

Clinical Study

Daily Controlled Consumption of an Electrokinetically Modified Water Alters the Fatigue Response as a Result of Strenuous Resistance Exercise

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Our objective was to assess the effects of consuming electrokinetically modified water (EMW) on fatigue attenuation and ratings of perceived exertion (RPE) following resistance exercise. A double-blind, placebo-controlled, two-arm trial was used in this investigation. Forty participants were randomly assigned to either an experimental or a placebo-control group. Participants consumed the EMW or placebo water daily for eighteen days prior to completing a fatigue protocol for the elbow flexors. The fatigue protocol consisted of a single bout of resistance exercise for the biceps brachii. Participants were tested for isometric strength before exercise and immediately following exercise. The maximal voluntary isometric contraction was used as the criterion measure for strength. To calculate the fatigue index, the postexercise maximal voluntary isometric contraction value was divided by the preexercise maximal voluntary isometric contraction value multiplied by 100. Also, ratings of perceived exertion (RPE) were assessed using the Borg scale. Fatigue indices and RPE were significantly lower for the experimental group compared to the control group ($P < 0.05$). Consuming EMW for eighteen days prior to high intensity resistance exercise can significantly enhance muscle contractile function by reducing muscle fatigue and RPE.

1. Introduction

Muscle fatigue during and after a single bout of high intensity exercise impairs physical performance and may increase the risk for musculoskeletal injury [1]. Muscle fatigue is observed as a progressive decline in muscular strength or force production during sustained isometric or isotonic exercise [2]. Fatiguing exercise has also been shown to cause a delayed onset of muscle soreness (DOMS) and prolonged strength loss during recovery [3, 4]. Strength loss can be observed immediately after the exercise bout and usually peaks somewhere between 24–48 hrs after exercise and can persist for several days up to a week [5, 6]. In most individuals DOMS appears as early as 24 hrs after exercise and peaks at 48 hrs and may persist for 3–5 days after exercise [7].

One major problem with persistent symptoms and prolonged strength loss after fatiguing exercise is that it can delay functional recovery [4, 5]. Reducing the onset and extent of muscle fatigue and DOMS during and after resistance exercise and/or sports performance is a major concern for athletes and sports medicine professionals. Recently, a modified isotonic saline solution, RNS60, which is generated through a process involving Taylor-Couette-Poiseuille (TCP) flow, has shown potent anti-inflammatory and cytoprotective effects across different disease states including allergic asthma and neuroinflammation [8–10] and is currently in an FDA-approved phase I/IIa safety study in healthy volunteers as well as patients with mild to moderate asthma. RNS60 is generated in a rotor/stator device by subjecting normal saline to TCP flow under elevated oxygen pressure [9]. TCP flow

combines intense local mixing with limited axial dispersion, providing large turbulent energy dissipation rates and surface-to-volume mixing ratios [11, 12]. The conditions used to make RNS60 maximize gas/liquid interface and energy transfer and generate a strong shear layer at the interface between the vapor and liquid phases near the rotor cavities, which correlates with the generation of small bubbles from cavitation [13].

RNS60 was shown experimentally to display direct cytoprotective and anti-inflammatory treatment effects through activation of the type IA phosphatidylinositol-3 (PI-3) kinase/Akt pathway and inhibition of NF- κ B [8]. In an animal model of multiple sclerosis, RNS60 treatment led to significant disease attenuation through shifting the balance of T cell subpopulations from an inflammatory to an anti-inflammatory profile [9]. The source of bioactivity in RNS60 is thought to involve the presence of charge-stabilized nanostructures (CSNs), which by virtue of their size, charge, and stability affect constituents of the cell membrane. Nanosized particles and bubbles have gained recent scientific attention, mainly for their use as contrast agents for biomedical imaging and as vehicles for drug delivery [14–16].

