipoprotein-B (Cat#: 03032574). LDL cholesterol concentrations were derived from TC, HDL and TG results. These analyses were performed by Mt Sinai Services (Mount Sinai Hospital, Toronto, ON, Canada) on serum samples.

**Glutathione Analysis**

Both reduced (GSH) and oxidized (GSSG) glutathione were measured from the same whole blood sample (with pre-added EDTA and NEM), and the same piece of muscle tissue. Muscle was homogenized in 50 mM Tris buffer with 20 mM boric acid (Sigma-Aldrich), 2 mM l-serine (Sigma-Aldrich), 20 μM acivicin (Enzo Life Sciences), and 5 mM N-ethylmaleimide (Sigma-Aldrich), pH at 8.0 with HCl. Analysis used a method adapted from Giustarini et al. [423] that was previously used by our lab [424-428]. All samples were analyzed using an Agilent HPLC 1100 (Agilent Technologies, Mississauga, ON, Canada) with a Zorbax C18 (4.6 mm x 150 mm, 5 μm) column (Agilent Technologies). Concentrations for both GSH and GSSG were calculated using standard concentration curves, and total glutathione (tGS) was calculated as GSH + 2*GSSG. For blood samples, results were expressed relative to hemoglobin concentration and reported in μmol/g Hb. For muscle samples, a sample of vastus lateralis tissue was reserved prior to acid deproteination for analysis of protein concentration using a Pierce BCA protein assay kit (Thermo Fisher Scientific), and results were expressed in relation to μmol $g^{-1}$ protein.

For GSH, 100 μL of blood was combined with 100μL of 15% trichloroacetic acid (TCA), and 70uL of homogenised muscle supernatant was combined with 7μL of 60% TCA before being shaken thoroughly and then centrifuged at 14,000g for 2 minutes. The deproteinized supernatants from this step were transferred to HPLC autosampler vials for column separation and then pumped under isocratic conditions using a 0.25% glacial acetic acid mobile phase with 6% acetonitrile at

a flow rate of 1.05mL/min$^{-1}$. The GSH-NEM conjugate peak was detected using a modular variable wavelength detector (UV monitoring) at 265nm wavelength.

For GSSG, 100µL of blood or 100µL of homogenised muscle supernatant was combined with 100µL of 15% perchloric acid before being shaken thoroughly and then centrifuged at 14,000g for 2 minutes. Then, 100µL of the deproteinized supernatant from this step was combined with 500µL of 0.5M NaOH, mixed thoroughly, and finally combined with 37.5µL 0.1% o-Pthalymide (Sigma-Aldrich) in methanol. This was incubated on a rocker in complete darkness for 15 minutes to create a GS-OPA conjugate. Following incubation, samples were transferred to HPLC autosampler vials for column separation and pumped under isocratic conditions using a mobile phase consisting of 25mM $Na_2HPO_4$ in HPLC-grade water with 15% methanol, at a flow rate of 0.5mL/min. The oxidized glutathione peak was detected by fluorescence-HPLC monitoring, involving excitation at 350nm, and emission detection at 420nm.

**Statistical Analysis**

Data analyses were performed while blinded to condition using GraphPad Prism (version 10.4.0; GraphPad Software Inc., San Diego, CA, USA) with $p \leq 0.05$ considered statistically significant. Replicate data from biochemical assays were assessed in a blinded fashion, and those with a CV of >15% were visually inspected, and a single data point was used instead of the average in cases of a clearly erroneous measurement. Of the 360 blood samples measured across all participants, 10 are missing from each biomarker analysis due to missed blood draws (HD = 3x 1h timepoint, 1x 4h timepoint, 2x 6h timepoint; LD = 1x 2h timepoint, 3x 6h timepoint). Due to analytical errors, complete data sets from four participants were missing for CRP (n=26 analysed) and one for LDL (n=29 analysed). Data from two participants were not included for IL-6 (n=28

analysed) due to being outliers (7.6 SDs and 13.2 SDs outside of the mean). Fifteen of a total of 120 FMD assessments were lost due to technical and visualization errors (e.g., issues with software, n=4; or movement artifact, n=11), of which 2 were HD pre-meal timepoints, 6 were 4h post-meal timepoints, 2 were LD pre-meal timepoints, and 5 were 4h post-meal timepoints. Data were checked for normality using skewness and kurtosis (±3 deemed acceptable) and non-normally distributed data were log-transformed (IL-6, Blood GSH:GSSG) for statistical analysis. Mixed-effects model (within-within repeated measures) using a Greenhouse-Geisser correction were used to assess interactions and main effects for time and dietary condition for the absolute concentrations of blood biomarkers (2x5 model), absolute concentrations of muscle glutathione measures (2x2 model) and vascular measures (2x2 model). To evaluate the overall postprandial response of blood biomarkers during the 6h period following meal consumption, the net incremental AUC (net iAUC) was calculated by subtracting the negative iAUC from the positive iAUC (which are the areas of excursion below and above the baseline (pre-meal) values, calculated using the trapezoid method) for each participant, which were then pooled and compared using a paired samples t-test. This method has previously been used to evaluate HFMC responses [16, 169]. Effect sizes were calculated when significant differences between dietary conditions were found, using Cohen's d to determine the magnitude of an effect independent of sample size. Small effect sizes are considered as $d < 0.2$, moderate effect sizes as $d = 0.2–0.8$, and large effect sizes as $d > 0.8$ [432].

## 6.4 Results

### Body Mass & Body Fat Percentage

Both body mass (Baseline HD: 84.7 ± 15.4kg, 6-Week HD: 85.1 ± 15.8kg, Baseline LD: 85.5 ± 15.1kg, 6-Week LD: 85.7 ± 15.6kg) and body fat percentage, measured using BIA (Baseline HD: 37.2 ± 7.0%, 6-Week HD: 37.7 ± 7.3%, Baseline LD: 38.0 ± 7.4%, 6-Week LD: 37.2 ± 7.6%) or ultrasound (Baseline HD: 34.6 ± 5.2%, 6-Week HD: 34.1 ± 5.2%, Baseline LD: 34.1 ± 4.9%, 6-Week LD: 34.1 ± 4.7%) were unchanged throughout the trial with no significant main effects or interactions for any measurement ($p>0.05$).

### Dietary Intake Assessment

Food diaries were collected from all 30 participants; however, due to inaccurate reporting by 4 participants (e.g., reported daily energy intake of <50% of estimated energy expenditure), data from n=26 were analyzed and reported (Table 2). Dietary intake was assessed at multiple timepoints throughout the study. Participant's 7-day food diaries recorded during weeks 1 and 6 of each intervention arm were combined to provide a representative sample of their nutritional intake during each intervention arm (**Table 13**). Paired sample t-tests indicated protein (d=0.489), saturated fat (d=0.899), calcium (d=3.021), Vitamin A (d=1.098) and Vitamin D (d=1.163) were significantly higher during the HD diet compared to the LD diet (all $p<0.05$), which was expected by trial design.

**Table 13.** Average daily dietary intake during the HD and LD intervention arms, based on analysis of 7-day food records (completed by participants during weeks 1 and 6 of each dietary intervention period, combined in table below).

| Nutrients | HD | LD | P-value | Cohens d |
|---|---|---|---|---|
| Energy (kcals) | 2091 ± 371 | 2050 ± 404 | 0.353 | |
| Protein (g) | **108.4 ± 25.2** | **95.0 ± 29.4** | **0.015** | **0.489** |
| Fat (g) | 86.2 ± 16.5 | 89.3 ± 19.2 | 0.287 | |
| Saturated Fat (g) | **33.5 ± 6.9** | **27.2 ± 7.2** | **<0.001** | **0.899** |
| Carbohydrate (g) | 225.1 ± 54.1 | 225.4 ± 53.2 | 0.975 | |
| Calcium (mg) | **1216 ± 252** | **567 ± 170** | **<0.001** | **3.021** |
| Magnesium (mg) | 238 ± 60 | 261 ± 66 | 0.052 | |
| Vitamin A (ug) | **445 ± 158** | **287 ± 128** | **<0.001** | **1.098** |
| Vitamin E (mg) | 7.1 ± 1.6 | 7.7 ± 2.2 | 0.060 | |
| Vitamin D (IU) | **199 ± 101** | **100 ± 66** | **0.0001** | **1.163** |
| Vitamin B12 (mg) | 4.3 ± 2.1 | 4.0 ± 2.4 | 0.484 | |

Data are displayed as mean ± SD, n=26. Significant differences ($p \leq 0.05$) bolded. Abbreviations: HD = high dairy; LD = low dairy; g = grams; IU = international units; kcals = kilocalories; kg = kilograms; mg = milligrams; ug = micrograms.

## Inflammatory Markers

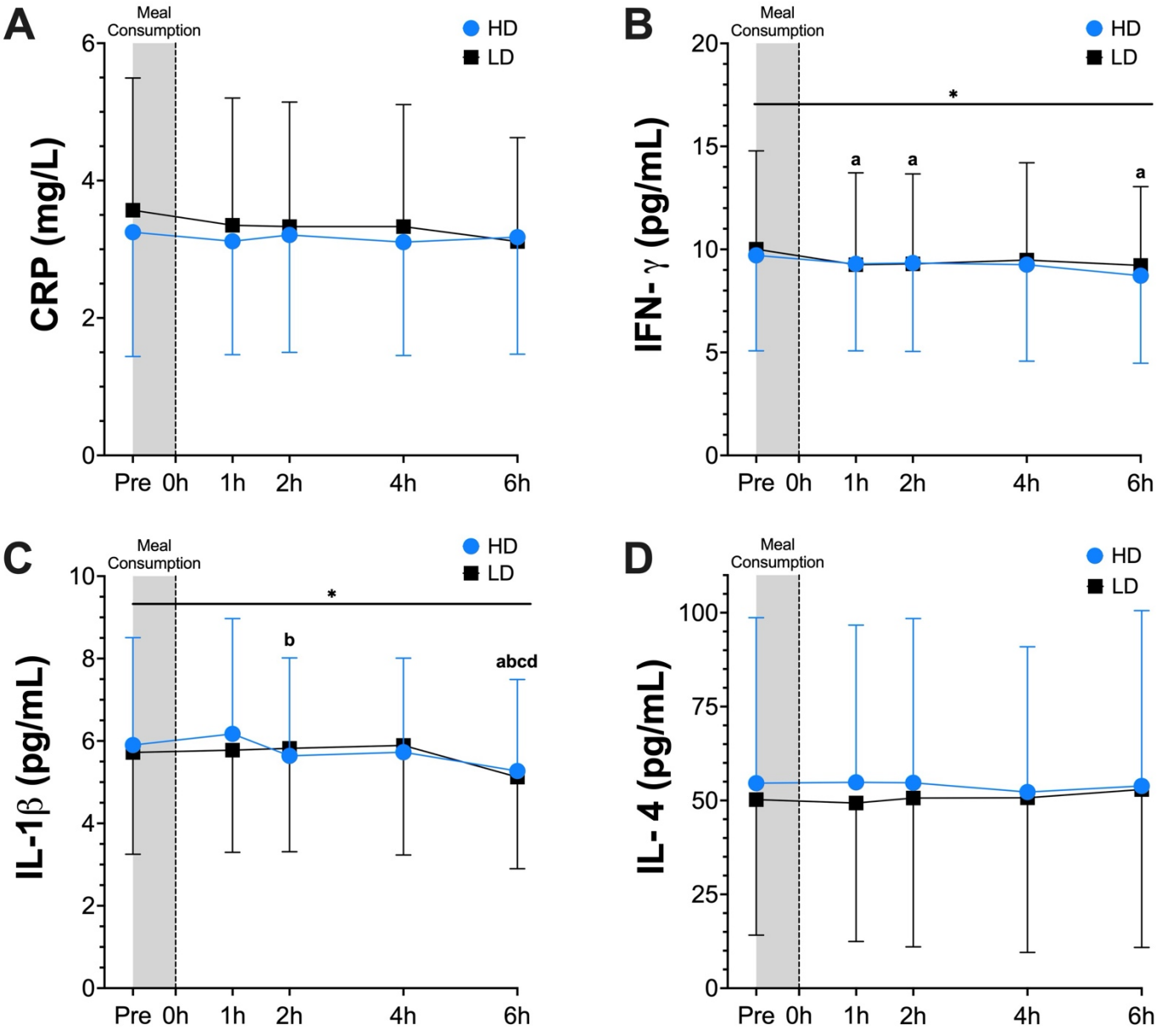
### Absolute Concentrations

Absolute concentrations for all inflammatory biomarkers assessed in both conditions are displayed in **Figure 21** (also displayed in **Supplementary Table 16**). There was a significant main effect of time for IL-6, with post-hoc testing showing the 6h concentration was significantly higher than the pre-meal (Δ0.49pg/mL; p=0.011), 1h post-meal (Δ0.50pg/mL; p=0.012) and 2h post-meal (Δ0.43pg/mL; p=0.005) concentrations, and that the 4h concentration was significantly higher than the 2h concentration (Δ0.16pg/mL; p=0.048).

There was a significant main effect of time for IFN-γ, with post-hoc testing showing that the pre-meal concentration was significantly lower than the 1h post (Δ-0.48pg/mL; p=0.014), 2h post (Δ-0.54pg/mL; p=0.019) and 6h post (Δ-0.79pg/mL; p=0.0002) concentrations.

There was also a significant main effect of time for IL-1β, with post-hoc testing showing that the 6h concentration was significantly lower than the pre-meal (Δ-0.47pg/mL; p=0.006), 1h post (Δ-0.55pg/mL; p=0.003), 2h post (Δ-0.32pg/mL; p=0.028) and 4h post (Δ-0.47pg/mL; p=0.0002) concentrations, and that the 2h concentration was significantly lower than the 1h concentration (Δ-0.25pg/mL; p=0.047).

Finally, there was a significant main effect of time for TNF-α, with post-hoc testing showing that the pre-meal concentration was significantly lower than the 1h post (Δ-0.63pg/mL; p=0.004), 2h post (Δ-0.69pg/mL; p=0.002), 4h post (Δ-0.69pg/mL; p=0.004) and 6h post (Δ-1.31pg/mL; p<0.0001) concentrations, and also that the 1h post (Δ-0.56pg/mL; p=0.0002), 2h post (Δ-0.45pg/mL; p=0.008) and 4h post concentrations (Δ-0.57pg/mL; p=0.001) were significantly lower than the 6h post concentration. There were no significant interactions or main effects observed for concentrations of CRP, IL-4 or IL-10 (all p>0.05).


A
Meal Consumption
HD
LD
CRP (mg/L)
0
2
4
6
Pre
0h
1h
2h
4h
6h
B
Meal Consumption
HD
LD
IFN- γ (pg/mL)
0
5
10
15
20
*
a
a
a
Pre
0h
1h
2h
4h
6h
C
Meal Consumption
HD
LD
IL-1β (pg/mL)
0
2
4
6
8
10
*
b
abcd
Pre
0h
1h
2h
4h
6h
D
Meal Consumption
HD
LD
IL- 4 (pg/mL)
0
25
50
75
100
Pre
0h
1h
2h
4h
6h

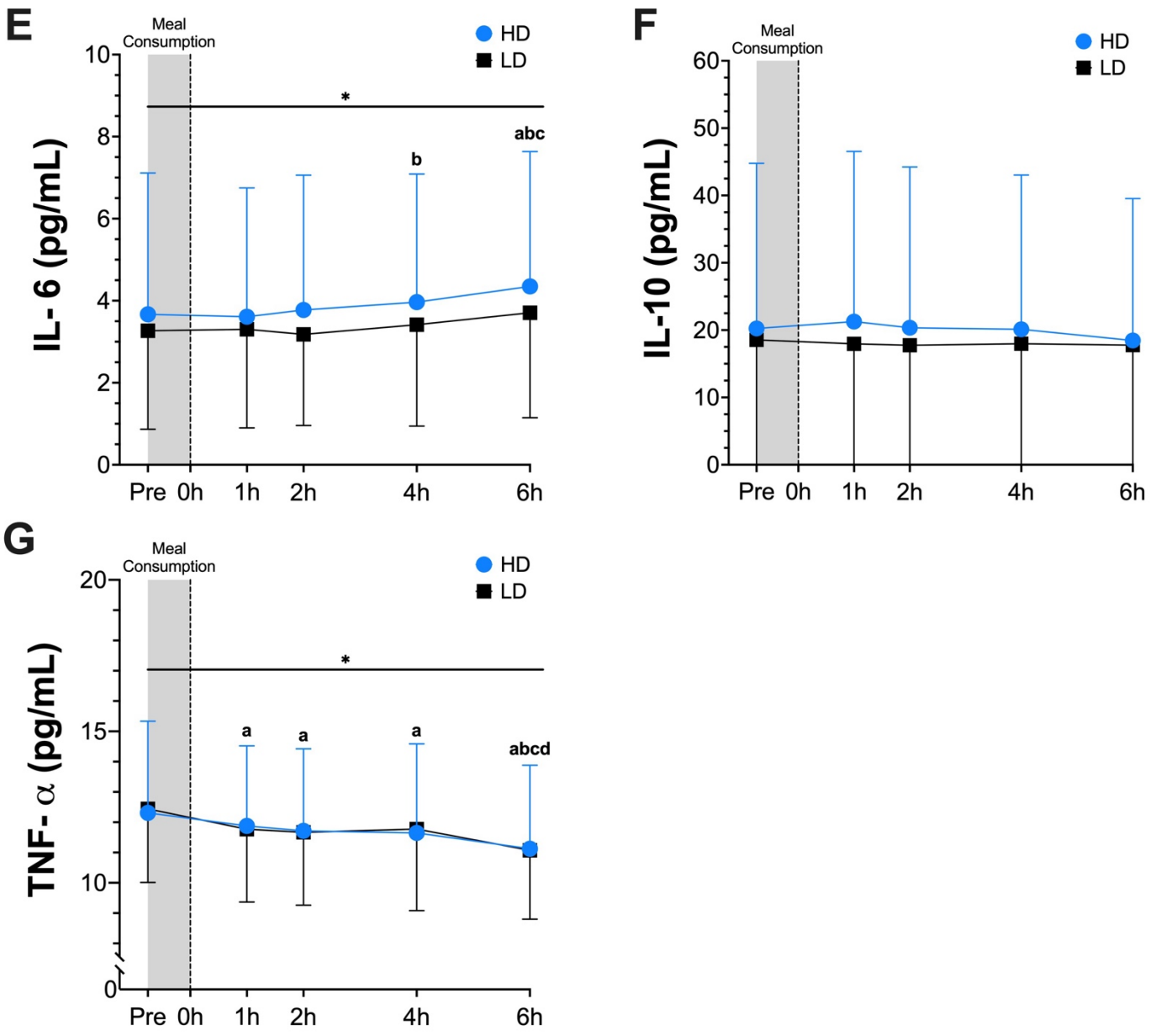
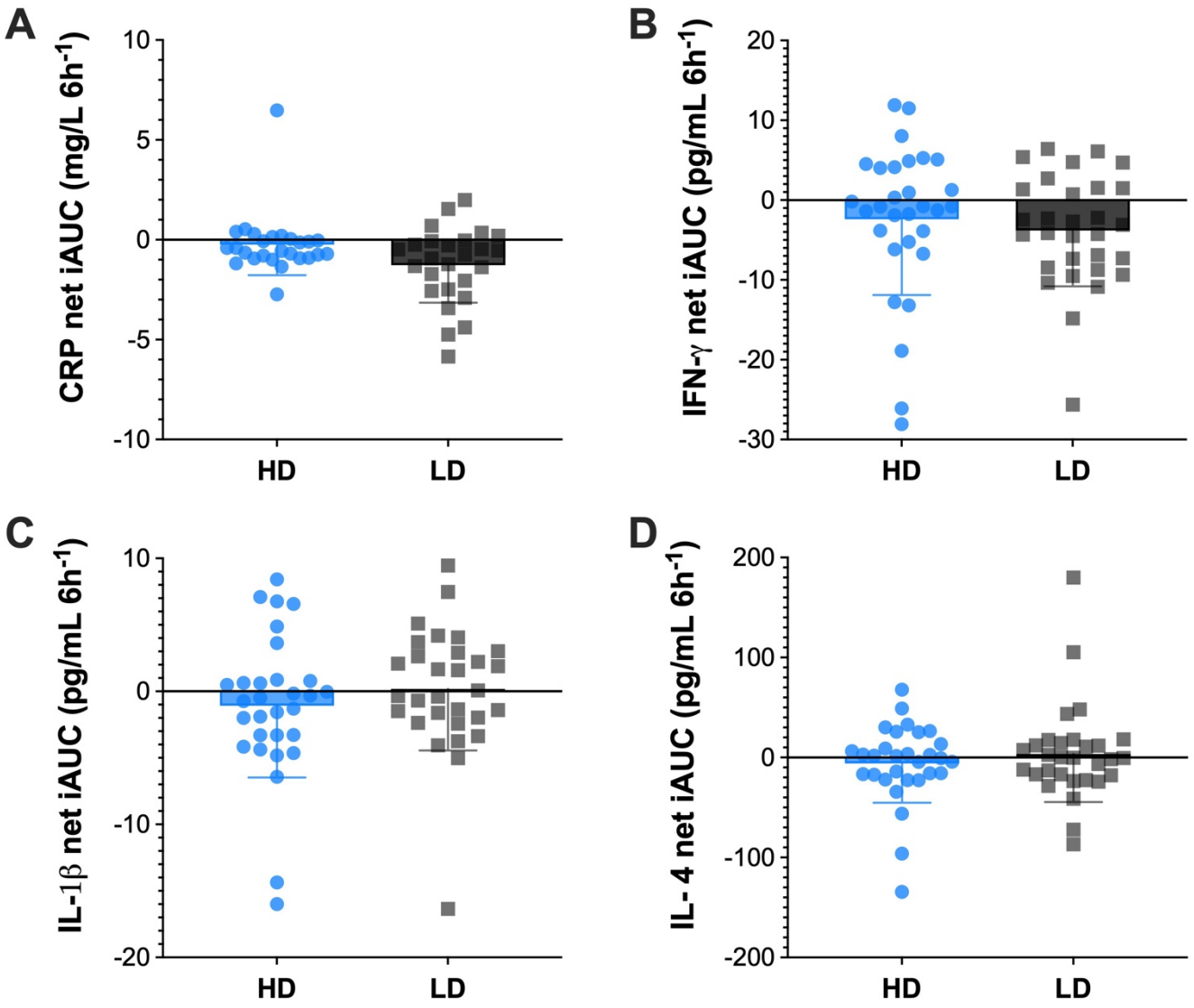



**Figure 21.** Absolute concentrations of inflammatory markers during the HFMC, taken pre-meal consumption and then again at 1h, 2h, 4h and 6h following meal completion for both the HD and LD diet arms. Data are displayed as mean ± SD. * = significant main effect of time ($p \leq 0.05$). Post-hoc analysis of time effects: **a** = significantly different from pre-meal; **b** = significantly different from 1h post; **c** = significantly different from 2h post; **d** = significantly different from 4h post. **A**: CRP = c-reactive protein (n=27); **B**: IFN-γ = interferon gamma (n=30); **C**: IL-1β = interleukin 1 beta (n=30); **D**: IL-4 = interleukin 4 (n=30); **E**: IL-6 = interleukin 6 (n=28); **F**: IL-10 = interleukin 10 (n=30); **G**: TNF-α = tumor necrosis factor alpha (n=30).

Net Incremental Area Under the Curve

The net iAUC of absolute concentration changes of each inflammatory marker were also calculated and compared between dietary conditions and are shown below (**Figure 22A – G**). There were no significant differences between conditions for CRP, IFN-γ, IL-1β, IL-4, IL-6, IL-10 or TNF-α ($p>0.05$ for all).



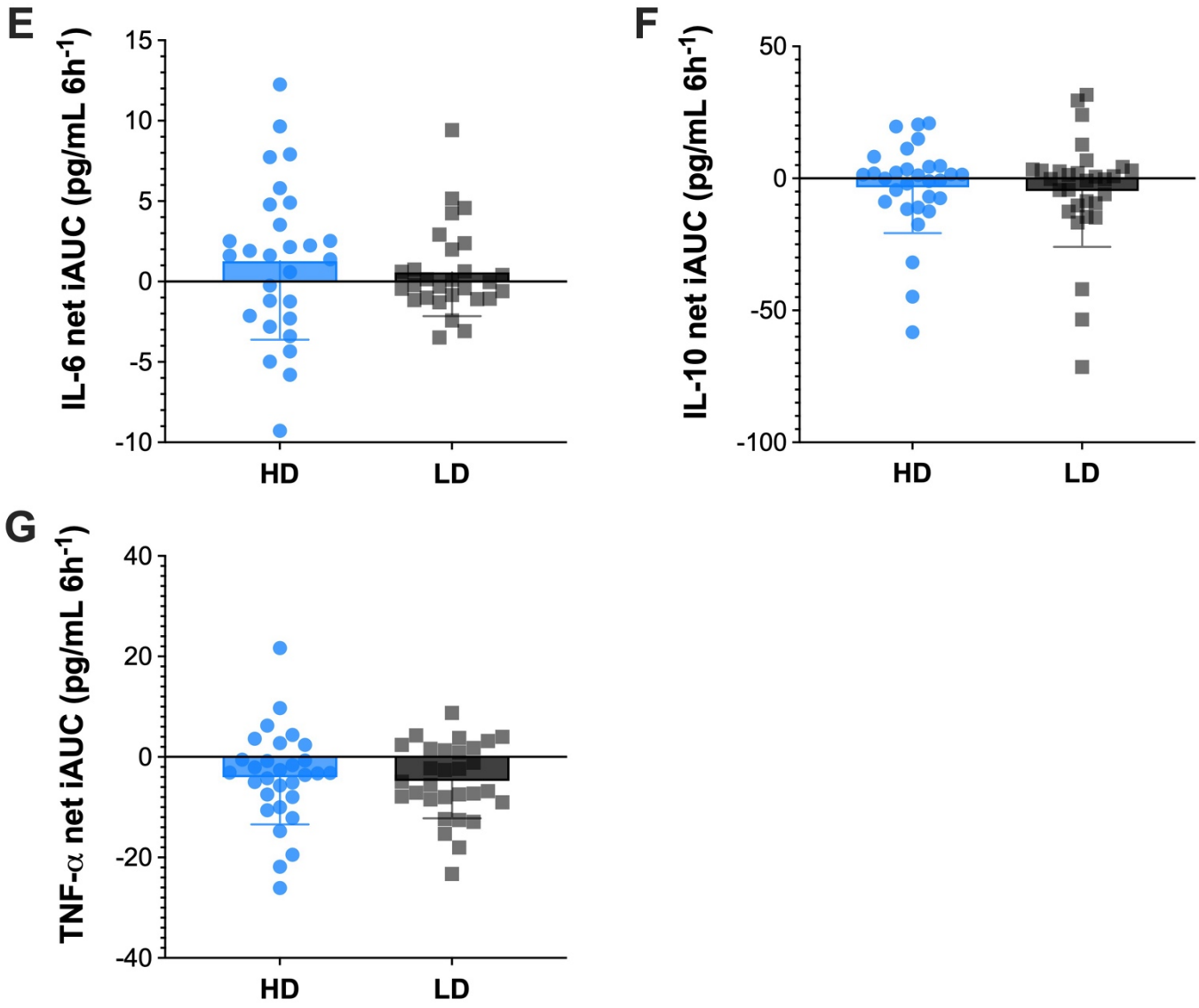



**Figure 22.** Net iAUC of inflammatory marker concentration changes across the HFMC during both the HD and LD trials. Data are displayed as mean ± SD. **A**: CRP = c-reactive protein (n=27); **B**: IFN-γ = interferon gamma (n=30); **C**: IL-1β = interleukin 1 beta (n=30); **D**: IL-4 = interleukin 4 (n=30); **E**: IL-6 = interleukin 6 (n=28); **F**: IL-10 = interleukin 10 (n=30); **G**: TNF-α = tumor necrosis factor alpha (n=30).

## Metabolic Markers

Absolute Concentrations

Absolute circulating concentrations for the metabolic biomarkers assessed in each dietary arm are displayed in **Figure 23** (also displayed in **Supplementary Table 17**).

There was a significant main effect of time for Apo-B, with post-hoc testing showing that the pre-meal concentration was higher than the 1h, 2h, 4h and 6h concentration (all $p<0.05$), and that the 6h concentration was significantly higher than the 4h concentration ($p=0.024$).

There was a significant main effect of time for glucose, with post-hoc testing showing that the 1h and 2h concentrations were significantly higher (both $p<0.0001$), and the 4h concentration was significantly lower ($p=0.004$) than pre-meal concentration, that the 1h concentration was significantly higher than the 2h ($p=0.035$), 4h and 6h concentrations (both $p<0.0001$), and the 2h concentration was significantly higher than the 4h and 6h concentrations (both $p<0.0001$).

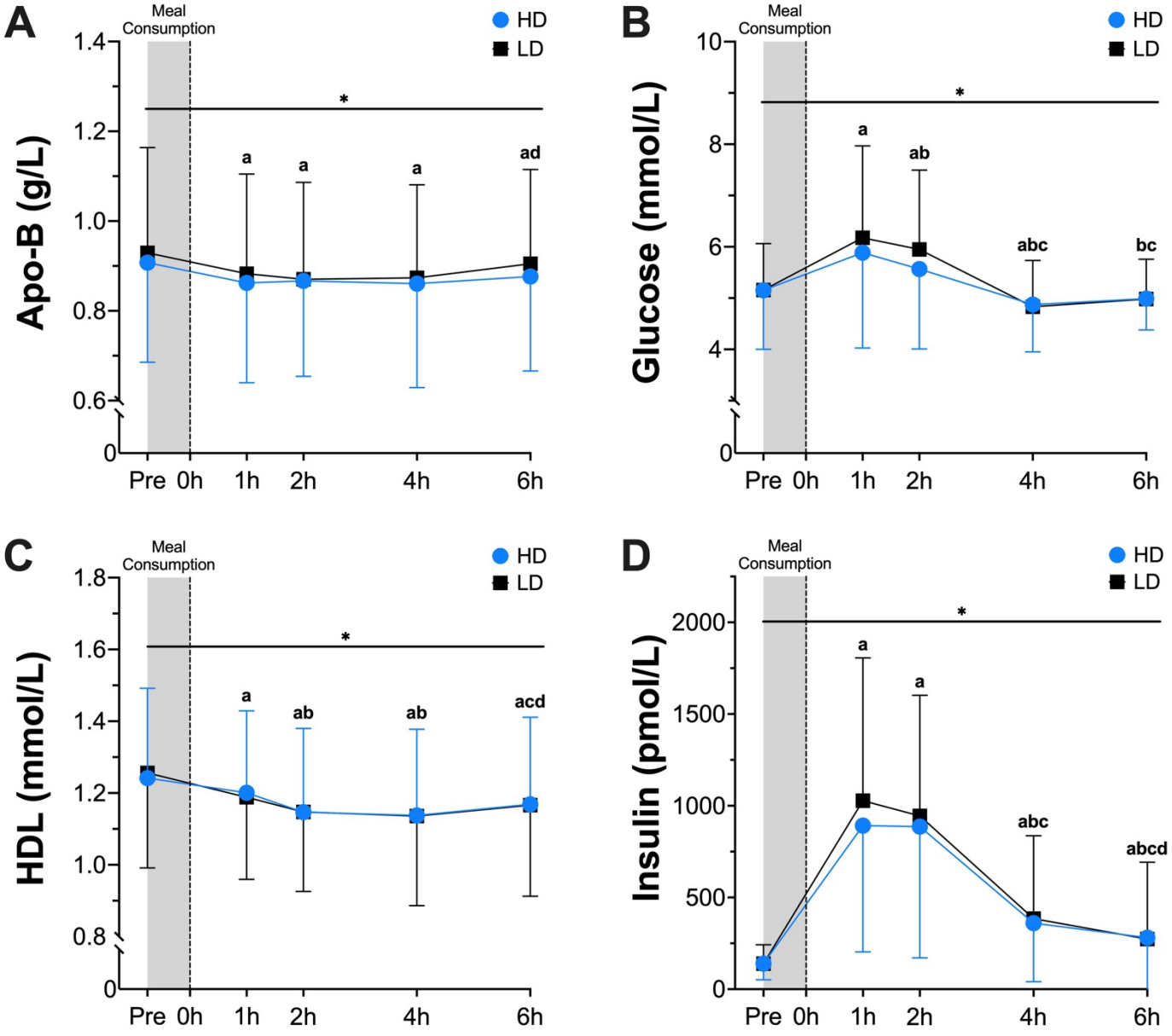
There was a significant main effect of time for HDL, with post-hoc testing showing that the pre-meal concentrations were significantly higher than the 1h, 2h, 4h and 6h concentrations (all $p<0.0001$), that the 1h concentration was significantly higher than the 2h and 4h concentrations (both $p<0.0001$) and that the 6h concentrations were significantly higher than the 2h ($p=0.015$) and 4h ($p<0.0001$) concentrations.

There was a significant main effect of time for insulin, with post-hoc testing showing that the pre-meal concentrations were significantly lower than the 1h, 2h, 4h and 6h concentrations (all $p<0.0001$), that the 1h concentration was significantly higher than the 4h and 6h concentrations (both $p<0.0001$), that the 2h concentrations were significantly higher than the 4h and 6h (both $p<0.0001$) concentrations, and that the 4h concentration was significantly higher than the 6h concentration ($p=0.018$).

There was a significant main effect of time for LDL, with post-hoc testing showing that the pre-meal concentrations were significantly higher than the 1h, 2h, 4h and 6h concentrations (all $p<0.0001$), that the 1h concentration was significantly higher than the 2h ($p<0.0001$) and 4h ($p=0.0001$) concentrations, that the 2h concentrations were significantly lower than the 4h ($p=0.007$) and 6h ($p<0.0001$) concentrations, and that the 4h concentration was significantly lower than the 6h concentration ($p=0.018$).

There was a significant main effect of time for TC, with post-hoc testing showing that the pre-meal concentrations were significantly higher than the 1h, 2h, 4h (all $p<0.0001$), and 6h ($p=0.016$) concentrations, that the 1h concentration was significantly higher than the 2h ($p=0.022$) and lower than the 6h ($p<0.0001$) concentrations, that the 2h concentrations were significantly lower than the 6h ($p<0.0001$) concentrations, and that the 4h concentration was significantly lower than the 6h concentration ($p<0.0001$).

Finally, there was a significant main effect of time for TG, with post-hoc testing showing that the pre-meal concentrations were significantly lower than at 1h, 2h, 4h and 6h (all $p<0.0001$), that the 1h concentration was significantly lower than at 2h and 4h (both $p<0.0001$), and that the 6h concentration was significantly lower than the 2h ($p=0.027$) and 4h ($p<0.0001$) concentration. There were however no significant interactions or trial effects for apo-B, glucose, HDL, insulin, LDL, TC or TG (all $p>0.05$).


A
Meal Consumption
HD
LD
Apo-B (g/L)
1.4
1.2
1.0
0.8
0.6
0
*
a
a
a
ad
Pre 0h 1h 2h 4h 6h
B
Meal Consumption
HD
LD
Glucose (mmol/L)
10
8
6
4
0
*
a
ab
abc
bc
Pre 0h 1h 2h 4h 6h
C
Meal Consumption
HD
LD
HDL (mmol/L)
1.8
1.6
1.4
1.2
1.0
0.8
0
*
a
ab
ab
acd
Pre 0h 1h 2h 4h 6h
D
Meal Consumption
HD
LD
Insulin (pmol/L)
2000
1500
1000
500
0
*
a
a
abc
abcd
Pre 0h 1h 2h 4h 6h

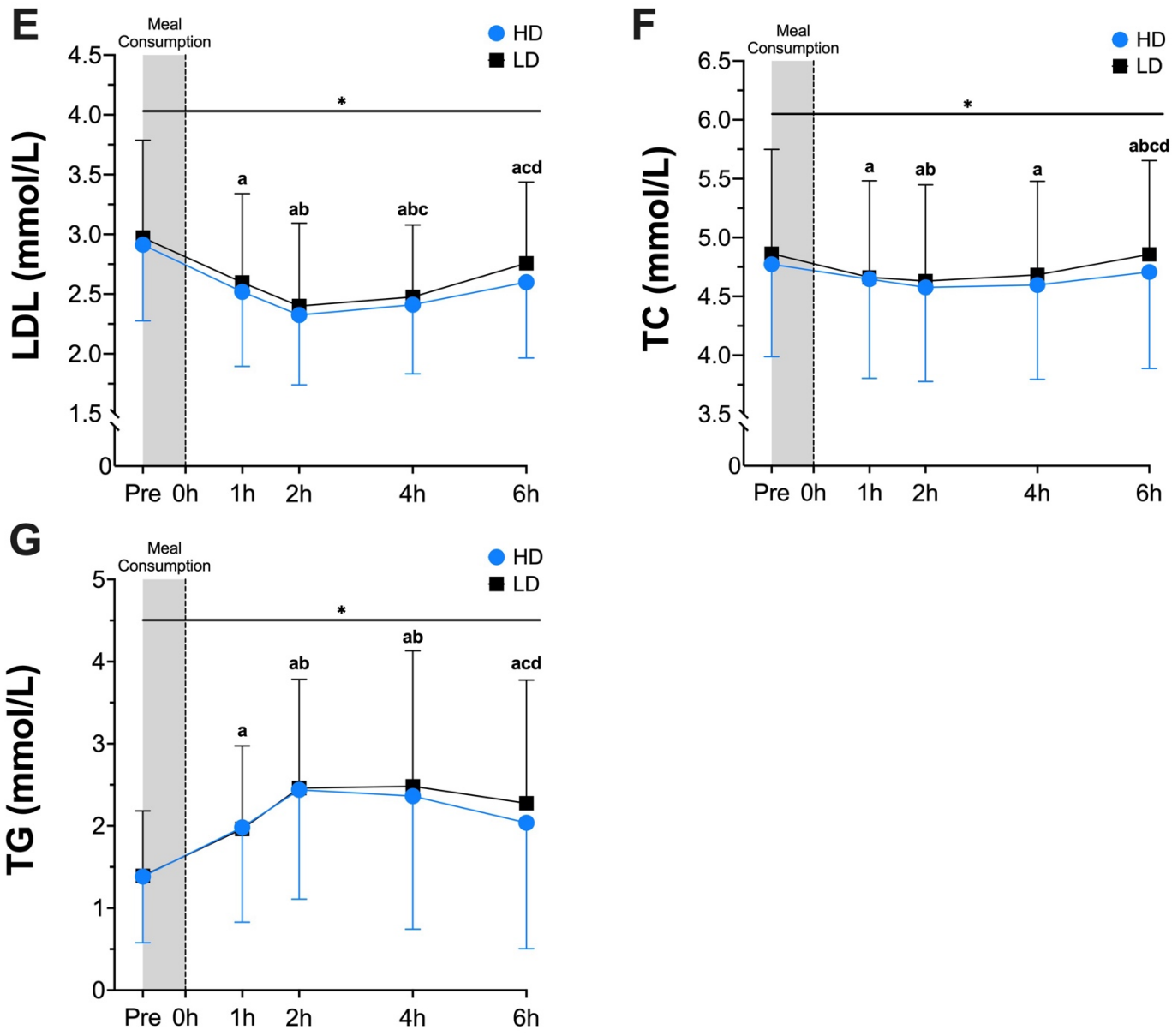
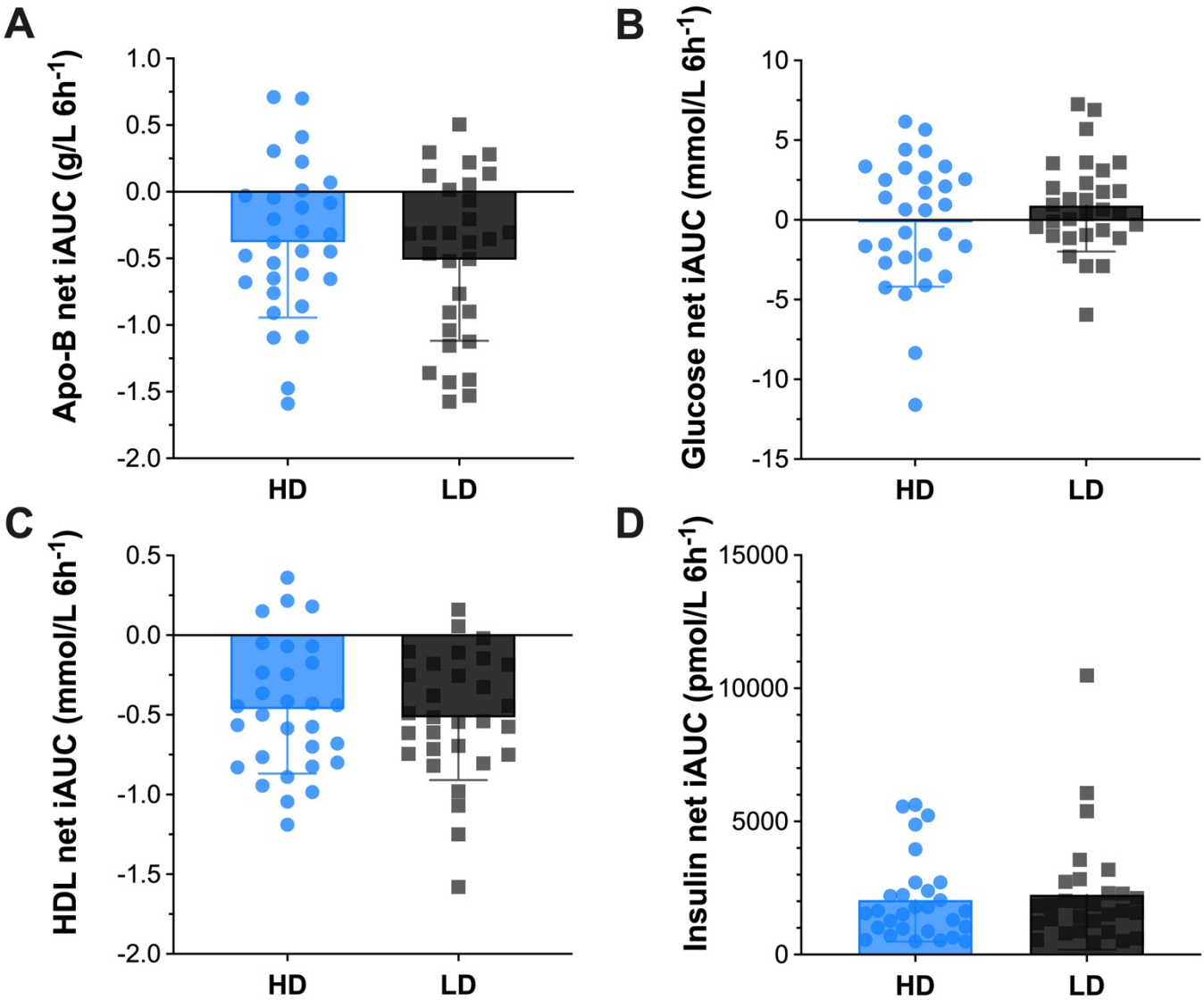



**Figure 23.** Absolute concentrations of metabolic markers measured during the HFMC, taken at pre-meal consumption and then again at 1h, 2h, 4h and 6h following completion of the meal, in both the HD and LD diet arms. Data are displayed as mean ± SD. * = significant main effect of time ($p \leq 0.05$). Post-hoc analysis of time effects: **A**: Apo-B = apolipoprotein b (n=30); **B**: blood glucose (n=30); **C**: HDL = high density lipoprotein (n=30); **D**: insulin (n=30); **E**: LDL = low density lipoprotein (n=29); **F**: TC = total cholesterol (n=30); **G**: TG = triglycerides (n=30).

