

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 – Of The Commonwealth System of Higher Education
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, MA
4. **Grant Contact Person’s Telephone Number:** 215.204.7655
5. **Grant SAP Number:** 4100050909
6. **Project Number and Title of Research Project:** Research Project 4: Mechanisms of Vascular Damage in Obesity
7. **Start and End Date of Research Project:** 09/01/12 – 08/31/13
8. **Name of Principal Investigator for the Research Project:** Rosario Scalia, M.D., PH.D.
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:

\$ 41,909.78

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, First Name	Position Title	% of Effort on Project	Cost
Liu Zhao, B.S.	Masters Student	100	30,000

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, First Name	Position Title	% of Effort on Project
Preston, Kyle	Graduate Student, RA	15
Scalia, Rosario	Professor	5

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
N/A		

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:
Integrative Mechanisms of Adipose Tissue Dysfunction	<input checked="" type="checkbox"/> NIH <input type="checkbox"/> Other federal (specify: _____) <input type="checkbox"/> Nonfederal source (specify: _____)	11/2012	\$ 892,000	\$892,000
	<input type="checkbox"/> NIH <input type="checkbox"/> Other federal (specify: _____) <input type="checkbox"/> Nonfederal source (specify: _____)		\$	\$
	<input type="checkbox"/> NIH <input type="checkbox"/> Other federal (specify: _____) <input type="checkbox"/> Nonfederal source (specify: _____)		\$	\$

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

Yes No

If yes, please describe your plans:

We anticipate that our recently funded NIH grant will provide a new area of investigation that will be continued in our laboratory. For instance, we are currently exploring the impact of nutrients overload (high fat meals) on inflammatory processes that occur in the adipose tissue. We believe that this is important and timely research that will be able to secure additional funds to our program via either NIH and/or private foundations such as the American Diabetes Association.

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

As stated above, we are interested in continuing this line of research. Obesity has become a major health challenge facing the US population. Obesity is associated with infiltration of circulating leukocytes cells into visceral fat depots. The inflamed adipose tissue becomes dysfunctional, produces cytokines, and contributes to insulin resistance, diabetes, cardiovascular disease, cancer and arthritis, whose incidence is gaining. All of these complications of obesity have been widely linked to inflammation. Even after accounting for lack of physical activity and genetic susceptibility, excessive food energy intake remains probably, the most common cause of obesity in western countries. Accordingly, we plan to continue our work to uncover novel mechanisms of adipose tissue inflammation in obesity with the overall goal of providing a framework for developing new therapeutic strategies to avert complications in the obese population of the USA.

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 No

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

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

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

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

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

Yes _____ No _____

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 _____ No _____

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

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 _____

If yes, please describe the collaborations:

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

Yes _____ No _____

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 (β) should not print as boxes (\square) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

The overall goal of this research project as listed in the original grant agreement is to understand the causal relationship between adiponectin and vascular inflammation. **The specific aims** were: *1) To study the effect of adiponectin on eNO/ROS balance and endothelial function in vivo; 2) To study the role of leukocyte-derived MPO in the endothelial protective action of adiponectin.*

Aim-1 was only partially completed. The results of studies obtained in Aim 1 have been submitted with our previous annual progress report where we demonstrated that adiponectin **a**) attenuates endothelial inflammation by reducing adhesion of circulating leukocytes to the vascular endothelium (leukocytes are the source of MPO, see Aim-2); **b**) reduces vascular oxidative stress by attenuating the effect of free radical signaling in endothelial cells, and **3**) increases mitochondrial abundance in endothelial cells.

The last and final section of results obtained pertain almost exclusively to Aim-2 and are reported below. These aims are also only partially completed and additional studies are now ongoing. These studies, which are still ongoing in our laboratory, are now supported from NIH funds, and we expect to generate a full-length manuscript within the current year of research.

During inflammation, neutrophils that adhere to vascular endothelium become activated, degranulate, and release, among other factors, the harmful oxidant, myeloperoxidase¹, an enzyme abundantly expressed in neutrophils, and to a lesser extent in monocyte and macrophages². Ligation of neutrophil PSGL-1 by endothelial expressed P- or E-selectin induces selectin-type specific phenotypic changes in circulating neutrophil that affect their adhesive behavior and degranulation ability^{1,3}. Our data in **Figure 1**, upper graph, demonstrate increased MPO content in the visceral fat of control mice following exposure to high-fat diet for 24 hours.

