2015

The Effects of Post-Exercise Consumption of a Kefir Beverage on Performance and Recovery During Intensive Endurance Training

Keely Virginia O'Brien
Louisiana State University and Agricultural and Mechanical College

THE EFFECTS OF POST-EXERCISE CONSUMPTION OF A KEFIR BEVERAGE ON PERFORMANCE AND RECOVERY DURING INTENSIVE ENDURANCE TRAINING

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Interdepartmental Program in
The School of Animal Sciences

by
Keely Virginia O'Brien
B.S., University of Tennessee, 2007
M.S., Louisiana State University, 2012
May 2015

ACKNOWLEDGEMENTS

Thanks are owed to Dr. Charles Boeneke and Dr. Laura Stewart for making this project possible, and to Dr. Kayanush Aryana, Dr. Witoon Prinyawiwatkul and Dr. Carol O'Neil, my doctoral committee members. I would like to acknowledge Laura Bobart and all of the time (and patience) she spent helping me in the lab. Thanks to all my professors and friends who made this an exciting and challenging experience. Finally, I would like to thank my family for their unwavering love and support.

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ABSTRACT

This study was designed to determine whether kefir accentuates the positive health benefits assessed by measures in fitness and/or body composition, as a measure of cardiovascular disease risk as well as the biomarker c-reactive protein. Sixty-seven adult males and females aged 18-35 years were assigned to one of four 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 weeks of structured endurance training while the active control groups maintained their usual exercise routine. Baseline physiological and exercise measurements were collected pre-intervention and post-intervention. Blood and saliva samples were analyzed for C-reactive protein, tumor necrosis factor-alpha and secretory immunoglobulin A. Instances of sickness and illness throughout the intervention were also examined. Additionally, each group was assigned to either a kefir or a calorie/macronutrient matched placebo beverage that was consumed twice per week. The endurance training protocol was effective as demonstrated by a significant improvement ($p<0.05$) in the 1.5-mile times of the endurance training groups. The endurance training groups also experienced a significantly higher ($p<0.05$) number of sicknesses than the active control groups. There were no significant interactions among groups with respect to physiological outcome variables with the exception of serum C-reactive protein. The endurance training group receiving a control beverage demonstrated a significant increase ($p<0.05$) in C-reactive protein following the 15 week training program while the endurance training group receiving a kefir beverage had no significant change in C-reactive protein. Although the reported sickness was not significantly different between

the endurance training group + kefir beverage and the endurance training group + control beverage, kefir supplementation may have been a factor in attenuating the increase in C-reactive protein 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 C-reactive protein.

CHAPTER 1. INTRODUCTION

1.1 Fermented Milk: History and Principles

Evidence of fermented milk production dates back to 7000 BCE with origins in the middle and far-east of Asia, making it one of the oldest methods of both temporary and long term food preservation. A further spreading east of these traditions, by way of Russia and Eastern Europe, by the Tartars, Mongols and Huns occurred during the expansion and conquests by these empires (Vasiljevic, et. al., 2008). The expansion of cultures that maintained livestock as a source of meat and dairy foods by the introduction of herds and traditional production methods, and subsequent industrialization of dairy food production, has led to a total worldwide domestic ruminant population of nearly three billion at the beginning of the twenty-first century (Weimer, 2001).

Cultures that were traditionally associated with herding or keeping livestock, including cattle, sheep, goats, mares or water buffalo, discovered and, over the course of many generations, refined the process of milk preservation. This ultimately led to the wide the range of fermented milks produced today, which are as diverse as the cultures that produce them. From the traditional sour milks of Eastern Europe to the hard salty cheeses first developed throughout the Mediterranean region, cultured dairy products have been a dietary staple for humans throughout recorded history, and although unaware of the microbiological processes involved, traditions and protocols were established within herding communities that ensured the methodologies and knowledge required to produce the flavors and textures associated with specific fermented dairy products were kept intact. Often, these practices were passed down from generation to generation within local communities, feudal states and monasteries (Caplice, et. al., 1999). Over

time, the tastes associated with fermented milks, such as the characteristic acidic flavor, may have also become associated with increased health and longevity, thus furthering its spread and increasing manufacture throughout and within ancient populations.

It was not until the late nineteenth century that scientists first began to take note that there were factors present in fermented milks that may be providing health benefits to the consumer that were beyond the scope of non-fermented milk. This realization sparked early microbiological work involving milk and fermented food products by numerous scientists, including Nobel Laureate Elie Metchnikoff, who correlated a large consumption of fermented milk to an above average life span. In populations of Bulgarian peasants consuming 'sour milk', he noticed that the average life span was eighty-seven years old, with one out of four living past one hundred years of age (Vasiljevic, 2008). Following suit, scientists such as Grigoroff (1905) and Rettger, et. al. (1914), as well as Metchnikoff himself (1905) were isolating bacteria from fermented milks and demonstrating that certain strains could survive and colonize the intestinal tract

Fermentation is defined as the conversion of carbohydrates to organic acids or alcohol and carbon dioxide, using bacteria and yeasts, or a combination thereof, under anaerobic conditions (Kosikowski, et. al., 1999). In milk, these fermentations most often occur as a result of the hydrolysis of lactose by lactic acid bacteria containing the enzyme β-galactosidase. Lactic acid bacteria prefer lactose as their source of carbon, and the end products can be exclusively lactic acid or a combination of lactic acid and other metabolic end products, such as acetic acid, carbon dioxide and hydrogen (Alfa-Laval, 1987). Yeasts, such as *Saccharomyces cerevisiae*, are also capable of fermenting lactose and other sugars and can be found in some fermented milks (Kwak, et. al., 1996). The

steps involved in the fermentation of glucose by lactic acid bacteria and yeasts are outlined in Figure 1.

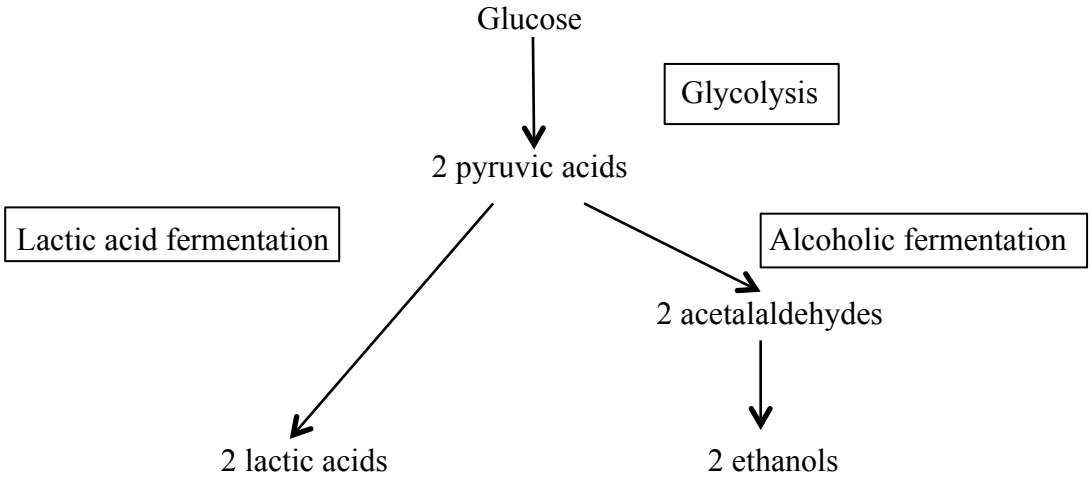

Figure 1. Lactic acid fermentation and alcoholic fermentation.

It also important to note that while the production of lactic acid from the fermentation of lactose contributes to the characteristic acidity associated with fermented milks, many interesting parallel post fermentation reactions often occur with other substrates, such as peptones, peptides and fatty acids to produce some of the distinctive flavors associated with cultured products.

1.2 Kefir Definition and Origin

Due to a growing consumer awareness and demand for foods with added or naturally occurring probiotics, a fermented milk called "kefir" is gaining in popularity and commercial production of kefir-like products has greatly increased over the past several years. Kefir is a naturally fermented milk beverage with a smooth and creamy texture and has an acidic and slightly alcoholic and yeasty taste; the presence of carbon dioxide gives a varying degree of effervescence (Farnsworth, 1999). In fact, the word kefir is derived from the Turkish word 'kef', which means pleasant taste (Kurmann, et.

al., 1992). Kefir originated in the Caucasus Mountains several centuries ago and was traditionally produced with caprine milk by inhabitants closely associated with the herding of goats and sheep.

Kefir has a rich history as it pertains to its genesis and spread throughout the regions of the Balkan and Caucasus regions of Eastern Europe; in fact, the origins of kefir predate written records (Rosell, 1932). Because of its ancient and apparently mysterious origin, kefir was known in antiquity as the 'Drink of the Prophet [Mohammad]' and the culture used to prepare it as the 'Grains of the Prophet Mohammad'. It was believed that the Prophet of Islam, Mohammad, was given the original kefir grains by the Angel Gabriel to be given to his followers, thus introducing kefir to the Orthodox Christians living in the mountainous regions of modern day Georgia (Margulis, 1996). A recent study by Yang, et. al. (2015) presented direct proteomic evidence for production of kefir that was found as an organic mass associated with the mummies of an Early Bronze Age (1980–1450 BCE) cemetery in Xinjiang, China.

1.3 Kefir Starter Culture

Kefir differs from other fermented milk products in its unique starter culture, which is an aggregation of many different bacteria and yeast species bound together in an exopolysaccharide (EPS) matrix produced by certain lactic acid bacteria in the presence of yeast (Figure 2). Farnsworth (1999) describes kefir grains as a mass of bacteria, yeasts, polysaccharides, and other products of bacterial metabolism, together with curds of milk proteins. The starter cultures, termed "grains", grow, propagate and pass their properties along to the following generations of grains (Simova, et. al., 2002).

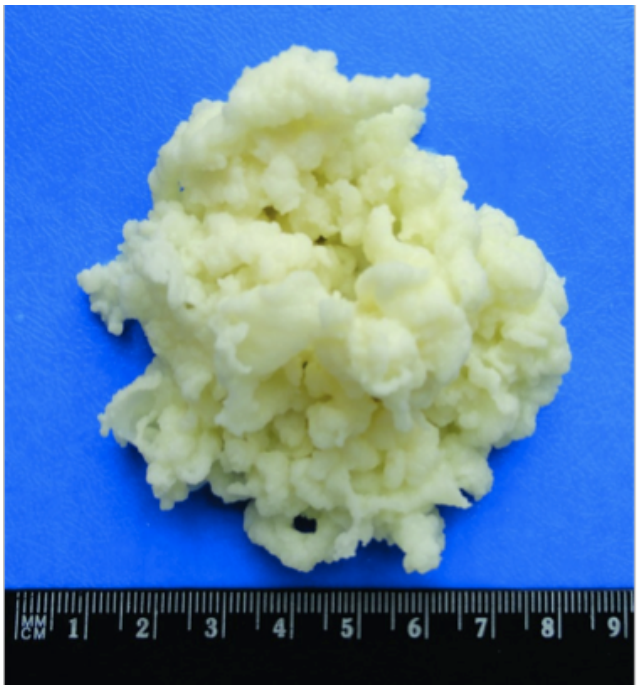

Figure 2. Macroscopic structure of kefir grains. (source: De Oliveira Leite, et. al., 2013: Creative Commons License CC BY-NC)

When describing the perpetuation of kefir grains by certain groups throughout history Rosell (1932) said, “One of the things that puzzle investigators in regard to the preparation of these milks [kefir] is that most of the races named are those who have kept kefir in its pure form. The method of their preparation was handed down as a precious inheritance from father to son in the families who concerned themselves with these products of ancient lineage, which, in a certain sense, may be said to constitute the ‘secret medicine’ of many countries.” It is the production of kefir and the propagation of the starter culture, using the traditional methods that preserve its defining characteristics, which have remained intact due to the preservation of the complex diversity and delicate balance of the microbial communities of the grains.

The fermentation of fresh milk is accomplished by the addition of the kefir grains, which may contain up to 50 bacterial species from genera including lactobacilli, lactococci, leuconostocs, acetobacter, enterococci and micrococci and up to 30 different yeast species

from genera such as kluvermyces and sacromyces; the strains are bound together by the exopolysaccharide kefiran, which is produced by the bacterial species *Lactobacillus kefiranofaciens* (Kwak, et. al., 1996; Diosma, et. al., 2014; Garofalo, et. al., 2015). The yellowish, white structures resemble small cauliflower florets and have a firm gel texture; the average kefir grain is approximately the size of a small marble with a weight of 0.5-1.5 grams (g), although individual grains can vary greatly in size and shape. The grains typically range in size from 0.3 to 3.5 centimeters (cm) in diameter; however some grains have been reported to grow much larger (Garrote, et. al., 1997). They are insoluble in water and ordinary solvents, and when immersed in milk the grains initiate the dual lactic acid and alcohol fermentations (Kosikowski, et. al., 1999). Zourari, et. al. (1988) reports the chemical composition of kefir grains as 890-900 grams/kilogram (g/kg) water, 2 g/kg lipid, 30 g/kg protein, 60 g/kg sugars and 7 g/kg ash.

Fermentation of milk with the kefir grains proceeds for approximately 24 hours. An initial drop in the pH is caused by the rapid growth of heterofermentative streptococci bacteria; the lowered pH favors the growth of lactobacilli, but causes the streptococci numbers to decline. The presence of yeasts in the kefir grains, along with the fermentation temperature (approximately 25°C) encourages the growth of aroma-producing heterofermentative streptococci. During the final stages of the initial incubation and during subsequent storage, growth of lactic acid bacteria is favored over growth of yeasts and acetic acid bacteria (Farnsworth, 2003 and Koroleva, 1988).

The microflora of kefir grains is remarkably stable, retaining its activity for years if preserved and incubated under the necessary physiological conditions required for the continued propagation the probiotic species (Simova, et. al., 2002). According to

Garrote, et. al. (1997), wet kefir grains will only retain activity for only 8-10 days if not inoculated into fresh milk, while dried grains retain activity for 12-18 months.

Studies have shown that grains from different geographic regions vary widely in composition, which can result in large variance in the finished kefir products (Marshall, et. al., 1984; Pintado, et. al., 1996; Simova, et. al., 2002; Wang, et. al., 2008). In addition, the microbiologic study of kefir is complicated by the constant evolution of identification and the related nomenclature of bacteria, often causing difficulties when comparing data between labs or with previous reports (Farnsworth, 1999).

In studies using electron microscopy to examine the structure and composition of kefir grains, Bottazzi, et. al. (1980) suggested that the yeasts and lactobacilli are not randomly distributed in the grain; the lactobacilli were associated with the periphery of the grain and the majority of yeasts located inside the grain. The source of the kefir grains for this study were only identified as "obtained from a commercial source", which may pose problems as grains from various regions may vary structurally in terms of distribution of the microflora as the species present varies between grains sourced from different sources/regions. In a 2005 study examining the microflora of Turkish kefir grains, Guzel-Seydem, et.al. found a ratio of 10^9:10^6 of lactic acid bacteria and yeasts, with lactobacilli species predominating, and no significant fluctuation during storage. However, previous studies on Irish kefir grains, performed by Rea, et. al. (1996) showed the contents of grains, reported in colony forming units per milliliter (cfu/ml) of lyophilized sample, to be 10^9 lactococci, 10^8 leuconostocs, 10^6 lactobacilli, 10^5 acetic acid bacteria and 10^6 yeasts (see Table 1).

Table 1. Average number of colony-forming units (CFUs) of probiotic microorganisms found in 1 gram (g) of traditionally produced kefir.

Genus	CFU per gram
Lactococcus	1,000,000,000
Leuconostocs	100,000,000
Lactobacillus	5,000,000
Yeasts	1,000,000
Acetobacter	100,000

(adapted from: Guzel-Seydem, et.al., 2005)

The weight percentage of kefir grains used to inoculate the milk has been shown to have a significant effect on the numbers of different microorganisms found in the finished product. When using a 1% by milk weight ratio of grains, lactobacilli and lactococci levels were found at the highest levels at the end of a 30 day storage period; when a 5% inoculate was used yeasts and acetic acid counts were highest after 72 hours (Irigoyen, et. al., 2005). Similarly, results by Koroleva (1988) demonstrated that the number of lactic acid bacteria tended to increase over time when lesser amounts of kefir grains were inoculated into the milk.

1.3.1 Microflora

Isolation and identification of the different strains of bacteria and yeasts present in kefir grains have traditionally been performed using culture-dependent methods, meaning that the probiotic species must be grown on selective media with identification being based on morphological and biochemical characteristics (Simova, et. al., 2002 and Wang, et. al., 2008). However, some studies have shown that many of the strains are very closely related and may pose problems when trying to isolate and identify individual strains (Micheli, et. al., 1999; Guzel-Seydim, et. al., 2005). More recent investigations have attempted to isolate strains based on genotype using polymerase chain reaction (PCR) combined with denaturing gradient gel electrophoresis (DGGE) (Wang, et. al., 2008; Tas, et. al., 2012; Garofalo, et. al. 2015). This culture-independent identification may be a useful in

analyzing complex microbial populations because this type of testing does not require prior separation of individual strains, as in culture-dependent identifications (Ercolini, 2004).

1.3.1.1 Lactobacilli

Lactobacilli are present in the largest amounts (65-85%) of the microbial population of kefir and kefir grains (Witthuhn, et. al., 2004). In a study examining the microflora of Brazilian kefir and kefir grains, Magalhas, et. al. (2011) also found lactobacilli species to be the predominant lactic acid bacteria type (78%) in kefir fermented with kefir grains, with lactococci comprising the majority of the remaining 28% of lactic acid species. Magalhas, et.al. (2011) also identified lactic acid bacteria isolates; *Lactobacillus paracasei* represented the largest and most commonly identified lactic acid bacteria isolate which represented 89 of 249 isolates. This was followed by *Lactobacilli parabuchneri* (41 isolates), *Lactobacilli casei* (32 isolates) and *Lactobacilli kefiri* (31 isolates) (see Table 2).

Table 2. Bacterial species commonly found in kefir and kefir grains.

Lactobacilli	**Lactococci**	**Acetic Acid Bacteria**
Lactobacillus kefir	*Lactococcus lactis subsp. lactis*	*Acetobacter aceti*
Lactobacillus kefiranofaciens	*Lactococcus lactis subsp. cremoris*	*Acetobacter pasteurians*
Lactobacillus rhamnosus		
Lactobacillus casei	**Streptococci**	**Other Bacteria**
Lactobacillus paracasei	*Streptococci thermophilus*	*Bacillus subtilis*
Lactobacillus fructivorans	*Streptococci durans*	*Micrococcus sp.*
Lactobacillus hilgardii		*Enterococcus durans*
Lactobacillus fermentum	**Leuconostocs**	
Lactobacillus viridescens	*Leuconostoc mesenteroides*	
Lactobacillus helveticus	*Leuconostoc kefir*	
Lactobacillus acidophilus	*Leuconostoc sp.*	
Lactobacillus delbruckii		
Lactobacillus brevis		
Lactobacillus plantarum		
Lactobacillus parakefir		
Lactobacilli parabuchneri		
Lactobacilli kefiri		

(adapted from: Witthuhn, et. al., 2004; Guzel-Seydim, et. al., 2005; Wang, et. al., 2008; Magalhas, et.al., 2011; Tas, et. al., 2012; Garofalo, et. al. 2015)

1.3.1.2 Lactococci

Magalhas, et. al. (2011) was only able to isolate one species, *Lactococcus lactis*, from Brazilian kefir and kefir grains; this particular species was identified in all 24 lactococci isolates from the total of 249 lactic acid bacteria isolates. In a 2005 study by Guzel-Seydim, et. al. a microbial enumeration and electron microscopy was performed on Turkish kefir and kefir grains, and although long, short and curved lactobacilli and yeasts were found in all samples, lactococci were not observed in any portion of the kefir grain. Guzel-Seydim, et. al. (2005) postulated that the presence of lactococci in the kefir but not in the grain samples might be caused by the unintentional removal of lactococci from the surface of the grains.

1.3.1.3 Yeasts

Magalhas, et. al. (2011) showed a majority of Brazilian kefir yeast isolates to be lactose-negative strains; specifically *Saccharomyces cerevisiae*, which made up 41 of 110 yeast isolates. Other non-lactose fermenting species isolated included *Kazachstania aerobia* (23 isolates) and *Lachancea meyersii* (15 isolates); these two species had been previously unreported in kefir and kefir grain studies (see Table 3). Yeasts, such as *Saccharomyces cerevisae,* contribute greatly to the sensory qualities of the kefir beverage, as they promote an alcoholic and yeasty aroma, as well as a refreshing taste (Magalhaes, et. al., 2011). It is also worth noting that the presence of non-lactose fermenting yeasts in kefir and kefir grains is dependent on the presence of other lactose fermenting species of bacteria and yeasts capable of hydrolyzing lactose (Simova, et. al., 2002).

