Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Temple University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010-12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Germaine A Calicat, MLA

4. **Grant Contact Person's Telephone Number:** 215-204-7655

5. **Grant SAP Number:** 4100050909

6. **Project Number and Title of Research Project**: Project 1 - *The Anabolic Role of Cannabinoid Receptors in Bone*

7. **Start and End Date of Research Project:** 7/1/10-6/30/11

8. **Name of Principal Investigator for the Research Project:** Mary E. Abood

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $ $43,311.48

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Marcu	Research Assistant (pre-doc)	100	$27,851

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____X____ No_____

If yes, please indicate the source and amount of other funds: Abood (PI) start-up funds

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____X____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
ROLES OF CB1/CB2 IN BONE CELL FUNCTION	☐XX NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	2/2011	$250,000	not funded
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____X____ No_____

If yes, please describe your plans: We plan to re-submit a R01 proposal on the roles of the CB1 and CB2 receptors in bone.

12. Future of Research Project. What are the future plans for this research project?
We will continue characterizing the signal transduction pathways initiated by CB1 and CB2 receptors, and plan to re-submit a R01 proposal on the roles of the CB1 and CB2 receptors in bone.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____X___ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	
Female				
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown			1	
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown			1	
Total			**1**	

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes____X_____ No_____

If yes, please list the name and degree of each researcher and his/her previous affiliation: Jahan Marcu, B.S. Previously from California Pacific Medical Center Research Institute

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

The formula funds allowed Jahan to train in bone cell biology. Approximately 10 million people over the age of 50 have been diagnosed with osteoporosis and 33.6 million more are estimated to have low bone mass (osteopenia); these are major health care problems. It is estimated that the direct health care costs from fractures related to osteopenia (hospitalizations, ER visits, physician visits, etc.) ranges from $12- $18 billion annually. This is clearly an area with unmet medical need.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No_____X____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____X____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

The Anabolic Role of Cannabinoid Receptors in Bone - The goal of the planned project is to define the anabolic role of cannabinoid receptors in bone. Recent studies have demonstrated the presence of endocannabinoids and their G protein-coupled cannabinoid receptors, CB1 and CB2, in the skeleton. Although it has become clear that this system is functional in bone, the precise mechanisms of action are only beginning to emerge. The planned studies will generate new information regarding the anabolic effects of cannabinoid receptors and mechanisms of action on osteoblast differentiation and function. Once we understand how cannabinoid receptors function to promote bone formation, this information will be helpful in developing new therapeutic strategies to selectively enhance bone formation in patients with clinically significant bone loss.

Specific Aims:
1) To determine intracellular signaling subsequent to ligand activation of CB1 and/or CB2 receptors in osteoblasts by using a combination of pharmacological and genetic tools to identify the receptors involved. The effects of cannabinoid agonists, antagonists/inverse agonists and related compounds on osteoblast proliferation, apoptosis and differentiation will be compared in cultures prepared from WT, CB1 KO, CB2 KO and CB1/2 dKO and GPR55 KO mice.
2) To identify the response of two key intracellular signaling pathways (ERK, and Akt) to the most efficacious agents (using Western blot analysis and immunocytochemistry).

Progress towards aims:
Specific Aim 1. Aim 1 was partially achieved. Results are stated below**.**
To examine the functional significance of CB1 and CB2 receptors on osteoblast differentiation, we established primary osteoblast cultures utilizing newborn wild-type calvaria. Measurement of alkaline phosphatase (ALP) activity was assessed to evaluate the effects of CB1/CB2 receptor deletion (CB1/2 KO mice) on osteoblast differentiation. As shown in figure 1, osteoblasts prepared from CB1/2 KO mice exhibited less differentiation that those prepared from WT mice, as documented by ALP staining (A,B) and activity (C,D). In Figures 1A and 1B, primary osteoblasts were isolated from the calvaria of 3-5 day old mice and stained for ALP activity. The images provide a qualitative assessment of matrix mineralization. The ALP staining was much more intense compared in wild type (WT) osteoblasts compared to the cells from CB1/2 KO animals. In Figure 1C, matrix levels of ALP were slightly lower in the CB1/2 KO primary osteoblasts than in WT, corroborating staining results. However, a defect in osteoblast differentiation could result in the ALP enzyme being released at high rates into media or serum, resulting in lower staining and matrix levels. Serum ALP activity was not significantly different between WT and CB1/2 KO cells. This suggests that there is less ALP production in CB1/2 KO mature osteoblasts.

