ACSM Northwest Student Research Award Program Application

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1. STUDY DESCRIPTION

Identification of early exercise training maladaptation markers through a 3-week lab-controlled training protocol

Rationale (not to exceed 400 words): Explain the problem that the proposed research will address, why the problem is important to address, and the new information the proposed research will provide.

Optimal physical performance is achieved through a tailored balance between training and recovery. When training load is not balanced with adequate recovery, various states of overtraining occur. Impaired exercise performance, without a clear mechanism (i.e., injury or illness) is the hallmark characteristic of overtraining.[1,2] The recovery time needed to reverse impaired performance is what determines the severity of overtraining.

Despite a large volume of literature, there are two glaring gaps concerning overtraining research: 1) the pathophysiological mechanisms of overtraining are not known and 2) there remains an absence of sensitive diagnostic criteria to identify overtraining or predict its impending occurrence. We believe these gaps are largely due to repetitive iterations of similar study designs and homogenous study populations (i.e., highly competitive male athletes). This is concerning as it has been reported that up to 64% of runners have experienced at least one episode of overtraining during their athletic careers.[1] The lack of diagnostic criteria coupled with limited prospective studies and similar study protocols have hindered our understanding of the progression of overtraining. When translating to sports medicine, these limitations inhibit our ability to effectively recognize and prevent training maladaptation.

We propose a 3-week lab-controlled training protocol designed to induce transient states of overtraining in recreationally active adult males and females. Compared to traditional field-based overtraining protocols, our lab-based protocol allows us to monitor significantly more variables in real-time as overtraining develops. Furthermore, our study includes recreationally active males and females, as opposed to typical cohorts consisting of high-level male athletes[3–7] or subjects previously diagnosed as overtrained.[8,9]

Recently, the burgeoning field of proteomics has begun to explore the biological mechanisms of overtraining with promising results. Novel field-based studies have identified multiple upregulated and downregulated proteins associated with impaired immune function following exhaustive exercise.[10–12] Our proposal aims to utilize proteomics, in conjunction with a cadre of physiological variables, to elucidate the biophysiological progression of training maladaptation through 3-week overtraining protocol.

Due to repetitive study designs and homogenous cohorts, the field of overtraining research has stagnated. Our lab-based overtraining protocol utilizes state-of-the-art proteomics in a novel population that will generate new insights regarding the pathological progression of overtraining. Findings from this proposal will be used to generate future, hypothesis-driven studies and supporting data for future proposals. It is hoped that this information will be useful in shaping future proactive approaches for detecting and preventing overtraining and the associated negative outcomes.

Specific Aims and Hypothesis (not to exceed 100 words): Clearly state the goals of the proposed research and summarize the expected outcomes of the research.

We hypothesize that following our lab-controlled training protocol, recreationally active males and females will become overtrained. Following an intense three-week exercise training protocol**,** subjects will be categorized into one of three groups determined by exercise performance outcomes: 1) adapted (no decrease in performance); 2) functionally overreached (performance decrements lasting <2 weeks); or 3) non-functionally overreached (performance decrements lasting >2 weeks.)

SA1: Determine inflammatory proteome activity among overtrained subjects following a 3-week intense overtraining protocol. We hypothesize that there will be distinct and significant protein production linked to acute immunse system activity in overtrained subjects following overload training.

Research Design and Target Completion Date (not to exceed 200 words): Briefly describe the overall research design and anticipated completion date.

Our study follows a repeated measures design lasting six weeks. Subjects are randomized into either an experimental or control group. Both groups perform weekly performance testing allowing within and between group comparisons. Experimental subjects undergo a three-week, progressive load training protocol followed by a three week recovery period. Control subjects attend the lab once weekly for six weeks and are asked to maintain a consistent lifestyle throughout the study duration. Experimental subjects with nonsignificant performance decrements (compared to control subjects) will be classified as adapted (AD.) Experimental subjects with significant performance decreases will be classified as functionally overreached (FOR) or non-functionally overreached (nFOR), depending on time to recovery.

At the time of this application we have completed data collection on six subjects (3 experimental, 3 control). Using high-throughput proteomics, we will analyze the proteome in two experimental subjects clearly experiencing training maladaptation, to detect immune system activity. As such, our completion date for this proposal would be quick; within a few months of receiving funding, we could process and analyze proteomic changes in our overtrained subjects.

