Influence of timing of milk consumption coupled with endurance training on body composition and endurance training adaptations in humans.

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Under the supervision of Drs. Brian D. Roy and Mike Plyley

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Abstract:
Consumption of low-fat milk (LFM) after resistance training has been shown to have positive influences on body composition and training adaptations; however, little research has examined the effects of LFM consumption following endurance training. The purpose of the study was to look at the effects of combining additional servings of LFM following endurance exercise on body composition, bone health, and training adaptations. 40 healthy males were recruited. Individuals were randomized into 4 groups – DEI (750mL LFM immediately post exercise), DEA (750mL LFM 4 hrs prior to or 6 hrs post exercise), CEI (750mL carbohydrate beverage immediately post-exercise), and CEA (750mL carbohydrate beverage immediately post-exercise). Participants took part in a 12-week endurance training intervention (1 h/day, 3 d/wk, ~60% max HR). 22 participants completed the study. Analysis showed significant increases in lean mass, spinal bone mineral content, relative VO2peak, and a decrease in Trap 5β across all groups (p < 0.05).
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>μU</td>
<td>Microunit(s)</td>
</tr>
<tr>
<td>25-(OH)D3</td>
<td>25-hydroxyvitamin D3</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin Converting Enzyme</td>
</tr>
<tr>
<td>ACSM</td>
<td>American Collage of Sports Medicine</td>
</tr>
<tr>
<td>ADP</td>
<td>Air-Displacement Plethsmography</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis Of Variance</td>
</tr>
<tr>
<td>BCAA</td>
<td>Branched-Chain Amino Acids</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone Mineral Content</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone Mineral Density</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BW</td>
<td>Body Weight</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>Calcium</td>
</tr>
<tr>
<td>CEA</td>
<td>Carbohydrate-Exercise-Alternate</td>
</tr>
<tr>
<td>CEI</td>
<td>Carbohydrate-Exercise-Immediate</td>
</tr>
<tr>
<td>CHO</td>
<td>Carbohydrate</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon Dioxide</td>
</tr>
<tr>
<td>COX-4</td>
<td>Cytchrome C Oxidase</td>
</tr>
<tr>
<td>CPR</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>CS</td>
<td>Citrate Synthesis</td>
</tr>
<tr>
<td>CSEP</td>
<td>Canadian Society of Exercise Physiology</td>
</tr>
<tr>
<td>DEA</td>
<td>Dairy-Exercise-Alternate</td>
</tr>
<tr>
<td>DEI</td>
<td>Dairy-Exercise-Immediate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual X-ray Absorptiometry</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FGF-23</td>
<td>Fibroblast Growth Factor 23</td>
</tr>
<tr>
<td>FFM</td>
<td>Fat Free Mass</td>
</tr>
<tr>
<td>FM</td>
<td>Fat Mass</td>
</tr>
<tr>
<td>g</td>
<td>Gram(s)</td>
</tr>
<tr>
<td>HD</td>
<td>High Dairy</td>
</tr>
<tr>
<td>HDL</td>
<td>High Density Lipoprotein</td>
</tr>
<tr>
<td>HR</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>h</td>
<td>Hour(s)</td>
</tr>
<tr>
<td>HRmax</td>
<td>Maximal Heart Rate</td>
</tr>
<tr>
<td>IL-1</td>
<td>Interleukin-1</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>IU</td>
<td>International Unit(s)</td>
</tr>
<tr>
<td>Kcal</td>
<td>Kilocalorie(s)</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram(s)</td>
</tr>
<tr>
<td>kJ</td>
<td>Kilojoule(s)</td>
</tr>
<tr>
<td>L</td>
<td>Litre(s)</td>
</tr>
<tr>
<td>LDL</td>
<td>Low Density Lipoprotein</td>
</tr>
<tr>
<td>LFM</td>
<td>Low Fat Milk</td>
</tr>
<tr>
<td>LM</td>
<td>Lean Mass</td>
</tr>
<tr>
<td>m</td>
<td>Meter(s)</td>
</tr>
<tr>
<td>min</td>
<td>Minute(s)</td>
</tr>
</tbody>
</table>
mg  Milligram(s)
ml  Millilitre(s)
mmol Milimole(s)
ng  Nanogram(s)
nmol Nanomole(s)
NWCR National Weight Control Registry
O₂  Oxygen
OPG Osteoprotegerin
PA  Physical Activity
PAR-Q Physical Activity Readiness Questionnaire
pg  Picogram(s)
PRO Protein
RAAS Rennin-Angiotensin-Aldosterone System
RANK Receptor Activator of Nuclear Factor Kappa-B
RANKL Receptor Activator of Nuclear Factor Kappa-B Ligand
RER Respiratory Exchange Ratio
rpm Revolutions Per Minute
TGR Triglycerides
TNF-α Tumor Necrosis Factor-Alpha
TRAP-5β Tartrate-Resistant Acid Phosphatase-5beta
W  Watt(s)
wk  Week(s)
y  Year(s)
Chapter 1

1.1 Introduction:

Obesity is an epidemic of modern society. Within Canada and across North America the majority of the population regardless of age, gender, or ethnicity is challenged with maintaining a healthy body weight. Obesity results from a caloric intake in excess of energy expenditure over a prolonged period of time, however; additional environmental, social, and genetic factors also have a role (1). An individual is considered to be overweight if their body mass index (BMI) falls between 25-29 kg/m², while an individual is considered obese when their BMI is ≥30 kg/m² (where BMI = body weight/height², in kg/m²) (2). The 2004 Canadian Community Health Survey reported 59% of the adult population, within Canada, to be overweight with 25% being obese (1). The rapid rise of obesity in recent years has taken an economic toll in many countries. In Canada alone, the annual economic impact of the overweight population is 2 billion dollars while the cost of obesity is estimated to be 4 billion dollars (2). This amounted to approximately 4.1% of Canada’s total health care expenditure in 2006, while the indirect costs totaled approximately $5 billion. Furthermore, the healthcare costs of obese individuals averages 18% higher for women and 15% higher for men when compared to normal weight individuals. These increased costs are based on hospitalization and physician costs resulting from chronic diseases and other medical conditions associated with an unhealthy body weight (2).

In addition to the direct economic burden of obesity itself, there is an additional burden associated with the many increased risk factors and chronic diseases associated with obesity. Some of these chronic diseases include coronary vascular disease, type-2 diabetes,
hypertension, dyslipidemia, coronary artery disease, stroke, osteoarthritis, and several types of cancer (1, 3-5). To put this into perspective, ~64% of the normal weight population reports having 1 medical condition while 21% report having 3 or more. Moreover, individuals who are obese are 2 times more likely to have 3 or more medical conditions (2). Obese individuals are 3-4 times more likely to have high blood pressure and diabetes, and 2 times more likely to have arthritis, rheumatism, urinary incontinence, and/or fibromyalgia (2). In addition to direct health risks, obesity is also reported to have a negative impact on overall quality of life. In a self-reported health status survey, 20% of obese individuals rated their quality of life as being fair or poor versus 10% for individuals with healthy body weight (2).

Since overweight individuals are at the great risk of becoming obese, this population represents a strategic group to target with dietary and exercise interventions that could help to reverse or attenuate the increase in body mass. Over the years, a number of lifestyle interventions, such as extreme and fad diets, workout videos and various other weight loss and exercise programs have targeted individuals who are overweight. However, a simple and effective lifestyle intervention for this population is increased daily physical activity (PA). This alone has been shown to lead to benefits in both body composition and overall health (6) as well as dramatically reduce the overall risk of mortality due to chronic diseases, including cardiovascular disease and cancer as well as others (7).

The 2006 Canadian Clinical Practice Guidelines on the Management and Prevention of Obesity in Adults and Children recommends both nutritional and PA based interventions, among others, to assist in the management and prevention of obesity (1). Their recommendations suggest an energy-reduced diet with regular PA. More specifically,
the nutritional intervention suggests a diet high in protein and low in fats, with the option of meal replacements for energy reduction. The exercise intervention specifies that long-term regular PA is important, with a gradual increase in duration for maximum benefit. The recommendations include 30 minutes per day, increasing to 60 minutes per day of PA. The specific recommendations for the American College of Sports Medicine (ACSM) and the Canadian Society of Exercise Physiology (CSEP) can be seen in the table below as compared to the intervention proposed by the current study.

**Table 1: Physical Activity Recommendations**

<table>
<thead>
<tr>
<th>Type</th>
<th>Time &amp; Intensity</th>
<th>ACSM</th>
<th>CSEP</th>
<th>Proposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic</td>
<td>Time</td>
<td>30 min/d; 5 d/wk (150 min)</td>
<td>25 min/d; 3 d/wk (75 min)</td>
<td>total 150 min/wk</td>
</tr>
<tr>
<td></td>
<td>Intensity</td>
<td>moderate</td>
<td>vigorous</td>
<td>moderate to vigorous</td>
</tr>
<tr>
<td>Strength</td>
<td>Time</td>
<td>2x per week</td>
<td>2x per week</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Intensity</td>
<td>moderate to high</td>
<td>___</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Nutritional intake of dairy foods may also play a possible role in weight management. The health benefits of milk have been well established - milk is considered to be a good source of protein, lipids, amino acids, vitamins, and minerals, while also containing carbohydrates (lactose) (8). In addition to weight management, milk has also been shown to have a positive influence on training adaptations, or physiological changes within the body brought on by training, through the natural combination of protein and carbohydrates found in milk (8, 9). Based on this and the knowledge of the benefit of PA
on health, body composition, and weight management (10, 11), we propose that a combination of increased PA through an endurance-training program, at a moderate intensity, and an increased intake of dairy, through low fat milk (LFM), would have positive health benefits in an overweight/inactive population. This is important as positive results could contribute to a realistic and affordable lifestyle intervention benefiting not only those at risk of becoming obese, but also the general population.

1.2 Body Composition

Body composition is the measurement of the relative proportions of fat and fat-free mass within the body (12, 13). More specifically, fat mass (FM) is a measure of the amount of fatty tissue, both essential and non-essential fats, within the body, while fat-free mass (FFM) is a measure of non-fatty tissue within the body, such as organs, bone, and muscle tissues (12). Non-essential fat found in the body is typically adipose tissue, while essential fat is the minimum amount of fat required for normal physiological function. Body composition has been shown to have an association with morbidity and mortality and is often used as an assessment of overall health and wellness (14-18).

1.2.1 Measurement of Body Composition

Current predictions estimate that the prevalence of obesity will double worldwide over the next 30 years (19). Increases in the prevalence of obesity can be attributed in part to the rapid increases in economic and industrialization across the world. This has led and continues to lead to changes in diet, including surpluses and easy access to subsidized foods, as well as an increasingly sedentary lifestyle worldwide (20). With the growing number of weight loss practices and techniques being designed to address this epidemic, accurate
measurements of body composition are critical for assessment and monitoring of individuals during interventions (21). While body mass (BM) and body mass index (BMI) measurements are useful for initial measurement and general assessment, they do not take into account individual variations, such as fat mass versus fat-free mass and the location of fat, or bone mineral density and content.

Historically, a variety of different methods have been used to estimate body composition - hydrostatic weighing, air displacement plethysmography, bioelectrical impedance analysis, and anthropometric measures such as sum of skin folds, girth measurements, and BMI. Traditionally, hydrostatic weighing has been considered as the gold standard of assessing body composition as it is based on densitometric methods (22). Hydrostatic weighing is based on the Archimedes principal to determine body volume and is done by submerging the subject in water while exhaling forcefully and completely. From this, the total body volume is determined by measuring the difference between the subject’s weight submerged in and out of water, which is then used to determine body density (22).

Though relatively accurate, there are several limitations of this method, including the time it takes to complete the measurement, the discomfort to the individual being tested, the difficulty that most individuals have in eliminating air in the lungs down to residual volume, and the inaccessibility for individuals of special populations. Furthermore, the method also relies on an accurate determination of residual volume of air in the lungs and airways. Lastly this method determines body density, so measures of body composition must be calculated based on predictive equations that convert the measure of body density to a value of body fat (21).
An example of air-displacement plethsmography (ADP) commonly used is the BodPod. Body density is measured through ADP by placing the individual into a chamber with a fixed air volume. The body volume is measured by calculating the air displaced when the subject is in the chamber. Body composition again is estimated by using prediction equations (22).

Bioelectrical impedance analysis is an easy, safe, non-invasive, and convenient way to estimate FFM and FM (23). Electrical currents are passed through the body between electrodes to estimate body composition. The resistance of the current differs depending on the amount of fat and lean tissue. The level of impedance is an indication of water and electrolyte composition in the body. From the data acquired using regression equations, lean tissue and body water volume as well as FFM and FM can be estimated (22).

Anthropometric measures of body composition are relatively inexpensive, non-invasive and convenient; however, the person conducting the measurements must be well trained and experienced. These measures include BMI, waste-to-hip ratio, circumference measurements, and skinfold analysis. As stated earlier, BMI is a measure of body weight relative to height^2. Though BMI is a good indicator of body composition among the general population, it does not take into account differences in different types of tissue, such as muscle versus fat. While BMI can be a good measure for general assessment one must be careful in how it is used as it is more a superficial measure, looking at the big picture, rather than separating the individual pieces of the puzzle (such as FM, FFM, water content, etc.). Because of this individuals can be incorrectly categorized, for example if you have two people of the same height and weight where one is a body builder and the other is overweight, both would be categorized as overweight through BMI regardless of the vast
differences in body composition. Additionally, waist circumference and waste to hip ratio measurements can be useful as a rapid and cost effective estimate of body composition analysis for unfit populations. However, such measures do not decipher between FFM and FM making it difficult to get a true understanding of body composition and track changes in body composition over time.

Skinfold measurements are a very common and relatively accurate alternative to more expensive and invasive means of body composition analysis. There are 7 commonly used skinfold sites that are used to assess body composition; they include the abdomen, triceps, chest, midaxillary, subscapular, suprailiac, and thigh.

Although all of the above methods are used as valid ways to analyze body composition, dual-energy x-ray absorptiometry (DXA) has more recently been considered as the gold standard for body composition analysis measurement (24) due to its ease of use in clinical settings, as well as its capacity to measure both whole body and regional measures of body mass and ability to quantify lean, fat, and bone mass (as well as bone mineral density, (BMD)) (25).

DXA was developed for use in analyzing body composition at a level sensitive enough to detect moderate changes in body composition with changes in body mass (26). The precision of DXA is 1-2% for BMD scans and 2-6% for body composition scans, thus allowing for the detection of changes in both body composition and BMD over time (26). The use of a 3-compartment model for analysis allows for whole body and/or regional measurement of fat, lean and bone mass (bone mineral content, BMC) (21, 26). Measurement of the lean mass includes proteins, glycogen, minerals, and water, including the water and organic minerals of bone (26). Measurement of fat, lean and bone mass are
measured directly and reported in grams, while derived measurements include BMD that is expressed per area measured (26). Moreover, results from regional body composition analysis can be expressed as a function of the tissue area measured or as a function of body weight.

Due to the accuracy and ease of use, DXA has quickly become the gold standard method of measuring body composition, as supported by Fisard et al.’s reported coefficient of variation of several measurements obtained through DXA (BM: 0.25%, LM: 1.0%, FM: 1.6%, % fat: 1.5%, and BMC: 1.0%) (21). Furthermore, the amount of radiation exposure to the participants that is received from a whole body DXA scan (~75 μSv) is relatively low when compared to annual doses from natural radiation (2400 μSv), as well as other x-ray techniques such as, dental (60.0 μSv), chest (50 μSv), and lumbar and lateral spine x-ray (820 μSv) (26). DXA is routinely used to monitor changes in body composition due to lifestyle interventions, such as weight loss programs.

