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:** Magee-Womens Research Institute and Foundation

2. **Reporting Period (start and end date of grant award period):** 1/1/13-12/31/13

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Cheryl A Richards, MBA

4. **Grant Contact Person's Telephone Number:** 412-641-8932

5. **Grant SAP Number:** 4100062212

6. **Project Number and Title of Research Project:** Project 3 - Preeclampsia and post-partum vascular function.

7. **Start and End Date of Research Project:** 1/1/13-12/31/13

8. **Name of Principal Investigator for the Research Project:** Carl Hubel, 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:

   $ 197,216.71

   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
Hubel, Carl	Principal Investigator	57%	$61,079
Myerski, Ashley	Research Technician	32%	$6,420

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
Brands, Judith	Collaborator	1%
Gandley, Robin	Collaborator	1%
Jeyabalan, Arun	Collaborator	1%
Weissgerber, Tracey	Collaborator	1%
Schmiedeknecht, Madeline	Student	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
Acquity UPLC H-Class System	In the past twelve months the Waters Acquity UPLC H-Class System (Ultra Performance Liquid Chromotography) has been used to measure: vitamin C, uric acid, L-arginine, asymmetric dimethylarginine, symmetric dimethylarginine, caffeine, paraxanthine, theobromine and theophylline in biological samples as part of various research projects. These analyses benefited research projects for Drs. Hubel, Roberts, Powers, Gandley, Jeyabalan, Holguin (Asthma Institute, Division of Pulmonary, Allergy, and Critical Care Medicine) and Morris (University of Pittsburgh HIV Lung Research Center, Division of Pulmonary, Allergy, and Critical Care Medicine).	$71,740.08

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:
Glycocalyx Syndecan-1 in Trophoblast Lipid Transport	xNIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	02/2014	$171,657	$ Application is pending
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

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

Yes____x_____ No_____

If yes, please describe your plans:

1) Dr. Hubel submitted an NIH Small Research Grant (R03), entitled "Glycocalyx Syndecan-1 in Trophoblast Lipid Transport" (17 February 2014). This leveraged data from both the 2012 Health Research Project and the 2011 Health Research Project ("Glycocalyx Syndecan-1 and Preeclampsia Pathogenesis").

2) An R01 submission to NIH/NICHD is planned for June 2014. Dr. Hubel and Dr. Robin Gandley will be co-Principal Investigators.

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

The IRB approved patient enrollment for this Project is ongoing, now supported by the PI's University of Pittsburgh Schools of Health Sciences Bridge funding, active until June 30, 2014. The intent is to enroll at least two more women with a history of preeclampsia and one woman with a history of uncomplicated pregnancy. Dr. Hubel intends to submit the summated findings for peer-reviewed publication by July 2014.

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				
Female	1			
Unknown				
Total	**1**			

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

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

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

    Yes_____ No___x_____

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

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

    Yes___x_____ No_____

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

    Dr. Hubel and Dr. Gandley have established collaborations with Dr. Judith Brands, post-doctoral fellow, and her mentor, Dr. John J. Pacella, Assistant Professor of Medicine, Cardiac Catheterization Laboratories, University of Pittsburgh School of Medicine. Dr. Brands performed the measurements of the sublingual circulation in postpartum women and helped to analyze the data obtained. This collaboration will continue with the planned R01 submission.

16. **Collaboration, business and community involvement.**

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

    Yes_____ No_____x_____

    If yes, please describe the collaborations:

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

Yes_____ No____x_____

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

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

Yes_____ No_____x_____

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

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

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

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

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

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

print as boxes (☐) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Research Objectives and Specific Aims:
Compared to women who had normal (uncomplicated) pregnancies, women who experienced preeclampsia (PE) in a pregnancy are at heightened (2- to 8-fold) risk of cardiovascular disease (e.g., hypertension, stroke, ischemic heart disease) in later life. The reasons for this remain unclear. Vascular stiffness (pulse wave velocity; PWV) and endothelium-dependent responsiveness of the brachial artery to an increase in shear stress (flow-mediated vasodilation; FMD) are indirect (surrogate) measures of later life cardiovascular disease risk in both healthy and diseased populations. In the postpartum state, FMD may be diminished in women with prior PE compared to women with prior normal pregnancy. However, the available data were obtained using flawed approaches. We will test how these vascular function variables, measured using current guidelines, are related to the status of the protective matrix that coats blood vessels (glycocalyx; GCX) by also measuring (a) urinary podocalyxin (a measure of renal podocyte GCX dysfunction) and (b) GCX width/integrity in microvessels of the tongue. We will study 18 non-pregnant women with a history of PE compared to 18 non-pregnant women with a history of normal pregnancy, 6 to 48 months after first pregnancy.

Aim 1: To test the hypothesis that brachial artery FMD is reduced and brachial-radial artery PWV is increased (adverse changes) in women with prior PE, compared to women with prior normal pregnancy, and determine whether the shear stimulus and the time to peak FMD are altered among women with prior PE.

Aim 2: To compare groups regarding sublingual capillary and venular GCX structural integrity and sublingual capillary density by sidestream dark field imaging. Compare maternal urinary podocalyxin as an indicator of renal glycocalyx damage/turnover.

Aim 3: To determine the relationship between vascular dysfunction, glycocalyx variables and circulating atherogenic lipids potentially implicated in GCX damage.

Substantial progress has been made toward completion of the Aims.

Progress Report for Specific Aim 1

Specific Aim 1: To test the hypothesis that brachial artery FMD is reduced and brachial-radial artery PWV is increased (adverse changes) in women with prior PE, compared to women with prior normal pregnancy, and determine whether the shear stimulus and the time to peak FMD are altered among women with prior PE.

All of the research activities described in this section were originally planned in the research proposal. There were no significant deviations from the research proposal. All Methods and Results reported here were supported by the 2012 Formula Health Research Grant.

Aim 1: Study Participant (Patient) Enrollment and Baseline Evaluation Methods:
Women were enrolled into the study performed at the Clinical and Translational Research Center (CTRC) at Magee-Womens Hospital. Final assignment of each patient to a pregnancy outcome diagnostic group was done after a complete review of patient clinical and laboratory data by the Preeclampsia Program Project (PEPP) Executive Committee.

Patients were selected from PEPP by reviewing records to ensure that patients who consented to be contacted for follow-up studies met the specific criteria for this study. Patients in the longitudinal arm of the PEPP had already participated in a postpartum visit at 6 months. This Formula Fund Project thus entailed a second postpartum visit for women who chose to participate, and Dr. Hubel has separate IRB approval (PRO12070072) for this study. *Inclusion criteria*: participated in the PEPP study; age 18-40; 6 to 24 months postpartum; had an uncomplicated pregnancy or was diagnosed with preeclampsia during the previous pregnancy in the PEPP study. Of note, chronic hypertension or renal disease prior to the index pregnancy were exclusion criteria.

