BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
Julian H. Lombard, Ph.D.	Professor
eRA COMMONS USER NAME (credential, e.g., agency login) Jlombard	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Texas - El Paso, El Paso, TX	B.A	05/69	Biological Sciences
Arizona State University, Tempe, AZ	M.S.	06/71	Zoology-Physiology
Medical College of Wisconsin, Milwaukee, WI	Ph.D.	05/75	Physiology
University of Virginia, Charlottesville, VA	Postdoctoral	06/77	Microcirculation

Personal Statement: My training, research experience, and collaborative relationships at the Medical College of Wisconsin (MCW) place our laboratory in a uniquely well-qualified position to employ an important and novel animal resource--the *Nrf2*$^{(-/-)}$ knockout rat lacking the major antioxidant and cell protective transcription factor NRF2—potentially involved either directly or indirectly in more than 200 different human diseases. In the present project, we will use the *Nrf2*$^{(-/-)}$ rat model to investigate an intriguing hypothesis regarding the mechanisms of endothelial dysfunction, oxidant stress, and impaired angiogenesis that occurs during ingestion of a high salt diet. Previous studies in our laboratory have shown that vascular oxidant stress and endothelial dysfunction during exposure to a high salt diet can be ameliorated by preventing salt-induced angiotensin II (ANG II) suppression. Studies in the laboratory of my longtime collaborator Dr. Andrew S. Greene have demonstrated the impaired angiogenic responses and microvascular rarefaction that occur during exposure to high salt diet can also be prevented by restoring normal plasma ANG II levels in the salt-fed animals. Identification of salt induced ANG II suppression as a major factor in causing salt-induced oxidant stress, endothelial dysfunction, and impaired angiogenic responses is contrary to conventional thinking that any reduction in ANG II levels is beneficial and opens up exciting new areas for investigation. Stemming from our proven track record in this area and our initial success with the *Nrf2*$^{(-/-)}$ mutant rats, we have developed the novel and intriguing hypothesis that the link between salt-induced ANG II suppression and vascular oxidant stress, endothelial dysfunction, and impaired angiogenesis is downregulation of NRF2-mediated antioxidant defenses—a novel mechanism that is not being investigated by any other laboratory in the world.

In light of the importance of understanding sex-related differences in cardiovascular disease and the relative scarcity of information regarding NRF2 antioxidant defenses in females, we will maximally utilize our novel rat resource by conducting studies in both male and female *Nrf2*$^{(-/-)}$rats and their wild type controls. We will also investigate a potentially “game-changing” therapeutic approach to vascular oxidant stress and impaired angiogenesis in low renin salt-sensitive hypertension, namely direct upregulation of the NRF2 antioxidant defense system. We are extremely excited about these studies in light of the pervasive importance of NRF2 in regulating antioxidant defenses, our novel rat genetic model, our compelling preliminary data, and the potential therapeutic and preventative benefits of directly upregulating this fundamental antioxidant defense system in conditions characterized by vascular oxidant stress and endothelial dysfunction.

I have had extensive training and experience in investigating cardiovascular physiology at all levels ranging from the whole body level to the microcirculation. I have been continuously funded by NIH since my appointment to the MCW faculty in 1977. In April 2014, I was the recipient of the Eugene M. Landis Award—the highest career award of the Microcirculatory Society. This wonderful award and my success in obtaining continuous funding for nearly 4 decades attest to the ability of my laboratory to direct and successfully conduct productive research at the cutting edge of cardiovascular physiology. However, these wonderful accomplishments would have been impossible without an unprecedented combination of resources, team work, collaboration and collegiality at the departmental and institutional level. Taken together, these unique and powerful assets of our research team strongly support the ability of our team to complete the important studies proposed in this exciting project.

