LONG TERM GRANT APPLICATION
Cover Sheet

Amount Requested: $2754.00

Project Information

McDonald, Chandler
Student Participant (Last, First)

Investigation of Serum Iron Related Biomarkers and Bone Density in ...
Project Title (10 words or less)

Aguilar-Alvarez, David
Faculty Mentor Name (last, first) Mail Code

Education Athletic Training and Nutrition
College (Weber State is the University, NOT college) Department

This project _X_ DOES/___ DOES NOT require review by the WSU Institutional Review Board for Human Subjects or the WSU Animal Care and Use Committee.

Student Signature — 10/1/18 Date

Project Mentor Signature — 9/20/18 Date Received by Mentor. Must be 10 business days before final deadline.

2805 Campus Mail — 8867 Phone Ext.

Undergraduate Research Committee Representative — 9/25/18 Date Received by URC Rep. Must be 5 business days before final deadline.

Faculty Mentor Department Chair — 10/1/18 Date

Please check if attended Research Proposal Workshop:

☑ Date Workshop attended January 8, 2018
(Please fill in the date of attendance)

LONG TERM GRANT APPLICATION
Budget Worksheet

BUDGET ITEM	Department or College Funds	Outside Agency Funds	Personal Funds	Undergrad. Research Funds	GRAND TOTAL
Materials	$100.00 Lab disposables -Pipette tips -Gloves -Alcohol swabs -Buffers -Syringes -Cryovials			**$2254.00** (Luminex/Magpix® Assay Kit)	$2354.00
Equipment	$15,000.00 xMAP Instrument MAGPIX for multiplex immunoassays $4,000.00 Refrigerated Micro-centrifuge (Use for multiple experiments not counted for grant total)				$19,000.00 (Use for multiple experiments not counted for grant total)
Research Scholarship (max request $2,500.00)		$1700.00 (*Abreila S. Hinckley Award*)		**$500.00**	$2200.00
Mileage to gather Data (.38 per mile)			$190.00 (*500 mi.*)		$190.00
GRAND TOTAL	$100.00	$1700.00	$190.00	**$2754.00**	$ 4,744.00

Project Description

It is well known that the dangers involved among male and female cross-country athletes include iron deficiency and a high rate of stress fractures,[7, 41] leading to absence in participation. Moreover, females in cross-country face a devastating hazard[1, 4, 12] – the female athlete triad – which includes a higher risk for stress fractures and future development of osteoporosis.[5, 16, 42, 44, 46] The likelihood of being categorized within the female athlete triad have shown to be significantly higher for cross-country athletes compared to other collegiate sports.[17, 28] Given this disparity, female cross-country athletes should also be repeatedly monitored for the criteria associated with the female athlete triad: menstrual dysfunction, low energy availability, and decreased bone mineral density.[28-29, 42] Nevertheless, since confounding variability of iron stores caused from menstruation in female athletes exist,[1, 4, 23] male assessment of iron deficiency remains unequivocally important.[7, 10-11, 17, 30]

Weber State University's Athletic Training and Nutrition department, alongside the School of Radiologic Sciences, is conducting a longitudinal cohort study to assess the following in male and female NCAA Division 1 cross-country athletes: (1) Serum ferritin (sFer) and hemoglobin; (2) Mandibular bone mineral density (BMD) through cone beam computed tomography (CBCT) and spine/pelvis BMD through dual energy X-ray absorptiometry (DEXA) scans; (3) Various serum inflammatory cytokines; (4) Serum bone biomarkers correlated to osteocyte activity.

Female and male cross-country athletes 18-25 years old have volunteered to participate in two blood draws, 2017 pre-season and 2018 pre-season, for year-to-year comparison of the above objectives. Blood samples have been analyzed at McKay Dee Hospital to satisfy the first objective. Additional samples have been aliquoted and stored in an ultra-low temperature freezer. Following the arrival of the bone biomarker analytes, I will perform a multiplex assay using a Luminex® MAGPIX® to analyze a set of bone biomarkers shown to correlate with osteocyte activity to satisfy the fourth objective. Likewise, I will assist on the analysis of various inflammatory cytokines also using multiplex technology to satisfy the third objective. Finally, BMD is being extrapolated from CBCT and DEXA scans that have been performed on the participant's mandible and spinal/pelvis region, respectively.

