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The Effects of Water with Anti-Inflammatory Capabilities (WAC) on Circulating Inflammatory Biomarkers at Rest and Following Resistance Exercise

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Abstract

Purpose: To determine the effects of waters with anti-inflammatory capabilities (WAC) influence circulating inflammatory biomarkers at rest and following resistance exercise. Methods: Participants completed a heavy lifting protocol and drank water with antiinflammatory capabilities for a total of six weeks. Blood samples were taken at different at 9 different time intervals to assess concentrations of anti-inflammatory markers and to quantify inflammatory biomarkers. Those time intervals included pre-AHREP 1 and post-AHREP 1, 24hr post-AHREP 1, 48-hr post-AHREP 1, 3-week check-up, pre-AHREP 2, post-AHREP 2, 24-hr post-AHREP 2, and 48-hr post-AHREP 2. Background: The biomarkers that were focused on were: IL-6 and TNF- α . Inflammation aims to eradicate the irritating agent and accelerate the regeneration of tissue, but it needs to be limited and/or regulated. There is the potential for water infused with natural bioactive berry volatile compounds to demonstrate preventive physiological benefits concerning systemic inflammation. The highest volatile concentration beverage will most likely demonstrate the most anti-inflammatory effect. Specific berries that contain antiinflammatory properties may ease systemic and chronic inflammation that may prevent or reduce local inflammation. There are commercial berry-flavored beverages but not with specific quantities of potentially protective ingredients like the potential berry-flavored with antiinflammatory capabilities (WAC). **Results:** Post-supplementation, immediately post-resistance exercise 2's IL-6 concentration was significantly greater than 48-hr post-resistance exercise 1 (P=0.013). No other significant time point differences were present (P>0.125). There were no time point differences in TNF- α during our study (P \geq 0.097). Conclusion: From this study, we can validate that the water infused with anti-inflammatory capabilities did work to improve the anti-inflammatory response.

Chapter 1: Introduction

An untrained individual will more than likely be familiar with a type of soreness after exposing their muscles to unacquainted training. This can also happen to trained individuals if they have overloaded their muscles through progression. What is this soreness? It is called Delayed-Onset of Muscle Soreness (DOMS). The experience of DOMS stems from soft tissue damage which drives the inflammatory response to produce swelling in this tissue (Quinlan & Hill, 2019). The sensation of sore, aching muscles is usually felt during movement or palpation and occurs a day or so after unaccustomed exercises (Smith, 1991). For a specific example, an individual who has never involved themselves in any type of exercise goes to the gym to train at a specific intensity for a specific amount of time, they will more than likely feel acute muscle soreness during or immediately after exercise. With DOMS, the symptoms of that individual will peak 24 to 72 hours after exercise.

This current study looks at circulating inflammatory biomarkers. Biomarkers are a tangible way to evaluate normal biological processes, pathogenic processes, and/or pharmacologic responses to a therapeutic intervention (Chatterjee & Butina, 2020). Quantifying muscle damage markers while assessing biomarkers of inflammation like capillary dilation, leukocytic infiltration, redness, heat, and pain in an athlete is pivotal to studying the potential source of inflammation and determining the action needed for athlete's health and optimal performance (Lee et al., 2017). Two circulating inflammatory biomarkers that are discussed are Interleukin-6 (IL-6) and Tumor Necrosis Factor Alpha (TNF- α). Based on the broad background given about these inflammatory biomarkers and DOMS, there could be a potential correlation between these inflammatory biomarkers and symptoms related to DOMS.

It is common knowledge that natural substances (volatile compounds), in particular: berries, hold anti-inflammatory properties that may be able to help counteract or combat chronic diseases. Systemic and chronic inflammation is recognized to intensify the four most costly chronic diseases (cancer, cardiovascular disease, arthritis, and kidney diseases). It is hypothesized that these specific berries that contain anti-inflammatory properties ease systemic and chronic inflammation that may prevent or reduce local and acute inflammation. Specific berries like cranberry, raspberry, and blackberry have plenty of nutritive and non-nutritive components such as vitamins, minerals, and especially polyphenols and volatiles (Battino et al., 2009). Fruit essences from these specific berries are important for this study because their phenolic and volatile compounds have higher concentrations of anti-inflammatory properties compared to just the juice from the berries. Fruit essences stand as a natural source of flavor expended to develop berry-flavored waters. There are commercial berry-flavored beverages but not with specific quantities of potentially protective ingredients like the potential berry-flavored water with anti-inflammatory capabilities (WAC). Natural volatile compounds are responsible for berry aroma; they are also more concentrated than compounds manufacturing color that is typically produced today. The berry-flavored WAC that will be used in the present study was colorless and strictly made from the gases of fruits (fruit essences) and was composed of 12% essence.