1.2.2 Fat Mass

The most commonly reported measurements of body fat are FM, FFM, and body fat percentage. FM refers to the portion of the body comprised of adipose tissue versus FFM, which is the portion of the body tissue free of adipose tissue or fatty cells. In looking at the “Load Capacity Model” proposed by Dulloo & Montani (27), adiposity contributes to the ‘metabolic load’ on the body while lean mass (or FFM), contributes to the ‘metabolic capacity’. In other words, excess adiposity is associated with negative effects on metabolic homeostasis, causing an increase in risk of cardiometabolic diseases (27). Lean mass can be associated with positive benefits, such as the maintenance of homeostasis and prevention of cardio-metabolic problems (27).
In addition to the amount of fat mass an individual has, the distribution of the FM is also an important factor to consider in risk assessment. Obesity can be classified into two subgroups, android obesity or gynoid obesity. Android obesity is characterized by fat distribution around the stomach and visceral organs, creating an “apple shape” in an individual. This is most commonly a characteristic in men, but can be seen in either sex, and is most associated with the many metabolic complications, as well as cardiovascular morbidity and mortality associated with obesity (28, 29). Gynoid obesity is characterized by a fat distribution around the hips, resulting in a “pear shape”, and is most frequently seen in women. Gynoid obesity has been shown to be not as strongly associated with metabolic complications (30).

In addition to fat distribution, obesity can be further categorized into hypertrophic obesity, which refers to an increased amount of fat in each fat cell, as well as hyperplastic obesity, which is characterized as an increase in the number of fat cells (30). Triglycerides are found in adipose tissue and are considered to be one of the major fuel reserves in the body. A lean adult will have up to 35 billion adipocytes, each containing ~0.4-0.6 μg of triglycerides, totaling about 130,000 kcals of stored energy. An obese individual can have up to 4 times as many adipocytes (~140 billion), each containing as many as 2 times the lipids in each cell (~0.8-1.2 μg triglyceride), totaling roughly 1 million kcals of stored energy (31). In terms of weight management, when an individual restricts his or her diet, the size of a fat cell can be reduced, but the number of fat cells is not reduced. Only through surgical liposuction is the overall number of fat cells reduced (30). The type of obesity in terms of cell number and size has been correlated with age of onset of obesity (30). This
can be important in establishing appropriate interventions for individuals targeting weight loss and control, and in the prevention of metabolic diseases later in life.

1.2.3 Lean Mass (Muscle Mass)

In contrast to FM, lean mass (LM) includes all other tissue types except fat. The various components that contribute to lean mass include: muscle mass, bone mass, as well as organs, and body water content. Many of these different components have been shown to be inter-dependent. For example, body weight has been correlated with bone mass density (BMD) (32). Similarly muscle mass and/or LM has also been associated with bone mass (33).

Muscle tissue is an important functional tissue within the human body. There are 3 types of muscle tissue: skeletal, smooth, and cardiac. For the purposes of this review, we will be focusing on skeletal muscle. The human body contains 400 skeletal muscles, which combined make up ~40-50% of total body weight (34, 35). The most important function of skeletal muscle is to allow the body to move freely and breathe. The 3 main functions of skeletal muscle include force generation for locomotion, force generation for postural support, and heat production during periods of cold stress (35). Skeletal muscle made up of muscle fibers (muscle cells), which can be separated into 2 main categories; slow twitch and fast twitch fibers. The 3 main types of muscle fibers include: type I, type IIa, and type IIb. Type I muscle fibers are also known as slow twitch or slow oxidative, they are metabolically more efficient and generate relatively lower amounts of force, but are more fatigue resistant. While Type IIa fibers are considered hybrid fibers with characteristics of both type I and II fibers. Generally, type II fibers are more glycolytic and powerful in nature, with type IIb being the most glycolytic and IIa the least glycolytic. Collectively, they work
to produce force, but they will adapt to the demands placed on them and transition to meet the needs imposed.

### 1.2.4 Bone Mass

Bones within the human skeleton can be classified into axial (skull, vertebral column, and rib cage) and appendicular (upper and lower limbs). Bone can also be classified by shape, long, short, flat, and irregular, as well as structure (spongy and compact). Bones serve essential functions including support, protection of internal organs, movement of the body, blood cell formation within the bone marrow inside the bone, and mineral and growth factor storage. For this review, we focus on the mineral component of bone and the production and storage of growth factors in bone. Bone serves as primary storage for minerals, such as calcium and phosphate, and is responsible for release of minerals into the bloodstream for distribution throughout the body. Besides mineral storage, bone is also important for production and storage of growth factors, such as insulin like growth factors, transforming growth factor, bone morphogenic proteins, as well as many others (36).

There are many important factors to consider when assessing bone health. Bone health is dependent on an adequate nutritional intake of calcium and other nutrients, as well as regular physical activity. A common clinical assessment of bone health is the measurement of bone mineral content (BMC) and bone mineral density (BMD) using DXA (37). BMC refers to the mineral content and is measured in grams. BMD is measured in grams per centimeter squared (g/cm²) (37). BMD is used to assess bone health and an individual’s risk of osteoporosis and fragility fracture. Overall, bone mass, and in turn
BMD, is ultimately determined by the metabolic activity of osteoblasts and osteoclasts within the bone tissue (37).

Because changes in BMD and BMC occur over long periods of time (1 year or longer), biochemical markers of bone cell activity, and thus bone turnover, can be measured to assess changes in bone metabolism over shorter periods of time. Blood and urine markers of bone turnover are used to acutely investigate bone health. Numerous markers in plasma can be assessed to provide insight into the overall metabolic changes in bone health. Some of these markers are osteocalcin, 25-hydroxyvitamin D (25[OH]D), OPG, RANK-L, FGF-23, TRAP-5β. Generally, bone markers can be classified into two categories, those that are associated with bone formation, and those that are associated with bone resorption. Osteocalcin is considered a marker of bone formation as it is produced by osteoblasts (38). Osteocalcin (a Gla protein) is the most abundant non-collagenous protein in bone, and both vitamin D and K are involved in its production (vitamin D) or post-translational processing (vitamin K). Increased concentrations of osteocalcin are thought to represent increased osteoblast activity, possibly increased bone formation. However, it is important to note that bone turnover is complex and regulation of osteoblasts and osteoclasts is interrelated (38).

Bone resorption is a process in which bone is degraded by osteoclastic activity, specifically the release of acids and proteases, and ultimately, mineral and bone matrix proteins are released into the circulation (39). Type-1 collagen is very abundant in bone and it provides a protein network that gives the bone its strength and framework. When bone is resorbed, various by-products are released into the blood. N-Telopeptides – a collagen crosslink that is formed through osteoclastic degradation of type 1 collagen is
considered a marker of the bone resorption process. Quantification of N-Telopeptides has been used in both research and clinical settings to estimate the rate of bone turnover. Tartrate-resistant acid phosphatase 5β (Trap-5β) is another marker of bone resorption, and thus higher osteoclastic activity. It is released by osteoclasts during bone resorption (40).

Some of the main cytokines that have been implicated in bone resorption include RANK, RANKL, and osteoprotegerin (OPG). RANK and RANKL are both found on the surface of osteoclasts and are critical in the activation of osteoclast precursors to an active osteoclast. In contrast, OPG inhibits the differentiation of osteoclast precursors into osteoclasts and also regulates the resorption activity of osteoclasts. The RANK, RANKL, OPG system is regulated by osteotropic hormones, which act to reduce parathyroid hormone and increase the ratio of OPG to RANKL (41). It is believed that osteoblasts are regulated primarily through the RANKL-RANK pathway, but other pathways are also likely involved. Interestingly, the ratio of RANKL to OPG has been found to be a major determinant of bone mass (41). Taking a closer look at the OPG-RANKL-RANK system, we know that OPG acts to inhibit osteoclast differentiation and activity, by interrupting signaling through sites where osteoblasts act to support osteoclastogenesis through cell-to-cell interaction, therefore playing a primary role in promoting osteoclastogenesis (42-44). Likewise, RANKL plays a key role in the stimulation of osteoclast differentiation, activity, and inhibition of osteoclast apoptosis (42, 45, 46). Thus, the OPG-RANKL-RANK system is important for modulating both the osteoblastic and osteoclastic lineage (42) and can contribute to a healthy skeleton (41).

Fibroblast growth factor 23 (FGF-23) has also been implicated in the regulation of bone metabolism. While FGF-23 is produced in many different tissues, the majority of
circulating FGF-23 is produced in osteocytes and some from osteoblasts. It appears that FGF-23 expression is regulated by vitamin D, phosphate, and possibly parathyroid hormone (47). FGF-23 may act on osteoblasts, where it may inhibit the maturation of these cells, and is also important in the maintenance of bone mineralization later in life (47). Research has shown that both an over expression, and an under expression of FGF-23 can result in impairment of bone biology (47). An excess of FGF-23 can negatively impact skeletal mineralization and this may be related to low levels of phosphorus and vitamin D (47).

Optimal vitamin D status, specifically circulating serum 25-hydroxyvitamin D (25(OH)D) concentrations, is important for bone and overall health. Supplementation of vitamin D has been shown to have a positive effect on BMD. Higher levels of serum 25(OH)D are associated with a higher BMD (48). Optimal concentrations have been shown to suppress serum parathyroid hormone (PTH), which promotes bone loss (48). Importantly, high concentrations have also been associated with a decreased risk for fracture. Optimal concentrations of 25(OH)D are important for bone health in young adults as well as the elderly. Recent research shows that the agreed upon optimal serum 25(OH)D level for bone health is between 50 and 80 nmol/l. Average vitamin D₃ intake per day must be at least 600IU to reach and maintain a 50nmol/l of serum 25(OH)D, with 800-100 IU of vitamin D₃ to reach 75 nmol/l of serum 25(OH)D (49).

1.2.4.1 Bone Health and Obesity

As previously stated, obesity is associated with an increased risk factor for several diseases. Interestingly, increased body weight and obesity has traditionally been associated with benefits to bone health (i.e., higher BMD), thought to occur due to increased
mechanical loading (50). However, more recent research suggests that excess fat accumulation is actually detrimental to bone mass. Cao provides an excellent review that supports this view - *Effects of Obesity on Bone Metabolism* (50).

Obesity is associated with chronic inflammatory responses, including abnormal cytokine production, increased acute-phase reactants, and activation of inflammatory signaling pathways, and as such, these disruptions may have adverse effects to bone health (50). Research has shown that chronic inflammation such as that caused by obesity, has negative influences on bone metabolism (50). This is believed to occur through several mechanisms, including an increased up-regulation of pro-inflammatory cytokines (TNF-alpha, IL-1, IL-6) (50-52). It is believed that through the RANKL-RANK-OPG pathway, these cytokines stimulate osteoclast activity, which, as previously stated, is associated with bone turnover (50).

Obesity may also alter bone metabolism through regulation of leptin and adiponectin (adipocyte-derived cytokines). Higher levels of leptin and lower levels of adiponectin are associated with aging (50, 53). In animal models, higher levels or overproduction of leptin can have negative effects on bone metabolism (54). Likewise, while adiponectin positively benefits bone metabolism through decreasing resorption and increasing bone mass (55), obese individuals are reported to have lower levels and this can negatively impact bone metabolism (50).

Habitual diets that are high in fat have been shown to contribute to low calcium absorption within the intestine, which in turn is detrimental to bone metabolism (50). Therefore, based on these various effects of obesity and high dietary fat intake, there are well-substantiated physiological mechanisms, which could lead to negative effects on bone
metabolism, despite the positive effects of obesity due to mechanical loading. Collectively, in individuals who are obese, it is likely that the underlying effects of obesity are actually negatively affecting bone metabolism more than the benefits of increased loading.

1.3 Physical Activity

Physical activity, exercise and physical fitness, are terms that are sometimes used interchangeably, but are actually very different by definition. Caspersen et al. (1985) provide the standard for current definitions of physical activity, fitness and exercise (56) used in similar studies today (57). Physical activity (PA) can be defined as “any bodily movement produced by skeletal muscles that result in energy expenditure” (56). While PA is a necessary part of daily living, the amount of PA is dependent on the individual and varies dramatically from person to person. Some authors have divided physical activity into categories of activity, such as sleeping, work, or leisure (58). Within these 3 categories, PA can further be divided into many sub-categories, including sport, conditioning, household tasks and others (56). Besides being categorized by activity, PA has also been categorized based on intensity of the activity: low, moderate, or heavy (56).

Though PA and exercise have been used interchangeably, there are differences. Exercise is PA, which is “planned, structured, repetitive, and purposive”, and intended for improvement and or maintenance of physical fitness (56). An example of exercise would be the use of strength and conditioning activities to enhance and maintain athletic performance.

Physical fitness on the other hand can be defined as “a set of attributes that people have or achieve”. An individual who is physically fit can be defined as having “the ability to carry out daily tasks with vigor and alertness, without undue fatigue and with ample
energy to enjoy leisure-time pursuits and to meet unforeseen emergencies” (President’s Council on Physical Fitness and Sports: Physical Fitness Research Digest, 56) Physical fitness can be subdivided into, health related fitness and athletic ability. Physical fitness is most frequently measured though cardio-respiratory analysis, body composition tests, muscular strength tests, muscular endurance and flexibility tests (56).

1.3.1 Physical Activity and Weight Management

Physical activity plays a key role in providing health benefits - enhancing general health and quality of life (59). More recently, physical inactivity has become a health-threatening aspect for much of the general population, and arguably, along with excess energy intake, is a major contributor to the obesity epidemic. As presented in a review by Jackicic (60), PA has been established as a key factor in weight loss, long-term weight maintenance, and prevention of weight gain, as well as general health related outcomes independent of weight loss (60).

Research has established that a balance between energy intake and energy expenditure is required for weight maintenance, and likewise a negative energy balance is required for weight loss (60). Most evidence shows a combination of proper diet and regular physical activity represents the most beneficial lifestyle intervention for weight loss. Although, the addition of PA alone has been shown to positively benefit short-term weight loss (61-63), results in a smaller decrease in body weight than is seen with combined diet and PA interventions, and is beneficial in reducing cardiometabolic risks (61). According to the U.S. Physical Activity Guidelines Committee Report (http://www.health.gov/PAguidelines/), weight loss from PA alone typically results in <3% or ~0.5-3 kg change in body weight. These numbers are consistent with other observations,
such as the National Institutes of Health and the National Heart, Lung, and Blood Institute (1998), and Wing et al. (64).

Physical activity has been well established to be a key factor in long-term weight loss and management (65). Research has shown that roughly 33-50% of weight lost due to dietary alterations is typical regained within the first 12-18 months of reaching target weight; however, with the addition of regular PA, weight loss is better maintained (64). Studies using data from the National Weight Control Registry (NWCR) show that a weight loss of 30lbs (maintained for at least 1 year) was strongly correlated with PA and the maintenance of weight loss. In a study by Klem et al. (66) analysis of data from the NWCR showed that individuals who participated in ~2,800 kcal/week of leisure-time activity were better able to maintain their weight loss (66). In contrast, those who were physically inactive and had lower energy expenditure were more likely to regain weight (67). Along with the initial weight loss and long-term maintenance, PA can be just as important in the prevention of weight gain and obesity (60). Thus, it is important to consider PA as a realistic and attainable lifestyle strategy that will assist with weight management and also promote overall health.

1.4 Physical Activity, Exercise, Fitness and Health

Endurance activity can be defined as sustained PA that causes a sustained elevation in heart rate. Endurance exercise has been proven to be an extremely effective form of exercise for improving overall health and wellness, including body composition and weight management. Endurance activities are sub-maximal prolonged activities, which
incorporate large muscle groups and are primarily dependent on oxidative metabolism (9). Endurance training involves repeated bouts of endurance exercise on subsequent days leading to adaptations within the body (68). There are many ways to assess the influence of endurance training on overall health and physical wellbeing - endurance capacity, performance, and training adaptations.

