Study Title:

Exercise Training in Children and Adults: Mechanisms of Allaying Inflammation in Asthma

Date:

10/06/2017

PROTOCOL NARRATIVE FOR EXPEDITED OR FULL COMMITTEE RESEARCH

University of California, Irvine
Institutional Review Board
Version: May 2011

IMPORTANT: CAREFULLY READ THE INSTRUCTIONS FOR EACH SECTION BEFORE COMPLETING THE PROTOCOL NARRATIVE.

WHEN CUTTING AND PASTING FROM ANOTHER APPLICATION OR PROTOCOL, PLEASE ENSURE THAT THE INFORMATION IS COMPLETE, SUPPLEMENTED WHERE NECESSARY, PASTED IN A LOGICAL ORDER, AND IS RELEVANT TO THE SPECIFIC SECTION.

NEED HELP? CONTACT THE HRP STAFF FOR ASSISTANCE.

HS#: 2012-9253
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Lead Researcher Name: DAN M. COOPER, MD
Study Title:
EXERCISE TRAINING IN CHILDREN AND ADULTS: MECHANISMS OF ALLAYING INFLAMMATION IN ASTHMA

NON-TECHNICAL SUMMARY

Provide a non-technical summary of the proposed research project that can be understood by IRB members with varied research backgrounds, including non-scientists and community members. The summary should include a brief statement of the **purpose of the research** and **related theory/data supporting** the intent of the study as well as a brief description of the **procedure(s) involving human subjects**. *This summary should not exceed ½ page.*

The goal of this research is to determine whether 10-weeks of exercise training can benefit asthmatic children and young adults with a history of exercise-induced bronchoconstriction (EIB). Physical activity is a double-edged sword for children and adults with asthma. On the one hand, exercise is a common trigger of wheezing (EIA—exercise induced asthma), occurring in as many as 80% of affected individuals. On the other hand, exercise and fitness training seem to benefit asthma control. The underlying causes responsible for EIA remain unknown. We will, for the first time, test hypotheses focused on mechanisms of exercise training, asthma and Exercise Induced Bronchoconstriction that involve the genomic changes of circulating white blood cells. We will also study an exciting newly discovered aspect of gene expression regulation in the white blood cells known as epigenetics: a process that takes place when genomic changes happen as a result of exposure to the environment. Our study is based on emerging exciting new data from this and other laboratories demonstrating that (1) white blood cells play an important role in bronchoconstriction[1,2] in children, (2) gene and cytokine expression in circulating white blood cells are abnormal in asthma,[3,4] and (3) brief exercise may change genomic and inflammatory-profiles of these cells.[5-7] Each participant will undergo a training regimen. The participants will come to our lab up to three times before the beginning of the training program, and up to three times immediately at the end of the exercise program to perform Exercise Fitness Session and Exercise Challenge Sessions. The visits may occur in any order, depending on the availability of the laboratory and timing. In the Exercise Fitness Session, the participant will perform a standard exercise test to determine fitness and will perform a series of pulmonary function tests. In addition, the participants will have two DXA scans to determine body composition (prior to

beginning the training sessions and after the training sessions have been completed). In one of the Exercise Challenge Sessions, the participants will have an indwelling venous catheter placed in an arm vein so that we can draw blood samples. In another Exercise Challenge Session we will perform non-invasive blood flow measurement. The participants will exercise using a protocol we have performed in our lab on hundreds of young adults and children in which the subject uses a stationary bicycle and completes a series of 10, 2-minute bouts of exercise interspersed with one-minute rest periods. The exercise-training intervention is built on effective social cognitive and self-determination approaches to health-behavior change. Physical activity is an essential component of growth and health in children, thus, this research will lead to improved clinical uses of exercise as preventive and adjunctive therapy in the current epidemic of childhood asthma.

SECTION 1: PURPOSE AND BACKGROUND OF THE RESEARCH

1. Describe **the purpose of the research** project and state the overall objectives, specific aims, hypotheses (or research question) and scientific or scholarly rationale for performing the study.
2. Provide the **relevant background information** on the aims/hypotheses (or research question) to be tested and the procedures/products/techniques under investigation.
3. Include a description of the **predictor and outcome variables**, as appropriate.
4. Include a critical evaluation of **existing knowledge**, and specifically identify the information gaps that the project intends to address.
5. Describe **previous research** with animals and/or humans that provides a basis for the proposed research. **Include references/citations**, as applicable.
   This section should not exceed 4 pages.

The goal of this research is to determine whether an exercise training intervention can benefit asthmatic children and adults with a history of exercise-induced bronchoconstriction (EIB). We will, for the first time, test hypotheses focused on mechanisms of asthma and EIB that involve genomic and epigenetic regulation of circulating leucocytes. In addition, we will gauge the effect of training on airway inflammation, asthma control, and muscle and cardiorespiratory fitness. Increasingly, asthma is viewed as a condition not just of the lungs but involving a broad range of impaired inflammatory response mechanisms at a systemic level. Our study is based on emerging exciting new data from this and other laboratories demonstrating that (1) first-responding innate immune cells (such as, neutrophils and monocytes) play key roles in bronchoconstriction[1,2] in children, (2) gene and cytokine expression in circulating neutrophils is abnormal in asthma[3,4] and (3) brief exercise alters genomic (including epigenetic) and inflammatory-functional profiles of these cells[5-7]. Collectively, these new insights lead to the premise that exercise training may benefit such children specifically by conditioning inflammatory control mechanisms in the leucocytes. Physical activity is an essential component of growth and health in children,[8,9] thus, this research will lead to improved clinical uses of exercise as preventive and adjunctive therapy in the epidemic of childhood asthma.

Specific Aims: Using a novel exercise training treatment intervention designed specifically to promote sustainability and generalizability in children and adults with chronic diseases like asthma, we plan to:

Primary Aims:

1. Elucidate key abnormal genomic (including epigenetic) responses to brief exercise in leucocytes of children and adults with persistent asthma
2. Identify the effect of exercise training on the key genomic exercise response mechanisms in leucocytes in children and adults with persistent asthma

Secondary Aim:

3. Assess the clinical impact of exercise training in children and adults with persistent asthma on: (a) exercise induced airway inflammation, (b) asthma control, and (c) cardiorespiratory fitness and muscle adaptation.

Hypotheses:

1. Leucocytes genomic, epigenetic, and functional regulation is abnormal in asthmatic individuals leading to a greater pro-inflammatory profile in response to a brief exercise challenge.
2. The exercise-training treatment will attenuate pro-inflammatory and intensify anti-inflammatory activity of leukocytes in response to brief exercise challenge.
3. The exercise-training treatment will diminish exercise-induced bronchoconstriction and its accompanying airway inflammation and improve asthma control.

Impact: Despite much recent progress in understanding asthma pathophysiology and the development of new therapies, the health care use associated with asthma, and the disruptions it causes to family and community life, have not decreased substantially.[10] The link between physical activity and asthma is strong, but remains enigmatic. Physical activity is a "double-edged sword" for the people with asthma. On the one hand, exercise is a common trigger of wheezing in asthma, occurring, in some studies, in as many as 80% of affected children.[11,12] On the other hand, exercise and fitness training seem to benefit asthma control in many affected children.[13-15]

As succinctly stated by Lucas and Platts-Mills,[16] *"It is our belief that an exercise prescription should be part of the treatment for all cases of asthma. The real question is whether prolonged physical activity and, in particular, outdoor play of children plays a role in prophylaxis against persistent wheezing. If so, the decrease in physical activity might have played a major role in recent increases in asthma prevalence and severity."* As recently noted by Voelkel and Spiegel[17], *"In spite of numerous attempts to control asthma by treatment with bronchodilators, steroids, antigen-directed desenstitization, and IgE-directed therapy, use of leukotriene receptor blockers and mast cell release inhibitors, a true control of the asthma syndrome with its multiple manifestations (exercise-induced, nocturnal, steroid-resistant, etc.), has so far eluded us. The reason is that…the disease… is perhaps much more an integrated system problem than only a bronchial problem."* The progress we have made in the past years supports the notion that EIB involves abnormalities in systemic, circulating leucocytes, particularly in response to exercise. Understanding these mechanisms will lead to improved use of exercise as adjunctive and preventive therapy in pediatric asthma.

Stevens and coworkers [18] recently published an eloquent and succinct assessment of the scale of the public health problem associated with childhood asthma, *"As many as 6.5 million children in the United States (or 8.9% of all children 0–18 years) have been diagnosed with asthma and are reported to currently have the disease.[19] The burden of asthma in childhood is tremendous, accounting for about 754,000 emergency department visits and 198,000 hospitalizations each year, and costing more than $850 million annually.[20] This burden is greater for vulnerable children, where rates of emergency visits and hospitalizations are double or triple among the poor, racial/ethnic minorities, and the uninsured."*[21-23] Clark and coworkers[10] recently noted in a review of the effectiveness of educational and behavioral asthma interventions, *"The paradox of asthma is that, although our understanding of its pathophysiological features and the therapies available for treating the disease have increased significantly over the past 2 decades, the prevalence of asthma, the health care use associated with it, and the disruptions it causes to family and community life have not decreased substantially. These facts suggest a need for interventions that enable individuals, families, communities, and health care providers to manage asthma and its consequences effectively."*

Critical exercise-asthma treatment issues remain enigmatic and poorly studied, ranging from rare but tragic instances of death due to EIB in asthmatic youth[24,25] to the lack of clinically validated paradigms of "return to play" following an exercise-associated asthma attack [26]. Despite the accepted clinical goal of ensuring that children with asthma fully participate in all

types of exercise, physical fitness and participation in physical activity have repeatedly been shown to be impaired in children with asthma.[27-29] Participation in school PE among children with asthma is reduced by as much as 40%.[30,31] Moreover, Conn and coworkers[32] recently discovered excessive use of electronic media in children with asthma, particularly in those with activity limitation. Whether these findings result from the *perception* of disability[33], or from poorly managed exercise-associated wheezing, is not known. But whatever the causes, ***reduced participation in physical activity is an ominous finding in a child with asthma.***

There is emerging data suggesting that exercise is beneficial for asthma in terms of disease control and pathogenesis. A growing number of animal studies (such as those by Pastva *et al.*[34] and Hewitt *et al.* [35,36]) examined how brief exercise and exercise training modulated subsequent lung inflammatory responses to ovalbumin (OVA) challenge in OVA-sensitized rats. These studies demonstrated a generally moderating effect of exercise on subsequent lung inflammatory responses to acute allergen challenges specifically by decreasing NF-κβ nuclear translocation and Iκβα phosphorylation thereby diminishing key pro-inflammatory (and possible neuroadrenergic) control pathways. In children, studies of the benefits of exercise and physical activity have yielded mixed results[37] (perhaps due to a number of the barriers to such research outlined below), but a recent study from Bonsignore et al.[38] concluded that exercise training in combination with anti-inflammatory therapy might synergize to attenuate airway response to methacholine challenge in asthmatic children.

