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The Acute Effects of a Maple Water Drink on Exercise Responses, Oxidative Stress and Inflammation in Overweight College Males

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Abstract The purpose of this study was to examine the acute effect of maple water on exercise responses and biomarkers of post-exercise inflammation and muscle damage in an overweight male college population. The initial study used a single blind, pre/post exercise design, where the participants (N=10) consumed maple water or placebo (355ml/12 fluid oz) prior to an incremental treadmill running protocol to exhaustion and returned one week later to consume the opposite treatment and repeated the maximal bout of exercise. During each exercise bout, finger-stick measures of blood glucose were taken, along with venipuncture measures for inflammatory markers, oxidative stress and muscle damage. Analysis of the data revealed a significant decrease in 2 anti-inflammatory markers IL-4 ($p<0.001$) and IL-10 ($p=0.026$), a significant decrease in 1 pro-inflammatory marker IL-12 ($p=0.022$), and a significant increase in oxygen consumption during exercise ($p=0.045$). Early outcomes indicate maple water has positive benefits for those that exercise in the areas of cardiovascular fitness and post exercise inflammation.

Keywords: *exercise performance, phytonutrients, inflammation, oxidative stress*

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1. Introduction

Energy drinks have taken their place in the world marketplace over the past 20 years, in large part, due to claims of increased exercise performance, mental awareness and memory, or improvement in affective state (mood). Studies have shown that during situations of mental [1,2,3] and physical stress [1], performance and mood can be maintained, if not improved due to the use of energy drinks [1,2]. These mental and physical exercise responses can be attributed in part to the moderately high levels of glucose and caffeine [1,2,3] that are a staple of the formulation of most energy drinks.

Conversely, sports drinks are formulated to replace lost fluids and electrolytes during training and competition by delivering carbohydrates and electrolytes throughout the body [4]. Sports drinks provide a proper hydration level prior to exercise, replace electrolytes and fluids lost through sweat during exercise, assist in thermoregulation throughout, and when combined with proper diet, restore the body post-exercise [5]. During periods of intense activity, especially in hot or humid environments, sports drinks are designed to deliver carbohydrates, electrolytes which can be absorbed quickly from the small intestine [4]. However, the carbohydrate and electrolyte concentration can vary greatly in both type (glucose, fructose, sucrose, or maltodextrin), carbohydrate amount (20 to 56g/L), and sodium concentration (0.46 to 1.2g/L) [6].

Typically, sports drinks are not diluted prior to ingestion, as this changes the concentration of the carbohydrate and sodium, thus delaying the delivery of energy and fluids. Any delay in fluid or electrolyte delivery affects hydration status and thermoregulation. A moderate decrease of body weight (2-3%) via fluid loss, or an increase in core body temperature (39°C) is associated with a significant decrease in physical performance [7]. Additionally, dehydration and increased body temperature are precursors to more serious acute health emergencies such as exertional heat stroke and rhabdomyolysis [8]. However, issues with caloric content, the use of food dyes, artificial sweeteners and gastrointestinal discomfort have caused moderate-to-high performance athletes to search for alternatives [9,10,11].

Maple water (MW) is water pulled up from the ground and filtered by the tree roots. The pure maple tree sap is collected directly from the maple tree, then the liquid is sterilized while maintaining the naturally occurring sugars, vitamins, and minerals that nourish the tree. It is an organic, vegan beverage with 30 calories per 12oz serving, approximately half the grams of carbohydrates (7g in maple water vs 14g in coconut water). It is naturally alkaline (pH 7.4) and contains more manganese than a cup serving of kale (MW 40% DV, kale 30% DV). MW contains naturally occurring polyphenolic compounds, antioxidants, prebiotics, electrolytes, and a Krebs cycle intermediate, malate [12,13,14]. The predominate occurring phenolic compounds found in maple water include lignans, flavonoids, coumarins and a stilbene [15,16,17].

While few previous studies exist on the efficacy of MW on exercise response measures, there are several studies examining the utility of other natural juices, in various combinations with electrolytes and carbohydrate concentrations. The purpose of this study was to investigate if drinking MW prior to a graded exercise test would improve exercise response when compared to a homemade maple flavored placebo. The researchers also investigated the efficacy of MW in reducing biomarkers associated with post-exercise inflammation and muscle damage. The researchers hypothesized that MW would have a beneficial effect on exercise responses. An added benefit would be that MW contains low levels of glucose, no caffeine, causing no GI-distress, and having electrolytes.

2. Materials and Methods

2.1. Participants

For this study, 10 apparently healthy college males were recruited out of the recreational center at a university in the southern United States. Study participants physiological data is summarized in Table 1.