Net Incremental Area Under the Curve

The net iAUC of absolute concentration changes of each metabolic marker were also calculated and compared between dietary conditions and shown below (**Figure 24A – G**). There were no significant differences between conditions for Apo-B, Glucose, HDL, Insulin, LDL, TC or TG ($p>0.05$ for all).



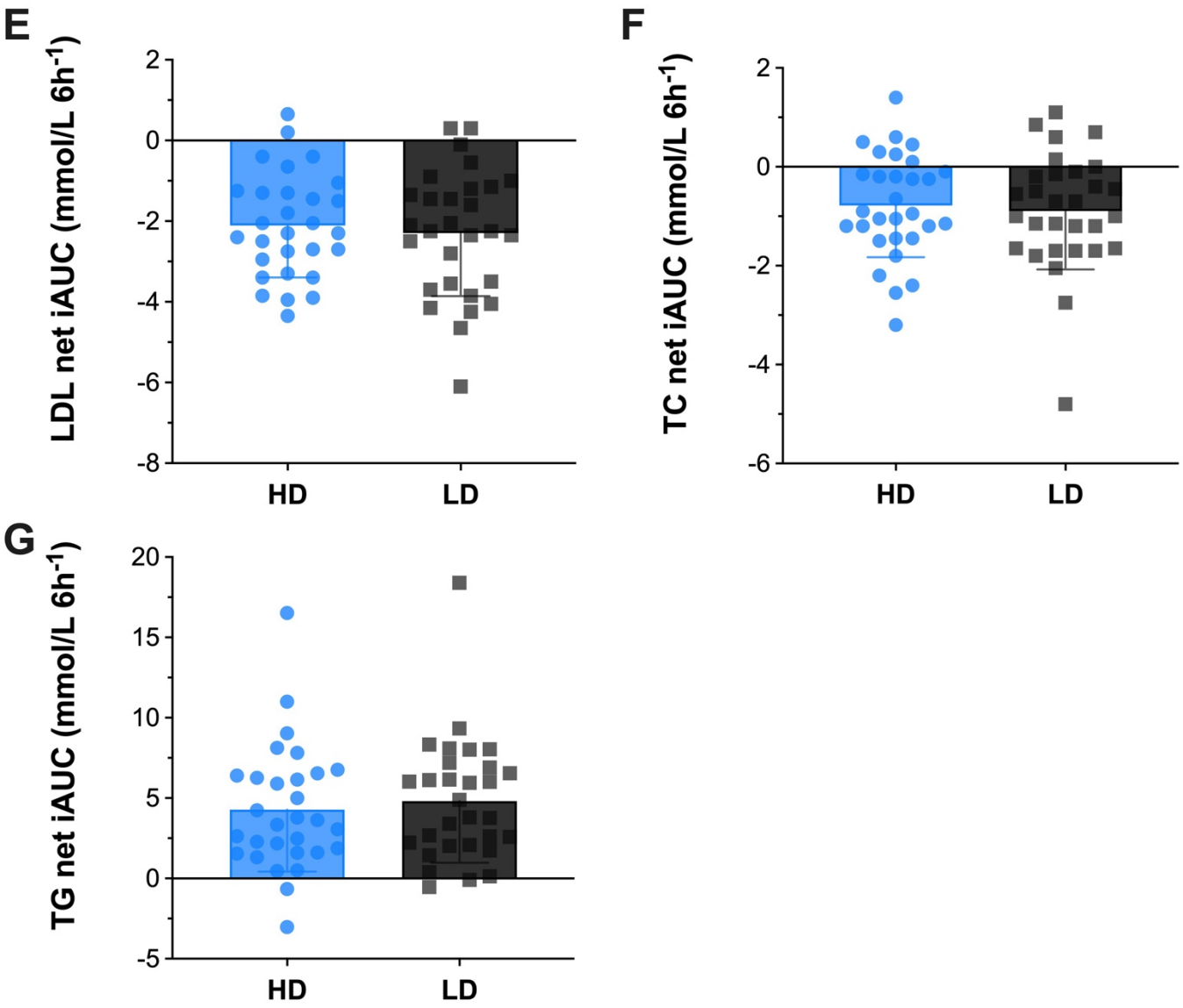



**Figure 24.** Net iAUC of metabolic biomarker concentration changes across the HFMC during both the HD and LD trials. Data are displayed as mean ± SD. **A**: Apo-B = apolipoprotein b (n=30); **B**: blood glucose (n=30); **C**: HDL = high density lipoprotein (n=30); **D**: insulin (n=30); **E**: LDL = low density lipoprotein (n=29); **F**: TC = total cholesterol (n=30); **G**: TG = triglycerides (n=30).

## Glutathione Markers

Absolute Concentrations

Absolute circulating blood concentrations of GSH, GSSG, tGS and GSH:GSSG ratio are displayed in **Figure 25 (**also displayed in **Supplementary Table 18**).

There was a significant main effect of time for blood tGS (p=0.021), with post-hoc testing showing that the 2h (p=0.040) and 4h (p=0.037) concentrations were significantly lower (both p<0.0001) than pre-meal, that 2h (p=0.043) and 4h (p=0.015) were significantly lower than 1h, and that 2h (p=0.047) and 4h (p=0.005) were significantly higher than 6h. There were no other significant interactions or main effects for the other blood glutathione markers (p>0.05 for all).

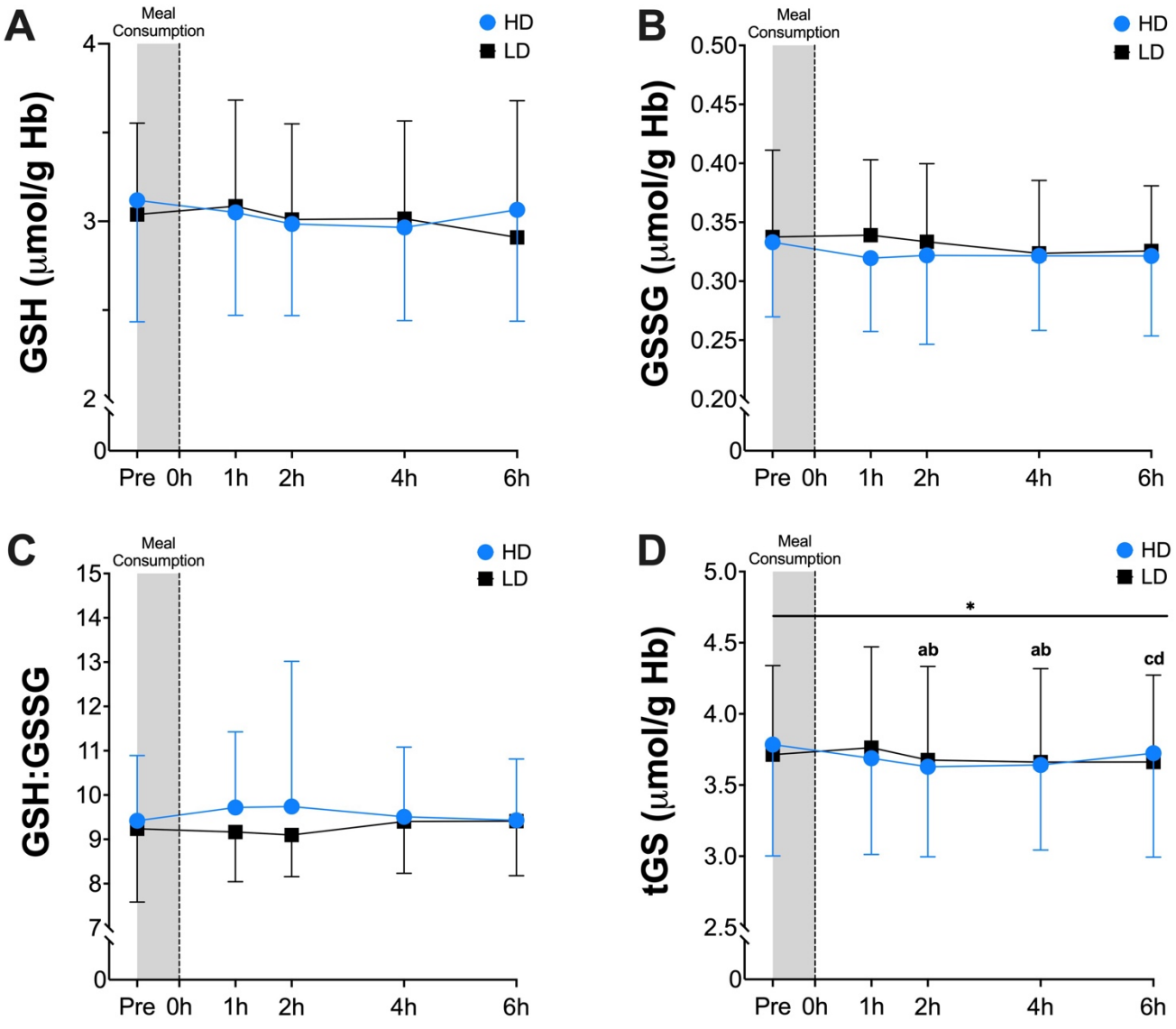


**Figure 25.** Absolute concentrations of glutathione markers during the HFMC, taken for whole blood at pre-meal consumption and then again at 1h, 2h, 4h and 6h following completion of the meal, in both the HD and LD diet arms. Data are displayed as mean ± SD. * = significant main effect of time ($p \leq 0.05$). Post-hoc analysis of time effects: **a** = significantly different from pre-meal; **b** = significantly different from 1h post; **c** = significantly different from 2h post; **d** = significantly different from 4h post. **A**: GSH = reduced glutathione (n=30); **B**: GSSG = oxidized glutathione (n=30); **C**: tGS = total glutathione (n=30); **D**: GSH:GSSG = reduced to oxidized glutathione ratio (n=30).

Absolute vastus lateralis muscle concentrations of GSH, GSSG, tGS and GSH:GSSG ratio are displayed in **Table 14**. There were no significant interactions or main effects for the muscle glutathione markers ($p > 0.05$ for all).

**Table 14.** Glutathione markers within the vastus lateralis muscle, measured pre-meal and 4h post-meal consumption in both the HD and LD dietary conditions.

| | | Pre-Meal | 4h Post | Time | Trial | Interaction |
|---|---|---|---|---|---|---|
| Muscle GSH (µmol/g protein) | HD | 14.7 ± 5.5 | 16.0 ± 5.1 | 0.750 | 0.487 | 0.571 |
| | LD | 16.8 ± 5.2 | 16.4 ± 6.4 | | | |
| Muscle GSSG (µmol/g protein) | HD | 1.11 ± 0.61 | 0.96 ± 0.46 | 0.579 | 0.302 | 0.505 |
| | LD | 1.18 ± 0.53 | 1.18 ± 0.48 | | | |
| Muscle GSH:GSSG | HD | 15.14 ± 6.74 | 19.23 ± 8.68 | 0.389 | 0.147 | 0.260 |
| | LD | 15.50 ± 4.20 | 14.32 ± 3.56 | | | |
| Muscle tGS (µmol/g protein) | HD | 16.90 ± 6.50 | 17.91 ± 5.70 | 0.862 | 0.453 | 0.664 |
| | LD | 19.19 ± 6.13 | 18.72 ± 7.17 | | | |

Data displayed as mean ± SD, n=9 for muscle measures. Significant differences ($p \leq 0.05$) bolded. Abbreviations: HD = high dairy; LD = low dairy; GSH = reduced glutathione; GSSG = oxidized glutathione; tGS = total glutathione; GSH:GSSG = reduced to oxidized glutathione ratio.

Net Incremental Area Under the Curve

The net iAUC of absolute concentration changes of whole blood glutathione markers were also calculated and compared between dietary conditions and are shown below (**Figure 26A – D**).

There were no significant differences between conditions for blood GSH, GSSG, tGS or GSH:GSSG ($p>0.05$ for all).

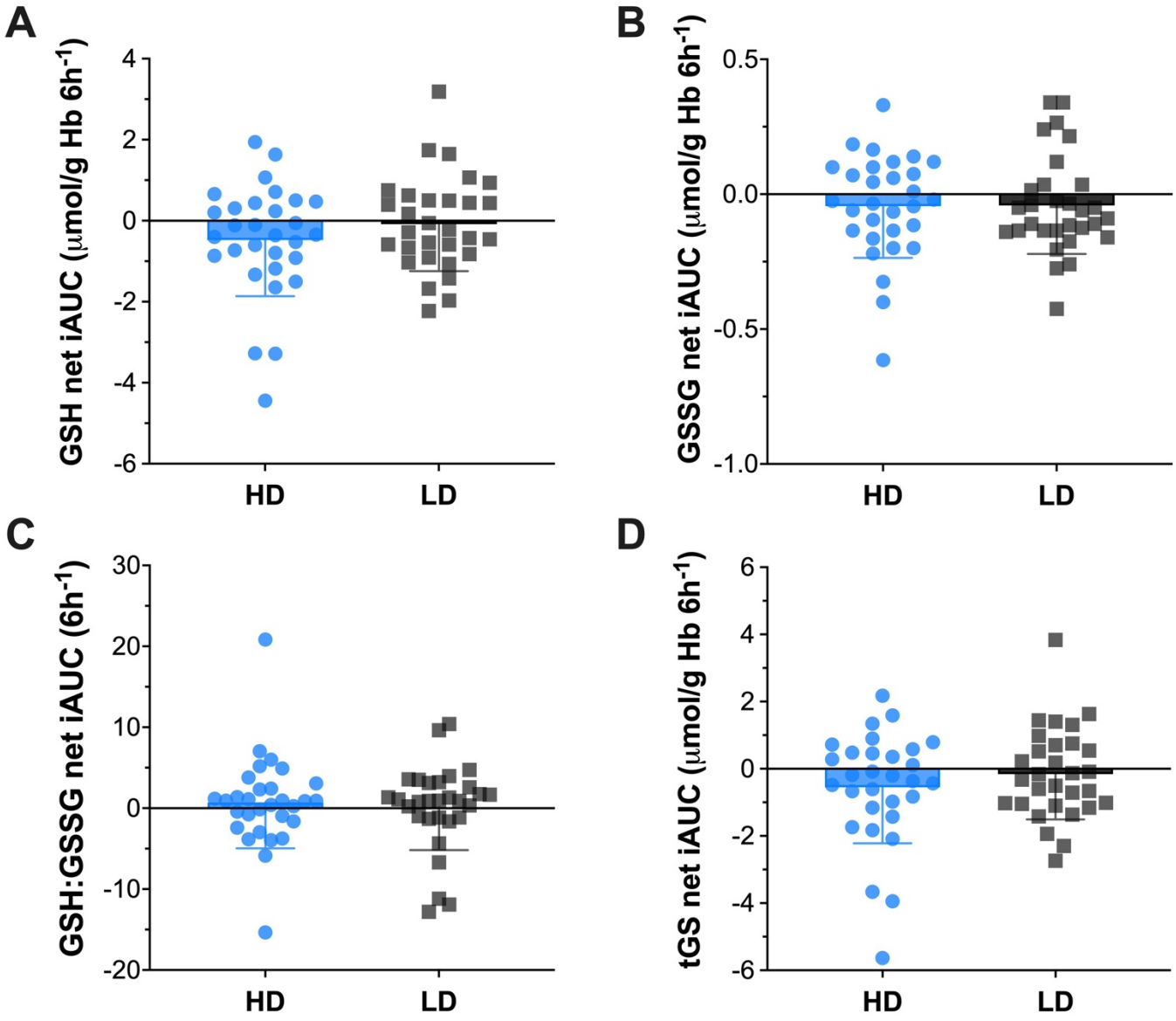



**Figure 26.** Net iAUC of glutathione biomarker concentration changes across the HFMC during both the HD and LD trials. Data are displayed as mean ± SD. **8A**: GSH = reduced glutathione (n=30); **8B**: GSSG = oxidized glutathione (n=30); **8C**: tGS = total glutathione (n=30); **8D**: GSH:GSSG = reduced to oxidized glutathione ratio (n=30).

**Vascular Measures**

Vascular measurement results are displayed in **Table 15**. There were significant main time effects for diastolic BP and max shear which both significantly decreased 4h postprandially (both $p<0.05$) and systolic BP which increased 4h postprandially ($p=0.006$). There were no significant interactions or main effects for carotid IMT and FMD% (both $p>0.3$).

**Table 15.** Vascular measurements taken pre-meal and 4h completion of the HFMC for both the HD and LD trial conditions.

| | | Pre-Meal | 4h Post | Time | Trial | Interaction |
|---|---|---|---|---|---|---|
| BP - Systolic (mmHg) | HD | 119 ± 10 | 122 ± 11 | **0.006** | 0.482 | 0.795 |
| | LD | 119 ± 12 | 124 ± 15 | | | |
| BP - Diastolic (mmHg) | HD | 75 ± 9 | 73 ± 8 | **0.046** | 0.734 | 0.949 |
| | LD | 75 ± 10 | 73 ± 9 | | | |
| Carotid IMT (cm) | HD | 0.050 ± 0.006 | 0.050 ± 0.007 | 0.929 | 0.211 | 0.806 |
| | LD | 0.052 ± 0.011 | 0.052 ± 0.010 | | | |
| FMD (%) | HD | 6.55 ± 4.37 | 6.33 ± 3.74 | 0.311 | 0.689 | 0.445 |
| | LD | 6.26 ± 3.83 | 6.91 ± 4.24 | | | |
| Max Shear | HD | 1301 ± 423 | 1062 ± 320 | **0.001** | 0.282 | 0.627 |
| | LD | 1402 ± 480 | 1180 ± 421 | | | |

Data are displayed as mean ± SD, n=30. Significant differences ($p\leq0.05$) are bolded. Abbreviations: HD = high dairy; LD = low dairy; BP = blood pressure; IMT = intima-media thickness; FMD = flow mediated dilation.

## 6.5 Discussion

This novel human study examined the effect of increased habitual dairy consumption on postprandial metabolic, inflammatory and glutathione responses of consuming a high-fat meal. Within the present study, the HFMC resulted in postprandial changes in some metabolic, inflammatory, glutathione and vascular measures over the 6h period following consumption of the HFMC meal but increasing habitual dairy consumption to 3 servings per day for the 6-weeks prior to the meal challenge did not modulate these responses at any individual timepoint, or across the postprandial period as a whole. These findings show that increasing mixed dairy consumption within the diet for a short-term period (6-weeks) provides neither beneficial nor detrimental effects on postprandial inflammation or metabolism in individuals with OW/OB and cardiometabolic dysfunction.

Assessing postprandial inflammatory responses is important considering that people in industrialised countries can spend over 16h per day in a postprandial fed state while awake [316, 327, 470]. Following the consumption of a high-fat meal, there can be a transient metabolic and inflammatory response [303, 304], which is typically greater in severity for individuals with OW/OB [305, 306]. Vascular damage can occur due to these postprandial perturbations, which has been implicated as a causal process in the development of CVD and other disease states [314-316]. Incorporation of dairy foods within the diet have been associated with both reductions in metabolic disease risk [9, 10, 29] and inflammation [5, 200, 323], but it is not yet well understood how habitual consumption of these foods may affect postprandial inflammatory and metabolic responses. We observed that IL-6 significantly increased following consumption of a high-fat meal (peaking at 6h post), but the responses were similar between the HD and LD diets. This was

reflected in the majority of the other metabolic, inflammatory, glutathione and vascular measures assessed, most of which changed over time but did not differ between dietary conditions.

In a similar study to ours, research by Pei and colleagues (2018) compared the effects of dairy yogurt *vs* semi-solid soy yogurt consumption on HFMC responses in premenopausal females both with and without OB [16]. In their study, participants consumed ~330g of either dairy or soy yogurt daily for 9-weeks, and then they also consumed yogurt immediately prior to a high-fat, high-carbohydrate meal (2 sausage, egg, and cheese sandwiches and 2 hash browns; ~960kcals, ~54% from dietary fat, ~34% from carbohydrates, ~12% from protein). They found that immediate pre-meal consumption of dairy yogurt reduced postprandial IL-6 concentrations compared to the soy yogurt in the females with OB only, possibly by mediating hyperglycemia and subsequent oxidative stress [16, 328]. In contrast, while we did observe the expected postprandial increase in IL-6, there were no significant differences in the response between the HD and LD diets. It seems likely that this is due to differences between our study designs. For example, the pre-meal yogurt that Pei's group provided may have acutely altered intestinal lipid absorption *via* formation of calcium soaps [165], altered the rate of gastric emptying which affects the magnitude and timing of postprandial glucose and insulin responses [172, 329], or through some other unknown form of glycemic modulation [16]. This is supported by findings from Mortensen et al. (2009) and Akhavan et al. (2010) who found that postprandial glucose responses were lower following consumption of whey protein immediately prior to the consumption of a high-fat meal [171, 172]. Unfortunately, neither of these two studies evaluated inflammatory responses, so we cannot determine whether alterations in these metabolic postprandial responses also relate to postprandial inflammation. Additional insight however can also be gained from studies that compared responses to high-fat meals either with or without dairy components.

Demmer and colleagues (2016) compared postprandial inflammatory and metabolic responses to a HFMC composed of palm oil with and without an added dairy fraction rich in milk fat globule membrane (MFGM) in individuals with OW/OB [17]. They found the addition of MFGM improved postprandial metabolic (reduced TC, LDL & insulin) responses, decreased soluble intracellular adhesion molecule (inflammatory marker) and increased the anti-inflammatory cytokine IL-10 [17]. In contrast, Rogers et al. (2017), found that meals containing MFGM did not elicit different postprandial inflammatory marker responses [330]. Schmid and colleagues (2015) evaluated the postprandial metabolic and inflammatory responses to high-fat meals with and without the inclusion of a mixture of dairy products (full-fat milk, cheese and butter) in healthy males and found no differences between the responses to these meals despite the dairy-containing meal having 30% greater calories [169]. Although there is some evidence that acute dairy intake either pre-meal or as part of a high-fat meal can modulate inflammation *via* changes in glucose/lipid absorption, these findings have not always been consistent. We did not observe any differences between the glucose, insulin or lipid responses between the HD and LD diets, which suggests that the 6-weeks of increased dairy consumption alone had little effect on postprandial digestion/absorption kinetics, which likely explains the lack of differences observed in the acute inflammatory response.

Another possible explanation for the differences between our findings and those within the literature may have been due to differences in the type of dairy provided, differences in the control/comparison group used in the study design, or differences in the population assessed (e.g. normal weight *vs.* OW/OB, mixture of sexes *vs.* female only) [471]. Pei and colleagues (2018) provided participants exclusively with yogurt compared to the variety of dairy products provided (2% milk, low-fat skyr yogurt, and full-fat cheddar cheese) in the current study, and compared that

to a soy yogurt instead of a habitual LD diet control [16]. In a different study carried out by Burton and colleagues (2017), it was found that 2-weeks of either 400g/d of probiotic yogurt or acidified milk reduced postprandial inflammation (IL-6, TNF-α and chemokine ligand 5) and altered gut microbiota when compared to 400ml/d of milk in healthy, normal weight males [472]. Unfortunately, they did not measure any inflammatory responses in a non-dairy control or habitual diet condition, making it difficult to compare to our study; however, it is interesting that there were such distinct differences in postprandial inflammatory biomarker responses between consumption of different types of dairy products. While our participants did consume yogurt as part of the intervention, they also consumed milk and cheese, so it is possible that the inclusion of these foods somehow attenuated the anti-inflammatory effects of yogurt consumption alone, perhaps due to differences in lipid composition/content, differences in bacterial cultures present or other physiochemical matrix influences on digestive properties. It is also possible that some of our participants may have had sub-clinical lactose intolerance. While we excluded participants with self-reported lactose intolerance, it is possible that some individuals had it without knowing, and that consumption of the milk (higher in lactose than yogurt [473]) may have altered inflammatory responses in these individuals.

Other acute inflammatory biomarker responses to food consumption have not always been consistent within the literature, with only IL-6 displaying consistent elevations following a HFMC [311, 474]. Other biomarkers such as TNF-α, CRP, and interleukins may either not respond acutely, or exhibit delayed responses which may not captured during a 6h sampling period [311, 474]. A review by Herieka & Erridge (2013) noted that plasma-based markers of postprandial inflammation are not always consistent and suggest that instead leukocyte-bound markers of inflammation may be a more reliable way to evaluate postprandial inflammatory responses [471].

Indeed, the other inflammatory biomarkers we assessed (TNF-α, IL-1β, IFN-γ) decreased over the course of the postprandial period. This was unexpected but has been observed previously following a HFMC [471, 475-477]. Furthermore, while insulin, glucose and TGs acutely increased across the postprandial period in our study, TC, LDL, HDL and Apo-b decreased. This has also been previously demonstrated following HFMC in other investigations [171, 311, 478]. During the HFMC, we did not control fluid intake, and the sodium content of the fast-food meal was high (~1600mg in 1000kcal meal), so it is possible that there were plasma volume increases over the postprandial period [479, 480]. These shifts may have contributed toward the reductions in some of the lipid and inflammatory blood marker concentrations that we observed. Utilising the same composition of high-fat meal and comparing results with studies performed in the same population is therefore important in order to be able to appropriately interpret the findings of HFMC studies.

We collected muscle biopsies within a subset of participants to evaluate changes in the antioxidant marker glutathione within both the circulation and muscle tissue, providing greater insight into how these acute postprandial responses may be affecting other physiological components. Reductions in skeletal muscle glutathione following a HFMC have been observed in both rats and humans [313, 481]. Anderson and colleagues (2009) found that individuals with BMI $\geq$40 kg/m$^2$ have lower skeletal muscle GSH and GSH:GSSG ratios in general, and that GSH:GSSG was reduced within the muscle in as little as 4h following the consumption of a high-fat meal in lean individuals [313]. Furthermore, Perez-Martinez et al. (2010) found that 12-weeks of a diet higher in MUFAs (concentrations of which are relatively high within dairy [40]) was able to enhance postprandial GSH and GSH:GSSG [482]. We did observe a reduction in tGS within the blood over the postprandial period, however, we did not see any difference in postprandial GSH:GSSG, no difference between the two dietary conditions, and no differences within any

glutathione related markers measured within the muscle. Anderson et al. (2009) only reported HFMC results in lean individuals, so differences in level of chronic oxidative stress/physiological dysfunction may explain why they observed a change within muscle, and we did not.

Six weeks of a HD diet did not significantly influence the FMD% response to a HFMC. In a meta-analysis of postprandial FMD responses, Fewkes and colleagues (2022) observed that adults >30 years old and those with greater cardiovascular risk had significantly smaller decrements in postprandial FMD after a HFMC suggesting that their vasculature becomes less responsive to acute fat intake potentially due to poorer endothelial health [483]. Therefore, it is possible that due to age and the presence of cardiometabolic risk factors, we were unable to detect the expected reductions in FMD% after the HFMC in our smaller cohort. Alternatively, it may have been some characteristic of the high fat meal or study design that affected our FMD results. Fewkes et al. (2022) identified that after controlling for factors such as age, BMI and health status, the fat content of a HFMC was only associated with FMD at 3h post consumption [483]. In addition, they recommend that a HFMC should provide 60% of energy from dietary fat to induce the most consistent FMD response. We evaluated postprandial FMD at 4h post-meal consumption, so may have missed the peak FMD change due to our sampling time. Furthermore, we provided 50% of TDEE from dietary fat in our meal, which perhaps reduced the magnitude of effect on the endothelium compared to a meal with a higher fat percentage, which may also contribute to the lack of postprandial differences observed. Sodium intake and/or fluid balance may also have affected vascular responses to the high-fat meal, with previous studies showing that high-salt meal consumption can suppress postprandial FMD and increase postprandial arterial stiffness [484, 485]. Nonetheless, we did observe a significant reduction in maximum shear stress and DBP with a significant increase in SBP at the 4h timepoint. These results suggest a greater pulse pressure

and potentially greater cardiac stroke volume and cardiac output after the HFMC. In light of no change in mean arterial pressure and greater cardiac output, we suggest a decrease in total peripheral resistance after the HFMC. Indeed, brachial artery diameter did increase after 4hrs of the HFMC. This increase in baseline diameter could have contributed to the observed reduction in maximal shear stress. In turn, this lower shear stress stimuli would lead to the expected reduction of FMD; however, this was not noted in the current study. Beyond the aforementioned limitations, it is important to also note that we did not control for hydration status or plasma volume.

Our study had several strengths and weaknesses which should be considered. Strengths include using a randomized crossover design to improve power and reduce the influence of interindividual variability on diets. We also used a realistic, ecologically valid intervention by providing different forms of wholefood dairy and recruited individuals likely to be at higher risk of developing cardiometabolic disease. Our participants had an average BMI of 32 $kg/m^2$ and a CRP level of 3.24 mg/mL (averaged across all measurement points), indicating that our population, on average, had OB and were in a state of LGCI (CRP of 3-10 mg/mL is considered high when used for clinical assessments [289, 486, 487]). We are also confident that participants maintained a stable lifestyle and consistently consumed the dairy products provided over the 6-week dietary period, as evidenced by the lack of differences between body mass or body fat percentage measures across timepoints, as well as the food records and logs provided by participants which indicated differences in some nutrients that are high in dairy products, namely, protein, saturated fat, calcium, Vitamin A, Vitamin D. Limitations of this study include significant heterogeneity, and possible lack of magnitude in cardiometabolic dysfunction within our participant pool. While we measured a number of cardiometabolic outcomes during screening, some participants were barely above the threshold for inclusion. Nine of our participants were aged 18-21 years old, so despite

being eligible, their younger age may have resulted in their cardiometabolic dysfunction and/or basal inflammation being lower than expected (causing a floor effect for inflammatory changes). Another limitation is that a subset of our participants (n=9) underwent a muscle biopsy during both of the HFMCs. While a sensitivity analysis did not determine any statistical differences, and the crossover design controlled for it within participants, it is possible that it may have altered physiological responses compared to those who did not receive a biopsy. We also did not control fluid intake or adjust for plasma volume shifts following consumption of the meal, which may have been altered due to the high-sodium intake of the meal provided. Finally, due to dietary restrictions, a third of our participants consumed a high-fat meal that did not contain meat. While we kept the total calories and percentage of calories from fat the same between the meal types, it is possible that the composition (e.g. saturated fat content, fatty acid type) may have affected the results of our study.

## 6.6 Conclusion

In conclusion, increased mixed dairy intake for 6-weeks did not improve postprandial inflammatory, metabolic, glutathione, or vascular measurements after consuming a high-fat meal. Literature shows that habitual incorporation of dairy products into one's diet may improve resting markers of LGCI [5, 200, 323], however, it does not appear to affect the postprandial response to a high-fat meal. Indeed, immediate, pre-meal dairy consumption may be required to attenuate postprandial oxidative stress and/or inflammatory responses to the meal, possibly *via* acute changes in glucose/lipid kinetics [16]. However, this may be an impractical feeding approach, hence why we sought to assess habitual intakes on postprandial responses. Future studies should continue to investigate the mechanisms through which dairy intake may modulate inflammation

and attempt to determine the time interval required between pre-meal dairy consumption and attenuated postprandial responses.

## 6.7 Supplementary Data Tables

**Supplementary Table 16.** Absolute concentrations of inflammatory markers measured during the HFMC, taken at pre-meal consumption and then again at 1h, 2h, 4h and 6h following completion of the meal, in both the HD and LD diet arms.

| | | Pre-Meal | 1h Post | 2h Post | 4h Post | 6h Post | Time | Trial | Interaction |
|---|---|---|---|---|---|---|---|---|---|
| CRP (mg/L) | HD | 3.25 ± 1.81 | 3.12 ± 1.65 | 3.21 ± 1.71 | 3.10 ± 1.65 | 3.18 ± 1.70 | 0.083 | 0.611 | 0.162 |
| | LD | 3.57 ± 1.92 | 3.35 ± 1.85 | 3.33 ± 1.81 | 3.33 ± 1.78 | 3.11 ± 1.51 | | | |
| IFN-γ (pg/mL) | HD | 9.71 ± 4.63 | **9.30 ± 4.22** | **9.34 ± 4.30** | 9.26 ± 4.68 | **8.72 ± 4.25** | **0.028** | 0.732 | 0.496 |
| | LD | 10.01 ± 4.78 | **9.26 ± 4.46 ^a** | **9.30 ± 4.36 ^a** | 9.48 ± 4.73 | **9.22 ± 3.82 ^a** | | | |
| IL-1β (pg/mL) | HD | 5.90 ± 2.61 | 6.17 ± 2 .80 | **5.64 ± 2.38** | 5.73 ± 2.28 | **5.27 ± 2.22** | **0.024** | 0.935 | 0.393 |
| | LD | 5.72 ± 2.47 | 5.78 ± 2.48 | **5.82 ± 2.51 ^b** | 5.89 ± 2.66 | **5.12 ± 2.22 ^abcd** | | | |
| IL-4 (pg/mL) | HD | 54.63 ± 44.06 | 54.84 ± 41.85 | 54.71 ± 43.74 | 52.23 ± 38.70 | 53.88 ± 46.69 | 0.369 | 0.122 | 0.361 |
| | LD | 50.22 ± 36.08 | 49.33 ± 36.89 | 50.64 ± 39.61 | 50.71 ± 41.19 | 52.94 ± 42.08 | | | |
| IL-6 (pg/mL) | HD | 3.67 ± 3.44 | 3.61 ± 3.14 | 3.78 ± 3.29 | **3.97 ± 3.12** | **4.35 ± 3.29** | **0.012** | 0.535 | 0.221 |
| | LD | 3.27 ± 2.40 | 3.30 ± 2.40 | 3.18 ± 2.22 | **3.41 ± 2.47 ^b** | **3.71 ± 2.56 ^abc** | | | |
| IL-10 (pg/mL) | HD | 20.25 ± 24.52 | 21.27 ± 25.27 | 20.35 ± 23.89 | 20.11 ± 22.94 | 18.46 ± 21.08 | 0.098 | 0.223 | 0.394 |
| | LD | 18.54 ± 21.80 | 17.97 ± 20.54 | 17.75 ± 20.38 | 17.98 ± 21.68 | 17.77 ± 22.25 | | | |
| TNF-α (pg/mL) | HD | 12.31 ± 3.03 | **11.88 ± 2.64** | **11.72 ± 2.71** | **11.65 ± 2.94** | **11.13 ± 2.75** | **<0.0001** | 0.907 | 0.947 |
| | LD | 12.44 ± 2.43 | **11.77 ± 2.41 ^a** | **11.67 ± 2.40 ^a** | **11.77 ± 2.69 ^a** | **11.07 ± 2.27 ^abcd** | | | |

Data are displayed as mean ± SD. Significant differences ($p \leq 0.05$) are bolded, n=30 for all except IL-6 which was n=28, and CRP which was n=27. Abbreviations: HD: high dairy; LD: low dairy; CRP: c reactive protein; IFN-γ: interferon gamma; IL-1β; interleukin 1 beta; IL-4: interleukin 4; IL-6: interleukin 6; IL-10: interleukin 10; TNF-α: tumor necrosis factor alpha. **a** = significantly different from pre-meal; **b** = significantly different from 1h post; **c** = significantly different from 2h post; **d** = significantly different from 4h post.

**Supplementary Table 17.** Absolute concentrations of metabolic markers measured during the HFMC, taken at pre-meal consumption and then again at 1h, 2h, 4h and 6h following completion of the meal, in both the HD and LD diet arms.

| | | Pre-Meal | 1h Post | 2h Post | 4h Post | 6h Post | Time | Trial | Interaction |
|---|---|---|---|---|---|---|---|---|---|
| Apo-B (g/L) | HD | 0.91 ± 0.22 | **0.86 ± 0.22** | **0.87 ± 0.21** | **0.86 ± 0.23** | **0.88 ± 0.21** | | | |
| | LD | 0.93 ± 0.23 | **0.88 ± 0.22 ^a** | **0.87 ± 0.22 ^a** | **0.87 ± 0.21 ^a** | **0.91 ± 0.21 ^ad** | **<0.001** | 0.615 | 0.578 |
| Glucose (mmol/L) | HD | 5.15 ± 1.15 | **5.89 ± 1.86** | **5.57 ± 1.56** | **4.87 ± 0.92** | **4.99 ± 0.61** | | | |
| | LD | 5.16 ± 0.91 | **6.18 ± 1.79 a** | **5.95 ± 1.55 ^ab** | **4.83 ± 0.90 ^abc** | **4.98 ± 0.78 ^bc** | **<0.001** | 0.190 | 0.196 |
| HDL (mmol/L) | HD | 1.24 ± 0.25 | **1.20 ± 0.23** | **1.15 ± 0.23** | **1.14 ± 0.24** | **1.17 ± 0.24** | | | |
| | LD | 1.26 ± 0.26 | **1.19 ± 0.23 ^a** | **1.15 ± 0.22 ^ab** | **1.14 ± 0.25 ^ab** | **1.17 ± 0.25 ^acd** | **<0.001** | 0.852 | 0.412 |
| Insulin (pmol/L) | HD | 139 ± 88 | **892 ± 689** | **886 ± 716** | **361 ± 320** | **280 ± 377** | | | |
| | LD | 139 ± 103 | **1,028 ± 778 ^a** | **945 ± 658 ^a** | **385 ± 453 ^abc** | **274 ± 419 ^abcd** | **<0.001** | 0.416 | 0.810 |
| LDL (mmol/L) | HD | 2.91 ± 0.64 | **2.52 ± 0.62** | **2.33 ± 0.58** | **2.41 ± 0.58** | **2.60 ± 0.63** | | | |
| | LD | 2.97 ± 0.81 | **2.60 ± 0.74 ^a** | **2.40 ± 0.69 ^ab** | **2.48 ± 0.60 ^abc** | **2.76 ± 0.68 ^acd** | **<0.001** | 0.496 | 0.841 |
| TC (mmol/L) | HD | 4.77 ± 0.79 | **4.65 ± 0.84** | **4.58 ± 0.80** | **4.60 ± 0.80** | **4.71 ± 0.82** | | | |
| | LD | 4.86 ± 0.89 | **4.66 ± 0.82 ^a** | **4.63 ± 0.82 ^ab** | **4.68 ± 0.80 ^a** | **4.86 ± 0.80 ^abcd** | **<0.001** | 0.459 | 0.224 |
| TG (mmol/L) | HD | 1.38 ± 0.81 | **1.98 ± 1.15** | **2.44 ± 1.33** | **2.36 ± 1.62** | **2.04 ± 1.53** | | | |
| | LD | 1.39 ± 0.79 | **1.96 ± 1.01 ^a** | **2.46 ± 1.33 ^ab** | **2.48 ± 1.65 ^ab** | **2.28 ± 1.50 ^acd** | **<0.001** | 0.459 | 0.224 |

Data are displayed as mean ± SD, n=30 except for LDL which was n=29. Statistically significant ($p \leq 0.05$) are bolded. Abbreviations: HD = high dairy; LD = low dairy; Apo-B = apolipoprotein B; HDL = high density lipoprotein; LDL = low density lipoprotein; TC = total cholesterol; TG = triglycerides. **a** = significantly different from pre-meal; **b** = significantly different from 1h post; **c** = significantly different from 2h post; **d** = significantly different from 4h post.

**Supplementary Table 18.** Absolute concentrations of glutathione markers during the HFMC, taken for whole blood at pre-meal consumption and then again at 1h, 2h, 4h and 6h following completion of the meal, in both the HD and LD diet arms.

| | | Pre-Meal | 1h Post | 2h Post | 4h Post | 6h Post | Time | Trial | Interaction |
|---|---|---|---|---|---|---|---|---|---|
| Blood GSH (µmol/g Hb) | HD | 3.12 ± 0.68 | 3.05 ± 0.58 | 2.98 ± 0.52 | 2.97 ± 0.53 | 3.07 ± 0.63 | 0.307 | 0.472 | 0.315 |
| | LD | 3.04 ± 0.51 | 3.08 ± 0.60 | 3.01 ± 0.54 | 3.01 ± 0.55 | 2.91 ± 0.77 | | | |
| Blood GSSG (µmol/g Hb) | HD | 0.333 ± 0.063 | 0.320 ± 0.062 | 0.322 ± 0.076 | 0.321 ± 0.063 | 0.321 ± 0.068 | 0.229 | 0.149 | 0.782 |
| | LD | 0.337 ± 0.074 | 0.339 ± 0.064 | 0.333 ± 0.066 | 0.324 ± 0.062 | 0.326 ± 0.055 | | | |
| Blood GSH:GSSG | HD | 9.42 ± 1.47 | 9.72 ± 1.71 | 9.74 ± 3.28 | 9.51 ± 1.57 | 9.43 ± 1.38 | 0.811 | 0.073 | 0.610 |
| | LD | 9.24 ± 1.65 | 9.17 ± 1.12 | 9.10 ± 0.94 | 9.40 ± 1.17 | 9.41 ± 1.23 | | | |
| Blood tGS (µmol/g Hb) | HD | 3.78 ± 0.78 | 3.69 ± 0.68 | **3.63 ± 0.63** | **3.64 ± 0.60** | **3.72 ± 0.73** | **0.021** | 0.825 | 0.464 |
| | LD | 3.71 ± 0.63 | 3.76 ± 0.71 | **3.68 ± 0.66** | **3.66 ± 0.66** | **3.66 ± 0.61** | | | |
| | | | | **^ab** | **^ab** | **^cd** | | | |

Data displayed as mean ± SD, n=30 for blood measures and n=9 for muscle measures. Significant differences ($p \leq 0.05$) bolded. Abbreviations: HD = high dairy; LD = low dairy; GSH = reduced glutathione; GSSG = oxidized glutathione; Hb = hemoglobin; tGS = total glutathione; GSH:GSSG = reduced to oxidized glutathione ratio. **a** = significantly different from pre-meal; **b** = significantly different from 1h post; **c** = significantly different from 2h post; **d** = significantly different from 4h post.

# Chapter 7: General Discussion

The overall objective of the research contained within this dissertation was to advance our understanding of the effects of dairy product consumption on bone, inflammation, metabolic and glutathione outcomes in different human populations in the context of either a high-fat meal or an exercise stressor. This research was the first to examine the effects of milk consumption on the acute bone biomarker response following high-impact, high-load exercise. It also was the first time that a short-term, ecologically valid, mixed dairy crossover nutrition intervention trial evaluated resting and postprandial inflammatory, metabolic and glutathione biomarkers in blood and in muscle tissue (for some endpoints). These well controlled human intervention trials generated novel experimental findings that will help progress our understanding of how dairy wholefoods can support improvements in physiology and health in different contexts.

To examine the effect of dairy consumption following two distinct physiological stressors (exercise and high fat meal challenge), two separate research studies were completed that were divided into four chapters. In **Study 1**, post-exercise milk or carbohydrate consumption (in random order) were provided to young female adults in a crossover design after the completion of a high-impact, high-load exercise bout. Circulating bone biomarkers were assessed in the hours and days following this stimulus and compared between nutritional interventions (**Chapter 3**). Then the responses of the bone and inflammatory markers (previously published [1, 298]) during this acute period were assessed using linear mixed modelling to determine if they were related to each other within the context of exercise and nutrition (**Chapter 4**). In partial alignment with our hypothesis, we found that consumption of milk blunted the relative post-exercise bone resorption response (CTX %change) compared to carbohydrate, but did not alter other bone turnover markers [1].