1.4.1 Endurance Capacity

Endurance capacity is the extent to which an individual has the potential to perform prolonged endurance exercise. There are many factors, central and peripheral, that affect the endurance capacity of an individual (69). They include thermoregulation, ability to conserve water, the individual’s ability to adapt to endurance exercise through substrate utilization, accumulation of lactic acid and reaching the lactic threshold, as well as oxygen consumption (70). At the muscular level, endurance capacity is enhanced by having a high oxidative capacity, an increased dependence on fatty acids as a substrate, increased muscle capillarization, and increased substrate utilization within the muscle fibers (69). Substrate utilization tends to vary according to muscle phenotype, meaning more oxidative muscles are likely to be more reliant on fat as a substrate, while more glycolytic muscles are more likely to rely on carbohydrate as a substrate (69). With training, endurance capacity can be increased, with the increases resulting from differing degrees of adaptation in the various factors mentioned previously.

1.4.2 VO$_2$max

One of the most commonly used ways to assess endurance capacity is through measuring VO$_2$max, or the maximum oxygen consumption of an individual, which
examines the body’s ability to deliver and utilize oxygen while working at the maximal level of activity (8, 54, 96). With chronic endurance training, an individual’s VO\(_2\)max can be increased; the amount that it can be increased varies on an individual basis. Increases in VO\(_2\)max resulting from exercise training can range from 2-50%, depending on the individual’s initial values and level of fitness (35, 71-73). The key principals of training that can be manipulated when creating a program to increase VO\(_2\)max, and in turn endurance capacity, are frequency, duration, and intensity of the program. A change in any or all of these factors can elicit a positive change in endurance capacity. General recommendations that have been described for increasing VO\(_2\)max include, engaging in endurance activity at an intensity between 50 to 85% VO\(_2\)max for between 20-60 minutes, 3-5 times per week (74). Generally, changes in VO\(_2\)max due to training are caused by improvements in either cardiac output (the delivery of blood) and/or arterio-venous oxygen difference (a-VO\(_2\) difference, the extraction and utilization of the oxygen in the blood at the tissues). More specifically, endurance training induced increases in VO\(_2\)max can be attributed to both an increase in stroke volume, influencing cardiac output, and an increase in a-VO\(_2\) difference due to changes in oxygen transport and uptake at the working muscle (35).

**1.4.3 Endurance Performance**

Endurance performance differs from endurance capacity in that endurance performance is a measure of an individual’s ability to perform endurance related exercise, and is measured as power (L/min), while an individual’s endurance capacity assesses one’s ability to perform an activity for an extended period of time, and is measured in total liters
consumed. Hence, endurance capacity is primarily related to an individual’s ability to perform at a given intensity or workload for an extended period of time without showing the signs and symptoms of fatigue.

Many different factors can contribute to fatigue resistance, and these factors vary depending on the intensity of the activity. A primary factor regarding an individual’s resistance to fatigue during endurance activities is the amount of glycogen stored in the muscles and the ability of the muscles to spare glycogen as an energy source (75). Muscle glycogen is a crucial factor in both endurance capacity as well as endurance performance. Glycogen is primarily stored in the muscles; the amount of glycogen stored in skeletal muscle can therefore influence endurance performance and endurance capacity (76). With intense endurance exercise or more prolonged endurance exercise, glycogen stores can become depleted leading to a decrease in performance and endurance capacity, respectively. With prolonged endurance exercise, fatigue can more often be attributed to glycogen depletion rather than other metabolic factors often associated with fatigue, such as high lactate concentrations or acidosis (76), which are more common with higher intensity activity.

The most practical way to assess endurance performance is through the use of a time trial. Using a time trial allows for a comparison over time and provides an opportunity to see improvements in performance. Most time trials are performed in such a way that the participant is instructed to complete a set distance or amount of work as fast as possible. This will allow for an evaluation of intensity and resistance to fatigue as the individual must pace him/her self to be able to complete the desired amount of work as quickly as possible. Jeukendrup et al. (77) compared two performance tests - one, time to exhaustion
and the other, a time trial to determine which was more reliable and reproducible. The results showed that a time trial was more reliable in terms of reproducibility, and in well-trained cyclists and tri-athletes, there did not appear to be a learning effect between tests (77). Similar results have been found by others, supporting the reliability of a time trial as the best measure of endurance performance (78-80).

1.4.4 Skeletal Muscle Adaptations

As indicated previously, the human body contains over 400 skeletal muscles, which serve many different functions (35). The primary functions of skeletal muscle include force generation for locomotion and postural support, and heat production during cold stress. Skeletal muscle fibers are classified based on structural and biochemical characteristics. In humans, skeletal muscle fibers are generally categorized into two main groups - fast-twitch and slow-twitch fibers (35, 81). On average, the ratio of fast to slow-twitch fibers within the body is fairly equal, however, the ratio can be influenced by genetics, blood levels of hormones, as well as patterns of PA (endurance vs. resistance) (82, 83). For our purposes, we will measure adaptations in markers of oxidative potential with training, and consider changes in muscle fiber type.

1.4.4.1 Training Induced Adaptations

Training can result in many different changes within skeletal muscle that ultimately result in an increase in the oxidative capacity of the muscle and a decrease in the oxidative stress, despite an increase in energy turnover. The oxidative capacity of the muscle is determined by a number of different factors, including but not limited to, the number of
mitochondria within muscle fibers and the number of capillaries surrounding the muscle fibers (35, 84). Increases in substrate turnover with training also increase the production of reactive oxygen species, and thus acutely increases markers of oxidative stress, which also lead to increases in the antioxidant systems within the fibers (35).

It has been well documented that endurance training leads to mitochondrial biogenesis (84). Furthermore, it also leads to an increase in the number of capillaries surrounding each fiber, allowing for more efficient delivery of oxygen and other nutrients to the muscle fibers during activity and recovery. Likewise, higher levels of myoglobin within skeletal muscle allows for improved oxygen delivery from the capillaries to the mitochondria (35). Several different biochemical markers of oxidative potential are routinely used to assess oxidative potential within skeletal muscle, including the activity and expression of citrate synthase (CS) and cytochrome C oxidase (COX-4) (85).

Studies have shown that large increases in the volume density of mitochondria are consistent with changes in the biochemical markers of oxidative metabolism, such as COX-4 (86). Research has also shown that skeletal muscle mitochondrial enzyme content increases with endurance training (86, 87). Increases in COX-4 activity are well accepted as indicating a change in the oxidative capacity of the muscle tissue (88). COX-4 is the final oxygen acceptor in oxidative phosphorylation, and as such, it is not surprising that with endurance exercise training, there is a positive correlation between the increase in COX and the increase in VO₂ peak per kg FFM (85). As stated previously, CS and COX-4 are indicators for oxidative potential within the muscle, and it has been well established that levels of CS and COX-4 increase with endurance exercise training. For example, Carter et al. showed an increase in CS of 41% and an increase in COX-4 of 26% with 7
weeks of endurance training (85). Similar increases have been shown in other training studies using protocols of similar length (86, 87, 89-91). These findings suggest that the amount of increase in the enzymes is directly related to not only the duration of training, but also the intensity and type of activity (86, 87, 89, 90).

1.4.4.2 Exercise Training Induced Angiogenesis

During exercise, there is a need for increased blood flow to working muscles, and an increase in cardiac output alone is not enough to meet the additional oxygen and nutrient requirements of the working muscles (92). There are two major adaptations that occur within the vascular system that are associated with exercise (84). They include an increase in flow capacity derived from an increase in the radius of large caliber vessels through vasodilation, and an increase in muscle capillary supply through angiogenesis (84). For the purpose of this review, we will focus on the role of angiogenesis. The primary function of the capillary network is to allow for exchange of gas and nutrients (such as O₂, CO₂, glucose, etc.) through diffusion between the vascular space and the muscle fibers. Angiogenesis refers to an increase in muscle capillary supply, i.e., an expansion of the capillary network to allow for an increase in blood flow and improvement in gas and nutrient exchange in the peripheral tissues of the body (84, 93). There are two primary mechanisms underlying angiogenesis: intussusception and sprouting (93). Intussusception occurs when one capillary splits into two from within the original capillary. This occurs through the formation of a divide along the luminal side of the capillary (93). The second mechanism is sprouting, which occurs when a new capillary forms from an existing one when activated endothelial cells branch out of an existing capillary. For the new capillary
to become active and functional, it must re-enter or connect to the existing capillary bed through either joining another capillary or joining a venule (93).

Research has shown that an increase in the capillary supply is associated with improvements in performance (94), as seen by an increase in the maximum oxygen consumption of muscle (93). Endurance training has been well established to result in an increase in muscle capillarization (95). Angiogenesis resulting from training is thought to result from a combination of growth/stimulatory factors (84), including hypoxia and shear stress (93). Research has shown that muscle capillarization is best expressed relative to muscle surface area (93), and that the capillary to fiber ratio varies depending on type of muscle fiber (84). Therefore, it is believed that the angiogenic responses induced from exercise training are fiber type dependent, which would suggest that signals for angiogenesis originate within contracting muscle fibers (84).

In summary, the plasticity of skeletal muscle fibers allows them to adapt in response to increases in PA. There are many structural and biochemical components of skeletal muscle that change with exercise, more specifically endurance exercise (96). As discussed, with endurance training, the oxidative capacity of a muscle has been shown to increase; this is accomplished through adaptations within the skeletal muscle leading to an increase in both mitochondrial content and muscle capillary supply (35, 84, 93). Many studies have found not only a high mitochondrial content in trained versus untrained individuals (97, 98), but also an increase in the capillary density (99, 100) and an increase in the capillary to fiber ratio as a result of training (96). These adaptations are important in allowing for improvements in substrate turnover and more efficient oxygen and nutrient delivery during
exercise training, ultimately leading to an increase oxidative and endurance capacity (84, 85).

1.4.4.3 Fiber Type Properties and Adaptations

Skeletal muscle fibers in humans are generally categorized as two main types - type II (fast-twitch) fibers and type I (slow twitch) fibers, based on their twitch characteristics. Fast-twitch fibers are also further broken down into two subgroups based on their metabolic characteristics, type IIb (fast twitch glycolytic fibers) and type IIa (fast twitch oxidative fibers) (35). Table 2 shows the properties of the different types of muscle fibers and their structural and functional characteristics. There has been significant debate as to whether training leads to a change in fiber type. While it was originally believed that there was no change in fiber type with training, subsequent research has shown that fiber type can be altered with training (35, 84, 85, 101). Changes seen in fiber type are often small and are always generally seen as fast to slow [i.e., type IIb → type IIa → type I] with endurance training (35).

Transitions in fiber type with training result from changes in the expression of contractile proteins (specifically myosin heavy chain (MHC) isoforms) and through an increase in oxidative capacity (101). As previously discussed, mitochondrial content increases with training, allowing for an increase in oxidative capacity (84, 102). Type IIb fibers rely primarily on glycolytic pathways for ATP production, while type IIa and type I fibers rely more on oxidative pathways (103). Along with the increase in mitochondrial content, it is believed that endurance training also effects MCH isoform expression as part of the shift in fiber type (101).
It is important to understand that overall, the beneficial effects of endurance training promote adaptations within skeletal muscle, which allow the muscle to become more efficient in substrate utilization and the production of ATP, which in turn, allows the muscle to be more resistant to fatigue (101). It is also possible that a better understanding of the signaling pathways associated with skeletal muscle adaptations could lead to new approaches for treating metabolic and cardiovascular diseases (101).

| Table 2: Skeletal Muscle Fiber Types: Structural and Functional Characteristics |
|-------------------------------------------------|-------------------|-------------------|-------------------|
| SLOW OXIDATIVE FIBERS (Type I)                  | FAST OXIDATIVE FIBERS (Type IIa) | FAST GLYCOLYTIC FIBERS (Type IIb) |
| METABOLIC CHARACTERISTICS                        | Slow              | Fast              | Fast              |


### Myosin ATPase activity

<table>
<thead>
<tr>
<th>Primary pathway for ATP synthesis</th>
<th>Aerobic</th>
<th>Aerobic (some anaerobic glycolysis)</th>
<th>Anaerobic glycolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myoglobin content</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Glycogen stores</td>
<td>Low</td>
<td>Intermediate</td>
<td>High</td>
</tr>
<tr>
<td>Recruitment order</td>
<td>First</td>
<td>Second</td>
<td>Third</td>
</tr>
<tr>
<td>Rate of fatigue</td>
<td>Slow (fatigue-resistant)</td>
<td>Intermediate (moderately fatigue-resistant)</td>
<td>Fast (fatigable)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ACTIVITIES BEST SUITED FOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endurance-type activities</td>
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<table>
<thead>
<tr>
<th>STRUCTURAL CHARACTERISTICS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
</tr>
<tr>
<td>Fiber diameter</td>
</tr>
<tr>
<td>Mitochondria</td>
</tr>
<tr>
<td>Capillaries</td>
</tr>
</tbody>
</table>

*Adapted from Power and Howley, 2009

### 1.5 Nutrition

Nutrition is integral for optimal health and longevity. Proper nutrition is an essential part of weight management and optimization training benefits. General dietary guidelines for individuals from the generally active to the elite athlete have been created as a basis to build a well-balanced and effective diet. Two important aspects of a dietary strategy include ensuring that energy needs are met and that the timing of ingestion of nutrients is appropriate. Research has shown that not consuming sufficient calories or consuming inadequate macronutrients can attenuate training adaptations (9, 104, 105). In addition, a
well-balanced and adequate diet consumed at the appropriate time can be key factor in enhancing training adaptations (106-114).

Energy intake and major nutrient needs for an active individual looking to maintain general fitness (exercise of 30-40 min duration, 3-4 times per week) should consume 1800-2400 kcal/day, i.e., 25-35 kcal/kg/day body weight (for a 50-80 kg individual). Depending on how active and intense an individual is training, this could increase up to 2500-8000+ kcal/day. One of the most important aspects of a diet is to insure the individual is consuming proper and adequate amounts of carbohydrate (CHO), protein (PRO), and fat. For an individual looking to attain general fitness, these amounts can typically be met though a normal diet which meets the recommended daily averages of each nutrient. To fulfill general health needs, diets should incorporate 45-55% CHO (3-5 g/kg/day), 10-15% PRO (0.8-1.0 g/kg/day), and 25-35% fat (0.5-1.5 g/kg/day) (105).

When considering CHO, the majority of dietary CHO should come from complex CHO with a low to moderate glycemic index (e.g., grains, starches, fruit, maltodextrin, etc.). Any excess CHO consumption is unnecessary when an individual is training for general fitness. Having adequate CHO in the diet is important for optimal endurance exercise performance. An intake of 7-10 g/kg over a 24-hour period should be adequate for optimal endurance performance (108). Research has shown that diets in this range of CHO intake are key in maximizing muscle glycogen content and they have been shown to improve endurance exercise performance (108, 115, 116).

An adequate intake of protein is also critical in optimizing the adaptive responses to training. The general recommendation for protein intake is 0.8-1.0 g/kg/day; however, there is evidence that some athletes require more than what is recommended for the general
population (110, 117, 118). Recommendations for endurance and power sport athletes during general training are 1.5-1.7 g/kg/day (119). Adequate intake of protein is critical to ensure a positive protein balance. Protein balance is a function of protein synthesis and protein degradation/catabolism. Not only does the amount of protein influence the responses to training, but also the types of dietary protein can differ according to source, amino acid profiles, and methods of processing or isolating the protein. These differences can influence the availability of amino acids and peptides, as well as the rate of metabolic influence of the protein. The highest quality sources of protein include light skinless chicken, fish, egg white, and skim milk (casein and whey). Fat is also an important aspect of the diet. Skeletal muscles store fat intramuscularly, in the form of triglycerides. Dietary fat helps to maintain energy balance, replenish intramuscular triglyceride stores, and provides adequate consumption of essential fatty acids. Intramuscular triglycerides are also useful as a source of energy during prolonged moderate intensity exercise (119).

