THE ACUTE EFFECTS OF MILK CONSUMPTION ON SYSTEMIC INFLAMMATION AFTER COMBINED RESISTANCE AND PLYOMETRIC EXERCISE IN YOUNG FEMALES

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Abstract

This thesis compared the inflammatory responses following post-exercise milk consumption versus an isoenergetic, isovolumetric carbohydrate beverage in young untrained females. We hypothesized that milk consumption would benefit markers of inflammation. Utilizing a crossover design, participants performed an acute bout of resistance/plyometric exercise followed by consumption of white skim milk (MILK) or a carbohydrate control (CHO). Blood samples were taken at baseline, 15min, 75min, 24h, and 48h post-exercise, and serum IL-1β, TNF-α, IL-6 and IL-10 were analyzed. There were no main effects or interactions for IL-1β or TNF-α. IL-6 increased 15min post-exercise vs. baseline (time effect). Between 24 and 48h, MILK and CHO had opposing effects on IL-10 (interaction), with MILK decreasing (from being higher at 24h) and CHO increasing (from being lower at 24h). Post-exercise milk consumption did not influence the absolute concentration of pro-inflammatory cytokines; however, there were divergent responses for the anti-inflammatory cytokine, IL-10, which warrants further exploration.

Dedication

To my Mom, Moira, who broke barriers as a woman in STEM and demonstrated the importance of participating in the scientific community.

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1-RM	1-repetition max
AUC	Area under the curve
BMI	Body mass index
CHO	Carbohydrate trial
CI	Confidence Interval
CK	Creatine Kinase
CRP	C-reactive Protein
CVs	Coefficients of variation
DHA	Docosahexaenoic acid
EIMD	Exercise-induced muscle damage
EPA	Eicosapentaenoic acid
IL-10	Interleukin-10
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
LPS	Lipopolysaccharide
MF	Milk fat
MILK	Milk trial
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
RCF	Relative centrifugal force
Reps	Repetitions
RM-ANOVA	Repeated measures analysis of variance
ROS	Reactive oxygen species
SE	Standard error
SFA	Saturated fatty acids
SSRI	Selective serotonin reuptake inhibitor
TNF-α	Tumor necrosis factor-alpha

Chapter One: Introduction

High-intensity, high-load, and unaccustomed exercise inflict damage onto the muscle, resulting in exercise-induced muscle damage (EIMD). EIMD occurs due to increased mechanical stress and tension on the muscle and metabolic alterations, including the production of reactive oxygen species (ROS) and inflammation (1–3). While EIMD is typically regarded as beneficial for muscle adaptation (4), it often results in a temporary reduction in muscle function, which can impede subsequent performance (1–3).

Following exercise, there is a transient inflammatory response, characterized by increases in both pro and anti-inflammatory cytokines (2,5–8). These cytokines act as messenger molecules that attract and activate leukocytes (white blood cells) to the damaged muscle to facilitate repair and regeneration (1,5,6,8,9). However, the inflammatory response typically results in additional damage to healthy tissue, secondary to the initial damage from the exercise bout (10–12). This secondary damage is due to the infiltration of neutrophils to the site of EIMD, resulting in the production of ROS and proteases, leading to more widespread (and untargeted) damage to the muscle (10–13). Thus, attenuating the post-exercise inflammatory response may reduce secondary damage, which will help alleviate the severity of EIMD.

Alleviating the severity of EIMD may result in a shorter recovery time, allowing individuals to return to optimal athletic performance or acts of daily living without impedance from muscle soreness (12,14). Further, reducing EIMD may improve training quality, increase training frequency, and enhance motivation and adherence to exercise leading to increased training adaptations (15). Many strategies have already been

employed for reducing EIMD, including stretching, pharmacology (i.e., anti-inflammatory drugs), massage, cryotherapy and nutrition (i.e., vitamin/mineral supplementation, antioxidants, protein) (12,15,16).

Milk and other dairy products represent functional whole foods that have been shown to benefit musculoskeletal and cardiometabolic health in different populations, including athletes, and those with chronic disease risk factors (17,18). These effects are related to their nutrient-dense profile, which includes high-quality protein (whey and casein), calcium, vitamin D, phosphorous and potassium (17,18). In addition, dairy products could be beneficial for reducing EIMD and post-exercise inflammation as many components of milk, including milk protein (whey and casein), magnesium, and components of milk fat (omega-3s and oleic acid [omega-9]) have anti-inflammatory properties (19). Indeed, cross-sectional studies in healthy adults (20,21), and those with cardiometabolic disease risk factors (22) and longer-term intervention studies among healthy adults with and without overweight/obesity (23–30) have shown reductions in systemic cytokines with chronic dairy consumption. Dairy products have also been shown, in some studies, to attenuate postprandial inflammation after consumption of a high-fat meal (25,31,32). Thus, dairy products could also positively influence the post-exercise inflammatory response.

Consuming milk following exercise has proven to be a beneficial strategy for enhancing rehydration and glycogen resynthesis and for stimulating muscle protein synthesis during the recovery period (15,33,34), likely related to the immediate provision of key nutrients (protein and carbohydrates) (34). Several studies that have examined post-exercise milk consumption, primarily in trained individuals or athletes, have shown

benefits for reducing EIMD, as evidenced by attenuated increases in creatine kinase (CK), reduced muscle soreness (assessed *via* visual analog scales or Likert scales), and preserved muscle function or performance (16,35,44,36–43). Given the anti-inflammatory properties of milk, the modulation of the inflammatory response may be one mechanism for these improvements in EIMD. However, limited research has directly examined the post-exercise inflammatory response following the consumption of white milk (35,45). Two studies compared the consumption of 500 mL of white milk and an isoenergetic carbohydrate beverage consumed immediately following sprint cycling (45) or interval sprints and plyometrics (35) in trained females, and found no differences in C-reactive protein (CRP) between trials from 2-72 h post-exercise (35,45). A third study provided 500 mL of white milk following simulated gameplay (soccer) and also compared the CRP response to an isoenergetic carbohydrate control (36). CRP was elevated in the milk trial vs. carbohydrate control at 2, 24 and 48 h post-exercise. While these studies begin to assess the post-exercise/nutrition inflammatory response, a major limitation of this work is the examination of just one inflammatory biomarker, CRP. The examination of several biomarkers that respond within the acute (48 h) post-exercise timeframe, would better characterize the inflammatory response (46). While CRP is a marker of chronic systemic inflammation (9,47), CRP does not typically change with acute exercise (9,47,48) or acute nutrition (without exercise), as illustrated in studies examining postprandial inflammation (49,50). Interestingly, two studies have investigated the effects of flavoured milk (strawberry or chocolate) consumed immediately post-exercise and 2 h post-exercise on multiple systemic cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6

(IL-6), and interleukin-10 (IL-10), in trained athletes (42) and untrained individuals (51) compared to isoenergetic carbohydrate and water controls. Neither study noted a difference between nutritional interventions for any systemic cytokine (42,51). However, the use of flavoured milk, due to the increased sugar content, may have masked a potential positive effect of milk, as foods with higher glycemic indices can increase postprandial inflammation and oxidative stress (52,53). Thus, research examining the effects of post-exercise white milk consumption on a comprehensive set of inflammatory biomarkers, following high-impact, high-load exercise is warranted.

Given the paucity of research in this area, especially with post-exercise nutrition, this thesis was undertaken to characterize and compare the acute systemic cytokine response (up to 48 h) of TNF-α, IL-1β, IL-6, and IL-10 following a single session of high-impact/load (resistance and plyometric) exercise and the post-exercise consumption of either skim milk (a whole-food dairy product) or an isoenergetic, isovolumetric, carbohydrate control drink (maltodextrin and water drink) in young, normal weight, females.

Chapter Two: Literature Review

2.1 The Immune System and Inflammation

The immune system, the body's defence against infection and injury, is a complex system involving cellular (leukocytes, more commonly known as white blood cells) and molecular (cytokines) signals (54). The immune system can be classified into two main components, innate and adaptive immunity (54). Innate immunity refers to inborn immunity and is comprised of neutrophils, monocytes, macrophages and natural killer cells (54). Innate immunity is the body's first line of defence following infection or injury, including EIMD (54). Conversely, adaptive immunity is acquired by exposure (54). The adaptive immune system consists of lymphocytes such as T and B cells (54).

Inflammation, a response of innate immunity, is characterized by the infiltration of leukocytes to an area of infection or damage, such as EIMD (54). This response is induced by cytokines, which are messenger molecules, produced by immune cells (and the muscle in the case of exercise) to attract and/or activate leukocytes to kill microbes or clear damaged tissue (54). Cytokines can be classified as either pro (i.e., TNF-α, IL-1β) or anti-inflammatory (i.e., IL-10), however, some cytokines, such as IL-6, can elicit both pro and anti-inflammatory effects (as will be explained in the following sections) (54). These pro and anti-inflammatory actions of the immune system must be balanced, as prolonged pro-inflammatory responses may lead to chronic inflammation and pathological conditions, and prolonged anti-inflammatory responses may lead to immunosuppression and greater susceptibility to infection (55).

2.2 The Immune Response to a Single Session of Acute Exercise

Following a single session of acute exercise, there is a rise in local (i.e., intramuscular) and systemic cytokines (1,5–11,56,57) and leukocyte populations (8,9). This post-exercise inflammatory response is thought to be induced by muscle damage (1,6), but it may also occur in the absence of overt muscle damage (2,11), as cytokines, IL-6 in particular, can be produced by the muscle during contraction (2,5,7). Typically, the extent of the inflammatory response is dependent on the level of muscle damage, with high levels of muscle damage inducing a greater response (2).

Muscle repair following EIMD can be broken down into two phases: 1) the clearing of damaged tissue, and 2) the regeneration of muscle. Neutrophils are the first leukocyte subtype (**Figure 1**) to increase in response to pathogens (58) and acute exercise (1,5,6,8,10,11), and play a critical role in clearing damaged tissue through phagocytosis (8,10,11). However, the release of ROS and other cytotoxic compounds (e.g. proteases) from neutrophils can result in more damage to the surrounding healthy tissue (10–13). Damage induced by the neutrophil response/activity is often referred to as secondary damage, as it occurs after the initial damage to the muscle caused by exercise (10–12). Following neutrophil infiltration into the muscle, macrophages begin to increase. Macrophages differentiate within the tissue from monocytes, their progenitor cells (59), as shown in Figure 1. Macrophages may also reside in tissues, including muscle, originating from embryonic progenitors and maintaining resident populations through local proliferation throughout adulthood (60). Measurement of monocyte populations in the blood following damaging activity (i.e., exercise) may be a more accessible and less invasive method (vs. muscle biopsies) to gain insight on macrophage infiltration into the muscle. Macrophages aid in the clearing of damaged tissue, but also facilitate muscle

repair, through the stimulation of growth factors (1,10–12). Macrophages can be classified into two phenotypes: M1 and M2 (59,61). It should be noted that the classification of macrophages as either M1 or M2 is a simplified framework (60). Within physiological conditions, macrophages, and their functions, exist on a spectrum between these extremes (60). For simplicity, macrophage phenotypes will be discussed using the M1/M2 framework as it is still common to do so within the exercise literature. The M1 phenotype is considered pro-inflammatory, as they secrete pro-inflammatory cytokines (e.g. TNF-α and IL-1β) (and produce ROS) which attract and recruit more immune cells to the area of muscle damage (13,61). M1 macrophages also assist neutrophils in the clearing/phagocytosis of damaged tissue (13,61). Further, M1 macrophages stimulate satellite cell proliferation (59,61). Satellite cells are often regarded as stem cells of skeletal muscle, as they contribute myonuclei to muscle fibres as an essential component of muscle regeneration (62). The M1 phenotype dominates 1-2 days following muscle injury (13). As recovery progresses, there is a rise in M2 macrophages, the anti-inflammatory macrophage phenotype, which is crucial for the repair and regeneration process of muscle (61). The rise in the M2 phenotype is partially modulated by the rise in IL-10 (13). M2 macrophages also secrete IL-10 and other anti-inflammatory cytokines (e.g. IL-4) promoting the production of the M2 phenotype (59,61). M2 macrophages also initiate the differentiation of satellite cells into skeletal muscle (59,61). Lymphocyte populations, such as T-cells and natural killer cells, (Figure 1) also rise post-exercise. These cells are recruited by M1 macrophages and play a key role in cytokine production, which orchestrates the inflammatory response (9,59). Since all aspects of the inflammatory response play key and intricate roles in muscle regeneration following

EIMD, blocking this response would be detrimental; however, attenuation of the pro-inflammatory response while augmenting the anti-inflammatory response to exercise may reduce secondary damage to the muscle and facilitate an earlier rise in M2 macrophage phenotypes, speeding recovery from EIMD.

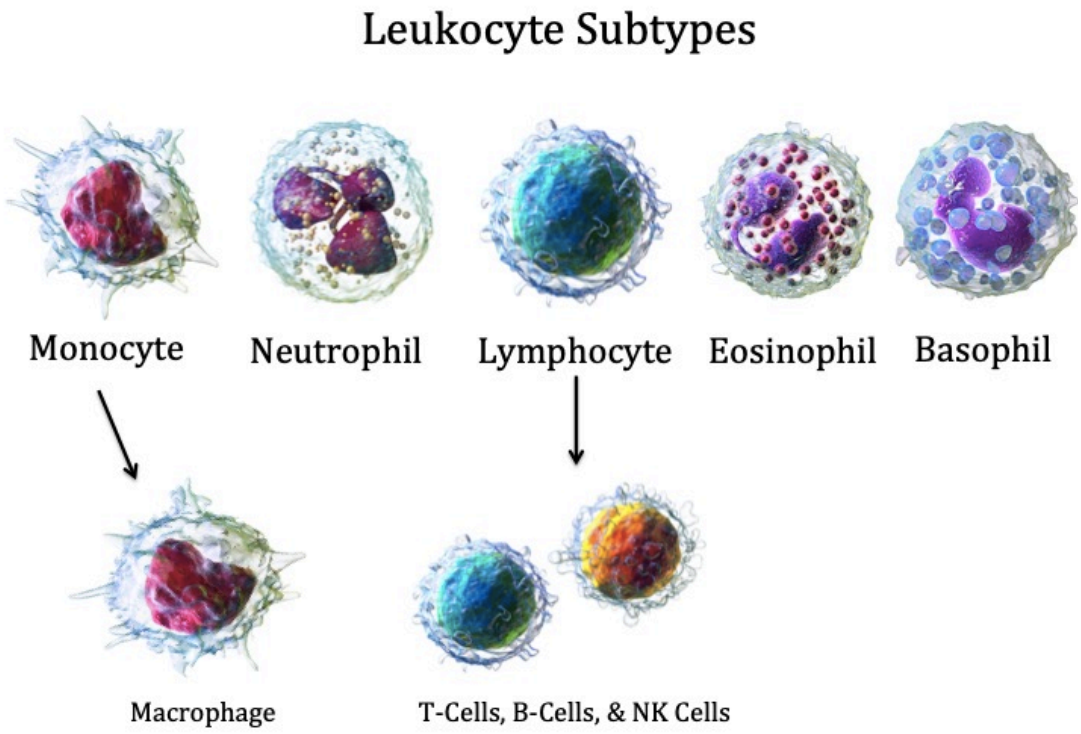

Figure 1: Leukocyte subtypes. Monocytes differentiate into macrophages when they enter tissues. Lymphocytes can be further classified into T-Cells, B-Cells, and Natural Killers. (Images from: Medical gallery of Blausen Medical 2014)

The extent of the inflammatory response has been shown to differ with the mode of exercise (6,57), type of muscle contraction (concentric vs. eccentric) (6,57), training status (trained/active, vs. untrained/sedentary) (6), and intensity and duration of the exercise bout (5–7,9,11,57). Eccentric protocols tend to be some of the more muscle-damaging modalities, eliciting larger inflammatory responses (6,57). Trained individuals often have a blunted inflammatory response due to muscle adaptation (57) and require

greater stress on the muscle to elicit damage and thus repair/regeneration (hence the need for periodized, overload and varied training protocols for athletes to improve their performance (63)). Studies have also shown that subsequent performance of the same exercise bout results in a blunted inflammatory response, a phenomenon known as the repeated bout effect (64). This is because the muscle/body adapts and rebuilds following damage, allowing it to better withstand similar subsequent damaging exercise protocols (64). Sex may not affect the post-exercise inflammatory response (65,66), however, exercise research in females is limited. Indeed in 2014, only 39% of research participants in exercise studies were female (67). As there are differences in the immune system between sexes (68), and estrogen has been shown to influence EIMD, due to its antioxidant capacity (69), research examining sex differences or females alone may be warranted.

2.2.1 Cytokine Response to Exercise

Cytokines are messenger molecules produced by many different cell types, including leukocytes, adipocytes, and skeletal muscle to regulate the inflammatory response. **Table 1** provides information on the four cytokines analyzed in this thesis including their classification (pro or anti-inflammatory), the cells that produce them, and their relevant actions. The interaction between cytokines and other immune cells is complex, as they can up-regulate and down-regulate one another, and their actions may be dependent upon the tissue from which they were produced (7,9,54). For example, IL-6 produced by the muscle acts in primarily an anti-inflammatory nature, while IL-6 produced by immune cells acts in a primarily pro-inflammatory nature (7,9,54). Due to these complex interactions, gaining a full understanding of the inflammatory response

requires, at the very least, measures of multiple cytokines in the blood (46). Furthermore, measurement of ratios of cytokines (e.g. TNF-α/IL-10) can provide a greater understanding of the balance between pro and anti-inflammatory actions (46,70).