To conduct the present study, there were two preliminary data studies plus another one that is accompanying the present study. The University of Arkansas Institutional Review Board (IRB) approved each of the preliminary studies and informed consent was obtained before each of the studies. The first study observed consumer acceptability and preference of three separate berry-flavored waters and three fruit essence concentrations. The second study determined how the body processes the volatile compounds and determined the optimal fruit essence concentrations for potential anti-inflammatory effects. The second study also examined the bioavailability and anti-inflammatory potential of berry volatiles in normal-weight individuals and individuals with obesity following consumption of a consumer-preferred WAC. The last study that will be accompanying the present study will examine the anti-inflammatory effects of the berry-flavored WAC. To examine the anti-inflammatory effects, systemic inflammation is required to be induced. The last study also determines the ability of the berry-flavored WAC to limit acute inflammation from the results of exercise heat stress in participants.

The purpose of this study was to determine the effects of waters with anti-inflammatory capabilities (WAC) on circulating inflammatory biomarkers at rest and following resistance exercise.

Chapter 2: Literature Review

Comparability of DOMS and Acute Inflammation

Before concluding how circulating inflammatory biomarkers are correlated to DOMS, there needs to be comparability between DOMS and acute inflammation. DOMS is under the umbrella of inflammation; they are not exclusive. DOMS can be considered to be under the term acute inflammation because it is a short inflammatory response, which promotes healing, has the fundamental symptoms that inflammation has like pain, swelling, and loss of function and they exhibit infiltration of immune cells, especially the macrophage (Smith, 1991). Biochemical markers, for instance, boost lysosomal activity that contributes to pathogens being phagocytized, promote tissue damage, and increase circulating levels of some acute-phase proteins (APP) (Smith, 1991). As a result of the production of cytokines like IL-6, APPs specifically emerge in the bloodstream in the early stages of inflammation or tissue injury (Smith, 1991). Like acute inflammation, DOMS has histological changes, these are microscopic changes in the structure of the cell during the first 72 hours after the activity (Smith, 1991).

Biomarkers Involved with Inflammation

It needs to be noted that biomarkers of muscle damage may contribute to some degree of DOMS and inflammation. Increased creatine kinase levels are a valuable biomarker for muscle damage because they catalyze the reversible conversion of ADP and PC which ultimately plays a part in the energy required for muscle contraction (Lee et al., 2017). Specific markers like skeletal muscle troponin 1 (TnI), skeletal muscle-specific enzymes, and markers indicating an oxidative stress-antioxidant response during muscle damage have been used to track muscle damage during exercise (Lee et al., 2017). TnI is an inhibitory subunit of troponin and regulates calcium during muscle contraction and relaxation (De Matteis et al., 2019). TnI also is a fast

fiber-specific serum marker for skeletal muscle damage, increases in the levels of the serum marker reflect muscle damage (De Matteis et al., 2019). Oxidative stress surfaces when there is a disparity between the production of free radicals and the body's antioxidant aptitude to hinder their damaging effects. This oxidative stress results in cell injury due to direct oxidation of cellular protein, lipid, and DNA or via cell signaling pathways that are responsible for accelerating atherogenesis. Oxidative damage is prolonged by cells and tissues that are unable to keep up with the free radical production (Yang et al., 2017).