There are several possible explanations for how dairy attenuated this catabolic bone response, including provision of calcium, protein or potentially through modulation of inflammation. Exercise can decrease serum ionized calcium *via* sweat losses and/or in support of other physiological requirements such as muscle contraction [379, 488]. Considering that bone is a calcium reservoir for the body, the bone resorption observed during/after exercise is likely a mechanism through which the body maintains serum calcium [379]. Indeed, studies involving pre-exercise calcium supplementation or calcium infusion during prolonged exercise have shown that bone resorption marker responses are attenuated [152, 379, 489]. Dairy products are high in calcium, and due to their WFM, may release calcium more slowly than supplementation, providing continuous supply to the serum and thus reducing the need for it to be leeched from bone. Thus, consumption of this wholefood may be an ideal tool for preventing exercise-induced bone resorption. We did not measure serum PTH or calcium losses, however, so we cannot be certain that potential alterations in calcium homeostasis occurred within our paradigm of high-impact, high load exercise. Future studies should measure these markers in addition to bone biomarkers and should also consider comparing the acute bone response following exercise to dairy products with different digestion/absorption kinetics and physicochemical properties (e.g., milk vs yogurt). Another possible mechanism is through dairy's ability to influence inflammation [5, 200, 323, 325]. During **Study 1**, inflammatory markers were also measured from the blood at the same timepoints and compared between the milk and carbohydrate condition [298]. The main findings from that investigation were that the late stage anti-inflammatory cytokine IL-10 responses differed between the groups, suggesting that milk may have enhanced recovery from the acute exercise bout compared to carbohydrate [298]. In recent decades, the literature has established a link between the immune system and bone (termed "osteoimmunology"), with cell and animal

studies highlighting a clear ability for inflammatory mediators to influence bone metabolism/turnover [371, 490]. To better understand associations between bone and inflammatory markers within the same participants, over the same time course, and in the context of exercise and nutrition, we performed a secondary analysis between bone and inflammatory markers using linear mixed models (**Chapter 4**). We found links between several different bone and inflammatory markers which seemed to be consistent with bone remodelling cycle physiology [64] and suggest that these systems interact within this exercise and nutrition paradigm. For example, our models generally found that proinflammatory and anti-inflammatory cytokines, were positively and negatively associated with catabolic bone biomarkers, respectively. Acutely blunting catabolic bone responses to exercise may have implications for acute exercise recovery provide long term benefits if continued consistently over time. If acute inflammatory responses are also involved with mediating bone responses/adaptations to exercise, then this may also inform exercise prescription or recovery strategies. For example, excessive blunting of post-exercise inflammation may reduce skeletal muscle anabolic responses/adaptations [491], so if the inflammatory and skeletal systems are related, perhaps this would also affect bone adaptations to training. Indeed, the cellular mechanisms that regulate bone adaptations are complex, especially when considered in relation to other systems/tissues. There are already many biomarkers that can be used to gain insight into these processes, but more are discovered/developed as our collective knowledge and technology advances. Recently there has been significant interest regarding signalling molecules derived from different tissues, and investigations into how they may crosstalk and bidirectionally regulate one another [381]. These include muscle derived myokines (e.g., IL-6, Irisin, IGF-1), bone derived osteokines (e.g., OC, SOST) and adipose tissue derived adipokines (e.g., leptin, resistin, adiponectin) [381]. In **Study 1**, we measured multiple bone markers in order

to gain a broader understanding of bone responses, but measuring markers known to crosstalk with the skeleton (in addition to the inflammatory markers measured) may have offered additional insight into the interconnections between these physiological systems. Future studies should also consider assessing both acute (hours to days) and longer term (weeks to months) responses and adaptations to combined dairy and exercise to see if improvements in short term responses consistently carry over to chronic beneficial bone health adaptions. Considering the interconnectedness of many of the physiological systems discussed, they should also consider measuring biomarkers from immune, muscle or fat cells to improve our understanding of responses throughout the entire body.

In **Study 2**, adult males and females with cardiometabolic dysfunction and elevated BMI completed two 6-week long dietary interventions in a crossover design that consisted of their habitual low dairy diet (≤1 serving per day), or a diet that included 3 servings per day of a mix of different dairy products, cheese, yogurt and milk. Inflammatory, metabolic and glutathione measurements were compared between conditions both at the beginning and end of the 6-week intervention periods while fasted (**Chapter 5**), as well as in the hours following the consumption of a high-calorie, high-fat meal at the end of each 6-week intervention period (**Chapter 6**). When comparing the fasted measures, we found that some inflammatory markers were favourably modulated in the dairy condition. This included the proinflammatory TNF-α, which decreased compared to the habitual condition, and the relative changes in anti-inflammatory IL-4 and GSH which were higher in the dairy condition. These results suggest that in as little as 6-weeks of mixed dairy consumption, individuals with OW/OB and cardiometabolic dysfunction may see mild attenuation of low-grade chronic inflammation. Dairy contains a wide variety of macro- and micronutrients which may have contributed to these effects [2]. They may induce

immunomodulatory effects from provision of dairy proteins and bioactive peptides [331], dairy fats (e.g. short-chain SFA, MUFAs and natural trans-FAs like CLA), micronutrients (e.g. calcium, magnesium, Vitamins A & D) or gut microbiome modulation [2]. Due to the nature of our study design, we are unable to determine which mechanism and/or active ingredient may have driven these responses, or whether it is a combination of several factors. This is a common feature when assessing the effects of wholefoods that are made up of many active and functional ingredients. Inflammation may also be affected *via* modulation of oxidative stress through provision of vitamins/minerals with enhanced ROS scavenging potential, or by supporting antioxidant systems such as with cysteine and glutathione [6]. We saw differences in relative change of GSH in the dairy group only, providing some evidence for the hypothesis that dairy can support antioxidant systems in individuals with cardiometabolic dysfunction who may have impairments in these systems [219]. Despite beneficial results in some inflammatory markers, it is worth noting that the effect sizes observed were small to moderate, and that only two out of the seven inflammatory markers assessed were affected by dairy consumption in our study, indicating that the magnitude of these benefits were relatively small. This is in line with the broader literature, with most meta-analyses indicating that the effect of dairy on inflammation is either neutral, or mildly anti-inflammatory [5, 200, 323, 325]. In terms of practical application of this intervention, we found that participants were able to achieve good compliance with consuming the amount and variety of dairy products offered, based upon discussion with them as well as upon the differences in nutrients associated with dairy that were observed between food diaries from each condition. They were also able to maintain body mass/composition and experienced no differences in lipid/metabolic markers over the 6-week intervention period even though they increased dietary saturated fat intake (by 6.3g on average) through dairy consumption. This indicates that, despite the free-living

conditions, the dietary counselling they received on how to exchange foods within their usual diet for dairy effectively allowed them to introduce these foods into the diet without deleterious consequences. Overall, small, persistent improvements in inflammation over a period of months to years has the potential to improve health and reduce the risk and/or delay disease development/progression. So even small changes, as are characteristic in nutrition studies, are beneficial.

Finally, previous studies have shown that pre-meal dairy (and dairy components such as whey or milk fat globule membrane) consumption could attenuate postprandial inflammation as well as lipid and glycemic spikes that can occur following consumption of a high-calorie, high-fat meal [16, 17, 172]. Considering the impracticality of consuming yogurt or whey protein immediately prior to a meal, we sought to determine if habitual dairy intake alone (i.e. without a dairy pre-load directly before the meal) could benefit postprandial inflammation and metabolism as this is a more practical and feasible way of eating. We hypothesized that the chronic consumption of dairy may have been able to provide a steady state of beneficial nutrients and build up some intracellular antioxidant 'reserve' that, when needed, could act to mitigate oxidative stress and inflammation following a high fat meal. Contrary to our hypothesis, we found no significant effects of increased habitual dairy consumption on postprandial inflammatory or metabolic responses in individuals with OW/OB and cardiometabolic dysfunction. It seems that the beneficial effects of dairy consumption on postprandial responses may be due to the acute modulation of nutrient absorption/digestion, resulting in attenuated hyperglycemia and subsequent oxidative stress [492]. If this is the prevailing mechanism, increased habitual consumption of dairy was unlikely to affect digestion/absorption and thus would explain the lack of benefit on postprandial inflammation in our study. Due to budget constraints, we were not able to measure

other oxidative stress markers (aside from the antioxidant glutathione) in this study, but considering that oxidative stress may initiate activation of inflammatory cascades [493], it would have been of interest to compare postprandial differences between dietary conditions (and to see if they matched the similar inflammatory responses we observed). It would also have been interesting to measure inflammation (IL-6 or oxidative stress markers within the muscle tissue collected, or within other tissues (e.g., with an adipose tissue biopsy) in order to see how they responded, and if upregulation of inflammatory pathways within them were congruent with the circulating markers assessed. Despite previous studies showing that changes in glutathione occur at the level of the muscle within 4h of high-fat meal consumption [313], we found no changes or effect of dietary condition on the glutathione measurements that were performed on muscle tissue samples. It is worth noting that our findings may have been influenced by methodological limitations of the study. For example, a subset of our participants (n=9) had a non-meat containing high-fat meal (i.e., a vegetable protein patty instead of a pork patty), and another subset (n=9) had a muscle biopsy performed prior to, and 4h following meal consumption. Possible differences in meal nutrient composition (e.g., SFA differences) or changes in basal inflammation induced by the biopsy process may have impacted the circulating/blood postprandial changes that we measured. While we did use a crossover design to mitigate these differences within participants, the fact that almost a third of our cohort experienced differences in meal challenge conditions may have been enough to alter the mean biomarker concentrations that were collected. We also studied both males and females within this trial, and our subgroup analysis suggested that there are possible differences between the effect of dairy intake on fasting inflammation. Ultimately, attempts were made to retain ecological validity within our study design, at the expense of tighter control and lower variability in our results. Both males and females, and meat-eaters and non-meat eaters exist

in the world, so by including this heterogeneity in our study population, our results may be more representative and applicable to the real world. Despite seeing no significant differences in postprandial responses, it was important to do our study the way we did to be able to determine whether dairy's beneficial postprandial effects were due to acute or chronic consumption. Considering some of the beneficial effects seen in the literature that may relate to dairy's ability to modulate digestion/absorption kinetics, it may be of interest to investigate if dairy consumption earlier in the day influences postprandial responses later on (e.g., investigating the adequate time interval between dairy-preload and meal, i.e., would morning yogurt consumption attenuate inflammatory responses of high-fat lunch or dinner?). We measured up to 6h postprandially, but it has been suggested that measuring glucose and triglyceride responses from HFMCs of longer durations (up to 8 h) may be valuable [494]. Indeed, several of our biomarkers (e.g., triglycerides, insulin, IL-6) did not appear to have returned to baseline by the 6h timepoint.

Determining the mechanism through which dairy exerts effects on human physiology can be challenging due to the amount and variety of nutrients and bioactive molecules that it provides. Milk has evolved to provide mammals with the nutrients required to survive and thrive, which is reflected in its complex food composition and matrix. With over 400 types of FAs, slow and fast digesting high quality proteins (including a vast array of peptides that seem to exert bioactive effects), high concentrations of essential micronutrients and the presence of bacterial cultures (in fermented varieties) that are all packaged/provided simultaneously, it is clearly difficult to elucidate the specific mechanisms involved [2, 41]. In fact, it seems likely that multiple nutrients within dairy are both interacting with our physiology upon absorption, as well as interacting with each other, leading to synergistic or permissive effects that may not be observed with the provision of single nutrients alone. While exploring the individual nutrients and their effects upon

consumption is useful, it is perhaps less important than developing interventions using foods people can easily consume to help improve outcomes relating to health and disease. Dairy products are readily available, relatively cheap and come in a variety of forms/flavours to better suit individual preferences and needs. If one (or multiple) specific nutrients such as milk fat globule membrane, bioactive peptides or calcium are found to positively influence health after being extracted, synthesised and provided as a supplement, this may be less feasible or more expensive than recommending a glass of milk. Especially considering that other essential nutrients will be provided alongside the target nutrient with milk. One reason that we opted to provide multiple dairy foods during **Study 2** was to be able to see the effects of a more realistic nutritional intervention on physiological responses. Also, 3 servings of a single food added to the diet is a lot for people to consume. Perhaps if we only provided one dairy product, like yogurt, we may have seen a stronger effect on mitigating inflammation and we would have been able to better consider potential mechanisms because yogurt is fermented, has bacterial cultures and is high in protein and calcium. Indeed, studies have shown a benefit of fermented foods on inflammation [472]. The trade-off with using a mixed food approach is that it is more realistic to consume a variety of foods regularly. Adherence and compliance with nutrition protocols can be challenging for the general population [495, 496], so developing strategies that people enjoy, are easy to follow and provide flexibility are important.

## 7.1 Future Directions and Recommendations

While the research completed herein answers some important questions, it has also raised a number of others that require further investigation:

1. Post-exercise milk intake was shown to blunt relative bone resorption responses after high-impact, high-load exercise. It may be valuable to perform a study that also involves a longer-term training component in order to determine how accurately acute bone biomarker responses translate into chronic adaptations. A previous study from our lab found that after the initial 7 days of resistance exercise and Greek yogurt consumption, CTX (bone resorption marker) was not increased, and in the same study, after 12-weeks of resistance training and Greek Yogurt consumption, CTX was still unchanged but P1NP (bone formation marker) was increased compared to a carbohydrate supplement control [11]. To build upon the findings from that study and the study presented in **Chapter 3**, it would be of interest to measure acute bone biomarker responses to exercise and dairy consumption both prior to and following a longer duration exercise training protocol (6 months+), and to also measure BMD or other static bone health measures. Theoretically blunting bone catabolism after exercise may be beneficial for accruing bone mass, but it should be confirmed by a study that involves consistent post-exercise dairy consumption for a longer duration, with assessment of both biomarkers and bone structure.
2. Continuing to investigate both mechanisms of acute dairy intake on bone responses, and ways to optimise these effects are of value. Future studies should consider measuring biomarkers such as PTH, inflammatory markers, myokines and adipokines alongside different exercise modalities. This will allow us to further enhance our understanding of possible mechanisms through which dairy is acting on bone. In addition, comparing dairy products with different compositions (e.g. milk vs. yogurt) or an individual nutrient compared to dairy (e.g. calcium supplementation vs. milk) may also allow greater insight into how different nutrients or matrix effects can alter physiological responses.

3. Our research found minor benefits to fasting inflammatory profiles in individuals with OW/OB and cardiometabolic dysfunction. Future studies should attempt to determine if a dose-response relationship exists, or if different combinations of dairy products are superior/inferior for health-related outcomes. This would allow us to determine whether consuming more than 3 daily servings imparts greater benefits, or if there is a minimum effective "dose" of dairy consumption required to receive benefits to inflammatory or skeletal responses.
4. Our subgroup analysis in **Chapter 5** found that increased dairy consumption attenuated TNF-α in females but not in males when the groups were analysed separately. It would be of interest to explore this potential sex difference in inflammatory profile responses to dairy consumption by using a larger comparative sample size of each sex.
5. Finally, we did not observe differences in circulating postprandial inflammatory markers after 6-weeks of either low or increased dairy consumption. Considering that dairy's benefits on high-fat meal responses may stem from acute pre-meal intakes, it may be of interest to investigate the time course over which dairy consumption can confer these effects. For example, would dairy consumption earlier in the day influences postprandial responses later, or do the benefits require consumption either immediately prior to, or alongside the high-fat meal.

# Chapter 8: Overall Conclusion

In summary, the collective findings from this research show that dairy intake may have beneficial effects on certain aspects of human physiology. In young healthy female adults, post-exercise milk consumption reduced relative markers of bone breakdown, but did not alter other bone turnover markers compared to carbohydrate. In this high-impact, high-load exercise and nutritional context, it also appeared that some bone and inflammatory markers were associated in ways that are consistent with the physiology of the bone remodelling response. In adults with OW/OB and cardiometabolic dysfunction, mixed dairy consumption for 6-weeks favourably altered some, but not all, fasted markers of inflammation and glutathione. Finally, increased consumption of dairy for 6-weeks did not seem to effect postprandial metabolic or inflammatory responses to a high-calorie, high-fat meal. The results from this dissertation show that wholefood dairy consumption can exert positive physiological effects, and that their inclusion into a varied and balanced diet is warranted.

# Reference List


1. Prowting, J.L., et al., *Acute effects of Milk vs. carbohydrate on bone turnover biomarkers following loading exercise in young adult females.* Frontiers in Nutrition, 2022. **9**: p. 840973.
2. Da Silva, M.S. and I. Rudkowska, *Dairy nutrients and their effect on inflammatory profile in molecular studies.* Molecular nutrition & food research, 2015. **59**(7): p. 1249–1263.
3. Bhardwaj, A., et al., *"Osteomicrobiology": the nexus between bone and bugs.* Frontiers in Microbiology, 2022. **12**: p. 812466.
4. Ferreira, A., et al., *Bone remodeling markers and bone metastases: From cancer research to clinical implications.* Bonekey Rep, 2015. **4**: p. 668.
5. Bordoni, A., et al., *Dairy products and inflammation: A review of the clinical evidence.* Critical reviews in food science and nutrition, 2017. **57**(12): p. 2497–2525.
6. Khan, I.T., et al., *Antioxidant properties of Milk and dairy products: a comprehensive review of the current knowledge.* Lipids in health and disease, 2019. **18**(1): p. 41.
7. Rizzoli, R., *Dairy products and bone health.* Aging Clinical and Experimental Research, 2021. **34**(1): p. 1–16.
8. Silva, F.M., et al., *Dairy product consumption reduces cardiovascular mortality: results after 8 year follow-up of ELSA-Brasil.* Eur J Nutr, 2022. **61**(2): p. 859–869.
9. Soedamah-Muthu, S.S. and J. De Goede, *Dairy consumption and cardiometabolic diseases: systematic review and updated meta-analyses of prospective cohort studies.* Current nutrition reports, 2018. **7**(4): p. 171–182.
10. Drouin-Chartier, J.-P., et al., *Systematic review of the association between dairy product consumption and risk of cardiovascular-related clinical outcomes.* Advances in Nutrition, 2016. **7**(6): p. 1026–1040.
11. Bridge, A.D., et al., *Consumption of Greek yogurt during 12 weeks of high-impact loading exercise increases bone formation in young, adult males–a secondary analysis from a randomized trial.* Applied Physiology, Nutrition, and Metabolism, 2020. **45**(1): p. 91–100.
12. Josse, A.R., et al., *Dairy product intake decreases bone resorption following a 12-week diet and exercise intervention in overweight and obese adolescent girls.* Pediatric Research, 2020. **88**(6): p. 1–7.
13. Josse, A.R., et al., *Diets higher in dairy foods and dietary protein support bone health during diet-and exercise-induced weight loss in overweight and obese premenopausal women.* The Journal of Clinical Endocrinology & Metabolism, 2012. **97**(1): p. 251–260.
14. Zemel, M.B., et al., *Effects of dairy compared with soy on oxidative and inflammatory stress in overweight and obese subjects.* The American journal of clinical nutrition, 2010. **91**(1): p. 16–22.
15. Stancliffe, R.A., T. Thorpe, and M.B. Zemel, *Dairy attentuates oxidative and inflammatory stress in metabolic syndrome.* The American journal of clinical nutrition, 2011. **94**(2): p. 422–430.
16. Pei, R., et al., *Premeal low-fat yogurt consumption reduces postprandial inflammation and markers of endotoxin exposure in healthy premenopausal women in a randomized controlled trial.* The Journal of nutrition, 2018. **148**(6): p. 910–916.

17. Demmer, E., et al., *Addition of a dairy fraction rich in milk fat globule membrane to a high-saturated fat meal reduces the postprandial insulinaemic and inflammatory response in overweight and obese adults.* Journal of nutritional science, 2016. **5**: p. e14.
18. Hariton, E. and J.J. Locascio, *Randomised controlled trials - the gold standard for effectiveness research: Study design: randomised controlled trials.* Bjog, 2018. **125**(13): p. 1716.
19. Musa-Veloso, K., et al., *Challenges in the design, interpretation, and reporting of randomized controlled clinical studies on the health effects of whole foods.* Applied Physiology, Nutrition, and Metabolism, 2021. **46**(9): p. 1152–1158.
20. Melse-Boonstra, A., *Bioavailability of micronutrients from nutrient-dense whole foods: Zooming in on dairy, vegetables, and fruits.* Frontiers in Nutrition, 2020. **7**: p. 101.
21. Park, Y.W. and G.F. Haenlein, *Milk and dairy products in human nutrition: production, composition and health*. 2013: John Wiley & Sons.
22. Silanikove, N., G. Leitner, and U. Merin, *The Interrelationships between Lactose Intolerance and the Modern Dairy Industry: Global Perspectives in Evolutional and Historical Backgrounds.* Nutrients, 2015. **7**(9): p. 7312–7331.
23. Auclair, O., Y. Han, and S.A. Burgos, *Consumption of milk and alternatives and their contribution to nutrient intakes among Canadian adults: evidence from the 2015 Canadian Community Health Survey—Nutrition.* Nutrients, 2019. **11**(8): p. 1948.
24. Health Canada. *Eating Well with Canada's Food Guide 2007*. 2007; Available from: https://www.canada.ca/en/health-canada/services/canada-food-guide/about/history-food-guide/eating-well-with-canada-food-guide-2007.html.
25. Health Canada. *Canada's Food Guide*. 2019; Available from: https://food-guide.canada.ca/en/.
26. Landi, N., S. Ragucci, and A. Di Maro, *Amino Acid Composition of Milk from Cow, Sheep and Goat Raised in Ailano and Valle Agricola, Two Localities of 'Alto Casertano'(Campania Region).* Foods, 2021. **10**(10): p. 2431.
27. Guetouache, M., B. Guessas, and S. Medjekal, *Composition and nutritional value of raw milk.* J Issues Biol Sci Pharm Res, 2014. **2350**: p. 1588.
28. Parada, J. and J.M. Aguilera, *Food microstructure affects the bioavailability of several nutrients.* J Food Sci, 2007. **72**(2): p. R21–32.
29. Thorning, T.K., et al., *Whole dairy matrix or single nutrients in assessment of health effects: current evidence and knowledge gaps.* The American journal of clinical nutrition, 2017. **105**(5): p. 1033–1045.
30. Fardet, A., et al., *Influence of food structure on dairy protein, lipid and calcium bioavailability: A narrative review of evidence.* Critical reviews in food science and nutrition, 2019. **59**(13): p. 1987–2010.
31. Rioux, L.-E. and S.L. Turgeon, *The ratio of casein to whey protein impacts yogurt digestion in vitro.* Food Digestion, 2012. **3**(1): p. 25–35.
32. Turgeon, S.L. and L.-E. Rioux, *Food matrix impact on macronutrients nutritional properties.* Food Hydrocolloids, 2011. **25**(8): p. 1915–1924.
33. Boirie, Y., et al., *Slow and fast dietary proteins differently modulate postprandial protein accretion.* Proceedings of the national academy of sciences, 1997. **94**(26): p. 14930–14935.

34. Caroli, A., S. Chessa, and G. Erhardt, *Invited review: Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition.* Journal of dairy science, 2009. **92**(11): p. 5335–5352.
35. Coneyworth, L.J., et al., *Geographical and seasonal variation in iodine content of cow's milk in the UK and consequences for the consumer´ s supply.* Journal of Trace Elements in Medicine and Biology, 2020. **59**: p. 126453.
36. Ibrahim, S.A. and R. Gyawali, *Lactose intolerance.* Milk and dairy products in human nutrition: Production, composition and health, 2013: p. 246–260.
37. Barr, S.I., *Perceived lactose intolerance in adult Canadians: a national survey.* Applied Physiology, Nutrition, and Metabolism, 2013. **38**(8): p. 830–835.
38. Dekker, P.J.T., D. Koenders, and M.J. Bruins, *Lactose-Free Dairy Products: Market Developments, Production, Nutrition and Health Benefits.* Nutrients, 2019. **11**(3): p. 551.
39. MacGibbon, A., *Composition and structure of bovine milk lipids.* Advanced dairy chemistry, volume 2: lipids, 2020: p. 1–32.
40. Unger, A.L., M. Torres-Gonzalez, and J. Kraft, *Dairy Fat Consumption and the Risk of Metabolic Syndrome: An Examination of the Saturated Fatty Acids in Dairy.* Nutrients, 2019. **11**(9): p. 2200.
41. Parodi, P.W., *Milk fat in human nutrition.* Australian Journal of Dairy Technology, 2004. **59**(1): p. 3.
42. Chouinard-Watkins, R., et al., *Dairy product consumption is associated with a lowering of linoleic acid within serum TAG in adolescent females with overweight or obesity: a secondary analysis.* British Journal of Nutrition, 2022. **127**(1): p. 68–77.
43. Bramanti, E., et al., *Separation and determination of denatured αs1-, αs2-, β-and κ-caseins by hydrophobic interaction chromatography in cows', ewes' and goats' milk, milk mixtures and cheeses.* Journal of Chromatography A, 2003. **994**(1-2): p. 59–74.
44. Chalupa-Krebzdak, S., C.J. Long, and B.M. Bohrer, *Nutrient density and nutritional value of milk and plant-based milk alternatives.* International dairy journal, 2018. **87**: p. 84–92.
45. Tang, J.E., et al., *Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men.* Journal of applied physiology, 2009. **107**(3): p. 987–92.
46. Anand, S., K. Som Nath, and M. Chenchaiah, *Whey and whey products.* Milk and Dairy Products in Human Nutrition: Production, Composition and Health, 2013: p. 477–497.
47. Rafiq, S., et al., *Chemical composition, nitrogen fractions and amino acids profile of milk from different animal species.* Asian-Australasian journal of animal sciences, 2016. **29**(7): p. 1022.
48. Auestad, N. and D.K. Layman, *Dairy bioactive proteins and peptides: a narrative review.* Nutrition reviews, 2021. **79**(Supplement_2): p. 36–47.
49. Ebringer, L., M. Ferenčík, and J. Krajčovič, *Beneficial health effects of milk and fermented dairy products.* Folia microbiologica, 2008. **53**(5): p. 378–394.
50. Pereira, P.C., *Milk nutritional composition and its role in human health.* Nutrition, 2014. **30**(6): p. 619–627.
51. Sumi, K., et al., *Nutritional value of yogurt as a protein source: digestibility/absorbability and effects on skeletal muscle.* Nutrients, 2023. **15**(20): p. 4366.
52. Fishbein, L., *Multiple sources of dietary calcium—some aspects of its essentiality.* Regulatory Toxicology and Pharmacology, 2004. **39**(2): p. 67–80.

53. Guéguen, L. and A. Pointillart, *The bioavailability of dietary calcium.* Journal of the American College of Nutrition, 2000. **19**(sup2): p. 119S–136S.
54. Itkonen, S.T., M. Erkkola, and C.J. Lamberg-Allardt, *Vitamin D fortification of fluid milk products and their contribution to vitamin D intake and vitamin D status in observational studies—a review.* Nutrients, 2018. **10**(8): p. 1054.
55. Health Canada. *Regulations Amending the Food and Drug Regulations (Nutrition Symbols, Other Labelling Provisions, Vitamin D and Hydrogenated Fats or Oils): SOR/2022-168*. 2022 [cited 2025; Available from: https://gazette.gc.ca/rp-pr/p2/2022/2022-07-20/html/sor-dors168-eng.html.
56. Health Canada. *Marketing Authorization for Vitamin D in Yogurt and Kefir: SOR/2024-88*. 2024 [cited 2025; Available from: https://gazette.gc.ca/rp-pr/p2/2024/2024-06-05/html/sor-dors88-eng.html.
57. Lamberg-Allardt, C., et al., *Vitamin D – a systematic literature review for the 5th edition of the Nordic Nutrition Recommendations.* Food & Nutrition Research, 2013. **57**(1): p. 22671.
58. Cheng, N. and A.R. Josse, *Dairy and Exercise for Bone Health: Evidence from Randomized Controlled Trials and Recommendations for Future Research.* Current Osteoporosis Reports, 2024. **22**(6): p. 502–514.
59. Sözen, T., L. Özışık, and N.Ç. Başaran, *An overview and management of osteoporosis.* European journal of rheumatology, 2016. **4**(1): p. 46.
60. Heaney, R.P., et al., *Peak bone mass.* Osteoporosis international, 2000. **11**(12): p. 985.
61. Weaver, C.M., et al., *The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations.* Osteoporosis international, 2016. **27**(4): p. 1281–1386.
62. Hightower, L., *Osteoporosis: pediatric disease with geriatric consequences.* Orthopaedic Nursing, 2000. **19**(5): p. 59.
63. Gao, C., et al., *Bone biomaterials and interactions with stem cells.* Bone research, 2017. **5**(1): p. 1–33.
64. Kenkre, J. and J. Bassett, *The bone remodelling cycle.* Annals of clinical biochemistry, 2018. **55**(3): p. 308–327.
65. Jilka, R.L., *Biology of the basic multicellular unit and the pathophysiology of osteoporosis.* Medical and pediatric oncology, 2003. **41**(3): p. 182–185.
66. Hauge, E.M., et al., *Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers.* Journal of Bone and Mineral Research, 2001. **16**(9): p. 1575–1582.
67. Eriksen, E.F., *Cellular mechanisms of bone remodeling.* Reviews in Endocrine and Metabolic Disorders, 2010. **11**(4): p. 219–227.
68. Franz-Odendaal, T.A., B.K. Hall, and P.E. Witten, *Buried alive: how osteoblasts become osteocytes.* Developmental dynamics: an official publication of the American Association of Anatomists, 2006. **235**(1): p. 176–190.
69. Florencio-Silva, R., et al., *Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells.* Biomed Res Int, 2015. **2015**: p. 421746.
70. Schaffler, M.B., et al., *Osteocytes: master orchestrators of bone.* Calcified tissue international, 2014. **94**(1): p. 5–24.
71. Civitelli, R., et al., *Intercellular junctions and cell-cell communication in bone.* Principles of Bone Biology, 2002: p. 287–302.

72. Arias, C.F., et al., *Bone remodeling: A tissue-level process emerging from cell-level molecular algorithms.* PloS one, 2018. **13**(9): p. e0204171.
73. Aguirre, J.I., et al., *Osteocyte apoptosis is induced by weightlessness in mice and precedes osteoclast recruitment and bone loss.* Journal of bone and mineral research, 2006. **21**(4): p. 605–615.
74. Dallas, S.L., M. Prideaux, and L.F. Bonewald, *The osteocyte: an endocrine cell… and more.* Endocrine reviews, 2013. **34**(5): p. 658–690.
75. Epsley, S., et al., *The Effect of Inflammation on Bone.* Frontiers in Physiology, 2021. **11**: p. 1695.
76. Takayanagi, H., *Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems.* Nature Reviews Immunology, 2007. **7**(4): p. 292–304.
77. Caetano-Lopes, J., H. Canhao, and J.E. Fonseca, *Osteoimmunology--the hidden immune regulation of bone.* Autoimmun Rev, 2009. **8**(3): p. 250–5.
78. Asagiri, M. and H. Takayanagi, *The molecular understanding of osteoclast differentiation.* Bone, 2007. **40**(2): p. 251–264.
79. Boyce, B.F. and L. Xing, *Functions of RANKL/RANK/OPG in bone modeling and remodeling.* Archives of biochemistry and biophysics, 2008. **473**(2): p. 139–146.
80. Martin, T.J., J.H. Gooi, and N.A. Sims, *Molecular mechanisms in coupling of bone formation to resorption.* Critical Reviews™ in Eukaryotic Gene Expression, 2009. **19**(1): p. 73–88.
81. McDonald, M.M., et al., *Osteoclasts recycle via osteomorphs during RANKL-stimulated bone resorption.* Cell, 2021. **184**(5): p. 1330–1347. e13.
82. Zhao, C., et al., *Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis.* Cell metabolism, 2006. **4**(2): p. 111–121.
83. Tang, Y., et al., *TGF-β1–induced migration of bone mesenchymal stem cells couples bone resorption with formation.* Nature medicine, 2009. **15**(7): p. 757–765.
84. Macsai, C.E., B.K. Foster, and C.J. Xian, *Roles of Wnt signalling in bone growth, remodelling, skeletal disorders and fracture repair.* Journal of cellular physiology, 2008. **215**(3): p. 578–587.
85. Clevers, H. and R. Nusse, *Wnt/β-catenin signaling and disease.* Cell, 2012. **149**(6): p. 1192–1205.
86. Hinson, J., P. Raven, and S. Chew, *12 - HORMONAL REGULATION OF PLASMA CALCIUM AND CALCIUM METABOLISM*, in *The Endocrine System (Second Edition)*, J. Hinson, P. Raven, and S. Chew, Editors. 2010, Churchill Livingstone. p. 147–159.
87. Brickley, M. and I. RACHEL, *Background to Bone Biology and Mineral Metabolism.* The Bioarchaeology of Metabolic Bone Disease, 2008: p. 21–41.
88. Dallas, S.L. and L.F. Bonewald, *Dynamics of the transition from osteoblast to osteocyte.* Annals of the New York Academy of Sciences, 2010. **1192**: p. 437.
89. Manolagas, S.C., *Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis.* Endocrine reviews, 2000. **21**(2): p. 115–137.
90. Sims, N.A. and T.J. Martin, *Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit.* BoneKEy reports, 2014. **3**: p. 481.
91. Ross, T.D., et al., *Integrins in mechanotransduction.* Current opinion in cell biology, 2013. **25**(5): p. 613–618.

92. Goodman, C.A., T.A. Hornberger, and A.G. Robling, *Bone and skeletal muscle: Key players in mechanotransduction and potential overlapping mechanisms.* Bone, 2015. **80**: p. 24–36.
93. Klein-Nulend, J. and A.D. Bakker, *Osteocytes: mechanosensors of bone and orchestrators of mechanical adaptation.* Clinical Reviews in Bone and Mineral Metabolism, 2007. **5**: p. 195–209.
94. Santos, L., K.J. Elliott-Sale, and C. Sale, *Exercise and bone health across the lifespan.* Biogerontology, 2017. **18**: p. 931–946.
95. Tu, X., et al., *Osteocytes mediate the anabolic actions of canonical Wnt/β-catenin signaling in bone.* Proceedings of the National Academy of Sciences, 2015. **112**(5): p. E478–E486.
96. Krishnan, V., H.U. Bryant, and O.A. MacDougald, *Regulation of bone mass by Wnt signaling.* The Journal of clinical investigation, 2006. **116**(5): p. 1202–1209.
97. Gardinier, J.D., et al., *PTH signaling mediates perilacunar remodeling during exercise.* Matrix Biology, 2016. **52**: p. 162–175.
98. Tobeiha, M., et al., *RANKL/RANK/OPG pathway: a mechanism involved in exercise-induced bone remodeling.* BioMed research international, 2020. **2020**: p. 6910312.
99. Yuan, Y., et al., *The roles of exercise in bone remodeling and in prevention and treatment of osteoporosis.* Progress in Biophysics and Molecular Biology, 2016. **122**(2): p. 122–130.
100. Gong, T., et al., *DAMP-sensing receptors in sterile inflammation and inflammatory diseases.* Nature Reviews Immunology, 2020. **20**(2): p. 95–112.
101. Roh, J.S. and D.H. Sohn, *Damage-associated molecular patterns in inflammatory diseases.* Immune network, 2018. **18**(4): p. e27.
102. Bar-Shavit, Z., *Taking a toll on the bones: regulation of bone metabolism by innate immune regulators.* Autoimmunity, 2008. **41**(3): p. 195–203.
103. Alonso-Pérez, A., et al., *Role of toll-like receptor 4 on osteoblast metabolism and function.* Frontiers in physiology, 2018. **9**: p. 504.
104. Nabipour, I., et al., *Relationships among serum receptor of nuclear factor-κB ligand, osteoprotegerin, high-sensitivity C-reactive protein, and bone mineraldensity in postmenopausal women: osteoimmunity versus osteoinflammatory.* Menopause, 2009. **16**(5): p. 950–955.
105. Steeve, K.T., et al., *IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology.* Cytokine & growth factor reviews, 2004. **15**(1): p. 49–60.
106. Liu, X.-H., et al., *Cross-talk between the interleukin-6 and prostaglandin E2 signaling systems results in enhancement of osteoclastogenesis through effects on the osteoprotegerin/receptor activator of nuclear factor-κB (RANK) ligand/RANK system.* Endocrinology, 2005. **146**(4): p. 1991–1998.
107. Lam, J., et al., *TNF-α induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand.* The Journal of clinical investigation, 2000. **106**(12): p. 1481–1488.
108. Zhao, B., *TNF and bone remodeling.* Current osteoporosis reports, 2017. **15**(3): p. 126–134.
109. Wang, L., et al., *Tumor necrosis factor-alpha inhibits osteogenic differentiation of pre-osteoblasts by downregulation of EphB4 signaling via activated nuclear factor-kappaB signaling pathway.* Journal of periodontal research, 2018. **53**(1): p. 66–72.

110. Ma, Q., et al., *NLRP3 inflammasome contributes to inflammation after intracerebral hemorrhage.* Annals of neurology, 2014. **75**(2): p. 209–219.
111. Agidigbi, T.S. and C. Kim, *Reactive oxygen species in osteoclast differentiation and possible pharmaceutical targets of ROS-mediated osteoclast diseases.* International journal of molecular sciences, 2019. **20**(14): p. 3576.
112. Ashtar, M., et al., *The Roles of ROS Generation in RANKL-Induced Osteoclastogenesis: Suppressive Effects of Febuxostat.* Cancers, 2020. **12**(4): p. 929.
113. Zhao, B. and L.B. Ivashkiv, *Negative regulation of osteoclastogenesis and bone resorption by cytokines and transcriptional repressors.* Arthritis research & therapy, 2011. **13**(4): p. 1–10.
114. Tang, M., et al., *Interferon-gamma-mediated osteoimmunology.* Frontiers in immunology, 2018. **9**: p. 1508.
115. Benedetti, M.G., et al., *The effectiveness of physical exercise on bone density in osteoporotic patients.* BioMed research international, 2018. **2018**: p. 4840531.
116. Michailidis, Y., et al., *Thiol-based antioxidant supplementation alters human skeletal muscle signaling and attenuates its inflammatory response and recovery after intense eccentric exercise.* The American journal of clinical nutrition, 2013. **98**(1): p. 233–245.
117. Gerstenfeld, L.C., et al., *Impaired fracture healing in the absence of TNF-α signaling: The role of TNF-α in endochondral cartilage resorption.* Journal of Bone and Mineral Research, 2003. **18**(9): p. 1584–1592.
118. Adamopoulos, I.E., *Inflammation in bone physiology and pathology.* Current opinion in rheumatology, 2018. **30**(1): p. 59.
119. Wang, F., et al., *Methods for bone quality assessment in human bone tissue: a systematic review.* Journal of Orthopaedic Surgery and Research, 2022. **17**(1): p. 174.
120. Dolan, E., et al., *The Influence of Nutrition Intervention on the P1NP and CTX-1 Response to an Acute Exercise Bout: A Systematic Review with Meta-Analysis.* Sports Medicine, 2024. **54**(11): p. 1–18.
121. de Bakker, C.M., et al., *Clinical evaluation of bone strength and fracture risk.* Current osteoporosis reports, 2017. **15**: p. 32–42.
122. Schini, M., et al., *Bone Turnover Markers: Basic Biology to Clinical Applications.* Endocrine Reviews, 2022. **44**(3): p. 417–473.
123. Kuo, T.-R. and C.-H. Chen, *Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives.* Biomarker research, 2017. **5**(1): p. 1–9.
124. Leeming, D., et al., *An update on biomarkers of bone turnover and their utility in biomedical research and clinical practice.* European journal of clinical pharmacology, 2006. **62**(10): p. 781–792.
125. Bhattarai, H.K., et al., *Vitamin D, Calcium, Parathyroid Hormone, and Sex Steroids in Bone Health and Effects of Aging.* Journal of Osteoporosis, 2020. **2020**(1): p. 9324505.
126. Rendina-Ruedy, E. and C.J. Rosen, *Parathyroid hormone (PTH) regulation of metabolic homeostasis: An old dog teaches us new tricks.* Molecular Metabolism, 2022. **60**: p. 101480.
127. Cranney, A., et al., *Parathyroid hormone for the treatment of osteoporosis: a systematic review.* Cmaj, 2006. **175**(1): p. 52–59.
128. Seeman, E., *From density to structure: growing up and growing old on the surfaces of bone.* Journal of bone and mineral research, 1997. **12**(4): p. 509–521.

129. Venken, K., et al., *Sex hormones, their receptors and bone health.* Osteoporosis International, 2008. **19**(11): p. 1517–1525.
130. Holick, M.F., *The role of vitamin D for bone health and fracture prevention.* Current Osteoporosis Reports, 2006. **4**(3): p. 96–102.
131. Lips, P., *Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications.* Endocrine reviews, 2001. **22**(4): p. 477–501.
132. Weiler, H.A., et al., *Vitamin D status of people 3 to 79 years of age from the Canadian health measures survey 2012–2019.* The Journal of Nutrition, 2023. **153**(4): p. 1150–1161.
133. Rizzoli, R., et al., *Benefits and safety of dietary protein for bone health—an expert consensus paper endorsed by the European Society for Clinical and Economical Aspects of Osteopororosis, Osteoarthritis, and Musculoskeletal Diseases and by the International Osteoporosis Foundation.* Osteoporosis International, 2018. **29**(9): p. 1933–1948.
134. Hidayat, K., et al., *The effects of dairy product supplementation on bone health indices in children aged 3 to 18 years: a meta-analysis of randomized controlled trials.* Advances in Nutrition, 2023. **14**(5): p. 1187–1196.
135. Huncharek, M., J. Muscat, and B. Kupelnick, *Impact of dairy products and dietary calcium on bone-mineral content in children: results of a meta-analysis.* Bone, 2008. **43**(2): p. 312–321.
136. Hidayat, K., et al., *The Effects of Milk Supplementation on Bone Health Indices in Adults: A Meta-Analysis of Randomized Controlled Trials.* Advances in Nutrition, 2022. **13**(4): p. 1186–1199.
137. Tai, V., et al., *Calcium intake and bone mineral density: systematic review and meta-analysis.* Bmj, 2015. **351**.
138. Shi, Y., et al., *Effects of dairy products on bone mineral density in healthy postmenopausal women: a systematic review and meta-analysis of randomized controlled trials.* Archives of Osteoporosis, 2020. **15**: p. 1–8.
139. Ma, D.F., et al., *Milk intake increases bone mineral content through inhibiting bone resorption: Meta-analysis of randomized controlled trials.* e-SPEN Journal, 2013. **8**(1): p. e1–e7.
140. Kottaras, S., et al., *Bone turnover markers and osteokines in adolescent female athletes of high-and low-impact sports compared with nonathletic controls.* Pediatric Exercise Science, 2022. **35**(1): p. 41–47.
141. Hong, A.R. and S.W. Kim, *Effects of resistance exercise on bone health.* Endocrinology and Metabolism, 2018. **33**(4): p. 435–444.
142. Volek, J.S., et al., *Increasing fluid milk favorably affects bone mineral density responses to resistance training in adolescent boys.* Journal of the American Dietetic Association, 2003. **103**(10): p. 1353–1356.
143. Gómez, A.L., et al., *Resistance training and milk-substitution enhance body composition and bone health in adolescent girls.* Journal of the American College of Nutrition, 2021. **40**(3): p. 193–210.
144. Josse, A.R., et al., *Body composition and strength changes in women with milk and resistance exercise.* Medicine & Science in Sports & Exercise, 2010. **42**(6): p. 1122–1130.
145. Huschtscha, Z., et al., *The effects of a high-protein dairy milk beverage with or without progressive resistance training on fat-free mass, skeletal muscle strength and power, and*

*functional performance in healthy active older adults: a 12-week randomized controlled trial.* Frontiers in nutrition, 2021. **8**: p. 644865.
146. Lee, S.M., S. Kim, and C.-g. Lim, *The effects of milk intake and whole-body vibration exercise on bone mineral density in elderly women in nursing homes.* Journal of physical therapy science, 2017. **29**(7): p. 1125–1128.
147. Dolan, E., et al., *The bone biomarker response to an acute bout of exercise: a systematic review with meta-analysis.* Sports medicine, 2022. **52**(12): p. 2889–2908.
148. Dolan, E., et al., *The bone metabolic response to exercise and nutrition.* Exercise and sport sciences reviews, 2020. **48**(2): p. 49–58.
149. Townsend, R., et al., *The effect of postexercise carbohydrate and protein ingestion on bone metabolism.* Translational Journal of the American College of Sports Medicine, 2017. **2**(20): p. 129–137.
150. Theocharidis, A., et al., *Effects of post exercise protein supplementation on markers of bone turnover in adolescent swimmers.* Journal of the International Society of Sports Nutrition, 2020. **17**(1): p. 1–11.
151. Aussieker, T., et al., *Collagen protein ingestion during recovery from exercise does not increase muscle connective protein synthesis rates.* Medicine and Science in Sports and Exercise, 2023. **55**(10): p. 1792.
152. Haakonssen, E.C., et al., *The effects of a calcium-rich pre-exercise meal on biomarkers of calcium homeostasis in competitive female cyclists: a randomised crossover trial.* PloS one, 2015. **10**(5): p. e0123302.
153. Kurgan, N., et al., *Twelve weeks of a diet and exercise intervention alters the acute bone response to exercise in adolescent females with overweight/obesity.* Frontiers in Physiology, 2023. **13**: p. 1049604.
154. Goodman, B.E., *Insights into digestion and absorption of major nutrients in humans.* Advances in Physiology Education, 2010. **34**(2): p. 44–53.
155. Mulet-Cabero, A.-I., et al., *Dairy structures and physiological responses: a matter of gastric digestion.* Critical Reviews in Food Science and Nutrition, 2020. **60**(22): p. 3737–3752.
156. Brown, J., et al., *Differential plasma branched-chain amino acid responses following the consumption of Greek-style yogurt and skimmed milk.* Applied Physiology, Nutrition, and Metabolism, 2023. **48**(7): p. 544–549.
157. Tipton, K.D., et al., *Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise.* American Journal of Physiology-Endocrinology and Metabolism, 2007.
158. Devries, M.C. and S.M. Phillips, *Supplemental protein in support of muscle mass and health: advantage whey.* Journal of food science, 2015. **80**(S1): p. A8–A15.
159. Trommelen, J., M.W. Betz, and L.J.C. van Loon, *The Muscle Protein Synthetic Response to Meal Ingestion Following Resistance-Type Exercise.* Sports Medicine, 2019. **49**(2): p. 185–197.
160. Dias, C.B., et al., *Postprandial lipemia: factoring in lipemic response for ranking foods for their healthiness.* Lipids in Health and Disease, 2017. **16**(1): p. 178.
161. Masson, C.J. and R.P. Mensink, *Exchanging saturated fatty acids for (n-6) polyunsaturated fatty acids in a mixed meal may decrease postprandial lipemia and markers of inflammation and endothelial activity in overweight men1–3.* The Journal of nutrition, 2011. **141**(5): p. 816–821.