The PEPP study staff asked postpartum women whether they were interested in participating in a second follow-up study. We attempted, when possible, to match controls and cases according to postpartum interval BMI, age, and race. Study visits were conducted during the early follicular phase of the menstrual cycle (days 1-7), or inactive phase if the subject was taking oral contraceptives (week with no pills or sugar pills). Subjects reported to the CTRC following an overnight fast. Subjects were also asked to avoid caffeine, alcohol, recreational illicit drugs, and strenuous exercise for 8 hours prior to the scheduled study visit.

After reporting to the CTRC, each patient completed a brief questionnaire about risk factors that might affect their risk of cardiovascular disease (e.g., exercise and sleep habits, breast feeding history, family history of heart disease). Prior to the vascular tests, a urine pregnancy test was performed to rule out current pregnancy. Blood pressure, pulse, weight, height and percent body fat (the latter obtained by bioelectric impedance) were measured. Spot urine and venous blood samples were obtained. Measurements of endothelium-dependent responsiveness of the brachial and radial arteries to an increase in shear stress (flow-mediated vasodilation) were performed. Peripheral vascular stiffness (pulse wave velocity) measurements and pulse wave analyses (augmentation data) were obtained.

Aim 1 Vascular Assessment Methods:
Hemodynamic data: The radial artery pulse was palpated and the tonometer placed on the strongest pulse site. Waveforms were collected until at least three had an operator index above 80, confirming a good quality signal. The SphygmoCor system software derives the central aortic pressure waveform noninvasively from the radial pulse wave.

The ejection of blood from the ventricle into the aorta generates an aortic pressure pulse. There is often a systolic shoulder on the ascending limb pressure curve coinciding with peak flow, followed by a rise in pressure to the systolic peak. This second increase in pressure is described as the ‘augmentation pressure’ (AP) and is primarily due to the reflected components of the original pressure pulse generated by ventricular ejection. AP is the

pressure difference between the first peak/shoulder and the second peak/shoulder. The amount of augmentation increases as the arteries stiffen. Augmentation index (AI) is the AP divided by the central pulse pressure. AI and AP are best compared when these data are normalized to a fixed heart rate. Calculated variables thus included aortic systolic and diastolic blood pressure (Table 2), aortic pulse pressure, mean arterial pressure (Table 2), AI, AP, AI normalized to a fixed heart rate of 75 beats per minute, and AP normalized to a fixed heart rate of 75 beats per minute.

Pulse wave velocity: With subjects in supine position, ECG leads were placed and vascular segment lengths measured along the body surface (heart-to-carotid and heart-to-femoral). A tonometer (SphygmoCor, AtCor Medical, Itasca, IL) was used to obtain pressure waveforms at the vascular sites. Using the SphygmoCor machine, ECG and pressure waveform data were simultaneously recorded for 30 seconds (100 Hz sampling rate) at each of the three sites. These data (carotid-to-fermoral PWV, an index of vascular stiffness) were analyzed off-line by a custom-developed MATLAB program.

Flow-mediated vasodilation: Brachial artery FMD was assessed in accordance with the most current (2011) guidelines. Continuous images in the dominant arm were obtained using a 10 MHz probe operating in B-Mode (GE Vivid i system). Blood velocity was simultaneously recorded by Doppler ultrasound, with the same probe operating at 4 MHz and an insonation angle <60°. The occlusion cuff was positioned on the forearm. The probe was proximal to the cuff. Simultaneous diameter and blood flow velocity images were obtained for 1 minute of baseline, 5 minutes of occlusion (cuff inflation to 200 mmHg), and 5 minutes post-release. Images were recorded continuously, using a digital capture system for later analysis using automated software (Quipu). Calculated values included: FMD; the shear stimulus for FMD (shear stress area under the curve from cuff release to the time of peak diameter); and low flow-mediated constriction (L-FMC). L-FMC is a measurement of vessel constriction above the blood pressure cuff. L-FMC occurs in some patient populations during occlusion and is postulated to be a response to reduced shear.

Aim 1: Study Participant (Patient) Enrollment and Baseline Clinical Evaluation Results: During the funding period, 17 women were enrolled into the study performed at the Clinical and Translational Research Center (CTRC) at Magee-Womens Hospital. Final assignment of each patient to a pregnancy outcome diagnostic group was done by the Preeclampsia Program Project (PEPP) Executive Committee. One participant was deemed to have proteinuria without gestational hypertension, and one participant had gestational hypertension but did not develop proteinuria. Therefore, these two individuals did not meet criteria for either the preeclampsia or uncomplicated (control) pregnancy study groups. The current comparison study groups consist of n=9 postpartum women with a history of uncomplicated pregnancy and n=6 postpartum women with a history of preeclampsia.

The pregnancy clinical characteristics of women who enrolled in the study are shown in Table 1. Postpartum (CTRC visit) clinical characteristics of these same women are given in Table 2. All postpartum study participants were non-smokers and not lactating.

By definition, women with preeclampsia had elevated late gestational blood pressures compared to women with uncomplicated pregnancy. Infant birthweights were also significantly reduced in women with preeclampsia.

Table 1. Clinical Pregnancy Data, Uncomplicated Pregnancy and Preeclampsia Groups

	Uncomplicated Pregnancy (n=9)	Preeclampsia (n=6)	P value
Age (years)	23.2 ± 2.7	25.0 ± 4.7	NS
BMI pre-pregnancy (kg/m^2)	32.5 ± 5.2	35.0 ± 9.2	NS
Gestational weeks at delivery	39.8 ±1.1	35.7 ± 5.5	NS
Early gestational BP (<20wks)			
Systolic (mm Hg)	112 ± 8	111 ± 9	NS
Diastolic (mm Hg)	68 ± 9	70 ± 8	NS
Pre-delivery BP:			
Systolic (mm Hg)	118 ± 11	149 ± 5	<0.01
Diastolic (mm Hg)	68 ± 5	86 ±6	<0.01
Infant Birthweight (g)	3146 ± 944	2543 ± 1307	<0.01
Placenta weight (g)	1022 ± 1215	654 ±199	NS
Infant length (cm)	51.8 ± 1.9	46.0 ± 12.7	NS
Infant head circumference (cm)	34.6 ± 1.3	30.8 ± 5.4	NS
Multiparous at index pregnancy (%)	33% (3/9)	67% (4/6)	NS
Cigarette smokers (%)	0%	0%	NS
Race (% black)	100%	100%	NS
Baby gender (% female)	44% (4/9)	67% (4/6)	NS

Note: Continuous variables are given as mean (SD); NS, not significant; BMI, body mass index

At approximately 1 year after pregnancy, the group of postpartum women who had experienced preeclampsia had significantly higher systolic, diastolic, and mean arterial blood pressures, on average, compared to postpartum women with a history of uncomplicated pregnancy (Table 2). No other baseline group differences were observed.

