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:** 27 - Glycosphingolipids and Diabetic Retinopathy

7. **Start and End Date of Research Project:** 07/08/2009-06/30/2011

8. **Name of Principal Investigator for the Research Project:** Mark Kester, PhD

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:

   $ 186,469

   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
Bewley	Faculty	10% Yr. 1	$12,635.36
Gianbuzzi-Tussey	Research Technician	40% Yr. 1; 83% Yr 2	$46,198.34

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
Kester	P.I.	2% Yr. 1; 2% Yr. 2

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
Participated in purchase of ABSciex 5600 Triple TOF	Proteomic, metabolomic, and lipidomic analyses	$62,389

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:
Studies to Verify Dysfunctional Toll-Like Receptor Signaling and Diabetic Retinopathy	☐NIH ☐ Other federal (specify:_) ☒Nonfederal source (specify: Juvenile Diabetes Cure Research PA Tax Check-Off Program)	November 2011	$178,619-$150,000 direct costs plus $28,619 indirect costs	$178,619

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:

NIH Competing Renewal being submitted March 2013.

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

To continue to use sophisticated mass spectrometry strategies to integrate the lipidomic "milieu" of diabetic retinopathy.

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	1			
Unknown				
Total	**1**			**1**

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

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

Xianming Wang, MS obtained his Master's Degree from Penn State University, Dept. of Pharmacology

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 Triple TOF 5600 has provided extensive proteomic, metabolomic, and lipidomic analyses for more than 20 separate PIs with multiple projects ranging from obesity to cancer to diabetes to lung development. The project also leveraged additional state and extramural foundation support.

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___x_____ No_____

If yes, please describe the collaborations:

Collaboration with University of Oklahoma (Drs. Pederson & Anderson) as well as continued collaboration with University of Michigan (Dr. Thomas Gardner).

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.

The *overall objective* of this project is to understand the molecular basis for vision impairment in diabetic retinopathy. The *specific objective* is to investigate the role of ceramide metabolism and glycosphingolipid-enriched lipid microdomains (rafts) in insulin signaling and cell survival in retinal neurons. The *rationale* for the project is based upon several novel observations, including: 1) diabetic retinas demonstrate decreased total ceramide levels; 2) with a concomitant increase in glucosylceramides concomitant with; 3) decreased insulin receptor signaling. We h*ypothesize* that diabetes increases glycosphingolipid production in the retina, which decreases activation of pro-survival signaling cascades contributing to cell death. The *significance* of this work is that identifying glycosphingolipids as a therapeutic target in diabetic retinopathy has the potential to lead to pharmacological and molecular interventions to prevent the progression of vision loss in patients with diabetes.

The original grant contained two specific aims, which are listed below. Over the entire period of the grant (07/08/2009-06/30/2011), we successfully completed both specific aims and these results were published in the following two manuscripts.

1.Fox TE, Young MM, Pedersen MM, Giambuzzi-Tussey S, Kester M, Gardner TW. Insulin signaling in retinal neurons is regulated within cholesterol-enriched membrane microdomains. Am J Physiol Endocrinol Metab. 2011 Mar;300(3):E600-9.

(Note: Even though the work reported upon in this article was not supported by the PA CURE Program, supplemental data from investigations unexpectedly synergized with the aims of the PA CURE grant.)

2. Fox TE, Bewley MC, Unrath KA, Pedersen MM, Anderson RE, Jung DY, Jefferson LS, Kim JK, Bronson SK, Flanagan JM, Kester M. Circulating sphingolipid biomarkers in models of type 1 diabetes. J Lipid Res. 2011 Mar;52(3):509-17.

Both of these publications serve to delineate the role of lipids in diabetic retinopathy. More importantly, The PA CURE funding allowed us to generate additional new unexpected data, which has been leveraged into the following new extramural support.

1. Studies to Verify Dysfunctional Toll-Like Receptor Signaling and Diabetic Retinopathy, PA Check-OFF program, DOH $178,619

For the review process, we provide the original two Specific Aims, the data demonstrating completion of the aims, as well as new data that supported additional funding opportunities.

Specific Aim One: Determine the consequence of glycosphingolipids on insulin signaling and cell death in ex vivo retinas. We will assess the effects of altered glycosphingolipid metabolism upon insulin signaling and resultant cell death in retinal explant models. Here, retinal explants

from C56BL/6 mice will be treated with inhibitors to glycosphingolipid metabolism and the effect on the phosphorylation of enzymes within the insulin signaling cascade assessed in addition to caspase 3 activity to assess cell death.

We have successfully completed the experiments outlined in Specific Aim 1. Specifically, we report that lipid microdomains, which are enriched in sphingolipids and glycosphingolipids, are critical mediators to insulin signaling. We also report that decreased caveolin expression within lipid microdomains contributes to dysfunctional insulin signaling in the diabetic retina.

Neuronal cell death is an early pathological feature of diabetic retinopathy. We showed previously that insulin receptor signaling is diminished in retinas of animal models of diabetes and that downstream Akt signaling is involved in insulin-mediated retinal neuronal survival. Further understanding of the mechanisms by which retinal insulin receptor signaling is regulated could have therapeutic implications for neuronal cell death in diabetes. We therefore investigated the role of cholesterol-enriched membrane microdomains to regulate PKC-mediated inhibition of Akt-dependent insulin signaling in R28 retinal neurons. We demonstrate that PKC activation with either a phorbol ester or exogenous application of diacylglycerides impairs insulin-induced Akt activation, whereas PKC inhibition augments insulin-induced Akt activation. To investigate the mechanism by which PKC impairs insulin-stimulated Akt activity, we assessed various upstream mediators of Akt signaling. PKC activation did not alter the tyrosine phosphorylation of the insulin receptor or IRS-2. Additionally, PKC activation did not impair phosphatidylinositol 3-kinase activity, phosphoinositide-dependent kinase phosphorylation, lipid phosphatase (PTEN), or protein phosphatase 2A activities. Thus, we next investigated a biophysical mechanism by which insulin signaling could be disrupted and found that disruption of lipid microdomains via cholesterol depletion blocks insulin-induced Akt activation and reduces insulin receptor tyrosine phosphorylation. We also demonstrated that insulin localizes phosphorylated Akt to lipid microdomains and that PMA reduces phosphorylated Akt. In addition, PMA localizes and recruits PKC isotypes to these cholesterol-enriched microdomains. We show that these lipid microdomains are necessary for insulin-stimulated insulin receptors and downstream signaling. We also demonstrate that Protein Kinase C-inhibition of insulin signaling occurs downstream of the insulin receptor through the impairment of the translocation of the pro-survival kinase Akt to these lipid microdomains. Taken together, these results demonstrate that both insulin-stimulated Akt signaling and PKC-induced inhibition of Akt signaling depend on cholesterol-enriched membrane microdomains, thus suggesting a putative biophysical mechanism underlying insulin resistance in diabetic retinopathy. These data are described in detail in the following published manuscript. (Fox TE, Young MM, Pedersen MM, Giambuzzi-Tussey S, Kester M, Gardner TW. Insulin signaling in retinal neurons is regulated within cholesterol-enriched membrane microdomains. Am J Physiol Endocrinol Metab. 2011 Mar;300(3):E600-9.)