Table 3. Species of yeasts reported in kefir grains of various origins.

Saccharomyces cerevisiae	*Candida friedrichi*	*Torulaspora delbrueckii*
Saccharomyces unisporus	*Candida friedrichii*	*Kazachstania aerobia*
Saccharomyces turicensis	*Candida pseudotropicalis*	*Brettanomyces anomalus*
Saccharomyces delbrueckii	*Candida tenuis*	*Lachancea meyersii*
Saccharomyces dairensis	*Candida inconspicua*	*Issatchenkia occidentalis*
Saccharomyces lactic	*Candida maris*	*Pichia frementans*
Saccharomyces spp	*Candida lambica*	
	Candida tannotelerans	
Kluyveromyces marxianus	*Candida valida*	
Kluyveromyces lactis	*Candida kefyr*	
	Candida holmii	

(adapted from: Simova, et. al., 2002; Magalhaes, et. al., 2011; Diosma, et. al., 2014; Garofalo, et. al., 2015)

According to Irigoyen, et. al. (2005), the levels of yeasts and acetic acid bacteria present in kefir are directly proportional to the quantity of grains inoculated. Interestingly, their study also found the levels of lactobacilli and lactococci to be inversely proportional to the amount of inoculate used; therefore, the number of microorganisms was higher when fewer kefir grains were used. This might be due to a more rapid initial increase in the amount of lactic acid bacteria in the kefir inoculated with the higher percentage of grains; the higher number of initial bacteria might cause a quick, sharp drop in pH which would kill some of the more acid sensitive strains, thus preventing their growth during storage and allowing for an increased proliferation over time of yeasts and other types of bacteria, such as micrococci and acetic acid bacteria. It has also been shown that lactic acid bacteria multiply less rapidly, and therefore, produce lactic and acetic acids more slowly when incorporated into a mixture containing yeasts than in a pure culture (Collar, 1996).

During refrigerated storage of kefir, lactic acid bacteria will begin to decrease, while the numbers of yeasts and acetic acid bacteria will remain fairly consistent (Irigoyen, et. al., 2005). Irigoyen, et. al. (2005) found no significant differences in yeast counts during a thirty day storage period at approximately 5°C; however, lactobacilli and lactococci

were shown to be significantly lower after thirty days of storage. These results differ from a similar study by Guzel-Seydim, et. al. (2005) that examined the microbiota of Turkish kefir and kefir grains; the microbial counts of the lactic acid bacteria did not decrease, and actually exhibited continued growth during and after 21 days of refrigerated storage. This can most likely be attributed to the ability of many of the yeast species present in kefir to metabolize some of the lactic acid produced by the bacteria, therefore enhancing the survivability of the lactic acid bacteria by the reduction metabolic end products.

1.3.2 Kefiran

EPS produced by some lactic acid bacteria have been the recent focus of research in various food industries as a beneficial additive for increasing viscosity in products, and the stipulated health benefits associated with bacterial EPS provide an added appeal to the consumer. Kefiran, an EPS produced and subsequently excreted by a certain strains of lactic acid producing bacteria found in kefir grains and kefir has been isolated, and its composition and chemical structure have been determined using methods such as acid and enzymatic hydrolysis and scanning electron microscopy (Figure 3). Although, at present, no studies isolating kefiran from the kefir beverage have been reported, Cerning, et. al. (1999) listed the range amount of EPS produced by lactic acid bacteria in fermented products as 25 to 890 mg/L.

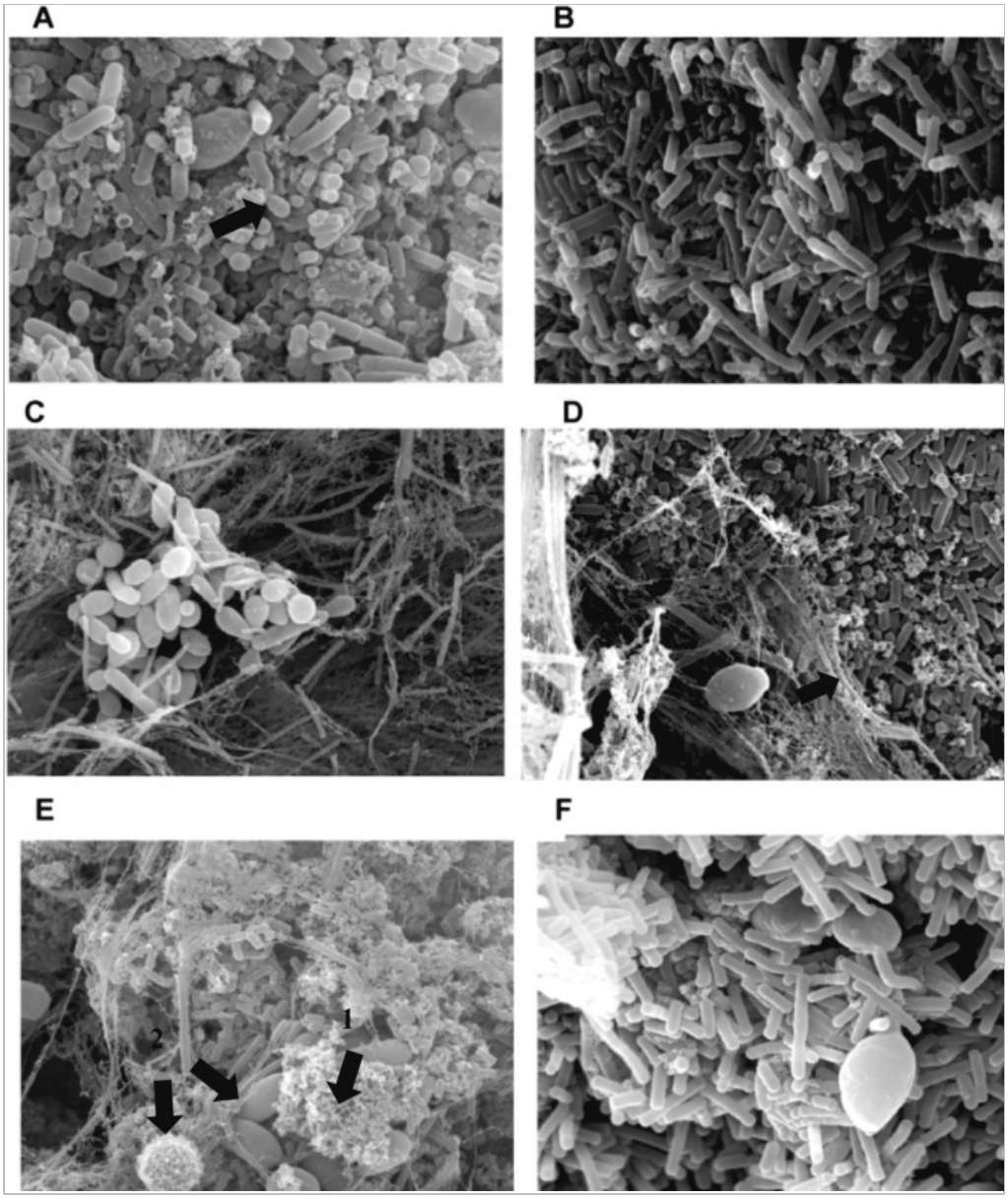

Figure 3. Scanning electron microscopy of Brazilian kefir grain microbiota. A, C, E: outer grain portions, B, D, F: inner grain portions. Arrows - Micrography A: cocci; Micrography D: fibrillar material - kefiran polysaccharide; Micrography E: arrow 1 - granular material - coagulated protein, arrow 2 - different yeast species. (source: De Oliveira Leite, et. al., 2013: Creative Commons License CC BY-NC)

The structure of kefiran was described by Riviere, et. al. (1967) as a water-soluble glucogalactan containing roughly equal amount of D-glucose and D-galactose residues. The production of this EPS is stimulated when *L. kefiranofaciens* grows in co-culture with *S. cerevisiae*, other yeasts and yeast extracts (Cheirsilp et al., 2003; Zajšek, et. al.,

2013; Pop, et. al., 2014). *Lactobacillus kefiranofacians*, the kefiran producing strain, comprises at least 24% of the dry material of the kefir grain. Although the chemical analysis preformed by Riviere, et. al. (1967) was accurate, the bacterium has, in recent years, been referred to as *Lactobacillus kefir* (Frengova, et, al., 2002) and most currently as*Lactobacillus kefiranofacians* (Cheirsilp, et. al., 2003). However, there are conflicting opinions in several independent studies regarding the naming of this bacterium and if the *Lactobacillus kefiran* and *Lactobacillus kefiranofacians* are the same strain or two different strains, with one or both producing kefiran in differing amounts (Kandler, et. al., 1983; Frengova, et. al., 2002; Rimada, et. al., 2002; Piermaria, et. al., 2009).

Different conditions such as the species present, the ratio of bacteria to yeasts, fermentation time, incubation temperature, storage time and other conditions can all affect the amount of EPS produced by kefiran producing strains (van Geel-Shutten, et. al., 1998). Mozzi, et. al. (1995) found that by increasing the time of fermentation up to 72 hours a significant reduction in kefiran synthesis was observed; these results were dependent both on the fermentation temperature (30, 37 and 42°C) and the strain of lactic acid bacteria employed.

The activity of the grains, or more precisely, the activity of the kefiran producing strains also plays a major role in the amount of kefiran present in the product. Schoevers, et. al. (2003) has reported a lowering of lactobacilli species present, as well as cellular activity, in some the kefir grains that have been subjected to prolonged storage without fresh milk or exposure to widely varying environmental conditions, such as fluctuating incubation temperatures or a change in milk type.

Because other environmental conditions such as temperature, pH, carbon and nitrogen content influence EPS production, control of fermentation protocols are important. Zajšek, et. al. (2013) established the optimal fermentation temperature to be 30°C, where EPS production from the kefir grains was maximal. Some yeast species present are also able to metabolize some of the lactic acid produced by the bacteria, therefore enhancing the survivability of the lactic acid bacteria by the reduction metabolic end products. EPS similar to kefiran have also been isolated, although in lesser amounts, from other lactic acid species such as *Lactobacillus reuteri* and *Lactococcus lactis* ssp. *cremoris* (van-Geel Schutten, et. al., 1998; Yang, et. al., 1999; Patel, et. al., 2012). Other examples of EPS use in the food industry are xanthan produced by *Xanthomonas campestris* and gellan from *Pseudomonas eloda* (Matsukawa, et. al., 2007; Patel, et. al., 2012).

1.4 Physiochemical and Sensory Properties of Kefir

The major end products, according to Kooman (1968), are approximately 0.8% lactic acid, 1.0% ethyl alcohol and carbon dioxide. Also present in smaller amounts are acetic acid, numerous volatile flavor compounds such as diacetyl and acetylaldehyde, exopolysaccharides, organic acids, and various vitamins and minerals. The optimum taste profile in kefir has a 3:1 diacetyl to acetylaldehyde ratio with a pH of 4.6 using milk with an initial fat content of no less than 3.0%, and although complex alcohols and acetone have also been identified as end products, they are not thought to be predominant factors in the flavor profile (Kosikowski, et. al., 1997; Kakisu, et. al., 2011).

The fat content of kefir may range from 0.5 to 3.0 percent, with solids not from fat from 8.0 to 11.0 percent (Kosikowski, et. al., 1999). This includes approximately

2.0 g of lactose per 100 g of kefir; the fermentation of milk by kefir grains results in approximately a 30% reduction in lactose (Alm, 1980). Alm (1980) examined lactose intolerant individuals demonstrating symptoms of abdominal distress and diarrhea following consumption of 500 ml of low fat milk and found that of the same quantity of fermented milk did not result in any symptoms.

The fat content of kefir will vary depending on the original fat content of the milk used (whole vs. skim, bovine vs. caprine), and the action of the microflora on milk fat can have significant effects on the lipid profile, especially after storage for a brief period (Gul, et. al., 2015). In a study involving the physiochemical analysis of milk, Irigoyen, et. al. (2005) found that the fat content of the finished kefir did not differ significantly from the fat content of the milk that the kefir was made from; however, oxidation of the fat molecules and the off-flavors associated with lipid oxidation can be counteracted to some degree by the microorganisms in kefir (as well as other fermented milks). The lactic acid bacteria consume oxygen providing a reducing effect, and during storage lipolysis, the breakdown of fats into glycerol and free fatty acids will also occur, contributing to an increase in short chain fatty acids (SCFA) (Alfa-Lavel, 1987).

Flavors attributed to higher levels of SCFA in milk can be very influential on flavor profile of fermented milks. For example, the release of capric acid, the most abundant saturated fatty acid found in goat milk, from the triglyceride will result in a distinctive goat milk flavor, often characterized as a “goaty” or “musky” flavor. The yeasts involved in kefir fermentation, particularly *Saccharomyces cerevisiae,* are also of importance when characterizing the sensory qualities of the kefir beverage, as they

promote a strong and typically yeasty aroma, as well as a refreshing and pungent, slightly alcoholic taste (Magahlaes, et. al., 2010).

1.5 Kefir Production Methods and Consumer Preferences

During kefir manufacture with the grains, the lactic acid fermentation slows considerably or stops as the pH declines, but the yeast fermentations continue allowing for an increase in ethanol production during storage. The secondary alcohol fermentations can lead to substantial changes in flavor as well as bulging or leaking packaging due to the continued production of carbon dioxide gas (Kwak, et. al., 1996; Sarkar, 2008).

Commercial kefir production utilizes a dry starter culture typically consisting of 8-12 species of bacteria and yeasts isolated from lyophilized kefir grains (or other sources). A new commercial starter, whether dry or from a liquid mother culture, must be added to each new batch of milk for kefir production. Studies preformed by Simova, et. al. (2002) demonstrated that traditionally produced kefir (with the starter grains removed) could not be used as a starter culture for kefir. The primary drawbackfor the large scale manufacture and marketing of traditionally produced kefir is that secondary alcohol fermentation often occurs at the distribution and storage phases, resulting in changes in flavor and taste because of the continued formation of ethanol and carbon dioxide gas (Kwak, et. al., 1996). This can result in swollen and leaking containers as the excess gas increases the internal pressure of the container and inconsistences in the final products.

A study by Beshkova, et. al. (2002) showed and increase in ethanol from 0.25% in traditional to 0.48% in commercial kefir, and an increase in CO_2 of 1.05g/l to 1.98 g/l. Kwak, et. al. showed that biostabilization of kefir can be achieved by using a starter

culture in which non-lactose fermenting yeasts, such as *Saccharomyces cerevisiae,* are present in substantial numbers. In fact, the standardization of the production of flavor compounds including lactic acid and ethanol has been achieved, for the most part, by the utilization of pure cultures as a starter for the commercial production of kefir. Gronnevik, et. al. (2011) showed that the levels of lactic acid and ethanol in Norwegian commercial kefir remained consistent during an 8-week storage period, with some volatile compounds fluctuating slightly; this was probably due to an increase in the yeast metabolism of bacterial intermediate or end products. This can be compared to studies performed on the physicochemical changes in milk during the production and storage of traditional kefir, which were found to be in flux and somewhat inconsistent between kefir made from differently sourced grains (Wszolek, et. al., 2001; Chen, et. al., 2005; Irigoyen, et. al., 2005; Magalhaes, et. al., 2011). The development of commercial starter cultures has allowed for widespread distribution of kefir and kefir products; however, the demand for traditionally produced kefir is rising, and methods for producing a consistent product with an adequate shelf life are being developed.

1.6 Health Benefits of Probiotics and Kefir

Similar to other fermented dairy foods supplemented with probiotic bacteria, 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 the milk (Figure 4).

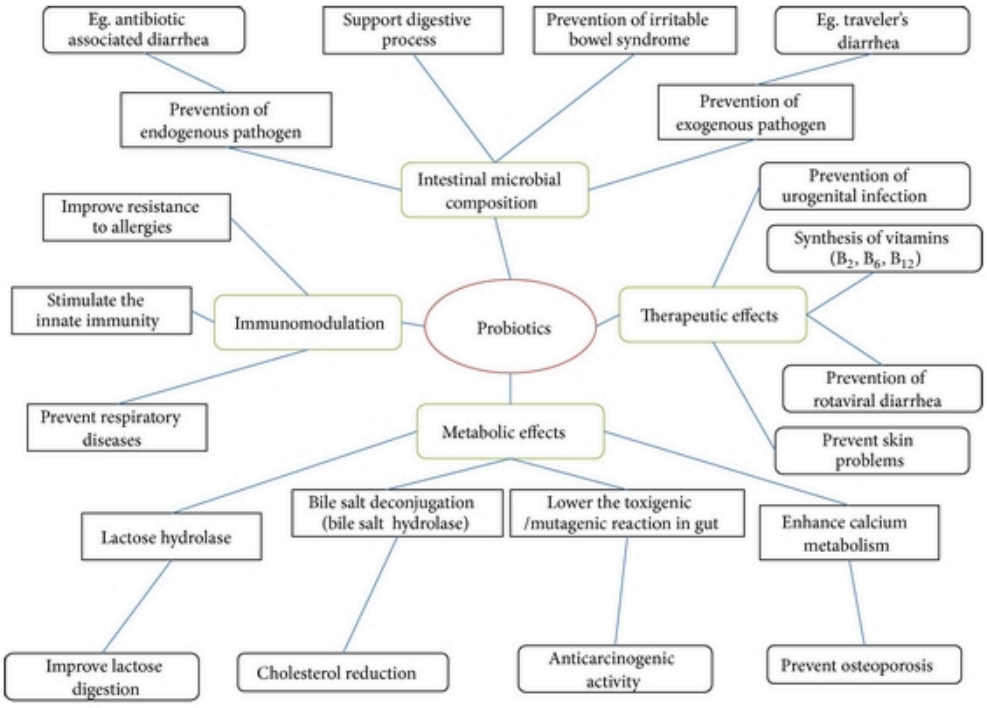

Figure 4. Proposed health benefits associated with the consumption of probiotics. (source: Anandharaj et al., 2014; distributed under the Creative Commons Attribution License)

In order for a probiotic to benefit human health it must have good technological properties, survive through the upper gastrointestinal tract and be able to function in the gut environment (Mattila-Sandholm, et. al., 2002). These properties, as well as many health benefits, have been examined, and kefir has demonstrated a wide array of positive effects such as antitumor and immunostimulating activity in animals (Quiros, et. al., 2005; Ghoneum and Gimzewski, 2014); these effects are only seen when the probiotics ingested are functioning properly in the intestinal mucosa (Figure 5).

Prebiotic and probiotic benefits incurred by the consumer include: competitive exclusion of pathogenic bacteria, increased absorption of nutrients, and well documented immunomodulating effects, such as the modification of the balance of immune cells in the intestinal mucosa (Vinderola, et. al., 2006; Maalouf, et. al., 2011; Medrano, 2011)

(see Figure 5). Probiotic organisms can exert their beneficial properties through two mechanisms: direct effects of the live microbial cells (probiotics) or indirect effects via metabolites of these cells (biogenics) (Vinderola, et. al., 2004). Biogenics are defined as food components that are derived from microbial activity which provide health benefits without involving the intestinal microflora (Takano, 2002).

