Fiscal Year:	FY 2014	**Task Last Updated:**	FY 02/06/2014
PI Name:	Vignaux, Guillaume F. Ph.D.		
Project Title:	Contribution of the Vestibular and Sympathetic Nervous Systems to Space-Induced Bone Loss		
Division Name:	Human Research		
Program/Discipline:	NSBRI		
Program/Discipline--Element/Subdiscipline:	NSBRI--Musculoskeletal Alterations Team		
Joint Agency Name:		**TechPort:**	No
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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Zip Code:	37232	**Congressional District:**	5
Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2011 NSBRI-RFA-11-01 Postdoctoral Fellowships
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No. of Post Docs:	1	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NSBRI
Contact Monitor:		**Contact Phone:**	
Contact Email:			
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Key Personnel Changes/Previous PI:			
COI Name (Institution):	Elefteriou, Florent (MENTOR/ Vanderbilt University)		
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Performance Goal Text:			

Task Description:	POSTDOCTORAL FELLOWSHIP Our main hypothesis is that the vestibular system participates to the maintenance of bone mineral density on Earth and its dysfunction under microgravity may contribute to the bone loss associated with space travel. Our preliminary findings have provided evidence to support our hypothesis. Bilateral vestibular lesion (VBX) using sodium arsanilate injections in rats led to significant bone loss associated to a decrease in osteoblasts number. Aims of this project are 1) to determine if VBX causes bone loss by activation of the sympathetic nervous system in our VBX model using beta-blocker-treated mice and mice lacking the beta-2 adrenergic receptors globally or specifically in osteoblasts; 2) to analyze the bone phenotype of mice devoid of vestibular gravity sensor (Het-/- mice), and 3) to test nitric oxide involvement in vestibular-related bone loss using a vestibular hair cells specific KO for Sod3. Our study may uncover a new pathway of bone regulation, a novel approach for the treatment of low bone mass diseases on Earth, and novel countermeasures to reduce risk of bone fracture in microgravity. During our first year of research we confirmed our previous results obtained in rats in a mouse model of vestibular lesion. Indeed 2-month old mice displayed reduced bone mineral density in femurs 1 month after VBX, as observed in rats. This result is important as it allows us to use genetically-modified mutant mice in future studies. We also investigated the SNS involvement in VBX-induced bone loss in mice. We found that daily propranolol treatment prevented VBX-induced bone loss, as it did previously in rats, and beta-2 adrenergic receptors KO mice femurs were resistant to VBX. Both results support the hypothesis of a SNS involvement in our model. We went further by using mice lacking the beta-2 adrenergic receptors specifically on osteoblasts. These mice were also resistant to VBX showing that the VBX-induced bone loss is a SNS mediated effect acting through the osteoblasts. Finally, no bone change was observed in 3-month old Het–/– mice (Nox3het/J) (mice lacking otoliths). Nox3 encodes a NADPH oxidase which is involved in the down-regulation of nitric oxide (NO) availability. Knowing that NO level in vestibular hair cells modulates vestibular signals and that it is an important neuromediator/neuromodulator of the vestibular response, we hypothesized that the combination of lack of otoliths and impaired neurotransmission possibly impact bone remodeling in a manner that is more complicated than anticipated. Aim 3 of this project was aimed at teasing apart the mechanism involved. We are currently breeding Sod3 flox mice that, in addition to results of aim 2, will help us to better understand the role of NO in vestibular response generation in hair cells.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Until 2012 there was no published data regarding the role of the vestibular system in bone biology. However, the presence of sympathetic nerves in the bone environment was reported more than 50 years ago (PMID: 13525457) and our laboratory has more recently shown, using genetic and pharmacologic approaches, that sympathetic nerves contribute to the regulation of bone homeostasis (PMID: 15724149). Osteoblasts express the beta-2 adrenergic receptor (ß2AR) and respond to catecholamines or pharmacological ßAR agonists with decreased proliferation (PMID: 18410742) and induction of RANKL expression, which leads to enhanced osteoclast formation and bone resorption. Propranolol, a ß1/ß2AR non-selective antagonist, inhibits these effects of sympathetic activation and protects from ovariectomy and unloading-induced bone loss in mice and rats (PMID: 12419242 , PMID: 15961387 , PMID: 17243867); in several retrospective studies, the use of ß-blockade was associated with increased BMD and decreased fracture rate in humans (PMID: 15724149 , PMID: 1919850 , PMID: 18622078). External factors also influence bone remodeling. In particular, gravity and inertial accelerations exert a range of mechanical stimulations on the skeleton that have an osteogenic effect demonstrated in birds, mice, rats, pigs, sheep and humans (PMID: 11541937 , PMID: 7153230 , PMID: 6699056 , PMID: 8368304 , PMID: 17185839). In contrast, mechanical unloading as a consequence of bed-rest or microgravity conditions causes bone loss in humans (PMID: 17396004) and rodents (PMID: 11541702), which is associated with suppressed bone formation