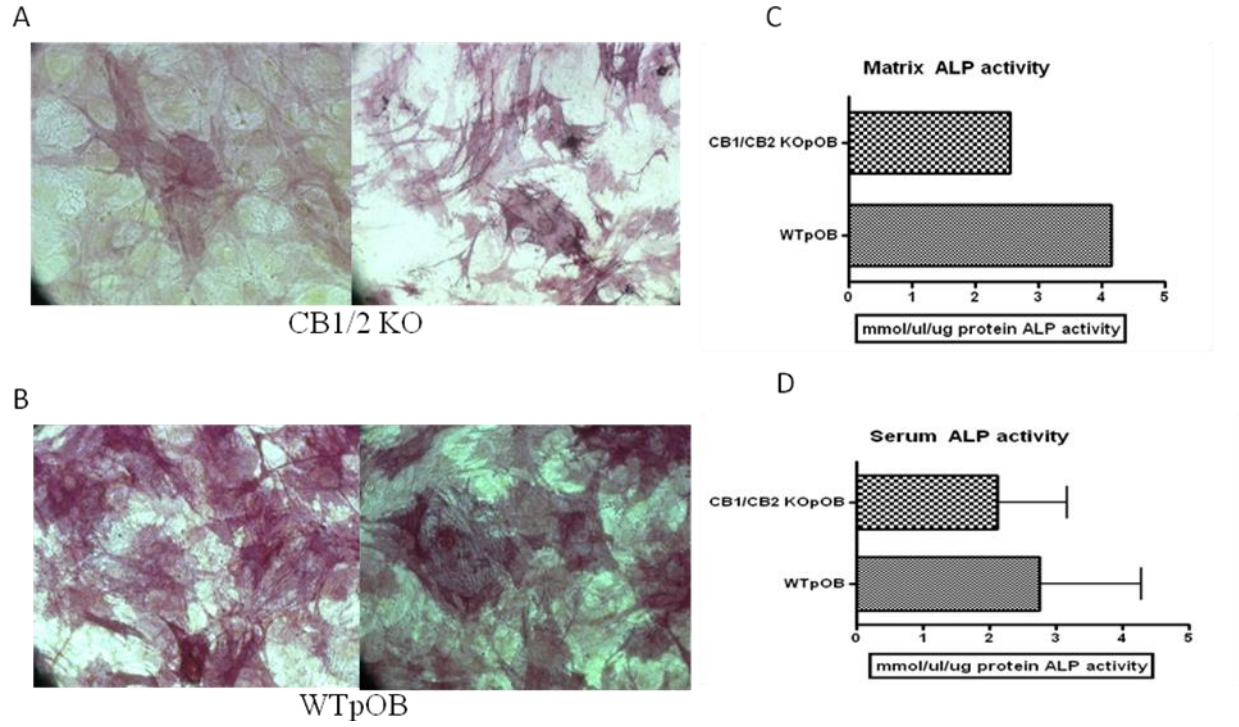

Figure 1. ALP Staining and ALP activity in Primary Osteoblasts prepared from KO or WT mice

Next, the ability of WT rat primary osteoblasts to proliferate in the presence of CB1 and CB2 receptor antagonists was assessed. As shown in Figure 2, treatment with a CB1 antagonist (SR141716) or a CB2 antagonist (SR144528) caused a significant, dose-dependent inhibition of cell proliferation.