One circulating inflammatory biomarker that is considered in the present study is IL-6, which is a cytokine derived from activated T lymphocytes that have many functions which include inflammation and the maturation of B cells. An important function of IL-6 is that it stimulates acute phase reactions (APR) which in turn keeps tissue damage at bay (Gabay, 2006). IL-6 has a dual effect; at the acute level it is a defense mechanism or anti-inflammatory but in chronic inflammation it is pro-inflammatory (Gabay, 2006). While TNF- α only has an average effect on the positive acute proteins, IL-6 stimulates the full spectrum of APPs (Heinrich et al., 1990). APR refers to a wide range of neuroendocrinal, physiological, and metabolic changes that are initiated immediately after the tissue is afflicted with an infection or injury (Liu & Ahearn, 2007). APP takes place when the innate immune system encounters something (like a stressful exercise protocol) and responds to it by activating certain immune cells, cytokines, and other pro-inflammatory markers to fight off what is stressing the system. The result is a generalized immune response that will cover most challenges to homeostasis (Heinrich et al., 1990). Proinflammatory cells are exactly what their name means; they are inflammatory cytokines which are a type of signaling molecule that is secreted from immune cells like Helper T cells and macrophages, and certain other cell types that promote inflammation (Zhang & An, 2007). The

other important circulating inflammatory biomarker is TNF- α . TNF- α is a strong proinflammatory cytokine because it participates in vasodilation, edema formation, and leukocyte adhesion of the epithelium and it is one of the cytokines that make up the APR (Zelová & Hošek, 2013). TNF- α also responds to IL-6 producing cells (Christiansen et al., 2013).

Pain and Soreness

Systemic inflammation means everything is inflamed, and there are high levels of immune cells circulating all over the body. Inflammation aims to eradicate the irritating agent and accelerate the regeneration of tissue. Inadequate or excessive inflammatory reactions can result in tissue damage and severe cases of organ failure and death, this is why the inflammation reaction should be limited and/or regulated (Zelová & Hošek, 2013). Pain is a factor under inflammation that can be good or bad. It is a personal, subjective experience influenced by cultural learning, influenced by how much attention is being paid to it and influenced by the situation itself among other psychological variables (Melzack & Katz, 2013). Pain and soreness are not one and the same. Soreness will make activities of daily living (ADLs) slightly uncomfortable but will subside in two to three days. The rate of muscle recovery from this soreness depends heavily on an individual's diet and sleeping patterns among other factors. Pain on the other hand will make ADLs difficult as well as limit ROM and the individual may stay in pain until it is treated. It is also important to note that pain does not need to be present to achieve muscle gains. In fact, pain may be a sign to hold off from participating in an activity. On another note, the intensity and duration of an exercise are important factors in DOMS (Cheung et., 2003). The intensity and duration of an exercise will be controlled in this study.

A Possible Result of WAC Juice and Inflammation

A potential outcome from the WAC study is that the water infused with natural bioactive berry volatile compounds may demonstrate preventive physiological benefits concerning systemic inflammation. Based on this, inflammatory biomarkers may be inhibited with berryflavored WAC juice. The highest volatile concentration beverage will most likely demonstrate the most anti-inflammatory effect. The overall objective of the WAC study is to establish the ability of berry-flavored infused with naturally occurring bioactive anti-inflammatory volatile compounds on reducing markers of inflammation and reducing risk factors associated with chronic disease.

Chapter 3: Methods

Experimental Study/Research Design

A true experimental research design was used, specifically a group pretest-posttest design. It is a true experimental research design because there are multiple controlled variables; the intensity of the heavy lifting protocol and the concentration of fruit essence in the WAC beverage are a couple of examples of what was consistent for all subjects.

Participants

Participants were recruited and were asked to complete a medical history form, as well as fill out an international physical activity questionnaire (IPAQ). Exclusion from the study was contingent on any medical conditions, such as kidney disease, diagnosed diabetes, Celiac, or Chron's disease; medications such as inhaled corticosteroids for allergies or asthma, or any anti-inflammatory medication; musculoskeletal injuries that would hinder the participant's ability to complete the study, as well as any participation in habitual resistance training. If the participant did not meet any exclusion criteria and completed the study with minimal risk, they were asked to sign an Informed Consent Form after reading and understanding the document, including what they were asked to do, and after they had any further questions regarding the study. Participants were asked to refrain from eating berries for 48 hours, from drinking alcohol for 24 hours, and from consuming caffeine for 12 hours prior to reporting for their baseline test, and all subsequent visits were held at the Exercise Science Research Center. For this study, there were a total of four subjects able to have their plasma analyzed for specific biomarker concentrations.

