Final Panel Summary

Solicitation:	**Research and Technology Development to Support Crew Health and Performance in Space Exploration Missions**
Proposal Number:	**07-HRP-2-0022**
Investigator:	**Mark Clarke**
Organization:	**University of Houston**
Title:	**Monitoring of Bone Loss Bio-Markers in Human Sweat ¿ a non-invasive, time efficient means of monitoring bone resorption markers under micro and partial gravity loading conditions.**
Consensus Score:	**76**

This Panel Summary expresses the consensus judgment of the Panel resulting from its discussion of both the proposal and the individual reviews.

Panel Summary: The panel scored this proposal as 'good'. It was enthusiastic about the concept of determining the usefulness of sweat as a potential fluid to indicate bone status in real time during space exploration and about the development of the sweat collection device identified in this proposal. The panel's enthusiasm was dampened by three concerns: 1) The preliminary data on ionized calcium in sweat would have been significantly strengthened (with little additional investment) if contemporaneous urine samples were also analyzed for ionized calcium, to determine if there was a relationship between the values for the two fluids. In particular, concern was raised as to whether the 10-fold range in sweat ionized calcium would be reflected in calcium values for urine; 2) It is suggested that the basic question of validating sweat as a monitor of bone status be better characterized in Phase One experiments by determining the sweat contents of a number of different bone markers in addition to ionized calcium and PYD; 3) Consistent with Item #2, it is suggested that the proposal focus solely on Phase One experiments to definitively determine how well sweat will serve as a measure of changes in bone status, before proceeding to the bed rest study and further development of the MSP device for bone marker analyses in space.

Preliminary Proposal Evaluations

Proposal Information

Proposal Title: Monitoring of Bone Loss Bio-Markers in Human Sweat: a non-invasive, time efficient means of monitoring bone resorption markers under micro and partial gravity loading conditions.

Proposal Number:07-HRP-2-0022

PI Name: Mark Clarke

PI Institution:UNIVERSITY OF HOUSTON SYSTEM

Scientific Merit: Strengths

Reviewer 1

This is a very well-written proposal that aims to validate the usefulness of monitoring biomarkers of bone loss in sweat as a non-invasive means to evaluate bone changes during space flight. Strengths include the following: 1) Use of an innovative device to collect liquid sweat by capillary action that is specifically amenable to collection in the space environment. 2) A strong set of preliminary data demonstrating a) use of the device to determine ionized Ca in sweat in 8 subjects and b) correspondence of the latter results to those obtained with a conventional closed pouch device. 3) Identification that changes in resorption markers in sweat have been shown by others to track changes in BMD in both hormone replacement therapy patients and osteoporosis patients, giving credibility to the approach of using sweat as a fluid to track bone changes during space flight. 4) Excellent laboratory facilities and resources, and clear descriptions of design for proposed studies.

Reviewer 2

The study design is well-developed with three phases of experiments that: 1) identify the best collection device and assess the predictive value of bone biomarkers in sweat to markers in urine and blood, as well as BMD; 2) evaluate the device in a bed rest study; and 3) develop a device that is operationally deployable. The project clearly aligns itself with two objectives in the current NASA NRA related to quantification of crew health associated with human space flight and development of technologies for monitoring and mitigating crew health. With regard to NASA's strategic road map, this proposal focuses on issues concerning bone loss and increased risk of fracture during space flight. The PI and co-investigator together demonstrate the expertise and experience to carry out all aspects of the project including sample collection, biological assays and the development of a device suitable for space flight. Furthermore, the facilities at the University of Houston, Laboratory of Integrated Physiology are quite impressive and well-equipped to carry out the proposed studies in Phase I. Testing the collection device under sedentary as well as moderate exercise conditions is a very important aspect of the project. Crewmembers typical day involved a variety of activities, some of which involved considerable physical exertion. Additionally, evaluation of the assays in the sweat of normal, highly trained, and constitutive bone loss groups is considered a strength of the application. The capability of the device to function under such conditions and predict alterations in bone metabolism under broad

range of metabolic circumstances is essential. Assuming that the analysis of biomarkers in sweat is shown to be predictive of biochemical markers of bone metabolism assessed in the urine and/or blood and that these alterations are predictive of changes in BMD, the technical readiness of this device and method of monitoring would be at a high level. Real time monitoring of bone biomarkers would undoubtedly provide the opportunity to customize countermeasures to meet the needs of individual crewmembers.

Reviewer 3

The proposed project presents a unique idea of evaluating bone remodeling through biochemical analysis of sweat using an innovative device already developed in the PI's lab. The proposal includes an excellent review of existing sweat collecting devices, and good presentation of the problems in existing technology (difficulty in estimation of the total volume, and difficulty in defining a reference chemical) PI conducted validation of the device through comparison among sweat, urine, and blood samples. A good set of specific questions/hypotheses are proposed. The PI has an excellent research record in space related biosciences with multiple inventions and patents). Preliminary data support the feasibility of the proposed study.