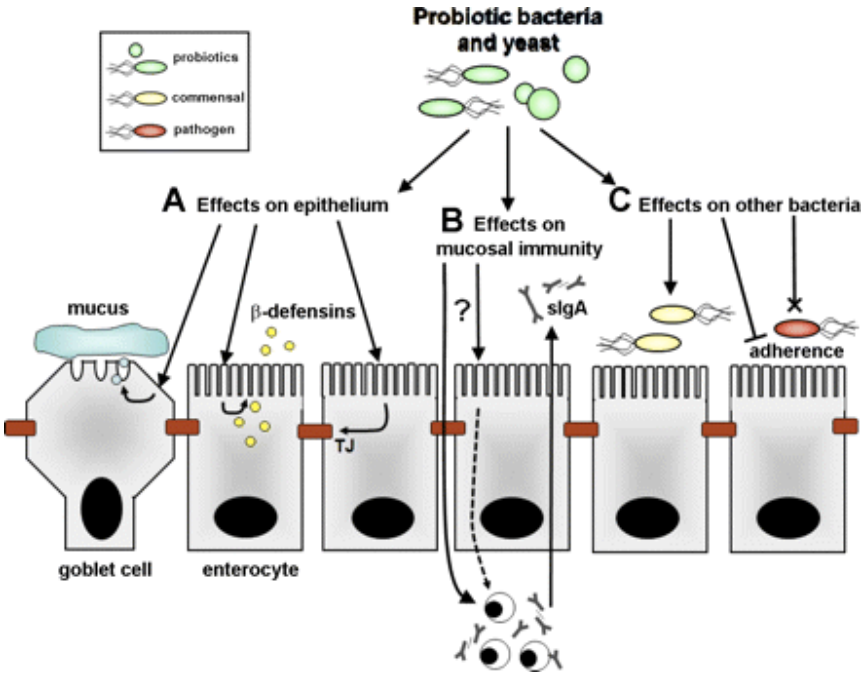

Figure 5. Effects of probiotic bacteria and yeast on intestinal epithelial barrier function. (source: Ohand, et. al., 2010: https://s100.copyright.com/AppDispatchServlet#formTop)

Several strains of *Lactobacillus delbrueckii* and *Streptococcus thermophilus* produce EPS (Hong and Marshall, 2001). In kefir, these loosely bound exopolysaccharides couls act as a stabilizer, preventing syneresis and graininess and provide a natural thickening effect (Cerning, 1990). In regards to health, bacterial exopolysaccharides could provide benefits such as aiding in bacterial adhesion to the lining of the gut and protection of probiotics during transit though the gastrointestinal tract.

Because the origins of lactic acid bacteria and yeasts are not human associated, they can be sensitive to the harsh conditions of the stomach and bile acids. This aspect becomes president when considering the health benefits incurred by the consumer of probiotic containing products. It has been shown that both the strain and the amount of probiotic species play a role in determining survival in the human gut (Elli, et. al., 2006 and Conway, et. al., 1987); and the adhesion to intestinal epithelial cells may play a critical role in the immunostimulating and immunomodulating effects of certain probiotic species. However, in vivo adhesion will most likely be influenced by both the normal microbiota and the specific probiotics included, but only few studies to date have directly examined the adhesion interactions of the probiotics and the intestinal mucosa (Ouwehand, et. al., 2000 and Collado, et. al., 2007).

In addition to the immunostimulating benefits of probiotics, kefir exhibits additional biological activities that include antibiotic and antifungal properties (Farnsworth, et. al., 2003); antioxidant action of probiotic bacteria towards numerous human pathogens has been also been observed in several studies (Zacconi, et. al., 2003; Medrano, et. al., 2006; Ismaiel, et. al., 2011). In a 2004 study, Rodrigues, et. al. demonstrated the antimicrobial and healing activity of kefir and kefiran extract; they showed that successful and faster wound healing occurred in rats when a topical kefiran mixture was used as alternative to antibiotics. Other studies have demonstrated that antibacterial, antimycotic and antitumor activity of cells increase when exposed to kefir and kefiran (Garrote, et. al., 2004; Micheli, et. al., 1999; Frengova, et. al., 2002).

1.7 Physiological Effects of Endurance Exercise

1.7.1 Metabolic Effects

Endurance exercise depletes the body's glucose stores (blood glucose, muscle and liver glycogen) and can have a catabolic effect on muscle proteins immediately following exercise. To most efficiently replenish energy stores and to maintain the functioning of skeletal muscles, adenosine triphosphate (ATP) is produced via the oxidative phosphorylation of carbohydrates, proteins and fats, as seen in Figure 6. During moderate physical activity intracellular glycogen and free fatty acids are the primary substrates utilized in the oxidative pathway; however, for endurance athletes, circulating glucose also provides a significant ATP source when exercising for prolonged periods of time (De Feo, et. al., 2003).

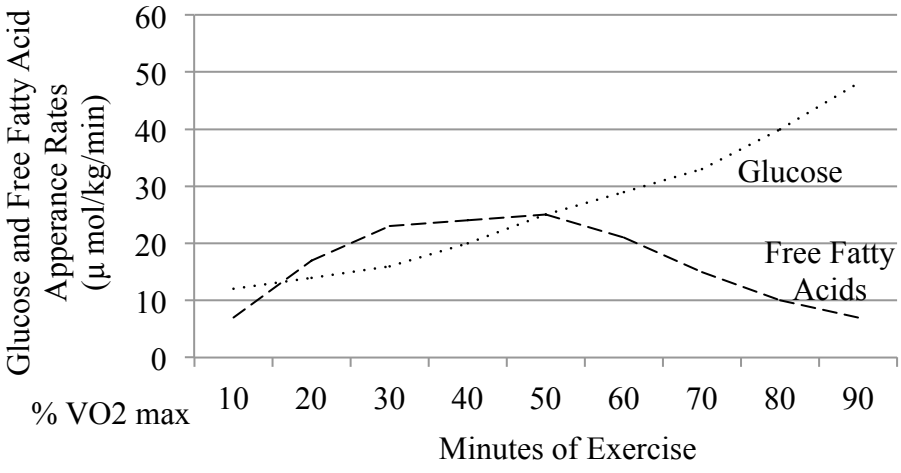

Figure 6. Rates of appearance of circulating glucose and free fatty acids (FFA) during exercise at graded intensities in humans. VO_{2max}: maximum oxygen uptake. (modified from: De Feo, et. al., 2003).

It has been well established that exercise can be a key strategy for improving health and is actually essential for good metabolic and cardiovascular function, of which insulin action is a primary component (Thyfault and Krogh-Madsen 2011), and the

beneficial effects of high intensity exercise have been demonstrated in several controlled studies (Babraj, et. al. 2009; Little, et. al. 2011). In order to optimize the metabolic, cardiovascular and psychological benefits that exercise can offer, Garber, et. al. (2011) advises people to perform a large volume (at least 30 min per day) of both moderate and vigorous intensity cardiorespiratory exercise on most days of the week, as well as strength and flexibility sessions 2–3 times per week. However, Metcalf, et. al. (2012) demonstrated that shorter and more intense exercise sessions could infer beneficial effects as well by conducting a 6-week exercise intervention consisting of very brief, manageable sessions; results showed that a short exercise intervention was associated with improved insulin sensitivity in sedentary young men, and improved aerobic capacity in men and women. When describing normal metabolic responses of individuals following exercise, differences between men and women must also be considered. By following a traditional aerobic exercise intervention in a large cohort, Boule, et. al. (2005) demonstrated that insulin sensitivity improved to a greater degree in men when compared with women, but the female participants had a higher baseline level of insulin sensitivity which may, in turn, impact subsequent metabolic training responses.

1.7.2 Immune Functioning

Prolonged intense exercise often results in perturbations in cellular aspects of immunity. Strenuous and eccentric exercise seems to exert the most prominent changes in immune parameters with extreme exercise, such as marathons and other frequently used training programs, being associated with a depression in immune function (Fitzgerald, 1991). In a 1992 study Keast, et. al. determined that regular, moderate training does seem to promote resistance to upper respiratory tract infection (URTI);

however, severe exertion, especially when coupled with mental stress, places athletes at increased risk for URTI (Nieman, 1994). A limitation to these types of epidemiologic studies on exercise and URTI is that they are based on self-reported symptoms rather than clinical verification and can often be unreliable (Hoffman-Goetz, et. al., 1995). Figure 7 shows the link between exercise associated immune changes and sensitivity to infection that may be explained by the so-called "open window" of altered immunity (Pederson and Toft, 2000).

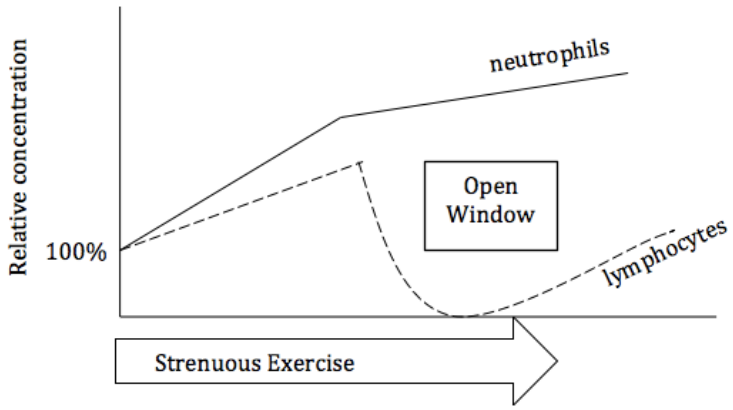

Figure 7. Model of the proposed neuroendocrinological mechanisms underlying the exercise induced "open window" in the immune system. (modified from: Pedersen and Toft, 2000).

Many of the mechanisms underlying exercise associated immune changes are multifactorial and include important neuroendocrinological factors such as adrenaline (epinephrine), noradrenaline (norepinephrine), growth hormone, and cortisol (Newsholme, 1994). The concentrations of these hormones typically increase during exercise and return to original values shortly after, but they also seem to exert effects on lymphocytes and neutrophils during the recovery period. Pederson and Toft (2000) hypothesized that during the first few hours following exercise, when immune

suppression is most pronounced, viruses and bacteria may gain a foothold, increasing the risk of subclinical and clinical infection.

1.7.3 Inflammation

Chronic inflammation is theorized to be the root cause for a number of associated diseases and disorders, such as chronic obstructive pulmonary disease, asthma, chronic heath failure, rheumatoid arthritis, diabetes mellitus type 1 and 2, inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis), inflammatory myopathies (e.g., idiopathic, polymyositis, dermatomyositis, inclusion body myositis), juvenile idiopathic arthritis and multiple sclerosis (Abbas, et. al., 2007; Ploeger, et. al., 2009; Hotamisligil, et. al., 2006). It has been theorized that the combination of an impaired immune response coupled with persistent immune stimulation may have a role in the low-grade systemic inflammation and altered cytokine balance, which may translate into increased cardiovascular diesase (CVD) risk (Stenvinkel, et. al., 2005; Adamopoulos, et. al, 2001).

Altered levels of cytokines are not only seen in inflammatory diseases; acute and chronic exercise can also have an effect on cytokine responses and inflammation in healthy individuals (Ploeger, et. al., 2009). Superficially, the body reacts to physical activity as it does during an acute, subclinical inflammatory response to a perceived pathological insult (Ostrowski, et. al., 1998), and pro- and anti-inflammatory cytokines are released into the circulation (Northoff, 1994).

The effects of regular or chronic exercise on basal levels of inflammatory markers have been used to recommend exercise as an anti-inflammatory therapy (Adamopoulos, 2002). However, compared to acute bouts of exercise, the effects of chronic exercise on inflammatory markers have been less investigated. Whereas exercise of moderate

intensity is associated with anti-inflammatory effects (Bruunsgaard, et. al., 2005), strenuous exercise can affect resting levels of inflammatory markers during and after a period of intensive training (Gleeson, et. al., 2006; Gokhale, et. al., 2007). In spite of the different backgrounds and symptoms related to the various diseases, all systemic chronic inflammation shares common characteristics, including elevated circulating levels of cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) under basal or resting conditions (Hotamisligil, 2006).

1.8 Biological Measures of Inflammation and Immune Functioning

The immune system's first lines of defense against microbial invasion are the external secretions that bathe mucosal surfaces (respiratory, intestinal and reproductive); secretory immunoglobulin A (sIgA) is a key component and is the dominant immunoglobulin present in mucosal secretions (King, et. al., 2003). IgA is secreted by mucosal plasma cells that are adjacent to the salivary glands; once secreted from the cell the dimeric IgA it is bound and transported by a polymeric immunoglobulin receptor through the salivary cells (King, et. al., 2003). The IgA dimer forms a complex with a fragment of the polymetric immunoglobulin receptor polypeptide and is released into the saliva as secretory IgA. Studies have shown that sIgA levels vary in response to stress, mood and emotional state (Cohen, et. al., 2003).

TNF-α, also known as cachectin, is a non-glycosylated polypeptide cytokine that is produced primarily by activated macrophages with a large percentage of TNF-α production being the result of stimulation of macrophages by lipopolysaccharide (Beutler, et. al., 1895). Therefore, TNF-α becomes an important mediator of the *in vivo* effects of lipopolysaccharide, and the inhibition of lipoprotein lipase and apidocyte gene

expression, in regards to metabolic activities, is strongly influenced by TNF-α (Dinerello, et. al., 2003). TNF-α is involved in various biological activities such as immunomodulating and proinflammatory activities by regulation of the production of antibodies by B cells and stimulation of cytotoxic T cells (Moldoveanu, et. al., 2000). Since TNF-α has also been shown to increase ubiquitin gene expression in skeletal muscle, it is possible that it causes muscle wasting by stimulating protein catabolism via the ubiquitin proteasome pathway (Stenvinkel, et. al. 2005). In a 2000 review, Shek and Shepard stated that strenuous exercise-induced elevations in interleukin-1β and TNF-α have been reported, but results from different studies appeared inconsistent, possibly due, in part, to differences in experimental design, timing of blood sampling, and cytokine assay sensitivity (Thomson, 1998; Adampopoulos, et. al., 2001; Himmerich, et. al., 2006).

C-reactive Protein (CRP) is formed primarily in hepatocytes with its rate of synthesis being primarily influenced by the cytokines involved in inflammatory processes, and because its half-life is estimated to be rather short (13-16 hours), serum CRP concentration can reflect acute infection, pneumonia and myocardial infarction (Cohen and Cohen, 2006). Several recent studies have demonstrated an association between inflammatory reactions and CVD such as both latent and chronic infections and arteriosclerosis. In fact, the CRP values found in Table 4 are often used by clinicians to assess systemic inflammation and are most notably used as a risk factor for CVD (Zulet, et. al., 2006). However, because no accepted normal ranges for adult populations have been established, CHD-Risk Level is used as a predictive measure only (Pearson et. al., 2003).

Table 4. CRP concentration in plasma and correlated risk of coronary heart disease.

C-Reactive Protein-Concentration for Plasma	Coronary Heart Disease-Risk Level
< 1 mg/l	low
1-3 mg/l	medium
> 3 mg/l	high

(modified from: Pearson, et. al., 2003

1.9 Sport Nutrition

According to the American College of Sports Medicine (ACSM) (2009), athletes should consume enough energy during periods of high-intensity and/or long-duration training in order to maintain body weight and health and to maximize training efforts. ACSM (2009) reports that low energy intakes can result in loss of muscle mass, menstrual dysfunction, loss of or failure to gain bone density, an increased risk of fatigue, injury and illness, and a prolonged recovery process. Recommendations include a carbohydrate intake of approximately 1.0-1.5 g per kg body weight during the first 30 minutes after exercise and again every 2 h for 4-6 h in order to adequately replace glycogen stores (ACSM, 2009) (Table 5).

Table 5. Macronutrient recommendations for athletes following exercise.

	Intake (per kg body weight)	Intake (per lb body weight)
Carbohydrate*	1.0-1.5 g	0.5-0.7 g
Protein	0.4-0.7 g	0.2-0.4 g

(adapted from: ACSM, 2009)

During exercise, carbohydrates maintain blood glucose levels and replace muscle glycogen. The ACSM carbohydrate recommendations for athletes range from 6 g/kg to 10 g/kg body weight (Table 5). The amount required depends on the athlete's total energy expenditure, type of training, sex, and other environmental conditions, such as extreme heat or cold. In an article examining the upper and lower limits of carbohydrate requirements for different types of athletic training, Jeukendrup (2013) concluded that

carbohydrate needs can reach much higher values (up to 90 g/h) for elite athletes exercising at higher absolute intensities or during an event lasting more than 2 hours.

Protein intake sufficient to maintain and increase muscle mass is necessary for optimal health and performance. Protein recommendations for endurance and strength-trained athletes range from 1.2-1.7 g/kg body weight (0.5-0.8 g/lb body weight). Consumption of protein provides essential amino acids required for the synthesis of body proteins. Amino acids also play key roles in the metabolic processes of many organs and tissues by serving as precursors and regulators in the production of metabolic mediators and compounds with regulatory biological activity e.g. hormones, neurotransmitters, DNA and RNA (Jeukendrup and Gleeson, 2010).

Fat is also important in the diet of athletes, not only as a source of energy, but because it provides fat-soluble vitamins and essential fatty acids. The ACSM (2009) recommends a fat intake ranging from 20-35% of the total energy intake and goes on to state that consuming less than 20% of energy from fat does not benefit performance.

Adequate fluid intake before, during, and after exercise is critical for health and optimal performance to avoid dehydration, with a goal of drinking enough fluid to avoid dehydration but not in excess of the sweating rate. For fluid intake following exercise the ACSM recommends 16-24 oz (450-675 mL) of fluid for every pound (0.5 kg) of body weight lost during exercise.

Before exercise, a meal or snack should provide sufficient fluid to maintain hydration and ease of digestion and be relatively low in fat and fiber in order to facilitate gastric emptying and to minimize gastrointestinal distress. The food consumed should be relatively high in carbohydrates to maximize maintenance of blood glucose, moderate in

protein, be composed of familiar foods, and be well tolerated by the athlete (ACSM, 2009). The recommendation of the ACSM for nutrition during exercise is for athletes to replace fluid losses and provide carbohydrates at a rate of approximately 30-60 g/hour (ACSM, 2009). Following exercise, ingestion of enough fluids, electrolytes and carbohydrates to replace muscle glycogen will ensure rapid recovery, and protein consumed after exercise will provide essential amino acids needed for repair and building of muscle tissue (Maguhan, 1997). Numerous studies have shown a 4 to 1 ratio of carbohydrates to protein to be the most beneficial macronutrient profile of a recovery food for athletes (Karp, et. al., 2008; Roy, et. al., 2008; Thomas, et. al., 2009). The effects of certain foods on intestinal health and overall well being of athletes in reducing and preventing elevated inflammatory response and instances of suboptimal immune functioning, as seen by measuring specific markers such as CRP, are also very important variables to consider for proper sports nutrition.

1.10 Probiotics and Exercise

Elevating and maintaining high levels of stress on the body, as often occurs with exercise, can cause acute and chronic disruption of immune functioning (Gleeson, et. al., 2006). In a review examining the relationship between probiotics and exercise, West, et. al. (2009) concluded that probiotic supplements modulate the intestinal microbial flora and offer promise as a practical means of enhancing gut and immune function in athletes.

Studies involving probiotics and athletes have demonstrated maintenance of gastrointestinal (GI) function and health by reducing the frequency and severity of GI issues including cramps, nausea, bloating and diarrhea during and following exercise (Berg, et. al., 1999). Attenuation of immunosuppressive effects of exercise have also

been demonstrated in athletes receiving an encapsulated probiotic supplement during training (Bishop, et. al., 2009). A similar study conducted by Kekkonen, et. al. (2007) showed a reduced susceptibility to illness especially in regards to an enhanced resistance to upper URTI in athletes consuming probiotics.

Zonulin is a novel protein that modulates intestinal permeability by disassembling the intercellular tight junctions (TJs) (Wang, et. al., 2001). This protein is most likely involved in the innate immunity of the gut and, when upregulated, appears to play a key role in the pathogenesis of autoimmune diseases, such as celiac disease (Sapone, et. al., 2006). Reduced gut permeability in athletes receiving a probiotic supplement during the course of a 14-week intensive cycling training course was demonstrated by a significantly lowered amount of zonulin in the feces of the probiotic group as compared to the placebo indicating reduced gut permeability in the probiotic supplemented group (Lamprechet, et. al., 2012). In addition, Lamprechet, et. al. (2012) concluded that probiotic supplementation beneficially affected TNF-α and exercise induced protein oxidation. Many probiotic strains are also able to stimulate the production of IgA by B cells, which help maintain intestinal humoral immunity by binding to antigens, thereby limiting their access to the epithelium (Delcenserie, et. al., 2009).

1.11 The Role of Dairy Foods in Sport Nutrition

Milk, supplemented with the recommended amount of carbohydrate, has been shown to be an ideal recovery beverage for athletes following bouts of resistance and endurance training as a way to attenuate some of the acute affects of strenuous activity (Roy, et. al., 2008; Thomas, et. al., 2009). In a 2007 study, Wilkenson found that milk-

based proteins promote muscle protein accretion to a greater extent than do soy-based proteins when consumed after resistance exercise.

The U.S. Department of Agriculture and U.S. Department of Health and Human Services 2010 Dietary Guidelines for Americans recommends milk as a healthful, protein and nutrient rich food source for people of all ages and activity levels. Milk is also a good source of calcium, phosphorous, vitamin D, riboflavin, and potassium (NIH, 2013). These nutrients play key roles in the development of strong bones and in restoring proper electrolyte balance and rehydration 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. 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 Dietary Guidelines for American's recommended 3 servings per day of low-fat dairy products (Heaney, 2013). According to a cross sectional diagnostic study performed by Srinivasan, et. al. (1998), the highest rates of lactose malabsorption were found in Asian populations, Native Americans and African Americans (60–100%) and lowest rates in people of northern European origin and the US white population (2–22%).