It is increasingly recognized that poorly controlled asthma in children can set the stage for lung disease in adulthood,[39] thus, efforts to improve fitness and asthma control in children and adolescents, a "critical period" of growth and development [40], are bound to have effects on health that last a lifetime.

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SECTION 2: ROLES AND EXPERTISE OF THE STUDY TEAM

List all study team members below.

1. Identify each **member's position** (e.g., Associate Professor, graduate or undergraduate student) and **department**, and describe his or her **qualifications, level of training and expertise.** Include information about relevant licenses/medical privileges, as applicable.
2. Describe each team member's **specific role and responsibility** on the study.
3. **Faculty Sponsors** - list as Co-Researchers and describe their role on the project; include oversight responsibilities for the research study.
4. Explain who will have **access to subject identifiable data**.
5. Indicate who will be **involved in recruitment, informed consent process, research procedures/interventions, and analysis of data.**

Lead Researcher:

Dan M. Cooper, MD: Chair, Department of Pediatrics, Associate Dean for Translational Science and Director of the UCI Institute of Clinical and Translational Science.
Dr. Cooper is a board certified pediatrician and pediatric pulmonologist who has been actively involved in NIH-funded research since 1978. Dr. Cooper's area of expertise is in the developmental biology of exercise response, and has published extensively in exercise testing, training programs in children and adolescents, hormonal response to exercise in both children and adults. Dr. Cooper has extensive experience in the recruitment of subjects, the consent process, exercise challenges in children, and in data analysis. As a Lead Researcher, Dr. Cooper is responsible for the entire conduct of the study, and he will supervise all aspects of the research project. He will have access to subject identifiable data, and will be involved in the consent process. Dr. Cooper will provide the medical supervision during the fitness assessment.

Co-Researchers:

Shlomit Radom-Aizik, PhD: Assistant Professor, Department of Pediatrics. Dr. Shlomit Radom-Aizik received her MSc. Degree in Physiology and Pharmacology from Tel Aviv University, Sackler Faculty of Medicine, in the field of exercise physiology, and completed her Ph.D dissertation in the Functional Genomics Unit at Sheba Medical Center, Tel Aviv University, Israel. She is currently the director of the Pediatric Exercise Research Center and Director of the Functional Genomics Core of UCI Institute for Clinical and Translational Science. Her research focuses mainly on analyzing the genomic responses of the immune system to exercise stimulus, using cutting edge technologies. She will supervise all aspects of the research project, will have access to subject identifiable data and will be involved in the consent process.

Frank Zaldivar, PhD: Dr. Zaldivar is an immunologist interested in inflammatory and infectious

diseases. He completed a four year NHLBI Post-Doctoral Fellowship in Hematology-Immunology. He is currently the Director of the UCI Bioassay Core. Dr. Zaldivar's area of expertise is in Flow Cytometry and Cytokine Biology/Molecular Immunology, and has published in these areas. Dr. Zaldivar will be involved in flow and cytokine data analysis, and will have access to subject identifiable data.

Fadia Haddad, PhD: Dr. Haddad received her Ph.D in 1989 in the field of Physiology and Biophysics from the University of California, Irvine. Her research focuses mainly on studying regulation of gene expression in mammalian cells using molecular biology approaches. Her expertise is in epigenetics, molecular and cellular adaptation in response to altered physical activity and in disease states. Currently Dr. Haddad is a Researcher and a Scientific Advisor for the Pediatric Exercise Research Center and for the UCI Institute for Clinical and Translational Science. She will be involved in data collection and analysis, and will have access to subject identifiable data.

Kimberley Lakes, Ph.D: Assistant Professor, Department of Pediatrics. As a scientist and child psychologist, Dr. Lakes have studied children's social, behavioral, and emotional development, and have the experience and skills needed to study the development of executive function in children. Dr. Lakes interest in exercise and how certain exercise programs, such as a traditional Taekwondo program, could promote self-regulation and executive function in children began during her doctoral studies and led to a publication (Lakes & Hoyt, 2004), a randomized trial involving an entire elementary school that compared traditional physical education to a Taekwondo physical education curriculum. Dr. Lakes will be involved in collecting and analyzing executive function data assessment. Dr. Lakes will not be consenting participants. She will have access to subject identifiable data.

Kim Lu, MD: Clinical Assistant Professor, Pediatric Exercise and Genomics Research Center, Department of Pediatrics. Dr. Lu completed her Fellowship in Pediatric Pulmonary at Johns Hopkins University in June of 2013. Dr. Lu is board certified in Pediatrics and Board eligible in Pediatric Pulmonary. Dr. Lu will be involved with subject recruitment, subject screening, consent, data collection and analysis. Dr. Lu will be involved in the consent process and will finalize consent. She will also have access to subject identifiable data.

Jen Jen Chen, MD: Clinical Assistant Professor, Pediatric Exercise and Genomics Research Center, Department of Pediatrics, University of California, Irvine. Dr. Chen obtained her medical degree from Oregon Health Sciences University and completed both her pediatric residency and pediatric pulmonary fellowship at the University of California, Irvine. Her research interests include evaluating the effects of exercise in pediatric population, such as evaluating physiologic response to various exercise protocols and delivering exercise through telemedicine. Dr. Chen will be providing medical supervision during the fitness assessment, and the exercise challenge. She will be involved in the consent process and will finalize consent. Dr. Chen will have access to subject identifiable data.

Nandini Kataria, MD is a Post-Doctoral Fellow in the UC Irvine Department of Pediatrics Pulmonology program. She is a board certified pediatrician. She completed her pediatrics residency at the University of Minnesota Children's Hospital. She also worked in the department of Pediatric Hematology/Oncology at the University of Minnesota as a junior scientist in the laboratory. As part of the fellowship training, she will spend her research time at UC Irvine. She will be developing protocols in the molecular laboratory for sample analysis. She will not be consenting participants and will have access to deidentified patient data.

Research Personnel:

Sigal Ben-Ad: Mrs. Ben-Ad holds a Bachelor degree in Physical Education from the Zinman College for Physical Education and Sport Sciences at the Wingate Institute, Israel. She served

as a lead trainer in the Paratrooper Battalion, Israel Defense Force (1981-1982), P.E teacher in Tel-Aviv, Israel (1983-1989), Mission Viejo, CA (1990-1993), Costa Mesa, CA (1993-1998). Since 1998 Mrs. Ben-Ad is an independent Personal Trainer and Pilates Instructor. In addition to her B.A degree in Physical Education, Mrs. Ben-Ad is a certified Personal Trainer, Pilates Instructor, and a Life Guard (Please see attached CV and certifications). She is also affiliated with IDEA; Health & Fitness Association. Mrs. Ben-Ad will be a research personnel, and adhere to all regulatory requirements on this IRB. Mrs. Ben-Ad will serve as a trainer in this study, and will have access to subject identifiable data.

Abraham Chiu, BS: Mr. Chiu is a graduate student. He will be present during the fitness tests of the subjects and will perform blood flow measurements. Mr. Chiu will have access to subject identifiable data. He will not be involved in subject recruitment or the informed consent process.

Bridgette Duarte, BS: Ms. Duarte holds a degree in Nutritional Science, and is currently RD eligible and is part of the Bio-nutrition Research Group for UCI ICTS- PERC. She has been involved in a large number of metabolic studies in children and adults. She is licensed in X-Ray Bone Densitometry and will be responsible for performing the DXA scans on the participants, the food records data collection and analysis. Ms. Duarte will not be consenting participants. She will have access to subject identifiable data.

Peter Horvath Ph.D. Dr. Horvath completed his graduate studies at the University of California, Irvine where he worked in Dr. Galassetti's laboratory at the Institute for Clinical and Translational Science in collaboration with PERC. In his research work, he investigated the effects of glucose, lipid, and a combination of glucose and lipid on the white blood cells of healthy, obese and type II diabetic subjects. Dr. Horvath is currently a 50% effort study coordinator in PERC. He will be involved in subject recruitment and consent process and will have access to subject identifiable data. He will also serve as administrative contact for this study.

Hoang Ngoc Pham, M.S- Mr. Pham received his Master of Science in Kinesiology from California State University Fullerton. He will be responsible for performing the exercise testing and training sessions. Mr. Pham will have access to subject identifiable data. Mr. Pham will not be consenting participants.

Annamarie Stehli is Principal Statistician in the Department of Pediatrics and a member of the UCI's Pediatric Exercise and Genomics Research Center (PERC). She obtained her MPH in Epidemiology/Biostatistics from the University of California, Berkeley. She has worked in the Department of Pediatrics since 2002, providing statistical and data management support on many inter-disciplinary projects. Ms. Stehli's research areas include exercise physiology, physiology of stress, neurodevelopmental disorders, maternal and child health, human growth trajectories, intervention studies, and longitudinal data analysis. Ms. Stehli will manage the database, have access to subject identifiable data, and will be involved with data analysis. Ms. Stehli will not be involved in recruitment or the consent process.

Robert Warren, is a PhD Student at the Beckman Laser Institute. He will be responsible for the operation of the Laser Speckle Imaging system. He will be present during the fitness tests of the subjects and will perform blood flow measurements. Mr. Warren will have access to subject identifiable data. He may have interaction with research subjects and access to deidentified research data, but he will not have access to subject PHI and medical records.
He will not be involved in subject recruitment or the informed consent process. He may have interaction with research subjects and access to deidentified research data, but he will not have access to subject PHI and medical records.

Student Interns

Pearl Law is a Bio 199 student. Ms. Law will be assisting with training activities related to this study at PERC. Assistance in training includes timing the training rotations and making sure the subjects adhere to the assigned rotations; taking notes of the duration of various rotation, and observing the training as directed by the trainer. The trainers are definitely first aid/CPR certified. Please note that the student assistants will never be alone with the subjects during training, they are not in charge of designing the training rotations, they are simply assisting under the direct supervision of the trainer who is first aid/CPR certified and always present in the same room/suite. She will also be assisting with data entry. Ms. Law will have access to subject identifiable information. She will not be involved with study recruitment or the informed consent process.

Other Research Personnel - ICTS and PERC Affiliated Nurses

ICTS nurses have much experience in exercise testing with patients with underlying heart and lung disease. ICTS nurses will not be consenting participants..