Additional studies which were not described in the Am J Physiol Endocrinol Metab. Manuscript demonstrates that caveolin expression within retinal membrane microdomains is dramatically decreased in two models of diabetic retinopathy (Ins2 Akita mouse and Strept-induced rat). More importantly, caveolin knockout animal's exhibit diminished physiological ocular responses as assessed by *in vivo* electro-retinagrams. Taken together these studies identify diminished caveolin expression as a target in diabetic retinopathy.

To further define the putative pathological roles of glucosylceramide synthase in diabetic retinopathy, we have expanded several of our glucosylceramide synthase (GCS) studies to explore potentially new roles within retinal cell types. As inflammation is an underlying event for diabetic retinopathy, we assessed the contribution of glycosphingolipids within a microglia cell line, BV-2. The specific objective was to assess a role for glycosphingolipids on NFκB signaling. Initially, we utilized a GCS inhibitor, AMP-Deoxynojirimycin (AMP-DNM), to determine the effect on lipopolysaccharide (LPS)-induced NFκB activation. AMP-DNM demonstrated a significant reduction in NFκB activity as well as a reduction in glycosphingolipid content. We also successfully utilized two other inhibitors of GCS, NB-DGJ and PPMP. NB-DGJ is structurally related to AMP-DNM, whereas PPMP is a structurally distinct inhibitor. To attempt to confirm this, we utilized an siRNA approach to diminish GCS expression. Despite sufficient knockdown of GCS expression, we were not able to observe the same result we observed with AMP-DNM. We are continuing to investigate the dichotomy between pharmacological and molecular approaches with this cell line.

With the use of PA CURE funds, we continued to evaluate other lipids that regulate insulin signaling within lipid microdomains. We demonstrated previously that pro-survival insulin receptor, PI3K-Akt, and p70 S6K signaling is diminished in models of diabetic retinopathy. As mammalian target of rapamycin (mTOR), an upstream activator of p70 S6Kinase is, in part, regulated by lipid-derived second messengers, such as phosphatidic acid (PA), we sought to determine if diminished mTOR/p70 S6Kinase signaling in diabetic retinas may reflect diminished PA levels. Alterations in PA mass from retinas of control and streptozotocin-induced diabetic rats were determined by mass spectrometry. The biochemical and biophysical mechanisms underlying the actions of PA on insulin-activated mTOR/p70 S6Kinase signaling were determined using R28 retinal neuronal cells. We demonstrated a significant decrease in PA in R28 retinal neuronal cells exposed to hyperglycemia as well as in streptozotocin-induced diabetic rat retinas. Exogenous PA augmented insulin-induced protection from interleukin-1β-induced apoptosis. Moreover, exogenous PA and insulin cooperatively activated mTOR survival pathways in R28 neuronal cultures. Exogenous PA colocalized with activated mTOR/p70 S6kinase signaling elements within lipid microdomains. The biochemical consequences of this biophysical mechanism is reflected by differential phosphorylation of tuberin at threonine 1462 and serine 1798, respectively, by PA and insulin, which reduce this suppressor of mTOR/S6Kinase signaling within lipid microdomains. These results identify PA-enriched microdomains as a putative lipid-based signaling element responsible for mTOR-dependent retinal neuronal survival. Moreover, diabetic retinal neuronal apoptosis may reflect diminished PA mass. Elevating PA concentrations and restoring mTOR signaling may be an effective therapeutic modality to reduce neuronal cell death in diabetic retinopathy. These data are described in detail in the recent publication: Fox et al Diabetes Diminishes Phosphatidic Acid in the Retina: A Putative Mediator for Reduced mTOR Signaling and Increased Neuronal Cell Death. IOVS 67. PMCID: PMC3478036. Taken together, PA CURE funds have been used to identify two distinct lipid-mediated biophysical mechanisms (diminished PA and increased glucosyl-ceramide content) by which insulin receptor activity is down-regulated in diabetic retinas.

Specific Aim Two: Determine the consequence of glucosylceramide synthase inhibition on the metabolic milieu. Our published studies identify glycosylceramide accumulation within lipid

rafts as a therapeutic target for diabetic retinopathy. These in vitro data support the hypothesis that inhibitors of glucosylceramide synthase may be effective in vivo therapeutic modalities. We will administer drugs via a systemic approach and directly assess clinical endpoints in a mouse model of diabetes. Taken together, these studies will provide novel insights into dysfunctional lipid metabolism in diabetes and may identify glycosylceramide accumulation as a therapeutic target for diabetic retinopathy.

We completed testing of the therapeutic efficacy of glucosylceramide synthase inhibition on diabetic retinopathy. Since we have shown glucosylceramide is upregulated in two distinct models of type 1 diabetes, the streptozocotin-induced diabetic rat model and the Ins2Akita, we sought to determine if inhibition of glucosylceramide synthase (GCS) would be efficacious for diabetic retinopathy. Initial studies using control animals revealed that the GCS inhibitor did not have any overt toxicology to the retina upon a dosing regimen of subconjunctival injection for 3 straight days. This lack of toxicology was determined by a DNA fragmentation ELISA-based assay (Roche Cell Death Detection kit) to assess drug-induced cell death. We next assessed the efficacy of this drug in a model of diabetes. Using 3 month STZ-induced diabetic rats, we again followed the same dosing regimen to inhibit GCS. Here, we observed a significant increase in DNA fragmentation in diabetic animals compared to controls. We however did not observe any alterations in DNA fragmentation with the GCS inhibitor. We are still in the process of determining if the inhibitor successfully decreased glucosylceramide levels in the diabetic retina and/or successfully reached the retina by utilizing mass spectrometry. We are also in the process of assessing several known biomarkers to determine if the inhibitor has any effect on their expression. As GCS inhibition has shown to be effective in type 2 models of diabetes, we are moving forward with a systemic study to determine the effect in type 1 models and peripheral complications. Importantly, we have recently obtained the tools and knowledge needed to further retina research by utilizing electroretinograms (ERGs) and have recently purchased a UTAS Visual Diagnostic System. The ERG will allow us to noninvasively assess retinal function and thus allow us to get a better understanding of the effects of therapeutics on the retina.