It is the absence of adequate hydrolysis of lactose in the small intestine (where the enzyme is normally active) that results in the passage of undigested lactose into the distal bowel where bacteria ferment the sugar, sometimes producing gas and symptoms such as

cramps, bloating, flatulence, and diarrhea (Tomba, et. al., 2012). When lactose malabsorption gives rise to symptoms, this is called 'lactose intolerance'. However, because a large portion of the lactose (≥30%) in milk is metabolized by lactic acid bacteria in the kefir culture, symptom free consumption of the kefir beverage by individuals with lactose sensitivities is possible, allowing milk to be a healthful addition to the diet.

In addition to the many beneficial nutrients intrinsic to the milk itself, healthful properties acquired by kefir during fermentation could be of importance to athletes, such as an increased bioavailability of the protein in milk (Magalhaes, et. al., 2011) and an enhanced micronutrient profile (Farnworth, 2003). In addition, several studies have shown the large amounts of medium and short chain fatty acids found in fermented milk are to aid in digestion and utilization of lipids (Kalser, 1971; Babayan, et. al., 1981; Fathi, et. al., 2015).

The capacity for probiotics to modulate perturbations in immune function after exercise highlight their potential for use in individuals exposed to high degrees of physical stress (West, et. al., 2009). The increased biomass of the microbes and the production of cellular proteins, peptides and free amino acids, that are subsequently released into the kefir beverage, further enhance the digestibility of milk and would make a kefir recovery beverage a good source of nutrients required for muscle synthesis and regeneration following activity.

1.12 Objectives

The objectives of this project were to:

1. Design an all natural, minimally processed post-exercise kefir based recovery beverage for athletes that meets 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, (3) contains live, active probiotic cultures at the time of consumption.
2. Determine the physiological responses to intensive exercise training and kefir supplementation by examining levels of established biological markers of inflammation and immune functioning
3. Provide sensory and product survey components implementing both affective and descriptive methodologies to (1) provide insight into the role of nutrition in exercise and current dietary practices in college students and student athletes, especially as they relate to dairy foods, (2) assess the current recovery protocols being implemented by the participants, (3) quantify participant consumption of fermented dairy products, (4) collect sensory data that can be used to further refine the formulation of the product with emphasis on acceptability post-exercise.

CHAPTER 2. MATERIALS AND METHODS

2.1 Experimental Design

A kefir beverage and a control beverage were administered in isocaloric amounts to 4 treatment groups: exercise training group supplemented with a non-fermented control beverage (n=11), exercise training group supplemented with a probiotic kefir beverage (n=14), active control supplemented with a non-fermented control beverage (n=21) and active control supplemented with a probiotic kefir beverage (n=21) (Table 6).

Table 6. Treatment group and sample size for subjects participating in a 15-week marathon training program and receiving a supplemental recovery beverage.

Subject Group	Treatment 1: Exercise	Treatment 2: Kefir	Sample Size
Exercise Training Control (ETC)	Exercise Training	Control Beverage	n=11
Exercise Training Kefir (ETK)	Exercise Training	Kefir Beverage	n=14
Active Control Control (ACC)	Active Control	Control Beverage	n=21
Active Control Kefir (ACK)	Active Control	Kefir Beverage	n=21

Throughout the intervention period, all subjects in all four groups completed weekly sickness, burnout, training/injury logs, diet records and sensory questionnaires relating to the beverages. Baseline measurements including height, weight, skinfold measurements and physical activity assessments were taken for all study participants. Subjects also were required to complete a 1.5-mile walk/run in the fastest possible time (Figure 8). Venus whole blood and plasma/serum samples (10 mL per sample) (20 mL total) were collected at the beginning (Pre) and at the end (Post) of the intervention period and were analyzed for levels of CRP, TNF-α and sIgA. This project was approved by the Louisiana State University Institutional Review Board (appendix A).

Pre-intervention tests	Post-intervention tests
Baseline resting measurements	Baseline resting measurements
Blood collection	Blood collection
Diet log	Diet log
1.5-mile walk/run	1.5-mile walk/run

Exercise Training + Kefir Beverage (ETK)
Exercise Training + Control Beverage (ETK)

I----------15 week intervention ----------I

Active Control + Kefir Beverage (ACK)
Active Control + Control Beverage (ACC)

Figure 8. Experimental design

2.2 Product Development

A kefir beverage and a control beverage were developed to meet the ACSM guidelines for recommended nutrition following endurance and resistance exercise. All products were manufactured, packaged and stored at the Louisiana State University Creamery (123 Dairy Science Building, Louisiana State University, Baton Rouge).

2.2.1 Fermented Milk Beverage

Pasteurized, non-homogenized, full fat cow's milk was fermented in either gallon sized lidded glass containers or 5 gallon lidded stainless steel milk pails by inoculation with approximately 30 grams 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 (Figure 9). 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 hours, or until a pH of 4.6 was reached. The kefir was then placed in refrigerated storage until formulation and packaging of the beverage.

The fruit base for the kefir beverage was processed separately and was subsequently blended with kefir several hours prior to consumption by the study participants. The fruit base included all ingredients, except the dairy portion; it was prepared using a VitaMix Commercial/Household Food Preparing Machine Model VM0100A. After combining the kefir and the fruit base, sixteen ounces of the product were portioned into clear plastic containers with snap on lids with a tamper evident seal. Each container was labeled with a number that corresponded to a coded system indicating the intended date of consumption and manufacture date. The containers were placed in refrigerated storage (6±5°C) until consumption. No other identifying information was on the container when presented to the participants.

2.2.2 Control Beverage

The non-fermented milk beverage was made using a VitaMix Commercial and Household Food Preparing Machine Model VM0100A. Lactaid®, an ultra-pasteurized homogenized lactose-free milk product, was used as the dairy portion of the control beverage.

The fruit base for the control beverage was processed separately and was blended with Lactaid® several hours prior to consumption by the study participants. The fruit base included all ingredients except the dairy portion; it was prepared using a VitaMix Commercial/Household Food Preparing Machine Model VM0100A. After combining the Lactaid® and its fruit base, sixteen ounces of the product were portioned into clear plastic containers with snap on lids with a tamper evident seal. The filled containers were placed in refrigerated storage (6±5°F) until consumption. Each container was labeled with a number that corresponded to a coded system indicating intended date of consumption and

manufacture date. No other identifying information was on the container when given to the subjects for consumption.

2.2.3 Quality Control Testing

To ensure the microbiological safety of the product 1 ml of each batch of the finished kefir was plated on *E. coli*/coliform Petrifilm™ (3M, St. Paul, WI); each batch of the fruit base was also tested. Finally, each batch of the final kefir beverage and the final control beverage was tested by plating 1 ml of the prepared products on *E. coli*/coliform Petrifilm™. The petrifilm was incubated aerobically at 32°C for approximately 24 hours. All beverages consumed by the participants had a coliform count of <1 cfu/ml. The percent titratable acidity (0.80±0.04 %) and pH (4.43±0.06) of each batch of kefir used in the kefir beverage was also assessed to ensure consistency of the finished product. Lactose levels in the kefir were determined using the Enzymatic/UV method (r-biopharm AG, Darmstadt, Germany). Percent lactose in the kefir was 3.49-3.56 % w/w, indicating a 30% reduction of lactose during fermentation. Quality control testing was conducted in the Dairy Science Laboratory (119 Dairy Science Building, Louisiana State University, Baton Rouge).

2.2.4 Nutritional Analysis

The nutrient profiles of the kefir beverage and the control beverage were identical and described in Table 7.

Table 7. Nutrient profile of recovery beverage given to endurance athletes participating in a 15-week marathon training program and an active control.

Serving size (ounces)	Calories (kcal)	Fat (g)	Sodium (mg)	Total Carbohydrate (g)	Fiber (g)	Sugar (g)	Protein (g)
16	220.16	5.4	71.68	66.56	2.8	30.72	10.24
12	165.12	4	53.76	50	2	23.04	7.7

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 (Table 8 and Table 9).

Table 8. Composition of a kefir beverage given to endurance athletes and an active control during a 15-week marathon training program. Weights, nutrients and probiotics are reported as mean values.

Bacteria (Lactobacillus, Lactococci, Streptococci) 10^{8}-10^{9} cfu/g

Yeasts 10^{6}-10^{7} cfu/g

	Weight	Calories	Fat	Sodium	Total Carbs	Fiber	Sugar	Protein
Kefir	672	448.5	25	338.5	34.5	0	34.5	26
Honey	72	219	0	3	225	1.8	60	0
Strawberries	128	46	0	1	10	3	6	1
Banana	354	315	1	3.5	56	9	43	3.5
Vanilla extract	13	37	0	1	1.5	0	1.5	0
Stevia	>1	0	0	0	0	0	0	0
Batch total	1239	1065.5	26	347	327	13.8	145	30.5
Per gram	1	0.86	0.021	0.28	0.26	0.011	0.12	0.04
Serving (12 oz)	192	165.12	4	53.76	50	2	23.04	7.7
Serving (16 oz)	256	220.16	5.4	71.68	66.56	2.8	30.72	10.24

Table 9. Composition of a control beverage given to endurance athletes and an active control during a 15-week marathon training program. Weights, nutrients and probiotics are reported as mean values.

	Weight	Calories	Fat	Sodium	Total Carbs	Fiber	Sugar	Protein
Lactaid®	672	448.5	25	338.5	34.5	0	34.5	26
Honey	72	219	0	3	225	1.8	60	0
Strawberries	128	46	0	1	10	3	6	1
Banana	354	315	1	3.5	56	9	43	3.5
Vanilla extract	13	37	0	1	1.5	0	1.5	0
Citric acid	>1	0	0	0	0	0	0	0
Batch total	1239	1065.5	26	347	327	13.8	145	30.5
Per gram	1	0.86	0.021	0.28	0.26	0.011	0.12	0.04
Serving (12 oz)	192	165.12	4	53.76	50	2	23.04	7.7
Serving (16 oz)	256	220.16	5.4	71.68	66.56	2.8	30.72	10.24

2.3 Subject Recruitment

The marathon training group was recruited from a Louisiana State University class, Kinesiology 4526: The Physiology of Endurance Training. The instructor of this course was Laura Stewart, Ph.D. In addition to traditional coursework, the students

enrolled in this class were training for a marathon and completing the below measurements as part of the course requirements. Students interested in joining the training group portion of this study were asked after the KIN 4526 class period if they would allow us to use data collected in the class for research purposes. Students were not penalized for not participating in the study. While there was 100 percent participation by the students in this course, any students who chose not to participate in this research study were offered an alternate assignment for course credit. The participants in this class were assigned randomly into two beverage treatment groups (ETC and ETK).

The subjects for the active control groups were recruited from a Louisiana State University sanctioned class, Kinesiology 3515: The Physiological Basis of Activity, also taught by Laura Stewart, Ph.D. The subjects in the active control group were randomly assigned to their beverage groups (ACC and ACK).

All subjects passed a required health screening and were between the ages of 18-35 years. General exclusion criteria for all subjects were as follows: the presence of disease conditions including diabetes, cardiovascular disease, cancer, human immunodeficiency virus, asthma, resting blood pressure >160/100 mm Hg, hospitalization within the last 6 months, plans to be away for more than 2 weeks in the next 9 months, significant weight loss in the past year (> 20 kg) or current use of weight loss medications, being pregnant or planning to become pregnant within the next 6 months.

2.4 Medical and Health History Screening

Subjects in the ETC and ETK first received permission to participate from a State licensed M.D. or D.O. (Appendix B). Medical clearance was not required for the ACC

and ACK because they were instructed to follow their normal workout routine without our supervision. All study participants completed a medical history form, Physical Activity Readiness Questionnaire (PAR-Q) and International Physical Activity Questionnaire (IPAQ) (Appendix B). The above screening and questionnaire were designed specifically with the participant's health in mind by: 1) preventing those with pre-existing conditions from participating in a running program/study and 2) allowing the researchers to become aware of any potential health issues. Subjects were also informed of the ingredients found in the product to eliminate any risk of health issues due to allergens and other food sensitivities. A signed waiver, indicating that food ingredients and information concerning possible allergens were disclosed, was required from all participants prior to inclusion in the study (Appendix B).

2.5 Pre-Intervention Data Collection: Baseline Resting Measurements

Baseline measurements (body composition, blood/saliva collection, questionnaires) were taken at the Nutrition and Human Assessment Laboratory, 252 Knapp Hall, Louisiana State University, Baton Rouge, LA. Subjects were instructed to refrain from exercising on the day of testing and to wear lightweight, comfortable clothing (including a sports bra and shorts underneath) that did not contain any metal. Upon arrival, the subjects were given the Participant Consent Form (Appendix C) and given time to review the document. Then, the experimental protocol was explained, and any questions posed by the subjects were answered.

2.5.1 Body Circumference/Body Composition

Skinfold measurements were taken in triplicate from three sites by a kinesiology graduate student, Laura Forney. For men the three sites measured were chest, abdomen

and thigh; for women the three sites measured were triceps, suprailiac and thigh. Measurements were taken by pinching the skin and then measuring with a spring-loaded Lange skinfold caliper (Cambridge Scientific Industries, Inc., Cambridge, MA) allowing the caliper to pinch the skin. Subjects were made aware of slight discomfort and possibly bruising on rare occasions experienced from the pinching and measuring of skinfolds. Hip and waist circumference was measured using a Gullick anthropometric tension tape (Patterson Medical, Cedarburg, WI). Subjects were then weighed barefoot on a digital platform scale and height was measured using a stadiometer.

2.5.2 Saliva Collection

Saliva was collected from all study participants. The subjects were asked to fast for at least four hours before the scheduled donation time. Briefly, subjects were instructed to passively drool by tilting their head forward, allowing saliva to pool on the floor of the mouth, and, without force, the pass the saliva into a sterile tube for 10 minutes or until four 1 ml polypropylene vials were filled. All samples were labeled with subject codes and elapsed time to completion was recorded for each participant. The filled sample vials were kept in wet ice during the collection period and then frozen in a laboratory chest freezer at -80°C until analysis.

2.5.3 Blood Collection

Venus whole blood (10 mL per sample) and plasma/serum samples (10 mL per sample) (20 mL total) were collected before (PRE) and following (POST) the intervention period from consenting study participants; the blood sample collection was not a mandatory measurement. Consenting participants were compensated $25 for each blood sample collection. Subjects arrived to the Nutrition and Human Assessment

Laboratory (252 Knapp Hall, Louisiana State University, Baton Rouge, LA) between 6:00 and 9:00 AM following an overnight fast and having refrained from exercise for the previous 72 hours. The subjects were seated quietly for 15 minutes before the blood samples were collected by a Registered Nurse, Nicole Petty. Aliquots designated for plasma were collected in a Vacutainor™ blood collection tube containing ethylenediaminetetraacetic acid (EDTA) (BD, Franklin Lakes, NJ), a strong anticoagulant, and the tubes were centrifuged (within 5 minutes following collection) for 15 minutes at 1000 x g and 4°C in order to separate the plasma from the cells. The plasma was transferred to microcentrifuge tubes, labeled with subject codes and stored in a laboratory chest freezer at -80°C until analysis. Aliquots to be used for analysis of serum components was collected in a Vacutainor™ blood collection tube containing a clot activator. The blood was allowed to clot by sitting at room temperature for approximately 60 minutes before it was centrifuged for 15 minutes at 1000 x g and 4°C.

2.5.4 Timed 1.5 Mile Run/Walk

The timed 1.5-mile walk/run served as the baseline exercise measurement for all subject groups. Subjects were instructed to cover 1.5 miles in the fastest possible time (walking was allowed, but the objective was to finish in the shortest amount of time). The elapsed time was called out and recorded (in minutes) as the subjects crossed the finish line.

2.6 Exercise Training

The marathon training program consisted of two supervised training sessions (running, swimming, strength training/drills) and one unsupervised training session each week during the 15-week training period (Appendix A). The unsupervised training

sessions were self-reported; however, the athletes were encouraged to participate in a student-led running group.

2.7 Kefir Supplementation

Kefir and control beverages were administered in isocaloric amounts to the ETK and ETC groups within 30 minutes after completion of a Tuesday and Thursday run at approximately 4:00 PM (Appendix A). Subjects in the ACK and ACC groups consumed the beverages during Tuesday/Thursday class periods scheduled from 1:40 to 3:00 PM. Beverage consumption was supervised, and all empty beverage bottles were returned to ensure the beverages were being consumed in full and also to identify any absent study participants (Figure 17). Participants who were absent during a scheduled class period or training session were given 24 hours to complete the training session (ETC and ETK) and to consume the beverage.

2.8 Sickness and Injury Questionnaires

Throughout the 15-week training period, subjects in both training and active control groups completed weekly reports that included a sickness questionnaire (instances, duration and severity of illness) and a training and injury recall log (type, severity, duration) (appendix E). The questionnaires were distributed to the participants at the beginning of each Tuesday class period and returned to the investigators the following Tuesday. Detailed instruction regarding proper completion of the questionnaires was provided at the beginning of the study.

2.9 Athlete Burnout Questionnaire

To assess the emotional response to training by athletes throughout the 15 week training program, the Athlete Burnout Questionnaire (ABQ) (Raedeke and Smith, 2001)

was used (Appendix E). The ABQ contains 15 items that assess three subscales of sport burnout: a reduced sense of accomplishment, emotional and physical exhaustion, and sport devaluation. Participants responded using an ordinal scale ranging from 1 to 5, with 1 denoting "almost never" and 5 denoting "almost always." The 1st and 14th items were reversed scored. The questionnaires were distributed to the participants at the beginning of each Tuesday class period and returned to the investigators the following Tuesday. Detailed instruction regarding proper completion of the ABQ was provided at the beginning of the study.

2.10 Sensory Surveys

Subjects were also asked to complete brief sensory questionnaires relating to the beverage and its sensory attributes (appendix F). Before beginning the intervention the study participants were given a survey containing questions relating to prior knowledge of sport nutrition and types of foods and beverages, if any, being consumed following exercise. The preliminary sport nutrition survey also contained questions regarding consumption of kefir and other fermented dairy products. The participants completed a product evaluation survey on the first day that they received their beverage as well as two additional times during weeks 4 and 10 of the intervention period. For this survey the participants were asked to evaluate the product and mark their response on a 9-point hedonic scale with 1=Dislike Extremely and 9=Like Extremely. Sensory attributes included on the product evaluation form included aspects of appearance, aroma, taste and overall liking. The third sensory evaluation was in the form of an exit survey; questions in this survey related directly to the participants willingness to buy this product,

perceived effects of this product on exercise recovery and overall experience of consuming the product.

2.11 Post-Intervention Data Collection

One week prior to the marathon event, participants in the training and the active control groups underwent post-training measures. Procedures for post-training measurements were identical to the protocol followed for the baseline resting and exercise measures as outlined in the Pre-intervention measurements (see Chapter 2.5).

2.12 Sample Analysis: Enzyme-Linked Immuno-Sorbent Assay (ELISA)

Wells of a microtiter plate were coated with polyclonal antibodies that were directed against the samples and standards. Samples and standards containing the target compound were added to the microtiter plate and the target compound in the samples was bound, in excess, to the coated polyclonal antibodies (the capture antibody) in the first incubation step; washing removed any unbound substances. Prior to a second incubation, an antibody specific to the target compound was added, binding to the target compound that was immobilized in the first incubation; another washing removed all unbound substances. The solid phase was then incubated with an enzyme and a substrate. The enzyme bonded to the target compound/antibody complex and acted upon the added substrate to produce a color of varying intensity that was, depending on the particular assay, either directly or inversely proportional to the amount of target compound present in the sample. Serum CRP was measured using an ELISA (Alpco Diagnostics, Salem, NH). TNF-α was measured using a TNF-α enzyme linked immune-sorbant assay (ELISA) (Alpco, Salem, NH). Salivary IgA was measured using a Salivary Secretory IgA Enzyme Immunoassay Kit (Salimetrics, State College, PA).

2.12.1 CRP ELISA

A wash buffer was made by diluting 50 ml of the wash buffer concentrate with 450 ml of deionized water, yielding a 1:10 solution. Crystals occurring in the wash buffer concentrate, due to high concentrations in stock solutions, were dissolved at 37°C in a warm water bath prior to dilution. The wash buffer remaining following completion of the assay was kept in refrigerated storage for use in subsequent CRP assays. The conjugate was diluted to a 1:100 solution by adding 100 microliters (µl) of the conjugate concentrate into 9900 µl of the previously diluted wash buffer. Plasma samples were diluted to 1:100 by adding 10 µl plasma to 990 µl of the sample buffer. A dilution factor of 100 was used to calculate the CRP concentration read off of the calibration curve generated for each assay.