Participants were eligible for participation in this study if they were male; enrolled in college; no current medication usage; normal dietary habits (no special diets); engaged in regular activity as self-reported on the Leisure and Physical Activity (LPA) survey [18]; and no excluding criteria for physical activity as self-reported on the PAR-Q+ 2017 [19]. The study group was physically active and engaged in regular exercise 3.6 days/week (± .7 days/week). However, the group's calculated BMI was 28, which placed them in the overweight category.

Table 1. Participant Physiological Measures

	Baseline
Age	22.9 ± 5.2 years
Height	180.1 ± 9.1 cm
Body Weight	90.7 ± 18.1 kg
Body Fat %	21.1 ± 0.9%

The participants gave written informed consent and reported to the Human Performance Lab for an initial visit, at which time anthropometric measures were collected. All participants gave written informed consent to take part in the study and the methods were reviewed and approved by the Institutional Review Board at the University of Louisiana at Lafayette (Approval SU17-13KNES).

2.2. Procedures

The authors followed a majority of the procedures we previously used in a study [20] of the effects of a pine bark extract on exercise response and post-exercise inflammation, oxidative stress, and muscle soreness and damage [20]. Participants were recruited from the student recreation center on campus. Once participants agreed to an initial visit appointment, participants gave written informed consent after having the experimental procedures explained to them. Afterwards participant height and weight were determined via stadiometer, and body fat percentage was measured using air displacement plethysmography (BodPod Gold Standard System, Rome, Italy). Participants were then scheduled for their subsequent visits, reminded to come to the lab on those visits in a fasting state, and not to exercise the day prior to each of two treatment visits.

The second visit began with an initial venipuncture blood draw, and then consumption of MW or Placebo. After waiting 1 hour to allow for digestion of the treatment, the participants then completed a graded exercise test, during which, finger stick measures of blood glucose (mg/dL) were taken every three minutes (max of 4 samples collected). A final venipuncture was performed one hour after the conclusion of exercise, and this concluded the second participant visit. The third visit was identical to the second, but the participant consumed the opposite beverage prior to exercise.

2.3. Assignment of Order

The initial study used single blind, pre/post exercise design, where the participants consumed MW or Placebo prior to a maximal bout of exercise and returned one week later to consume the opposite treatment. Participants were randomly assigned to an order of treatment (MW or Placebo) via computerized randomizer program (www.random.org), so that 5 participants would consume MW first and 5 would consume the maple-flavored water placebo on the initial exercise trial day. Both the MW and Placebo were pre-package into unique, coded bottles and provided to participants prior to the exercise trial. There was a 1-week washout period between exercise trial visits.

2.4. Treatments

The treatments for the study consisted of 12 fluid ounces (355ml) of pure MW (Drink Simple Maple Water, Drink Maple LLC) or an identical volume of distilled water flavored with maple extract to mimic the smell and taste. This quantity was selected for the treatment as this is the size of the commercially produced MW product available to the public. To determine antioxidant capacity of the maple water, it was compared to Pomegranate juice (POM Wonderful, LLC), Lemon Juice (RealLemon, Keurig Dr. Pepper Inc) and Coconut Water (Zico Beverages LLC) using a commercially available total antioxidant capacity kit (Cell Biolabs, San Diego, CA). The assay demonstrated that Pomegranate Juice had 18.1 fold higher, Lemon Juice had 11.3 fold higher, and Coconut water had 8.1 fold higher Trolox equivalent antioxidant capacity as compared to Maple water.

2.5. Exercise Protocol

Participants engaged in a graded exercise test until failure on a computerized treadmill interfaced with a metabolic cart (Parvo Medics TrueOne 2400, Parvo Medics, Salt Lake City, UT). VO_{2max} was calculated via expired gas measurement using the Modified Bruce protocol for the exercise test. The Modified Bruce protocol begins at 2.74 km/h at a 0% grade, increases to 5% grade in stage 2, then increases both in speed and grade every three minutes beginning with stage 3 (when it then matches the Standard Bruce protocol). Measure of glucose were taken in 3-minute intervals, during transitions between Bruce stages, via dermal puncture of the participants' fingers. A maximum of 4 samples was collected per participant, as none of the participants were able to complete the 5^{th} stage before ending the exercise test. Blood glucose and blood samples were collected and analyzed in real-time during activity using a glucometer (HemoCue, Brea, CA).

The collected blood from venipuncture was later analyzed via chemiluminescent assay (Quansys Biosciences, West Logan, Utah), for inflammatory markers, and by colorimetric assay to measure muscle damage.