Recall, cytokines act as chemoattractants by bringing leukocytes to the site of muscle damage following exercise. Thus, the measurement of systemic/blood cytokines is an accessible, valuable, and acceptable way to characterize the inflammatory response and make inferences about the local response within the muscle post-exercise (without taking a biopsy). Examination of systemic/circulating leukocytes and specifically their intracellular cytokines may provide additional information about the source of cytokine release following exercise and allow for greater inference into the muscle inflammatory response. For further information about the muscle inflammatory response and microenvironment, muscle biopsies could also be acquired.

Table 1: Summary of select cytokines: source of production and relevant actions

Cytokine	**General Classification**	**Produced By**	**Relevant Actions**
Interleukin-6 (IL-6)	Both pro and anti-inflammatory	Muscle (during contraction) – anti-inflammatory (2,5,7,57,71–74)	Reduces TNF-α and IL-1β; Triggers release of IL-10 (7,9)
		Adipocytes, macrophages and monocytes – pro-inflammatory (54)	Synthesis of acute-phase proteins (e.g. C-reactive protein in the liver) (54) and attraction/activation of neutrophils (11)
Tumor Necrosis Factor-alpha (TNF-α)	Mainly pro-inflammatory	Mast cells (59), macrophages and T-cells (54)	Activate neutrophils, synthesis of acute-phase proteins (C-reactive protein), initiation of apoptosis (54)
Interleukin-1B (IL-1β)	Mainly pro-inflammatory	Macrophages, endothelial cells and epithelial cells (54)	Synthesis of acute-phase proteins (CRP), T_H17 differentiation (54)
Interleukin-10	Mainly anti-	Macrophages,	Inhibition of IL-12 TNF-α and

(IL-10)	inflammatory	dendritic cells and T-cells (54)	IL-1β production, decrease expression of co-stimulators and MHC II (54), inhibit the production of TNF-α and IL-1β (7,9,54).

The following sections will provide insight into the influence of IL-6, TNF-α, IL-1β, and IL-10 following acute exercise; including a single bout of resistance and high-intensity exercise, including plyometrics, in humans.

2.2.2 Interleukin-6 (IL-6)

IL-6 is the first cytokine to increase in the systemic circulation following acute exercise, often seen before a rise in neutrophils or other leukocyte subgroups (6–8,57). Although IL-6 can be released from adipocytes and monocytes, many researchers attribute the muscle as the main source of IL-6 during exercise (5,7,57,71–74). Researchers also hypothesize that the initial rise in IL-6 occurs in response to muscle contraction during exercise, rather than muscle damage per se (2,4,7). Especially considering IL-6 release immediately following exercise is not always greater in eccentric exercise compared to concentric exercise, despite eccentric exercise eliciting greater muscle damage (75). In contrast, in the absence of exercise, and following the consumption of a high-fat meal, IL-6 may be released from adipocytes and monocytes (76). Following exercise, secondary rises in IL-6 are seen a few hours into recovery and may be attributed to release from immune cells to aid the muscle repair processes (77). The source of IL-6 is important as the actions of IL-6 depend on the source of release; muscle-derived IL-6 acts in an anti-inflammatory nature, inhibiting TNF-α and inducing IL-10 release, while immune cell-derived IL-6, released by leukocytes (e.g. monocytes

and M1 macrophages), acts primarily in a pro-inflammatory nature, inducing CRP release (7,9,54) and recruiting neutrophils to the source of muscle damage (Table 1) (11).

Some studies have investigated the source of IL-6 release post-exercise to determine the implications of its response (71–74). Starkie et al., (73) examined the percentage of IL-6^+ monocytes using flow cytometry, following a 2 h cycling protocol at 70% VO_2 peak in endurance-trained males. Post-exercise IL-6^+ monocytes were reduced by 44%, even though circulating IL-6 increased 400% post-exercise, suggesting that monocytes are not the source of IL-6 post-exercise (73). Similar findings were seen using flow cytometry after prolonged endurance exercise (in trained individuals), with increases in systemic IL-6 levels, and reductions in IL-6^+ monocyte counts (71), while no change was seen in IL-6^+ monocytes following 30min of heavy cycling (in recreationally active males) despite increases in systemic IL-6 levels (74). Another study examined the arterial-femoral venous difference of IL-6 following 5 h of one-legged dynamic knee extensor exercise at 40% peak power (72). The researchers noted that arterial plasma IL-6 concentration increased 1809% during exercise, and the arterial-femoral venous difference in the exercised leg followed the same pattern of release, indicating that skeletal muscle is likely the main source of IL-6 release (72). Cumulatively, these results suggest that muscle, rather than monocytes, is the main source of IL-6 release during exercise, indicating that it may be acting in an anti-inflammatory manner during and immediately post-exercise.

Within the systemic circulation, most studies observe an increase in IL-6 concentration within 1 h post-exercise (65,66,86–89,78–85) and as discussed, this early increase in IL-6 is likely released from the muscle (71–73,77). However, some studies

have found no increase in IL-6 post-exercise (90–96). The lack of post-exercise increase in IL-6 in these studies may be related to the specific timepoints assessed. For example, two of these studies had their first post-exercise blood draw at 12 h (91) and 24 h (95) post-exercise and no blood draw within the first few hours following exercise, likely missing the transient response. Also, some of these studies examined shorter exercise protocols (15-20 min), consisting of only one upper-body exercise (92,95,96). These exercise protocols likely recruit less muscle mass in comparison to full-body exercise protocols, or exercise protocols incorporating lower body (i.e., larger muscle groups) exercises. As the muscle is thought to be a major source of IL-6 release post-exercise, muscle mass recruitment is a key factor in determining the magnitude of the IL-6 response (57). Further, IL-6 has been shown to have greater increases during fasted exercise or exercise following glycogen depletion, due to its role in increasing glucose uptake by the muscle (97–99). Indeed, exercise following carbohydrate ingestion has been shown to attenuate post-exercise increases in systemic IL-6 (77,99). Performance of exercise in the fed state may explain why some studies found no increase in IL-6 immediately post-exercise (93,94,96). Training status may also affect the IL-6 response immediately post-exercise due to the role of muscle-derived IL-6 in increasing glucose uptake during exercise (75). As trained individuals are less reliant on glucose as fuel during exercise (and have greater muscle glycogen stores), trained individuals typically have a blunted response compared to untrained individuals (75). Together, these studies indicate IL-6 increases during exercise and can be captured within 1 h post-exercise (likely muscle-derived). Further, the magnitude of the increase may be influenced by the amount of muscle mass recruited (more muscle mass equates to greater increases),

glycogen availability (lower glycogen availability may lead to greater increases), and training status (highly trained individuals may have a blunted response).

Several studies have also noted elevated levels of IL-6 during later stages of recovery (2-24+ h, i.e., not the initial rise) (78,79,83,84,87,100). One study examining an isokinetic resistance exercise protocol in trained males noted IL-6 levels peaked at 6 h post-exercise, however, this was the last sampling time during the acute exercise intervention (79), thus it can not be determined if levels continued to rise or returned to baseline shortly thereafter. Some studies noted a return to baseline levels by 24 h post-exercise (83,89), while others found levels to be elevated for up to 24-72 h (78,87,100). The extent of the response may be due to the modality of exercise as those with prolonged responses tended to have particularly damaging exercise protocols consisting of plyometric or eccentric exercise (78,100). Gordon et al., (87) examined IL-6 in young and middle-aged adults following 8 sets of 10 repetitions (reps) of isokinetic leg extension. The researchers observed a significant time effect for IL-6, with elevated levels (compared to baseline) 30 min post-exercise, as well as 2, 24, and 48 h post-exercise (87). However, there was no significant difference in IL-6 at 1 h post-exercise compared to baseline (87). This study may be highlighting the two distinct rises (i.e., the biphasic response) of IL-6, the immediate/initial rise in IL-6 from myocytes, and the immune-mediated rise in IL-6 that occurs during recovery (as early as 2 h post-exercise). However, this study did not include any measurement of leukocytes or their intracellular cytokines, thus the source of cytokine release cannot be determined.

2.2.3 Tumor Necrosis Factor Alpha (TNF-α)

TNF-α is a pro-inflammatory cytokine and a part of the acute phase response of inflammation (54). The release of TNF-α is one of the earliest responses to muscle damage and results in the recruitment of neutrophils to the muscle (59). TNF-α is also released by the M1 macrophage phenotype (pro-inflammatory) and T-cells following muscle damage (6–8,54,59,101). The rise in TNF-α in the muscle following exercise attracts immune cells to the source of damage, exacerbating the immune response (59). However, TNF-α also plays an important role in regeneration, as it attracts satellite cells and promotes satellite cell proliferation while inhibiting satellite cell differentiation (13,59).

Although some studies have found increases in TNF-α in the systemic circulation between 0-30 min following resistance or high-intensity exercise (66,86,93,102), other studies have reported no change (94–96,100,103–105). Bazgir et al., found a tendency for TNF-α to decrease immediately post-exercise in young athletes and untrained individuals following a concentric (i.e., not as damaging) exercise protocol, and a significant decrease in TNF-α among untrained individuals immediately following an eccentric exercise protocol (105). The decrease, or tendency to decrease, of TNF-α, post-exercise may be due to the initial rise in IL-6. This early rise in IL-6 is hypothesized to be muscle-derived, acting in an anti-inflammatory nature and can therefore inhibit the production of TNF-α (7,9,77). Additionally, exercise in the fed state leads to an attenuated rise in IL-6 due to the availability of glucose for fuel during exercise (97–99). Of the three studies that observed a rise in TNF-α post-exercise, two provided a standardized breakfast of whole-grain bread with butter or peanut butter, 2% milk fat (MF) yogurt or milk, a fruit

(banana or apple) and a beverage (coffee or tea) (66) or a low-protein, low-carbohydrate meal (7 g protein, 3 g carbohydrate, 13 g fat) (102), and one study did not mention if exercise was performed in a fed or fasted state (93). With the attenuation of IL-6 due to pre-exercise feeding, TNF-α may have been less inhibited and this could explain why these studies found increases in TNF-α post-exercise. Due to the inhibition of TNF-α by muscle-derived IL-6, and the role of TNF-α in the recruitment of neutrophils to clear muscle damage, it may be reasonable to anticipate a rise in TNF-α during mid-recovery (2-12 h post-exercise), similar to immune-derived IL-6. However, only two studies noted elevated levels at 2-3 h post-exercise (resistance, and 2 h cycling, respectively) (102,103). Cumulatively, these studies suggest that TNF-α may not change or may decrease immediately following fasted exercise, but increases may be observed at 2-3 h post-fasted exercise. However, little is known about the influence of post-exercise nutrition on the response of TNF-α.

2.2.4 Interleukin 1-Beta (IL-1β)

IL-1β is also a pro-inflammatory cytokine that is present in the early inflammatory response (6,54,57). IL-1β is produced mainly by M1 macrophages and acts to attract additional M1 macrophages and T-cells to the source of muscle damage (54,59). Although IL-1β is produced and secreted at the site of muscle damage, it may not be released systemically (70,106). A study compared the cytokine concentrations within the interstitial fluid of muscle to plasma concentrations in rats during endotoxemia (administration of lipopolysaccharide (LPS) to mimic a bacterial infection) (106). The researchers observed an increase in IL-1β in the interstitial fluid, but no change within the plasma, leading them to believe that myocytes and immune cells within the muscle were

producing IL-1β and the release into the systemic circulation is tightly regulated (106). Thus, IL-1β concentration may increase post-exercise within the muscle, but changes may not be seen within the systemic circulation. Within humans, studies have found an increase in IL-1β mRNA expression 2 h post-exercise (9-fold) returning to baseline by 24 h following 45 min of lower body exercises (intensity not specified) (83), and a 2-fold increase in muscle mRNA at 72 h post-exercise, following 3 sets of 8 reps at 80% 1 rep max (1RM) and one set to volitional failure for three lower body exercises (107). The second study noted a more prolonged change in IL-1β mRNA (107) which may be due to the intensity of the protocol, but the intensity was not specified in the first study. However, systemic concentrations of IL-1β were not evaluated in either study to allow for comparison between local and systemic responses. Thus, the notion that intramuscular IL-1β increases during exercise but is not released into the systemic circulation following resistance exercise has not been fully explored in humans, but important exceptions may relate to exercise modality (explained below).

Most human studies found no increase in circulating IL-1β following resistance or high-intensity exercise (81,93,95,96). One study found increases in IL-1β mid-exercise and immediately post-exercise, which quickly returned to baseline (within 15min) in active males following intense/exhaustive leg press (85). This quick return to baseline may explain why other studies find no change in IL-1β with exercise, as its increase/detection in the systemic circulation may be very transient and difficult to capture following exercise. Chatzinikolaou et al., (78) found increased levels of IL-1β immediately following a plyometric exercise protocol, but levels were undetectable at all other timepoints (24, 48, 72, 96, and 120 h post-exercise) in subjects who were

resistance-trained, but naïve to plyometric exercise. This may be related to the mode of exercise, as plyometrics involves eccentric, high-impact contractions which typically results in greater muscle damage (6,57). Greater muscle damage may lead to a larger increase of IL-1β within the muscle, which may result in a release (spilling out) into the systemic circulation. Kouvelioti et al., (66) found similar results in young untrained adults to Chatzinikolaou et al., (78) with increased IL-1β concentration 5 min after high-intensity interval running and cycling (combined modalities) and no difference relative to baseline by 1 h. Again, this may be a function of the high-intensity exercise protocol and the untrained/recreationally active sample. Interestingly, one study in untrained males found IL-1β to fall below baseline at 6 h post-exercise, continuing to decrease for 5 days following an eccentric contraction-based resistance exercise protocol (100). However, the exercise bout was performed 10 minutes following 1RM testing (100) thus the study did not have a washout period between 1RM testing and the exercise trial which is customary in exercise interventions. The performance of the exercise bout following the 1RM testing could have put additional stress on participants, altering the inflammatory response to the exercise bout (i.e., a carry-over effect). Together these studies suggest IL-1β may not change within the systemic circulation following exercise, but when the bout is particularly strenuous, a transient increase may be detected shortly following exercise.

2.2.5 Interleukin 10 (IL-10)

IL-10 is an anti-inflammatory cytokine, produced mainly by M2 macrophages and T-cells (54). IL-10 release can also be stimulated by the release of muscle-derived IL-6 and acts to inhibit the release of pro-inflammatory cytokines (TNF-α and IL-1β) (7,9,54). Importantly, IL-10 promotes the production of M2 macrophages, which is crucial for

muscle regeneration as the M2 phenotype initiates differentiation of satellite cells, and secretes growth factors and anti-inflammatory cytokines (13,59). Thus, IL-10 is not a part of the acute phase response to exercise; rather the increase in IL-10 is typically seen farther into the recovery process (24 h+ post-exercise) as it initiates the second phase of muscle damage repair (i.e. regeneration) (13).

As IL-10 is not typically part of the initial/immediate response to exercise, many studies find no change in IL-10 immediately following acute exercise. (81,83,85,93,94,100,103,107,108). However, a few studies did see early changes in IL-10 with increases (66,108), or decreases (96,103) 30 min to 1 h post-exercise. Hirose et al., (96) examined the inflammatory response following 2 bouts of eccentric exercise of the elbow flexors (6 sets of 5 reps at 40% 1RM, 2 min rest between sets), separated by four weeks, in untrained males. The researchers found decreases in circulating IL-10 immediately following exercise, with greater decreases following the first bout of exercise compared to the second bout (96); an indication of the repeated bout effect (64,96). The researchers also saw an increase in IL-10 4 days after the first bout of exercise (35.9% increase from baseline) (96). Interestingly, this increase appeared earlier and was of greater magnitude in the second bout of exercise (135% at 6 h post-exercise), indicating an anti-inflammatory adaptation to exercise (96). Further research should continue to explore this short-term adaptation to exercise, specifically examining systemic and/or intramuscular leukocyte responses to gain insight into the local response. Smith et al., (100) observed a trending increase in IL-10 vs. baseline at 48 h ($p = 0.07$), and a significant increase at 72 h post-resistance exercise, which remained elevated at 6 days in untrained males, indicating a sustained anti-inflammatory response to a single

bout of exercise. These findings indicate that IL-10 may not increase immediately post-exercise but rather a day or more post-exercise and elevated levels may persist for several days, exhibiting an anti-inflammatory effect.

2.2.6 Summary of the Cytokine Response to Acute High-Intensity Exercise

A summary of findings reporting the magnitude and timing of cytokine changes following an acute bout of resistance or high-intensity exercise (including plyometrics) can be seen in Table 2.