Prior to use in the assay all reagents and samples were allowed to come to room temperature (18-26°C) and were mixed thoroughly. To begin, the microtiter strips were washed 5 times by dispensing 250 µl of diluted wash buffer into each well. After the final washing step the residual wash buffer was removed by tapping/blotting the plate on clean, absorbent paper towels. To duplicate wells, 100 µl of each sample standards and controls were added. The plate was covered tightly and incubated for 1 hour at room temperature on a horizontal mixer.

After the incubation period the contents of each well were discarded by quickly inverting the microtiter plate, and the wells were washed 5 times by dispensing 250 µl wash buffer into each well. The residual wash buffer was removed by tapping the plate on absorbent paper towels. Following washing 100 µl conjugate was added to each well. The plate was covered tightly and allowed to incubate for another hour at room

temperature on a horizontal plate mixer. After the incubation period the contents of each well were discarded, and the wells were washed 5 times by dispensing 250 µl bash buffer into each well. The residual wash buffer was removed by tapping the plate on absorbent paper towels. Addition of 100 µl of substrate into each well was followed by a 10-20 minute incubation at room temperature in the dark. Because the intensity of the color change was temperature sensitive, the color change was observed and the reaction was stopped once good differentiation is achieved. The reaction was stopped by addition of 50 µl of stop solution, which was mixed thoroughly by gently tapping the side of the plate.

The absorption was determined immediately following completion of the assay with a microplate reader set to a detection wavelength of 450 nanometers (nm). A 4-parameter-algorithm was used to calculate the results. A linear ordinate was used for optical density and a logarithmic abscissa was used for concentration with a zero calibrator specified for values less than 1 (e.g. 0.0001). The values obtained from the calibration curve were multiplied by 100 in order to calculate the CRP concentration in the plasma samples.

2.12.2 TNF-α ELISA

The provided Human TNF-α (Hu TNF-α) standard was reconstituted to 2000 picograms per milliliter (pg/mL) with standard diluent buffer. The solution was swirled gently to mix and allowed to sit for 10 minutes to ensure complete reconstitution. After 10 minutes 0.300 mL of the reconstituted standard to a tube containing 0.300 mL of the standard diluent buffer and was labeled 1000 pg/mL Hu TNF-α Mix. Then 0.300 mL of the standard diluent buffer was added to each of six 1.5 mL sample tubes labeled 500,

250, 125, 62.5, 31.2 and 15.6 pg/mL Hu TNF-α. These were the standards used to produce the standard curve.

The standards were made from the first dilution: 0.300 mL of the contents were transferred from the provided standard to a tube containing 0.300 mL of the standard diluent buffer and the respective serial dilutions were made. A tube containing 0.300 mL of the standard diluent buffer only was used as the 0 pg/mL standard for the purposes of constructing the standard curve. The streptavidin horse-radish peroxidase (streptavidin-HRP) was prepared by adding 120 µL of the concentrate with 12 mL of streptavidin-HRP diluent. The diluted solution was labeled streptavidin-HRP working solution. The wash buffer concentrate (25X) was allowed to reach room temperature and was mixed well to ensure that any precipitated salts were re-dissolved. One part of the wash buffer concentrate (25X) was mixed with 24 parts of deionized water. Working wash buffer was prepared by diluting by adding 50 mL of wash buffer concentrate with 1,200 mL deionized water.

All reagents were allowed to reach room temperature and the liquids gently mixed before use. First, 100 µL of the standard diluent was added to zero wells and 100 µL of the standards, samples and controls were added to the microtiter wells. Then, 50 mL of incubation buffer was added to the wells containing the standards and the serum samples. The plate was covered and incubated at room temperature for 2 hours. Following incubation, the solution was thoroughly decanted from the wells and discarded. The wells were then washed 4 times. The working wash buffer solution was put into a squirt bottle and the plate was flooded with wash buffer, completely filling all the wells. The plate was inverted and tapped dry on clean paper towels after each wash. Each well, except the

chromogen blank, received 100 µL of biotinylated anti-TNF-α (biotin conjugate) solution and the plate was gently tapped on its side to mix. The plate was covered and incubated at room temperature for 1 hour. Following incubation, the solution was again decanted and discarded and the plate was washed 4 times. Each well, except the chromogen blank, received 100 µL of streptavidin-HRP working solution. The plate was covered for incubation at room temperature for 30 minutes. Following incubation, the plate was decanted and washed 4 times, and 100 µL of the stabilized chromogen was added. Upon the addition of the stabilized chromogen the liquid in the wells began to turn blue. The plate was then incubated in the dark for 30 minutes. After the final incubation 100 µL of the stop solution was added to each well and the plate was tapped gently to mix. The stop solution caused the solution in the wells to change from blue to yellow.

The absorbance of each well was read at 450 nm. The plate reader was calibrated against a chromogen blank composed of 100 µL each of stabilized chromogen and stop solution. The plates were read immediately after adding the stop solution.

2.12.3 SIgA ELISA

All reagents were allowed to come to room temperature (approximately 25°C) and were mixed thoroughly before use by inverting each bottle several times. The microtiter plate was also allowed to come to room temperature at this time. Precipitated matter in the concentrated wash buffer was re-dissolved by heating the concentrate bottle to 40°C for approximately 10 minutes; the concentrate was allowed to return to room temperature before use in the assay. The wash buffer was prepared by adding 100 ml of the 10X concentrate into 900 ml of deionized water to create a 1:10 dilution.

SIgA diluent was prepared by diluting the 50 ml of the sIgA diluent concentrate into 200 ml of deionized water to make a 1:5 dilution. The lyophilized stop solution was reconstituted with 10 ml of deionized water and was allowed to sit undisturbed for 10 minutes before mixing well.

To prepare the sIgA standards 6 microcentrifuge tubes were labeled 1 to 6; 15 µl of the provided 600 µg/ml standard (tube 1) was added to tube 2 and 30 µl of the sIgA diluent was added into tubes 2 through 6. The standards were serially diluted as follows: 15 µl of the 600 µg/ml standard was added to tube 2 and the tube was briefly vortexed, 15 µl of the solution from tube 2 was pipetted into tube 3. This dilution was continued for tubes 4, 5 and 6 yielding final concentrations of 600 µg/ml, 200 µg/ml, 66.7 µg/ml, 22.1 µg/ml, 7.4 µg/ml, and 2.5 µg/ml.

The samples were vortexed followed by centrifuging for 10 minutes at 3000 revolutions per minute (rpm) for 15 minutes to remove any mucins that could interfere with antibody binding in the sample wells. From the clear portion 25 µl of each sample was pipetted from the centrifuged samples into small microcentrifuge tubes labeled with the identity of each saliva sample and containing 100 µl of the prepared sIgA diluent to create a 1:5 dilution. Each tube was lightly vortexed to mix. Finally, 10 µl of the standards, controls, and 1:5 diluted unknowns were added to separate 12 x 75 mm capped tubes containing 4 ml of the sIgA diluent, and 10 µl of the sIgA diluent was added to a zero tube. All tubes were vortexed to mix.

An initial incubation with the conjugate was performed in the tubes containing the standards, controls and unknown samples. They were inverted several times to mix and were incubated at room temperature for 90 minutes. Before transfer to the assay plate the

tubes were inverted again, and 50 µl from each standard, control and sample tube was added to the respective well on the microtiter plate. An amount of 50 µl of the sIgA diluent was added to each well. The plate was covered and incubated at room temperature for 90 minutes with continuous shaking at 400 rpm. Following incubation the plate was washed and decanted 6 times by adding 300 µl of the wash buffer into each well. The plate was blotted on clean paper towels. To each well 50 µl of the provided 3,3',5,5'-tetramethylbenzidine (TMB) solution was added with a multichannel pipette. The plate was then mixed on a horizontal plate mixer for 5 minutes at 500 rpm and incubated for an additional 40 minutes at room temperature in the dark. With a multichannel pipette, 50 µl of stop solution was added to each well. The plate was then mixed on the horizontal mixer for approximately 3 minutes at 500 rpm, until all the wells turned from green to yellow.

Immediately following completion of the assay the plate was placed in a microplate reader at 450 nm. The average optical densities (OD) for each set of duplicate sample wells, the percent of SIgA bound (B/Bo) for each standard, control and sample was determined by dividing the average OD (B) by the average OD for the zero (Bo). The concentrations of the controls and unknowns were determined by interpolation by logistics using a 4-parameter non-linear regression curve fit (Gen5 Data Analysis Software, Winooski, VT). The concentrations of unknown saliva samples were multiplied by 5 to obtain the final SIgA concentrations in µg/ml. Samples that had values greater than 600 µg/ml were tested again, in subsequent assays, by preparing the samples with a higher dilution ratio and re-running for more accurate results.

2.13 Statistical Analysis

All statistical analysis was carried out in JMP Pro 11 (SAS Software, Cary, NC). A 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, TNF-α and sIgA samples were tested in duplicate. Concentrations of CRP were log transformed to adjust for normality for statistical analysis. Mean burnout scores and standard deviations were computed for each burnout subscale to determine the student-athletes' burnout levels. A series of one-way between-subject ANOVAs and independent t-tests were computed to identify and further examine any significant differences in burnout levels. Data from sensory testing was also analyzed using JMP Pro 11 (SAS Software, Cary, NC). For the sensory testing, ANOVA was done to analyze the questions with the 9-point hedonic scale (Peryam and Pilgrim, 1957). A frequency count was used to analyze the subject responses to Yes/No questions presented in the preliminary and exit surveys.

CHAPTER 3. RESULTS AND DISCUSSION

3.1 Endurance Training

The effect of exercise training and kefir supplementation on 1.5-mile times is shown in Figure 9. There was a significant time x training group interaction (p = 0.0124) (Figure 21) with the ET groups (ETK and ETC) experiencing an average of 4.11% improvement in 1.5-mile time (Table 10).

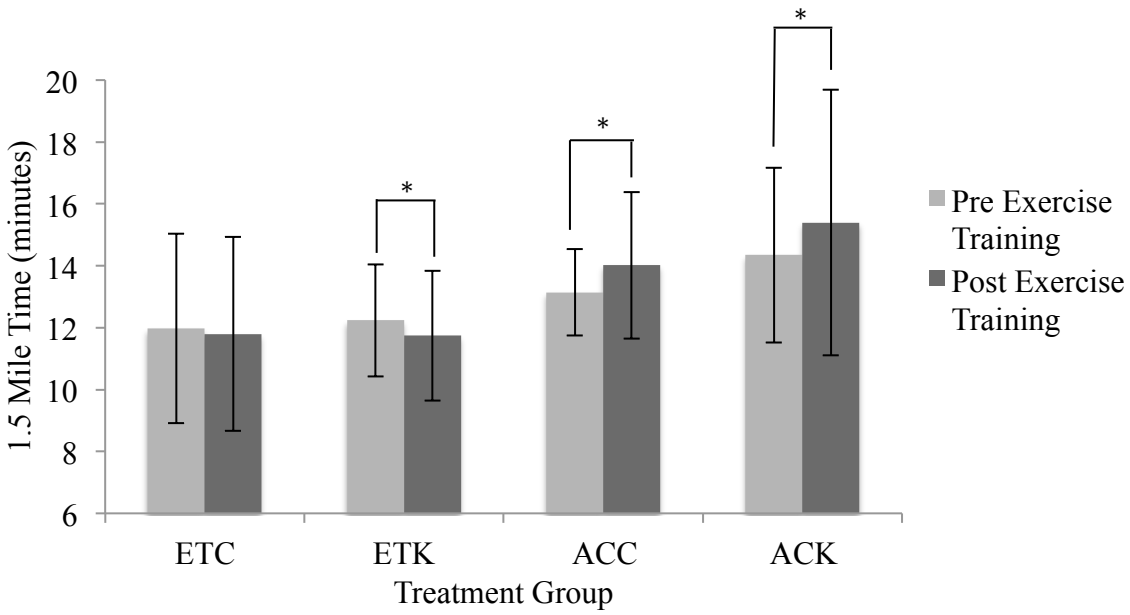

Figure 9. 1.5-mile time for subject groups before and after the 15-week intervention period. *Means for each bar differ significantly (P < 0.05). 1.5-mile times are reported as mean ± SD. Pre exercise training and post exercise training values were collected at the beginning and the conclusion of a 15-week marathon training program. ETC=Exercise Training + Control Beverage; ETK=Exercise Training + Kefir Beverage; ACC=Active Control + Control Beverage; ACK= Active Control + Kefir Beverage.

There was a significant ($p<0.05$) improvement in the 1.5-mile times with the ETK group having a Pre time of 12.24 minutes and Post time of 11.74 minutes, resulting in a 30 second improvement for the group. The ETC group had an average 1.5-mile time of 11.98 minutes at the beginning of the 15-week protocol (Pre) and 11.79 at the end of the intervention period (Post). While the ET groups had no significant change (ETC) or

significantly ($p>0.05$) improved 1.5-mile times (ETK), both the ACC and the ACK groups had significantly ($p<0.05$) slower times Pre-intervention as compared to Post-intervention. The ACC group had an average Pre time of 13.14 minutes and a post time of 14.02 minutes, which was a significant ($p<0.05$) increase in 1.5-mile time by 46 seconds. The ACK group had an average Pre time of 14.35 minutes and an average post time of 15.39 minutes resulting in a significant ($p<0.05$) increase in 1.5-mile time by 62 seconds.

Table 10. Average percent change in 1.5-mile walk/run times for endurance training athletes and an active control consuming a kefir recovery beverage and a control beverage.

Treatment Group	Percent change in 1.5-mile time
ETC	-1.6909[a]
ETK	-4.3174[a*]
ACC	9.1068[b]
ACK	6.9827[b]

[ab]Group means not connected by a common letter are significantly different ($p < 0.05$).
[*]Significant improvement ($p<0.05$) in mean1.5-mile time. ETC=Exercise Training + Control Beverage; ETK=Exercise Training + Kefir Beverage; ACC=Active Control + Control Beverage; ACK= Active Control + Kefir Beverage.

The improvement in mean 1.5-mile time in the ETK group demonstrated the effectiveness of the training program in achieving the desired results. The endurance capacity i.e. aerobic capacity is most the often and most accurately measured by the maximum oxygen uptake (VO_2max) during a graded exercise (treadmill) test. However, testing the VO_2max of individual subjects can be both costly and time consuming. The 1.5-mile walk/run test has been shown as a valid measure to estimate VO_2 max and is commonly used in a college setting when it is necessary to test a large number of people at one time (Larsen, et. al., 2002).

While accepted as a suitable VO_{2max} predictor in lieu of a graded exercise test (Jeukendrup, et. al. 2010), there are several factors that may limit the accuracy of the 1.5-mile walk/run test such as the undetermined accuracy and validity of the 1.5-mile protocol between male and female populations (Kline, et. al., 1987). Also, although participants were instructed to "complete the course in as fast a time as possible" untrained individuals may not have been able to maintain a proper pace resulting in a 1.5-mile time that does not represent the actual endurance capacity.

3.2 Body Density and Percent Body Fat

The skinfold measurement equations estimated body density, which was then used to estimate percent fat. The two generalized equations used to calculate body density were from Jackson and Pollock (1978) for men and Jackson, Pollock, and Ward (1980) for women (Table 11).

Table 11. Equations used for the calculation of body density and percent body fat based on skinfold data collected during resting baseline measurements.

	Body Density	Skinfold Measurements
Females	$1.0994921 - 0.0009929*\Sigma + 0.0000023*\Sigma^2 - 0.0001392*age$	triceps, suprailiac, thigh
Males	$1.1093800 - 0.0008267*\Sigma + 0.0000016*\Sigma^2 - 0.0002574*age$	chest, abdominal, thigh
	Percent Body Fat✣	Σ = sum of skinfold measurements
	[(4.570 / Body Density) - 4.142] * 100	✣ percent body fat equation for males and females

Body density was calculated by the Body density of the participants before and after the 15-week training period is reported in Figure 10. No significant differences ($p>0.05$) in body density were found between or within groups.

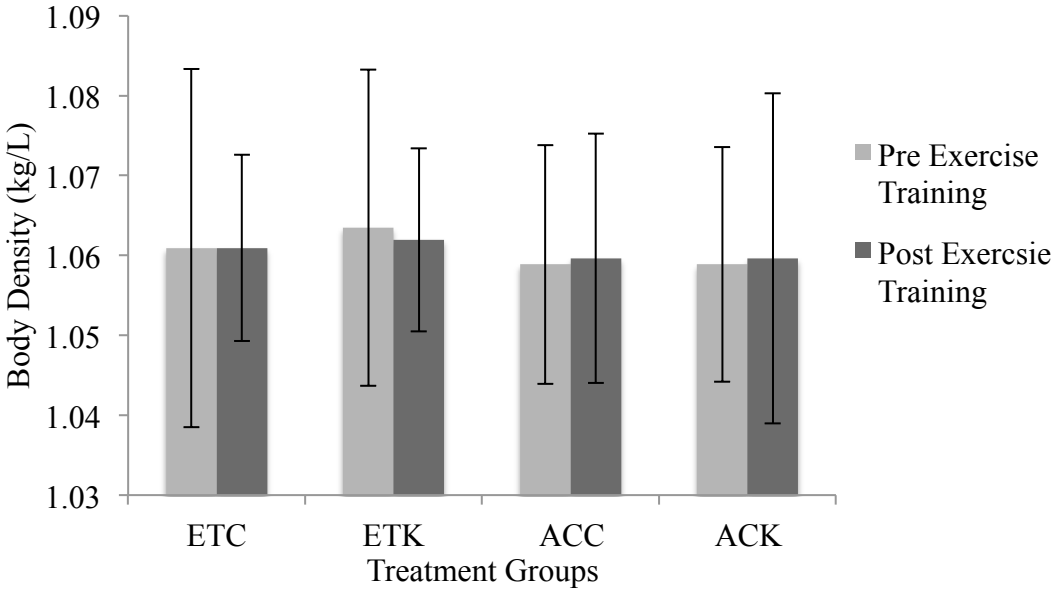

Figure 10. Body density of study participants before and after the 15-week intervention period. There were no significant ($p>0.05$) differences in density body found between treatment groups or pre-intervention and post-intervention. Body density is reported as mean ± SD. Pre exercise training and post exercise training values were collected at the beginning and the conclusion of a 15-week marathon training program. ETC=Exercise Training + Control Beverage; ETK=Exercise Training + Kefir Beverage; ACC=Active Control + Control Beverage; ACK= Active Control + Kefir Beverage.

There were two calculation steps for determining percent fat: determining body density and then using body density to estimate percent fat. The equation used for estimating percent fat from body density was the Brozek (2006) formula (see Table 11). Percent body fat of the participants before and after the 15-week training period is reported in Figure 11. No significant differences ($p>0.05$) were found between or within groups in regards to variations in body fat percentage. These results, indicating that the participants in this project had no significant fluctuations in weight during the course of the 15-week protocol, suggest that the kefir beverage and the control beverage did not differ in their effect on the body composition of participants over the course of the intervention. The no change in mean body composition of participants in the ETC and ETK suggest that they were maintaining a diet sufficient in calories to maintain a

consistent body composition during training, with the supplementation with a dairy based recovery beverage possibly contributing to this consistency.

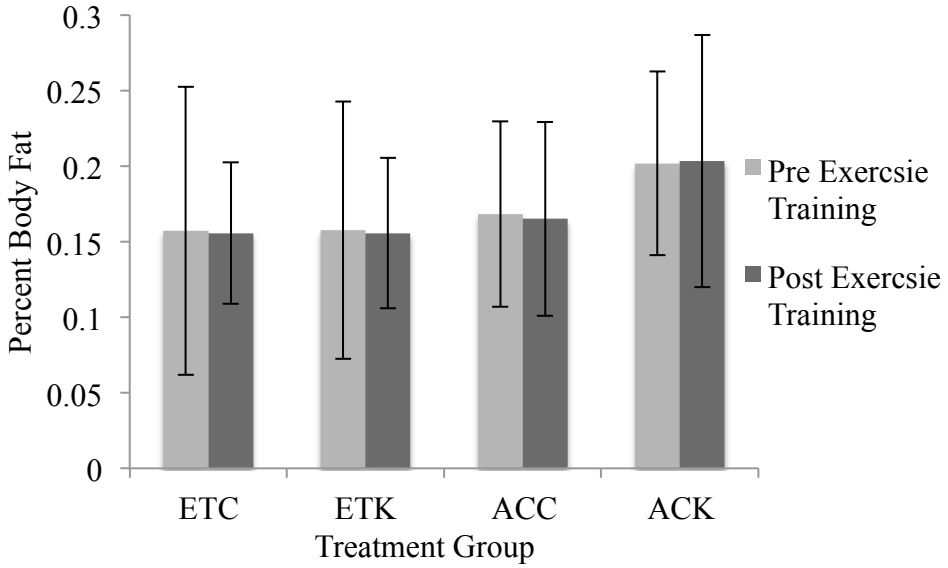

Figure 11. Percent body fat of study participants before and after the 15-week intervention period. There were no significant ($p>0.05$) differences in percent body fat found between treatment groups or pre-intervention and post-intervention. Body fat percent is reported as mean ± SD. Pre exercise training and post exercise training values were collected at the beginning and the conclusion of a 15-week marathon training program. ETC=Exercise Training + Control Beverage; ETK=Exercise Training + Kefir Beverage; ACC=Active Control + Control Beverage; ACK= Active Control + Kefir Beverage.