Table 2. Clinical Characteristics, Non-pregnant Women 6-24 Months After Uncomplicated or Preeclamptic Pregnancy (described in Table 1)

	Prior uncomplicated pregnancy (n=9)	Prior preeclampsia (n=6)	P value
Age (years)	24.7 ± 2.6	26.8 ± 4.7	NS
Days post delivery	367 ± 151	471 ± 211	NS
Body mass index (kg/m^2)	34.7 ± 3.8	34.0 ± 10.0	NS
Body fat (%, by bioimpedance)	44.1 ± 4.4	40.4 ± 13.3	NS
Systolic BP (mm Hg)	92 ± 11	114 ± 8	<0.01
Diastolic BP (mm Hg)	65 ± 8	84 ± 12	<0.01
Aortic pulse pressure (mm Hg)	28 ± 6.1	30 ± 8	NS
Mean Arterial pressure (mm Hg)	76.9 ± 8.5	98.5 ± 10.5	<0.01
Resting heart rate (bmp)	68 ±9	71 ± 12	NS

Note: Continuous variables are given as mean (SD); NS, not significant; BMI, body mass index; Heart rate, beats per minute (bpm).

Aim 1: Vascular Assessment Results:

It was not possible to obtain vascular measurements on several of the study participants. In certain instances, we were not able to obtain acceptable measurement signals or were not able to collect data with acceptable standard deviations, primarily due to the high prevalence of obesity (interference from adipose tissue) in our recruitment population. The vascular data tables indicate the number of patients, by study group, corresponding to each measured variable.

Hemodynamic data: Both AI and AP are estimates of arterial stiffness. AP, normalized to a fixed heart rate of 75 beats per minute, was obtained from five women with previous preeclampsia (mean mm Hg ± SD: 5.0 ±3.1) and eight with previous uncomplicated pregnancies (2.3 ±2.7) (P=0.12). AI, normalized to a fixed heart rate of 75 beats per minute (mean % ± SD), was (16.4 ±8.2) and (6.8 ±10.5) (P=0.09) for these groups, respectively. Although not significant, the mean values are consistent with the hypothesis that arterial compliance is reduced (stiffness increased) in postpartum women who have experienced preeclampsia. As shown in Table 2, the women with prior preeclampsia also had higher mean systolic and diastolic blood pressures compared to the controls.

Pulse wave velocity: PWV from carotid to femoral arteries is the gold standard for assessment of arterial stiffness. This noninvasive technique is difficult to measure with tonometry when there is excessive adipose tissue over the femoral artery. We found it frequently impossible to palpate the femoral artery, and obtain a strong and reproducible waveform, in obese patients (body mass index > 30). Due to the high prevalence of obesity in our recruitment population, we were not able to collect PWV data with acceptable standard deviations from three prior preeclampsia patients. The other three prior preeclampsia patients yielded PWV data (mean m/s ± SD) of 5.46 ± 2.18. Values from six controls were 3.60 ± 1.72. While not significantly different between groups (P=0.20), values

are in the direction of increased stiffness in women with a history of preeclampsia. We have been targeting recruitment of women with BMI < 30 to ensure representation across the BMI spectrum and increase sample size.

Flow-mediated vasodilation: The material transfer agreement between University of Pittsburgh and the Mayo Clinic was completed in September 2013, allowing our collaborator, Tracey Weissgerber (now Assistant Professor at the Mayo Clinic), to analyze these data. Brachial and radial artery FMD data were successfully collected on all but one study participant. However, not all data were of analyzable quality. In most instances, waveforms were of insufficient quality, a problem primarily with women of BMI >30. Table 3 lists, by patient group, the overall current results and sample size for each measured variable.

Table 3: FMD-related data.

Brachial artery data	Prior uncomplicated pregnancy	Prior preeclampsia	P value
Brachial baseline diameter (mm)	3.1±0.12 (n=7)	3.2±0.57 (n=4)	NS
Brachial occlusion diameter (mm); during last minute of occlusion	3.1±0.12 (n=7)	3.2±0.57 (n=4)	NS
Brachial peak diameter (mm); post cuff release	3.3±0.08 (n=7)	3.4±0.58 (n=4)	NS
Time from cuff release to peak dilation, brachial artery (s)	49.3±20.0 (n=7)	40.7±27.3 (n=4)	NS
Shear rate area under the curve, brachial artery	20290±9898 (n=7)	18809±7542 (n=4)	NS
Brachial flow-mediated dilation (FMD) (%)	7.58±4.06 (n=7)	6.87±2.68 (n=4)	NS
Brachial low flow-mediated constriction (L-FMC) (%)	-0.16±1.2 (n=7)	0.89±0.29 (n=4)	NS
Radial Artery Data			
Radial baseline diameter (mm)	1.95±0.22 (n=5)	2.22±0.042 (n=3)	NS
Radial occlusion diameter (mm); during last minute of occlusion	1.93±0.27 (n=5)	2.17±0.042 (n=3)	NS
Radial peak diameter (mm); post cuff release	2.06±0.27 (n=5)	2.36±0.047 (n=3)	NS
Time from cuff release to peak dilation, radial artery (s)	38.4±19.6 (n=5)	47.0±18.3 (n=3)	NS
Shear rate area under the curve, radial artery	18388±6483 (n=5)	20016±10245 (n=3)	NS
Radial flow-mediated dilation (FMD) (%)	5.30±2.75 (n=5)	6.53±3.26 (n=3)	NS
Radial low flow-mediated constriction (L-FMC) (%)	-1.50±3.08 (n=5)	-2.27±0.12 (n=3)	NS

As an index of vascular health, brachial artery FMD provides a noninvasive bioassay for *in vivo* endothelial function. For example, FMD is significantly attenuated after intra-arterial administration of a nitric oxide synthase blocker (e.g., L-NMMA). Nitric oxide is an anti-atherosclerotic molecule, and decreased nitric oxide bioavailability is a hallmark of pro-atherogenic states. Poor FMD may predict future cardiovascular events. For the FMD tests, we used ultrasound to measure vessel diameter and blood velocity. The blood pressure cuff was inflated to induce lower arm ischemia for 5 minutes. Ischemic vessels below the cuff typically dilate. When the cuff is released, dilated vessels below the cuff cause an increase in blood flow to the lower arm. This increase in flow passes through the vessel *above the cuff*, causing shear stress. Healthy vessels dilate above the cuff in response to shear stress (increased % FMD). Shear stress = [blood viscosity x (blood velocity/vessel diameter)]. Shear rate = (velocity/diameter). The current data (Table 3) suggest that brachial and radial FMD do not differ between women with a history of preeclampsia and women with a history of uncomplicated pregnancy 1 year postpartum. However, we have not yet achieved the sample size (15/group) estimated as necessary to detect a significant difference.