Table 2: Summary of the systemic cytokine response following an acute bout of high-intensity exercise, without nutrition

Cytokine	Acute response to exercise
IL-6	• Increase within 1 h post-exercise (65,66,86–89,78–85). (hypothesised to be muscle-derived (5,7,57,71–74) • Initial increase is 2-fold or greater (65,82,84,87–89). • Possible secondary increase (78,79,87), beginning at 2 h post-exercise (87) (hypothesized to be immune-derived (77))
TNF-α	• May not change immediately following exercise (95,96,100,103–105). • Possible decrease following eccentric exercise (105) • May increase in mid-recovery (2-3 h) (102,103)
IL-1β	• May not change following exercise (81,82,93,96,108). • Possible increase within 5 min post-exercise during particularly strenuous exercise (66,78).
IL-10	• No change immediately post-exercise (81,83,85,93,94,100,103,107,108). • Increases late in recovery phase (1+ day post-exercise) and can remain elevated for several days (96,100).

2.2.7 Benefits of Modulating the Post-exercise Inflammatory Response

While the post-exercise inflammatory response plays a critical role in regeneration and repair of muscle following EIMD, attenuation of the pro-inflammatory response and augmentation of the anti-inflammatory response to exercise could prove beneficial. Attenuating post-exercise inflammation could reduce secondary damage

inflicted by leukocytes, ROS and proteases (109–111). While augmenting the anti-inflammatory response to exercise may initiate an earlier rise in M2 macrophage phenotypes, which is the critical point for initiating muscle regeneration (13,59). This may be of particular interest in older individuals or individuals with chronic inflammatory conditions, who experience exacerbated pro-inflammatory responses following exercise (112,113), which may delay muscle repair, leading to longer recovery times. Ultimately, attenuating the pro-inflammatory response and augmenting the anti-inflammatory response could speed recovery and allow athletes to return to training or competition at optimal performance more quickly, or simply allow individuals to return to exercise and/or daily activities without being impeded by muscle soreness or reduced muscle function (12). Nonetheless, when targeting post-exercise inflammation, it is crucial to find a balance between optimizing recovery and facilitating repair (110,111). While several strategies have been employed to enhance recovery and post-exercise inflammation, including cryotherapy, massage, and pharmacological interventions (e.g., non-steroidal anti-inflammatory drugs), nutritional provisions may be a simple, viable and cost-effective solution. In this regard, whole food dairy products, including milk, may be of particular interest due to their favourable nutrient profile and anti-inflammatory and antioxidant properties (19,114).

2.3 Milk: Implications on Inflammation

2.3.1 Anti-inflammatory Properties of Milk and Dairy Products

Dairy products, and their constituent nutrients, have been shown to have anti-inflammatory and antioxidant properties (19,115,116) which may be particularly important for the modulation of the inflammatory response. Indeed, cross-sectional

studies examining healthy men and women have found lower levels of pro-inflammatory markers, including CRP, TNF-α and IL-6, to be associated with increased dairy consumption (20–22). Further, systematic reviews have shown that chronic consumption of milk and dairy products elicit significant anti-inflammatory effects (reducing systemic concentrations of CRP, TNF-α, and IL-6) in healthy adults and those with metabolic syndrome or diabetes (46,114). Molecular studies indicate that dairy proteins, whey and casein, as well as calcium, magnesium, and Vitamin D, all present in milk, have anti-inflammatory properties **(Figure 2)** (19). Milk also contains monounsaturated fatty acids, including oleic acid (18:1 n-9), and small amounts of polyunsaturated fatty acids, including omega-3 fatty acids, which have all been shown to exhibit anti-inflammatory properties *in vitro* (Figure 2) (19). Despite this, popular media often regards dairy as pro-inflammatory, mostly due to the presence of saturated fatty acids and allergenic dairy proteins (in a small sub-set of the population). While molecular studies have shown that isolated long-chain saturated fatty acids, such as palmitic (C16:0) and stearic (C18:0) acid, (approximately 41% of milk fat) exhibit a pro-inflammatory effect (Figure 2) by activating nuclear factor-kappa B, which initiates transcription of many inflammatory cytokines (IL-6, TNF-α, and IL-1β) (19), the effects of these and other saturated fats within the dairy nutrient matrix itself may have differing effects. Intriguingly, a prospective study of 5209 adults examining the association between the intake of saturated fat from different foods and cardiovascular disease (CVD) risk found a reduced risk of CVD with dairy saturated fat intake, while an increased risk with saturated fat intake from meat (117). Thus, we cannot generalize the health effects of dairy constituents in isolation or within other food matrices to whole-food dairy products, such

as milk (118,119). The dairy matrix, that is, the connections between nutrients within dairy foods, is crucial to consider as nutrient-nutrient interactions and food synergy may elicit differing effects than the individual components or these same components within other foods (such as meat) (120).

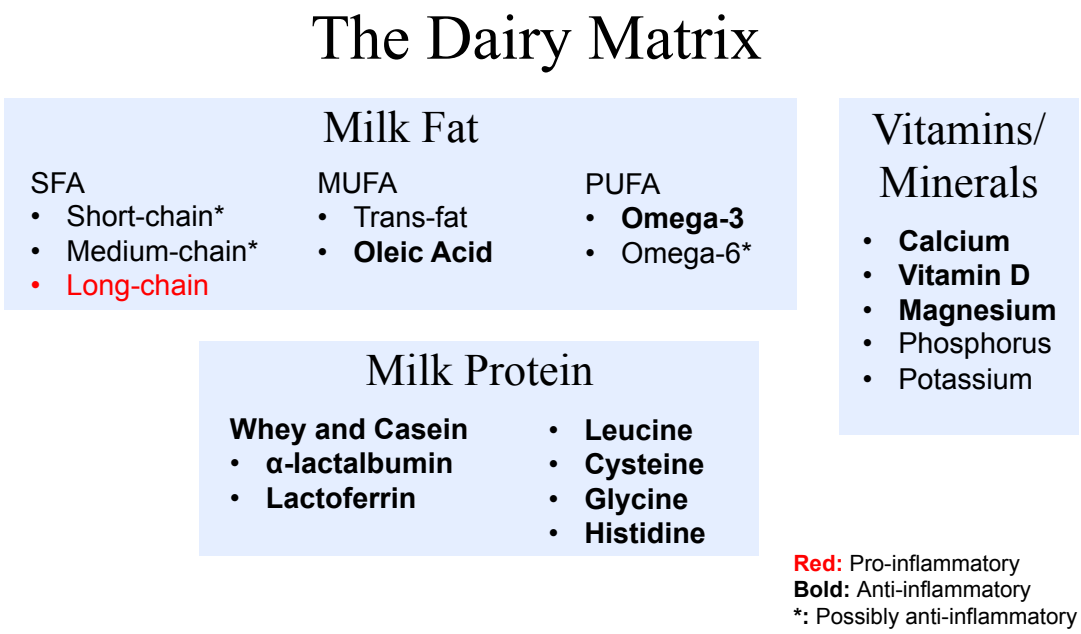

Figure 2: Breakdown of the dairy matrix, detailing the anti-inflammatory and pro-inflammatory constituents as shown in isolated molecular studies. Abbreviations: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

2.3.2 Milk and Milk Protein Consumption Post-exercise

Studies investigating post-exercise milk or milk protein consumption in humans (typically trained athletes or active individuals) have generally observed positive effects on improving subsequent performance (35,36,121,122,37–44) and strength measures (16,35–37,123,124), reducing muscle soreness (16,37,39,45,121,122,124), and attenuating increases in CK (16,37,122,125,126) compared to carbohydrate beverages

and/or water controls. However, little research has investigated the influence of post-exercise milk consumption on markers of acute inflammation (35,36,42,45,51).

Three studies have examined the impact of post-exercise white milk consumption on inflammation in trained females (35,36,45). All three studies provided either 500 mL of white 1%MF milk or an isoenergetic carbohydrate control immediately following a high-intensity interval exercise protocol with (35) and without plyometrics (45) or simulated team-sport exercise (soccer) (36). Blood draws were taken at baseline, 2, 24, 48, and 72 h post-exercise to examine one inflammatory marker, CRP (35,36,45). Two of the studies observed no difference in CRP between nutritional interventions (35,45). While the last study noted CRP was elevated in the milk trial vs. the carbohydrate trial at 2, 24, and 48 h post-exercise, despite benefits of milk on improving knee extension and flexion strength, and 5 m sprint performance (vs. the carbohydrate control) at 24 and 48 h post-exercise (36). Due to the complex interaction of cytokines and their ability to up-regulate and down-regulate one another (Table 1), the examination of only one inflammatory marker and no cytokines severely impacts the ability to characterize the inflammatory response (46). Further, while two of the aforementioned studies noted increases in CRP at 24 h post-exercise (vs. baseline) (36,45), other studies observed no change in CRP following resistance or high-intensity exercise, with (35) and without nutrition (87–89,127). Moreover, feeding studies examining postprandial inflammation without exercise observe no influence of acute nutrition on CRP (49,50). Thus, CRP may not be an appropriate measure when examining the influence of post-exercise nutrition on the inflammatory response.

The influence of flavoured milk (chocolate or strawberry), consumed following exercise, on multiple cytokines has been investigated (42,51,128). One study, in a parallel design, examined the influence of strawberry milk (providing 0.9 g of carbohydrates/kg body weight and 0.4 g of protein/kg body weight; n = 8) consumed immediately following a resistance exercise protocol and at 2 h post-exercise vs. an isoenergetic carbohydrate control (providing 1.3 g of carbohydrate/kg of body weight; Gatorade; n = 9) and a water control (Crystal Lite and water - no nutritional value; n = 9) in untrained individuals (51). The researchers found no differences between groups, but a significant effect of time for IL-6 and TNF-α, IL-6 was elevated at 6 h post-exercise, and TNF-α decreased immediately post-exercise (51). They noted no nutritional effect or main effect of time for IL-1β, or differences between trials for muscle soreness, CK, or strength measures (51). However, participants took part in an initial exercise bout the night before the resistance exercise protocol (to deplete glycogen stores) which could have impacted the inflammatory response to the resistance exercise protocol (51). Moreover, their power to detect between-group differences may have been low. A similar study examined chocolate milk consumed immediately post-exercise and 2 h post-exercise, compared to a control matched for carbohydrates and fat content (dextrose with canola oil) and a sugar-water control (Splenda and water with non-caloric flavouring) in trained individuals (n = 10) using a crossover design (42). The volume of chocolate milk provided was stratified by subjects' weight, those who weighed <64 kg received 500 mL, those between 64-77 kg received 600 mL, and those >77 kg received 700 mL. They found no nutritional effect, but an effect of time for TNF-α and IL-6 with increases observed immediately post-exercise (42). The researchers found no change in IL-10 post-exercise (42). However,

they noted a significantly faster performance of a timed trial (40km cycle) after 4 h of recovery with chocolate milk compared to the carbohydrate and water control (42). This difference could not be explained by glycogen resynthesis, as they initially hypothesized, as there was no difference in glycogen resynthesis between the chocolate milk and carbohydrate trial. Cumulatively, these studies found no anti-inflammatory effect of flavoured milk consumed post-exercise, however, the potential anti-inflammatory effects of the milk could have been masked by the higher sugar content and glycemic index of the flavoured milk (52) making small differences between cytokine concentrations difficult to detect, especially with small sample sizes. Hence why studies investigating the inflammatory response with white milk post-exercise are warranted.

Studies examining milk protein (i.e., whey, casein) ingestion post-exercise have shown anti-inflammatory effects (121,129,130). One study found reductions in TNF-α with milk protein (combination of amino acid isolates) consumption in resistance-trained males compared to a placebo with no protein, following a lower-body resistance exercise protocol (129). McKinlay et al., (121) examined the consumption of whey protein compared to an isoenergetic carbohydrate beverage and a water control consumed after high-intensity interval swimming in adolescent athletes and measured IL-6, IL-10 and TNF-α. The researchers observed reductions in muscle soreness with whey protein consumption vs. water, but no differences between the carbohydrate and the whey protein groups, or between the carbohydrate and the water groups at 24 h post-exercise (121). No significant effects were seen for IL-6 or TNF-α; however, a significant increase in IL-10 was observed in the whey protein group compared to the water control at 8 h post-exercise, but there was no difference between the carbohydrate group and the whey

protein group (121). Similarly, Kerasioti et al., (130) found that IL-10 levels were 118% higher with consumption of a carbohydrate-whey protein cake compared to an isoenergetic carbohydrate cake at 4 h following a 2 h cycling protocol (60-65% VO_2 max) in active males, although it was not statistically significant. The researchers also saw significant reductions in IL-6 and CRP in the protein group at 4 h post-exercise (130). Cumulatively these three studies indicate a possible positive benefit of dairy protein consumption on different cytokines. Of note, the effect of post-exercise milk or dairy protein on the anti-inflammatory IL-10 response is particularly intriguing and warrants further exploration.

Interestingly, a recent study by Russo et al., (128) provided participants 3 equal boluses of either 2%MF chocolate milk (providing 1.2 g/kg of body weight of carbohydrate, and 0.4 g/kg of body weight of protein), or a chocolate flavoured dairy milk-based beverage (i.e. reconstituted dairy protein drink; providing 1.2 g/kg of body weight of carbohydrates) every 10 min starting 30 min into recovery from a 2 h high-intensity interval exercise protocol in highly trained endurance athletes (n = 9). They found no effect of time or interaction for IL-6, TNF-α, or IL-10, but a significant interaction for IL-1β, whereby the concentration was significantly higher at 4 h in the reconstituted dairy protein drink compared to chocolate milk (128). This study may provide a reason to believe that milk, as a whole food, may be superior to dairy constituents (e.g., dairy protein) at reducing inflammatory indices following exercise, further research is warranted.

2.3.2.1 Timing of Milk Consumption

The timing of milk consumption relative to the exercise bout may influence the post-exercise inflammatory response. In a study by Cockburn et al., (131) different timings of milk protein consumption were investigated and compared to a water control. This study compared the effects of a milk protein-based drink (1000 mL, 707 kcal, CHO: 118.2 g, Fat: 16.4 g, Protein: 33.4 g) administered once but at three different timepoints (pre-exercise, immediately post-exercise, or 24 h post-exercise) vs. a water control. The exercise protocol consisted of unilateral eccentric-concentric knee flexion using an isokinetic dynamometer (131). While the study did not directly measure inflammation, the authors did examine markers of muscle damage including CK, muscle soreness, peak torque, and reactive strength (measured as performance of a maximal height jump performed with the shortest contact time following a drop jump) at 24, 48, and 72 h post-exercise (131). Consumption of milk protein immediately following exercise attenuated the rise in CK at 48 and 72 h post-exercise compared to the water control, and attenuated ratings of muscle soreness at 48 h compared to pre-exercise milk protein consumption and the water control (131). When the authors examined measures of strength, milk consumption immediately post-exercise resulted in the smallest decrease in peak torque at all timepoints (24, 48, and 72 h post-exercise) compared to all other trials, and attenuated reductions in reactive strength compared to pre-exercise consumption and the water control (131). Therefore, this study suggests that the consumption of milk immediately post-exercise may be most beneficial for attenuating EIMD, however, inflammation was not assessed.

2.3.2.2 Dosage of Post-exercise Milk Consumption

The dose of post-exercise milk consumption also varies between studies. Most previous research studies examining milk for post-exercise recovery have provided 500 mL (35–39,43,45), as it provides roughly 20 g of protein, which is known to be an optimal dose for stimulating muscle protein synthesis (132). As no study has determined the optimal volume of milk required to reduce post-exercise inflammation to aid recovery, 500 mL of milk has become a standard and well-tolerated bolus volume. Cockburn et al., (123) compared consumption of 500 mL and 1000 mL of semi-skimmed milk with a 1000 mL water control, consumed immediately following muscle-damaging exercise (6-sets of 10 reps unilateral eccentric-concentric knee flexion using an isokinetic dynamometer). The researchers found no difference between either milk trial in terms of muscle function or CK, but a possible benefit of 500 mL of milk vs. 1000 mL on muscle soreness in the first 48 h post-exercise (123). While 1000 mL delivered in one bolus did not appear to be more effective than one bolus of 500 mL, the administration of two 500 mL servings of milk, at two separate timepoints (separated by at least 1 hour), post-exercise may be advantageous because it provides two independent stimuli (i.e., two chances for beneficial nutrient consumption) and may be better tolerated by participants compared to 1000 mL at one time. Indeed, providing subjects with a 500 mL serving of milk at two feeding occasions has been used previously to maximally stimulate protein synthesis (121,133,134) and enhance recovery (i.e., markers of EIMD) (16,41,42,51,126,135). Thus, two 500 mL servings of milk administered after exercise may help to reduce the effects of EIMD to a greater extent than a single bolus of 500 mL or 1000 mL.

2.3.3 Summary: Post-exercise Milk Consumption

In summary, studies examining the consumption of milk protein-based recovery drinks/foods show some beneficial effects for attenuating the post-exercise inflammatory response compared to carbohydrate (129,130) or water controls (121); however, research examining the use of milk, as a whole food, is scarce and has limitations (35,36,42,45,51). Specifically, some studies have used chocolate milk (42,51) which, due to its higher sugar content and higher glycemic index, may negatively affect the inflammatory response (52). While studies investigating white milk have only examined one inflammatory marker (CRP) (35,36,45). Due to the complex interaction of cytokines and immune cells, it is critical to measure multiple inflammatory markers over an appropriate post-exercise period (i.e., ≥24 h) to gain an understanding of the post-exercise inflammatory response, and how different nutritional provisions may alter this response (46). Thus, there is a need for research to examine post-exercise white milk consumption and characterize the inflammatory response using a comprehensive set of inflammatory markers.