The equations used to determine body density have withstood the test of numerous cross-validation studies, with standard errors ranging from 3.6% to 3.8%, and are routinely used in body density assessment by sport and health professionals (Jeukendrup, et. al., 2010).

However, a limitation to this formula is that it assumes the density of fat-free mass to remain constant across the population when it in fact varies. The actual percent body fat tends to be somewhat higher than the measured percent fat in the lean, muscular individual and the opposite effect in obese individuals (Brožek, et. al., 2006). Table 12 lists current accepted ranges for body fat percentages and their descriptive categories.

Table 12. Descriptive categories for ranges of body fat percentage ranges in women and men issued by The American Council of Exercise.

Description	Women	Men
Essential fat	10-13%	2-5%
Athletes	14-20%	6-13%
Fitness	21-24%	14-17%
Average	25-31%	18-24%
Obese	>32%	>25%

(adapted from: American Council on Exercise, 2009)

There were 12 participants out of 67, with the majority in the ETC and ETK groups, with very small percentages of body fat (>3-5%); this may, in part, be due to the fact that most of the participants in the ETC and ETK groups, as well as some participants in the ACC and ACK groups, were trained endurance runners or involved in athletics requiring low body fat i.e. gymnastics, cheerleading, cycling.

3.3 C-Reactive Protein

There were no significant interactions ($p>0.05$) with respect to all other outcome variables of biological inflammatory and immune markers with the exception of serum CRP. The ETC group had a significant increase ($p<0.05$) in CRP levels following training as seen in Figure 12. There was no significant increase ($p>0.05$) in the level of CRP in the ETK, ACC or ACK groups. A possible trend was observed for a kefir CRP interaction with the ETK and ACK groups experiencing percent increases in CRP following training of 21.18% and 5.45% as compared to the ETC and ACC (64.71%, 22.36%).

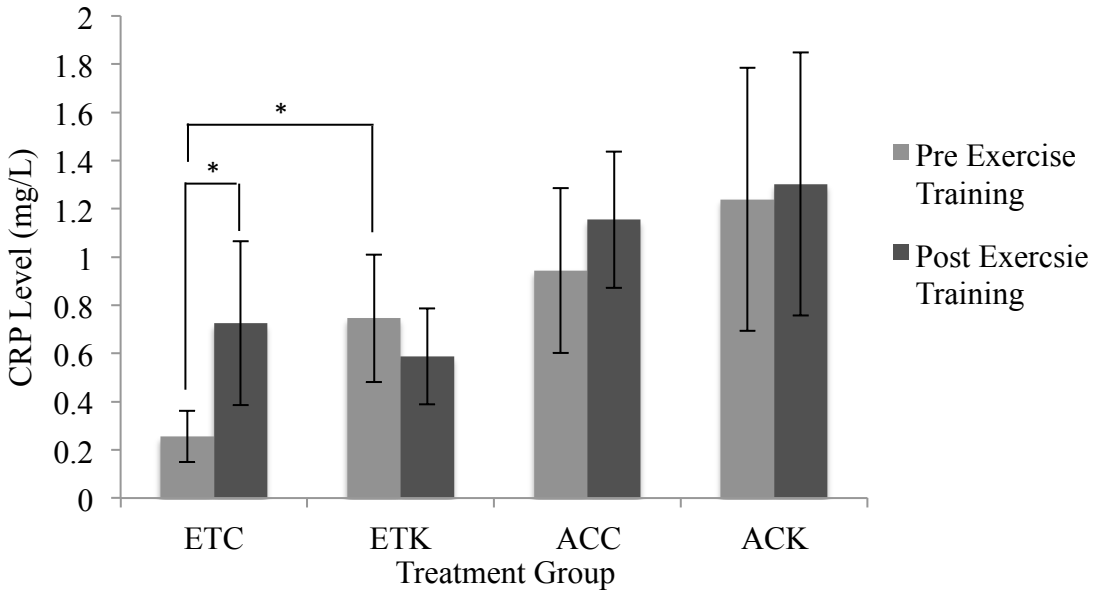

Figure 12. Effect of endurance training and kefir supplementation on serum C-reactive protein (mg/L). *Means for each bar differ significantly ($P < 0.05$). Data corresponds to the average ± SD of the results of subjects from 4 separate groups. Pre exercise training and post exercise training values were collected at the beginning and the conclusion of a 15-week marathon training program. ETC=Exercise Training + Control Beverage; ETK=Exercise Training + Kefir Beverage; ACC=Active Control + Control Beverage; ACK= Active Control + Kefir Beverage.

The training program resulted in significantly increased ($p<0.05$) CRP levels in the ETC. This increase could possibly be attributed to increased inflammation in the body due to the strenuous training regime. The CRP level in the ETK group following training was not significantly different than the initial CRP level. The CRP level in the ETK group following training may indicate that supplementation with the kefir beverage could have a beneficial effect in regards to reducing the amount of exercise induced inflammation as measured by circulating CRP. The effect of exercise on CRP was examined by Stewart, et. al. in a 2007 study using active and previously inactive training groups. Their results showed that both groups had a significant decline in serum CRP from pre- to post-training, with the most marked decrease in serum CRP (58%) occurring in the previously inactive group. The participants in the ETC and ETK groups had been

exercising prior to this study in preparation for the endurance training which may have influenced a trend towards lower initial resting CRP levels.

Individuals with a level of serum CRP of <l mg/L a considered to be at low risk for cardiovascular disease (CVD), individuals with values from 1–3 mg/L at moderate risk and greater than 3 mg/L are at high risk for CVD (see Table. 4). Based on these risk factors and the CRP means Pre and Post, we see a definite correlation between trained and untrained individuals in respect to overall risk for CVD. Average serum CRP concentrations in ACC both Pre and Post intervention (1.24 mg/L and 1.30 mg/L) were in the moderate-risk category. Average value for the ACC group Pre intervention (0.944 mg/L) was on the very high end of the low risk category, and the Post intervention average CRP level (1.15 mg/L) rose to the moderate risk category. The ETC and ETK groups both had average CRP levels (ETC: 0.255 mg/L and 0.725 mg/L; ETK: 0.746 mg/L and 0.588 mg/L) that started at and remained in the low risk for CVD category Pre and Post intervention.

These possible effects of kefir on attenuating CRP in s individuals participating in strenuous exercise programs may indicate a suppression of pro-inflammatory pathways caused by increased stress on the body. Supplementation with kefir could also be stimulating an anti-inflammatory response and anti-oxidative pathways in participants following training which might also explain the lack of CRP elevation post-intervention in the ETK group.

3.4 Tumor Necrosis Factor-α

There were no significant ($p<0.05$) interactions among groups with respect to levels of TNF-α. The levels of TNF-α showed no significant ($p<0.05$) change between

samples collected Pre-intervention and Post-intervention within the treatment groups (Figure 13).

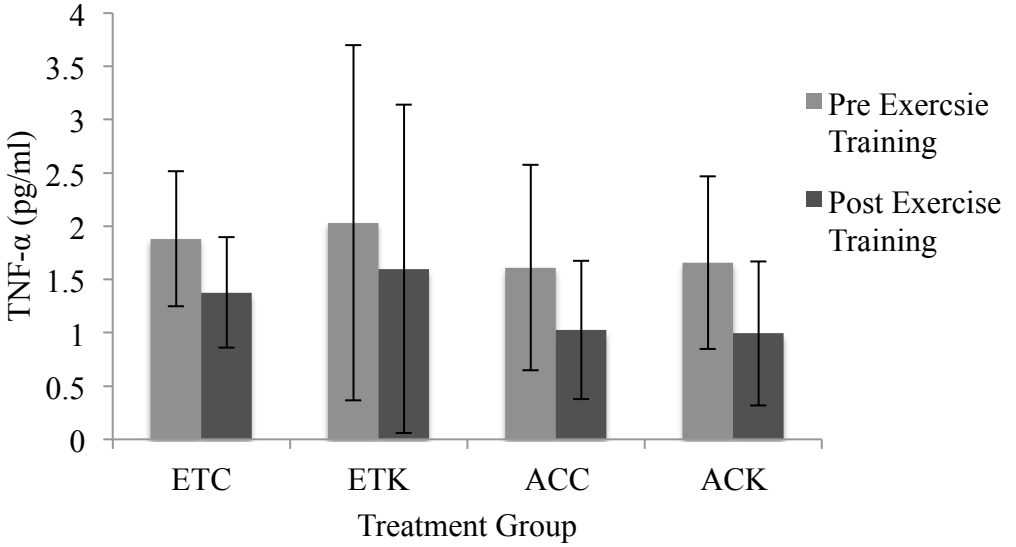

Figure 13. Effect of endurance training and kefir supplementation on TNF-α (pg/ml). There were no significant ($p>0.05$) differences in TNF-α found between treatment groups or pre-intervention and post-intervention. Blood samples were collected before and after completion of the training/intervention period. Data correspond to the average ± SD of the results of subjects from 4 separate groups. Pre exercise training and post exercise training values were collected at the beginning and the conclusion of a 15-week marathon training program. ETC=Exercise Training + Control Beverage; ETK=Exercise Training + Kefir Beverage; ACC=Active Control + Control Beverage; ACK= Active Control + Kefir Beverage.

Previous studies examining the levels of soluble circulating TNF-a in plasma have yielded varying results. An average TNF-α of 5.7 for 24 women ages 19 to 39 was found in one study (Bullo, et. al., 2002) while another study including 307 women ages 19-49 was reported a TNF-a 10.30 with values ranging from 5.45-19.86 (Himmerick, et. al., 2006). The normal value for college age active women and men is most likely reflected an average TNF-α level, as the levels of circulating TNF-α typically trend upward during aging (Bruunsgaard, et. al., 2002).

While some cytokines, such as interleukin-6, are strongly associated with CRP and other inflammatory biomarkers, the association between TNF-α and CRP is rather

weak (Tsukui, et. al., 2000). This suggests that circulating levels of maybe influenced by a number of different factors and that circulating TNF-α levels may not reflect biologic activity at the tissue level. Several studies have demonstrated significant increases in circulating cytokines, including TNF-α, when samples were collected immediately following strenuous activity (King, et. al., 2000; Bullo, et. al., 2002; Himmerich, et, al., 2003). The serum samples in this study were collected from rested participants having abstained from activity for 24 hour prior to collection, which may have been a factor in the failure of this study to demonstrate significant training effects of TNF-α. Because the groups had no significant differences ($p>0.05$) in the level of TNF-α pre-intervention and post-intervention or between groups, no conclusions could be made regarding the effects of probiotics on attenuating the elevation expected following a high intensity training session. However, it has been shown that deleterious effects of TNF-alpha and IFN-gamma on epithelial function can be prevented by probiotic treatment and the effect of bacteria on intestinal epithelial function may justify their use in inflammatory disorders (Resta-Lenert and Barrett, 2006).

3.5 Frequency of Sickness and Injury

The total reported sicknesses are reported in Figure 14. ETK (79 sicknesses) and ETC (64 sicknesses) had a much higher number of reported sicknesses than ACC (20 sicknesses) and ACK (19 sicknesses) even though the sample sizes for ETC (n=10) and ETK (n=13) were much lower than ACC (n=21) and ACK (n=21). The frequency of sickness was found to be significantly higher ($p<0.05$) in ETK and ETC as compared to ACK and ACC (Figure 14). There were no significant differences found in frequency of sickness between ETK and ETC or between ACK and ACC.

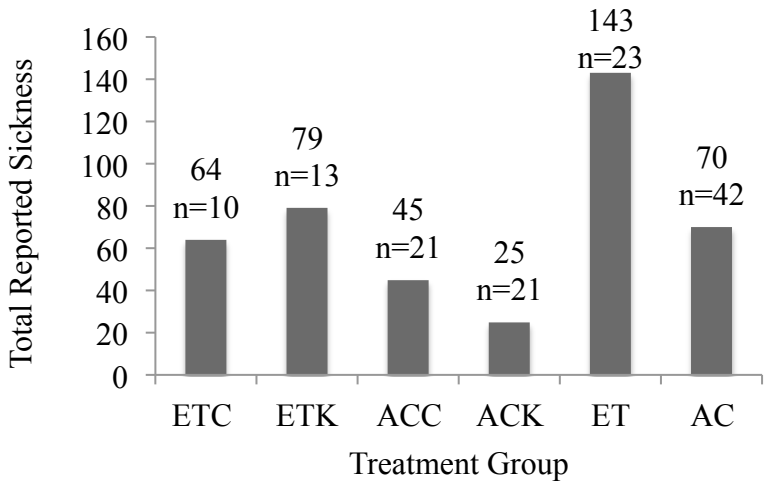

Figure 14. Total reported sickness during the 15-week intervention period. ETC=Exercise Training + Control Beverage; ETK=Exercise Training + Kefir Beverage; ACC=Active Control + Control Beverage; ACK= Active Control + Kefir Beverage; ET=ETC+ETK; AC=ACC+ACK

The average reported sicknesses (Table 13) of the ETC (6.4±4.08) and ETK (5.64±3.93) groups were significantly higher than the ACC (2.34±2.79) and ACK (1.31±1.16) groups due to well documented perturbations in immune functioning following intensive training.

Table 13. Average incidences of sickness for endurance training athletes and an active control consuming a kefir recovery beverage and a control beverage during a 15-week marathon training program.

Treatment group	Mean	SD
ETC	6.40[a]	4.08
ETK	5.64[a]	3.93
ACC	2.34[b]	2.79
ACK	1.31[b]	1.167

[ab]Pairs not connected by a common letter are significantly different ($p < 0.05$). ETC=Exercise Training + Control Beverage; ETK=Exercise Training + Kefir Beverage; ACC=Active Control + Control Beverage; ACK= Active Control + Kefir Beverage.

Many of the exercise-induced changes in immunity are similar to changes associated with other forms of stress, such as sleep deprivation, aging and psychological stress. Pederson, et. al. (2000) concluded that because the underlying mechanisms involved in exercise and stress induced inflammation are multifactorial and include both

neuroendocrinological and metabolic factors, nutritional supplementation may protect against the increased risk of infection in the recovery period after strenuous exercise. If probiotics can improve immunity in athletes, then they may also be suitable for improving immunity in other individuals exposed to regular stress, such as the military and shift workers (Vinderola, 2004). Results from this study indicate that while the instances of illness do indeed increase with an intensive training load, an a trend indicating possible attenuation of the severity or frequency of sickness in athletes could be, in part, due to the benefits incurred by kefir consumption.

The total reported injuries are reported in Figure 15. ETK (70 injuries) and ETC (98 injuries) had a much higher number of injuries than ACC (20 injuries) and ACK (19 injuries) even though the sample sizes for ETC (n=10) and ETK (n=13) were much lower than ACC (n=21) and ACK (n=21).

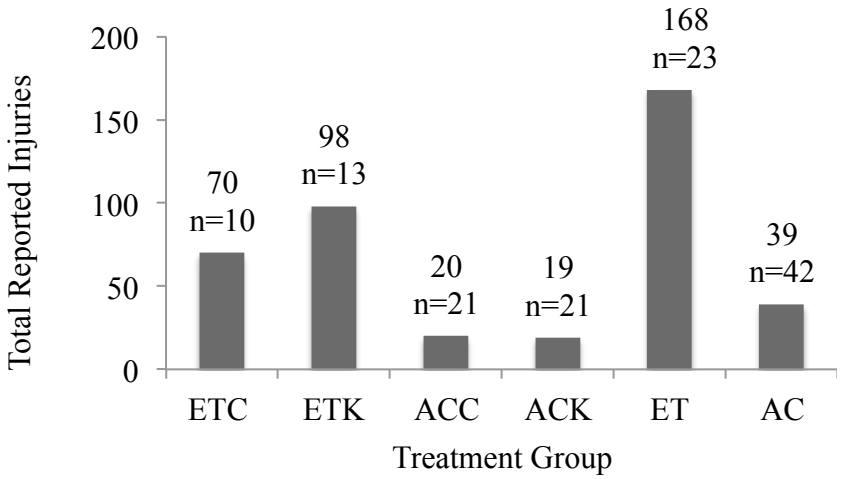

Figure 15. Total reported injury during the 15-week intervention period. ETC=Exercise Training + Control Beverage; ETK=Exercise Training + Kefir Beverage; ACC=Active Control + Control Beverage; ACK= Active Control + Kefir Beverage; ET=ETC+ETK; AC=ACC+ACK

The average number of reported injuries for ETC and ETK were significantly ($p>0.05$) higher than average number of injuries reported by ACC and ACK. There were no significant differences found in frequencies of sickness and injury pre-intervention as compared to post-intervention between ETK and ETC or between ACK and ACC.

Table 14. Average number of injuries for endurance training athletes and an active control consuming a kefir recovery beverage and a control beverage during a 15-week marathon training program.

Treatment group	Mean	SD
ETC	7.357[a]	2.309
ETK	6.500[a]	3.616
ACC	1.455[b]	1.657
ACC	0.9523[b]	1.700

[a]Pairs not connected by a common letter are significantly different ($p < 0.05$). ETC=Exercise Training + Control Beverage; ETK=Exercise Training + Kefir Beverage; ACC=Active Control + Control Beverage; ACK= Active Control + Kefir Beverage.

The significantly higher ($p<0.05$) incidences of injury in the ETC and ETK groups as compared to the ACC and ACK groups (Table 14) was expected due to the increases likelihood of sport related injury accompanying long distance running programs.

3.6 Athlete Burnout

The Athlete Burnout Questionnaire (ABQ) (Raedeke and Smith, 2001) consists of 15 sport specific items with each question being "How often do you feel this way?" Athletes were asked to rate the extent to which they experienced each item in relation to their participation motives on a 5-point Likert scale with 1 = almost never to 5 = almost always (Appendix E). The ABQ had three 5-item subscales assessing 3 key dimensions of burnout: Reduced sense of accomplishment (e.g., "It seems that no matter what I do, I don't perform as well as I should"); Emotional and physical exhaustion (e.g., "I feel so tired from my training that I have trouble finding energy to do other things");

Devaluation of sport participation (e.g., "The effort I spend participating in my sport would be better spent doing other things").

There were no significant ($p>0.05$) interactions found among groups with respect to athlete burnout and attitudes about exercise and sport (Table 15).

Table 15. Three measures of athlete burnout in athletes and an active control consuming a kefir recovery beverage and a control beverage during a 15-week endurance training program.

	Emotional and Physical Exhaustion	Sense of Accomplishment	Sport Devaluation
ETC	1.821	1.911	1.642
ETK	2.076	2.150	1.631
ACC	1.801	1.915	1.647
ACK	1.904	1.912	2.043

Three 5-item subscales in the Athlete Burnout Questionnaire assessing 3 key dimensions of burnout were used. There were no significant ($p>0.05$) differences in the athlete burnout measures found between treatment groups. Values are reported as mean value of participation motives on a 5-point Likert scale with 1 = almost never to 5 = almost always. ETC=Exercise Training + Control Beverage; ETK=Exercise Training + Kefir Beverage; ACC=Active Control + Control Beverage; ACK= Active Control + Kefir Beverage.

The three measures of athlete burnout (Emotional and Physical Exhaustion, Sense of Accomplishment, Sport Devaluation) showed no significant ($p>0.05$) differences between the four treatment groups or between the training participants (ETC and ETK combined) and the exercise controls (ACC and ACK combined).