We hypothesize that sFer will increase and BMD will decrease from year-to-year, and hemoglobin's variance will correlate with sFer. We hypothesize that mandibular BMD extrapolated from CBCT will correlate with bone biomarker levels that demonstrate osteocyte activity. We hypothesize that an inverse relationship exists between the level of inflammatory cytokines and sFer. Finally, we hypothesize that there may exist a relationship between sFer and BMD for this specific population.

Within the first objective, sFer and hemoglobin will be measured to assess each athlete's iron status.[9] Iron deficiencies (ID), including iron deficient anemia, have shown to decrease physical performance as it is a key component of the oxidative metabolic process.[1, 12, 33, 35-36] Endurance athletes have shown to pose a greater risk in developing iron deficiency[10] when compared to other athletes due to several key factors including repetitive foot-strike hemolysis[39] and inadequate iron intake.[11] Continual assessment is needed to ensure Weber State University's male and female cross-country athletes are achieving an adequate iron intake to prevent ID and improve performance.[8-12, 38]

Due to the nature of the distance runner's work load,[27, 34, 40] BMD has shown to be high at the calcaneal region[21] but low throughout the spine and pelvis, attributing to an increased risk of stress-fractures at these sites.[7, 30] When evaluating the biomechanical principles of a cross-country athlete with consideration of Wolff's Law, these findings are logically consistent. It is important to continually assess the BMD of the cross-country population since literature has shown cross country athletes are at higher risk for BMD loss[44] due to repetitive, low-impact,[3, 16] and unidirectional loading patterns,[40] tendency for disordered eating,[4, 46] and a high training volume[17] attributing to decreased energy availability.[35]

Assessing BMD via CBCT has been shown to be an effective measure in accurately predicting BMD when compared to other gold-standard methods.[2, 18-19, 22] Under the second objective, the participants will undergo dentistry CBCT to help determine BMD measured at the individual's mandible in Weber State University's department of dental hygiene. Mandibular BMD has shown to be an effective method of predicting skeletal osteoporosis.[18] DEXA scans will also be performed off-campus at Ogden Clinic to evaluate spinal and pelvis BMD. However, due to the limited scope of assessing BMD at these sites while considering that the occurrence of tibia and femur stress fractures is among the most common injury to long-distance runners, more assessments on BMD are needed.[41]

Iron's role in oxidative phosphorylation to produce energy is fundamental.[1, 12, 33, 43] All metabolic pathways are reduced during ID, resulting in an increase in other energy pathways to compensate for the decreased ATP production in the citric acid cycle.[26] Since inflammatory cytokines, alongside hepcidin activity,[9] have shown to play a pivotal role with iron's homeostasis and regulation,[14-15, 33, 37, 45] it noteworthy of assessment. As outlined in the third objective, various inflammatory cytokines will be quantitatively determined through multiplex assays.

Outlined within the fourth and final objective is analysis of various bone biomarkers shown to correlate osteocyte activity in various ways.[6, 13, 24] As previously discussed, CBCT and DEXA scans are effective tools to assess BMD, however the scope of site-specific areas are too narrow for a confident assessment of other locations shown to be troublesome for endurance-trained populations.[7, 20, 30, 46]

Moving forward with the stated objectives will serve to benefit the division 1 cross-country athletes at Weber State University, especially since this athletic population poses the greatest risk of injury related to ID. Assessing multiple biomarkers, proteins, and minerals in conjunction with BMD will also further our understanding of possible correlations.[25, 32] My role in the project will be undergraduate support which will include collecting informed consents, blood samples post-venipuncture, plasma aliquoting, analysis of objectives (1), (3), and (4) via multiplex assays, statistical analyses, abstract and article composition, and presentations at local and national conferences including UCUR, NCUR, ACSM Annual and Regional, Departmental Honors Research Conference (DHRC), and Nutrition 2019.