In addition to a well balanced diet, it is also important to consider certain dietary nutrients to ensure adequate intake for bone health. Prince, Langford, & Liporace have reviewed the essential nutrients to ensure bone health and their availability in the typical North American diet (120). Table 3, adapted from Prince, Langford, & Liporace, provides an outline of the essential nutrients for bone health, as well as the common food sources from which they can be incorporated in the diet to ensure adequate intake, it is important to note that some recommended intakes are gender and age specific.

Table 3: Nutrients for Bone Health (Men age 19+)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>RDA Prince, Langford, &amp; Liporace</th>
<th>RDA IOM</th>
<th>RDA HC</th>
<th>Food Source</th>
</tr>
</thead>
</table>
While it is important to ensure a well balanced diet and adequate intake of macronutrients, it is not always feasible to find the time it takes to cook a full balanced meal or get all the proper nutrients immediately after a workout. However, there are whole food options, which help to provide essential and additional macronutrients. Milk is one of these proposed options, naturally fulfilling adequate requirements for both CHO and PRO post exercise. Consuming adequate macronutrients immediately post-exercise is vital in refueling, as well as prompting training adaptations - this aspect will be discussed further in the following sections.

### 1.5.1 Timing of Intake

Research has shown that the timing of macronutrient intake can influence post-exercise metabolism (108). It has been well established that the consumption of CHO immediately post-endurance exercise is critical in optimizing the rate of glycogen
resynthesis (108, 111, 116). There has also been a great deal of research investigating CHO consumption immediately post-resistance training, which has shown decreases in myofibrillar protein degradation, increases in protein synthesis (109), and increases in whole body protein synthesis (107). The timing of macronutrient intake is important to consider with training as it can influence protein metabolism, as well as overall protein balance. With exercise, there are alterations in protein metabolism, an increase in protein synthesis, and a decrease in protein degradation, which benefits whole body protein balance. Alterations in the timing of protein intake have been shown to help maintain a more positive protein balance (108). Not only has the timing been shown to benefit protein metabolism post-exercise, but research has also shown an improvement in time to exhaustion during subsequent endurance exercise bouts when macronutrients are consumed immediately post-exercise (108, 121).

With the numerous and obvious benefits associated with consuming adequate macronutrients immediately post-exercise, it seems as though this should be a common and accepted practice. However, this is not the case, likely a consequence of the fact that logistically not everyone has the time or the resources available to consume a full and balanced meal immediately after PA. Therefore, many athletes turn to easier and more cost effective ways of consuming post-training nutrition. Many commercial drinks are marketed as post-recovery beverages and vary in composition and consistency. Low fat milk represents an easily accessible and effective alternative to ensuring adequate consumption of appropriate macronutrients during recovery from exercise. Milk is a readily accessible and reasonably inexpensive nutrient dense alternative to highly priced sports recovery
drinks, complicated protein and carbohydrate shakes which must be mixed and made, or consuming a full meal post-training.

### 1.5.2 Low Fat Dairy/Milk

One strategy to provide adequate post-exercise macronutrient intake is through provision of a beverage that contains these nutrients. There are several commercially available sports drinks that are marketed to athletes with the claim that they help in training adaptations and recovery. These drinks typically are made up of 2-10% carbohydrate (typically in the form of glucose, sucrose, fructose, and glucose polymers), as well as electrolytes (sodium and potassium), which help replace those lost in sweat, promote intestinal absorption of glucose and water, and enhance post-exercise rehydration (75). Recent research has shown that the combination of protein and carbohydrate, when compared to carbohydrate alone, in a recovery beverage following exercise, helps to aid in the increase of skeletal muscle protein synthesis and whole body protein synthesis (122), as well as potentially aiding in the increase in exercise capacity (75). Interestingly, milk has been shown to be beneficial in aiding in recovery and adaptation to training (9, 123) as it is very nutrient dense, containing carbohydrates, proteins, electrolytes and other macro and micronutrients. More specifically, skim milk (LFM) has characteristics, similar to commercially available sports drinks, with the added benefit of being a good source of protein (9, 123). For a thorough review, see Milk: the New Sports Drink? A Review (9).

The health benefits of milk and dairy consumption have been extensively researched and reviewed (11). Dairy products are considered to be a good source of protein, lipids, amino acids, vitamins, and minerals. In addition to containing carbohydrates (lactose) in similar quantities to commercially available sports drinks (maltodextrin,
glucose), milk also contains the proteins casein and whey (ratio of 3:1) (11). The presence of the proteins casein and whey in milk leads to more sustained digestion, absorption, increased feelings of satiety, and increasing delivery of amino acids to muscles. This also leads to a more sustained elevation of blood amino acid concentrations following absorption, aiding in maintenance and stimulation of PRO synthesis during recovery (124).

Interestingly, some of the peptide fragments resulting from the digestion of milk proteins have unique biological effects, which may help to facilitate adaptations gained through endurance training (124, 125). Milk also contains high concentrations of electrolytes. The majority of electrolytes found in milk are stored as minerals that are released upon digestion. Milk has been found to be rich in potassium, calcium, and phosphorus, while also containing smaller amounts of sodium, selenium and iron. The electrolytes found in milk aid in recovery of those lost through sweat during exercise, and also contributing to whole body fluid recovery following exercise (9, 123).

1.5.2.1 Milk and endurance training

The majority of research that has examined the interaction of an increased dairy intake and PA has focused on resistance training (9), with very little research investigating the interaction of milk and endurance training. There is limited research into the possible benefit of combining higher milk intake with endurance exercise. Studies have shown that milk consumed during or following prolonged exercise helps contribute to the reduction of whole body protein breakdown, and contributes to protein synthesis and increase in protein oxidation (126).

Skim milk contains ~32 g of protein per liter (11), and this protein has a high biological value and has been shown to be a good source of essential amino acids (11). The
peptide fragments that result from the digestion of milk protein have been shown to have a wide array of different properties, such as antimicrobial, antioxidant, immuno-modulation, anti-carcinogenic, and many others (127). As already mentioned, casein and whey are two of the major proteins found within milk. Casein alone makes up roughly 80% of the proteins found in milk, and it appears to assist in facilitating the absorption of calcium in from milk in humans (11).

The bioactive proteins and peptides found in milk contribute to moderating many regulatory processes within the body (11). One example of a bioactive peptide found within milk is a peptide fragment that has been show to have antihypertensive effects through the inhibition of angiotensin-converting enzyme (ACE). Other peptides have opioid-like activities, as well as antithrombotic properties and aiding in binding minerals (128).

Milk proteins also contain a high abundance of branched chain amino acids. These amino acids have been well documented to be critical substrates for protein synthesis as well as suppressing protein catabolism. Overall, the essential amino acids (AA) have been found to play a bigger role in muscle protein synthesis than non-essential (129). More specifically, the branched chain amino acid (BCAA) leucine has been shown to be involved in stimulating muscle protein synthesis through initiation of the insulin-signaling pathway (130, 131). After digestion of whey, insulin release is stimulated, which aids in reducing blood glucose concentrations (11). For further information regarding health benefits associated with the components of milk see the table below and refer to Bovine Milk in Human Nutrition – A Review (11).

Milk is a natural source of ACE inhibitors. It is believed that peptides within milk have anti-hypertensive effects, which may result from inhibition of angiotensin-
converting enzyme (ACE) (11, 128). ACE inhibitors are known to decrease the local vascular resistance of arterioles and large conductance vessels, helping to improve blood flow in muscles, as well as benefiting the redistribution of cardiac output towards muscle (69). ACE inhibition has also been shown to improve angiogenesis in skeletal muscles leading to the expansion of the capillary network and helping to improve substrate and oxygen delivery to peripheral tissues (e.g., working skeletal muscle during exercise).
Table 4: Composition of Non-Fat Milk (750mL)

<table>
<thead>
<tr>
<th>Milk Component</th>
<th>Concentration (750mL, Skim Milk)</th>
<th>% RDI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Proximates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy</td>
<td>274 kcal</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>26.62 g</td>
<td>0.66 g/kg, 56g/d</td>
</tr>
<tr>
<td>Total lipid (fat)</td>
<td>1.41 g</td>
<td>Not Determined</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>37.98 g</td>
<td>130 g</td>
</tr>
<tr>
<td>Fiber</td>
<td>0 g</td>
<td>38 g</td>
</tr>
<tr>
<td>Sugar</td>
<td>37.98 g</td>
<td>&lt; 25% total energy</td>
</tr>
<tr>
<td><strong>Minerals</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>1597 mg</td>
<td>800 mg</td>
</tr>
<tr>
<td>Iron</td>
<td>0.31 mg</td>
<td>6 mg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>86 mg</td>
<td>330 mg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>101 mg</td>
<td>580 mg</td>
</tr>
<tr>
<td>Potassium</td>
<td>1300 mg</td>
<td>4.7 g</td>
</tr>
<tr>
<td>Sodium</td>
<td>407 mg</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Zinc</td>
<td>3.13 mg</td>
<td>9.4 mg</td>
</tr>
<tr>
<td><strong>Vitamins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>7.8 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td>Thiamin</td>
<td>0.282 mg</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>1.096 mg</td>
<td>1.1 mg</td>
</tr>
<tr>
<td>Niacin</td>
<td>0.689 mg</td>
<td>12 mg</td>
</tr>
<tr>
<td>Vitamin B-6</td>
<td>0.313 mg</td>
<td>1.1 mg</td>
</tr>
<tr>
<td>Folate</td>
<td>39 µg</td>
<td>320 µg</td>
</tr>
<tr>
<td>Vitamin B-12</td>
<td>2.98 µg</td>
<td>2.0 µg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>1073 IU</td>
<td>625 µg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.08 mg</td>
<td>12 mg</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>368 IU</td>
<td>10 µg</td>
</tr>
<tr>
<td><strong>Lipids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatty Acids (saturated)</td>
<td>0.916 g</td>
<td></td>
</tr>
<tr>
<td>Fatty Acids</td>
<td>0.368 g</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>16 mg</td>
<td></td>
</tr>
</tbody>
</table>

* data from United States Department of Agriculture; (USDA) http://fnic.nal.usda.gov
Though controversial, recent research has shown the possibility of a relationship between polymorphism in the gene encoding in ACE and physical performance in humans (69). ACE converts angiotensin I to II, which are vasoconstrictors involved in vascular tone (69). The two different phenotypes of ACE appear to be related to physical performance. The I allele has been associated with reduced levels of ACE activity in serum and tissues. This has been connected to increased endurance performance, as well as an improvement in responses of adaptation to physical training (69). The D allele has been associated with a higher ACE activity. Research has shown power-oriented and elite short-distance athletes have higher levels of D allele compared to sedentary individuals.

Though there is yet to be an established clear link between endurance performance and ACE activity, it has been hypothesized that there is an association between the I allele and endurance performance. Research suggests that the I allele combined with a decrease in ACE activity could be the key factor relating to an increased enhancement of metabolic efficiency as a response to endurance training (132). A study by Bahi et al. (69) shows that in humans, a higher frequency of I allele is associated with lower ACE activity, which is found primarily in individuals who are high level endurance trained athletes. The study also found that renin-angiotensin-aldosterone system (RAAS) and angiotensin II levels, which are activated during exercise, play a primary role in physiological responses of electrolytic balance and cardiovascular function. The ACE genotype is important in determining an individual’s capacity to endurance training adaptations. It has been reported that athletes who carry the II genotype exhibit the greatest beneficial effect of endurance training on efficiency of muscular contraction. Habouzit et al. examined the influence of ACE activity on the adaptive responses of skeletal muscle training (132). They
hypothesized that chronic ACE inhibition, when combined with endurance training, would help to improve endurance performance. The study found that a decrease in ACE activity actually enhanced the response to endurance training without changing the muscles oxidative capacity or contractile phenotype (132).

Therefore, there is growing evidence that milk may be beneficial in aiding in skeletal muscle adaptations, and it provides an easily accessible and inexpensive alternative to popular sport drinks and protein shakes for post-workout macronutrient consumption (9).

1.5.2.2. Milk & Body Composition

In addition to benefiting training adaptations, recent evidence has shown that increased milk intake may be beneficial in facilitating improvements in body composition and overall health (133). Additionally, consumption of milk has been associated with positive effects on body composition and weight loss through the calcium and mineral mix that is found in milk (11, 133, 134). This may facilitate a decrease in the accumulation of body fat and potentially aid in acceleration of weight and fat loss with energy-restricted diets.

While there are inconsistencies among the various studies that have looked at the association between dairy consumption and body weight, an inverse effect of dairy consumption and body weight (135, 136), waist circumference, and body composition (134, 137, 138) is generally observed. Studies have also indicated that increasing calcium intake to 1200 mg/d is associated with decreases in BMI. Overall, it appears that calcium has been suggested to have a leading role in the beneficial influences of dairy consumption on body composition and body weight regulation (134). However, when increased dairy intake is
compared to increased calcium intake, it appears that there are other components of dairy that act with calcium to beneficially influence body composition and body weight (134).

Although the mechanisms underlying the effects dairy intake has on body composition and weight management are not completely known, a number of potential mechanisms have been suggested and cited as being plausible (134). One of the more popular suggestions is that increased dietary calcium has an effect on intracellular calcium and lipid absorption. Increased intracellular calcium in adipocytes altars lipid metabolism leading to increased turnover, while dietary calcium impacts adipocyte lipid metabolism and may decrease fatty acid absorption from the gastrointestinal tract (134, 139). Besides the dietary calcium obtained through dairy products, other components within milk, including protein and fat along with their metabolites, have been shown to impact weight control (134). Furthermore, there is evidence showing whey protein and casein may be involved in the regulation of body weight through inducing satiety (140). For further review of this topic, refer to the 2011 review “Associations Between Dairy Consumption and Body Weight: A Review of the Evidence and Underlying Mechanisms”, by Dougkas et al. (134).

Another potential mechanism by which dairy products, such as milk, contribute to body composition and weight management is through its effects on appetite regulation. Research has proven that diets high in protein are found to be more satiating than those that are low in protein (112, 141). Milk naturally contains both whey protein and casein, and whey protein is rapidly digested and absorbed, leading to its classification as a “fast protein”. Such proteins appear to be more effective in suppressing hunger within the first 90 minutes following consumption. Casein is considered to be a “slow protein” due to the
greater time required for digestion and absorption, and it appears to assist with appetite suppression beyond 150 minutes following consumption (112, 141).

The carbohydrates and fats within dairy products may also contribute to appetite regulation. Lactose, the only carbohydrate found in milk, is a low glycemic index CHO (142). Low glycemic index carbohydrates have been proven to increase satiety and decrease energy intake through effects on blood glucose concentrations and insulin responses, as well as stimulating bioactive peptides found within the stomach (143, 144). Fat contributes to appetite regulation through slowing gastric emptying time, as well as stimulating gastrointestinal hormones (145).

The evidence relating dairy products, such as milk, to weight regulation and body composition is of great importance. Such findings suggest that increasing dairy intake may represent an easily accessible and affordable lifestyle intervention, which can be used by almost anyone from elite athletes to the overweight and obese individuals to help control body weight and promote improvements in body composition. With the worldwide obesity epidemic, it is imperative that we take action to try to prevent obesity and the associated chronic diseases. The inclusion of increased milk consumption appears to be a simple, and possibly effective way of promoting improved health, body weight, and weight management in individuals who are not lactose intolerant or have allergies to these food products.