B. Positions and Honors:

PROFESSIONAL POSITIONS:

1974-1977 Postdoctoral Fellow, (Laboratory of Brian R. Duling, Ph.D.) Dept. of Physiology, University of Virginia School of Medicine, Charlottesville, VA
1977-1981 Assistant Professor, Dept. of Physiology, Medical College of Wisconsin, Milwaukee, WI
1981-1988 Associate Professor, Dept. of Physiology, Medical College of Wisconsin, Milwaukee, WI
1988-Present Professor, Dept. of Physiology, Medical College of Wisconsin, Milwaukee, WI

AWARDS AND OTHER PROFESSIONAL ACTIVITIES:

Established Investigator Award -- American Heart Association, 1985-1990
Fellow -- Council for High Blood Pressure Research, American Heart Association
Fellow, APS Cardiovascular Section
Program, Nominating, and Executive Committees, Microcirculatory Society, USA
President, Microcirculatory Society, USA; President, Midwest Physiological Society
Chairman: Cardiovascular Regulation 2, Vascular Biology and Blood Pressure Regulation 1, and SURF Review Groups, American Heart Association, National Center.
Program Committee: 24th European Conference on Microcirculation—Amsterdam, Netherlands
Scientific and Organizing Committee: 1st International Symposium on Hypertension: From Laboratory Bench to Clinical Settings; Osijek, Croatia
Program Committee—American Physiological Society
Outstanding Mentor Award, MCW Graduate School of Biomedical Sciences
Distinguished Service Award, Medical College of Wisconsin
Chairman-8th World Congress for Microcirculation, August 2007
2014 Eugene M. Landis Award—Microcirculatory Society USA

Federal Government. Public, and Editorial Advisory Committees:

Member: Experimental Cardiovascular Sciences (ECS) Study Section-DRG,
Editorial Board - *Microcirculation, Endothelium and Lymphatics*;
Editorial Board - *Microcirculation*
Editorial Board - *American Journal of Physiology (Heart and Circulatory Physiology)*;
Associate Editor: *American Journal of Physiology (Heart & Circulatory Physiology)*

C. Contributions to Science:

Mechanisms of Altered Vascular O_2 Sensitivity in Hypertension: it is well known that vascular resistance is elevated in all forms of hypertension, and arteries of hypertensive animals exhibit an increased sensitivity to multiple vasoconstrictor stimuli, e.g., norepinephrine. Much less is known about vessel responses to local regulators of active tone, e.g. increases in O_2 availability, in hypertension. Early studies in my laboratory demonstrated an increased sensitivity of arterioles to elevated PO_2 in the earliest stages of multiple forms of hypertension including spontaneously hypertensive rats, reduced renal mass hypertensive rats, and Dahl salt sensitive rats. Importantly, this enhanced sensitivity to elevated PO_2 is present before other vascular alterations occur in these forms of hypertension. Subsequent studies in my laboratory identified a role for cytochrome P450 (CYP450) metabolites of arachidonic acid in mediating enhanced arteriolar O_2 sensitivity in hypertensive animals. I directed the studies of vascular O_2 sensitivity personally. Collaborative work with the laboratory of Dr. Allen W. Cowley, Jr. correlated these findings with whole body hemodynamic changes in the early and established stages of the reduced renal mass-salt loading model of volume-expanded hypertension; and a highly profitable collaboration with Dr. Camille Falck at the University of Texas Southwestern Medical School was instrumental in identifying the role of CYP 450 metabolites of arachidonic acid (specifically 20-HETE) in mediating the enhanced responses to elevated PO_2 in microvessels of hypertensive animals.

a. Lombard, J.H., M.E. Hess, and W.J. Stekiel. Neural and local control of arterioles in spontaneously hypertensive rats. *Hypertension* 6:530-535, 1984.

b. Lombard, J.H., M.E. Hess, and W.J. Stekiel. Enhanced response of arterioles to oxygen during development of hypertension in SHR. *Am. J. Physiol*. 250:H761-H764, 1986.
c. Lombard, J.H., C. Hinojosa-Laborde, and A.W. Cowley, Jr. Hemodynamics and microcirculatory alterations in reduced renal mass hypertension. *Hypertension* 13:128-138, 1989.
d. Frisbee, J.C., J.R. Falck, and J.H. Lombard. Contribution of cytochrome P450-4A ω-hydroxylase to altered arteriolar reactivity with high salt diet and hypertension. *Am. J. Physiol.* 278:H1517-H1526, 2000.

