Cardiovascular diseases remain the leading cause of death in the US for both men and women. Research in this field is of critical importance to the health of our community and our nation. The overall goal of the Cardiovascular Center (CVC) is to enable laboratory discoveries to move forward in the form of new treatments, products, preventions and cures to those who suffer from diseases of the heart, blood vessels, kidneys, and lungs.

The CVC plays a vital role as a campus incubator to stimulate the exchange of new ideas and to nurture the development of novel research programs that foster the understanding of the underlying basic mechanisms of cardiovascular diseases and the translation of this basic knowledge into improved diagnostics, prevention, and therapeutics for the citizens of Wisconsin and beyond. The laboratories of 20 investigators reside within the confines of the CVC, and many more Faculty are affiliated with our Center throughout the MCW campus. More than 100 clinicians and more than 50 basic scientists from various departments conduct research with cardiovascular implications.

Over the past year, progress has been made in coalescing scientific Affinity Groups among the members of the CVC thereby fostering interdisciplinary collaborations in areas that have been defined as focus areas for growth at MCW such as heart failure and heart tissue regeneration. Work is now in progress to better align the focus of CVC research with those areas of strength within the MCW clinical service lines of cardiology and surgery, both adult and pediatric. Toward that end, over the past year, CVC has co-recruited a number of new Faculty, including Jennifer Strande, M.D., Ph.D. (a cardiologist in the division of Cardiovascular Medicine), Paul Goldspink, Ph.D. (cardiac physiology), Aron Geurts, Ph.D. (cardiovascular genomics) and Scott Levick, Ph.D., (cardiovascular pharmacology). In order to support the expanding research efforts of the CVC, an additional 5000 square feet of new laboratory space has been developed and a number of the existing laboratories have undergone renovations to provide better utilization of existing space. Greater space has been developed to accommodate students and research fellows, and a newly created Conference Center that enables seating up to 125 and is divisible for small group meetings, has been constructed.

These activities represent important advancements toward the overall mission of the CVC “to further develop strong and nationally recognized interdisciplinary cardiovascular research programs at the Medical College of Wisconsin and to promote comparable excellence in clinical care and education while developing community outreach.”

Allen Cowley, Ph.D.
Created in 1992, the Cardiovascular Center (CVC) is focusing on the prevention, detection, treatment and cure of the large family of cardiovascular diseases.

Under the direction of Dr. Cowley, the CVC is administered by the staff: (from left to right): Allen W. Cowley Jr., PhD, Jane Brennan Nelson, Jean-Francois Liard, MD, PhD, Joanna McCormick, Cecily Burk, April Mays

The Cardiovascular Center offers a number of services to its Faculty and to the MCW campus, including the following cores: Information Technology, Engineering, and Microscopy.

IT core: (left to right) Andrew J. Milbrath, Greg McQuestion, Pedro Mendez

Engineering core: David Eick, Mike Kloehn

Microscopy core: Glenn Slocum
The Cardiovascular Center in 2010-2011

Financials

Cardiovascular Center —— FY 2011

### Annual Revenue Sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCW Central Funds</td>
<td>$465,062</td>
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<tr>
<td>Dean Program Development</td>
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<tr>
<td>Philanthropic</td>
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<tr>
<td><strong>Total Revenues</strong></td>
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This past year has been marked by the recruitment of several new Faculty:

- Paul Goldspink, PhD, Associate Professor, Physiology
- Jennifer Strande, MD, PhD, Assistant Professor, Cardiovascular Medicine
- Aron Geurts, PhD, Assistant Professor, Physiology
- Scott Levick, PhD, Assistant Professor, Pharmacology

The CVC primary Faculty whose research is described in this annual report together with Dr. Cowley combine for a total of $12,915,367 in NIH funding as listed in NIH RePORTER (fiscal year total cost).

Extensive renovations were conducted in the CVC, with the creation of several new facilities, including over 5000 square feet of laboratory space for basic scientists, a surgical facility suite, a stem cell culture facility, a 125-seat conference room, a dedicated room for a new confocal microscope purchased by the CVC, a freezer farm, and the consolidation of core equipment in rooms protected by electronic card readers.