A technology similar to that used to produce RNS60 was used to make a beverage suitable for human consumption and has led to the development of electrokinetically modified water (EMW). EMW is manufactured from reverse osmosis water with an added mineral content of calcium chloride, magnesium chloride, and potassium bicarbonate and an elevated oxygen concentration. No other chemicals are added. EMW is manufactured in a similar manner to RNS60. While its potential effects on intracellular signaling pathways affected by RNS60 have not been directly examined, recent evidence has shown that oral consumption of the EMW positively influenced training adaptations in humans undergoing a treadmill exercise regimen [17]. Subjects consumed the EMW or placebo beverage for 14 days followed by the completion of a 60-minute treadmill exercise bout at a relative exercise intensity of 75% maximal oxygen consumption (VO_2max). Subjects who consumed the EMW were shown to have lower ratings of perceived exertion (RPE) at 15 and 50 minutes of exercise as well as a 5% increase in VO_2max levels determined by the modified Astrand protocol [17]. In a related study, EMW consumption significantly reduced the extent of muscle damage and soreness, as well as postexercise inflammation after a single bout of high intensity eccentric exercise compared to subjects who consumed unprocessed water [18]. In addition to its functional improvements and protective effects, consumption of the water-based beverage may be used as a means to prevent fluid loss and maintain hydration during and after exercise.

Based on the early performance test results in humans showing positive effects on muscle function and cytoprotection as a result of consuming EMW [17, 18], we conducted a study to determine whether daily, oral consumption of EMW alters the fatigue response during a single bout of resistance exercise compared to consuming unprocessed water, which served as a placebo control. We hypothesized that oral consumption of the EMW will effectively attenuate muscle fatigue and lower ratings of perceived exertion (RPE) in

response to sustained high intensity resistance exercise for the biceps brachii.

2. Materials and Methods

2.1. Participants. Forty healthy, physically active males and females volunteered to participate in the trial. Participant demographics and descriptive statistics are listed in Table 1. Participants were required to be nonsmoking and free of nutritional supplements for a minimum of six weeks. Participants were excluded if they reported any previous exposure to the EMW or regular involvement in a weight-training program within the last six weeks for the upper extremity. The criterion for “regular involvement” was equal to or greater than 2 times per week of weight training. Participants were also excluded if they had a prior history of injury to the neck, shoulder, or elbow region of the dominant limb, a reported history of a bacterial infection, and/or the use of anti-inflammatory medication within the last six weeks. Participants were instructed to maintain normal exercise and dietary habits and not to begin any weight loss program throughout their participation in the study. The study was approved by the Western Institutional Review Board (Olympia, WA) Ethics Committee, and written informed consent was obtained from each participant according to the declaration of Helsinki.

2.2. Design. A double-blind, placebo-controlled, two-arm trial was used in this investigation. Participants were randomly assigned to either experimental ($n = 20/\text{group}$) or a placebo-control group ($n = 20/\text{group}$). Participants allocated to the experimental group consumed the electrokinetically modified water (EMW), while the placebo-control group consumed unprocessed water. Participants consumed EMW or unprocessed water daily for 18 days prior to completing a fatigue protocol. On day 19, participants completed a fatigue protocol for the elbow flexors. Isometric strength measurements of the biceps brachii were performed before exercise and again immediately following the fatigue protocol. Ratings of perceived exertion were self-reported by each participant after exercise. Refer to Figure 1 for a timeline of the experimental protocol. All testing sessions were completed at the same time of day to avoid circadian effects.

2.3. Electrokinetically Modified Water. The electrokinetically modified water (EMW) was manufactured from reverse osmosis water with an added mineral content of 1.84 mg/L calcium chloride, 1.84 mg/mL magnesium chloride, and 1.17 mg/mL potassium bicarbonate and oxygen at a final concentration of ≥ 45 ppm by processing in a rotor/stator device at 4°C (patent #6,386,751). Placebo-control water was made from the same source water by adding the same minerals without processing in the rotor/stator device. The EMW and placebo-control water were packaged in identical 500 mL plastic bottles, and participants were instructed to consume a daily prescribed dose of the beverage (EMW or placebo) based on body weight categories: < 59.1 kg = 2, bottles/day (b/d); 59.1–72.7 kg = 3 b/d; 72.7–86.4 kg = 4 b/d; 86.4–100 kg = 5 b/d; > 100 kg = 6 b/d.