Mechanisms of Salt-Induced Vascular Oxidant Stress: It is well known that hypertension is associated with endothelial dysfunction and vascular oxidant stress. In recent years, there is an increasing focus on the detrimental effects of high salt diet, not only on the cardiovascular system (independent of the elevated blood pressure), but in many other pathological conditions as well. In this area of research, my laboratory has shown that high salt (HS) diet leads to vascular oxidant stress and impaired relaxation of resistance arteries and microvessels in multiple animal models, and identified a paradoxical role of angiotensin II (ANG II) suppression in mediating oxidant stress and impaired vascular relaxation in salt-fed rats and hamsters. Our finding that low levels of plasma ANG II lead to vascular oxidant stress and endothelial dysfunction that is strikingly similar to that associated with elevated ANG II levels is extremely novel from a conceptual standpoint; and could cast a new light on findings in human populations showing endothelial dysfunction with high salt diet and increased mortality in salt-sensitive human patients (even if they fail to develop hypertension). Very important recent findings are: **1)** that the protective effects of low dose ANG II infusion (to restore normal plasma ANG II levels) in salt-fed animals are mediated via activation of the AT_1 receptor with ultimate activation of the ERK 1/2 signal transduction pathway; **2)** that low dose ANG II infusion restores endothelial function and reduces vascular oxidant stress by activating the AT_1 receptor; **3)** that the protective effect of infusing angiotensin (1-7) to ameliorate endothelial dysfunction in salt-fed rats is mediated via different mechanisms than the protective effect of low dose ANG II infusion, but that the signal transduction pathways converge at the level of the ERK 1/2 signal transduction pathway; and, most recently, **4)** that the protective effects of low dose ANG II infusion to ameliorate endothelial dysfunction are lost in mutant rats lacking the master antioxidant and cell protective transcription factor NRF2. I personally directed all studies of the effect of high salt diet on vascular reactivity in oxidant stress, and have also had a long-standing and highly valuable collaboration with Dr. Andrew Greene, Director of the Bioengineering and Biotechnology Center at MCW, in investigating the role of salt-induced ANG II suppression in contributing to reduced microvessel density (microvascular rarefaction) independent of the elevated blood pressure. Our studies of the detrimental effects of salt-induced ANG II suppression represent novel "outside the box" thinking that is in direct opposition to conventional and stereotypical thinking that lowering ANG II levels is always beneficial. These findings may be especially relevant to the therapeutic management of low renin forms of salt-sensitive hypertension; and our emerging work indicating that restoration of normal plasma ANG II levels upregulates antioxidant defenses by activating the NRF2 system may be especially important and relevant, as it raises the possibility of beneficial effects of direct upregulation of endogenous antioxidant defense mechanisms as a potential therapeutic approach.

a. Weber, D.S., and J.H. Lombard. Angiotensin II AT_1 receptors preserve vasodilator reactivity in skeletal muscle resistance arteries. *Am. J. Physiol.* 280:H2196-H2202, 2001.
b. Zhu J, J. Friesema, I. Drenjancevic-Peric, R.J. Roman and J.H. Lombard. High salt diet impairs vascular Ca^{2+} signaling and nitric oxide production via ANG II suppression in rat aorta. *Am J Physiol (Heart Circ. Physiol.)* 291:H929-H938, 2006.
c. McEwen, S.T., S.F. Balus, M.J. Durand, and J.H. Lombard, J.H. Angiotensin II maintains cerebral vascular relaxation via EGF receptor trans-activation and ERK 1/2. *Am. J. Physiol. (Heart and Circulatory Physiology)* 297: H1296-H1303, 2009.
d. Durand, M.J., G. Raffai, and J.H. Lombard. Angiotensin (1-7) and low dose angiotensin II infusion reverse salt-induced endothelial dysfunction via different mechanisms in rat middle cerebral arteries. *Am. J. Physiol. (Heart and Circulatory Physiology)* 299 (4):H1024-H1033, 2010.

