UNIVERSITY of HOUSTON

COMMITTEES FOR THE PROTECTION OF HUMAN SUBJECTS

December 12, 2007

To Whom It May Concern

Dear Sir/Madam:

This is to confirm that Dr. Mark S. F. Clarke has submitted an application to the University of Houston Committee for the Protection of Human Subjects entitled, "Monitoring of Biomarkers of Bone Loss in Human Sweat – A Non-invasive, Time Efficient Means of Monitoring Bone Resorption Markers under Micro and Partial Gravity Loading Conditions." This application is in support of a grant proposal by the same title.

This application will be reviewed by a subcommittee of the Committee for the Protection of Human Subjects on Friday, January 11, 2008. Information regarding the committee's decision will be forwarded to the investigator within 7 to 10 working days after the meeting.

The University of Houston is in compliance with the requirements of protection of human subjects (45 CFR 46) under federalwide assurance number FWA00005994.

Please contact Debra L. Comeaux if any additional information is required at (713) 743-9215 or by email at dcomeaux@uh.edu.

Sincerely,

John P. Gaa/dcomeaux

John P. Gaa, Ph.D., Chairman
Committee for the Protection of Human Subjects (COM1)
University of Houston

PROJECT TITLE: Monitoring of Biomarkers of Bone Loss in Human Sweat – a non-invasive, time efficient means of monitoring bone resorption markers under micro and partial gravity loading conditions.

PROJECT INVESTIGATORS: Mark. S. F, Clarke, Ph.D. (Principal Investigator); Daniel O'Connor, Ph.D. (Co-Investigator).

PROJECT GOAL: We propose to validate the concept that the rate and extent of unloading-induced bone loss in humans can assessed by monitoring the levels of two bone resorption markers in sweat, namely ionized calcium and collagen break-down products (i.e. pyridinoline cross-links). 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) in order to define the relationship between levels of these biomarkers in the respective biological samples. Bone mineral density (BMD) measures will also be incorporated in the long-term studies in order 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.

RELEVANCE TO GOALS AND OBJECTIVES OF NRA

This proposal is responsive to Objectives #1 and #3 of the present NRA as listed in Section I.B.3, namely 1) *Quantification of the crew health and performance risks associated with human space flight for the various exploration missions and 3) Development of technologies to provide mission planners and system developers with strategies for monitoring and mitigating crew health and performance risks.* In addition, this proposal is responsive to two specific goals of the HRP element of the NRA contained in Section A.2.A, namely *1) novel technologies that provide for real-time, in-flight monitoring of bone turnover during long-duration spaceflight and 2) innovative means to measure and monitor net bone calcium loss during long-duration space-flight.* With regard to NASA's strategic road map, this proposal focus on issues described in Risk Factors 9 & 10 concerning bone loss and increased risk of fracture during space flight. The technologies that will be tested for the collection of sweat and subsequent analysis of ionized calcium and collagen cross-links have a Technical Readiness Level of 2-3 with actual commercial devices being available in one instance and functioning prototypes being available in the other.

BACKGROUND INFORMATION

Bone loss during space flight, defined as a reduction in bone mineral density and structural integrity of primarily trabecular bone, is a well documented event (Collet, Uebelhart et al. 1997; LeBlanc, Shackelford et al. 1998; Carmeliet, Vico et al. 2001; Smith and Heer 2002; Smith, Wastney et al. 2005). Bone loss is a serious issue with regard to crew health and safety, especially on extended duration missions that involve physical activity in environments other than microgravity (i.e. Lunar and Mars exploration-class missions) where the increased risk of fracture upon reloading may have a significant impact on mission success. To date, two countermeasure approaches have been suggested to prevent bone loss during space flight, namely physical stimulation of the skeletal system (i.e. whole body/regional vibration or resistive loading) (Rubin, Turner et al. 2001; Smith, Davis-Street et al. 2003; Lee, Cobb et al. 2004; Shackelford, LeBlanc et al. 2004; Cavanagh, Licata et al. 2005; Blottner, Salanova et al. 2006; Gilsanz, Wren et al. 2006) or pharmaceutical intervention (i.e. bisphosphonate drugs) (LeBlanc, Driscol et al. 2002; Cavanagh, Licata et al. 2005). Both approaches appear to successfully prevent some of the bone loss that occurs in ground-based models of space flight, with the resistive exercise countermeasure approach having the dual utility of being beneficial in preventing loss of muscle mass during unloading (Bamman, Clarke et al. 1998; Clarke, Bamman et al. 1998). However, the long-term effects of vibration training or bisphosphonate treatment on numerous physiological systems including bone itself are as yet not fully understood (Santini, Vincenzi et al. 2002; Abercromby, Amonette et al. 2007; Palomo, Liu et al. 2007).

Bone loss during space flight occurs as a result of reduced levels of musculoskeletal loading that in turn alters the balance between bone formation and bone resorption (i.e. the normal bone remodeling process) in favor of bone resorption (Smith, Nillen et al. 1998; Smith, Wastney et al. 2005; LeBlanc, Spector et al. 2007). Similar bone loss (as a consequence of increased bone resorption and decreased bone formation) occurs in terrestrial settings, such as during prolonged bed-rest unloading (Zerwekh, Ruml et al. 1998; LeBlanc, Spector et al. 2007) or diseases such as osteoporosis (Lin and Lane 2004), resulting in a reduction in bone mineral density (BMD) and structural integrity. The gold standard measurement for assessing BMD is dual X-ray absorptiometry (DXA) (Economos, Nelson et al. 1999), a method that requires bulky equipment, well-trained personnel, and an extended assessment/monitoring period to allow significant changes in BMD to be detected at the level of resolution provided for by DXA. Structural integrity and by extension bone strength is estimated using peripheral quantitative computerized tomography (pQCT) (Wachter, Augat et al. 2001). As such, the operational usefulness of the DXA technology, and to a lesser extent pQCT in the space flight environment is limited for a variety of reasons, including significant up-mass, volume and power issues. In addition, the extended period (i.e. months) required to detect small changes in BMD by DXA precludes the use of this technology as a realistic means of providing the information required to justify modifying an ineffective bone loss countermeasure prescription during a mission.

One of the central challenges faced in the manned space program is providing real-time biomedical monitoring of crew members in order to assess the efficacy and effectiveness of countermeasures designed to combat musculoskeletal de-conditioning (Feeback and Clarke 2006) induced by extended space flight. This challenge is especially problematic in the case of bone loss. Regardless of the ultimate countermeasure or countermeasures that will be deployed to combat bone loss during space flight it is essential that both their efficacy and effectiveness be validated/monitored not only in terrestrial analogs of altered gravity exposure (i.e. micro- or partial gravity loading) but also during actual extended space flight/planetary exploration. As such, the development of non-invasive, non-radiological techniques that can be used to provide real-time monitoring of bone loss and more importantly the effects of bone loss countermeasures during space flight and planetary exploration is critical to crew health and performance as well as mission success.

A more recent approach to assessing bone loss other than DXA or pQCT is the monitoring of biomarkers known to be associated with the particular stages of the bone remodeling process (Young 2003). During bone resorption for example, calcium and collagen cross-links are released as a result of osteoclast activity, whereas during bone formation increased levels of bone-specific alkaline phosphatase and osteocalcin are produced due to increased osteoblast activity (Pagani, Francucci et al. 2005). Elevated or decreased levels of these respective biomarkers can be detected in the blood and urine of individuals undergoing bone loss (Maimoun, Couret et al. 2002; Smith, Wastney et al. 2005) and are correlated with changes in BMD over time (Smith, Nillen et al. 1998; Stepan and Vokrouhlicka 1999). In addition, these biomarkers are also sensitive to increased mechanical loading of bone, with bone resorption markers going down and bone formation markers going up in normal individuals who are for example undergoing resistive exercise training (Fujimura, Ashizawa et al. 1997; Shackelford, LeBlanc et al. 2004). This approach has been successfully used to monitor the efficacy of pharmaceutical treatment in patients being treated for osteoporosis (Pagani, Francucci et al. 2005), as well as being correlated with decrements in BMD in individuals undergoing extended bed rest (LeBlanc, Driscol et al. 2002) and in astronauts during space flight (Smith, Nillen et al. 1998; Smith, Wastney et al. 2005). More importantly, significant changes in bone resorption biomarkers are observed within days of unloading and remain elevated through-out the unloading period, well before any changes in BMD can be measured (Smith, Nillen et al. 1998; LeBlanc, Spector et al. 2007). As such, real-time monitoring of biomarkers of bone loss, such as calcium and/or collagen cross-links, may provide the information required not only to determine the degree/rate of bone loss during space flight or partial planetary gravity but also to monitor and modify if necessary the effects of in-flight bone loss countermeasures such as the Advanced Resistive Exercise Device (aRED).