Chapter Three: Objectives and Hypotheses

3.1 Objectives:

The overall objective of this thesis was to characterize and compare the acute effects (up to 48 h) of milk consumption (MILK) versus an isoenergetic, isovolumetric carbohydrate drink (CHO) on markers of systemic inflammation (TNF-α, IL-1β, IL-6 and IL-10) following a single bout of combined high-intensity resistance and plyometric exercise in young, normal weight, adult females.

The specific objectives include:

A. to examine the influence of post-exercise MILK or CHO consumption on the absolute concentration of the aforementioned cytokines over the entire trial (baseline to 48 h);
B. to compare the influence of MILK vs. CHO on the entire post-exercise inflammatory response (15 min to 48 h) using area under the curve (AUC) calculations;
C. to assess the relative concentration of cytokines at the completion of the trial (i.e., at 48 h).

3.2 Hypotheses:

It was hypothesized that post-exercise milk consumption would positively affect the inflammatory response to acute exercise compared to an isoenergetic, isovolumetric carbohydrate drink.

The specific hypotheses include, relative to post-exercise carbohydrate consumption, milk consumption will:

A. attenuate the pro-inflammatory response, while enhancing the anti-inflammatory response to exercise, specifically, milk will:
    i. attenuate the secondary rise of IL-6 (immune-derived).
    ii. attenuate the rise in IL-1β post-exercise, with levels returning to baseline values more quickly.
    iii. enhance the rise of IL-10 post-exercise.
B. reduce AUC for pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and augment the AUC for the anti-inflammatory cytokine, IL-10, between 15 min- 48 h.
C. attenuate markers of inflammation at the end of the acute trial (48 h).

Chapter Four: Methods

4.1 Participants

Thirteen healthy, university-aged (18-25 years) females were recruited from York University and Brock University (Ontario, Canada) using approved posters (Appendix A) posted on campus and to social media (e.g., Facebook), through classroom presentations, and by word of mouth. Participants were screened to ensure they met the inclusion criteria, which included, no existing medical conditions, normal body mass index (BMI; 18.5-24.9 kg/m^2), not regularly undergoing a resistance training program in the last 6 months, and no known allergies to dairy protein or lactose intolerance. A health and screening questionnaire was used to verify eligibility for participation in the study (Appendix D). Participants who met the eligibility criteria were informed about the study protocol and then provided written informed consent. This research study was approved by both York University and Brock University's Human Research Ethics Boards and was registered at www.clinicaltrials.gov (NCT03615989).

4.2 Pre-experimental Trial Testing

4.2.1 Baseline Measurements

Before the first trial, participants arrived at the laboratory to have their baseline measurements taken. Height was measured without shoes, using a stadiometer, and reported to the nearest cm. Percent body fat (%) was assessed via bioelectrical impedance analysis (BIA; InBody 520 bioelectrical impedance analysis system; Biospace Co. Inc. Los Angeles, CA, USA). The Godin Shephard Leisure Time Physical Activity Questionnaire was used to assess participants' current physical activity level, and the

Physical Activity Readiness Questionnaire (2018 Par-Q+) was administered to ensure each participant was safe to engage in physical activity.

4.2.2 Familiarization Session

Before the first trial, participants completed an exercise familiarization session with a certified personal trainer (a graduate student) to become aware of the exercises they were to perform during each trial and to determine their voluntary 1 repetition maximum (1-RM) for chest press, seated row, and leg press. To ensure safety, participants completed a warm-up by lifting low loads (30-50% of their perceived 1-RM, as per the BORG Ratings of Perceived Exertion Scale) for 8-10 reps. 1 RMs were determined using a 5 or less RM protocol and calculated using the O'Connor calculation [1-RM = weight × (1+ (0.025 × reps))]. Specifically, during the test, the weight for each exercise was progressively increased to ensure volitional failure at 5 reps or less within 4 successive sets (this improves the accuracy of the O'Connor calculation (136,137)). 1RM was then estimated with the O'Connor calculation using the weight from the set with the lowest number of completed reps (138). The use of this predictive equation has been validated in females (136,139,140).

4.3 Experimental Trials

This study utilized a crossover design. Following the pre-study testing, participants completed two trials in a randomized order: 1) exercise + carbohydrate (CHO), and 2) exercise + milk (MILK). If participants were not on hormonal contraceptives (n = 8), the trials were separated by a minimum of 4-weeks. This washout period was selected to allow for the scheduling of both trials to be 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. Before each trial, participants were asked if they were currently experiencing any symptoms of illness, and if necessary, the trial was rescheduled. Participants were asked to refrain from alcohol consumption and exercise for 48 h before and after the trial. Participants reported to the lab after an overnight fast (minimum eight hours) on the exercise day.

4.3.1 Exercise Protocol

During both acute exercise trials, participants completed the same resistance and plyometric exercise protocol, including the number of sets, reps, and loads lifted (when applicable), but the number of reps and loads lifted varied between individuals based on the results of their 1-RM test. All exercise protocols were conducted by a certified personal trainer and began between 8:30 and 9:30 am, at two university campus gyms, either York University's Tait McKenzie Centre or Brock University's The Zone. Each exercise bout (~60 min) consisted of plyometric exercises including broad jumps, pogo jumps, altitude drops, and explosive lunges (~200 impacts/session), and resistance exercises including chest press, leg press, and seated row (3-4 sets/exercise, 8-12 reps/set, at roughly 75% 1-RM) using selectorized resistance exercise machines in the gym. Participants could consume water *ad libitum* throughout the exercise trial.

4.3.2 Supplement Protocol

Following exercise, participants and their trainers walked back to the laboratory and consumed their first trial drink within 5-10 minutes after cessation of exercise. They consumed either 555 mL of white skim milk (**Table 3**) or an isoenergetic, isovolumetric, carbohydrate drink (CHO). This volume of milk was chosen because it is commonly used

within the literature and well-tolerated following exercise (35–39,43,45). The CHO drink consisted of 52.7 g of maltodextrin mixed with water and calorie-free, fruit-flavoured sweetener (Mio) to increase palatability. A second, identical, trial drink was consumed 1 h after consumption of the first trial drink (**Figure 3**). All trial drinks were consumed within 5 min and participants were shown a timer to ensure completion of the drink within the allotted time. Participants could also consume water *ad libitum* during the post-exercise recovery phase.

Table 3: Approximate macronutrient breakdown of one bolus of the trial drink.

	MILK	**CHO**
Volume (mL)	555	555
Calories (kcal)	200	200
Carbohydrates (g)	29	50
Protein (g)	20	0
Fat (g)	0	0

4.3.3 Blood Sample Collection

Venous blood samples were collected from a vein located in the antecubital fossa of each participant's arm by trained study personnel (Drs. A. Josse or, on occasion, V. Jamnik) using a standardized venipuncture technique at baseline, 15 min, 75 min, 24 h, and 48 h post-exercise (Figure 3). For both trials, approximately 16 mL of blood (1 green top vacutainer collection tube (6 mL sodium heparin plasma), 1 red top (6 mL serum) and 1 lavender top (4 mL EDTA plasma)) was collected at each timepoint on the exercise day (baseline, 15 min, and 75 min post-exercise). On the days following the exercise bout (i.e., for the 24 and 48 h post-exercise blood draws), approximately 12 mL of blood was collected; 1 green top (6 mL sodium heparin plasma) and 1 red top (6 mL serum) vacutainer collection tube. Blood samples in the green and lavender top collection tubes

(plasma) were kept at 4°C for 25-30 min before being centrifuged (Rotina 38R, Hettich, Tuttlingen, Germany) at 1300 relative centrifugal force (RCF) g at 4°C for 15 min. Blood samples in the red top collection tubes (serum) sat at room temperature to clot for 25-30 min before being centrifuged (1300 RCF g at 4°C for 15 min). Serum and plasma were then aliquoted into multiple small (1.5 mL) Eppendorf cryotubes and stored at -80°C until all trials were completed.

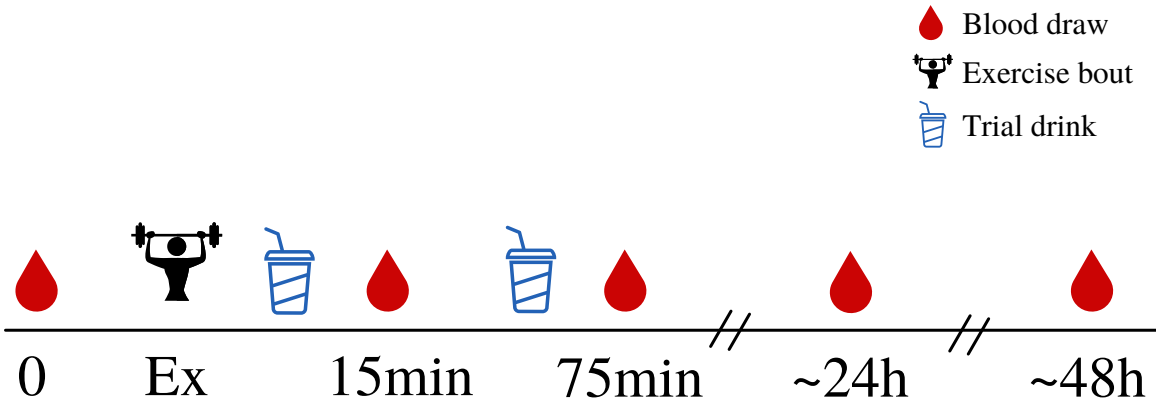

Figure 3: An overview of the experimental trial. Participants arrived for a baseline, rested, fasted blood draw and then participated in the exercise protocol. Following exercise, participants consumed the first trial drink (either MILK or CHO) and underwent a post-exercise blood draw immediately following drink consumption (15 min post-exercise). A second, identical, trial drink was provided at 1 h post-first drink consumption, accompanied by a second blood draw immediately following second drink consumption (75 min post-exercise). Subsequent blood draws occurred at 24 and 48 h following exercise, in the fasted state.

4.3.3.1 Assessing Changes in Hematocrit

After each blood draw on the exercise day, at 3 timepoints: baseline, 15 min and 75 min post-exercise, hematocrit was measured from the lavender top (EDTA plasma) collection tubes. Hematocrit levels were measured in triplicate by the same investigator to correct for exercise-induced changes in plasma volume, if necessary. Microhematocrit tubes with heparin (60 mm pre-calibrated microhematocrit tubes, VWR) were filled with

whole blood, sealed, and spun in a microhematocrit centrifuge (Haematokrit 210, Hettich, Tuttlingen, Germany), at 20000 RCF for 5 min, to separate the red blood cells from the plasma. Each tube was read using the standard evaluation disk, and all three hematocrit values were recorded and averaged to determine a final measure of hematocrit.

4.3.4 Dietary Analysis

Participants were asked to keep their diet as consistent as possible across both trials. During each trial, participants completed a two-day food diary where they recorded everything they ate and drank starting the day of exercise. Dietary intakes were analyzed using the ESHA Food Processor Program (Food Processor SQL, ESHA Research, Salem, OR.).

4.4 Cytokine Analysis

Serum concentrations of IL-6, TNF-α, IL-1β, and IL-10 were analyzed by *Eve Technologies* (Calgary, AB; https://www.evetechnologies.com) in duplicate using a microbead multiplex assay kit (Milliplex MAP Human high sensitivity T cell panel HSTCMAG-28SK, Millipore Corp, MA, USA). Post-analytical analysis, ratios of pro-inflammatory to anti-inflammatory cytokines (TNF-α/IL-10, IL-1β/IL-10, and IL-6/IL-10) were also assessed.

4.5 Statistical Analysis

One participant was unable to complete a 48 h blood draw during their CHO trial, so the last obtained measure (24 h post-exercise) was carried forward for all cytokines. One participant was removed from the IL-6 analysis as their values were greater than three standard deviations from the mean. Average coefficients of variation (CVs) for each

cytokine were 8.7% for IL-6, 5.6% for TNF-α, 7.5% for IL-1β, and 8.0% for IL-10. For one participant's MILK trial, there were high duplicate CVs (>40%) for IL-6 values across four out of five blood draws (all blood draws, except the 75 min post-exercise), for all data points in this trial the values that most aligned with the mean pattern of change were included in the analysis. In addition, in cases where the duplicate CV was greater than 20%, the value that was most aligned with the mean pattern of change for that cytokine was chosen (6 data points).

Prior to the analysis, variables were assessed for normality, by examining skewness and kurtosis z-scores. IL-6, TNF-α, IL-10, TNF-α/IL-10, IL-1β/IL-10, IL-6/IL-10 were not normally distributed and were therefore log-transformed, improving normality. Values in figures are presented as mean ± standard error (SE). If the variable was log-transformed, values are presented as geometric mean and 95% confidence interval (CI). Two-way repeated-measures analysis of variances (RM-ANOVA; within-factor time: baseline, 15 min, 75 min, 24 h, 48 h post-exercise; within-factor trial: CHO, MILK) were conducted on the absolute concentrations of each cytokine (Objective A), and cytokine ratios to assess main effects (time and trial) and time x trial interactions. A two-way RM-ANOVA (within-factor time: baseline, 15 min, 75 min post-exercise; within-factor trial: CHO, MILK) was also conducted to assess changes in hematocrit over time on the exercise day. A two-way RM-ANOVA (within-factor time: baseline, 15 min, 75 min, 24 h, 48 h post-exercise; within-factor trial number: trial 1, trial 2) was conducted to assess the effect of trial order (i.e., the influence of the repeated bout effect) on each cytokine. If any analysis violated the assumption of sphericity, based on Mauchly's test of sphericity, the Greenhouse Geisser correction factor was used. Following a significant

main effect for time and/or time x trial interaction, post-hoc analyses (paired t-tests) were conducted to detect differences over time and between groups.

To assess only the post-exercise cytokine response, as per objective B in this thesis, the net area under the curve (AUC) was calculated from relative percent change to baseline values. Percent change values were calculated by subtracting the baseline concentration from each timepoint and then dividing by an individual's baseline concentration. Paired t-tests were performed to assess differences between the post-exercise net AUC of the CHO and MILK trials. In accordance with objective C in this thesis, one-tailed paired t-tests were used to analyze relative percent change (to baseline) values at 48 h only, between the CHO and MILK trials to compare the response at the completion of the trial. For reference, two-tailed t-tests are also presented. Percent change values were log-transformed if they were not normally distributed (IL-6, TNF-α, IL-10) and are presented as mean ± SE. Paired t-tests were also used to compare resistance exercise volume (sets × reps × load) completed during the exercise sessions and to assess all dietary variables between the two trials.

Significance for all tests was set at $p < 0.05$. Trends are also described when $p \leq 0.12$. The AUC analyses were completed using Prism 9.0.2 for macOS (GraphPad software, San Diego, California, USA). Other statistical analyses were completed using SPSS version 27.0 (SPSS, Chicago, Illinois, USA).

4.6 COVID-19: Issues and Implications related to this MSc Thesis Project

This human research study was affected by the COVID-19 pandemic. The COVID-19 pandemic began in March 2020. Due to the restrictions imposed at York University on all face-to-face human research (starting March 2020 and ongoing into July

2021), recruitment for this study was unable to continue, and data collection was unable to be completed as we had originally planned. This unfortunately contributed to a reduction in our sample size (n = 13), when we were planning on recruiting n = 25. Additionally, due to the pandemic and related restrictions and timing, cytokine analyses for this thesis were outsourced to *Eve Technologies*, whereas under usual circumstances, the candidate (E. Fraschetti) would be responsible for carrying out the analyses herself.

Chapter Five: Results

5.1 Participants

Baseline characteristics are shown in **Table 4**. In terms of randomization, six participants completed the MILK trial before the CHO trial, while the remaining seven participants completed the CHO trial before the MILK trial. Trials were separated by an average of 6.1 ± 1.8 weeks.

Table 4: Participant baseline characteristics (n=13).

Variable	Value
Age (y)	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

Note: all values are mean ± SD. BMI: body mass index.

5.2 Exercise Data

In both the CHO and MILK trials, all participants completed 198 impacts/jumps per exercise session. Additionally, there was no difference in resistance exercise volume (sets × reps × load) performed per exercise session between trials (CHO: 5487 ± 1482 kg; MILK: 5345 ± 1485 kg; p = 0.25).

5.3 Dietary Variables

Differences in dietary outcomes between trials are reported in Tables 5 and 6. Two participants did not return their food logs for both trials; therefore, dietary results are displayed for n=11. Table 5 displays the dietary intakes with the trial drinks included and Table 6 displays the dietary intakes without the trial drinks included.

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.

Dietary Variable	CHO	MILK	p-value
Energy intake (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**
Selenium (mcg)	60 ± 36	62 ± 33	0.91

Note: Values are mean ± SD; n=11

Table 6: 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) excluding the provided trial drinks.