Related to adiponectin, data shown in **Figure 2** demonstrate reduced adiponectin mRNA copies in the mesenteric VAT of mice given a high-fat meal. Interestingly, this phenomenon was not observed in MPO deficient mice (**Figure 2**), which has further prompted us to test the effect of MPO on adiponectin secretion by adipocytes, as stated in the original grant agreement. Data reported in **Table 1** clearly demonstrate that incubation of 3T3-L1 adipocytes drastically reduced adiponectin secretion, in the absence of any significant change in cell viability or density. In agreement with these data, others have observed acute reduction in adipose tissue adiponectin mRNA levels during pulmonary allergic reactions⁵, a condition in which granulocytes become highly activated.

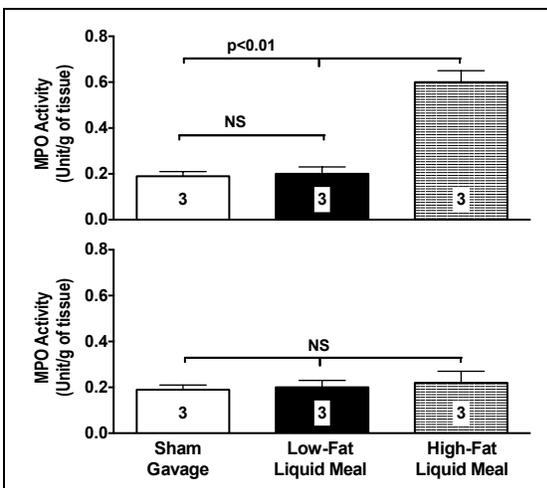


Figure 1. Quantification of MPO content in VAT (upper graph) and SAT (bottom graph) of mice given free access to high-fat diet for 24 hours. MPO was measured according to methods previously published by the PI⁴. Values are Means \pm SE. Numbers at the bottom of bars are the number of mice studied in each group.

One of the main harmful byproduct of MPO is hypochlorous acid (HOCl), a powerful oxidant that is able to initiate modification reaction to signaling proteins⁶. It has been demonstrated that

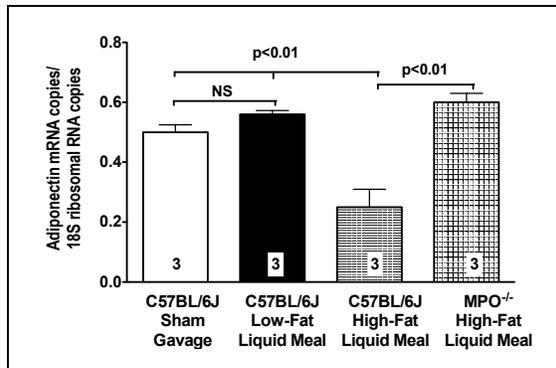


Figure 2. Quantification of adiponectin mRNA copies content in mesenteric VAT of mice given free access to high-fat diet for 24 hours. Adiponectin mRNA expression was measured by quantitative real-time RT-PCR and normalized for expression of 18S rRNA. Values are Means±SE. Numbers at the bottom of bars are the number of mice studied in each group.

MPO-derived HOCl increases activity of MMPs via the cysteine switch conserved among the MMPs and ADAMs⁷.