162. Bonham, M.P., et al., *Lipidomic profiling of chylomicron triacylglycerols in response to high fat meals.* Lipids, 2013. **48**: p. 39–50.
163. Clemente, G., et al., *Effects of different dairy products on postprandial lipemia.* Nutrition, Metabolism and Cardiovascular Diseases, 2003. **13**(6): p. 377–383.
164. Vors, C., et al., *Modulating absorption and postprandial handling of dietary fatty acids by structuring fat in the meal: a randomized crossover clinical trial1 2 3.* The American Journal of Clinical Nutrition, 2013. **97**(1): p. 23–36.
165. Lorenzen, J.K., et al., *Effect of dairy calcium or supplementary calcium intake on postprandial fat metabolism, appetite, and subsequent energy intake.* The American journal of clinical nutrition, 2007. **85**(3): p. 678–687.
166. Hansson, P., et al., *Meals with similar fat content from different dairy products induce different postprandial triglyceride responses in healthy adults: a randomized controlled cross-over trial.* The Journal of nutrition, 2019. **149**(3): p. 422–431.
167. Drouin-Chartier, J.-P., et al., *Differential impact of the cheese matrix on the postprandial lipid response: a randomized, crossover, controlled trial<sup>†</sup>.* The American Journal of Clinical Nutrition, 2017. **106**(6): p. 1358–1365.
168. Tholstrup, T., et al., *Does fat in milk, butter and cheese affect blood lipids and cholesterol differently?* Journal of the American College of Nutrition, 2004. **23**(2): p. 169–176.
169. Schmid, A., et al., *Inflammatory and metabolic responses to high-fat meals with and without dairy products in men.* British journal of nutrition, 2015. **113**(12): p. 1853–1861.
170. Kjølbæk, L. and A. Raben, *The impact of dairy matrix structure on postprandial lipid responses.* Proceedings of the Nutrition Society, 2024. **83**(1): p. 9–16.
171. Mortensen, L.S., et al., *Differential effects of protein quality on postprandial lipemia in response to a fat-rich meal in type 2 diabetes: comparison of whey, casein, gluten, and cod protein.* The American journal of clinical nutrition, 2009. **90**(1): p. 41–48.
172. Akhavan, T., et al., *Effect of premeal consumption of whey protein and its hydrolysate on food intake and postmeal glycemia and insulin responses in young adults.* The American journal of clinical nutrition, 2010. **91**(4): p. 966–975.
173. McDonald, J.D., et al., *Dairy milk, regardless of fat content, protects against postprandial hyperglycemia-mediated impairments in vascular endothelial function in adults with prediabetes by limiting oxidative stress responses that reduce nitric oxide bioavailability.* The Journal of Nutritional Biochemistry, 2019. **63**: p. 129–139.
174. Mariotti, F., et al., *Casein compared with whey proteins affects the organization of dietary fat during digestion and attenuates the postprandial triglyceride response to a mixed high-fat meal in healthy, overweight men.* The Journal of nutrition, 2015. **145**(12): p. 2657–2664.
175. Feeney, E.L., et al., *Dairy matrix effects: response to consumption of dairy fat differs when eaten within the cheese matrix—a randomized controlled trial.* The American Journal of Clinical Nutrition, 2018. **108**(4): p. 667–674.
176. Hjerpsted, J., E. Leedo, and T. Tholstrup, *Cheese intake in large amounts lowers LDL-cholesterol concentrations compared with butter intake of equal fat content123.* The American journal of clinical nutrition, 2011. **94**(6): p. 1479–1484.
177. Nestel, P., A. Chronopulos, and M. Cehun, *Dairy fat in cheese raises LDL cholesterol less than that in butter in mildly hypercholesterolaemic subjects.* European journal of clinical nutrition, 2005. **59**(9): p. 1059–1063.

178. Biong, A.S., et al., *A comparison of the effects of cheese and butter on serum lipids, haemostatic variables and homocysteine.* British journal of nutrition, 2004. **92**(5): p. 791–797.
179. Timon, C.M., et al., *Dairy consumption and metabolic health.* Nutrients, 2020. **12**(10): p. 3040.
180. Thorning, T.K., et al., *Milk and dairy products: good or bad for human health? An assessment of the totality of scientific evidence.* Food & nutrition research, 2016. **60**(1): p. 32527.
181. Artaud-Wild, S.M., et al., *Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. A paradox.* Circulation, 1993. **88**(6): p. 2771–2779.
182. Sacks, F.M., et al., *Dietary fats and cardiovascular disease: a presidential advisory from the American Heart Association.* Circulation, 2017. **136**(3): p. e1–e23.
183. Siri-Tarino, P.W., et al., *Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease.* The American journal of clinical nutrition, 2010. **91**(3): p. 535–546.
184. de Oliveira Otto, M.C., et al., *Dietary intake of saturated fat by food source and incident cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis.* Am J Clin Nutr, 2012. **96**(2): p. 397–404.
185. Goldbohm, R.A., et al., *Dairy consumption and 10-y total and cardiovascular mortality: a prospective cohort study in the Netherlands–.* The American journal of clinical nutrition, 2011. **93**(3): p. 615–627.
186. Michaëlsson, K., et al., *Milk intake and risk of mortality and fractures in women and men: cohort studies.* Bmj, 2014. **349**: p. g6015.
187. Zheng, H., et al., *Metabolomics investigation to shed light on cheese as a possible piece in the French paradox puzzle.* Journal of agricultural and food chemistry, 2015. **63**(10): p. 2830–2839.
188. Petyaev, I.M. and Y.K. Bashmakov, *Could cheese be the missing piece in the French paradox puzzle?* Medical hypotheses, 2012. **79**(6): p. 746–749.
189. Elwood, P.C., et al., *The consumption of milk and dairy foods and the incidence of vascular disease and diabetes: an overview of the evidence.* Lipids, 2010. **45**(10): p. 925–939.
190. Huth, P.J. and K.M. Park, *Influence of dairy product and milk fat consumption on cardiovascular disease risk: a review of the evidence.* Advances in nutrition, 2012. **3**(3): p. 266–285.
191. De Goede, J., et al., *Dairy consumption and risk of stroke: a systematic review and updated dose–response meta-analysis of prospective cohort studies.* Journal of the American Heart Association, 2016. **5**(5): p. e002787.
192. Drouin-Chartier, J.-P., et al., *Comprehensive Review of the Impact of Dairy Foods and Dairy Fat on Cardiometabolic Risk.* Advances in Nutrition, 2016. **7**(6): p. 1041–1051.
193. de Goede, J., et al., *Effect of cheese consumption on blood lipids: a systematic review and meta-analysis of randomized controlled trials.* Nutrition Reviews, 2015. **73**(5): p. 259–275.
194. Christensen, R., et al., *Effect of calcium from dairy and dietary supplements on faecal fat excretion: a meta-analysis of randomized controlled trials.* Obesity reviews, 2009. **10**(4): p. 475–486.

195. Soerensen, K.V., et al., *Effect of dairy calcium from cheese and milk on fecal fat excretion, blood lipids, and appetite in young men123.* The American Journal of Clinical Nutrition, 2014. **99**(5): p. 984–991.
196. Lorenzen, J.K. and A. Astrup, *Dairy calcium intake modifies responsiveness of fat metabolism and blood lipids to a high-fat diet.* British Journal of Nutrition, 2011. **105**(12): p. 1823–1831.
197. Dunne, S., et al., *The effects of saturated fat intake from dairy on CVD markers: the role of food matrices.* Proceedings of the Nutrition Society, 2024. **83**(4): p. 236–244.
198. Astrup, A., et al., *WHO draft guidelines on dietary saturated and trans fatty acids: time for a new approach?* Bmj, 2019. **366**.
199. Leeuwendaal, N.K., et al., *Fermented foods, health and the gut microbiome.* Nutrients, 2022. **14**(7): p. 1527.
200. Ulven, S.M., et al., *Milk and dairy product consumption and inflammatory biomarkers: an updated systematic review of randomized clinical trials.* Advances in Nutrition, 2019. **10**(suppl_2): p. S239–S250.
201. Mittal, M., et al., *Reactive oxygen species in inflammation and tissue injury.* Antioxidants & redox signaling, 2014. **20**(7): p. 1126–1167.
202. Bréchard, S., S. Plançon, and E.J. Tschirhart, *New insights into the regulation of neutrophil NADPH oxidase activity in the phagosome: a focus on the role of lipid and Ca2+ signaling.* Antioxidants & redox signaling, 2013. **18**(6): p. 661–676.
203. Ives, A., et al., *Xanthine oxidoreductase regulates macrophage IL1β secretion upon NLRP3 inflammasome activation.* Nature communications, 2015. **6**(1): p. 1–11.
204. Bellissimo, C.A. and C.G. Perry, *Regulation of Skeletal Muscle Reactive Oxygen Species During Exercise*, in *The Routledge Handbook on Biochemistry of Exercise*. 2020, Routledge. p. 51–70.
205. Sies, H. and D.P. Jones, *Reactive oxygen species (ROS) as pleiotropic physiological signalling agents.* Nature reviews Molecular cell biology, 2020. **21**(7): p. 363–383.
206. Roberts, C.K. and K.K. Sindhu, *Oxidative stress and metabolic syndrome.* Life sciences, 2009. **84**(21-22): p. 705–712.
207. Senoner, T. and W. Dichtl, *Oxidative stress in cardiovascular diseases: still a therapeutic target?* Nutrients, 2019. **11**(9): p. 2090.
208. Hurrle, S. and W.H. Hsu, *The etiology of oxidative stress in insulin resistance.* Biomedical journal, 2017. **40**(5): p. 257–262.
209. Keaney Jr, J.F., et al., *Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study.* Arteriosclerosis, thrombosis, and vascular biology, 2003. **23**(3): p. 434–439.
210. Furukawa, S., et al., *Increased oxidative stress in obesity and its impact on metabolic syndrome.* The Journal of clinical investigation, 2017. **114**(12): p. 1752–1761.
211. DeMarco, V.G., et al., *Cytokine abnormalities in the etiology of the cardiometabolic syndrome.* Current hypertension reports, 2010. **12**(2): p. 93–98.
212. Sen, C.K., *Oxidants and antioxidants in exercise.* Journal of applied physiology, 1995. **79**(3): p. 675–686.
213. Ng, C.F., et al., *The rate of cellular hydrogen peroxide removal shows dependency on GSH: mathematical insight into in vivo H2O2 and GPx concentrations.* Free radical research, 2007. **41**(11): p. 1201–1211.

214. Halprin, K.M. and A. Ohkawara, *The measurement of glutathione in human epidermis using glutathione reductase.* J invest dermatol, 1967. **48**(2): p. 149–52.
215. Hammarqvist, F., et al., *Skeletal muscle glutathione is depleted in critically ill patients.* Critical care medicine, 1997. **25**(1): p. 78–84.
216. Luo, J.-L., et al., *Skeletal muscle glutathione after surgical trauma.* Annals of surgery, 1996. **223**(4): p. 420.
217. Reid, M. and F. Jahoor, *Glutathione in disease.* Current Opinion in Clinical Nutrition & Metabolic Care, 2001. **4**(1): p. 65–71.
218. Townsend, D.M., K.D. Tew, and H. Tapiero, *The importance of glutathione in human disease.* Biomedicine & pharmacotherapy, 2003. **57**(3-4): p. 145–155.
219. White, A.C., V.J. Thannickal, and B.L. Fanburg, *Glutathione deficiency in human disease.* The Journal of Nutritional Biochemistry, 1994. **5**(5): p. 218–226.
220. Choromańska, B., et al., *The impact of hypertension and metabolic syndrome on nitrosative stress and glutathione metabolism in patients with morbid obesity.* Oxidative Medicine and Cellular Longevity, 2020. **2020**: p. 1057570.
221. Picklo, M.J., E.K. Long, and E.E. Vomhof-DeKrey, *Glutathionyl systems and metabolic dysfunction in obesity.* Nutrition Reviews, 2015. **73**(12): p. 858–868.
222. Wu, G., et al., *Glutathione metabolism and its implications for health.* The Journal of nutrition, 2004. **134**(3): p. 489–492.
223. Li, Y., G. Wei, and J. Chen, *Glutathione: a review on biotechnological production.* Applied microbiology and biotechnology, 2004. **66**(3): p. 233–242.
224. Minich, D.M. and B.I. Brown, *A review of dietary (phyto) nutrients for glutathione support.* Nutrients, 2019. **11**(9): p. 2073.
225. Richie, J.P., et al., *Randomized controlled trial of oral glutathione supplementation on body stores of glutathione.* European journal of nutrition, 2015. **54**(2): p. 251–263.
226. Søndergård, S.D., et al., *The effects of 3 weeks of oral glutathione supplementation on whole body insulin sensitivity in obese males with and without type 2 diabetes: A randomized trial.* Applied Physiology, Nutrition, and Metabolism, 2021. **46**(ja): p. 1133–1142.
227. Atkuri, K.R., et al., *N-Acetylcysteine—a safe antidote for cysteine/glutathione deficiency.* Current opinion in pharmacology, 2007. **7**(4): p. 355–359.
228. McPherson, R.A. and G. Hardy, *Clinical and nutritional benefits of cysteine-enriched protein supplements.* Current Opinion in Clinical Nutrition & Metabolic Care, 2011. **14**(6): p. 562–568.
229. Bounous, G. and P. Gold, *The biological activity of undenatured dietary whey proteins: role of glutathione.* Clin Invest Med, 1991. **14**(4): p. 296–309.
230. Bounous, G., G. Batist, and P. Gold, *Immunoenhancing property of dietary whey protein in mice: role of glutathione.* Clin Invest Med, 1989. **12**(3): p. 154–61.
231. Karelis, A., et al., *Effect of cysteine-rich whey protein (immunocal®) supplementation in combination with resistance training on muscle strength and lean body mass in non-frail elderly subjects: a randomized, double-blind controlled study.* The Journal of nutrition, health and aging, 2015. **19**(5): p. 531–536.
232. Luo, J.-L., et al., *Surgical trauma decreases glutathione synthetic capacity in human skeletal muscle tissue.* American Journal of Physiology-Endocrinology And Metabolism, 1998. **275**(2): p. E359–E365.

233. Bounous, G., *Whey protein concentrate (WPC) and glutathione modulation in cancer treatment.* Anticancer research, 2000. **20**(6): p. 4785–4792.
234. Grey, V., et al., *Improved glutathione status in young adult patients with cystic fibrosis supplemented with whey protein.* Journal of Cystic Fibrosis, 2003. **2**(4): p. 195–198.
235. Micke, P., et al., *Oral supplementation with whey proteins increases plasma glutathione levels of HIV-infected patients.* European journal of clinical investigation, 2001. **31**(2): p. 171–178.
236. Nguyen, D., et al., *Effect of increasing glutathione with cysteine and glycine supplementation on mitochondrial fuel oxidation, insulin sensitivity, and body composition in older HIV-infected patients.* The Journal of Clinical Endocrinology & Metabolism, 2014. **99**(1): p. 169–177.
237. Bumrungpert, A., et al., *Whey protein supplementation improves nutritional status, glutathione levels, and immune function in cancer patients: A randomized, double-blind controlled trial.* Journal of medicinal food, 2018. **21**(6): p. 612–616.
238. Gutman, J.B. and P.A. Kongshavn, *Cysteine/cystine-rich undenatured whey protein supplement in patients' pressure ulcers outcomes: an open label study.* Journal of wound care, 2019. **28**(Sup7): p. S16–S23.
239. McCarty, M.F. and J.J. DiNicolantonio, *An increased need for dietary cysteine in support of glutathione synthesis may underlie the increased risk for mortality associated with low protein intake in the elderly.* Age, 2015. **37**(5): p. 1–12.
240. Abbas, A.K., A.H. Lichtman, and S. Pillai, *Basic immunology: functions and disorders of the immune system*. 2015: Elsevier Health Sciences.
241. Furman, D., et al., *Chronic inflammation in the etiology of disease across the life span.* Nature medicine, 2019. **25**(12): p. 1822–1832.
242. Calder, P.C., et al., *Dietary factors and low-grade inflammation in relation to overweight and obesity.* British Journal of Nutrition, 2011. **106**(S3): p. S1–S78.
243. Bulló, M., et al., *Systemic inflammation, adipose tissue tumor necrosis factor, and leptin expression.* Obesity research, 2003. **11**(4): p. 525–531.
244. Park, H.S., J.Y. Park, and R. Yu, *Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-α and IL-6.* Diabetes research and clinical practice, 2005. **69**(1): p. 29–35.
245. Festa, A., et al., *The relation of body fat mass and distribution to markers of chronic inflammation.* International journal of obesity, 2001. **25**(10): p. 1407–1415.
246. Bastard, J.P., et al., *Recent advances in the relationship between obesity, inflammation, and insulin resistance.* Eur Cytokine Netw, 2006. **17**(1): p. 4–12.
247. Hotamisligil, G.S., *Inflammation and metabolic disorders.* Nature, 2006. **444**(7121): p. 860–867.
248. Lacativa, P.G.S. and M.L.F.d. Farias, *Osteoporosis and inflammation.* Arquivos Brasileiros de Endocrinologia & Metabologia, 2010. **54**(2): p. 123–132.
249. Castellani, P., E. Balza, and A. Rubartelli, *Inflammation, DAMPs, tumor development, and progression: a vicious circle orchestrated by redox signaling.* Antioxidants & redox signaling, 2014. **20**(7): p. 1086–1097.
250. Biswas, S.K., *Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox?* Oxidative medicine and cellular longevity, 2016. **2016**: p. 5698931.

251. Villarreal-Molina, M.T. and B. Antuna-Puente, *Adiponectin: Anti-inflammatory and cardioprotective effects.* Biochimie, 2012. **94**(10): p. 2143–2149.
252. Chaturvedi, A.K., et al., *Evaluation of multiplexed cytokine and inflammation marker measurements: a methodologic study.* Cancer epidemiology, biomarkers & prevention, 2011. **20**(9): p. 1902–1911.
253. Scheller, J., et al., *The pro- and anti-inflammatory properties of the cytokine interleukin-6.* Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 2011. **1813**(5): p. 878–888.
254. Velazquez-Salinas, L., et al., *The role of interleukin 6 during viral infections.* Frontiers in microbiology, 2019. **10**: p. 1057.
255. Chen, Y.-L., et al., *Correlation between serum interleukin-6 level and type 1 diabetes mellitus: A systematic review and meta-analysis.* Cytokine, 2017. **94**: p. 14–20.
256. Starr, M.E., B.M. Evers, and H. Saito, *Age-associated increase in cytokine production during systemic inflammation: adipose tissue as a major source of IL-6.* Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences, 2009. **64**(7): p. 723–730.
257. Petersen, A. and B. Pedersen, *The role of IL-6 in mediating the anti inflammatory.* J physiol pharmacol, 2006. **57**(Suppl 10): p. 43–51.
258. Leuchtmann, A.B., et al., *Interleukin-6 potentiates endurance training adaptation and improves functional capacity in old mice.* Journal of cachexia, sarcopenia and muscle, 2022. **13**(2): p. 1164–1176.
259. Nash, D., et al., *IL-6 signaling in acute exercise and chronic training: Potential consequences for health and athletic performance.* Scandinavian Journal of Medicine & Science in Sports, 2023. **33**(1): p. 4–19.
260. Zegeye, M.M., et al., *Activation of the JAK/STAT3 and PI3K/AKT pathways are crucial for IL-6 trans-signaling-mediated pro-inflammatory response in human vascular endothelial cells.* Cell Communication and Signaling, 2018. **16**: p. 1–10.
261. Bin Dhuban, K., et al., *Signaling through gp130 compromises suppressive function in human FOXP3+ regulatory T cells.* Frontiers in immunology, 2019. **10**: p. 1532.
262. Orange, S.T., et al., *The exercise IL-6 enigma in cancer.* Trends in Endocrinology & Metabolism, 2023. **34**(11): p. 749–763.
263. Keller, C., et al., *Effect of exercise, training, and glycogen availability on IL-6 receptor expression in human skeletal muscle.* Journal of Applied Physiology, 2005. **99**(6): p. 2075–2079.
264. Gray, S.R., M. Robinson, and M.A. Nimmo, *Response of plasma IL-6 and its soluble receptors during submaximal exercise to fatigue in sedentary middle-aged men.* Cell Stress and Chaperones, 2008. **13**(2): p. 247–251.
265. Walshe, I., et al., *The reliability of the IL-6, sIL-6R and sgp130 response to a preloaded time trial.* European journal of applied physiology, 2010. **110**(3): p. 619–625.
266. Jostock, T., et al., *Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses.* European journal of biochemistry, 2001. **268**(1): p. 160–167.
267. Akbari, M. and V. Hassan-Zadeh, *IL-6 signalling pathways and the development of type 2 diabetes.* Inflammopharmacology, 2018. **26**(3): p. 685–698.
268. Oh, D.Y., et al., *Increased macrophage migration into adipose tissue in obese mice.* Diabetes, 2012. **61**(2): p. 346–354.

269. Amano, S.U., et al., *Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation.* Cell metabolism, 2014. **19**(1): p. 162–171.
270. Apovian, C.M., et al., *Adipose macrophage infiltration is associated with insulin resistance and vascular endothelial dysfunction in obese subjects.* Arterioscler Thromb Vasc Biol, 2008. **28**(9): p. 1654–9.
271. Kraakman, M.J., et al., *Blocking IL-6 trans-signaling prevents high-fat diet-induced adipose tissue macrophage recruitment but does not improve insulin resistance.* Cell metabolism, 2015. **21**(3): p. 403–416.
272. Zelová, H. and J. Hošek, *TNF-α signalling and inflammation: interactions between old acquaintances.* Inflammation research, 2013. **62**: p. 641–651.
273. Cawthorn, W.P. and J.K. Sethi, *TNF-α and adipocyte biology.* FEBS Letters, 2008. **582**(1): p. 117–131.
274. Sullivan, K.E., *Regulation of inflammation.* Immunologic Research, 2003. **27**(2): p. 529–537.
275. Dinarello, C.A., *Immunological and inflammatory functions of the interleukin-1 family.* Annual review of immunology, 2009. **27**(1): p. 519–550.
276. Akdis, M., et al., *Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: Receptors, functions, and roles in diseases.* Journal of Allergy and Clinical Immunology, 2016. **138**(4): p. 984–1010.
277. Agostini, L., et al., *NALP3 forms an IL-1β-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder.* Immunity, 2004. **20**(3): p. 319–325.
278. Ghanbari, M., et al., *Interleukin-1 in obesity-related low-grade inflammation: From molecular mechanisms to therapeutic strategies.* International Immunopharmacology, 2021. **96**: p. 107765.
279. Huang, L.-Y., et al., *Interferon Family Cytokines in Obesity and Insulin Sensitivity.* Cells, 2022. **11**(24): p. 4041.
280. Schmidt, F.M., et al., *Inflammatory cytokines in general and central obesity and modulating effects of physical activity.* PloS one, 2015. **10**(3): p. e0121971.
281. Munteanu, C. and B. Schwartz, *The relationship between nutrition and the immune system.* Frontiers in nutrition, 2022. **9**: p. 1082500.
282. Chatterjee, P., et al., *Regulation of the Anti-Inflammatory Cytokines Interleukin-4 and Interleukin-10 during Pregnancy.* Frontiers in Immunology, 2014. **Volume 5 - 2014**.
283. Shaikh, P.Z., *Cytokines & their physiologic and pharmacologic functions in inflammation: A review.* International Journal of Pharmacy & Life Sciences, 2011. **2**(11).
284. Brown, M.A. and J. Hural, *Functions of IL-4 and control of its expression.* Critical Reviews™ in Immunology, 2017. **37**(2-6).
285. Howard, M. and A. O'Garra, *Biological properties of interleukin 10.* Immunology today, 1992. **13**(6): p. 198–200.
286. Opal, S.M., J.C. Wherry, and P. Grint, *Interleukin-10: potential benefits and possible risks in clinical infectious diseases.* Clinical infectious diseases, 1998. **27**(6): p. 1497–1507.
287. Du Clos, T.W., *Function of C-reactive protein.* Annals of Medicine, 2000. **32**(4): p. 274–278.
288. Hage, F.G. and A.J. Szalai, *C-reactive protein gene polymorphisms, C-reactive protein blood levels, and cardiovascular disease risk.* Journal of the American college of cardiology, 2007. **50**(12): p. 1115–1122.

289. Ellulu, M.S., et al., *Obesity and inflammation: the linking mechanism and the complications.* Archives of medical science: AMS, 2017. **13**(4): p. 851.
290. Sproston, N.R. and J.J. Ashworth, *Role of C-reactive protein at sites of inflammation and infection.* Frontiers in immunology, 2018. **9**: p. 754.
291. Allen, J., Y. Sun, and J.A. Woods, *Chapter Fourteen - Exercise and the Regulation of Inflammatory Responses*, in *Progress in Molecular Biology and Translational Science*, C. Bouchard, Editor. 2015, Academic Press. p. 337–354.
292. Peake, J.M., et al., *Muscle damage and inflammation during recovery from exercise.* Journal of applied physiology, 2017. **122**(3): p. 559–570.
293. Liu, C.-C. and J.M. Ahearn, *Acute-phase proteins and inflammation: immunological and clinical implications*, in *Measuring Immunity*. 2005, Elsevier. p. 131–143.
294. Kaspar, F., et al., *Acute-phase inflammatory response to single-bout HIIT and endurance training: a comparative study.* Mediators of inflammation, 2016. **2016**: p. 5474837.
295. Kouvelioti, R., et al., *Cytokine and Sclerostin Response to High-Intensity Interval Running versus Cycling.* Medicine and science in sports and exercise, 2019. **51**(12): p. 2458–2464.
296. Kurgan, N., et al., *Cytokines, Adipokines and Bone Markers at Rest and in Response to Plyometric Exercise in Obese vs Normal Weight Adolescent Females.* Frontiers in endocrinology, 2020. **11**: p. 971.
297. Mezil, Y.A., et al., *Response of bone turnover markers and cytokines to high-intensity low-impact exercise.* Med Sci Sports Exerc, 2015. **47**(7): p. 1495–502.
298. Fraschetti, E.C., et al., *The acute effects of milk consumption on systemic inflammation after combined resistance and plyometric exercise in young adult females.* Nutrients, 2022. **14**(21): p. 4532.
299. Brenner, I., et al., *Impact of three different types of exercise on components of the inflammatory response.* European journal of applied physiology and occupational physiology, 1999. **80**(5): p. 452–460.
300. Nieman, D.C., et al., *Variance in the acute inflammatory response to prolonged cycling is linked to exercise intensity.* Journal of Interferon & Cytokine Research, 2012. **32**(1): p. 12–17.
301. Petersen, A.M. and B.K. Pedersen, *The anti-inflammatory effect of exercise.* J Appl Physiol (1985), 2005. **98**(4): p. 1154–62.
302. Gonzalo-Encabo, P., et al., *The role of exercise training on low-grade systemic inflammation in adults with overweight and obesity: A systematic review.* International journal of environmental research and public health, 2021. **18**(24): p. 13258.
303. Jackson, K.G., et al., *Acute effects of meal fatty acids on postprandial NEFA, glucose and apo E response: implications for insulin sensitivity and lipoprotein regulation?* British journal of nutrition, 2005. **93**(5): p. 693–700.
304. Aljada, A., et al., *Increase in intranuclear nuclear factor κB and decrease in inhibitor κB in mononuclear cells after a mixed meal: evidence for a proinflammatory effect.* The American journal of clinical nutrition, 2004. **79**(4): p. 682–690.
305. van Dijk, S.J., et al., *Responses to high-fat challenges varying in fat type in subjects with different metabolic risk phenotypes: a randomized trial.* PloS one, 2012. **7**(7): p. e41388.
306. Adamska, E., et al., *Intake of meals containing high levels of carbohydrates or high levels of unsaturated fatty acids induces postprandial dysmetabolism in young overweight/obese men.* BioMed Research International, 2015. **2015**(1): p. 147196.

307. O'Keefe, J.H. and D.S. Bell, *Postprandial hyperglycemia/hyperlipidemia (postprandial dysmetabolism) is a cardiovascular risk factor.* The American journal of cardiology, 2007. **100**(5): p. 899–904.
308. Nappo, F., et al., *Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals.* Journal of the American College of Cardiology, 2002. **39**(7): p. 1145–1150.
309. Miglio, C., et al., *Antioxidant and inflammatory response following high-fat meal consumption in overweight subjects.* European Journal of Nutrition, 2013. **52**(3): p. 1107–1114.
310. Schwander, F., et al., *A dose-response strategy reveals differences between normal-weight and obese men in their metabolic and inflammatory responses to a high-fat meal.* The Journal of nutrition, 2014. **144**(10): p. 1517–1523.
311. Gregersen, S., et al., *Inflammatory and oxidative stress responses to high-carbohydrate and high-fat meals in healthy humans.* Journal of nutrition and metabolism, 2012. **2012**(1): p. 238056.
312. Cardona, F., et al., *Circulating antioxidant defences are decreased in healthy people after a high-fat meal.* British Journal of Nutrition, 2008. **100**(2): p. 312–316.
313. Anderson, E.J., et al., *Mitochondrial H 2 O 2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans.* The Journal of clinical investigation, 2009. **119**(3): p. 573–581.
314. Bansal, S., et al., *Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women.* Jama, 2007. **298**(3): p. 309–316.
315. Cavalot, F., et al., *Postprandial blood glucose is a stronger predictor of cardiovascular events than fasting blood glucose in type 2 diabetes mellitus, particularly in women: lessons from the San Luigi Gonzaga Diabetes Study.* The Journal of Clinical Endocrinology & Metabolism, 2006. **91**(3): p. 813–819.
316. Klop, B., et al., *Understanding postprandial inflammation and its relationship to lifestyle behaviour and metabolic diseases.* International journal of vascular medicine, 2012. **2012**: p. 947417.
317. Donath, M.Y., D.T. Meier, and M. Böni-Schnetzler, *Inflammation in the pathophysiology and therapy of cardiometabolic disease.* Endocrine reviews, 2019. **40**(4): p. 1080–1091.
318. Koenig, W., et al., *C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992.* Circulation, 1999. **99**(2): p. 237–242.
319. Pradhan, A.D., et al., *C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus.* Jama, 2001. **286**(3): p. 327–334.
320. Ridker, P.M., et al., *Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men.* Circulation, 2000. **101**(15): p. 1767–1772.
321. Tamakoshi, K., et al., *The metabolic syndrome is associated with elevated circulating C-reactive protein in healthy reference range, a systemic low-grade inflammatory state.* International journal of obesity, 2003. **27**(4): p. 443–449.
322. Franceschi, C. and J. Campisi, *Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases.* Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences, 2014. **69**(Suppl_1): p. S4–S9.

323. Moosavian, S.P., et al., *Effects of dairy products consumption on inflammatory biomarkers among adults: A systematic review and meta-analysis of randomized controlled trials.* Nutrition, Metabolism and Cardiovascular Diseases, 2020. **30**(6): p. 872–888.
324. Nieman, K.M., B.D. Anderson, and C.J. Cifelli, *The effects of dairy product and dairy protein intake on inflammation: a systematic review of the literature.* Journal of the American College of Nutrition, 2021. **40**(6): p. 571–582.
325. Labonte, M.-E., et al., *Impact of dairy products on biomarkers of inflammation: a systematic review of randomized controlled nutritional intervention studies in overweight and obese adults.* The American journal of clinical nutrition, 2013. **97**(4): p. 706–717.
326. Zemel, M.B. and X. Sun, *Dietary calcium and dairy products modulate oxidative and inflammatory stress in mice and humans.* The Journal of nutrition, 2008. **138**(6): p. 1047–1052.
327. Burdge, G.C. and P.C. Calder, *Plasma cytokine response during the postprandial period: a potential causal process in vascular disease?* British journal of nutrition, 2005. **93**(1): p. 3–9.
328. Mah, E. and R.S. Bruno, *Postprandial hyperglycemia on vascular endothelial function: mechanisms and consequences.* Nutrition research, 2012. **32**(10): p. 727–740.
329. Opinto, G., A. Natalicchio, and P. Marchetti, *Physiology of incretins and loss of incretin effect in type 2 diabetes and obesity.* Archives of physiology and biochemistry, 2013. **119**(4): p. 170–178.
330. Rogers, T.S., et al., *The role of a dairy fraction rich in milk fat globule membrane in the suppression of postprandial inflammatory markers and bone turnover in obese and overweight adults: an exploratory study.* Nutrition & Metabolism, 2017. **14**(1): p. 36.
331. Ricci-Cabello, I., M. Olalla Herrera, and R. Artacho, *Possible role of milk-derived bioactive peptides in the treatment and prevention of metabolic syndrome.* Nutrition reviews, 2012. **70**(4): p. 241–255.
332. Turpeinen, A.M., et al., *Antihypertensive effects of bioactive tripeptides—a random effects meta-analysis.* Annals of Medicine, 2013. **45**(1): p. 51–56.
333. Ringseis, R., et al., *Peptides and hydrolysates from casein and soy protein modulate the release of vasoactive substances from human aortic endothelial cells.* Biochimica et Biophysica Acta (BBA)-General Subjects, 2005. **1721**(1-3): p. 89–97.
334. Rousseau-Ralliard, D., et al., *Inhibitory effect of αS1-and αS2-casein hydrolysates on angiotensin I-converting enzyme in human endothelial cells in vitro, rat aortic tissue ex vivo, and renovascular hypertensive rats in vivo.* Journal of Dairy Science, 2010. **93**(7): p. 2906–2921.
335. Aihara, K., H. Ishii, and M. Yoshida, *Casein-derived tripeptide, Val-Pro-Pro (VPP), modulates monocyte adhesion to vascular endothelium.* Journal of Atherosclerosis and Thrombosis, 2009. **16**(5): p. 594–603.
336. Enomoto, H., et al., *Glycation and phosphorylation of α-lactalbumin by dry heating: Effect on protein structure and physiological functions.* Journal of Dairy Science, 2009. **92**(7): p. 3057–3068.
337. Håversen, L., et al., *Lactoferrin down-regulates the LPS-induced cytokine production in monocytic cells via NF-κB.* Cellular immunology, 2002. **220**(2): p. 83–95.

338. Mattsby-Baltzer, I., et al., *Lactoferrin or a fragment thereof inhibits the endotoxin-induced interleukin-6 response in human monocytic cells.* Pediatric Research, 1996. **40**(2): p. 257–262.
339. Garcia-Macedo, R., et al., *Glycine increases mRNA adiponectin and diminishes pro-inflammatory adipokines expression in 3T3-L1 cells.* European journal of pharmacology, 2008. **587**(1-3): p. 317–321.
340. Spittler, A., et al., *Immunomodulatory effects of glycine on LPS-treated monocytes: reduced TNF-α production and accelerated IL-10 expression.* The FASEB journal, 1999. **13**(3): p. 563–571.
341. Kanikarla-Marie, P. and S.K. Jain, *L-Cysteine supplementation reduces high-glucose and ketone-induced adhesion of monocytes to endothelial cells by inhibiting ROS.* Molecular and cellular biochemistry, 2014. **391**: p. 251–256.
342. Hasegawa, S., et al., *Cysteine, histidine and glycine exhibit anti-inflammatory effects in human coronary arterial endothelial cells.* Clinical & Experimental Immunology, 2012. **167**(2): p. 269–274.
343. Ohira, H., et al., *Butyrate attenuates inflammation and lipolysis generated by the interaction of adipocytes and macrophages.* Journal of atherosclerosis and thrombosis, 2013. **20**(5): p. 425–442.
344. Zapolska-Downar, D., et al., *Butyrate inhibits cytokine-induced VCAM-1 and ICAM-1 expression in cultured endothelial cells: the role of NF-κB and PPARα.* The Journal of nutritional biochemistry, 2004. **15**(4): p. 220–228.
345. Murumalla, R.K., et al., *Fatty acids do not pay the toll: effect of SFA and PUFA on human adipose tissue and mature adipocytes inflammation.* Lipids in health and disease, 2012. **11**: p. 1–9.
346. Granados, N., et al., *Distinct effects of oleic acid and its trans-isomer elaidic acid on the expression of myokines and adipokines in cell models.* British journal of nutrition, 2011. **105**(8): p. 1226–1234.
347. Cullberg, K., et al., *Effects of LPS and dietary free fatty acids on MCP-1 in 3T3-L1 adipocytes and macrophages in vitro.* Nutrition & diabetes, 2014. **4**(3): p. e113–e113.
348. Soto-Vaca, A., et al., *Differential effect of 14 free fatty acids in the expression of inflammation markers on human arterial coronary cells.* Journal of agricultural and food chemistry, 2013. **61**(42): p. 10074–10079.
349. Månsson, H.L., *Fatty acids in bovine milk fat.* Food & nutrition research, 2008. **52**: p. 10.3402/fnr. v52i0. 1821.
350. Gebauer, S.K., et al., *Effects of ruminant trans fatty acids on cardiovascular disease and cancer: a comprehensive review of epidemiological, clinical, and mechanistic studies.* Advances in nutrition, 2011. **2**(4): p. 332–354.
351. Zhu, Y., et al., *Calcium and 1α, 25-dihydroxyvitamin D3 target the TNF-α pathway to suppress experimental inflammatory bowel disease.* European journal of immunology, 2005. **35**(1): p. 217–224.
352. Westphal, S., et al., *Postprandial lipid and carbohydrate responses after the ingestion of a casein-enriched mixed meal.* The American journal of clinical nutrition, 2004. **80**(2): p. 284–290.
353. Pei, R., et al., *Evidence for the effects of yogurt on gut health and obesity.* Critical reviews in food science and nutrition, 2017. **57**(8): p. 1569–1583.

354. Putt, K.K., et al., *Yogurt inhibits intestinal barrier dysfunction in Caco-2 cells by increasing tight junctions.* Food & function, 2017. **8**(1): p. 406–414.
355. Song, Y., et al., *Magnesium intake and plasma concentrations of markers of systemic inflammation and endothelial dysfunction in women.* The American journal of clinical nutrition, 2007. **85**(4): p. 1068–1074.
356. Marcotorchino, J., et al., *Vitamin D reduces the inflammatory response and restores glucose uptake in adipocytes.* Molecular nutrition & food research, 2012. **56**(12): p. 1771–1782.
357. Cannell, J.J., W.B. Grant, and M.F. Holick, *Vitamin D and inflammation.* Dermato-endocrinology, 2014. **6**(1): p. e983401.
358. Azizieh, F., K.O. Alyahya, and R. Raghupathy, *Association between levels of vitamin D and inflammatory markers in healthy women.* Journal of Inflammation Research, 2016: p. 51–57.
359. Krajewska, M., et al., *Vitamin D effects on selected anti-inflammatory and pro-inflammatory markers of obesity-related chronic inflammation.* Frontiers in Endocrinology, 2022. **13**: p. 920340.
360. Mocchegiani, E. and M. Malavolta, *Role of zinc and selenium in oxidative stress and immunosenescence: implications for healthy ageing and longevity*, in *Handbook on immunosenescence*. 2009, Springer. p. 1367–1396.
361. Fardet, A. and E. Rock, *In vitro and in vivo antioxidant potential of milks, yoghurts, fermented milks and cheeses: a narrative review of evidence.* Nutrition research reviews, 2018. **31**(1): p. 52–70.
362. Power, O., P. Jakeman, and R. FitzGerald, *Antioxidative peptides: enzymatic production, in vitro and in vivo antioxidant activity and potential applications of milk-derived antioxidative peptides.* Amino acids, 2013. **44**(3): p. 797–820.
363. Tamanna, N., et al., *The effect of short-term methionine restriction on glutathione synthetic capacity and antioxidant responses at the whole tissue and mitochondrial level in the rat liver.* Experimental gerontology, 2019. **127**: p. 110712.
364. Choi, I.-Y., et al., *Milk Intake Enhances Cerebral Antioxidant (Glutathione) Concentration in Older Adults: A Randomized Controlled Intervention Study.* Current Developments in Nutrition, 2021. **5**(Supplement_2): p. 900–900.
365. Deth, R., et al., *Clinical evaluation of glutathione concentrations after consumption of milk containing different subtypes of β-casein: results from a randomized, cross-over clinical trial.* Nutrition journal, 2015. **15**(1): p. 1–6.
366. Asemi, Z., et al., *Effect of daily consumption of probiotic yogurt on oxidative stress in pregnant women: a randomized controlled clinical trial.* Annals of Nutrition and Metabolism, 2012. **60**(1): p. 62–68.
367. Ejtahed, H.S., et al., *Probiotic yogurt improves antioxidant status in type 2 diabetic patients.* Nutrition, 2012. **28**(5): p. 539–543.
368. Lopez-Miranda, J., C. Williams, and D. Lairon, *Dietary, physiological, genetic and pathological influences on postprandial lipid metabolism.* British Journal of Nutrition, 2007. **98**(3): p. 458–473.
369. Clemente-Suárez, V.J., et al., *Global impacts of western diet and its effects on metabolism and health: A narrative review.* Nutrients, 2023. **15**(12): p. 2749.
370. Walsh, M.C. and Y. Choi, *Biology of the RANKL–RANK–OPG system in immunity, bone, and beyond.* Frontiers in immunology, 2014. **5**: p. 511.