The CVC Scientific Advisory Committee was again of great help to us in achieving our objectives and will continue to provide guidance. This Committee is made of the following MCW Faculty:

- Ellis Avner, MD, Associate Dean of Research, Children's Research Institute, Professor, Pediatrics
- Joseph Besharse, PhD, Chair of Cell Biology, Neurosciences and Anatomy
- William Campbell, PhD, Chair of Pharmacology
- Michael Cinquegrani, MD, Chief of Cardiovascular Medicine
- Stephen Duncan, PhD, Professor of Cell Biology
- Andrew Greene, PhD, Director, Bioengineering and Biotechnology Center
- David Gutterman, MD, Professor in Cardiology, Senior Associate Dean for Research
- David Harder, PhD, Professor of Physiology, Associate Dean for Research Mentoring
- Howard Jacob, PhD, Director of the Human and Molecular Genetics Center
- Elizabeth Jacobs, MD, Chief of Pulmonary and Critical Care Medicine
- Michael E. Mitchell, MD, Associate Professor Surgery, Division of Cardiothoracic Surgery
- Ramani Ramchandran, PhD, Investigator Children’s Research Institute, Associate Professor, Pediatrics
- David Waltier, MD, Chair of Anesthesiology
- Gilbert White, MD, Director of Research of Blood Research Center
- Michael Widlansky, MD, Assistant Professor, Cardiovascular Medicine

Further details about the CVC are provided on the CVC website at [http://www.mcw.edu/cvc.htm](http://www.mcw.edu/cvc.htm) and on the Kidney Disease Center website at [http://www.mcw.edu/kdc](http://www.mcw.edu/kdc).
Characterization of adenosine receptors: Dr. Auchampach and his team are focused on the study of adenosine receptors, or proteins on the surface of cells that recognize adenosine and related compounds. Adenosine is produced by cells when the breakdown of adenosine triphosphate (ATP) is increased during energy utilization. Hence, adenosine levels are raised when a cell is stressed, activated, or when there is not enough oxygen in the cells, as in ischemic heart disease and myocardial infarction.

There are four different types of cell-surface receptors for adenosine. Dr. Auchampach’s research studies the function of two of the receptor subtypes that have only recently been discovered, the $A_2B$ and $A_3$ adenosine receptors. His group is testing the hypothesis that the $A_3$ adenosine receptor helps minimize muscle damage and reduce inflammatory processes that occur during cardiac ischemia, whereas the $A_2B$ adenosine receptor that has low binding affinity for adenosine promotes fibrotic responses in the cardiovascular system contributing to the development of heart failure and hypertensive disease. The research is aimed at developing new drug therapies that target receptors for adenosine as potential treatments for patients with coronary artery disease. In particular, it tests the hypothesis (see Figure below) that activating the $A_3$ adenosine receptor in cardiomyocytes protects against ischemic injury by improving mitochondrial function and reducing apoptosis, whereas activating the $A_3$ adenosine receptor in immune cells during reperfusion is protective by suppressing inflammatory responses.

Cardiac regeneration: in collaboration with colleagues at MCW and the Medical College of Georgia, Dr. Auchampach’s research group is also investigating the therapeutic potential of stem cells for regenerating heart tissue that has been damaged following myocardial infarction. Using embryonic and induced pluripotent stem cells, the hypothesis is being tested that transplantation of stem cells that have been pre-differentiated to specific cardiac lineages (i.e., cardiomyocytes or vascular precursor cells) will reduce the potential for tumor formation and improve functional recovery of the damaged heart due to the incorporation of functional, electrically coupled myocytes into the myocardium.