It should be noted that constitutively expressed ADAM17 has two highly conserved cysteine-X-X-cysteine motifs, which are targets of redox modification, and hydrogen peroxide appears to activate ADAM17 via this mechanism⁸. Moreover, HOCl appears to inactivate TIMP metalloproteinase inhibitor 1 (TIMP-1) via modification of the N-terminal cysteine, which is also conserved among TIMPs⁹. Tissue inhibitor of metalloproteinase-3 (TIMP3) is a critical endogenous ADAM17 inhibitor. TIMP3 activity is reduced in type 2 diabetes, which is associated with enhanced ADAM17 activity¹⁰. TIMP3 over-expression in macrophage prevented adipocyte inflammation and insulin resistance in

mice fed high-fat diet¹¹. Therefore, we have now hypothesized that with fatty meals MPO rapidly increases ADAM17 activity in adipocytes via either direct HOCl modification of its cysteine switch and/or HOCl modification of TIMP3 N-terminal cysteine residue. This is now ongoing research in our laboratory. In support of this working hypothesis, data in **Figure 3** show that MPO and HOCl increase ADAM 17 activity leading to TNF α production in 3T3-L1 adipocytes *in vitro*. ***This process, if confirmed, can help explain two related aspects of adipocyte dysfunction in nutrients overload conditions, i.e., the increased production of cytokines and the loss of adiponectin function.***

Table 1. Effect of MPO on production rate of adiponectin from 3T3-L1 adipocytes.	
24 h (ng/ml per 24H)	
Control	289±54
50 nmol/L MPO	130±32 (p<0.01 vs control)
Adiponectin concentration in the culture media was measured in triplicates by radioimmunoassay (mouse adiponectin radioimmunoassay kit; Linco Research, St Charles, MO).	

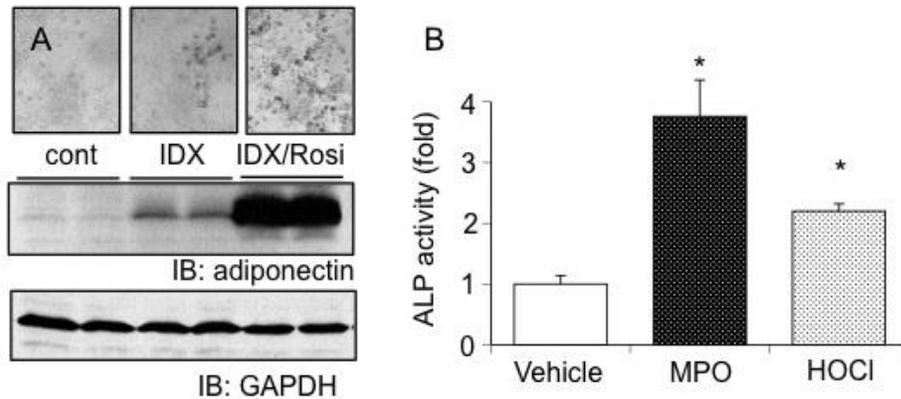


Figure 3. **A:** 3T3L1 cells were cultured with control medium (DMEM+10%FCS), standard differentiated medium (IDX: 100 nM insulin+250 nM dexamethasone +0.5 mM IBMX), or modified differentiation medium (IDX+1 μ M rosiglitazone) for 3 days. Expression of adiponectin was analyzed by immunoblotting. **B:** ADAM17 activation was assessed in 3T3L1 adipocytes. Differentiated 3T3L1 cells as in A were infected with adenovirus encoding proTNF α -ALP (300 moi) for 2 days. The cells were stimulated with 50 nM MPO or 1 mM HOCl for 1 h as indicated under serum-free condition, and secreted medium ALP activity was determined (N=3-4). *p<0.05.

References

1. Lorant DE, Topham MK, Whatley RE, McEver RP, McIntyre TM, Prescott SM and Zimmerman GA. Inflammatory roles of P-selectin. *Journal of Clinical Investigation*. 1993;92:559-70.
2. Koefler HP, Ranyard J and Pertcheck M. Myeloperoxidase: its structure and expression during myeloid differentiation. *Blood*. 1985;65:484-91.
3. Lorant DE, Topham MK, Whatley RE, McEver RP, McIntyre TM, Prescott SM and Zimmerman GA. Inflammatory roles of P-selectin. *The Journal of clinical investigation*. 1993;92:559-70.
4. Scalia R, Murohara T, Delyani JA, Nossuli TO and Lefer AM. Myocardial protection by N,N,N-trimethylsphingosine in ischemia reperfusion injury is mediated by inhibition of P-selectin. *J Leukoc Biol*. 1996;59:317-24.
5. Shore SA, Terry RD, Flynt L, Xu A and Hug C. Adiponectin attenuates allergen-induced airway inflammation and hyperresponsiveness in mice. *J Allergy Clin Immunol*. 2006;118:389-95.