371. Tsukasaki, M. and H. Takayanagi, *Osteoimmunology: evolving concepts in bone–immune interactions in health and disease.* Nature Reviews Immunology, 2019. **19**(10): p. 626–642.
372. Nakashima, T., et al., *Evidence for osteocyte regulation of bone homeostasis through RANKL expression.* Nature medicine, 2011. **17**(10): p. 1231–1234.
373. Nakashima, T. and H. Takayanagi, *Osteoimmunology: crosstalk between the immune and bone systems.* Journal of clinical immunology, 2009. **29**(5): p. 555–567.
374. Snelling, A.M., et al., *Modifiable and nonmodifiable factors associated with osteoporosis in postmenopausal women: results from the Third National Health and Nutrition Examination Survey, 1988–1994.* Journal of women's health & gender-based medicine, 2001. **10**(1): p. 57–65.
375. Lennox, G.M., et al., *Non-modifiable risk factors for stress fractures in military personnel undergoing training: a systematic review.* International journal of environmental research and public health, 2021. **19**(1): p. 422.
376. Wilson-Barnes, S.L., S.A. Lanham-New, and H. Lambert, *Modifiable risk factors for bone health & fragility fractures.* Best Practice & Research Clinical Rheumatology, 2022. **36**(3): p. 101758.
377. Leblanc, A.D., et al., *Bone mineral loss and recovery after 17 weeks of bed rest.* Journal of bone and mineral research, 1990. **5**(8): p. 843–850.
378. Robling, A.G., A.B. Castillo, and C.H. Turner, *Biomechanical and molecular regulation of bone remodeling.* Annu. Rev. Biomed. Eng., 2006. **8**(1): p. 455–498.
379. Kohrt, W.M., et al., *Maintenance of serum ionized calcium during exercise attenuates parathyroid hormone and bone resorption responses.* Journal of Bone and Mineral Research, 2018. **33**(7): p. 1326–1334.
380. Ha, H., et al., *Reactive oxygen species mediate RANK signaling in osteoclasts.* Experimental cell research, 2004. **301**(2): p. 119–127.
381. Kirk, B., et al., *Muscle, bone, and fat crosstalk: the biological role of myokines, osteokines, and adipokines.* Current Osteoporosis Reports, 2020. **18**(4): p. 388–400.
382. Wolff, I.v., et al., *The effect of exercise training programs on bone mass: a meta-analysis of published controlled trials in pre-and postmenopausal women.* Osteoporosis international, 1999. **9**(1): p. 1–12.
383. Behringer, M., et al., *Effects of weight-bearing activities on bone mineral content and density in children and adolescents: a meta-analysis.* Journal of Bone and Mineral Research, 2014. **29**(2): p. 467–478.
384. Marques, E.A., J. Mota, and J. Carvalho, *Exercise effects on bone mineral density in older adults: a meta-analysis of randomized controlled trials.* Age, 2012. **34**(6): p. 1493–1515.
385. Cerqueira, É., et al., *Inflammatory effects of high and moderate intensity exercise—a systematic review.* Frontiers in physiology, 2020. **10**: p. 489354.
386. Moldoveanu, A.I., R.J. Shephard, and P.N. Shek, *The cytokine response to physical activity and training.* Sports medicine, 2001. **31**(2): p. 115–144.
387. Scott, J.P., et al., *Effect of fasting versus feeding on the bone metabolic response to running.* Bone, 2012. **51**(6): p. 990–999.
388. Rantalainen, T., et al., *Short-term bone biochemical response to a single bout of high-impact exercise.* Journal of sports science & medicine, 2009. **8**(4): p. 553.
389. Babraj, J.A., et al., *Human bone collagen synthesis is a rapid, nutritionally modulated process.* Journal of Bone and Mineral Research, 2005. **20**(6): p. 930–937.

390. Benjamini, Y. and Y. Hochberg, *Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing.* Journal of the Royal Statistical Society: Series B (Methodological), 2018. **57**(1): p. 289–300.
391. Hardy, R. and M. Cooper, *Bone loss in inflammatory disorders.* Journal of Endocrinology, 2009. **201**(3): p. 309–320.
392. Qi, Z., W. Liu, and J. Lu, *The mechanisms underlying the beneficial effects of exercise on bone remodeling: Roles of bone-derived cytokines and microRNAs.* Progress in Biophysics and Molecular Biology, 2016. **122**(2): p. 131–139.
393. Feghali, C.A. and T.M. Wright, *Cytokines in acute and chronic inflammation.* Front Biosci, 1997. **2**(1): p. d12–d26.
394. Chang, M.K., et al., *Osteal tissue macrophages are intercalated throughout human and mouse bone lining tissues and regulate osteoblast function in vitro and in vivo.* The Journal of Immunology, 2008. **181**(2): p. 1232–1244.
395. Hofbauer, L.C., et al., *Osteoprotegerin production by human osteoblast lineage cells is stimulated by vitamin D, bone morphogenetic protein-2, and cytokines.* Biochemical and biophysical research communications, 1998. **250**(3): p. 776–781.
396. Weitzmann, M.N., et al., *Increased production of IL-7 uncouples bone formation from bone resorption during estrogen deficiency.* The Journal of clinical investigation, 2002. **110**(11): p. 1643–1650.
397. Li, Y., et al., *B cells and T cells are critical for the preservation of bone homeostasis and attainment of peak bone mass in vivo.* Blood, 2007. **109**(9): p. 3839–48.
398. Weitzmann, M.N., *The Role of Inflammatory Cytokines, the RANKL/OPG Axis, and the Immunoskeletal Interface in Physiological Bone Turnover and Osteoporosis.* Scientifica, 2013. **2013**(1): p. 125705.
399. Schlagheck, M.L., et al., *Cellular immune response to acute exercise: Comparison of endurance and resistance exercise.* European journal of haematology, 2020. **105**(1): p. 75–84.
400. Tsukasaki, M., et al., *OPG production matters where it happened.* Cell Reports, 2020. **32**(10): p. 108124.
401. Takeuchi, T., H. Yoshida, and S. Tanaka, *Role of interleukin-6 in bone destruction and bone repair in rheumatoid arthritis.* Autoimmunity Reviews, 2021. **20**(9): p. 102884.
402. Kishimoto, T., *Interleukin-6: discovery of a pleiotropic cytokine.* Arthritis Res Ther, 2006. **8 Suppl 2**(Suppl 2): p. S2.
403. Ellingsgaard, H., P. Hojman, and B.K. Pedersen, *Exercise and health — emerging roles of IL-6.* Current Opinion in Physiology, 2019. **10**: p. 49–54.
404. Nara, H. and R. Watanabe, *Anti-Inflammatory Effect of Muscle-Derived Interleukin-6 and Its Involvement in Lipid Metabolism.* International Journal of Molecular Sciences, 2021. **22**(18): p. 9889.
405. Blanchard, F., et al., *The dual role of IL-6-type cytokines on bone remodeling and bone tumors.* Cytokine & Growth Factor Reviews, 2009. **20**(1): p. 19–28.
406. Hashizume, M., N. Hayakawa, and M. Mihara, *IL-6 trans-signalling directly induces RANKL on fibroblast-like synovial cells and is involved in RANKL induction by TNF-α and IL-17.* Rheumatology, 2008. **47**(11): p. 1635–1640.
407. Palmqvist, P., et al., *IL-6, leukemia inhibitory factor, and oncostatin M stimulate bone resorption and regulate the expression of receptor activator of NF-kappa B ligand,*

*osteoprotegerin, and receptor activator of NF-kappa B in mouse calvariae.* Journal of immunology (Baltimore, Md.: 1950), 2002. **169**(6): p. 3353–3362.
408. Hu, X., et al., *Expression of RANKL by peripheral neutrophils and its association with bone mineral density in COPD.* Respirology, 2017. **22**(1): p. 126–132.
409. Fadda, S., et al., *Serum levels of osteoprotegerin and RANKL in patients with rheumatoid arthritis and their relation to bone mineral density and disease activity.* The Egyptian Rheumatologist, 2015. **37**(1): p. 1–6.
410. Gilbert, L., et al., *Inhibition of osteoblast differentiation by tumor necrosis factor-α.* Endocrinology, 2000. **141**(11): p. 3956–3964.
411. Baek, K., et al., *TNF-α upregulates sclerostin expression in obese mice fed a high-fat diet.* Journal of cellular physiology, 2014. **229**(5): p. 640–650.
412. Kouvelioti, R., et al., *Response of sclerostin and bone turnover markers to high intensity interval exercise in young women: does impact matter?* BioMed research international, 2018. **2018**.
413. De Heredia, F.P., S. Gómez-Martínez, and A. Marcos, *Obesity, inflammation and the immune system.* Proceedings of the Nutrition Society, 2012. **71**(2): p. 332–338.
414. Khanna, D., et al., *Obesity: A Chronic Low-Grade Inflammation and Its Markers.* Cureus, 2022. **14**(2): p. e22711.
415. Klein, S., et al., *Clinical implications of obesity with specific focus on cardiovascular disease: a statement for professionals from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism: endorsed by the American College of Cardiology Foundation.* Circulation, 2004. **110**(18): p. 2952–2967.
416. Grosso, G., et al., *Anti-inflammatory nutrients and obesity-associated metabolic-inflammation: state of the art and future direction.* Nutrients, 2022. **14**(6): p. 1137.
417. Dumas, A.-A., et al., *A systematic review of the effect of yogurt consumption on chronic diseases risk markers in adults.* European Journal of Nutrition, 2017. **56**(4): p. 1375–1392.
418. Moyen, A., et al., *Relative Validation of an Artificial Intelligence–Enhanced, Image-Assisted Mobile App for Dietary Assessment in Adults: Randomized Crossover Study.* Journal of Medical Internet Research, 2022. **24**(11): p. e40449.
419. Ross, R., et al., *Canadian 24-Hour Movement Guidelines for Adults aged 18–64 years and Adults aged 65 years or older: an integration of physical activity, sedentary behaviour, and sleep.* Applied physiology, nutrition, and metabolism, 2020. **45**(10): p. S57–S102.
420. Grundy, S.M., et al., *Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition.* Circulation, 2004. **109**(3): p. 433–8.
421. Kang, S., et al., *Validity of the portable ultrasound BodyMetrix™ BX-2000 for measuring body fat percentage.* Sustainability, 2020. **12**(21): p. 8786.
422. Friedewald, W.T., R.I. Levy, and D.S. Fredrickson, *Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge.* Clinical chemistry, 1972. **18**(6): p. 499–502.
423. Giustarini, D., et al., *Analysis of GSH and GSSG after derivatization with N-ethylmaleimide.* Nature protocols, 2013. **8**(9): p. 1660.

424. Turnbull, P.C., M.C. Hughes, and C.G. Perry, *The fatty acid derivative palmitoylcarnitine abrogates colorectal cancer cell survival by depleting glutathione.* American Journal of Physiology-Cell Physiology, 2019. **317**(6): p. C1278–C1288.
425. Hughes, M.C., et al., *Impairments in left ventricular mitochondrial bioenergetics precede overt cardiac dysfunction and remodelling in Duchenne muscular dystrophy.* The Journal of physiology, 2020. **598**(7): p. 1377–1392.
426. Hughes, M.C., et al., *Early myopathy in Duchenne muscular dystrophy is associated with elevated mitochondrial H2O2 emission during impaired oxidative phosphorylation.* Journal of cachexia, sarcopenia and muscle, 2019. **10**(3): p. 643–661.
427. Delfinis, L.J., et al., *Muscle weakness precedes atrophy during cancer cachexia and is linked to muscle-specific mitochondrial stress.* JCI insight, 2022. **7**(24).
428. Fuller, K.N., et al., *Oral combined contraceptives induce liver mitochondrial reactive oxygen species and whole-body metabolic adaptations in female mice.* The Journal of Physiology, 2022. **600**(24): p. 5215–5245.
429. Thijssen, D.H., et al., *Assessment of flow-mediated dilation in humans: a methodological and physiological guideline.* Am J Physiol Heart Circ Physiol, 2011. **300**(1): p. H2–12.
430. Glickman, M.E., S.R. Rao, and M.R. Schultz, *False discovery rate control is a recommended alternative to Bonferroni-type adjustments in health studies.* Journal of Clinical Epidemiology, 2014. **67**(8): p. 850–857.
431. Benjamini, Y., A.M. Krieger, and D. Yekutieli, *Adaptive linear step-up procedures that control the false discovery rate.* Biometrika, 2006. **93**(3): p. 491–507.
432. Cohen, J., *A power primer.* Psychological bulletin, 1992. **112**(1): p. 155–159.
433. Labonté, M.-È., et al., *Dairy Product Consumption Has No Impact on Biomarkers of Inflammation among Men and Women with Low-Grade Systemic Inflammation.* The Journal of Nutrition, 2014. **144**(11): p. 1760–1767.
434. Pei, R., et al., *Low-fat yogurt consumption reduces biomarkers of chronic inflammation and inhibits markers of endotoxin exposure in healthy premenopausal women: a randomised controlled trial.* British Journal of Nutrition, 2017. **118**(12): p. 1043–1051.
435. Hauner, H., et al., *Plasma concentrations of soluble TNF-alpha receptors in obese subjects.* International journal of obesity, 1998. **22**(12): p. 1239–1243.
436. Medzhitov, R., *Origin and physiological roles of inflammation.* Nature, 2008. **454**(7203): p. 428–435.
437. van Meijl, L.E. and R.P. Mensink, *Effects of low-fat dairy consumption on markers of low-grade systemic inflammation and endothelial function in overweight and obese subjects: an intervention study.* British journal of nutrition, 2010. **104**(10): p. 1523–1527.
438. Opal, S.M. and V.A. DePalo, *Anti-inflammatory cytokines.* Chest, 2000. **117**(4): p. 1162–1172.
439. Vannier, E., L.C. Miller, and C.A. Dinarello, *Coordinated antiinflammatory effects of interleukin 4: interleukin 4 suppresses interleukin 1 production but up-regulates gene expression and synthesis of interleukin 1 receptor antagonist.* Proceedings of the National Academy of Sciences, 1992. **89**(9): p. 4076–4080.
440. Penedo, L.A., et al., *Intake of butter naturally enriched with cis9, trans11 conjugated linoleic acid reduces systemic inflammatory mediators in healthy young adults.* The Journal of nutritional biochemistry, 2013. **24**(12): p. 2144–2151.
441. Lorea Baroja, M., et al., *Anti-inflammatory effects of probiotic yogurt in inflammatory bowel disease patients.* Clinical & Experimental Immunology, 2007. **149**(3): p. 470–479.

442. Rooney, M., et al., *Bovine dairy products and flow mediated dilation (FMD): a systematic review of the published evidence.* Eur J Nutr, 2025. **64**(2): p. 66.
443. Ballard, K.D., et al., *Acute effects of ingestion of a novel whey-derived extract on vascular endothelial function in overweight, middle-aged men and women.* Br J Nutr, 2013. **109**(5): p. 882–93.
444. Fekete Á, A., et al., *Whey protein lowers blood pressure and improves endothelial function and lipid biomarkers in adults with prehypertension and mild hypertension: results from the chronic Whey2Go randomized controlled trial.* Am J Clin Nutr, 2016. **104**(6): p. 1534–1544.
445. Fekete Á, A., et al., *Whey protein lowers systolic blood pressure and Ca-caseinate reduces serum TAG after a high-fat meal in mildly hypertensive adults.* Sci Rep, 2018. **8**(1): p. 5026.
446. Vasilopoulou, D., et al., *Reformulation initiative for partial replacement of saturated with unsaturated fats in dairy foods attenuates the increase in LDL cholesterol and improves flow-mediated dilatation compared with conventional dairy: the randomized, controlled REplacement of SaturatEd fat in dairy on Total cholesterol (RESET) study.* Am J Clin Nutr, 2020. **111**(4): p. 739–748.
447. Markey, O., et al., *Postprandial Fatty Acid Profile, but Not Cardiometabolic Risk Markers, Is Modulated by Dairy Fat Manipulation in Adults with Moderate Cardiovascular Disease Risk: The Randomized Controlled REplacement of SaturatEd fat in dairy on Total cholesterol (RESET) Study.* J Nutr, 2021. **151**(7): p. 1755–1768.
448. Cortés-Valencia, A., et al., *Dairy consumption and subclinical atherosclerosis: A cross-sectional study among middle-aged Mexican women.* Nutr Metab Cardiovasc Dis, 2021. **31**(6): p. 1747–1755.
449. Machin, D.R., et al., *Effects of non-fat dairy products added to the routine diet on vascular function: a randomized controlled crossover trial.* Nutr Metab Cardiovasc Dis, 2015. **25**(4): p. 364–9.
450. Otani, H. and I. Hata, *Inhibition of proliferative responses of mouse spleen lymphocytes and rabbit Peyer's patch cells by bovine milk caseins and their digests.* Journal of Dairy Research, 1995. **62**(2): p. 339–348.
451. Eriksen, E., et al., *Effect of milk proteins and their hydrolysates on in vitro immune responses.* Small Ruminant Research, 2008. **79**(1): p. 29–37.
452. Wong, C., et al., *Effects of purified bovine whey factors on cellular immune functions in ruminants.* Veterinary Immunology and Immunopathology, 1997. **56**(1-2): p. 85–96.
453. Cross, M.L. and H. Gill, *Immunomodulatory properties of milk.* British Journal of Nutrition, 2000. **84**(S1): p. 81–89.
454. McGregor, R.A. and S.D. Poppitt, *Milk protein for improved metabolic health: a review of the evidence.* Nutrition & metabolism, 2013. **10**: p. 1–13.
455. Dugan, C.E., et al., *Dairy consumption lowers systemic inflammation and liver enzymes in typically low-dairy consumers with clinical characteristics of metabolic syndrome.* Journal of the American College of Nutrition, 2016. **35**(3): p. 255–261.
456. Wennersberg, M.H., et al., *Dairy products and metabolic effects in overweight men and women: results from a 6-mo intervention study.* The American journal of clinical nutrition, 2009. **90**(4): p. 960–968.

457. Al-Harthi, L., et al., *The impact of the ovulatory cycle on cytokine production: evaluation of systemic, cervicovaginal, and salivary compartments.* Journal of Interferon & Cytokine Research, 2000. **20**(8): p. 719–724.
458. Brännström, M., et al., *Variations in peripheral blood levels of immunoreactive tumor necrosis factor alpha (TNFalpha) throughout the menstrual cycle and secretion of TNFalpha from the human corpus luteum.* Eur J Obstet Gynecol Reprod Biol, 1999. **83**(2): p. 213–7.
459. Angstwurm, M.W., R. Gärtner, and H.L. Ziegler-Heitbrock, *Cyclic plasma IL-6 levels during normal menstrual cycle.* Cytokine, 1997. **9**(5): p. 370–374.
460. Cifuentes, M., et al., *Low-grade chronic inflammation: a shared mechanism for chronic diseases.* Physiology, 2025. **40**(1): p. 4–25.
461. Lecube, A. and C. López-Cano, *Obesity, a diet-induced inflammatory disease*. 2019, MDPI. p. 2284.
462. Panahi, S., et al., *Yogurt, diet quality and lifestyle factors.* Eur J Clin Nutr, 2017. **71**(5): p. 573–579.
463. Bouzo, V., et al., *Evaluation of the diet tracking smartphone application Keenoa™: a qualitative analysis.* Canadian Journal of Dietetic Practice and Research, 2021. **83**(1): p. 25–29.
464. Mifflin, M.D., et al., *A new predictive equation for resting energy expenditure in healthy individuals.* The American journal of clinical nutrition, 1990. **51**(2): p. 241–247.
465. Bergström, J., *Percutaneous needle biopsy of skeletal muscle in physiological and clinical research.* Scandinavian journal of clinical and laboratory investigation, 1975. **35**(7): p. 609–616.
466. Shanely, R.A., et al., *Human skeletal muscle biopsy procedures using the modified Bergström technique.* Journal of visualized experiments: JoVE, 2014(91): p. 51812.
467. Hughes, M.C., et al., *Mitochondrial bioenergetics and fiber type assessments in microbiopsy vs. Bergstrom percutaneous sampling of human skeletal muscle.* Frontiers in Physiology, 2015. **6**: p. 360.
468. Bonafiglia, J.T., et al., *A comparison of pain responses, hemodynamic reactivity and fibre type composition between Bergström and microbiopsy skeletal muscle biopsies.* Current Research in Physiology, 2020. **3**: p. 1–10.
469. Malik, M., et al., *Heart rate variability: Standards of measurement, physiological interpretation, and clinical use.* European heart journal, 1996. **17**(3): p. 354–381.
470. Meessen, E.C., et al., *Human postprandial nutrient metabolism and low-grade inflammation: a narrative review.* Nutrients, 2019. **11**(12): p. 3000.
471. Herieka, M. and C. Erridge, *High-fat meal induced postprandial inflammation.* Molecular nutrition & food research, 2014. **58**(1): p. 136–146.
472. Burton, K.J., et al., *Probiotic yogurt and acidified milk similarly reduce postprandial inflammation and both alter the gut microbiota of healthy, young men.* British Journal of Nutrition, 2017. **117**(9): p. 1312–1322.
473. Alm, L., *Effect of Fermentation on Lactose, Glucose, and Galactose Content in Milk and Suitability of Fermented Milk Products for Lactose Intolerant Individuals.* Journal of Dairy Science, 1982. **65**(3): p. 346–352.
474. Emerson, S.R., et al., *Magnitude and timing of the postprandial inflammatory response to a high-fat meal in healthy adults: a systematic review.* Advances in Nutrition, 2017. **8**(2): p. 213–225.

475. Teeman, C.S., et al., *Postprandial lipemic and inflammatory responses to high-fat meals: a review of the roles of acute and chronic exercise.* Nutrition & metabolism, 2016. **13**: p. 1–14.
476. Payette, C., et al., *Sex differences in postprandial plasma tumor necrosis factor–α, interleukin-6, and C-reactive protein concentrations.* Metabolism, 2009. **58**(11): p. 1593–1601.
477. Nestel, P., et al., *Circulating inflammatory and atherogenic biomarkers are not increased following single meals of dairy foods.* European journal of clinical nutrition, 2012. **66**(1): p. 25–31.
478. Rifai, N., J.R. Merrill, and R.G. Holly, *Postprandial effect of a high fat meal on plasma lipid, lipoprotein cholesterol and apolipoprotein measurements.* Annals of clinical biochemistry, 1990. **27**(5): p. 489–493.
479. Bonfils, P.K., et al., *The influence of high versus low sodium intake on blood pressure and haemodynamics in patients with morbid obesity.* Journal of Hypertension, 2013. **31**(11).
480. Visser, F.W., et al., *Rise in Extracellular Fluid Volume During High Sodium Depends on BMI in Healthy Men.* Obesity, 2009. **17**(9): p. 1684–1688.
481. Andrich, D.E., et al., *A short-term high-fat diet alters glutathione levels and IL-6 gene expression in oxidative skeletal muscles of young rats.* Frontiers in Physiology, 2019. **10**: p. 372.
482. Perez-Martinez, P., et al., *Postprandial oxidative stress is modified by dietary fat: evidence from a human intervention study.* Clinical Science, 2010. **119**(6): p. 251–261.
483. Fewkes, J.J., et al., *A single, high-fat meal adversely affects postprandial endothelial function: a systematic review and meta-analysis.* Am J Clin Nutr, 2022. **116**(3): p. 699–729.
484. Dickinson, K.M., P.M. Clifton, and J.B. Keogh, *Endothelial function is impaired after a high-salt meal in healthy subjects.* The American journal of clinical nutrition, 2011. **93**(3): p. 500–505.
485. Dickinson, K.M., et al., *Postprandial effects of a high salt meal on serum sodium, arterial stiffness, markers of nitric oxide production and markers of endothelial function.* Atherosclerosis, 2014. **232**(1): p. 211–216.
486. Tangvarasittichai, S., S. Pongthaisong, and O. Tangvarasittichai, *Tumor necrosis factor-A, interleukin-6, C-reactive protein levels and insulin resistance associated with type 2 diabetes in abdominal obesity women.* Indian Journal of Clinical Biochemistry, 2016. **31**(1): p. 68–74.
487. Kushner, I. and M.J. Antonelli, *What should we regard as an "elevated" C-reactive protein level?* Annals of internal medicine, 2015. **163**(4): p. 326.
488. Barry, D.W. and W.M. Kohrt, *Acute effects of 2 hours of moderate-intensity cycling on serum parathyroid hormone and calcium.* Calcified Tissue International, 2007. **80**: p. 359–365.
489. Wherry, S.J., et al., *Maintaining serum ionized calcium during brisk walking attenuates the increase in bone resorption in older adults.* Bone, 2021. **153**: p. 116108.
490. Guerrini, M.M. and H. Takayanagi, *The immune system, bone and RANKL.* Archives of biochemistry and biophysics, 2014. **561**: p. 118–123.
491. Lundberg, T.R. and G. Howatson, *Analgesic and anti-inflammatory drugs in sports: Implications for exercise performance and training adaptations.* Scandinavian journal of medicine & science in sports, 2018. **28**(11): p. 2252–2262.

492. Ballard, K.D., et al., *Low-fat milk ingestion prevents postprandial hyperglycemia-mediated impairments in vascular endothelial function in obese individuals with metabolic syndrome.* J Nutr, 2013. **143**(10): p. 1602–10.
493. Chatterjee, S., *Chapter Two - Oxidative Stress, Inflammation, and Disease*, in *Oxidative Stress and Biomaterials*, T. Dziubla and D.A. Butterfield, Editors. 2016, Academic Press. p. 35–58.
494. Berry, S.E., et al., *Human postprandial responses to food and potential for precision nutrition.* Nature Medicine, 2020. **26**(6): p. 964–973.
495. Glanz, K., *Compliance with dietary regimens: Its magnitude, measurement, and determinants.* Preventive Medicine, 1980. **9**(6): p. 787–804.
496. Gibson, A.A. and A. Sainsbury, *Strategies to Improve Adherence to Dietary Weight Loss Interventions in Research and Real-World Settings.* Behavioral Sciences, 2017. **7**(3): p. 44.

# Appendices

## Appendix 1 - Study 1 Ethical Approval

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|---|---|
| **Certificate #:** | **2019-045** |
| **Initial Approval:** | **01/31/19-01/31/20** |
| **Amendments:** | **Amendment approved: 11/28/19**<br>**2nd Amendment approved 04/15/20**<br>**3rd Amendment approved: 09/11/20** |
| **Renewals:** | **02/07/20-02/07/21** |
| **Current Approval Period:** | **02/07/20-02/07/21** |

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### ETHICS AMENDMENT APPROVAL

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

**From:** Alison M. Collins-Mrakas, Sr. Manager and Policy Advisor, Research Ethics
*(on behalf of Veronica Jamnik, Chair, Human Participants Review Committee)*

**Date:** Friday, September 11, 2020

**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

With respect to your research project entitled, **"Cre-Ex Study: Does Creatine Augment the Acute Effect of Exercise and Dairy on Bone Turnover?",** the committee notes that, as there are no substantive changes to either the methodology employed or the risks to participants in and/or any other aspect of the research project, a renewal of approval re the proposed amendment(s) to the above project is granted.

Any further 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.

Ongoing research – research that extends beyond one year – must be renewed prior to the expiry date.

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".**

Please note that prior to commencing any research activities, researchers are advised to review the latest updates on research involving human participants at:
*https://www.yorku.ca/research/researchers-faqs/*

Should you have any questions, please feel free to contact me at: 416-736-5914 or via email at: acollins@yorku.ca.

Yours sincerely,

Alison M. Collins-Mrakas M.Sc., LLM
Sr. Manager and Policy Advisor,
Office of Research Ethics

## Appendix 2 - Study 2 Ethical Approval

YORK
UNIVERSITÉ
UNIVERSITY

OFFICE OF
RESEARCH
ETHICS (ORE)
3rd Floor,
309 York Lanes

4700 Keele St.
Toronto ON
Canada M3J 1P3
Tel 416 736 5914
Fax 416 736-5512
www.research.yorku.ca

| | |
|---|---|
| **Certificate #:** | **2021-177** |
| **Approval Period:** | **10/12/21 – 10/12/22** |

# ETHICS APPROVAL

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

**From:** Alison M. Collins-Mrakas, Director, Research Ethics
*(on behalf of You-ta Chuang, Chair, Human Participants Review Committee)*

**Date:** Tuesday, October 12, 2021

**Title:** **Influence of increased dairy product consumption on markers of inflammation and cardiometabolic disease - A crossover study**

**Risk Level:** ☐ Minimal Risk ☒ More than Minimal Risk

**Level of Review:** ☐ Delegated Review ☒ Full Committee Review

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I am writing to inform you that this research project, **"Influence of increased dairy product consumption on markers of inflammation and cardiometabolic disease - A crossover study"** has received ethics review and approval 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 approval 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".**

**Please note** that in response to the ongoing changes due to the pandemic, researchers are required check the *YuBetter website* (Section: Coming to Campus) for updates as there may be changes to protocol requirements.

Should you have any questions, please feel free to contact me at: 416-736-5914 or via email at: acollins@yorku.ca.

Yours sincerely,

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

## Appendix 3 - Study 2 Consent Form

YORK UNIVERSITÉ UNIVERSITY
School of Kinesiology and Health Science

# Informed Consent Document

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

**Study Name**: Influence of increased dairy product consumption on markers of inflammation and cardiometabolic disease - A crossover study

**SHORT TITLE:** "DRIVE (Dairy Research on Inflammatory & Vascular Endpoints) Study"



| Investigators | Department | Contact | Email |
|---|---|---|---|
| **Dr. Andrea Josse (PI)** | KAHS, York University | (416) 736-2100 ex.30038 | ajosse@yorku.ca |
| **Dr. Christopher Perry** | KAHS, York University | | cperry@yorku.ca |
| **Dr. Heather Edgell** | KAHS, York University | | edgell@yorku.ca |
| **Dr. Lauren Skelly** | KAHS York University | | skellyle@yorku.ca |
| **Dr. David Wright** | HK, University of Guelph | | dcwright@uoguelph.ca |
| **Joel Prowting (student)** | KAHS, York University | (416) 736-2100 ex.33990 | jprowt@yorku.ca |
| **Emily Fraschetti (student)** | KAHS, York University | | |



**Purpose of the Research:**
The purpose of this study is to determine whether 6-weeks of increased dairy consumption can reduce inflammation and other markers of chronic disease while fasted or following a high-fat meal.

**Inclusion criteria:**

- 18 - 70 years old
- Body mass index (BMI) ≥ 25 kg/m²
- ≤ 2 structured exercise sessions/week
- Habitual low dairy consumption (≤ 1 serving/day)
- Having at least two other metabolic risk factors based on clinical guidelines, including:
  - Elevated blood pressure (≥130/≥85 mm Hg)
  - Impaired fasting glucose (≥5.6 mmol/L) measured using a finger prick sample
  - Impaired fasting triglycerides (≥1.7 mmol/L) or high-density lipoprotein (<1.03 mmol/L for males, <1.3 mmol/L for females) measured using a finger prick blood sample.
  - Increased waist circumference (≥102 cm for males and ≥88 cm for females).
  - Borderline high fasting low-density lipoprotein (≥3.5 mmol/L) or total cholesterol (≥5.2 mmol/L).

**Exclusion criteria:**

- Allergy to dairy foods, diagnosed lactose intolerance or an aversion to foods provided during the study (i.e., a fast-food breakfast meal which may contain eggs, hash browns, meat [vegetarian option available] and English muffins, donuts/pastries).
- Previous history of diabetes and/or related cardiovascular disease
- The use of *multiple* medications for managing lipids, glucose and/or blood pressure

**What You Will Be Asked to Do:**
First, to determine your initial eligibility to participate in this study, you were asked to complete a health questionnaire, a physical activity questionnaire and a dairy intake questionnaire (phone screening). Informed consent is now being sought (using this form). Following this, you will be asked to provide a finger prick blood sample (details below).

Based on your results, if you are eligible, you will be assigned to complete two 6-week dietary interventions in random order, with at least a 4 week "wash out" period (or your habitual eating) in

between. The two dietary interventions are: 1) Your normal, low-dairy diet; and 2) A higher dairy diet where we will provide you with 3 servings per day of dairy foods every 2 weeks. Prior to beginning either 6-week diet intervention, you will be asked to attend the laboratory at York University after an overnight fast (8+ hours) to undergo some baseline tests. These tests include assessment of your body composition, a blood sample, and other measures of your blood vessel function. There is also an option to collect 2 muscle biopsy samples per trial. All of these tests, including waist circumference and blood pressure determination will be repeated at the end of each 6-week intervention.

**Detailed Description of testing procedures:**

Anthropometry (height, weight, waist circumference and hip circumference):

- Your height will be measured using a stadiometer. You will be instructed to stand up straight with your shoes off.
- Your weight will be measured using a standard electronic weighing scale.
- Your waist circumference will be measured at the umbilicus and hip circumference measured at the widest portion of the buttocks using a standard, retractable, non-metallic tape measure under the clothing in accordance with the WHO guidelines.

Body Composition (Bio Electrical Impedance and possibly also the Bodymetrix [ultrasound]):

- BIA: Your body fat percentage will be measured through a method called "Bio Electrical Impedance" (BIA). You will be asked to stand on a device and hold onto the handles. This device sends a tiny electric current through your body to determine the resistance of different tissues and ultimately provide your body fat percentage estimate. The current is very minimal, and you will not feel it at all. This measurement is routinely done in laboratory and is very safe. This will take a few minutes to complete.
- Bodymetrix: Your body fat thickness at 3 different sites on your body will be assessed using a Bodymetrix portable ultrasound machine that emits high-frequency sound waves through the body tissues. A thin layer of ultrasound gel will be applied to the wand head and then placed perpendicular to the point of skin contact at each of the seven observation sites. These sites include the chest, halfway along your torso, above the hip bone, abdominal area, thigh, back of upper arm, and below your shoulder blade. The wand will take a measure for roughly 5 seconds at a time.

Blood sampling (fingerpick and venipuncture):

- During the in-person screening visit, a fasted, rested finger-prick blood sample will be taken to measure your blood glucose and blood fats. A capillary blood glucose lancing device will be used on one of your fingers to produce a small blood droplet (2-3ml) that can be used for this analysis. You will be asked to prick your own finger.
- During the baseline testing (2 times in total – at the start of each diet phase), blood will be collected from your arm (front part of your elbow using a standard blood drawing technique and taken by a trained professional). During this blood draw, 20-25ml of blood will be taken. We ask that you arrive to our laboratory (BC 123) for testing after an overnight fast (minimum 8 hours), but you may drink water. We also ask that you refrain from exercise for at least 48 hours prior and that you not take any over the counter pain medication for 12 hours prior to your blood sample. If you take a daily prescription medication, we will discuss this with you. We will use your blood to measure different molecules within the blood including fats and inflammation markers. Once blood is collected, it will be transferred to special tubes that will then be stored in a freezer for later analysis.
- Both blood tests will be conducted and/or overseen by a qualified person to whom these controlled acts have been delegated by a regulated health professional (i.e. a medical doctor).

Vascular measures (blood pressure, carotid artery distensibility, and flow-mediated dilation):

- Blood pressure (BP) will be taken at the brachial artery (upper arm) using an Omron 907XL Professional Digital Blood Pressure monitor while you are at rest and in a lying down position. The machine automatically takes 3 serial blood pressure measures and averages them. At the same time as your BP is being measured, an ultrasound probe will be placed on your neck (with

gel) to image your carotid artery. There are no risks associated with these measures.

- Flow Mediated Dilation (FMD): A blood pressure cuff will be placed around your forearm and an ultrasound probe will be used to measure your brachial artery in your upper arm. Once equipped, baseline measurements will be taken for 2 minutes and then the blood pressure cuff around your forearm will be inflated for 5 minutes causing occlusion of the blood flow below it. The cuff will then be deflated causing the blood to flow again, and measurements will be taken for 2 more minutes. There are no risks associated with these measures. You may find the occlusion and the initial release of the cuff uncomfortable.

Leg/Thigh Muscle Tissue Samples

- At the end of each 6-week dietary intervention, during the follow up testing session, you will have the option to have 2 small muscle biopsy samples taken from the same leg/thigh, for a total of 4 muscle biopsy samples (2 at the end of each trial) over the 16-week study period. Each muscle biopsy sample will be taken from the side of the upper leg (thigh). One muscle biopsy sample is the size of half a pea (thought to amount to ~1/10,000th of the total muscle mass in your thigh). It will be taken at rest while you lie in a supine position at the following timepoints: 1) before consumption of the high fat meal, 2) 4h-5h after consuming the high fat meal.
- **NOTE:** Tissue samples are not required from all participants. In addition to the other measures, you can voluntarily decide, upon signing of the consent form, whether to be part of either:
    - **Subset 'A'** – you will not provide muscle tissue samples at any point but will complete the rest of the outlined study measures.
    - **Subset 'B'** – you consent to providing 4 muscle tissue samples at various timepoints in the study in addition to completing the rest of the outlined study measures.

Food records, body weight and activity monitoring:

- You will be asked to complete a 7-day food diary before and at the end of each 6-week intervention. You will need to record everything that you eat and drink over these periods, either through a paper food record or a mobile/tablet app called Keenoa. The records will be analyzed by a Registered Dietitian (RD) to assess your normal nutrient intake levels and then to provide suggestions on how to incorporate the dairy food into your diet (i.e., replacing other foods to prevent increased calorie intake and weight gain).
- During each of the two 6-week diet periods, you will be asked to complete two additional 3-day food diaries (during week 2 and week 4).
- During each of the two 6-week diet periods, you will also be asked to complete two International Physical Activity Questionnaires (IPAQ) to briefly assess your activity levels, and you may be asked to record your steps.
- You will be asked to record your bodyweight at home weekly during the study.

Questionnaires:

- During your initial baseline assessment, you will be asked to complete a screening/health questionnaire to document your general health, individual and family history of disease and medication use.
- Also, at this time, you will also be asked to complete a specific dairy food questionnaire to provide details regarding your dairy food consumption.
- You will be asked to fill in the IPAQ, as described above.

Following baseline testing, you will meet (either virtually or in-person) with the RD to discuss each diet arm. Specifically, if you are on the dairy diet, you will receive advice on how to incorporate these dairy foods into your diet by replacing other foods of similar energy content so as to not increase your total energy intake and body weight. You will meet with the RD at several other times throughout the study. During each 6-week diet period, you will be asked to keep your physical activity levels constant and to not adopt any major dietary changes during the study.

At the end of each 6-week intervention diet, you will be asked to return to York University, as mentioned above, to repeat the initial set of tests performed at baseline. In addition to the fasted tests, we will also

carry out a number of tests after asking you to consume a high-fat test meal (i.e., a fast-food breakfast). We will place an intravenous catheter into your arm vein at the front of your elbow which allows us to collect blood samples at different time points for around 6 hours after you have eaten the meal. This method of blood sampling is instead of performing serial venipunctures. Although, we can do this if you'd prefer. We will also measure your blood vessel function before and at 4 hours after the meal.

**Detailed Description of additional testing procedures:**
Meal challenge: following your fasted measurements (detailed above), you will be asked to consume a high-fat breakfast meal (e.g., from Tim Horton's, Starbucks) consisting of approximately 800-1400 kcals (approx. 50% of your recommended daily energy requirement). An example of this meal (subject to change) would be 2 sausage and egg McMuffins, 2 hash browns and water.

Intravenous (IV) catheter procedure: Following your fasted measurements (detailed above), an IV catheter will be placed into the vein of your non-dominant arm using a standard technique and standard materials. This will allow us to collect regular blood samples from you without having to use a new needle (and puncture) every time. Although, if you'd prefer serial venipunctures, this is also possible. After the catheter is inserted, a 20ml sample of blood will be taken. Additionally, 1, 2, 4, and 6 h blood samples following the high-fat meal will also be taken. This blood sampling procedure will be conducted by a qualified person to whom these controlled acts have been delegated by a regulated health professional (i.e., a medical doctor). Around 100ml of blood will be withdrawn during each follow up session. With the initial screening finger-prick test (2-3ml of blood), baseline venipuncture blood draw (20ml of blood) and two follow up sessions using the catheter (100ml x 2 of blood), we estimate that a total of 223ml of blood will be taken in total over the entire 16+-week study period.

**Time Commitment**

| | **Estimated Time Commitment** |
|---|---|
| **Initial screening:** explanation of study protocol, signing of informed consent form, eligibility assessment: questionnaires, finger prick blood sample, completion of 7-day food record | 90 min (1.5 hours) + 15 min/day for 7-days (1.75 hours) |
| **Baseline testing 1:** anthropometry, body composition, venous blood draw, blood vessel function, meeting with RD | 75 min (1.25 hours) |
| **Diet 1 for 6-weeks:** weekly body weight measurement, 3-day food records, PA records in weeks 2 and 4, 7-day food record in week 6 | 15 min/day for 13-days over 6 weeks (3.25 hours) |
| **Repeat testing 1:** anthropometry, body composition, venous blood draw, blood vessel function, high-fat meal challenge, intravenous blood sampling, muscle biopsy (x2 – optional) | 480 min (8 hours) |
| 4(+) week **washout** period | - |
| **Baseline testing 2:** anthropometry, body composition, venous blood draw, blood vessel function, meeting with RD, 7-day food record in week -1. | 75 min (1.25 hours) |
| **Diet 2 for 6-weeks:** weekly body weight measurement, 3-day food records, PA records in weeks 2 and 4, 7-day food record in week 6. | 15-20 min/day for 13-days over 6 weeks (3.25 hours) |
| **Repeat testing 2:** anthropometry, body composition, venous blood draw, blood vessel function, high-fat meal challenge, intravenous blood sampling, muscle biopsy (x2 – optional) | 480 mins (8 hours) |
| **Total Time Commitment** | **28+ hours over 16+ weeks (approx. 1.8 hours per week)** |

**Risks and Discomforts**: Although this in unintended, you may experience some uneasiness or anxiety due to the personal nature of the questions asked on the questionnaires and/or the body composition determination (assessment of body fat). We will do our best to minimize these feelings.
The potential physical risks involved in participation include:

a) Blood sample collection: You may experience slight pain, and/or tingling, and/or bruising in the area after the blood samples (finger prick, venipuncture, and venous IV-catheter). In some instances, you may unexpectedly feel faint or dizzy 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. Also, it is 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.

b) Vascular measurements: You may experience discomfort associated with the FMD measures. As described, FMD determination involve short-term (5 min) occlusion of a cuff around your arm. This may result in tingling or a slight warm/burning sensation in your fingertips. Once the cuff is released, it may result in an uncomfortable blood-rushing feeling. This will all resolve shortly after the cuff is released.

c) Muscle Biopsy Collection: Please refer to the attached form on pg. 8-9 entitled *"Description of Medical Procedures"* for a complete description of the medical procedures to be performed during the study and the potential risks and discomforts associated with these procedures.

d) As this research requires face-to-face interaction, in our current state, there is a potential risk of contracting COVID-19. As a research team we will be following strict health and safety guidelines to minimize the spread of COVID-19 and expect you to follow public health directives as well. Lab surfaces will be cleaned frequently and after use to reduce the risk of contracting COVID-19. We (researchers and participants) will all be required, until further notice, to use personal protective equipment, such as face coverings at all times, and physical distancing of 2 meters will be maintained unless not possible.

e) This study may use the *Zoom* video calling/chat platform to collect data, which is an externally hosted cloud-based service. When information is transmitted over the internet privacy cannot be guaranteed. There is always a risk your responses may be intercepted by a third party (e.g., government agencies, hackers). Further, while York University researchers will not collect or use IP addresses or other information which could link your participation to your computer or electronic devices without informing you, there is a small risk with any platform such as this of data that is collected on external servers falling outside the control of the research team. If you are concerned about this, we would be happy to make alternative arrangements (where possible) for you to participate, perhaps *via* telephone. Please contact a member of the research team for further information. Please note that it is the expectation that participants agree not to make any unauthorized recordings of the content of a meeting/data collection session.

f) The food tracking phone/device application to be used is called *Keenoa*. It does not collect your personal identifiable information. Rather, through your interaction, it collects photos of your food and drink for the researchers to assess. We can also read any comments you may leave for us through the app, and we can leave comments for you too to address. The use of your Apple ID or Google Play ID will allow you to initially download the app and create a *Keenoa* account without providing any personal identifying information. Then, we will be able to send you your unique six-digit participant ID to identify and connect your food diary to you. When information is transmitted over the internet privacy cannot be guaranteed. There is always a risk your responses may be intercepted by a third party (e.g., government agencies, hackers), although your identity will not be revealed because only the research team has the ID code key to link your food diary with your personal information. Please contact a member of the research team for further information.