Based on these observations and the well-established benefits of exercise on health, body composition and weight management (10, 16), we propose that a combination of an endurance training program and supplementation of low-fat milk may have synergistic health benefits. If this hypothesis is correct, positive results could contribute to a realistic
and affordable lifestyle intervention for not only those at risk of becoming obese, but also for the general population. Figure 1 shows the benefits of PA as an intervention and the changes in risk factors, which lead to an overall decreased risk of the incidence of cardiovascular diseases and mortality.

**Figure 1: Benefits to Physical Activity Interventions**

Adapted from: Lakka & Bouchard. HEP. 170;163, 2005

- **Weight Loss**
  - Changes in Risk Factors
    - Improved insulin sensitivity
    - Improved glucose tolerance
    - Risk of type 2 diabetes
    - HDL cholesterol
    - Triglycerides
    - Improve endothelial function
    - Risk of thrombosis
    - Adiposity
    - Abdominal fat mass

- **Physical Activity & Dairy Consumption**

Incidence of cardiovascular diseases, metabolic disease,

Bouchard. HEP. 170;163, 2005
2.1 Statement of Problem

What is the influence of combining increased low-fat milk consumption with increased physical activity (endurance exercise) in an overweight population? And secondly, is there an added benefit to timing of consumption of the low-fat milk?

2.2 Purpose

The purpose of this study was to examine the effects of combining increased habitual dairy intake (in the form LFM) with increased PA in the form of endurance training. More specifically, we will be examining adaptations to endurance training, in terms of changes in body composition (fat, lean and fat mass), markers of aerobic fitness and performance (VO$_2$peak, time trial), and biochemical markers of bone health. Additionally, the timing of intake was be investigated to determine if intake immediately following training facilitated more significant improvements in adaptations, compared to intake at an alternate time of the day.

Positive findings would provide a basis for developing an effective and realistic lifestyle intervention that can be easily adopted by individuals striving to maintain a healthy body weight. Maintaining a healthy body weight is important for maintaining overall health and preventing chronic diseases that could lead to morbidity or premature mortality. Along with potential health benefits that could be associated with this study, positive findings could also bring further awareness of LFM as a viable post-exercise beverage. Most importantly, the interventions represent inexpensive, realistic and attainable lifestyle changes for individuals who are at risk of becoming obese. Establishing such interventions is of critical importance as the prevalence of obesity is rapidly rising across North America and throughout the world.
2.3 Hypotheses

The overall hypothesis is that dietary supplementation with LFM immediately post-endurance training will help to facilitate both physiological and health-related adaptations. More specifically, we hypothesize that dietary supplementation with 3 servings (750mL) of LFM within one hour of completion of daily exercise will facilitate positive changes in body composition, as well as physiological and health related adaptations when compared to 750mL LFM supplemented (a) 4+ hours before, or (b) 6+ hours post-exercise, or when compared to supplementation with an iso-caloric carbohydrate-based energy beverage consumed (c) immediately following training (within one hour) or either (d) 4+ hours before exercise or (e) 6+ hours post-exercise.

2.4 Methods

2.4.1 Participants

Sample size calculations, based on studies by Josse et al. (146) and Zemel et al. (147), indicated that we needed to recruit 56 healthy, university-aged males; however, only 40 were recruited into the study. Participants were chosen on the basis of being sedentary, and overweight (BMI>25), and did not routinely participate in endurance-type activities. This population was targeted as they are at the greatest risk of becoming obese, and also have the greatest potential to be influenced by the lifestyle modifications that were used in the current study. The PA and nutritional strategies combined in this study were lifestyle modifications that have been previously discussed, both of which contribute to improvements in overall health in similar populations when administered in isolation.
2.4.2 Study Design

Figure 2: Methods

2.4.2.1 Testing

The study included pre-testing, a twelve-week supervised endurance-training program, and post-testing. The participants’ first visit to the lab consisted of a detailed explanation of the study and obtaining informed consent. Participants also completed a food frequency questionnaire, which established their habitual dietary intake of dairy based on foods. They also completed a brief medical history questionnaire and a Physical Activity Readiness Questionnaire (PAR-Q) to insure that they were able to safely take part in the exercise program. Finally, the participants’ height and weight were measured and body mass index (BMI) was calculated. BMI was used to verify that the participants were classified as overweight. Once prospective participants were screened to insure that they
met the required inclusion criteria, and were able to safely participate in the training program, they were invited to participate in the remaining aspects of the study.

Upon acceptance into the study, participants were asked to return a few days later to complete a maximal oxygen consumption test (VO$_2$peak). The VO$_2$ peak test was completed using a cycle ergometer (Excalibur Sport, Lode BV, The Netherlands), and a MOXUS Modular VO$_2$ System (AIE Technologies, Pittsburg, PA). The cycle ergometer was chosen because of its ease of use across the general public and its use in many training studies similar to this one (61, 62, 64, 108, 148, 149). Upon arrival at the lab, participants were familiarized with the protocol and fitted to the bike, provided with a heart rate monitor (Polar, Wearlink, Stamford, CT), and fitted with a mouthpiece. The protocol that was used included a two-minute, self paced warm-up with resistance set to 80 watts. At the end of the two-minute warm-up the test began, beginning at 80 watts, resistance increased by 20 watts every 2 minutes until volitional exhaustion, followed by a cool down at a decreased workload for 5 min or until the participant had recovered. The test termination criteria used were similar to that previously described by Pritchett et al. (8). Termination of the VO$_2$peak test occurred when one or more of the following criteria was achieved: the participant requested the test be stopped for any reason, the participant reached voluntary exhaustion, the participant displayed any signs or symptoms that required the test be stopped, the participant was unable to maintain the required workload, or it was unsafe for the participant to continue (150). Verification of VO$_2$peak included a review of the following variables: a respiratory exchange ratio (RER) of $>$1.1, a minute ventilation (VE) of $>$ 100 L/min, a plateau of oxygen consumption (VO$_2$) with a continued increase in workload, or
achieving a heart rate >85% of age predicted maximum heart rate. At least two of these criteria were required to establish a base VO\textsubscript{2}peak (150).

Between the second and third visits, each participant was asked to complete a four-day diet record, including three weekdays and one weekend day. Each participant’s diet record was then analyzed using dietary analysis software (Nutritionist Pro, Version 2.2, First DataBank Inc., San Bruno, CA, USA). Using this information, the participants were divided into two categories based on daily habitual milk intake, the categories included: low (<1 serving per day) or moderate (1-2 servings per day). We then attempted to stratify and randomize participants into one of four groups based on an equal distribution of the two categories of milk intake (low or moderate), age, and fitness. The four experimental groups were as follows: Dairy-Exercise-Immediate (DEI), Dairy-Exercise-Alternate (DEA), Carbohydrate-Exercise-Immediate (CEI), and Carbohydrate-Exercise-Alternate (CEA).

The participants’ third visit took place at the McMaster University Medical Center; the participant’s underwent a whole body DXA scan (Lunar, GE, Pewaukee, WI) for body composition, BMC, and BMD. Following the DXA scan, each participant underwent a resting skeletal muscle biopsy (~80mg) from the vastus lateralis muscle (~15 centimeters proximal to the knee joint). The biopsy was performed under a local anesthetic using a custom suction-modified Bergström needle (5-mm diameter). Part of the muscle sample was imbedded in OCT and frozen for later histochemical analysis and then stored at -86°C. Additionally, a fasting blood sample was collected from each participant during this visit from the antecubital vein, and collected into heparinized and serum separator vials for isolation of plasma and serum, respectively. Both the plasma and serum vials were left
to stand at room temperature for 30 minutes, and then were centrifuged at 1750g for 10 minutes. Following centrifugation, the resulting plasma and serum samples were collected and stored at -86°C for later analysis. It is important to note, participants were asked to fast overnight, samples were taken early in the morning and participants were given time to eat a standardized meal prior to the muscle biopsy.

During the next, and last pre-test visit to the lab, the participants completed a self-paced cycling time trial. The time trial was preformed on a Lode cycle ergometer (Groningen, NL). Prior to the start of the trial, the participants were allowed to warm up as desired. Once the participant was sufficiently warmed up, they were asked to complete 200kJ of work as fast as possible. Throughout the duration of the time trial, the participants were not given information on time, power output, or pedal frequency, and no verbal encouragement was given during the test. The participants were only provided with feedback regarding the amount of work (kJ) completed every 25kJ.

2.4.2.2 Training

The training intervention consisted of a 12-week progressive endurance-training program. The first week of training began at 50% of the maximum heart rate, and was increased to 60% of the maximum heart rate after the initial 3 weeks of training. Training took place for 1 h/day, 5 d/wk (Monday through Friday) for the duration of the 12-week training program. All training was completed on a Monark Ergomedic 828 E bicycle ergometer (Monark Exercise AB; Vansbro, SE) at the Brock University Heart Institute. Graduate or senior undergraduate students with experience in personal training and prescription supervised all of the training. The appropriate workload for each participant
was determined and monitored using heart rate based off ranges specified for each individual from the VO$_2$peak test.

The drinks were administered to participants in plain unmarked bottles and, for the DEI and CEI groups, were consumed in the presence of study personnel in charge of administering the drinks. Participants were blinded to the groups and subjects were asked not to discuss the different beverages with other participants. The DEI group participants had their habitual diets supplemented with 750mL (3 additional servings) of LFM, which was consumed within one hour of completing the exercise session each day. The DEA group supplemented their habitual diet with 750mL of LFM, which was consumed at least 4 hours prior to, or 6 hours after, the exercise session. The CEI group had their habitual diet supplemented with a carbohydrate-based beverage that was isocaloric to the LFM supplement, and was consumed within one hour of exercise. The CEA group consumed the same carbohydrate-based beverage as the CEI group, but it was consumed at least 4 hours prior to, or 6 hours post-exercise.

It should be noted that the DEA and CEA groups consumed their beverages at least 4 hours before, or 6 hours after, exercise to insure that it was a dietary supplement, and did not have an effect on recovery or any potential adaptations associated with exercise. The beverage consumed by the dairy groups was 3 servings or 750mL of fat free milk totaling 270 kcal. The CHO groups consumed a drink consisting of 60g maltodextrin mixed into 750 mL of water totaling 240 kcal. Both drinks contained ~20mL fat free vanilla flavoring in an effort to disguise the beverage and to prevent any bias between subjects.

At the conclusion of the 12-week training program, post-testing consisted of the same testing as the pre-testing (VO$_2$max test, self-paced time trial, blood samples, muscle
biopsy, DXA scan, and 4-day food log). All post-testing was completed between 48-72 hours after the training session to prevent any acute effects of exercise from altering final results. The post-testing diet record was completed during the final week of training.

2.5 Analysis

2.5.1 Muscle Analysis:

A piece of the muscle biopsy (approximately 20 mg) was sectioned (10μm) and mounted on slides. The slides were stained for fiber type (also used for determination of fiber area) using an Azure A stain, and for identification of muscle capillaries (7) (see staining methods - Appendix A). Note: the capillary supply data are not being presented in this thesis, further explanation can be found in the discussion section.

2.5.2 Blood Analysis:

The blood samples were separated into serum and plasma samples prior to analysis. Serum total cholesterol, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and triglycerides (TRG) were measured using standard clinical procedures (Clinical Chemistry, McMaster University).

Plasma samples were also analyzed for biochemical markers of bone health: OC, and 25-hydroxyvitamin D (25[OH]D), OPG, RANKL, Trap-5β and FGF-23 using commercially available ELISA kits.

2.5.3 Statistical Analysis:
A 3-way analysis of variance (repeated measures) by condition, time, and pre/post, was used to analyze all data, and a Tukey HSD test was used to establish pair-wise differences for all significant interactions observed.

The number of participants for each group (N=12) was previously established using power calculations that were based on observations of changes in body composition (146, 151). Josse et al. (149) observed a mean change in the fat mass between groups of 1.3 kg. Using the standard deviation of the residuals - 0.50 kg, the sample size was calculated assuming a power of 0.90 and an alpha of 0.05. This calculation predicted a sample requirement of N=10 per group. Zemel et al. (151) conducted a study where supplemental dairy was added to a calorie-restricted diet in an obese adult population. The intervention diet included 3 additional servings of dairy per day over a time period of 24 days. The differences in fat mass observed between groups was 2.35 kg, the estimated standard deviation of the residuals was 1.1 kg. Assuming a one-tailed test, a power of 0.90 and an alpha of 0.05, these data indicated a sample size for each group of N=9. Therefore, we attempted to achieve a target sample size of N=14 per group, which allowed for a 30-40% drop out rate, while still allowing for adequate statistical power.
3. Results

3.1 General Observations

Forty participants who met all inclusion exclusion criteria, as previously outlined, were recruited. 17 participants were unable to complete the study for various reasons, such as location of training and change in semester, leaving 23 total participants who completed the protocol. Upon completion of the study, data of one participant was excluded due to a pulmonary illness obtained towards the end of the study, which affected his ability to complete the required post-testing. In total, data from 22 participants were used for the analysis reported here.

At baseline, there were no significant differences between the four experimental groups in terms of age, body mass, and initial VO2peak (Table 5).

<p>| Table 5: Baseline characteristics for individuals by group |</p>
<table>
<thead>
<tr>
<th>Groups:</th>
<th>DEI (n=6)</th>
<th>DEA (n=5)</th>
<th>CEI (n=6)</th>
<th>CEA (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>23.7 ± 2.5</td>
<td>20.6 ± 2.5</td>
<td>24.4 ± 3.0</td>
<td>29.4 ± 3.5</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>79.5 ± 3.5</td>
<td>83.5 ± 5.6</td>
<td>90.6 ± 6.0</td>
<td>86.2 ± 4.2</td>
</tr>
<tr>
<td>Absolute VO2 (ml/min)</td>
<td>3737.7 ± 214.5</td>
<td>3286.8 ± 194.4</td>
<td>3927.0 ± 375.0</td>
<td>3099.0 ± 404.2</td>
</tr>
<tr>
<td>Relative VO2 (ml/kg/min)</td>
<td>46.9 ± 1.9</td>
<td>40.0 ± 5.0</td>
<td>43.8 ± 7.5</td>
<td>35.3 ± 4.0</td>
</tr>
</tbody>
</table>

Observation: No significant difference observed.
Abbreviations: yrs: years, kg: kilograms, DEI: Dairy Exercise Immediate, DEA: Dairy Exercise Alternate, CEI: Carbohydrate Exercise Immediate, CEA: Carbohydrate Exercise Alternate.
Participants: DEI: n=6, DEA: n=5, CEI: n=6, CEA: n=4 – note DEI group 1 participant was excluded as an outlier, total n=21

The four-day diet logs revealed no significant differences in participants’ habitual diets pre- or post-training, and there were no differences between any of the experimental groups (Table 5). Additionally, when the experimental beverages were included in the post-diet analysis, no significant differences were found between the different groups (Table 6).
Table 6: Total Energy Intake in Kcal (Pre & Post)

<table>
<thead>
<tr>
<th>Groups:</th>
<th>DEI (n=7)</th>
<th>DEA (n=4)</th>
<th>CEI (n=6)</th>
<th>CEA (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Energy Intake (Kcal)</td>
<td>2845.4 ± 424.3</td>
<td>1930.8 ± 350.8</td>
<td>2214.6 ± 296.5</td>
<td>2622.4 ± 244.5</td>
</tr>
<tr>
<td>CHO</td>
<td>1500.4 ± 223.5</td>
<td>945.4 ± 170.9</td>
<td>987.0 ± 181.3</td>
<td>1354.0 ± 109.9</td>
</tr>
<tr>
<td>Fat</td>
<td>830.4 ± 102.1</td>
<td>819.9 ± 131.5</td>
<td>820.2 ± 212.3</td>
<td>899.5 ± 147.4</td>
</tr>
<tr>
<td>PRO</td>
<td>423.3 ± 75.4</td>
<td>303.4 ± 50.7</td>
<td>356.5 ± 56.6</td>
<td>406.6 ± 75.4</td>
</tr>
</tbody>
</table>

Observations: No significant difference observed.
Participants: DEI: n=7, DEA: n=4, CEI: n=6, CEA: n=4, note-1 participant was removed from the DEI group due to no post data.

