Title: Gut Microbiota of Ultrarunners Prior to and Following a 161 km Ultramarathon Race

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Assistant Professor and Chair Health and Human Performance
The College of Idaho
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Abstract of Proposal

Introduction

Gastrointestinal symptoms plague upwards of 96% of ultrarunners with 35% of ultrarunners whom drop out reporting GI symptoms (16). GI issues come in many different forms, and can generally be separated into upper (bloating, cramps, nausea, vomiting) and lower GI issues (cramps, flatulence, side ache, diarrhea, bloody stool) (14). Nausea is the most common GI symptom. The nausea associated with GI issues during ultras is accompanied by endotoxemia, or the movement of gut bacteria into the blood stream (16). However, strategies to reduce nausea and other GI symptoms are lacking.

Our guts contain millions of microbes which are collectively called the microbiota. Their collective genetic material is the microbiome. The gut microbiota diversity and quantity has an important and increasingly established role in health and disease (11). For instance, the microbiota of the gut alter metabolism, neuronal functioning, skeletal muscle function, immune function and importantly for ultrarunners, GI function. The gut microbiota can help improve GI barrier integrity and function through specific metabolic byproducts. Exercise and diet both alter the composition of the gut microbiota and altered compositions of the microbiota are found in elite athletes and people with differing levels of cardiorespiratory fitness. Nutritional supplements which support specific microbes are able to reduce gut disruption to the GI barrier and improve performance in some endurance events (< 2 hours).

Given the role of microbiome intersecting with GI issues, exercise performance, and diet it seems plausible that the microbiome of ultrarunners may change during an ultra-marathon, be related to GI symptoms such as nausea, and have an effect on performance. The development of dietary strategies for optimal microbiome composition prior to and following ultra-marathons maybe necessary, but first we need to characterize the changes in the microbiome following an ultra-marathon.

Purpose

This study will determine how the microbiome of ultrarunners changes during a race and whether starting microbiome composition or changes in microbiome composition are associated with specific GI symptoms (nausea) and performance (finishing) during the race. This study will be first of it’s kind to study how acute extreme exercise may alter the microbiome diversity and quantity. While this study is very much preliminary in nature it will generate information from which specific microbiota compositions can be identified as protective against exercise induced GI problems. Follow up studies plan to use probiotics to prevent or limit GI symptoms.

Methods

400 registered runners from Western States 100 will be given the opportunity to participate in the study. A total of 24 runners will be recruited based on sample sizes in similar studies (17). Stool samples will be collected in a 48 hour window prior to racing and 24 hour period following racing and given to researchers onsite for proper handling. DNA will be isolated from the stool samples and sent to an independent laboratory for standard 16S sequencing to characterize the diversity. Standard bioinformatic analysis will help of the Bioinformatics Core at The College of Idaho (see letter of support) will identify diversity of the gut microbiome pre and post-race. Regression analysis will primarily analyze the relationship between starting and changing microbiomes and GI symptoms (via questionnaire) and finishing times/rates.
Narrative of Proposal

Specific Aims.
Gastrointestinal (GI) problems plague a majority of ultrarunners with nausea being the most prevalent complaint of finishers and non-finishers (16). While the exact cause of nausea is likely multifactorial (heat, blood flow, diet, etc.), several studies show that during prolonged endurance exercise there is an increase in endotoxemia, or movement of bacteria from the gut to the blood, which is associated with nausea (3, 17). Endotoxemia is caused by a “leaky” gut, or impaired GI barrier function. GI barrier dysfunction occurs during heavy exercise, but can be improved through nutritional probiotic supplementation (10). Increasingly, studies are linking gut barrier function and the gut microbiota.

The gut microbiota plays an important role in metabolism, neuronal functioning, skeletal muscle function, immune function and GI distress (11). Changes in the microbiota composition are associated with changes in exercise, diet, and psychological stress. Beneficial microbiome profiles are seen in elite athletes and those with higher cardiorespiratory fitness (5). Support of the microbiota by supplementing with probiotics shows improved performance over shorter (< 2 hour exercise bouts) in some, but not all, studies (12)(15). Together the data suggest that ultra-runner may benefit from a specific gut microbiome composition from improved gut barrier function which may lower endotoxemia and nausea prevalence.

This research project has three specific aims which can be independently tested.