TABLE 1: Participant characteristics for the EMW and PLA groups.

	Gender	Age (years)	Height (cm)	Weight (kg)	BMI
EMW	10 men, 10 women	23.1 ± 3.0	170.0 ± 9.3	72.5 ± 13.8	24.6 ± 3.0
PLA	9 men, 11 women	24.0 ± 4.3	174.8 ± 8.2	72.5 ± 13.2	23.6 ± 3.2

Values listed as mean ± SD; EMW: electrokinetically modified water; PLA: placebo water.

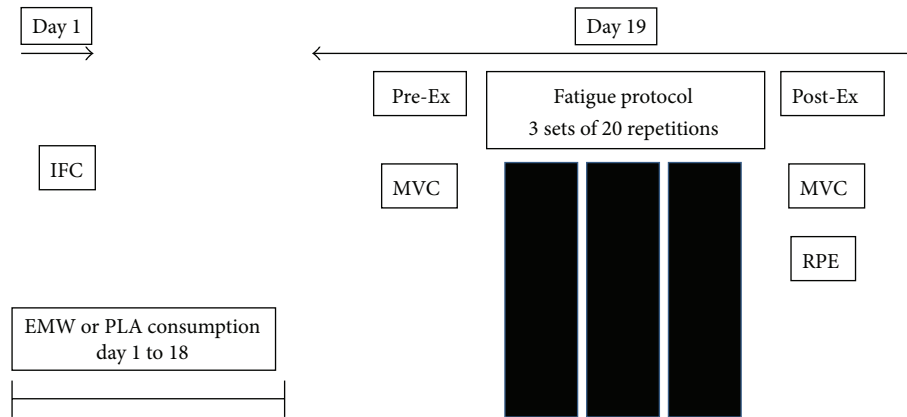


FIGURE 1: Flow chart demonstrating the time line for the experimental protocol. IFC-informed consent.

2.4. Fatigue Protocol. The fatigue protocol consisted of a single bout of resistance exercise for the biceps brachii muscle. An isokinetic testing and exercise device (Kin-Com 125 AP, Isokinetic International, Chattanooga, TN, USA) provided resistance during arm exercise. Prior to the fatigue protocol each participant completed a series of stretching exercises involving all of the major muscle groups of the upper extremity. Participants were then seated in the Kin-Com with their dominant arm secured at their side in 90° elbow flexion. Initially, the participant's maximal voluntary isometric contraction (MVC) was measured (N-m) and then the subject began the exercise protocol. The fatigue protocol required participants to perform repeated maximal shortening (concentric) and lengthening (eccentric) actions for the biceps brachii muscle group. The angular velocity was set at 45°/sec for concentric actions and 60°/sec for eccentric actions. Each participant completed 3 sets of 20 repetitions and was instructed to perform the arm repetitions "as hard as they can." Participants were given a 1-minute recovery period between sets. Verbal encouragement was provided by the investigator during the fatigue protocol.

2.5. Isometric Torque and Fatigue Index. Maximal voluntary isometric contraction (MVC) was assessed using the Kin-Com dynamometer. The MVC is the peak isometric torque produced during a static muscle contraction and was used as the criterion measure for isometric strength. Participants were seated with their dominant arm placed at their side in 90° elbow flexion. Each participant performed three maximal voluntary isometric contractions of the dominant arm. Each contraction was held for five seconds. A 30-second recovery period was provided between contractions. The most forceful contraction of the three values was recorded as peak torque in N-m. The MVC was measured before exercise (baseline)

and again immediately following the fatigue protocol and the scores were used to calculate the fatigue index. The fatigue index (FI) is the percent decline in MVC from beginning to the end of the exercise fatigue protocol and was calculated for each participant by subtracting the postexercise MVC value from the preexercise MVC value and then dividing the difference score by the preexercise MVC value multiplied by 100.