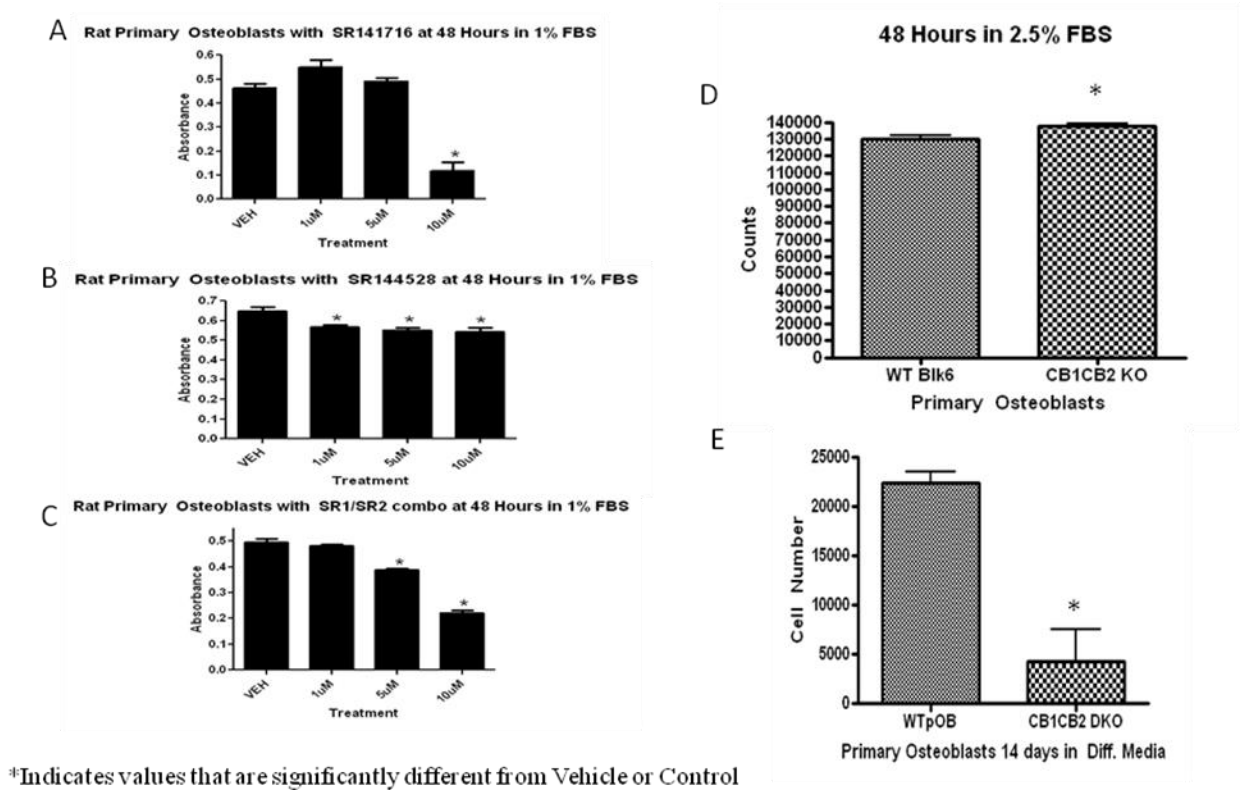

Fig 2. Cell Proliferation experiments in Primary Osteoblasts

Under low serum conditions the CB1 antagonist SR141716 was able to significantly reduce absorbance of proliferating osteoblasts (a measure of cell proliferation) within 48 hours (Fig 2A). The CB2 antagonist did not significantly alter cell proliferation in primary osteoblasts (Fig 2B). When applied in combination, the antagonists reduced cell proliferation similar to the application of the CB1 antagonist alone. Under normal conditions (no differentiation factors) CB1/2 KO osteoblasts isolated from 3-5 day old mice produced a significantly higher amount of proliferation compared to WT (Fig 2D). However, this effect was reversed when the cells were challenged with differentiation factors (Fig 2E). The results indicate that there may be a defect in cell proliferation during maturation of osteoblasts isolated from CB1/2 KO animals.