**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; (4) receive nutritional counselling by a professional (registered dietitian). Your own individual results or group results can also be provided to you upon request.

In addition, upon completion of the study, you will be remunerated for your time (If you withdraw from the study, remuneration will be prorated based upon the number of weeks completed per diet intervention):

- Participants in **subset 'A'** will receive up to $200 ($100 per completed diet intervention) for your participation.
- Participants in **subset 'B'** will receive up to $400 ($100 per completed diet intervention, $200 for completion of all 4 biopsies) for your participation.

**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 remuneration for the time completed. In the event you withdraw from the study, unless you indicate otherwise (and we destroy your samples), your samples and data may be used in our final analysis. Should you wish to withdraw your data after the study, you will have the option to do so before completion of the data analysis. We reserve the right to withdraw participants from the study at any time, however you will still receive renumeration for the time completed.

**Confidentiality**: Once you begin the study, you will be given a personal participant identification number. From this point on, we will 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. In addition, 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-investigators and student investigators directly involved with the study 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. Paper information will be kept safely for 5 years after publication, at which time, this information will be destroyed. Electronic deidentified databases will be kept indefinitely and anonymized data may be deposited in an online open repository, as is sometimes requested by funders and academic journals before publication. 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. It is important to note that the data collected in this research project may be used – in an anonymized form - by members of the research team or by other researchers in subsequent research investigations exploring similar lines of inquiry. Such projects may still be required to undergo ethics review by institutional Research Ethics Boards, and any secondary use of anonymized data, particularly by the research team, will be treated with the same degree of confidentiality and anonymity as in the original research project.

**COVID-19 Specific Consent Information – If applicable:** All research activities will take place at York University campus, under the jurisdiction of York Region Public Health Services. We are taking all safety precautions to reduce the risk of spread of COVID-19 and expect you (as a research participant) to follow public health directives as well.

Only research involving participants considered "low risk" may proceed at this time. If you feel that you are from a vulnerable group with respect to COVID-19, please discuss your participation with the researchers before consenting. You are under no obligation to participate and nothing bad will happen if you change your mind about participating in the research.

As you will be coming onto York University campus to fulfill the research, the following safety protocols must be followed, as per Occupational Health and Safety:

- Take appropriate precautions (e.g., face covering / mask) if taking public transportation and entering public indoor spaces.
- Wash your hands upon coming onto campus / entering a building. Hand sanitizer will be made available to you.
- Physical distancing will be maintained as best as possible. If this is not possible (i.e., when taking specific measurements or blood samples), face coverings are recommended to be worn, and additional precautions will be taken by researchers as deemed appropriate (e.g., face shields, gloves, lab coats, etc.). We will provide you with a disposable mask, if needed.

**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 Joel at jprowt@yorku.ca and/or (416) 736-2100 (33990). or the main supervisor of this research, Dr. 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.

**Informed Consent:**

This research has received ethics review and approval by the Ethics Review Committee, which is a 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.

☐ **Subset 'A':** I choose to be assigned to the participant group that will not receive biopsies.

☐ **Subset 'B':** I choose to be assigned to the participant group that will receive 4 biopsies.

**Signature**_____ **Date**_____
Participant

**Signature**_____ **Date**_____
Principal Investigator or designate

## ADDITIONAL DESCRIPTION OF MEDICAL PROCEDURES – Muscle Biopsies

The study in which you are invited to participate involves optional muscle biopsy sampling. Prior to making any decisions, you are asked to read this form which outlines the potential medical risks inherent to this procedure. Please note that the muscle biopsy procedure does not increase the risk of COVID-19 above the previously stated risk associated with face-to-face research. In addition, we ask that you complete the attached "Participant Screening Questionnaire" (Page 10), which is designed to identify any medical reason that might preclude your participation in this aspect of the study.

**Muscle Biopsy Procedure:**
Dr. Christopher Perry (School of Kinesiology and Health Science) will perform the muscle biopsy procedure. It involves the removal of a small piece of muscle from your outer thigh (quadriceps) using a small, sterile hollow needle. Dr. Perry is a physiologist, has been trained by physicians and has performed these procedures numerous times previously at York.

**Detailed description of the Muscle Biopsy Procedures and potential risks:**
The percutaneous Bergstrom needle muscle biopsy procedure involves the removal of a small piece of muscle tissue using a sterile hollow needle which is ~4-5 mm in diameter. This procedure involves two injections. First, the area over the muscle to be sampled is cleaned with an antiseptic solution and a small amount (1.5-5 ml) of local anesthetic ("freezing"; the same as what is used in the dentist's office) is injected into and under the skin over the vastus lateralis (outer thigh) muscle. Second, a ~5 mm long incision will be made through the skin and fascia over the biopsy site. There may be a small amount of bleeding from the incision, but this is minimal. The sterile biopsy needle is inserted into the biopsy site. A small piece of muscle (~80-200mg, i.e., smaller than an eraser at the end of a pencil) will quickly be obtained and then the needle will be removed. The muscle is quickly removed from the needle over a sterile work area nearby. This sampling procedure comprises a single biopsy timepoint and requires 1 to 2 minutes in total. During the time that the actual biopsy sample is being taken (~2-3 sec), the sensation of pressure in the muscle is felt, and on some occasions, this is moderately uncomfortable for a few seconds (similar to a strong muscle twitch or a 'cramp'). However, the discomfort passes quickly, and you should be quite capable of getting up and moving around afterward.

Following the removal of the muscle sample, the biopsy site is closed with a sterile Steristrip or with stitches joining the skin edges of the incision and wrapped with a tensor bandage and ice pack for 15 minutes. You will be given extra Steristrips and will be briefed about care of the biopsy section. You will be asked to refrain from excessive muscle use for the remainder of the day. Once the anesthetic wears off, your leg may feel tight in the local area of the sampling and often there is the sensation of a bruise or "charlie horse". This is normal. You should not take any aspirin-based medicine for 24 hours following the experiment as this can promote bleeding in the muscle. However, other analgesics such as Advil (ibuprofen) or Tylenol (acetaminophen) are acceptable alternatives. It is also beneficial, but not required, to keep the limb elevated when you are sitting, and the periodic application of an ice pack will help to reduce any swelling and residual soreness. The following day, the muscle may feel slightly 'bruised' upon certain movements such as going down stairs. Any tightness in the muscle usually disappears within 1-2 days. You should be able to routinely begin your usual activities and exercise immediately with muscles other than the biopsied muscle and can resume normal activities with the biopsied muscle within 2 days. In order to allow the incision to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 2-3 days. Daily showers are acceptable, but baths, swimming, saunas, etc. should be avoided. After a shower the incision area should only be tapped dry. The Band-Aid can be changed at any time if needed and removed after 3-4 days.

**Potential Risks:** This biopsy technique is routinely used in physiological research, and complications are rare provided that proper precautions are taken. However, there is a risk of internal bleeding at the site of the biopsy, which can result in bruising and temporary discoloration of the skin or a small hematoma, all of which have a high chance of resolving within days. On occasion, a small lump of fibrous tissue may form under the site of the needle insertion, but this normally disappears within 2-3 months. As with any biopsy, there is also a slight risk of infection; however, this risk is virtually eliminated through proper

cleansing of the area (additional supplies will be provided to you upon departure from the lab). If the incision does not heal within a few days or you are in any way concerned about swelling and redness, please contact us immediately. In very rare occasions, there can be damage to a superficial sensory nerve which will result in temporary numbness in the area. There is also a theoretical but small risk of damage to a small motor nerve branch leading to the muscle which is being sampled and would likely have no impact on day-to-day activities. There is also a small chance that you will be allergic to the local anesthetic.

You will be excluded from participation in this part of the study if you are taking aspirin or any anticoagulant medication ('blood thinners') in order to avoid additional risks of bleeding complications.

**The possibility of a medical emergency:** In the unlikely event of a medical emergency, Dr. Robert Laham (the physician overseeing these procedures at York) is fully aware of the procedure and our research study in general. He is on call and willing to address any unexpected emergency situations. Dr. Robert Laham can be reached at Appletree Medical Centre, York Lanes Mall, York University, through a direct line at 416-479-8649. We (the investigators) will follow standard York Emergency Procedures if Dr. Laham cannot be reached at his direct line. Specifically, 911 will be called directly (9-911 from a local university extension) followed by calling Security Control Centre at Ex. 33333 for assistance.

**MUSCLE HEALTH RESEARCH CENTRE – BIOPSY PRE-SCREENING QUESTIONNAIRE**

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

Name: _____ Date: _____

1. In the past 14 days, have you experienced any of the following symptoms? (New or worsening cough, shortness of breath or difficulty breathing, temperature equal to or over 38°C, feeling feverish, chills, fatigue or weakness, muscle or body aches, new loss of smell or taste, headache, gastrointestinal symptoms [abdominal pain, diarrhea, vomiting], feeling unwell).

YES NO

2. In the past 14 days, have you been in contact with anyone who is sick or who has a confirmed case of COVID-19?

YES NO

3. In the past 14 days, have you travelled outside of Canada?

YES NO

4. When you receive an impact to your muscle, do you develop bruises easily?

YES NO

5. Are you currently taking any medication (including aspirin or anticoagulant 'blood thinners') or have you taken any medication in the last two days?

YES NO

If yes, please list the medication and dose: _____

6. Have you previously participated in a study that involved having muscle biopsies taken?

YES NO

7. Have you ever had a negative or allergic reaction to local freezing (e.g., during dental procedures)?

YES NO

Reviewed by:

Name: _____ Date: _____

# Appendix 4 – Testing and Analysis SOPs

## 4.1 Fingerprick Screening SOP

**EQUIPMENT** - turn on/set up (Video Guide: https://youtu.be/L-iH_DFsDFA)

Cholestech:

- Run: "Optics check cassette"
- Turn on printer
- Monthly: Calibrate using pipette, add control high and low to two different cartridges

**SUPPLIES** – collect and lay out:

- Remove Cholestech cartridge from fridge (at least 15 minutes prior to use)
- Fingerprick supplies:
    a. Sharps Bin
    b. Absorbent cloth area
    c. Glove
    d. Alcohol swab
    e. Gauze
    f. Band-Aid
    g. Disposable lancets
    h. Capillary tube + black plunger
    i. Cholestech cartridge

**Protocol**

1. Explain procedure to participant
    a. "Assessing blood glucose and lipids using a small droplet of blood from your finger. Similar to how a type 1 diabetic checks their blood glucose."
2. Ask if participant is comfortable pricking own finger, or if they want assistance from researcher
3. Ask if they prefer a particular finger/hand (can be slightly sore after, so may want to choose non-dominant hand if they don't have a preference)
4. Ask if hands are cold. If so, warm up under warm tap first
5. Ensure supplies are laid out, don gloves. Open Cholestech cartridge, put plunger into capillary tube (red side).
6. Use alcohol swab to sanitize finger, wait for it to air dry
7. Unscrew protective "cap/insert" of lancet. Place against top/side of the end of the finger (not the pad of the finger, as skin is thickest here). Push firmly against skin to prick finger
8. Immediately dispose of lancet in sharps bin
9. Using gauze, wipe first droplet of blood away from site

10. Squeeze finger to enlarge the droplet (I recommend doing this below table so gravity can assist). Simultaneously place capillary tube into/against droplet to begin drawing blood up. Try to keep end of capillary tube within blood droplet the entire time to reduce air bubbles. Maintain constant pressure and/or squeeze finger proximally-distally if required to continue blood flowing
    a. Note: if blood is not drawing into capillary tube or stops before filling to more than ~2/3rds of the way up, may have an air bubble/coagulation. Stop procedure on that finger and instruct participant that it needs to be done again on new finger
11. When capillary tube is full (to black line), remove from droplet and cover prick site with gauze. Instruct participant to apply pressure with gauze
12. Use plunger to evacuate capillary tube blood into well within Cholestech cartridge (should see the blood seep into white material within cartridge well)
13. Press run on Cholestech machine, insert cartridge into slot (line up black line of cartridge with black line of insert area)
14. Takes ~5 minutes or so to run
15. Return to participant, check they are ok. Look at finger. If excess blood, wipe off with alcohol swab, then apply band aid

**Tidy Up & Put Away**

- **EQUIPMENT** - turn off:
    - Cholestech + printer

- **SUPPLIES**:
    - Blood:
        - Sharps in sharps bin
        - Anything that touched blood in biohazard bag/bin
        - Anything else in normal garbage
        - Wipe down surface with Lysol

## 4.2 Venepuncture & IV Insertion SOP

**SUPPLIES** – collect and lay out:

- Venepuncture supplies:
    a. Sharps Bin
    b. Absorbent cloth area
    c. Gloves
    d. Alcohol swab
    e. Gauze
    f. Band-Aid
    g. Tourniquet
    h. Needle (21g or butterfly)
    i. Vacutainer (labelled)
    j. Vacutainer holders
- IV specific supplies
    k. [IV] IV catheter
    l. IV] Extension tubing
    m. [IV] Q-syte
    n. [IV] Tegaderm
    o. [IV] Saline syringe

**Protocol**

1. Explain procedure to participant, ask if any history of fainting/other issues
2. Don gloves
3. Prepare needle if needed (unravel tube, prepare vacutainer holder etc.)
4. Roll up sleeve, initial check of site
5. Sanitize area for at least 10 seconds, rub vigorously with alcohol swab
6. Apply tourniquet 2-3" above antecubital fossa
7. Determine which vein to choose, palpate if necessary (re-sanitise if so)
8. At 15-30 degree angle, insert needle into vein

**If venepuncture only:**

9. If flash occurs (butterfly), insert vacutainer (Yellow/Red top, then Green top, then Purple top) and wait until they fill. Remove tubes and invert 10-15 times if coated (e.g., Green and Purple)
10. Release tourniquet
11. Swiftly remove needle and apply gauze to site, apply pressure (ask participant to take over)
12. Cap needle and dispose in sharps container

**If IV insertion:**

9. If flash occurs, advance catheter into vein. Withdraw needle (or press button if spring loaded). Cap needle and dispose in sharps container
10. Apply pressure above, quicky attach extension tubing (with Q-syte attached to opposite end) into catheter
11. Release tourniquet
12. Apply Tegaderm to secure catheter, tape tubing to arm, wipe up blood with alcohol swab if required
13. Screw in vacutainer holder to Q-syte, then insert required vacutainers (Yellow/Red top, then Green top, then Purple top) and wait until they fill. Remove tubes and invert 10-15 times if coated (e.g., Green and Purple)
14. Remove vacutainer holder
15. Use sterile saline syringe to flush the extension tube/catheter until clear. Lock off extension tube as pushing saline in to prevent back flow
16. Check/flush catheter every 30 mins, and after blood collection

## 4.3 Muscle Biopsy SOP

## Preparation

### 1-3 Days Prior:

- Clean Bergstrom needles – rinse in Baxedin, clean with soapy water, rinse thoroughly then dry
- Autoclave (Vacuum cycle #4 or #5)
    - 6 Bergstrom needles in sterile bags
    - 3-4 silver bowls in green towel
    - 3-4 green towels
- Gather/check:
    - Cidex (sterilizer) spray bottle
    - Biohazard & sharp bin
    - Bed paper covering + paper towels
    - Disinfectant soap or kitchen sponge with antibacterial soap – for scrub up
    - Juice box/water
    - After care ziploc:
        - Alcohol swabs x10
        - Tegaderm x8
        - Steri strips x8
        - Compression tensor bandages x2
        - Printed instructions

- Lay out on side table in following order:
    - 1) Sterile gloves + spare
    - 2) Autoclaved sterile green cloth for the "sterile field" cart + extras
    - 3) Autoclaved silver bowl wrapped in green towel
    - 4) Stanhexidine bottle
    - 5) 4-6 Sterile gauze packets
    - 6) 2mL or 5mL syringe x3
    - 7) 25g needle x3
    - 8) Xylocaine
    - 9) Alcohol swabs x4
    - 10) Scalpel x3
    - 11) 3x Bergstrom needle per biopsy (6/day on HFMC days) = enough for back ups
    - 12) Large suction syringe and tubing
    - 13) Yellow tip for syringe suction x3

- 14) Steri strips
- 15) Tegaderm
- 16) Tensor bandage
- Apparatus for muscle processing:
    - Kidney dish
    - Cutting area
    - Filled Ice box
    - Liquid nitrogen dewer
    - Cryotubes (poke 2-3 holes in top with needle)
    - Ice packs

**Protocol**

1. Wipe down and sanitize area – bed, table, cart
2. Lay out all items in order required
3. Chris "scrubs up" hands
4. Sterile gloves – assistant passes to Chris
5. Towel for sterile area – assistant unwraps for Chris to take and lay onto cart
6. Silver bowl – assistant unwraps for Chris to place on cart
7. Stanhexidine – assistant pours ½ inch into silver bowl
8. Gauze – assistant opens and hands over to Chris (4-6x pieces)
9. Sanitization of thigh with stanhexidine by Chris using gauze
10. 5mL Syringe – assistant opens for Chris to take
11. 25g Needle – assistant opens for Chris to take
12. Xylocaine – assistant sanitizes top of bottle with alcohol swab, holds upside down at angle with both hands, Chris inserts needle and takes 0.5cc of xylocaine
13. Inject xylocaine and wait ~5-10 minutes
14. Scalpel – assistant opens scalpel for Chris to take
15. Bergstrom needle – assistant opens the Bergstrom needle for Chris to take and put on cart with gauze over it + Chris hands the plunger component to 2nd assistant
16. Chris double checks the numbers on the Bergstrom apparatus + assesses to make sure it's not sticking to itself/listens for the sound
17. Chris asks participant about feeling sharpness of scalpel and then makes a ~1 cm incision if numb
18. Assistant puts the tip of suction syringe into the needle as Chris hold its – careful not to touch gloves
19. Assistant creates suction by pulling the syringe when Chris gives the signal

20. Chris makes 3-5 cuts, rotating each time and hands Bergstrom off directly to assistant 2 (careful not to touch gloves) as he puts gauze and pressure over the incision site for 5-10 minutes
    - Simultaneous Tissue Processing by assistant 1 & 2 (work on top of ice box, go as fast as possible)
        - Eject tissue from Bergstrom needle chamber. Can use saline syringe to flush out via hole in the top too
        - Saline or PBS on sample to remove blood
        - Use scalpel to cut/separate into ~50mg sized pieces
        - Transfer to cryovials, cap and drop in liquid nitrogen
        - Immediately move to -80 freezer
21. Assistant 1 returns and opens the steri strips for Chris who applies it
22. Assistant 1 opens the Tegaderm for Chris who applies it
23. Apply ice, pressure and elevate leg for ~15 minutes
24. Explain after care procedure, including how to clean and keep area sterile, how to apply steri strips + Tegaderm, signs of infection and what to do if they occur etc.
25. Clean up
    - Bergstrom needles + parts go into Baxedin to soak for ~15 mins
        - Then rinse thoroughly with soapy water, can use a brush to get into needle if needed
        - Rinse with running water
        - Will then autoclave before next use
    - Everything else disposed of in correct place (e.g., scalpel in sharps, needle in sharps, anything blood in biohazard)
    - Wipe down area thoroughly with Cidex

## 4.4 HPLC Glutathione Analysis SOP

Day(s) before checklist:

- Check stock/supplies and expiry dates
    - HPLC grade water
    - Methanol (MeOH)
    - Acetonitrile (MeCN)
    - Glacial acetic acid (aka ethanoic acid, $CH_3COOH$)
    - TRIS-BSA (+ these if more needs to be made)
    - 0.2M N-Ethylmaleimide (NEM) (powdered NEM if more needs to be made)
    - Sodium Phosphate Dibasic ($Na_2HPO_4$)
    - Trichloroacetic acid (TCA)
    - Perchloric acid (PCA)
    - OPA-1 (Phthalaldehyde)
    - NaOh
    - Drabkins Reagent/Hemoglobin assessment kit for blood GSH
    - Powdered GSH & GSSG
    - Check column (Zorbax Eclipse XDB-C18, Cat# 993967-902 for $917)
- Label tubes:
    - Eppendorf's for standards
    - HPLC vials

Day of checklist:

- Make up TRIS-BSAN
    - Thaw 0.2M NEM stock from freezer, add 25ul NEM per 1ml TRIS-BSA
- Make up mobile phase
- Make up standards for standard curve
- For GSH:
    - Transfer tubes into HPLC Vials
- For GSSG:
    - Incubate with OPA for 15 minutes before transfer tubes to HPLC vials

## Step 1: Turn on HPLC (bottom left corner of each module) & select method

- Computer – Lab Solutions -> HPLC UV (GSH) or RF (GSSG) -> File -> Open Method File -> Methods -> Prowting GSH or GSSG -> Download and Close
- HPLC, turn on in U shape from top left, down and then around to top right:
    - Liquid Chromatograph (LC-30AD)
    - Autosampler (SIL-30AC)
    - Communications Bus Module (CBM-20A)
    - Detector
        - GSH = UV/VIS detector (SPD – 20A)

        - GSSG = Fluorescence detector (RF-20A xs)
    - Column Oven (CTO-20AC) = add your column the correct way (flows from bottom to top, arrows denote this)

## Step 2: Prepare buffers & reagents

- TRIS-BSA – Remove from fridge (place on mix tray)
- NEM 0.2M – Remove from freezer (place on mix tray)
- Mobile Phase:
    - Solvent A:
        - GSH = Add 2.5mL glacial acetic acid into 997.5mL HPLC grade water, then pH to 3.1 (add 2M NaOH or more glacial acetic acid), filter
        - GSSG = Add 1.77g $Na_2HPO_4$ into 425mL HPLC grade water, mix well with rod, pH to 6.0 using phosphoric acid ($H_3PO_4$), add 75mL Methanol (MeOH)
    - Solvent B: 100 % Acetonitrile
    - Solvent D: 50/50 Methanol:H2O
    - Make sure rinse lines also filled with 50:50 MeOH:H2O
    - Also check LC-30AD section and make sure its topped up (HPLC grade water daily, or can do 10% isopropyl alcohol to 90% water for week)

- Each run = 15 mins, so can calculate how much of each mobile phase is needed depending on how many samples (with some extra to be safe). For GSH, around 13 mins is 100% A (87% of total) and 2 mins is 100% B (13% of total)
- E.g. 96 samples + 5 standards = 101
- 101 * 15 mins per sample = 1515 mins (or 25.25h) continuous @ 1.05mL per min = 1591mL total
- 1591 * 0.87 = 1318mL Mobile Phase A. Make 1.5 – 2L.
- 1591 * 0.13 = ~207mL Solvent B. Add 300-500mL
- Will also keep running after finishing the run, so plan for when you come back to the lab if run overnight to make sure the reservoirs don't run dry

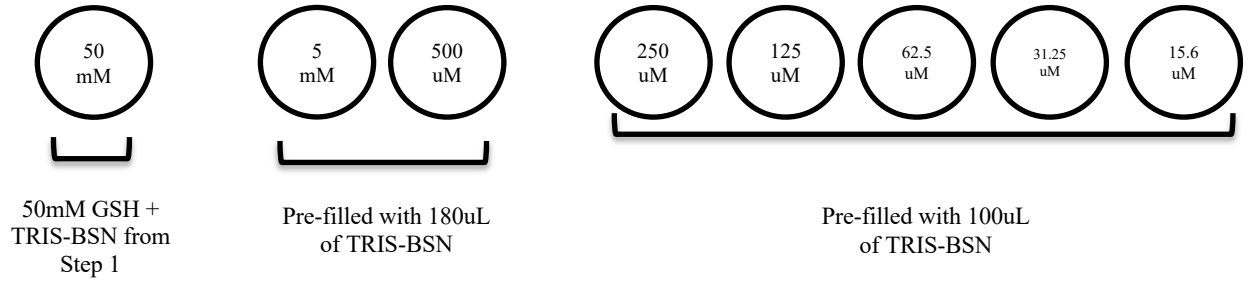
## Step 3: Pre-clean and prime

- Auto purge before use (~5 mins line A, B, D and 10 mins autosampler and rinse)
- Prime/clean:
    - 100% line D for 5 mins
    - 75% D to 25% B for 5 mins
    - 50% D to 50% B for 5 mins
    - 25% D to 75% B for 5 mins
    - 100% B for 5 mins
    - Swap to real mobile phase – 94% A and 6% B and let run until ready

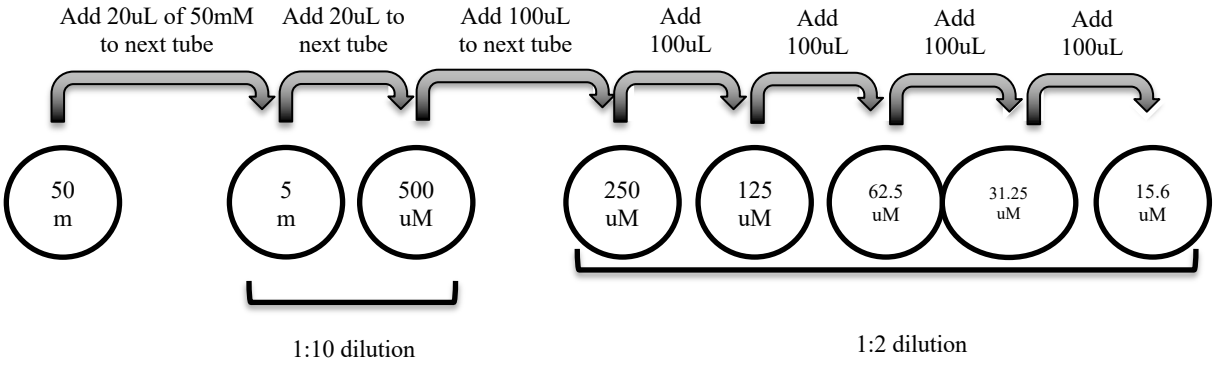
## Step 4: Standard preparation and runs

**GSH Standard** - every day, run a 4 point standard (250uM GSH, 62.5uM GSH, 31.25uM GSH, 15.6uM GSH)

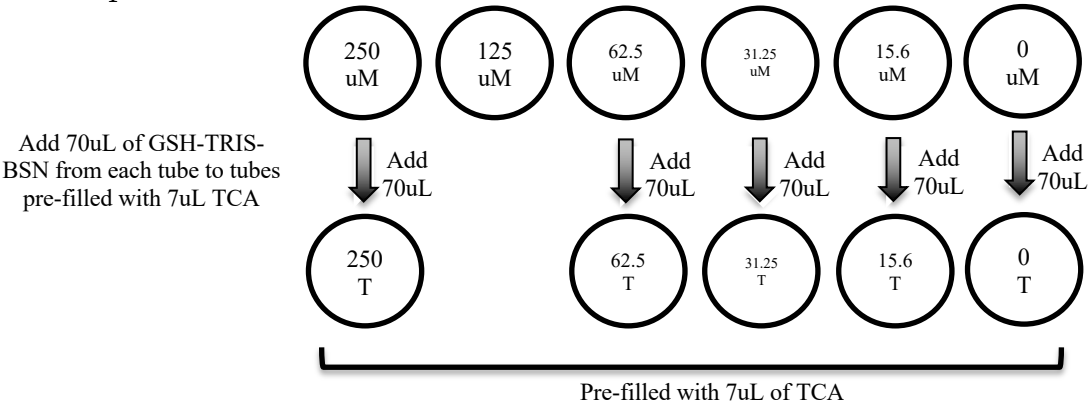
- Pre-step: Add 75uM of NEM to 3mL TRIS-BSA to get TRIS-BSN (wrap in foil)
- Step 1: Make 50mM GSH in TRIS-BSAN
    - Need 15.366mg per 1mL (X actual/15.366 = actual mL needed) – do not exceed 1.5mL (or won't fit in tube)
- Step 2: Set up Eppendorf tubes with TRIS-BSN as follows




- Step 3: Dilute samples as follows: 50mM GSH -> 5mM GSH -> 0.5mM (500uM) GSH -> 250uM GSH -> 125uM GSH -> 62.5uM GSH -> 31.25uM GSH -> 15.6uM GSH
    - **Vortex thoroughly each time, new tips each time**



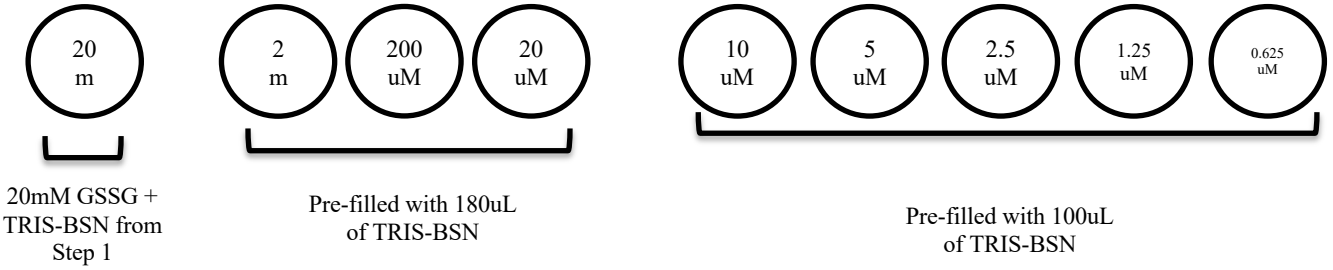

- Step 4: Take 70ul of 250uM GSH, 62.5uM GSH, 31.25uM GSH, 15.6uM GSH tubes, and transfer to new tubes pre-filled with 7ul TCA



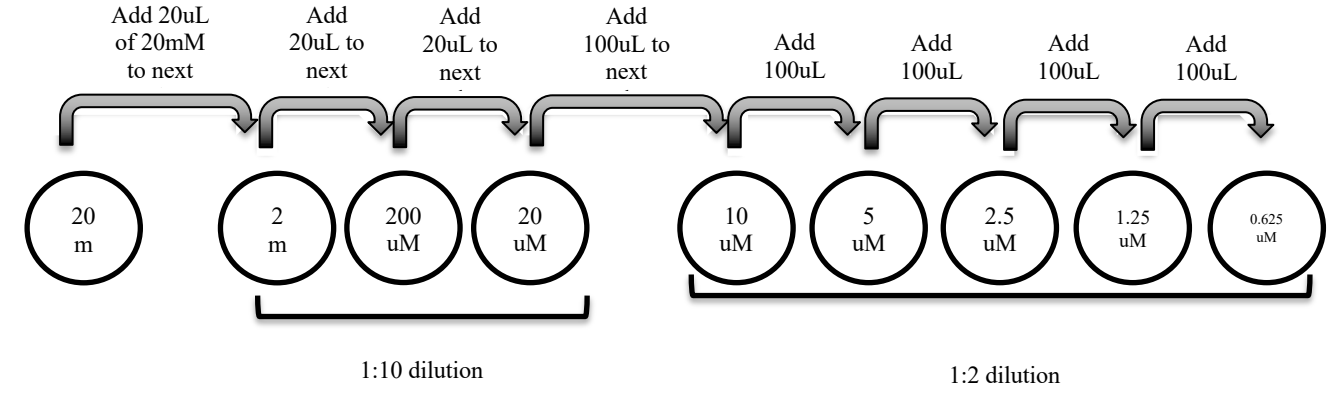
- Step 5: Transfer GSH-TCA tube to HPLC vial, ready to run

**GSSG Standard** - every day, run a 4 point standard (20uM, 10uM GSSG, 2.5uM GSSG, 1.25uM GSSG, 0.625uM GSSG)

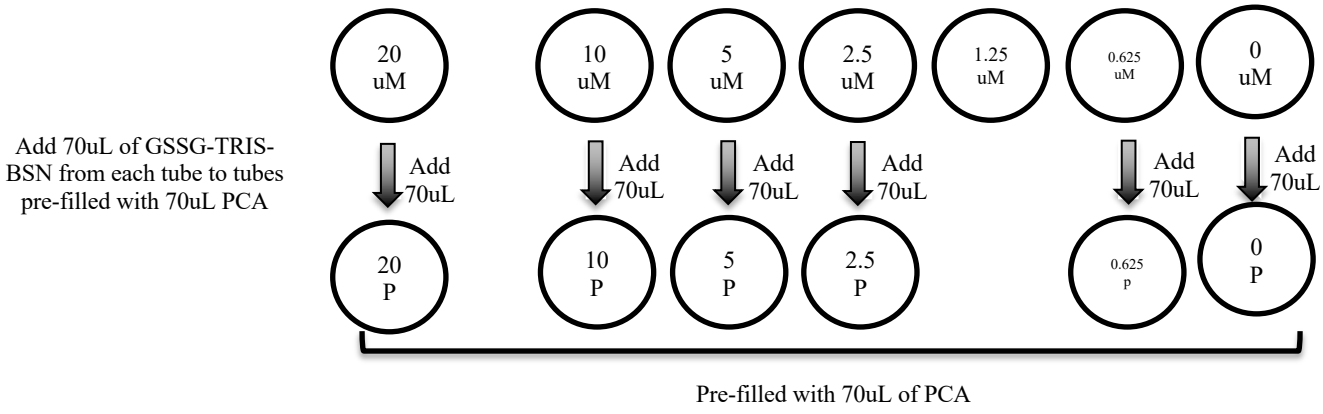
- Pre-step: Add 75uM of NEM to TRIS-BSA to get TRIS-BSN (wrap in foil)
- Step 1: Make 20mM GSSG in TRIS-BSAN
    - Need 12.25mg per 1mL (X actual/12.25 = actual mL needed) – do not exceed 1.5mL
- Step 2: Set up Eppendorf tubes with TRIS-BSN as follows



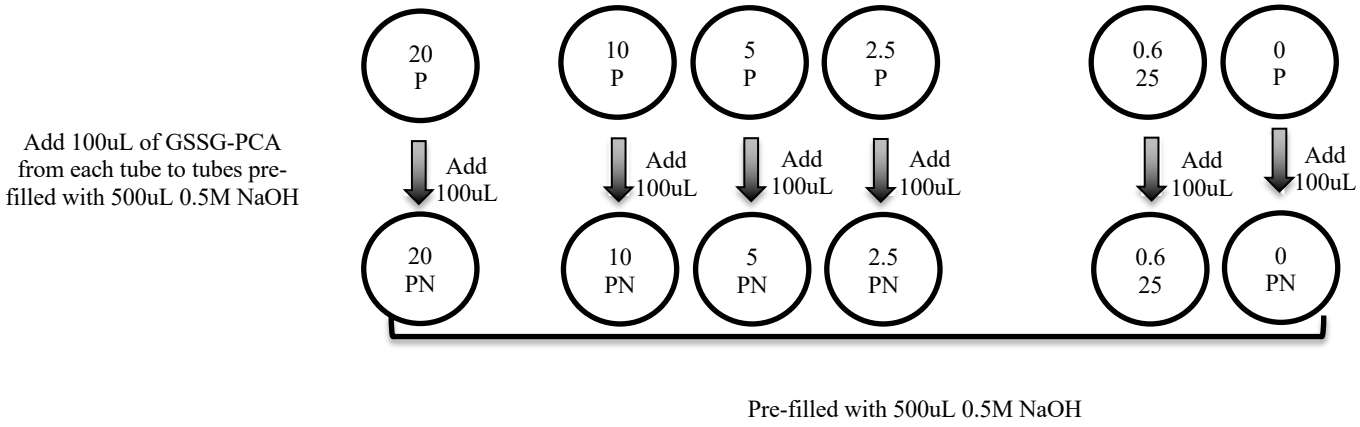

- Step 3: Dilute samples as follows: 20mM GSSG -> 2mM GSSG -> 200uM GSSG -> 20uM GSSG -> 10uM GSSG -> 2.5uM GSSG -> 1.25uM GSSG -> 0.625uM GSSG
    - **Vortex thoroughly each time, new tips each time**




- Step 4: Take 70ul and transfer to new tubes pre-filled with 70ul PCA



- Step 5: Transfer 100uL from GSSG-PCA tube to new tube with 500ul 0.5M NaOH




- Step 6: Add in 37.5ul of 0.1% OPA-MeOH (our fluorophore, which is stored in -20°C freezer) to PCA-NaOH tubes. Incubate standards in the dark for at least 15 minutes.

- Step 7: After incubation, transfer 200uL to HPLC vials, ready for run.

## Step 5: Sample preparation

**For Whole Blood:**

**GSH:**

1) Thaw blood on rocker
2) Add 100uL NEM-treated whole-blood sample to 100ul TCA15; shake each sample vigorously and centrifuge it at 14,000g for 2 min at room temperature.
3) Add supernatant to HPLC vials
4) Load 50 ul of standard into HPLC vials.
5) Add all in to machine and run (Prowting GSH)
6) Measure hemoglobin concentration in blood samples (those with anticoagulant and NEM) according to the manufacturer's instructions for the Drabkin's reagent.

**GSSG:**

1) Add 100uL NEM-treated whole-blood sample to 100uL TCA15; shake the sample vigorously and centrifuge it at 14,000g for 2 min at room temperature.
2) Add supernatant to 500uL of 0.5M NaOH
3) [DARK] Add in 37.5ul of 0.1% OPA (fluorophore, stored in -20°C freezer)
4) Incubate samples in the dark for at least 15 minutes on rocker
5) After incubation, transfer to HPLC vial, ready for run.
6) Measure hemoglobin concentration in blood samples (those with anticoagulants and NEM) according to the manufacturer's instructions (for the Drabkin's reagent).

**For Muscle**

- Label and prepare 6 tubes per muscle sample (allows measurement of protein, GSH and GSSG all from same sample this way):
    - Tube1 for muscle homogenate
    - Tube 2 for protein assay
        - 5ul homogenate into 45ul TRIS-BSN buffer
        - Poke hole in top of tube
    - Tube 3 with TCA
        - 7ul TCA into tube
    - Tube 4 PCA
        - 70ul PCA into tube
    - Tube 5 labeled GSH
        - Poke hole in top of tube
    - Tube 6 labeled GSSG
        - Add 500ul 0.5M NaOH

- <u>Muscle Prep:</u>
    - Homogenize tissue in TRIS-BSAN at a 1mg-10ul ratio (e.g., 20mg muscle to 200uL TRIS-BSN),
        - leave on ice until all samples are done
        - Try and get at least 145ul final volume, otherwise need to adjust volumes of TCA and PCA, not a big deal, but adds time
    - Spin samples at 800g for 10 minutes at 4 degrees
    - Add supernatant to tube 1
- Take from tube 1:
    - 5ul tube 1 -> tube 2 (protein tube)
        - Freeze in liquid nitrogen or -80
    - 70ul tube 1 -> tube 3 (TCA tube, for GSH)
        - Put onto rocker
    - 70ul tube 1 -> tube 4 (PCA tube, for GSSG)
        - Put onto rocker
    - Vortex tubes 3 and 4
- Spin TCA and PCA tubes at 20,000g for 5minutes at 4$^{o}$C
- Carefully take tubes out of centrifuge, be careful not to dislodge pellet
- From tube 3: Take off supernatant, and transfer to tube 5 (GSH tube, ready to be ran)
- From tube 4: take 100ul of tube 4 -> tube 6 (GSSG tube, thaw, add OPA-1, ready to be ran)
- Freeze

Determine protein from samples using tube 2 (protein tube) with protein assay (BCA) to express results relative to protein.

## Step 6: Running analysis to determine GSH or GSSG

**GSH determination (UV)**

- GSH flow rate at 1.05ml/min
- Sample detected using UV detector (on HPLC stack) at 265nm
- 94% Mobile phase A, 6% acetonitrile
    - Protocol name on HPLC is Prowting GSH (or TurnbullGSH)
- Take GSH tube, transfer total volume into HPLC vial.
- Have machine insert 10ul of sample into machine
    - GSH elutes as two peaks right before NEM spike because the configuration of GSH (3 amino acids) can be built in two ways depending on the specific amino acid configuration. Use 2$^{nd}$ peak because 1$^{st}$ peak is sometimes influenced by other compounds depending on the tissue being analyzed

**GSSG determination (fluorescent)**

- Excitation/Emission 350/420nm
- Flow rate at 0.5 ml/min
- Sample elutes as a single peak
- Injection volume: 50ul
- Protocol is set up as Prowting GSSG (or TurnbullGSSG)
- Take tube 6 out of freezer
- When thawed, add in 37.5ul of 0.1% OPA (our fluorophore, which is stored in -20$^{o}$C freezer)
- Incubate samples in the dark for at least 15 minutes
- After incubation, transfer to HPLC vial, ready for run.