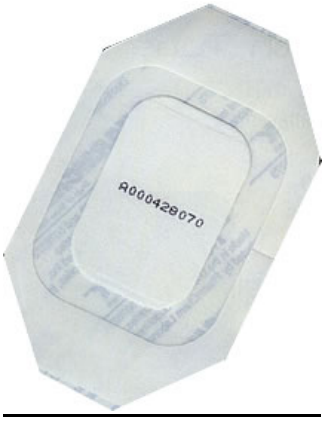
From an operational perspective however, the collection of either blood or urine samples during a mission requires a significant commitment of crew time, as well as posing additional technical challenges with regard to the biochemical analysis of these samples. These technical challenges primarily surround the physical/fluidic issues associated with analyzing a liquid sample in micro- or partial gravity environments, rather than the actual underlying biochemistry utilized to measure the biomarker. With these operational limitations in mind, the ideal technique for measuring biomarkers of bone loss would employ an approach that fulfills the following criteria: ***(1) the biological sample would be collected from a crew member in a non-invasive manner requiring little or no crew interaction; (2) once collected the sample could be analyzed during space flight using a standard biochemical technique previously validated in terrestrial laboratories; (3) the data generated using this biochemical technique would be valid relative to the wider clinical norms and longitudinal data generated in space flight analog studies.***

Collection of sweat as a source of physiologically relevant biomarkers has previously been used to monitor both clinical and human performance outcomes. Sweat has also been used to monitor levels of a number of drugs, including methamphetamine (Barnes, Smith et al. 2007), benzodiazepines, opiates (Kintz, Tracqui et al. 1996), caffeine (Delahunty and Schoendorfer 1998) and nicotine (Kintz, Henrich et al. 1998). Human sweat has been shown to contain significant amounts of a number of clinically relevant biomarkers, including calcium (O'Toole, Johnson et al. 2000; Palacios, Wigertz et al. 2003; Rianon, Feeback et al. 2003), collagen cross links (i.e. pyridinoline) (Sarno, Powell et al. 1999; Sarno, Sarno et al. 2001), cortisol, growth hormone, insulin-like growth factor-1 and a variety of interleukins to name a few (Marques-Deak, Cizza et al. 2006). In addition, monitoring of biomarkers contained in the sweat of soldiers has received significant interest as means of assessing nutritional status and stress levels under battlefield conditions (Cizza, Eskandari et al. 2004). The possibility that biosensors attached to the skin, designed to collect, analyze and transmit the concentration of specific biomarkers to a remote monitoring station is of great interest to the military and potentially to NASA during planetary exploration.

In humans, three types of sweat glands produce sweat, namely the apocrine, apoeccrine and eccrine sweat glands. The apocrine and apoeccrine sweat glands are confined to the axillary and perineal regions and produce sweat containing a fatty material called sebum. The eccrine glands are distributed over the whole body surface, produce liquid sweat and are primarily responsible for thermo-regulation of the body via evaporation of this fluid from the surface of the skin. The structure of the eccrine sweat gland consists of secretory coil and a duct that opens to the skin. The secretory coil secretes a plasma-like fluid, the amount of fluid being produced being dependent on the degree of sympathetic nervous system stimulation of the gland. Low levels of sympathetic stimulation result in the vast majority of the fluid being reabsorbed before reaching the duct, resulting in a low sweating rate. However, if sympathetic stimulation levels are high as is the case during stressful situations, increased physical activity or increased body temperature, large amounts of fluid are secreted by the secretory coil and reach the duct before being reabsorbed, resulting in an increase in the volume of sweat produced and sweating rate. As such, the total amount of biomarker secreted in sweat in a fixed period of time is dependent on not only the circulating levels of the biomarker but also the volume of sweat produced and the sweating rate. As such, it is still unclear if the total amount of biomarker secreted in sweat produced under sedentary conditions is equivalent to that produced under active conditions when normalized for sweat volume.

To date, only two means of collecting human sweat for subsequent biomarker analysis have been reported. The first is the use of a non-permeable plastic or wax layer (Parafilm™) attached to the skin with an adhesive patch where sweat pools between the skin and the layer and is then collected when the layer is removed. This technique is also known as a closed pouch collection device and collects unadulterated liquid sweat. The second technology utilizes an absorbent cellulose patch attached to the skin with an adhesive layer and is commercially available under the name OsteoPatch™ or PharmChek™ (Figure 1). This technology relies on collection of sweat by evaporation of the water component of sweat while dissolved analytes and biomarkers are retained within the cellulose material. The OsteoPatch™ or PharmChek™ sweat patches are FDA-approved medical devices for the qualitative detection of a number of drugs and their metabolites. This technology has also been used to monitor bone resorption markers (i.e. free pyridinoline cross-links) in both hormone replacement therapy patients and osteoporosis patients being treated with alendronate, where changes in the level of resorption markers in sweat directly correlated with true changes in BMD (Sarno, Sarno et al. 2001).

Figure 1 OsteoPatch™/ PharmChek™ absorptive pad for collection of sweat.



Although the OsteoPatch™/ PharmChek™ sweat patch technology is simplest of the two published sweat collection methods, it requires removal of analytes/biomarkers from the cellulose pad

by rehydration/resolvation prior to quantification. This is achieved by adding a known amount of distilled water to the cellulose pad followed by removal of the reconstituted sweat by centrifugation prior to biomarker analysis. As the actual volume of liquid sweat collected by the OsteoPatch™/ PharmChek™ is unknown, biomarker concentration is determined by normalization to the inorganic phosphate content of the reconstituted sample. However, previous studies that have directly collected liquid sweat using a non-permeable layer/closed pouch device indicate that the inorganic phosphate content of sweat differs depending on both the sweating rate and hydration state of the subject (Hayden, Milne et al. 2004). Although the OsteoPatch™/ PharmChek™ patch allows for calculation of the total amount of a biomarker secreted in the sweat produced over time by a defined area skin, it does not provide an accurate measure of sweat volume. The OsteoPatch™/ PharmChek™ patch has previously been used to determine sweat calcium excretion in exercising normals and bed-rest subjects (Rianon, Feeback et al. 2003) where the low amounts of calcium collected using the Osteopatch™ patch required the use atomic absorption flame photometry analysis (a technique unsuitable for deployment in space) for quantification. However, our own preliminary studies relative to calcium levels (and by extension other biomarkers) in unadulterated liquid sweat (rather than elution from a cellulose patch) indicate that biomarker recovery from the OsteoPatch™ patch may be much less efficient than previously reported in the literature (*see Preliminary Data Section below*).

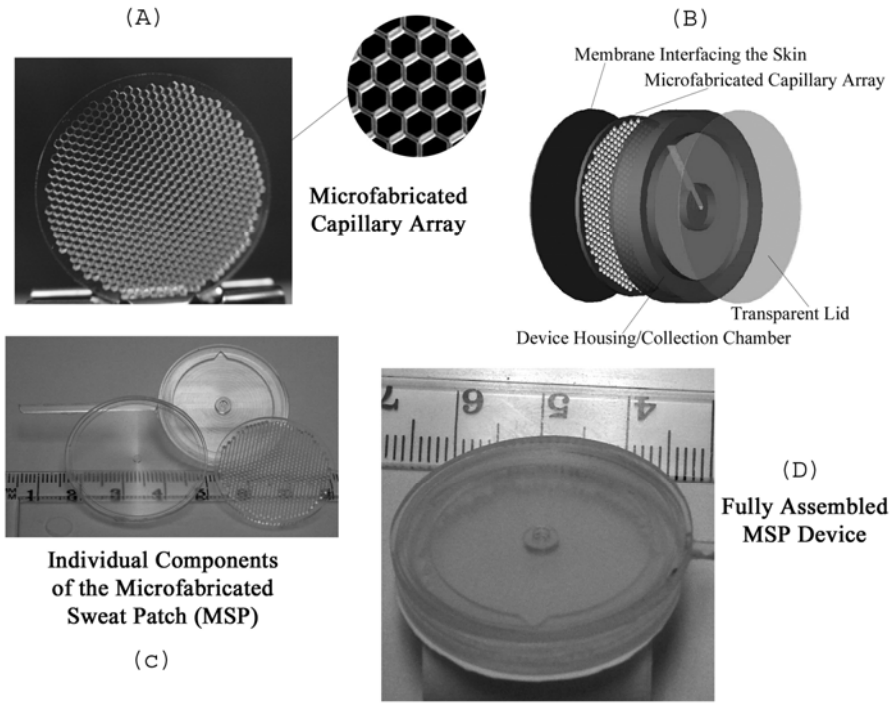
In general, sweat is a physiological sample that can be collected in a non-invasive and time efficient manner and may prove to be ideal sample for monitoring a number of biomarkers of bone loss during a space mission. However, existing collection technologies such as the OsteoPatch™/ PharmChek™ patches or closed pouch devices do not lend themselves to use in micro- or partial gravity environments due the fluidic collection/fluidic handling/phase separation issues commonly experienced in altered gravity conditions. For example, the non-permeable layer collection technique requires pooling of sweat in a catch pocket for later collection by suction, whereas the OsteoPatch™/ PharmChek™ technology requires accurate rehydration with a pre-determined volume of distilled water followed by sample collection by centrifugation.

