



The effects of postexercise consumption of a kefir beverage on performance and recovery during intensive endurance training

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ABSTRACT

This study was designed to determine whether kefir accentuates the positive health benefits assessed by measures in fitness, body composition, or both, as a measure of cardiovascular disease risk as well as the biomarker C-reactive protein (CRP). Sixty-seven adult males and females aged 18 to 24 yr were assigned to 1 of 4 groups: (1) endurance training + control beverage, (2) endurance training + kefir beverage, (3) active control + control beverage, or (4) active control + kefir beverage. The exercise groups completed 15 wk of structured endurance training while the active control groups maintained their usual exercise routine. Additionally, each group was assigned to either a kefir or a calorie/macronutrient matched placebo beverage that was consumed twice per week. No significant interactions were found among groups with respect to outcome variables with the exception of serum CRP. The endurance training was effective in improving 1.5-mile (2.41 km) times and kefir supplementation may have been a factor in attenuating the increase in CRP that was observed over the course of the intervention period. This preliminary study suggests that kefir may be involved in improving the risk profile for cardiovascular disease as defined by CRP.

Key words: kefir, probiotics, exercise, inflammation, C-reactive protein

INTRODUCTION

Endurance exercise has repeatedly demonstrated positive health benefits, such as improved cardiovascular functioning and helping to maintain a healthy BW. However, exercise can deplete the body's glucose stores (blood glucose, muscle, and liver glycogen) and can have catabolic effects on muscle proteins immediately

following exercise. In several studies, milk, with the addition of an appropriate amount of carbohydrate, has been shown to be an ideal recovery beverage for athletes following bouts of endurance training as a way to attenuate some of the acute effects of strenuous activity (Karp et al., 2006; Roy, 2008; Thomas et al., 2009). The American College of Sports Medicine (ACSM) recommends the following: carbohydrate intake: 1.0 to 1.5 g/kg of BW (0.5–0.7 g/lb), and protein intake: 0.4 to 0.7 g/kg of BW (0.2–0.4 g/lb; ACSM and ADA Joint Position Statement, 2009). Other studies show a ratio of 4 to 1 carbohydrates to protein to have beneficial results in recovery if consumed within 2 h following exercise (Manninen, 2006; Thomas et al., 2009). Milk is also a good source of calcium, phosphorus, potassium, and vitamin D, which aid in developing strong bones and restoring proper electrolyte balance and hydration following exercise. Although can milk play an important role in sport nutrition, athletes with lactose sensitivities are unable to consume lactose-containing dairy products, such as unfermented milk, without some degree of gastrointestinal discomfort (Tomba et al., 2012). The production of lactase by mucosal cells in the intestinal epithelium decreases with age in most humans, particularly in individuals of East Asian and African descent (Suchy et al., 2010), and it is estimated that 65 to 85% of adults of these races lack sufficient lactase to digest the lactose that would accompany the 3 servings per day of low-fat dairy products recommended by the Dietary Guidelines for Americans (Rowlands et al., 2007).

Increased stress on the body, as occurs with exercise, can also cause acute and chronic disruption of immune functioning and increased inflammation (Oktealden et al., 1992; Pals et al., 1997; Ji, 2002; de Oliveira et al., 2011). C-reactive protein (CRP) is a biomarker that is often used to assess both acute and chronic inflammation. Muscle damage during intensive exercise typically results in elevated CRP levels in the blood (Mackinnon, 2000). Because probiotics have demonstrated positive effects on reducing chronic inflammation in the body, they would likely have a similar effect on exercised-induced inflammation.

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Kefir is a naturally fermented milk beverage containing a mixed microbial culture of lactic acid bacteria and yeasts. Characteristics include a smooth and creamy texture and an acidic, slightly alcoholic and yeasty taste with varying levels of effervescence (Muir et al., 1999). Health aspects attributed to the consumption of kefir, include, but are not limited to, improved lactose utilization, anti-carcinogenic activity, control of intestinal infections, and improved nutritional quality of milk (Vinderola et al., 2006; Medrano et al., 2011). Studies involving probiotics and athletes have demonstrated maintenance of gastrointestinal function and health, attenuation of immunosuppressive effects, reduced susceptibility to illness, enhanced resistance to upper-respiratory tract infections, and reduced gut permeability (Kekkonen et al., 2007; West et al., 2009; Cox et al., 2010; Gleeson et al., 2011; Lamprecht et al., 2012). However, the effects of probiotic supplementation following exercise have not been examined when using fermented milk as the delivery method for probiotics. The capacity for probiotics to modulate perturbations in immune function following exercise, along with the enhanced digestibility of fermented milk, would make a kefir-based recovery beverage a good source of nutrients required for muscle synthesis and regeneration following activity. The objective of this project was to design an all-natural, minimally processed postexercise kefir-based recovery beverage for athletes to meet 3 specific criteria: (1) follows the ACSM guidelines for endurance athlete nutrition following exercise, (2) has a significantly reduced lactose content to allow for consumption by lactose-sensitive athletes, and (3) contains live, active probiotic cultures at the time of consumption. A second objective was to determine the physiological responses to intensive exercise training and kefir supplementation by examining levels of established biological markers of inflammation and immune functioning.