"Overtraining" is defined by Buggett (1998) as "a non-deliberate long-term decrement in performance capacity resulting from a failure to recover adequately from an accumulation of training and non-training stress". It is common for elite athletes, especially young and inexperienced competitors, to "push themselves too far, overreach, constantly experience extreme tiredness on a regular basis and never realize their full potential or achievement capability (Gould and Dieffenbach, 2002). In athletes, burnout can be a result of excessive training and insufficient recovery following training. Kellman (2002) describes this type of overreaching as a deliberate part of the training and

recovery process needed to achieve desired performances, with performance incompetence being a temporary effect (Lehmann, et. al., 1999). This short-term lowered performance capacity state can be restored with the help of a brief recovery period (Meeusen, et. al., 2006). In contrast to functional overreaching, non-functional overreaching e.g. overtraining often results in athlete burnout due to a combination of excessive overload and inadequate recovery and can lead to severe psychological and physiological symptoms. Because no significant differences were observed in ABQ scores between ETC and ETK and no burnout was observed, the beverages provided to the athletes may have aided in recovery and preventing burnout due to overtraining.

3.7 Consumer Survey and Sensory Analysis

In all groups, the vast majority of participants believed that food would aid in recovery following exercise, with only 4 participants in the AC groups (ACC and ACK) responding "not sure". When asked how long following exercise food is typically consumed, responses were varied, with most reporting eating within an hour following exercise, and, when asked what they ate at that time, the two most common responses were "smoothie" and "shake", as reported by 23 of the total 67 participants. Other common responses were "gatorade/powerade" (4 responses), "granola bar" (3 responses) and "dinner" (3 responses). Four participants in the ET group (n=25) and 5 participants in the AC group (n=42) reported consumption of dairy products (milk, yogurt, whey protein) following exercise.

Based on responses to questions regarding preexisting post-exercise recovery programs with a food component, information regarding current exercise practice and implementation of a recovery program was assessed. The majority of participants in all

groups reported that they were currently exercising at moderate intensity at least 2 times per week. Five participants in the ET groups (n=25) and 7 out of the AC groups (n=42) were following an exercise recovery program. For the participants using food to aid in exercise recovery, common responses were "smoothie" and "protein shake" (23 responses).

Prior to participation in this study, only 6 participants had heard of kefir (N=67), and 2 individuals had previously consumed kefir. Finally, the participants were asked about current yogurt and other fermented milk consumption with the response choices being: Never, Once a week, Two times a week, and More than two times per week (Table 16).

Table 16. Reported consumption of yogurt and other fermented dairy products by college students (age 18-30 yrs) participating in a marathon-training program and an active control.

Treatment group	Never	Once a week	Two times per week	> Two times per week
ECT	5	2	2	5
ETK	5	1	2	7
ACC	5	9	3	4
ACK	4	12	3	4
Total (N=67)	19	24	10	20

ETC=Exercise Training + Control Beverage; ETK=Exercise Training + Kefir Beverage; ACC=Active Control + Control Beverage; ACK= Active Control + Kefir Beverage.

The goals of the participant survey component of this research were to provide insight into the perceptions of the role of nutrition in exercise and current dietary practices in college students and student athletes especially as they relate to dairy foods. From these results, it is clear that the majority of the college students participating in this study have limited knowledge regarding recovery from exercise and sport nutrition. Although there were a few students in the ETK and ETC using dairy products (milk or yogurt) as part of a recovery program involving food, the introduction of a dairy food

following exercise was a new recovery implement for a large majority of participants. The reported rates of fermented dairy consumption indicated that 30% of the participants were consuming more than 2 servings per week. The majority (51%) of the participants reported yogurt consumption once or twice a week, and the remainder 29% reported no consumption of fermented dairy products.

Results of the product evaluation survey are reported in Figure 17. No significant differences ($p<0.05$) were found between the kefir beverage and the control beverage for any of the sensory attributes. This indicates that the control beverage was successfully matched to the kefir beverage in regards to flavor, texture, appearance and overall acceptability, which was necessary to maintain the subject blinded experimental design.

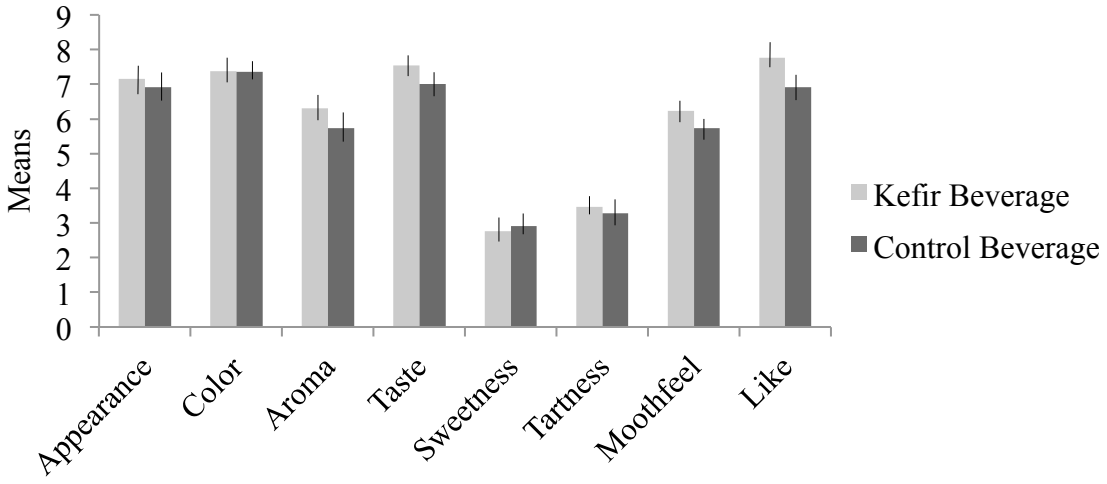

Figure 16. Sensory analysis of kefir recovery beverage and control.

The overall acceptance and perceived benefits of the recovery beverages provided was assessed in an exit survey (Table 17). Results indicate a very positive response to both beverages overall indicating that majority of participants would buy this product before and after knowing about possible probiotic benefits from consumption.

Table 17. Product acceptability and consumer intent of purchase for a kefir based post-exercise recovery beverage and a control beverage by athletes participating in a 15-week marathon training program and an active control.

	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

3.8 Limitations

Due to problems encountered with the Secretory IgA Enzyme-Linked-Immuno-Assay test kit (Salimetrics, State College, PA), many of the saliva samples needed to be reanalyzed. However, there was not enough saliva supernatant to complete the sIgA assay; therefore, no results are available for average sIgA levels for the treatment groups. The donation of blood samples from the study participants was not compulsory and several students from each group did not participate in this portion of the research; in addition, three students who gave blood at the beginning of the experimental period did not participate in the post intervention period, and although all groups maintained a size of n=8, this lowered sample size could have influenced the results of the statistical analysis, especially because of the high degree of deviation between the participants within each group. Because a relatively small sample size was collected for testing the number of assays performed per participant sample was limited. Several samples that needed to be re-run due to higher than expected CVs of duplicate samples were unavailable for subsequent assays; the number of samples in the ETC and ETK groups dropped to 6 and 7, respectively. The sample size limitations prevented valid statistical analysis of these groups for sIgA. ELISA methods are useful because they provide high

specificity and good standardizability between and within assays. Unfortunately, cytokine assay results may vary due to the methods used for cytokines detection and their sensitivities, interferences due to different drugs used, and the effect of concomitant pathologies (Szegedi, et. al. 2003).

Compliance with and beverage consumption was not a major issue. However, although instructed not to discuss beverage, several participants did compare sensory aspects such as levels of sweetness and color and made attempts to determine which beverage (kefir or control) they were consuming. One subject was removed from the study due to 3 missed beverage treatments. Adherence to the training protocol also proved challenging for several participants in the ETK and ETC groups.

For this project, the inability to control for variables such as consumption of other fermented foods or probiotics and other dietary components may have had influenced the action of the probiotics. Excessive caffeine and alcohol use has also been shown to have deleterious effects on training, as they can prevent proper rehydration due to increased diuresis (Burke, et. al., 1997). However, the effect of probiotics on alcohol consumption and gut homeostasis has demonstrated some interesting results. In a 2009 study by Forsyth, et. al., rats were gavaged with alcohol twice daily for 10 weeks; the alcoholic rats were also treated with once daily gavage of either 2.5×10^7 live *L. rhamnosus* Gorbach–Goldin (LGG) or vehicle (V). They found that reduced alcohol-induced gut leakiness and significantly blunted alcohol-induced oxidative stress and inflammation in both intestine and the liver. Another similar study found that Short-term oral supplementation with *B. bifidum* and *L. plantarum 8PA3* was associated with restoration of the altered bowel flora and the frequency of endotoxemia associated with

chronic alcohol consumption and demonstrated an improvement in alcohol-induced liver injury (Kirpich, et. al., 2008).

A wash out period A group of participants instructed not to consume probiotics containing foods for a certain period of time leading up to the intervention i.e. wash out period, or a group of subjects not regularly consuming probiotics or diary products would have better elucidated the probiotic effects of kefir in combination with a training intervention. In addition, a water control for baseline measurements and would especially help to determine if the consumption this product versus no recovery food would have a significant effect; however, a third treatment group (water only) was not possible due to low subject numbers.

This research protocol did not control for antibiotic use by the participants during illness. Although antibiotic use was not reported by the participants, there was most likely prevalent antibiotic use, especially in the ETC and ETK groups as they had many more instances of sickness as compared to the control. The dosage, type and time receiving antibiotics needed to be assessed to determine the existence of any interactive effects of antibiotic with exercise training and kefir supplementation.

CHAPTER 4. CONCLUSIONS AND FUTURE WORK

This study demonstrates the potential for a kefir recovery beverage as an appealing source of nutrients and probiotics for athletes as an exercise recovery beverage and for non-athletes as a healthful food product. The significant reduction of milk sugars by lactic acid bacteria and yeasts during fermentation and the breakdown of proteins into bioactive peptides and free amino acids most likely increased digestibility, as the beverage was well tolerated by all of the athletes following intense exercise. The milk based sports recovery drinks in the present market are processed to increase shelf life and to remove lactose; however, the development of an all natural, minimally processed beverage formulated with naturally occurring probiotics will provide athletes of all ages and skill levels with a healthy alternative to other currently available products.

The effects of kefir on CRP levels in athletes and how this translates to the mediation of inflammation and immunity via changes in gut health by using keifr following intense activity may aid in understanding the role of probiotics and other fermented foods as they relate to human health. Results of this study suggest that supplementation with kefir during intensive exercise training could possibly attenuate symptoms or reduce instances of some of the negative side-effects of exercise by helping to regulate the damage caused by inflammation and oxidative stress.

An important next step in understanding the mechanisms involved in the immunomodulating and anti-inflammatory effects of consuming kefir following activity will be further characterization of gut microbiota of various exercising populations, including individuals with diseases such as cancer and diabetes. Using genomic sequencing to determine if any significant variations exist in the gut microflora between

subjects consuming kefir post-exercise would further elucidate the effects and possible uses of kefir in training, recovery and maintenance of optimal health. Another necessity would be exploration into the relationship between other bioactive components in milk, particularly those found in fermented milks, such as bacteriocins, organic acids and exopolysaccharides, and human health.

Improvement and innovation of processing techniques for fermented products, with an emphasis on new packaging strategies for dairy products containing live, active cultures will be crucial for the success of this product on a larger scale. The design of a packaging system for fluid dairy products containing heterofermentative bacteria and yeasts allowing a controlled release of excess gas while maintaining a level of carbonation in the product will be required for a recovery beverage of this type to be successful in the current food market. In, summary, this study provided useful information regarding acceptability of a kefir based post-exercise recovery beverage by athletes and demonstrated an attenuation of exercise induced inflammation following kefir consumption. Continued exploration of the therapeutic properties of kefir as it relates to achieving and maintaining optimal health during athletic training will be necessary to adequately assess the potential benefits of including kefir in a post-exercise recovery plan.

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APPENDIX A – IRB APPROVAL FORM

Institutional Review Board
Dr. Robert Mathews, Chair
131 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.6792
irb@lsu.edu | lsu.edu/irb

ACTION ON PROTOCOL APPROVAL REQUEST

TO: Charles Boeneke
Animal Science

FROM: Robert C. Mathews
Chair, Institutional Review Board

DATE: January 3, 2013
RE: **IRB#** 3335

TITLE: The Effects of Post-Exercise Consumption of a Kefir Beverage on Performance and Recovery During Intensive Endurance Training

New Protocol/Modification/Continuation: New Protocol

Review type: Full __X__ Expedited ___ **Review date:** 12/14/2012

Risk Factor: Minimal _____ Uncertain __X__ Greater Than Minimal_____

Approved* ____X____ **Disapproved** _____

Approval Date: 12/14/2012 **Approval Expiration Date:** 12/13/2013

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: 80

Protocol Matches Scope of Work in Grant proposal: (if applicable) N.A.

***Approval Note: Your study is not to begin until your Certificate of Confidentiality is approved and on file with the LSU-BR Institutional Review Board**

By: Robert C. Mathews, Chairman _____

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. SPECIAL NOTE:

**All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.fas.lsu.edu/osp/irb*

APPENDIX B – TRAINING DOCUMENTS
1.1 The Physiology of Endurance Training: Course Flyer

KIN 4526: The Physiology of Endurance Training

Goals:

→ to understand core concepts of endurance physiology

→ to provide the unique opportunity to apply these principles by performing standard physiological tests on your very own endurance training model-YOU!

T, TH
3:20 – 4:30

Target Race: The Kentucky Derby Marathon in Louisville, Kentucky on April 27, 2013

3 Academic Credit Hours

If interested, contact Laura Stewart, PhD at stewart6@lsu.edu

APPEXDIX B – TRAINING DOCUMENTS
1.2 Marathon Training Plan

Marathon Training Plan (Distances in Miles)									
Week	Week Beginning	Mon	Tues	Wed	Thur	Fri	Sat	Sun	Total (miles/wk)
1	14-Jan	Rest	3	3	3	Rest	6	cross	15
2	21-Jan	Rest	3	4	3	Rest	7	cross	17
3	28-Jan	Rest	3	4	3	Rest	8	cross	18
4	4-Feb	Rest	3	4	3	Rest	7	cross	17
5	11-Feb	Rest	3	5	3	Rest	10	cross	21
6	18-Feb	Rest	3	6	3	Rest	12	cross	24
7	25-Feb	Rest	3	5	4	Rest	11	cross	23
8	4-Mar	Rest	4	7	4	Rest	14	cross	29
9	11-Mar	Rest	4	8	5	Rest	16	cross	33
10	18-Mar	Rest	4	9	5	Rest	18	cross	36
11	25-Mar	Rest	5	8	5	Rest	15	cross	33
12	1-Apr	Rest	5	8	5	Rest	20	cross	38
13	8-Apr	Rest	5	10	5	Rest	12	cross	32
14	15-Apr	Rest	5	6	5	Rest	8	cross	24
15	22-Apr	Rest	3	4	2	Rest	RACE		

Cross = Cross Training 30 min -1 hr of Walking, Biking or Swimming

APPENDIX C – CONSENT FORMS
2.1 Research Consent Form – Product Information

REASEARCH CONSENT FORM

I, _____, agree to participate in the research entitled "The Effects of Post-Exercise Consumption of a Fermented Milk Beverage on Performance and Recovery During Intensive Endurance Training" which is being conducted by the School of Animal Sciences at Louisiana State University, phone number (225) 578-3241.

I understand that participation is entirely voluntary and I can withdraw my consent at anytime with the results of the participation returned to me, removed from the experimental records, or destroyed.

The following points have been explained to me:

1. In any case, it is my responsibility to report prior to participation to the investigators any allergies I may have.

2. The reason for the research is to gather consumer information of a dairy product made from cultured milk, whole fruits, natural sweeteners and other natural ingredients and flavorings.

3. Participation entails minimal risks: The only risk that can be envisioned is an allergic reaction to the frozen product manufactured from cow's milk, strawberry, banana, honey, vanilla extract, citric acid and stevia. However, because it is known to me beforehand what type of food is to be tested, the situation can normally be avoided.

4. The results of this participation will be confidential and will not be released in any individual identifiable form without my prior consent unless required by law.

5. The investigator will answer any further questions about that research, either now or during the course of the project.

The study has been discussed with me and all of my questions have been answered. I understand that additional questions regarding this study should be directed to the investigators. In addition, I understand that research at Louisiana State University, which involves human participation, is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Robert C. Matthews, Institutional Review Board at (225) 578-8692. I agree with the terms above and acknowledge I have been given a copy of the consent form.

_____ _____

Signature of Investigator Signature of Participant

Date:_____ Witness:_____

APPENDIX C – CONSENT FORMS
2.2 Participant Consent Form – Explanation of Research Protocols

Participant Consent Form

1. Study Title: The Effects of Post Exercise Consumption of a Fermented Milk Beverage on Performance and Recovery During Intensive Endurance Training

2. Performance Site: Louisiana State University Baton Rouge, LA 70803

3. Investigators: The following investigators are available for questions about this study, M-F, 8:00 a.m.- 4:30 p.m. Principle Investigator: Charles Boeneke, Ph.D. 225-578-4383 Co-investigators: Keely O'Brien. M.S. 225-578-4382 Laura K. Stewart, Ph.D. 225-578-3549

4. Purpose of the Study: The purpose of this research project is to examine the effects of post workout supplementation with a milk beverage on subjects participating in a 15-week intensive marathon training program.

5. Subject Inclusion: Participants in the control and training groups must be at least 18 years of age and currently enrolled students or are employees at LSU. Subjects in the training group must have medical clearance following a complete comprehensive physical from a state licensed M.D or D.O. The cleared student must provide documentation of permission by returning a signed "Physician Approval Form" after the physical and before participating in the study. Students in the active control group must complete a physical activity readiness questionnaire (PAR-Q) before participating. You must be healthy and between the ages of 18-35 years. General exclusion criteria for all subjects are as follows: the presence of disease conditions including diabetes, cardiovascular disease, cancer, HIV, asthma, resting blood pressure > 160/100mmHg, hospitalization within the last 6 months, plans to be away for more than 2 weeks in the next 9 months, significant weight loss in the past year (> 20 kg) or current use of weight loss medications, being pregnant or planning to become pregnant within the next 6 months. Information regarding product ingredients and possible allergens will be provided to you; subjects with allergies or sensitivities to product ingredients will be excluded.

6. Number of subjects=

Milk beverage 1 + marathon training group: n=20 Milk beverage 2 + marathon training group: n=20

Milk beverage 1 + active control: n=20 Milk beverage 2 + active control: n=20

Total: N=80 7. Study Procedures:

Product Development and Manufacture: Products will be manufactured in the LSU Creamery, a licensed dairy processing facility.

Baseline: Before the 13 week training program begins, you will have baseline measurements taken. This will include basic baseline measurements such as body weight, height, waist hip ratio, resting metabolic rate and blood sample collection. An assessment of your physical fitness will be estimated by having you perform a 1.5 mile run/walk test. This test will require you to complete 1.5 miles at a maximal effort. A physical fitness questionnaire will be administered and a dietary log will be kept for one week prior to the start of the program. An ingredient list will be provided to you prior to consumption of the product to make you aware of any possible allergens and a product liability waiver will be collected.

13 Week Intervention Program: At the start of the program, you will be provided with a suggested training program (training group only). If you choose not to follow this training program, you will not be penalized. Twice weekly you will be given a 12-ounce portion of a smoothie type beverage. Throughout the 15-week training period, both groups will fill out a training log. This should take about 20 minutes weekly to complete. Participants in the active control group will be instructed to continue their own personal training programs and participants in both groups will continue to complete weekly training and diet logs, and sickness inventory questionnaires.

Post-Training: One week prior to the marathon event, you will undergo post-training measures. Procedures for post-training measurements will be identical to the protocol followed for preliminary measures.

8. Benefits:

While no guarantee of benefits can be made, you will be given training and nutrition instruction and a body composition analysis at no cost to you. You may also learn more about your physical capacity for endurance and should significantly improve your aerobic fitness. Subjects will be provided a comparison of his/her pre-training and post-training results at the end of the semester. An optional paid blood collection will be given with $25 being paid following each of a total of two collections.

9. Risks/Discomforts:

Product Consumption: As with any food product, there is a chance that you may have certain allergies or food sensitivities to ingredients included in the product. All ingredients will be disclosed to you prior to consumption of the product to alert you of any potential health concerns regarding food allergens. You will be required to sign a waiver acknowledging that you have been informed of this risk prior to the consumption of the product.