Aletha Diane Capobianco, R.N. is a clinical nurse II at the ICTS and has had much experience with phlebotomy and screening procedures. Diane will have access to patient identifiable data, will take vital measurements, draw blood, and, if necessary, will administer the rescue bronchodilator.

Melanie Meton, R.N. is a clinical nurse II at the ICTS and has had much experience with phlebotomy and screening procedures. Melanie will have access to patient identifiable data, will take vital measurements, draw blood, and, if necessary, will administer the rescue bronchodilator.

Connie Parido, R.N. is a clinical nurse II at the ICTS and has much experience with phlebotomy and screening procedures. Connie will have access to patient identifiable data, will take vital measurements, draw blood, and, if necessary, will administer the rescue bronchodilator.

Other personnel not engaged in research*; performing duties related to their expertise; listed as personnel responsible for the medical supervision of subjects:

In addition to Dr. Cooper, **Dr. Chen** will be providing medical supervision during the fitness assessment. Drs. Chen is Board Certified Pediatricians who is a Post-Doctoral Fellow in the UC Irvine Department of Pediatrics Pulmonology program. As Board Certified Pediatricians (n.b., all Fellows accepted into the Program are Board Certified Pediatricians), they are well-experienced in the management of asthma as this is a common pediatric disease. Asthma care is a required component of all pediatric residencies. These are all individuals who are seeking additional training in the field of pediatric pulmonary medicine because of their devotion to pulmonary diseases in children. They are well qualified for the purposes outlined in this research. As part of their training, these pediatricians rotate through UC Irvine Medical Center, CHOC Children's Hospital, and Miller Children's Hospital. They spend two days per month as part of Dr. Cooper's Pulmonary Clinic at CHOC. In addition, their research time (approximately 12 months of the 3-year fellowship) is spent at UC Irvine.

SECTION 3: RESEARCH METHODOLOGY/STUDY PROCEDURES

A. Study Design and Procedures

1. Provide a **detailed chronological description of all study activities** (e.g., pilot testing, screening, intervention/interaction/data collection, and follow-up) and **procedures**.

Include an explanation of the study design (e.g., randomization, placebo-controlled, cross-sectional, longitudinal, etc.)

a. Indicate how much **time will be required of the subjects**, per visit and in total for the study.
b. Indicate the **setting where each procedure will take place**/be administered (e.g. via telephone, clinic setting, classroom, via email). ***Note:*** *If any of the procedures will take place at off-campus locations (e.g., educational institutions, businesses, organizations, etc)* ***Letters of Permission*** *are required.*
c. If a procedure will be completed more than once (e.g., multiple visits, pre and post survey), indicate **how many times** and the **time span** between administrations.

2. **For studies that involve routine (standard of care) medical procedures:** Make clear whether procedures are being done for clinical reasons or for study purposes, including whether the procedures are being done more often because of the study. Use the following guidelines to determine the extent to which standard procedures and their associated risks need to be described in protocol:
   a. If the standard procedure is not explicitly required by the study protocol, the protocol need not describe that procedure or its risks.
   b. If the standard procedure is a main focus of the study (e.g., one or more arms of a randomized study is standard) or is explicitly required by the study protocol, the protocol must include a full description of the procedure and its risks.]
3. It is **strongly recommended** that you include a table of visits, tests and procedures. Tables are easier to understand and may help to shorten long repeated paragraphs throughout the narrative.
4. If study procedures include collecting **photographs, or audio/video recording**, specify whether any subject identifiable information will be collected and describe which identifiers will be collected, if any.
5. Describe how the **subject's privacy will be protected** during the research procedures. ***Note****: This is not the same as confidentiality (see the Privacy and Confidentiality web page).*
6. Be sure to submit **data collection instruments** for review with your e-IRB Application (e.g., measures, questionnaires, interview questions, observational tool, etc.).

A summary of the research activities can be found in Table 1 at the end of this section.

This is a training program that can last up to 12 weeks and includes:

1. Testing the participants in the UCI Pediatric Exercise Research Center (PERC) (at the beginning and again at the end of the training program).
2. Ongoing training will continue outside of UCI with professional personal trainers
3. Collecting saliva to measure stress and inflammatory mediators (prior to the beginning, in the middle and again at the end of the training program).
4. Monitor exercise intensity levels in the field, during an exercise session and physical activity levels over full 7 days (weeks 1, middle of the training program and the last week of the training program).
5. Executive Function assessment (at the beginning and again at the end of the training program).
6. Dietary log book (at the beginning and again at the end of the training program).

Before and at the end of the training intervention the subjects will arrive to the Pediatric Exercise Research Center, for up to 3 sessions every time, to perform the following assessments:

Baseline assessments

1) Fitness Assessment Session. Participants will undergo a comprehensive clinical history and physical examination with determination of Tanner stage, vitals and anthropometric

measurements including DXA-determined body composition and bone density. Aerobic fitness will be assessed using incremental cycle-ergometer exercise test to determine peak VO2.

2) Exercise Challenge Session + blood draws.

3) Exercise Challenge Session + blood flow measurements.

The same sessions will be repeated for each subject once the training regimen is complete, and may be held in any order.

The Fitness Assessment Session and the Exercise Challenge Session will take place no less than 48 hours apart and no more than four weeks apart.

A portion of these visits may take place at the Beckman Laser Institute (BLI). The BLI is located next to the PERC/ICTS Exercise Lab. The BLI is providing numerous pieces of equipment that will be used for blood flow measurements.

Fitness Assessment Session-before and after the training program

- Measurements of height, weight, body composition (percent body fat), temperature and blood pressure. The PERC/ICTS staff has extensive experience in these measurements in children and great care is expended in stadiometer and scale calibration and use.
- Children will be asked to fill a standard self-assesssment Tanner staging questionnaire; this questionnaire is currently used by our group in several UCI IRB apporved studies, and a copy is appended to this application.
- Dual X-ray Absorbiometry (DXA) – Each participant will have to perform 2 DXA assessments: 1 DXA in week 1, prior to beginning the training sessions, and 1 DXA in week 12, after completion of 10 week training. For each DXA, subjects will undergo a total of 3 scans: a complete body scan, a hip scan, and spine scan. Total and regional body composition will be measured by dual-energy X-ray absorptiometry (DXA) using a Hologic densitometer. Measurement of body composition using DXA is based on the exponential attenuation due to absorption by body tissues of photons emitted at two energy levels (40 and 70 keV). Subjects will be asked to lie on their backs on a padded table wearing normal clothes with metal objects removed. The counter moves in a raster pattern above the subject's body from head to foot and counts attenuation rates of photons emitted from the X-ray source within the table. The total dose for a scan is less than several hours of background exposure (1-4 millirem per DXA scan). DXA provides estimates of the following parameters: bone mineral densities (BMD; g/cm2), soft-tissue attenuation ratios, fat and lean tissue weights (g), and bone mineral weight. These parameters can be recorded for the whole body or in any number of defined anatomical regions of interest. DXA scanning involves x-ray dose at minimal levels. Technicians do not wear shielding, nor do subjects require shielding during the procedure. Female subjects capable of having children (regardless of age) will be asked to provide a small urine sample prior to the DXA scan. Results of the pregnancy test will be disclosed to either the parent or the legal guardian with the child's permission and/or the participant for subjects less than 18 years of age. Results of the pregnancy test will be disclosed to female subjects 18 years of age or older.
- Exercise testing will be performed at the UCI PERC/ICTS Applied Physiology/Human Performance Core Laboratory. To measure cardiorespiratory responses to exercise and assess fitness, we will use a ramp-type progressive exercise test on an electronically braked cycle ergometer (SensorMedics Ergoline 800S, Yorba Linda, CA) (1), through a method previously designed for children and adolescents (2). The work rate will increase by 10 W per min (or adjusted according to the subject's age and fitness level) so that the total exercise time will roughly equal 8-12 min, and each subject will exercise to the limit of his or her tolerance. During the exercise, the participant will breath through a mouthpiece, a noseclip will be worn, and measurements of their breath will be taken. Gas

exchange will be measured breath-by-breath using a metabolic cart (SensorMedics VMax 229, Yorba Linda, CA). The metabolic cart measures inhaled as well as exhaled carbon dioxide and oxygen by use of a mouthpiece and noseclip allowing gas exchange measures in the breath. After exercise, participant's heart rate will be monitored until it reached pre-exercise values. If any faintness or dizziness is reported, participants are asked to lay down and are then allowed to leave after 30-min of cessation of symptoms. Gas exchange will be measured breath-by-breath (3). Multiple Pulmonary Function Tests (PFT's) will be performed prior to and following the exercise challenge. This approach has been used extensively in our laboratory in children, adolescents and adults.

- Diet: During the week of physical activity evaluation, participants will complete a three-day food record, to be analyzed by the UCI PERC bionutrition using the Nutrition Data Sytsem for Research (University of Minnesota, Minneapolis, MN), a specialized software containing a nutrient profile of over 18,000 foods.

Exercise Challenge Session and Blood Draws--before and after the training program
The trainers are aware that they are responsible for the children's safety. All parents will be strongly encouraged to attend the training session with their child. The trainer is aware of abuse reporting procedures.

- The exercise session will occur no less than 48 hours and no later than 4 weeks apart from the **Fitness Assessment Session.** . Participants will arrive in the morning after an 8 hour fasting. Participants will be able to continue to use their long-term asthma control medications during the period in which they are required to fast. An indwelling catheter will be placed in an antecubital vein of each subject by trained physicians and/or nurses with specific experience in pediatric procedures of this nature. If requested, a cream will be used to numb the site for the catheter placement to reduce discomfort. The indwelling catheter will allow multiple blood draws without the possible adrenergic stimulation associated with multiple needle sticks.
- After a 20 min rest, PFTs and baseline blood draw will be acquired and exercise can begin. Exercise will consist of a series of 10, 2-min bouts of constant work rate cycle ergometry with 1-min resting intervals between each exercise bout. The work rate will be individualized for each subject, and equivalent to the work rate corresponding to 50% of the difference between the anaerobic or lactate threshold (LT--determined non-invasively from the ramp test) and peak VO2. We use this approach to ensure that the exercise input is standardized to physiological indicators of each individual subject's exercise capability.
- During exercise and for the 60 min following exercise additional blood samples will be acquired. The total amount of blood withdrawn at each timepoint will be up to 80 ml, or ~ 5 tablespoons for children and up to 160 ml for adults (no more than 5ml/Kg), amounts we routinely collect in children and adults without causing discomfort. If any faintness or dizziness is reported, participants are asked to lay down and are then allowed to leave after 30-min of cessation of symptoms. Collected blood samples will be anlyzed for glucoreguolatory hormones, pro-/anti-inflammatory cytokines, growth factors, markers of oxidative stress, gene and microRNA expression studies and leukocytes functional assays.
- Assessment of Exhaled NO: Participants will be asked to blow for approximately 5 seconds before and after exercise for analysis of the composititon of expired gases found in the breath. As noted, our Co-Researcher has extensive experience in the measurement of NO in the exhaled breath in healthy controls and asthmatics. Assessment of exhaled breath NO represents a novel and exciting approach toward understanding airway inflammation.