To overcome these issues, we have recently developed a miniaturized, rapid, non-invasive, gravity-independent technique for collection of human sweat. This technique utilizes a micro-fabricated array of capillary tubes arranged in a disc form, manufactured from biocompatible, low-protein binding materials, known as the Micro-fabricated Sweat Patch (MSP) device (see Figure 2). This device was originally developed under the auspices of a NASA/JSC CDDF award for advanced technology development. The initial project was carried out in conjunction with the PI at the Center for Advanced Microstructures and Device (CAMD) at the Louisiana State University and focused on development of the techniques and optimal materials for fabrication of the device. The MSP device is fabricated using "state-of-the-art" polymer-based x-ray lithography techniques known as LIGA and was designed to be mass produced once validated. The MSP device is attached to the surface of the skin using an adhesive patch during the sweat collection protocol. A pre-determined volume of unadulterated liquid sweat (i.e. up to 650 μl) can be collected either at rest or during exercise using the MSP device by a ***gravity independent*** means, namely capillary action. The unadulterated liquid sweat sample is then recovered from the MSP capillary array by centrifugation into a collection tube containing predetermined amounts of analysis reagents. In addition, a second generation MSP device that utilizes sensor elements embedded within the device itself (rather than removal of the sample by centrifugation prior to analysis) to determine biomarker concentration (for example calcium membrane selective electrodes) is envisioned and forms the later project goals described in this proposal. These goals center on the use of ion-specific electrode technology to detect calcium in sweat samples and the use of molecular imprinting technology to develop a solid state PYD biosensor element. The ability to fabricate the MSP device so that a pre-determined volume of sweat contacts the sensor allows determination of an accurate concentration value for the analyte of interest.

PRELIMINARY STUDIES

Our preliminary studies have focused on testing and comparing the ability of the first generation MSP device (Figure 2) to collect a sample of unadulterated liquid sweat relative to samples collected using a non-permeable plastic layer/closed pouch device. A second goal of these preliminary studies was to compare the levels of a physiologically relevant biomarker (i.e. calcium) in a sweat sample collected using these two different collection methods. Testing was carried out using sweat collected from the right forearm of both male and female subjects participating in moderate physical activity (i.e. active sweating during soccer practice). Either an MSP device or a closed pouch device was secured using an adhesive pad to the skin of the right forearm of each subject. After 20 min of active sweat collection, the closed pouch device was removed. Unadulterated liquid sweat was collected by direct aspiration from the closed pouch device and dispensed into the same type of sample tube used for collecting sweat samples from the MSP device. In the case of the MSP devices, samples were collected directly by centrifugation into the sample tube.

Figure 2 Micro-fabricated Sweat Patch (MSP) Device. (A) micro-fabricated capillary array manufactured using the LIGA process. (B) diagrammatic representation of the MSP components. (C) disassembled MSP components. (D) fully assembled MSP device. (Scale – cm).



Sweat samples were then directly assayed for calcium content using a commercially available micro-titer plate format calcium assay based on the production of a colorimetric end product. Ionized calcium in triplicate samples (5 µl volume each) of sweat was determined using a commercially available colorimetric assay available from BioAssay Systems (Hayward, CA). The assay can detect ionized calcium in a linear fashion in the range of 25–2000 µg/ml (62 µM – 50 mM range). Calcium concentration was determined by comparison to a standard curve generated on the same micro-titer plate using ionized calcium standards supplied by the manufacturer. Calcium levels in sweat collected from a total of thirteen subjects varied from approximately 140 µg/ml to 1350 µg/ml (i.e. 3.5mM to 34mM concentration) during active sweating using either the closed pouch device (Figure 2) or MSP

device (Table 1). Total sweat volume produced during the 20 min exercise period was not determined during this protocol. At present it is unknown whether or not the variation observed in sweat calcium concentrations between subjects was due solely to differences in sweating rate between individuals or constitutive differences in the amount of calcium being secreted by the individual.

As can be seen in Figure 2 and Table 1, a wide range of calcium concentrations in sweat were detected in different individuals, ranging from approximately 140 ug/ml Ca2+ to 1350 ug/ml Ca2+ (3.5mM to 34mM). This figure is in stark contrast to the much smaller amounts of calcium excreted in sweat collected using the Osteopatch™ device, reported as a daily loss of around 35 mg/day Ca2+ from sweat (normalized for total skin surface area) or approximately 23 ug Ca2+/24 hr collection period in a single Osteopatch™ device (Rianon, Feeback et al. 2003). Although our preliminary data was generated from sweat collected during a 20 min period of exercise (i.e. active sweating) whereas the previously reported data was generated from a 24 hr period of evaporative sweat collection under sedentary conditions, the large discrepancy (i.e. at least one order of magnitude) in the amount of calcium detected was a concern.

Figure 2 illustrates the calcium content of fore-arm sweat collected from eight individuals after 20 min of active sweating using a closed pouch device followed by direct aspiration of sweat from the device. All sweat was collected from the right forearm.

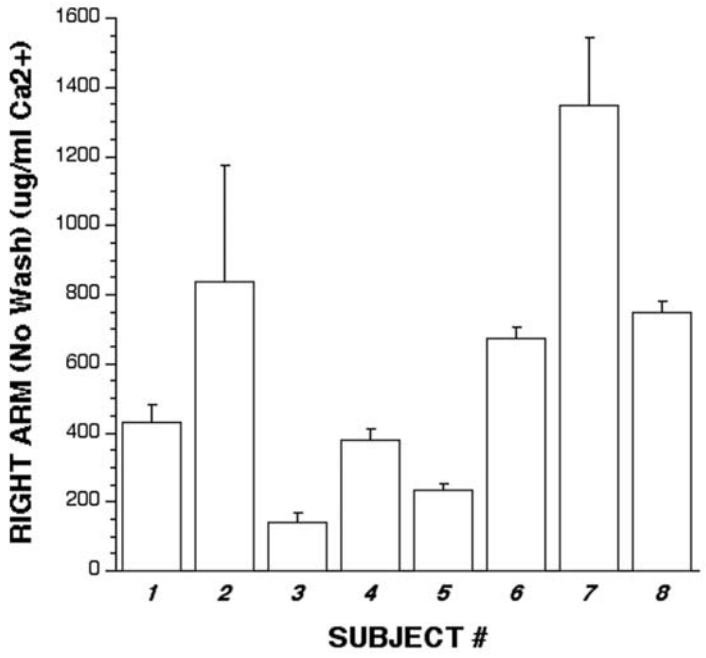
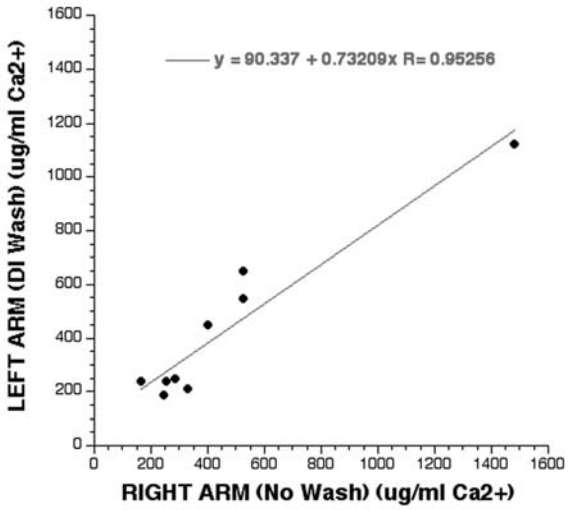


Table 1 Calcium (Ca2+) Concentration in Sweat Collected from Exercising Subjects using MSP Devices.