The dietary food-logs were also analyzed for vitamin D and calcium intake. At baseline, participants had an average intake of 140 IU of vitamin D per day in their habitual diets, and there was no difference between groups before and after training. However, when the intervention beverage was included in the analysis, significant differences were observed between the groups post-training. Post-hoc analyses showed that the dairy groups had a significantly higher intake of vitamin D than the CHO groups (p<0.001), and the intake of the dairy groups was significantly higher post-intervention than at baseline (Table 7).

Despite the differences in dietary intake of vitamin D post-training, no significant differences in the circulating concentration of 25-(OH)D3 were observed for the different groups or time points. There was a trend for the CEA group to have a lower serum 25-(OH)D3 level, but this cannot be attributed to the intervention; it could, in part, be due to a smaller sample size for the group (n=4).
Table 7: Dietary Intake of Vitamin D and Calcium

<table>
<thead>
<tr>
<th>Groups:</th>
<th>DEI (n=5)</th>
<th>DEA (n=7)</th>
<th>CEI (n=6)</th>
<th>CEA (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Vitamin D w/out</td>
<td>93.7 ± 171.7 ± 103.7 ± 125.6 ± 123.8 ± 172.0 ± 166.2 ± 155.7 ±</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplement (IU)</td>
<td>17.9</td>
<td>76.8</td>
<td>36.2</td>
<td>38.2</td>
</tr>
<tr>
<td>Vitamin D (IU) w/ Supplement</td>
<td>93.7 ± 471.7 ± 103.7 ± 425.6 ± 123.8 ± 172.0 ± 166.2 ± 155.7 ±</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium w/out</td>
<td>735.7 ± 98.2 ± 741.6 ± 134.9 ± 134.9 ± 164.2 ± 1008.0 ± 1027.5 ± 836.2 ± 851.5 ±</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplement</td>
<td>± 138.7</td>
<td>± 134.9</td>
<td>± 69.8</td>
<td>± 121.4</td>
</tr>
<tr>
<td>Calcium w/</td>
<td>735.7 ± 98.2 ± 741.6 ± 1623.8 ± 741.6 ± 1642.5 ± 1008.0 ± 1027.5 ± 836.2 ± 851.5 ±</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplement</td>
<td>± 138.7</td>
<td>± 134.9</td>
<td>± 69.8</td>
<td>± 121.4</td>
</tr>
</tbody>
</table>

Observation: Significant increases in dairy groups seen when beverages were added to post testing for calcium and vitamin D.

Abbreviations: IU: international units; DEI: Dairy Exercise Immediate; DEA: Dairy Exercise Alternate; CEI: Carbohydrate Exercise Immediate; CEA: Carbohydrate Exercise Alternate.

Participants: DEI: n=6, DEA: n=7, CEI: n=6, CEA: n=4

* supplement refering to the intervention beverage

As was seen with serum vitamin D, daily dietary calcium intake was the same for the different experimental groups at baseline, and remained similar following the intervention - when the dietary intervention was not included. However, when the intervention beverages were considered in the analysis, there was a significant increase in dietary calcium intake within the milk groups, compared to the CHO groups (p<0.05), with an overall higher calcium intake per day post-intervention than at baseline (p<0.001) for these groups (Table 7).

Figure 3: Change in Serum 25(OH)D3
Observation: No significant difference observed.

Abbreviations: nmol/L: nanomoles per liter, DEI: Dairy Exercise Immediate, DEA: Dairy Exercise Alternate, CEI: Carbohydrate Exercise Immediate, CEA: Carbohydrate Exercise Alternate. Participants: DEI: n=6, DEA: n=7, CEI: n=6, CEA: n=4

3.2 Functional Adaptations (Capacity [VO2peak], Performance [TT])

Aerobic capacity was assessed through measurement of VO2peak pre- and post-training. There was a significant increase in relative VO2peak in all groups from pre- to post-training (p < 0.05). There were no significant differences between groups or across time when the VO2peak values were analyzed in absolute terms (ml/min). There was a trend towards significance when VO2peak was analyzed relative to lean mass (ml/kg/min), with post-test values being higher than pre-test across all groups (p= 0.072) (Table 8).

Table 8: Individual VO2peak
### Groups:

<table>
<thead>
<tr>
<th>Groups</th>
<th>DEI (n=6)</th>
<th>DEA (n=5)</th>
<th>CEI (n=6)</th>
<th>CEA (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>3832.7 ± 310.7</td>
<td>3887.3 ± 181.3</td>
<td>3237.0 ± 208.6</td>
<td>3927.0 ± 375.0</td>
</tr>
<tr>
<td>Post</td>
<td>3267.2 ± 310.7</td>
<td>3887.3 ± 181.3</td>
<td>3267.2 ± 208.6</td>
<td>3267.2 ± 302.5</td>
</tr>
<tr>
<td>Pre</td>
<td>3900.0 ± 3237.0</td>
<td>4300.0 ± 310.7</td>
<td>3237.0 ± 208.6</td>
<td>3267.2 ± 302.5</td>
</tr>
<tr>
<td>Post</td>
<td>3099.0 ± 3267.2</td>
<td>4300.0 ± 310.7</td>
<td>3237.0 ± 208.6</td>
<td>3267.2 ± 302.5</td>
</tr>
<tr>
<td>VO₂ / total BM</td>
<td>46.7 ± 20.0</td>
<td>49.5 ± 2.3</td>
<td>41.9 ± 5.6</td>
<td>43.8 ± 2.3</td>
</tr>
<tr>
<td>(ml/kg/min)</td>
<td>40.5 ± 5.6</td>
<td>47.7 ± 6.0</td>
<td>35.3 ± 4.0</td>
<td>37.6 ± 3.3</td>
</tr>
<tr>
<td>VO₂ / lean mass</td>
<td>66.8 ± 2.9</td>
<td>66.9 ± 1.0</td>
<td>57.5 ± 3.0</td>
<td>69.4 ± 2.8</td>
</tr>
<tr>
<td>(ml/kg/min)</td>
<td>66.9 ± 1.0</td>
<td>57.5 ± 3.0</td>
<td>60.0 ± 3.0</td>
<td>52.3 ± 2.8</td>
</tr>
</tbody>
</table>

**Observations**: Significant increase in relative VO₂peak across all groups from pre to post training.

**Abbreviations**: IU: international units, DEI: Dairy Exercise Immediate, DEA: Dairy Exercise Alternate, CEI: Carbohydrate Exercise Immediate, CEA: Carbohydrate Exercise Alternate.

**Participants**: DEI: n=6, DEA: n=7, CEI: n=6, CEA: n=4

Endurance performance was assessed with a time trial before, and after, training. Despite decreases in time to completion across all groups from pre- to post-training (delta time to completion: DEI= -3.57±2.90, DEA= -2.22±1.82, CEI= -0.57±1.89, CEA= -0.94±1.34), there were no significant differences found by group or time. Likewise, there were no significant differences for the average power output during the time trials between the groups or over time (Figure 3 & 4).

### 3.3 Body Composition

There were no significant differences in total body mass, fat mass, or lean mass between the different groups before, or following the intervention. However, a significant main effect for time was observed, in which a significant increase in lean mass was observed over the course of the study for all experimental groups (p < 0.05) (Figure 5).
Figure 4: Time Trial: Change in Average Power (watts) by Group

Observations: No significant difference observed.
Abbreviations: DEI: Dairy Exercise Immediate, DEA: Dairy Exercise Alternate, CEI: Carbohydrate Exercise Immediate, CEA: Carbohydrate Exercise Alternate.
Participants: DEI: n=6, DEA: n=7, CEI: n=6, CEA: n=4

Figure 5: Time to Completion of Time Trial
Observations: No significant difference observed.
Abbreviations: min: minute, DEI: Dairy Exercise Immediate, DEA: Dairy Exercise Alternate, CEI: Carbohydrate Exercise Immediate, CEA: Carbohydrate Exercise Alternate.
Participants: DEI: n=6, DEA: n=7, CEI: n=6, CEA: n=4

There were no significant differences in whole BMD and BMC between groups or across time (Table 9). However, when BMC and BMD were analyzed by body region (legs, trunk, arms, upper body, lower body, spine), spinal BMC was found to be significant across time, with BMC values increasing (p<0.05) from pre- to post-training (Figure 6).

Table 9: Whole Body BMC and BMD

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DEI (n=5)</td>
<td>DEA (n=7)</td>
<td>CEI (n=6)</td>
<td>CEA (n=4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD (g)</td>
<td>1.256 ± 0.059</td>
<td>1.244 ± 0.054</td>
<td>1.286 ± 0.047</td>
<td>1.286 ± 0.047</td>
<td>1.274 ± 0.045</td>
<td>1.284 ± 0.045</td>
<td>1.269 ± 0.045</td>
<td>1.266 ± 0.045</td>
</tr>
<tr>
<td>BMC (g/cm²)</td>
<td>3193.1 ± 155.5</td>
<td>3094.3 ± 182.6</td>
<td>3294.1 ± 208.0</td>
<td>3304.5 ± 212.3</td>
<td>3281.1 ± 227.3</td>
<td>3400.6 ± 228.0</td>
<td>3302.4 ± 200.3</td>
<td>2209.8 ± 140.0</td>
</tr>
</tbody>
</table>

Observations: No significant differences observed.
Figure 6: Changes in Body Composition by Group

(A) Observations: Significant main effect for lean mass, shown by an increase from pre to post training in all groups. Abbreviations: DEI: Dairy Exercise Immediate, DEA: Dairy Exercise Alternate, CEI: Carbohydrate Exercise Immediate, CEA: Carbohydrate Exercise Alternate.

Participants: DEI: n=7, DEA: n=5, CEI: n=6, CEA: n=4
3.4 Blood: Biochemical Markers of Bone Health

Serum markers of bone health were analyzed pre- and post-training. There were no differences between groups or over time for osteocalcin (OC), osteoprotegerin (OPG), or osteopontin (OPN) (Table 10). Serum concentrations of FGF-23 were below the level of detection for the Magpix® detection system. For Trap-5b, no differences were observed between groups before, or after, training (Table 10). However, a small, but statistically significant main effect was observed for Trap-5b in which all-experimental groups exhibited a decline following the training (p=0.04).

3.5 Muscle Fiber Type and Fiber Area

There were no significant differences between groups or over time for muscle fiber type or fiber area (Table 11).
Table 10: Markers of Bone Health between Groups

<table>
<thead>
<tr>
<th>Groups:</th>
<th>DEI (n=7)</th>
<th>DEA (n=5)</th>
<th>CEI (n=6)</th>
<th>CEA (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Trap 5β (U/L)</td>
<td>3.75 ± 0.46</td>
<td>3.80 ± 0.49</td>
<td>3.74 ± 0.73</td>
<td>3.40 ± 0.34</td>
</tr>
<tr>
<td>OPG (pg/ml)</td>
<td>606.9 ± 621.7</td>
<td>472.0 ± 466.8</td>
<td>541.7 ± 547.0</td>
<td>3.74 ± 0.68</td>
</tr>
<tr>
<td>OC (pg/ml)</td>
<td>37433 ± 38116</td>
<td>35528 ± 33100</td>
<td>31283 ± 29703</td>
<td>30895 ± 28776</td>
</tr>
<tr>
<td>OPN (pg/ml)</td>
<td>31538 ± 30021</td>
<td>28232 ± 29438</td>
<td>26408 ± 27000</td>
<td>24148 ± 24148</td>
</tr>
<tr>
<td>FGF23 (pg/ml)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Observations: Significant main effect for trap-5β, shown by a decrease in all groups following training.

Abbreviations: Trap 5β: Titrate-Resistant Acid Phosphate 5 beta, OPG: Osteoprotegerin, OC: Osteocalcin, OPN: Osteopontin, pg/ml: picograms per milliliter, U/L: units per liter, N/A: not applicable (numbers undetectable), DEI: Dairy Exercise Immediate, DEA: Dairy Exercise Alternate, CEI: Carbohydrate Exercise Immediate, CEA: Carbohydrate Exercise Alternate. Participants: DEI: n=7, DEA: n=5, CEI: n=6, CEA: n=4

Table 11: Percent Change in Muscle Fiber Type by Group

<table>
<thead>
<tr>
<th>Groups:</th>
<th>DEI (n=7)</th>
<th>DEA (n=4)</th>
<th>CEI (n=5)</th>
<th>CEA (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>% Type I</td>
<td>32.6 ± 6.83</td>
<td>31.3 ± 4.79</td>
<td>40.6 ± 4.83</td>
<td>34.6 ± 0.78</td>
</tr>
<tr>
<td>% Type II</td>
<td>67.4 ± 6.83</td>
<td>68.7 ± 4.79</td>
<td>59.4 ± 4.83</td>
<td>65.3 ± 0.64</td>
</tr>
</tbody>
</table>

Observations: No significant differences observed.

Abbreviations: BMC: bone mineral content, BMD: bone mineral density, DEI: Dairy Exercise Immediate; DEA: Dairy Exercise Alternate; CEI: Carbohydrate Exercise Immediate; CEA: Carbohydrate Exercise Alternate. Participants: DEI: n=6, DEA: n=4, CEI: n=5, CEA: n=4, note: 2 post muscle samples were indistinguishable when stained and therefore removed from analysis.
4. Discussion

The primary purpose of this study was to examine the effects of combining increased dairy intake and endurance training on body composition, endurance training adaptations, and biochemical markers of bone health in an overweight population. We hypothesized that the increased dairy intake groups would demonstrate augmented physiological and health related adaptations when compared to the CHO groups. Additionally, we had hypothesized that the dairy immediate group would show greater benefit when compared to the dairy alternate group.

The most interesting finding was that there was an increase in LM, which occurred in all groups post-training when compared to baseline. Additionally, training resulted in an increase in relative VO$_2$peak across all groups compared to baseline. Despite these changes, none of the hypotheses were supported, as several outcomes remained unchanged from baseline to post-training, and a lack of significant changes were observed across all experimental groups.

4.1 Energy Expenditure and Diet Composition

Health Canada’s Food Guide suggests 2 servings of milk or alternatives per day for individuals 19-50 and 3 servings a day for individuals 51 and over (http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/basics-base/quantit-eng.php). Similarly, the United States Department of Agriculture recommends a daily intake of 3 cups of dairy per day for men and women age 19 and up (http://www.choosemyplate.gov/food-groups/dairy-amount.html). Our intervention involved supplementation with 750 mL of LFM after each training session in the dairy groups, either immediately after training, or 4 hours before or 6 hours after training. This amount was used to insure that those participants in the dairy
groups were consuming slightly more than recommended for dairy each day. Prior to the present study, the participants were asked to complete a food frequency questionnaire to verify that their dairy intake was not already above the general recommended daily intakes. Interestingly, at baseline and post-testing, there were no significant differences between groups for dairy intake. Additionally, no group met the recommended servings for dairy, as stated by Canada’s Food Guide, prior to the start of the study (DEI: 0.47±0.14, DEA: 0.53±0.19, CEI: 0.50±0.10, CEA: 0.85±0.08). When diets were analyzed again post-training, without taking into account the intervention beverages, participants still did not meet the recommended intake for dairy (DEI: 0.75±0.19, DEA: 0.50±0.07, CEI: 0.72±0.14, CEA: 0.63±0.13) and again, there were no significant differences in dairy intake by group or time point. Therefore, the 750mL of LFM was adequate to bring the participants in the dairy groups to the recommended dietary intake of dairy only on the days they participated in the training. Additionally, the RDA for both Vitamin D and Calcium were met with the addition of the milk supplement in both dairy groups (Table 7).

