Milk and Exercise in childhood Obesity

EFFECT OF MILK AND EXERCISE IN CHILDHOOD OBESITY: PROTEIN METABOLISM, CARDIOVASCULAR HEALTH AND INFLAMMATION

By

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**LAY ABSTRACT**

The aim of this thesis was to determine if milk and exercise work together to: promote body fat loss while maintaining protein balance and muscle in overweight adolescents; increase fitness and strength; and assess changes in inflammatory markers and disease risk factors. A 7-day diet with 1-hour exercise sessions was performed with milk or a carbohydrate drink. Protein balance was measured by a urine marker. Pre and post the following measures were used: cycling test for power; weight lifting test for strength; blood for disease markers; and blood pressure. After the week, the milk group were in a more positive protein balance, maintained more muscle, had a greater loss in body fat, greater increase in power and lower blood pressure compared to the carbohydrate group. Some of the inflammatory markers increased in both groups. Exercise combined with the nutrients from milk can have a positive effect on adolescent health.

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**ABSTRACT**

The aim of this thesis was to determine the synergistic effect of milk and exercise to: promote fat loss while maintaining protein balance and muscle; improve cardiovascular fitness and strength; and to evaluate the short-term adaptations of the inflammatory system and metabolic risk factors in overweight adolescents (boys ages 11-15 years, girls ages 9 to 13 years). A 7-day dietary intervention with 1-h intense exercise was used with randomization to milk (MILK: n=26, 8 male, 18 female) or carbohydrate beverage (CONT; n=29, 12 male, 17 female) post exercise. Both groups received a diet based on their resting energy expenditure. To determine whole body protein balance (WBPB), participants consumed 15N-glycine. Subjects performed a maximal cycling test to determine changes in power and 1-repetition maximum testing to determine changes in strength. Blood was taken to evaluate glucose and insulin; tumour necrosis factor-alpha (TNF-α), interleukin-6 and c-reactive protein ; and blood pressure was measured pre and post intervention. Although body weights did not change, the MILK group maintained more muscle (-0.2 ± 0.6 vs. -0.7 ± 0.8 kg, p<0.01) and had a greater loss in body fat (-0.4 ± 1 vs. 0.5 ± 1.0 %, p<0.006). The changes in body composition were supported by a greater WBPB after training in the MILK group (1.64 ± 1.1 vs. 0.84 ± 0.6, p<0.001). Power increased only in the MILK group with an increase of 0.13 watts/kg (p<0.05) with an increase in V02max that approached significance(p=0.06). Improvements in strength and the blood risk factors were not different between the groups. There was a greater decline in mean arterial pressure in the MILK group

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(-3 ± 6 mmHg vs. 2 ± 7 mmHg, p< 0.04). The exercise intervention led to an increase in TNF-α in both groups (0.3 ± 0.7 pg/ml vs. 0.5 ± 0.7 pg/ml, p<0.001). These data support the consumption of milk after exercise in the early stages of pediatric obesity treatment.

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Lastly, I would like to dedicate this thesis to my Uncle Eldon who passed away during writing of this document. He said to me that “there should be another doctor in this family.” It appears that this may actually ‘at long last’ happen.

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**PREFACE**

The work presented here is a sandwich style thesis. There is a general introduction to the concepts relating to experiments performed. Chapters 2, 3 and 4 constitute manuscripts that have been submitted for publication. Preceding each manuscript chapter is a preface detailing the significance to the thesis as a whole and contributions of each author to the work. Some of the content found in the background section in Chapter 1 may be repeated in other chapters. Work was conducted from 2009 to 2015.

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**LIST OF ABBREVIATIONS**

|  |  |
| --- | --- |
| **1RM:** | one repetition max |
| **APHV:** | age of peak height velocity |
| **B:** | protein breakdown |
| **BIA:** | bio-electrical impedance analysis |
| **BPM:** | beats per minute |
| **CONT:** | control group |
| **CVD:** | cardiovascular disease |
| **CRF:** | cardio-respiratory fitness |
| **CRP:** | c-reactive protein |
| **DASH:** | Dietary Approaches to Stop Hypertension |
| **DXA:** | dual-energy x-ray absorptiometry |
| **DRI:** | Dietary Reference Intakes |
| **E:** | Urinary nitrogen excretion |
| **FFM:** | fat free mass |
| **GI:** | gastrointestinal |
| **HIT:** | high intensity training |
| **HTN:** | hypertension |
| **HR:** | heart rate |
| **I:** | dietary nitrogen intake |
| **IL-6:** | interleukin-6  xvi |
| **LBM:** | lean body mass |
| **MAP:** | mean arterial pressure |
| **MILK:** | experimental milk group |
| **NB:** | nitrogen balance |
| **PMP:** | peak mechanical power |
| **PSMF:** | protein sparing modified fast |
| **Q:** | nitrogen flux |
| **RD:** | Registered Dietitian |
| **RDA:** | Recommended Dietary Allowance |
| **RER:** | resting energy expenditure |
| **RPE:** | rate of perceived exertion |
| **RPM:** | revolutions per minute |
| **RQ:** | respiratory quotient |
| **RMR:** | resting metabolic rate |
| **S:** | protein synthesis |
| **TNF-α:** | tumour necrosis factor-alpha |
| **TTE:** | time to exhaustion |
| **T2DM:** | type 2 diabetes mellitus |
| **V02max** | maximal oxygen consumption |
| **WBPB** | whole body protein balance |

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**CHAPTER 1**

**General introduction and objectives**

**I INRODUCTION AND OBJECTIVES**

**1.1. The problem of childhood obesity**

Childhood obesity in Canada and around the world is causing alarm and urgent calls to action by international, national, and local agencies (Ontario Medical Association, 2005; Tremblay MS, Katzmarzyk PT, & Willms JD, 2002; Dabelea D, Bell RA, & D'Agostino RB Jr., 2007). Obese children are at a considerably higher risk for Type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) – conditions that may be prevented by regular physical activity (Singh R, Shaw J, & Zimmet P, 2004; Kelsey MM, Zaepfel A, Bjornstad P, & Nadeau KJ, 2014; Vasconcellos F et al., 2014). In Canada, for example, the Ontario Medical Association has expressed that they “*are very concerned that we may be raising the first generation of children who will not outlive their parents”* (Ontario Medical Association, 2005) as demonstrated in Figure 1*.* Rapid increases in childhood obesity over the last three decades (Tremblay MS et al., 2002) have resulted in rising rates of insulin resistance (Kim G & Caprio S, 2013), and 25% of obese youth presenting to weight management programs today have pre-diabetes (Sinha R, Fisch G, & Teague B, 2002; Wiegand S et al., 2004), a risk factor for developing T2DM. Ninety percent of children diagnosed with pediatric primary hypertension are obese (Flynn J, 2013) which plays a role in the atherosclerotic plaque formation seen in cardiovascular disease (Montero D, Walther G, Perez-Martin A, Roche E, & Vinet A, 2012; DeBoer MD, 2013). Visceral adipocytes secrete inflammatory cytokines that play a role in the above health concerns (Visser M, Bouter LM, McQuillan G, Wener MH, & Harris TB, 2001).

Figure 1: Health consequences of pediatric obesity



Treatment programs for childhood obesity must therefore focus on reversing these significant metabolic consequences. A systematic review of randomized controlled trials for the treatment of childhood obesity (Summerbell CD et al., 2004) concluded that although the evidence was not strong, interventions with the most promise (based on weight loss) incorporate nutritional counseling and physical activity programs (Epstein LH, Wing RR, Koeske R, & Valoski A, 1985), reward reduction in sedentary activities (Epstein LH, Valoski A, & Vara LS, 1995) and include behavioural therapy components (Epstein LH, Myers MD, Raynor HA, & Saelens BE, 1998). Similar conclusions were reached in the published Canadian Clinical Practice Guidelines for the Prevention and Treatment of Obesity in Adults and Children (Lau DC & Obesity Canada Clinical Practice Guidelines Steering Committee Expert Panel, 2007). A key factor in this relationship is physical activity. In light of Canada’s guidelines and on the heels of physical activity recommendations from the United States (Williams AJ et al., 2013), it was timely to conduct this trial, which helped inform *how* physical activity should be prescribed for obese youth (Spear BA, Barlow SE, & Ervin C, 2007). After exercise there is a negative net protein balance as muscle is catabolized for the physical activity and protein synthesis cannot keep pace with protein breakdown (Balagopal P, 1998). This imbalance needs to be restored with the consumption of nutrients. When protein and carbohydrate are consumed post exercise, the catabolism of muscle during exercise can be avoided (Rasmussen BB, Tipton KD, Miller SL, Wolf SE, & Wolfe RR, 2000). In particular, the constituents of milk provided post exercise can promote favourable changes in body composition with increases in lean mass gain and loss of total and visceral fat in overweight adults on a weight reducing diet (Josse AR, Atkinson SA, Tarnopolsky MA, & Phillips SM, 2011). In youth, the problem is how do you impose the necessary energy deficit to eliminate body fat without negatively affecting the processes of growth needed to conserve lean mass, including muscle and bone (Amador M, Ramos L, Morono M, & Hermelo M, 1990). Therefore, the objectives of this study were to determine the effects of short-term exercise training with and without milk post exercise in overweight youth. The specific effects included:

1) whole body protein balance;

2) anthropometrics including weight, waist circumference, percent body fat and lean

body mass;

3) fitness indicators such as a) maximum oxygen consumption and time to exhaustion in exercise tests and b) muscle strength during weight lifting protocols;

4) metabolic health including mean arterial pressure (MAP), insulin, glucose and Homeostatic Model Assessment (HOMA); and

5) inflammatory profile including C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6).

# 1.2. Childhood obesity, protein metabolism and lean mass

Protein is an essential component of all tissues, and its turnover involves a dynamic process of degrading old proteins and constructing new ones. The equilibrium of this remodeling process (synthesis and breakdown) leads to protein balance, allowing for cell growth (Balagopal P, 1998). Obesity is a condition that disturbs protein metabolism. Compared to non-obese children, obese children have 45 percent higher protein turnover with higher whole body protein synthesis and breakdown (Balagopal P et al., 2003; Schutz Y, Rueda-Maza C, Zaffanello M, & Maffeis C, 1999). However, when these variables are adjusted for fat-free mass (FFM), the difference is no longer apparent. Obese individuals have elevated levels of FFM to sustain the disproportionate adipose tissue (Schutz Y et al., 1999). It is approximated that FFM may account for as much as 50 percent of the excess weight (Dietz W & Hartung R, 1985b). Adolescents who develop obesity earlier in life, infancy as compared to childhood, have even greater lean tissue deposition (Cheek DB, Schultz RB, Parra A, & Reba RC, 1970).

Despite this supplementary FFM, it is necessary during adolescence to maintain or expand FFM for a number of reasons. First, adolescence is characterized by a rapid onset of growth, second only to the first year of life. Protein anabolism occurs to support the gain in lean tissue required, especially for skeletal muscle (Bitar A, Fellmann N, & Vernet J, 1999). In fact, weight loss in the course of the pubertal spurt has been “advised against” due to a reduction in linear growth velocity (Amador M et al., 1990; Dietz W & Hartung R, 1985a). Secondly, “inappropriate” weight reduction results in an alteration in the resting metabolic rate (RMR) due to loss of FFM. The RMR is the energy expended by bodily tissues to sustain body function at rest. It is dependent on various factors but is principally related to the quantity of FFM (Stiegler P & Cunliffe A, 2006). Indeed in adults, those who preserve the most FFM have less weight regain after ceasing a weight loss program (Westerterp-Plantenga MS, Lejeune MPGM, Nijs I, van Ooijen M, & Kovacs EMR, 2004).

Thus it is prudent to supply the overweight adolescent with the suitable diet and exercise protocol to maintain protein balance for preservation of muscle and growth but at the same time expend sufficient calories to reduce excess adiposity, which is often the source of metabolic complications. What constitutes the appropriate activity pattern and diet composition as well as the amalgamation of the two aspects is discussed below.

# 1.2.1 Physical activity treatment and lean mass

*Aerobic Exercise:* There is a paucity of data on the effects of aerobic exercise, independent of diet adjustments, on protein balance in children in general and in overweight children, in particular. In a study with healthy children, Bolster et al (Bolster DR, Pikosky M, McCarthy LM, & Rodriguez NR, 2001) evaluated seven subjects aged 8 to 10 years of age. The subjects undertook a walking program five days a week for 45 to 60 minutes with intermittent rest periods. Protein turnover was evaluated with the 15N-glycine stable isotope methodology. This technique involves oral consumption of a stable isotope and measurement of the marker in urine which allows for calculation of protein synthesis, flux and breakdown. After six weeks, there was a considerable reduction in protein synthesis and protein breakdown. The authors deemed that the negative protein balance was induced by the enhancement in energy expenditure, as energy and protein intakes did not change over the study period. Lean body mass did not change (Bolster DR et al., 2001).

In obese children, it has not been established if protein balance is modified with aerobic exercise but alteration in FFM with aerobic activity has been researched. Although it has been demonstrated that FFM increased with exercise, the majority did not involve a control group (Sasaki J, Shindo M, Tanaka H, Ando M, & Arakawa K, 1987; Gutin B, Ramsey L, & Barbeau P, 1996; Ferguson MA et al., 1999). In boys, it is expected that FFM will increase consistently between ages 6 to 11 years and during the course of adolescence (Wells JCK, 2003) by normal growth and development. Therefore it is challenging to resolve if the increase in lean body mass was the same, less or more then would be expected at this time in development. In a study that did employ a control group, the treatment group underwent a 40 minute session of exercising on cardio machines and playing games at 70 to 75% of max heart rate for five days per week for four months. The control group was requested not to modify activity levels. Despite this advisement, their levels declined (as summer progressed to fall and winter during the four months), but FFM in kg increased. The children in the treatment segment also had an increase in FFM, and it was significantly more than the control group. Unfortunately, the outcomes are confounded by the reduction of exercise of the control children (Owens S, Gutin B, & Allison J, 1999). The same research group, using subjects as their own controls in a cross-over design, observed an increase in FFM during the exercise component of the study which they attributed to normal growth (Ferguson MA et al., 1999). Diets were not analyzed in any of the aforementioned FFM studies, which could have accounted for changes in lean body mass.

*Resistance Exercise:* Resistance training is a series of progressive exercises that enhance a person’s capacity to exert muscular force against resistance (Sothern MS, Loftin JM, & Udall JN, 2000a). Historically, resistance training in children was viewed as unproductive and unsafe. However recently, the American College of Sports Medicine and the Canadian Society for Exercise Physiology advocate that children can participate in weight training if part of a supervised and well-designed program (Behm DG, Faigenbaum AD, Falk B, & Klentrou P, 2008). It can also be included in successful multidisciplinary obesity treatment programs for pre-adolescents and adolescents (Schwingshandl J, Sudi K, Eibl B, Wallner S, & Borkstein M, 1999; Benson AC, Torode ME, & Fiatrone Singh MA, 2008; Sothern MS, Loftin JM, & Udall JN, 1999). Adherence to weight training programs can be better than walking programs. For example, the number of participants that completed a one-year exercise program was 79% with weight lifting and only 21% in those who walked, suggesting that overweight youth may be more likely to sustain resistance-type activities (Sothern MS et al., 2000a).

The effects of resistance exercise on protein use in children have not been extensively studied. Pikosky et al (Pikosky M, Faigenbaum A, Westcott W, & Rodriguez N, 2002) examined 11 children of varied adiposity and had them undertake six weeks of resistance training, twice per week for 30 to 40 minutes. Protein synthesis and breakdown decreased. Although net protein utilization did not decrease significantly, it approached a net loss (p=0.07). Diets were held constant. It was not apparent whether the overweight children’s results were analogous to the healthy weight children in the study.

There are a small number of studies regarding the effect of resistance training (without aerobic activity or dietary changes) on FFM. Without controls or dietary information, a few have indicated that FFM does not change with resistance training (McGuigan MR, Tatasciore M, Newton RU, & Pettigrew S, 2009; Benson AC et al., 2008). In contrast, Shaibi et al (Shaibi GQ, Cruz ML, & Ball GD, 2006) had adolescent males randomly assigned to either a twice per week resistance training program or non-exercising control group for sixteen weeks. Lean body mass increased appreciably in both groups but was not statistically different between the groups.

*Aerobic Exercise and Resistance Exercise Combined:* There are only a few studies in which aerobic activity and resistance training have been united (devoid of dietary changes) to determine if the combination changes lean body mass (DeStefano RA, Caprio S, Fahey JT, Tamborlane WV, & Goldberg B, 2000; Watts K, Beye P, & Siafarikas A, 2004; Bell LE, Watts K, & Siafarikas A, 2007). None had non-treatment groups or controlled for diet. Two found no change in FFM and one established an increase, but the results could not be distinguished from normal growth.

*High Intensity Training:* Although there is no universal definition, high intensity training (HIT) refers to repetitive sessions of short intermittent exercise. A single effort may last a few seconds or up to a few minutes. Multiple efforts are separated by several minutes of rest with low-intensity exercise. It is not performed against resistance but usually involves cycling or running and significant hypertrophy does not occur (Gibala MJ & McGee SL, 2008). Tjonna et al (Tjonna AE, Stolen TO, & Bye A, 2009) has used this technique with overweight adolescents. The subjects were randomized to either aerobic interval training twice per week for three months or to a group with one hour of activity (not described) 3 times per week. Lean mass increased in both but was not statistically different between the two groups. A criticism of the paper was that the HIT group consumed less protein for the duration of the study (Tjonna AE et al., 2009).

The interesting aspect of HIT is its similarity to the natural physical activity patterns of children. When a child engages in physical activity, it tends to be in short bursts of relatively high intensity, with the majority of time spent in very low intensity activities (Bailey RC et al., 1995). This kind of physical activity carries connotations of “fun” for the child and may increase the likelihood of sustaining physical activity, which is essential to the long-term management of childhood obesity (Alexander SA, Frohlich KL, & Fusco C, 2014).

Based on the available literature, the impact of exercise training on protein metabolism in obese adolescents is not clear. Protocols that incorporate different types of exercise, including aerobic, resistance, and HIT, should be studied, because variety in physical activity may be more likely to lead to sustainability of behavior in this population (Alexander SA et al., 2014). The problem is that we do not know the short-term impact of a combination of these activity types on protein turnover.