Aron Geurts, PhD

Through genetic engineering in rats, the Geurts’ lab, along with colleagues in the Human and Molecular Genetics Center and the Department of Physiology, has made recent progress unraveling some of the complex genetics of hypertension and kidney failure. Recent large-scale genetic studies in humans have identified many genes which play likely, yet poorly understood roles in risk and progression of diseases like hypertension and chronic kidney disease. This has shifted the focus of the Geurts’ lab and colleagues to understanding these specific genes as they may relate to these diseases. The laboratory rat is the preferred model to study diseases like hypertension and renal failure because it is highly physiologically similar to humans. In the past few years, the Geurts’ lab has developed several technological approaches to modify the genome of rats to disrupt or ‘knock out’ specific genes, something that was not previously possible. This technological breakthrough has been named as one of the Top 10 scientific breakthroughs in both 2009 and 2010 by “The Scientist” and “Science” magazines, respectively.
An effort is underway with other MCW laboratories to use these new approaches to knock out in the laboratory rat 100 genes which have been identified in human studies. These models can then be studied to see if disrupting these genes plays a potential role in hypertension or kidney failure which lends support to the human findings and provide an animal model to understand gene function. The initial results are promising as knocking out one gene, Sh2b3, which has been studied for several years in mice but never appreciated for its role in disease, demonstrates significant protection from both hypertension and kidney failure, and possibly other disease traits. This suggests that this is a very important gene in human disease which we can now study further. It is the first time that a human disease gene candidate has been experimentally validated in a rat model of disease.

The figure below illustrates the power of the zinc-finger nuclease (ZFN) technology in rats as used by Dr. Geurts’ lab for one of many genes it can be applied to. A gene called Rab38 controls trafficking of vesicles inside cells. When this gene is mutated in the rat using ZFN technology, it causes a pigmentation defect leading to the fawn color that is evident in a subset of the animals. Importantly, we have worked with Howard Jacob’s lab to validate that this gene is also controlling trafficking in the tubules of the kidney and mutations in this gene can contribute to the end stage renal failure – a disease that affects millions of people worldwide.

Dr. Goldspink’s lab is presently investigating the influence of insulin like growth factor-1 (IGF-1) isoform peptides on the resident stem/progenitor cells populations within the heart. They are exploiting their findings in a number of different ways. They (and collaborators from two other institutions) have developed a technology that provides a microscopic physical scaffold to deliver both the physical cues for tissue growth combined with the capacity to deliver peptide therapeutics. They believe this “biomimetic” approach can be used to optimize stem cell therapy either alone or in conjunction with autologous stem cells. This novel approach of delivering implantable cell-sized “biomimetic devices” to instruct resident stem cells to enhance natural tissue repair and regeneration, is also adaptable to other tissues as well as the heart. Based on this technology, Dr. Goldspink has formed an early stage biomedical device company (Cell Habitats, Inc.) aimed at developing an easy-to-administer, micro device that allows the natural repair and regeneration of damaged tissue. Its first application is to restore normal cardiac function after a heart attack.

The image below shows immunofluorescence staining of small Troponin I positive cells (shown with arrows) in the heart of MGF E-domain peptide treated mice following myocardial infarction. Split image collected at 40X magnification on Nikon A1 confocal microscope. Green is an antibody against Troponin I, red is cell membrane stained with wheat germ agglutinin, blue is nuclei stained with DAPI.
Dr. Gutterman’s group is involved in a number of projects. The first one is a study of ballerinas, who experience cardiovascular and bone health problems. Twenty-two professional ballet dancers volunteered to determine the prevalence of the 3 components of the female athlete triad (disordered eating, menstrual dysfunction, low bone mineral density) and their relationships with brachial artery flow-mediated dilation. Seventeen dancers (77%) had evidence of low/negative energy availability. Thirty-two percent had disordered eating. Thirty-six percent had menstrual dysfunction. Twenty-three percent had evidence of low bone density. Sixty-four percent had abnormal brachial artery flow-mediated dilation. All of the 3 components of the triad plus endothelial dysfunction were present in 14% of the subjects. In conclusion, endothelial dysfunction was correlated with reduced bone mineral density, menstrual dysfunction, and low serum estrogen. These findings may have profound implications for cardiovascular and bone health in professional women dancers. They were recently published by A.Z. Hoch et al. in Clin. J. Sport Med. 2011; 21:119–125.

Another project examined the hypothesis that hydrogen peroxide ($\text{H}_2\text{O}_2$) serves as the endothelium-dependent transferrable hyperpolarization factor (EDHF) in human coronary arterioles (HCAs) to shear stress. Two HCAs were cannulated in series, with a donor intact vessel upstream and an endothelium-denuded detector vessel downstream. Results showed that flow induced endothelial production of $\text{H}_2\text{O}_2$, which acts as the transferrable EDHF activating large conductance $\text{Ca}^{2+}$-activated $\text{K}^+$ channels on the smooth muscle cells. This was published by Y. Liu et al. in Circ. Res. 2011; 108:566-573.