Procedures

All visits to the lab were completed between the hours of 0700 and 0900 to ensure each participant arrived fasted. For baseline testing, occurring on Day 0, participants determined their 1 repetition maximum (1RM) back squat using standardized protocols set forth by the National

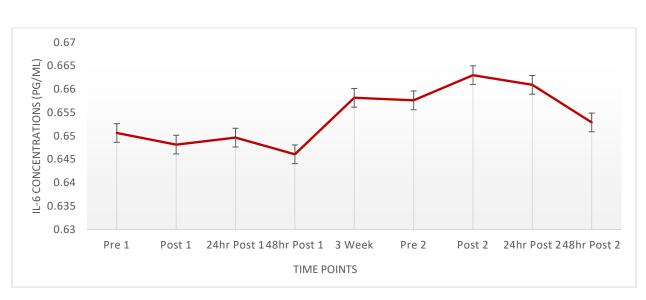
Strength and Conditioning Association. Prior to baseline testing, participants received a dualenergy X-ray absorption scan to determine their body composition. On Day 7, participants reported to the lab and gave a blood sample from their antecubital vein. A trained phlebotomist placed a venous catheter in the vein of the participant. After baseline blood was drawn, participants consumed 250 mL of berry-flavored WAC. They then underwent a standardized warm-up, which consisted of 5 minutes at a self-selected pace on a treadmill, followed by two sets of 10 bodyweight squats, 10 glute bridges, and 10 lunges on each leg. Following the warmup, participants completed an acute heavy resistance exercise protocol (AHREP), which consisted of a 6x10 repetition maximum (10RM) back squat, with two minutes of rest in between sets. The present protocol was chosen due to its effectiveness in eliciting a large inflammatory response. In other studies, the working set load was estimated by taking 75% of the 1RM weight, which estimates 10RM. During exercise, at each 15-minute interval, and immediately postexercise, participants drank 250 mL of cooled berry-flavored WAC. Throughout the exercise protocol, core body temperature, heart rate, and skin temperature were assessed. It is important to assess these because trials were to be arrested if participants reported signs/symptoms of impending heat illness or if they reached the body temperature cutoff or a greater than 90% of age-predicted heart rate maximum. Immediately following completion of the exercise, participants gave another blood sample, before a standardized cool down where they were asked to walk at a comfortable pace on a treadmill. On Day 8, approximately 24 hours after completion of AHREP1, participants returned to the lab for another blood draw. Before leaving the lab, they were given 42 bottles containing 16.9 oz. of berry-infused water, which they were instructed to drink 2 times per day, starting after they left the lab (this was supplementation day 0, denoted as S0). On Day 9 (S1), approximately 48 hours after completion of AHREP1, the participants

returned to the lab for another blood draw. On Day 29 of the study (S21), participants returned to the lab for a "compliance check." They received another 40 drinks, as well as gave another blood sample. On S41, Day 50 of the study, participants returned to the lab and gave a blood sample. They then underwent a standardized warm-up. Following the warmup, participants completed a second AHREP. Immediately following completion of the exercise, participants gave another blood sample, before a standardized cool down where they were asked to walk at a comfortable pace on a treadmill. On Day 51, approximately 24 hours after completion of AHREP2, participants returned to the lab for another blood draw. On Day 52, approximately 48 hours after completion of AHREP2, the participants return to the lab for the last blood draw. At this point, the participation of subjects was concluded, and participants were compensated for their efforts.

Descriptive Statistical Analysis

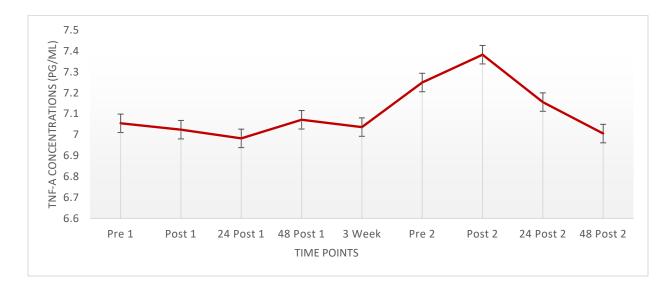
Through the blood samples, IL-6 and TNF- α were assessed using commercially available kits called the Thermo Scientific Human TNF- α and IL-6 Enzyme-linked Immunosorbent Assay (ELISA). A repeated-measures ANOVA was used to analyze data for the time point differences. Significance was set a-priori at P < 0.05.

Chapter 4: Results



Of the 15 subjects who participated, only 4 subjects' plasma samples were randomly selected to be analyzed.