2.6. Perceived Exertion. Ratings of perceived exertion (RPE) were measured using the Borg scale [19]. After completing the fatigue protocol, the participant was asked to choose the number that best described their perceived level of exertion or effort for the active muscle (RPE-AM) over the course of the entire exercise session [20]. The active muscle was the biceps brachii muscle. The Borg scale ranged from 6 to 20, where 6 refers to "no exertion at all" and 20 refers to "maximal exertion."

2.7. Statistical Analysis. Descriptive data generated for each outcome variable was reported as mean ± SD. Fatigue indices and RPE scores were analyzed using independent (unpaired) *t*-tests with statistical significance set *a priori* at $P < 0.05$. All data analyses were performed using IBM SPSS Statistics for Windows 19.0 (IBM Corp, Armonk, NY).

3. Results

3.1. Participant Characteristics and Baseline Measures. Table 1 data indicates that both groups were generally well proportioned for gender, as well as chronologically and anthropometrically. Preexercise baseline measures for strength and RPE were not significantly different between groups ($P > 0.05$).

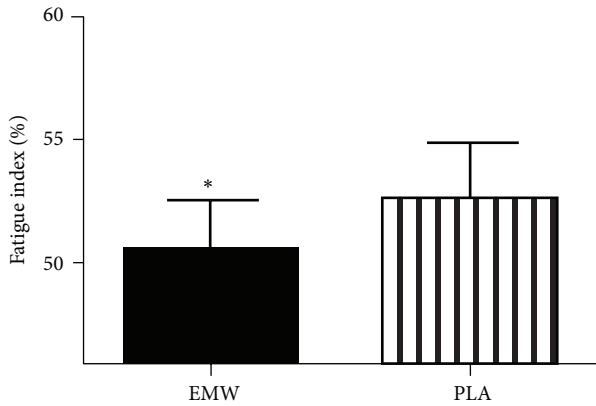


FIGURE 2: Fatigue indices show that participants who consumed unprocessed water (PLA) lost significantly more isometric strength as a result of the exercise protocol than participants who consumed the EMW ($t_{(38)} = 3.38$; $P = 0.002$). Values are reported as means (\pm SD).

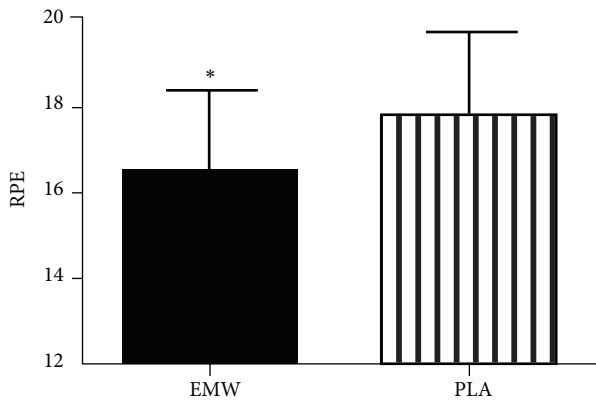


FIGURE 3: Participants who consumed unprocessed water (PLA) reported significantly higher ratings of perceived exertion immediately after the exercise protocol than participants who consumed the EMW ($t_{(38)} = 2.31$; $P = 0.026$). Values are reported as means (\pm SD).

3.2. Strength Loss. The experimental group had a smaller decrease in MVC (N-m) from preexercise to immediately postexercise compared to the control group (Table 2). Absolute differences from preexercise to immediate postexercise MVC scores between the experimental group (19.4 N-m) and the control group (24.1 N-m) indicated a 20% greater absolute MVC loss within the control group.

3.3. Fatigue Index. The fatigue index (%) was significantly lower for the experimental group compared to the control group indicating that the group that consumed the EMW displayed less strength loss pre- to postexercise ($t_{(38)} = 3.38$; $P = 0.002$, Figure 2).

3.4. Ratings of Perceived Exertion. Ratings of perceived exertion scores indicated that the fatigue protocol was perceived as less effortful by the experimental group than by the control group ($t_{(38)} = 2.31$; $P = 0.026$, Figure 3).