Aim 1: Characterize changes in the gut microbiota composition after a 161 km race.

Hypothesis: That the gut microbiota composition will become less diverse from prerace to postrace in a 161 km race.

We propose to measure microbiota composition via 16S ribosomal RNA sequencing with well-established and standard approaches in stool samples collected within 48 prior to racing and within 24 hours of finishing a 161 km race.

Aim 2: Determine whether an athlete’s microbiota composition prior to racing is associated with performance or GI symptoms during a 161 km race.

Hypothesis: Those athletes with a more diverse gut microbiota composition will have lower prevalence of GI issues in general, and nausea specifically, and have improved performance.

We propose to stratify our data based on specific microbiota compositions PRIOR to the race to and analyze for differences in GI symptoms or performance.

Aim 3: Determine whether the degree to which changes in microbiota composition from prerace to postrace occur are associated with performance or GI symptoms during a 161 km race.

Hypothesis: Those athletes with less change in microbiota composition from prerace to postrace will have lower prevalence of GI issues (and nausea) and be more likely to finish.

We propose to stratify our data based on specific and degree of microbiota composition CHANGES during the race to analyze for differences in GI symptoms or performance.

Benefits: The data collected here will be highly specific and relevant for ultrarunners in the future. If we identify a specific gut microbiota composition that reduces the likelihood of nausea symptoms then attempts to favorably alter microbiota through changes in diet, probiotic supplementation, or lifestyle are viable strategies to improve performance, enjoyment, and health in ultramarathons.

Background and Significance
Gastrointestinal Issues are Major Issues Effecting Ultrarunners. Gastrointestinal (GI) distress is a major cause of poor performance and failure to finish ultra-marathon events. Many different types of GI
distress exist, including upper (stomach, nausea, vomiting) and lower (cramps, flatulence, loose stool) GI symptoms. At Western States 96% runners have some sort of GI symptoms and 43.9% of finishers and 35.6% of non-finishers report that GI symptoms altered their race performance (16). Nausea is the most common GI issue effecting 86% and 90.5% of the finishers and non-finishers at Western States (16).

*Nausea is related to Gut Permeability.* One potential cause of the nausea is an increase in the luminal permeability in the GI tract which will allow bacteria to translocate from the gut to the circulation and induce an inflammatory response. Indeed, endurance exercise is known to disrupt GI barrier function, potentially through ischemia or hypoperfusion (3). In agreement, 81% of Comrades Marathon have measurable endotoxemia and those who took more than 8 hours had higher levels of endotoxemia (3), suggesting ultrarunners maybe at higher risk. Indeed, ultrarunners whom experience nausea symptoms are more likely to have blood markers of endotoxemia than runners without nausea (11). Thus, gut barrier function is disrupted during long endurance exercise events, which is related to the nausea.

*The Gut Microbiota is Important for Overall Health and Gut Permeability.* Every surface of the human body is host to a large variety and number of microbes, which collectively are called the microbiota or microbiome when their genetics are considered. In the gut at least 5 different phyla of microbiota exist with 160 species of microbes. The most abundant phyla *Firmicutes* and *Bacteroidetes* make up 90% of all the microbes in the gut. However, an additional 100 species exist and dynamically change in response to aging, diet, and exercise. These microbes play an important role in health in general with the ability to neutralize drugs, promote gut health through mobility and barrier function, interact with the immune system, modify neurotransmitters locally and systemically, and alter metabolism (4). Gut microbiota can improve the barrier function of epithelial gut cells though an increased production of metabolites such as short chain fatty acids such as butyrate which can also be used for energy by exercising skeletal muscle (2).

*Exercise Alters the Microbiome.* Exercise reduces the risk of 35 different chronic diseases and is a powerful regulatory gut health (4). Exercise training increases the diversity of commensal gut microbiota and reduces the amount of pathogenic bacteria (5). People with higher levels of cardiorespiratory fitness have a different microbiota composition, which includes enriched microbiota species which produce butyrate (15). While exercise alters the microbiota composition, altering the microbiota composition can also alter exercise and immune function.

*Probiotics May Improve Performance and Health in Runners.* Probiotic supplements improve microbiota health in general and increase the prevalence of specific microbe species. Runners randomized to 4 weeks (double blind, cross over) of probiotic treatment perform better in the heat with lower levels of inflammation compared to placebo (7). Furthermore, marathon runners taking probiotics during training had less severe GI issues post-marathon race (18). Lastly, probiotics may improve immune function and reduce the upper respiratory infections in healthy physical activity adults (6).

