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Article Title: Effects of Short-Term Exercise Training With and Without Milk Intake on Cardiometabolic and Inflammatory Adaptations in Obese Adolescents

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Abstract

There is some evidence that a combination of factors can reduce inflammation and associated metabolic risk factors. We studied the early cardiometabolic and inflammatory adaptations to a short-term exercise intervention with and without milk 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 levels were not different between the groups post training. Glucose was reduced over time in both groups (-9±13 mg/dl MILK and -6±14 mg/dl CONT, p<0.05) but not different between groups. There was a greater decrease in mean arterial pressure (MAP) in the MILK group (-3±6 mmHg MILK vs. 2±7 mmHg CONT, p<0.04). Milk provided post-exercise did not affect 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 in both groups (0.27±0.7 pg/ml MILK and 0.48±0.6 pg/ml CONT, p<0.001). The early adaptations to a short-term exercise intervention in obese adolescents include a reduction in MAP 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 (52). 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) (52). These pro-inflammatory markers are associated with cellular dysfunction, the development of insulin resistance and atherosclerotic plaque formation, contributing to cardiovascular disease (7;34). 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 (20;36;37). 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 (20;36;37). It is known that a healthy diet is required for immune balance and regulation (1). 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 (28), folic acid (40) or vitamin C and E (9).

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 (18;19;42). 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 (46;56;57) but this has not been demonstrated in all reports (43;54). In a recent review, it was noted that due to methodological limitations in existing studies, further studies are warranted (24). To our knowledge, an exercise program that includes milk and its effects on inflammation and metabolic health has not been tested in overweight youth (46). 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 study on protein metabolism (Gillis et al. under review). The single-blind randomized trial involved overweight obese adolescent males (ages 11 to 15 years) and females (ages 9 to 13 years) who were randomized to one of two beverages after exercise. Both groups were provided with a measured daily diet that met their dietary energy requirements (including their experimental beverages) compared to recommendations for age and gender (47). Over 7 days, they performed moderate and high intensity cycling and resistance training for one hour. High intensity cycling involved 6-15 second bouts at 100% of their determined VO2peak with an active 1 minute recovery between bouts, and moderate cycling was performed at 50% of VO2peak. Resistance training involved three sets of 10 repetitions with a range of muscle groups at 70 percent of their 1RM. One group (MILK) received 500 ml of skim chocolate milk (52 grams carbohydrate, 0 grams fat, 16 grams protein post exercise) and the other (CONT) received an isocaloric milk-free carbohydrate beverage (32 grams carbohydrate, 8 grams fat, 0 grams protein) that had similar taste to chocolate milk. Written informed consent was obtained from participants and ethics
approval was received by the Hamilton Health Sciences/Faculty of Health Sciences research 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. Pre and post blood pressure measurements were taken two days before and two days after the 7-day protocol.

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 (31).

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 (56) and Zemel 2010 (57). 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**

**Adherence to protocol:** Of the provided dietary energy, 98% was consumed, and 99% of the seven exercise sessions were attended.

**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 in both groups but no time effects for IL-6 or CRP (Figure 1).
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.

Changes in dietary intake can have a significant effect on blood pressure but most research in children has been longitudinal or cross-sectional (3;35;41;55). However, in a randomized controlled trial (RCT) (4), 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 (4). Milk is rich in potassium and magnesium (53), 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 (10). In children, CRP has been the most researched inflammatory marker with links to hypertension (44). 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 (44). There is lack of agreement in the literature as to whether exercise training alone over several months, without weight or fat loss, can reduce blood pressure in overweight children (12). Most research groups have indicated no change in diastolic blood pressure while systolic blood pressure has been
shown to decrease with exercise alone in a meta-analysis of a few studies (12). What makes our data compelling is that when exercise was combined with a healthy diet including milk, blood pressure was reduced to a greater extent than a healthy lifestyle without milk. The attenuation in diastolic blood pressure was greater than is seen in longer duration studies (12).

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 (6;23;53). Similar to our study, St-Onge and colleagues (45), 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 (45). 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 (27). Acutely, there are altered insulin responses to high intensity exercise in obese in comparison to normal and overweight individuals in the fed state. Obese children will have a decrease in insulin immediately after exercise with a rise 30 minutes post exercise (49). Yet others have shown that a single bout of resistance exercise can improve insulin sensitivity for 24 hours post exercise (22) but may only occur with those who are non-exercisers prior to the test (38). Short term exercise programs (e.g. 7 weeks) improve insulin sensitivity but it is not clear the effect is due to weight or fat loss (11). There are also inconclusive data on which exercise mode, duration, intensity and frequency affect insulin (11;22).
Insulin resistance and diabetes are inflammatory conditions analogous to hypertension (5;7). Others have shown that CRP is associated with increased insulin and insulin resistance (25). Although CRP is considered a general marker of immune activation, it is only weakly correlated with other cytokines (15). Thus TNF-α and IL-6 can be independent markers of insulin resistance and have been associated with metabolic health in obese youth (8;14). We did not find that the consumption of additional milk for one week resulted in reduced inflammatory markers more than a non-dairy carbohydrate beverage as others have found in adults (43;50;54). In contrast, studies from Zemel (46;56) showed that TNF-α, IL-6 and CRP decreased when adults consumed four cups of milk for 24 weeks. Yet Van Mejil (51) 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 (24). 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 (16;39). Obese children have a higher acute response than healthy weight children to this stress response immediately after and two hours post a VO₂peak test (32). In adults, aerobic and resistance exercise training over several months are effective at attenuating this acute response (16;39). 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. We acknowledge that short-term changes may be different than longer-term changes, in terms of how inflammatory markers respond to an increase in daily exercise. What makes our study unique is that we trained the obese youth daily with an intense protocol while others have used...
exercise on average three to four times per week with moderate intensity over 8 to 24 weeks (20;33;36). Indeed, we are also aware of much shorter term studies (consisting of either exercise or diet manipulation) that appear to show rapid changes. For example, in work by Zemel, a healthy diet caused a reduction in inflammatory markers in one week in overweight adults (57); and Izadpanah found that moderate intensity exercise performed daily in overweight children caused a decrease in inflammatory markers in just two weeks (17). In this way, we were interested in studying the early changes in inflammatory markers in response to an intense, 7-day exercise protocol.

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 (17). 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 participants in the aforementioned study had two and a half hours of playing games at a more moderate tempo (17). Over-training in elite athletes can exacerbate the inflammatory response to exercise (2). Studies that employ high intensity interval training are usually performed only three times per week (21;26;48). 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? This one-week ‘proof of principle’ intervention was important to undertake as dietary intake and activity patterns can be altered in longer term trials, which often lack rigorous experimental control and experience inevitable changes in growth and development that occur as an adolescent matures (13;29;30).

One strength of the current study was that participants were recruited evenly throughout the year and we were able to tightly control energy intake and expenditure.

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 causes an elevation in TNF-α in obese adolescents with no change in CRP or IL-6. These inflammatory markers are not affected by short-term milk consumption. However, milk can lower blood pressure compared to individuals who consume a milk-free beverage post exercise.
Reference List


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Figure 1: Effects of exercise and beverage on inflammatory markers pre and post intervention. Presented as mean and standard deviation, A) TNF-α, *significant time effect in both groups p<0.001; B) IL-6; and C) CRP.
Table 1: Inflammatory and Metabolic Risk Factors

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

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