Currently, I am under the tutelage of Dr. David Aguilar-Alvarez, who serves as my director and mentor for the project. Dr. Aguilar-Alvarez is the Principle Investigator for the proposed project and has overseen all my trainings – collecting informed consents, samples, aliquoting, operation of Luminex® MAGPIX® multiplex assays – except statistical analysis. My trainings in statistics has been from previous courses – MATH 1040: Introduction to Statistics, and ESS 3600: Measurement/Statistics in ES – from current and former WSU professors, Dr. Cora Neal and Dr. W. Matt Denning, respectively. I am CITI stage-1 Health Professions and Human Physiology Researcher certified (exp. 07/2020) and am OSHA Bloodborne Pathogens certified (exp. 01/2019).

References

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Project Methods & Timeline

The design of the study is a WSU IRB approved, longitudinal cohort analyzing the participant's pre-season blood serum biomarkers year-to-year. Two blood draws have been conducted on each subject at the beginning of the cross-country season: once in September 2017, and again in September 2018. The study will be conducted at three locations: blood samples have been taken via certified phlebotomist venipuncture in the Nutritional Biochemistry Lab (SW 133), CBCT has been performed in the dental hygiene department through the Dentsply Sirona GALILEOS® Comfort^{PLUS} 3D Imaging CBCT, and DEXA scans are currently being given at the Ogden Clinic.

Male and female cross-country athletes, ages 18-25 years old, from Weber State University (a NCAA Division 1 Institution) have been selected on a volunteer basis. Power analysis done with a related study showed around 22 participants will be necessary. As it now stands, samples have been collected from 20 individuals that qualify for participation. To satisfy power analysis, further collections may be requested by those who have participated in 2017 and in 2018.*

Independent variables in the study includes the blood serum's biomarker measures. Thus far, the biomarkers to be analyzed are as follows:

ACTH	Insulin	Osteopontin	Sclerostin (SOST)
DKK1	Leptin	Osteoprotegerin	
FGF-23	Osteocalcin	PTH	

Analyses of the biomarkers listed above will be compared with the BMD of the mandible, pelvis, and spine for each athlete following CBCT and DEXA scans. Iron profiles including ferritin and hemoglobin, along with white blood cells, will be analyzed concurrently. Cross-comparisons will be made between iron profiles and BMD since current findings are inconclusive and targeted to special populations.

Following the results of the statistical analyses, an abstract will be drafted, and a research poster will be created with the expectation for presentation at several research conferences. As a student expected to graduate with departmental honors, I have committed to present at the departmental honors research conference, UCUR, NCUR, ACSM Annual, and Nutrition 2019. An abstract will be soon submitted to these presentation organizations well before the expected deadlines.

Project Timeline	**Completion Deadline**
Order Materials	Oct 8, 2018
Recruit Participants	*Completed (Read above)
Blood sample collection	*Completed (Read above)
Mandibular CBCT	Completed
Perform inflammatory cytokine Luminex® analysis	Oct 5 – Oct 6, 2018
Perform bone biomarker Luminex® analysis	Oct 12 – Oct 13, 2018
Perform Statistical Analyses of Data	Oct 15 – Oct 16, 2018
Submit Abstract	Oct 22, 2018
Poster Presentations	Nov 9, 2018 ; Feb 14-16, 22, 2019 May 28 – Jun 1, 8-11, 2019
Article Submission to Peer Journals	Jun 5, 2019

Flouroluminecent magnetic beads used in multiplex assays for the assessment of biomarkers is a cutting-edge technology that has dramatically reduced cost and time. Fortunately, Weber State University has acquired a Luminex® MAGPIX® – an instrument that maximizes these benefits. The industry standard for measuring biomarkers before multiplex assays consisted of measuring only one analyte at a time, taking around 14 hours for the analysis of one biomarker of interest. With the use of the Luminex® MAGPIX®, we can now effectively measure up to 40 analytes at one time to dramatically reduce this time and cost.

Budget Explanation		
Materials/Supplies	**Amount**	**Cost**
Price per plate (plus tax) *MilliporeSigma® Cat. No. HBNMAG-51K*	40 samples (20 for year 2017 and 20 for year 2018)	$2254.00 (incl. 7% sales tax of $148.00)
Research Stipend	100+ hours	$500.00
Grand total:		**$2754.00**

MilliporeSigma® Human Bone Assay Kit (*Cat. No. HBNMAG-51K*) is the only company to offer all ten analytes of interest offered for Luminex® MAGPIX®. Neither R&D Systems®, Bio-Rad®, Thermo Fisher Scientific, OriGene®, eBioscience®, Active Motif®, or Affymetrix® had the capability to customize a multiplex kit to contain all ten analytes of interest – with the majority of the companies only offering between four to eight of the analytes at a similar price. Thus, the customized bone biomarker catalog ordered through MilliporeSigma® is the most pragmatic and cost effective.