**1.2.2 Reduction of calories and lean mass**

Although numerous studies have evaluated reduction of calories and subsequent effects on body fat, only a few have covered the effects on protein balance or FFM as outlined below. Diets extremely low in calories negatively affect protein metabolism in obese adolescents (Merrit RJ, Bistrian BR, Blackburn GL, & Suskind RM, 1980; Pencharz PB, Motil KJ, Parsons HG, & Duffy BJ, 1980). In both of these studies, the Protein Sparing Modified Fast (PSMF) was used. This extreme regime provides approximately 900 calories soley in the form of protein (2 to 2.5 grams protein/ kg ideal body weight), no added fat, and adequate fluid intake and vitamin/mineral supplements (Bell L, Chan L, & Pencharz PB, 1985). After one week, protein balance declined (Pencharz PB et al., 1980). However, there was considerable inter-individual variation. At four weeks, half of the subjects did not achieve protein balance nor did it recover over the total three months. After completing the PSMF, the subjects followed a more moderate diet plan, balanced with all the food groups, to either maintain or continue weight loss. Total body nitrogen continued to decline for a full year (Pencharz PB et al., 1980). In other studies, using more balanced, but still very low calorie diets (approximately 1000 kcal), fat free mass either did not increase or increased modestly (0.43 kg) (Schwingshandl J et al., 1999; Woo KS, Chook P, & Yu CW, 2004).

Direct comparisons between very low calorie diets and other dietary strategies that are not as extreme have not been undertaken in obese adolescents. Adults on very low calorie diets lose more weight at the initiation of the program but gain the majority back, thus in the long term are not successful (Marinilli Pinto A et al., 2008). Using 16 children between the ages of 8 to 10 years, over six weeks, Ebbeling et al (Ebbeling CB & Rodriguez NR, 1998) had subjects moderately reduce calories from their diets. Despite being less severe, net protein balanced declined due to a decrease in protein synthesis, with no change in breakdown. There was also a slight decline in FFM.

In summary, few studies have focused on reducing calories and lean mass in obese children. But the available evidence does suggest that significant changes to diet alone could have major impact on lean mass development. The potential impact on long-term growth is unknown. However, it seems prudent to explore the role of appropriate energy intake (i.e., that which meets needs), along with physical activity-induced energy deficit and level of protein intake.

**1.2.3 Combined exercise and dietary changes and lean mass**

Ebbeling et al. (Ebbeling CB & Rodriguez NR, 1999) undertook a protein balance study that reviewed the additive effects of diet plus exercise. Obese 8 to 10 year old children had a modest decrease in calories, enough to lose less than 0.5 kg per week, for six weeks. Protein synthesis, breakdown, net turnover, nitrogen flux and nitrogen balance all decreased significantly over that time. The diet was maintained but exercise, walking five days per week for a subsequent six weeks, was added. Protein synthesis, breakdown and nitrogen flux increased above baseline but net turnover and nitrogen balance remained at the lower levels. There was only a slight reduction in FFM over the entire 12-wk study.

Another research group (Balagopal P, 1998) investigated moderate losses of weight from minor dietary changes and modest exercise starting at 20 minutes and progressing to 45 minutes three times per week in adolescents. Leucine flux, which is an indicator of whole body protein breakdown, decreased over the three-month study. The non-oxidative portion of leucine disposal, which indicates whole body protein synthesis, did not change. In total, there was an overall reduction in protein turnover. FFM did increase. This study incorporated an obese control group into the design who were not educated on lifestyle changes. They did not have any variation in whole body protein turnover or FFM over the study period (Balagopal P, 1998).

When the PSMF or a very low calorie diet is combined with aerobic and resistance exercise, an increase in lean body mass can be observed after ten weeks (Schwingshandl J et al., 1999; Sothern MS, Vdall Jr JN, Suskind RM, Vangas A, & Blecker V, 2000b). However, it is uncertain if this was a result of growth or the treatment program. In contrast, when Woo et al (Woo KS et al., 2004) combined resistance and aerobic exercise with a 900 to 1200 kcal diet, FFM did not change. In another exercise (aerobic and resistance) combination study, with a reduction in calories of 15 to 20%, FFM did not change over nine months (Lazzer S, Boirie Y, & Poissonnier C, 2005).

Notwithstanding the methodological issues of the above studies as it relates to growth and development, the available evidence does seem to point to the benefit of combining increased physical activity with an appropriate dietary strategy. Because these studies have been longer-term (weeks to months), it was important to step back and evaluate the short term responses of protein turnover to combinations of nutrients and bouts of exercise. With this new understanding of the role of specific nutrients and functional foods in enhancing the benefit of acute bouts of exercise, this study will give us the confidence to design the best possible longer-term intervention trials. From a clinical perspective, learning how to make the most out of what exercise obese adolescents do engage in would be of great significance.

# 1.2.4 Focus on Protein Intake: Quality, Quantity and Timing

The current recommendations for protein requirements in adolescents, based on Dietary Reference Intakes (DRI), were developed through studies on protein balance in healthy weight children and adults (National Academy of Sciences, 2009). For the ages nine through 13 years, using total body potassium as a marker, 292 Caucasian children were evaluated (Ellis KJ, Shypailo RJ, Abrams SA, & Wong WW, 2007). Energy intake and activity levels were not assessed. The Estimated Average Requirement is “estimated” by the factorial method. This method approximates the nitrogen losses and the amount accreted during periods of growth. The protein requirement is then estimated by extrapolating the losses to zero balance point in which protein (nitrogen) needs are equal to the obligatory protein (nitrogen) lost. The miscalculation in this method is that the association between protein intake and nitrogen retention is curvilinear denoting that the efficiency of the nitrogen retention becomes less as the zero balance point is reached. To indicate the need for 97.5% of the population, the Recommended Dietary Allowance (RDA) is determined at 0.95 g/kg/ day or 34 g of protein. In contrast for adolescents, ages 14 to 18 years, the maintenance nitrogen requirement was taken from adults plus an additional amount to comprise the needs for growth, as again determined by the factorial method. Using a meta-analysis, 235 adults, again at a healthy weight, were studied (Rand WM, Pellett PL, & Young VR, 2003), and the RDA was determined to be 0.85 g/kg/day or 52 g of protein. The authors discussed a major limitation to their data that makes the requirement questionable for obese individuals. It is accepted that nitrogen metabolism and nitrogen balance vary depending on energy intake. The subjects that were evaluated had intakes that matched their energy expenditure. But one third of the variation in nitrogen balance can be attributed to variations in energy intake (National Academy of Sciences, 2009).

It is conceivable that protein balance can occur with a low energy intake (Millward DJ, 2004). In malnourished children, a high fat, low protein diet results in lower increases in height compared to an isoenergetic high protein diet (Malcolm LA, 1970). The provision of extra protein not only increased height but mobilized fat to decrease total body fat. There is marked variability in nitrogen utilization during rapid weight gain. Changes in the composition of deposited tissues can determine growth rates with different energy intakes affecting adipose and lean tissue magnitudes. In animals losing weight, a positive nitrogen balance can be achieved with high protein low energy intakes (Coyer PA, Rivers JPW, & Millward DJ, 2009).

The other contentious issue with the RDA for protein is whether exercise can increase the requirements for protein. This is particularly debated in the adult literature but will not be argued here (Gibala MJ, 2007). Male adolescent soccer players who trained seven to nine hours per week were in positive nitrogen balance but consumed 1.68 g/kg body weight per day, which is clearly above the RDA. They had a control group of non-active adolescents who also consumed an analogous amount of protein but were also in similar nitrogen balance indicating the more active soccer players did not require additional protein to sustain their physical activity (Boisseau N, Le Creff C, Loyens M, & Poortmans JR, 2002). The authors resumed their line of research and provided soccer players with diets of varying amounts of protein (1.4, 1.2 and 1.0 g/kg body weight) to determine the amount that was required for nitrogen balance over three balance periods (four days in length) in descending amounts of protein. Energy intake was adjusted for body weight, as there was a broad range. The subjects were in nitrogen balance with 1.4 g/kg body weight of protein, and it was significantly correlated with protein intake and energy balance (Boisseau N, Vermorel M, Rance M, Duche P, & Patureau-Mirand P, 2007).

To revisit the studies listed above (Section 3.3 Combined exercise and dietary changes and lean mass), these investigations reduced energy intake, but usually took care to supply protein intakes above the RDA, on average 1.1 g/kg body weight (with protein intakes even greater in the PSMF) (Balagopal P et al., 2003; Schwingshandl J et al., 1999; Sothern MS et al., 1999; Pencharz PB et al., 1980; Woo KS et al., 2004; Ebbeling CB et al., 1998; Ebbeling CB et al., 1999; Lazzer S et al., 2005). Thus despite high quantities of protein being provided, or at least above the RDA, nitrogen balance declined in a number of studies. The type and timing of protein has been shown to facilitate protein balance and FFM as described below. Evaluation of these studies is relevant in the context of using dietary strategies to optimize protein balance following exercise in overweight youth.

*Short-term Studies:* Muscle protein synthesis is stimulated above resting levels with exercise in adults. Muscle protein breakdown also rises with resistance exercise in the fasted state (Phillips SM, Tipton KD, Aarsland A, & Wolf SE, 1997). This catabolism can be avoided with intake of amino acids (Biolo G, Tipton KD, Klein S, & Wolfe RR, 1997). When a balanced amino acid mixture was infused at rest and three hours after one bout of leg resistance exercise, there was an increase in protein synthesis with no change in muscle breakdown. Accordingly, the impact on muscle growth (i.e., lean mass) must be due to an interactive effect of exercise and nutrient intake. At rest, the amino acid infusion increased protein synthesis by 150% but after amino acids and exercise were amalgamated it increased 200%. The mechanism proposed by the authors was that exercise stimulated protein synthesis that caused intracellular amino acids to be incorporated into protein at an increased rate. Thus, there was a transient drop in protein breakdown to restore intracellular amino acids. The supplementary infusion of amino acids helped maintain the basal levels of amino acids (Biolo G et al., 1997). The window needed for this increased protein metabolism appears to be one to three hours after exercise (Rasmussen BB et al., 2000).

Furthermore, the type of protein has an influence on muscle hypertrophy. Proteins such as whey and soy are rapidly digested and stimulate protein synthesis. In contrast, casein protein causes a slower and longer lasting rise in plasma amino acids and suppresses protein breakdown (Boirie Y et al., 1997). Thus, a combination of fast and slow protein should support muscle anabolism. Indeed, casein and whey promoted muscle protein balance after resistance exercise in healthy adults (Tipton KD et al., 2004). They were randomized to a placebo, casein or whey protein drink one hour after a heavy leg resistance exercise bout. Net amino acid balance became positive after the two protein drinks but remained negative for the placebo. This indicated that single proteins can stimulate muscle protein synthesis (Tipton KD et al., 2004).

Carbohydrate post exercise can also improve muscle protein balance in adults (Borsheim E et al., 2004). After ingesting a drink with 100 g carbohydrate one-hour post exercise verses a non-caloric placebo drink, net protein balance improved to some extent with the carbohydrates mostly due to decreased muscle protein breakdown, but did not reach positive values. The authors felt the reported effects of amino acid administration had a stronger benefit for muscle building. Collectively, protein and carbohydrate seem to have a positive effect on protein metabolism. Rasmussen et al (Rasmussen BB et al., 2000) randomly gave healthy weight men and women either a treatment beverage with 6 g essential amino acids and 35 grams sucrose or a flavored placebo beverage one or three hours post exercise. There was increased muscle protein synthesis with the essential amino acid drink but not with the placebo. Muscle protein breakdown did not change with either beverage. Thus net balance increased with both drinks but was significantly greater in the protein/carbohydrate drink. Carbohydrates cause a rise in insulin levels and this hormone is a requisite for protein synthesis. The interaction of insulin, amino acid availability and resistance exercise caused the heightened protein anabolism (Rasmussen BB et al., 2000).

Fluid bovine milk contains approximately 80% casein and 20% whey so is capable of providing a balance in protein synthesis and breakdown. It also contains carbohydrate (Elliott TA, Cree MG, Sanford AP, Wolfe RR, & Tipton KD, 2006). In fact, milk consumption after one bout of resistance exercise has been shown to enhance protein metabolism in adults (Elliott TA et al., 2006). Greater muscle protein accretion and positive nitrogen balance were reported with milk as opposed to soy. The two intact proteins provided equal quantities of essential amino acids, but there was dissimilarity in digestion rates. Milk provides a slower delivery of amino acids to the muscle. Thus the muscle is able to pick up amino acids over an extended period to enhance muscle building. The rapid digestion of soy protein results in faster delivery of amino acids from the gut to the liver so there is an increase in amino acids available for synthesis of serum proteins and urea (Bos C, Metges CC, & Gaudichon C, 2003).

While the above studies focus on protein metabolism in adults, they provide a relevant discussion of the interaction between acute bouts of exercise and protein intake among children. Research in children is restricted to the whole body level due to the ethical restraints of muscle biopsies, although the novelty in this thesis was to investigate potentially similar effects of milk in obese children. In a series of experiments, Dietz et al (Dietz W & Schoeller DA, 1982; Dietz W & Wolfe RR, 1985) determined the protein balance of adolescents on a weight reduction diet (number of calories not stated) with differing macronutrient composition. The diets were isonitrogenous (1.5 grams of protein/kg body weight) but differed in the remaining calories with either 1 gram glucose per kg ideal body weight or made isocaloric with fat. Both diets caused a negative protein balance and loss of FFM on a weekly and cumulative basis but the losses were more substantial with the diet not containing carbohydrate. Clearly, more research is needed to compare the effects of combinations of nutrients versus single nutrients on protein metabolism in obese youth.

*Longer-term studies:* In adults,outcomes of milk consumption on exercise for an extended period have also been completed by two research groups. Walberg Rankin et al (Walberg Rankin J et al., 2004) had untrained young men consume either milk or a carbohydrate electrolyte beverage immediately following each resistance workout over 10 weeks. Muscular strength and FFM increased in both groups equally. In contrast, Hartman et al (Hartman JW et al., 2007) concluded that milk was superior to soy and a maltodextrin beverage (that was isoenergetic with milk and soy). Drinks were consumed immediately after exercise with a subsequent beverage one hour later. This timing of beverages was to allow for the intial quick acting whey protein to initiate protein synthesis, and the long-acting casein protein to sustain protein synthesis. The milk group had significantly greater gains in FFM and type I and II muscle fibres after 12 weeks of training. A point of major interest in this study, in regards to obesity, is that the milk group had a greater loss of fat mass compared to the other beverage groups. In a study with obese young women, when milk was consumed post exercise, there was a higher loss of trunk fat and visceral adipose tissue than those without dairy (Josse AR et al., 2011). If similar results could be demonstrated in obese adolescents, this would represent a significant advance in clinical practice by highlighting the importance of exercise and nutrient interactions on weight management.

Many large population based cross-sectional studies have evaluated the relationship between dairy and body composition in children. Youth who consume more dairy have been reported to have a lower incidence of obesity (Spence LA, Cifelli CJ, & Miller GD, 2011). Very few clinical trials have been conducted to specifically assess the effects of milk on body proportions. Most rather had a goal to investigate the effects of calcium supplementation or milk on bone health and development. In most of these studies, the subjects were a healthy body weight with a paucity of trials with obese participants (Spence LA et al., 2011). In randomized controlled trials that included obese youth, differences in weight and body fat were not evident when milk was consumed, but FFM was not always evaluated (Spence LA et al., 2011). In one study, overweight children, had an increase in FFM when 453 grams of milk were added to their diets (Albala C et al., 2008). However, the intervention group also removed 711 grams of sweetened beverages from their diets with the control group adding sweetened beverages that led to an additional 91 kcal per day being added to the control group over 16 weeks. Thus it was not clear if the control group's attenuated increase in lean body mass accumulation was due to lack of milk or increase in sweetened beverages. The positive effects on lean body mass and growth occurred despite no changes in body fat in this trial in the intervention group (Albala C et al., 2008).

Finally, most of the trials did not have participants exercise in a controlled, supervised manner (Albala C et al., 2008; St-Onge MP, Goree LL, & Gower B, 2009; Kelishadi R et al., 2009; Ghayour-Mobarhan M, Sahebkar A, & Vakili R, 2009). Though in one study, participants who had supervised resistance training exercise had no significant changes in body composition with milk compared to water consumption after the exercise over six months (Lambourne K et al., 2013). However, despite more energy being consumed by the milk drinkers they did not gain additional weight or body fat compared to the post exercise water group which would support providing milk after exercise (Lambourne K et al., 2013).

The potential for a unique exercise and milk intervention to help improve weight management for obese youth is clinically relevant and scientifically interesting. However, we first needed a study to evaluate short-term effects on protein turnover before longer-term studies can be implemented appropriately. The number of children that have been evaluated is minimal and dietary and exercise techniques employed have lead to negative protein balance. See Table 1 for review of previous work on protein balance with N-glycine in children that leads credence to this statement that protein balance in children with dietary and exercise modifications requires more study.

Table 1.1: Protein balance in children by N-glycine methodology

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Study | Number of Subjects | Weight Classification | Method | Results |
| A. | 8 | Healthy weight | -Increase moderate exercise  -no diet change | -lower protein synthesis, breakdown and balance |
| B. | 11 | Varying adiposity | -Increase resistance training  -no diet change | -lower protein synthesis and breakdown |
| C. | 16 | Overweight | -same activity  -lower calories | -lower protein synthesis and balance |
| D | 5 | Overweight | -increase moderate activity  -lower calories | -lower protein synthesis, breakdown and balance |

1. Bolster DR, Pikosky M, McCarthy LM, & Rodriguez NR, 2001
2. Pikosky M, Faigenbaum A, Westcott W, & Rodriguez N, 2002
3. Ebbeling CB & Rodriguez NR, 1998
4. Ebbeling CB & Rodriguez NR, 1999

**1.2.5 The Role of Milk for Lean Mass in Overweight Youth: A Theoretical Model**

Dairy products are widely recommended in the healthy diets of adolescents, yet despite this recommendation, 85% of the population is not consuming enough (DaSilva MS & Rudkowska I, 2014). In preschool age, childhood and adolescence, there have been correlational studies in which weight and percent body fat are negatively related to intake of dairy products (Skinner JD, Bounds W, & Carruth BR, 2003; Carruth B & Skinner JD, 2001; Novotny R, Daida YG, Acharya S, Grove JS, & Vogt TM, 2004). A potential mechanism behind this observation is that calcium has a role in adipocyte metabolism. Increasing this nutrient suppresses calcitrophic hormones which facilitate the change of dietary energy from adipose tissue to lean body mass (Zemel MB & Miller SL, 2004). In an intervention trial with adults, when calcium was supplementary to a hypocaloric diet, superior weight and fat loss were achieved on higher doses. However, even higher weight and fat deficits were noticed with a high dairy intake (Zemel MB, Richards J, Milstead A, & Campbell P, 2005). Thus there are likely other constituents of milk which affect nutrient repartitioning. Branch chain amino acids which are rich in whey protein, have been implicated (Zemel MB et al., 2004). The benefits of protein in satiety management should not be ignored (Bendtsen LQ, Lorenzen JK, Bendsen N, Rasmussen C, & Astrup A, 2013). A decrease in hunger with protein is mediated through gastrointestinal (GI) hormones such as cholescystokinin, peptide YY, ghrelin and others. Acutely, the whey protein in milk decreases hunger due to its fast release in the blood and subsequent release of GI hormones. However, the milk protein casein appears to decrease energy intake over seven days but there is no clear evidence that whey or casein is superior at weight loss (Bendtsen LQ et al., 2013). If the correct form of protein and carbohydrate can be provided, such as what is contained in milk, it may facilitate positive protein balance in growing adolescents who are attempting to manage their weight.