Together the data strongly argue that: 1) GI symptoms in general, and nausea specifically is a cause of decreased ultramarathon performance, and 2) Nausea is related to GI health and gut permeability. 3) The microbiota play an important role in gut health. 4) The microbiota is altered by exercise. 4) Altering the microbiota through probiotics can lead to increased performance and health.

However, whether the microbiota composition is altered acutely by a 161km ultramarathon or whether the microbiota starting or end race composition is important for performance and GI symptoms is unknown.
We propose that a 161 km ultramarathon race will alter the microbiota of runners and that starting microbiota compositions will predict the likelihood of GI symptoms which may influence the likelihood of a runner to finish.

**Preliminary Data**

While we do not have any preliminary data in microbiota samples, high throughput metagenomics and microbiota identification analysis using next generation sequencing is inexpensive and relatively easy given the number of tools which exist for analysis (9). Furthermore, our College of Idaho campus was granted an INBRE award from the National Institute of Health, part of which is meant to train faculty in bioinformatics. I would plan to use this resource to attend a workshop on analysis of microbiome/microbiota sequencing data (see letter of support from Bioinformatics Core Director – Dr. Luke Daniels).

I also have experience in so called “-omics” analysis. Specifically of large metabolomics datasets with regard to changes in diet and age (13) and human plasma microRNA sequencing data sets (17). Thus, I am confident that I can conduct the analysis and that the data will lead to preliminary data for additional funding of follow up studies.

**Experimental Design and Methodology**

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<td>IRB Approval From College of Idaho (ongoing)</td>
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**Subjects:** We will include information regarding the study and the opportunity to enroll in this study with other material distributed to 400 Western States runners registered for the 2018 race. We will enroll the first 24 participants and an additional 6 subjects will on a “back up” list should the other runners drop out prior to race day. No special preference will be given to the demographic breakdown of the participants. Based on similar studies we believe that the study is appropriately powered given it’s exploratory and descriptive nature and it’s similarity to other studies on ultrarunners and GI issues (16, 17). We are in the process of obtaining IRB approval at The College of Idaho and do not anticipate any problems to carrying out the sample collection in the field and sample preparation in the labs at College of Idaho which are Biohazard 2 rated. (see letter attached from IRB chair).

**Sample Collection:** Subjects will be asked to collect two samples. One, the first stool samples within 48 hours of race start. The second, the first stool sample 24 hours post race. Stool will be collected in DNA/RNA Shield™ Fecal Collection Tube (Zymo Research) which contains a scoop attached to the cap and DNA/RNA shield which keeps DNA samples stable at room temperature for 2 years (and RNA for 1 year). Once collected all samples will be coded to ensure anonymity in the analysis and reporting of the data.

**GI Symptoms and Race Day:** Runners will be given surveys to assess GI symptoms and race day nutrition during the race as others have done (16).

**DNA Isolation and Analysis:** DNA will be isolated from stool samples using ZymoBIOMICS™ DNA Miniprep Kit (ZymoBIOMICS) which is specifically made for downstream microbiota analysis. DNA
will then be sent to an external laboratory for 16S sequencing of microbial DNA V1-V3 regions. The 16S rRNA sequence is amplified which are then used to identify unique genomic sections of different microbes. The relative abundance of the microbiota community will be identified from the V1-V3 regions which offer greater assessment of microbiota diversity in the MiSeq platform (6). The MiSeq analysis using the Illuminia platform is robust (1) and common among many different Core facilities listed on Science Exchange (ScienceExchange.com) which will be used for analysis. Furthermore additional funding will be sought out through alternative sources to run metagenomics shotgun sequencing (more expense than 16S profiling) to determine the relative abundance of different gene functions of the microbiome.

Statistical Analysis: Regression analysis will be done to control for various confounding factors.

Dissemination of Knowledge: I plan to write 1-3 manuscripts from the data collected with a target of Journal of Applied Physiology. I plan to present the data at 2019 Medicine and Science in Ultra-Endurance Sports conference, ACMS regional meeting in 2018, and ACSM national meeting in 2019. In addition I plan to write an article in UltraRunning Magazine highlighting the findings.