Scientific Merit: Major Weaknesses

Reviewer 1

One potentially major weakness was noted: Results of Phase One Experiment 3 may demonstrate no correlation of selected sweat markers with changes in BMD, generating a 'fatal flaw' in the set of proposed studies. That is, a rationale would no longer exist for proceeding to Phase Two, the bed rest study, and maybe also Phase Three, adaptation of the sweat collecting device to automated monitoring of the sweat markers for evaluation of bone changes in space.

Reviewer 2

No major weaknesses identified by this reviewer.

Reviewer 3

Although the experimental plan is well thought out, no good research plan for understanding large variations among individuals is presented. Although there is a good correlational relationship between left and right samples, large variations in the calcium concentration among individuals are reported. Those variations might make it difficult to interpret the data.

Scientific Merit: Minor Weaknesses

Reviewer 1

Minor weaknesses include the following: 1) Analyses for calcium in blood and urine are not as well described as for sweat. That is, it appears that ionized calcium will be determined in sweat whereas total calcium will be determined in blood plasma/serum. If ionized calcium will be analyzed in the blood plasma/serum compartment, the approach needs to be better clarified. Also, if total calcium in blood will be determined, it can be projected that little correlation will exist between sweat concentrations of ionized calcium, which differ at least 6-fold from one person to another (Fig 2, Table 1), and total blood calcium, which is highly controlled and should not differ from one person to another by more than 10 to 20%. 2) Study groups appear to need further

consideration. For example, 18- to 30-y-olds define the group with 'stable BMDs', but BMD levels continue to increase until at least the mid-twenties. In addition, for the group that includes elderly females, how will the group be defined with respect to years since menopause, presence of existing osteoporosis?

Reviewer 2

The primary concern with this very well-developed application is that it is very ambitious for a 3-year project. Phase 1 is obviously critical to the success of Phase 2 and 3 due to the development of the collection device, the ability to detect and normalize PYD and ionized calcium in sweat under various conditions, and the predictability of these measures relative to change in BMD over a 6 month study period will be accomplished under this first phase. While it is appreciated that the next logical step would be to test this method of monitoring alteration in bone metabolism in a bed rest study, such tests may be more appropriate for subsequent projects to follow. Focusing on Phase 1 and addressing some of the issues relevant to normalization of the data and evaluation of other more potentially sensitive markers should be considered. The preliminary data provided demonstrate that calcium can be detected in sweat with the assay proposed; however, the very high values for calcium and wide range of values in the sweat reported raise a significant question as to how predictive sweat is in non steady-state scenarios. Additionally, a significant concern relative to space flight as well as with physical exertion is how hydration status will affect this type of the data. Cephalid fluid shifts known to occur in microgravity alter fluid balance/homeostasis. Normalizing values to Pi per the manufacturer's recommendation or total volume or rate may be helpful, but it is questionable that this will fully address the issue. This is a very important issue that has yet to be addressed based on the preliminary data provided. Clear rationale is not provided for the selection of ionizing calcium and free PYD as perhaps the most sensitive markers of bone metabolism. It is assumed that free PYD was selected based on the previous work by Sarno M et al., 1999 and 2001. Are there other indicators of bone metabolism that are more sensitive that could be tested in this protocol? Notably in Experiment 3, bone-specific alkaline phosphatase which is usually measured in the blood is proposed in blood and urine. Although bone loss in space is attributed to an increase in primarily bone resorption, the ability to assess bone resorption relative to bone formation would be very novel. Perhaps the potential for assessing BASP in sweat should be considered on some level in earlier experiments. A minor concern is that this project represents an ambitious project put forward by a team of researchers that do not demonstrate a previous history of collaboration. There is some concern from this reviewer that this project in its current form may be understaffed.

Reviewer 3

Human subjects: not well presented about the plan and approval status. Phase 1: In experiment 3 the hypothesis is focused on the hip and lumber region. The rationale is not well spelled out. There is an apparent lack of description regarding how to compare the samples from sweat, urine, and blood.

Scientific Merit: Overall Evaluation

Reviewer 1

The strengths of this proposal outweigh the weaknesses. The innovation of the sweat collection device, with the vision to develop a single unit in Phase Three that both collects and analyzes the

sweat is attractive. The PI clearly combines an interest in scientific research with a capability for innovative applications.

Reviewer 2

This is a strong application that has identified an area of work with great potential for providing a real time monitoring system of bone metabolism. Given the concerns relative to the preliminary findings and normalization of the data, scaling the project back in terms of focus to Phase 1 would enhance the opportunity to achieve the intended results.

Reviewer 3

This is a strong proposal from a PI with a good research record in the field of space-related biology. The idea of using sweat is rather unique and promising. The research environment is excellent. A concern (cited in the major weakness section) is variations among individuals. It is recommended that the PI provides a research plan for data interpretation considering those variations.

Additional Comments (Optional)

Reviewer 1

Proposal provides excellent budget justification.

Reviewer 2

No comments given.

Reviewer 3

A collaborative work with NASA/JSC has been done as an award for advanced technology development.