Substantial discrepancies in FMD protocols exist across different laboratories, and there is also wide inter-subject variability in hyperemic shear stress (the stimulus for FMD). One can normalize the measured vasodilation response to the applied stimulus; in other words, dividing the peak FMD by the shear stress area under the curve (individual area until peak FMD). To date, however, no differences in shear rate area under the curve have been observed (Table 3).

The degree of radial artery vasoconstriction during reduced flow caused by wrist occlusion L-FMC was recently proposed as a vascular function test. Mean radial artery L-FMC in healthy subjects is 2 to 7%, and radial L-FMC may be diminished in hypertensive patients compared with healthy subjects. Radial artery L-FMC also occurs in healthy pregnant women, with mean values within the range reported for men and women. In contrast, brachial artery diameter did not change during forearm occlusion among non-pregnant women (Weissgerber TL et al. J Appl Physiol, 2010). Small, but statistically significant, decreases in brachial L-FMC were observed in pregnant women (J Appl Physiol, 2010). We *hypothesized that*, compared to women with a previous uncomplicated pregnancy, radial artery, but not brachial artery, L-FMC will be diminished among postpartum women with previous preeclampsia. Although, with limited sample size to date, this hypothesis does not appear to be supported (Table 3). The reason for the relatively low radial artery L-FMC (~2%) in the current study, compared to the literature, is unknown but might relate to the high prevalence of obesity in our study population.

Progress report for Specific Aim 2

Specific Aim 2: To compare groups regarding sublingual capillary and venular GCX structural integrity and sublingual capillary density by sidestream dark field imaging. Compare maternal urinary podocalyxin as an indicator of renal glycocalyx damage/turnover.

All of the research activities described in this section were originally planned in the research proposal. There were no deviations from the research proposal. All Methods and Results reported here were supported by the 2012 Formula Health Research Grant.

Aim 2 Methods:

After baseline clinical data, pulse wave velocity and augmentation data were obtained (Aim 1), the sublingual capillary density/glycocalyx measurements were completed (Aim 2). The glycocalyx lining the apical surface of the vascular endothelium is a major determinant of vascular endothelial function. The glycocalyx limits access of red blood cells (RBCs) to the endothelial cell membrane; the red cell penetrable region is known as the perfused boundary region (PBR) (Figure 1). Glycocalyx damage can result in an *increased* PBR, which can be measured noninvasively in the sublingual microvasculature. We tested the *hypothesis that the PBR is increased in non-pregnant women with a history of preeclampsia compared to non-pregnant women with a history of uncomplicated pregnancy (controls*).

Imaging of the sublingual microvasculature was performed using a sidestream-darkfield (SDF) MicroScan Video Microscope (MicroVision Medical, Wallingford, PA) connected to GlycoCheck acquisition and analysis software (GlycoCheck BV, Maastricht, The Netherlands). The SDF camera uses green light-emitting diodes (540nm) to detect the hemoglobin of passing RBCs. The software has been developed to calculate the dynamic lateral RBC movement into the glycocalyx of the microcirculation (expressed as PBR). The method of calculation is fully automated to ensure unbiased analysis of the glycocalyx.

Briefly, the camera was placed under the tongue for approximately 2 minutes. Pressure on the oral mucosa was limited as much as possible to avoid obstruction of normal flow. The software then automatically identified all measurable sublingual microvessels and defined small vascular segments every 10 μm along the length of these vessels. Subsequently, a sequence of 40 frames was recorded in time, containing on average 300 vascular segments. The observer moved the SDF imaging unit to a different location for another recording session of 40 frames, until a minimum total of 3,000 vascular segments were recorded. After these measurements, 21 line markers were placed at an interval of 0.5 μm around all vascular segments. For each vascular segment, 840 radial intensity profiles were tested (21 line markers x 40 frames) for the presence of RBCs and signal quality. The program only selected vessels that were well perfused. From these intensity profiles, the software gave an RBC width distribution of each individual vascular segment, from which the median RBC width (P50 RBCW; Figure 1) and the outer edge of the RBC-perfused lumen (Dperf; the outward-most location ever penetrated by RBCs) were determined (Figure 1). Because the PBR is present at both sides of the RBC column, it was calculated using the equation: PBR = [(Dperf−P50 RBCW)/2]. Next, the calculated PBR values, classified according to their corresponding RBC column width values (between 5 and 25 μm), were averaged to provide a

single average PBR value for each patient. As shown in Figure 1, glycocalyx damage is known to increase the PBR of the glycocalyx. The program also determined whether an RBC was present on every line marker that had been placed on all the detected microvessels. The number of line markers on which an RBC was detected is given as a percentage of the total number of placed line markers and as the *RBC filling percentage*. RBC filling is known to be reduced in poorly perfused microvessels.

Aim 2 Results:

Vessel segments were classified in 1-µm-wide P50 RBCW classes (x-axis) and median PBR values (y-axis) were determined for each 1-µm diameter class (Figure 2) before calculating the average PBR over the 5- to 25-µm P50 RBCW range. The number of vessel segment measurements per P50 width class was at least 100. A greater PBR signifies a diminished ability of the glycocalyx matrix to exclude red cells (i.e., a functionally thinner glycocalyx). We compared the PBR of sublingual microvessels in four of the non-pregnant women with prior preeclampsia and nine women with prior uncomplicated pregnancy 1 year postpartum. *The PBR, averaged across the major range of vessel sizes (5-25 microns)*, was significantly ($P< 0.01$) increased (greater red cell penetration; reduction in glycocalyx barrier capacity) in women with prior preeclampsia (n=4; average PBR µm ± SD: 2.7±0.07) compared to controls (n=9; 2.2±0.18). The magnitude of the increase in PBR (signifying poor functional glycocalyx barrier property) in postpartum women with prior preeclampsia is similar to that recently reported in end stage renal disease vs. healthy controls (Dane M.J.C. et al. Clin J Am Soc Nephrol, 2014 [EPub ahead of print] doi: 10.2215/CJN.08160813). This may reflect maternal constitutional disease and/or lasting adverse effects of preeclampsia on microvessels.