2.6. Blood Collection

Blood donated by the participants during the study was collected in 7.5 ml serum separator tubes. This was allowed to stand at room temperature for 15 minutes then centrifuged at 4°C at 3500 rpm for 10 minutes. Supernatant was removed and stored in micro-centrifuge tubes for later analysis.

Serum was analyzed for oxidative stress levels via lipid peroxidation of malonaldehyde (MDA) with a TBARS colorimetric assays (Cayman Chemicals; Ann Arbor, MI). Additionally, serum was tested for Creatine Kinase activity via colorimetric assays to examine muscle damage (Sigma-Aldrich; St. Louis, MO). The absorption endpoints of these assays were read with a BioTek ELX 808 microplate reader with Gen5 software for data analysis (BioTek Instruments; Winooski, VT).

A multiplex chemiluminescent assay (Quansys, Logan, UT) was run to examine inflammation (IL-1α, IL-4, IL-5, IL-10, IL-12, IL-23, TNF-α). At the conclusion of the assay procedures the plate was imaged with a CCD imager (18 megapixel) and the data were analyzed with Qview Pro Software (Quansys Biosciences; Logan, UT).

2.7. Statistical Analysis

The principal investigator entered all data into JMP 15.0 pro software at the conclusion of the study. Descriptive data such as height, body weight, and body composition are presented as mean ± SD (standard deviation). Data were grouped according to research question and analyzed via two-way, repeated measures ANOVA and are presented as F statistic (F) and probability (p value). Statistical significance was set *a priori* at alpha <0.05. Post-hoc analysis was conducted where necessary via non-parametric means (Chi-squared test) provided data deviated from a normal distribution.

3. Results

3.1. Multiplex Inflammatory Panel

To Repeated measures ANOVA analysis revealed a significant treatment by group interaction for two anti-inflammatory cytokines IL-4 and IL-10, both of which decreased after exercise in the maple water group. There was also a significant treatment by group interaction one pro-inflammatory cytokine, IL-12, which decreased after exercise in the maple water group. The results of the entire multiplex panel are included in Table 2.

Table 2. Summary of Maple Water vs. Placebo on Inflammatory Panel

Treatment	Time	Marker	Mean	*SD*	Two-Way ANOVA
Maple Water	Pre	IL-1a	7.150	5.01	
(pg/mL)	Post	IL-1a	7.290	6.82	
Placebo	Pre	IL-1a	9.760	11.29	
	Post	IL-1a	6.910	6.61	p=0.287
Maple Water	Pre	IL-4	0.010	0.005	
(pg/mL)	Post	IL-4	0.003	0.004	
Placebo	Pre	IL-4	0.006	0.004	
	Post	IL-4	0.010	0.001	p<0.001*
Maple Water	Pre	IL-5	0.273	0.032	
(pg/mL)	Post	IL-5	0.262	0.032	
Placebo	Pre	IL-5	0.309	0.159	
	Post	IL-5	0.297	0.138	p=0.934
Maple Water	Pre	IL-10	2.252	0.537	
(pg/mL)	Post	IL-10	1.745	0.623	
Placebo	Pre	IL-10	1.951	0.721	
	Post	IL-10	2.066	0.733	p=0.026*
Maple Water	Pre	IL-12	4.323	1.227	
(pg/mL)	Post	IL-12	3.895	1.081	
Placebo	Pre	IL-12	3.429	0.936	
	Post	IL-12	4.158	1.277	p=0.022*
Maple Water	Pre	IL-23	3.730	2.499	
(pg/mL)	Post	IL-23	3.450	1.621	
Placebo	Pre	IL-23	4.419	2.838	
	Post	IL-23	4.326	1.536	p=0.851
Maple Water	Pre	TNF-a	7.918	3.319	
(pg/mL)	Post	TNF-a	8.037	3.472	
Placebo	Pre	TNF-a	7.938	3.922	
	Post	TNF-a	7.577	3.728	p=0.724

*Significant at $p \leq 0.05$.

3.2. Creatine Kinase (CK), Oxidative Stress and Blood Glucose

There were no significant differences between maple water and placebo groups for CK or oxidative stress for all time points. Additionally, there were no treatment*time effects for any blood markers. However, repeated measure ANOVA did reveal a significant effect of treatment by group at the final Blood Glucose measure (F=4.10, p=0.026). The final blood glucose measure was 83.25±11.12 mg/dL for the maple water treatment and 104.00±14.79 mg/dL for the placebo. The results for the exercise biomarkers are included in Table 3.