We have also begun studies to obtain a better understanding of GCS in diabetes and normal retinal physiology. These studies take advantage of a LoxP-flanked GCS gene, which will allow us to selectively eliminate GCS expression in target tissues. Our initial goals are to selectively knockout GCS expression in endothelial cells (Cre recombinase under the control of a Cdh5 promoter) to assess the influence on vascular permeability and/or leukocyte adhesion within the retinal vasculature. Similarly, retinal neuron-specific knockout (Cre recombinase under control of Nes promoter) will also be utilized. Glial cell knockouts are also a possibility, but are beyond our present scope. These approaches will allow for more targeted hypothesis testing of the roles of GCS than a systemic pharmacological inhibition approach. Presently, we are still establishing our mouse colony and are seeking funding to address these roles of GCS.

We have also quantified sphingolipid biosynthesis in these diseased retina as well as the mechanisms responsible for their altered content. Increased de novo sphingolipid synthesis has been implicated in many inflammatory responses and to contribute to disease pathogenesis. We have begun studies to examine this biosynthetic pathway and a potential contribution to elevated glycosphingolipid flux. Our mass spectrometry-based studies have revealed that ceramides are predominantly enriched with C16 and C18 fatty acids. In analysis of the ceramide synthases that

regulate this pathway by qRT-PCR demonstrated that CerS4 is the predominant isoform of the retina. CerS1, CerS2, CerS5, CerS6 are also expressed, but at much lower levels. CerS3 was undetectable. Glycosphingolipids are also enriched with C16 and C18 fatty acids, but at a lesser percentage with an increase in longer chain and unsaturated fatty acids. Further studies will examine if the retina preferentially metabolizes longer chain and unsaturated sphingolipids to glycosphingolipids and determine the role of CerS4 in retinal sphingolipid metabolism. These studies will allow us to further understand the pathways that regulate alterations in glycosphingolipids in diabetic retinopathy.

One of the major outcomes of completion of this AIM has been the ability to define the diabetic milieu for diabetic retinopathy. These data were published in: Fox TE, Bewley MC, Unrath KA, Pedersen MM, Anderson RE, Jung DY, Jefferson LS, Kim JK, Bronson SK, Flanagan JM, Kester M. Circulating sphingolipid biomarkers in models of type 1 diabetes. J Lipid Res. 2011 Mar;52(3):509-17. In this manuscript, we further defined the sphingolipid milieu for diabetes by examining the sphingolipid content of the plasma, as well as the liver and heart. Briefly, we demonstrate two overarching events. First, we demonstrate that circulating promitogenic sphingosine-1-phosphate, a ceramide-metabolite, is elevated in two distinct models of type 1 diabetes. Secondly, we demonstrate that an omega-9 fatty acid, nervonic acid, is diminished within the sphingolipid class of lipids. Plasma ceramides were elevated only in the STZ-induced diabetic rat, but not the Ins2Akita mouse. Despite this difference, further examination of the fatty acid profiles of both models reveals a significant decrease in the omega-9, 24:1 fatty acid (nervonic acid) esterified to ceramide. This specific fatty acid decrease was also observed in the sphingomyelin and cerebroside classes of sphingolipids. The difference of the ceramide alteration between the STZ-diabetic rats and Ins2Akita diabetic mice may be due to a higher basal level of insulin in the mice compared to the rats. It should also be noted that the Ins2Akita mice do not become hyperlipidemic like the STZ-diabetic rats. Further studies are underway to delineate what reductions of nervonic acid may mean for diabetes and diabetic complications such as retinopathy.

As a high-fat/poor diet can lead to obesity and insulin resistance, we next determined if such a diet had an effect on nervonic acid containing sphingolipids in controls. We also assessed the effect of the high fat diet in the Ins2Akita diabetic mice to determine how these animals respond to the high fat diet-induced stress. After 10 weeks on the high fat diet, both controls and diabetic mice showed significantly elevated sphingomyelins, cerebrosides and ceramides of almost all fatty acid species with one exception. The exception was again, a decrease in nervonic acid. The decrease in diabetes for the 24:1 containing sphingomyelin and cerebroside classes was exacerbated with the high fat diet.

As sphingolipids can be found packaged onto lipoprotein complexes in the plasma, this would potentially implicate the liver as a contributory factor in altered sphingolipids in circulation. From the Ins2Akita diabetic liver, we observed a similar decrease in 24:1. Similarly, as with the diet-induced changes in the plasma, a high fat diet also induced significant reductions in 24:1 in the liver. Again, while we observe this diminished nervonic acid containing sphingolipids, as observed in the plasma, the high fat diet led to large increases in several types of sphingolipids. Note that we did not observe changes in liver So1P. This may reflect a source of elevated So1P other than the liver.

To further understand this diminished fatty acid content of the plasma, we examined the fatty acid profile of the total lipids present in the plasma of the STZ-induced diabetic rat. By GC-FID analysis of total fatty acids, we observed diminished 24:1. Interestingly, while the percentage of 24:1 in free or esterfied form is relatively low compared to other fatty acids in the total lipid pool, the percentage of 24:1 present on sphingolipid classes is considerably higher. This suggests that nervonic acid is selectively esterfied to sphingolipids rather than glycerol-containing lipids.

Though our focus is the retina, we do remove other tissues from our animal studies to maximize the data that we can obtain. Specifically, we also examined control and diabetic kidney samples. The “gestalt” of these data is that there is an upregulation of several phosphatidylcholine-, phosphatidylethanolamine-, and triglyceride-species containing saturated palmitic and stearic acids and polyunsaturated docosahexanoic acid (DHA) in diabetics. The increase in the polyunsaturated DHA is intriguing as the retina behaves in the opposite manner, with diabetes reducing DHA-containing lipids. Consistent with our recently published paper on reduced nervonic acid-containing sphingolipids in the plasma, heart and liver of a type 1 diabetic model, we also show that nervonic acid containing sphingomyelin is diminished in the kidney. Interestingly, we also saw an increase in a sphingomyelin that contains a d18:2 backbone. This subtype of sphingolipids is poorly explored in the field and demonstrates the importance of "omic-" based approaches to shed light on metabolites that are typically overlooked. We have also started collecting samples to be able to expand our studies once we obtain sufficient biological replicates based on patient parameters. These studies will be expanded to encompass a broader spectrum of lipids to obtain a better understanding of diabetes-induced alterations to lipid metabolism.

We have also employed a Metabolomics approach to assess diabetic complications. In our efforts to delineate the underlying biochemical changes and identify novel therapeutic targets, we have continued to utilize combinatorial lipidomic and metabolomic approaches to quantify diabetes-induced changes. Unbiased/untargeted retina profiling has revealed several alterations of metabolites. The alterations that contribute the most to the differences between control and diabetic retinas are diabetes-induced elevation of glycerophosphocholine (GPC, a product of phosphatidylcholine breakdown), the branched-chain amino acids leucine and isoleucine, and the polyols, sorbitol and fructose. Additional metabolites that are altered include pantothenate, sialic acid, glutamine and glutathione. These metabolites are involved in lipid metabolism, glycosylation of lipids and prevention of oxidative damage, respectively. The sialic acid pathway is being examined further for our glycosphingolipid studies. The product of GCS can be further metabolized, which can include the addition of sialic acid to form gangliosides.

