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:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814-935-1081

5. **Grant SAP Number:** # 4100047645

6. **Project Number and Title of Research Project:** 30 - Diabetic Changes in Contractile Proteins and Contractility in Arterial versus Venous Grafts

7. **Start and End Date of Research Project:** 9/1/2009 – 6/30/2011

8. **Name of Principal Investigator for the Research Project:** Lakshman Segar, PhD
   (PI officially changed name from Lakshman Sandirasegarane to Lakshman Segar on November 8, 2010.)

9. **Research Project Expenses**.

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

   $46,528

   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
None			

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
Segar	Principal Investigator	4%
Pyla	Postdoctoral fellow	10%
Kozak	Collaborator	1%

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
None		

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_____ No____X_____

If yes, please indicate the source and amount of other 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:
Vascular Phenotypic Regulation by Growth Factors, Insulin, and Glucose	■NIH - NHLBI ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	Orig.sub 11/2009 Awarded 7/26/2010	$1, 250,000 (direct costs)	$1,034,387 (direct costs)

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:

My plan is to submit a proposal aimed at understanding the effects of antihypertensive drugs on vascular remodeling in diabetic patients.

12. Future of Research Project. What are the future plans for this research project?

The future plan is to study the alterations in vascular contractility in diabetic patients with uncontrolled hyperglycemia.

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				1
Unknown				
Total				**1**

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

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

    Yes_____ No____X_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

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.

    Our laboratory has well-equipped facilities to perform isometric tension measurements (contractility studies) using PowerLab and Bridge Amplifier. This equipment (organ bath system) was instrumental in collecting the data pertaining to contractility studies using arterial and venous tissues.

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 agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). 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.

Project goals, objectives and specific aims (as contained in the grant agreement):
The *long-term goal* of this project is to gain new understanding into the contractile properties of arterial *vs.* venous grafts. The *specific objective* is to investigate the relationship between contractile protein levels and the contractile responses in arterial *vs.* venous grafts in diabetic patients. Saphenous vein grafts have been shown to have worse coronary outcomes compared with left internal mammary artery (LIMA) grafts. We *hypothesize* that contractile proteins and/or contractile responses in vein grafts are compromised to a greater extent compared with arterial grafts. The *Specific Aim* of this project will determine diabetic and nondiabetic changes in contractile protein levels and contractile responses using saphenous vein and LIMA tissues from patients undergoing coronary artery bypass grafting surgery (CABG). The *experimental approach* will utilize saphenous vein and LIMA tissues to determine: i) total proteins; ii) contractile proteins such as SM α-actin, smooth muscle myosin heavy chain, calponin, and caldesmon by immunoblot analysis; and iii) serotonin-induced contractility by isometric tension measurements. The *rationale* for this Aim is that these studies will reveal diabetic *vs.* nondiabetic alterations in contractile proteins that regulate smooth muscle contractility. The *overall outcome* of this Aim is that these studies would constitute a translational approach to predict enhanced restenosis due to dysregulated contractile proteins/contractility as a function of arterial *vs.* venous grafts.

Summary of the progress made in achieving the goals, objectives and aims: Left internal mammary artery (LIMA) and saphenous vein (SV) segments were obtained from nondiabetic (n = 6) and diabetic (n = 1) patients undergoing coronary artery bypass grafting surgery (CABG).

Methods, Results, Data Analysis and Interpretation:

I) Histological/morphological analysis**:** To determine the morphological differences between human left internal mammary artery (LIMA) and saphenous vein (SV) segments, we performed hematoxylin and eosin (H&E) staining using these vascular tissue segments as an initial step.

Methods: Sections of LIMA and SV segments were cut and stained with hemotoxylin/eosin (H&E). The histological sections of LIMA and SV segments were then examined using light microscopy to evaluate the distinct differences in morphology.