Specific Aim 2. To identify the response of two key intracellular signaling pathways (ERK, and Akt) to the most efficacious agents (using Western blot analysis and immunocytochemistry). Specific Aim 2 was achieved in part.

Cannabinoid receptors signal in bone: CB1,CB2, and GPR55. In Figure 3A, the effect of the CB1 antagonist SR141716 was examined in the MC3T3 osteoblastic cell line. Below figure 3A, a representative western blot of a SR141716 timecourse for pERK is shown. Blockade of the CB1 receptor with SR141716 produced a rapid increase in the activity of pERK which returned to near basal levels after about 1 hour. Blockade of the CB2 receptor did not cause a noticeable difference in ERK activity (Figure 3B). The putative cannabinoid receptor GPR55 was targeted with the agonist lysophosphatidylinositol (LPI), which caused a small increase in pERK activity, which did not appear to return to baseline even after 3 hours (Figure 3C).

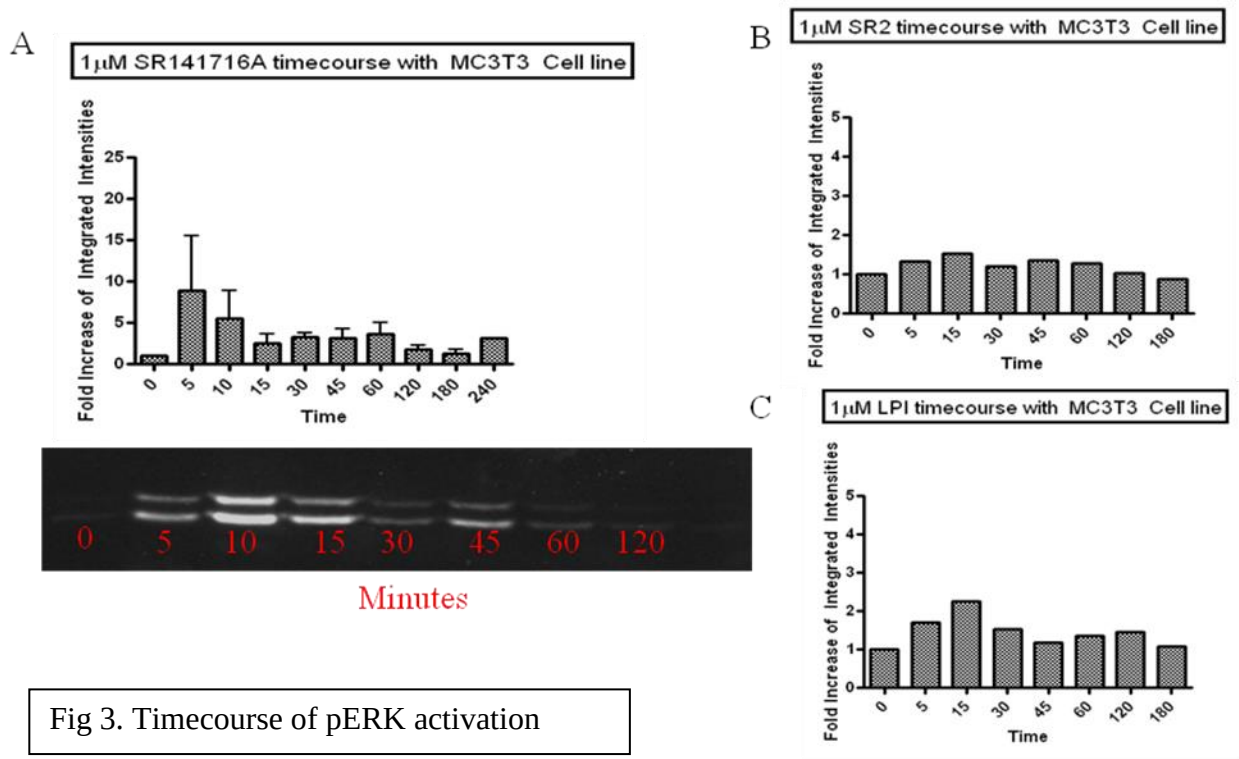

Fig 3. Timecourse of pERK activation

Nonpsychotropic Cannabinoids May Alter ERK and AKT. Activation of CB1 receptors is largely responsible for the psychotropic effects of marijuana and delta-9-tetrahydrocannabinol (THC). We wanted to investigate whether nonpsychotropic agonists may stimulate signaling pathways important for bone growth; these compounds would be more desirable for further therapeutic development in the treatment of bone diseases. Interestingly, we found the CB2-selective agonist, HU308, was able to stimulate pERK activity (Fig. 4A). This suggests that activation of the CB2 receptor may be beneficial for bone growth, in line with our observations that receptor antagonists did not affect cell proliferation. The nonpsychotropic plant cannabinoid agonist, cannabidiol, stimulated pAKT activity (Fig 4B).

THC May Alter ERK Activity. Shown in figure 5 is a representative western blot from a 2 hour timecourse of pERK activation following treatment of the MC3T3 osteoblast cell line. THC stimulated pERK activity until about 45 minutes, where activity began to noticeably drop off. THC and other synthetic cannabinoids remain the most highly abused illicit narcotics. Investigating the effect of compounds such as THC, may provide information for identification

of bone disorders due to the abuse of cannabinoid drugs, potentially allowing effective interventions in drug abusing populations.