Taken together, these studies demonstrate that a combinatorial metabolomic/lipidomic mass spectrometry approach can be used to define alterations in diabetic tissues, including retina, as well as to define and validate metabolic targets for therapeutic intervention. Of particular importance, is the novel finding that diminished nervonic acid levels in diabetic tissues and plasma could indicate that a preventative, nutritional regimen to replenish omega-9 fatty acids in diabetic patients could be a low-cost alternative to traditional pharmaceutical approaches.

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. Circulating sphingolipid biomarkers in models of type 1 diabetes.	Fox TE, Bewley MC, Unrath KA, Pedersen MM, Anderson RE, Jung DY, Jefferson LS, Kim JK, Bronson SK, Flanagan JM, Kester M.	J Lipid Res; 52(3):509-17. PMCID: PMC3035687	August 2010	☐Submitted ☐Accepted XPublished
2. Diabetes Diminishes Phosphatidic Acid in the Retina: A Putative Mediator for Reduced mTOR Signaling and Increased Neuronal Cell Death.	Fox TE, Young MM, Pedersen MM, Han X, Gardner TW, Kester M	Investigative Ophthalmol & Visual Sci.;53(11):7257-67 PMCID: PMC3478036	March 2011	☐Submitted ☐Accepted XPublished

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

Yes_____ No____x_____

If yes, please describe your plans:

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.

Based upon PA CURE funding, we have identified several lipid-based markers of diabetic retinopathy that have the potential to serve as "druggable" targets.

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.

We have identified omega 9 fatty acids (nervonic acid), nutritional supplementation as a putative preventative treatment of diabetic retinopathy.

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__x_____ No_____

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: Method and system for altering dysfunctional lipid flux in diabetic complications

b. Name of Inventor(s): M. Kester and T. Fox

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

   Diabetic complications, including diabetic retinopathy, are often associated with dysfunctional lipid metabolism. We have recently demonstrated that animal models of diabetes have increased glycosphingolipid flux as well as accumulation of diglycerides. These changes in lipid profiles are associated with diabetic complications. This invention disclosure describes several modalities to restore lipid homeostasis and minimize diabetic complications.

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 x_____ No____

Provisional patent filed before start of TSF PA CURE grant

If yes, indicate date patent was filed: 06/2005

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 x__
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 x__

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 x__

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___x_____ No_____

If yes, please describe your plans:

Continuing discussion with pharmaceutical companies.

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.*

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Kester, Mark	G.Thomas Passananti Professor of Pharmacology
eRA COMMONS USER NAME (credential, e.g., agency login) Mxk38@psu.edu	

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
State University of New York at Stonybrook, NY	B.S.	05/1976	Biology, Economics
State University of New York at Buffalo, NY	Ph.D.	05/1982	Cell Biology

A. Personal Statement

Mark Kester is the G. Thomas Passananti Professor of Pharmacology at Penn State Hershey College of Medicine. Before accepting this prestigious endowed professorship, he was a Distinguished Professor of Pharmacology as well as Interim Chair of Pharmacology. Dr. Kester is also Director of the Penn State Center for NanoMedicine and Materials, which reports to Penn State Hershey Cancer Institute, the Penn State Huck Institute of the Life Sciences and the Penn State Materials Research Institute. Dr. Kester's research interests include the design, characterization and validation of nanotechnologies for targeted drug delivery. His laboratory has evaluated nanoliposomes, nanodendrimers and nanocolloids as effective drug delivery vehicles for pharmacological and molecular agents. Recent work focuses on nontoxic nanoscale systemic delivery systems for hydrophobic pro-apoptotic lipids as well as siRNAs that target mutated tumorigenic proteins. Dr. Kester has consulted with, or founded, several companies that have the license to his nano"Solutions". In addition, Dr.Kester is a co-author of *Integrated Pharmacology*, published by Elsevier, Ltd., which was just recognized as a "highly commended textbook" by the British Medical Society.

B. Positions and Honors

Positions

1982-1984 Research Associate, The University of Illinois at Chicago, Chicago, IL; Michael Barany, M.D., Ph.D., Professor of Biological Chemistry

1984-1986 Research Instructor, The University of Texas Health Science Center at San Antonio, San Antonio, TX; Donald Hanahan, Ph.D., Professor of Biochemistry

1986-1995 Assistant Professor, Medicine/Physiology, Case Western Reserve University, Cleveland, OH

1995-1997 Associate Professor, Medicine/Physiology, Case Western Reserve University, Cleveland, OH

1997-2001 Associate Professor, Dept. Pharmacology, Penn State Univ. College of Medicine, Hershey, PA

2001-2006 Professor, Department of Pharmacology, Penn State Univ. College of Medicine, Hershey, PA

2002-2003 Interim Chair, Dept. Pharmacology, Penn State University College of Medicine, Hershey, PA

2005-pres. Professor, Department of Cellular and Molecular Physiology, Penn State University College of Medicine, Hershey, PA

2006-2007 Distinguished Professor of Pharmacology, Penn State University College of Medicine, Hershey, PA

2007-pres. G.Thomas Passananti Professor of Pharmacology, Penn State University College of Medicine
2010-pres. Director, Penn State Center for Nanomedicine and Materials
2011-pres. Co-Leader, Experimental Therapeutics, Penn State Hershey Cancer Institute, Hershey, PA
2012-pres. Joint appointment, Professor, Material Science and Engineering, The Pennsylvania State University