Results, Data analysis and Interpretation:

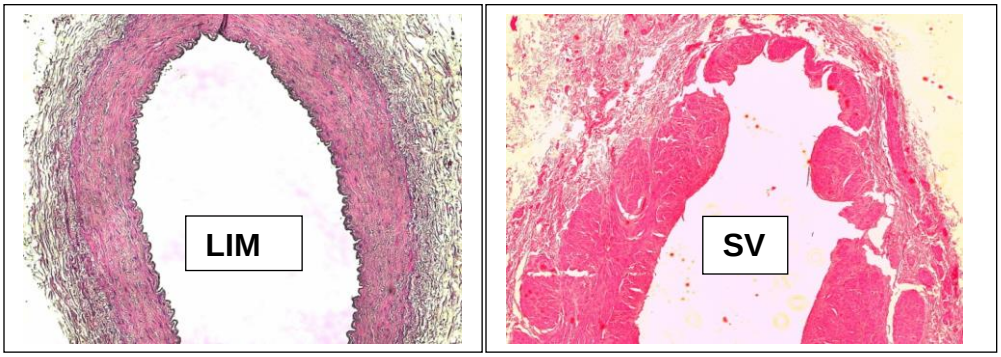

Fig. 1: H&E staining of human left internal mammary artery (**LIMA**) and saphenous vein (**SV**).

The analysis of H&E stained cross sections of LIMA revealed that the arterial tissue is rich in smooth muscle cells compared with the venous tissue (SV). Notably, LIMA tissue clearly showed the following layers: internal elastic lamina on the luminal side; and smooth muscle in the medial layer.

After observing the unique differences in morphology between the arterial tissue (LIMA) and the venous tissue (SV), we optimized the conditions to determine smooth muscle α-actin (SM α-actin) expression using the LIMA tissue, as illustrated in the following subsection.

II) Immunohistochemical (IHC) analysis: To determine the expression of smooth muscle α-actin (SM α-actin) protein in left internal mammary artery (LIMA) segments, we performed immunohistochemical analysis using the primary antibody specific for SM α-actin.

Methods: LIMA tissue segments were fixed in 10% neutral buffered formalin for 24 hours. The tissue was paraffin embedded and sectioned as 7 µm sections. The slides with tissues were washed in xylene 2 times for 5 minutes (for deparaffinization). Next, the tissue was rehydrated by performing the following wash steps. 2 times for 3 minutes in 100% ethanol, 3 minutes in 95% ethanol, 3 minutes in 70% ethanol, 3 minutes in 50% ethanol and running tap water for 5 minutes. Heat-mediated antigen retrieval was done by heating slides in 10 mM sodium citrate buffer, pH 6.00 at 95-100^{0}C for 20 minutes. The slides were removed from heat and allowed to stand at room temperature for 5 minutes and then kept in running cold tap water for 5 minutes. The slides were then rinsed in TBS-T for 5 minutes and blocked in 10% goat serum with 1% BSA in TBS-T for 1 hour at room temperature. The slides were drained and incubated with primary antibody in 1% BSA in TBS for 1 hour. The slides were rinsed in 1X TBS-T four times for 5 minutes. Endogenous peroxidase activity was blocked by incubating the slides in 0.3% H_2O_2 in TBS for 15 minutes. For enzymatic detection, slides were incubated with HRP conjugated secondary antibody in TBS with 1% BSA for 1 hour at room temperature. The cells were washed in TBS 3 times for 5 min. The tissue was developed with chromogen (3-Amino-9-ethyl carbazol) for 5 min at room temperature. The reaction was stopped by rinsing in running tap water for 5 min. The tissue was then mounted in aqueous mounting medium

Results, Data analysis and Interpretation:

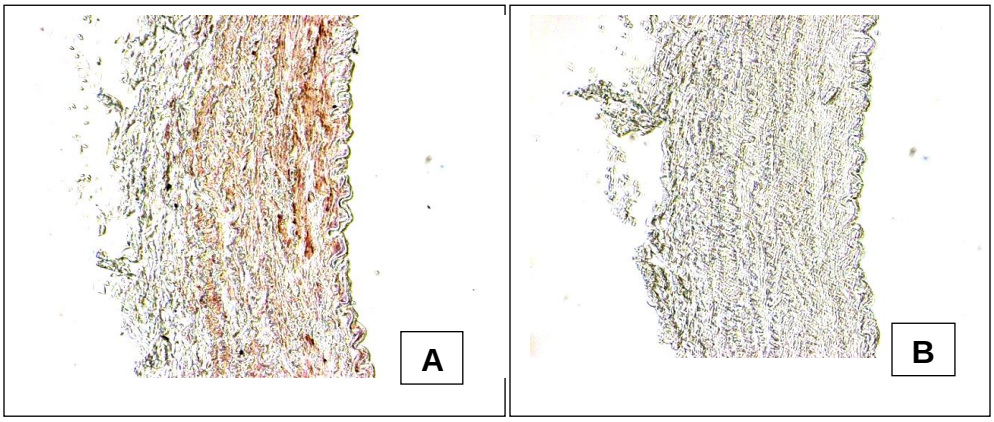

Fig. 2: Immunohistochemical analysis of SM α-actin expression in paraffin-embedded human left internal mammary artery (LIMA) cross sections. Panel A shows SM α-actin expression in smooth muscle cells as detected by horse radish peroxidase staining. Panel B shows the tissue cross-section without SM α-actin staining (negative control). The data shown are representative of 2 separate experiments.