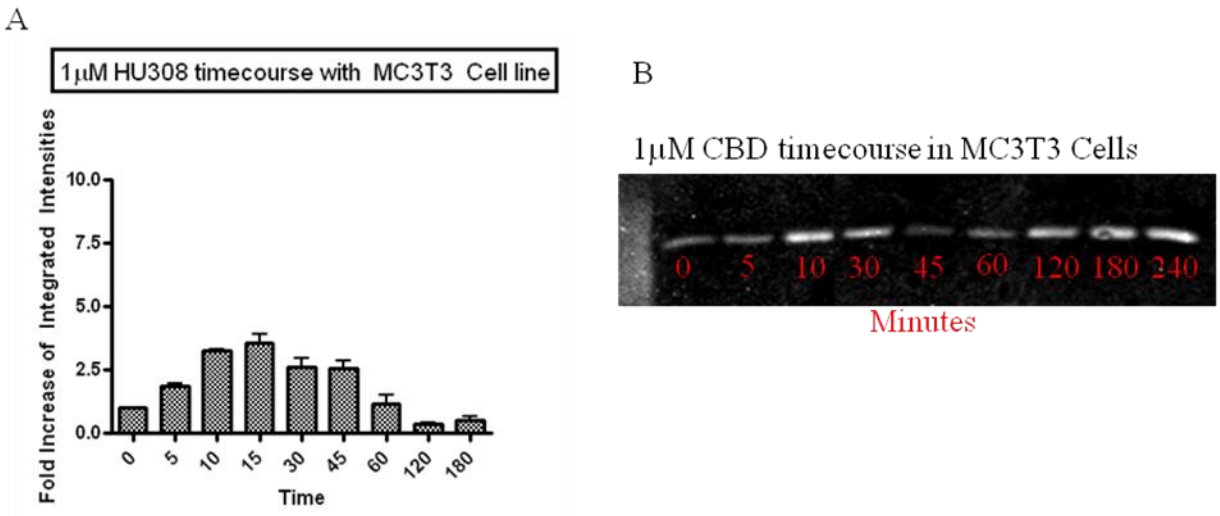

Figure 4. Non-psychotropic cannabinoids alter ERK and AKT activity

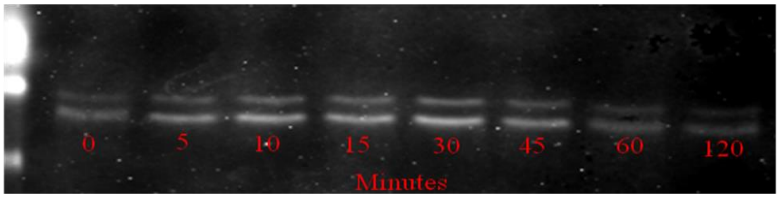

Figure 5. 1 μM THC activates pERK in MC3T3 osteoblasts cells

Recently, an interest in the role of endogenous cannabinoid signaling in bone has surfaced. Non-psychotropic cannabinoids, such as CBD and HU308 may have the potential to treat a wide variety of bone pathologies, without the negative effects of THC. However, THC and other synthetic cannabinoids remain the most highly abused illicit narcotics. We have begun investigating the effects of endogenous, non-psychotropic, and widely abused cannabinoids on the signaling cascades in bone. We sought to elucidate molecular mechanisms of cannabinoid on bone. To study the role of endogenous cannabinoids, we used CB1 and CB1 antagonists. Additionally, we isolated primary osteoblasts from WT and CB1/2 KO neonatal pups, and examined the cells for alterations in ALP staining and cell proliferation. Our data may lead to a better understanding of bone biology, may aid with drug development for bone diseases, and may provide information for identification of bone disorders due to the abuse of cannabinoid drugs, potentially allowing effective interventions in drug abusing populations.

Materials and Methods:

Osteoblast Isolation and Culture

Primary immature osteoblasts were isolated from the calvaria bones of 3-5 day old animals by sequential collagenase digestions. Prior to experiments, the cells were cultured in a 75cm^2 flasks

in standard α-MEM media. The cells were left to adhere and proliferate until the flask was nearly confluent before using in experiments.

Proliferation Assays with Primary Osteoblasts