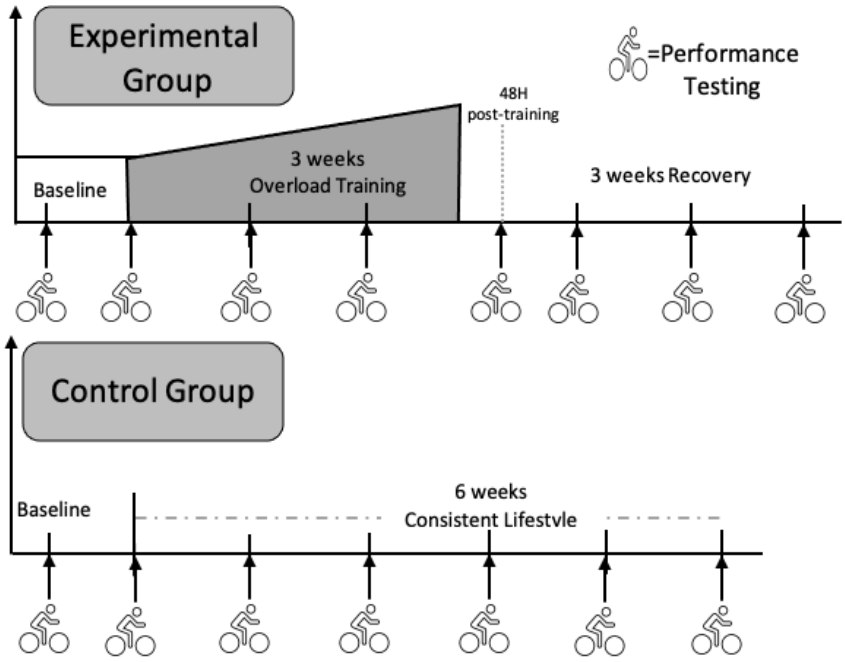

Figure 1: 3-week overtraining protocol followed by 3 weeks recovery. Each week training sessions increase in intensity or duration.

Methodology (not to exceed 400 words): Concisely describe the procedures and analyses used to accomplish the specific aims of the research. Include how the data will be collected, analyzed, and interpreted.

Subject delineation. *Control subjects* are asked to maintain a consistent lifestyle including exercise and dietary habits. The variation shown in control subjects for variables of interest will be used to calculate the smallest worthwhile change (SWC),[4,13] and will be used to determine thresholds for significant performance changes (i.e., overtraining) in the experimental group. Table one includes a list of performance variables, among others, that will be utilized to determine exercise performance, chiefly aerobic capacity (VO_{2max}), total work completed (TWC), and time to exhaustion (TTE).

Experimental subjects will complete a training protocol consisting of six training visits per week for three weeks, followed by performance testing once weekly for three weeks (recovery). Following training, subjects showing performance variation within the SWC will be classified as “adapted,” while subjects with performance variations larger than the SWC will be classified as overtrained.

Plasma Protein isolation/Proteomics Analysis. Each performance testing day, venous blood will be collected, centrifuged, and the plasma stored at -80°C. Plasma will be prepared for analysis by performing a protein crash using 100uL of specimen and 500uL acetonitrile (5:1 v/v ratio) in a microcentrifuge tube. The samples will be incubated for 60 min at 4°C to precipitate proteins. The samples will be centrifuged at 16,000 x g for fifteen minutes. The supernatant will be transferred to a new tube, evaporated, and reconstituted in solvent. Following preparation, plasma proteins will be sent to the Washington State University Tissue Imaging, Metabolomics and Proteomics Laboratory for analysis. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) will be used to identify protein production linked to immune system activity in maladapted subjects. STRING is a database of known and predicted physical/functional protein associations based on genomic context, high-through put experiments, co-expression and previous knowledge.[14]

Proteomic activity at post-training and post-recovery time points will be compared to baseline protein levels to indicate any changes to immune system activity. We intend to measure all samples through data independent acquisition (DIA) mode. This generates a record of all detectable fragments of peptides combining the advantages of selected reaction monitoring (reproducible) and shotgun analysis (high throughput).[10]

Variables of Interest within Overtraining Protocol		
Domain	**Variables of Interest**	**Notes**
Metabolic	**Oxygen uptake (aerobic capacity)** CO_2 Production Lactate, Glucose, specific Gravity (urine)	
Pulmonary	Ventilation, tidal Volume	
Cardiovascular	Maximum Heart Rate, Heart Rate Recovery, Oxygen Pulse, Hematocrit	
Performance	**Peak Power Output** **Total Work Completed (TWC)** Time-to-exhaustion (TTE) Handgrip Dynamometry	This is a two-bout graded exercise Test Protocol allowing confirmation of metabolic data (i.e. VO2 peak vs. VO2 max achievement) and allows additional performance measures (TWC & TTE)
Psychological/Mood	Profile of Mood States (POMS) Sleep Hygiene (Actigraph)	
Psychophysiological	RPE breathing; RPE legs	
Immunological/biochemical	Cytokines (weekly Plasma) Proteins (weekly plasma) Proteins (daily Urine)	
Endocrine	Cortisol; Testosterone	
Ratio Data	HR: Power Output HR: Lactate HR: RPE RPE: Power Output	
Dietary	Carbohydrate, Protein, Lipid calories consumed, Total Caloric Intake	Dietary surveys once weekly using ASA-24

Table 1: Variables of Interest

2. LITERATURE OF MAJOR SIGNIFICANCE

Include a concise list of no more the 15 key references related to the proposed research.