Figure 1. Post-supplementation, immediately post-resistance exercise 2 IL-6 concentration was significantly greater than 48-hr post-resistance exercise 1 (P=.013). No other significant time point differences were present (P \ge .125).



There were no time point differences in TNF- α during our study (P \geq .097).

This study aimed to establish the effects of waters with anti-inflammatory capabilities on circulating inflammatory biomarkers at rest and following resistance exercise. As a result, there were higher concentrations of IL-6 in the plasma of the four subjects after the 6 weeks of WAC supplementation. However, circulating levels of TNF- α was not significantly decreased or increased in the four subjects. It was originally thought that TNF- α would decrease due to the WAC supplementation, yet the concentration change was negligible.

The study demonstrated that circulating levels of IL-6 increased in response to 6 weeks of WAC supplementation and resistance exercise. The results of the higher concentration of IL-6 after exercise are similar while also different from previous literature. Several studies have demonstrated that circulating levels of IL-6 increase in response to strenuous physical activity (Keller et al., 2001). However, there is various literature that exhibits the reduction of IL-6 with supplementation of berry essences (Battino et al., 2009). From this study, IL-6 acts as an anti-inflammatory biomarker which means that at an acute level there will be high concentrations of IL-6 and therefore stimulates an immune response that will be activated to maintain homeostasis.

The study also demonstrated that circulating levels of TNF- α do not change in response to the same 6 weeks of WAC supplementation and resistance exercise. The results of the insignificant change in TNF- α levels after supplementation and exercise are different from previous literature. One study demonstrated that a daily high dose of fruit-based anthocyanins for 8 weeks reduced concentrations of TNF- α in older adults with MCI (do Rosario et al., 2021). From this study, TNF- α remains neutral even though it is supposed to be a pro-inflammatory biomarker. Why didn't TNF- α levels insignificantly change?

Our WAC is much more concentrated with polyphenols as well as colorless compared to the current drinks that are manufactured like sports drinks with color and made from fruit juices, not fruit essence. Because the water is infused with natural bioactive berry volatile compounds it demonstrated some preventive physiological benefits that concern acute systemic inflammation.

Limitations

Our study had some limitations within which our findings needed to be interpreted carefully. The results of this study may not be completely generalizable because there was a time constraint that would not allow us to analyze more than four subjects' plasma out of the 15 subjects that participated in the entirety of the study. Another limitation is that it is difficult to determine whether the increase of IL-6 and neutral levels of TNF- α were strictly due to the resistance exercise or the supplementation of WAC. The study was not able to set up a control group (participants who drank just water) like originally planned due to the time constraint, because of this we cannot positively confirm if the participant inflammatory biomarkers were influenced by WAC or the acute resistance exercise alone. The increase in IL-6 concentrations and neutral levels of TNF- α could be influenced by numerous factors. If the study were to analyze more plasma samples, there could be definite and significant changes in the two inflammatory biomarkers. The study focuses on the effects of WAC on the two inflammatory biomarkers at nine time points with respect to resistance exercise and does not evaluate the subjectiveness of delayed onset of muscle soreness.

Conclusion

By analyzing the concentrations of two different inflammatory biomarkers, IL-6 and TNF- α , we can support within limits and relatively that the water infused with antiinflammatory capabilities did improve the anti-inflammatory response. While not having a control group and an exceedingly small sample size limits the generalizability of the results, this research provides new insight into the anti-inflammatory capabilities of fruit essences as well as illustrates IL-6 as an anti-inflammatory biomarker, but also raises the question of why TNF-α did not decrease as we hypothesized. Based on these conclusions, practitioners should consider a control group and a bigger sample size to test. To better understand the implications of these results, future studies could measure the other factors influencing these inflammatory biomarker concentration levels as well as instill more post-time points to evaluate the inflammatory biomarkers over a longer period of time. Future studies could also measure the ability of berryflavored infused with naturally occurring bioactive anti-inflammatory volatile compounds to reduce risk factors associated with chronic disease. Further research is needed to truly determine the effects of water infused with anti-inflammatory capabilities on circulating inflammatory biomarkers. These findings may challenge existing assumptions of IL-6 being pro-inflammatory at an acute level as well as dispute how strong of a pro-inflammatory TNF- α actually is.

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