Role of Cytochrome P450-ω Hydroxylase Enzymes in Microvascular O_2 Sensing and Altered Vascular Reactivity in Hypertension: It is well known that changes in oxygen availability affect active force development by blood vessels. Resistance arteries and arterioles are exquisitely sensitive to changes in

vascular oxygen levels. The mechanisms of vascular oxygen sensitivity have been intensely debated for decades. In conjunction with the laboratories of Drs. David R. Harder, John R. Falck, and Richard J. Roman, we have produced compelling evidence that arachidonic acid metabolites of the cytochrome P450 (CYP450) - ω hydroxylase system, specifically 20-HETE, play an important role in vascular oxygen sensing. Our studies have also shown that upregulation of the CYP450 metabolites with elevated dietary salt intake in Sprague-Dawley rats and, especially, in the Dahl salt sensitive rat model of low renin salt-sensitive hypertension in humans, plays a major role in contributing to altered sensitivity of arterioles and resistance arteries to changes in PO_2, norepinephrine, and increases in intravascular pressure (myogenic responses) in those animal models. These findings should provide new and important insight into the mechanisms of altered vascular reactivity in multiple forms of hypertension.

a. Frisbee, J.C., R.J. Roman, J.R. Falck, and J.H. Lombard. 20-HETE modulates myogenic activation of skeletal muscle arteries from hypertensive Dahl-SS rats. *Am. J. Physiol.* 280: H1066-H1074, 2001.
b. Kunert, M.P., R.J. Roman, M. Alonso-Galicia, J.R. Falck, and J.H. Lombard. Cytochrome P-450 ω-hydroxylase: A potential O_2 sensor in rat arterioles and skeletal muscle cells. *Am. J. Physiol.* 280:H1840-H1845, 2001
c. Wang J, Roman RJ, Falck JR, de la Cruz L, and Lombard JH. Effect of high-salt diet on CYPP450-4A ω-hydroxylase expression and active tone in mesenteric resistance arteries. *Am J Physiol* 288:H1557-H1565, 2005.
d. Lukaszewicz, K. M., J.R Falck, V. L. Manthati, and J. H. Lombard. Introgression of Brown Norway CYP4A genes onto the Dahl Salt-Sensitive Background Restores Vascular Function in SS-5^{BN} Consomic Rats. *Clinical Science* 124:333-342, 2013.

Mechanisms of Vascular Dysfunction in Disease-Sensitized Rat Genetic Models: The most recent (and ongoing) studies in our laboratory have investigated the mechanisms of altered vascular reactivity in specialized disease-sensitized rodent genetic rat models—primarily the Dahl salt sensitive rat model of human low renin salt sensitive hypertension and, to a lesser extent, the fawn hooded hypertensive rat. During the course of these studies, we found that chronically low ANG II levels lead to endothelial dysfunction and oxidant stress in resistance arteries of Dahl salt-sensitive rats even when the animals are normotensive and maintained on a low salt diet. We also showed that the AT_1 receptor mediates the protective effect of low dose ANG II infusion to restore vascular relaxation and reduce vascular oxidant stress in SS rats (similar to its effects in salt-fed Sprague-Dawley rats and golden hamsters). Our more recent studies have focused on the effect of chromosomal substitutions and mutations of specific genes on vascular phenotypes. Of particular note, we have found that substitution of chromosome 13 (SS.13^{BN} consomic rats) or portions of chromosome 13 carrying a normally functioning renin gene from either the Brown Norway or Dahl salt-resistant (R) rat ameliorates endothelial dysfunction in the Dahl SS rat genetic background. Another surprising finding is endothelial dysfunction is ameliorated in SS rats during diet induced obesity (which raises ANG II levels) while consumption of a high fat diet leads to endothelial dysfunction in SS.13^{BN} consomic rats carrying a normally functioning renin gene from the Brown Norway rat. We now have compelling evidence that the unexpected protective effect of low dose ANG II infusion to restore vascular function in SS rats (and also salt fed Sprague-Dawley rats and golden hamsters) is due to upregulation of antioxidant defenses such as Cu/Zn SOD; and that exposure to chronically low levels of plasma ANG II plays a crucial role in endothelial dysfunction and vascular oxidant stress in the Dahl salt sensitive rat model of low renin salt-sensitive hypertension in humans. Because angiotensin converting enzyme inhibitors and angiotensin receptor blockers are less effective in low renin forms of hypertension than in disorders associated with elevated ANG II levels, our current line of investigation should provide valuable insight into more effective therapeutic approaches toward the treatment of various forms of hypertension in individual patients. Although I was directly responsible for the studies of altered vascular reactivity, a highly valuable (and ongoing) collaboration with the MCW rat genomics group including Drs. Howard Jacob and Aron Geurts has been instrumental to the success of these very important studies.