**1.3 Childhood obesity and cardiovascular fitness**

Cardio-respiratory fitness (CRF) is the ability of the body to deliver and utilize oxygen to the working muscles during sustained exercise (Savva SC et al., 2013). A high CRF in early adolescence is associated with half the incidence of overweight and obesity during growth into late adolescence (Savva SC et al., 2013). Hypertension, hypercholesterolemia and a pro-inflammatory state is associated with a low CRF in youth (Carnethon MR, Gulati M, & Greenland P, 2005; Barbeau P, Litaker M, & Woods KF, 2002) which is mediated through adiposity. It is not clear if the intensity of activity performed by obese youth determines level of CRF due to the inconsistency of definition of thresholds for moderate and vigorous activity. In observational studies, the amount of vigorous physical activity appears to be a significant predictor of adiposity and fitness level (Parikh T & Stratton G, 2011). However, in more controlled interventions, vigorous and moderate intensity exercise can influence CRF (Parikh T et al., 2011). Quantifying CRF by 1-metabolic equivalents reveals a reverse relationship between CRF and mortality from cardiovascular and all-cause mortality in large scale epidemiological research in adults (Kodama S, Saitko K, & Tanaka S, 2009). As level of pediatric CRF moderately tracks into adulthood, strategies to increase fitness at an early age are warranted (Van Oort C et al., 2013; Soric M, Gostovic MJ, Hocevar M, & Misigoj-Durakovic M, 2014).

**1.3.1 Cardiovascular fitness and physical activity**

**High and moderate intensity aerobic exercise and resistance training**

Although there are a plethora of research groups that have exercised obese children to determine effects on weight and adiposity, the effects of exercise on maximal oxygen consumption (V02max) in exercising obese children is more limited (Gutin B et al., 1996; DeStefano RA et al., 2000; Tjonna AE et al., 2009; Barbeau P et al., 2007). Despite cardiovascular fitness having a genetic component with highly variable inter-individual differences ranging between 20 and 90 % (Hagberg JM, Moore GE, & Ferrell RE, 2001), training, devoid of dietary changes, can have a positive influence on V02max (Tjonna AE et al., 2009; Barbeau P et al., 2007; Gutin B et al., 1996; DeStefano RA et al., 2000). In as little as two days per week for three months, high intensity interval treadmill training can increase V02max by 3 ml/kg/min in overweight/obese adolescents (Tjonna AE et al., 2009). Those with a lower initial V02max may have even greater elevations as those with a VO2max of 22 ml/kg/min had an increase of 5.8 ml/kg/min over three months of varied training equipment working at their anaerobic threshold (DeStefano RA et al., 2000). The intensity level of the exercise may be important as moderate activity at 50-60% of VO2max does not always have a significant effect on Vo2max. Despite exercising five times per week at a moderate pace, children had similar Vo2max increments than a non-exercising lifestyle education group (Gutin B et al., 1996; Blaak EE, Westerterp KR, Bar-Or O, Wouters LJM, & Saris WHM, 1992).

When resistance training is combined with aerobic exercise in a circuit format, fitness at sub-maximal and maximal levels increases (Watts K et al., 2004; Wong PCH et al., 2008; Davis JN et al., 2011). This occurs even if the aerobic exercise is undertaken at a moderate level (Farpour-Lambert NJ et al., 2009). Thus resistance exercise has an effect on aerobic fitness. Indeed, when overweight children were randomized to either a control group, medium to high intensity aerobic training or resistance training group, those undertaking resistance training had a mean increase in VO2max of 8 ml/kg/min over three months which was not different than the aerobic exercise group of 9 ml/kg/min with no change in the controls (Lee S et al., 2012).

Thus it appears that intense aerobic activity and resistance activity combined could benefit the CRF of obese youth.

**1.3.2 Fitness, physical activity and diet**

There are limited data but changes in dietary intake alone do not appear to affect Vo2max (Becque MD, Katch VL, Rocchini AP, Marks CR, & Moorehead C, 1988; Yu CCW et al., 2008). However, it is difficult to determine the effect of the diet, as the exercise groups also did not have an improvement in Vo2max with the moderate activity prescribed in these studies (Becque MD et al., 1988; Yu CCW et al., 2008). However, Lee (Lee S et al., 2012) had adolescent males randomized to either a diet only group or two different exercise groups. The resistance training and aerobic activity groups had an increase in VO2max. However, the control group on a weight maintenance diet did not have a significant change in fitness levels pre and post the three month trial. It is noteworthy that those undertaking moderate exercise combined with a very low calorie diet (800 calories mainly made up of protein) do have an increase in VO2max (Sothern MS et al., 2000b). It is uncertain what component of the diet may have caused this increase or whether it was solely due to the moderate exercise performed. When dietary changes are added to physical activity interventions to lose weight, similar trends are noted with moderate intensity activity coupled with moderate dietary restriction (0.45 to 0.90 kg/week loss) resulting in no change in VO2max (Becque MD et al., 1988; Epstein LH et al., 1989). However, there was not a control group that did not exercise to compare with in this study so firm conclusions could not be reached.

**1.3.3 Aerobic exercise, resistance training and milk**

In adults, milk consumption has also been shown to improve exercise performance in healthy adults (Karp JR et al., 2006b; Lunn WR, Pasiakos SM, & Colletto MR, 2012; Thomas K, Morris P, & Stevenson E, 2009; Ferguson-Stegall L, McCleave EL, & Ding Z, 2011a; Cockburn E, Stevenson E, Hayes PR, Robson-Ansley P, & Howatson G, 2010; Pritchett K, Bishop P, Pritchett R, Green M, & Katica C, 2009; Lee JKW, Maughan RJ, Shirreffs SM, & Watson P, 2008). Acutely, milk protein appears to increase time to exhaustion in a sub-maximal cycling test (Karp JR et al., 2006b; Lunn WR et al., 2012; Thomas K et al., 2009); improve performance times in a 40km time trial (Ferguson-Stegall L et al., 2011a) and peak torque and strength in knee extension exercise (Cockburn E et al., 2010), compared to carbohydrate beverages or water. However, not all researchers have shown acute performance improvements with milk provision post exercise (Pritchett K et al., 2009; Lee JKW et al., 2008).

In one longer training study with overweight women, using a sub-maximal fitness test protocol, cardiovascular fitness improved in a similar manner between the groups with and without milk (Josse AR et al., 2011). This has not been evaluated in obese youth.

There is a paucity of studies, particularly in youth, investigating the effects of milk on resistance training-induced gains in strength (Walberg Rankin J et al., 2004; Hartman JW et al., 2007). Although one research group saw an improvement in seated row and hamstring curl in overweight women (Josse AR et al., 2011), strength does not appear to increase in most exercise routines with training with milk post exercise in adults (Walberg Rankin J et al., 2004; Hartman JW et al., 2007), or in non-obese and obese adolescents, compared to other beverages (Volek JS et al., 2003; Lambourne K et al., 2013). Given the paucity of data in obese adults and adolescents, determining the effects of milk with exercise training are warranted. It is not clear if other components of the diet had an impact on strength or cardiovascular fitness in the aforementioned studies as none of the studies included regimented diets to ensure that the participant groups were consuming similar calories, carbohydrates and protein throughout the day, particularly in the pre-exercise time period, as this can also impact performance (American Dietetic Association, Dietitians of Canada, American College of Sports Medicine, Rodriguez NR, & DiMarco NM Langleys S, 2009). Therefore it is necessary to provide participants with a specific timed diet to ensure it was the milk having the effect and not some other component of the diet consumed. It would be scientifically and clinically interesting to study if one food component when combined with exercise could have a positive effect on fitness and strength and lead to a more concrete prescription for exercise in pediatric obesity and health.

**1.4 Childhood obesity and inflammation**

**1.4.1 Chronic inflammation in pediatric obesity**

Adipose tissue is no longer recognized solely as a storage site for lipids but also functions as an endocrine and immune centre (Huang CJ, Zourdos MC, Jo E, & Ormsbee MJ, 2013). It is the site of essential hormones, cytokines, chemokines, growth factors and complement proteins. These substances control the homeostasis of the whole body and assist in regulation of food intake, energy expenditure, glucose and lipid metabolism, insulin sensitivity, angiogenesis, vascular remodeling and cell growth (Lee H, Lee IS, & Choue R, 2013). Secretary molecules from adipocytes and hepatocytes, such as pro-inflammatory cytokines TNF-α, IL-6 and CRP, have a pathophysiological role in obesity and co-morbidities (Huang CJ et al., 2013; Visser M et al., 2001).

During excessive weight gain, the influx and storage of excess lipids in adipocytes perturbs normal cell function and results in the hyper-secretion of inflammatory molecules into the circulation (Huang CJ et al., 2013). The pathological growth of adipose tissue is associated with inadequate vascularization and hypoxia (Xu H, 2013). As of 20 years ago, it has been widely established that obesity is a state of low-grade systemic inflammation in adults (Huang CJ et al., 2013) and children (Schwarzenberg SJ & Sinaiko AR, 2006), and this chronic state has deleterious effects on multiple organs (Li ZY, Wng P, & Miao CY, 2011). These include liver, muscle, hypothalamus and pancreas which leads to T2DM and cardiovascular disease (Li ZY et al., 2011; Xu H, 2013).

**1.4.2** **Inflammation diet and exercise**

When overweight children undertake exercise intervention trials, without dietary modifications, the chronic elevation of inflammatory cytokines such as CRP, TNF-α and IL-6 are not reduced when weight and/or body fat are maintained or elevated post training (Murphy EC-S et al., 2009; Kelly AS, Steinberger J, Olson TP, & Dengel DR, 2007; Nassis GP et al., 2005). It is equivocal whether a decrease in fat mass post training causes a reduction in inflammatory markers due to limited data in overweight children (Meyer AA, Kundt G, Lenschow U, Schuff-Werner P, & Kienast W, 2006; Farpour-Lambert NJ et al., 2009) and may be more related to the length or volume of training performed. Meyer et al (Meyer AA et al., 2006) had obese adolescents exercise aerobically for half a year, 3 times per week for 60 to 90 minutes. They noted a trend in reduction of fat mass (p=0.064) with a significant decrease in BMI and CRP. Alternatively, 8 year old children exercised for less volume (50 minutes, 3 times per week) for only three months. Despite a decrease in percent fat and an increase in lean body mass, CRP was not altered. This study used a combination of aerobic and resistance training (Farpour-Lambert NJ et al., 2009).

It is known that a healthy diet is required for immune balance and regulation (Barbaresko J, Koch M, Schulze MB, & Nothlings U, 2013). However, it is evident that when diet is the only variable altered that most foods and/or nutrients individually, such as nuts (Maranhao PA et al., 2011), folic acid (Pena AS et al., 2007) or vitamin C and E (Engler MM et al., 2003), do not alter inflammatory markers, in particular CRP, in overweight children.

Although the data are limited, weight and/or fat loss is the basis for a decrease in CRP in adolescent intervention trials (Kelishadi R, Hashemi M, Mohammadifard N, Asgary S, & Khavarian N, 2008; Roberts CK, Chen AK, & Barnard RJ, 2007; Kaufman CL et al., 2008). These interventions included expert advice on dietary recommendations but did not provide the actual food to the participants. So it is uncertain if the reduction in calories as a whole or the calories from particular foods provided the stimulus for the amelioration in inflammatory markers. This adds further rationale for the current thesis in providing adequate calories to obese youth when making change to decipher the precise basis for the improvement in inflammatory health.

**1.4.3 Inflammation, protein and milk**

It is not clear if protein, and in particular the milk protein, improves the inflammatory profile in animals and humans. In obese mice and rat models when the amino acid leucine is provided in water, there is no effect on body weight or food intake in these animals. However, there is an improvement in TNF-α levels in adipose tissue (Macotela Y et al., 2011). But others dispute this finding and report no change in TNF-α unless accompanied by endurance exercise (Torres-Leal F Fonseca-Alaniz MH et al., 2011). Because these animals increased muscle mass and decreased weight from the weight loss diet/exercise protocol, it is not clear if the improvement in inflammatory markers was due to the change in anthropometrics or the leucine supplementation (Torres-Leal F Fonseca-Alaniz MH et al., 2011). When TNF-α and IL-6 are infused into animals and humans, protein balance is disrupted with a decrease in protein synthesis and increase in protein degradation (Hoshino E et al., 1991; van Hall G et al., 2008). But when overall dietary intake is considered, it is clear that the protein balance changes are due to the lack of availability of nutrients, dietary energy or both. Thus amino acid availability is important (van Hall G, 2012).

In adults, there is some evidence that when milk is provided as part of a weight loss diet that inflammatory markers can improve in as little time as one week (Zemel MB & Sun X, 2008; Zemel MB, 2002; Stancliffe RA, Thorpe T, & Zemel MB, 2011b; vanMeijl LEC & Mensink RP, 2010), but this has not been demonstrated in all reports (Rosado JL et al., 2011; Wennersberg MH et al., 2009) . In a recent review, it was noted that due to methodological factors in existing studies, further studies are warranted (Labonte ME, Couture P, Richard C, Desroches S, & Lamarche B, 2013). Of the eight studies evaluated, only one assessed the change in the inflammatory profile as the main outcome variable and thus had the statistical power to determine the effects of a milk intervention on inflammation. In this study (Stancliffe RA et al., 2011b), there was an improvement in the inflammatory markers which was independent of changes in adiposity over seven days.

Potential mechanisms for the improvement in inflammation with milk consumption include: 1) the suppressing of calcitriol by calcium which reduces reactive oxygen species and circulating malondialdehyde expression; 2) lactoferrin protein in milk having anti-inflammatory effects; 3) bioactive compounds in milk, which are small proteins that are stimulated with digestion, suppressing the oxidative stress and inflammatory response of the renin-angiotensin system in adipose tissue; and 4) milk having a high concentration of leucine which promotes protein synthesis and inhibits degradation so alteration in energy partitioning between adipose tissue and muscle (Zemel MB et al., 2008; Labonte ME et al., 2013). To our knowledge, this has not been tested in overweight youth and in particular the short term synergistic effects of diet and exercise protocols are warranted given the ambiguity in the literature.

**1.5 Metabolic consequences of obesity**

As chronic inflammation is associated with adverse metabolic health, the provision of milk with a healthy diet and structured exercise program could potentially improve factors such as hypertension, hyperglycemia and insulin resistance (Stancliffe RA et al., 2011b).

**1.5.1 Hypertension**

Hypertension in childhood affects 30% of obese children (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). Hypertension is not only caused by the chronic inflammatory state but from the insulin resistance and hyperinsulinemia often seen in pediatric obesity. These sequela can activate the renal sympathetic nervous system causing sodium and water retention and increased blood pressure. Furthermore, excess fat can compress the kidney, decreasing blood flow so sodium is reabsorbed and blood pressure increases (Flynn J, 2013).

The effects of exercise alone on blood pressure in children are inconsistent due to the heterogeneity of subjects and protocols. Generally, aerobic and strength training does not play a role in reducing blood pressure in children as is seen in the adult literature (Pescatello L et al., 2004). However, there is some evidence that there is an effect in upper BMI percentiles (Garcia-Hermosa A, Saavedra JM, & Escalante Y, 2013). In a recent meta-analysis in obese children, exercise protocols that include exercise programs three times per week for greater than 60 minutes can have a moderate yet positive effect on systolic blood pressure. To reduce diastolic blood pressure, programs less than 12 weeks need a frequency of greater than three times per week to be effective (Garcia-Hermosa A et al., 2013).

Changes in dietary intake can have a significant effect on blood pressure but most research in children has been longitudinal or x-sectional (Couch SC & Daniels SR, 2006; Moore LL et al., 2012; Rangan AM et al., 2012; Yuan WL et al., 2013). In a randomized controlled trial (RCT) (Couch SC et al., 2008), the Dietary Approaches To Stop Hypertension (DASH) trial, tested a diet high in fruits, vegetables and low fat dairy (2 servings per day) plus low in red meat and refined carbohydrates. Blood pressure was reduced with this diet but it was not clear which nutrients, group of nutrients or particular foods caused the positive effect. Changes in blood pressure were correlated with an increase in potassium, magnesium, fruit and milk intake and decrease in fat consumption (Couch SC et al., 2008). Milk is rich in potassium and magnesium (Weaver CM, 2014), and thus milk has the potential to exert positive effects on blood pressure.

Furthermore, potassium, calcium and magnesium are nutrients that re-calibrate action potentials in muscle and thus determine the tonic nature of smooth muscle cells in the vascular system (McCartney DMA & Byrne DG, 2014). Riboflavin and folate help lower blood pressure through activation of nitrous oxide that can dilate vessels and decrease peripheral resistance and thus blood pressure (McCartney DMA et al., 2014). Vitamin D facilitates the absorption of calcium in the gastrointestinal tract and also improves insulin resistance (McCartney DMA et al., 2014).

Lifestyle programs that concurrently increase activity and improve healthy eating have the capacity to reduce blood pressure irrespective of effects of the program on adiposity (Cai L et al., 2014). Therefore, it would be appealing to test the effects of milk in conjunction with an exercise program on blood pressure.

**1.5.2 Insulin Resistance and Blood Glucose Balance**

Prior to the development of T2DM, obese youth can develop beta cell dysfunction and peripheral insulin resistance (Fedewa MV, Gist NH, Evans EM, & Dishman RK, 2014). Dysglycemia, hyperinsulinemia and insulin resistance affect lipid and glucose homeostasis (Decsi T & Molnar D, 2003). Fasting hyperglycemia incidence is high in childhood obesity and has been estimated at 25 % with hyperinsulinemia affecting 54 % of obese children (Decsi T et al., 2003).