Dietary Variable	CHO	MILK	p value
Energy intake (kcal)	1757 ± 627	1673 ± 616	0.46
Protein (g)	67 ± 27	61 ± 28	0.09
Carbohydrate (g)	221 ± 91	212 ± 102	0.55
Fat (g)	68 ± 30	68 ± 24	0.97
Vitamin D (IU)	47 ± 54	48 ± 50	0.96
Calcium (mg)	561 ± 374	473 ± 266	0.18
Iron (mg)	10 ± 4	10 ± 6	1.00
Magnesium (mg)	194 ± 145	202 ± 113	0.76
Potassium (mg)	1768 ± 1379	1914 ± 1316	0.35
Selenium (mcg)	60 ± 36	62 ± 33	0.91

Note: Values are mean ± SD; n=11

There were no differences between the habitual diets of the participants during the trials when trial drinks were removed ($p>0.05$ for all variables; Table 6). However, some differences for intakes of protein and related dairy nutrients were apparent when the trial drinks were included. Thus, these differences were due to the study design.

5.4 Individual Trial Circumstances and Situations

One individual completed an additional exercise (lying leg curl) in comparison to the other participants, but this was kept consistent between their trials. One participant experienced pain in their shoulder while exercising and was unable to complete all sets of the chest press exercise during their second trial (MILK). In addition, this participant felt unwell after the exercise bout and consumed only ¾ of their first post-exercise trial drink (MILK) but was able to consume the entire second trial drink (consumed 1 h post-first trial drink). Another participant's first post-exercise blood draw was delayed by 20 min due to technical difficulties. Lastly, one participant started a new medication (selective serotonin reuptake inhibitor (SSRI)), between trial visits, and completed the CHO trial approximately two months following the start of the medication. Some evidence suggests that SSRIs can reduce systemic inflammatory cytokine concentrations at rest, specifically reducing IL-6, TNF-α, IL-1β and IL-10 within 5-6 weeks (141), however, the effect of SSRIs on the post-exercise inflammatory response is unclear. Their data were included in the analyses.

5.5 Cytokine Responses

There was no trial order effect for any cytokine (main effect of order, $p>0.05$; interaction, $p>0.05$). Since there was no change in hematocrit following exercise (main effect of time, $p>0.05$), cytokine values were not corrected for plasma volume shifts.

5.5.1 The Influence of Post-exercise Nutrition on Absolute Concentrations of Cytokines (Objective A)

There was a significant main effect of time for the absolute concentration of IL-6 ($p=0.02$; $n=12$; **Figure 4A**), with no main effect of trial ($p=0.67$) or time x trial interaction ($p=0.37$). Specifically, the concentration of IL-6 was higher at 15 min post-exercise than all other timepoints (post-hoc tests, $p<0.05$), suggesting a transient increase from baseline at 15 min and a return to baseline by 75 min post-exercise. There was also a trend for 24 h to be elevated compared to baseline (post-hoc test, $p=0.07$). For TNF-α, there was no main effect of trial ($p=0.98$) or time ($p=0.27$), or a time x trial interaction ($p=0.81$) for the absolute concentrations (**Figure 4B**). For IL-1β, there was no main effect of trial ($p=0.31$) or time ($p=0.46$) for the absolute concentrations. However, there was a trend ($p=0.12$) towards an interaction, where the absolute concentration of IL-1β at 48 h appeared to be lower (relative to baseline) in the MILK trial but not different in the CHO trial (**Figure 4C**). For IL-10, there was a significant time x trial interaction for the absolute concentration ($p=0.02$), with a trending main effect of time ($p=0.12$), but no main effect of trial ($p=0.76$; **Figure 4D**). In the MILK trial, IL-10 was elevated at 15 min compared to 75 min (post-hoc test, $p=0.04$). In the CHO trial, there was a trend for IL-10 to be elevated at 15 min compared to 75 min (post-hoc test, $p=0.09$) and compared to baseline (post-hoc test, $p=0.12$). Between 24 and 48 h, the concentration of IL-10 changed in opposite directions in the MILK and CHO trials. In the MILK trial, IL-10 decreased from 24 h to 48 h (-0.41pg/mL; post-hoc test, $p=0.01$), and at 48 h was trending lower than baseline (post-hoc test, $p=0.09$) and 15 min (post-hoc test, $p=0.08$). In the CHO trial, IL-10 increased from 24 h to 48 h (+0.61pg/mL; post-hoc test, $p=0.04$),

and 24 h was lower than 15 min (post-hoc test, p=0.04) while 48 h was trending to be greater than baseline (post-hoc test, p=0.12).

5.5.2 The Influence of Post-exercise Nutrition on the Overall Cytokine Response to Exercise and Drink Ingestion (Objective B)

AUC analysis revealed no differences between trials for IL-6 (p=0.37; **Figure 5A**), TNF-α (p=0.35; **Figure 5C**), IL-1β (p=0.22; **Figure 5E**), or IL-10 (p=0.24; **Figure 5G**), suggesting no differential influence of MILK on the overall post-exercise cytokine response compared with CHO. The relative percent changes (to baseline) over time of each cytokine, used for the AUC analyses, are also presented for IL-6 (**Figure 5B),** TNF-α (**Figure 5D**), IL-1β (**Figure 5F**), and IL-10 (**Figure 5H**).

5.5.3 Relative Change in Cytokine Concentrations at 48 h Post-exercise (Objective C)

In accordance with our hypothesis that MILK will attenuate the inflammatory response at 48 h, one-tailed t-tests on the percent change to baseline at 48 h revealed significant differences in the relative concentration of IL-1β and IL-10 (p=0.049, **Figure 6C**; and p=0.032, **Figure 6D**, respectively) and a trending differences for IL-6 and TNF-α (p=0.058, **Figure 6A** and p=0.08, **Figure 6B**, respectively), with higher relative concentrations in the CHO trial compared with the MILK trial. (For reference, two-tailed t-tests: IL-6 p=0.12; TNF-α p=0.17; IL-1β p=0.10; IL-10 p=0.06).

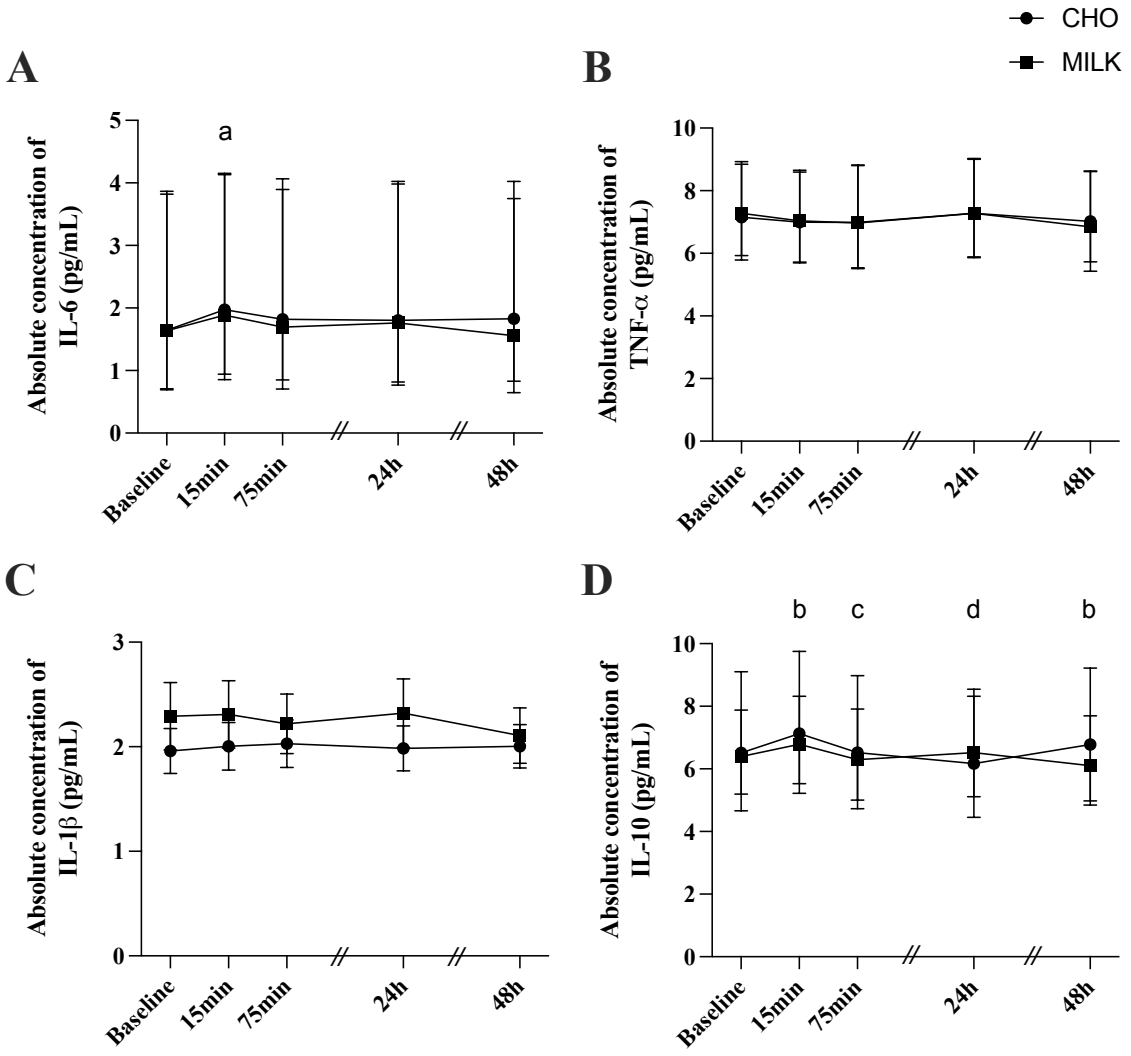

Figure 4: Absolute serum concentrations for IL-6 (A), TNF-α (B), IL-1β (C) and IL-10 (D) at baseline, 15 min, 75 min, 24 h and 48 h post-exercise in the CHO and MILK trials. Following a main effect of time for IL-6 (p=0.02) post-hoc analysis for combined groups: "a" denotes a significant difference from all other timepoints at 15 min. Following a time x trial interaction for IL-10 (p=0.02), post-hoc analysis: "b" denotes a significant difference at 15 min and 48 h vs. 24 h in CHO, "c" denotes a significant difference at 75 min vs. 15 min in MILK, and "d" denotes a significant difference at 24 h vs. 48 h in MILK. Values are presented as geometric mean ± 95% CI for log-transformed variables (i.e., IL-6, TNF-α and IL-10) and mean ± SE for IL-1β.

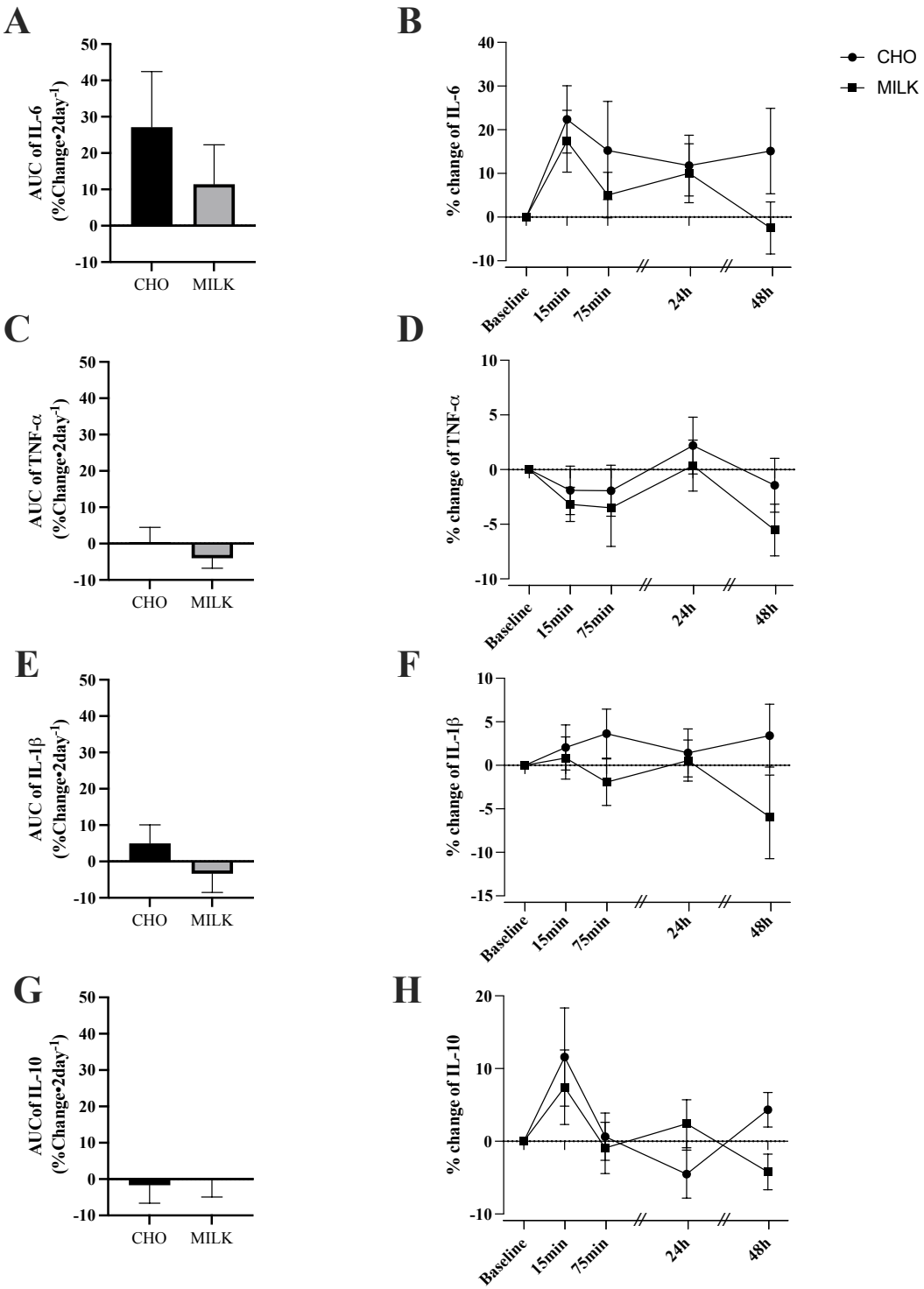

Figure 5: Net AUC for the relative percent change (to baseline) in IL-6 (A), TNF-α (C), IL-1β (E) and IL-10 (G) over 2 days post-exercise (15 min to 48 h) in the CHO and MILK trials. The percent change over time in IL-6 (B), TNF-α (D), IL-1β (F) and IL-10 (H), used for the AUC analyses in the CHO and MILK trials, is also shown. Values are mean ± SE.

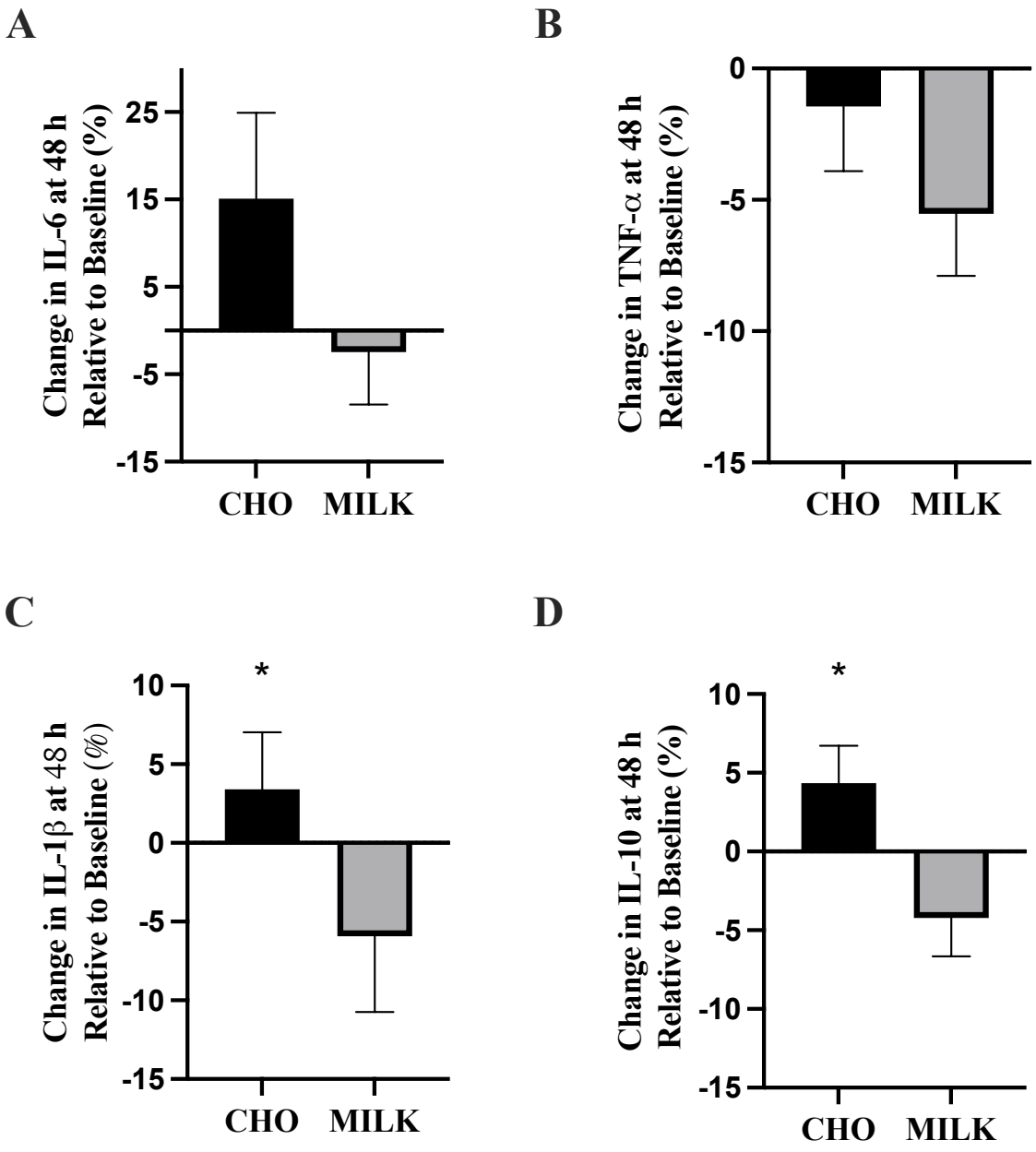

Figure 6: Comparison of the relative percent change (to baseline) in IL-6 (A), TNF-α (B), IL-1β (C) and IL-10 (D) at 48 h post-exercise in the CHO and MILK trials. Percent change values were log-transformed for IL-6, TNF-α and IL-10. Symbol (*) denotes a significant difference between trials for one-tailed t-tests ($p<0.05$). Values are mean ± SE.