6. Rossmann C, Rauh A, Hammer A, Windischhofer W, Zirkl S, Sattler W and Malle E. Hypochlorite-modified high-density lipoprotein promotes induction of HO-1 in endothelial cells via activation of p42/44 MAPK and zinc finger transcription factor Egr-1. *Arch Biochem Biophys*. 2011;509:16-25.
7. Fu X, Kassim SY, Parks WC and Heinecke JW. Hypochlorous acid generated by myeloperoxidase modifies adjacent tryptophan and glycine residues in the catalytic domain of matrix metalloproteinase-7 (matrilysin): an oxidative mechanism for restraining proteolytic activity during inflammation. *The Journal of biological chemistry*. 2003;278:28403-9.
8. Wang Y, Herrera AH, Li Y, Belani KK and Walcheck B. Regulation of mature ADAM17 by redox agents for L-selectin shedding. *J Immunol*. 2009;182:2449-57.
9. Wang Y, Rosen H, Madtes DK, Shao B, Martin TR, Heinecke JW and Fu X. Myeloperoxidase inactivates TIMP-1 by oxidizing its N-terminal cysteine residue: an oxidative mechanism for regulating proteolysis during inflammation. *J Biol Chem*. 2007;282:31826-34.
10. Cardellini M, Menghini R, Martelli E, Casagrande V, Marino A, Rizza S, Porzio O, Mauriello A, Solini A, Ippoliti A, Lauro R, Folli F and Federici M. TIMP3 is reduced in atherosclerotic plaques from subjects with type 2 diabetes and increased by SirT1. *Diabetes*. 2009;58:2396-401.
11. Menghini R, Casagrande V, Menini S, Marino A, Marzano V, Hribal ML, Gentileschi P, Lauro D, Schillaci O, Pugliese G, Sbraccia P, Urbani A, Lauro R and Federici M. TIMP3 overexpression in macrophages protects from insulin resistance, adipose inflammation, and nonalcoholic fatty liver disease in mice. *Diabetes*. 2012;61:454-62.

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
- 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. (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). For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

- Project 01 – Smith – Three cases of isolated
- Project 01 – Smith – Investigation of NEB1 deletions

Project 03 – Zhang – Molecular profiling of aromatase

Project 04 – Bates – Neonatal intensive care

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):
				<input type="checkbox"/> Submitted <input type="checkbox"/> Accepted <input type="checkbox"/> Published
				<input type="checkbox"/> Submitted <input type="checkbox"/> Accepted <input type="checkbox"/> 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:

All data On MPO/adiponectin reported in this final progress report will be organized in manuscript once some histology studies on adipose tissue of mice fed a high-fat diet will be completed. We anticipate submitting the manuscript before the Fall of 2014.

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.

None

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 _____

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.*

NAME Rosario Scalia	POSITION TITLE Professor
eRA COMMONS USER NAME (credential, e.g., agency login)	

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/Y Y	FIELD OF STUDY
University of Catania, Italy	MD	1986	Medicine
Universities of Catania, Cagliari and Florence	Ph.D.	1991	Vascular Biology
Thomas Jefferson University	Postdoc.	1995	Physiology

A. Personal Statement. The focus of my laboratory is to understand the mechanisms by which the dysfunctional endothelium orchestrates leukocyte-endothelium interactions and leukocyte trafficking in organ tissues. Endothelial bound leukocytes disrupt endothelial function itself, damage the vascular wall in its entirety, and cause organ dysfunction and failure. Since the beginning of my independent career, I have investigated the pathogenetic contribution of leukocyte-endothelium interactions to the endothelial dysfunction and microvascular damage of insulin resistance and hyperglycemia. My work was one of the first to demonstrate the critical role of endothelial nitric oxide in the regulation of cell surface expression of endothelial cell adhesion molecules such as P-selectin and VCAM-1. Another line of ongoing research in my lab has first uncovered the role of the calcium-dependent protease μ -calpain in the endothelial dysfunction, leukocyte trafficking and endothelial hyper permeability of the insulin resistant diabetic vasculature. Recent publications have also linked the calpain system to well-established mediators of diabetic complications such as PKC and Angiotensin. In collaboration with Dr. George King's laboratory at the Joslin Diabetes Center, my lab has contributed to the understanding of the role that the endothelial expressed insulin receptor plays in leukocyte-endothelium interaction and vascular