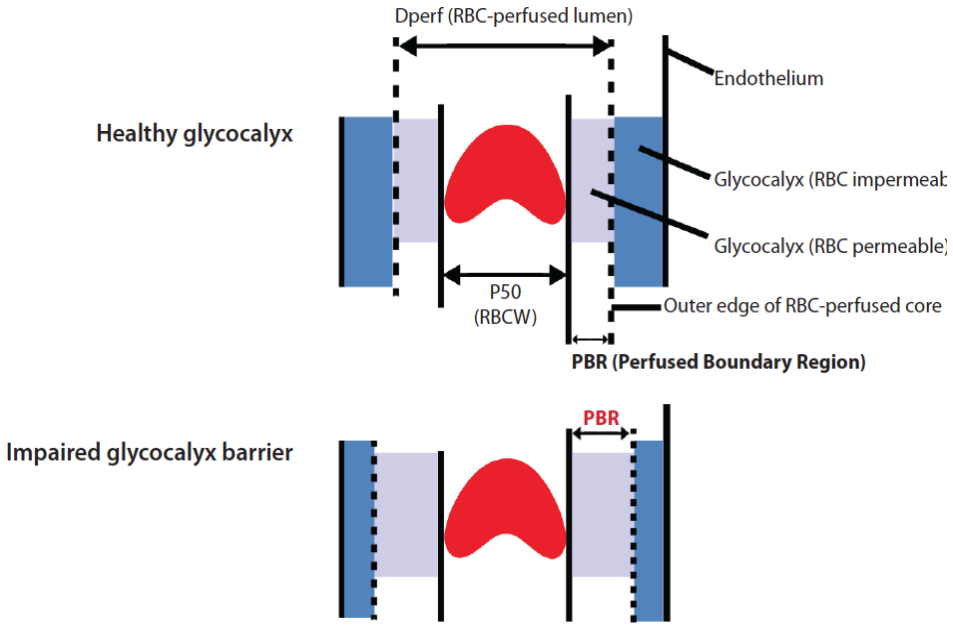

Figure 1: Portrayal of the perfused boundary region (PBR) in a healthy microvessel (top) and in diseased microvessel (bottom); from http://www.glycocheck.com/solutions.php

The mean ± SD PBR value (for each vessel size recorded) for patient groups is given in Figure 2. Significant differences were observed across the span of PBR widths at specific P50 (vessel width) values.

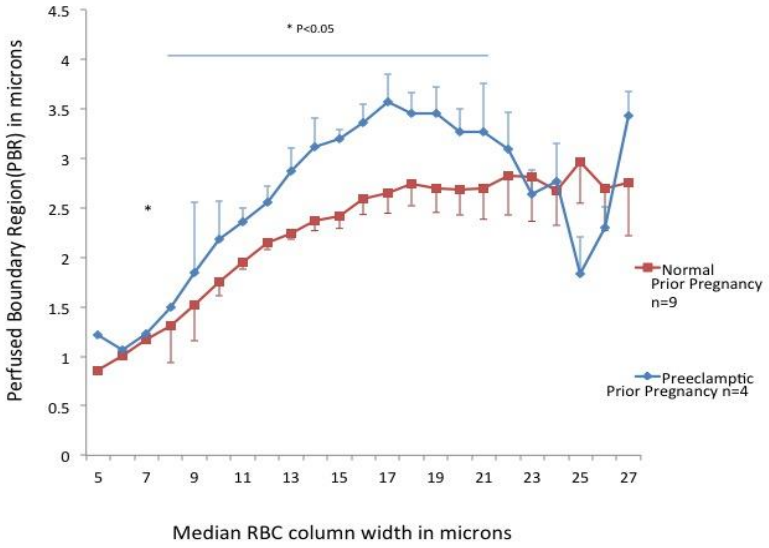

Figure 2: Sidestream darkfield analysis of the sublingual microvasculature of postpartum women with a history of normal pregnancy outcome or preeclampsia. Asterisks denote significant differences between preeclampsia (n=4) and control (n=9) postpartum groups (P<0.05) for a span of individual PBR widths at specific P50 (vessel width) values. PBR is the zone of the glycocalyx into which RBCs can penetrate. Larger penetration into a vessel of comparable RBC column width indicates a more permeable glycocalyx (indicative of a less healthy glycocalyx).

Red blood cell filling percentage. RBC filling is reduced in poorly perfused microvessels. Postpartum women with prior preeclampsia exhibited a significantly (P<0.03) lower RBC filling percentage (greater percentage of microvessel segments lacking red cells) (65.1± 4.3) compared to postpartum women with uncomplicated pregnancy outcome (72.7 ± 5.2).

To our knowledge we are the first to report this index of glycocalyx disruption accompanying poor microvessel perfusion, higher blood pressures, and increased plasma triglycerides (please see also Aim 3) 1 year after pregnancy in women with a history of preeclampsia.

Urine samples have been successfully collected from all postpartum women. Maternal urinary podocalyxin, an indicator of renal glycocalyx damage/turnover, will be measured in these samples in batch after enrollment has ended. This will eliminate inter-assay variability.

Progress report for Specific Aim 3

Specific Aim 3: To determine the relationship between vascular dysfunction, glycocalyx variables and circulating atherogenic lipids potentially implicated in GCX damage.

Aim 3 Methods:
Further recruitment and analysis of FMD data will allow us to determine the relationship between vascular dysfunction and glycocalyx variables. The Ultra Performance Liquid Chromatography system has been set up and been calibrated/validated and is in use by several Magee-Womens Research Institute Investigators. To minimize assay variability, the circulating bioactive lipids potentially implicated in GCX damage and urinary podocalyxin will be measured in batch. Maternal plasma total triglycerides, total cholesterol and total free (non-esterified) fatty acids were measured at 20 weeks of gestation and in samples obtained at the 1 year postpartum CTRC visit.

With the exception of mid-pregnancy lipid measurements, all of the research activities described in this section were originally planned in the research proposal. The lipid measurements at mid-pregnancy (20 weeks') were performed after we realized that the plasma samples were available. The purpose of this deviation from the original research proposal was to see if pregnancy lipids correlate with vascular function measures in the post-partum state.