SUBJECT ID	MEAN Ca2+ (ug/ml)	Standard Deviation
A	232	22
B	641	28
D	368	31
E	247	17
G	1235	168

In order to ensure that these surprisingly large amounts of excreted calcium where not associated with contamination of our sweat samples with exogenous calcium sources, such as soap or detergent residues on the skin, a direct comparison of sweat collected from the left and right forearms of the same individual using a closed pouch device was made, with the left forearm being washed with distilled water and then dried before placement of the closed pouch device. Sweat was collected in this fashion from nine additional male and female subjects participating in moderate physical activity (i.e. active sweating during soccer practice), again with a wide range of sweat calcium concentrations being detected (Figure 3). However, when the amount of calcium contained in the paired sweat samples from washed and unwashed arms were compared, a high degree of correlation (R= 0.953) between calcium concentration from left and right arms, washed and unwashed respectively, was observed (Figure 3), with no significant differences in calcium concentrations between right and left forearm sweat samples being detected (paired t-test - two-tailed; p = 0.483).

Figure 3 illustrates the effect of washing the skin with de-ionized water prior to sweat collection. Sweat was contemporaneously collected from both the left and right forearms of a total of nine individual using a closed pouch device. In order to ensure that the calcium signal was associated with sweat, rather than for example soap product contamination, one arm was washed with distilled water and dried prior to pad placement.



Our preliminary data indicates that unadulterated liquid sweat, collected under moderate exercise conditions from young healthy adults using either a closed pouch device or an MSP device, contains significantly more excreted calcium than that previously reported using an evaporative sweat collection technique (i.e. OsteoPatch™/ PharmChek™ patches) (Rianon, Feeback et al. 2003). This discrepancy may be due to significantly more calcium being excreted during active sweating as compared to sweating under sedentary conditions, or, a significant retention of calcium within the cellulose matrix of the OsteoPatch™/ PharmChek™ patches following rehydration, that in turn leads to a significant underestimation of the amount of calcium excreted in sweat. Our preliminary data indicate that at least one biomarker of bone loss (i.e. excreted calcium) can be detected in sweat and that the levels of this biomarker are high enough to detect using a simple colorimetric assay based on the well-characterized chemistry use to determine clinical levels of calcium in both blood and urine.

PROJECT PLAN

We propose to validate the concept that the rate and extent of unloading-induced bone loss in humans can assessed by monitoring the levels of two bone resorption markers in sweat, namely ionized calcium and collagen break-down products (i.e. pyridinoline cross-links). Preliminary data in healthy adults indicates that excreted calcium levels under active sweating conditions can be easily quantified using a simple colorimetric assay. Initial testing will be carried out in three populations (at rest and during activity) that have different constitutive levels of bone remodeling, namely normal healthy individuals, air-force cadets undergoing regular heavy resistance training and healthy elderly individuals. 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) in order to define the relationship between levels of these biomarkers in the respective biological samples. Bone mineral density (BMD) measures will also be incorporated in the long-term studies in order 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.

Our proposal is divided into three phases lasting approximately 12 months per phase.

Phase One will involve direct comparison of three different sweat collection techniques, namely the closed pouch collection device, the OsteoPatch™/ PharmChek™ device and the MSP device with regard to measurement of ionized calcium and free pyridinoline in sweat. Sweat will be collected under resting and light exercise conditions from three populations that have different constitutive levels of bone remodeling, namely normal healthy individuals, air-force cadets undergoing regular heavy resistance training and healthy elderly individuals. Calcium and free pyridinoline levels in contemporaneous samples of sweat, blood and urine will be compared. Sweat will be collected from the skin surface of the right forearm for a period of 4 hr under sedentary conditions and 20 min under light exercise conditions (i.e. bicycle ergometry at 30% of predicted VO2max). Quantitation of ionized calcium will be achieved using a standard commercially available colorimetric assay. The use of a calcium-selective electrode technology (the I-Stat system previously validated and utilized on-orbit) for analyzing sweat calcium concentration will also be investigated. Pyridinoline levels in sweat will be quantified using a commercially available ELISA assay (Quidel Corp, San Diego, USA). Alternatively, an antibody capture-based Time-of-Flight Mass Spectrometry (TOFMS) approach to quantifying free pyridinoline will also be investigated (Clarke, Weinberger et al. 2002).

PHASE ONE of the project will consist of three separate experiments designed to test the listed hypotheses.

PHASE ONE: Experiment 1

Specific Aims*:* To investigate the relationship between the amount of calcium and PYD secreted in unadulterated sweat collected using a closed pouch device (CPD) depending on whether sweat is produced under exercising or non-exercising conditions. A secondary goal is to determine if calcium or PYD levels excreted in sweat are predictive of the amount of calcium or PYD present in the plasma or excreted in a contemporaneous urine samples or a 24 hr urine sample.

Hypotheses

(1) Is there a significant difference in the concentration of calcium or PYD in sweat obtained under sedentary or moderate exercise conditions?

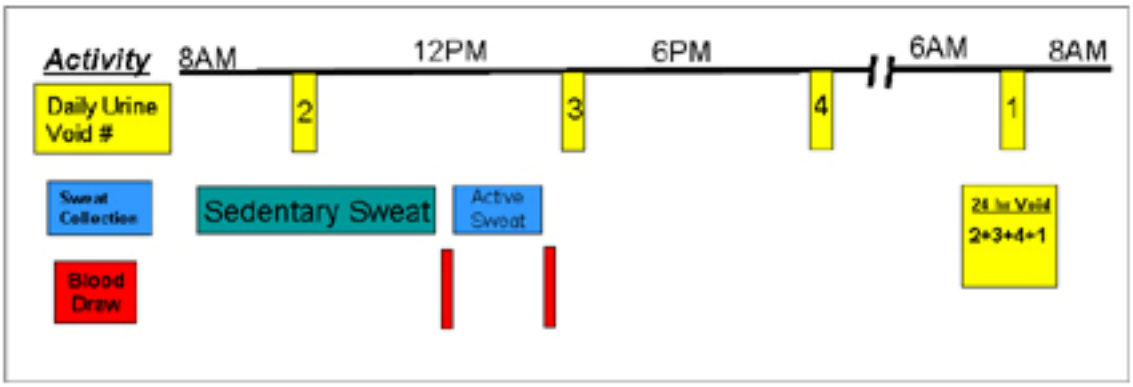
(2) Are the total amounts of calcium and PYD secreted in sweat the same if normalized for either total sweat volume collected or sweat rate (volume/time)?
(3) Is sweat calcium or PYD concentration (from sedentary or exercising periods) predictive of the amount of either analyte excreted in the urine collected in a contemporaneous fashion?
(4) Is sweat calcium or PYD concentration (from sedentary or exercising periods) predictive of the total amount of either analyte excreted in the urine over a 24 hr period?
(5) Is sweat calcium or PYD concentration (from sedentary or exercising periods) predictive of the plasma concentration of either analyte?

Experimental Design

Sample Size: We used a power analysis to determine a sample size of 15 subjects for Experiment 1. The power analysis was based on the criterion that the sweat measures will predict the subjects' urine and blood measures within an interval that is less than ±5% of the population standard deviation, which we identified as clinically acceptable accuracy. For example, the population standard deviation for 24 hour urine calcium is approximately 35 mg. A sample of fifteen subjects would allow for determining whether the predictions using sweat measures are within ±1.7 mg of the 24 hour urine calcium measures. Specifically, the sample size was estimated for a type I error rate (α) of 0.01 and a type II error rate (β) of 0.01 (power = $1 - \beta = 99\%$) by computing the noncentrality parameter ($\Phi = 11.07$) for the noncentral F distribution under the assumption that the ratio of prediction error (δ) to population standard deviation (σ) is $\delta/\sigma \leq 0.05$; i.e., the prediction error is less than 5% of the population standard deviation.