Anticipated Problems

Sample Collection: While sample collection and storage should be easy pre-race, the post-race collection maybe more difficult giving the time differences of finishers and when samples are available. However, others have shown that large community based collections of samples via the mail are possible as an alternative collection method (17).

Underpowered: The proposed study is designed with a focus on Specific Aim 1 as that is most appropriately powered. Aims 2 and 3 are more subject to random variation in the prevalence of GI issues experienced during the race. While we would like to focus on Aim 1, we are not opposed to collecting prerace samples from 48 participants to better associate initial microbiota composition with performance outcomes. Either way we plan to apply for additional funds through the Western States 100 foundation (see attached letter).

Data Analysis: While I have expertise in other types of –omics analysis I may need to collaborate with other scientist who specialize in the microbiota composition analysis. I plan to use the INBRE funding mechanism (attached letter) to attend a conference or visit potential collaborators to develop expertise in this area.

References and Citations


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Matthew Laye, PhD
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mlaye@collegeofidaho.edu

Education:
2004-2009 University of Missouri, Ph.D in Medical Physiology
Dissertation: The Effects of Physical Activity on Adipose Tissue Metabolism and DNA Methylation
Supervisor: Dr. Frank Booth
1999-2003 University of California – Davis, Bachelor of Science with Honors, in Exercise Biology

Positions and Employment
2017- Chair Health and Human Performance, The College of Idaho (Caldwell, ID)
2015 - Assistant Professor, The College of Idaho (Caldwell, ID).
2012 - 2015 Senior Postdoctoral Fellow, Buck Institute for Research on Aging.
2009 - 2012 Junior Group Leader and Post-Doctoral Fellow, Centre for Inflammation and Metabolism, University of Copenhagen, Rigshospitalet (Danish National Hospital).
2004-2009 PhD Student, University of Missouri.
2004 Exercise Physiologist Technician, Western Human Nutrition Research Center. Davis, CA.
2003 Undergraduate Research Assistant in Ozone Research, University of California – Davis.
2003 Undergraduate Student Advisor: Department of Exercise Biology, University of California – Davis.

Research Experience
2015 – Assistant Professor of Health and Human Performance. The College of Idaho (Caldwell, ID)
1) Develop behavioral interventions to alter physical activity levels of members of the community through use of wearable technology.
3) Develop methods to study interaction between psychological and physical fatigue.
2012 – 2015 Postdoctoral Fellow. The Buck Institute for Research on Aging (Novato, CA). Laboratory of Pankaj Kapahi PhD.
I use adaptable model systems to understand how the metabolome changes with aging. I use R for metabolomics data analysis. Screen nutraceuticals that can be used in humans for immediate clinical interventions.
2009 – 2012 Junior Group Leader. Centre for Inflammation and Metabolism. Rigshospitalet (Copenhagen, DK) Laboratory of Bente Klarlund Pedersen MD PhD.
Worked with various cohorts of patients including exercise training in prostate cancer patients. Used basic molecular approaches in both invasive (muscle biopsies) and non-invasive (plasma) approaches. Established adipose tissue biopsy and culture methods. Developed methods to measure microRNA in human plasma. My group consisted of 2 PhD students, MD, and technician.
2004- 2009 PhD Student, Department of Medical Physiology and Pharmacology. University of Missouri. (Columbia, MO) Laboratory of Frank Booth PhD. Using a novel model of physical inactivity we examined how adipose tissue and muscle fatty acid and mitochondrial metabolism respond to sedentary lifestyle. Developed DNA methylation assays for skeletal muscle.
2004 Exercise Physiologist. Western Human Nutrition Research Center (Davis, CA). Performed exercise testing, physical activity assessment instrument calibration, body composition, and subject orientation for multi Principle Investigators

Grants/Funding Support
Current
2017-  Idaho Space Grant Consortium. Travel Grant (2000 USD).
2015 - 2017  The College of Idaho.  Startup Funds. PI (5000 USD)

Past
2012-2015  NIH – 5F32AG044065, Post-doctoral F32, Salary (154,000 USD)
Highly Conserved 4E-BP Dependent Secreted Proteins from Human and Fly Skeletal Muscle

2011-2013  Lundbeckfonden, Consumables, 500000 DKK (90000 USD).
Regulation of microRNA Expression by DNA Methylation in Healthy and Type 2 Diabetic, and Exercised Skeletal Muscle