As shown in Panel A, the brown color indicates α-actin staining by smooth muscle cells present in the medial layer, intercalated between the internal elastic lamina (IEL) and elastin fibers. Thus, LIMA tissues will provide a suitable model for the morphological determination of contractile proteins.

III) Western blot (WB) analysis: To determine the expression of contractile proteins such as smooth muscle a-actin (SM a-actin) and calponin in left internal mammary artery (LIMA) and saphenous vein (SV) segments, we performed western blot analysis using the primary antibody specific for SM a-actin or calponin.

Methods: For whole tissue protein extraction, 50-60 mg of LIMA or SV tissue segments were homogenized with four volumes of HES buffer (20 mM HEPES, 1 mM EDTA and 250 mM sucrose, pH 7.4) containing protease inhibitor cocktail (Thermo Scientific, Rockford, IL). Homogenization was performed using Tissue Lyser LT (Qiagen) at a setting of 40 Hz for 3 minutes. The homogenized tissues were mixed with urea solubulization buffer (8M urea, 50 mM Tris, 5% SDS and 350 mM DTT) at a fixed ratio of 2.5:1. The above mixture was allowed to stand at room temperature for 1-2 hours with intermittent vortexing and sonication to ensure complete solubulization of proteins. The samples were then centrifuged at 600xg for 10 min to remove tissue debris. The protein concentration in the supernatants was determined using 2-D Quant kit (GE health care). The supernatants (10-15 µg protein each) of LIMA and SV tissue homogenates were electrophoresed using pre-cast 4-12% NuPage mini-gels (Invitrogen), and the resolved proteins were transferred to nitrocellulose membranes (Hybond C, GE Healthcare). The membranes were blocked in 5% nonfat milk, and probed with the primary antibodies specific for SM α-actin and calponin. After extensive washes, the immunoreactivity was detected using specific HRP-conjugated secondary antibodies followed by enhanced chemiluminescence (Amersham Biosciences).

Results, Data analysis and Interpretation:

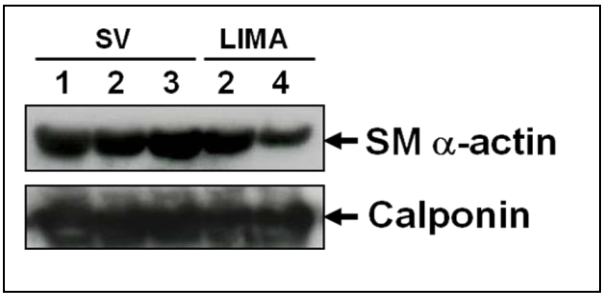

Fig. 3. Western blot analysis of contractile proteins in SV and LIMA tissue segments.

SV-1: nondiabetic; **SV-2**: diabetic; **SV-3**: nondiabetic
LIMA-2: diabetic; **LIMA-4**: nondiabetic

As shown in Fig. 3, the expression of contractile proteins such as SM α-actin and calponin was observed in both SV and LIMA segments. It appears that diabetes does not affect the expression of SM α-actin and calponin at the protein level. Further studies are clearly warranted to determine whether diabetes affects the promoter activity of SM α-actin and calponin.

IV) Optimization studies for vascular tissue contractility - comparison between serotonin (5HT) and phenylephrine (PE)-induced increase in smooth muscle contractility: Due to the confounding variables (e.g., hypertension, obesity, smoking) associated with diabetic and nondiabetic human subjects, we used rat aorta to optimize the conditions for vascular contractility studies prior to using LIMA and SV segments from coronary bypass surgery patients.