As stated previously, the intervention involved three different time points for beverage intake - one in which the beverage was consumed immediately post-exercise compared to either 4 hours before, or 6 hours, after training was completed. We had hypothesized that the group consuming the LFM beverage immediately after training would see the greatest benefits and adaptations when compared to the alternate group. This hypothesis was based on research showing that the timing of macronutrient intake can influence post-exercise metabolism (106-108, 110). As milk is a whole food providing key macronutrients, we were interested to see if there would be any added benefit from a specific timing of the intake. Our analysis did not support this hypothesis, as there were no
significant differences between the various time points for the ingestion of milk. There are a number of different reasons that may have contributed to our observations, such as the test population used in the study, and how/when the data were recorded. Most studies to date, that have looked at the timing of the nutritional intake, have focused on the effects of the timing of macronutrient intake on performance, or examined changes in energy balance and protein (PRO) metabolism over a shorter study period (106-108, 110, 111). For example, Roy et al. (108) examined the effects of the timing of macronutrient intake on PRO metabolism, energy balance, and endurance exercise performance over 7 days. In contrast, the current intervention was 12 weeks, and focused more on potential changes that would be seen during subsequent exercise and in the hours immediately following the exercise rather than the long-term effects in a more highly trained group of athletes. Therefore, it may be possible that our study and data collection were too broad in nature to account for changes that may have been noticed had we incorporated a narrower focus, or been able to collect data immediately after the exercise periods. Additionally, many studies that have investigated the timing of the intake have focused on much more athletic or active populations (106-108, 110, 111). The participants in the current study were essentially inactive prior to the study, and it is possible that any subtle differences attributed to timing were overshadowed by the changes seen in all groups from baseline due to the relatively drastic change in lifestyle and the large relative increase in PA that occurred in the current population. Therefore, future research should attempt to determine if responses would be different in a more active population compared to a sedentary population.

4.2 Observed Changes in Body Composition
One of the primary hypothesized outcomes of this study was to see improvements in body composition (é lean mass/ê fat mass). Measurements of body composition included fat mass, lean mass, BMD, and BMC. Measurements were obtained through a DXA scan at baseline and post-training. We hypothesized a decrease in fat mass, resulting in a decrease in the fat mass to lean mass ratio. Additionally, we hypothesized there would be positive changes in BMD and BMC with our protocol. However, none of these changes were observed. There were no significant differences observed at any time point or between groups for changes in lean mass, fat mass, BMC, or BMD. Interestingly there was a significant increase across all groups in lean mass from baseline to post-training. Though there was no significant difference seen for whole body BMC or BMD directly, there was a positive effect seen for BMC in the spine with training and Trap-5β, also with training (discussed in section 4.2.3).

4.2.1 Body Weight and Fat Mass

There are several potential factors that may have contributed to our observed results for body weight and fat mass. While it would be expected that an overweight individual would significantly decrease body weight and fat mass with endurance training, the scientific literature suggests that PA interventions alone typically result in minimal changes in body weight, with changes typically totaling less than 3% (0.5-3.0 kg) (Physical Activity Guidelines Report (http://www.health.gov/PAGuidelines/). These observations are consistent with what others have observed, including the National Institutes of Health and the National Heart, Lung, and Blood Institute, as well as several studies (60-63, 152). Wing et al. (61) looked at 3 lifestyle interventions (diet alone, diet + exercise, exercise alone) over the course of a 6-month intervention period. They observed that the diet and exercise
combined group lost the most weight (10.4%), followed by the diet alone (9.1%), and finally the exercise alone group (2.1%). These observations are important to note, as our study included a relatively significant lifestyle change, with the increase in PA and only a minor change in diet.

Hagan et al. (62) completed a study similar to ours using an intervention period of 12-weeks. This intervention resulted in significantly less weight loss overall, with the changes in the exercise alone group comparable to what we observed in the current study over a similar intervention period. Similar to the Wing et al. study, the diet and exercise combined group saw the greatest loss in weight (11.4%), followed by the diet alone group (7.2%), and then the exercise alone group, which exhibited the smallest weight loss (0.6%) (55). Our study resulted in changes at the lower end or just below the range observed in their study (DEI: 0.26 ± 0.70 kg, DEA: 0.56 ± 1.24 kg, CEI: 0.20 ± 1.16 kg, CEA: 0.46 ± 0.89 kg) with an overall average change of 0.37 ± 1.0 kg. While there is considerable support showing the beneficial effects of exercise on body composition and weight management, it is the addition of a dietary intervention in combination with the increased PA that appears to be the most effective in aiding changes in body weight and fat mass (10, 11). While we did incorporate a supplemental beverage as part of our training protocol, this addition represented only a minor component of the participants overall habitual diet. Furthermore, there are a growing number of studies that support our hypothesis that milk can have a positive influence on body composition (10, 11, 13, 125-127); however, it is conceivable that the duration of the intervention was inadequate to elicit significant changes. Perhaps the inclusion of a more intensive dietary intervention, in addition to the
exercise and drink supplementation, would have had a greater impact on the body composition results.

4.2.2 Lean Mass

The significant increase in lean mass observed in all of the intervention groups in this study, was somewhat unexpected. Many endurance-training studies that have used a similar exercise intervention generally report decreases in fat mass and body weight as opposed to increases in lean mass. For example, Willis et al. (153) looked at overweight and obese middle-aged individuals in an attempt to determine the optimal mode of exercise for weight loss and changes in body composition. Participants were split into 3 groups - an aerobic training group, a resistance-training group, and a combined group who performed both. Results showed the aerobic training and combined groups had a significantly greater decrease in total body mass and fat mass when compared to the resistance training group; in addition, the resistance training group and combined group demonstrated significant increases in lean mass when compared to the aerobic training group. Furthermore, Stuart et al. (154) showed that 8-weeks of endurance training on cycle ergometers (in a population similar to that used in the current study), did not lead to any changes in lean mass (Stuart et al. (154): n=18, BMI = 28-35; our study: n=22, BMI = 23-35).

Increases in lean mass are more commonly reported with resistance training interventions. Several studies have shown a substantial increase in lean mass if resistance training was combined with supplemental LFM intake (155, 156). For example, Hartman et al. (155) used a resistance training intervention as opposed to endurance training. The study was conducted over the course of 12 weeks, with training 5 days per week. Groups were split into a LFM group, a CHO group, and a soy group. Beverages were consumed
immediately after training (500mL) and again 1 hour after training (500mL), with the total intake being 1000mL of the assigned intervention beverage. Results showed increases in lean mass across all groups, but the most significant increases were observed in the milk group (control: 3.7%, Soy: 4.4%, Milk: 6.2%). Our results were similar in that all groups showed an increase in lean mass - DEI: 0.99%, DEA: 0.99%, CEI: 0.99%, CEA: 0.98%, however there was no added benefit for beverage type.

The reasons underlying the lack of effect of milk on lean mass in the current study remains unclear; however, a possible explanation may be related in part to the specific participant population. The participants used in the current study were completely untrained and essentially sedentary prior to the start of the study. Therefore, the increase in activity with training may have led to the increases in lean mass observed in the present study. Perhaps, if our population had been more active, the increase in lean mass would not have been realized. Donges et al. (148) reported similar results to those observed in our study. However, it is important to note that the mode of exercise used in their study was resistance training rather than endurance training, and their study looked at the effects of single-mode vs. duration matched concurrent exercise training on body composition in overweight sedentary middle-aged men. The study included three intervention groups, concurrent exercise training (%50 resistance, 50% endurance), endurance training (40-60 min on a cycle ergometer), and resistance training (10 exercises 3-4 sets x 8-10 reps).

Interestingly, while our intervention was most similar to the endurance-training group, our results across all groups, matched most closely to the resistance-training group (Table 13).

Table 12: Comparison of Results: Doungus et al., 2013 vs. the Current Study

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>EET</th>
<th>RET</th>
<th>DEI</th>
<th>DEA</th>
<th>CEI</th>
<th>CEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Mass (kg)</td>
<td>-1.9 ± 0.7</td>
<td>0.2 ± 0.2</td>
<td>0.3 ± 0.7</td>
<td>0.6 ± 1.2</td>
<td>0.2 ± 1.2</td>
<td>0.5 ± 0.9</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>-4.5 ± 1.6</td>
<td>-2.8 ± 1.1</td>
<td>0.7 ± 0.9</td>
<td>-0.2 ± 0.7</td>
<td>-1.3 ± 1.1</td>
<td>0.8 ± 0.7</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>-0.8 ± 0.7</td>
<td>1.5 ± 0.6</td>
<td>0.9 ± 0.5</td>
<td>0.7 ± 1.1</td>
<td>0.9 ± 0.2</td>
<td>1.1 ± 1.1</td>
</tr>
</tbody>
</table>

Abbreviations: kg: kilograms; EET: endurance exercise group; RET: resistance exercise group; DEI: Dairy Exercise Immediate; DEA: Dairy Exercise Alternate; CEI: Carbohydrate Exercise Immediate; CEA: Carbohydrate Exercise Alternate.

Interestingly, Ferguson-Stegall et al. (149) completed a study in which they observed results similar to those of the current study, in terms of increases in lean mass. The study looked at the effects of chocolate milk consumption post-endurance exercise in terms of its effects on VO$_{2\text{max}}$ and body composition. The study was a double blind trial consisting of 4.5 weeks of endurance training for 60 minutes per day, 5 days per week at 75-80% VO$_{2\text{peak}}$. There were 3 intervention groups in the study - a chocolate milk group, a CHO group, and a placebo group. While it is important to note the duration of the study was shorter and the intensity slightly higher than the present study, their results showed an increase in lean mass in the resistance-training group similar to those seen across all groups in the present study. Perhaps an increase in statistical power would be beneficial to better understand our observations in terms of the observed changes in lean mass. Alternatively, a study of greater duration and/or intensity may be necessary when working with sedentary individuals to establish the long-term gains directly associated with the intervention/exercise training beyond any early initial gains from the lifestyle change. The recruitment of different population groups, such as groups that are sedentary, slightly active, and more active, within a single study could provide more insight on such changes in lean mass.

4.2.3 Bone Health

The interventions in the current study had no significant effects on whole body BMC or whole body BMD. However, when the data were broken down regionally, there
was a significant main effect noted for time in terms of spine BMC, showing an increase in all groups from baseline to post-training. One possible reason for the lack of change in whole body BMC and BMD could have been the study duration. Sahni et al. (157) looked at the effects of milk and yogurt consumption and its effects on BMD over a prolonged period of time. The study showed that dairy intake has a positive association with hip and spine BMD; however, this study followed the participants for approximately 12 years, using food frequency questionnaires as opposed to the current study which followed participants for only 12 weeks and involved no subsequent long term follow up. Interestingly, some other dairy intervention studies have not shown any effects on BMC or BMD. For example, Hinton et al. (158) showed no difference in changes in BMD and BMC when participants were on a 24-week dietary intervention that met dairy intake recommendations compared to a diet that did not meet the recommendations. Even though the dairy group had a significantly greater intake of calcium, protein, and vitamin D, similar to the observed differences between beverage groups in the current study, there were no significant differences in whole body BMC or BMD. The authors suggest that one reason for the lack of effect on bone mineral may be due to the time that is needed to detect changes in bone mass, and that any possible differences between the groups may have been better detected after a longer duration. Additionally, the study only looked at whole body bone mass and content, but suggested that if regional measures had been taken, such as hip, spine, and wrist, site-specific alterations may have been detected. This statement is somewhat consistent with results of the current study, in that our observations of a significant main effect for BMC within the spine, which showed an increase in all groups from baseline to post-training. Based on these data and given the length of our study in comparison, it is
likely that 12 weeks was inadequate to elicit any significant changes in BMD and BMC at the whole body level that could be detected by DXA. Future studies should consider longer interventions to determine the combined effect of endurance training and increased milk intake on BMC and BMD.

It has been well established that biochemical markers of bone health change in advance of changes in BMC and BMD (159, 160). As such, they are often used as surrogate markers of possible coming adaptations in BMC and BMD. In the current study, we did assess blood samples for an array of biochemical markers indicative of bone health. These markers included osteocalcin, 25-hydroxyvitamin D, OPG OPN, FGF-23, and Trap-5β. Generally, it is well accepted that exercise and sport have a positive impact on the RANK-RANKL-OPG pathway (149). It is important to note that while most exercise interventions positively benefit the RANK-RANKL-OPG system, extreme intensities may have negative effects on bone metabolism.

This area of study is relatively novel, and further work is needed to better understand the effects of exercise on bone metabolism and the biochemical markers of bone turnover. Previous research in this area has led to many inconsistencies being observed (161-164). For example, one study incorporated 32 weeks of resistance training in 47 healthy adults and observed significant increases in spinal and proximal femoral BMD; however, when bone metabolism markers were analyzed, including TNF-α, OC, OPF and RANKL, no significant differences were seen. Again, except for a small but significant main effect seen for Trap-5β (all groups exhibiting a decrease post-training (p=0.04)), our study resulted in no significant changes in any of the metabolic bone markers in either group or time point. Additionally, the levels of FGF-23 were below the level of
detection for the kit that was used for analysis. It is possible that, similar to BMD and BMC, an intervention of greater than 12-weeks would be needed to see any significant changes in most of these markers, or alternatively, an intervention that was more intense and involved more stress on bone, such as running, with its repeated loading and impact on bone would be more useful in producing effects. The slow turnover rates of bone as a tissue needs to be considered in study design, and as a result, most studies in this area usually involve much longer intervention periods, such as 6-8 months (161-164) compared to the 12-week intervention used in our study. Therefore, it is recommended that future studies should follow participants for a longer duration to be able to better assess changes in bone health. Thus, our results remain inconclusive as to the effects of increased dairy consumption and endurance training on BMD and BMC. However, it is important however, to note the change in Trap-5β, which cannot be attributed to the addition of the intervention beverage, since similar decreases were seen in all groups. The change does support a benefit of exercise on Trap-5β, which is a marker of osteoclast activity. Although most research has examined resistance training and its effects, other studies have shown similar decreases in Trap-5β post training (165, 166). This is important as changes in biochemical markers of bone can be seen prior to changes in BMC and BMD, suggesting that an intervention of longer duration may lead to changes in BMC and BMD as hypothesized.