Free fatty acids were quantitatively determined by an enzymatic, colorimetric method developed by Wako Pure Chemical Industries, Ltd. The coefficient of variation between runs was 6.5%. Cholesterol and triglycerides were measured in duplicate by colorimetric techniques, using commercial kits from Pointe Scientific (Canton, MI); the average coefficients of variation between runs ranged from 5.3 percent to 8.4 percent. Relationships of vascular and clinical variables were assessed by Pearson's correlation coefficient.

Mid-pregnancy maternal plasma samples were available (mean gestational age 20 weeks; range 15-23 weeks). This time period was at least 4 weeks before clinically evident preeclampsia. Mid-pregnancy plasma triglycerides, but not cholesterol or total free (non-esterified) fatty acid, concentrations were significantly higher in the group of women who subsequently developed preeclampsia (Table 4).

Table 4. Mid-gestational lipids, Uncomplicated Pregnancy and Preeclampsia Groups

	Uncomplicated Pregnancy (n=9)	Preeclampsia (n=5)	P value
Gestational age at venipuncture (mid-pregnancy blood sample) (weeks)	20.3 ± 1.3	19.0 ± 3.2	NS
Maternal cholesterol (mg/dL)	202 (175-220)	180 (164-263)	NS
Maternal triglyceride (mg/dL)	102 (76-109)	136 (108-171)	P<0.05

Maternal free fatty acids (mmol/L)	0.31 (0.15-0.41)	0.23 (0.17-0.33)	NS

Note: Continuous variables are given as mean (SD) or median (interquartile range; IQR); NS, not significant. Early gestational blood pressures are the average of gestational blood pressures measured before 20 weeks' gestation.

Cholesterol and triglyceride values were not significantly different between the groups 6-24 months after pregnancy (Table 5). We have purchased an additional kit for free fatty acid measurements.

Table 5. Plasma Lipid Data, Non-pregnant Women 6-24 Months After Uncomplicated or Preeclamptic Pregnancy

	Prior uncomplicated pregnancy (n=9)	Prior preeclampsia (n=6)	P value
Cholesterol (mg/dL)	105 (77-139)	132 (88-148)	NS
Triglyceride (mg/dL)	48 (37-62)	40 (28-64)	NS

Note: Continuous variables are given as mean (SD) or median (interquartile range; IQR); NS, not significant; BMI, body mass index; Heart rate, beats per minute (bpm).

Several potentially relevant relationships were observed between vascular dysfunction, blood lipids, and glycocalyx variables. RBC filling percentage (increased in well-perfused microvessels) correlated negatively (inversely) with the glycocalyx PBR averaged over the 5-25 micrometer range of microvessels (r=-60, P<0.04). RBC filling (in the postpartum state) correlated negatively with plasma triglycerides measured at 20 weeks of pregnancy (r=-0.69, P<0.01). Glycocalyx PBR averaged over the 5-9 micrometer range of microvessels (PBR 5-25) in the postpartum state and over the 10-19 micrometer range (PBR 10-19) in the postpartum state correlated positively with plasma triglycerides measured at 20 weeks of pregnancy (r=0.70, P<0.01 and r=0.58, P<0.04, respectively). This is of interest given that pregnancy may amplify or unmask atherogenic lipid profiles that may be less evident in young women in the postpartum state.

Milestones:

Milestone(s) for 1/1/2013-6/30/2013: We will complete ~50% of the recruitment and data acquisition and begin preliminary analyses for Aims 1 and 2.

During this first reporting period, we successfully enrolled and studied 11 patients. This represents less than the target of 50% (n=18) of the recruitment.

For reasons beyond our control, the material transfer agreement between the University of Pittsburgh and the Mayo Clinic (application initiated December, 2012) was not completed until September 2013. Our collaborator, Tracey Weissgerber (now Assistant Professor at the

Mayo Clinic) was able to analyze/interpret the flow-mediated vasodilation data accumulated prior to September 2013 (inclusive of 1/1/2013-6/30/2013) only after the material transfer agreement was completed.

Milestone(s) for 7/1/2013-12/31/13: We will complete >90% of targeted recruitment. We will complete statistical analyses and Aim 3, present data at a national meeting and submit the aggregate project results for publication in a peer-reviewed scientific journal(s).

During this second reporting period, we successfully enrolled and studied 6 patients. This represents less than the target of 50% (n=18) of the recruitment.

Beyond the 17 patients enrolled and studied during the 1/1/2013-12/31/2013 funding period, an additional 19 eligible women were identified. Of these 19, 15 either did not reply to our contact or were unable to be contacted. Three patients had another pregnancy and were thus ineligible on that basis. One patient reported being unable to get sufficient time off from work.

A further reason for the shortfalls included loss of our Preeclampsia Program Project recruitment staff in October 31, 2013. Although we received a percentile score of 12.0, the Preeclampsia Program Project (PI: Hubel, CA) was not renewed by NIH. We were not able to support our recruiting staff after October 31, 2013. We trained laboratory technician Ashley Myerski (at Magee-Womens Research Institute) to perform the tasks of patient contact, recruiting, and scheduling. The study lost 2 months of recruitment time in the process.

We are endeavoring to continue recruitment to increase the study sample size. There are currently three newly identified postpartum women who have agreed to participate in the study and are scheduled to visit the CTRC for vascular tests in March 2014. We believe we will thereby reach a sufficient sample size for publication of our sublingual glycocalyx data (which is already statistically significant).

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, and an abbreviated title of the publication. 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):
1. None				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published

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

Yes____x_____ No_____

If yes, please describe your plans:

Dr. Hubel intends to submit the summated findings for peer-reviewed publication by July 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____x_____

If yes, please describe your plans:

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

Carl A. Hubel, PhD
Associate professor
Magee-Womens Research Institute and Dept. OB/GYN & Reproductive Sciences, University of Pittsburgh

Personal Statement:

I have devoted my career to understanding the cardiovascular adaptations that occur during pregnancy and the maternal-placental interactions that converge to generate the vascular endothelial cell dysfunction that underlies the pregnancy disorder preeclampsia. My work has supported the hypothesis that abnormal maternal lipid metabolism and reactive oxygen and nitrogen species are involved in the pathogenesis of preeclampsia. I have contributed to evidence that metabolic aberrations persist/reemerge in women with preeclampsia, potentially explaining both the risk of preeclampsia and the elevated risk of future cardiovascular disease in this group of women. Although much research has focused on identifying circulating damaging agents contributing to endothelial dysfunction in preeclampsia, we are now also honing in on a potential first-line, fragile target— the endothelial and syncytiotrophoblast glycocalyx (apical surface layer). We propose that glycocalyx dysfunction is a critical aspect of placental and maternal vascular dysfunction leading to clinical preeclampsia, and a potential target for therapy.