Methods: Thoracic aorta was isolated from wistar rats weighing 250-300 g. The aorta was cut into 2-mm rings and mounted in oxygenated tissue baths for isometric tension measurements (as shown in Fig. 4). The aortic rings mounted in organ bath chambers were initially subjected to 2 g of resting tension and allowed to equilibrate for ~2 hr. After the attainment of steady-state basal tone, the ring preparations were exposed to 80 mM KCl to verify similar increases in the contractile response in all rings. After the washout of KCl, stimulation with 1 µM phenylephrine (PE) resulted in an increase in contractility in all rings. In PE-pre-contracted rings, complete removal of endothelium was assessed by the lack of relaxation with 1 µM acetylcholine (ACh). The rings were then rinsed three times with Krebs buffer to restore the tension to the precontracted level. Subsequently, the rings were maintained under resting tension for an additional 30 minutes. The cumulative concentration-response for serotonin (5HT)-induced smooth muscle contractility was then compared with phenylephrine (PE).

Left: An image depicting the oxygenated organ bath system with tissue in place between the force transducer and the fixed glass anchor. See tissue circled in red.

Right: An image of the organ bath system, including the force transducer, fine adjustment knobs, glass chambers, fixed glass anchors, and fluid catch pans.

Fig. 4. Organ bath system for isometric tension measurements.

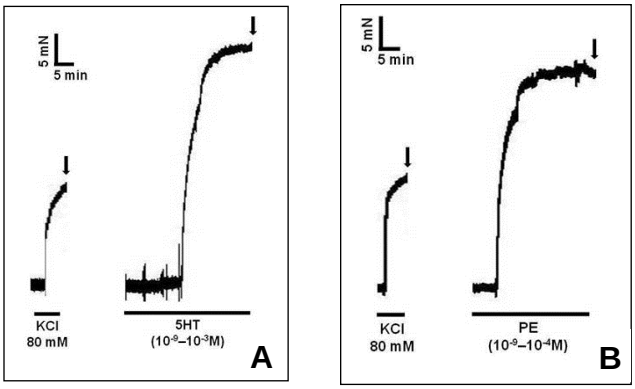

Fig. 5. Serotonin (5HT) *vs.* phenylephrine (PE)-induced increase in arterial smooth muscle contractility. The data shown are representative of 4 separate experiments. Note: KCl was used to assess tissue viability before and after the completion of concentration- response studies.

Stimulation of aortic rings with cumulative concentrations of 5HT (10^{-8} to 10^{-3} M) resulted in significant increases in smooth muscle contractility with an EC_{50} value of -6.29 ± 0.15 (log M) and the maximal responsiveness of 248.2 ± 11.3 % (Emax) (Fig. 5A, Table 1). In parallel, stimulation of aortic rings with cumulative concentrations of PE (10^{-9} to 10^{-4} M) resulted in significant increases in smooth muscle contractility with an EC_{50} value of -8.34 ± 0.18 (log M) and the maximal responsiveness of 206.9 ± 6.7 % (Emax) (Fig. 5B, Table 1). Although 5HT shows a much higher Emax value, it is apparent from the EC_{50} values that 5HT is relatively less potent in inducing contractile response in the arterial tissue, compared with PE. It is therefore equally important to determine whether similar responses are observed with 5HT and PE in the venous tissue.

V) Serotonin (5HT) and phenylephrine (PE)-induced increase in smooth muscle contractility in human saphenous vein – comparison with arterial tissue contractility: In this study, we determined the cumulative concentration-response for 5HT and PE-induced contractility using human saphenous vein (SV) segments.

Methods: Human saphenous vein segments were cut into 2-mm rings and mounted in oxygenated tissue baths for isometric tension measurements (as shown in Fig. 4). The ring preparations mounted in organ bath chambers were initially subjected to 2 g of resting tension and allowed to equilibrate for ~2 hr. After the attainment of steady-state basal tone, the ring preparations were exposed to 80 mM KCl to verify similar increases in the contractile response in all rings. After the washout of KCl, stimulation with 1 µM phenylephrine (PE) resulted in an increase in contractility in all rings. In PE-precontracted rings, complete removal of endothelium was assessed by the lack of relaxation with 1 µM acetylcholine (ACh). The rings were then rinsed three times with Krebs buffer to restore the tension to the pre-contracted level. Subsequently, the rings were maintained under resting tension for an additional 30 minutes. The cumulative concentration-response for serotonin (5HT)-induced smooth muscle contractility was then compared with phenylephrine (PE).

Table 1: Comparison of contractile responses between venous and arterial tissues.