Experimental Protocol: Subjects (15 normal healthy male and female adults, 18 – 30 years old, all activity levels, BMI within normal range recruited from the undergraduate university population) will be asked to batch collect their urine for a period of 24 hr beginning with their second urine void on the day of the experiment and ending with their first urine void the next morning. Each urine void over the course of the 24 hours will be collected in a separate disposable container to allow determination of individual void volume and biomarker (i.e. Ca2+ and PYD) concentration prior to pooling of the sample to obtain a composite 24hr void for analysis. Subjects will wear a closed pouch device (CPD) on their right forearm for a period of 4 hr to collect a sweat sample under sedentary conditions beginning immediately after their first urine void on the day of the experiment. At the end of 4 hr, sweat will be collected from the patch, its volume determined, aliquoted into smaller volumes and frozen at -80^{o}C for later biochemical analysis. At this time, a sample of whole venous blood will be collected from the right antecubital vein by standard venipuncture technique for the preparation of a plasma sample. A new CPD will be placed on the same region of the subject's right forearm and sweat will be collected for a period of 20 minutes while the subject exercises at a light to moderate level. Exercise will consist of stationery bicycling (subject's choice) at a work-load of 30% of their age-predicted VO2max. At the end of the exercise session an additional sample of whole venous blood will be collected from the left antecubital vein by standard venipuncture technique for the preparation of a plasma sample.

Timeline



Comparisons

Calcium and PYD concentrations in sedentary sweat versus active sweat.
Calcium and PYD concentrations in sedentary sweat versus urine Void 2.
Calcium and PYD concentrations in active sweat versus urine Void 3.
Calcium and PYD concentrations in sedentary/active sweat versus plasma calcium and PYD concentrations.
Calcium and PYD concentrations in sedentary/sweat versus 24 hr urine void.

PHASE ONE: Experiment 2

Specific Aims: To investigate any differences in the amount of calcium and PYD detected in unadulterated sweat collected using a closed pouch device (CPD) (criterion measure), sweat collected using the micro-fabricated sweat patch (MSP) or the OsteoPatch™/ PharmChek™ device (OPD). This data will be used to determine the most accurate and efficient sweat collection device for determining sweat biomarker concentration. A secondary goal is to simultaneously collect sweat samples from the same region of both forearms to determine if anatomical location impacts the amount of analyte secreted in sweat.

Hypotheses

(1) Are there significant differences in the amount of calcium or PYD detected in sweat collected using the three devices obtained under sedentary or exercise conditions?
(2) Are there significant differences in the amount of calcium or PYD detected in sweat collected using the three devices obtained under sedentary or exercise conditions between the right and left forearms?

Experimental Design

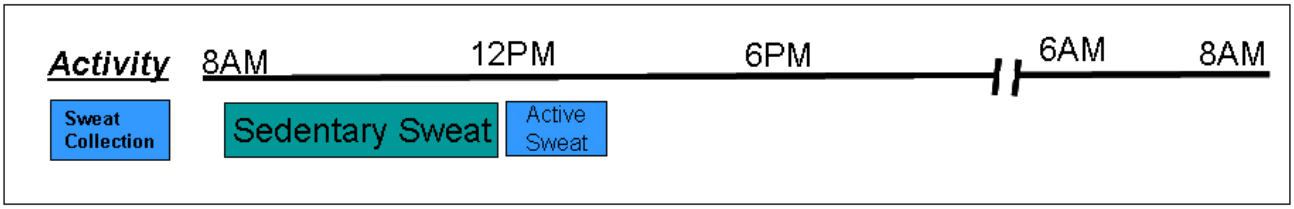
Sample Size: A sample size of 24 subjects for Experiment 2 was determined by a power analysis methodology commonly used in designing bioequivalence studies comparing two methods of measurement (see, for example, Zhang, 2003). The power analysis was based on the criterion that the differences between any two sweat collection methods will be less than the within-subject measurement error for each method. Specifically, the number of subjects required in this within-subjects analysis was computed by

$$n = (t_{1-\alpha,2n-2} + t_{1-(1-c)\beta,2n-2})^2(\sigma_i/[\Delta-\theta])^2$$

where σ_i is the within-subject standard deviation (i.e., the repeatability index, or measurement error), Δ is the magnitude of the tolerance interval for clinically useful comparison, and θ is the magnitude of the tolerance limit of absolute differences between methods. The ratio σ_i/Δ, measurement error to tolerance, is the critical parameter function for determining the sample size; in the proposed study this ratio was $\sigma_i/\Delta \geq 1.1$ (i.e., the tolerance interval Δ between methods was always smaller than meaurement error σ_i). The solution of this equation requires an iterative algorithm to converge on an integer value for n, the number of subjects required to ensure that the tolerance interval and absolute tolerance boundaries are not violated.

Experimental Protocol: As the OPD uses an absorptive/evaporative process to collect sweat constituents, sweat volume for calculation of calcium and PYD concentrations in sweat will be calculated using inorganic phosphorus (Pi) as a volume marker (the manufacturer's recommended method). Both the IPP and MSP collect unadulterated liquid sweat so a direct volumetric measurement will be made for calculation of calcium and PYD concentrations in both these samples. Sweat will be collected from subjects under sedentary and exercising conditions using the same methodology and time-line as detailed in Experiment 1 except that urine and plasma samples will not be collected. CPD, MSP and OPD collection devices will be simultaneously worn on the same anatomical regions of right and left forearms, the devices being placed next to each other on the skin in random order. Sweat will be simultaneously collected using the OP, IPP and MSP devices under both sedentary and exercising conditions.

Timeline



Comparisons

Calcium and PYD concentrations in sweat collected using the CPD, MSP and OPD under sedentary conditions.
Calcium and PYD concentrations in sweat collected using the CPD, MSP and OPD under exercise conditions.
Calcium and PYD concentrations between forearms in sweat collected using the CPD, MSP and OPD under sedentary conditions.
Calcium and PYD concentrations between forearms in sweat collected using the CPD, MSP and OPD under exercise conditions.

PHASE ONE: Experiment 3

Specific Aims: To investigate the relationship between sweat, urine and blood levels of calcium, PYD (bone resorption) and bone specific alkaline phosphatase (BSAP) (bone formation – urinary and blood, unknown if present in sweat) relative to bone mineral density (BMD) over a six month period in three separate populations having different constitutive levels of bone remodeling. These three populations will be young healthy individuals (control group - normal bone remodeling, stable BMD), air-force cadets undergoing regular heavy resistance training (increased bone formation, increasing BMD) and

healthy elderly individuals (increased bone resorption, decreasing BMD). BMD measures (hip and lumbar regions) will be performed at the beginning, at three months and again at the end of the study to assess overall BMD changes in each population. The relationship between sweat, blood and urine biomarkers of bone remodeling (collected on a monthly basis) and BMD will be investigated to determine the predictive value of the biomarkers for changes in BMD.

Hypotheses

(1) Can sweat, blood or urine biomarkers of bone remodeling (calcium, PYD, BSAP) be used to predict the rate of change in BMD of the hip or lumber region?
(2) Which source of biomarkers (sweat, urine or blood) is the most predictive (i.e., accurate) measure of changes in BMD?
(3) How accurately can the amounts of biomarkers of bone remodeling found in sweat predict changes in BMD?
(4) Which type of sweat sample (sedentary or active) is most predictive of changes in BMD?