MATERIALS AND METHODS

A kefir beverage was developed that meets the ACSM guidelines for recommended nutrition following endurance and resistance exercise manufactured at the Louisiana State University Creamery. Pasteurized, non-homogenized, full-fat cow milk was fermented in either gallon-sized lidded glass containers or 5-gallon lidded stainless-steel milk pails by inoculation with approximately 30 g of kefir grains (Cultures for Health, Sioux Falls, SD) per gallon of milk. During the fermentation process, the kefir grains were contained in unbleached cotton tea bags. The bags were sterilized in boiling water before addition of the kefir grains. The milk was allowed to ferment at 25°C for approximately 24 h, or until a pH of 4.6 was reached. The kefir was then placed

in refrigerated storage until formulation and packaging of the beverage. Lactaid, an ultra-pasteurized homogenized lactose-free milk product, was used as the dairy portion of the control beverage.

A fruit base for the kefir and control beverage was processed separately and was subsequently blended with kefir or Lactaid several hours before consumption by the study participants. The fruit base included all of the beverage ingredients except the dairy portion; it was prepared using a VitaMix Commercial/Household Food Preparing Machine Model VM0100A (VitaMix Corp., Cleveland, OH). After combining the kefir and the fruit base, 16 ounces (454 g) of the product was portioned into clear plastic containers with snap-on lids with a tamper-evident seal. Each container was labeled with a number that corresponded to subject code; no other identifying information was on the container when presented to the participants. The containers were placed in refrigerated storage ($6 \pm 5^\circ\text{C}$) until consumption. The ingredients and macronutrient profiles for the kefir beverage and the control beverage were identical with the exception of the type of milk used (kefir or Lactaid; Table 1). Nutritional analysis for the products and ingredients was performed using The Food Processor Nutrition and Fitness Software (ESHA Research, Professional Nutritional and Analysis Software, Salem, OR). Macronutrient contents for each ingredient were generated by The Food Processor software, and the total macronutrient composition for each beverage was calculated from those values. Sensory questionnaires were administered during the intervention period to determine the likeability of the beverages by the participants and the intent to purchase before and after knowing about potential probiotic effects.

This study was approved for use of human subjects by the Louisiana State University Institutional Review Board (#3335). Subjects were recruited from the student population at Louisiana State University. Subjects

Table 1. Nutrient compositions of a kefir beverage and a control beverage given to endurance athletes and an active control during a 15-wk marathon training program

Item	Kefir beverage	Control beverage
Serving size (oz)	16	16
Calories (kcal)	220.16	220.16
Fat (g)	5.4	5.4
Sodium (mg)	71.68	71.68
Total carbohydrate (g)	66.56	66.56
Fiber (g)	2.8	2.8
Sugar (g)	30.72	30.72
Protein (g)	10.24	10.24
LAB ¹ (cfu/serving) ²	10^9 – 10^{10}	0
Yeasts (cfu/serving)	10^7 – 10^8	0

¹Lactic acid bacteria.

²Colony forming units per 16-oz (454-g) serving.

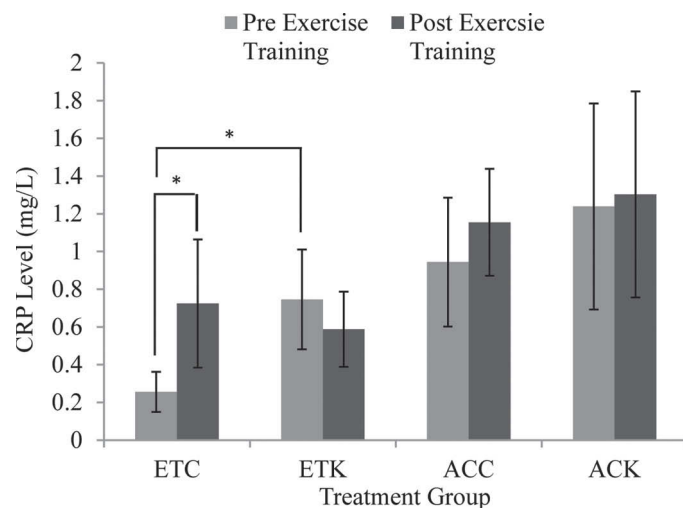
Table 2. Product acceptability and consumer intent of purchase for a kefir-based postexercise recovery beverage and a control beverage by athletes participating in a 15-wk marathon training program and an active control

Item	Acceptability		Will purchase this product before knowing about probiotics		Will purchase this product after knowing about probiotics			Did consuming this product following exercise aid in recovery?		
	Yes	No	Yes	No	Yes	No	Maybe	Yes	No	Maybe
Kefir beverage	35	1	30	6	26	1	11	11	2	22
Control	32	0	26	6	27	0	5	9	1	22

were healthy and between the ages of 18 to 35 yr. General exclusion criteria for all subjects were as follows: the presence of disease conditions including diabetes, cardiovascular disease, cancer, HIV, asthma, resting blood pressure >160/100 mmHg, hospitalization within the last 6 mo, plans to be away for more than 2 wk in the next 9 mo, significant weight loss in the past year (>20 kg) or current use of weight loss medications, and being pregnant or planning to become pregnant within the next 6 mo. A total of 67 participants were recruited. The treatment groups and sample sizes were exercise training + kefir beverage (**ETK**; $n = 13$), exercise training + control beverage (**ETC**; $n = 10$), active control + kefir beverage (**ACK**; $n = 21$), and active control + control beverage (**ACC**; $n = 21$).