Human saphenous vein	**log EC_{50} (M)**	**Emax** (%)
Serotonin (5-HT)	-7.183	211.56
Phenylephrine (PE)	-6.185	157.22
Arterial tissue control (rat aorta)	**log EC_{50} (M)**	**Emax** (%)
Serotonin (5-HT)	-6.29 ± 0.15	248.2 ± 11.3
Phenylephrine (PE)	-8.34 ± 0.18	206.9 ± 6.7

The data shown in Table 1 reveal that stimulation of human saphenous vein (SV) ring preparations with cumulative concentrations of 5HT (10^{-8} to 10^{-3} M) resulted in significant increases in smooth muscle contractility with an EC_{50} value of -7.183 (log M) and the maximal responsiveness of 211.56 % (Emax). In parallel, stimulation of SV ring preparations with cumulative concentrations of PE (10^{-9} to 10^{-4} M) resulted in significant increases in smooth muscle contractility with an EC_{50} value of -6.185 (log M) and the maximal responsiveness of 157.22 % (Emax). Of importance, 5HT shows a much higher Emax value in both venous and arterial tissues, compared with PE. However, it is apparent from the EC_{50} values that 5HT is relatively more potent in inducing contractile response in the venous tissue (human saphenous vein), compared with that observed in the arterial tissue control group.

Although we obtained sufficient amounts of human saphenous vein (SV) tissue segments for contractility studies, we received comparably less amounts of tissue with regard to LIMA. To address this problem, we are optimizing the conditions to handle 0.5 mm (instead of 2 mm) ring

preparations to further determine the changes in arterial *vs.* venous tissue contractile response in diabetic states.

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 or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) 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. None				☐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:

We are planning to submit the manuscript on contractility studies to a suitable journal by 2013.

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.

After optimizing the conditions for smooth muscle contractility (isometric tension measurements), we have observed that serotonin is much more potent in inducing contractile response in human saphenous vein, compared with rat arterial control group. Of importance, serotonin is implicated in thrombogenic vasoconstriction and vasospasm.

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

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.*

Dr. Segar is no longer at Penn State University; see Biosketch below.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
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NAME	POSITION TITLE
Segar, Lakshman (legally changed from Sandirasegarane, Lakshman on November 8, 2010)	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) LSEGAR	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Madras, India	B.Sc.	05/78	Chemistry
University of Madras, India	M.Sc.	04/81	Biochemistry
University of Saskatchewan, Canada	Ph.D.	05/95	Pharmacology
University of Saskatchewan, Canada	Postdoctoral	09/94 - 06/96	Physiology
University of British Columbia, Vancouver, BC	Postdoctoral	07/96 – 06/98	Pharmaceutical Sciences

A. Personal Statement

The overall goal of the proposed research is to gain new understanding into the mechanisms of atherosclerosis. The specific objective is to investigate how platelet-derived growth factor (PDGF) regulates glucose transport and insulin receptor signaling to influence phenotypic changes in vascular smooth muscle cells (VSMCs). The experimental models for the current proposal include: i) human aortic VSMCs in culture; ii) mouse model of arterial injury; and iii) arterial *vs* venous grafts from diabetic and nondiabetic patients. Based on prior experience in animal studies, vascular tissue/cell culture techniques, contractility studies, and signal transduction mechanisms, the PI will be directly responsible for the overall administration of the project. He will play a critical role in key experiments that include VSMC phenotypic characterization *in vitro* and *in vivo*, isometric tension measurements using saphenous vein and internal mammary artery ring preparations, immunocytochemical and immunohistochemical analyses, VSMC transfections with cDNAs and siRNAs, western blotting, immunoprecipitation, *in vitro* kinase assays, EMSAs, ChIP assays, luciferase assays, and glucose transport and metabolism. He will also be responsible for the training and management of the postdoctoral fellow and the research technician engaged in achieving the specific aims outlined in the research proposal. In addition, he will receive the required assistance from the collaborators, Dr. Mark Kozak, Dr. Jason K. Kim, Dr. John Y. Jun, and Dr. Ian A. Simpson. Furthermore, the PI will play a major role in the design and interpretation of data and preparation of manuscripts and reports.