Primary cells from rats were plated at 2,000 cells/well in MEM with 10% FBS. After 24 hours the cells were washed with plain media, and the media was replaced with MEM with 1%FBS and cells were treated with 1,3,5,7 and 10 µM of SR141716A, SR144528, or a combination of the two compounds. Cells culture was stopped at 24 and 48 hours post treatment. Cell proliferation was measured using a WST-1 proliferation kit from Caymen (Cat# 10008883). To measure the number of mature osteoblasts, primary cells from WT and CB1/CB2 KO mice were plated at 2,000 cells/well in 96 well plates. After 24 hours the media was replaced with MEM containing β-glycerol phosphate (10mM) and ascorbic acid (50ug/ml). The media with differentiation factors was changed every 2-3 days. After 14 days, the proliferation differentiated or mature osteoblasts were measured number using a WST-1 proliferation kit from Caymen (Cat# 10008883). To measure the proliferation in undifferentiated ostoeblasts, primary cells from WT and CB1/CB2 KO mice were plated at 4,000 cells/well in 96 well plates in 2.5%FBS. After 48 hours, the media was removed and cells proliferation was measured using a CyQuant kit from Invitrogen (Cat# C35006). A calibration curve was also generated to determine cells numbers (Data not shown).

ALP staining

Primary immature osteoblasts were seeded at 100,000 cells/well into 12 well plates. After 24 hours the media was replaced with MEM containing β-glycerol phosphate (10mM) and ascorbic acid (50 µg/ml). The media with differentiation factors was changed every 2-3 days. After 14 days, the cells were washed and stained for alkaline phosphatase using the ALP staining kit from Sigma-Aldrich (Cat# 86R-1KT).

ALP activity

Media and cell lysates from 96 well plates were analyzed for ALP activity after 14 days of differentiation with the LabAssay ALP kit from Wako (Cat# 291-58601).

Time course experiments

MC3T3 cells were seeded into 10mm dishes at 200,000 cells/dish in MEM with 10%FBS. The cells were transferred to 1%FBS at least 24 hours prior to experimentation. Cells were washed with HBSS before receiving 1 µM of a cannabinoid (dissolved in DMSO) in HBSS. Cells were harvested at various times using a protease (Thermo Sci Cat#78425) and phosphatase (Thermo Sci Cat#78420) inhibitor cocktail.