Whenever exercise tests are being conducted on participants with asthma, there is always a licensed physician present at the center. Should a participant experience an asthma attack during the exercise testing, the participants will respond as they have been prescribed by their personal

physician. As a precaution, the on-site physician will be the one to provide the order for the bronchodilator (if need) before it is administered to the participant.

Measurement of Endothelial Function- Blood Flow Measurments
Both visits described above will make use of a variety of non-invasive optical measurements of endothelial/vascular function and tissue oxygenation. These measurements are described below, and will take place during both fitness and a second exercise challenge sessions.

- A non-invasive measurement of endothelial function (blood flow and level of blood oxygenation measurments) will be performed with a modified hyperemic test. This is a very simple procedure in which a laser doppler sensor is applied to the volar aspect of the hand, at the 1st finger distal metacarpal surface. The cuff of a blood pressure machine is then placed around the wrist, and a computer-controlled manometer inflates the cuff to 200 mmHg, and then rapidly releases it. After the cuff is deflated, blood flow in the hand transiently increases, and the characteristics of this increases in flow (amount of peak flow, rapidity to reach this peak, etc.) are related to the health of the vascular system, and can be accurately measured by the laser doppler sensor. This measurement will occur during testing at the PERC/ICTS Exercise Lab, but NOT during exercise.
- An additional measurement modality we will use is Laser Speckle Imaging (LSI). This is a device that has been developed at the Beckman Laser Institute, and it consists of a non-contact single-wavelength light source coupled to a CCD camera. The laser will be projected on the participant's hand, and the reflected light will be collected by the camera. Using this technique, we will be able to record and analyze local heterogeneities in blood flow in the skin microcirculation. This data will be compared to the results obtained by the laser doppler sensor. This measurement modality will only be used at Beckman Laser Institute, and NOT during exercise
- In addition to the Periflux system, we will perform measurements of tissue content of hemoglobins, water, and lipids using Diffuse Optical Spectroscopy (DOS), a system developed at the Beckman Laser Institute of UC-Irvine This instrument uses light in the near-infrared range of wavelengths to measure tissue optical properties in a safe, non-invasive manner. The pulse oximeter is a similar commercial instrument that has found wide clinical acceptance. This optical device uses two wavelengths, one red and the other near-infrared, to measure the arterial hemoglobin saturation (SaO2) of blood. DOS probe will be placed on the volunteer's upper extremity, lower extremity, or abdomen for a short duration (less than 5 minutes) before, during, and after exercise. DOS will be used to monitor tissue optical properties both during exercise testing (on the upper extremity, lower extremity, or forehead) at the PERC/ICTS Exercise Lab, and in static conditions at BLI (on the arm, leg, or abdomen).
- Body composition (% body fat) will be assed via portable bioelectrical impedance assessment (InBody720), a commonly used method for estimating body composition, and in particular body fat. This portable device requires the subject to stand on the machine while measures of body mass, percent body fat and lean body mass are being done. Age and height will be inputted into the machine, followed by a 2-minute analysis in which the child simply stands on the machine.
- There are two devices that will be used that are considered DOS devices. One is the above-described BLI-fabricated device. Another is the TRS-20, developed by Hamamatsu Photonics. It works on similar principles, and will be used in the same way as described above.

Training Sessions

Training will continue in community venues and in the Pediatric Exercise Research Center with professional personal trainers with a minimum of Bachelor degree in a Wellness/Exercise Science related field, P.E teachers or personal trainers certified by an accredited fitness

organization such as ACSM, NSCA, NASM or ACE. The training sessions will occur three times a week up to 10 weeks, in small groups of 3-6 subjects.

Those who participate in this study will have been cleared to participate in all sports, including school P.E. and extramural sports such as club soccer and basketball. In schools, children with asthma must have a release form for the use of a bronchodilator use when needed. These medicines can be determined by school officials. Increasingly, schools are permitting students to carry medication themselves.

The parent/legal guardian of the child participant will be instructed to bring his/her child's bronchodilator with them to the exercise training session (for adult participants, they will be instructed to bring their bronchodilator to the exercise training session). Before each training session begins, the trainer will ensure that the participant has his/her bronchodilator readily available prior to beginning exercise activities. Should an exercise-induced bronchonstriction episode occur, participants will be instructed to respond as they have been prescribed by their personal physician. If necessary, the trainer will contact 9-1-1. All personal trainers will be required to have completed CPR certification (BLS).

Included in the training of our professional personal trainers will be for them to be aware of and familiar with asthma signs and symptoms (please note that this is more training that coaches typically receive). All trainers will tell the participant to let him/her know if they are experiencing symptoms. Before each training session begins, the trainer will ensure that the participant has his/her bronchodilator readily available prior to beginning exercise activities. Should an exercise-induced bronchonstriction episode occur, participants will be instructed to respond as they have been prescribed by their personal physician. If necessary, the trainer will contact 9-1-1. All personal trainers will be required to have completed CPR certification (BLS). Additionally, we will have an on call physician that can be reached if any problems occur or questions arise. Parents will be encouraged to accompany their children during the exercise session and remain near. **If the participant becomes uncomfortable, they will be instructed to stop the exercise at any point.** Trainers will be instructed not to push the children if they feel uncomfortable at any time.

If any emergencies should occur during these training sessions, the personal trainer will be instructed to call 9-1-1.

Training Program

The exercise training sessions will include 5 minute warm up, 5 minute cool down (at the end) and a combination of aerobic and resistance exercises. In weeks 1 and 2 aerobic exercises will be performed for 15-20 minutes and will include the utilization of an ergometer (e.g., stationary bicycle, treadmill, Stairmaster and rowing machine) and or playing small group games, stationary punching bag and adapted ball games (e.g., basketball, soccer, badminton). The duration of the aerobic exercises will be gradually increased and will get up to 45 minutes by weeks 8-10. In addition to the aerobic exercises, participants will be performing resistance exercises (e.g., light free weights, band exercises, pushups, and pull-ups). These exercises will be done in sets, starting from law number (4-6) of repetition within each set, and will gradually increase to 10-12 repetition in a set. The number of sets will also be gradually increased based on the participant strength. Resistance exercises will take approximately 20-25 minutes in the first few weeks and gradually decrease in time in parallel to the increase in time for the aerobic exercises. Each training session will last approximately an hour.

Collecting saliva to measure stress and inflammatory mediators:

Weeks one, in the middle of the training program and the last week of training

The hypothalamic-pituitary-adrenal (HPA) axis is a major homeostatic system which under basal conditions maintains a circadian rhythm and is activated in response to stress. Measurement of salivary cortisol has provided a great deal of experimental information on activity of the HPA axis in health and disease and on the involvement of cortisol in the mechanisms determining our reactivity and adaptation to environmental changes. A relatively noninvasive technique, salivary sampling has the dual advantage over blood sampling of (i) permitting cortisol to be measured without contamination of results by needle stress, and (ii) extending investigation beyond experimental laboratory studies through the ease of home sampling (4).

Saliva will be collected 3 times a day (3 samples) over two consecutive days (total of 6 samples). Two baseline values followed by one additional sample of salivary collections: T0-In bed: when waking up before brushing teeth, T2, 30 min after waking up and before brushing teeth, and T3-before dinner. This will be repeated the following day. Saliva collection kits will be sent home. Parents/guardians will assist their children to collect the samples.

We will also collect saliva before and after the exercise challenges that will be performed at PERC/ICTS Performance Lab, at the beginning and the end of the program.

Assessment of physical activity over 7 days:

This will occur during week one, in the middle of the training program and in the last week of training program.

Actigraph assesses movements resulting from muscle force as well as accelerations due to changes in the position of the sensor in the gravitational field (5). Actigraphy has become a valuable clinical and research tool to objectively evaluate sleep, daytime activity, and circadian activity rhythms in health and disease (6, 7).

Actigraphs will be given by the trainers in the training session at the beginning, middle and the end of exercise program. After the training session, subjects will take the ActiGraphs home and put them on for 7 days. Actigraphs are worn on the waist with an elastic band. Subjects /parents will be asked to fill activity diaries for each of the seven days, recording the time they woke up, the time they went to bed, and time they removed the actigraph. As well as major activity events during the day (e.g., PE in school, soccer, taking a walk, biking, etc.)

Assessment of exercise intensity during an exercise session

Heart rate monitors (watches worn on the wrist) will be used by the trainers during the training sessions to asses exercise intensity.

Executive Function assessment

Executive Function assessment will be done using Hearts & Flowers tasks (Adele Diamond Laboratory, 8, 9). These executive function tasks are administered via touch-screen computers and will require 5 minutes per participant. See http://www.devcogneuro.com/eftasks.html for detailed descriptions of tasks. Dr. Kimberley Lakes will supervise the assessment before, in the middle and after the training program.

Using the Blood Donor Program

In order to set up the leukocyte genomic and functional assays and for cell culture experiments that are looking at the leukocyte genomic and functional response to various stimuli, we will use human whole blood from healthy individuals that will be drawn by ICTS nurses as part of the UCI ICTS Blood Donor Program. No code linking back to identifiers will be created or accessed.