B. Positions and Honors

Positions and Employment

1982-1989 **Demonstrator,** Department of Biochemistry, Jawaharlal Institute of Post-graduate Medical Education and Research, Directorate General of Health Services, Pondicherry, India

1989-1990 **Scientific Officer-cum-Tutor,** Department of Biochemistry, Jawaharlal Institute of Post-graduate Medical Education and Research, Directorate General of Health Services, Pondicherry, India

1990-1994	**Graduate Student,** Department of Pharmacology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
1992-1993	**Graduate Teaching Assistant,** Department of Pharmacology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
1994-1996	**Post-doctoral Fellow,** Dr. P.V. Sulakhe's laboratory, Department of Physiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
1996-1998	**Post-doctoral Fellow,** Dr. Jack Diamond's laboratory, Division of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, B.C., Canada
1998-2000	**Research Associate,** Dr. Mark Kester's laboratory, Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA
2000-2006	**Assistant Professor,** Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA
06/2006-12/2008	**Assistant Professor** (tenure track), Department of Medicine and Penn State Heart & Vascular Institute, Pennsylvania State University College of Medicine, Hershey, PA
01/2008-03/2010	**Core Lab Director,** General Clinical Research Center, Penn State Heart & Vascular Institute, Pennsylvania State University College of Medicine, Hershey, PA
01/2009-08/2011	**Assistant Professor** (tenure track), Departments of Medicine & Pharmacology and Penn State Heart & Vascular Institute, Pennsylvania State University College of Medicine, Hershey, PA
08/2011-Present	**Associate Professor** (tenure track), Program in Clinical and Experimental Therapeutics, Department of Clinical and Administrative Pharmacy, University of Georgia College of Pharmacy, Augusta, GA

Professional Memberships

2008-Present	Member, American Heart Association
2008-Present	Member, American Society for Pharmacology and Experimental Therapeutics
2013-	Member, American Physiological Society
2012 (Fall)	Member, 2012 Vascular Endothelial Biology 2 Peer Review Committee, American Heart Association
2013 (Spring)	Member, 2013 Vascular Wall Biology Basic Science 5 Peer Review Committee, American Heart Association
2013 (Spring)	Adhoc Reviewer, Diabetes UK, London, UK

Honors

1990-1994	Graduate Scholarship, College of Graduate Studies and Research, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
1996-1998	Post-doctoral Fellowship, Health Services Utilization and Research Commission, Saskatoon, Saskatchewan, Canada
1999-2004	Beginning Grant-In-Aid awards (twice) from the American Heart Association, Pennsylvania-Delaware Affiliate
2005	Grant-In-Aid awards from three National Diabetes Research funding agencies: NIDDK (NIH/R21), JDRF (Regular Research Grant), and ADA (Innovation Award); - First Rank in the ADA Innovation Award category