Aerobic activity and resistance training can help regulate blood glucose (Gutin B & Owens S, 2011). Although exerting a small to moderate effect, physical activity is beneficial in improving fasting insulin and insulin resistance (Fedewa MV et al., 2014). These changes can occur without changes in body fat, lean body mass or weight (McCormack SE, McCarthy MA, & Harringon SG, 2013). However, it was noted in a meta-analysis that the effects of exercise mode, duration, intensity and frequency on insulin levels in children remains to be determined (Fedewa MV et al., 2014).

A decrease in dietary fat and/or a high dietary fibre intake increases insulin sensitivity in obese children. However, it is not clear if the improvement was due to the food component or weight loss in subjects (Levy-Marchal C et al., 2010). As in hypertension, diets that encourage fruits, vegetables, dairy products, whole grains and lean meats, have a positive effect on insulin resistance (Calton EK, James AP, Pannu PK, & Soares MJ, 2014) but do not point to the individual food or nutrient having the greatest effect.

Recent reviews have indicated that the evidence of the benefit of dairy on metabolic health is limited by appropriately powered RCT's and results are conflicting with milk and/or milk products having variable effects on glucose homeostasis and insulin resistance (Weaver CM, 2014; Kratz M, Baars T, & Guyenet S, 2013; DaSilva MS et al., 2014). St-Onge and colleagues (St-Onge MP et al., 2009), found that those randomized to four cups of milk compared to one cup per day had no difference in fasting glucose or insulin. However, with a glucose challenge test, the high milk group can have an improvement in insulin usage (St-Onge MP et al., 2009). More research is required given the paucity of data in this area in children and adolescents. Interestingly, in a longitudinal trial, middle aged-women who consumed high dairy in adolescence had a lower risk of T2DM and especially if the high dairy intake of adolescence persisted into adulthood (Malik VS et al., 2011).

**1.6 Summary**

It is evident that there is a need to undertake a trial to facilitate a decrease in adiposity in obese children while maintaining essential growth and development processes. It has been well established that lifestyle interventions have modest success, and there are inherent gaps in the literature. Thus novel treatment options are necessary. However, it is premature to undertake an extended trial without first having precise and fundamental measurements of intake and output. There is a theoretical basis and postulated mechanisms that dairy can improve health indices above bone structure alone. This thesis addressed the question of whether milk combined with intense exercise could improve protein balance, anthropometric variables and metabolic complications in the obese adolescent.

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**CHAPTER 2**

**Title: Milk combined with short-term high-intensity exercise training improves body fat and whole body protein balance in overweight adolescents**

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**PREFACE**

***Significance to thesis***

A negative energy balance should be beneficial to overweight youth for loss of fat mass, but may also impose unwanted reductions in lean mass, which need to be conserved during growth. As fluid milk contains proteins that can help improve protein balance, the goals of this study were to: measure whole body protein balance in response to short-term physical activity-induced energy deficit in overweight adolescents; and to compare the effect of post-exercise milk intake versus isocaloric carbohydrate intake on whole body protein balance (WBPB) in response to short-term physical activity-induced energy deficit. The uniqueness of this protocol was that all foods, in specified portion sizes, and test beverages were provided to participants during the trial. Furthermore, exercise was monitored objectively and all exercise sessions were supervised by trained researchers.

***Authors’ contributions***

Linda J. Gillis contributed to the design of the study, performed all experiments and data collection, organized team of volunteers and research assistants, acted as the Registered Dietitian (RD) for dietary analysis, performed all statistical analyses, wrote the initial draft of the manuscript and worked on refining this draft and the revisions based on editorial review. She also collaborated on the grant for funding for this project.

Brian W. Timmons contributed to the design of the study and refined the draft and revisions. He also collaborated on the grant for funding. Stuart M. Phillips and Stephanie A. Atkinson refined this draft and revisions based on editorial review. The 15N–enrichment of urinary ammonia was determined using isotope ratio mass spectrometry (Metabolic Solutions Incorporated, Nashua, New Hampshire, USA).

**Abstract**

Rising rates of pediatric obesity require dietary and exercise regimes that allow for growth and maintain muscle. The aim of this study was to determine the synergistic effect of milk consumption and exercise on whole body protein balance (WBPB) in overweight adolescents. A 7-day dietary intervention with high intensity exercise was employed to generate a small energy deficit through daily 1-h exercise sessions . Participants were randomized to receive milk (MILK; n=26) post exercise or isoenergetic servings of a carbohydrate beverage (CONT; n=29). Both groups received a diet based on their resting energy expenditure. To determine WBPB, participants consumed oral doses of 15N-glycine with urine being collected at four time points. Milk consumption continued for two days post intervention. The MILK group maintained more fat free mass (FFM) (-0.2 ± 0.6 and -0.7 ± 0.8 kg, MILK and CONT respectively, p<0.01) and had a greater change in percent body fat (-0.4 ± 1 and 0.5 ± 1.0 %, MILK and CONT respectively, p<0.006). WBPB was greater after training 7 days in the MILK group compared with controls (1.64 ± 1.1 vs. 0.84 ± 0.6, MILK and CONT respectively, p<0.001). In the ensuing two days, the MILK group continued to be in a more positive WBPB compared to the controls. These data support the consumption of milk after exercise to aid in WBPB and preservation of FFM in the early stages of obesity treatment.

Keywords: protein balance, pediatric, obesity treatment, milk, fat free mass, exercise

**Milk combined with short-term high-intensity exercise training improves whole body protein balance and maintains fat free mass in overweight adolescents**

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**Conflict of Interest Statement**

The authors declare no conflict of interest.

**Introduction**

A recent systematic review (Sbruzzi G et al., 2013) concluded that although the evidence was not strong, pediatric obesity interventions with the most promise (based on weight loss) incorporated nutritional counseling and physical activity programs (Epstein LH et al., 1985), reward reduction in sedentary activities (Epstein LH et al., 1995) and included behavioural therapy components (Epstein LH et al., 1998; Golan M, Fainaru M, & Weizman A, 1998; Warschburger P, Fromme C, Petermann F, Wotjalla N, & Oepen J, 2001). A key factor in this relationship is physical activity, and it is important to determine how best to optimize the combination of physical activity and nutrition prescription for obese youth (Spear BA et al., 2007).

Protein turnover provides for a dynamic remodeling of protein. In childhood, the balance between protein synthesis and breakdown needs to be positive to allow for growth

(Balagopal P, 1998). Adolescence is characterized by rapid somatic growth, and protein anabolism occurs to support the gain in lean tissue required, especially for skeletal muscle (Bitar A et al., 1999). In fact, weight loss during pubertal growth is inadvisable due to a possible reduction in linear growth velocity (Amador M et al., 1990; Dietz W et al., 1985a). Secondly, rapid weight reduction results in an alteration in the resting metabolic rate due to loss of FFM (Stiegler P et al., 2006). Indeed, adults who preserve the most FFM during weight loss show less weight regain (Westerterp-Plantenga MS et al., 2004). Obesity is, however, a condition that disturbs protein metabolism in youth, as obese children have higher protein turnover than their non-obese counterparts (Balagopal P et al., 2003; Schutz Y et al., 1999). Thus, it is prudent to develop diet and exercise recommendations suitable for the maintenance of positive protein balance for preservation of muscle and growth while at the same time allowing the child to expend sufficient calories to reduce excess adiposity, which is often the source of metabolic complications. Although limited (Ebbeling CB et al., 1999; Balagopal P et al., 2003; Lazzer S et al., 2005), the available evidence supports the benefit of combining increased physical activity with an appropriate dietary strategy to maintain protein balance in overweight youth.

Bovine milk contains approximately 80% casein and 20% whey so is capable of providing a balance in protein synthesis and breakdown, and milk consumption after a single bout of resistance exercise enhances protein balance in adults (Elliott TA et al., 2006). Further, consumption of milk, versus a carbohydrate (Josse AR, Atkinson SA, Tarnopolsky MA, & Phillips SM, 2001; Hartman JW et al., 2007) or soy beverage (Josse AR et al., 2001; Hartman JW et al., 2007), for 12 to 16 weeks of training resulted in significantly greater gains in FFM. Additionally, a major point of interest, in regards to obesity, is that the milk group had a greater loss of fat mass compared to the other beverage groups. While the above studies focus on protein metabolism in adults, they provide a rationale for further investigation of the interaction between exercise and protein intake as a viable option for use in pediatric populations. We were particularly interested in the short-term effects to facilitate the knowledge of the magnitude of exercise and dietary energy required to allow for muscle development, when a potential longer trial is executed.

Thus, the objective of this study was to determine the effects of short-term exercise training on whole body protein balance (WBPB) with and without milk intake in overweight youth. We hypothesized that overweight youth would experience a greater WBPB in response to short-term exercise training that would be enhanced with post-exercise milk intake.

**Materials and methods**

*Study design*: This study was a single-blinded randomized, controlled, parallel intervention study involving overweight adolescent males and females. Participants consumed sufficient energy to meet requirements while undertaking a 7-day exercise training protocol involving resistance exercise, along with high and moderate intensity cycling exercise. Participants were randomly assigned to perform the training with post-exercise consumption of chocolate milk or an isoenergetic carbohydrate drink. The study protocol is illustrated in Figure 1.

*Participants*: Overweight participants were recruited from the waiting list of a hospital-based obesity treatment program or from community advertisements. Overweight was defined as a body mass index (BMI) ≥85th percentile for age and sex (Harrington DM, Staiano AE, Broyles ST, Gupta AK, & Katzmarzyk PT, 2013). Boys ages 11 to 15 years and girls ages 9 to 13 years were included to represent an age range approximating the ages of peak height velocity (APHV) (Mirwald RL, Baxter-Jones AD, Bailey DA, & Beunen GP, 2002). To be eligible for this study, participants had to be routinely consuming ≤1 serving of dairy per day with no regular participation in resistance training. Exclusion criteria included an allergy to dairy protein or diagnosed lactose intolerance, Type 2 Diabetes, or taking medications that affected appetite. Participants agreed to refrain from taking dietary supplements during the study and avoid performing strenuous physical activity outside of the training sessions. Ethics approval was obtained from the joint McMaster University and Hamilton Health Sciences Ethics Review Board and informed consent was obtained from the participant and one parent.

*Preliminary Sessions and Dietary Analysis:* Upon recruitment into the study, each participant was randomly assigned to the milk group (MILK) or control group (CONT). This randomization occurred separately for boys and girls but in a counterbalanced manner so as to have equal numbers of boys and girls in each group. Participants completed a standard screening questionnaire to document medical history, medication and supplement use and physical activity level and were given a 3-day food record to complete. Participation began with a 2-week baseline period (Day -19 to -5, Figure 1) in which body weight was measured at the beginning and end. If the difference in measurements was less than 2%, participants continued with the study. If the difference was greater than 2%, the children were considered not to be in a weight stable condition and would not continue.

*Visit 1 (Day -3, Figure 1):* Anthropometrics were performed that included height, weight and waist circumference and resting energy expenditure (REE) was assessed using a ventilated hood system. Standing and sitting height were measured in stocking feet using a Harpenden Stadiometer with a precision of 0.1 cm, weight to the nearest 0.01 kg in a t-shirt and shorts using an electronic scale, and waist circumference two inches above the umbilicus with a weighted tape measure. Puberty was determined by participant-assessed Tanner Staging (Marshall WA & Tanner JM, 1969; Marshall WA & Tanner JM, 1970). The APHV was estimated according the equation of Mirwald (Mirwald RL et al., 2002). Body composition was assessed by dual-energy X-ray absorptiometry (DXA) (Hologic Discovery, Waltham, MA) and bio electrical impedance analysis (BIA) (InBody520 Body Composition Analyzer, Cerritos, California) was utilized to examine changes in body fat before and after the 7-day exercise training period. At pre-treatment, the whole body fat measurements obtained with BIA and DXA were similar and had a high correlation of

r = 0.6, p < 0.05. Therefore, BIA was used to determine change in body fat, thereby reducing exposure of each participant to DXA radiation. Participants then met with a Registered Dietitian (RD) to complete a 24-h diet recall and food frequency questionnaire. Prior to leaving the laboratory, the participants were instructed to collect a baseline urine sample at 8 pm that evening and then consume a single oral dose of 15N-glycine (2 mg/kg body weight) mixed with water. They collected all urine output for the next 12 hours (overnight).

*Visit 2 (Day -2, Figure 1):* This visit was used to evaluate aerobic fitness via an incremental cycling test with determination of peak mechanical power (PMP) according to published methods (Bar-Or O & Rowland TW, 2004). Strength was determined with a one-repetition maximum for upper and lower body strength exercises. These evaluations were used to determine exercise workloads during the 7-day exercise training protocol. For each participant, the RD generated a diet plan that provided a constant energy intake for the 7-day exercise-training period. This energy intake was equal to the requirements based on assessment of REE plus a physical activity factor to account for the training to be performed. Thus, it was anticipated that each participant would experience a reduction in energy intake from their habitual intake (which we assumed would be in surfeit), but would not enter an energy restricted state where requirements were not being met (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005). Protein intake as part of energy consumed without factoring in the experimental beverages was the same for both groups and equal to 1.0 g/kg body weight or 17% protein by calorie, as proposed by Ebbeling (Ebbeling CB et al., 1999). However, when the 3 extra servings of chocolate milk in the MILK group were considered, total protein intake was ultimately higher in the MILK group (1.4 g/kg body weight). To enhance compliance and convenience for families, a checklist and the food (portioned out and weighed) necessary to follow the dietary regimen were provided.

*7-day exercise training protocol (Days 1 to 7, Figure 1):* Forty-eight hours after Visit 2, participants began their 7-day exercise training protocol. Each day, participants arrived at the laboratory at 6 pm with their food list that was checked daily to ensure compliance to the dietary recommendations. Any uneaten food was returned, measured and accounted for. Each training session involved 60 minutes of exercise, which included resistance exercises for the upper and lower body, along with short bouts of high-intensity cycling and a longer bout of moderate-intensity cycling. Exercises were performed in a circuit training format to reduce boredom. The exercise routine commenced with a five minute warm up of low intensity cycling. The resistance training exercises consisted of bicep curl, triceps extension, one arm dumbbell row, one dumbbell front raise, lat pull, bench press, leg extensions, squats and lunges. These resistance exercises varied from day to day so that the same muscle group was provided ~48-h of rest, but the variation was the same for each group. Each participant performed two sets of 10 repetitions of each exercise during the circuit. The high-intensity cycling involved 6, 15-second intervals at 100% PMP followed by 1-minute of light cycling at 30 watts. The moderate intensity exercise involved 20 minutes of continuous cycling at 50% PMP. A five-minute cool down of low-intensity cycling completed the training session.

Upon arrival to the laboratory on Day 1 and Day 7 of the 7-day exercise training protocol, each participant provided a spot urine for background 15N-enrichment of urinary ammonia. At 8 pm, they consumed a single oral dose of 15N-glycine (2 mg/kg body weight) mixed with water before leaving the laboratory, and collected all urine output for the next 12 hours (overnight) including the first urine of the next morning.

*Experimental beverages*: Immediately after each training session, participants consumed the first of their 3 beverages; 60 min later they consumed the 2nd beverage; the 3rd beverage was consumed the next morning at 8 am. The MILK group received fat-free chocolate milk as their experimental beverage plus 100 g of low fat yogurt. The CONT group consumed a beverage consisting of D-glucose as a 9% solution and was isoenergetic to the chocolate milk (MILK 250 ml = 578 kJ, 8 g protein, 26 g carbohydrate and 0 g fat vs. CONT 250 ml = 535 kJ, 0 g protein, 16 g carbohydrate and 4 g fat). The control beverage was mixed so that it had similar visual, taste and olfactory characteristics as the chocolate milk, making participants blind to the beverage they were consuming. The controls also received the 100 mg of low fat yogurt.

*Final Assessment Visit (Day 9, Figure 1):* Forty-eight hours following the last training session, a final assessment visit was completed. During this visit, procedures completed in Visit 1 were repeated, including height, weight, body fat assessment by BIA, and waist circumference. Participants continued their standardized diet from the last training session through to the final assessment visit. A final assessment of WBPB was made with participants consuming a single oral dose of 15N-glycine and then collecting all urine output for the next 12 hours.

*Measurement of protein turnover:* Urinary nitrogen excretion (E) was determined from urea and creatinine and losses from other sources (e.g., hair, feces, miscellaneous) were assumed at 0.023% of body weight (Todd KS, Butterfield GE, & Howes Calloway D, 1984) and 15N–enrichment of urinary ammonia was determined using isotope ratio mass spectrometry (Metabolic Solutions Incorporated, Nashua, New Hampshire, USA). Correction for background enrichment was computed and applied to yield a true tracer (Tr) to tracee (t) ratio to determine nitrogen flux (Q). Nitrogen intake (I) was determined by analysis of the protein in the 3-day food record for Day 3 and from compliance records for assessments made on Days 1, 7, and 9 (Figure 1). Nitrogen flux (Q), protein synthesis (S), protein breakdown (B) and whole body protein balance (WBPB) were calculated as presented below where d denotes the oral dose of glycine.

15N (d = g glycine × 0.1972)

Q [g⋅(kg⋅d)-1] = [d/corrected Tr/t ratio/12 h x 24 h/body weight]

S [g⋅(kg⋅d)-1] = [Q-(E/12h x 24 h/body weight)] × 6.25 g protein/g N

B [g⋅(kg⋅d)-1] = [Q-(I/12 h x 24h/body weight)] × 6.25 g protein/g N

WBPB [g⋅(kg⋅d)-1] = S-B

*Assessment of Nitrogen-containing compounds:* Nitrogen balance (NB) was taken as the difference between intake and excretion.

*Sample Size:* Although we were limited by a lack of similar studies in overweight youth to calculate sample size, we used published data in addition to our own unpublished observations using the 15N-glycine methodology. Based on the work of Hartman et al. (Hartman JW et al., 2007), the daily difference in WBPB is estimated as 0.24 g⋅(kg⋅d)-1, with the assumption that 100% of the positive protein balance was going toward accretion of lean mass. Based on our own unpublished work, expected difference in WBPB in children following a controlled diet with imposed sessions of exercise is ~0.25 g⋅(kg⋅d)-1. We calculated (Dawson B & Trapp RG, 2004) that a sample size of 15 participants per group would allow us to detect a difference between beverage groups in WBPB of 0.24 g/kg·day–1 with ~80% power at an alpha level of 5%. The sample was doubled to include males and females for a total of 60 participants.

## Data Analysis: The change scores in WBPB and anthropometric variables were analyzed with 2-way ANOVAs with 1 between factor (beverage group) and 1 within factor (time) with gender and age of peak height velocity as covariates. As there were no gender differences all data were collapsed into the two specified beverage groups. Data transformations were made when the data was not normally distributed. Chi-square analysis was used to determine proportionality in supplement use between groups.