Westerns

Protein content of cellular extracts was determined using Bradford reagent and an identical amount of protein from each extract was analyzed on 10% polyacrylamide gels for SDS-page analysis. Levels of pERK and ERK were determined using a monoclonal antibody (1:5000 Sigma-Aldrich M8159) and a polyclonal antibody (1:1000; Cell Signaling 9102). AKT was determined using a polyclonal antibody (1:1000 dilution; Cell Signaling Cat#9275). The levels RUNX2 were determined with a polyclonal antibody (1:200; Santa Cruz BioTech Cat#10758). Membranes were incubated with the appropriated primary and secondary antibodies (Odyssey Cat# 926-32210, 926-32221, and 926-32211) and analyzed on a LICOR machine to generate integrated intensity values of activated proteins. Background levels of inactivated ERK or actin were used as a control for each individual value.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X____No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X____ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last

name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____X____ No_____

If yes, please describe your plans:

When more studies are completed, we plan to publish these results.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be

single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Our studies have generated new information regarding the anabolic effects of cannabinoid receptors and mechanisms of action on osteoblast differentiation and function. As we understand how cannabinoid receptors function to promote bone formation, this information will be helpful in developing new therapeutic strategies to selectively enhance bone formation in patients with clinically significant bone loss.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None (not yet)

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:

Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____x_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*
- 2 page NIH Biosketch appended

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
Abood, Mary E.	Associate Professor of Anatomy and Cell Biology Associate Professor, Center for Substance Abuse Research
eRA COMMONS USER NAME (credential, e.g., agency login) ABOODM	

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
Carleton College, Northfield, MN	B.A.	1979	Chemistry
University of California, San Francisco	Ph.D.	1986	Pharmacology
Stanford University, Stanford, CA	Post-Doc	1989	Molecular Biology

A. Personal Statement. The goal of the proposed project is to study the role of cannabinoid receptors in bone cell function. I have been involved in the characterization of cannabinoid receptors for 20 years. Most of my research has been on the structure and function of cannabinoid receptors, including localization, characterization of the downstream signaling pathways and identification of additional receptors on which cannabinoids exert their actions. In summary, I have a demonstrated record of successful and productive research projects on cannabinoid receptor function.

B. POSITIONS AND HONORS.

Positions and Employment

1989 - 1990 Research Associate, Pritzker Laboratory, Stanford University
1990 - 1996 Assistant Professor, Department of Pharmacology and Toxicology
School of Medicine, Medical College of Virginia
1996- 1999 Associate Professor with tenure, Department of Pharmacology and Toxicology
Virginia Commonwealth University
1999- 2007 Scientist, California Pacific Medical Center Research Institute
1999-present Affiliate Associate Professor, Department of Pharmacology and Toxicology
Virginia Commonwealth University
2007-2008 Senior Scientist, California Pacific Medical Center Research Institute
2008-2011 Associate Professor with tenure, Department of Anatomy and Cell Biology and
Center for Substance Abuse Research, Temple University
2011- Professor, Department of Anatomy and Cell Biology and
Center for Substance Abuse Research, Temple University

Other Experience and Professional Memberships

1989-present Member, Society for Neuroscience
1991-present Member, International Cannabinoid Research Society (Elected Secretary, 2001-2004, Elected
Treasurer, 2009-2011)
1993-present Member, American Society for Pharmacology and Experimental Therapeutics
1992-present Member, College on Problems of Drug Dependence
2005 Reviewer, Study Section, National Institutes of Health ZRG1 BDCN-F
2005-2009 Member, MNPS Study Section Member, National Institutes of Health

2006-present Member, IUPHAR Receptor Nomenclature Committee for Cannabinoid Receptors

Honors

1984 ARCS Scholar (Achievement Awards for College Scientists)
1986 National Research Service Award, Public Health Service Department of Health and Human Services
1987 - 1989 Individual National Research Service Award, Public Health Service Department of Health and Human Service