**Running GSH & GSSG:**

- Add HPLC tubes to autosampler, make note of locations
- Quick batch and label each (can check here that settings are correct, to sample 10uL etc.)
- Run

## Step 7: Clean Machine & Shut Down

- Move probe from A to D
- Change to 25% B, 75% A for 10 mins
- Change to 50% B, 50% A for 10 mins
- Change to 100% B for 10 mins, leave lines in this way unless long shutdown

- Shutdown:
    - Turn off pump
    - Close software
    - Turn off machines

## Step 8: Post-run Analysis

- Lab solutions -> post run -> browser -> open method file (may need to create compound table in wizard, should be set up on "Prowting GSH" method though)
- Data tab (bottom left) -> drag in standards -> change dropdown to "standard calc point"
- Drag data files in, should auto calculate based on standard curve you set
- File -> Export Quantitative results
- (David Yadzi on YouTube for tutorials)

### How to make reagents:

- **EDTA (stable 2 weeks @ 4 degrees**) = add 75mg powder to 1mL water

- **0.2M NEM (stable 3 months @ -20 degrees)** = add to standards and blood to prevent auto-oxidation of GSH to GSSG. To make:
    - Add 250.25mg NEM powder to 10mL dd$H_20$

- **TRIS-BSA =** homogenization buffer with Trizma Base + Boric Acid/Serine/Acivicin to inhibit γ-glutamyltranspeptidase from metabolizing GSH:GSSG (aka prevent GSH breakdown). To make:
    - 200mL HPLC grade water
    - 50mM Trizma Base: 1.212g – (MW=121.14g)
    - 20mM Boric Acid: 0.248g – (MW=61.83g)
    - 2mM L-serine: 0.042g – (MW=105.69g)
    - 20uM Acivicin (-20 freezer): 0.714mg (MW=178.57)
    - pH to 8 with HCL then filter

- **TRIS-BSAN (stable 1 month at -20)**= TRIS-BSA and NEM combination
    - Thaw 0.2M NEM from freezer
    - Add 25uL NEM per 1mL TRIS-BSA (typically do 75uL for 3mL TRIS-BSA)

- **TCA60 (12 months)** = Add 60g TCA to 100mL ddH20
    - **TCA15** = Add 10mL TCA60 to 30mL ddH2O

- **PCA15** = For 40mL, add 8.571 of PCA70 (in bottle) to 31.429 ddH2O
- **NaOH 0.5M =** add 4g into 200mL ddH2O

**Mobile Phase Solvents:**

- **GSH Mobile Phase = Glacial Acetic Acid**: Solvent A. Prepare fresh daily.
  - Add 2.5mL of glacial acetic acid into 997.5mL HPLC grade water (or 1.25mL into 498.75mL)
  - pH to 3.1:
    - To bring up, add a couple of drops of 2M NaOH
    - To bring down, add a couple of drops of glacial acetic acid
- **Acetonitrile** = Solvent B, typically pre-made and bought
- **GSSG Mobile Phase** = Solvent A for GSSG mobile phase. Prepare fresh daily.
    - Add 1.775g $Na_2HPO_4$ to 425mL of dd$H_20$
    - pH to 6 with phosphoric acid
    - Add 75mL HPLC MeOH (methanol)
- **50:50 Methanol:H2O =** Solvent D (and rinse)

**Troubleshooting Notes**

- Check reservoirs are full (rinse bottles and LC30-AD internal bottle)
- Clean lines with MeOH:H2O, run for longer (~30 mins). Can try back flushing (turn column around) to try clear debris
- Deep clean with water or diluted bleach (look up specific guidelines for this) overnight at low flow (0.5ml/min). Take column out (never run only water through it).
- pH and filter mobile phases
- Make/get new reagents
    - TRIS
    - NEM
    - EDTA
    - Mobile Phases
    - OPA-1
    - Methanol
- Thoroughly mix each stage (vortex and shake)
- Check machine – possible pump failure (leaks) or bulb failure (moving baseline)
    - Lamp check
    - Wavelength check
- Check Pats notebook, Shivs draw (at desk) in Perry Lab
- Check Giurastini et al. (2013) [423]
- Contact Jocey at Mandel

## 4.5 Body Composition SOP

**Height**

1) Remove shoes
2) Raise stadiometer arm
3) Have participant stand with their back to it
4) Instruct them to stand up straight and take a breath, then step forward
5) Record value on stadiometer (number found at middle point, where arm extends from)

**Weight**

1) Remove shoes and bulky/heavy clothing
2) Step onto scale
3) Move big weight to as close as possible (can ask what they think they weigh to get in the ballpark)
4) Move smaller scale at top until balance is even
5) Record this value

**Waist Circumference**

1) Remove bulky clothing, if comfortable, raise shirt up
2) Place tape measure in line with belly button
3) Pull firm but not extremely tight
4) Record measurement

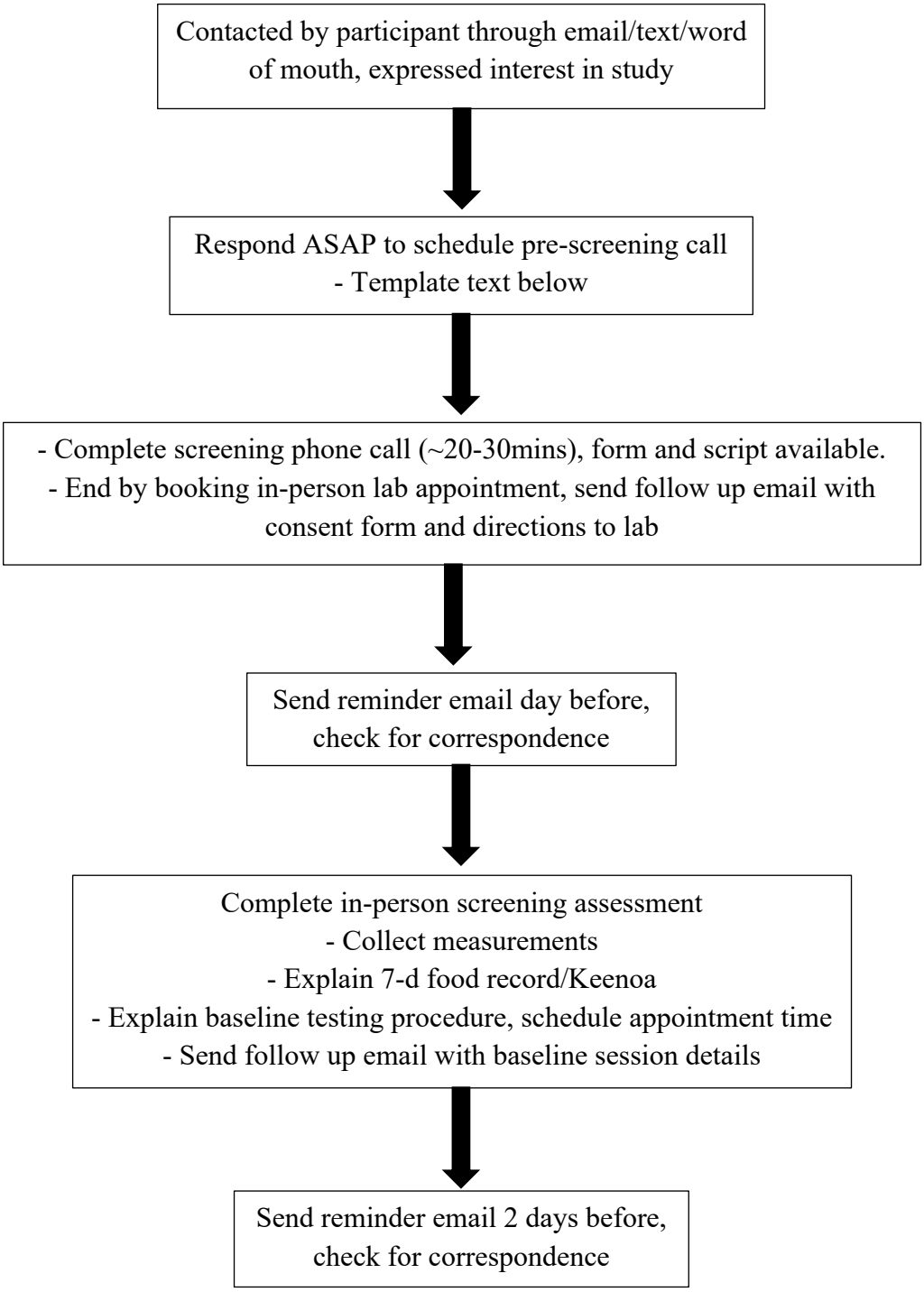
**BIA**

1) Remove shoes and socks
2) Hold handles
3) Input data on machine (height, weight, age, gender etc.)
4) Record values – FFM, FM, BF%

**BodyMetrix**

1) Plug in device to computer, open software
2) Create participant in system
3) Input information – age, height, weight, gender etc.
4) Select Jackson and Pollock 3-site
5) Mark the sites with pen/dot
6) Put ultrasound gel on end of probe
7) Place perpendicular on skin fold site, firm pressure but do not compress hard
8) Hold button on device until steady signal, press again to record skinfold thickness
9) Do all 3 sites, then repeat

### 4.6 Participant Communication SOP



**Initial Email Response Template**

Hi [Insert Participant Name],

Thank you for your interest in participating in our new and exciting research study to investigate the effects of dairy consumption on inflammation and health measures! Before we can enroll you in the study, we need to complete an initial assessment to determine your eligibility for the study.

The first component of this screening process can be completed via phone or zoom call and will involve us asking you some questions regarding your current health status, normal weekly dairy consumption, and some other lifestyle-related questions. I have attached an invitation letter providing an overview of the study, but during the phone call we can provide you with a more detailed rundown/overview of the study, as well as answer any questions about it that you may have. This call will last approximately 20-30 minutes.

Please let me know the best day and time this week for you to complete this call, as well as your contact details (either phone number or email address for a zoom meeting).

Thank you very much for your time, and I hope you have a wonderful rest of the day!

Kind Regards,

Joel Prowting, PhD(c)
Dietary Research on Inflammation and Vascular Effects (DRIVE) Study Team
**(Attach Invitation Letter)**

**In-Person Screening Schedule Template**

Hi [Participant Name],

Thanks again for taking the time to do the phone call screening today, it was a pleasure to meet you!

As mentioned during the call, I have attached a copy of the **study consent form.** This contains all of the details involved with participating in the study. If possible, please read it over before attending the in-person screening visit. We will then be able to go through it with you as well as answer any further questions you may have.

The next step is for you to attend our laboratory to complete some additional in-person screening measures, including measurement of your body composition, blood pressure, and fasting blood glucose/lipid levels (through a fingerprick droplet blood test). We ask that you attend the lab in a fasted state (i.e., **no food, drink (water is fine), or caffeine** within 8h prior to your visit) and to wear/bring comfortable, loose-fitting/athletic clothing.

This visit will last approximately 30 - 45 minutes and be completed in our laboratory located on the York University Keele campus. We will meet you at the east side entrance of the "Calumet College" building. I have included a document with directions and a map of the location.

When would be a good day/time for you to attend next week?/**or confirm date/time discussed during the phone call.** We usually recommend sometime in the morning considering that the blood test needs to be completed while fasted.

Thanks again for your time, and I'm looking forward to meeting you in person! If you need to reschedule for any reason, or have any trouble finding the building/entrance, you can contact me through this email address, or text/call my cell at: (519) 999-9780.

Kind Regards,

Joel Prowting, PhD(c)
DRIVE Study Research Team
**(Attach Study Consent Form)**
**(Attach lab/campus directions)**
**(Attach COVID-19 info/screening link)**

**24h Prior Reminder Email Template (In-Person Screening)**

Hi **[Participant Name],**

Your in-person laboratory screening visit is scheduled for tomorrow at **[Insert Time]**. Please reply to this email or contact us at: **[Insert Researcher Phone Number]** if you cannot attend or need to reschedule for any reason.

As a reminder, you will be attending our laboratory to complete some additional screening measures, including measurement of your body composition, blood pressure and fasting blood glucose and lipid levels (via fingerprick assessment). We ask that you attend the lab in a fasted state (i.e., no food, drink (water is fine) or caffeine prior to your visit) and wear/bring comfortable, loose-fitting/athletic clothing.

This visit will last approximately 30 minutes and be completed in our laboratory in the Norman Bethune College building (Room 123) on the York University Keele campus. I have included a document with directions and a map to the location. Prior to attending campus, please ensure you have proof of double COVID-19 vaccination and have completed the screening questionnaire at the following link:

Please let us know if you have any additional questions, but if not, we'll see you tomorrow!

Kind Regards,
**[Researcher Name]**

**In-person Screening Follow Up Email**

Hi **[Participant Name],**

It was great to meet you today, thanks again for completing the in-person screening component of this study!

As I mentioned earlier, the next component of the study requires you to complete a 7-day food record to allow for our Registered Dietician, Sarah, to assess your usual diet and confirm that you are eligible. She will be reaching out with you shortly to book a virtual consultation in order to walk you the food record process. I have included her on this email, so you will have each other's contact information in case you have any questions regarding the food record.

You can also find the link to the food tracking app Keenoa here:
**[Insert Link]**

Following completion and assessment of the 7-day food record, you will officially be eligible to begin the study! The next step would then be to attend our laboratory again to complete the baseline testing visit. Details are found in the consent form that we provided you, but briefly, would involve us measuring your body composition, vascular measurements and acquiring a venous blood sample. We once again ask that you attend the lab in an overnight fasted (8h minimum) state (i.e., no food, drink (water is fine), caffeine or pain medication prior to your visit) and wear/bring comfortable, loose-fitting/athletic clothing. Please also refrain from alcohol consumption or intensive exercise/activity during the two days prior to your session.

This visit will last approximately 45 minutes and will again be completed in our laboratory in the Norman Bethune College building (Room 123) on the York University Keele campus. Prior to attending campus, please ensure you have proof of double COVID-19 vaccination and have completed the screening questionnaire at the following link:

Your session is currently scheduled for **[Insert Date]** at **[Insert Time]**. We will send a reminder email the day prior, but if you cannot attend or need to reschedule the appointment, please either email us back **[Insert Researcher Email Address]** or text/call us at **[Insert Researcher Phone Number].**

Thanks again for your time!

Kind Regards,
**[Researcher Name]**
**(Attach Keenoa guide documents/video link)**
**(Attach COVID-19 info/screening link)**

**48h Prior Reminder Email Template (Baseline Testing)**

Hi **[Participant Name],**

Your baseline testing session is scheduled for two days from now **[Insert Date]** at **[Insert Time]**. Please reply to this email or contact us at: **[Insert Researcher Phone Number]** if you cannot attend or need to reschedule for any reason.

As a reminder, you will be attending our laboratory so we can take some measurements prior to your 6-week dietary period. Further details are found in the consent form that we provided you, but briefly, will involve us measuring your body composition, vascular measurements and acquiring a venous blood sample. We once again ask that you attend the lab in an overnight fasted (8h minimum) state (i.e., no food, drink (water is fine), caffeine or pain medication prior to your visit) and wear/bring comfortable, loose-fitting/athletic clothing. Please also refrain from alcohol consumption or intensive exercise/activity over the next two days.

This visit will last approximately 45 minutes and will again be completed in our laboratory in the Norman Bethune College building (Room 123) on the York University Keele campus. Prior to attending campus, please ensure you have proof of double COVID-19 vaccination and have completed the screening questionnaire at the following link:

Please let us know if you have any additional questions, but if not, we'll see you soon!

Kind Regards,
**[Researcher Name]**

**Baseline Testing Follow Up Email (Dairy)**

Hi **[Participant Name],**

Thank you for attending the baseline testing session today, you did great!

The next phase of the study is the 6-week dietary intervention phase. During this time, we will supply you with dairy products, and ask you to consume 3 servings per day (replacing other foods in your diet with them). At various time points during this 6-weeks, we will ask you to complete a physical activity questionnaire, track your body weight and to record your food intake using a phone/tablet app (Keenoa link here: **[Insert Link])** or paper food log.

We will contact you the day before these questionnaires are required to remind you. For the food assessment, our Registered Dietician Sarah will contact you and monitor your results through the app. If at any time you have questions, you can contact myself or Sarah through the contact information included at the bottom of this email.

At the end of the 6-week period, you will return to the lab to be re-tested as well as to complete the high-fat meal challenge. This is currently scheduled for **[Insert Date]** at **[Insert Time]** and will require you to be in the lab for around 7h. We will send a reminder email the day prior, but if you cannot attend or need to reschedule the appointment, please either email us back **[Insert Researcher Email Address]** or text/call us at **[Insert Researcher Phone Number].**

Thanks again for your time and we appreciate your continued participation in the study!

Kind Regards,
**[Researcher Name]**

**Baseline Testing Follow Up Email (Habitual)**

Hi **[Participant Name],**

Thank you for attending the baseline testing session today, you did great!

The next phase of the study is the 6-week dietary phase. During this time, you will continue to consume your normal diet and follow your normal lifestyle. At various time points during this 6-weeks, we will ask you to complete a physical activity questionnaire, track your body weight and to record your food intake using a phone/tablet app (Keenoa link here: **[Insert Link])** or paper food log.

We will contact you the day before these questionnaires are due to help to remind you. For the food assessment, our Registered Dietician Sarah will contact you and monitor your results through the app. If at any time you have questions, you can contact myself or Sarah through the contact information included at the bottom of this email.

At the end of the 6-week period, you will return to the lab to be re-tested as well as to complete the high-fat meal challenge. This is currently scheduled for **[Insert Date]** at **[Insert Time]** and will require you to be in the lab for around 7h. We will send a reminder email the day prior, but if you cannot attend or need to reschedule the appointment, please either email us back **[Insert Researcher Email Address]** or text/call us at **[Insert Researcher Phone Number].**

Thanks again for your time and we appreciate your continued participation in the study!

Kind Regards,
**[Researcher Name]**

**High Fat Challenge Reminder Email Template**

Hi **[Participant Name],**

Your 6-week follow up testing session is scheduled for two days from now **[Insert Date]** at **[Insert Time]**. Please reply to this email or contact us at: **[Insert Researcher Phone Number]** if you cannot attend or need to reschedule for any reason.

As a reminder, you will be attending our laboratory so we can take some measurements following your 6-week dietary period. Further details are found in the consent form that we provided you, but briefly, we will:

- Repeat the baseline testing measurements again (body composition, vascular measurements, blood sample)
- If you chose to opt in, perform a fasted muscle biopsy
- Consume a high fat meal (McDonalds breakfast)
- You will remain in the lab for the following 6-hours as we monitor you and take additional measurements during this period (vascular measures, blood sample, biopsy)

We once again ask that you attend the lab in an overnight fasted (8h minimum) state (i.e., no food, drink (water is fine), caffeine or pain medication prior to your visit) and wear/bring comfortable, loose-fitting/athletic clothing. Please also refrain from alcohol consumption or intensive exercise/activity over the next two days.

This visit will last approximately 8 hours and will again be completed on the York University Keele campus. We will have an iPad available, but we recommend bringing additional materials/activities during this period.

Please let us know if you have any additional questions, but if not, we'll see you soon!

Kind Regards,
**[Researcher Name]**

## 4.7 Screening Visit SOP

Preparation

1) Turn on (calibrate if needed) equipment – cholestech + printer
2) Gather supplies
    a. Remove cholestech cartridge from fridge
    b. Fingerprick supplies:
        i. Absorbent cloth area
        ii. Gloves
        iii. Alcohol swab
        iv. Gauze
        v. Bandaid
        vi. Lancet
        vii. Capillary tube + plunger
        viii. Cartridge
    c. Tape measure
    d. [In Calumet] Blood pressure machine + cuffs
3) Print all forms/documents – CRF, informed consent, Keenoa guide

**Participant Arrival & Data Collection**

4) Greet participant at Calumet
5) Confirm phone screening questions complete
6) Sit down and go through consent form/answer questions – **confirm it is signed**
7) Measure seated blood pressure twice, 1 min rest in between
8) Take participant down to Bethune lab
9) Measure height, weight, waist circumference
10) Explain self fingerprick protocol, help if necessary, collect sample, second person run it on cholestech

**Explanation of Next Steps**

11) Keenoa:
    a. Help them download app if needed
    b. Walk through inputting a snack/food item.
    c. Provide training guide and that dietitian will contact them
12) Explain next steps, pencil in baseline testing date (at least 7 days after)
13) Explain that we'll check results for eligibility after food diary data collected and be in touch to confirm session
14) Escort them out of the building
15) Send follow up email

## 4.8 Baseline Visit SOP

Preparation

1) **EQUIPMENT** - turn on/set up:
    a. BodyMetrix – set up participant profile
    b. Centrifuge settings (4°C + 1300 RCF for 15 minutes)

2) **SUPPLIES** – collect and lay out:
    a. Blood:
        i. Have pipettes (P1000, P200, P20) and tips (1000 & 200) ready
        ii. Fill 4 Eppendorf tubes with NEM and EDTA for GSH analysis
            1. 0.5mL of blood = 11.5mL of K3 EDTA + 50mL of NEM
            2. Mix well or vortex
        iii. Venipuncture supplies:
            - Absorbent cloth area
            - Gloves
            - Alcohol swab
            - Gauze
            - Band-Aid
            - Tourniquet
            - Sharps Bin
            - Butterfly Needle
            - Vacutainers - Red (4mL) x2, EDTA (4mL) x2, Na Heparin (4mL) x1
            - Eppendorf/cryo tubes
                a. **~20 Labelled** (4x GSH, 4x Serum, 4x Na Heparin Plasma, 8x EDTA Plasma; see Tube Labelling SOP for details)
    b. Body Composition:
        i. [Calumet]
            1. Tape measure
            2. BodyMetrix machine + laptop + ultrasound gel (+ paper towels to wipe off gel)
            3. Shorts and t-shirt
    c. Vascular
        i. [Calumet] Confirm Tania set up and ready (Heather's Lab)

    d. Dairy food (if required) – 2 weeks supply:
        **i.** Milk = **2 x 2L cartons (~16 days)**
        **ii.** Yogurt = **5 x 650g containers (~14 days)**
        **iii.** Cheese = **4 x multipacks (~16 days)**

3) Print all forms/documents for day:
    a. Data collection form
    b. Bodyweight log (if they have scales at home)
    c. 7-day food diary (if not using Keenoa)

d. Dairy package:
    i. Dairy checklist habit tracker
    ii. Tips and tricks
    iii. Exchange list & sample meals

**Participant Arrival & Data Collection**

Pre-check/greet:

4) Greet participant at Calumet
5) Sit down, confirm pre-test check questions, and explain testing session

Vascular:

6) Escort to heathers lab for vascular measure assessment (~22 – 30 minutes)
7) Collect from Tania, take participant to Calumet office for BodyMetrix

Body Composition:

8) Check if male/female tester preferred, perform BodyMetrix assessment
    a. Jackson & Pollock 3-site (See SOP for more details)
9) Escort participant down to Bethune, then measure weight & BIA body fat% on Tanita scale (See SOP for more details)

Blood Draw:

10) Complete blood draw
    1. From first red tube, immediately move 0.5mL to GSH eppendorfs (pre-coated with 11.5ul of EDTA, 50ul NEM) and invert 8 times to mix. Place in -20°C in freezer (move to -80°C after visit is complete)
    2. Invert purple (EDTA) and green (Na Heparin) tubes ~8 times immediately upon collection
    3. Move purple and green tubes to centrifuge and spin immediately to get plasma
    4. Leave red tube to clot for minimum of 30 mins (ideally 60 mins)

    Can be done after participant has left:

    1. Spin red tube(s) in centrifuge to get serum
    2. Use serum from second red tube for lipid assessment
        a. Pipette left over serum into Eppendorf, transfer from Eppendorf to afinion cartridge (careful to avoid generating bubbles), run in analyzer
    3. Pipette all remaining serum/plasma into labelled eppendorfs
    4. Initially place upright into -20 C in Bethune
    5. Move to -80°C in Heather's lab (either in box or Ziploc bags)

**Explanation of Next Steps**

Habitual 6-Week Diet

11) Continue with normal diet and lifestyle

12) Schedule check in visits + food diary collection:
    a. Week 0: track with Keenoa for next 7-days
    b. Week 2: schedule appointment for IPAQ + BIA, remind of 3-day Keenoa
    c. Week 4: schedule appointment for IPAQ + BIA, remind of 3-day Keenoa
    d. Week 6: track with Keenoa for 7-days prior to HFMC

Dairy 6-week Diet

13) **[Sarah Consultation]** Dairy = replace foods in diet with 1 serving each of milk, yogurt and cheese per day:
    i. Use measuring cup to show how much to consume – give to them to take
    ii. Give refrigerated bag with 1 or 2 weeks supply of dairy
    iii. Give dairy package
    iv. Keenoa review – take photo pre and post just for dairy
14) Schedule check in visits + food diary collection:
    a. Week 0: track with Keenoa for next 7-days
    b. Week 2: schedule appointment for IPAQ + BIA, remind of 3-day Keenoa + pick up Dairy
    c. Week 4: schedule appointment for IPAQ + BIA, remind of 3-day Keenoa + pick up Dairy
    d. Week 6: track with Keenoa for 7-days prior to HFMC

15) Escort them out of the building
16) Confirm all data has been recorded on CRF form, store in filing cabinet in Calumet office
17) Send follow up email with confirmed dates for future visits

**Tidy Up & Put Away**

18) **EQUIPMENT** - turn off:
    a. BodyMetrix + laptop, Centrifuge
19) **SUPPLIES**:
    a. Blood:
        i. Sharps in sharps bin
        ii. Anything that touched blood in biohazard bag/bin
        iii. Anything else in normal garbage
        iv. Wipe down surface with Lysol
    b. Body Composition:
        i. Clean ultrasound gel off BodyMetrix probe, put away in box

## 4.9 Meal Challenge Visit SOP

Preparation

1) **EQUIPMENT** - turn on/set up:
    a. BodyMetrix – set up participant profile
    b. Centrifuge settings (4°C + 1300 RCF for 10 minutes)

2) **SUPPLIES** – collect and lay out:
    a. Blood:
        i. Have pipettes (P1000, P200, P20) and tips (1000 & 200) ready
        ii. Fill 20 Eppendorf tubes with NEM and EDTA for GSH analysis
            1. 0.5mL of blood = 11.5mL of K3 EDTA + 50mL of NEM
            2. Mix well or vortex
        iii. Venipuncture supplies:
            - Absorbent cloth area
            - Gloves
            - Alcohol swab
            - Gauze
            - Band-Aid
            - Tourniquet
            - Sharps Bin
            - Catheter needle (some butterfly's just in case)
            - Extension tubing
            - Q-syte
            - Saline syringes
            - Tube & Eppendorf racks x5
            - Vacutainers for each time point (~20 total)
                - Baseline - Red (4mL) x2, EDTA (6mL) x1, Na Heparin (4mL)
                - 1h - Red (4mL) x2, EDTA (6mL) x1, Na Heparin (4mL) x1
                - 2h - Red (4mL) x2, EDTA (6mL) x1, Na Heparin (4mL) x1
                - 4h - Red (4mL) x2, EDTA (6mL) x1, Na Heparin (4mL) x1
                - 6h - Red (4mL) x2, EDTA (6mL) x1, Na Heparin (4mL) x1
            - Eppendorf/cryo tubes for each time point (~90 total)
                a. (4x GSH, 4x Serum, 4x Na Heparin Plasma, 6x EDTA Plasma; see Tube Labelling SOP for details)
                b. 18 for each: Baseline, 1h, 2h, 4h, 6h
    b. Body Composition:
        i. [Calumet]
            1. Tape measure
            2. BodyMetrix machine + laptop + ultrasound gel (+ paper towels)
            3. Shorts and t-shirt
    c. Vascular
        i. [Calumet] Confirm Tania set up and ready (Heather's Lab)
3) Print all forms/documents for day:

a. Data collection form
b. Schedule
c. SOPs

**Participant Arrival & Data Collection**

Pre-check/greet:

4) Greet participant at Calumet
5) Sit down, confirm pre-test check questions, and explain testing session

Vascular (Baseline):

6) Escort to heathers lab for vascular measure assessment (~22 – 30 minutes)
7) Collect from Tania, take participant to Calumet office for BodyMetrix

Body Composition:

8) Check if male/female tester preferred, perform BodyMetrix assessment
    a. Jackson & Pollock 3-site (See SOP for more details)
9) Escort participant down to Bethune, then measure weight & BIA body fat% on Tanita scale (See SOP for more details)

Blood Draw (Baseline):

10) Insert indwelling venous catheter for blood draws
    5. From first red tube, immediately move 0.5mL to GSH eppendorfs (pre-coated with 11.5ul of EDTA, 50ul NEM) and invert 8 times to mix. Place in -20°C in freezer (move to -80°C after visit is complete)
    6. Invert purple (EDTA) and green (Na Heparin) tubes ~8 times immediately upon collection
    7. Move purple and green tubes to centrifuge and spin immediately to get plasma
    8. Leave red tube to clot for minimum of 30 mins (ideally 60 mins)
    9. Spin red tube(s) in centrifuge to get serum
    10. Pipette all remaining serum/plasma into labelled eppendorfs
    11. Initially place upright into -20 C in Bethune then move to -80°C in Heather's lab

[Optional] Muscle Biopsy (see SOP)

HFMC:

11) Provide participant with meal (Tim Hortons and/or Starbucks meals, see HFMC guide document)
12) Record time started and time finished (no more than 10 minutes to eat it)

Blood Draw (HFMC = 1h, 2h, 4h, 6h post-prandial):

13) Indwelling venous catheter flushed after draw & every 30-minutes
    a. 30-min, 90-min, 150-min, 180-min, 210-min, 270-min, 300-min, 330-min
14) Blood taken at following time points:
    a. 1h post-prandial
    b. 2h post-prandial

c. 4h post-prandial
d. 6h post-prandial

15) Repeat post-sample processing steps for each time point:
    a. From first red tube, immediately move 0.5mL to GSH eppendorfs (pre-coated with 11.5ul of EDTA, 50ul NEM) and invert 8 times to mix. Place in -20°C in freezer (move to -80°C after visit is complete). Sit for 30-60 mins, centrifuge, pipette serum into eppendorfs, freeze
    b. Invert purple (EDTA) and green (Na Heparin) tubes ~8 times immediately upon collection, centrifuge immediately, pipette plasma into eppendorfs, freeze
    c. Move to -80°C by end of day

Vascular (4h post):

16) Assist Tania in moving equipment from Calumet to Bethune
17) Ensure quiet and dark for duration of test (25 – 30 mins)

Biopsy (4h post)

After care:

- Ensure catheter removed successfully and sanitized area, provide food/drink
- Give biopsy care instructions + care kit

**Explanation of Next Steps**

4-Week Washout

18) Continue with normal diet and lifestyle for next 4-weeks, minimal contact from us
19) Schedule baseline 2 visit date
20) Escort them out of the building
21) Confirm all data has been recorded on CRF form, store in filing cabinet in Calumet office
22) Send follow up email with confirmed dates for future visits

**Tidy Up & Put Away**

23) **EQUIPMENT** - turn off:
    a. BodyMetrix + laptop, Centrifuge
24) **SUPPLIES**:
    a. Blood:
        i. Sharps in sharps bin
        ii. Anything that touched blood in biohazard bag/bin
        iii. Anything else in normal garbage
        iv. Wipe down surface with Lysol
        v. Confirm all samples in -80
    b. Body Composition:
        i. Clean ultrasound gel off BodyMetrix probe, put away in box

## 4.10 High Fat Meal SOP

To calculate TDEE, use the Mifflin St Jeor formula (found here: https://www.omnicalculator.com/health/bmr or https://www.inchcalculator.com/mifflin-st-jeor-calculator/). Can find age, height and weight from the master data collection sheet

Sandwich Options:

- **Tim Hortons Biscuit Sandwich with Sausage:** 530 kcal, 35 g fat, 34 g CHO, 20 g protein.
- **Tim Hortons Sandwich without cheese**: 480 kcal, 32 g fat, 33 g CHO, 17 g protein.
- **A&W Beyond Meat Burger (base)**: 500 kcal, 29 g fat (52%), 40g CHO (32%), 22 g protein (17%).
- **A&W Beyond Meat Burger (No ketchup 15kcal, mustard 9kcal, pickles 5kcal, tomato 5kcal, lettuce 1kcal, red onion 5kcal)**: 460 kcal, 28 g fat (55%), 32g CHO (29%), 20 g protein (17%).

Hashbrown Options:

- **Tim Hortons Hashbrowns:** 117.7 kcal, 6 g fat, 15.3 g CHO, 1.3 g protein.
- **A&W Hashbrowns:** 180kcal, 11g fat (55%), 18g CHO (40%), 2 protein (4%)

Donut Options:

- **Chocolate Glazed Timbit:** 80 kcal, 3.5 g fat, 11 g CHO, 1 g protein.
- **Old Fashioned Glazed Timbit:** 80 kcal, 3.8 g fat, 10.8 g CHO, 0.9 g protein.
- **Sour Cream Glazed Timbit:** 92.8 kcal, 5 g fat, 11.2 g CHO, 0.9 g protein.
- **Old Fashioned Plain Timbit:** 63.6 kcal, 3.8 g fat, 6.5 g CHO, 0.9 g protein.
- **Honey crueller** = 320kcals, 21g fat (59%), 30g CHO (38%), 2g pro (3%)

Misc (remove from online calc if needed):
**Fast food Egg** = 100kcal, 8g fat, 0.5g CHO, 6g protein
**Processed cheese slice** = 50kcal, 4g fat, 1g CHO, 3g protein

## Regular HF Meals

**800 kcal:** 1 Sausage Biscuit sandwich (no cheese), 1 hashbrown, 3 timbits.
*838 kcal, 49.4g (53%) fat, 80.7g (36%) CHO, 21g (10%) protein.*

**1000 kcal:** 1 Sausage Biscuit sandwich (no cheese), 2 hashbrowns, 4 timbits.
*1034 kcal, 58g (50%) fat, 104g (41%) CHO, 23g (9%) protein.*

**1200 kcal:** 1 Sausage Biscuit sandwich (no cheese), 2 hashbrowns, 7 timbits.
*1275 kcal, 70.6g (50%) fat, 139.2g (44%) CHO, 25.9g (8%) protein.*

**1400 kcal:** 2 Sausage Biscuit sandwich (no cheese), 1 hashbrown, 5 timbits.
*1478 kcal, 89 g fat (54%), 135.3g (37%) CHO, 42.8g (12%) protein.*

# Lacto-Ovo/Halal Meals

**1000 kcal:** 1x A&W Beyond Meat Burger + 2 A&W Hashbrown + 3 Chocolate Timbits
1060 kcal, 60.5 g fat (51%), 101g CHO (38%), 27 g protein (11%).

**1200 kcal: :** 2 A&W beyond meat burger + 1 A&W hashbrown + 2 timbits
1230kcals, 75g fat (55%), 95g CHO (31%), 44g protein (14% protein)
.
**1400 kcal:** 2 A&W beyond meat burger + 1 A&W hashbrown + 1 honey crueller
1420kcals, 88g fat (56%), 112g CHO (32%), 44g protein (13% protein)

Discontinued Oct 2023
**Starbucks Beyond Meat Sandwich**: 390 kcal, 19 g fat, 31 g CHO, 25 g protein.
**Starbucks Beyond Meat Sandwich (no cheese)**: 340 kcal, 15 g fat, 30 g CHO, 22 g protein.
**Starbucks Beyond Meat Sandwich (no cheese, no egg)**: 240 kcal, 7 g fat, 29.5 g CHO, 16 g protein.

# Appendix 5 - Data Collection Forms

## 5.1 Initial Phone Screening Form

Name of potential subject:

How did you hear about the study?:

**Please explain the following about the study to the potential participant:**

"- This is a nutrition study with a focus on health markers that will take a minimum of **16-weeks** to complete. We are looking to investigate the effects of increasing dairy consumption on measures of health, such as inflammation levels, blood fat levels and blood vessel function."

- If you are eligible to participate, you will be asked **to consume dairy products** (yogurt, milk and cheese) for a 6-week period during the study. You will also be asked to record your food intake using a 3 or 7-day food diary at various times throughout the study as well as to attend our lab a minimum of **4 times** to have measurements taken.

- As part of this study, we will take some tests which include the assessment of your height, weight, waist/hip circumference, body composition, a blood sample, and measures of your blood vessel function. Blood samples will be used to measure several physiological markers of health. We are interested to see how these measures will change when your daily dairy consumption increases. If you choose, we can share these data with you.

- We would like to reiterate that we do require a **commitment** from you if you choose to participate in this study. It is very important that you **do not miss appointments** for the study and that you try your best to follow the instructions provided by the research personnel. We will tell you, the key to success in a study like this is **compliance** and **adherence** to the protocol. We have a lot of experience in running studies like these."

"Do you have any questions?"

"Are you interested in being **formally screened** for the study right now? I would require you to answer a few questions which will take about 5 minutes. Or would you rather think about the information I just gave you and call us back when/if you would like to be screened?"

Interviewer: _____ Date of Contact/Call: _____

**Researcher:** **Date & Time:**

| **1. Participant Details** | | | |
|---|---|---|---|
| Name: | | | |
| Telephone #: | | Email: | |
| Preferred Contact method? | \| \| Telephone \| \| Zoom \| \| Email | | |
| Age: | | \| \| **18 - 70 years old?** | |
| Date of Birth: | | | |
| Sex: | | | |
| Self-Identified Ethnicity: | | | |
| Height: | | | |
| Weight: | | | |
| Calculated BMI: | | \| \| **≥ 25 kg/m$^2$?** | |
| **2. Exercise/Activity Level** | | | |
| How many times per week do you perform structured exercise (not just walking)? | | | |
| | | \| \| **≤ 2 structured weekly sessions?** | |
| Type of activity and duration (per day or week): | | | |
| | | | |
| **3. Supplement consumption** | | | |
| Do you consume nutrition supplements (e.g., | \| \| Yes* | \| \| No | |

| | | |
|---|---|---|
| Multivitamin, Calcium, Vitamin D, Iron, B12 etc.) | | |
| If YES, please specify the: | | |
| Types: | | |
| Amount: | | |
| Frequency (per day or average per week): | | |
| *** (Ask if they would be willing not to take these for the duration of the study. If they agree, they need at least a 2-week washout before we start any pre-measurements.)** | | |

| **4. Health & Lifestyle Questions** | | |
|---|---|---|
| Do you smoke? | \| \| Yes | \| \| No |
| 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 have an allergy to dairy/milk protein? | \| \| Yes | \| \| No |
| Do you have Celiac Disease? | \| \| Yes | \| \| No |
| Do you have any other gastrointestinal | \| \| Yes | \| \| No |

| | | |
|---|---|---|
| diseases or condition? | | |
| Have you ever had any bone, joint or muscle injury? | \| \| Yes | \| \| No |
| Have you ever had any major joint instability or ongoing chronic pain such as in the knee, back or elbow? | \| \| Yes | \| \| No |
| **5. 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, e.g., birth control, antidepressants or micronutrients are ok): | | |
| | | |
| **6. 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? | | |
| **If the participant is over the age of 40:** | | |
| Has the length of your menstrual cycle changed? If yes, has your cycle become longer (greater or equal to 7 days) persistently (for the last 10 cycles)? | | |
| | | |
| Are you currently experiencing any vasomotor symptoms (such as hot flashes or night sweats)? If yes, how long have you been experiencing these symptoms? | | |
| | | |

| |
|---|

| | |
|---|---|
| **6.** As a participant in this study you will be required to consume several servings of dairy products every day for a 6 wk period.<br><br>**Are you willing to incorporate dairy products into your diet?** | |
| \| \| Yes | \| \| No |
| **7.** As a participant in this study **you will be required** to consume a "high-fat test meal" (i.e. fast food restaurant breakfast meal) to allow us to see how your body responds**.**<br><br>**Are you willing to eat a fast-food restaurant meal?** | |
| \| \| Yes | \| \| No |

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

**If YES** → It looks like you have met all of our inclusion criteria. The next step is to complete the **Dairy Consumption Questionnaire** and then schedule an **in-person screening appointment** with you to come into the Study Office to discuss the consent form and have a few more screening measurements taken. This appointment should take no more than 45 minutes.

**If NO** → Unfortunately, you do not meet the inclusion criteria for the study based on your answer about "X". At this time, we cannot include you in the study. Thank you for your interest. If the inclusion criteria changes in future, would you like us to reach out to you? | | Yes | | No

## 5.2 Dairy Consumption Questionnaire

Instructions

This questionnaire is about dairy products and will be used to make an estimation of the dairy products you consumed the last month. The following remarks are important and should be considered during the answering of the questionnaire.

- Answer each question as best you can by ticking the appropriate check box (to the left of each option). Estimate if you are not sure. A guess is better than leaving a blank.

- The questions cover **the last month**, this means the last 4 weeks. This includes both weekdays and weekends.

- The products you drank or ate at birthdays, weddings, receptions, etc. **should** be taken into account as well.

- If you had a completely different diet than normal for example due to illness or holiday for the last month, consider the month before the last month to fill in the questions.