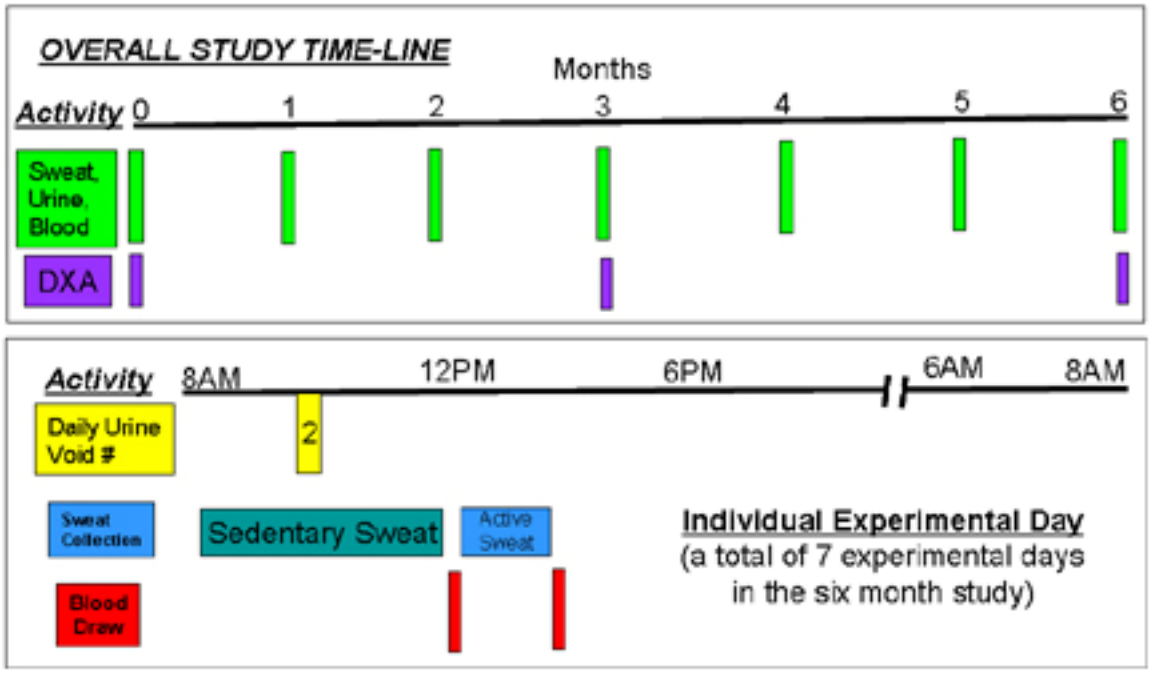
Experimental Design

Sample Size: We will use a total sample size of 45 subjects, 15 subjects in each group, for Experiment 3. The sample size was based on the criterion that the sweat, blood, and urine measures will predict the subjects' BMD within an interval that is less than ±5% of the population standard deviation. Specifically, the sample size was estimated for a type I error rate (α) of 0.01 and a type II error rate (β) of 0.01 (power = $1 - \beta = 99\%$) under the assumption that the ratio of prediction error (δ) to population standard deviation (σ) is $\delta/\sigma \leq 0.05$ for three repeated measures of each measure and three repeated measures of BMD, assuming a population correlation (ρ) among the measures of 0.75. A mixed model analysis will be used to test the Phase One: Experiment 3 hypotheses. Sweat, blood, and urine measures will be modeled as time-varying within-subjects fixed effects predicting BMD, group will be modeled as a time-invariant fixed effects covariate, and fixed interaction effects for measures (sweat, blood, urine) by time will be included. All measures will be centered on the Time 1 values. Initial BMD and change in BMD over time and their covariance will be modeled as random effects, varying by subject. The test of the hypotheses will consist of tests of contrasts created in the within-subjects fixed interaction effects matrix. The model's covariance random effect will be tested to determine if variance in initial status affects the hypothesized relations of the changes in the sweat, blood, and urine measures to the change in BMD over time.

Experimental Protocol: Both male and female participants will be recruited to each experimental group. The control group will consist of 18-30 year old college students who are considered untrained and have an activity level of 0 to 1 on the University of Houston Self Reported Activity Scale. The constitutive bone formation group will be recruited from the freshmen class of the University of Houston's Air-force ROTC Detachment 003 who undergo regular organized physical activity training as part of their academic curriculum (see letter of support from Colonel Philip Bossert, Commanding Officer, Air-force ROTC Detachment 003, University of Houston). The constitutive bone loss group will be recruited from members of the Senior Fitness Initiative-TEXERCISE-Houston group (see letter of support from Dr. Joel Bloom, Chair of TEXERCISE Houston, Senior Fitness Initiative of the City of Houston's Mayors Wellness Council. All participants will receive DXA scans of the hip and lumbar region at the beginning of the study, again after three months and at the end of the six month study period. BMD for the head of the trochanter and lumbar vertebrae (L1-L4) will be determined using an Hologic DXA scanner located in the Department of Health and Human Performance at the University of Houston. Sweat, urine and blood samples will collected on a total of seven experimental days during the study (at the beginning of the study and then at monthly intervals until the end of the six month

study). Participants will be asked to provide a sample of their second urine void of the day on each experimental day, a sedentary sweat sample, an active sweat sample and a blood sample immediately before and after the active sweat sampling time point. Sweat samples will be collected using the sweat collection device deemed the most accurate with regard to biomarker determination and easy of use based on data analysis of **Experiments 1 and 2**. Blood samples will be drawn using standard veni-puncture technique as described above. Sweat, blood and urine samples will be aliquoted into smaller volumes and frozen at -80^{o}C for batch analysis of calcium, PYD and BSAP levels at the end of the study.

Timeline



Comparisons

Calcium, PYD and BSAP concentrations in sweat, blood and urine collected under sedentary conditions compared to BMD measures.

Calcium, PYD and BSAP concentrations in sweat, blood and urine collected under exercise conditions compared to BMD measures.

Comparison of regional BMD measures to Calcium, PYD and BSAP concentrations in sweat, blood and urine collected under sedentary and active conditions.

Based on the data generated in Phase One, a decision on ***(1)*** the most efficient/accurate means of collecting sweat for monitoring of bone resorption markers relative to the criterion measures (blood and urine levels) and BMD values and **(2)** the most suitable method of sweat collection for use in partial micro- or partial gravity conditions will be made for further testing in **Phase Two**.

PHASE TWO of this project will involve testing of the sweat collection technology and collection conditions (active or non-active conditions) chosen in **PHASE 1** in a NASA co-ordinated bed-rest and/or lunar gravity study conducted at GRC-UTMB facility in Galveston. Sweat samples from bed-rest subjects will be collected as determined by our preliminary studies in Phase One, analyzed for calcium, PYD and BSAP and this data will be correlated with the standard bone loss measures

collected during these studies (i.e. blood and urine bone turnover biomarkers, BMD by DXA or pQCT) in order to validate the use of sweat biomarker analysis as a means of monitoring bone loss suitable for space flight.

PHASE THREE of this project will involve the development of an operationally deployable sweat collection device suitable for sweat sample collection in microgravity or partial gravity conditions. This effort will incorporate the development of a sweat collection device that allows collection and analysis of sweat for biomarkers of bone turnover, such as calcium and PYD, within a single device. We will draw on our significant expertise in the development of macro-fluidic and micro-fluidic biosensors for use in microgravity (Clarke and Feeback 1999; Clarke and Feeback 2004; Singh, Desta et al. 2005; Gazda, Schultz et al. 2007) for use in developing this technology if Phase One and Phase Two of this project prove successful. Fabrication and design facilities will be provided by the Center for Advanced Micro-structures and Devices at the Louisiana State University on a contract basis under the direction of the Principal Investigator. Phase Three of this project may run concurrently with Phase Two of this project depending on the results from Phase One and access to bed-rest patients.

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PROJECT ADMINISTRATION/MANAGEMENT APPROACH

The Principal Investigator, Dr. Mark Clarke, will be responsible for the overall direction of the project including the day to day supervision of the two requested graduate students including their training in sample collection and biochemical analysis. Dr. Daniel O'Connor will be responsible for experimental design issues/data analysis and provide additional supervision of the requested graduate students. Although University of Houston CPHS approval of the overall complete project is pending (Jan 2008) (see UH-CPHS Letter), each individual experimental element (i.e. blood, urine and sweat collection protocols, exercise protocols and DXA scanning) employed in this project already enjoy CPHS approval as standard procedures performed within the Laboratory of Integrated Physiology under "stand-alone" CPHS approved blanket protocols.

LABORATORY FACILITIES

The Principal Investigator is a founding member of the Laboratory for Integrated Physiology (LIP) in the Department of Health and Human Performance at the University of Houston. The LIP is a modern human performance research laboratory covering approximately 6000 sq. feet which has been split into three main areas and houses a multi-disciplinary team of faculty members working in variety of

research fields. The Principal Investigator, Dr. Mark Clarke and the Co-Investigator, Dr. Daniel O'Connor have complete access to all equipment and resources located in the LIP.
The first main laboratory area contains equipment focused in the exercise physiology research area, including strength/conditioning equipment, aerobic capacity (VO2max) testing equipment, a virtual-reality bicycle ergometer, near-infra red spectroscopy (NIRS) equipment designed to non-invasively monitor muscle/tissue blood flow, a whole body vibration platform for studying bone/muscle adaptations to vibration stimulus and strength testing equipment. This area also has several smaller side rooms in which are located a phlebotomy/minor surgical procedures room, store room and graduate student offices.