Table 1. Study Overview—Timeline, Measures, Activities, and Outcome Variables

Time	*Measure/Activity*	*Description*
Weeks 1		
PERC/ICTS Lab: Fitness Assessment (Approximately 2 hours)	Clinical Assessment	• Clinical History of Asthma • Asthma questionnaire • Vitals and anthropometric measures (blood pressure, temperature, height and weight) • Pubertal stage (by questionnaire), and pregnancy test for eligible females.
	Progressive Exercise Testing	• Ramp type protocol to asses peak VO_2 (aerobic fitness) • Pre- and post-exercise Exhaled NO measurements • Pre- and post-exercise PFTs • Pre- and post-exercise blood flow measurements • Pre- and post-exercise saliva collection
	DXA	• Body composition (lean and fat mass)
	Executive Function Test	• Executive function tasks – 5 min of specific computer games
PERC/ICTS Lab: Exercise Challenge(separate at least 48 hours from the Fitness Assessment visit) (Approximately 3 hours)	Exercise Challenge on a cycle ergometer (10 x 2-min bouts @ ~70% peak work rate, with 1-min intervals)	• Blood draw* (before, immediately after, and one-hour after the exercise challenge). • Pre- and post-exercise Exhaled NO measurements • Pre- and post-exercise PFTs • Pre- and post-exercise saliva collection
PERC/ICTS Lab: Exercise Challenge (Approximately 2 hours)	Exercise Challenge on a cycle ergometer (10 x 2-min bouts @ ~70% peak work rate, with 1-min intervals)	• Pre- and post-exercise blood flow measurements • Pre- and post-exercise PFTs
At Home	Nutritional Assessment	24-h food recalls over 3 days
Week 2		
At Home	Actigraph	• Assessment of physical activity over 7 days
		•
At Home	Saliva Collection	• 3 times a day (3 samples) over two consecutive days
Weeks 2-11		
In the Field (Approximately 1 hour per session for up to 30 sessions)	Exercise-Training	• Personal trainers, small-groups, community venues, tailored training sessions x 3 weekly

	Compliance	• Attendance at exercise sessions • Exercise intensity will be assessed using heart rate (HR) monitors
Week 6-7		
At Home	Actigraph	• Assessment of physical activity over 7 days
At Home	Saliva Collection	• 3 times a day (3 samples) over two consecutive days
Week 11		
At Home	Actigraph	Assessment of physical activity over 7 days
At Home	Saliva Collection	3 times a day (3 samples) over two consecutive days
Weeks 12		
PERC/ICTS Lab: Fitness Assessment (Approximately 2 hours)	Clinical Assessment	• Clinical Evaluation • Vitals and anthropometric measures (blood pressure, temperature, height and weight)
	Progressive Exercise Testing	• Ramp type protocol to asses peak VO_2 (aerobic fitness) • Pre- and post-exercise Exhaled NO measurements • Pre- and post-exercise PFTs • Pre- and post-exercise blood flow measurements • Pre- and post-exercise saliva collection
	DXA	• Body composition (lean and fat mass)
	Executive Function Test	• Executive function tasks – 5 min of specific computer games
PERC/ICTS Lab: Exercise Challenge(separate at least 48 hours from the Fitness Assessment visit) (Approximately 3 hours)	Exercise Challenge on a cycle ergometer (10 x 2-min bouts @ ~70% peak work rate, with 1-min intervals)	• Blood draw* (before, immediately after, and one-hour after the exercise challenge). • Pre- and post-exercise Exhaled NO measurements • Pre- and post-exercise PFTs • Pre- and post-exercise saliva collection
PERC/ICTS Lab: Exercise Challenge (Approximately 2 hours)	Exercise Challenge on a cycle ergometer (10 x 2-min bouts @ ~70% peak work rate, with 1-min intervals)	• Pre- and post-exercise blood flow measurements • Pre- and post-exercise PFTs
		•
At Home	Nutritional Assessment	• 24-h food recalls over 3 days
		•

*Primary Outcome Variable: Gene expression in Leukocytes

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B. Statistical Analysis Plan

1. **Variables of Interest** - Clearly identify the primary outcome(s) and key factor(s) of interest.
2. **Statistical goal** - State the statistical goal(s) of the study (e.g., Comparison of group means, estimation of the proportion of success, estimation of variability for future study design, etc.).
3. **Statistical approach** - Describe the statistical approach(es) to be used to address the study's statistical goal(s) (e.g., T-test to compare means, confidence interval estimate(s), etc.). ***Note:*** *Required for ICTS SRC review.*
4. **Secondary analyses** - Clearly state any secondary analyses to be performed including secondary outcomes and comparison groups along with the statistical methods that will be used to perform the secondary analyses. ***Note:*** *Required for ICTS SRC review.*

If a statistical analysis plan is not appropriate for your study design, please describe a non-statistical plan for assessing your study results.

Aim 1: *Elucidate key abnormal genomic and epigenetic responses to brief exercise in leukocytes of children and adults with moderate-to-severe asthma.*

Aim 2: *Identify the effect of training on the key genomic and epigenetic exercise response mechanisms in leukocytes in children and adults with moderate-to-severe asthma.*
Genomic expression profile is the **primary outcome variable** for Aims 1 and 2. Gene expression data will be normalized, log-transformed, and quality-checked and filtered across all arrays from all study subjects. Pre- to end-exercise change and before- to after-training change will be calculated for each subject and each probe set. Probe sets that pass filtration and belong to

selected functional pathways will be examined for group difference using 2-sample t-test and further adjusted with false discovery rate (FDR) controlling adjustments as described by Benjamini and Hochberg.[150]

Leukocyte cell-function profile is the **secondary outcome variable** for Aims 1 and 2. Each outcome variable will be treated as an individual hypothesis. The primary analysis will apply 2-samplet-test to examine group difference in exercise effect and in training effect. The secondary analysis will utilize mixed model to evaluate group difference in training and exercise effect together, adjusting for possible confounding factors such as gender, BMI percentile, and fitness level.

Aim 3: *Assess the clinical impact of exercise training in children and adults with persistent asthma on: (a) exercise induced airway inflammation, (b) asthma control, and (c) cardiorespiratory fitness and muscle adaptation.*

Outcomes from Aim 3 are considered as secondary outcome variables. We will apply paired t-tests for before- to after-training change. Furthermore, we will employ linear regression model to examine the effect of covariates such as gender, asthma severity (ACQ), and lean muscle tissue response to exercise-training (as determined by DXA).

SECTION 4: SUBJECTS (PERSONS/CHARTS/RECORDS/SPECIMENS)

A. Number of Subjects (Charts/Records/Biospecimens)

1. Indicate the **maximum number of subjects to be recruited/consented** on this UCI protocol. This is the number of potential subjects you may need to recruit to obtain your target sample size. This number should include projected **screen failures and early withdrawals**. ***Note:*** *The IRB considers individuals who sign the consent form to be "enrolled" in the research.*
2. For **Mail/Internet surveys** include the number of people directly solicited.
3. If the study involves use of **existing charts, records, biospecimens**, specify the maximum number that will be reviewed/tested to compile the data or the sample population necessary to address the research question.

The maximum number of subjects to be recruited/consented on this protocol is 240. This number includes screen failure and early withdrawals (see table 2 for breakdown in subjects for each group).

4. Of the maximum number of subjects listed above, indicate the **target sample size** for the study. This is the number of subjects expected to complete the study or the number necessary to address the research question.
5. *For social/behavioral research*, the maximum sample size is often similar to the target sample size. If the **maximum sample size** is significantly greater (i.e., ≥ 1.5x) than the **target sample size** provide a justification.
6. For studies where multiple groups of subjects will be evaluated, **provide a breakdown per group** (e.g. controls vs. experimental subjects; children vs. adults; by age group).

Of the maximum number of participants, our target sample size is 200. Below is a table with a breakdown in the number of participants per group.

Table 2: Subject number break down per group for maximum number to be recruited and target number of participants.

	Maximum to be Recruited			Target Participants	
	Control	Asthma		Control	Asthma
Mid and late pubertal boys (Tanner 3-5) age 8 - 17 years	35	35		30	30
Mid and late pubertal girls (Tanner 3-5) age 8 - 17 years	35	35		30	30
Young adult women aged 18-35	25	25		20	20
young adult men aged 18-35	25	25		20	20
Total number of subjects	**240**			**200**	

7. For **multi-center research**, indicate the overall sample size for the entire study (across all sites).

[X] Not applicable - This study is not a multi-center research study.

8. Explain **how the overall target sample size was determined** (e.g., power analysis; precision estimation).
9. Demonstrate that the **target sample size will be sufficient** to achieve the study goal and should coincide with the statistical approach **described in Section 3B**.
10. **Sources and information** of assumed group effects and variability should be supplied (e.g., pilot data; data from related literature).

To accomplish our aims, we will recruit 70 asthmatics and 70 gender-, age- and fitness-matched controls (as noted, below the 50th percentile of appropriate normative data) to participate in the training course. Given an anticipated dropout rate (based on our experience in recruiting subjects in this region) of about 10-15%, we expect to have complete samples around 60 participants in each group of children. The power analyses were based on PASS 2008 (Hintze, J. (2008). NCSS, LLC. Kaysville, Utah. www.ncss.com).

For the adult groups we will recruit 50 asthmatics and 50 gender, age and fitness-matched controls. We expect to have complete samples around 40 participants in each group.

For the primary outcome in gene expression, we will include about 3000 probe sets belonging to functional pathways of interest in asthma and exercise, as described above and in the Preliminary Studies Section. After filtration, around 1000 to 2000 probe sets will be left for analysis. Table 3 in the Human Subjects Section shows the power and effect size that can be detected from microarray data using a 2-sided 2-sample t-test for a range of: (1) probe sets **to examine** and (2) significant probe sets **to detect** (with FDR at 0.05 level). With the same power, a smaller effect size can be detected with a larger sample size. Effect size is defined as the expected log-transformed group difference divided by the within-group standard deviation. Our previous data showed that about 5% of the probe sets have an effect size over 0.9.

Table 4 shows the power analysis for the secondary outcome variables (e.g., in Aim 1, leukocyte function; in Aim 2, change in exhaled NO after the exercise challenge). The effect size is defined as the expected group difference divided by the within-group standard deviation for 2-sample t-test and as the mean difference between before- and after-training divided by the standard deviation of the difference for paired t-test.

Table 3. Power Analysis for the Primary Outcome Variables

Number of Probe Sets to Examine		1000				2000			
Number of Probe Sets to Detect		10	50	100	300	10	50	100	300
Sample Size	Power	Effect Size							
40 per group	90%	1.1	1.0	0.9	0.8	1.2	1.0	1.0	0.9
	80%	1.0	0.9	0.8	0.7	1.1	1.0	0.9	0.8
	70%	1.0	0.8	0.8	0.7	1.0	0.9	0.8	0.7
60 per group	90%	0.9	0.8	0.8	0.7	0.9	0.8	0.8	0.7
	80%	0.8	0.7	0.7	0.6	0.9	0.8	0.7	0.7
	70%	0.8	0.7	0.6	0.5	0.8	0.7	0.7	0.6

Table 4. Power Analysis for the Secondary Outcome Variables

	Sample size per group for 2-sided 2-sample t-test			Sample size for 2-sided paired t-test		
	40	50	60	40	50	60
Power	Effect Size			Effect Size		
70%	0.562	0.502	0.457	0.403	0.358	0.326
80%	0.634	0.566	0.516	0.454	0.404	0.368
90%	0.734	0.665	0.597	0.526	0.468	0.425

B. Inclusion and Exclusion Criteria

1. Describe the **characteristics and provide justification** for inclusion of the proposed subject population. At a minimum include information about the age and gender of the study population.
2. Describe **different subject groups** (e.g., students and teachers; control group and treatment group(s), children and adults) separately.

The study population will consist of 60 children with Asthma (boys and girls, 8-17y/o) and 40 adults with Asthma (men and women, 18 yrs -35y/o) who: have been determined to be in good health by pre-participation sports physical perfomed at PERC, have no evidence of disease or disability that would impair participation in a vigorous physical activity program, have been diagnosed with persistent asthma according to criteria by NHLBI, have a clinical history of suggesstive of exercise induced bronchoconstriction, have less than 50% fitness as determined from our own normative data collected in our lab (N=95), and with BMI percentile < 95^{th} .

The control groups will be age, gender, and fitness matched with asthmatic participants, and consist of 60 children (boys and girls, 8-17yrs) and 40 adults (men and women, 18yrs -35y/o) who have been determined to be in good health by preparticipation history and physical examiniation performed at PERC, have no evidence of disease or disability that would impair performance of cycle ergometer exercise, and BMI< 95^{th} percentile.

3. Provide the **inclusion and/or exclusion criteria** for the proposed subject population, as applicable.

[ ] Not applicable – This is not a clinical investigation and/or the characteristics of the population sufficiently describe the proposed subject population.

Inclusion criteria Subjects with Asthma

- Determined to be in good health by preparticipation history and physical examination performed at PERC
- No evidence of disease or disability that would impair participation in a vigorous physical activity program
- Diagnosed with persistent asthma according to criteria by NHLBI
- Clinical history suggestive of exercise induced bronchoconstriction
- VO2max <55 ml O2/kg/min and habitual physical activity corresponding to lifestyles from sedentary to moderately physically active, with exclusion of intense physical training
- BMI less than 95th percentile

Exclusion criteria for Subjects with Asthma

- Asthmatic participants who experience more than **two** asthma attacks a week
- Asthmatic participants who have been admitted to the ER/hospitalized due to an asthma attack more than **two** times in the past month
- If there is any evidence that the participant (adult and child) or that the child's/family is unfamiliar with the bronchodilator or any other asthma medications.
- Use of illegal drugs or abuse of alcohol
- Other conditions that preclude exercise (such as neuromotor disease, heart disease, major endocrine abnomalities or any other condition that would prevent a child from participating in vigorous physical activity for a training program)
- Very high physical fitness (>55 ml O2/kg/min VO2max) or habitual physical activity, corresponding to an elite athlete training regimen.
- Pregnancy or breastfeeding

Inclusion criteria for Control Subjects

- Determined to be in good health by preparticipation history and physical examination performed at PERC.
- No evidence of disease or disability that would impair performance of cycle ergometer exercise.
- VO2max <55 ml O2/kg/min and habitual physical activity corresponding to lifestyles from sedentary to moderately physically active, with exclusion of intense physical training
- BMI less than 95th percentile

Exclusion criteria for Control Subjects

- Regular use of anti-inflammatory medications parenterally, orally, or as inhaled agents.
- Regular use of bronchodilators
- Use of illegal drugs or abuse of alcohol
- Very high physical fitness (>55 ml O2/kg/min VO2max) or habitual physical activity, corresponding to an elite athlete training regimen
- Pregnancy or breastfeeding

4. If **exclusion** is based on age, gender, pregnancy/childbearing potential, social/ethnic group, or language spoken (e.g., Non-English Speakers), **provide a scientific rationale**.

This study will be limited to Children aged 8-17 years and Young Adults aged 18-35. The choice of studying young children/adolescents is due to the lack of information regarding the effect of acute bout of exercise and exercise training, in this specific age group on leukocyte function, genomic, and epigentic responses. Few studies have been performed on adults looking at some aspects that we cover in this study, but results may not apply to children/adolescents, who present marked differences with adults. The young adults are going to serve as a reference group to those in 8-17 age bracket.

The effects of DXA to an unborn fetus, should a female subject be pregnant, are unknown, therefore pregnant individuals will be excluded.
To ensure protection of the subjects rights and safety, consent and assent forms will be carefully explained to them and to their authorized representatives. The study team has extensive experience in working with children and adults. It is also explicitly stated in the consent forms that participation in this protocol is voluntary.

An equal number of male and female participants will be included in the asthmatic and healthy groups. Different ethnic/racial groups will be included attempting to reproduce their respective distribution within the Orange County population, as per the latest report from the US Census Bureau. English and Spanish speaking subjecst will be recruited.

SECTION 5: RECRUITMENT METHODS AND PROCESS

A. Recruitment Methods

Please check **all** applicable recruitment methods that apply to the study. Place an "**X**" in the bracket **[ ]** next to the recruitment method.

[ ] This study involves no direct contact with subjects (i.e., use of existing records, charts, specimens)
- ***Skip to Section 6.***

[X] UCI IRB approved advertisements, flyers, notices, and/or media will be used to recruit subjects. ***Submit advertisements for IRB approval.***
- Passive Recruitment - Potential subjects initiate contact with the study team.
- ***Complete Question 5B - Explain where recruitment materials will be posted.***

[ ] The study team will recruit potential subjects who are unknown to them (e.g., convenience sampling, use of social networks, direct approach in public situations, random digit dialing, etc.)
- Active Recruitment – Researchers contact potential subjects.
- ***Complete Question 5B.***

[ ] The UCIMC Clinical Trials web page will be used. ***Submit the UCIMC Standard Research Recruitment Advertisement for IRB approval.***
- Passive Recruitment - Potential subjects initiate contact with the study team.
- ***Skip to Section 6.***

[ ] The study will be listed on Clinicaltrials.gov. ***Note:*** *This is required for all clinical trials.*

- Passive Recruitment - Potential subjects initiate contact with the study team.
- **Skip to Section 6.**

[ ] The UCI Social Sciences human subject pool will be used. ***Submit the Social Science Human Subject Pool Recruitment Advertisement for IRB approval.***

- Passive Recruitment - Potential subjects initiate contact with the study team.
- ***Skip to Section 6.***

[ ] Study team members will contact potential subjects who have provided permission to be contacted for participation in future research studies.

- Active Recruitment – Researchers contact potential subjects.
- ***Complete Question 5B – Explain when and how these individuals granted permission for future contact; provide the IRB protocol numbers, if applicable.***

[ ] Study team members will approach their own patients, students, employees for participation in the study.

- Active Recruitment – Researchers contact potential subjects.
- ***Complete Question 5B.***

[X] Study team members will send UCI IRB approved recruitment materials (e.g., recruitment flyer, introductory letter) to colleagues asking for referral of eligible participants.*

- Passive Recruitment – Potential subjects initiate contact with the study team or
- Active Recruitment – Colleagues get permission from interested individuals to release contact information to researchers. Researchers contact potential subjects.
- ***For Active Recruitment, complete Question 5B.***

**Note:* *Additional requirements for using this recruitment method are included in the Protocol Narrative instructions.*

[ ] Study team members will provide their colleagues with a UCI IRB approved introductory letter. The letter will be signed by the treating physician and sent to his/her patients to inform them about how to contact study team members.

- Passive Recruitment - Potential subjects initiate contact with the study team.
- The IRB approved letter must be sent by the treating physician.
- The study team does not have access to patient names and addresses for mailing.
- ***Skip to Section 6.***

[ ] UCI study team members will screen UCIMC medical records to determine subject eligibility and approach patients directly about study participation.*

- Active Recruitment – Researchers contact potential subjects.
- ***Complete Appendix T to request a partial waiver of HIPAA Authorization.***
- ***Complete Question 5B.***

* **Note** *Additional requirements for using this recruitment method are included in the Protocol Narrative instructions.*

[ ] Other Methods: <indicate the recruitment method(s) here>

- ***Complete Question 5B, as applicable.***

B. Recruitment Process

1. Based on the methods checked above, describe and provide **details of the recruitment process** (i.e. when, where, by whom and how potential subjects will be approached, e.g. screening medical charts, findings subjects during routine patient visits, etc.).
2. If you will recruit by mail, e-mail, or phone, explain how potential subjects' **contact information will be obtained**.
3. If active recruitment methods will be used (i.e., researchers will make direct contact with subjects for the purpose of recruitment), explain how the individual's **privacy will be protected**. ***Note:*** *This is not the same as confidentiality (see the Privacy and Confidentiality web page).*

Participants will be recruited through electronic ads, flyer distribution, and through other studies being conducted on campus. Specifically, electronic ads will be placed in the UCI E-mail list-server. Flyers and provision of direct information to potential subjects will take place in schools and asthma clinics, pediatric endocrinology clinics of the UCI Medical Center, the Children's Hospital of Orange County, Harbor-UCLA General Hospital, the Orange County Kaiser pediatric endocrinology practice, and local health associations such as Latino Health Access and P.A.D.R.E. Flyers will also be placed in the waiting room at the ICTS in Orange and at Hewitt Hall (main campus). Lastly, , personnel for other studies utilizing the same study population may be provided with flyers to hand to their participants and study participants can then call if they would like more information about this study.

SECTION 6: INFORMED CONSENT PROCESS

1. Specify **how consent will be obtained** and describe the specific **steps for obtaining informed consent**.
2. Include information about **when and where** consent will take place and the **length of time** subjects will be given to decide whether they wish to participate.
3. If study team members will approach their own patients, students, or employees for participation in the study, explain what precautions will be taken to **minimize potential undue influence or coercion**, and **how compromised objectivity will be avoided**.
4. If children are involved in this study, please describe the **parental permission** process and the **child assent** process.
5. Be sure to **submit the consent/assent document(s)** with your e-IRB Application (i.e. Study Information Sheet, Recruitment script, Consent Form, etc.).
6. If this study involves the creation, use, or disclosure of Protected Health Information (PHI), specify the process for **obtaining HIPAA Authorization**. Be sure to submit the HIPAA Research Authorization form with your e-IRB Application.

Check all that apply:

[X] Written (signed) informed consent will be obtained from subjects. Signed informed consent, parental permission, and/or child assent will be obtained from subjects, as applicable. ***Describe the informed consent process.***

[ ] **Requesting a waiver of written (signed) informed consent** (i.e., signed consent will not be obtained). Informed consent, parental permission and/or child assent will be obtained from subjects, as applicable. **Explain how informed consent will be obtained.** ***Complete Appendix P.***

[ ] **Requesting a waiver of informed consent** (i.e., consent will not be obtained). ***Complete Appendix O. Skip to Section 7.***

Potential participants will be informed of the project and its goals by key personnel. For adolescents and children, consent will be obtained from their parents or legal guardians and assent will be obtained from the children themselves. Our staff will meet with potential volunteers either at the Pediatric Exercise Research Center or at one of the two ICTS clinical centers. We meet with potential volunteers and their parents/guardians in a private consulting room where there is no disturbance or distraction from other ongoing clinical activities. Each child and appropriate guardian will meet with designated study staff and the protocol will be explained in detail. It is our experience that this process requires about 20-30 minutes. We then ask the potential study volunteer and his or her guardians to review the written Consent form. Our staff indicates that they will be outside the consulting room should the child or guardian(s) have questions. This process usually takes about 10 minutes. We then ask the potential volunteer and guardians if they have additional concerns or questions and whether or not they wish to participate in the study. The whole process typically takes about 45 minutes.