4.3 Functional adaptations

It is well established that endurance training results in improvements in functional work capacity and aerobic capacity (70-72). We hypothesized that our training intervention would result in a significant decrease in the time to completion of a time trial, and to an increase in VO2peak.
The intervention used in the current study did lead to an increase in aerobic fitness, as all groups showed significant increases in VO$_2$peak relative to total body mass. Since the increase was similar between all groups (Table 7), the increase can be attributed to the training component of the study and not the drink interventions. The increases seen in the present study are consistent with what we would expect to see with a low to moderate intensity aerobic training intervention (60-62, 65, 167). Interestingly, combining chocolate milk intake with aerobic training has been previously observed to lead to greater increases in VO$_2$peak (149). Although we saw no significant differences between groups, the observed increases were similar to those seen across the intervention groups in the Ferguson-Stegall et al. study (149). This study compared the effects of providing CHO + PRO, in the form of chocolate milk vs. a CHO based drink, and a placebo after training. The training protocol was somewhat similar to ours, consisting of 4.5 weeks of training, 5 days per week, 60 min per day, at 75-80% VO$_2$peak. While the population used in their study was similar - recreationally active untrained participants, i.e., individuals who had not worked out more than 3 hours a week over the last 2 years, we studied individuals who were sedentary and not training at all. Ferguson-Stegall et al. showed a significant increase in VO$_2$peak in the chocolate milk group, and proposed that the largest increase in VO$_2$peak was seen in the chocolate milk group, likely the result of an enhanced albumin synthesis caused by the CHO + PRO mix found in milk which resulted in an increase in plasma volume and a corresponding increase in stroke volume and in turn cardiac output (149). Unfortunately, there were no actual measurements taken for stroke volume or cardiac output, and therefore suggestions are only based on speculation. Future studies should incorporate further serum analysis to expand upon this possible mechanism.
While we did expect to see an increase in VO\textsubscript{2}peak over the course of the intervention, some of the present data were not accurate due to an unpredictable system error within the VO\textsubscript{2} collection software. As a result, some data were incomplete for some participants and therefore, the raw data had to be used to calculate approximate values for the final VO\textsubscript{2}peak values. Had we been able to collect complete and accurate raw data for each individual, the measurements would have been useful for more than just the final values of VO\textsubscript{2}peak. For instance, the raw data for total oxygen uptake and maximal oxygen uptake, as well as workload reached, and time to exhaustion could have been used to provide additional information in regards to performance and efficiency.

A decrease in the time to complete the time trial, and an increase in the average power output during the time trial from baseline to post-training were observed in all our groups. However, no differences were observed between the different groups. Research has repeatedly shown that following training, an individual’s time to completion should decrease (70-73). The reliability and reproducibility of a time trial as a measurement of performance is supported by many studies (70-73). There are several potential contributing factors that explain the results of the current study. It is possible that the participant’s results may have been skewed due to a learning effect, which could have occurred from pre- to post-testing. Based on analysis of the results, and through observation, several participants who self-paced at a relatively high intensity became exhausted early in the pre-test chose a slightly lower, more sustainable pace during the post-test, which contributed to the slower or similar times to completion seen for some participants during post-test assessment. Jeukendrup et al. (77) have examined the use of a time trial as a measurement tool, and found time trials to be reliable and reproducible test, with little to no learning effects. It is
important to note however, that the participants involved in the Jeukendrup study were all very well trained cyclists, who would have been familiar with pushing themselves and were familiar with the concept of self-pacing at higher exercise intensities. This difference in population may contribute to the differing results. In the future, when working with a sedentary population, it may be beneficial to run a series of familiarization trials in advance of all pre-tests (specifically, the time trial and VO$_2$peak, in this case) to try to minimize any learning effect within the actual study.

### 4.4 Muscular Adaptations

Histochemical analysis of skeletal muscle samples was performed to examine adaptations within the muscle over the course of the investigation. Specifically, we analyzed changes in muscle fiber type and fiber area. We hypothesized a shift in fiber type towards Type I, and an increase in fiber diameter, with the greatest increases in the DEI group. Previous research has shown that muscle fiber type can be altered with training (34, 83, 85, 87, 168-170), and that these changes are typically small, and that fiber transitions are generally from fast to slow, i.e., from Type II to Type I, with endurance training (35). Despite the well-established observations of fiber changes with endurance training, our hypothesis was not supported as no significant differences were observed between groups or across time for fiber type or fiber area from baseline to post-training. Upon careful reflection and reconsideration of the literature, our observations may not actually be completely unexpected given the intensity of the workload and duration of our study. Coggan et al. (171) looked at the effects of endurance training on skeletal muscle adaptations in men and women 60-70 years of age. The authors suggest that the minimal changes in skeletal muscle adaptations seen in other studies may be attributed to an
inadequate training stimulus during the training intervention period. In their study, Coggan et al. examined 23 healthy participants who took part in a 9-12 month intervention, which incorporated 45 min/d walking or jogging at 80% maximal heart rate, 4 days/week (171). Similar to our study, there was no noticeable difference in the percentage of Type I muscle fibers; there was however, a significant decrease in type 2b and a significant increase in Type IIa fibers. If we had been able to distinguish between Type IIa and Type IIb fibers, we may have seen similar differences, indicating a slight shift overall from fast to slower fiber type. Future studies should incorporate longer interventions and higher exercise intensities, which might elicit greater changes.

In addition to fiber type, we also looked at fiber area. We hypothesized that with endurance training, muscle fiber area would increase. Previous research has shown that with training muscle fiber area increases (172, 173). There has been a great deal of research showing increases in muscle fiber size occur with resistance training, as well as with endurance training studies. Again referring to the study by Coggan et al., significant increases in the cross sectional area of the muscle fibers were noted for both Type I (12% increase) and Type IIb (10% increase) fibers following 9-12 months of endurance training (171). Farup et al. (174) completed a study similar to the current study in duration, also using endurance training or resistance training with a population of untrained men. Fourteen untrained men were split into two intervention groups. The training program was 10-weeks in duration, with training 3 days per week, and the endurance training was performed on a cycle ergometer. Following 10-weeks of training, no significant changes in muscle fiber cross-sectional area was observed. Again, the analysis of the data from the current study showed no significant difference from baseline to post-training in any group,
suggesting that a longer duration or a higher intensity workload should be considered for future studies, when examining changes in cross-sectional fiber area.

We attempted to analyze capillary density using a histochemical staining procedure as outlined by Rosenblatt et al. (175). After early success with practice samples using the staining procedures, we were unable to achieve clear consistent staining of the actual samples. Despite repeated efforts and modifications of the procedure, the capillaries were indistinguishable. Additionally, we considered alternate staining techniques, however, there was an insufficient amount of tissue remaining to undertake alternative methods.

**Implications and Strengths**

The current study investigated the potential of combining realistic lifestyle interventions for maintaining a healthy body composition while giving added health and training benefits to a population at risk for becoming obese. However, following our analysis, the hypotheses proposed at the onset of the study remain inconclusive, but the results do offer a good starting point for the development of additional studies in this area of research. A major strength of this study was the intervention group that was targeted, adult males who were overweight and sedentary, as they represent a large portion of the general population, making the findings very applicable to a larger proportion of the general population than if we had focused on athletes or more active individuals. By targeting the overweight population, we also targeted a group most at risk for becoming obese and developing diseases associated with obesity.

**Limitations**
A major and very significant limitation to the current study was the dropout rate of the participants. Prior to recruitment, the number of participants needed per group was established using power calculations. These calculations were based on observations in changes of body composition reported by Josse et al. (146) and Zemel et al. (147). Sample sizes were estimated assuming a power of 0.90 and an alpha of 0.05 (for further explanation refer back to section 2.5.3). Ultimately, we concluded a sample size of N=14 per group would be required, (a total N=56 subjects) to allow for a conservative drop out rate of 30-40%, while still allowing for adequate statistical power. Due to limitations beyond our control, the actual number of participants recruited was only 40, as opposed to the desired 56, and only 22 participants completed all of the training and testing sessions (dropout rate of 45%). There are several factors that possibly contributed to this - injuries, changes in course scheduling, difficulties arising from the location of the training site, and non-compliance. Based on these observations, future studies should acknowledge that the drop rate of studies of this nature could be significantly greater than suggested in the literature, and therefore, a greater number of subjects need to be recruited to achieve the appropriate number of subjects to achieve statistical significance. Our final N of 22 resulted in power calculations being done with a power of 0.5 and the effect size for main outcomes ranging between 0.02-0.3. This resulted in a final power calculation of less than 0.01. Post-data analysis, an a priori power analysis was done to determine the required sample size to obtain a power of 0.80 based on the current data. This analysis indicated that a total of 238 participants would be needed to achieve a power of 0.80.

Another notable limitation was the difficulty encountered with the faulty metabolic cart. Inaccuracy and technical difficulties with the metabolic cart led to inconsistent results,
forcing us to calculate the VO$_2$peak results for each participant using raw data and the Fick equation. Additionally, we found that with both the VO$_2$peak test and the time trial, there was an apparent learning effect by some participants, which potentially affected the reliability and validity of the results. It is also worth noting that for the VO$_2$peak test, many participants who achieved a plateau in VO$_2$ in the first test, were unable to push as hard in the post-test, and may have stopped before reaching true exhaustion. This may be attributed to the population studied, as sedentary individuals may be less likely to have the desire to push to their limit, compared to competitive or trained individuals. One suggestion for future studies is to have participants undergo multiple familiarization trials in an attempt to reduce or eliminate such issues during actual testing.

**Future Directions**

Though it is difficult to suggest future research directions based on this study due to the inconclusive results, there are a number of recommendations that can be made to improve upon the current study. First, to better investigate exercise and dietary interventions and their affect on bone, longer interventions are required, with a minimum intervention of 6 months. The current study was too short in duration to see significant changes in bone mass and several of the bone markers. Second, future studies in this population would benefit from a larger sample size to accommodate a higher than expected dropout rate. Finally, any future studies should also examine the benefits of an increased dairy intake on physiological variables in recreationally active individuals compared to trained competitive athletes.
Conclusions

There were no significant differences across time or by group for body mass, fat mass, BMD, BMC, most metabolic markers of bone health, time trial, or with muscle fiber type or area. Furthermore, there was no difference due to timing of beverage intake. Based on the study results, and acknowledging the limitations of having a small sample size, there was no added benefit of supplementing increased dairy consumption (LFM), coupled with low/moderate intensity endurance training on physiological adaptation, compared to a carbohydrate beverage coupled with endurance training, in overweight, sedentary individuals. We found no significant added benefit in terms of changes in fat mass, lean mass, or in measures of endurance performance (time trial), aerobic capacity (VO₂peak), skeletal muscle adaptations (muscle fiber type or fiber area), or bone adaptations (BMC, BMD). Training did however result in an increase in VO₂peak in each group from pre- to post-testing. There was also an unexpected, significant increase in lean mass across all groups from pre- testing to post-testing.

In conclusion, 12 weeks of endurance training, 1 hr/day, 5 days/wk, at 60% max hr, combined with 750mL does not appear to facilitate physiological or health related adaptations (body composition, endurance performance, aerobic capacity, skeletal muscle adaptations, or markers of bone health) in overweight, sedentary university aged males.
References


68. Lee IM. Dose-response relation between physical activity and fitness: Even a little is good; more is better. JAMA. 2007 May 16;297(19):2137-9.


Appendix A: Histochemical Staining

A.1. Haematoxylin and Eosin (H&E)

The H&E stain stains the sarcolemma and nuclei blue, fibers pink, and connective tissue a lighter pink. This stain was used to check orientation of the muscle sample on the piece of cork to be sure it was oriented as a cross section and not longitudinally.

Reagents:

1. Harris’ Haematoxylin:
   - Harris’ haematoxylin powder: 21.5 g
   - Absolute alcohol: 10.0 mL
   - Distilled water: 200.0 mL
   - Add 4% glacial acetic acid just before use

2. Eosin (1%):
   - Eosin: 1.0 g
   - Distilled water: 100 mL

3. Acid-Alcohol (0.2%):
   - HCl (concentrated): 100.0 μL
   - Alcohol: 50.0 mL

Procedure:

1. Incubate in Harris haematoxylin solution: 3 minutes
2. Rinse in cold tap water: 2 minutes
3. Differentiate in 0.2% acid-alcohol until pink: as needed
4. Re-blue in Harris’ haematoxylin solution: as needed
5. Place in 1% Eosin: 15-20 sec
6. Wash quickly in distilled water
7. Dehydrate:
   - 80% alcohol: 2 minutes
   - 90% alcohol: 2 minutes
   - 100% alcohol: 2 minutes
A.2. Azure A Metachromatic Dye-ATPase Stain for Fiber Typing

**Reagents:**

1. **Basic Medium:**
   - Glycine 3.96 g
   - Calcium Chloride 4.20 g
     Sigma (C3306)
   - Sodium Chloride 3.80 g
   - Sodium Hydroxide 1.90 g
   - dH₂O bring to 1000 mL volume

   *Store at 0-4°C for 90 days at pH 9.4 (adjust pH with 5N or 1 N HCL or 1 N NaOH)*

   **Note:** Calcium Chloride may cause precipitate. If this occurs make the Basic Medium without Calcium Chloride, adjust the pH to 9.4 then slowly add Calcium Chloride.

2. **Acid Medium**
   - Sodium Acetate (82.03 g) 3.90 g
     Sigma (S2889)
   - Potassium Chloride 3.70 g
     Sigma (P4504)
   - dH₂O bring to 500 mL

   *Store at 0-4°C for 90 days*

3. **Tris Buffer**
   - Trizma base 12.10 g
     Sigma (TRS001.1)
   - Calcium Chloride (dehydrate) 2.60 g
   - dH₂O 950 mL

   *Adjust pH to 7.8 with 5 N HCl and bring to 1000mL with dH₂O*

4. **1% Calcium Chloride (dihydrate)**
   - CaCl₂ 10.0 g
   - dH₂O bring to 1000 mL

5. **Incubation Medium**
   - ATP 0.255 g
   - Basic Medium 150 mL

   *Bring to a pH of 4.5
   **Make fresh daily*
6. **Pre Incubation Wash**
   - 1% Calcium Chloride 150 mL

7. **Pre Incubation Medium**
   - Acid medium 150 mL
   *Bring to pH 4.5

**Procedure:**

1. Remove slides from fridge and allow to warm to room temperature
   *Slides must be dry

2. Add slides to pre-incubation medium (4.5 pH) 2 min

3. Run through 2 rinses in Tris Buffer 2 min (each)

4. Incubate in Incubation Medium 25 min

5. Rinse in 1% Calcium Chloride solution 3x4 dips (each)
   *change solution as shown:
   a. 4 dips
   b. 4 dips
   c. 4 dips

6. Rinse in Azure A 10 sec

7. Rinse under running dH₂O 10 sec

8. Dehydrate slides rapidly as follows: 5 dips (each)
   a. 95% Ethanol
   b. 100% Ethanol
   c. 100% Ethanol

9. Place in Xylene (Use fume hood) 5 min

10. Mount with paramount
A.3. Capillary and Fiber Type Stain (Rosenblatt J. et al. 1987)

**Preparation of Solutions:**

1. **Fixative (5% formalin buffered at pH 7.6)**
   
   Formaldehyde Solution (40%)  
   50 ml  
   Na cacodylate (MW 160) (0.144 M)  
   31 g  
   CaCl₂ (MW 147) (0.068 M)  
   10 g  
   Sucrose (MW 342) (0.332 M)  
   115 g  

2. **Incubation Medium**
   - Mix just prior to use, add *Solution 1* to *Solution 2*
     a. *Solution 1*  
        Gelatin  
        300 mg  
        0.1 m tris (hydroxymethyl)aminomethane maleate buffer  
        20 mL  
     
     b. *Solution 2*  
        ATP  
        25 mg  
        0.06 M lead nitrate (prepared daily)  
        3 ml  
        0.068 M Calcium Chloride  
        5 ml  
        double distilled water  
        20 ml  

   *Make fresh daily*  
   *Adjust to pH 7.2 prior to use with NaOH*  

3. 2% ammonium sulfide (v/v)

**Procedure:**

1. Place slides in Coplin Jars, refrigerate at 4°C for 30 min prior to staining.
2. Fix for 5 min at 4°C in buffered fixative (5% formalin buffered at pH 7.6)
3. Rinse in distilled water (15 successive fillings of Coplin Jar)
4. Incubate with incubation medium for 60 min at 37°C in a shaker bath
5. Rinse in distilled water (15 times)
6. Develop in 2.0% ammonium sulfide (v/v) for 1 min.
7. Dehydrate in successive alcohol baths  
   a. 5 dips 95% ethanol  
   b. 5 dips 100% ethanol  
   c. 5 dips 100% ethanol
8. Clear in xylene (5 min or 2 min)