inflammation *in vivo*. More relevant to this application, the PI's was one of the first to study the impact of adiponectin in the regulation of adipocyte function, microvascular endothelial function, cell adhesion molecules, and eNOS activity. To implement these challenging integrative physiology studies my laboratory has acquired state-of-the-art *in vivo*, *ex vivo* and *in vitro* techniques. The lab currently uses: **1)** spinning-disk confocal intravital microscopy to study microvascular function in the intact animal under conditions of monitored blood flow and systemic blood pressure; **2)** wire-myography to measure vascular reactivity in isolated resistance arterioles and capacitance venules; **3)** polarographic electrodes to measure real-time levels of endothelial nitric oxide; hydrogen sulfide, and hydrogen peroxide; **4)** standard biochemistry and molecular methodologies to measure RNA and protein expression levels; **5)** Flow cytometry to measure expression of cell adhesion molecules in isolated cells; **6)** cell isolation techniques to obtain primary culture of endothelial cells and adipocytes from relevant animal models.

B. Positions

- 09/86-09/88 Resident Physician of Cardiology Cardiology and Pharmacology, University of Catania, Italy
- 09/88-05/92 Resident Physician and PhD Student Clinical Pharmacology, University of Catania, Italy
- 06/92-03/94 Post-Doctoral Fellow, Pharmacology, University of Catania, Italy
- 03/94-10/94 Post-Doctoral Fellow, Pharm Mol Biochem., Thomas Jefferson University, Philadelphia, PA USA
- 01/95-06/99 Research Associate, Physiology Thomas Jefferson University, Philadelphia, PA USA
- 07/99-06/04 Assistant Professor, Physiology, Thomas Jefferson University, Philadelphia, PA USA
- 07/04-01/09 Associate Professor, Physiology, Thomas Jefferson University, Philadelphia, PA USA
- 02/09-06/10 Associated Professor, Temple University, Philadelphia, PA USA
- 07/10 Professor, Temple University, Philadelphia, PA USA.

Present Membership

American Physiological Society, American Association for the Advancement of Science, America Diabetes Association, American Hearth Association, Shock Society

Review Panels

- 2002-2007 American Diabetes Association Grant Review Panel (Member)
- 2003-2005 American Hearth Association, Mid-Atlantic Affiliate (Member)
- 2006 NIH/ ZDK1 GRB-G J1
- 2007 JDRF Innovative Grant Review for the Complications Study Section
- 2008 NIH/ZHL 1 PPG-J (M2)
- 2009 Juvenile Diabetes Foundation
- 2009 Diabetes UK (Ad-hoc reviewer)
- 2009 Telethon (European Community; Ad-hoc reviewer)
- 2010 NIH/IPOD Study Section (Temporary member)
- 2010 NIH RFA Review Meeting – ZHL1 CSR-W (S1)
- 2010-2016 American Diabetes Association Grant Review Panel (Member)

2011-2015 American Heart Association, Mid-Atlantic Affiliate (Member)

2011 *NIH Study Section ZDK1 GRB-N (M2)*

2011 *NIH ZDK1 GRB-J O1 Special Emphasis Panel Editorial Board*

2012-2014 NIH/IPOD Study Section (Temporary member)

2010-2015 American Diabetes Association Grant Review Panel (Member)

Reviewer For Scientific Journals: American Journal of Physiology, American Journal of Respiratory and Critical Care Medicine, ATVB, British Journal of Pharmacology, Diabetes, Circulation, Circulation Research, European Journal of Pharmacology, Journal of Molecular and Cellular Cardiology, Journal of Endocrinology, Journal of Endotoxin Research, Journal of Pharmacology and Experimental Therapeutics, FASEB Journal, Transplantation.