**Results**

Fifty-five overweight adolescents completed the study (20 males/35 females). All participants were weight stable after the two week run-in phase and continued in the study. One-third of the participants in each subject group were on supplements prior to starting the study which included vitamin D, vitamin C, iron, omega-3 fatty acid and multivitamin/mineral supplements. There was no difference between the groups in the number of those consuming supplements (MILK n=8, CONT n=10, p = 0.1). There were 3 in the CONT group and 2 in the MILK group on vitamin D. All but two participants stopped the supplements during the trial (one in CONT remained on daily iron and one in CONT remained on daily vitamin D).

Five participants did not complete the full protocol and missing values were imputed using mean values based on group designation (n=3 in the MILK and n=2 in the CONT groups, respectively). Of the 50 remaining participants, adherence rate to the training sessions was high with a 99% compliance rate to the seven training sessions. Baseline characteristics of participants are reported in Table 1. Despite randomized assignment to the MILK and CONT groups, initial weight and lean body mass in kg were significantly different between the groups at baseline. Therefore, those variables were used as covariates in analyses, but did not alter the outcomes. The CONT group was also more mature as indicated by estimated years from the APHV in both sexes and Tanner stage in girls. The participants' reported habitual milk intake, taken from the food frequency questionnaire, was low in both groups (5 cups per week) at initiation of the study and there was no difference between the two groups.

Heart rates achieved during resistance training, high intensity and moderate cycling training averaged 145, 174, and 148 beats per minutes, respectively and were not different between the two groups.

*Dietary adherence:* Compliance to the diets was high in both groups at 98% of provided energy in the MILK group and 97% in the CONT group (Table 2). By design, the MILK group consumed more protein than the CONT group. Compared to recommendations, (estimated energy requirement for each participant compared to their age, gender and low activity level (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005), the diet was to provide calories slightly below recommendations. Based on subjects’ individual RMR and activity factor, the percent of energy requirements the participants consumed (based on compliance data) was 94±10 percent which was 127 calories less than recommendations. This mild deficit was not different between the groups.

*Anthropometry:* The MILK group experienced a significantly greater reduction in percent body fat (Figure 2) but lost less FFM (Figure 3). There was no significant difference in body weight change following training between groups (-0.7 ± 0.7 kg in MILK and 0.5 ± 0.3 kg in CONT, p<0.3). There was a greater change in waist circumference in the MILK group compared to the CONT group which approached significance (-1.2 ± 3 in milk and 0.3 ± in control, p=0.06).

*Whole body protein turnover:* Indices of nitrogen and protein turnover are reported in Table 3. Nitrogen balance was greater after 7 days of training in the MILK group compared with the CONT group. In the ensuing two days, without training (Post), the MILK group continued to be in a more positive NB compared to the CONT group. Individually, protein breakdown and synthesis were not different between groups; however, the MILK group was in a more positive WBPB than the CONT group after 7 days of training and to a lesser extent 2 days Post training (Table 3).

**Discussion**

We report that the post-exercise consumption of flavoured milk resulted in a synergistic effect with short-term exercise training to improve WBPB and beneficial changes in body composition such as body fat and FFM in overweight male and female adolescents compared to an isoenergetic non-milk beverage. The controlled dietary intake and supervised daily exercise protocol in this study were followed well by the participants and lending credence to the use of these techniques for adolescent obesity treatment, particularly during the initiation of treatment.

In order to remain active, it is important that obese youth enjoy the activities they are undertaking to help with long term compliance. The interesting aspect of high intensity training (HIT) is its similarity to the “stop-and-go” nature of children’s physical activity patterns (Bailey RC et al., 1995). This may increase the likelihood of sustaining physical activity, which is essential to the long-term management of childhood obesity (Tjonna AE et al., 2009). Resistance training has been included in successful obesity treatment programs for youth with better adherence than a walking program (Schwingshandl J et al., 1999; Benson AC et al., 2008; Sothern MS et al., 1999). The present study is unique in that three modes of exercise were utilized in the protocol. It was evident from the high compliance to the daily activity that obese youth were capable of sustaining this level, and average heart rates indicated they were exercising at a high enough rate to improve cardiovascular fitness (Molnar D & Livingstone B, 2000). It still needs to be determined if compliance to this exercise protocol could be sustained longer term.

All participants irrespective of diet treatment group were in positive WBPB at all time points in the study. This may reflect the healthy diets that both groups consumed with recommendations close to the dietary reference intakes for energy and protein. This contrasts with a previous study in seven healthy children in which a considerable reduction in protein synthesis and protein breakdown occurred with a walking program (Bolster DR et al., 2001). The authors (Bolster DR et al., 2001) deemed that the negative protein balance was induced by the enhancement in energy expenditure, as energy and protein intakes did not change over the study period. In obese children, it has not been established if protein balance is modified with aerobic exercise. Although it has been demonstrated that FFM increases with exercise training, the majority of studies have not employed a control group to ascertain the influence of normal growth and development and do not analyze dietary intake (Sasaki J et al., 1987; Gutin B et al., 1996; Ferguson MA et al., 1999; Wells JCK, 2003). With resistance training, protein synthesis and breakdown decreased in response to six weeks of resistance training, twice per week in 11 children (Pikosky M et al., 2002). Although net protein utilization did not decrease significantly, there was a trend towards a net loss of protein (p=0.07). It is possible that resistance training only twice per week was not sufficient to induce changes in protein turnover. Tjonna et al. found that HIT with overweight adolescents did not result in changes in FFM versus controls (Tjonna AE et al., 2009); however, in that study the HIT group consumed less protein for the duration of the study.

A contentious issue with the Recommended Dietary Allowance (RDA) for protein in adolescents (0.85 g of protein /kg/day) is whether regular exercise can increase the requirements for protein. Nitrogen balance was reached when adolescent soccer players consumed 1.4 g/kg body weight of protein (Boisseau N et al., 2007). This is clearly above the RDA for protein of 0.85 g of protein /kg/day. In our study, the MILK group consumed 1.4 g/kg protein with the CONT group consuming 1.0 g per kg which represented ~12 grams/day of additional protein in the MILK group, although energy intake was the same. We believe that the milk protein itself caused the improvement in protein balance, as it has been demonstrated that milk protein is superior to other proteins, such as soy protein, for muscle hypertrophy

(Hartman JW et al., 2007). It is not clear if the extra protein provided to the MILK group was the reason for the improvements in protein balance or whether some other constituent of the milk was responsible for the effects observed (Zemel MB, 2002; Phillips SM & Van Loon LJC, 2011). It could also be that the timing of protein consumption in close temporal proximity to the exercise was important.

Even with a moderate reduction in calories in healthy weight children, net protein balance declines due to a decrease in protein synthesis which can cause a reduction in FFM (Ebbeling CB et al., 1998); however, the potential impact on long-term growth of such a practice is unknown. Notwithstanding, we recommend that it is practical to explore the role of an added nutrient-dense source of protein with appropriate energy intake along with a physical activity-induced energy deficit since energy restriction alone may not be prudent. For example, Ebbeling (Ebbeling CB et al., 1999) reported that obese children with a modest decrease of 500 kcal/d had reduced protein synthesis, breakdown, net turnover, nitrogen flux and nitrogen balance. With the addition of exercise to the same program (walking 5 d/wk) protein synthesis, breakdown and nitrogen flux increased above initial pre-study values, but nitrogen balance remained at the lower levels (Ebbeling CB et al., 1999). We propose that our data, and those of Ebbeling (Ebbeling CB et al., 1999), demonstrate that in the face of short-term exercise training, exercise is likely critical, when consuming insufficient energy from the diet, in maintaining WBPB in both groups.

Many large population based cross-sectional studies have evaluated the relationship between dairy and body composition in children. Youth who consume more dairy have been reported to have a lower incidence of obesity (Spence LA et al., 2011). However, in prospective randomized controlled trials with obese youth, differences in weight and body fat were not evident when milk was consumed, but FFM was not always evaluated (Spence LA et al., 2011). In one study, overweight participants, had an increase in FFM when two cups of milk were consumed (Albala C et al., 2008). Finally, most of the trials did not have participants exercise in a controlled, supervised manner (Albala C et al., 2008; St-Onge MP et al., 2009; Kelishadi R et al., 2009; Ghayour-Mobarhan M et al., 2009). Participants who followed a similar protocol to ours with respect to milk consumption post-exercise had no significant changes in body composition after resistance exercise compared to water consumption over six months (Lambourne K et al., 2013). It is difficult to compare this study to our results since the participants did not follow a specific dietary protocol and exercised only three times per week. Nonetheless, despite more energy being consumed by the milk drinkers they did not gain additional weight or body fat compared to the post exercise water group, which adds further confirmation of our results (Lambourne K et al., 2013).

A limitation of this study was that despite randomization, there were differences in some of the anthropometric variables between the two groups and needed to be controlled for statistically. Fortunately, the main variable, WBPB, was similar between the two groups at baseline and after one day of training. Thus changes at the end of treatment reflected effects from the treatment and not the participant body size variances.

Our study demonstrated that obese youth are able to undertake one hour of intense cycling and resistance training daily over a one-week time period and assists in providing protocol guidelines for longer term trials. When combined with flavoured milk consumption following each exercise session, this short-term exercise training resulted in an improved WBPB, maintenance in FFM and change in body composition, compared to individuals who consume an isoenergetic milk-free beverage. We consider the responses to this intervention to represent a significant advance in clinical practice by highlighting the importance of exercise and nutrient interactions early in the treatment of weight management in obese adolescents.

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**Table 1**: Baseline MILK and CONT characteristics of participants

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristic | MILK (n=26) | CONT (n=29) | p value |
| Sex (males/females) | 8/18 | 12/17 | -- |
| Age (years) | 11 (1.5) | 12 (1.5) | 0.09 |
| Height (cm) | 152 (11) | 159 (10) | \*0.01 |
| Weight (kg) | 66 (20) | 77 (17) | \*0.03 |
| Fat-free mass (kg) | 38 (9) | 45 (9) | \*0.005 |
| Waist circumference (cm) | 89 (14) | 94 (12) | 0.3 |
| Body Fat (% via DXA) | 39 (8) | 39 (6) | 0.9 |
| Body Fat (% via BIA) | 40 (7) | 40 (7) | 0.9 |
| Body Mass Index | 28 (5) | 30 (5) | 0.08 |
| RMR (percent predicted) | 102 (8) | 97 (9) | \*0.03 |
| RMR (kJ) | 6643 (1009) | 7024 (1055) | 0.2 |
| Boys APHV (years) | 12(0.3) | 12 (0.8) | 0.5 |
| Boys Years from APHV | -1.4 (0.8) | -0.18 (1.1) | \*0.03 |
| Boys Tanner (score) | 2 (1) | 2.6 (0.8) | 0.2 |
| Girls APHV (years) | 11 (0.7) | 11 (0.7) | 0.2 |
| Girls Years from APHV | -0.05 (1.3) | 0.8 (1.3) | \*0.06 |
| Tanner (score) | 2.6 (1.1) | 3.3 (0.7) | \*0.05 |

MILK = milk group, CONT = control group, Presented as means ± (standard deviation)

DXA=dual x-ray absorptiometry, BIA=bio electrical impedance, RMR = resting metabolic

rate, APHV= age of peak height velocity, \*p<0.05

**Table 2**: Compliance to meal plan

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Diet Component | MILK  Amount consumed | CONT  Amount consumed | P value | MILK percent consumed of provided food | CONT percent consumed of provided food | p value |
| Calories (kJl) | 7363  (8205) | 7455  (7577) | 0.7 | 98 (3) | 97 (4) | 0.4 |
| Protein (g) | 88 (9) | 75 (100) | \*0.001 | 97 (3) | 95 (6) | 0.06 |
| Protein (g/kg) | 1.4 (0.3) | 1 (0.20) | \*0.001 | 97 (3) | 95 (6) | 0.06 |
| Fat (g) | 47 (9) | 55 (6) | \*0.001 | 98 (4) | 97 (5) | 0.3 |
| Carbohydrate (g) | 259 (25) | 259 (26) | 0.9 | 97 (3) | 98 (7) | 0.4 |
| Calcium (mg) | 1120 (214) | 375 (55) | \*0.001 | 96 (14) | 97 (8) | 0.7 |

Presented as means ± (standard deviation)

\*significantly different at p< 0.05

MILK = milk group, CONT = control group

Table 3: Nitrogen balance and protein turnover in MILK and CONT groups during the intervention

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Pre | | P value | Day 1 | | P value | Day 7 | | P value | Post | | P value |
| Protein Variable | MILK | CONT |  | MILK | CONT |  | MILK | CONT |  | MILK | CONT |  |
| E (g/kg/day) | 0.18  (0.07) | 0.17  (0.06) | 0.6 | 0.2  (0.2) | 0.13  (0.08) | \*0.05 | 0.09  (0.04) | 0.07  (0.03) | \*0.004 | 0.11  (0.07) | 0.10  (0.04) | 0.3 |
| I (g/kg/day) | 0.22  (0.06) | 0.21  (0.05) | 0.9 | 0.23  (0.05) | 0.16  (0.04) | \*0.0001 | 0.25 (0.05) | 0.18 (0.02) | \*0.0001 | 0.24  (0.05) | 0.16  (0.04) | \*0.0001 |
| NB  (g/kg/day) | 0.03  (0.07) | 0.04  (0.2) | 0.9 | 0.03  (0.14) | 0.02  (0.09) | 0.8 | 0.16  (0.06) | 0.11  (0.02) | \*0.0002 | 0.13  (0.09) | 0.07  (0.05) | \*0.001 |
| B (g/kg/day) | 2.15  (3) | 1.73  (2.9) | 0.6 | 2.66  (4.1) | 3.2  (7) | 0.7 | 2.18  (3.4) | 7.7  (2.4) | 0.2 | 5.12  (13.3) | 3.12  (6.9) | 0.5 |
| S (g/kg/day) | 2.59  (2.9) | 2.22  (1.5) | 0.5 | 3.02  (4.3) | 3.48  (7.2) | 0.8 | 4.14  (3.4) | 9.08  (24) | 0.3 | 6.8  (13.4) | 4.0  (7.0) | 0.3 |
| WBPB (g/kg) | 0.44  (0.9) | 0.04  (2.5) | 0.9 | 0.36  (1.8) | 0.27  (1.2) | 0.8 | 1.96  (0.7) | 1.38  (0.3) | \*0.0002 | 1.64  (1.1) | 0.84  (0.6) | \*0.001 |

MILK = milk group, CONT = control group, Presented as means ± (standard deviation), \*p<0.05

E=nitrogen excretion, I=nitrogen intake, NB=nitrogen balance; B=protein breakdown, S=protein synthesis, WBPB=whole body

protein balance

Day -19 -5 -3 -2 1 2 3 4 5 6 7 9

Tasks DI DI DI DI DI DI DI DI DI DI

MQ EX

1RM

CI CI CI CI CI CI CI

AP AP AP

15N 15N 15N 15N EX 1RM

**Figure 1.** Study Protocol.

DI, Diet Instruction/Evaluation; MQ, Medical Questionnaire; AP, Anthropmetrics; CI,

Circuit Training; EX, Peak Cycling Power; 1RM, Strength Testing; 15N, Urine

Collection of 15N-glycine.



**Figure 2.** Change in percent body fat between MILK and CONT groups. Values

presented as mean and standard deviation. \*p< 0.006.



**Figure 3.** Change in FFM between MILK and CONT groups. Values presented

as mean and standard deviation. FFM, fat-free mass. \*p< 0.01

**CHAPTER 3**

**Title: Milk and short-term high intensity training improves aerobic power**

**in obese youth**

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**PREFACE**

***Significance to thesis***

Overweight adolescents have a low level of cardiovascular fitness and increasing activity can improve this health concern. Nutrition, in the form of milk post-exercise, may enhance an exercise effect as demonstrated in healthy adults in glycogen depletion exercise trials, where milk consumed post-exercise improved time to exhaustion. Milk may also improve strength when consumed post resistance training in obese adults. To our knowledge, such roles for milk have not been researched in overweight youth. In Chapter 2, it was demonstrated that milk preserves muscle mass better than a non-milk carbohydrate beverage. This chapter expanded on this evidence to determine if milk consumed post-exercise led to functional changes in cardiovascular fitness and strength.

***Authors’ contributions***

Linda J. Gillis contributed to the design of the study, performed all experiments and data collection, organized team of volunteers and research assistants, acted as the RD for dietary analysis, performed all statistical analyses, wrote the initial draft of the manuscript and worked on refining this draft and the revisions based on editorial review. She also collaborated on the grant for funding for this project.

Brian W. Timmons contributed to the design of the study and refined the draft and revisions and collaborated on the grant for funding. Stuart M. Phillips refined this draft and revisions based on editorial review.

**Abstract**

**Purpose:** Given the rise in pediatric obesity an optimal dietary and exercise regime that improves fitness and cardiovascular health needs to be elucidated. Thus the aim of this study was to evaluate if there was a synergistic effect of milk versus an isoenergetic control beverage, during short-term combined resistance and high intensity training to improve exercise capacity and strength in overweight children. **Methods:** The study design was a single-blind randomized, controlled, parallel intervention with a 7-day protocol of mild energy deficit brought about by daily one-hour exercise sessions (weight training and high intensity cycling). One group (n=26) received chocolate milk post exercise (MILK) and the other (n=29) received isoenergetic servings of a carbohydrate beverage (CONT). Accelerometry was carried out over the seven days to ensure a similar quantity of daily activity. Before and after the intervention, participants underwent a maximal aerobic cycling test to determine VO2peak, peak mechanical aerobic power and time to exhaustion (TTE), as well as 1-repetition maximum strength testing. **Results:** Peak mechanical power increased only in the MILK group with an increase of 0.13 watts/kg (p<0.05) with trends towards an increase in VO2peak of 1.2 ml/kg/min (p=0.06), but not TTE (p=0.1) over the 7 days. Strength increased over the intervention but with no differences between groups. **Conclusion:** These data support the consumption of milk with short-term exercise training during a mild exercise-induced energy deficit to promote early improvements in aerobic power in obese adolescents.