I believe that requesting the amount of $500.00 for a research stipend is a fair and justifiable when considering the number of hours required to complete the project. When evaluating the time commitment of 100+ hours required to complete the project versus requested amount, the research stipend would reward me with a pay-rate less than the State of Utah's current minimum wage of $7.25 (i.e., less than $5 per hour). An outline of the time commitment from 2017 to present per activity is as follows:

Task	**Time commitment (hours)**
Protocol Training	4
Collecting Samples and Aliquoting	6
Luminex® MAGPIX® Training	12
Running the Experiment	72
Cataloguing Data	6
Statistical Analyses	5
Lab Cleaning	2
Abstract, Poster, Article Composition, Presentations	350+
Grand Total	**100+ hours**

Granting the requested amount for the research stipend is crucial. My ability to work in other areas of employment is very limited due to the time commitment to execute the project.

Funding for this project will bode well for my outlook to admission advisors at a doctoral of physical therapy institution I am planning to attend. Furthermore, it will also bode well with my outlook and gratitude to the OUR office and my future assistance towards the WSU Alumni Association.

LONG TERM GRANT APPLICATION
Additional Questions

1. What funding have you received from OUR in the past? Where has your previous project been disseminated?

   I have not received any OUR grant funding in the past. This is my first application for project funding.

2. Is this project part of a required course? If so, please indicate the support (monetary and in-kind) provided for this project by the academic department.

   This project is part of the required course NUTR 4520 Directed Undergraduate Nutrition Research. Academic support can be found under the Project Methods & Timeline section, whereas monetary support can be found in the next question.

3. What additional sources of funding have been solicited? Is your department willing/able to fund any equipment they will be retaining?

   No additional funding sources have been solicited since due to recent protocol developments. All necessary equipment – including the Luminex® MAGPIX®, centrifuge, fridge, freezer, vortex, ultrasonic, etc. – in addition to disposables and safety materials has been purchased through the Athletic Training and Nutrition (ATN) department.

4. Where do you plan to disseminate the results of this project?

   I have planned on presenting this project via poster presentation at the following conferences:

   - Honors Department Research Conference (Nov 2018) at Weber State University
   - UCUR 2019 at Weber State University
   - NCUR 2019 at Kennesaw State University, Kennesaw, GA
   - Nutrition 2019 in Baltimore, MD
   - ACSM (American College of Sports Medicine) Annual Conference 2019 in Orlando, FL

5. If you are requesting a Research Scholarship, please list all significant time commitments (5+ hours per week) that you expect to maintain over the duration of your project including, for example, class and work schedules.

   See previous section titled Budget Explanation (page 12 of 16)

LONG TERM GRANT APPLICATION
Faculty Recommendation Form

Student Name (last, first): _McDonald, Chandler_____

Project Title: _Investigation of Serum Iron Related Biomarkers and Bone Density in NCAA Division I Cross Country Men and Women Athletes_

Mentor Directions: After carefully reviewing the proposal and assessing both the viability of this project and the qualifications of the student requesting funding, answer the questions found below. Please expand the sections as necessary (**do not attach separate letter**). If the project involves the use of human subjects or protected animals, be sure the student secures IRB or ACUC approval. If the project receives funding, it is your responsibility to work closely with the student, monitor the ongoing progress of the project and budget, and evaluate the project's results. Failure to do so will jeopardize funding for this project and any future projects.

1. How long and in what capacity have you known this student?

I have known Chandler since for two years. Chandler have taken classes with me and have worked as our nutrition biochemistry laboratory manager performing health assessment on study participants. He also, helped by providing supplemental instruction in our introductory classes and have been an incredible asset for our program. Since I met Chandler we have been discussing research opportunities and came with the idea for this project.

2. Briefly describe the proposed project. Is this part of a larger research project? Is this part of a course? If so, how is the project apart from the nature and scope of activities normally taken for the course (Please attach a copy of your course syllabus)?