The exercise components of this research were conducted in conjunction with the School of Kinesiology at Louisiana State University. The training sessions consisted of supervised long-distance runs occurring twice weekly that lasted for the period of time it took for completion of the running course. The ACC and ACK groups were instructed to follow their normal workout routine without supervision. The kefir beverage and the control beverage were administered in isocaloric amounts to the subjects within 30 min after each training session. Throughout the training period, subjects in both training and active control groups completed weekly sickness, burnout, training/injury logs, diet record, and sensory questionnaires relating to the beverage. Baseline measurements (heart rate, blood pressure, body composition, questionnaires) in both training and control groups were taken, and skinfold measurements were taken 3 times from 7 sites (chest, midaxillary, triceps, subscapular, abdomen, suprailiac, and thigh) by a trained exercise physiologist. Subjects also were required to complete a 1.5-mile walk/run in the fastest possible time. Venous whole blood (10 mL per sample) and plasma/serum samples (10 mL per sample; 20 mL total) and were collected preintervention and postintervention from all study participants following an overnight fast and having refrained from exercise for the previous 72 h. The subjects were seated quietly for 15 min before the blood samples were collected by

a registered nurse. Aliquots used for analysis of serum components were collected in a Vacutainer blood collection tube containing a clot activator. The blood was allowed to clot by sitting at room temperature for approximately 60 min before it was centrifuged for 15 min at $1,000 \times g$ and 4°C . Serum samples were analyzed for levels of CRP. Serum CRP was measured using an ELISA (Alpco Diagnostics, Salem, NH). All statistical analysis was carried out in JMP Pro 11 (SAS Institute Inc., Cary, NC). A multivariate ANOVA (MANOVA) was used to identify significant interactions ($P < 0.05$). Group means and standard deviations were calculated for all descriptive and outcome variables. Student's t -tests were performed post hoc for any significant differences detected at the $\alpha = 0.05$ level. All CRP samples were tested in duplicate, and concentrations of CRP were log-transformed to adjust for normality for statistical analysis.

**Figure 1.** Effect of endurance training and kefir supplementation on serum C-reactive protein (CRP; mg/L). *Means for each bar differ significantly ($P < 0.05$). Data correspond to the average \pm standard deviation of the results of subjects from 4 separate groups. Preexercise training and postexercise training values were collected at the beginning and the conclusion of a 15-wk marathon training program. ETC = exercise training + control beverage; ETK = exercise training + kefir beverage; ACC = active control + control beverage; ACK = active control + kefir beverage.

RESULTS AND DISCUSSION

A significant time \times training group interaction ($P = 0.0124$) was observed with the exercise training groups, with ETK and ETC experiencing an average of 4.11% improvement in 1.5-mile time. The 2 groups not participating in the exercise training, ACK and ACC, showed no significant variation in 1.5-mile time. Body composition measurements (body density and percent body fat) had no significant change preintervention or postintervention for any of the treatment groups. No significant differences were found between any of the treatment groups with regard to body composition. No significance interactions were found among groups with respect to all other outcome variables with the exception of serum CRP. The endurance training was effective in improving 1.5-mile times, indicating the effectiveness of the training protocol in improving performance.

Intensive endurance training caused a significant ($P < 0.05$) increase in CRP levels in the ETC group, demonstrating that the training program resulted in an increased level of inflammation in the body as indicated by the elevated CRP level (Figure 1). No significant increase was present in the level of CRP in the ACC or ACK groups. The lack of a significant ($P < 0.05$) increase in level of CRP the ETK group, as seen in the ETC group, may indicate attenuation of exercise-induced inflammation following training, as measured by circulating CRP. Survey results also indicate a very positive response to both beverages (kefir and control) overall, indicating that majority of participants would buy this product before and after knowing about possible probiotic benefits from consumption (Table 2).

Enhanced digestibility of kefir following activity, due to the reduction of lactose by fermentation, will allow for consumption by athletes with lactose sensitivities. The effects of kefir on intestinal health and overall well-being of athletes in reducing and preventing the marked increase in levels of inflammatory markers such as CRP are promising and warrant further investigation. The kefir recovery beverage, designed to meet the nutrient requirements following endurance training and containing naturally occurring probiotics, provided athletes with a convenient and healthy dairy food product to include in a sport nutrition plan.

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