- If you don't use a product at all, fill in 'Not this month'. Always give an answer

**QUESTION 1 – Drinking Milk**

| | |
|---|---|
| 1a. How often did you drink **milk as a beverage** (NOT in coffee, NOT in cereal)? *(Please do not include chocolate milk, hot chocolate and flavored milk or* yogurt*)* | |
| | Not this month **(go to question 2)** |
| | 1 day per month or less |
| | 2-3 days per month |
| | 1 day per week |
| | 2-3 days per week |
| | 4-5 days per week |
| | 6-7 days per week |
| 1b. Each day you drank milk as a beverage, how much did you usually drink? | |
| | Less than 1 cup (8 ounces) |
| | 1-1.5 cups (8 to 12 ounces) |
| | More than 1.5 cups (12 ounces) |
| 1c. How often was the milk reduced-fat or fat-free? | |
| | Almost never or never |
| | About ¼ of the time |
| | About ½ of the time |
| | About ¾ of the time |
| | Almost always or always |

| **Additional Info:** |
|---|
| |
| |

**QUESTION 2 – Milk with Cereals**

| 2a. Do you consume **milk with cereals**? | |
|---|---|
| | Not this month **(go to question 3)** |
| | 1 day per month or less |
| | 2-3 days per month |
| | 1 day per week |
| | 2-3 days per week |
| | 4-5 days per week |
| | 6-7 days per week |
| 2b. Each time **milk was added to your cold cereal**, how much was usually added? | |
| | Less than 1 cup (8 ounces) |
| | 1-1.5 cups (8 to 12 ounces) |
| | More than 1.5 cups (12 ounces) |
| 2c. How often was the milk **reduced-fat** or **fat-free**? | |
| | Almost never or never |

| | |
|---|---|
| | About ¼ of the time |
| | About ½ of the time |
| | About ¾ of the time |
| | Almost always or always |
| **Additional Info:** | |
| | |
| | |

**QUESTION 3 – Milk with Tea or Coffee**

| 3a. How often do you drink **coffee or tea with milk**? | |
|---|---|
| | Not this month **(go to question 4)** |
| | 1 day per month or less |
| | 2-3 days per month |
| | 1 day per week |
| | 2-3 days per week |
| | 4-5 days per week |
| | 6-7 days per week |
| 3b. Each time **milk was added to your coffee or tea**, how much was usually added? | |

| | |
|---|---|
| | Less than 1 tablespoon |
| | 1 to 3 tablespoons |
| | More than 3 tablespoons |
| 3c. How often was the milk **reduced-fat** or **fat-free**? | |
| | Almost never or never |
| | About ¼ of the time |
| | About ½ of the time |
| | About ¾ of the time |
| | Almost always or always |
| **Additional Info:** | |
| | |
| | |

**QUESTION 4 – Drinking Chocolate or Flavoured Milk**

| | |
|---|---|
| 4a. How often did you drink **chocolate/flavoured milk as a beverage** (including hot chocolate)? | |
| | Not this month **(go to question 5)** |
| | 1 day per month or less |
| | 2-3 days per month |

| | |
|---|---|
| | 1 day per week |
| | 2-3 days per week |
| | 4-5 days per week |
| | 6-7 days per week |
| 4b. Each day you drank **chocolate/flavoured milk as a beverage**, how much did you usually drink? | |
| | Less than 1 cup (8 ounces) |
| | 1-1.5 cups (8 to 12 ounces) |
| | More than 1.5 cups (12 ounces) |
| 4c. How often was the chocolate/flavoured milk **reduced-fat** or **fat-free**? | |
| | Almost never or never |
| | About ¼ of the time |
| | About ½ of the time |
| | About ¾ of the time |
| | Almost always or always |
| **Additional Info:** | |
| | |
| | |

**QUESTION 5 – Drinking Yogurt**

| | |
|---|---|
| 5a. How often did you drink **yogurt as a beverage**? | |
| | Not this month **(go to question 7)** |
| | 1 day per month or less |
| | 2-3 days per month |
| | 1 day per week |
| | 2-3 days per week |
| | 4-5 days per week |
| | 6-7 days per week |
| 5b. Each day you drank **drink yogurt**, how much did you usually drink? | |
| | Less than 1 cup (8 ounces) |
| | 1-1.5 cups (8 to 12 ounces) |
| | More than 1.5 cups (12 ounces) |
| 5c. How often was the drink yogurt **reduced-fat** or **fat-free**? | |
| | Almost never or never |
| | About ¼ of the time |
| | About ½ of the time |

| | |
|---|---|
| | About ¾ of the time |
| | Almost always or always |
| **Additional Info:** | |
| | |
| | |

**QUESTION 6 – Eating Yogurt**

| 6a. How often did you eat **yogurt**? | |
|---|---|
| | Not this month **(go to question 8)** |
| | 1 day per month or less |
| | 2-3 days per month |
| | 1 day per week |
| | 2-3 days per week |
| | 4-5 days per week |
| | 6-7 days per week |
| 6b. Each day you ate **yogurt**, how much did you usually eat? | |
| | Less than ½ cup or less than 1 container |
| | ½ to 1 cup or 1 container |

| | |
|---|---|
| | More than 1 cup or more than 1 container |
| 6c. How often was the yogurt you ate **reduced-fat** or **fat-free**? | |
| | Almost never or never |
| | About ¼ of the time |
| | About ½ of the time |
| | About ¾ of the time |
| | Almost always or always |
| **Additional Info:** | |
| | |
| | |

**QUESTION 7 – Eating Pudding or Custard**

| | |
|---|---|
| 7a. How often did you eat **pudding or custard**? | |
| | Not this month **(go to question 10)** |
| | 1 day per month or less |
| | 2-3 days per month |
| | 1 day per week |
| | 2-3 days per week |

| | |
|---|---|
| | 4-5 days per week |
| | 6-7 days per week |
| 7b. Each day you ate **pudding or custard**, how much did you usually eat? | |
| | Less than 1 cup (8 ounces) |
| | 1-1.5 cups (8 to 12 ounces) |
| | More than 1.5 cups (12 ounces) |
| 7c. How often was the pudding or custard you ate **reduced-fat** or **fat-free**? | |
| | Almost never or never |
| | About ¼ of the time |
| | About ½ of the time |
| | About ¾ of the time |
| | Almost always or always |
| **Additional Info:** | |
| | |
| | |

**QUESTION 8 – Eating Whipped Cream**

| | |
|---|---|
| 8a. How often did you eat **whipped cream**? | |
| | Not this month |
| | 1 day per month or less |
| | 2-3 days per month |
| | 1 day per week |
| | 2-3 days per week |
| | 4-5 days per week |
| | 6-7 days per week |
| 8b. Each time you ate **whipped cream**, how much did you usually eat? | |
| | Less than 1 tablespoon |
| | 1 to 3 tablespoons |
| | More than 3 tablespoons |
| 8c. How often was the whipped cream you ate **reduced-fat** or **fat-free**? | |
| | Almost never or never |
| | About ¼ of the time |
| | About ½ of the time |

| | |
|---|---|
| | About ¾ of the time |
| | Almost always or always |
| **Additional Info:** | |
| | |
| | |

**QUESTION 9 – Eating Ice Cream or Frozen Dairy**

| 9a. How often did you eat **ice cream or frozen dairy**? | |
|---|---|
| | Not this month |
| | 1 day per month or less |
| | 2-3 days per month |
| | 1 day per week |
| | 2-3 days per week |
| | 4-5 days per week |
| | 6-7 days per week |
| 9b. Each time you ate **ice cream or frozen dairy**, how much did you usually eat? | |
| | Less than 1 cup (8 ounces) |
| | 1-1.5 cups (8 to 12 ounces) |

| | |
|---|---|
| | More than 1.5 cups (12 ounces) |
| 9c. How often was the ice cream or frozen dairy you ate **reduced-fat** or **fat-free**? | |
| | Almost never or never |
| | About ¼ of the time |
| | About ½ of the time |
| | About ¾ of the time |
| | Almost always or always |
| **Additional Info:** | |
| | |
| | |

**QUESTION 10 – Eating Cheese**

| | |
|---|---|
| 10a. How often did you eat **cheese** (including low-fat; including on cheeseburgers or in sandwiches or subs)? | |
| | Not this month **(go to question 12)** |
| | 1 day per month or less |
| | 2-3 days per month |
| | 1 day per week |
| | 2-3 days per week |

| | |
|---|---|
| | 4-5 days per week |
| | 6-7 days per week |
| 10b. Each time you ate **cheese**, how much did you usually eat? | |
| | Less than ½ ounce or less than 1 slice |
| | ½ to 1½ ounces or 1 slice |
| | More than 1½ ounces or more than 1 slice |
| 10c. How often was the cheese you ate **reduced-fat** or **fat-free**? | |
| | Almost never or never |
| | About ¼ of the time |
| | About ½ of the time |
| | About ¾ of the time |
| | Almost always or always |
| **Additional Info:** | |
| | |
| | |

**QUESTION 11 – Eating Cottage Cheese**

| |
|---|
| 11a. How often did you eat **cottage cheese**? |

| | |
|---|---|
| | Not this month **(go to question 9)** |
| | 1 day per month or less |
| | 2-3 days per month |
| | 1 day per week |
| | 2-3 days per week |
| | 4-5 days per week |
| | 6-7 days per week |
| 11b. Each day you ate **cottage cheese**, how much did you usually eat? | |
| | Less than 1 cup (8 ounces) |
| | 1-1.5 cups (8 to 12 ounces) |
| | More than 1.5 cups (12 ounces) |
| 11c. How often was the cottage cheese you ate **reduced-fat** or **fat-free**? | |
| | Almost never or never |
| | About ¼ of the time |
| | About ½ of the time |
| | About ¾ of the time |
| | Almost always or always |

| **Additional Info:** |
|---|
| |
| |

**QUESTION 12 – Eating Cream Cheese**

| 12a. How often did you eat **cream cheese**? | |
|---|---|
| | Not this month **(go to question 13)** |
| | 1 day per month or less |
| | 2-3 days per month |
| | 1 day per week |
| | 2-3 days per week |
| | 4-5 days per week |
| | 6-7 days per week |
| 12b. Each time you ate **cream cheese**, how much did you usually eat? | |
| | Less than 1 tablespoon |
| | 1 to 3 tablespoons |
| | More than 3 tablespoons |
| 12c. How often was the cream cheese you ate **reduced-fat** or **fat-free**? | |

| | |
|---|---|
| | Almost never or never |
| | About ¼ of the time |
| | About ½ of the time |
| | About ¾ of the time |
| | Almost always or always |
| **Additional Info:** | |
| | |
| | |

**QUESTION 13 – Eating Sour Cream**

| 13a. How often did you eat **sour cream**? | |
|---|---|
| | Not this month **(go to question 11)** |
| | 1 day per month or less |
| | 2-3 days per month |
| | 1 day per week |
| | 2-3 days per week |
| | 4-5 days per week |
| | 6-7 days per week |

| | |
|---|---|
| 13b. Each time you ate **sour cream**, how much did you usually eat? | |
| | Less than 1 tablespoon |
| | 1 to 3 tablespoons |
| | More than 3 tablespoons |
| 13c. How often was the sour cream you ate **reduced-fat** or **fat-free**? | |
| | Almost never or never |
| | About ¼ of the time |
| | About ½ of the time |
| | About ¾ of the time |
| | Almost always or always |
| **Additional Info:** | |
| | |

**This is the last question, thank you very much for completing this questionnaire!**
**Sent Full Consent Form?:** | | Yes | | No

**Date of scheduled In-person visit:**

| **General Comments** |
|---|
| |
| |

## 5.3 In-Person Screening Collection Form

**Interviewer:**
**Date & Time:**

| | | |
|---|---|---|
| **1. Informed Consent** | ☐ Yes, signed ☐ No | |
| **2. Vascular** | | |
| Blood Pressure #1: | | |
| Blood Pressure #2: | | |
| Mean Blood Pressure: | | ☐ **≥ 130/85 mmHg** |
| **3. Anthropometry** | | |
| Height (cm): | | |
| Weight (kg): | | |
| BMI (kg/m$^2$): | | ☐ **≥ 25 kg/m$^2$?** |
| Waist Circumference (cm): | | ☐ **≥ 102cm males**<br>**≥ 88cm females** |
| **4. Fingerprick Fasted Blood Sample** | | |
| Blood Glucose (mmol/L) | | ☐ **≥ 5.6 mmol/L** |
| Total Cholesterol (mmol/L) | | ☐ **≥ 5.2 mmol/L** |
| LDL (mmol/L) | | ☐ **≥ 3.5 mmol/L** |
| HDL (mmol/L) | | ☐ **< 1.03 mmol/L males**<br>**< 1.30 mmol/L females** |
| Triglycerides (mmol/L) | | ☐ **≥ 1.7 mmol/L** |

# Final Eligibility Assessment

| **Inclusion Criteria** | | |
|---|---|---|
| 1. Age 18 - 70 years | ☐ Yes | ☐ No |
| 2. Activity Level ≤ 2 structured exercise sessions/week | ☐ Yes | ☐ No |
| 3. BMI ≥ 25 kg/m2 | ☐ Yes | ☐ No |
| 4. Habitual dairy consumption ≤ 1 serving/day | ☐ Yes | ☐ No |
| 5. At least two other metabolic risk factors from list: | | |
| Increased waist circumference | ☐ Yes | ☐ No |
| Elevated blood pressure | ☐ Yes | ☐ No |
| Impaired fasting glucose | ☐ Yes | ☐ No |
| Impaired fasting TG or HDL | ☐ Yes | ☐ No |
| Borderline high fasting LDL or TC | ☐ Yes | ☐ No |
| **Exclusion Criteria** | | |
| 1. Allergy to dairy foods, diagnosed lactose intolerance or an aversion to foods provided during the study (i.e. a fast-food breakfast meal - eggs, hash browns, meat and English muffins) | ☐ Yes | ☐ No |
| 2. Previous history of diabetes and/or related cardiovascular disease | ☐ Yes | ☐ No |
| 3. The use of multiple medications for managing lipids, glucose and/or | ☐ Yes | ☐ No |

| | | |
|---|---|---|
| blood pressure | | |

| Check |
|---|

1. Experimental protocol explained? ☐ Yes ☐ No
2. Informed consent signed? ☐ Yes ☐ No
3. All screening questions completed? ☐ Yes ☐ No
4. Keenoa or 7-day food diary explained and provided? ☐ Yes ☐ No
5. Availability over the next 16-weeks? ☐ Yes ☐ No
6. **Subject eligible to be randomized?** ☐ Yes ☐ No

| Randomization |
|---|

1. High Dairy Diet ☐ First ☐ Second
2. Control Diet ☐ First ☐ Second

| **General Comments** |
|---|
| |
| |
| |

## 5.4 Baseline Testing Collection Form

**Tester(s):**
**Date & Time:**

| Pre-test Check |
|---|

1. 8+ hour overnight fast (water excepted)? ☐ **Yes** ☐ No

2. Strenuous exercise in preceding 48h? ☐ Yes ☐ **No**

3. Alcohol consumption in preceding 24h? ☐ Yes ☐ **No**

4. Caffeine or pain medication (e.g., NSAIDs) in preceding 12h? ☐ Yes ☐ **No**

5. Approximate day of menstrual cycle:

6. Changes in medication or health status since last visit? ☐ Yes ☐ **No**

7. Prescription medication taken? ☐ Yes ☐ No

If yes to 5 or 6, please state which **medications/conditions are changed, by how much, and what time today prescription medications were taken**:

| |
|---|
| |

**Eligible to proceed?**

☐ **Yes** ☐ No

| | | |
|---|---|---|
| **1. Vascular Measures** | | |
| 5-minutes rest? | ☐ Yes ☐ No | |
| Initial Blood pressure (mmHg): | ☐ Left Arm ☐ Right Arm | |
| | **1st:** | **2nd:** |
| **Carotid Landmark (cm):** | | |
| **2. Body Composition – BodyMetrix** | | |
| Thigh (Both) | **Landmark (cm):** | **Thickness (mm):** |
| Chest (Male) | **Landmark (cm):** | **Thickness (mm):** |
| Abdomen (Male) | **Landmark (cm):** | **Thickness (mm):** |
| Suprailiac (Female) | **Landmark (cm):** | **Thickness (mm):** |
| Triceps (Female) | **Landmark (cm):** | **Thickness (mm):** |
| Overall Body Fat %: | | |
| **3. Body Composition – Tanita Scale BIA** | | |
| Weight (kg): | | |
| BIA Body Fat %: | | |
| Fat Mass (kg): | | |
| Fat Free Mass (kg): | | |
| **4. Venipuncture Blood Sample** | | |
| Phlebotomist: | ☐ JP ☐ AJ ☐ Other: | |
| Arm used: | ☐ Left ☐ Right | |

| | | |
|---|---|---|
| # of attempts: | ☐ 1 ☐ 2 ☐ 3 ☐ 4 | |
| Tubes taken?: | ☐ 1st Red ☐ 3rd Green ☐ 5th Purple<br>☐ 2nd Red ☐ 4th Purple | |
| Time of day taken? | | |
| Time of day centrifuged? | **Plasma:** | **Serum:** |
| Pipetted into how many eppendorfs? | 4x GSH, actual # =<br>4x Serum, actual # =<br>8x EDTA Plasma, actual # =<br>4x NaHep Plasma, actual # = | |
| Frozen & stored in -80°C? | | |
| Additional comments about blood sample? | | |

| **5. Afinion Blood Lipid Assessment** | | | |
|---|---|---|---|
| Total Cholesterol: | | LDL: | |
| HDL: | | Triglycerides: | |
| Non-HDL: | | Chol/HDL: | |

6-Week Dietary Intervention Checklist

1. Provided 3 servings/d of dairy for 2 weeks? ☐ **Yes** ☐ No ☐ N/A

2. Keenoa/food record sheet & bodyweight log given? ☐ **Yes** ☐ No

**3. Date for future visits scheduled?** ☐ **Yes** ☐ No

**Future Visit Dates**

| | |
|---|---|
| Week 0: (7-day Keenoa + IPAQ) | |
| Week 2: (3-day Keenoa + IPAQ, + BIA) | |
| Week 4: (3-day Keenoa + IPAQ, + BIA) | |
| Week 6: (7-day Keenoa) | |
| High Fat Meal Challenge 1: | |
| Baseline 2 (after 4 week washout) | |
| High Fat Meal Challenge 2: | |

| **Mifflin St Jeor Calculation for HFMC** | |
|---|---|
| RMR | |
| TDEE | |

| **General Comments** |
|---|
| |
| |
| |

**5.5 HFMC Collection Form (Visit 3 & 5)**

**Tester(s):**
**Date & Time:**
**Current Diet Period:** ☐ 1 ☐ 2 **Current Condition:** ☐ A ☐ B

| Pre-test Check |
|---|

1. 8+ hour overnight fast (water excepted)? ☐ **Yes** ☐ No
2. Strenuous exercise in preceding 48h? ☐ Yes ☐ **No**
3. Smoking or alcohol consumption in preceding 24h? ☐ Yes ☐ **No**
4. Caffeine or pain medication (e.g., NSAIDs) in preceding 12h? ☐ Yes ☐ **No**
5. Approximate day of menstrual cycle: ☐ **N/A**
6. Currently have (or within last 7-days) cold/illness symptoms? ☐ Yes ☐ **No**
7. Biopsy questions asked? ☐ Yes ☐ **No** ☐ **N/A**
8. Changes in medication or health status since last visit? ☐ Yes ☐ **No**
9. Prescription medication taken? ☐ Yes ☐ No

If yes to 6, 8 and/or 9, please state which **medications/conditions are changed, by how much, and what time today prescription medications were taken**:

| | |
|---|---|
| | |
| | |
| | |

**Eligible to proceed?**

☐ **Yes** ☐ No

| **Baseline Fasted Measures** | | |
|---|---|---|
| **1. Vascular Measures** | | |
| 5-minutes rest? | ☐ Yes ☐ No | |
| Initial Blood pressure (mmHg): | ☐ Left Arm ☐ Right Arm | |
| | **1st:** | **2nd:** |
| **Carotid Landmark (cm):** | | |
| Vascular measure actual start times: | | |
| **2. Body Composition – BodyMetrix** | | |
| Thigh (Both) | **Landmark (cm):** | **Thickness (mm):** |
| Chest (Male) | **Landmark (cm):** | **Thickness (mm):** |
| Abdomen (Male) | **Landmark (cm):** | **Thickness (mm):** |
| Suprailiac (Female) | **Landmark (cm):** | **Thickness (mm):** |
| Triceps (Female) | **Landmark (cm):** | **Thickness (mm):** |
| Overall Body Fat %: | | |
| **3. Body Composition – Tanita Scale BIA** | | |
| Weight (kg): | | |
| BIA Body Fat %: | | |
| Fat Mass (kg): | | |

| | | | |
|---|---|---|---|
| Fat Free Mass (kg): | | | |
| **4. Blood Sample** | | | |
| Phlebotomist: | ☐ JP ☐ AJ ☐ AR ☐ Other: | | |
| Arm used: | ☐ Left ☐ Right | | |
| # of attempts: | ☐ 1 ☐ 2 ☐ 3 ☐ 4 | | |
| Catheter placed successfully? | ☐ Yes ☐ No | | |
| Tubes taken?: | ☐ Red 4mL ☐ 3rd Green 4mL ☐ 5th Purple 4mL<br>☐ Red 4mL ☐ 4th Green 6mL ☐ 6th Purple 6mL | | |
| Time of day taken? | | | |
| Pipetted into how many eppendorfs? | ☐ GSH x4 ☐ EDTA x5<br>☐ NaHep x4 ☐ Serum x4 | | |
| Centrifuged, pipetted, and frozen Pre samples? | ☐ Yes ☐ No | | |
| Additional comments about veins/ blood draw/ sample? | | | |
| **5. Biopsy** | | | |
| Biopsy Participant? | ☐ Yes ☐ No | | |
| Leg Used | ☐ Left ☐ Right | | |
| Lidocaine Time Given: | | Lidocaine mL Given: | |
| Biopsy Time: | | Approx Tissue Weight: | |
| Sample in -80? | Done ☐ | | |
| Biopsy Additional Notes | | | |

| | |
|---|---|

| High Fat Meal Challenge Measures | | | |
|---|---|---|---|
| Estimated TDEE (Mifflin St Jeor) | | | |
| Which HFMC used? | ☐ Lacto-Ovo ☐ Regular<br><br>☐ 800kcals ☐ 1000kcals ☐ 1200kcals<br>☐ 1400kcals ☐ 1600kcals | | |
| Actual food given: | ☐ Starbucks Beyond Meat Sandwich<br>☐ Tim Hortons Sausage Biscuit Sandwich (No Cheese)<br>☐ Tim Hortons Hashbrowns<br>☐ Tim Hortons Timbits<br>☐ Other?: | | |
| Time meal started: | | Time meal finished: | |
| Timer(s) set? | ☐ Yes ☐ No | | |
| **1h Blood Sample** | | | |
| 30-min saline flush? | Done ☐ | Time: | |
| Extra saline flush? (delayed meal) | Yes ☐ No ☐ | Time: | |
| 60-min (**1h**) post tubes taken? | ☐ Red 4mL ☐ 3rd Green 4mL ☐ 5th Purple 4mL<br>☐ Red 4mL ☐ 4th Green 6mL ☐ 6th Purple 6mL | | |
| Time of day taken? | | | |
| Pipetted into how many eppendorfs? | ☐ GSH x4 ☐ EDTA x5<br>☐ NaHep x4 ☐ Serum x4 | | |
| 90-min saline flush? | Done ☐ | Time: | |
| Centrifuged, pipetted, and frozen 1h-post samples? | Done ☐ | | |
| **2h Blood Sample** | | | |
| 120-min (**2h**) post tubes taken? | ☐ Red 4mL ☐ 3rd Green 4mL ☐ 5th Purple 4mL<br>☐ Red 4mL ☐ 4th Green 6mL ☐ 6th Purple 6mL | | |

| | | | |
|---|---|---|---|
| Time of day taken? | | | |
| Pipetted into how many eppendorfs? | ☐ GSH x4 ☐ EDTA x5<br>☐ NaHep x4 ☐ Serum x4 | | |
| 150-min saline flush? | Done ☐ | Time: | |
| Centrifuged, pipetted, and frozen 2h post samples? | Done ☐ | | |
| 180-min saline flush? | Done ☐ | Time: | |
| 210-min saline flush? | Done ☐ | Time: | |
| **4h Blood Sample** | | | |
| 240-min (**4h**) post tubes taken? | ☐ Red 4mL ☐ 3rd Green 4mL ☐ 5th Purple 4mL<br>☐ Red 4mL ☐ 4th Green 6mL ☐ 6th Purple 6mL | | |
| Time of day taken? | | | |
| Pipetted into how many eppendorfs? | ☐ GSH x4 ☐ EDTA x5<br>☐ NaHep x4 ☐ Serum x4 | | |
| Centrifuged, pipetted, and frozen 4h post samples? | Done ☐ | | |
| **4h Vascular Measures** | | | |
| 5-minutes rest? | ☐ Yes | | |
| Resting blood pressure: | | | |
| Vascular measure actual start time: | | | |
| **4h Biopsy** | | | |
| Biopsy participant? | ☐ Yes ☐ No | | |
| Leg Used | ☐ Left ☐ Right | | |
| Lidocaine Time Given: | | Lidocaine mL Given: | |
| Biopsy Time: | | Approx Tissue Weight: | |

| | |
|---|---|
| Sample in -80? | Done ☐ |
| Biopsy Additional Notes | |

| **6h Blood Sample** | | |
|---|---|---|
| 270-min saline flush? | Done ☐ | Time: |
| 300-min saline flush? | Done ☐ | Time: |
| 330-min saline flush? | Done ☐ | Time: |
| 360-min (**6h**) post tubes taken? | ☐ Red 4mL ☐ 3rd Green 4mL ☐ 5th Purple 4mL<br>☐ Red 4mL ☐ 4th Green 6mL ☐ 6th Purple 6mL | |
| Time of day taken? | | |
| Pipetted into how many eppendorfs? | ☐ GSH x4 ☐ EDTA x5<br>☐ NaHep x4 ☐ Serum x4 | |
| 17) Centrifuged, pipetted, and frozen 6h post samples? | ☐ Yes ☐ No | |
| All eppendorfs labelled and moved to -80°C freezer? | Done ☐ | |
| Catheter removed & participant complete? | Done ☐ | |

| **Next Steps:** | | | |
|---|---|---|---|
| Baseline 2: | | | |
| HFMC Visit 2: | | | |
| Study Complete | Done ☐ | $ compensation received? | |

| General/Additional Comments |
|---|
| |
| |
| |
| |

| Biopsy Pre-Screening Questionnaire | |
|---|---|
| Your responses to this questionnaire are confidential and you are asked to complete it for your own health and safety. If you answer "YES" to any of the following questions, please give additional details in the space provided and discuss the matter with one of the investigators. You may also refuse to answer any of the questions. | |
| **1) In the past 14 days, have you experienced any of the following symptoms?**<br>(New or worsening cough, shortness of breath or difficulty breathing, temperature equal to or over 38°C, feeling feverish, chills, fatigue or weakness, muscle or body aches, new loss of smell or taste, headache, gastrointestinal symptoms [abdominal pain, diarrhea, vomiting], feeling unwell). | ☐ Yes ☐ No<br>Details: |
| **2) In the past 14 days, have you been in contact with anyone who is sick or who has a confirmed case of COVID-19?** | ☐ Yes ☐ No |
| **3) In the past 14 days, have you travelled outside of** | ☐ Yes ☐ No |

| | |
|---|---|
| **Canada?** | |
| **4) Are you currently taking any medication (including aspirin or anticoagulant 'blood thinners') or have you taken any medication in the last two days?** | ☐ Yes ☐ No<br>Medication & Dose: |
| **5) When you receive an impact to your muscle, do you develop bruises easily?** | ☐ Yes ☐ No |
| **6) Have you previously participated in a study that involved having muscle biopsies taken?** | ☐ Yes ☐ No |
| **7) Have you ever had a negative or allergic reaction to local freezing (e.g., during dental procedures)?** | ☐ Yes ☐ No |
| **8) (If second HFMC) Following your last muscle biopsy, were there any issues/concerns/questions you have?** | |
| **Reviewed by (sign & date):** | |
| **Cleared to proceed from Dr Perry?** | |

**The possibility of a medical emergency:** In the unlikely event of a medical emergency, Dr. Robert Laham (the physician overseeing these procedures at York) is fully aware of the procedure and our research study in general. He is on call and willing to address any unexpected emergency situations. Dr. Robert Laham can be reached at Appletree Medical Centre, York Lanes Mall, York University, through a direct line at 416-479-8649. We will follow standard York Emergency Procedures if Dr. Laham cannot be reached at his direct line. Specifically, 911 will be called directly (9-911 from a local university extension) followed by calling Security Control Centre at Ex. 33333 for assistance.

# Appendix 6 - Participant Hand Outs

## 6.1 Muscle Biopsy Care Instructions

**Muscle Biopsy Procedure:** Dr. Christopher Perry (School of Kinesiology and Health Science) will perform the muscle biopsy procedure. It involves the removal of a small piece of muscle from your outer thigh (quadriceps) using a small, sterile hollow needle. Dr. Perry is a physiologist, has been trained by physicians and has performed these procedures numerous times previously at York.

**Detailed description of the Muscle Biopsy Procedures and potential risks:** The percutaneous Bergstrom needle muscle biopsy procedure involves the removal of a small piece of muscle tissue using a sterile hollow needle which is ~4-5 mm in diameter. This procedure involves two injections. First, the area over the muscle to be sampled is cleaned with an antiseptic solution and a small amount (1.5-5 ml) of local anesthetic ("freezing"; the same as what is used in the dentist's office) is injected into and under the skin over the vastus lateralis (outer thigh) muscle. Second, a ~5 mm long incision will be made through the skin and fascia over the biopsy site. There may be a small amount of bleeding from the incision, but this is minimal. The sterile biopsy needle is inserted into the biopsy site. A small piece of muscle (~80-200mg, i.e., smaller than an eraser at the end of a pencil) will quickly be obtained and then the needle will be removed. The muscle is quickly removed from the needle over a sterile work area nearby. This sampling procedure comprises a single biopsy timepoint and requires 1 to 2 minutes in total. During the time that the actual biopsy sample is being taken (~2-3 sec), the sensation of pressure in the muscle is felt, and on some occasions, this is moderately uncomfortable for a few seconds (similar to a strong muscle twitch or a 'cramp'). However, the discomfort passes quickly, and you should be quite capable of getting up and moving around afterward.

Following the removal of the muscle sample, the biopsy site is closed with a sterile Steristrip or with stitches joining the skin edges of the incision and wrapped with a tensor bandage and ice pack for 15 minutes. You will be given extra Steristrips and will be briefed about care of the biopsy section (additional information below). You will be asked to refrain from excessive muscle use for the remainder of the day. Once the anesthetic wears off, your leg may feel tight in the local area of the sampling and often there is the sensation of a bruise or "charlie horse". This is normal. You should not take any aspirin-based medicine for 24 hours following the experiment as this can promote bleeding in the muscle. However, other analgesics such as Advil (ibuprofen) or Tylenol (Acetaminophen) are acceptable alternatives. It is also beneficial, but not required, to keep the limb elevated when you are sitting, and the periodic application of an ice pack will help to reduce any swelling and residual soreness. The following day, the muscle may feel slightly 'bruised' upon certain movements such as going downstairs. Any tightness in the muscle usually disappears within 1-2 days. You should be able to routinely begin your usual activities and exercise immediately with muscles other than the biopsied muscle and can resume normal activities with the biopsied muscle within 2

days. In order to allow the incision to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 2-3 days. Daily showers are acceptable, but baths, swimming, saunas, etc. should be avoided. After a shower the incision area should be patted dry. Band-Aids can be changed at any time if needed and removed after 3-4 days.

**Potential Risks:** This biopsy technique is routinely used in physiological research, and complications are rare provided that proper precautions are taken. However, there is a risk of internal bleeding at the site of the biopsy, which can result in bruising and temporary discoloration of the skin or a small hematoma, all of which have a high chance of resolving within days. On occasion, a small lump of fibrous tissue may form under the site of the needle insertion, but this normally disappears within 2-3 months. As with any biopsy, there is also a slight risk of infection; however, this risk is virtually eliminated through proper cleansing of the area (additional supplies will be provided to you upon departure from the lab). If the incision does not heal within a few days or you are in any way concerned about swelling and redness, please contact us immediately. In very rare occasions, there can be damage to a superficial sensory nerve which will result in temporary numbness in the area. There is also a theoretical but small risk of damage to a small motor nerve branch leading to the muscle which is being sampled and would likely have no impact on day-to-day activities. There is also a small chance that you will be allergic to the local anesthetic.

You will be excluded from participation in this part of the study if you are taking aspirin or any anticoagulant medication ('blood thinners') in order to avoid additional risks of bleeding complications.

**The possibility of a medical emergency:** In the unlikely event of a medical emergency, Dr. Robert Laham (the physician overseeing these procedures at York) is fully aware of the procedure and our research study in general. He is on call and willing to address any unexpected emergency situations. Dr. Robert Laham can be reached at Appletree Medical Centre, York Lanes Mall, York University, through a direct line at 416-479-8649. We (the investigators) will follow standard York Emergency Procedures if Dr. Laham cannot be reached at his direct line. Specifically, 911 will be called directly (9-911 from a local university extension) followed by calling Security Control Centre at Ex. 33333 for assistance.

**Specific biopsy aftercare instructions:**

The biggest risk following a muscle biopsy is an infection. To minimize the risk of infection PLEASE carefully follow the instructions for care of the biopsy (biopsies) below.

1. ELEVATE, apply mild PRESSURE (wrap using the tensor bandage provided) and ICE over the biopsy area while keeping the Tegaderm intact – 2-3 times a day for 10-15 minutes each time on the day of and 1-2 days after the biopsy.

2. Keep biopsy area CLEAN and DRY.

a. Daily: clean biopsy area with the alcohol wipes provided using a circular motion starting right around the incision site and moving away from the incision site. Reapply steristrips and Tegaderm after cleaning.
b. Leave Tegaderm on while showering. After showering, immediately pat the biopsy area dry (Tegaderm should prevent water reaching the incision). Can then remove, clean (see above) and apply new steristrips and Tegaderm.
c. No baths, jacuzzi, whirlpool, swimming, saunas, or any prolonged water immersion for at least 3-4 days after the biopsy.

3. Check the incision site for redness, itching, swelling, a hot sensation, bleeding, or yellowish discharge. If you see this, then contact Dr. Chris Perry and seek medical attention from a physician immediately.

4. Muscle tightness and/or moderate muscle soreness, similar to muscle soreness after a big run or workout is normal 1-3 days after the biopsy and will pass. Light activity following the biopsy, such as walking or light cycling, can help reduce this stiffness/soreness.

5. If the incision is painful, apply ice over the biopsy area as instructed above. If pain relief is needed, take Tylenol or Ibuprofen (Motrin) as directed by the manufacturer provided you can tolerate the medication. Aspirin should not be taken as it can result in increased bleeding.

If you have any concerns or experience redness, itching, swelling, a hot sensation, or bleeding at the incision site, or severe muscle soreness, then immediately remove the stitch, clean the biopsy as thoroughly as possible and call **Dr. Chris Perry** at: **+1 (289) 264-7587**

If you cannot contact Dr. Perry and you have a concern with the biopsy (biopsies) then remove the stitch, clean the biopsy as thoroughly as possible and go to a physician's office or urgent care. Call 911 or visit the emergency room in case of an emergency such as excessive/continuous bleeding.

**6.2 Participant Dairy Nutrition Guide**

**1) ONE CUP (~250ml) of 2% Lactantia milk to be consumed daily.**


**2) TWO STICKS of cracker barrel cheese (~40g) to be consumed daily.**


**3) ONE CUP (~200g) of Siggi's Skyr Yogurt to be consumed daily**


# Tips and Tricks for Incorporating Dairy Products!

- Pour milk over cereal
- Blend milk or yogurt into a smoothie
- Try your yogurt with fruit and nuts
- Take your cheese on the go as a quick snack, or make up a cheese plate with fruit and crackers
- Use your cheese in any recipe that would normally require cheese (omlettes, tacos etc.)
- Replace common snack foods (chips, candy, nuts, fruit) with dairy products when you are hungry

# Exchanges Handout

Use this guide to keep your diet at the same level of calories when you add the study dairy. The following are the calorie amounts for your dairy products:

- **One cup 2% milk = 120 kcal.**
- **200 g 2% Skyr = 190 kcal**
- **42 g cheese = 160 kcal.**

The exchange amounts are given precisely, but you can round for convenience.

**Grains and Starches**

| **Food Item** | **Serving Size 150 kcal** | **Serving Size 200 kcal** |
|---|---|---|
| **BREADS** | | |
| Sliced bread, various types | 1.67 medium slice | 2.22 medium slice |
| Chapati, roti, prata | 1.32 medium (7 inch) | 1.75 medium (7 in.) |
| English muffin | 1.16 | 1.55 |
| Pita bread | 0.96 medium | 1.28 medium |
| Tortilla | 1.01 (8 inch) | 1.34 (8 inch) |
| Bagel | 0.54 medium | 0.72 medium |
| Bun, hamburger or hotdog | 1.07 | 1.43 |
| Croissant | 0.58 medium | 0.77 medium |
| Pizza crust | 1.90 oz (53 g) | 2.53 oz (71 g) |
| Plain roll | 1.29 medium | 1.72 medium |
| **CEREALS** | | |

| | | |
|---|---|---|
| Bran cereals | 0.83 cup | 1.11 cup |
| Hot cereals | 0.74 cup | 0.99 cup |
| Shredded Wheat | 1.92 biscuits | 2.56 biscuits |
| Cheerios, Rice Krispies | 1.42 cups | 1.91 cups |
| Corn Flakes | 1.52 cups | 2.02 cups |
| Granola | 0.29 cup | 0.38 cup |
| **CRACKERS** | | |
| Ryvita | 4.3 pieces | 5.7 pieces |
| Wasa | 3.4 pieces | 4.5 pieces |
| Soda crackers | 12 crackers | |
| Matzoh | 1.4 pieces | 1.8 pieces |
| Melba toast | 8 pieces | 11 pieces |
| Triscuit | 7 pieces | 10 pieces |
| **GRAINS AND LEGUMES** | | |
| Barley, pearled, cooked | 0.78 cup | 1.04 cups |
| Buckwheat, cooked | 0.97 cup | 1.30 cups |
| Bulghur, cooked | 0.99 cup | 1.32 cups |
| Corn, kernel | 1.38 cups | 1.83 cups |
| Couscous, cooked | 0.85 cup | 1.13 cups |
| Millet, cooked | 0.71 cup | 0.95 cup |
| Rice, cooked (brown & white) | 0.60 cup | 0.81 cup |
| Quinoa, cooked | 0.68 cup | 0.90 cup |
| Black beans, chickpeas, kidney beans, lentils, navy beans, split peas, cooked | 0.66 cup | 0.88 cup |
| Hummus | 0.35 cup | 0.47 cup |
| **PASTA** | | |
| Pasta, cooked (whole wheat & white) | 0.72 cup | 0.96 cup |
| Chow Mein noodles | 0.71 cup | 0.95 cup |
| Egg noodles | 0.68 cup | 0.91 cup |
| Rice noodles | 0.79 cup | 1.05 cups |
| **SWEET/STARCHY VEGETABLES** | | |
| Plantain, cooked, mashed | 0.70 cup | 0.93 cup |
| Potatoes, boiled, baked | 0.75 cup | 1.00 cup |
| Potatoes, mashed | 0.57 cup | 0.76 cup |
| Sweet potato | 0.95 cup | 1.27 cups |
| Yam | 0.97 cup | 1.29 cups |
| Beets | 1.76 cups | 2.35 cups |
| Peas | 1.21 cups | 1.61 cups |
| Rutabaga | 2.08 cups | 2.78 cups |
| Squash | 1.83 cups | 2.44 cups |
| Tomatoes, canned, stewed | 2.27 cups | 3.03 cups |

**Fruits**

| | | |
|---|---|---|
| Apple, pear | 1.60 medium | 2.13 medium |
| Apricot, plum | 9 (apricot), 5 (plum) | 12 (apricot), 7 (plum) |
| Banana | 1.43 medium | 1.91 medium |
| Blackberry, cranberry, raspberry, strawberry, gooseberry | 3.13 cups | 4.17 cups |
| Elderberry, mulberry, blueberry, currants, partridge berry, cloudberry, bakeapple, Saskatoon berry | 1.77 cups | 2.38 cups |
| Cherries | 1.55 cups | 2.06 cups |
| Grapes, red/green | 1.44 cups | 1.92 cups |
| Melon, papaya, mango | 2.21 cups (melon/papaya), 1.51 cups (mango) | 2.94 cups (melon/papaya), 2.02 cups (mango) |
| Orange, grapefruit, tangerine | 2.46 medium orange, 1.40 medium grapefruit, 3.26 medium tangerine | 3.28 medium orange, 1.87 medium grapefruit, 4.35 medium tangerine |
| Peach, nectarine | 2.59 medium | 3.42 medium |
| Pineapple, fresh | 1.83 cups | 2.44 cups |

**Milk & Alternatives**

| | | |
|---|---|---|
| Buttermilk | 0.99 cup | 1.32 cups |
| Evaporated milk | 0.44 cup | 0.59 cup |
| Milk, all types (cow's/goat's/sheep's) | 1.23 cups | 1.64 cups |
| Soy milk | 1.44 cups | 1.92 cups |
| Soy milk (chocolate/strawberry) | 0.98 cup | 1.31 cups |
| Yogurt, plain | 1.01 cups | 1.34 cups |

**Meat & Alternatives**

| | | |
|---|---|---|
| Whole egg | 1.95 large eggs | 2.60 large eggs |
| Canned tuna, salmon, shellfish | 0.57 cup | 0.76 cup |
| Fish fillets or steaks | 2.58 oz (72 g) | 3.45 oz (97 g) |
| Black beans, chickpeas, kidney beans, lentils, navy beans, split peas, cooked | 0.66 cup | 0.88 cup |
| Beef, chicken, emu, game, goat, goose, ham, lamb, pheasant, pork, turkey, veal, quail | 2.05 oz (58 g) | 2.74 oz (77 g) |
| Ground meats | 0.27 cup | 0.36 cup |
| Deli meats, back bacon | 2.24 slices | 2.99 slices |
| Peanut butter | 1.56 Tbsp | 2.08 Tbsp |
| Firm tofu, tempeh | 0.41 cup (tofu), 0.47 cup (tempeh) | 0.55 cup (tofu), 0.63 cup (tempeh) |

**Fats**

| | | |
|---|---|---|
| Avocado | 0.66 avocado | 0.88 avocado |
| Bacon | 4 slices | 5 slices |
| Butter | 1.49 Tbsp | 1.98 Tbsp |
| Cream, half & half | 0.50 cup | 0.67 cup |
| Margarine, non hydrogenated, regular | 1.49 Tbsp | 1.98 Tbsp |
| Mayonnaise, regular | 1.61 Tbsp | 2.15 Tbsp |
| Nuts, seeds, tahini | 2.72 Tbsp, 1.69 Tbsp (tahini) | 3.64 Tbsp, 2.25 Tbsp (tahini) |
| Oils | 1.26 Tbsp | 1.68 Tbsp |
| Salad dressing | 3.19 Tbsp | 4.26 Tbsp |
| Sour cream | 5.36 Tbsp | 7.14 Tbsp |

**Other**

| | | |
|---|---|---|
| Cake, no icing | 1.60 oz (45 g) | 2.13 oz (60 g) |
| Cookies | 2 medium (2 1/4 in diam) | 2.67 medium |
| Frozen yogurt | 0.58 cup | 0.77 cup |
| Granola bars | 1.11 bar | 1.48 bar |
| Ice cream, any flavour | 0.55 cup | 0.73 cup |
| Snack chips, all varieties | 1 oz (28 g) | 1.33 oz (37 g) |
| Chocolate candy | 0.97 oz (27 g) | 1.30 oz (36 g) |
| Gummy candy | 1.49 oz (42 g) | 1.98 oz (55 g) |

## Sample Menu 1 (2000 kcal)

**Breakfast:**
**One cup of 2% milk**
One and a half cups of Fibre One cereal
Black coffee
One banana

**Snack:**
One apple
1 tbsp walnuts or nut of choice

**Lunch:**
**Cheesy Vegetable Omelette (see recipe) with toast**
OR
**Best-Ever Grilled Cheese (see recipe) with raw veggies**
Two cups of soup, stew or curry of your choice

**Snack:**
**One red scoop of Skyr yogurt (can use in a smoothie-- see recipe)**

**Dinner:**
Two cups of lentil soup
Two cups of salad (arugula, cucumber, tomato, green onion, etc.) topped with 1 tsp sunflower seeds (or choice of seed), 2 t olive oil, balsamic/lemon to taste
2.5 oz lean meat of choice
One cup of brown rice, squash, sweet potato, etc.
One pear

## Sample Menu 2 (2000 kcal)

**Breakfast:**
**One cup of 2% milk**
Two slices of whole-wheat toast
Omelet with egg whites (6 tbsp), onions, spinach, mushrooms
Black coffee
One cup of mixed berries (could make a smoothie with the milk)

**Snack**
One apple
1 tbsp walnuts or nut of choice

**Lunch:**
1.5 cups of Homemade Chili with **2 cheese sticks (grated)**
Two cups of salad (arugula, cucumber, tomato, green onion, etc.) topped with 1 tsp sunflower seeds (or choice of seed), 2 t olive oil, balsamic/lemon to taste, 1/2 tsp Parmesan cheese
Two handfuls of whole-grain crackers (Triscuits, Mary's Organic Crackers, etc.)

**Snack:**
One pear

**Dinner:**
Two cups of steamed kale, chard or collards
5 oz meat of choice
Two cups of Roasted Root Vegetables (see recipe)
**One red scoop of Skyr yogurt**

## Berry Smoothie Recipe

1 frozen banana (can be fresh)
1 cup frozen mixed berries
**1 cup 2% milk OR 200 g Skyr yogurt (do not use both)**
Water, juice or nondairy beverage to thin out as needed

Blend all ingredients together until it reaches desired consistency.

Adapted from: https://wholefully.com/berry-smoothie/

## Cheesy Vegetable Omelet

2 eggs
2 tsp milk
1/4 tsp salt
dash of pepper
1 Tbsp chopped tomatoes
1 Tbsp chopped green peppers
Small handful broccoli florets, finely chopped
**2 sticks Black Diamond Cheddar cheese, shredded**
1/2 Tbsp butter or margarine

Cut up your veggies. Crack eggs into a bowl and whisk with milk, salt and pepper.

Place an 8 inch pan over medium-high heat; add butter or margarine.

When the butter or margarine is sizzling, add egg mixture. It should take about 4-5 minutes to cook through.

Add half the grated cheese (1 stick) to one size of the omelet; cover with the vegetables and the rest of the grated cheese. Carefully flip the uncovered side over the covered side; push down with spatula and cook for another minute.

Adapted from: https://brooklynfarmgirl.com/vegetable-farm-omelette/

## "Best-Ever" Grilled Cheese

2 slices bread
2 Tbsp mayonnaise (use less/light if desired)
1 Tbsp butter or margarine
**2 sticks Black Diamond Cheddar cheese, shredded**
Ground black pepper

Place bread on a cutting board and spread mayonnaise over top side of each slice (this will give it crunch). Heat a small skillet over medium; add half of butter or margarine.

When it melts, place one slice of bread (mayo side down) in skillet; top with cheese and season with pepper. Top with second slice of bread (mayo side up).

Turn sandwich after about 4 minutes and add rest of butter or margarine to the skillet. Press down on the sandwich; cook until golden brown and cheese melted.

Adapted from: https://www.bonappetit.com/recipe/best-ever-grilled-cheese