Positions and Honors:

Current Employment:

2004-present	Secondary Appointment, Dept. Environmental and Occupational Health, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA
2009-Present	Associate Professor, Department of Obstetrics, Gynecology & Reproductive Sciences, University of Pittsburgh, School of Medicine, Pittsburgh, PA

Honors and National/International Academic Service:

1988 American Heart Association, Egan Post-doctoral Fellowship, Vermont Chapter, 1988; PHS Institutional NRSA, "Training in Smooth and Cardiac Muscle Biology", University of Cincinnati, 1991; PHS Individual NRSA, "Endothelin and Ion Regulation in Coronary Smooth Muscle", Post-doctoral Fellowship, University of Cincinnati / University of Pittsburgh, 1992; Irene McLenahan Young Investigators' Research Fund, "Oxidative Modification of Low-Density Lipoprotein in Preeclampsia", Magee-Womens Hospital, Pittsburgh, 1995; Ad Hoc Reviewer (Temporary Member) NIH CSR, Pregnancy & Neonatology (PN) Study Section, 2004, 2005, 2009; Ad Hoc Reviewer, Special Emphasis Panel (SEP) for the National Center for Research Resources (NCRR), Clinical Centers in Research Excellence (CCRE) at Research Centers in Minority Institutions (RCMI), 2004; SEP for NCRR-NIH, General Clinical Research Center Site

Visit Team-University of Oklahoma Health Sciences Center GCRC, 2004; SEP for NCRR-NIH, General Clinical Research Center Site Visit Team University of Vermont and Fletcher Allen Health Care GCRC, 2005; Advisory Panel member: NIH/NICHD workshop, "Preeclampsia: A Pressing Problem", Bolger Center, Potomac, MD, 2006; NIH SEP, Endocrinology, Metabolism Nutrition and Reproductive Sciences (EMNR) IRG, 2010; Ad Hoc Reviewer for UK agencies (SPARKS, NHSScotland, The Wellcome Trust, Tommy's, The Baby Charity, Science Foundation Ireland), 2000,2002,2004,2007-08,2011-12; NICHD SEP ZHD1 DSR-Z(LK), 2013; Ad Hoc Reviewer, Canadian Institutes of Health Research- Clinical Research, 2013; NIH ZRG1 CB-L 55 Developmental Pharmacology SEP, Dec. 2013.

Relevant Publications (15 of 58):

Hubel CA, McLaughlin MK, Evans RW, Hauth BA, Sims CJ, Roberts JM. Fasting serum triglycerides, free fatty acids and malondialdehyde are increased in preeclampsia, are positively correlated, and decrease within 48 hours postpartum. Am J Obstet Gynecol. 1996;174:975-982.

Hubel CA, Lyall F, Gandley RE, Roberts JM. Small low-density lipoproteins and vascular cell adhesion molecule (VCAM-1) are increased in association with hyperlipidemia in preeclampsia. Metabolism. 1998;47(10):1281-1288.

Hubel CA, Snaedal S, Geirsson RT, Roberts JM, Ness RB, Arngrímsson R. Dyslipoproteinemia in postmenopausal women with a history of eclampsia. Br J Obstet Gynaecol. 2000;107:776-784.

Shibata E, Rajakumar A, Powers RW, Larkin RW, Gilmour C, Crombleholme WR, Ness RB, Roberts JM, Hubel CA. Soluble fms-like tyrosine kinase 1 (sFlt-1) is increased in preeclampsia but not in small for gestational age pregnancies: Relationship to circulating placental growth factor (PlGF). J Clin Endocrinol Metab. 2005;90:4895-4903

Ramirez RJJ*, Hubel CA*, Novak J, DiCianno JR, Kagan VE, and Gandley RE. Moderate ascorbic acid deficiency increases myogenic reactivity of arteries from pregnant but not virgin ascorbate-dependent rats. (*co-first author) Hypertension. 2006;47:454-460.

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Hubel CA, Wallukat G, Wolf M, Herse F, Rajakumar A, Roberts JM, Markovic N, Thadhani R, Luft FC, Dechend R. Agonistic angiotensin II type 1-receptor autoantibodies in postpartum women with a history of preeclampsia. Hypertension. 2007;49(3):612-617

Hubel CA, Powers RW, Sneadal S, Gammill HS, Ness RB, Roberts JM, Arngrimsson R. C-reactive protein is elevated 30 years after eclamptic pregnancy. Hypertension. 2008;51(6):1499-1505. PMC2398699

Gandley RE, Rohland J, Zhou Y, Shibata E, Harger GF, Rajakumar A, Kagan VE, Markovic N, and Hubel CA**.** Increased myeloperoxidase in the placenta and circulation of women with preeclampsia. Hypertension. 2008;52(2):387-393. PMC2735015

Shibata E, Hubel CA*, Powers RW, von Versen-Hoeynck F, and Roberts JM. Placental system A amino acid transport is reduced in pregnancies with small for gestational age (SGA) infants but not in preeclampsia with SGA infants. (*corresponding author) Placenta. 2008;29(10):879-882. PMC2703008

Bainbridge SA, Roberts JM, von Versen-Höynck F, Koch J, Hubel CA. Uric acid attenuates trophoblast invasion and integration into cell monolayers. Am J Physiol Cell Physiol. 2009;297(2):C440-450. PMC2724097

Luppi P, Powers RW, Verma V, Edmunds L, Plymire D, Hubel CA. Maternal circulating $CD34^{+}VEGFR\text{-}2^{+}$ and $CD133^{+}VEGFR\text{-}2^{+}$ progenitor cells increase during normal pregnancy but are reduced in women with preeclampsia. Reprod Sciences 2010;17:643-652. PMC2893245

Schreurs MPH, Hubel CA, Bernstein IM, Jeyabalan A, Cipolla M. Increased oxidized low-density lipoprotein causes blood-brain barrier disruption in early-onset preeclampsia through LOX-1 FASEB J. 2013;27(3):1254-1263. PMC3574277

Weissgerber TL, Rajakumar A, Myerski A, Edmunds LR, Powers RW, Roberts JM, Gandley RE, Hubel CA. Vascular Pool of Releasable Soluble VEGF Receptor-1 (sFLT1) in Women with Previous Preeclampsia and Uncomplicated Pregnancy. J Clin Endocrinol Metab 2014 [EPub ahead of print]. *This research was supported by the State of Pennsylvania Health Research Formula Fund, (1/1/2012 – 12/31/2012; C.A. Hubel, PI) "Glycocalyx Syndecan-1 and Preeclampsia Pathogenesis*" .

Catov, JM, Bertolet M, Chen Y, Evans RW, Hubel CA. Nonesterified fatty acids and spontaneous preterm birth: factor analysis to identify patterns of risk. American J Epidemiol [in press].

Judith Brands, PhD

November 2009-present:

Post-doc at the Center for Ultrasound Molecular Imaging and Therapeutics at University of Pittsburgh Medical Center (UPMC).

Project: 'The endothelial glycocalyx: A novel cardiovascular therapeutic target'. Supervisor: J. Pacella, MD, FACC Collaborating investigator: H. Vink, PhD

• Used intra-vital microscopy (cremaster) to study whether pharmacologic rebuilding of glycocalyx improves microvascular function and perfusion in the setting of post-ischemic reperfusion.

• Studied cell free layer as mechanism for improved microvascular perfusion observed after infusion of drag reducing polymers.

• Set up and run a clinical study to determine sublingual glycocalyx measurement as predictor for coronary artery disease.

• Used microvascular sonothrombolysis, or ultrasound (US) induced clot dissolution (microvascular sonothrombolysis) in rat hindlimb.

July 2006-June 2009:

PhD student

Maastricht University

PhD student at the Department of Medical Physics, University of Amsterdam (2004-2006) and the Department of Physiology, Maastricht University (2006-present). Project: 'Role for agonist-induced glycocalyx modulation in coronary flow regulation and oxygen exchange. Application of tracer dilution for determination of glycocalyx volume'

Relevant publications:

Brands J, van Haare J, Vink H, Vanteeffelen JW. Whole-body recruitment of glycocalyx volume during intravenous adenosine infusion. Physiol Rep. 2013 Oct;1(5):e00102.

Brands J, Kliner D, Lipowsky HH, Kameneva MV, Villanueva FS, Pacella JJ.

New insights into the microvascular mechanisms of drag reducing polymers: effect on the cell-free layer. PLoS One. 2013 Oct 4;8(10):e77252.

VanTeeffelen JW, Brands J, Janssen BJ, Vink H. Effect of acute hyaluronidase treatment of the glycocalyx on tracer-based whole body vascular volume estimates in mice. J Appl Physiol 2013 May;114(9):1132-40.

Robin E. Gandley
Assistant Professor
Department of Environmental and Occupation Health, University of Pittsburgh

Dr. Gandley has significant expertise examining process of oxidative stress arising from both free metals and reduced antioxidant capacity. She has incorporated biochemical techniques, cell culture and isolated arterial tissues and blood from both animal models as well as clinical samples to establish mechanisms of oxidative stress and vascular dysfunction during pregnancy and preeclampsia. Dr. Gandley focused on reproductive toxicology related to metals and pregnancy after her graduate work at the University of Maryland at Baltimore. As a post-doctoral fellow, she focused on the vascular system and mechanistic studies of pregnancy-related hypertension. Dr. Hubel and Dr. Gandley have collaborated for many years focusing on mechanisms of endothelial dysfunction related to the pathophysiology of preeclampsia. This project is a logical extension of their recent work. Dr. Gandley has significant experience using both patient samples and animal models to examine changes in vascular function during pregnancy.

Positions and Employment

1987-1988	Student Internship, Department of Energy, Bruceton, PA
1989-1990	Laboratory Manager, Department of Biochemistry, University of Maryland.
1989-1993	Research Assistant, Program in Toxicology, University of Maryland
1993-1999	Postdoctoral Fellow, Department of Obstetrics/Gynecology and Reproductive Sciences, University of Pittsburgh
1999-2000	Research Assistant Professor, Department of Obstetrics/Gynecology and Reproductive Sciences, University of Pittsburgh
1999-Present	Assistant Investigator, Magee-Womens Research Institute
2000-2002	Assistant Professor, Department of Obstetrics/Gynecology and Reproductive Sciences, University of Pittsburgh
2002-Present	Assistant Professor, Department of Environmental and Occupation Health, University of Pittsburgh

Relevant Publications:

Weissgerber TL, Rajakumar A, Myerski AC, Edmunds LR, Powers RW, Roberts JM, Gandley RE, and Hubel CA. (2013) Vascular pool of releasable soluble VEGF receptor-1 (sFlt) in women with previous preeclampsia and uncomplicated pregnancy. J Clin. Endocrin & Met Online publication doi:10.1210/jc.2013-3277.

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Dr. Jeyabalan is a physician-scientist in Maternal-Fetal Medicine Division at Magee-Womens Hospital, University of Pittsburgh. She has been involved in a number of clinical research projects in the arena of high risk obstetrics including preeclampsia and intrauterine growth restriction. Dr. Jeyabalan is active in the laboratory at Magee-Womens Research Institute investigating cardiovascular and renal adaptations to pregnancy and preeclampsia and has a number of peer-reviewed publications in this field. She served as the principal investigator of the Clinical Data Core component of the preeclampsia program project grant titled “Mechanisms of preeclampsia: impact of obesity”. Dr. Jeyabalan successfully recruited over 700 women in early gestation and successfully studied many of these women across gestation and up to 24 month post-delivery. Dr. Jeyabalan is clinically active and serves as the medical director of the

Maternal-Fetal Medicine outpatient services, the Obstetric Specimen Procurement Unit, as well as the Clinical and Translational Research Center at Magee-Womens Hospital. Currently, 50% of Dr. Jeyabalan's time is committed to research and 50% to clinical medicine.

Relevant Publications:

Jeyabalan A, Kerchner LJ, Fisher MC, McGuane JT, Doty KD, Conrad KP. Matrix metalloproteinase-2 activity, protein, mRNA and tissue inhibitors in small arteries from pregnant and relaxin-treated nonpregnant rats. Journal of Applied Physiology 2006; 100(6):1955-63. PMID16484357.

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Dr. Weissgerber's research interests focus on the relationships between vascular health and adaptation to pregnancy, pregnancy outcome, and later life risk of cardiovascular disease. Dr. weissgerber is also interested in the potential for interventions, including exercise, to modify these relationships and improve pregnancy outcome, and maternal and fetal health.

Relevant publications:
Weissgerber TL, Rajakumar A, Myerski A, Edmunds LR, Powers RW, Roberts JM, Gandley RE, Hubel CA. Vascular Pool of Releasable Soluble VEGF Receptor-1 (sFLT1) in Women with Previous Preeclampsia and Uncomplicated Pregnancy. J Clin Endocrinol Metab 2014 [EPub ahead of print]. This research was supported by the State of Pennsylvania Health Research Formula Fund, (1/1/2012 – 12/31/2012; C.A. Hubel, PI) "Glycocalyx Syndecan-1 and Preeclampsia Pathogenesis".
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