Keywords: maximal exercise testing, pediatric, overweight, weight training, dairy

**Introduction**

Obesity is a condition that disturbs protein metabolism. Compared to non-obese, obese have ~45 percent higher protein turnover with higher whole body protein synthesis and breakdown (Balagopal P et al., 2003; Schutz Y et al., 1999). Obese individuals tend to have elevated levels of fat-free mass (FFM) proportionate to their increased adipose tissue mass (Schutz Y et al., 1999). The provision of protein post exercise has the potential to improve protein balance and exercise performance in overweight individuals. Indeed, in overweight pre-menopausal women, a high protein, high dairy diet resulted in greater strength gains after sixteen weeks in some resistances training exercises compared to an adequate protein yet low dairy diet (Josse AR et al., 2011). However, cardiovascular fitness with a sub-maximal fitness test protocol improved in a similar manner between the groups (Josse AR et al., 2011).

The benefits of milk consumption on muscle protein synthesis and muscle protein breakdown have been observed among adults (Phillips SM, Hartman JW, & Wilkinson SB, 2005). Milk consumption has also improved exercise performance in healthy adults. Acutely, milk protein appears to increase time to exhaustion in a sub-maximal cycling test (Karp JR et al., 2006a; Lunn WR et al., 2012; Thomas K et al., 2009); improve performance times in a 40km time trial (Ferguson-Stegall L et al., 2011a) and peak torque and strength in knee extension exercise (Cockburn E et al., 2010), compared to supplementation with a carbohydrate beverage or water; however, such findings are not universal (Pritchett K et al., 2009; Lee JKW et al., 2008). There are few studies, particularly in youth, investigating the effects of milk on resistance training-induced gains in strength (Walberg Rankin J et al., 2004; Hartman JW et al., 2007) and other measures of fitness. Strength does not increase with training when milk is consumed post exercise in healthy weight adults (Walberg Rankin J et al., 2004; Hartman JW et al., 2007). Furthermore adolescents that consumed milk post resistance exercise showed equivalent improvements in strength as those consuming of juice (Volek JS et al., 2003); nonetheless, the participants in that study were not obese and were not provided with a specific or standardized dietary plan (Volek JS et al., 2003). It is not clear if other components of the diet had an impact on strength or cardiovascular fitness in the aforementioned studies as none included regimented diets to ensure that the participant groups were consuming similar calories, carbohydrates and protein throughout the day particularly in the pre-exercise time period, as this can also impact performance (American Dietetic Association et al., 2009).

Given the paucity of data in overweight youth, the conflicting evidence in adults and the lack of stringent dietary monitoring in most studies, the objectives of the current study were to determine if short-term exercise training and milk intake could provide a synergistic effect on maximal and sub-maximal aerobic fitness and measures of strength in obese adolescents. We hypothesized that the adolescents who consumed milk with exercise would have a greater increase in cardiovascular fitness and muscle strength compared to a carbohydrate beverage.

**Methods**

We report here the secondary outcomes of a project in which we studied the impact of short-term exercise training and milk intake on whole body protein balance. Baseline characteristics of the participants are summarized in Table 1. Written informed consent was obtained from participants and ethics approval was received by the joint McMaster University and McMaster Children’s Hospital ethics board. Briefly, this was a single-blind randomized, parallel control intervention involving obese adolescent males and females. Participants were provided with sufficient energy to meet their estimated requirements taken from resting metabolic rate measurements including a factor for the exercise to be performed. They undertook a 7-day supervised exercise training protocol that included moderate and high intensity cycling and resistance training over a 60 min session. Diets were identical between groups except the MILK group consumed three cups of chocolate milk daily while the CONT group had three cups of an isoenergetic chocolate-flavoured milk-free carbohydrate beverage. To ensure compliance to diet and training protocols, food was measured out for participants to consume, and they wore accelerometers to measure total activity output during the week.

*Measures of maximal aerobic fitness:* Two days prior and two days after the 7-day training, aerobic fitness was assessed using the McMaster All-Out Progressive Continuous Cycling Test (Bar-Or O et al., 2004) on an electromagnetically braked cycle ergometer (Corival: Lode Groningen, The Netherlands). Workload was increased every 2 min in constant increments. Each participant was instructed to pedal at a steady pace (60 rpm) until they were unable to continue, despite vigorous verbal encouragement. Breath-by-breath gas exchange was monitored throughout the test using a calibrated metabolic cart (Vmax29; SensorMedics, Palm Springs CA, USA). Heart rate (HR) was measured using a Polar heart rate monitor (Polar Electro Oy, Kempele, Finland) and recorded every minute. Rating of perceived exertion (RPE) during the test was evaluated every two minutes and at the conclusion of the cycling test using Borg’s 6-20 scale. We defined VO2peak as the highest measured 30-s at exhaustion. Participants were deemed to have attained VO2peak if two of the following three criteria were reached: HR ≥ 185 bpm; respiratory exchange ratio ≥ 1.1; and an inability to maintain a cadence of 60 rpm despite strong verbal encouragement.

*Measures of sub-maximal fitness:* To assess sub-maximal fitness, HR at RPE of 15 and power output at a HR of 150 bpm during the McMaster Cycling test were calculated. Values were extrapolated from linear regression analysis between the HR and RPE and the power output and HR relationships, respectively.

*Measures of strength:* Strength was determined by a voluntary one-repetition maximum (1-RM) lifting test. After a light warm-up exercise, a demonstration of proper weight training technique was provided to the participants. Subsequently for each individual muscle group (e.g. biceps), the participants would lift a dumbbell at a low weight (1:1:1 cadence) and rate on a scale of 1 to 10 their difficulty with lifting the dumbbell and report if they felt the weight could be increased. If the reported value was less than 10, the trained instructor would increase the amount of weight lifted until the participant reported they could not lift the dumbbell further if the amount of weight were to be increased. One minute of rest was provided between each increase in weight lifted. If the instructor felt the final lift was not performed with proper form, the previous weight lifted would be the 1-RM recorded, and the lifting would not advance for that muscle group.

*Physical activity:* To ensure participants in both groups were engaging in similar levels of physical activity during the intervention period, uniaxial Actigraph GT1M accelerometers (Fort Walton Beach, Florida, USA) were worn over the right hip at all times except during water activities and sleeping. Participants were given a log book to record when the device was put on and removed to verify activity performed. Three-second epochs were utilized to capture the habitual physical activity usually carried out by youth (Bailey RC et al., 1995). Actigraph files were downloaded with ActiLife software (Pensacola, Florida, USA), and time spent in sedentary, light, moderate and vigorous activity and total step counts was determined. Physical activity intensities were calculated from cut-points developed by Evenson 2008 (Evenson KR, Catellier DJ, Gill K, Ondrak KS, & McMurray RG, 2008).

*Statistical analysis:* Baseline fitness and strength values were compared using unpaired Student’s t-tests. To determine the effects of milk intake and short-term exercise training on fitness variables an analysis of variance with between (beverage) and within (time) factors was run with sex and age of peak height velocity as covariates. As there were no sex-based differences all data were collapsed into the two specified beverage groups.

**Results**

*Measures of maximal aerobic fitness:* Maximal fitness variables prior to commencing the intervention were not different between the groups (Table 2). Post intervention, the MILK group had a higher power output in watts/kg (p<0.05) with a trend towards a higher (p=0.06) but not TTE (p=0.1) (Figures 1-3). When power output and VO2peak were expressed per FFM, they were not different.

*Measures of sub-maximal aerobic fitness*: Sub-maximal fitness variables prior to commencing the intervention were not different between the groups (Table 2). Sub-maximal variables (HR at RPE 15 and watts/kg at a HR of 150 bpm) did not change over time or between groups.

*Measures of strength*: Although strength with each of the weight training exercises increased significantly over time in absolute values (Table 3), when expressed per kg of FFM, they were no longer significant. Leg extension and squat increased to a greater extent in the CONT group in absolute terms but not when expressed per kg of FFM. There were no other strength change differences between the two groups.

*Physical activity:* There were 4 MILK and 6 CONT participants who wore the accelerometer for less than four days and/or less than 10 hours per day and thus their data are not included in accelerometer analysis. At least 6 days of data were available for 93% of participants. According to the accelerometer output, mean daily minutes of sedentary time (491± 110 vs. 488 ±108 min/day, p=0.9), light activity (148 ± 36 vs.150 ± 33 min/day, p=0.9), moderate activity (30 ± 10 vs.31 ± 12 min/day, p=0.7), vigorous activity (16 ± 6 vs.19 ± 15 min/day, p=0.4) and step counts per day (8011 ± 2058 vs.8151 ± 2925 steps/day, p=0.9) during the intervention period were similar in the MILK and CONT groups, respectively.

*Training Variables:* The average HR achieved during resistance training (142 ± 2 vs. 146 ± 3, p=0.07); high intensity training (MILK 174 ± 3 vs. 173 ± 4, p=0.6); and moderate training (MILK 150 ± 6 vs. 147 ± 5, p=0.4) components of the intervention were not different between the MILK and CONT groups, respectively.

**Discussion**

Some studies have examined the potential benefits of milk consumption on exercise performance but, to our knowledge, none have been conducted in obese children. We found that the consumption of milk post exercise during a one-week training period in overweight youth improved measures of aerobic power to a greater extent than consumption of an isoenergetic carbohydrate beverage. However, while both groups gained strength we did not observe an effect of beverage on these gains. As opposed to previous trials our study had numerous strengths, which included daily supervised training by experienced investigators; objective daily physical activity measurements; provision of food and experimental beverages; and high compliance to training sessions and beverage consumption.

The effects of milk intake on exercise performance have been investigated using different performance paradigms. Using a time to exhaustion (TTE) test consumption of bovine milk (containing ~17g of protein) compared to non-milk placebo beverages improves performance (Ferguson-Stegall L et al., 2011a; Lunn WR et al., 2012; Karp JR et al., 2006a; Thomas K et al., 2009). When the amount of milk protein is substantially less, the improvement in time to exhaustion with milk is not observed (Lee JKW et al., 2008). Many of the moderate intensity testing trials were performed after glycogen depletion which may be difficult for overweight youth to tolerate (Karp JR et al., 2006a; Thomas K et al., 2009; Ferguson-Stegall L et al., 2011a). In contrast, when athletes were tested at a higher intensity the TTE was not found to be different when chocolate milk is consumed verses a non- protein beverage (Pritchett K et al., 2009). In our study, there was a trend towards a longer time to exhaustion in those who consumed milk. It is possible that there was an adaptation over the seven days of daily training that allowed those consuming milk to store more muscle glycogen. Greater glycogen stores may have led to differences in maximal output but not during submaximal workloads when the reliance on muscle glycogen is lower (Tarnopolsky MA et al., 1997; Aucouturier J, Baker JS, & Duche P, 2008).

It is not clear if milk provided post-exercise acutely or chronically improves strength. Acutely, the consumption of milk immediately and 24 hours post exercise appears to improve knee extension torque and reactive strength index compared to water (Cockburn E et al., 2010). Milk reduces delayed onset muscle soreness (Cockburn E et al., 2010); however, the lack of an appropriate carbohydrate- and protein-containing placebo limits the interpretation of this observation (Cockburn E et al., 2010). Others have shown that decreases in quadriceps isokinetic peak torque 72 hours post exercise are not different when milk, a carbohydrate placebo or water are consumed (Wojcik JR, Walberg-Rankin J, Smith LL, & Gwazdauskas FC, 2001). Over several months, consumption of milk post resistance exercise does not improve strength in non-obese untrained adults more than a non-milk carbohydrate beverage (White KM, Bauer SJ, Hartz KK, & Balridge M, 2009; Hartman JW et al., 2007; Walberg Rankin J et al., 2004) and only in bench press in overweight females (Josse AR, Tang JE, Tarnopolsky MA, & Phillips SM, 2010). Overweight individuals have potential greater strength changes than healthy weight active individuals (Schranz N, Tomkinson G, & Olds T, 2013). However, there were no significant 1RM strength differences when milk was consumed in overweight adolescents post exercise in the current study. Conceivably, if the study had been sustained longer than one week, the smaller reduction in FFM in the MILK group compared to the CONT group (as we reported previously) would have led to greater strength gains in the MILK group over time. This is one of the limitations in the study in that the protocol followed was brief and thus we were unable to determine if FFM losses would continue in the CONT group. A second limitation is that the increases in strength in both groups would almost certainly have been due to a learning effect and thus a longer-term intervention would have been beneficial.

In conclusion, we found that chocolate milk consumption combined with short-term exercise training improved aerobic power with a trend towards a greater increase in VO2peak compared to consumption of an isocaloric carbohydrate beverage. However, there was no impact of beverage type on strength gains.

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**Conflict of Interest**

SMP has received funding from the US National Dairy Council and Dairy Farmers of Canada for previous research. There are no professional relationships with companies or manufacturers that will benefit from the results by any of the authors. The results of the present study do not constitute endorsement by ACSM.

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|  |  |  |  |
| --- | --- | --- | --- |
| Characteristic | MILK (n=26) | CONT (n=29) | p value |
| Sex (males/females) | 8/18 | 12/17 | -- |
| Age (years) | 11 (1.5) | 12 (1.5) | 0.09 |
| Height (cm) | 152 (11) | 159 (10) | \*0.01 |
| Weight (kg) | 66 (20) | 77 (17) | \*0.03 |
| FFM1 (kg) | 38 (9) | 45 (9) | \*0.005 |
| Waist circumference (cm) | 89 (14) | 94 (12) | 0.3 |
| Body Fat (% via DXA1) | 39 (8) | 39 (6) | 0.9 |
| Body Mass Index | 28 (5) | 30 (5) | 0.08 |

Table 1: Baseline MILK and CONT characteristics

Displayed as mean (standard deviation)

\*significantly different at p< 0.05

1 FFM-fat free mass, DXA=dual x-ray absorptiometry

|  |  |  |  |
| --- | --- | --- | --- |
| Variables | MILK | CONT | p value |
| Resting HR1 (bpm) | 96 (9) | 93 (12) | 0.2 |
| Maximal HR (bpm) | 181 (13) | 180 (13) | 0.8 |
| Power output, (Watts) | 112 (34) | 128 (40) | 0.1 |
| Power output (Watts/kg) | 2 (0.4) | 2 (0.4) | 0.9 |
| Power output (Watts/FFM1 in Kg) | 3 (0.8) | 2.8 (0.5) | 0.5 |
| RPE1 (arbitrary units) | 18 (1) | 19 (2) | 0.3 |
| RER1 | 1 (0.07) | 1.1 (0.08) | 0.2 |
| VO2-max (ml/kg/minute) | 24 (5) | 24 (6) | 0.9 |
| VO2-max (ml/kg FFM/minute) | 42 (8) | 41 (6) | 0.4 |
| Time to exhaustion (min) | 10 (2) | 10 (2) | 0.9 |
| HR at RPE-15 (bpm) | 154 (14) | 155 (13) | 0.8 |
| Power output at HR-150 (Watts/kg) | 1 (0.3) | 1.1 (0.2) | 0.2 |

Table 2: Pre Cardiovascular Fitness Variables between MILK and CONT

Displayed as mean (standard deviation)

HR= heart rate, FFM=fat free mass, RPE-rate of perceived exertion, RER=respiratory exchange ratio

No between group differences.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 1 RM Exercise (Kg) | Pre MILK | Pre CONT | Post MILK | Post CONT |
| Triceps Extension | 19 (8) | 19 (8) | 22 (9) | 22 (8) |
| Front Raise | 5 (1) | 6 (2)\* | 5 (1) | 6 (3)\* + |
| Bench Press | 17 (13) | 21 (12) | 20 (12) | 24 (12) + |
| Leg Extension | 33 (17) | 35 (20) | 36 (17) | 45 (25) +# |
| Lunge | 10 (5) | 11 (8) | 11 (5) | 14 (9) + |
| Squat | 11 (6) | 12 (8) | 12 (5) | 16 (9) +# |
| Biceps | 6 (2) | 7 (3) | 6 (2) | 8 (3) + |
| Arm Row | 11 (5) | 13 (8) | 12 (4) | 15 (8) + |
| Lat Pull | 28 (23) | 32 (14) | 26 (11) | 33 (12) |

Table 3: Strength differences between MILK and CONT at baseline and over time

\* Significantly different between groups, +significantly different over time, #interaction between groups over time (p<0.05)

Note when reported per kg of LBM, the group, time and group x time effects are no longer significant.

**Captions for figures**

Figure 1: Peak mechanical aerobic power pre and post intervention

Presented as mean and standard deviation

\*p<0.05

Figure 2: pre and post intervention

Presented as mean and standard deviation

\*p=0.06 (not significantly different)

Figure 3: Time to exhaustion pre and post intervention

Presented as mean and standard deviation

TTE=time to exhaustion

\*p=0.10 (not significantly different)

Figure 1



Figure 2



Figure 3



**CHAPTER 4**

**Title:** Effects of short-term exercise training with and without protein intake on inflammatory markers in obese adolescents

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**PREFACE**

***Significance to thesis***

Overweight adolescents have metabolic complications such as hyperinsulinemia, hyperglycemia, insulin resistance and hypertension which are related to a chronic inflammatory state. Acutely, exercise can escalate inflammatory markers but are attenuated with longer term training of eight to twelve weeks. It is not known at what point during the training regime this attenuation occurs. Thus the goal of this manuscript was to undertake a short-term training protocol (one week) to determine if inflammatory makers would be diminished and whether this correspondingly led to a lowering of metabolic risk factors. The second purpose was to determine if fluid milk, in conjunction with the exercise program, could have superior effects than a non-milk carbohydrate beverage, as milk has been shown to help reduce inflammation, insulin resistance and hypertension.

***Authors’ contributions***

Linda J. Gillis contributed to the design of the study, performed all experiments and data collection unless noted below, organized team of volunteers and research assistants, acted as the RD for dietary analysis, performed all statistical analyses, wrote the initial draft of the manuscript and worked on refining this draft and the revisions based on editorial review. She also collaborated on the grant for funding for this project.

Brian W. Timmons contributed to the design of the study and refined the draft and revisions. He also collaborated on the grant for funding for this project.

Stuart M. Phillips refined this draft and revisions based on editorial review.

Maple Liu performed experiments and data collection particularly in laboratory analysis of inflammatory markers. She worked on refining the draft of the manuscript and revisions based on editorial review.

Nick Persadie performed experiments and data collection particularly in laboratory analysis of glucose and insulin. He worked on refining the draft of the manuscript and revisions based on editorial review.

**Abstract**

We studied the early cardiometabolic and inflammatory adaptations to a short-term exercise intervention with and without milk consumption in obese adolescents. Fifty-four adolescents were randomized to consume milk post exercise (MILK) or a carbohydrate beverage (CONT) during one-week of daily exercise. Insulin and glucose levels were not different between the two groups post training; however, there was a greater decrease in mean arterial pressure in the MILK group (-3 ± 6 mmHg in MILK vs. 2 ± 7 mmHg in CONT, p< 0.04). Milk provided post-exercise did not affect the inflammatory markers C-reactive protein (CRP), tumour necrosis factor- α (TNF-α) or interleukin-6 (IL-6). The exercise intervention led to an increase in TNF-α (p< 0.001). No significant changes in IL-6 or CRP occurred in response to exercise training. The early adaptations to a short-term exercise intervention in obese adolescents include a reduction in mean arterial pressure and an increase in some inflammatory markers.