5.5.4 Cytokine Ratios

Ratios of TNF-α/IL-10, IL-1β/IL-10, and IL-6/IL-10 were investigated and no main effects or interactions were observed ($p>0.05$; **Table 7**). However, there was a

trending main effect of time for TNF-α/IL-10 (p=0.09), such that the ratio appeared to decrease at 15 min post-exercise.

Table 7: Cytokine ratio responses following the exercise protocol in the CHO and MILK trials.

Ratio	Trial	Baseline	15 min	75 min	24 h	48 h	RM-ANOVA p-values		
							Trial	Time	Int
TNF-α/ IL-10	CHO	1.10 (0.8-1.6)	0.98 (0.7-1.4)	1.07 (0.7-1.6)	1.18 (0.8-1.8)	1.03 (0.7-1.5)	0.75	0.09	0.22
	MILK	1.14 (0.9-1.5)	1.04 (0.8-1.4)	1.11 (0.8-1.5)	1.12 (0.8-1.5)	1.12 (0.8-1.5)			
IL-1β/ IL-10	CHO	0.28 (0.2-0.4)	0.26 (0.2-0.4)	0.29 (0.2-0.4)	0.30 (0.2-0.4)	0.28 (0.2-0.4)	0.19	0.27	0.44
	MILK	0.32 (0.2-0.4)	0.31 (0.2-0.4)	0.32 (0.2-0.4)	0.32 (0.2-0.4)	0.31 (0.2-0.4)			
IL-6/ IL-10	CHO	0.27 (0.1-0.6)	0.30 (0.1-0.6)	0.30 (0.1-0.6)	0.32 (0.2-0.6)	0.29 (0.1-0.6)	0.80	0.13	0.86
	MILK	0.28 (0.1-0.6)	0.30 (0.1-0.6)	0.30 (0.1-0.7)	0.30 (0.1-0.6)	0.27 (0.1-0.6)			

Note: Values are geometric mean (95% CI)

Chapter Six: Discussion

The present study within this thesis is the first study, to our knowledge, to compare the effects of post-exercise white milk versus carbohydrate consumption on a set of systemic cytokines following a single bout of high-impact resistance and plyometric exercise. We demonstrated an influence of post-exercise nutrition on the IL-10 response during the later phase of the acute trial, where from 24 to 48 h post-exercise, the concentration of IL-10 decreased and increased in the MILK and CHO trials, respectively. We also observed a similar increase between trials over time (main time effect and no interaction effect) in the concentration of IL-6, suggesting an influence of exercise but not nutrition. However, the nutrition and exercise protocol did not appear to alter absolute concentrations of IL-1β or TNF-α throughout the trials (Objective A). There were also no differences in the net relative post-exercise response between trials (Objective B). Lastly, the relative change in IL-1β and IL-10 at 48 h, was significantly lower in the MILK trial compared to the CHO trial, while the relative change in TNF-α and IL-6 was trending lower in the MILK trial, which may indicate an attenuated inflammatory response with post-exercise milk consumption (Objective C). Given our reduced sample size (due to COVID-19), and the exploratory nature of objective C, these effects warrant further investigation.

6.1 Individual Cytokine Responses

6.1.1 IL-6

Within the present study, we observed a significant 22% and 17% (combined mean of 20%) increase in the concentration of IL-6 at 15 min post-exercise compared to all other times in the CHO and MILK trials, respectively. The timing of this increase is

consistent with findings in the literature, with most exercise studies reporting an increase in IL-6 within 1 h post-exercise (65,66,86–89,78–85). At 75 min post-exercise, we found that the levels had returned to baseline, indicating a transient response. Indeed, Heavens et al., (65) observed a significant increase in systemic IL-6 concentration immediately following high-intensity resistance exercise in males and females. At 15 min post-exercise, levels remained elevated in the males, but among females, levels began to decrease and were not different from baseline. As the initial rise in IL-6 is hypothesized to be muscle-derived in response to muscle contraction (5,7,57,71–74), it is logical to assume that IL-6 will no longer be produced/released from the muscle following cessation of exercise (i.e., muscle contraction). Thus, the initial response in IL-6 is highly transient and needs to be captured quickly following cessation of exercise (within 15 min).

Most studies illustrate a 2-fold or greater increase in IL-6 shortly after the cessation of exercise (65,82,84,87–89), while the present study observed only a 17-22% increase. This potential 'blunted' effect in our study may be due to the timing of the blood sample at the end of the exercise protocol, where the time between the true cessation of exercise and blood collection was ~15 min vs. immediately/5 min post-exercise as seen in many other studies (65,66,87,92–94,96,103–105,108,78–83,85,86). Blood samples were collected 15 min post-exercise because the exercise protocol needed to be carried out in a different location on campus (Tait Mackenzie Centre at York or The Zone at Brock) from the blood sampling (Bethune Building at York or Welch Hall at Brock), and the trial drink was consumed before the first post-exercise blood sample.

Since this initial post-exercise blood draw was later in this study compared to others, the IL-6 concentrations may already be coming down from their post-exercise peaks.

While the early post-exercise rise in IL-6 is thought to be muscle-derived IL-6, increases in IL-6 during recovery (beginning at 2 h) may be attributed to its release from immune cells in response to exercise-induced muscle damage (77). Gordon et al., (87) observed an increase in IL-6 30 min following isokinetic leg extension in young and middle-aged adults (combined groups), and levels returned to baseline by 1 h. At 2 h post-exercise, the researchers observed a secondary rise in IL-6, and levels continued to rise and were elevated compared to baseline, at 24 and 48 h post-exercise (87). This study may be capturing the two distinct rises in IL-6 post-exercise, the initial increase of muscle-derived IL-6 in response to muscle contraction and the subsequent increase in immune-derived IL-6 in response to muscle damage. Other studies have also reported elevated IL-6 levels between 2-24+ h post-exercise (78,79,83,84,87,100). In the present study, there was a trend ($p=0.07$) for IL-6 to be elevated at 24 h post-exercise compared to baseline in both trials (Figure 4A). This secondary rise in IL-6 may be indicative of a rise in immune cell-derived IL-6 (77), which acts in a pro-inflammatory nature (54), in contrast to the initial rise/muscle-derived IL-6, which acts in an anti-inflammatory nature (5,7,57). Multiple blood samples between 2-24 h post-exercise in future investigations may aid in better capturing this secondary rise in IL-6. Future research should also examine systemic leukocyte populations and the cytokines they release to determine the true source of IL-6, as these data will shed light on the actions (pro or anti-inflammatory) of IL-6.

6.1.2 TNF-α

We observed no changes over time or between trials for the absolute concentrations of TNF-α. This is congruent with the literature, as studies examining the response of TNF-α following exercise (with and without post-exercise nutrition) often observe no change in TNF-α shortly following exercise (0-30 min post) (94–96,100,103–105,121,128). However, following other inflammatory challenges, such as a high-fat and/or refined carbohydrate meal, TNF-α has been shown to increase (76). Muscle-derived IL-6 acts in an anti-inflammatory nature and can suppress the initial production of TNF-α (**Figure 7**) (7,9,77). Therefore, the decrease we observed in TNF-α post-exercise (Figure 4B), although not statistically significant, may be attributed to the rise in IL-6 at 15 min post-exercise. Other studies have also found TNF-α to decrease post-exercise (with and without post-exercise nutrition) (51,95,96,100,105,121). Considering the role of TNF-α in recruiting neutrophils to the site of EIMD (59), and that TNF-α is produced by M1 macrophages (pro-inflammatory, dominating in the first 24 h+ of recovery) (6–8,59,101), TNF-α may increase in mid-stages of recovery, similar to immune-derived IL-6. Two studies noted elevated levels of TNF-α at 2-3 h post-exercise, following resistance exercise (102) and 2 h of cycling in trained individuals (103). As the present study had no sampling periods between 75 min and 24 h we are unable to detect changes in the concentration of TNF-α between these times. Examination of TNF-α in mid-stages of recovery (2-8 h) may be beneficial to understand the temporal change in TNF-α following exercise, with and without nutrition. Further, in the present study, the relative change in TNF-α was trending lower in the MILK trial compared to CHO at 48 h,

which may indicate a positive benefit of milk at 48 h (discussed further in the following sections).

6.1.3 IL-1β

While we observed no main effects of trial or time for IL-1β in the present study, we did observe a trend towards a significant interaction ($p=0.12$) where at 48 h post-exercise IL-1β appeared to be lower relative to baseline in the MILK trial and unchanged in the CHO trial (Figure 5C). This may indicate a positive benefit of post-exercise milk consumption at 48 h, which will be further discussed in the following sections. Despite this potential interaction effect (trending), similar studies report no change in IL-1β following resistance or high-intensity interval exercise during initial recovery (post-exercise to 1 h) (81,93) and extended recovery (up to 96 h post-exercise) (95,96), but none of these studies assessed the combined effect of nutrition and exercise. In contrast, some studies have noted increases in IL-1β following particularly strenuous/damaging exercise (i.e. high-impact or eccentric exercise), without nutrition (66,78,85). One study noted an increase in IL-1β during and immediately following resistance exercise in active males, however, by 15 min levels were not different from baseline (85). This finding indicates a highly transient response of IL-1β following exercise and may explain why the present study found no increase at 15 min post-exercise, despite the exercise bout being of high intensity. While IL-1β, secreted primarily by M1 macrophages, plays a role in the recruitment of M1 macrophages and T-cells to the area of damage following exercise (54,59), measuring the concentration within the systemic circulation may not reflect the local response (70,106). The release of IL-1β in the muscle is thought to be tightly regulated, as evidenced by increases in IL-1β in the interstitial fluid of the muscle

but no change in plasma concentration following the administration of LPS in rats (106). Further, exercise studies in humans have noted increases in IL-1β muscle mRNA post-exercise (83,107), which may indicate exercise-induced production of IL-1β within the muscle, however, levels of mRNA may not always reflect the amount of protein produced (142). Moreover, it is unclear how post-exercise nutrition, specifically white milk, could modulate this response.

6.1.4 IL-10

We observed divergent responses in the absolute concentration of IL-10 between trials. Specifically, the two trials had opposing effects between 24 and 48 h. In the MILK trial, IL-10 decreased at 48 h compared to 24 h, and in the CHO trial, IL-10 increased between these timepoints (Figure 4D). This rise in IL-10 in the CHO trial between 24-48 h may be indicative of the rise in M2 (anti-inflammatory) macrophages and a reduction in M1 (pro-inflammatory) macrophages (Figure 7), a key aspect of the initiation of muscle regeneration following EIMD (13,59), which would suggest that muscle regeneration processes are likely beginning at ~48 h in the CHO trial. In the MILK trial, however, IL-10 decreased from 24 to 48 h, and at 48 h there is an attenuation of all inflammatory indices, which may indicate the end of the inflammatory response and muscle repair processes (discussed below) but IL-10 appears to increase earlier. Thus, we speculate that the IL-10 concentration may rise earlier (sometime between the 75 min and 24 h post-exercise timepoints (Figure 4D)) within the MILK trial, indicating an earlier onset of muscle regeneration, and a benefit of milk on improving recovery time. This speculative earlier rise in the anti-inflammatory macrophage phenotype (M2) may be the result of reduced secondary damage (elicited by ROS and proteases produced by neutrophils) to

the muscle in the initial phase of recovery. However, as we did not take any blood samples between 75 min and 24 h post-exercise we do not know the true magnitude or timing of the increase in IL-10 in the MILK trial. Future research examining timepoints between 1 to 24 h and 24 to 48 h post-exercise will allow for greater characterization and understanding of how post-exercise consumption of milk and CHO influence IL-10.

We also demonstrated a significant difference in the relative change at the 48 h timepoint between the trials, with a lower relative concentration of IL-10 in the MILK compared to the CHO trial (Figure 6D). This may indicate a negative effect of milk at 48 h as a lower relative concentration of the anti-inflammatory cytokine, IL-10, may indicate a pro-inflammatory state, however, given that the other pro-inflammatory cytokines were also lower at 48 h (significantly lower in IL-1β and trending lower in TNF-α, IL-6) and there were no changes in cytokine ratios (Table 7) during the trials it is more likely that the reduction in IL-10 at this time is related to reductions in the other cytokines, as the interactions between cytokines are complex and work to regulate one another (7,9,54). Specifically, increasing IL-10 can blunt the production and release of TNF-α and IL-1β (Figure 7). As discussed previously, we speculate IL-10 concentration increased earlier within the MILK trial (between 75 min and 24 h), thus at 48 h, the production of IL-10 may have already counteracted the pro-inflammatory cytokine response. Further, the reduction of all inflammatory cytokines in the MILK trial (vs. increases in the CHO trial) may indicate the completion of the inflammatory response to exercise. This may then illustrate a benefit of milk at 48 h for reducing inflammatory indices compared to CHO and speeding acute recovery, but more research is needed to confirm.

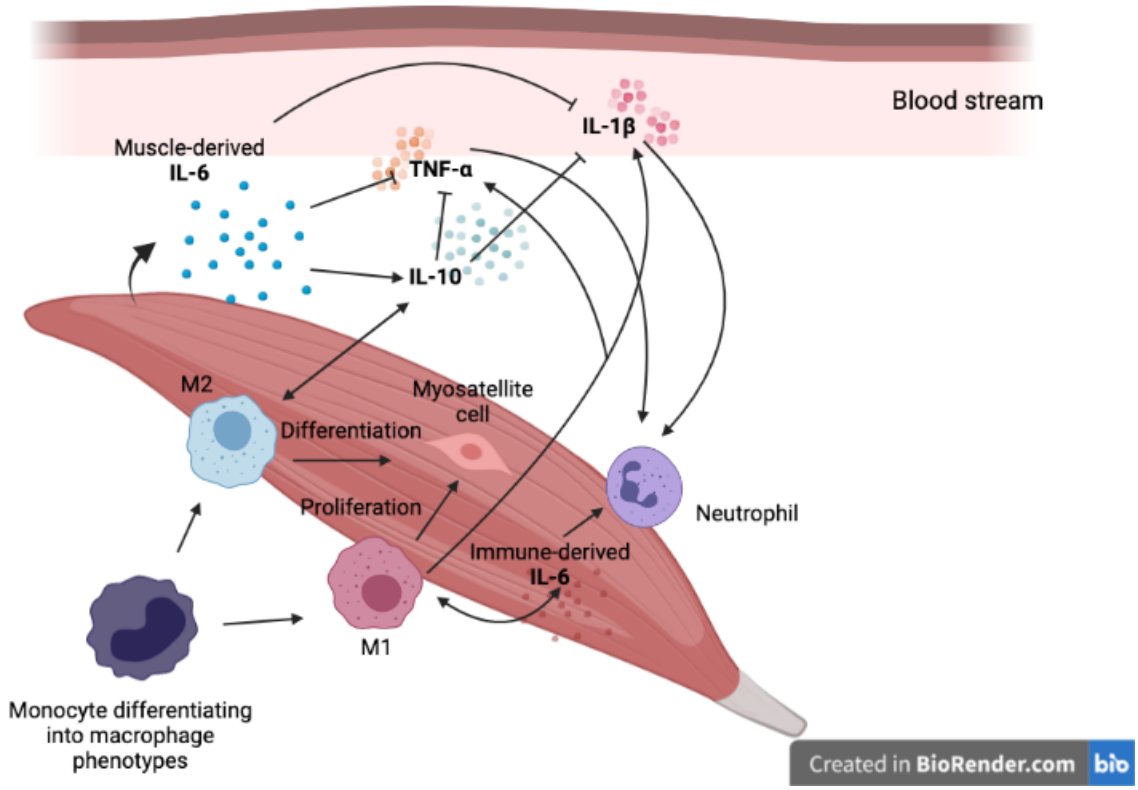

Figure 7: Depiction of the complex interactions between key immune cells and muscle following exercise that elicits muscle damage. Created with BioRender.com.