Honors

1972-1976 Regents Scholarship – SUNY Stonybrook
1976 Economics Honor Society, SUNY Stonybrook
1978 Graduate Research Access Development Grant – SUNY Buffalo
1981 University Student Teaching Award – SUNY Buffalo
1982-1984 Muscular Dystrophy Association Post-doctoral Fellowship
1984 Midwest Medical Research Forum, Award Winner, University of Illinois at Chicago
1985-1986 American Heart Association, Texas Affiliate, A.N. Rocco Fellowship
1988-1989 Kidney Foundation of Ohio, John Lowey Award
1990-1995 National Institutes of Health, FIRST Award
1993 American Society of Nephrology, Travel Award
1994 Kidney Foundation of Ohio, Medical Advisory Board
1996 Award of Tenure, The Pennsylvania State University
2000 National Institutes of Health Study Section, Site Visit, PO2 DJ 958349-07, Dec. 2000
2000-2003 National Institutes of Health Study Section, Physiological Chemistry, Ad Hoc Member
2001 National Institutes of Health Grant Review, Ad hoc member, COBRE Awards
2001 Award of Teaching Excellence – Presented by Class of 2004, Penn State College of Medicine
2002-pres. Editorial Board, Archives of Biochemistry and Biophysics
2002 NIH National Cancer Institute, Subcommittee D, Reporter
2004-2006 Graduate Student Association Award of Teaching Excellence
2004-2008 Graduate Student Teaching Award, Penn State University
2003 Guest Editor, Archives of Biochem. & Biophy. (The Next Generation of Sphingolipid Strategies)
2004 NIH Study Section Site Visit, P01 CA106494-01
2005-pres. NIH Study Section, Biochemistry and Biophysics of Membranes, Ad Hoc Member
2006 Cancer Nanobiology Think Tank, CCR Nanobiology Program, NCI Frederick, NIH
2007 Mini Symposium Organizer, Charleston Ceramide Conference, Monterey, CA
2007 NIH Study Section, NCI subcommittee F (T32 and K99/R00)
2007 NIH National Cancer Institute, NCI-F Training Review Committee, Ad hoc member
2008 Keynote Speaker, International Conference on Nanotechnology, Abu Dhabi, United Arab Emirates
2009 Invited Speaker, Gastro 2009, World Congress of Gastroenterology, London, UK
2009 National Institutes of Health Study Section, RC1 applications (ZRG1 OTC K(58)R), Ad hoc Member
2003-pres. International Advisory Council, International Ceramide Conference
2010 NCI Cancer Nanotechnology Training (R25) and Career Development Award (K99/R00)
2010 RC1 Applications Special Emphasis Panel ZCA1 RTRB-2 (M1)
2010 Invited Speaker, Third Annual Unither Nanomedical and Telemedical Technology Conference, Quebec, Canada
2010 National Institutes of Health Study Section Special Emphasis Panel/Scientific Review Group 2010/08 ZRG1 CB-B (55) R

2010 Nelson W. Taylor Lecture in Materials, Penn State
2011 Plenary lecture, International Sphingolipid Meeting, Villars, Switzerland
2011 NIDDK Board of Scientific Counselors
2011 Outstanding Achievement in Innovation Award, Penn State University, Inaugural Award Winner
2011 National Institutes of Health Study Section, Special Emphasis Panel (ZRG1 BDCN-W (03)
2011 NIDDK Editorial Review Board, Type 2 Diabetes Impact Award (ZDK1 GRB J 01)

C. Selected peer-reviewed publications (in chronological order).

1. Bourbon NA, Yun J, Berkey D, Wang Y, Kester M. Inhibitory actions of ceramide upon PKC-epsilon/ERK interactions. Am J Physiol Cell Physiol 2001; 280(6):C1403-11.
2. Edwards L, Fishman D, Horowitz P, Bourbon N, Kester M, Ernsberger P. The I1-imidazoline receptor in PC12 pheochromocytoma cells activates protein kinases C, extracellular signal-regulated kinase (ERK) and c-jun N-terminal kinase (JNK). J Neurochem 2001; 79(5):931-40.
3. Sandirasegarane L, Kester M. Enhanced stimulation of Akt-3/protein kinase B-gamma in human aortic smooth muscle cells. Biochem Biophys Res Commun 2001; 283(1):158-63.
4. Bourbon NA, Sandirasegarane L, Kester M. Ceramide-induced inhibition of Akt is mediated through protein kinase Czeta: implications for growth arrest. J Biol Chem 2002; 277(5):3286-92.
5. Yun JK, Kester M. Regulatory role of sphingomyelin metabolites in hypoxia-induced vascular smooth muscle cell proliferation. Arch Biochem Biophys 2002; 408(1):78-86.
6. Kester M, Kolesnick R. Sphingolipids as therapeutics. Pharmacol Res 2003; 47(5):365-71.
7. Reiter CE, Sandirasegarane L, Wolpert EB, Klinger M, Simpson IA, Barber AJ, Antonetti DA, Kester M, Gardner TW. Characterization of insulin signaling in rat retina in vivo and ex vivo. Am J Physiol Endocrinol Metab 2003; 285(4):E763-74.
8. Stover T, Kester M. Liposomal delivery enhances short-chain ceramide-induced apoptosis of breast cancer cells. J Pharmacol Exp Ther 2003; 307(2):468-75.
9. Stahl JM, Sharma A, Cheung M, Zimmerman M, Cheng JQ, Bosenberg MW, Kester M, Sandirasegarane L, Robertson GP. Deregulated Akt3 activity promotes development of malignant melanoma. Cancer Res 2004; 64(19):7002-10.
10. Stover TC, Sharma A, Robertson GP, Kester M. Systemic delivery of liposomal short-chain ceramide limits solid tumor growth in murine models of breast adenocarcinoma. Clin Cancer Res 2005; 11(9):3465-74.
11. Antonetti DA, Barber AJ, Bronson SK, Freeman WM, Gardner TW, Jefferson LS, Kester M, Kimball SR, Krady JK, LaNoue KF, Norbury CC, Quinn PG, Sandirasegarane L, Simpson IA. Diabetic retinopathy: seeing beyond glucose-induced microvascular disease. Diabetes 2006; 55(9):2401-11.
12. Basile M, Lin R, Kabbani N, Karpa K, Kilimann M, Simpson I, Kester M. Paralemmin interacts with D3 dopamine receptors: implications for membrane localization and cAMP signaling. Arch Biochem Biophys 2006; 446(1):60-8.
13. Fox TE, Finnegan CM, Blumenthal R, Kester M. The clinical potential of sphingolipid-based therapeutics. Cell Mol Life Sci 2006; 63(9):1017-23.
14. Fox TE, Han X, Kelly S, Merrill AH, 2nd, Martin RE, Anderson RE, Gardner TW, Kester M. Diabetes alters sphingolipid metabolism in the retina: a potential mechanism of cell death in diabetic retinopathy. Diabetes 2006; 55(12):3573-80. PMCID: PMC2141542
15. Fox TE, Houck KL, O'Neill SM, Nagarajan M, Stover TC, Pomianowski PT, Unal O, Yun JK, Naides SJ, Kester M. Ceramide recruits and activates protein kinase C zeta (PKC zeta) within structured membrane microdomains. J Biol Chem 2007; 282(17):12450-7.
16. Abcouwer SF, Shanmugam S, Gomez PF, Shushanov S, Barber AJ, Lanoue KF, Quinn PG, Kester M, Gardner TW. Effect of IL-1beta on survival and energy metabolism of R28 and RGC-5