1. Meeusen R, Duclos M, Foster C, et al. Prevention, diagnosis, and treatment of the overtraining syndrome: Joint consensus statement of the european college of sport science and the American College of Sports Medicine. *Medicine and Science in Sports and Exercise*. 2013;45(1):186-205. doi:10.1249/MSS.0b013e318279a10a
2. Cadegiani FA, Kater CE. Novel insights of overtraining syndrome discovered from the EROS study. *BMJ Open Sport and Exercise Medicine*. 2019;5(1):1-11. doi:10.1136/bmjsem-2019-000542
3. Bellenger CR, Thomson RL, Robertson EY, et al. The effect of functional overreaching on parameters of autonomic heart rate regulation. *European Journal of Applied Physiology*. 2017;117(3):541-550. doi:10.1007/s00421-017-3549-5
4. Bellinger PM, Sabapathy S, Craven J, Arnold B, Minahan C. Overreaching Attenuates Training-induced Improvements in Muscle Oxidative Capacity. *Medicine and science in sports and exercise*. 2020;52(1):77-85. doi:10.1249/MSS.0000000000002095
5. Hausswirth C, Louis J, Aubry A, Bonnet G, Duffield R, le Meur Y. Evidence of disturbed sleep and increased illness in overreached endurance athletes. *Medicine and Science in Sports and Exercise*. 2014;46(5):1036-1045. doi:10.1249/MSS.0000000000000177
6. Bourdillon N, Nilchian M, Millet GP. Photoplethysmography Detection of Overreaching. *Medicine and Science in Sports and Exercise*. 2019;51(4):701-707. doi:10.1249/MSS.0000000000001836

7. Tanskanen MM, Kyröläinen H, Uusitalo AL, et al. Serum sex hormon-binding globulin and cortisol concentrations are associated with overreaching during strenuous military training. *Journal of strength and conditioning research*. 2011;25(3):787-797.
8. Cadegiani FA, Kater CE. Basal hormones and biochemical markers as predictors of overtraining syndrome in Male athletes: The EROS-Basal study. *Journal of Athletic Training*. 2019;54(8):906-914. doi:10.4085/1062-6050-148-18
9. Ackerman KE, Paula Pinto A, Ribeirão Preto P, Anthony Hackney BC, Cadegiani FA, Kater CE. Eating, Sleep, and Social Patterns as Independent Predictors of Clinical, Metabolic, and Biochemical Behaviors Among Elite Male Athletes: The EROS-PREDICTORS Study. *Frontiers in Endocrinology | www.frontiersin.org*. 2020;1:414. doi:10.3389/fendo.2020.00414
10. Nieman DC, Groen AJ, Pugachev A, Vacca G. Detection of functional overreaching in endurance athletes using proteomics. *Proteomes*. 2018;6(3). doi:10.3390/proteomes6030033
11. Antona GD', Louis J, Roberts MD, et al. Proteomic Markers of Non-functional Overreaching During the Race Across America (RAAM): A Case Study. Published online 2019. doi:10.3389/fphys.2019.01410
12. Nieman DC, Groen AJ, Pugachev A, et al. Proteomics-Based Detection of Immune Dysfunction in an Elite Adventure Athlete Trekking Across the Antarctica. *Proteomes*. 2020;8(1). doi:10.3390/proteomes8010004
13. Aubry A, Hausswirth C, Louis J, Coutts AJ, Buchheit M, le Meur Y. The development of functional overreaching is associated with a faster heart rate recovery in endurance athletes. *PLoS ONE*. 2015;10(10):1-16. doi:10.1371/journal.pone.0139754
14. Szklarczyk D, Morris JH, Cook H, et al. The STRING database in 2017: Quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Research*. 2017;45(D1):D362-D368. doi:10.1093/nar/gkw937

3. DETAILED BUDGET

Direct costs only. ACSM Northwest does not pay indirect costs as part of the ACSM Northwest Student Award Program. Funds cannot be used to cover faculty or technician salaries, or graduate student stipend. If your budget exceeds the amount awarded by ACSM Northwest, explain how the remainder of funds will be acquired in order to complete the research.

Equipment: itemize and include justification.

n/a	$0

Supplies: itemize and include justification.

$1,260 for proteomics analysis through the Washington State Unviersity Tissue Imaging, Metabolomics, and Proteomics Laboratory (6 samples at $210/sample.) **Other costs: $40** Bood collection supplies (e.g. gloves, lancets)	$1,300

Travel: conference, mileage/airfare, accommodations, and include justification.

n/a	$0

Participant Costs: break down number of participants, fee per subject and include justification.	
n/a	$0
Other Expenses: itemize and include justification.	
n/a	$
Additional Budget Information: If necessary, indicate how the project will be completed/who will provide additional funding if your budget exceeds the amount awarded by ACSM Northwest. *Keep in mind ACSM Northwest will be awarding* ***two awards up to a combined amount of $2000.***	
Our proposed budget requests funding to perform proteomic analysis at baseline, immediately post-training, and post-recovery for two subjects (6 analyses total). Should full funding not be available, we would adjust our funding request to allow for three proteomics analyses in one subject costing $630 without any additional supplies.	$630
Total Direct Costs for the Project Period	$1,300