6.2 Post-Exercise Milk Consumption

Other studies examining dairy consumption following exercise have illustrated a possible benefit of milk protein (i.e., whey protein) on IL-10 during the mid-stages of recovery (4-8 h post-exercise). Specifically, using a parallel design in competitive adolescent swimmers, McKinlay et al., (121) observed significantly greater absolute increases in IL-10 after post-exercise whey protein consumption vs. a water control at 8 h post-exercise. However, there was no significant differences between the whey and carbohydrate group or the carbohydrate and water group (such that the IL-10 response to CHO was intermediate between whey and water) (121). The researchers also hypothesized that this elevation in IL-10 in the whey protein group may be attributed to

the switch from M1 to M2 macrophage phenotypes (121). It is important to note that the timing of the rise in M2 macrophages in the trained adolescent swimmers examined by McKinlay et al. (121) may differ in comparison to untrained adults. Also, the intensity and impact of swimming may differ from that in our protocol, which could implicate the post-exercise cytokine response. A second study investigated the consumption of a whey protein cake vs. a carbohydrate control following prolonged cycling in 9 trained adults males using a cross-over design (130). The authors observed higher levels of IL-10 (118% difference) at 4 h post-exercise following the consumption of whey vs. the control, however, the difference between trials was not statistically significant (130). As the present study did not include sampling times between 75 min and 24 h, we are unable to detect changes/differences in IL-10 that may be occurring during these mid-stages of recovery. It is important to note that both studies (121,130) used a component of milk (i.e., whey protein) as the supplement, rather than a whole food, which may have also affected the inflammatory response. Another similar study by McKinlay et al. (143) using a cross-over design in 13 trained adolescent females demonstrated that Greek yogurt consumption (i.e., a whole-food dairy product; 3 x 160 g/d) following a high-intensity soccer training camp for 5 days resulted in a significant increase in IL-10 concentration compared to an isoenergetic CHO pudding. Importantly, in this study, IL-10 was not measured acutely post-exercise *per se* but rather at rest and the day following (~12 h after the last exercise session) the end of the intense 5-day training camp (143). Nonetheless, this finding may indicate that the anti-inflammatory response, specifically IL-10, is benefited by whole-food dairy products. Considering the dairy matrix, which along with milk protein (whey and casein) contains important vitamins, minerals and bioactives with

anti-inflammatory properties (e.g., calcium, vitamin D, magnesium (19)), and the potential likelihood of additive or synergistic influences of nutrient-to-nutrient interactions (118–120), it is possible that milk, as a whole food, may be more efficacious at benefiting the inflammatory response in comparison to whey protein alone. Indeed, one study examined the influence of 2%MF chocolate milk vs. a reconstituted dairy-milk-based beverage (powered milk protein reconstituted in water), consumed after 2 h of high-intensity interval exercise, on markers of inflammation in highly trained athletes (128). The researchers observed no main effects or interactions for IL-6, TNF-α, or IL-10, but a possible benefit of chocolate milk vs. the milk protein beverage on attenuating IL-1β at 4 h post-exercise (128). This may indicate a greater benefit for whole food dairy products (vs. milk protein) on inflammatory markers when consumed post-exercise.

Aside from some of those studies mentioned above, a limited number of studies have investigated the influence of post-exercise milk consumption on indices of inflammation (35,42,45,51,128). Of these studies, three investigated white milk compared to an isoenergetic carbohydrate control consumed after high-intensity interval exercise with (35) and without plyometrics (45) or after stimulated gameplay (soccer) (36) in trained female athletes. While one study observed elevated CRP in the MILK trial compared to the carbohydrate control at 2 h, 24 h and 48 h (36), the other studies found no differences in acute CRP concentrations between trial drinks (35,45). Evaluation of only one inflammatory marker (and no cytokines) severely limits the ability to characterize the inflammatory response. Since we did not measure CRP in the present thesis, we cannot make direct comparisons to these studies. Further studies are needed (building upon ours) to better characterize the inflammatory response post-exercise with

white milk consumption. Other investigations that examined different types of milk reported no differences in IL-6 and TNF-α (42,51), IL-1β (51) or IL-10 (42) between flavoured milk (strawberry or chocolate) and a carbohydrate or water control. However, utilization of 'flavoured milk' may mask the anti-inflammatory effects of milk due to the higher sugar content and glycemic index (52). Cumulatively, these limitations make it difficult to compare findings to the present study. Nonetheless, many studies have observed significant benefits of post-exercise milk or milk protein consumption on acutely improving subsequent exercise/sport performance (35,36,121,124,37–44) and muscle strength (16,35–37,122–124), reducing muscle soreness (16,35,37,39,122) and attenuating the rise in CK (16,37,122,126), while other studies illustrate a neutral (i.e. not different from the control) effect of milk on performance (35,51,126,135,144), muscle soreness (38,45,51,126,145) or CK (35,38,42,45,51,145). No study, to our knowledge, has found a negative effect of consuming milk post-exercise on any performance-related variable. Given that our study is the first to examine post-impact exercise white milk consumption on a set of inflammatory cytokines, our results begin to shed light on a potential beneficial acute inflammatory effect of white milk compared to isoenergetic, isovolumetric CHO consumption in young untrained females. Further investigation that specifically examines cytokine concentrations within mid-stages of recovery (2-12 h post-exercise) with a greater sample size is required.

6.3 Implications

Among athletes, it may not be beneficial to interfere with the post-exercise inflammatory response or attenuate EIMD as these processes are integral for muscle adaptation. Indeed, athletes are advised against taking high-dose antioxidants (146) and

anti-inflammatory drugs (147) to maximize adaptation to a training stimulus (110). However, the anti-inflammatory/antioxidant effects of whole food nutritional interventions, such as milk (or other dairy products) are less potent than these high-dose supplements or pharmacological interventions and often contain other vital nutrients that promote musculoskeletal adaptation and recovery. Milk contains many nutrients that are beneficial for recovery and refuelling from exercise and that facilitate training adaptations, including carbohydrates (for glycogen resynthesis), high-quality protein (whey and casein; for stimulating muscle protein synthesis), calcium and vitamin D (to support bone and musculoskeletal health), and electrolytes (to enhance rehydration) (15,33,34). Thus, nutritional interventions with a whole-food focus that promote the intake of key vitamins and minerals (e.g., calcium, vitamin D) may be ideal (110,146). Further, it is important to note that in some instances it may be advantageous for athletes to focus on acutely reducing EIMD and the post-exercise inflammatory response (110). For example, during a competition where the athlete is participating in many events/games in one day or over multiple days enhancing recovery time may be critical to ensure optimal performance (110,146). Ultimately, when providing food/nutritional supplements it is important to find a balance between speeding recovery and facilitating adaptation (110,111). Additional research is required to determine if post-exercise milk consumption can achieve this balance.

Chronic inflammatory health conditions, as well as general ageing, can be accompanied by low-grade systemic inflammation and higher basal levels of systemic cytokine concentrations, including TNF-α and IL-6 (112,113,148). Persistent elevation of circulating cytokines and inflammation can contribute to several pathophysiological

outcomes/risk factors leading to chronic disease (148) but also to catabolism of the muscle, resulting in muscle atrophy (13,77). Exacerbated inflammatory responses post-exercise have been observed among older adults (113) and those with inflammatory conditions (112). Specifically, a recent review noted greater increases in lymphocytes and neutrophils following exercise (various modalities and intensities) in older adults compared to young adults (113), suggesting an aggravated inflammatory response post-exercise. A systematic review examining post-exercise inflammation in individuals with inflammatory conditions found levels of IL-6 and counts of total leukocytes and lymphocytes remained significantly elevated compared to healthy controls (whose levels had already returned to baseline within 120 min) (112), indicating a prolonged response among those with chronic inflammation. Ultimately, these exacerbated inflammatory responses to exercise may lead to slower muscle repair resulting in longer recovery times (13). While chronic exercise positively affects systemic inflammation (i.e., reducing pro-inflammatory cytokine concentrations at rest and enhancing immunity), and muscle mass accretion (112,113,148), these prolonged pro-inflammatory responses following acute exercise can impair muscle protein synthesis and trigger muscle protein breakdown, promoting muscle wasting (13). Sustained inflammation may also result in greater neutrophil recruitment due to elevated pro-inflammatory cytokines (Figure 7), inducing more secondary damage at the muscle via the production of ROS and proteases (10–12). In addition, there may be a delayed rise in M2 macrophages with sustained inflammation. Given the M2 phenotype is a key factor in muscle regeneration, triggering satellite cell differentiation (Figure 7), this delay could further impair recovery (13). Thus, attenuating the extent of the inflammatory response to acute exercise, among those with chronic

inflammation, may provide benefits for improving recovery and muscle repair, reducing muscle wasting (13), and ensuring inflammation is not amplified (112). Indeed, training studies in older adults have indicated greater muscle mass accretion (149) and improvements in strength (150) when multi-ingredient anti-inflammatory supplements (made up of whey protein, B-vitamins, select amino acids etc.) were consumed in conjunction with exercise. Given the results of these studies and the findings of the present study, there is reason to believe that consumption of anti-inflammatory foods or supplements, such as milk, following exercise may be beneficial for attenuating inflammatory indices, which may be of particular benefit to populations with chronic inflammation or older adults to enhance recovery and muscle regeneration. Moreover, with appropriate post-exercise nutrition, the chronic benefits of exercise (including benefits on systemic inflammatory markers and immunity) may be able to be achieved in these populations for which most exercise may otherwise be contraindicated due to exacerbation of their symptoms or detrimental long recovery times/muscle weakness.

6.4 Strengths and Limitations

6.4.1 Strengths

Our study utilized a crossover design and thus, each participant completed both a MILK and CHO trial. This study design helped to reduce the variability that is often inherent when examining systemic cytokines, as each individual served as their own control. This approach likely improved our ability to detect differences between trials. Second, the use of whole-food, white milk, improves the generalizability and practical application of our findings since whole foods are more readily available (and less expensive) for consumption compared to specialized supplements. Moreover, nutrients

are typically consumed within foods and complex matrices, rather than in isolation. These matrices, particularly the dairy matrix (which contains multiple nutrients and constituents in various quantities (18,114,118)), have been shown to have additive or synergistic effects and may provide greater benefits on body composition, cardiometabolic disease risk and bone health in comparison to the individual constituents. Further, while the use of protein isolates, such as whey protein, following exercise may be a common practice among athletes or trained individuals, it may not be accessible, feasible or convenient for casual exercisers or older populations. Protein isolates may also lack other nutrients that are important for certain populations to consume (e.g., older adults also need vitamin D and calcium to support their skeletal health (151) which can be found in whole-food dairy products but not necessarily in a whey protein supplement). Lastly, this study is the first to investigate the influence of white milk on post-exercise inflammation using multiple inflammatory markers. As noted previously, examination of only one marker severely limited the ability of previous studies to comment on the influence of milk on the inflammatory response. Our study advances the field by beginning to characterize a more comprehensive inflammatory response following post-exercise milk consumption.

6.4.2 Limitations

The main limitation of this thesis relates to the abrupt stopping of the study before its completion and further restrictions on in-house analyses due to the global pandemic of COVID-19. Unfortunately, this severely impacted our study numbers as we were intending to recruit a sample size of n=25. As such, we may be unable to appropriately detect differences in cytokine concentrations between the CHO and MILK trials. Hence why, in the current thesis, we decided to report statistical trends and conduct one-tailed

exploratory analyses. Another limitation is that we did not investigate any other markers of muscle damage, such as muscle soreness (e.g., visual analog scales) or measures of strength (e.g., Biodex) or performance (e.g., jump height). The current body of literature examining milk consumption post-exercise is focused almost exclusively on these markers of recovery; however, no study has assessed markers of recovery and a set of inflammatory markers with white milk consumption. In the future, this type of research should include a muscle soreness questionnaire and/or performance tests to better characterize the effect of the exercise protocol and nutritional intervention on EIMD and post-exercise inflammation and allow for a better comparison with the current literature. Lastly, our ability to detect differences between trials may be limited by our sampling times. As we have no blood sample(s) between 75 min and 24 h post-exercise, we were unable to detect and characterize any impact of our intervention between these timepoints. Future research should consider additional sampling times within the mid-stages of recovery, especially considering the findings of dairy protein on IL-10 concentrations between 4-8 h post-exercise (121,130). For example, sampling in 2 h increments, beginning at 2 h post-exercise and continuing up to 8-12 h, may provide greater insight and characterization of the acute inflammatory response. Of note, the current sampling protocol was developed to minimize participant burden, which will also need to be taken into consideration in the design of future studies. Despite these limitations, the results of our study provide crucial data to begin to characterize the inflammatory response when white milk is consumed post-exercise.

6.5 Future Directions

Additional research is required to further characterize the inflammatory response following post-exercise milk consumption. First, the current study, or a variation of it, should be conducted with more participants. Second, the examination of peripheral blood leukocytes would allow for greater characterization of the inflammatory response and stronger inference about the local response (e.g., inference about the different stages of muscle damage and repair). Recently, two studies have investigated the influence of post-exercise chocolate milk consumption on leukocyte populations (128,152). While the first study found no difference in total leukocyte or neutrophil counts between the chocolate milk and water control (152), the second study found increased neutrophil counts with 2%MF chocolate milk compared to a reconstituted dairy protein beverage at 4 h following a high-intensity interval exercise protocol (128). This may be a negative effect of chocolate milk, as greater neutrophil counts may lead to greater secondary damage, however, no blood samples were taken after 4 h post-exercise (128). Future research should examine leukocytes/neutrophils (as well as cytokines) at timepoints up to or beyond 48 h. Further, examination of systemic leukocyte populations (and their cytokine productions/secretions) using flow cytometry (as opposed to HemoCue and a Coulter Counter, as in (128,152)) would provide a more intricate measure of cell counts and changes within leukocyte subpopulations following exercise, as the flow cytometer uses multiple lasers and involves optimization of the fluorophores for examination of up to 12 different antibody markers (extra or intra-cellular) allowing for greater classification of leukocyte subtypes and cytokines that are produced within these cells. For example, using flow cytometry, monocytes can be further classified into classical, pro-inflammatory,

monocytes ($CD14^+CD16^-$) and non-classical ($CD14^-CD16^+$) monocytes (102). Furthermore, flow cytometry allows for the examination of single cells and thus, one can measure intracellular cytokine production of different circulating immune cells to determine the source of cytokine release. The ability to determine the source of cytokine release is especially important when examining IL-6 because the source of IL-6 determines its action (i.e., pro-inflammatory (primarily from immune cells – monocytes, M1 macrophages and neutrophils) or anti-inflammatory (primarily from muscle)). Collecting muscle biopsies would also allow for examination of the local leukocyte response and provide direct insight into the phases of repair and regeneration following EIMD. It is also prudent to investigate other dairy products, such as yogurt. Different food forms (solid, liquid, semi-solid) have different absorption kinetics (118), which have been shown to result in different postprandial amino acid responses (greater serum amino acid concentrations with yogurt vs. milk) (153). Thus, the kinetics of the anti-inflammatory properties in dairy may differ between dairy products and elicit differing effects (in magnitude or timing) on post-exercise inflammation. Further, fermented dairy products are of particular interest as they provide additional health benefits through the regulation of the gut microbiome and synthesis of bioactive compounds that can influence the immune system and inflammatory pathways (154). For example, fermentation of dairy products results in the production of lactic acid, which has been shown to reduce pro-inflammatory cytokine production, and ROS activity in the intestines (154). Given the different physical features and nutritional matrices of whole-food dairy products, further examination and comparison of different dairy products on the post-exercise inflammatory response is warranted.

6.6 Conclusion

The present study begins to characterize the inflammatory response to post-impact exercise white milk consumption in young, healthy females. There were no differences in the absolute concentrations of IL-6, TNF-α, and IL-1β between trials, suggesting no influence of white milk on the absolute concentrations of pro-inflammatory cytokines compared with CHO. We observed divergent responses between 24 and 48 h for the anti-inflammatory cytokine, IL-10, between the MILK and CHO trials. Further research should be conducted to investigate these different responses, with additional blood sample collection timepoints between 75 min and 24 h as well as between 24 and 48 h post-exercise. We also observed a possible benefit of MILK compared to CHO on attenuating the relative change in inflammatory indices at 48 h post-exercise, but there were no significant differences in the net AUC for relative change between trials for any cytokine. It should be noted that due to the COVID-19 pandemic and the cessation of human research at York University, our study was likely underpowered to properly assess potential differences in inflammatory markers, nonetheless, several statistical trends were apparent which warrant further exploration. Thus, additional research investigating various aspects of the inflammatory response as well as measures of muscle soreness and/or performance/strength following post-exercise white milk consumption is needed.

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Appendices

Appendix A: Approved Recruitment Poster

Cre-Ex Study

Are you a female between the ages of 18-30 years with limited weightlifting experience?