Finally, Dr. Gutterman’s group conducts research involving the age-dependence of flow-induced dilation of the coronary arterioles in man. Human coronary arterioles (~150 μm internal diameter) were obtained at surgery from patients with or without coronary artery disease (CAD) and prepared for videomicroscopy. After constriction with endothelin-1, vasomotor responses to flow were evaluated in the presence, absence or combination of the cyclooxygenase (COX) inhibitor indomethacin and the nitric oxide synthase inhibitor L-nitro-arginine methyl ester. The following age groups were examined: children 0–18 years; adults 19–55 years; older adults >55 years without CAD, and adults with CAD (mean age: 64±8.2 years). It was found that flow-induced dilation in children is mediated mostly by COX; whereas, during the aging process or in the presence of CAD its contribution is diminished. These novel findings may have clinical implications for the use of COX inhibitors in children and young adults. This was published by N.S. Zinkevich et al. in Circulation 2010, 122, 21, A15764.
David Harder, PhD

Dr. Harder’s lab explores the dynamic control of cerebral blood flow and the interactive actions of cells which both require and impact delivery of oxygen to the brain. Astrocytes play a crucial role in sensing and transmitting neuronal signals to nearby cerebral microvessels to regulate cerebral blood flow and thus delivery of oxygen and nutrients to match the metabolic demand of activated neurons. Studies are conducted to define the signaling pathways impacting blood flow to metabolically active neurons. The hypothesis is that signaling pathways between astrocytes and the cerebral microvasculature are initiated via metabolites which act through second messengers, some of which require trigger $\mathrm{Ca}^{2+}$ and/or phosphorylation, i.e. protein kinase C, serine/threonine protein kinase (Akt), mitogen-activated protein kinases (P38), extracellular-signal-regulated kinases (ERKs), and inositol trisphosphate ($\mathrm{IP}_3$). Among the cellular intermediates are epoxyeicosatrienoic acids (EETs), 20-hydroxyeicosatetraenoic acid (20-HETE), adenosine triphosphate (ATP), adenosine, nitric oxide (NO) and others. Finally, plasma membrane ion channels comprise an important group of the functional triggers mediating integration between neurons, astrocytes and the microvasculature. A systematic study of the signaling mechanisms mediating the functional role of astrocytes in regulating cerebral blood flow to match metabolic demand of neurons is of critical importance for understanding the mechanism regulating the cerebral circulation in different conditions. A recent publication from Dr. Harder’s lab demonstrated that adenosine works through generation of free radicals (Gebremedhin et al., Journal of Cerebral Blood Flow and Metabolism, 2010; 30: 1777–1790). Dr. Harder also just published his 2009 Wiggers Award lecture on the topic of the mechanisms of autoregulation of cerebral blood flow (American Journal of Physiology 2011; 300(5):1557-1565).

The Figure presented below was published in the Wiggers Award lecture. It shows changes in morphology of astrocytes and cerebral capillary endothelial cells when co-cultured: a) formation of capillary-like structures (arrow) double labeled with Dil-Ac-LDL (red) and GFAP (green) in the co-culture of astrocytes and cerebral capillary endothelial cells; b) formation of capillary-like structures (arrow) triple labeled by PECAM-1 (green), GFAP (red), and DAPI (blue) in the co-culture of astrocytes and cerebral capillary endothelial cells; c) double immunolabeling of blood vessels and astrocytes with PECAM-1 (green) and GFAP (red) in a section from normal rat cortex showing astrocytes form foot processes that impinge on vessels (arrows); d) cells (*) forming tube identified by DAPI staining (e) in co-culture exhibit punctate staining of connexin 43 on their cell-cell borders (arrow). Arrow points to area shown in the insert at higher magnification; f) cells (*) outside tubes in the same co-culture as in d show moderate to light cytoplasmic staining of connexin 43.
**John Imig, PhD**