C. SELECTED PEER-REVIEWED PUBLICATIONS (Selected from over 75 peer-reviewed publications)

1. Kapur A, Zhao P, Sharir H, Bai Y, Caron MG, Barak LS, Abood ME. Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. J Biol Chem. 284:29817-27, 2009. [Faculty of 1000 Biology Selection].
2. Zhao P, Sharir H, Kapur A, Cowan A, Geller EB, Adler, MW, Seltzman HH, Reggio PH, Heynen-Genel S, Sauer M, Chung TDY, Bai Y, Chen W, Caron MG, Barak, LS* and Abood ME*. Targeting of the orphan receptor GPR35 by Pamoic Acid: a Potent Activator of ERK and βarrestin2 with antinociceptive activity, Molecular Pharmacology, 78: 560-568, 2010. *co-contributing authors [special commentary accompanies the article].
3. Sharir H, Abood ME. Pharmacological Characterization of GPR55, A Putative Cannabinoid Receptor. Pharmacol Ther. 126:301-13, 2010.
4. Heynen-Genel S, Dahl R, Shi S, Sauer M, Hariharan S, Sergienko E, Dad S, Chung T, Stonich D, Su Y, Caron M, Zhao P, Abood ME, Barak LS. Antagonists for the Orphan Receptor GPR35. In: NIH Molecular Libraries. Probe Reports from the Molecular Libraries Program [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2010. [updated 2010 Oct 4].
5. Kotsikorou E, Lynch DL, Abood ME, Reggio PH. Lipid Bilayer Molecular Dynamics Study of Lipid-Derived Agonists of the Putative Cannabinoid Receptor; GPR55. Chem Phys Lipids. 164:131-43, 2011.
6. Brailoiu GC, Oprea TI, Abood ME*, Brailoiu E*. (2011) Intracellular cannabinoid CB1 receptors are activated by anandamide. J Biol Chem 2011 June 30 [Epub ahead of print] [Faculty of 1000 Biology Selection].
7. Kotsikorou E, Madrigal K, Hurst D, Sharir H, Lynch D, Heynen-Genel S, Milan L, Chung T, Seltzman H, Bai Y, Caron MG, Barak LS, Abood ME, Reggio PH (2011) Identification of the GPR55 agonist binding site using a novel set of high potency GPR55 selective ligands. Biochemistry, 2011 Jun 1 [Epub].

D. RESEARCH SUPPORT

1 R21 DA029432-01 Abood (PI) 9/30/09-9/29/2011
Pamoic acid analogues as potent GPR35 agonists inducing antinociception.
The specific aims of this proposal are: To propose a strategy to identify predominantly commercially available small molecules towards the objective of identifying a useful molecular probe for GPR35. Optimizing these novel compounds will allow the characterization of GPR35 biology in vitro and in animal models of pain.

1R01 DA023204-5 Abood (PI) 06/15/07-05/31/2012
Molecular Characterization of GPR35 and GPR55, Putative Cannabinoid Receptors
The goal of the proposed project is to understand the functional features of two putative cannabinoid receptors GPR55 and GPR35. We propose to elucidate the molecular signature

of cannabinoid receptors by defining how GPR55 and GPR35 recognize and respond to cannabinoids.
Role: PI

5 P50DA05274-22 Dewey (PI) 09/01/2007-06/30/2012
Cloning and characterization of cannabinoid receptors.
The cloning of a cannabinoid receptor affords the opportunity to examine the binding properties of these receptors in transfected cell lines and to compare the structure-activity relationships of binding with those of whole animal pharmacology.
Role: PI, Project 2

2 P01 DA09158-14 Makriyannis, Alex, (P.I.) 06/01/2007- 05/31/2012
Endocannabinoid Active Sites as Therapeutic Targets
We are testing compounds created by Dr. Makriyannis in our existing mutant and wild-type receptor expression systems.
Role: Co-PI