The ICTS Clinical Research Center is modeled after a doctor's office. There is a comfortable waiting area with a receptionist who monitors information flow in the unit. Names of individuals are used discreetly by the receptionist. All charts remain in the control of the receptionist, nurses, physicians and key personnel.

The study team will comply with the HIPAA obligations associated with this study and will attach the "Authorization for Release of Personal Health Information (Protected Health Information) for Research Purposes – Exercise Training in Children and Adults: Mechanisms of Allaying Inflammation in Asthma' and, if applicable, the "Authorization for Release of Personal Health Information (Protected Health Information) for Research Purposes – **Optional Studies**" to the adult as well as parental informed consent document when consenting subjects".

7. **Non-English Speaking Participants:** In order to consent subjects who are unable to read and speak English, the English version of the consent form must be translated into appropriate languages once IRB approval is granted.

Check all that apply:

[ ] Not applicable - Only individuals who can read and speak English are eligible for this study.

[X] The English version of the consent form will be translated into appropriate languages for non-English speaking subjects once IRB approval is granted. An interpreter will be involved in the consenting process. ***Note:*** *The IRB must officially stamp the translated consent forms.*

[ ] Requesting a short form consent process. ***Complete Appendix Q.***
The short form process will be used for the following languages:
[ ] All non-English languages
[ ] All non-English languages except Spanish
[ ] Other languages (specify): <Type here>

SECTION 7: RISK ASSESSMENT AND POSSIBLE BENEFITS

Note: Review of the instructions for this section is strongly recommended.

A. Risk Assessment

Place an "**X**" in the bracket **[ ]** next to the level of review (based upon the investigator's risk assessment).

[X] This study involves greater than minimal risk to subjects and requires **Full Committee review.**

[ ] This study involves no more than minimal risk and qualifies as **Expedited research**. ***Provide justification below for the level of review and for the applicable Expedited Category(ies) that you have chosen:***

B. Risks and Discomforts

1. Describe the **risks/potential discomforts** (e.g., physical, psychological, social, economic) associated with **each** intervention or research procedure.
2. Describe the expected frequency (i.e., **probability**) of a given side effect or harm and its severity (e.g., mild, moderate, severe).
3. If subjects are **restricted from receiving standard therapies** during the study, describe the risks of those restrictions.
4. If collecting identifiable private information, address the risk of a **potential breach of confidentiality**.

All procedures will be supervised by PERC-ICTS nurses and/or study personnel.

The possible risks and/or discomforts associated with the procedures in this study include:

BLOOD DRAWING – Blood draw may cause pain, bruises, bleeding or very rarely infection. Approximately 5-10% of healthy subjects experience some dizziness, feeling faint or nausea associated with catheter placement (vaso-vagal responses). All blood draws will be performed by trained individuals, and subjects will be carefully monitored by study members. We have performed over 800 such procedures in children and adults at PERC-ICTS with no major complications.

DXA – Radiation risk from DXA scanner. The UCI radiation safety committee will review this study. The DXA scanner uses x-rays to quantify body composition measurements. Study participants will be exposed to a dose of between 1 to 4 millirem per DXA scan. To minimize risks associated with DXA scan, female subjects of childbearing potential (regardless of chronological age) will be given a urine pregnancy test prior to enrollment and prior to the DXA scan. Pregnant female subjects shall be excluded from the study.

EXERCISE TEST– Exercise testing can cause sweating, muscle soreness, feelings of breathlessness, and nausea or dizziness. In rare instances, exercise tests may cause chest pain, tightness, or a change in vital signs. In children and adults with asthma, exercise can cause bronchoconstriction. We have performed over 1,000 exercise tests as described in the protocols with no major complications.

EXHALED BREATH COLLECTION – Exhaled breath collection can cause transient fatigue.

OXIMETER – Potential for mild local discomfort experienced due to placement of the pulse oximeter on a finger.

MEASURMENT OF ENDOTHELIAL FUNCTION (blood flow measurements) - Inflation of cuff may cause discomfort or bruising.

DIFFUSE OPTICAL SPECTROSCOPY (DOS) - This measurement poses very little risk to patients and investigators. This same general instrumentation has been used in several human clinical studies at UCI (#95-563, #01-1924, #02-2306, #01-2011) without incident. Similar instrumentation has also experienced widespread clinical use in the literature. Near-infrared light does not ionize biological tissue and poses no significant health risk. Since water absorption is low within this spectral range, local heating of the tissue is also minimal. Burns and heat damage are highly unlikely. The optical powers we will use in this study are all far less than those used with surgical lasers meant to incise, ablate, and coagulate tissue. The measurement itself is painless, and does not cause any significant discomfort. The risks of the TRS device and similar or lower than those for DOS.

LASER SPEKCLE IMAGING (LSI) - The maximum power of the Speckle light source is 10-50 mW, which is comparable to halogen-bulb household flashlights. This intensity poses no risk to human tissues.

Growth and Development Questionnaire Risks- Participants may feel uncomfortable while answering the growth and development questionnaires about changes in puberty (i.e. under arm, facial and pubic hair growth, breast development, and monthly periods).

BODY COMPOSITION- There is no known risk associated with this device

POTENTIAL BREACH OF CONFIDENTIALITY – We acknowledge that a breach of confidentiality is an associated risk of participation in this study.

UNFORSEEN RISKS – We acknowledge that there may be unforeseen risks to the participants due to his/her participation in the research study.

5. Discuss what steps have been taken and/or will be taken to **prevent and minimize** any risks/ potential discomforts to subjects (address physical risks as well as other risks such as the potential for a breach of confidentiality). Examples include: designing the study to make use of procedures involving less risk when appropriate; minimizing study procedures by taking advantage of clinical procedures conducted on the subjects; mitigating risks by planning special monitoring or conducting supportive inventions for the study.

CONFIDENTIALITY - Protections against risks to privacy of individuals or confidentiality of the data will be taken in accordance with usual human subjects' research procedures, including: ensuring that all study personnel have completed adequate training in conduct of human subjects' research, coding all data so that participants' data are suitably confidential, and obtaining all data in circumstances that preserve participants' privacy.

BLOOD DRAWING - To minimize the risks associated with blood drawing, all blood draws will be performed by trained individuals and subjects will be carefully monitored by study members.

DXA - To minimize risk associated with DXA scan, female subjects of childbearing potential (regardless of chronological age) will be given a urine pregnancy test prior to enrollment and prior to the DXA scan. Pregnant and breastfeeding female subjects shall be excluded from the study.

EXERCISE TESTING - To minimize risks associated with exercise test, resting heart rate and blood pressure are obtained prior to exercise. Heart rate and ECG are monitored during the exercise. The subject is instructed to stop exercise at any time during the test whenever he/she desires. If a vaso-vagal response occurs, the exercise is stopped and the subject will be placed in a recumbent position until heart rate and blood pressure normalize. If wheezing or other symptoms of exercise-induced bronchospasm occur during the exercise test, the test will be stopped. We have available at the PERC/ICTS Human Performance Laboratory treatment consisting of supplemental oxygen and/or bronchodilator. These will be administered as appropriate by the physician or nurse onsite. Recommendations will be made in these instances for long-term follow-up care

Should an adverse event occur during exercise testing while at the UCI PERC/ICTS, the physician on-call for exercise testing will be alerted. The on-call physicians have a response time of approximately 5 minutes.

Proper warm-up and other safety procedures will be instituted to minimize the possibility of musculoskeletal injuries. A member of the study team (MD or RN) will be present during the exercise test.

MEASURMENTS OF ENDOTHELIAL FUNCTION/DOS/LSI - Minimizing risks – The procedure has been adapted to pediatic populations by reducing the inflation time of the blood pressure cuff. While in fact an equivalent procedure in adults is normally performed with inflation time of 5 min or more, in our study inflation time for children will be only 60 seconds; greatly reducing the risk of discomfort or bruising. With this modifications, the procedure is successfully perfomed in children at other institutions (i.e. Univ. of Chicago, where it was developed). The addition of Laser Speckle Imaging and Diffuse Optical Spectroscopy measurements should not add any additional discomfort.

UNFORSEEN RISKS - The researchers will comply with UCI's Adverse Events/Unanticipated Problems reporting policy and procedures. Unanticipated adverse events: A physician and medical personnel are on call for all aspects of the study and will respond according to the event. The IRB will be notified of the event. If it is determined that a serious unanticipated adverse event has occurred, then the study will be modified or terminated. All adverse events and unanticipated problems will be reported according to the UCI IRB reporting requirements via the electronic AE/UP submission module.

C. Potential Benefits

1. Discuss the potential benefits that may accrue **directly to subjects.** ***Note:*** *Compensation is not a benefit. Do not include it in this section.*

[ ] There is no direct benefit anticipated for the subjects.

OR

The possible benefits the subject may experience from the procedures described in this study include the following: subjects may learn that they have exercise-induced asthma and this can be treated. Also, the subject may get information about his/her level of fitness, bone mineralization, and percent body fat which can help them in guiding their own programs to improve fitness if necessary.

2. Describe the **potential societal/scientific benefit(s)** that may be expected from this study.

The information gained from this project will benefit children and adults who suffer from asthma, by better understanding the effects of exercise and exercise training on asthma management.

D. Risk/Benefit Assessment

Explain why the study risks are reasonable in relation to the **potential benefits** to subjects and society.

The procedures involved in this research have been performed by thousands of children and young adults in our laboratory including those with disease and disability. Fortunately, we have never experienced any major complications. There are potential benefits to the participants in learning their own fitness levels, bone mineralization, and percent body fat which can help them in guiding their own programs to improve fitness if necessary. The research can shed light on mechanisms that could eventually improve the way we care for children and adults.

The risks in this study are few and are outweighed by the potential benefits of exercise.

SECTION 8: ALTERNATIVES TO PARTICIPATION

1. Describe the **standard or usual care** activities at UCI (or study site) that are available to prospective subjects who do not enroll in this study, as applicable.
2. Describe other **appropriate alternative procedures** to study participation that are available to prospective subjects.
3. If no alternatives exist, indicate that the only alternative is non-participation

[X] No alternatives exist. The only alternative to subjects is not to participate in the study.