Table 3. Summary of Maple Water vs Placebo on Blood Markers

Treatment	Blood Marker	Time	Mean	*SD*	Treatment by Group Post-Exercise Effect
Maple Water	CK	Pre	119.39	61.365	
	(U/L)	Post	113.024	55.441	
Placebo	CK	Pre	99.332	77.722	
	(U/L)	Post	100.464	81.959	p=0.693
Maple Water	MDA	Pre	0.599	0.369	
	(μM)	Post	0.656	0.338	
Placebo	MDA	Pre	0.692	0.369	
	(μM)	Post	0.613	0.326	p=0.055
Maple Water	Blood Glucose	Pre	100.6	10.047	
	(mg/dL)	3 mins	103.5	14.199	
		6 mins	102.2	12.882	
		Post	83.25	11.117	
Placebo	Blood Glucose	Pre	96.6	8.682	
	(mg/dL)	3 mins	96.1	7.078	
		6 mins	91.4	16.043	
		Post	104	14.799	p=0.026*

*Significant at $p \leq 0.05$.

3.3. Oxygen Consumption during Exercise Trials

One-way analysis of groups for VO_2max demonstrated a significant increase (p=0.045) in the maple water group (42.01±7.58mlO_2/kg/min) versus placebo (35.6±8.62mlO_2/kg/min) in oxygen utilization during the graded exercise test.

3.4. Antioxidant Capacity of MW, other Beverages

Post-hoc analysis on the antioxidant capacity of maple water to raw pomegranate juice, lemon juice and coconut water. Raw pomegranate juice was found to have the highest Trolox equivalents at 100μm, followed by lemon juice (61μm), coconut water (45μm) and maple water at (6μm).

4. Discussion

In this study, the researchers investigated if drinking MW prior to a treadmill exercise test would improve exercise response when compared to a homemade maple flavored placebo. The researchers also investigated the efficacy of MW in reducing post-exercise biomarkers associated with inflammation and muscle damage. The researchers hypothesized that maple water would have the same or greater purported benefits of commercially formulated energy and sports drinks, but with the added benefit of containing substantially lower levels of glucose, no caffeine, no dyes, and a similar composition of electrolytes. When examining MW side-by-side with commercial energy drinks, the researchers believed MW would compare favorably in both results and by a lack of adverse effects that occasionally occur with energy drinks [21].

The present study found a statistically significant improvement in VO_{2max} on the treadmill test for the MW group vs placebo group. The significant increase in rate of oxygen consumption during the exercise event is consistent with many previous studies on the efficacy of energy drinks and running performance [22-26]. It should be noted that all participants in the study (which was counterbalanced in order) achieved a higher maximum oxygen consumption in the MW condition as compared to the Placebo. The authors can speculate that the carbohydrate in the MW might be a reason why the subjects performed better during the MW trial. It is also possible that the reduced inflammation and oxidative stress levels in the MW condition contributed to better aerobic performance. While the findings from previous studies on energy drinks can be attributed to an ergogenic effect from caffeine, glucose, and taurine, maple water does not contain these elements. Kalman et al. (2012) examined the utility of coconut water, coconut water concentrate, and bottled water against a carbohydrate-electrolyte sports drink in a single 60-minute bout of treadmill running [27]. No differences were noted in the measures of exercise performance, specifically in time to exercise exhaustion, between the four interventions. Lagowska et al (2017) studied 11 well-trained rowers over two sessions of 80 minutes of strenuous, sports-specific activity. The researchers found no difference in measures of performance and hydration in well-trained rowers when comparing a commercial sports drink against a beverage comprised of salt, banana, pineapple juice, lemon juice and honey [28]. In a similar study, water, sports drink, or sugarcane juice were substituted for energy drinks. 15 male cyclists pedaled at 70% of VO_2max on three different visits to the lab. Every 20 minutes, the participants gave a blood sample and were given 3ml/kg of body weight of either water, sports drink, or sugarcane juice. At the conclusion of exercise, the participants were given a bolus of 200ml of the same fluid. The researchers demonstrated no significant differences between the three groups for total exercise time, and plasma volume [29].

While MW is similar to coconut water, the ergogenic effects demonstrated by maple water in this study may be due to factors related to it having a common electrolyte and mineral profile. Previous studies on the rehydration capacity of MW revealed a 6-fold higher osmolality and a notable difference in electrolytes versus the maple-flavored control beverage, similar in composition to the control in this study [12]. Investigation into the soil of Southeastern Quebec and Northwest New England, where the trees (*Acer saccharum* M. "Common Sugar Maple") used to collect the sap for MW are located reveals electrolytes, specifically, K, Ca, Mg make up a majority of the nutrient content located in the soil of the area [30]. When examined closer, the sugar maple tree contains minerals (K, Ca, Mg, P, Na, Fe and Cu) in larger quantities than its regional cousin, the red maple

(*Acer rubrum* L.) [1]. Investigations of functional foods has demonstrated in mouse models that supplementation of polyphenols before uphill exhaustive running may increase endurance capacity by upregulating blood glucose and muscle glycogen levels [32]. While MW did improve performance quality during exercise, Matias et al. found that the electrolyte content of MW was relatively inadequate in terms of ability to rehydrate after moderate activity [12].

Intense exercise causes a significant release of pro-inflammatory cytokines and free radicals from activated leukocytes and leads to muscle damage and tissue injury [33-36]. In inflammatory conditions, anti-inflammatory responses are also induced to re-establish basal conditions. An example of this occurs when IL-10, IL-4 and IL-1 receptor antagonist (IL-1ra) are released into circulation to suppress the production of pro-inflammatory cytokines, typically IL-1β, IL-6 and tumor necrosis factor (TNF)-α. The later three pro-inflammatory cytokines being the most influential in acute inflammation [35,37,39-41]. Cellular immunity is activated by IL-12, with a two-fold function as an immunomodulary cytokine (as IL-12p70) that induces cellular immunity as protection against pathogens such as viruses and intracellular bacteria [34]. Additionally, IL-12 (as IL-12p40) acts as anti-inflammatory cytokine, similar to IL-4 and IL-10 which block action and production of other pro-inflammatory and immunomodulatory cytokines (Table 3). It is this way that IL-12 serves as a functional bridge between early nonspecific innate resistance and subsequent antigen-specific adaptive immunity [39-42]. However, many studies suggest cytokine response is related to exercise intensity and duration, as opposed to exercise-induced muscle damage [34,38-41,43]. Endurance exercise also blocks cellular immune response and causes susceptibility to infections by increasing plasma levels of IL-12p40 and IL-4 [38,39,44,45]. As such, the cytokine response due to MW in this study requires further examination to determine a dose-response effect with extension of exercise duration and intensity.

MW demonstrated in this study the ability to decrease oxidative stress post-exercise, which is in stark contrast to the findings with commercially available energy drinks. A 2013 study by Zeidán-Chuliá et al. found that “energy drinks with guarana and caffeine, alone or in combination with taurine, may exert neurotoxicological effects in part by disruption of redox homeostasis” in vitro models [46]. A follow-up study in 2017 by Mansy et al. administered an energy drink to four groups of rats. Three groups were given a progressively larger dose of energy drink that replicated mimicked low (0.4ml/100g body weight/day), moderate (1.1ml/100g body weight/day) and high (2.2ml/100g body weight/day) levels of human consumption of energy drinks. produced noticeable renal and hepatic damage in rats exposed to energy drinks for 12 weeks, this was attributed to increased free radicals and oxidative stress [47]. These two studies [46,47] demonstrate a need for an alternative ergogenic product to energy drinks. MW has no negative effects of blood glucose and amplifies the body’s antioxidant capacity, and our early data indicates that MW improves exercise responses, while amplifying the body’s ability to recognize post-exercise damage. Further exploration into MW should examine it ability to potentially repair what damage has been done.

To further explain the unique effects of MW on oxidative stress, the researchers conducted post-hoc analysis on the antioxidant capacity of maple water to raw pomegranate juice, lemon juice and coconut water. Raw pomegranate juice was found to have the highest Trolox equivalents at, followed by lemon juice, coconut water and maple water. This finding is consistent with a previous study (Matias et al, 2019) of maple water demonstrated a 4-fold higher antioxidant potential versus a control of placebo maple-flavored water [12]. One proposed mechanism of MW’s antioxidant effects is that it either works through direct endogenous compounds or indirectly through manganese, of which maple water has 40% of the Reference Daily Intake (RDI) [34,35]. RDI is used in the labeling of food and supplements produced in the United States and Canada, and represents the daily intake level that is considered sufficient to meet the requirements of 97-98% of healthy individuals in every demographic in the United States.

5. Conclusions

Due to the unique findings in this study and the limited sample size, the findings warrant further investigation. It should be noted that some of the observed responses could in part be due to the limited fitness levels of the participants. Early outcomes indicate maple water has positive benefits for those that exercise in the areas of cardiovascular fitness and post exercise inflammation. Further examination should examine the effect amount of MW consumed has on the measures from this study, including long-term use on measures of body mass and composition. Future study of the protective effects of MW should engage participants in longer bouts of intense exercise to determine if there is dosage effect of exercise associated with antioxidant effects. Additional study is needed to refine the mechanisms behind the exercise and antioxidative phenomenon.

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Statement of Competing Interests

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