retinal neurons. Invest Ophthalmol Vis Sci 2008; 49(12):5581-92.
17. Altinoglu EI, Russin TJ, Kaiser JM, Barth BM, Eklund PC, Kester M, Adair JH. Near-infrared emitting fluorophore-doped calcium phosphate nanoparticles for in vivo imaging of human breast cancer. ACS Nano 2008; 2(10):2075-84.
18. Houck KL, Fox TE, Sandirasegarane L, Kester M. Ether-linked diglycerides inhibit vascular smooth muscle cell growth via decreased MAPK and PI3K/Akt signaling. Am J Physiol Heart Circ Physiol 2008; 295(4):H1657-68. PMCID: PMC2593499
19. Kester M, Heakal Y, Fox T, Sharma A, Robertson GP, Morgan TT, Altinoglu EI, Tabakovic A, Parette MR, Rouse SM, Ruiz-Velasco V, Adair JH. Calcium phosphate nanocomposite particles for in vitro imaging and encapsulated chemotherapeutic drug delivery to cancer cells. Nano Lett 2008; 8(12):4116-21.
20. Morgan TT, Muddana HS, Altinoglu EI, Rouse SM, Tabakovic A, Tabouillot T, Russin TJ, Shanmugavelandy SS, Butler PJ, Eklund PC, Yun JK, Kester M, Adair JH. Encapsulation of organic molecules in calcium phosphate nanocomposite particles for intracellular imaging and drug delivery. Nano Lett 2008; 8(12):4108-15.
21. O'Neill SM, Olympia DK, Fox TE, Brown JT, Stover TC, Houck KL, Wilson R, Waybill P, Kozak M, Levison SW, Weber N, Karavodin LM, Kester M. C(6)-Ceramide-Coated Catheters Promote Re-Endothelialization of Stretch-Injured Arteries. Vasc Dis Prev 2008; 5(3):200-210. PMCID: PMC2783594
22. Pearlman E, Johnson A, Adhikary G, Sun Y, Chinnery HR, Fox T, Kester M, McMenamin PG. Toll-like receptors at the ocular surface. Ocul Surf 2008; 6(3):108-16.
23. Stover TC, Kim YS, Lowe TL, Kester M. Thermoresponsive and biodegradable linear-dendritic nanoparticles for targeted and sustained release of a pro-apoptotic drug. Biomaterials 2008; 29(3):359-69.
24. Sun Y, Fox T, Adhikary G, Kester M, Pearlman E. Inhibition of corneal inflammation by liposomal delivery of short-chain, C-6 ceramide. J Leukoc Biol 2008; 83(6):1512-21.
25. Tran MA, Gowda R, Sharma A, Park EJ, Adair J, Kester M, Smith NB, Robertson GP. Targeting V600EB-Raf and Akt3 using nanoliposomal-small interfering RNA inhibits cutaneous melanocytic lesion development. Cancer Res 2008; 68(18):7638-49.
26. Tran MA, Smith CD, Kester M, Robertson GP. Combining nanoliposomal ceramide with sorafenib synergistically inhibits melanoma and breast cancer cell survival to decrease tumor development. Clin Cancer Res 2008; 14(11):3571-81.
27. Zolnik BS, Stern ST, Kaiser JM, Heakal Y, Clogston JD, Kester M, McNeil SE. Rapid distribution of liposomal short-chain ceramide in vitro and in vivo. Drug Metab Dispos 2008; 36(8):1709-15.
28. Ganapathi SB, Kester M, Elmslie KS. State-dependent block of HERG potassium channels by R-roscovitine: implications for cancer therapy. Am J Physiol Cell Physiol 2009; 296(4):C701-10. PMCID: PMC2670659
29. Heakal Y, Kester M. Nanoliposomal short-chain ceramide inhibits agonist-dependent translocation of neurotensin receptor 1 to structured membrane microdomains in breast cancer cells. Mol Cancer Res 2009; 7(5):724-34. PMCID: PMC2732571
30. Kline CL, Shanmugavelandy SS, Kester M, Irby RB. Delivery of PAR-4 plasmid in vivo via nanoliposomes sensitizes colon tumor cells subcutaneously implanted into nude mice to 5-FU. Cancer Biol Ther 2009; 8(19):1831-7.
31. Matters GL, Harms JF, McGovern CO, Jayakumar C, Crepin K, Smith ZP, Nelson MC, Stock H, Fenn CW, Kaiser J, Kester M, Smith JP. Growth of human pancreatic cancer is inhibited by down-regulation of gastrin gene expression. Pancreas 2009; 38(5):e151-61. PMCID: PMC2704379
32. Salli U, Fox TE, Carkaci-Salli N, Sharma A, Robertson GP, Kester M, Vrana KE. Propagation of undifferentiated human embryonic stem cells with nano-liposomal ceramide. Stem Cells Dev 2009; 18(1):55-65.
33. Adair JH, Parette MP, Altinoglu EI, Kester M. Nanoparticulate alternatives for drug delivery.

ACS Nano 2010; 4(9):4967-70.
34. Barth BM, Gustafson SJ, Young MM, Fox TE, Shanmugavelandy SS, Kaiser JM, Cabot MC, Kester M, Kuhn TB. Inhibition of NADPH oxidase by glucosylceramide confers chemoresistance. Cancer Biol Ther 2010; 10(11):1126-36. PMCID: PMC3047104
35. Barth BM, Sharma R, Altinoglu EI, Morgan TT, Shanmugavelandy SS, Kaiser JM, McGovern C, Matters GL, Smith JP, Kester M, Adair JH. Bioconjugation of calcium phosphosilicate composite nanoparticles for selective targeting of human breast and pancreatic cancers in vivo. ACS Nano 2010; 4(3):1279-87. PMCID: PMC2894697
36. Chapman JV, Gouaze-Andersson V, Messner MC, Flowers M, Karimi R, Kester M, Barth BM, Liu X, Liu YY, Giuliano AE, Cabot MC. Metabolism of short-chain ceramide by human cancer cells--implications for therapeutic approaches. Biochem Pharmacol 2010; 80(3):308-15. PMCID: PMC2883648
37. Fox TE, Kester M. Therapeutic strategies for diabetes and complications: a role for sphingolipids? Adv Exp Med Biol 2010; 688:206-16.
38. Ganapathi SB, Fox TE, Kester M, Elmslie KS. Ceramide modulates HERG potassium channel gating by translocation into lipid rafts. Am J Physiol Cell Physiol 2010; 299(1):C74-86. PMCID: PMC2904259
39. Liu X, Ryland L, Yang J, Liao A, Aliaga C, Watts R, Tan SF, Kaiser J, Shanmugavelandy SS, Rogers A, Loughran K, Petersen B, Yuen J, Meng F, Baab KT, Jarbadan NR, Broeg K, Zhang R, Liao J, Sayers TJ, Kester M, Loughran TP, Jr. Targeting of survivin by nanoliposomal ceramide induces complete remission in a rat model of NK-LGL leukemia. Blood 2010; 116(20):4192-201. PMCID: PMC2993625
40. Wijesinghe DS, Allegood JC, Gentile LB, Fox TE, Kester M, Chalfant CE. Use of high performance liquid chromatography-electrospray ionization-tandem mass spectrometry for the analysis of ceramide-1-phosphate levels. J Lipid Res 2010; 51(3):641-51. PMCID: PMC2817594
41. Winter JN, Fox TE, Kester M, Jefferson LS, Kimball SR. Phosphatidic acid mediates activation of mTORC1 through the ERK signaling pathway. Am J Physiol Cell Physiol 2010; 299(2):C335-44. PMCID: PMC2928642
42. Barth BM, E IA, Shanmugavelandy SS, Kaiser JM, Crespo-Gonzalez D, Divittore NA, McGovern C, Goff TM, Keasey NR, Adair JH, Loughran TP, Claxton DF, Kester M. Targeted Indocyanine-Green-Loaded Calcium Phosphosilicate Nanoparticles for In Vivo Photodynamic Therapy of Leukemia. ACS Nano 2011; 5(7):5325-5337.
43. Barth BM, Cabot MC, Kester M. Ceramide-based therapeutics for the treatment of cancer. Anticancer Agents Med Chem 2011; 11(9):911-9.
44. Fox TE, Bewley MC, Unrath KA, Pedersen MM, Anderson RE, Jung DY, Jefferson LS, Kim JK, Bronson SK, Flanagan JM, Kester M. Circulating sphingolipid biomarkers in models of type 1 diabetes. J Lipid Res 2011; 52(3):509-17. PMCID: PMC3035687
45. Fox TE, Young MM, Pedersen MM, Giambuzzi-Tussey S, Kester M, Gardner TW. Insulin signaling in retinal neurons is regulated within cholesterol-enriched membrane microdomains. Am J Physiol Endocrinol Metab 2011; 300(3):E600-9. PMCID: PMC3279305
46. Hankins JL, Fox TE, Barth BM, Unrath KA, Kester M. Exogenous ceramide-1-phosphate reduces lipopolysaccharide (LPS)-mediated cytokine expression. J Biol Chem. 2011;286(52):44357-66. PMCID: PMC3247944.
47. Heakal Y, Woll MP, Fox T, Seaton K, Levenson R, Kester M. Neurotensin receptor-1 inducible palmitoylation is required for efficient receptor-mediated mitogenic-signaling within structured membrane microdomains. Cancer Biol Ther 2011; 12(5):427-35. PMCID: PMC3219081
48. Heakal Y, Kester M, Savage S. Vemurafenib (PLX4032): An Orally Available Inhibitor of Mutated BRAF for the Treatment of Metastatic Melanoma. Ann Pharmacother 2011; 45(11):1399-405. PMID: 22028422
49. Jiang Y, DiVittore NA, Kaiser JM, Shanmugavelandy SS, Fritz JL, Heakal Y, Tagaram HR,

Cheng H, Cabot MC, Staveley-O'Carroll KF, Tran MA, Fox TE, Barth BM, Kester M. Combinatorial therapies improve the therapeutic efficacy of nanoliposomal ceramide for pancreatic cancer. Cancer Biol Ther 2011; 12(7):574-85. PMCID: PMC3218384

50. Liao A, Broeg K, Fox T, Tan SF, Watters R, Shah MV, Zhang LQ, Li Y, Ryland L, Yang J, Aliaga C, Dewey A, Rogers A, Loughran K, Hirsch L, Jarbadan NR, Baab KT, Liao J, Wang HG, Kester M, Desai D, Amin S, Loughran TP, Jr., Liu X. Therapeutic efficacy of FTY720 in a rat model of NK-cell leukemia. Blood 2011; 118(10):2793-800. PMCID: PMC3172796
51. O'Neill SM, Yun JK, Fox TE, Kester M. Transcriptional regulation of the human neutral ceramidase gene. Arch Biochem Biophys 2011; 511(1-2):21-30. PMID: 21531200
52. O'Neill SM, Houck KL, Yun JK, Fox TE, Kester M. AP-1 binding transcriptionally regulates human neutral ceramidase. Arch Biochem Biophys 2011; 511(1-2):31-9. PMID: 21530485
53. Ryland LK, Fox TE, Liu X, Loughran TP, Kester M. Dysregulation of sphingolipid metabolism in cancer. Cancer Biol Ther 2011; 11(2):138-49. PMID: 21209555
54. Tabakovic A, Kester M, Adair JH. Calcium phosphate-based composite nanoparticles in bioimaging and therapeutic delivery applications. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2012; 4(1):96-112.
55. Tagaram HR, Divittore NA, Barth BM, Kaiser JM, Avella D, Kimchi ET, Jiang Y, Isom HC, Kester M, Staveley-O'Carroll KF. Nanoliposomal ceramide prevents in vivo growth of hepatocellular carcinoma. Gut 2011; 60(5):695-701. Highlighted in Nature Reviews 8:4, 2011. PMID: 21193455
56. Takahashi Y, Meyerkord CL, Hori T, Runkle K, Fox TE, Kester M, Loughran TP, Wang HG. Bif-1 regulates Atg9 trafficking by mediating the fission of Golgi membranes during autophagy. Autophagy 2011; 7(1):61-73. PMCID: PMC3039731
57. Avella DM, Li G, Schell TD, Liu D, Zhang SS, Lou X, Berg A, Kimchi ET, Tagaram HR, Yang Q, Shereef S, Garcia LS, Kester M, Isom HC, Rountree CB, Staveley-O'Carroll KF. Regression of established hepatocellular carcinoma is induced by chemoimmunotherapy in an orthotopic murine model. Hepatology. 2012;55(1):141-52. PMCID: PMC3243781.
58. Barth BM, Gustafson SJ, Hankins JL, Kaiser JM, Haakenson JK, Kester M, Kuhn TB. Ceramide kinase regulates TNFalpha-stimulated NADPH oxidase activity and eicosanoid biosynthesis in neuroblastoma cells. Cell Signal. 2012;24(6):1126-33. PMCID: PMC3338860.
59. Fox TE, Young MM, Pedersen MM, Han X, Gardner TW, Kester M. Diabetes Diminishes Phosphatidic Acid in the Retina: A Putative Mediator for Reduced mTOR Signaling and Increased Neuronal Cell Death. Invest Ophthalmol Vis Sci. 2012;53(11):7257-67. PMCID: PMC3478036.
60. Kaiser JM, Imai H, Haakenson JK, Brucklacher RM, Fox TE, Shanmugavelandy SS, Unrath KA, Pedersen MM, Dai P, Freeman WM, Bronson SK, Gardner TW, Kester M. Nanoliposomal minocycline for ocular drug delivery. Nanomedicine. 2012. Mar 28. [Epub ahead of print] PMID: 22465498
61. Morad SA, Levin JC, Shanmugavelandy SS, Kester M, Fabrias G, Bedia C, Cabot MC. Ceramide-antiestrogen nanoliposomal combinations--novel impact of hormonal therapy in hormone-insensitive breast cancer. Mol Cancer Ther. 2012 11(11):2352-61. PMCID: PMC3495995.
62. Tabakovic A, Kester M, Adair JH. Calcium phosphate-based composite nanoparticles in bioimaging and therapeutic delivery applications. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2012;4(1):96-112.
63. Tacelosky DM, Creecy AE, Shanmugavelandy SS, Smith JP, Claxton DF, Adair JH, Kester M, Barth BM. Calcium phosphosilicate nanoparticles for imaging and photodynamic therapy of cancer. Discov Med. 2012;13(71):275-85. PMID: 22541615
64. Watters RJ, Kester M, Tran MA, Loughran TP, Jr., Liu X. Development and use of ceramide nanoliposomes in cancer. Methods Enzymol. 2012;508:89-108. PMID: 22449922
65. Watters RJ, Fox TE, Tan SF, Shanmugavelandy S, Choby JE, Broeg K, Liao J, Kester M, Cabot

MC, Loughran TP, Liu X. Targeting Glucosylceramide Synthase Synergizes with C(6)-Ceramide Nanoliposomes to Induce Apoptosis in NK Leukemia. Leuk Lymphoma. 2012 Nov 26. [Epub ahead of print]

66. Young MM, Takahashi Y, Khan O, Park S, Hori T, Yun J, Sharma AK, Amin S, Hu CD, Zhang J, Kester M, Wang HG. Autophagosomal membrane serves as platform for intracellular death-inducing signaling complex (iDISC)-mediated caspase-8 activation and apoptosis. J Biol Chem. 2012;287(15):12455-68. PMCID: PMC3320995.
67. Young MM, Kester M, Wang HG. Sphingolipids: Regulators of the Crosstalk between Apoptosis and Autophagy. J Lipid Res. 2012 Nov 13. [Epub ahead of print]

D. Research Support

Ongoing Research Support

5 R01 CA133525-05 (Loughran, Thomas P.) 3/1/2008-1/31/2013
NIH/NCI
"Targeted Therapeutics of LGL Leukemia Utilizing Ceramide Nanoliposomes"
The major goal of the project is to utilize nanotechnology to deliver hydrophobic therapeutics for treatment of leukemia.

5 R01 EY018336-03 (Kester, Mark) 8/1/2009-7/31/2013
NIH/NEI
"The Role of Glycosphingolipids in Diabetic Retinopathy"
The overall objective of the proposed project is to understand the molecular basis for vision impairment in diabetic retinopathy. The specific objective is to investigate the role of ceramide metabolism and glycosphingolipid-enriched lipid microdomains (rafts) in insulin signaling and cell survival in retinal neurons.

5 R01 CA098472-09 (Loughran, Thomas P.) 7/1/2011-4/30/2016
NIH/NCI
"Survival Mechanisms of Leukemic NK Cells"
The long-term goal of this project is to develop better therapeutics for NK-LGL leukemia which is an incurable illness.

Planning Grant (Kester/Staveley-O'Carroll) 11/1/2011-4/30/2013
The V Foundation for Cancer Research
"Novel Therapeutic Approaches to Gastrointestinal Malignancies"
The overall goal of the program project is to engineer non-cationic immuno-targeted nanoliposomes that can deliver anti-fibrotic, anti-viral or anti-mitogenic agents systemically.

SAP 4100059196 (Kester, Mark) 6/1/2012-5/31/2014
PA Department of Health
"Therapeutic Delivery of siRNA Using Calcium Phosphate NanoJackets for Improved Cancer Treatment"
The overall goal of the proposed project is to enable an investigational new drug (IND) application to the FDA for regulatory review.

SAP #4100059259 (Kester, Mark) 7/1/2012-6/30/2014
PA Department of Health
"Studies to Verify Dysfunctional Toll-Like Receptor Signaling in Diabetic Retinopathy"
The major objectives to be addressed with this proposal are to identify altered TLRs in the diabetic retina and to validate that specific TLRs contribute to diabetic retinopathy.

1 R01 CA165335-01A1 (Staveley-O'Carroll, Kevin F.) 7/19/2012-6/30/2017
NIH
“An Orthotopic Murine Model of HCC: Immunotolerance and Prevention”
The overall objective of this proposal is to elucidate the FDA-approved chemotherapeutic agent sunitinib enhances T cell-mediated immunotherapy of HCC.

Completed Research Support (previous 3 years)

(Kester) 07/08/09-06/30/11
PA Tobacco Settlement Fund (Research Startup)
“Glycosphingolipids and Diabetic Retinopathy”
A proposal to provide additional data for a recently review grant to the American Diabetes Assocation entitled, "The Role of Glycosphingolipids in Diabetic Retinopathy"

4-2007-231 (Gardner) 05/01/07-09/30/10
Juvenile Diabetes Foundation – Center Grant
JDRF Center for Mechanisms and Intervention of Diabetic Retinopathy at PSU
Project 4 (Kester – PI Project 4): Ocular Drug Delivery
The goal of this project is to design and optimize nanotechnology delivery systems for diabetic retinopathy.

#4100045897 (Kester) 01/01/09-12/31/10
PA Department of Health
The Role of Glycosphingolipids in Diabetic Retinopathy
The role and consequences of decreased caveolin content in models of diabetic retinopathy is investigated.

R01 GM077391-02 Supplement (Kester) 09/07/09-01/31/11
NIH/John Wayne Cancer Institute (Cabot)
“Ceramide, Membrane Glycolipids and Glycoprotein Expression”
Dr. Kester will collaborate on Dr. Cabot’s studies investigating the role of glucosylceramide synthase as a novel "drugable" target for multi-drug resistance in cancer. "drugable" target for multi-drug resistance in cancer. Dr. Kester will apply his nano "solutions" to selectively deliver pharmaceutical and molecular agents that target glucosylceramide synthase.

5 R01 HL076789-05 (Kester, Mark) 8/1/2006-5/31/2012
NIH/NHLBI
“PKC zeta as a Target for Ceramide”
The goals of this project are to identify ceramide-enriched lipid rafts as the site at which PKC zeta is phosphorylated and optimally activated by upstream kinases, as well as to characterize and validate ceramide-binding domains or clefts on PKC zeta. These studies will elucidate the biophysical and biochemical mechanisms by which inflammatory cytokine receptor-induced ceramide formation limits cellular proliferation in models of inflammatory vascular diseases.

7-09-BS-98, Yr. 3 (Kester, Mark) 7/1/2009-6/30/2012
American Diabetes Association
“Therapeutic modulation of glycosphingolipid metabolism in a model of Type 1 Diabetes”
The therapeutic role of pharmacologically manipulating glucosylceramide content is investigated in models of diabetic retinopathy.