The second main laboratory area contains equipment primarily focused in the motor control/motor performance area, including a "state-of-the–art" motion capture camera system based on reflective marker technology, a portable instrumented walk-way for gait analysis, a permanent walkway with embedded force-plates for gait and posture analysis and assorted surface EMG systems for studying neuromuscular activation during movement and/or muscle contraction. This area also houses a Biodex II Isokinetic Strength Testing system, several motorized treadmills and two partial weight support systems. These weight support systems are used to study the effects of different levels of loading on gait patterns in a variety of subject groups, including Parkinson's patients, orthopedic patients and astronauts. This area also houses a number of unique pieces of equipment in which LIP personnel have played a major design role. These include a body lift with an integrated foot vibration plate for use in studying orthostatic intolerance and lower limb neuromuscular activation in spinal cord patients, a horizontal sled lower limb maximal isometric strength testing device used in elderly, obese and very young populations and a variety of dynamic foot pressure devices used in normal, elderly and spinal cord injured populations to probe the utility of propioceptive pathways in maintaining lower limb neuromuscular pathways. This area also houses a number of graduate student offices.

The third main laboratory area is equipped as a modern biochemistry laboratory capable of performing a wide variety of biochemical and physiological testing. Specific equipment housed in this area includes a Coulter CBC blood analyzer, a robotic liquid handling system for high throughput analysis, an automated micro-titer plate washer, a Spectromax UV/Visible micro-titer plate reader, a Spectromax Gemini Fluorescent micro-titer plate reader, a LC analysis system, a blood lactate analyzer, tissue homogenizers, a high capacity SpeedVac liquid sample concentrator and a muffle furnace for dry ashing of samples prior to lipid biochemistry analysis. General laboratory equipment includes Class I and Class II chemical and biological safety hoods, three refrigerated bench-top centrifuges, a refrigerated bench-top high speed micro-centrifuge, two separate Milli-Q Ultra pure water systems and assorted balances (analytical and large scale). Attached to the biochemistry laboratory area is a 600 sq feet tissue culture suite housing a Class II Baker SG-600 laminar air-flow tissue culture hood, two CO_2 tissue culture incubators, a Motic inverted phase contrast microscope, a refrigerated bench-top micro-centrifuge and a shaking incubator. The tissue culture laboratory is also equipped with a Flexcell Mechanical Strain Tissue Culture System that allows the investigation of defined levels of either static or dynamic load on a variety of cell types in culture, and a Rotating Cell Culture System that allows the generation of three dimensional tissues constructs of a variety of cell types including gut or airway epithelial cells and bone cells.

In addition, the Principal Investigator also has full access to equipment and resources located in the Life Sciences Laboratories at NASA-Johnson Space Center under the auspices of a bilateral Space Act Agreement (SAA-URA-1-2004) between NASA-Johnson Space Center and the University of Houston.

The Laboratory of Integrated Physiology (LIP) can be found on the web at http://grants.hhp.coe.uh.edu/lip/ and a description of the wide range of research projects being carried out in the Department of Health and Human Performance can be found on the web at http://www.hhp.uh.edu/research/research.cfm.

BUDGET JUSTIFICATION

The Principal Investigator and Co-Investigator are requesting complete or partial summer salary support respectively for all three years of the proposal. In addition, two full-time graduate student researchers have been requested for all three years (12 month/year) of the project. The heavy time commitment associated with recruitment and testing of human subjects in Year 1 of the project (Phase One) requires two graduate student assistants in order to keep the project within its time-line as well as faculty over-sight by the PI and Co-I. During this period (Phase One) three major studies employing a total of 84 participants will be carried out. One of these studies will last a total of six months with a total of 45 subjects tested on seven different occasions. As such, these graduate students will integral to the smooth functioning of the project. In Phase Two (integration into a NASA-sponsored bed-rest study) these students will be responsible for the day to day sample collection and analysis required.

As part of the Phase One, 30 participants will receive a total of 90 DXA scans. The Department of Health and Human Performance has a DXA facility located within the department which will be utilized for this study. The PI (as member of the HHP faculty) can utilize this facility at a reduced cost, namely $85 per subject regardless of the number of scans as compared to $120 per scan for non-department associated studies. These fees are used to support system maintenance and repair, as well as operator costs and certification activities.

A total of 1275 individual samples will be collected in Phase One of this project, of which each will be analyzed in duplicate for both calcium and PYD. It is envisaged that an additional 400 samples will be generated as part of the Phase Two bed-rest component of the project depending on the number of subjects recruited to a particular bed-rest campaign. This generates a significant cost associated with immuno-analysis ELISA kits for PYD. We have negotiated a reduced price for bulk buying of these kits from $740 per kit to $690 per kit from the manufacturer (Quidel Corp, San Diego, USA). The large number of sample analyses are reflected in the materials/consumables costs of the budget, including the cost of maintaining the plate washer equipment required for the ELISA assay (already present in the LIP).

A I-Stat selective ion electrode system has been requested in Year 3 of the budget in order to modifiy the system with regard to the development of a monolithic sweat collection device based upon this technology.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Clarke, Mark S.F.	Associate Professor, Dept of Health and Human Performance, University of Houston

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Manchester Metropolitan University; Manchester, England	MI Biol (Pharm)	1987	Pharmacology
Manchester Metropolitan University; Manchester, England	Ph.D.	1991	Cell Biology/Biochemistry
Harvard Medical School, Boston	Post-doc	1991-92	Cell Biology

Positions and Employment

1991-92 Postdoctoral Fellow, Department of Cell Biology & Anatomy, Harvard Medical School.
1992-94 Research Fellow, Department of Cell Biology & Anatomy, Medical College of Georgia.
1994-96 National Research Council Resident Research Fellow, NASA/Johnson Space Center.
1996-01 Senior Staff Scientist, Universities Space Research Association, NASA/Johnson Space Center.
1998-date Assistant Adjunct Professor, Department of Pharmacol. & Tox., Medical College of Georgia.
2000-date Assistant Adjunct Professor, Department of Mol. Phys. and Biophysics, Baylor College of Medicine.
2001-date Associate Professor, Department of Health and Human Performance, University of Houston.

Honors and Awards

Recipient of The Young Investigator Award, 16th Gray Conference, (Vasculature as a Target in Tumor Therapy), September 1990, Manchester, England.
National Research Council Postdoctoral Research Fellowship tenable at the National Aeronautics and Space Administration, Johnson Space Center, Houston, Texas (1994-1996).
NASA Space Act Award: "Development of an Impact Mediated Loading (IML) Device for Cell Transfection" (1997)
NASA Space Act Award: "Development of an Unilateral/Unidirectional Cell Stretching Device" (1997).
NASA Space Act Award: "Directional Acceleration Vector Driven Displacement of Fluids (DAVD-DOF)" (2003).

Selected publications (in chronological order 13 of 35).

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Gazda, D.B., Schultz, J.R., **Clarke, M.S.F.** (2007) Liquid Metering Centrifuge Sticks (LMCS): A Centrifugal Approach to Metering Known Sample Volumes for Colorimetric Solid Phase Extraction (C-SPE). *(ICES Journal, in press)*
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Clarke, M.S.F., Khakee, R. and McNeil, P.L. (1993) Loss of cytoplasmic basic fibroblast growth factor from physiologically wounded myofibers of normal and dystrophic muscle. J. Cell Science 106; p121-133..
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Inventions/Patents

New Invention: Centrifuge Operated Slide Stainer (1998) (**Clarke, M.S.F.** and Feeback, D.L.) (U.S. Patent # 6008009)
New Invention: Unidirectional Cell Stretcher (1998) (**Clarke, M.S.F.** and Feeback, D.L.) (U.S. Patent # 6107081)
New Invention: Impact mediated loading of macromolecules (1997) (**Clarke, M.S.F.**, Vanderburg, C.R. and Feeback, D.L.) (U.S. Patent # 6221666).
New Invention: Directional Acceleration Vector Driven Displacement of Fluids (DAVD-DOF) (2004) (**Clarke, M.S.F.** and Feeback, D.L.) (U.S. Patent # 6.743, 632).
New Invention: Production of Stable Aqueous Dispersions of Single Wall Carbon Nanotubes (SWCNT's) (2005) (**Clarke, M.S.F.** et al.) (U.S. Patent # 6,878,361).
New Invention: Dispersion of Single Wall Carbon Nanotubes (SWCNT's) into Aqueous Solutions (2005) (**Clarke, M.S.F.**) (U.S. Patent # 6,896,864).
New Invention: Micro-fabricated Volumetric Device for Collection of Biological Fluids. (**Clarke, M.S.F.** and Feeback, D.L.) (Patent filed 3/05, status pending).
New Invention: Method and apparatus for a three dimensional tissue culture model of bone (**Clarke, M.S.F.,** Sundaresan, A and Pellis, N.R.) (Patent filed 3/06, status pending).