Help us determine if dairy, creatine and exercise influences bone and inflammation

- **Up to 3 TRIALS; 3 VISITS PER TRIAL**
- **10-14 TOTAL HOURS**
- **SUPPLEMENT INTAKE (CREATINE+MILK or MILK or CARBOHYDRATE) + EXERCISE**
- **BODY COMPOSITION DETERMINATION**
- **BLOOD SAMPLES**
- **$20 COMPENSATION PER TRIAL**

Contact us at: cre.exstudy@gmail.com or by phone (416) 736-2100 x20222

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

*should prospective participants choose to interact with this post on Facebook, their privacy and confidentiality may be compromised
Approved by the York University Research Ethics Board (ORE) File# (2019-045)

Appendix B: Informed Consent Form

Informed Consent Form

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

Study Name: Cre-Ex Study: Does milk and/or creatine augment the acute effect of exercise and dairy on bone turnover and inflammation?

Researcher name:

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Dr. Lauren Skelly	KAHS, York University		skellyle@yorku.ca

Purpose of the Research:
The purpose of this study is to determine whether dairy in combination with creatine supplementation will positively impact loading exercise-induced bone cell activity in healthy young females more than dairy and exercise or exercise alone. In addition, this study will also determine if consumption of milk post-exercise influences the acute inflammatory response to exercise.

Study Intervention
As a participant in this study you will be asked to complete up to 3 different acute exercise and nutritional supplement trials. Each trial will be assigned to you in random order. The three trials are: 1) exercise+carbohydrate (CHO), 2) exercise+milk (Milk), and 3) exercise+milk+creatine (Cre). Below you will find instructions for completing each supplement trial. The whole study will take a maximum of 8-12 weeks to complete as each supplement trial will be separated by 2-4 weeks. During this time, you will be asked to maintain your habitual diet and to refrain from taking any vitamin/mineral supplements, calcium-fortified products or protein supplements. Following the exercise bout, you will also be asked to refrain from any additional exercise until after the last blood draw at 48 hours.

Instructions for Each Trial:

1) **Exercise and Carbohydrate (CHO) group.** Carbohydrate will be consumed as a beverage. Calorie-free flavour can be added if desired (CHO+water and flavouring).
   Step 1: You will be asked to arrive at the lab (BC 121) at York University after an overnight fast and rate your baseline muscle soreness using a muscle soreness scale and a baseline blood sample (max 20ml).
   Step 2: You will then be asked complete a supervised resistance and plyometric (jumping) exercise bout.
   Step 3: Immediately after exercise you will be asked to consume a carbohydrate beverage.
   Step 4: You will then be asked to complete a blood sample 5 minutes after exercise (max 20ml) and another blood sample 1 hour after exercise (max 20ml).

Step 5: You will then be asked to consume an additional carbohydrate beverage 1 hour after the exercise bout.
Step 6: You will be asked to rate your muscle soreness using a muscle soreness scale before the two post-exercise blood samples (in step 4).
Step 7: You will be asked return to the lab (BC 121) at York University after an overnight fast, 24 hours and 48 hours later to complete the last 2 blood samples (max 20ml each) and rate your muscle soreness using a muscle soreness scale

2) Exercise and Milk (Milk) group
*****Follow the same instructions above but skim milk will replace the carbohydrate beverage for this trial group.

3) Exercise, Milk and Creatine (Cre) group
Step 1: You will be asked to consume creatine powder for 6 days prior to your exercise trial. This is the "loading phase", which is necessary to possibly see an appropriate effect of creatine on bone. You will be asked to consume creatine, 5g 4 times a day: at breakfast, lunch, a mid-afternoon snack and dinner during the loading phase. You will be provided with all of the creatine in a Ziploc freezer bag with a 5-gram scoop for portioning. On the 7th day, you will be asked to come to the lab (BC 121) at York University for the exercise trial.
Step 2: You will be asked to arrive at the lab (BC 121) at York University after an overnight fast and a baseline blood sample (max 20ml).
Step 3: You will then take a dose of creatine (5g) and complete a supervised resistance and plyometric (jumping) exercise bout.
Step 4: Immediately after exercise, you will consume skim milk plus 5g creatine.
Step 5: You will then complete a blood sample 5 minutes after exercise (max 20ml) and another blood sample 1 hour after exercise (max 20ml).
Step 6: You will then be asked to consume skim milk again at 1 hour after the exercise bout.
Step 8: You will be asked to consume a maintenance dose of creatine (5g) with your evening meal on that day and the next day.
Step 9: You will be asked to return to the lab (BC 121) at York University after an overnight fast, 24 hours and 48 hours later to complete the last 2 blood samples (max 20ml each).

Some participants will be asked to only complete the carbohydrate and milk trials. Not the creatine trial.

What is Creatine?
Creatine is a molecule that is naturally made and is found within your body. It aids in energy production. When taken as a supplement, it can provide additional benefits to muscle and potentially bone tissue. Creatine supplementation has been shown to increase muscle strength making it a popular supplement for those looking to increase muscle function and performance. Creatine has been extensively researched and is safe for your consumption. If you have any further questions about creatine, more information can be provided upon request.

Acute Exercise Bout
As part of each acute supplement trial, you will be asked to perform one bout of exercise. Exercise will take place in the morning hours between 8 and 11am at York University either in the Tait McKenzie Center, or another lab. The bout will consist of resistance exercise (e.g. bench press, lat pulldown, squats, leg press) combined with plyometric (jumping) exercise (e.g. box jumps, drop-box jumps, frog jumps). Each session will last for approximately 60 minutes. Each exercise session will be facilitated by a competent personal trainer and/or a trained kinesiology student who will ensure safety and correct technique. Please ensure you are properly dressed for the exercise sessions. This includes running shoes, shorts/jogging pants and a t-shirt/tank-top. You may also bring a water bottle. We ask that you maintain your current pre-study physical activity patterns during the study and refrain from starting any new forms of physical activity until

the study is complete. Due to the nature of the exercise bout, muscle soreness is likely to occur the evening and day or two following. Researchers will contact you the first and/or second day after each exercise bout to ensure there are no issues or concerns.

Description of Testing Procedures

1. Questionnaires

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

2. Familiarization Session

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

3. Food diaries

You will be asked to complete a 3-day food diary for each trial. The 3 days include the day before the exercise bout, the day of the exercise bout and the day after the exercise bout. For one of the trials (exercise+milk+creatine (Cre), a food diary will be kept for the whole loading phase as well as the 3 days surrounding the exercise bout. We ask that you try to consume a similar diet/eating pattern during this time for all supplement trials. This will allow us to accurately assess your current nutrient intake. We ask that these diaries be filled out truthfully and with as much detail as possible. We encourage any questions you may have about these diaries and we will go over how to fill them out with you prior to you filling out your own.

4. Blood collection

We would like to collect blood from your arm (front part of elbow using a standard technique, and taken by a trained professional) 5 times per supplement trial. During each supplement trial, a maximum of 20ml of blood will be taken: upon arrival to the lab, 5 minutes post-exercise, 1 hour post-exercise, 24 hours post-exercise and 48 hours post-exercise. This is a total of 100ml of blood (over 3 days) for each supplement trial. We ask that you arrive to our laboratory (BC 121) 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 to your first blood sample and until after the last blood draw at 48 hours. We will use your blood to measure different bone markers that reflect activity inside the bone and/or inflammatory markers that are related to muscle soreness. Once blood is collected, it will be transferred to special tubes. These tubes will then be stored in a freezer for later analysis. **Note**: You may also leave the lab to use the washoom or grab water briefly during the hour between the 5 minutes post-exercise and 1 hour post-exercise blood draws.

5. Anthopometry and Body Composition

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

What You Will Be Asked to Do in the Research:

Maximal Time Commitment

Treatment Number	**Acute trial 1**	**Acute Trial 2**	**Acute trial 3**
Initial meet, signing of consent form, questionnaires	(60 mins)		
Familiarization Session (Exercise Intro, body comp, 1rm)	(60 mins)		
Day 1 - Exercise (60-90 mins), Blood Sample (90 mins)	(180 mins)	(180 mins)	(180 mins)
Day 2 - 24 Hour Blood Sample	(15 mins)	(15 mins)	(15 mins)
Day 3 - 48 Hour Blood Sample	(15 mins)	(15 mins)	(15 mins)
Food Diary	(30 mins)	(30 mins)	(30 mins)
Total Per acute trial:	**6 hs**	**4 hs**	**4 hs**

Estimate of total time commitment in hours to complete initial familiarization session and all 3 acute trials: ~ 14 hours. If you are asked to complete only 2 trials, your time commitment will be 10 hours.

Upon completion of all trials or withdrawal, you will be compensated $20 for each completed trial.

Risks and Discomforts:
There is little direct risk to you, except for the potential increased risk of exposure to COVID19, although this risk is low. Please see below for additional details. You may experience some uneasiness or anxiety due to the personal nature of the questions asked on the questionnaires. We assure you that this is nothing to be anxious about and we will do our best to minimize these feelings. The only foreseeable physical risks involved in participation include a) potential muscle soreness/discomfort after completing the exercise bout which may take 2-3 days to subside. This is completely normal; b) you may experience slight pain, and/or tingling, and/or bruising in the area after the blood samples. In some instances, you may unexpectedly feel faint or lose consciousness if looking at the needle or blood. It is also possible that a hematoma (bruise) could form under the skin beside the puncture site. With this in mind, 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. c) know that there is a potential to have stomach pain, nausea and/or cramping if large amounts of creatine are taken at once, however; this risk is minimal if you follow the creatine loading directions, we provide you. d) potential increased risk of exposure to COVID19.

Specific COVID19-related risk: As this research requires face-to-face interaction between human participants and researchers, there is a risk of contracting COVID19. As a research team at our institution (York University) we will be following strict health and safety guidelines to minimize the potential exposure of COVID19 and expect all research participants to follow public health directives. All lab surfaces will be cleaned frequently and after each use. Researchers and participants will be required to use personal protective equipment, such as face coverings, and physical distancing of 2 meters will be maintained unless not possible. You will be provided with your own disposable face covering and hand sanitizer while in the lab with us.

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

Voluntary Participation and Withdrawal: Your participation in the study is completely voluntary and you may choose to stop participating at any time. Your decision not to volunteer, to stop participating, or to refuse to answer particular questions will not influence the nature of the ongoing relationship you may have with the researchers or study staff, or the nature of your

relationship with York University either now, or in the future. If you decide to stop participating, you may withdraw without penalty, financial or otherwise, and you will still receive the promised inducement. In the event you withdraw from the study, all associated data collected will be immediately destroyed wherever possible. Should you wish to withdraw after the study, you will have the option to also withdraw your data up until the analysis is complete.

Confidentiality:
Once you begin the study, you will be given a personal participant identification number. All research related documents will only use only this ID number to identify you and your data, not your name. This way, those who may be analyzing your online/computer data will not be able to link it to you. However, we will be collecting your personal contact information for scheduling purposes during the trial. Also, considering the issues with COVID19, we must retain your contact information for contact tracing purposes, so we can let you and Public Health know if you may have been exposed to COVID19 at our research site. Contact information will be kept separate from data collected though the research study to allow for de-identification of the research data. You maintain your right to withdraw from the study at any time, including research data (if applicable). If you do withdraw, we will continue to retain your contact information but will only give it to Public Health if required for contact tracing purposes. As such, we cannot guarantee anonymity as your personal contact information identifies you as a participant in our study. However, your personal information will not be linked to your data. The identification key and all information collected about you during this study will remain confidential and will be stored in locked offices and on secured computers to which only the principal investigator, co-investigator and student investigator will have access to. Unless you choose otherwise, all information you supply during the research will be held in confidence and unless you specifically indicate your consent, your name will not appear in any report or publication of the research. Results of this study will be made available to scientists, though publication in scientific journals, but your name and personal data will not appear on its own. We will only report group information. Electronic and paper information will be kept safely for 5 years after publication (April 2025), at which time, all information will be destroyed. In addition, you will have access to your own results, as well as results from the group when our analysis is completed and available. Please let us know if you would be interested in receiving the results. Confidentiality will be provided to the fullest extent possible by law. The data collected in this research project may be used – in an anonymized form - by members of the research team in subsequent research investigations exploring similar lines of inquiry. Such projects will still undergo ethics review by the HPRC, our institutional REB. Any secondary use of anonymized data by the research team will be treated with the same degree of confidentiality and anonymity as in the original research project.

COVID-19 Specific Consent Information
All research activities will take place at York University campus, under the jurisdiction of Toronto Public Health. We are taking all safety precautions to reduce the risk of spread of COVID19 and expect all research participants 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 COVID19 effects (e.g., senior, immuno-compromised), 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.

Because 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:

- Screening – as per requirements for persons coming onto campus.
- Take appropriate precautions (e.g. face covering / cloth mask) if taking public transportation and entering public indoor spaces.
- Wash your hands upon coming onto campus / entrance to building. Hand sanitizer will be made available to you.

- Physical distancing will be maintained, at all times, and if not possible wear a face covering / cloth mask. Otherwise we will provide you with PPE.

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

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

Legal Rights and Signatures:

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

Signature _____ **Date** _____
Participant

Signature _____ **Date** _____
Principal Investigator

Appendix C: Health and Screening Questionnaire

Screening Questionnaire

Name: _____

Potential ID #: _____ Date: _____

D.O.B: _____

Telephone #:_____

e-mail address: _____

Best way to contact you? _____

ID#: _____ Date:_____

Age:_____ (**must be 18 – 30 years**)

Height: _____ cm

Weight:_____ kg

Calculate BMI (kg/m^2):_____

BMI (**must be Normal BMI**): _____

Underweight	16	18.5
Normal (healthy weight)	18.5	25
Overweight	25	30
Obese	30	+

Are you on any form of medicinal birth control? **YES**/NO
Name of medication: _____

Exercise frequency:
How many times per week do you perform structured exercise?

_____
(**[~ < twice/week] to be eligible**)

How often do you engage in any weight-lifting or other types of training with the goal to build muscle or increase physical performance (i.e. plyometric training) long enough to work up a sweat (heart beats rapidly)? Circle the one that applies:

OFTEN SOMETIMES RARELY NEVER

Protein supplements consumption:
Do you consume protein supplement products? YES/**NO**

If YES, please specify the types, amount and frequency (servings per day or average per week):
_____
___
_____
___

Do you consume Calcium and/or Vitamin D supplements and/or multivitamins? YES/**NO**

If YES, amount and frequency:
_____

Does the potential subject meet inclusion criteria thus far? YES/NO.

If YES → Continue with the questions.
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 reason is because they consume too much supplementary protein (within reason) or a multivitamin/vitamin D/calcium supplement, ask them if they would be willing to not consume these things. If yes, then they can be eligible, BUT they need at least a 2-week washout before we start any pre-measurements.**

Additional Screening Questions:

Are you lactose intolerant (diagnosed by doctor)? YES/**NO**

Do you believe that you may be lactose-intolerant or lactose-sensitive? YES/**NO**

Do you have an allergy to dairy/milk protein? YES/**NO**

Do you have any other food allergies? YES/**NO**

How often do you currently consume Dairy Products?

Never Once/week Two-three/ week Four-or-more/week

Have you supplemented with creatine previously? YES/**NO**

Are you a non-smoker? **YES**/NO

Do you consume alcohol on a regular basis (at least every couple of days)? YES/NO

Have you ever had any bone, joint or muscle injury (ligament sprains, muscle strains or bone fractures)? YES/**NO**

If YES, when/how did this occur? _____

If YES, how was this treated (i.e. surgery, physiotherapy?)

_____

If YES, does this currently affect your physical ability (i.e. any limitations)?

_____

Have you ever had any major joint instability or ongoing chronic pain (such as) in the knee, back, elbow or ankle? YES/**NO**

If YES, when/how did this occur? _____

If YES, how was this treated (i.e. surgery, physiotherapy?)

_____

If YES, does this currently affect your physical ability (i.e. any limitations)?

_____

Do you have arthitis or any spinal conditions? YES/**NO**

If YES, when/how did this occur? _____

If YES, how was this treated (i.e. surgery, physiotherapy?)

_____

If YES, does this currently affect your physical ability (i.e. any limitations)?
_____

Other Medical Conditions:

Heart disease/condition	YES/**NO**
Gastrointestinal 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**

Do you take any prescription medication (not birth control)? YES/NO

Name and reason: _____
(Anything prescribed by a doctor, micronutrients are ok)

Do you take any medication that may affect Bone or Muscle? YES/NO
_____
Such as: cortisone, prednisone, Prozac.

Do you take any over-the-counter medications/supplements/vitamins (not calcium, vitamin D and/or multivitamin)? YES/NO

Name and reason: _____

Do you fear confined spaces or have claustrophobia? YES/NO

Eligible: YES/NO/consult with coordinator

If this potential subject may be ineligible, please consult with a study coordinator before determining final eligibility and before booking an in-person screening visit.

If NO, give reason: _____

If YES, date: _____

Additional Comments Below:

Appendix D: Permission to use BioRender Figures

Publishing a Thesis

Where your thesis will be published has a direct relationship to the account type you need when exporting your figures!

Written by Shane Williams
Updated over a week ago

If your thesis is going to be uploaded to a University database or library we do not consider this as a published thesis. Therefore, you can definitely use our free basic version for this.

If your thesis will be published in an article or journal (open access included), we do consider this a published and recommend upgrading to our paid premium subscriptions to obtain the publishing license.

Here is a breakdown of where you can use your figures based on the plans: