

Fiscal Year:	FY 2010	Task Last Updated:	FY 06/23/2010
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Project Title:	Monitoring of Bone Loss Biomarkers in Human Sweat: A Non-Invasive, Time Efficient Means of Monitoring Bone Resorption Markers under Micro and Partial Gravity Loading Conditions		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Biomedical countermeasures		
Joint Agency Name:		TechPort:	Yes
Human Research Program Elements:	(1) HHC: Human Health Countermeasures		
Human Research Program Risks:	(1) Bone Fracture: Risk of Bone Fracture due to Spaceflight-induced Changes to Bone (2) Osteo: Risk Of Early Onset Osteoporosis Due To Spaceflight		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Comments:			
Project Type:	GROUND	Solicitation / Funding Source:	2007 Crew Health NNJ07ZSA002N
Start Date:	05/20/2008	End Date:	11/20/2011
No. of Post Docs:	0	No. of PhD Degrees:	0
No. of PhD Candidates:	1	No. of Master' Degrees:	0
No. of Master's Candidates:	1	No. of Bachelor's Degrees:	0
No. of Bachelor's Candidates:	0	Monitoring Center:	NASA JSC
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Flight Program:			
Flight Assignment:	NOTE: New end date is 11/20/2011 per NSSC information (Ed., 5/31/2011)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	O'Connor, Dan (University of Houston)		
Grant/Contract No.:	NNX08AQ37G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:

We propose to validate that the rate and extent of unloading-induced bone loss in humans can be assessed by monitoring the levels of two bone resorption markers in sweat, namely ionized calcium and collagen break-down products. Initial testing will be carried out in three populations (at rest and during activity) that have different constitutive levels of bone remodeling, namely young normal healthy individuals, air-force cadets undergoing regular heavy resistance training and healthy elderly individuals. All groups will include both male and female participants. Biomarker concentration will be determined in contemporaneous samples of sweat, blood and urine collected during both short (24 hr) and long-term studies (six months) to define the relationship between biomarkers levels in the respective biological samples. Bone mineral density (BMD) measures will be incorporated in the long-term studies to test the predictive value of sweat biomarkers with regard to overall bone remodeling. Future testing will utilize subjects undergoing bed-rest simulations of micro- or partial gravity loading conditions. Several different sweat collection techniques will be investigated to determine the most appropriate and efficient means of sample collection suitable for deployment during a space flight mission. These experiments will also include investigation of the most appropriate biomarker analysis techniques that allow for future deployment in micro- or partial gravity environments. This near-real-time monitoring approach may also provide the information required to justify modifying an ineffective bone loss countermeasure prescription during a mission. One of the approaches tested will be a novel, micro-fabricated fluid collection capillary array, known as the micro-fabricated sweat patch (MSP) device, specifically developed for use in microgravity. The MSP technology was initially developed because of its potential to become an autonomous, solid-state collection/analysis device worn on the skin of an astronaut requiring little or no crew interaction to perform its monitoring function.

Rationale for HRP Directed Research:**Research Impact/Earth Benefits:**

Loss of bone mass, density and structural integrity is a significant health risk in a variety of populations such as the elderly, post-menopausal women, young female athletes and astronauts. Such changes in overall bone quality lead to a greater risk of bone fracture and potentially a reduced rate of bone healing after injury. The ability to monitor biomarkers of bone remodeling (e.g. ionized calcium, collagen cross links) using sweat as an analytical sample provides an attractive alternative to the more invasive and costly measures presently employed such as a bone density scans by DXA, 24 hour urine collection protocols or whole blood analyses. The development of a non-invasive, skin-mounted monitoring device which allows the quantitation of ionized calcium and/or collagen cross links in sweat will allow bone loss to be monitored in a wide variety of terrestrial populations that to date have not easily been monitored outside of a clinical setting. This particular project focuses on validating the concept that sweat analysis can be used as a non-invasive means of monitoring bone loss in crew members during periods of mechanical unloading under altered gravitational conditions. In addition, this project is also investigating the best technical approach to collecting a sweat sample which is specifically applicable to the space-flight environment while utilizing well-accepted, clinically validated analytical methods. Development of a technology capable of real-time monitoring of biomarkers of bone loss that satisfies the criteria required for use in the space-flight environment (i.e. non-invasive/non-intrusive, passive, small, light-weight, low power) has many direct applications in various populations here on Earth.

Our initial studies in Year 1 of this project indicated that there was significant individual variation in the amount and rate of sweating observed between subjects based on: 1) the anatomical site sampled (i.e. fore-head, shoulder, fore-arm); 2) level of activity (i.e. "active" sweating during exercise; and 3) the concentration of biomarkers relative to the onset of "active" sweating. In addition, the existing sweat collection technology previously employed to collect sweat (i.e. absorptive pad) exhibited significant analytical issues based on sub-optimal analyte retention and analyte extraction efficiency.

To overcome these difficulties we developed a standardized sweat collection protocol in which subjects performed a defined exercise protocol at a fixed level of intensity (i.e. 30 min of aerobic exercise at an intensity of 40% of their age-predicted $\text{VO}_{2\text{max}}$) under similar environmental conditions and hydration status. Sweat samples were collected at the fore-head using micro-fabricated polymer capillary arrays which collect sweat as an unadulterated liquid sample. This approach allows both determination of the volume of sweat produced and has the advantage of minimal residual analyte retention within the array due to its polymer construction unlike the existing absorptive sweat collection technology. Sweat samples were initially collected at discrete time intervals to determine when during exercise-induced sweating did biomarker concentration reach "steady-state" levels. These experiments indicated that biomarker concentrations (i.e. Ca^{2+} and T-CCL) in "active" sweat reached a "steady state" after 15 min of exercise period regardless of the volume of sweat produced by a particular individual. In addition, analyte concentration (i.e. T-CCL) in actively produced sweat was found to be highly correlated ($R = 0.92$) with plasma levels of the same analyte during the period sweat was being collected. However, comparison of analyte concentration produced during actively produced sweat and 24 hr urine voids in the same individual indicated, that although related in a linear fashion ($R = 0.61$), that sweat analyte excretion during the 15 min period of exercise was not predictive of total 24hr urine analyte excretion. As 24 hr urine void collection provides an integrated value for whole body biomarker excretion rates, comparison of the amount of biomarker excreted during a 15 min period of "active" sweating from 7.5cm of skin surface at the fore-head may be inappropriate and thus account for the poor correlations observed between total calcium and T-CCL in sweat versus 24 hr urine voids.

As the overall goal of this project is to determine whether or not excretion of bone loss biomarkers in sweat is predictive of bone loss biomarker levels detected in 24 hr urine voids, we revisited the possibility of collecting a sweat sample over a 24 hr period in order to provide a more appropriate comparison. During Year 1 of this project we investigated the accuracy of the existing absorptive patch sweat collection technology with regard to analyte concentration relative to unadulterated liquid sweat samples collected in a contemporaneous fashion at the same anatomical site. These data indicated that the cellulose matrix of the absorptive patch posed significant analytical problems. These issues were that the cellulose matrix: 1) contained significant amounts of endogenous calcium signal; 2) resulted in significant retention of sweat-derived T-CCL that could not be extracted from the matrix using the standard method (i.e. extraction in ddH_2O); and 3) that extraction efficiency from the matrix was variable between identical samples.

Task Progress:

To address these analytical issues we investigated the use of two new absorptive materials, a glass fiber material and a polypropylene woven material, for the collection of sweat over a 24 hr period. Our initial experiments focused on the extraction efficiency of four bone loss biomarkers of interest, namely calcium, T-CCL, NTx and osteocalcin (OST). We also modified the extraction protocol to utilize acidified ddH_2O (pH 5.0) which theoretically would enhance the solubility of these biomarkers in the extraction buffer and reduce ionic interactions within the matrix of the absorptive material. Unlike the original cellulose absorptive pad technology, efficient recovery of known amounts of all analytes was achieved using our modified extraction method from both the glass fiber and woven polypropylene materials.

Utilizing glass fiber absorptive pads (7.5cm² in area) and our modified extraction protocol, we have now collected sweat samples from subjects over a period of 24 hr at three separate anatomical locations, the shoulder, hip and fore-arm. We also collected 24hr urine voids from these subjects during the same 24 hr period. To date we have collected and analyzed samples from 8 subjects and have an additional 12 subjects enrolled in the study. When the total amount of calcium excreted in sweat over a 24 hr period into a 7.5cm² glass fiber absorptive pad was compared to the total amount of calcium excreted in a 24hr urine void obtained from the same individual, our preliminary results indicate that there is a near linear correlation between the two values in sweat collected from the shoulder ($R = 0.94$) and fore-arm ($R=0.91$) and contemporaneous 24 hr urine voids. A similar relationship between 24hr sweat T-CCL and 24hr urine void T-CCL levels was observed at the shoulder ($R = 0.99$) in these subjects.

Our data concerning the relationship between both calcium and T-CCL levels detected in 24hr sweat samples as compared to those detected in 24hr urine voids from the same individual suggests that bone loss biomarker concentration in the criterion sample (i.e. 24 hr urine void) can be accurately predicted from that found in a sweat sample collected over the same 24 hr period using an absorptive pad technology. The use of a glass fiber absorptive pad and modified extraction buffer ensures consistent and efficient analyte extraction from the pad. We are presently expanding the number of subjects in our data set and also including NTx analysis in that data set. From an operational perspective, we are also investigating a number of separate approaches to performing the liquid-based extraction procedure under microgravity conditions and testing the ability of the iSTAT analysis technology (previously flown and validated on Shuttle and ISS) to perform the sample analysis.

Bibliography Type:

Description: (Last Updated: 03/08/2018)