The proposed project is part of the *Investigation of Serum Iron Related Biomarkers and Bone Density in NCAA Division 1 Cross Country Men and Women Athletes* study. In this project, we will be collaborating with Professor Taylor Ward from Radiological Sciences to analyze the effect of training on bone related disorders. We plan to evaluate if cross-country training affects bone mass density and elucidate potential mechanisms underlying these effects. This will include the potential role of iron status and specific cytokines in bone density. Chandler will be measuring serum bone biomarkers that we hope to associate with athletes bone density.

3. Give an assessment of the project's significance to the student's discipline and of the project's educational and/or professional benefit to the student.

Chandler is also planning to pursue a physical therapist degree following completion of his undergraduate degree. This project will enhance his application status in that pursuit and gain him experience on dealing with human trials.

4. Comment on the qualifications of the student to successfully complete this project, both in terms of the project's scope and its time frame.

Chandler is a very smart and well-rounded student, who can communicate scientific and health related information into easy to understand concepts and practical applications very efficiently. Chandler has a strong interest in nutritional science and its application to the public. He also have dedicated time to collect samples and recruit participants for this study. He demonstrates rational, logical and critical thinking required to write scientific papers and have shown to be very organized with his time during our collaboration. I have no doubt Chandler will generate an excellent product out of this research in a timely manner.

5. Comment on the justification and appropriateness of the project budget, including the necessity of a Research Scholarship (if requesting one).

The project budget will cover the assay expenses and research scholarship. The nutrition program will cover lab disposables and the equipment necessary for this project. The stipend requested is to cover the time that Chandler will need to take off his usual employment to be trained in the methodology to conduct the assay, develop an adequate protocol, train other students on the use of the equipment and perform all other scholarship related activities such as writing a research article about our findings. Given the expenses involved with these protocols, the budget is appropriate as outlined in the project expenses.

6. Describe your role in the project.

Chandler approached to me with very interesting research ideas on the fall of 2016. He have been previously collaborating with Dr. Saori Hanaki. We talked about the possible research pathways we could take based on the given samples available and the equipment required. Chandler got very interested in bone biomarker research and layed out a possible hypothesis. After that, I just facilitated the equipment necessary to perform this research. My role will continue as a mentor and facilitator of the necessary tools for Chandler success.

7. Include anything else that you think will be helpful to the committee in evaluating this application.

Projects like this one could result in the establishment of a fruitful mutual collaboration with the department of radiological sciences at WSU, which in terms will aloud other undergraduate students to benefit from this partnership.

This project X DOES ___ DOES NOT require review by the WSU Institutional Review Board for Human Subjects or the WSU Animal Care and Use Committee.

Project Mentor Signature _____ Date 10/1/18

2805 Campus Mail Code

8867 Phone Extension

WEBER STATE UNIVERSITY
Institutional Review Board

August 20, 2018

David Aguilar-Alvarez
1435 Village Drive
Department: 2805 ATN
Ogden, UT 84408

Dear Dr. Aguilar-Alvarez,

The addendum letter for the study: IRB # 17-ED-004.
Approval date: 8/25/17.
Status: Expedited
You have been granted a one year extension on the study entitled "Investigation of Serum Iron Related Biomarkers and Bone Density in NCAA Division 1 Cross Country Men and Women Athletes".

You have one year from the date above to complete the data collection or submit another continuation letter.

Respectfully,

Natalie A. Williams

Natalie A. Williams, Ph.D.
Chair, Institutional Review Board, Education Subcommittee

Shopping Cart

Oct 01, 2018

Customer Name :
Email :

Millipore Products

Product	Qty/Pack	Shipping/Availability	Quantity	Total
MILLIPLEX MAP Human Bone Magnetic Bead Panel - Bone Metabolism Multiplex Assay (HBNMAG-51K) Selected Analytes: ACTH,Insulin,Leptin,Osteopontin,FGF-23,DKK1,Osteoprotegerin,Osteocalcin,Sclerostin (SOST),PTH	96-well plate		1	USD 2,106.00

Your Subtotal USD 2,106.00

For general questions please contact our customer service:

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MA

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400 Summit Drive
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Telephone: 978-715-4321
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