C. Selected Peer-reviewed Publications (Selected from 23 peer-reviewed publications)

Most relevant to cardiovascular and neuronal biology

1. **Sandirasegarane, L.** & Gopalakrishnan, V. (1994). Limitations of the radioreceptor assay of inositol 1,4,5-trisphosphate in vanadate-treated cell suspensions. *Biochemical Journal,* 298, 511-512.
2. **Sandirasegarane, L.,** Herman, R.J. & Gopalakrishnan, V. (1994). High glucose attenuates peptide agonist-evoked increases in cytosolic free $[Ca^{2+}]^i$ in rat aortic smooth muscle cells. *Diabetes,* 43(8), 1033-1040.
3. **Sandirasegarane, L.** & Gopalakrishnan, V. (1995). Vanadate increases cytosolic free calcium in rat aortic smooth muscle cells. *Life Sciences,* 56(7), PL169-PL174.
4. **Sandirasegarane, L.** & Diamond, J. (1999). The nitric oxide donors, SNAP and DEA/NO, exert a negative inotropic effect in rat cardiomyocytes which is independent of cyclic GMP elevation. *Journal of Molecular and Cellular Cardiology,* 31(4), 799-808.
5. **Sandirasegarane, L.,** Charles, R., Bourbon, N. & Kester, M. (2000). NO regulates PDGF-induced activation of PKB but not ERK in A7r5 cells: implications for vascular growth arrest. *American Journal of Physiology Cell Physiology,* 279(1), C225-C235.
6. Charles, R., **Sandirasegarane, L.,** Yun, J., Bourbon, N., Wilson, R., Rothstein, R.P., Levison, S.W. & Kester, M. (2000). Ceramide-coated balloon catheters limit neointimal hyperplasia after stretch injury in carotid arteries. *Circulation Research,* 87(4), 282-288.
7. **Sandirasegarane, L.** & Kester, M. (2001). Enhanced stimulation of Akt-3/protein kinase B-γ in human aortic smooth muscle cells. *Biochemical Biophysical Research Communications,* 283(1), 158-163.
8. Bourbon, N.A., **Sandirasegarane, L.** & Kester, M. (2002). Ceramide-induced inhibition of Akt is mediated through protein kinase Cζ: implications for growth arrest. *Journal of Biological Chemistry,* 277(5), 3286-3292.
9. Reiter, C.E, **Sandirasegarane, L.,** Wolpert, E.B., Klinger, M., Simpson, I.A., Barber, A.J., Antonetti, D.A., Kester, M. & Gardner, T.W. (2003). Characterization of insulin signaling in rat retina *in vivo* and *ex vivo*. *American Journal of Physiology Endocrinology and Metabolism,* 285, E763-E774.
10. Biswas, S.K., Zhao, Y., Nagalingam, A., Gardner, T.W. & **Sandirasegarane, L.** (2008). PDGF- and insulin/IGF-1-specific distinct modes of class IA PI 3-kinase activation in normal rat retinas and RGC-5 retinal ganglion cells. *Investigative Ophthalmology & Visual Science* 49(8), 3687-3698 (PMC Journal – In Process).
11. Biswas, S.K., Zhao, Y. & **Sandirasegarane, L**. (2009). Imatinib induces apoptosis by inhibiting PDGF- but not insulin-induced PI 3-kinase/Akt survival signaling in RGC-5 retinal ganglion cells. *Molecular Vision* 15, 1599-1610. PMCID: PMC2728561
12. Zhao, Y., Biswas, S.K., McNulty, P.H., Kozak, M., Jun, J.Y. & **Segar, L.** (2011). PDGF-induced vascular smooth muscle cell proliferation is associated with dysregulation of insulin receptor substrates. *American Journal of Physiology Cell Physiology,* 300: C1375-C1385. PMCID: PMC3118627.
13. Jun, J.Y., Ma, Z., & **Segar, L.** (2011). Spontaneously diabetic $Ins2^{+/Akita}$:ApoE-deficient mice exhibit exaggerated hypercholesterolemia and atherosclerosis. *American Journal of Physiology Endocrinology and Metabolism,* 301: E145-E154. PMCID: PMC3129838.
14. Jun, J.Y., Ma, Z., Pyla, R., & **Segar, L.** (2012). Leptin treatment inhibits the progression of atherosclerosis by attenuating hypercholesterolemia in type 1 diabetic $Ins2^{+/Akita}$:$apoE^{-/-}$ mice. *Atherosclerosis* 225: 341-347. PMCID: PMC3502687
15. Pyla, R, Poulose, N., Jun, J.Y., & **Segar, L.** (2013). Expression of conventional and novel glucose transporters, GLUT1, -9, -10, -12, in vascular smooth muscle cells. *Am J Physiol:Cell Physiol*. 304: C574-C589.

Research Support

Ongoing Research Support

1031RE262380 Segar (PI) 10/01/12 - 06/30/13
R. C. Wilson Pharmacy Fund - UGA College of Pharmacy Foundation Fund
Identification of Glucose Transporters that Transport Fructose in Vascular Smooth Muscle Cells
The overall goal of this project is to determine the contribution of GLUT5, GLUT9, and GLUT12 toward fructose uptake in vascular smooth muscle cells.
Role: PI

R01 HL097090 Segar (PI) 08/01/10-11/30/15
Vascular Phenotypic Regulation by Growth Factors, Insulin, and Glucose
The overall goal of this project is to determine the role of platelet-derived growth factor in vascular glucose metabolism in insulin-resistant states.
Role: PI

Completed Research Support

No Number Assigned Segar (PI) 07/08/09-06/30/11
PSU Bridge Grant - PA Dept. of Health; Tobacco Settlement Fund
Diabetic changes in contractile proteins and contractility in arterial *vs* venous grafts
The overall goal of this project is to determine the extent of changes in contractile proteins and contractile responses between arterial and venous grafts in diabetic and nondiabetic patients undergoing coronary artery bypass grafting surgery.
Role: PI

No Number Assigned Segar (PI) 12/01/09-04/30/11
Pennsylvania Lions Sight Conservation and Eye Research Foundation NO COST EXTENSION
Diabetic Changes in Nuclear MAP Kinase and PI 3-Kinase/Akt Signaling in the Retina
The overall goal of this project is to determine the extent of changes in nuclear MAP kinase and PI 3-Kinase/Akt signaling in the diabetic retina.
Role: PI