**Key words:**  adolescent obesity, high intensity training, milk, inflammation, blood pressure

**Introduction**

A hallmark of pediatric obesity is the presentation of chronic systemic inflammation, which is thought to stem primarily from adipose tissue (Visser M et al., 2001). Visceral adipocytes secrete inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which in turn can stimulate hepatic production of c-reactive protein (CRP) (Visser M et al., 2001). These pro-inflammatory markers are associated with cellular dysfunction, the development of insulin resistance and atherosclerotic plaque formation, contributing to cardiovascular disease (Montero D et al., 2012; DeBoer MD, 2013). Thus, interventions designed to reduce systemic inflammation are warranted.

There is debate over the extent to which exercise training interventions can reduce systemic inflammation in overweight children. Exercise intervention trials, without dietary modifications, generally do not have an anti-inflammatory effect on cytokines such as CRP, TNF-α and IL-6 when the intervention is not accompanied by decreases in weight and/or body fat (Murphy EC-S et al., 2009; Kelly AS et al., 2007; Nassis GP et al., 2005). However, the available exercise training studies involving youth have had durations of at least 8 to 12 weeks, and understanding of the initial alterations in the cytokine milieu remain unknown (Murphy EC-S et al., 2009; Kelly AS et al., 2007; Nassis GP et al., 2005). It is known that a healthy diet is required for immune balance and regulation (Barbaresko J et al., 2013). However, it is evident that when diet is the only variable altered that most foods and/or nutrients individually do not alter inflammatory markers, in particular CRP, in overweight children such as nuts (Maranhao PA et al., 2011), folic acid (Pena AS et al., 2007) or vitamin C and E (Engler MM et al., 2003).

Although the data are limited, it is apparent that in lifestyle programs (induction of a negative energy balance through diet and exercise), changes in CRP will only occur when the adolescents show reduced weight and/or fat loss (Kelishadi R et al., 2008; Roberts CK et al., 2007; Kaufman CL et al., 2008). These interventions included expert advice on dietary recommendations but did not provide the actual food to the participants. So it is uncertain if the reduction in energy intake as a whole or the energy from particular foods provided the stimulus for the amelioration of inflammatory markers. In adults, there is some evidence that when milk is provided as part of a weight loss diet that inflammatory markers can improve in as little as one week (Zemel MB et al., 2008; Zemel MB, Sun X, Sobhani T, & Wilson B, 2010; Stancliffe RA, Thorpe T, & Zemel MB, 2011a; vanMeijl LEC et al., 2010) but this has not been demonstrated in all reports (Wennersberg MH et al., 2009; Rosado JL et al., 2011). In a recent review, it was noted that due to methodological limitations in existing studies, further studies are warranted (Labonte ME et al., 2013). To our knowledge, a weight loss program that includes milk and its effects on inflammation and metabolic health has not been tested in overweight youth (Stancliffe RA et al., 2011a). We hypothesized that a diet including post-exercise milk consumption, versus an isoenergetic carbohydrate beverage, provided to participants with a structured exercise program would improve inflammatory markers (CRP, IL-6 and TNF-α) and metabolic factors (blood pressure, glucose, insulin and Homeostatic Model Assessment, HOMA) over a one-week time period. Specifically we were interested in the early adaptations that occur with a structured exercise program with and without protein intake in the form of milk.

**Methods**

This study evaluated secondary outcomes from a previous study on protein metabolism. The single-blind randomized trial involved overweight obese adolescent males and females. Participants were provided with a daily diet that met their dietary nutrient needs. Over 7 days, they performed moderate and high intensity cycling and resistance training over one hour. One group received chocolate milk post exercise (MILK) and the other (CONT) received a milk-free carbohydrate beverage. Written informed consent was obtained from participants and ethics approval was received by the joint McMaster University and McMaster Children’s Hospital ethics board.

Blood pressure: Systolic and diastolic blood pressures were measured by three trained investigators via a sphygmomanometer using a cuff size appropriate for obese individuals. Three values were taken and averaged while sitting in a resting position. Mean arterial pressure was calculated as diastolic blood pressure multiplied by one third of the difference between systolic blood pressure and diastolic blood pressure.

Insulin and glucose: The participants provided a twelve hour fasting blood sample at 9am in the morning. Blood was taken from the antecubital vein to determine glucose and insulin prior to starting the study (2 days before the first training session) and on the last day of the study (2 days after the last training session). Glucose was analyzed with a calorimetric assay (Cat. No. 10009582, Cayman Chemical, Ann Arbor Michigan), and insulin was analyzed with a high sensitivity enzyme-linked immunosorbent assay (ELISA) (KAQ1251, Invitrogen, Burlington, ON). The calculated intra-assay coefficient of variation was 3 and 12% for glucose and insulin, respectively. HOMA was calculated as (glucose x insulin)/405 (Matthews DR et al., 1985).

Inflammatory markers: From the same fasting blood samples collected pre and post training, IL-6, TNF-α and CRP were measured with ELISA kits (R&D systems, Minneapolis, MN): IL-6 (Cat. No. HS600B), TNF-α (Cat. No. HSTA00D), and CRP (Cat. No. DCRP00). The calculated intra-assay coefficient of variation was 6, 5 and 4% for IL-6, TNF-α and CRP respectively.

Statistics: To ensure the sample size was adequate for evaluation of early adaptations in inflammatory markers, a sample size calculation was performed using the work of Zemel 2008 (Zemel MB et al., 2008) and Zemel 2010 (Zemel MB et al., 2010). In these studies, the standard deviation (SD) and difference between the means could be calculated over a one-week trial. The marker with the greatest SD and difference between the means was IL-6 with minimum of 44 subjects (22 each group) required for significance. General well-being was determined and those with active rhinitis or those on antibiotics were excluded from the inflammatory biomarker data. One of the female participants was started on antibiotics during the trial so was excluded from analysis leaving 54 participants (20 males and 34 females). After randomization, 25 participated in the MILK group and 29 in the CONT group. At baseline, t-tests were performed to ensure similar levels of cardiometabolic and inflammatory markers between groups. Treatment variables were analyzed with 2-way ANOVAs with 1 between factor (beverage group) and 1 within factor (time) and sex as a covariate. As there were no sex-based differences all data were collapsed into the two specified beverage groups.

**Results**

Blood pressure: There was a decline in diastolic blood pressure and MAP, but not systolic blood pressure, in the MILK group compared to the CONT group (Table 1).

Glucose and insulin: Although the MILK group had numerically greater reductions in glucose, insulin and HOMA, these reductions were not statistically different than the CONT group. Over time, there was a significant reduction in glucose in both groups (Table 1).

Inflammatory markers: There were no significant differences between the MILK and CONT groups for the three inflammatory markers studied. However, there was a significant time effect for TNF-α increasing from pre to post (Figure 1). There were no time effects for IL-6 (Figure 2) or CRP (Figure 3).

**Discussion**

Intense daily exercise in obese adolescents over one week has effects on some markers of inflammation with a significant increase in TNF-α. However, some of the disease risk factors associated with inflammation were improved such as blood glucose. When milk is included in a healthy diet, there are no differences between the MILK group or CONT group in inflammatory or disease risk factors except for MAP which is significantly decreased with milk consumption combined with exercise.

Hypertension in childhood affects 30% of obese children (National high blood pressure education program working group on high blood pressure in children and adolescents, 2004). Changes in dietary intake can have a significant effect on blood pressure but most research in children has been longitudinal or cross-sectional (Couch SC et al., 2006; Moore LL et al., 2005; Rangan AM et al., 2012; Yuan WL et al., 2013). However, in a randomized controlled trial (RCT) (Couch SC et al., 2008), the Dietary Approaches To Stop Hypertension (DASH) trial, tested a diet high in fruits and vegetables, low fat dairy (2 servings per day), low in red meat and refined carbohydrates. Blood pressure was reduced with the DASH diet but it was not clear which nutrients, group of nutrients or particular foods caused the positive effect. Changes in blood pressure were correlated with an increase in potassium, magnesium, fruit and milk intake and decrease in fat consumption (Couch SC et al., 2008). Milk is rich in potassium and magnesium (Weaver CM, 2014), and in our study the only differences in diet were due to milk alone, and not other foods; thus, we speculate that the combination of exercise and MILK is what resulted in the reduction in blood pressure. There is growing evidence that hypertension is a pro-inflammatory condition with increased levels of CRP, IL-6 and TNF-α (Feber J & Ahmed M, 2010). In children, CRP has been the most researched inflammatory marker with links to hypertension (Sacheck J, 2008). It is questionable whether the CRP is simply increased due to obesity, independent of blood pressure. However, those children with the duality of obesity and hypertension have the highest CRP levels (Sacheck J, 2008).

In adults, recent reviews have indicated that the evidence of the benefit of dairy on metabolic health is limited by appropriately powered RCT's and results are conflicting with milk and/or milk products having variable effects on glucose homeostasis and insulin resistance (Kratz M et al., 2013; DaSilva MS et al., 2014; Weaver CM, 2014). Similar to our study, St-Onge and colleagues (St-Onge MP et al., 2009), found that those randomized to four cups of milk compared to one cup per day had no difference in fasting glucose or insulin. However, with a glucose challenge test, the high milk group had an improvement in insulin usage (St-Onge MP et al., 2009). Our HOMA values while numerically lower in the MILK group post-training were not statistically different than the CONT group. It is plausible that numerous years of obesity into adulthood are required to see significant alterations in fasting glucose, insulin and HOMA with dairy consumption. More research is required given the paucity of data in this area in children and adolescents. Interestingly, in a longitudinal trial, middle aged-women who consumed high dairy in adolescence had a lower risk of type II diabetes and especially if the high dairy intake of adolescence persisted into adulthood (Malik VS et al., 2011).

Insulin resistance and diabetes are inflammatory conditions analogous to hypertension (DeBoer MD, 2013; Dandona P, Ghanim H, Chaudhuri A, Dhindsa S, & Kim SS, 2010). Others have shown that CRP is associated with increased insulin and insulin resistance (Lambert M et al., 2004). Although CRP is considered a general marker of immune activation, it is only weakly correlated with other cytokines (Herder C et al., 2007). Thus TNF-α and IL-6 can be independent markers of insulin resistance and have been associated with metabolic health in obese youth (Decsi T et al., 2003; Gupta A, Ten S, & Anhalt H, 2005). Similar to our study, in a cross sectional study with 519 adolescents, fasting glucose was not associated with IL-6 and TNF-α and fasting insulin and HOMA were associated with IL-6 and not TNF-α (Herder C et al., 2007). Variable results between studies could be related to the level of obesity or other risk factors for disease such as lipid levels (Herder C et al., 2007). We did not find that the consumption of additional milk for one week resulted in reduced inflammatory markers more than a non-diary carbohydrate beverage as others have found in adults (Wennersberg MH et al., 2009; Rosado JL et al., 2011; Van Loan MD et al., 2011). In contrast, studies from Zemel (Stancliffe RA et al., 2011a; Zemel MB et al., 2008) showed that TNF-α, IL-6 and CRP decreased when adults consumed four cups of milk for 24 weeks. Yet Van Mejil (van Meijl LEC & Mensink RP, 2010) found a decrease in TNF-α but not in IL-6 or CRP. The disparity in results between research groups could be attributed to the lack of homogeneity in type of dairy product, fat percentage or sugar added to the milk. The difference in low versus high dairy could also impact results as those that differed by greater than or equal to 3 cups of milk showed improvement in inflammatory markers (Labonte ME et al., 2013). However, this was not replicated in the current study and could be due to the stability of body weight in each group over the one week training period.

Acute exercise causes a stress response with an increase in cortisol and adrenaline which in turn temporarily increases IL-6 and TNF-α, particularly with intense activity (Huang CJ et al., 2013; Nimmo MA, Leggate M, Viana JL, & King JA, 2013). Obese children have a higher acute response than healthy weight children to this stress response immediately after and two hours post a VO2peak test (McMurray RG et al., 2007). In adults, aerobic and resistance exercise training over several months are effective at attenuating this acute response (Huang CJ et al., 2013; Nimmo MA et al., 2013). It is not clear how long this acute elevation in inflammatory markers with exercise persists and the length of time an individual needs to train in order to attenuate this response. In a comparable designed study to the current study, overweight children and adolescents had a decrease in inflammatory markers IL-6 and TNF-α with a supervised daily activity program and provision of food over two weeks (Izadpanah A et al., 2012). In the current study, it is not clear why TNF-α increased after one week of training but could be that our participants performed intense bouts of cycling and resistance training while the Izadpanah participants had two and a half hours of playing games at a more moderate tempo(Izadpanah A et al., 2012). Over-training in elite athletes can exacerbate the inflammatory response to exercise (Cooper DM, Radom-Aizik S, Schwindt C, & Zaldivar F, 2007). Studies that employ high intensity interval training are usually performed only three times per week (Leggate M et al., 2012; Kessler HS, Sisson SB, & Short KR, 2012; Stensvold D, Slordahl SA, & Wisloff U, 2012). Thus was the daily intensive exercise over a one week period in previously sedentary individuals too high of a stress leading to increases in the inflammatory markers?

Future work could potentially compare one week of intense daily exercise versus three days per week of intense activity to see if in fact the daily exercise was counterproductive for inflammation or an issue of inadequate recovery time. The other question to examine is if this inflammatory response is detrimental over time as glucose decreased in both groups and MAP decreased when milk was consumed as part of the diet. The interrelationship between inflammation and metabolic disease still needs further clarification.

In conclusion, a daily intensive exercise protocol over one week has effects on the inflammatory profile of obese adolescents with elevations in TNF-α. These early changes in markers are not different with or without milk. However, milk can lower blood pressure compared to individuals who consume a milk-free beverage post exercise.

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Table 1: Inflammatory and Metabolic Risk Factors

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Factor | MILK pre | CONT pre | MILK post | CONT post |
| Glucose (mg/dl) | 95 (10) | 93 (10) | 86 (14) | 87 (11)\* |
| Insulin (uIU/ml) | 47 (23) | 47 (13) | 42 (20) | 48 (24) |
| HOMA | 11 (6) | 11 (2) | 9 (5) | 10 (5) |
| Systolic blood pressure (mmHg) | 112 (7) | 114 (9) | 109 (9) | 114 (8) |
| Diastolic blood pressure (mmHg) | 71 (4) | 68 (5) | 67 (6) | 68 (5)\*\* |
| MAP (mmHg) | 84 (4) | 83 (5) | 81 (7) | 84 (5)\*\* |

MILK = milk group, CONT = control group

HOMA = Homeostatic Model Assessment, MAP = mean arterial pressure

Presented as mean (standard deviation)

\*Time effect significantly different at p<0.05

\*\*Interaction effect significantly different at p<0.05

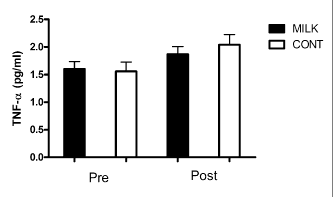


Figure 1: Effects of exercise and beverage on TNF-α pre and post intervention

MILK=milk group, CTRL=control group,

Presented as mean and standard deviation

Significant time effect p<0.001

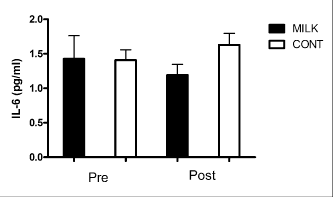


Figure 2: Effects of exercise and beverage on IL-6 pre and post intervention

MILK=milk group, CTRL=control group,

Presented as mean and standard deviation

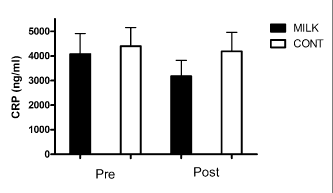


Figure 3: Effects of exercise and beverage on CRP pre and post intervention

MILK=milk group, CTRL=control group,

Presented as mean and standard deviation

**CHAPTER 5**

**General discussion and conclusions**

**5.0 General Discussion and Conclusions**

Adolescence is a period in life that requires nutrients for rapid growth and alterations in development. A lack of dietary energy during this significant life phase can impair this stage and thus should be avoided (Amador M et al., 1990). However, in the case of obese adolescents, if they do not alter their diets and activity patterns, it can cause detrimental co-morbidities and premature mortality (Morrison JA, Friedman LA, & Gray-McGuire C, 2007). This thesis assisted in the prescription that is required to help maintain total body weight and lean body mass while reducing body fat. Moreover, it aided in the reduction of some of the metabolic complications associated with childhood obesity such as hypertension.

The key factors that substantiated these results is that the: entire diet was provided and surpluses and deficits were calculated; training sessions were supervised with an individual research coach with intensity of activity monitored via heart rate; and overall activity levels were monitored via an objectively measured technique.

**5.1 Significance of the studies**

One of the key findings was that flavoured milk, when consumed post exercise, improved protein balance in overweight youth. This in turn helped maintain muscle mass which is key to long term weight maintenance. When the intense activity sessions ceased for three subsequent days, the participants remained in positive protein balance with milk alone. However, the balance was lower than when the exercise and milk coincided together. There is an abundance of evidence that eating healthier and being more active can help improve anthropometric profiles (Hoelscher DM, Kirk S, Ritchie L, & Cunningham L, 2013), but it is important to look beyond this simple equation. The timing of nutrients, particularly those found in milk, with exercise have superior benefits (Phillips SM et al., 2011). Prior to this thesis, there was insufficient research on the effects of milk post exercise on protein balance in the pediatric population. In two previous investigations that had obese children reduce calories through dietary changes and exercise, the participants had a lower protein turnover post treatment (Ebbeling CB et al., 1998; Ebbeling CB et al., 1999) which was deleterious to maintenance of lean body mass (Ebbeling CB et al., 1999). The participants in these studies lost weight likely due to the reduced caloric intake (Ebbeling CB et al., 1998; Ebbeling CB et al., 1999). In this thesis, the subjects ate close to their recommendations for age and sex (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005) and weight did not change. It was the milk consumption that assisted in the maintenance of lean body mass and total body weight with loss of body fat. It is known that acutely protein and carbohydrate post exercise improves overall protein balance (Phillips SM et al., 2011).