a. Drenjancevic-Peric I and Lombard JH. Reduced ANG II and oxidative stress contribute to impaired vasodilation in Dahl salt sensitive rats on low salt diet. *Hypertension* 45 [Part 2] 687-691, 2005.
b. Drenjancevic-Peric, I. A.S. Greene, and J.H. Lombard. Restoration of cerebral vascular relaxation in renin congenic rats by introgression of the Dahl R renin gene. *Am. J. Hypertension* 23:243-248, 2010.

c. Durand, M.J., C. Moreno, A.S. Greene, and J.H. Lombard. Impaired relaxation of cerebral arteries in the absence of elevated salt intake in normotensive congenic rats carrying the Dahl salt-sensitive renin gene. *Am. J. Physiol. (Heart and Circulatory Physiology)* 299 :H1865-H1874, 2010.
d. Beyer, A.M., G. Raffai, B. Weinberg, K. Fredrich, and J.H. Lombard. Dahl salt-sensitive rats are protected against vascular defects related to diet-induced obesity. *Hypertension* 60:404-410, 2012.

List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/48016475/

D. Research Support: Ongoing and Completed within the past 3 years.

Ongoing Research Support:

1R21OD018309-01 Lombard (PI) 04/15/2014-1/31/2016
Oxidant Stress in the Nrf2 Knockout Rat
The goal of this study is to phenotype basic cardiovascular and renal parameters in rats in which the Nrf2 gene has been eliminated utilizing TALEN technology.
Role: PI (10% Effort)

2R56HL065289-13A1 Lombard (PI) 09/01/2014 – 8/31/2015
High Salt Diet, Angiotensin II, and Microvessel Dilation
The goal of this study is to determine the mechanisms of the impaired relaxation of cerebral and skeletal muscle resistance arteries that occurs when normotensive animals are subjected to an elevated dietary salt intake.
Role: PI (25% Effort)

P50 GM094503-1 Beard (PI) 08/11/2011 – 07/31/2016
The Virtual Physiological Rat Center for the Study of Complex Disease

The goal of this project is to utilize computational biology approaches to understand the contribution of altered physiological mechanisms to complex disease, employing sensitized rat models.

Role: Project Leader—Project 1 (10% Effort)

Completed Research Support (Last 3 Years):

R01 HL72920-08 Lombard (PI) 04/01/2007-03/31/2012
Microvessel O_2 Responses in Salt-Sensitive Hypertension
The goal of this project is to determine the role of 20-HETE and cytochrome P450 ω-hydroxylase in mediating the enhanced response of microvessels to elevated PO_2 in salt sensitive hypertension.
Role: PI

Pending Research Support

R1R21-OD021874-01-A1 Lombard (PI) 04/01/2016-03/31/2018
Development of a Mas Receptor Mutant Rat
The goal of this project is to develop and phenotype a mutant rat straining lacking functional Mas1 receptors for angiotensin (1-7) in the Dahl salt-sensitive genetic background.
Role: PI (10% Effort)