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Daniel P. O'Connor	Assistant Professor of Biostatistics
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Rice University	BA	1991	Human Performance and Health Sciences
Texas Woman's University	MS	1993	Physical Therapy
University of Houston	PhD	2002	Kinesiology

A. Positions and Honors

Positions and Employment

1993-1994 Research Physical Therapist, Fondren Orthopedic Group, Houston, TX
1994-1998 Supervisor of Rehabilitation Department, Texas Orthopedic Hospital, Houston, TX
1998-2000 Research Coordinator, Joe W. King Orthopedic Institute, Houston, TX
2000-2004 Adjunct Instructor, Physical Therapy Department, Texas Woman's University, Houston, TX
2001-2004 Adjunct Lecturer, Kinesiology Department, Rice University, Houston, TX
2001-2007 President, Joe W. King Orthopedic Institute, Houston, TX
2007- Assistant Professor of Biostatistics, Department of Health and Human Performance, University of Houston, Houston, TX

Other Experience and Professional Memberships

1986- National Athletic Trainers' Association (NATA)
1998-2000 Associate Editor, Journal of Rehabilitation Outcomes Measurement
2001- Manuscript reviewer, Journal of Orthopaedic and Sports Physical Therapy
2004 Ad Hoc Editorial Board Member, Journal of Orthopaedic and Sports Physical Therapy
2005 Reviewer, Archives of Physical Medicine and Rehabilitation
2005 Reviewer, Arthritis Care and Research
2006-2007 Second Vice President, Southwest Athletic Trainers' Association
2006- American College of Sports Medicine (ACSM)
2007- Reviewer, Medicine and Science in Sports and Exercise
2007- Reviewer, Journal of Orthopaedic Trauma
2007- Reviewer, BMC Health Services Research

Honors

Second Runner-Up, Journal of Athletic Training, Kenneth L. Knight Award for Outstanding Research Manuscript, 2005.

B. Selected Peer-Reviewed Publications

(Selected from 46 peer-reviewed publications.)

1. Edwards TB, Sabonghy EP, Elkousy H, Warnock KM, Hammerman SM, O'Connor DP, Gartsman GM. Glenoid component insertion in total shoulder arthroplasty: Comparison of three techniques for drying the glenoid prior to cementation. Journal of Shoulder and Elbow Surgery. 2007;16(3 Suppl):S107-S110.

2. Brinker MR, O'Connor DP. Ilizarov treatment of infected nonunions of the distal humerus after failure of internal fixation: an outcomes study. Journal of Orthopaedic Trauma. 2007;21:178-184.
3. Brinker MR, O'Connor DP, Pierce P, Spears JW. Payer type has little effect on rate of operative treatment and surgeons' work-intensity. Clinical Orthopaedics and Related Research. 2006;451:257-262.
4. Woods GW, Calder CT, O'Connor DP. Continuous femoral nerve block versus intraarticular injection for pain control after ACL reconstruction. American Journal of Sports Medicine. 2006;34:1328-1333.
5. Gartsman GM, Elkousy HA, Warnock KM, Edwards TB, O'Connor DP. Radiographic comparison of pegged and keeled glenoid components. Journal of Shoulder and Elbow Surgery. 2005;14:252-257.
6. O'Connor DP, Laughlin MS, Woods GW. Factors related to additional knee injuries after anterior cruciate ligament injury. Arthroscopy. 2005;21:431-438.
7. Szabo I, Buscayret F, Edwards TB, Nemoz C, O'Connor DP, Boileau P, Walch G. Radiographic comparison of two different glenoid preparation techniques in total shoulder arthroplasty. Clinical Orthopaedics and Related Research. 2005;431:104-110.
8. O'Connor DP. Comparison of two psychometric scaling methods for ratings of acute musculoskeletal pain. Pain. 2004;110:488-494.
9. Gartsman GM, O'Connor DP. Arthroscopic rotator cuff repair with and without arthroscopic subacromial decompression: a prospective, randomized study. Journal of Shoulder and Elbow Surgery. 2004;13:424-426.
10. Brinker MR, O'Connor DP. The incidence of fractures and dislocations referred for orthopaedic services in a capitated population. Journal of Bone and Joint Surgery (American). 2004;86:290-297.
11. Brinker MR, O'Connor DP, Pierce P, Woods GW, Elliott MN. Utilization of orthopaedic services in a capitated population. Journal of Bone and Joint Surgery (American). 2002;84:1926-1932.
12. Brinker MR, Cuomo JS, Popham GJ, O'Connor DP, Barrack RL. An examination of bias in shoulder scoring instrument among healthy collegiate and recreational athletes. Journal of Shoulder and Elbow Surgery. 2002;11:463-469.
13. O'Connor DP, Brinker MR, Woods GW. Preoperative health status among patients with four common knee conditions treated with arthroscopy. Clinical Orthopaedics and Related Research. 2002;395:164-173.
14. O'Connor DP, Jackson AS. Predicting physical therapy visits needed to achieve minimal functional goals after arthroscopic knee surgery. Journal of Orthopaedic and Sports Physical Therapy. 2001;31:340-358.

C. Research Support

Completed Research Support

Kinamed, Inc., Edwards (PI)
September 2003 – December 2005
Accuracy of a Total Shoulder Arthroplasty Implant Guidance System
Role: Co-Investigator

The Committee on State Societies, American Academy of Orthopaedic Surgeons, Brinker (PI)
October 2003 – November 2005
Intensity of Orthopaedic Treatment as a Function of Diagnosis, Patient Demographics, and Payer Type
Role: Co-Investigator

U N I V E R S I T Y of H O U S T O N

College of Education
Department of Health and Human Performance

3855 Holman St. Garrison Rm 104
Houston, TX 77204-6015

UH

Dr. Mark Clarke
University of Houston
Department of Health & Human Performance
104 Garrison Building
Houston, Texas 77204-6015

December 5, 2007

Dear Dr. Clarke,

In response to your request for senior aged subjects for your research project, I propose that active members of TEXERCISE –Houston be contacted through our initiative. I will contact Ms. Mary Ann Chambers, one of our Senior Exercise Program Leaders, and work out the details with her. I understand that you will need a group of at least 15 active senior citizens and that all data collection protocols will be minimally-invasive (blood, sweat and urine samples, DXA scans, approved by the University of Houston's CPHS and conducted by trained personnel under your direction.

One of the foci of the City of Houston's Mayor's Wellness Council is assessment of exercise of our citizens across the lifespan. Your proposed research meets the criteria of the Senior Fitness Initiative---TEXERCISE-Houston, and I wholeheartedly advocate the focus of this research and look forward to discussing further details, such as data collection time frames, transportation etc. with you. We will need to work closely with the instructors to assure participant safety and compliance.

Sincerely,
J.A. Bloom

Joel A. Bloom, Ph.D. MSE
Chair- TEXERCISE Houston
Senior Fitness Initiative of the City of Houston's Mayors Wellness Council

From: Bossert, Philip [mailto:pbossert@Central.UH.EDU]
Sent: Thursday, November 29, 2007 3:35 PM
To: Mark Clarke
Subject: RE: Potential Research Project

Mark

I support this project. I'll be in NJ next week for some training and have asked the cadets to see how many volunteers we could get for your project next August.

I'll let you know on 10 Dec when I get back.

Thanks for asking us.

R/
Phil

PHILIP A. BOSSERT, JR., Col, USAF
3855 Holman St., Garrison Room 109
University of Houston
Houston, TX 77204-5048
713-743-4932/3707
FAX 713-743-4113

From: Mark Clarke [mailto:Mark.Clarke@mail.uh.edu]
Sent: Tuesday, November 27, 2007 5:31 PM
To: Bossert, Philip
Subject: Potential Research Project

Dear Colonel Bossert,

I wonder if we could schedule a 10 minute meeting sometime tomorrow or Thursday to discuss a research project that I am developing in which I would like to recruit some of your cadets.

The project is a NASA project which focuses on understanding bone loss in astronauts. Terrestrial tests of bone loss included dual X-ray scans of bone (DXA) and measurement of biochemical markers of bone loss (calcium and collagen cross-links) in blood or urine. None of these technologies lend themselves to deployment during space flight or on extended space missions.

The involvement of the cadets would be in the validation phase of the project and be over a period of 6 months, would require three whole body scans for body composition/bone density and involve the collection of sweat from the forearm and a standard blood sample/urine sample on a monthly basis. I believe that your cadets undergo a program of physical training throughout their tenure with you and as such an incoming class would be an ideal group to track increases in bone density as their training status increases.

My question is "Is this project something that you could advocate your cadets being involved in".

Regards

Mark