and a mild increase in resorption. Weight-bearing pressure is sensed by proprioceptive sensors in the joints, capsules, ligaments, muscles, tendons and skin, while the gravito-inertial acceleration (generating the weight) is sensed by otoliths and visceral graviceptors (PMID: 22579804). The otolith system, which is part of the vestibular system in the inner ear, is the main sensory organ of gravity and linear accelerations. Its contribution to the regulation of posture, respiration, heart rate and blood pressure in animals (PMID: 12787870 , PMID: 14660474 , PMID: 15640755 , PMID: 21921247) and humans (PMID: 9173934 , PMID: 12111293) is well-documented and supported by anatomical projections from vestibular nuclei to autonomic centers of the brainstem (PMID: 7611512 , PMID: 18809392). Vestibular inputs contribute to changes in sympathetic nerve outflow during movements and postural changes that follow stimulation of the vestibulo-sympathetic reflex (PMID: 9728081 , PMID: 9416585 , PMID: 10896872 , PMID: 22946097), as demonstrated by the orthostatic hypotension observed in cats with bilateral vestibular destruction (PMID: 15475594). In 2013 our laboratory reported evidence for a role of the vestibular system in bone homeostasis control in rats (PMID: 23553797). Using an established model of bilateral vestibular lesions and microtomographic and histomorphometric bone analyses, we showed that induction of bilateral vestibular lesion in rats generates significant bone loss, which is restricted to weight-bearing bones and associated with a significant reduction in bone formation. Importantly, this bone loss was not associated with reduced locomotor activity or metabolic abnormalities, was accompanied with molecular signs of increased sympathetic outflow, and could be prevented by the ß-blocker propranolol. Collectively, these data suggest that the homeostatic process of bone remodeling has a vestibulo-sympathetic regulatory component and that vestibular system pathologies might be accompanied by bone fragility. The vestibular system can be affected in several ways. Thus our work might be helpful for patients after direct damages of the vestibular system, such as idiopathic vestibular areflexia, vestibular neuritis, Menière's disease, labyrinthectomy, vestibular nerve section, or the use of ototoxic antibiotics, or with a vestibular disorder subsequent to an ischemic stroke, a neurotoxic pathology or a neural tumor.

Task Progress:	SPECIFIC AIM 1: Determine if vestibular lesion causes bone loss by activation of the sympathetic nervous system. - The first step of this study consisted in evaluating the effect of a bilateral vestibular lesion (VBX) on femoral bone mass. Using micro-CT analyses on 2-month old mice, we demonstrated that VBX induces a significant decrease in femoral bone mass one month after the lesions. Histomorphometric analyses revealed that this bone loss was associated with a decrease in bone formation whereas bone resorption was not affected. - The second step was to determine whether the sympathetic nervous system (SNS) mediates this VBX-induced bone loss. Using 2-month old beta-2 adrenergic receptors KO mice, we detected no bone change in BV/TV 1 month after VBX. Moreover, daily propranolol treatment had no effect on WT-sham mice but completely blunted VBX-induced bone loss in WT-VBX mice. Finally the SNS involvement in the VBX-induced bone loss was further supported by the increase in Ucp1 gene expression in brown adipose tissue (a marker of the SNS activity). - No change in BV/TV was observed 1 month after VBX in 2-month old beta-2 adrenergic receptors osteoblasts-specific KO mice. These results suggest that VBX-induced sympathetic outflow to bone triggers bone loss via its effect on beta-2 adrenergic receptors expressing osteoblasts. SPECIFIC AIM 2: Analyze the bone phenotype of mice devoid of vestibular gravity sensor. We used 3-month old Het–/– mice (Nox3het/J), lacking otoliths (gravity sensors), in order to mimic the decrease in vestibular stimulation in space. Micro-CT analyses revealed no bone changes in these mice compared to WT. Because of the lack of otoliths, we assumed that these mice should present a decrease in vestibular inputs and bone loss. However, Nox3 encodes a NADPH oxidase in hair cells which is involved in the downregulation of nitric oxide (NO) availability. Knowing that NO level in vestibular hair cells modulates the vestibular signals and that it is an important neuromediator/neuromodulator of the vestibular response, a constitutive high NO level in hair cells might explain our results in this model (aim 3 will help to clarify this). Another possible explanation is that the time point analyzed is not optimal. Younger and older mice will thus be analyzed. SPECIFIC AIM 3: Test nitric oxide involvement in vestibular-related bone loss. The Sod3flox/flox mice are currently breeding. A cre-adenovirus will be injected in the inner ear to inactivate Sod3 specifically in the vestibular system. The bone phenotype will then be analyzed by micro-CT and histomorphometry. Tomato reporter mice are currently tested to track cre virus infection and identify optimal titer.
Bibliography Type:	Description: (Last Updated: 04/12/2016)
Articles in Peer-reviewed Journals	Vignaux G, Besnard S, Ndong J, Philoxène B, Denise P, Elefteriou F. "Bone remodeling is regulated by inner ear vestibular signals." J Bone Miner Res. 2013 Oct;28(10):2136-44. http://dx.doi.org/10.1002/jbmr.1940 ; PubMed PMID: 23553797 , Oct-2013
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