TABLE 2: Maximal voluntary contraction (N-m) values for the EMW and PLA groups.

	EMW	PLA
Preexercise	38.5 \pm 19.0	46.5 \pm 26.0
Postexercise	19.1 \pm 10.0	22.4 \pm 14.0

Values listed as mean \pm SD; EMW: electrokinetically modified water; PLA: placebo water.

4. Discussion

Our findings demonstrate that oral consumption of an electrokinetically modified water (EMW) beverage can improve skeletal muscle function. Consuming the EMW 18 days prior to high intensity resistance exercise attenuated muscular fatigue and subjectively made the strenuous resistance exercise feel less taxing.

4.1. Strength Loss. In the present study, the group that consumed unprocessed water was found to have a 20% greater absolute loss of isometric strength after the arm resistance exercise protocol compared to the group that consumed the EMW. The preexercise to postexercise differences in strength loss indicate that the group who consumed EMW experienced less muscle fatigue during the exercise protocol when compared to the placebo-control group. Fatigue and strength loss during protracted muscle activity have been postulated to increase the risk of strain injury. Garrett et al. [21] were able to demonstrate in a rat model that a skeletal muscle can absorb up to 50% more energy when eccentrically loaded as opposed to when the muscle is passively stretched. Similarly, Mair et al. [1] were able to show that fatigued muscles absorb less force than nonfatigued muscles and concluded that the decrease in the capability of the muscle to absorb force is associated with a reduction in contractile function. Therefore, it can be surmised that a fatigued muscle is vulnerable to strain injury due to a diminished ability of the muscle to absorb forces when actively contracting. Our findings indicate that consuming EMW may protect an exercising muscle from damage by limiting the onset and extent of fatigue and therefore the strain on the active muscle.

4.2. Perceived Exertion. Our finding of attenuated strength loss in subjects who consumed EMW was paralleled by lower session RPE assessed using the Borg scale. RPE is a subjective means to measure fatigue and is used extensively in human performance research laboratories as well as in clinical practice settings to subjectively gauge the level of stress and perceived exertion an individual is experiencing during strenuous exercise [22]. In the present study, the group that consumed unprocessed water rated their level of perceived exertion 7% higher than the group that consumed the EMW. The perception of physical exertion or effort during high intensity exercise is a psychological construct that is closely related to fatigue, a known physiological construct [19]. Cooper et al. [17] also reported lower RPE at 15 and 50 minutes of exercise as well as a 5% greater level of maximal oxygen consumption in a subgroup of male subjects who

consumed the EMW for 14 days prior to completing a 60-minute submaximal treadmill exercise test. Exactly how the EMW attenuates the mediating effect of perceived exertion has not been elucidated, and therefore further investigation is needed.

The perception of physical exertion during voluntary muscle work comes from multiple sensory inputs originating in muscle and joint structures that are under mechanical, chemical, and thermal stress [23, 24]. The physiological factors that mediate perceptions of exercise intensity and workload are not exactly known; however, a possible link may exist between impairments in muscle contractile function and altered sensory feedback from muscle and joint receptors. Fatigue-induced impairments in muscle contractile function could trigger stronger neurophysiological “distress” signals from surrounding peripheral afferents including local nociceptors [25]. Impaired blood flow and increased lactate levels in exercising muscle and blood may trigger sensations of pain and discomfort, thus elevating perceptions of exertion during arm exercise [25, 26]. Other peripheral sources mediating perceptions of exertion may include shifts in energy substrate availability and thermal stress [25, 27]. Utter et al. [28] found that marathon runners who consumed a carbohydrate beverage were able to run at a higher intensity while reporting lower RPE during the race when compared to runners who consumed a placebo beverage.