2011  Augusten Fonden, Consumables, 200000 DKK (40000 USD). Marathon Runners

2010-2012  Forskningsråd Sundhed og Sygdom Post-Doctoral Fellowship, 1130000 DKK (208114 USD) salary plus research
Regulation of microRNA Expression by DNA Methylation in Healthy and Type 2 Diabetic Skeletal Muscle

2008-2009  American College of Sports Medicine Pre-Doctoral Research Grant (5000 USD research support)
Epigenetic Regulation of Genes by Exercise

2008-2009  American Heart Association Pre-Doctoral Fellowship (stipend support)
Physical Activity Induced Changes in DNA Methylation of Key CVD-Related Genes in Mouse Skeletal Muscle

2004-2008  University of Missouri. Life Science Fellow (stipend support),

Honors and Leadership Positions
2012-2015  Postdoctoral Association, Vice President and Academic Training Committee, The Buck Institute for Research on Aging

2008-2009  Outstanding Graduate Student, Department of Medical Pharmacology and Physiology, University of Missouri

2007-2008  Vice President of the Medical Pharmacology and Physiology Graduate Student Association

11/2007  M. Harold Laughlin Scholarship, University of Missouri – College of Veterinary Sciences

5/2007  Travel Award for Experimental Biology, Graduate Professional Council, University of Missouri

1/2007  Travel Stipend for PhD Course: Inflammation and Metabolism, Copenhagen, Denmark

1/2006  Travel Award from Keystone Symposia

1999-2003  Graduation with Honors, University of California – Davis

Reviewing Duties

Professional Organizations
2004 –  American Physiological Society
2004 - American College of Sports Medicine

Consulting/Writing
2015 - UltraRunning Magazine. “Science of Ultrarunning Column”
2015 - Consultant. GU Energy

Selected Peer Reviewed Publications from 32 total, 6 first, 4 last, H factor 19:


10-31-2017

Ultra Sports Science

To whom it may concern:

Matt Laye has submitted a grant proposal to you to look at gastrointestinal microbiome composition in ultrarunners both before and after a 100 mile running endurance event. He has requested to perform the study at the Western States 100 mile Endurance Run (WSER 100) in 2018.

We support his research efforts and will allow him to solicit potential candidates for his study at the WSER 100 in 2018. We will also be willing to match any funding grant from Ultra Sports Science of up to $5000 to support his research efforts.

Feel free to contact me if you have any questions.

Sincerely,

John N. Diana, MD
Director of Research, WSER
Jdiana10@aol.com or research@wser.org
707-812-2639
Dear Sports Science Grant Reviewers,

I am writing in support of Dr. Matthew Laye’s grant “Gut Microbiota of Ultrarunners Prior to and Following a 161 km Ultramarathon Race”. This project sounds very interesting and something that the College of Idaho will support. More specifically, the project is in line with one of our specific project goals at the College, increasing education and research opportunities for students in areas of study that involve bioinformatics projects.

As the Bioinformatics Coordinator at our campus (an NIH-INBRE grant funded position) I have a budget to support funding for bioinformatics experiments and for training faculty and students in this area. Past projects have included supporting faculty for travel/training for conferences/workshops, purchasing imaging & data analysis software, computer hardware, and wet lab reagents that contribute to experiments that generate “big data” type datasets (genomics, transcriptomics). In the past we have not spent our entire budget and thus is it extremely likely that we will provide additional support for many aspects of Dr. Laye’s project should he need it.

Personally, Dr. Laye and I are beginning to embark on another microbiome study, which should provide further opportunities to obtain expertise in analysis in data obtained from this study. If you have any questions about my support of this project please feel free to contact me by email (ldaniels@collegeofidaho.edu) or by phone (208-459-5893).

Sincerely,

R. Luke Daniels, Ph.D.
Associate Professor of Biology
The College of Idaho
October 26, 2017

To whom it may concern:

I have briefly reviewed the project summary for Dr. Matt Laye’s proposal entitled *Microbiota of Ultrarunners*. The College of Idaho IRB will be able to review the protocol in a timely manner and I can confirm that our campus has the facilities needed to work with human stool samples.

Sincerely,

Ann Koga, Ph.D.
Dept. of Biology
Chair, Institutional Review Board