SECTION 9: ADVERSE EVENT REPORTING/MANAGEMENT AND COMPENSATION FOR INJURY

A. Adverse Events and Unanticipated Problems

1. Indicate that you are familiar with **UCI's Adverse Events/Unanticipated Problems** reporting policy and procedures. See http://www.research.uci.edu/ora/hrpp/adverseexperiences.htm for details.

[ ] **Although this study involves no interaction/intervention with research subjects** (i.e., involves the use of records, charts, biospecimens) an unanticipated problem may still occur (e.g., a breach in confidentiality), the researchers are aware of UCI's Unanticipated Problems involving Risk to Participants or Others reporting policy and procedures and will comply with this policy.

[X] **This study involves interaction/intervention with research subjects.** The researchers are aware of UCI's Unanticipated Problems involving Risk to Participants or Others reporting policy and procedures and will comply with this policy.

2. **If this study involves interaction/intervention with research subjects,** explain how the research team will **manage adverse events and unanticipated problems** that may occur during the study or after completion of the study (i.e., provide a plan).

[ ] Not applicable - This study involves **no interaction/intervention** with research subjects (i.e., involves the use of records, charts, and/or biospecimens).

OR

Should an adverse event occur during exercise testing while at the UCI PERC-ICTS, the physician on-call for exercise testing will be alerted. The on-call physicians have a response time of approximately 5 minutes.

The researchers will comply with UCI's Adverse Events/Unanticipated Problems reporting policy and procedures. Unanticipated adverse events: A physician and medical personnel are on call for all aspects of the study and will respond according to the event. The IRB will be notified of the event. If it is determined that a serious unanticipated adverse event has occurred, then the study will be modified or terminated. All adverse events and unanticipated problems will be reported according to the UCI IRB reporting requirements via the electronic AE/UP submission module.

B. Compensation for Injury

For **Full Committee protocols**, explain how costs of treatment for research related injury will be covered.

[ ] Not applicable - This study involves no more than minimum risk and qualifies as **Expedited research**.

[X] Researchers are familiar with and will follow UC policy regarding treatment and compensation for injury. If subjects are injured as a result of being in the study, UCI will provide necessary medical treatment. The costs of the treatment may be covered by the University of California, the study sponsor, or billed to subject or the subject's insurer just like other medical costs, depending on a number of factors. The University and the study sponsor do not normally provide any other form of compensation for injury.

[ ] Other: <Type here>

SECTION 10: PARTICIPANT COSTS

1. If subjects or their insurers will be charged for study procedures, **identify and describe those costs.**
2. Explain why it is **appropriate to charge those cost** to the subjects or their insurers. Provide supporting documentation as applicable (e.g., FDA Device letter supporting charges).

[ ] Not applicable - This study involves no interaction/intervention with research subjects (i.e., involves the use of records, charts, biospecimens).

[X] There are no costs to subjects/insurers.

OR

SECTION 11: PARTICIPANT COMPENSATION AND REIMBURSEMENT

1. If subjects will be compensated for their participation, explain **the method/terms of payment** (e.g., money; check; extra credit; gift certificate).
2. Describe the **schedule and amounts of compensation** (e.g., at end of study; after each session/visit) including the total amount subjects can receive for completing the study.
3. Specify whether subjects will be **reimbursed for out-of pocket expenses**. If so, describe any requirements for reimbursement (e.g., receipt).

Note: *Compensation should be offered on a prorated basis when the research involves multiple sessions.*

[ ] Not applicable - This study involves no interaction/intervention with research subjects (i.e., involves the use of records, charts, biospecimens).

[ ] No compensation will be provided to subjects.

[ ] No reimbursement will be provided to subjects.

All subjects will receive the same reimbursments at each of the 6 visits in the following manner:

Fitness Assessment Session 1: $25
Exercise Challenge Session+ Blood Flow Measurments1: $25

Exercise Challenge Session + Blood Draws 1: $50

Fitness Assessment Session 2: $25
Exercise Challenge Session+ Blood Flow Measurements 2: $25
Exercise Challenge Session+ Blood Draws 2: $100

Reimbursements will be paid in the form of cash.

SECTION 12: CONFIDENTIALITY OF RESEARCH DATA

1. Indicate all identifiers that may be included in the research records for the study. Check all that apply:

   Note: *If this information is being derived from a medical record; added to a medical record; created or collected as part of health care, or used to make health care decisions it qualifies as PHI under HIPAA. The subject's HIPAA Research Authorization is required or a waiver of HIPAA Authorization must be requested (Appendix T).*

[ ] No subject identifiers are obtained (i.e., researchers will not collect information that can link the subjects to their data)

OR

[X] Names	[ ] Social Security Numbers	[ ] Device identifiers/Serial numbers
[X] Dates*	[X] Medical record numbers	[ ] Web URLs
[X] Postal address	[ ] Health plan numbers	[ ] IP address numbers
[X] Phone numbers	[ ] Account numbers	[ ] Biometric identifiers
[X] Fax numbers	[ ] License/Certificate numbers	[ ] Facial Photos/Images
[X] Email address	[ ] Vehicle id numbers	[ ] Any other unique identifier

[X] Other (Specify all): birth date, ethnicity, sex, parent or guardian name.

* birth date, treatment/hospitalization dates

2. Explain how data will be **recorded.**

Check all that apply:

[X] Paper documents/records
[X] Electronic records/database
[ ] Audio recording
[ ] Video recording
[ ] Photographs
[X] Biological specimens
[ ] Other(s) (specify): <Type here>

3. Indicate **how data will be stored, secured** including paper records, electronic files, audio/video tapes, biospecimens, etc.

Note: *If the research data includes subject identifiable private information and/or Protected Health Information, the storage devices or the electronic research files must be encrypted.*

Electronic Data (check all that apply):
[X] Coded data; code key is kept separate from data in secure location.
[ ] Data includes subject identifiable information. ***Note:*** *Encryption software is required.* (Provide rationale for maintaining subject identifiable info): <Type here>
[X] Data will be stored on secure network server.
[ ] Data will be stored on stand alone desktop computer (not connected to network/internet)
[ ] Other (specify here): <Type here>

Hardcopy Data, Recordings and Biospecimens (check all that apply):
[X] Coded data; code key is kept separate from data in secure location.
[ ] Data includes subject identifiable information (Provide rationale for maintaining subject identifiable info): <Type here>
[X] Data will be stored in locked file cabinet or locked room at UCI/UCIMC.
[X] Data will be stored locked lab/refrigerator/freezer at UCI/UCIMC.
[ ] Other (specify here): <Type here>

Data on Portable Devices:
4. Describe the **portable device(s) to be used** (e.g. laptop, PDA, iPod, portable hard drive including flash drives).
5. Specify whether **subject identifiable data** will be stored on the device. If so, **justify why** it is necessary to store subject identifiers on the device.

Note: *Only the "minimum data necessary" should be stored on portable devices as these devices are particularly susceptible to loss or theft. If there is a necessity to use portable devices for initial collection of identifiable private information, the portable storage devices or the research files* ***MUST BE ENCRYPTED****, and subject identifiers transferred to a secure system as soon as possible.*

[X] Not applicable – No study data will be maintained on portable devices.

OR

Data Access:
6. Specify who, besides the entities listed below, will have **access to subject identifiable private data and records**.
7. If there is a **code key**, specify who on the research team will hold the key, and who will have access to the key.
8. If publications and/or presentations will include **subject identifiable information**, specify where the data will be **published and/or presented** and address how the study team will obtain permission from subjects.

Note: *Authorized UCI personnel such as the research team and the IRB, the study sponsor (if applicable), and regulatory entities such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP), may have access to study records to protect subject safety and welfare. Any study data that identifies the subjects should not be voluntarily released or disclosed without the subjects' separate consent, except as specifically required by law. Publications and/or presentations that result from this study should not include subject identifiable information; unless the subject's separate consent has been obtained.*

[ ] Not applicable – No subject identifiers will be collected.
[X] Not applicable – Only the entities listed above will have access to subject identifiable private data and records.

Data Retention:

9. Explain **how long subject identifiable research data** will be **retained**. The data may include a code with a separate code key or the data may include subject identifiers.

Notes:

- ***If more than one of the options below is applicable*** *[e.g., the study involves children],* ***records should be kept for the longer period.***
- *Research documentation involving Protected Health Information (PHI) should be retained for six years (e.g., IRB documentation, consent/assent forms –* ***NOT*** *the actual PHI). Investigators must destroy PHI at the earliest opportunity, consistent with the conduct of this study, unless there is an appropriate justification for retaining the identifiers or as required by law.*

[ ] Not applicable. No subject identifiable research data will be retained.

[ ] Destroy once data collection is completed

[ ] Destroy at the earliest opportunity, consistent with the conduct of this research. Specify timeframe: <Type here>

[ ] Destroy after publication/presentation

[ ] Maintain for approximately <Type here> years. (e.g., 3 months, etc.)

[X] Maintain in a repository indefinitely. Other researchers may have access to the data for future research. Any data shared with other researchers, will not include name or other personal identifying information. Note: ***Appendix M is required.***

[X] Research records will be retained for seven years after all children enrolled in the study reach the age of majority [age 18 in California] as this study includes children .

[ ] Research records will be retained 25 years after study closure as this study involves in vitro fertilization studies or research involving pregnant women.

[ ] As this is a FDA regulated study, research records will be retained for two years after an approved marketing application. If approval is not received, the research records will be kept for 2 years after the investigation is discontinued and the FDA is notified.

[X] Other: Data will be stored for future research. The indirect link to the subjects (ie code and/or key) will be removed/destroyed once data collection is complete.

Data Destruction:

10. If audio or video recordings will be taken, specify the **timeframe for the transcription and/or destruction of the audio and video recordings.**
11. If photographs will be collected, specify the **timeframe destruction of photographs.**

[X] Not applicable – No audio/video recordings or photographs will be collected.
[ ] Audio or video recordings transcribed; specify time frame: <Type here>
[ ] Audio or video recordings destroyed; specify time frame: <Type here>
[ ] Audio or video recordings maintained indefinitely
[ ] Photographs destroyed; specify time frame: <Type here>
[ ] Photographs maintained indefinitely

Certificate of Confidentiality:

12. Specify whether a Certificate of Confidentiality (COC) has been or will be requested from the NIH. If yes, explain in what situations personally identifiable information protected by a COC will be disclosed by the UCI study team.

Note: *If the COC has been secured a copy of the COC Approval Letter should accompany the IRB application or be provided to the IRB upon receipt.*

[X] Not applicable – No COC has been requested for this study.