4.3. Mechanism of Action. While the exact mechanism of action by which the EMW attenuates strength loss and perceived exertion is unclear, we speculate that exercise-induced muscle fatigue is mitigated by EMW consumption through a reduction of sarcolemma disruption or reduced impairment on neuromuscular function. In humans, the EMW has shown functional and cytoprotective [18] effects that are reminiscent of the effects of a therapeutic saline solution, RNS60, manufactured by a similar process. In either case, no traditional active ingredient is added to the fluid. Instead, both fluids are generated using a process involving TCP flow, which produces strong, controlled turbulence and cavitation events in the fluid. Research on RNS60 suggests that its bioactivity is based on the presence of nanobubble-based physical structures stabilized by an electrical double-layer at the liquid/gas interface [8–10]. The exact nature of these nanostructures is under investigation and the descriptive term “charge-stabilized nanostructures” has been used to refer to these entities.

We recently reported that subjects who consumed the EMW for 18 days prior to completing a single bout of high intensity eccentric exercise had significant reductions in the extent of muscle damage and postexercise inflammation compared to subjects who consumed unprocessed water [18]. The group that consumed the EMW displayed lower blood levels of creatine kinase and high sensitivity CRP at 48 h and 96 h after exercise [18], thus indicating that less cell damage and inflammation occurred as a result of the exercise protocol. The bioactivity of RNS60 has been linked to effects on intracellular signaling pathways that influence cell stability and function [8–10]. Using a mouse model of Parkinson’s disease, Khasnavis et al. [8] showed that RNS60 improved

locomotor function and reduced inflammation and neuronal degeneration in animals challenged with methyl-phenyl-tetrahydropyridine intoxication compared to a controlled saline. Therefore, a plausible explanation for our findings is that daily controlled consumption of EMW improved muscle cell integrity during strenuous exercise, thereby preserving contractile function of the exercising muscle. This allowed subjects to complete the exercise protocol with lower levels of fatigue and less perceived exertion. Blood lactate and glucose levels were not monitored in the present study. Therefore, more research is necessary to determine what metabolic sources are directly involved in mediating peripheral perceptions of exertion in exercising limbs as well as the underlying mechanisms by which the EMW is able to preserve contractile function during resistance exercise.

4.4. Practical Applications. Reducing the extent of strength loss during prolonged exercise is important for athletes involved in training and competition by sustaining their work capacity and lessening their risk for musculoskeletal injury. Oral consumption of the EMW is a novelty for athletes and physically active individuals alike in that the beverage can be consumed not only as an aid to maintain hydration levels, but additionally with the intent of attenuating strength loss as a result of intense resistance exercise. Thus, the combined fluid replacement/hydration and functional improvements of consuming EMW can benefit these individuals by moderating the physiological and psychological stress of intense exercise training and competition.

From a dose-response perspective evidence shows that positive ergogenic and protective effects occur when pre-fatigue and exercise consumption periods are between 14 and 18 days [17, 18]. Besides this very short window of consumption time, we do not know if abbreviated or extended wash-in periods have a significant effect on the size of the ergogenic effects. Future studies should investigate whether the effects are dose-dependent and potentially if optimal dosing parameters are discernable for the EMW.

4.5. Limitations. Study limitations include the use of only one dosing schedule for subjects and the use of a small muscle mass for testing (biceps brachii). Also, the study participants were physically active; however, they were not considered to be elite or highly trained competitive athletes, thus limiting the generalizability of the results. The generalizability of the research findings could be increased by examining the possibility of a differential sex effect of EMW consumption. More research is necessary to substantiate our findings and to delineate the molecular events involved. Our study suggests a novel application for the EMW and reproducing the positive results of this study and the one by Cooper et al. [17] will extend our knowledge of the types of psychophysical responses that can be expected from consuming the EMW. Future studies should be designed to incorporate the use of larger muscle groups, and thus larger muscle mass, as well as using more dynamic resistance exercises such as Wingate cycle ergometry testing. We also recommend expanding the number and types of outcome measures as well as designing

studies that focus more closely on the benefit as it relates to the volume of the EMW consumed.

5. Conclusions

Oral consumption of EMW for 18 days prior to high intensity resistance exercise can significantly reduce muscular fatigue and RPE during strenuous exercise. The combined effects of fatigue attenuation and lower RPE may decrease the risk for musculoskeletal injury as well as enhance postexercise recovery mechanisms.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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