Milk is an exemplar protein source as it contains both a fast acting protein (whey) which stimulates protein synthesis and a slow protein (casein) which keeps amino acids, in particular leucine, present in the blood in the post exercise protein synthesis condition Jakubowicz D & Froy O, 2013; Lambourne K et al., 2013). In order to remove the carbohydrate from milk (glucose) in the blood and prevent hyperglycemia, insulin is released which also stimulates protein synthesis (Jakubowicz D & Froy O, 2013; Lambourne K et al., 2013). Milk additionally affects the incretin system and bioactive peptides which all play a role in insulin excretion and post prandial hyperglycemia (Jakubowicz D et al., 2013). Besides the anabolic effect of milk proteins, calcium has an effect on weight. Calcium and/or milk supplementation: alter lipid metabolism with a shift to lipolysis in fat cells; and increase fecal fat loss from β-lactoglobulin; which leads to an increase in thermogenesis (Tremblay A & Gilbert JA, 2009; Sousa GDT et al., 2012). Furthermore, the whey protein has also been implicated in improvements in energy metabolism-related hormones. For example, whey can help improve leptin resistance and lower ghrelin levels (Sousa GDT et al., 2012). Despite these purported mechanisms, in other randomized controlled trials in children and adolescents, milk does not improve the anthropometric profile (Spence LA et al., 2011). This thesis adds to the literature that the timing of the milk beverage may be crucial, at least in the first week of exercise. Having the milk consumed post exercise is a successful strategy in adult obese females for improvement in lean body mass (Josse AR et al., 2010). The current thesis extends this finding to males as there were no significant differences in protein balance between the girls and boys.

In pediatric weight loss programs, goals to increase healthy foods are more successful at keeping weight off than suggesting youth decrease unhealthy foods (Epstein LH, Paluch RA, Beecher MD, & Roemmich JN, 2008). It was not clear if specific foods or food groups would benefit obese individuals. Thus the current study adds to the literature with the promotion of milk, as a positive early change in overweight youth.

The second key finding was that milk and exercise can improve cardiovascular power to a greater extent than exercise followed by a non-protein beverage. Examining if milk and exercise can improve fitness using a Vo2max power test has not been carried out previously in youth. Over six weeks in older adults, an increase in Vo2max with a water based beverage supplemented with milk proteins can be achieved (Robinson MM, Turner SM, Hellerstein MK, Hamilton KL, & Miller BF, 2011). Other work in healthy physically fit adults has involved longer time to exhaustion tests with glycogen depletion that would be difficult to undertake in an overweight adolescent. However, these studies concur that milk can improve performance in exercise tests (Karp JR et al., 2006a; Lunn WR et al., 2012; Thomas K et al., 2009; Ferguson-Stegall L, McCleave EL, & Ding Z, 2011b).

Several potential mechanisms have been proposed for how milk can enhance performance. Similar to the anthropometric benefits of milk, the positive effect of leucine and carbohydrate in stimulating protein synthesis could potentially lead to training adaptations (Phillips SM et al., 2011). Indeed, the consumption of chocolate milk aides in glycogen re-synthesis that would be beneficial for subsequent exercise sessions (Ferguson-Stegall L et al., 2011b). Milk additionally restores fluid balance in a similar or superior manner than water and sport beverages (Phillips SM et al., 2011). Finally, there is a decrease in exercise induced muscle damage and delay in muscle soreness when milk is consumed pre and post exercise (Cockburn E et al., 2010).

If youth perceive that exercise takes less effort, as what occurs when fitness improves, they are more likely to continue to be active (Kirschenbaum DS & Gierut K, 2013). The improved fitness with an improved body fat loss in one week could empower them to continue with their healthy lifestyle improvements. It may likewise give them confidence that they are physically and mentally able to perform intense activity (Steele MM, Daratha KB, Bindler RC, & Power TG, 2011).

The third key finding is that milk improves blood pressure. This was not unexpected as reported in large scale retrospective and prospective trials, there is an association between lack of dairy products and hypertension (Lamarche B, 2009). Results of intervention trials using the DASH diet which includes milk consumption point to its benefits as well (Yuan WL et al., 2013). The current thesis adds to the literature as milk was the only variable altered in the diet that included an abundance of fruits and vegetables and low salt intake also found in the DASH diet. Calcium from dairy products has been implicated as one of the nutrients that aids in the attenuation of blood pressure through its suppression of 1,25-dihyroxyvitamin D which normally increases peripheral vascular resistance (Lamarche B, 2009). Casein, whey and bioactive peptides inhibit angiotensin-1-converting enzyme in the kidneys which also aid in reducing vessel resistance (Lamarche B, 2009). The current thesis provided auxiliary information in that milk and exercise can improve mean arterial pressure in a brief timed trial.

The fourth key finding is that an increase in some inflammatory markers can occur with daily intense activity over one week in obese children, and milk does not act differently than a non-milk carbohydrate beverage in this effect. One other research group has studied this question in adults over a short-term time period (also one week) without weight loss and found the opposite result that milk caused an acute reduction in TNF-α and IL-6. However, this was in comparison to a soy beverage, and it was not noted if the participants exercised and to what extent during the trial (Zemel MB et al., 2010). Others have shown that exercise causes an amplification in some inflammatory markers using acute exercise protocols (Huang CJ et al., 2013; Nimmo MA et al., 2013). This is related to an amplified stress response with increased cortisol and adrenaline which occurs to a even greater extent in obese individuals (McMurray RG et al., 2007). Training trials over longer periods have had reductions in the inflammatory milieu which have been hypothesized to be related to milk having: bioactive peptides that inhibit angiotensin converting enzyme; antimicrobial effects; and abilities in reducing oxidative stress (Jakubowicz D et al., 2013; Sousa GDT et al., 2012). Longer term trials are complicated by the fact that the participants are placed on hypocaloric diets and the loss of weight could be the cause of the attenuation of markers and not the actual treatment (Zemel MB et al., 2008). Thus this thesis adds to the literature in that short-term changes to the inflammatory attenuation response of exercise do not occur over one week.

**5.2 Current hypotheses and future experiments**

There are various research avenues that can be explored from this study in the future. First, it is evident that it needs to be determined if daily intense activity in obese children, not accustomed to vigorous activity, is detrimental to metabolic health or a normal transient elevation of inflammatory markers. Thus one could test the current daily exercise protocol in comparison with the more frequently utilized activity protocol of three times per week (Montero D et al., 2012). Zemel’s group noted that CRP was not reduced at 7 days but was at 28 days with exercise and milk (Zemel MB et al., 2010). Inflammatory markers could be measured each day over two to four weeks to determine when the markers would be attenuated.

This trial was one week in duration so could be extended to determine if there would be long term beneficial effects of milk and exercise, conceivably a 6 to 12 month trial. Other health indicators such as cholesterol, fatty liver and other common health concerns of obese adolescents could also be evaluated (Larson-Ode K, Frohnert BI, & Nathan BM, 2009).

It was demonstrated that milk was responsible for the improvements in anthropometric profiles, fitness and metabolic health as this was the only distinct variable between the two participant groups. However, it is not known if the milk reacted in conjunction with other healthy foods provided for the effect. It would have been clinically interesting to have had a third experimental group that received milk post exercise with the typical "unhealthy" diet of an obese child. For example, a diet low in fruits and vegetables, and high in saturated and trans fat and added sugars (Gillis LJ & Bar-Or O, 2003). This could aid in determining if the two previous studies in youth that provided milk after exercise, and did not find benefits to the participant’s health, were related to other unhealthy components contained in their diets (Volek JS et al., 2003; Lambourne K et al., 2013). Teasing out what nutrients or foods acted with the milk would take further testing.

The current thesis demonstrated that youth from 9 to 15 years of age will attend an intense exercise and diet protocol daily over one week as compliance to the protocol was remarkably high. Perceived self-efficacy in exercise and diet is believed to be a prerequisite for behaviour change and for maintenance of that behaviour change (Bandura A & Schunk D, 1981; Bohman B, Nyberg G, Sundblom E, & Elinder LS, 2014). Youth who have more confidence in their ability to perform physical activity and make healthy diet choices have a lower level of adiposity (Steele MM et al., 2011). As early weight loss in a pediatric obesity treatment program is linked to treatment response (Goldschmidt AB et al., 2011), an improvement in skills by an intensive one week program could provide further empowerment for future success in goal setting and behaviour change (Kirschenbaum DS et al., 2013). This theory has not been researched in the literature and warrants further analysis.

**5.3** **Conclusion**

This thesis presented a novel approach to the treatment of pediatric obesity with societal and clinical implications and furthermore provided results that helped clarify a divergence of outcomes in the literature. It stepped beyond the recommendation of exercise and a healthy diet (Hoelscher DM et al., 2013) and discovered the synergistic effect of exercise and milk. Consuming milk post exercise results in a preservation of lean body mass through enhanced protein balance. Weight remains stable with a reduction in body fat which allows for future growth and development. Metabolic health is altered in obesity (Larson-Ode K et al., 2009), and this thesis demonstrated the unique combination of milk and exercise in reducing blood pressure. However, one week is too short to see a reduction in the chronic inflammation of obesity, and in fact some markers increase demonstrating that the acute rise in inflammatory factors from exercise (Huang CJ et al., 2013) is sustained over one week. The current thesis revealed the short term benefits of milk and exercise in treating pediatric obesity and set the stage for future research in this area.

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**Appendix A**

Milk Food Frequency Questionnaire

Milk Food Frequency Questionnaire

Subject ID#: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

NOTE: check the box that applies.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Questions** | **Serving Frequency** | | | | |
| How often do you: | **Never** | **1-2 times per month** | **1-2 times per week** | **1 time per day** | **2 or more times per day** |
| Drink milk as a beverage or with cereal (please include chocolate milk)? |  |  |  |  |  |
| Drink meal replacement, energy, or high-protein beverages such as Instant Breakfast, Ensure, Slim-Fast, Sustacal or others? |  |  |  |  |  |
| Eat cheese (including brick cheese, sliced cheese on cheeseburgers or in sandwiches or subs)? |  |  |  |  |  |
| Eat yogurt (NOT including frozen yogurt) |  |  |  |  |  |
| Eat ice cream? |  |  |  |  |  |
| Drink orange juice with added calcium? |  |  |  |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Questions** | **Serving Size** | | | |
| Each time you: | **None** | **Less than 1 cup** | **1 to 1½ cups** | **More than 1½ cups** |
| Drank milk as a beverage, how much did you usually drink? |  |  |  |  |
| Added to milk your cold cereal, how much was usually added? |  |  |  |  |
| Drank meal replacement beverages, how much did you usually drink? |  |  |  |  |
| Ate ice cream, how much did you usually eat? |  |  |  |  |
| Drank orange juice with added calcium , how much did you drink? |  |  |  |  |
|  | **None** | **Less than 100ml**  **(Less than**  **1 container)** | **100ml (1 container)** | **More than 100ml**  **(1 or more containers)** |
| Ate yogurt, how much did you usually eat? |  |  |  |  |
|  | **None** | **Less than ½ ounce or less than 1 slice** | **½ to 1½ ounces or 1 slice** | **More than 1½ ounces or more than 1 slice** |
| Ate cheese, how much did you usually eat? |  |  |  |  |

**Appendix B**

Diet Plan

Milk with Exercise Study Sample Meal Plan

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Meal | | Day 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Break-fast | Smoothie-yogurt, chocolate milk, frozen banana, whole wheat toast, margarine | Raisins, Corn Bran Cereal, Almonds, chocolate milk | Shreddies, pear, chocolate milk | Whole wheat toast, peanut butter, orange, chocolate milk | Tortilla shell, peanut butter, banana, chocolate milk | Shreddies, fruity cheerios, cantaloupe, chocolate milk | Raisins, corn bran cereal, almonds, choc- olate milk |
| Snack | Fruit to go bars | Orange | Applesauce | Grapes | Applesauce | Water-melon | Plums |
| Lunch | Whole wheat bread, tuna, mayo-nnaise, celery, fruit roll up | Whole Wheat Bread, chicken, mayo-nnaise, lettuce, grapes, chocolate chip granola bar, carrots | Pita, pork, taztziki sauce, apple, cucumber, thinsation cookies | Tortilla shell, chicken, caesar salad, straw-berries, rice krispie square | Bagel, hummus, carrot sticks, orange, thinsation cookies | Egg salad sandwich (egg with mayo-nnaise), whole wheat bread, carrots, grapes, rice krispie square | Tortilla, chicken, salsa, lettuce, melon, thin-sation cookies |
| Snack | apple | Yogurt tube | Yogurt, strawberries | Carrots, celery, peppers, ranch salad dressing | Yogurt Tube | Celery, peppers, cucumber, ranch salad dressing | Yogurt |
| Supper | Hamburger, bun, ketchup, tossed salad with mandarin oranges, sunflower seeds, salad dressing, veggie kabobs-zucchini, tomatoes, green pepper | Pork tenderloin with chutney, green beans, mashed potato, margarine, tossed salad, Italian salad dressing | Chicken, mustard, bread crumbs, parmesan cheese, peas, rice, tomatoes, margarine | Fish, lemon, canola oil, spicy potatoes, corn on the cob, margarine | Scrambled eggs, brown beans, toast, margarine, tossed salad, Italian salad dressing | Shake and bake chicken nuggets, caesar potato slices, peas | Steak, broccoli, garlic bread, caesar salad |
| Snack | Corn Bran, Chocolate milk | Apple dipped in cinnamon and sugar, chocolate milk | Celery, carrots, ranch dressing, chocolate milk | Yogurt, bran buds,choc-olate milk | Water-melon, chocolate milk | Potato chips, chocolate milk | Fruit to go bars, chocolate milk |

250 ml CONT beverage (in place of chocolate milk)

Mix 3 tablespoons non-dairy creamer, 1 teaspoon cocoa, 1-1/2 tablespoons chocolate syrup and 200 ml water.

Heat in microwave stirring until dissolved. Cool before serving.

**Appendix C**

Exercise Plan

MILK Study Circuit Routine

Subject ID# \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

NOTE: a 1min rest period is followed by the cycle ergometer

**Day 1:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Warm Up |  | | | | | |
| 1 RM | Biceps Curl | Cycle ergometer Bout #1 | One Arm Row | Cycle ergometer  Bout #2 | Lat. Pull | Cycle ergometer  Bout #3 |
| Set #1 |  |  |  |  |  |  |
| Set #2 |  |  |  |  |  |  |
| Moderate Ex |  | | | | | |
| Cool Down |  | | | | | |

**Day 2:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Warm Up |  | | | | | |
| 1 RM | Triceps Extension | Cycle ergometer  bout #1 | One Arm dumbbell Front Raise | Cycle ergometer  Bout #2 | Bench Press | Cycle ergometer  Bout #3 |
| Set #1 |  |  |  |  |  |  |
| Set#2 |  |  |  |  |  |  |
| Moderate Ex |  | | | | | |
| Cool Down |  | | | | | |

**Day 3:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Warm Up |  | | | | | |
| 1 RM | Leg Extension | Cycle ergometer  bout #1 | Lunge | Cycle ergometer  Bout #2 | Squat | Cycle ergometer  Bout #3 |
| Set #1 |  |  |  |  |  |  |
| Set#2 |  |  |  |  |  |  |
| Moderate Ex |  | | | | | |
| Cool Down |  | | | | | |

**Day 4:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Warm Up |  | | | | | |
| 1 RM | Biceps Curl | Cycle ergometer  bout #1 | One Arm Row | Cycle ergometer  Bout #2 | Lat. Pull | Cycle ergometer  Bout #3 |
| Set #1 |  |  |  |  |  |  |
| Set#2 |  |  |  |  |  |  |
| Moderate Ex |  | | | | | |
| Cool Down |  | | | | | |

**Day 5:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Warm Up |  | | | | | |
| 1 RM | Triceps Extension | Cycle ergometer  bout #1 | One Arm dumbbell Front Raise | Cycle ergometer  Bout #2 | Bench Press | Cycle ergometer  Bout #3 |
| Set #1 |  |  |  |  |  |  |
| Set#2 |  |  |  |  |  |  |
| Moderate Ex |  | | | | | |
| Cool Down |  | | | | | |

**Day 6:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Warm Up |  | | | | | |
| 1 RM | Leg Extension | Cycle ergometer  bout #1 | Lunge | Cycle ergometer  Bout #2 | Squat | Cycle ergometer  Bout #3 |
| Set #1 |  |  |  |  |  |  |
| Set#2 |  |  |  |  |  |  |
| Moderate Ex |  | | | | | |
| Cool Down |  | | | | | |

**Day 7:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Warm Up |  | | | | | |
| 1 RM | Biceps Curl | Cycle ergometer  bout #1 | One Arm Row | Cycle ergometer  Bout #2 | Lat. Pull | Cycle ergometer  Bout #3 |
| Set #1 |  |  |  |  |  |  |
| Set#2 |  |  |  |  |  |  |
| Moderate Ex |  | | | | | |
| Cool Down |  | | | | | |

**Appendix D**

Additional data requested from external reviewer during defense

Table 1: Anthropometric changes in milk and control groups

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Variable | Pre | | Post | | Change pre to post | |
|  | MILK | CONT | MILK | CONT | MILK | CONT |
| Fat mass (kg) | 27 (12) | 32 (11) | 25 (10) | 31 (9)\* | -2 (6) | -1 (7) |
| Percent fat | 40 (7) | 40 (7) | 39 (6) | 40 (7) | -0.4 (1) | 0.5 (1)\* |
| Fat free mass (kg) | 38 (8) | 45 (9) | 38 (9) | 45 (2) | -0.2 (0.7) | -0.7 (0.8)\* |
| Percent fat free mass | 60 (7) | 60 (7) | 61 (6) | 60 (7) | 1.2 (3) | 0.2 (3) |

\*milk group significantly different than control group at p < 0.05

Mean (standard deviation)

MILK = milk group and CONT =control group

Measurements obtained via bioelectrical impedance

Table 2: Additional bone and diet data

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Variable | Pre | | Post | | Change pre to post | |
|  | MILK | CONT | MILK | CONT | MILK | CONT |
| Vitamin D Intake (IU) | 144 (60) | 144 (87) | 383 (16) | 92 (14)\* | 241 (62) | -52 (86)\* |
| Bone mineral content (g) | 1527 (370) | 1849 (464)\* | N/A | N/A | N/A | N/A |
| Bone mineral density (g/cm2) | 0.92 (0.1) | 0.98 (0.1) | N/A | N/A | N/A | N/A |

\*milk group significantly different than control group at p < 0.05

Mean (standard deviation)

MILK = milk group and CONT =control group

Vitamin D intake at pre from 3-day food records

Vitamin D intake at post from provided diet minus foods not eaten

Bone mineral content and bone mineral density obtained from DXA measurement

N/A = not measured at post