Exercise Training & Testing: As with any exercise program, there is a chance that you will experience muscle soreness, fatigue, or even injuries such as sprains or strains. There is also a remote risk of a heart attack or stroke and in very rare cases, death. Precautions

to minimize this risk have been taken by requiring medical clearance to participate as well as completion of a health history questionnaire. Your cooperation in obtaining approval from your physician as well as providing honest answers in completing the health history form will decrease this risk.

Plasma and Whole Venous Blood Collection: There is risk of bruising and soreness at the site of blood collection, and the remote risk of fainting.

In addition to the risks listed above, you may experience a previously unknown risk or side effect.

10. Injury/Illness: In the unlikely event of injury or medical illness resulting from the above procedures, contact **Laura Stewart, Ph.D., 225-578-3549.** You will be referred for treatment, but the expense of medical treatment will be your responsibility. No compensation is available in case of study-related illness or injury.

11. Right to Refuse: You may choose not to participate or to withdraw from the study at any time without penalty; however there will be a loss of any benefit to which you might otherwise be entitled, including monetary compensation for blood sample collection.

12. Privacy: Your identity will remain confidential unless disclosure is required by law. In other words, data will be kept confidential unless release is legally compelled. All data collected will be handled only by the investigators and kept in a secure location. Results of the study may be published using group means only and names or identifying information will not be included in the publication.

13. Financial Information: There is no cost to you, and compensation will be given if you

participate in the blood collection portion of the study. $25 will be paid following each sample collection.

14. Signatures: The study has been discussed with me and all my questions have been answered. I may direct additional questions regarding study specifics to the investigators. If I have any questions about subjects' rights or other concerns, I can contact Robert C. Matthews, Institutional Review Board at (225) 578-8692. I agree to participate in the study described above and acknowledge the investigator's obligation to provide me with a signed copy of this consent form.

_____ Participant's Signature Date

_____ Witness Signature Date

_____ Investigator Signature Date

APPENDIX C – CONSENT FORMS
2.3 Physician Approval Form for Participation in Marathon Training

Physician Approval Form
Kinesiology 4526
The Physiology of Endurance Training

This physical must be completed by a Louisiana State licensed M.D. or D.O. only

I have completed a full comprehensive physical examination of _____ *(print student's full name)* as required for him/her to participate in Kinesiology 4526 (The Physiology of Endurance Training). I understand that the student will be engaging in an intensive running and conditioning program which will involve optional participation in a course-designated Marathon during the Spring 2012 semester. Based on my physical findings, I am making the following recommendation related to the demands of the course:

____ Patient is cleared to participate **without restriction**.
____ Patient is cleared to participate with the following restrictions or guidelines.
_____
_____ Patient is **not** cleared to participate.

Physician Signature: _____
Physician Name (Print):_____ _
Date: _____
Address: _____
Phone: _____
Fax: _____
Email: _____

If there are questions or concerns, please contact:

Laura K. Stewart, Ph.D.
Department of Kinesiology
112 Long Fieldhouse
Louisiana State University
Baton Rouge, LA 70803
Phone: 225.578.3549
Fax: 225.578.3680
stewart6@lsu.edu

Please return this completed form to Laura Stewart, Ph.D.

APPENDIX D - PHYSICAL ACITIVTY ASSESSMENT FORMS
3.1 Physical Activity Readiness Questionnaire (PAR-Q)

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT _____ WITNESS _____
or GUARDIAN (for participants under the age of majority)

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Supported by: Health Canada Santé Canada

continued on other side...

APPENDIX D – PHYSICAL ACTIVITY ASSESSMENT FORMS
3.2 International Physical Activity Questionnaire (IPAQ)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days. Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days. Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

APPENDIX E – RECURRING SURVEYS
4.1 Baseline Measurement Worksheet

BASELINE MEASUREMENTS

ID_____ Date_____

Age_____ Sex_____ Height_____ Weight_____

Date of last menstrual period _____

Waist _____ Hip _____ W/H Ratio _____

Skinfold measurements

Male:

Chest _____ _____ _____

Abdominal _____ _____ _____

Thigh _____ _____ _____

Female:

Triceps _____ _____ _____

Suprailiac _____ _____ _____

Thigh _____ _____ _____

Blood collection _____

APPENDIX E – RECURRING SURVEYS
4.2 Weekly Self-Report Injury Questionnaire

ID _____ Date_____

Weekly Self-Report Injury Questionnaire

Use this log to record any injury which you may have experienced during the past week of training. Record the severity score (1-4) and the impact score (A-D) by each symptom in the column corresponding to the date.

INJURY	DATE	DATE	DATE	DATE	DATE	DATE	DATE	DATE
Shin Splints								
Patellofemoral syndrome								
Pulled hamstring								
IT Band syndrome								
Ankle Sprain								
Plantar Fasciitis								
Achilles Tendonitis								
Arch Pain								
Knee Pain								
Hip Pain								
Groin Pain								
Blisters								
Other:								
Did you visit the doctor? Yes/No								
Were there changes in your daily routine? Yes/No								
Was your training affected by the injury? Yes/No								

APPENDIX E – RECURRING SURVEYS
4.3 Weekly Sickness Self-Assessment Questionnaire

Weekly Respiratory Symptom Self-Assessment Questionnaire

1. Use your daily respiratory symptom self-assessment questionnaire to summarize the following symptoms which you may or may have not have experienced within the last week. Record the severity score (1-4) and the impact (A-D) by each symptom in the column corresponding to the date. *For example: insert a "2, B" in column "11/10" row "cough" if on the 10th of November you experienced a mild cough that reduced the time or distance of your activities.

Symptom	DATE	DATE	DATE	DATE	DATE	DATE	DATE	DATE
Cough								
Sneezing								
Sore Throat								
Nasal Discharge								
Stuffy Nose								
Headache								
Tiredness								
Chilliness								
Shaking Chills								
Fever								
Hoarseness								
Aching muscle or joints								
Watery or burning eyes.								
Did you visit the doctor? Yes/No								
Were there changes in your daily routine? Yes/No								
Was your training effected by illness? Yes/No								

2. Please report the number of hours spent training and the average intensity in each of the following activities during the past week.
   a. = low intensity, easy workouts
   b. = moderate intensity, moderate workouts
   c. = high intensity, intense workout

Mode of Activity	Weekly Training Time	Average Intensity of Training
Swimming		
Cycling		
Running		
Other Physical Activities		

APPENDIX E – RECURRING SURVEYS
4.4 Athlete Burnout Questionnaire (ABQ)

Date: _____ ID:_____

Please read each statement carefully and rate *how often* you experienced each item *since the last week*. Substitute your specific sport (e.g., marathon training/running) when reading the generic term [*sport*].

	HOW OFTEN: Almost never	Rarely	Some times	Frequently	Almost always
Statements:					
1. I'm accomplishing many worthwhile things in [*sport*].	1	2	3	4	5
2. I feel so tired from my training that I have trouble finding energy to do other things.	1	2	3	4	5
3. The effort I spend in [*sport*] would be better spent doing other things.	1	2	3	4	5
4. I feel overly tired from my [*sport*] participation.	1	2	3	4	5
5. I am not achieving much in [*sport*].	1	2	3	4	5
6. I don't care as much about my [*sport*] performance as I used to.	1	2	3	4	5
7. I am not performing up to my ability in [*sport*].	1	2	3	4	5
8. I feel "wiped out" from [*sport*].	1	2	3	4	5
9. I am not into [*sport*] like I used to be.	1	2	3	4	5
10. I feel physically worn out from [*sport*].	1	2	3	4	5
11. I feel less concerned about being successful in [*sport*] than I used to.	1	2	3	4	5
12. I am exhausted by the mental and physical demands of [*sport*].	1	2	3	4	5
13. It seems that no matter what I do, I don't perform as well as I should.	1	2	3	4	5
14. I feel successful at [*sport*].	1	2	3	4	5
15. I have negative feelings toward [*sport*].	1	2	3	4	5

Subscales:

- Reduced sense of accomplishment: 1(REV), 5, 7, 13, 14(REV)
- Emotional/Physical exhaustion: 2, 4, 8, 10, 12,
- Devaluation: 3, 6, 9, 11, 15

APPENDIX F – SENSORY EVALUATION FORMS
5.1 Preliminary Sport Nutrition Survey

ID_____ Date_____
Sample_____

1. Will consuming food following exercise aid in recovery? Yes ( ) No ( ) Not sure ( )

2. How long after exercise do you eat or drink anything other than water?

0-30 minutes	30-60 minutes	60-90 minutes	>90 minutes
( )	( )	( )	( )

3. What do you consume at that time? _____

4. Do you currently follow a post exercise recovery program including food? Yes ( ) No ( )

5. If so, briefly describe_____

6. Have you heard of or consumed kefir? Yes ( ) No ( )

7. If so, briefly define_____

8. How often do you consume yogurt or other fermented dairy products (such as sour cream, buttermilk, kefir, etc.)?

Never	Once a week	Two times a week	More than two times per week
( )	( )	( )	( )
1	2	3	4

APPENDIX F – SENSORY EVALUATION FORMS
5.2 Product Evaluation Survey

Sample_____ Date_____

PLEASE EVALUATE THE PRODUCT AND CHECK THE SPACE THAT BEST REFLECTS YOUR FEELING ABOUT THE PRODUCT

1. How would you rate the overall **APPEARANCE** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )
1	2	3	4	5	6	7	8	9

2. How would you rate the **COLOR** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )
1	2	3	4	5	6	7	8	9

3. How would you rate the **AROMA** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )
1	2	3	4	5	6	7	8	9

4. How would you rate the **TASTE** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )
1	2	3	4	5	6	7	8	9

5. How would you rate the **SWEETNESS** of this product?

Too weak		Just about right		Too strong
( )	( )	( )	( )	( )
1	2	3	4	5

7. How would you rate the **TARTNESS/SOURNESS** of this product?

Too weak		Just about right		Too strong
( )	( )	( )	( )	( )
1	2	3	4	5

8. How would you rate the **MOUTHFEEL/CONSISTANCY** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )
1	2	3	4	5	6	7	8	9

9. OVERALL, how do you "**LIKE**" this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )
1	2	3	4	5	6	7	8	9

APPENDIX F – SENSORY EVALUATION FORMS
5.3 Exit Survey

Sample_____ Date_____

1. Overall, is this product acceptable? Yes ( ) No ( )

2. Would you **BUY** this product if it were commercially available? Yes ( ) No ( )

3. Would you **BUY** this product if you knew it contained probiotics and prebiotics with health benefits?

   Yes ( ) No ( ) Maybe ( )

4. Do you feel that this product aided your ability to recover following an intense bout of exercise?

   Yes ( ) No ( ) Maybe ( )

5. Briefly describe your overall experience with consuming this product during the training period

_____
_____
_____
_____

APPENDIX G – RAW DATA
6.1 JMP Output

Fit Group

Oneway Analysis of Sickness By GROUP

Oneway Anova
Summary of Fit

Rsquare	0.360867
Adj Rsquare	0.330432
Root Mean Square Error	2.918949
Mean of Response	3.179104
Observations (or Sum Wgts)	67

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
GROUP	3	303.07412	101.025	11.8570	**<.0001***
Error	63	536.77662	8.520		
C. Total	66	839.85075			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
ACC	21	2.14286	0.63697	0.870	3.4157
ACK	22	1.13636	0.62232	-0.107	2.3800
ETC	10	6.40000	0.92305	4.555	8.2446
ETK	14	5.64286	0.78012	4.084	7.2018

Std Error uses a pooled estimate of error variance

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.63895	0.05

LSD Threshold Matrix

Abs(Dif)-HSD				
	ETC	ETK	ACC	ACK
ETC	-3.4449	-2.4322	1.2976	2.3258
ETK	-2.4322	-2.9114	0.8422	1.8730
ACC	1.2976	0.8422	-2.3772	-1.3435
ACK	2.3258	1.8730	-1.3435	-2.3225

Positive values show pairs of means that are significantly different.

Connecting Letters Report

Level		Mean
ETC	A	6.4000000
ETK	A	5.6428571
ACC	B	2.1428571
ACK	B	1.1363636

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
ETC	ACK	5.263636	1.113244	2.32584	8.201433	**<.0001***
ETK	ACK	4.506494	0.997935	1.87299	7.139998	**0.0002***
ETC	ACC	4.257143	1.121496	1.29757	7.216718	**0.0018***
ETK	ACC	3.500000	1.007133	0.84222	6.157777	**0.0050***
ACC	ACK	1.006494	0.890512	-1.34353	3.356512	0.6724
ETC	ETK	0.757143	1.208560	-2.43219	3.946475	0.9232

Oneway Analysis of Injury By GROUP

Oneway Anova
Summary of Fit

Rsquare	0.528986
Adj Rsquare	0.506557
Root Mean Square Error	2.720064
Mean of Response	3.283582
Observations (or Sum Wgts)	67

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
GROUP	3	523.49073	174.497	23.5846	**<.0001***
Error	63	466.12121	7.399		
C. Total	66	989.61194			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
ACC	21	0.95238	0.59357	-0.234	2.1385
ACK	22	1.45455	0.57992	0.296	2.6134
ETC	10	6.50000	0.86016	4.781	8.2189
ETK	14	7.35714	0.72697	5.904	8.8099

Std Error uses a pooled estimate of error variance

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.63895	0.05

LSD Threshold Matrix

Abs(Dif)-HSD				
	ETK	ETC	ACK	ACC
ETK	-2.7131	-2.1149	3.4485	3.9281
ETC	-2.1149	-3.2102	2.3078	2.7897
ACK	3.4485	2.3078	-2.1643	-1.6877
ACC	3.9281	2.7897	-1.6877	-2.2152

Positive values show pairs of means that are significantly different.

Connecting Letters Report

Level		Mean
ETK	A	7.3571429
ETC	A	6.5000000
ACK	B	1.4545455
ACC	B	0.9523810

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
ETK	ACC	6.404762	0.938511	3.92808	8.881449	**<.0001***
ETK	ACK	5.902597	0.929940	3.44853	8.356666	**<.0001***
ETC	ACC	5.547619	1.045082	2.78970	8.305541	**<.0001***
ETC	ACK	5.045455	1.037392	2.30783	7.783082	**<.0001***
ETK	ETC	0.857143	1.126214	-2.11488	3.829167	0.8716
ACK	ACC	0.502165	0.829836	-1.68773	2.692063	0.9301

Oneway Analysis of Emotional Physical Exhaustion By GROUP

Oneway Anova

Summary of Fit

Rsquare	0.026843
Adj Rsquare	-0.0195
Root Mean Square Error	0.637305
Mean of Response	1.895513
Observations (or Sum Wgts)	67

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
GROUP	3	0.705810	0.235270	0.5793	0.6308
Error	63	25.587945	0.406158		
C. Total	66	26.293756			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
ACC	21	1.80062	0.13907	1.5227	2.0785
ACK	22	1.90433	0.13587	1.6328	2.1759
ETC	10	1.82154	0.20153	1.4188	2.2243
ETK	14	2.07683	0.17033	1.7365	2.4172

Std Error uses a pooled estimate of error variance

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.63895	0.05

LSD Threshold Matrix

Abs(Dif)-HSD				
	ETK	ACK	ETC	ACC
ETK	-0.63567	-0.40248	-0.44104	-0.30407

ACK	-0.40248	-0.50709	-0.55863	-0.40938
ETC	-0.44104	-0.55863	-0.75213	-0.62526
ACC	-0.30407	-0.40938	-0.62526	-0.51902

Positive values show pairs of means that are significantly different.

Connecting Letters Report

Level		Mean
ETK	A	2.0768348
ACK	A	1.9043282
ETC	A	1.8215385
ACC	A	1.8006216

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
ETK	ACC	0.2762132	0.2198912	-0.304069	0.8564956	0.5939
ETK	ETC	0.2552964	0.2638694	-0.441042	0.9516352	0.7683
ETK	ACK	0.1725066	0.2178830	-0.402476	0.7474895	0.8579
ACK	ACC	0.1037066	0.1944288	-0.409382	0.6167951	0.9506
ACK	ETC	0.0827898	0.2430587	-0.558630	0.7242100	0.9863
ETC	ACC	0.0209169	0.2448604	-0.625258	0.6670920	0.9998

Oneway Analysis of Sense of Accomplishment By GROUP

Oneway Anova

Summary of Fit

Rsquare	0.049931
Adj Rsquare	0.004689
Root Mean Square Error	0.555059
Mean of Response	2.046499
Observations (or Sum Wgts)	67

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
GROUP	3	1.020070	0.340023	1.1036	0.3543
Error	63	19.409690	0.308090		
C. Total	66	20.429761			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
ACC	21	1.91513	0.12112	1.6731	2.1572
ACK	22	2.16795	0.11834	1.9315	2.4044
ETC	10	1.91078	0.17553	1.5600	2.2615
ETK	14	2.14964	0.14835	1.8532	2.4461

Std Error uses a pooled estimate of error variance

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha

2.63895	0.05

LSD Threshold Matrix

Abs(Dif)-HSD				
	ACK	ETK	ACC	ETC
ACK	-0.44165	-0.48246	-0.19405	-0.30147
ETK	-0.48246	-0.55363	-0.27089	-0.36762
ACC	-0.19405	-0.27089	-0.45204	-0.55844
ETC	-0.30147	-0.36762	-0.55844	-0.65507

Positive values show pairs of means that are significantly different.

Connecting Letters Report

Level		Mean
ACK	A	2.1679540
ETK	A	2.1496387
ACC	A	1.9151293
ETC	A	1.9107809

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
ACK	ETC	0.2571731	0.2116911	-0.301470	0.8158160	0.6198
ACK	ACC	0.2528247	0.1693372	-0.194048	0.6996974	0.4477
ETK	ETC	0.2388578	0.2298162	-0.367616	0.8453319	0.7271
ETK	ACC	0.2345094	0.1915135	-0.270886	0.7399044	0.6138
ACK	ETK	0.0183153	0.1897645	-0.482464	0.5190948	0.9997
ACC	ETC	0.0043484	0.2132604	-0.558436	0.5671325	1.0000

Oneway Analysis of Sport Devaluation By GROUP

Oneway Anova
Summary of Fit

Rsquare	0.085363
Adj Rsquare	0.041809
Root Mean Square Error	0.637774
Mean of Response	1.772923
Observations (or Sum Wgts)	67

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
GROUP	3	2.391639	0.797213	1.9599	0.1291
Error	63	25.625606	0.406756		
C. Total	66	28.017245			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
ACC	21	1.64727	0.13917	1.3692	1.9254
ACK	22	2.04300	0.13597	1.7713	2.3147
ETC	10	1.64195	0.20168	1.2389	2.0450

ETK	14	1.63055	0.17045	1.2899	1.9712

Std Error uses a pooled estimate of error variance

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.63895	0.05

LSD Threshold Matrix

Abs(Dif)-HSD				
	ACK	ACC	ETC	ETK
ACK	-0.50746	-0.11773	-0.24084	-0.16295
ACC	-0.11773	-0.51940	-0.64133	-0.56399
ETC	-0.24084	-0.64133	-0.75269	-0.68545
ETK	-0.16295	-0.56399	-0.68545	-0.63614

Positive values show pairs of means that are significantly different.

Connecting Letters Report

Level		Mean
ACK	A	2.0430017
ACC	A	1.6472694
ETC	A	1.6419464
ETK	A	1.6305478

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
ACK	ETK	0.4124539	0.2180433	-0.162952	0.987860	0.2420
ACK	ETC	0.4010553	0.2432375	-0.240837	1.042947	0.3593
ACK	ACC	0.3957323	0.1945719	-0.117734	0.909198	0.1867
ACC	ETK	0.0167216	0.2200529	-0.563988	0.597431	0.9998
ETC	ETK	0.0113986	0.2640635	-0.685452	0.708250	1.0000
ACC	ETC	0.0053230	0.2450406	-0.641327	0.651973	1.0000

VITA

Keely Virginia O'Brien graduated *Magna Cum Laude* from the University of Tennessee at Chattanooga in 2007 earning a bachelor's degree in biological sciences with a minor in chemistry. At UTC Keely served as the president of the Biology Honor Society, Beta Beta Beta and was a member of several academic and service honor societies including Mortar Board and Greenspaces. In 2006 and 2007 she was awarded Outstanding Chemistry Laboratory Teaching Assistant. After completing this degree Keely owned and operated a small business manufacturing cultured food products such as sauerkraut, kim chi, kombucha and kefir. A passion for food and nutrition led to the study of fermented dairy products in the master's program in The School of Animal Sciences at Louisiana State University. She was awarded a Graduate Assistantship during her years of study at LSU and received her Masters Degree in 2012. When not studying or working, Keely enjoys spending time with her family, Jeff and Emily Fair, fermenting and cooking locally raised foods, outdoor activities and tending to her garden.