Dr. Imig works on therapeutically promising new compounds for combating hypertension and cardiovascular disease. With a team of Wisconsin and Texas scientists, he has recently discovered a promising new avenue that they strongly believe can be further developed to treat these diseases. The researchers are Abdul Hye Khan, William B. Campbell and John D. Imig, from the Medical College of Wisconsin, and Vijaya L. Manthati, Jawahar L. Jat, and John R. Falck from the University of Texas Southwestern Medical Center. The findings were discussed in April 2011 at the Experimental Biology meeting (“Novel Epoxyeicosatrienoic Acid Analogs Increase Sodium Excretion and Lower Blood Pressure in Hypertension”). Key to the research is the role of endothelial cells, which line the inside of the blood vessels and the heart. Endothelial cells produce arachidonic acid metabolites, a class of fatty acids that have biological actions beneficial for cardiovascular health. This production occurs through three primary enzymatic pathways. Two of these pathways, the cyclooxygenase and the lipoxygenase pathways, have been successfully targeted for the treatment of inflammation, pain, fever, and asthma. The third enzymatic pathway is the cytochrome P450 pathway that produces epoxyeicosatrienoic acids (EETs) as major biologically active metabolites. EETs are endothelial-derived factors that significantly influence cardiovascular function. EETs can dilate blood vessels, lower blood pressure and have additional biologic actions including anti-inflammatory and anti-platelet aggregator activity. These biological activities have made EETs a very attractive therapeutic target for cardiovascular diseases.

For the last several years this research team has developed and synthesized an array of EET analogs, or chemical compounds that act as EETs. These EET analogs have been tested for beneficial cardiovascular actions. For this study, 35 different EET analogs were screened for their ability to dilate blood vessels. The screening produced five EET analogs that would be further examined to determine their ability to lower blood pressure in animal models of hypertension. Two of the five EET analogs administered to hypertensive animals effectively lowered blood pressure and reduced kidney injury. This work is a major step forward in developing novel EET analogs for the treatment of cardiovascular disease.

**Elizabeth Jacobs, MD, MBA**

Dr. Jacobs and her colleagues are working on a project with important implications for the diagnosis and treatment of ischemia-reperfusion (IR) lung injuries. IR lung injuries are commonly encountered clinically in conditions such as crush injury to the chest, lung transplantation and others. While the role of mitochondrial reactive oxygen species (mtROS) in IR injuries is generally accepted, less information is available regarding lung IR injury than cardiac or cerebral IR where the density of mitochondria is much higher. Dr. Jacobs will employ an in vivo, rodent survival model of unilateral lung injury and cutting-edge imaging technology to monitor and understand the role of mitochondrial complex I. Using this model, Dr. Jacobs and her colleagues have excellent evidence that mitochondrial dysfunction drives lung IR injury. By varying the severity of IR and tracking changes in lung mitochondrial bioenergetics in the first 2-72 hours, they will develop a profile which predicts recoverability by 7 days. Furthermore, they introduce two absolutely novel methods to quantify apoptosis or an altered redox status in vivo at the bedside, which they will correlate with measures of mitochondrial function.

The first method is in vivo SPECT/CT imaging,
using $^{99m}$Tc-duramycin to detect apoptosis. Its use in rats is demonstrated in Figure 3, which illustrates that duramycin (DU) uptake is increased in IR lungs by single photon emission computed tomography (SPECT). $^{99m}$Tc-duramycin was administered through an IV line and images acquired at steady-state. Without relocation of the rat, an injection of $^{99m}$Tc-MAA was made and rats were reimaged. $^{99m}$Tc-MAA was used as a pulmonary perfusion marker as the particles range in size from 10-40 microns and lodge in the pulmonary capillaries in proportion to flow. The Figure below shows representative parametric SPECT images, depicting DU uptake normalized to flow (based on MAA), of a control rat (3i) and one rat each subjected to IR of the left lung 24 (3ii) and 48 (3iii) hours earlier. The left-right ratio of flow normalized $^{99m}$Tc-duramycin uptake was increased by $\sim 35\%$ 24 hours after IR and greater than $60\%$ 48 hours after IR (n=1 each IR, n=3 control).

The second novel method introduced by Dr. Jacobs and her colleagues is optical imaging to detect the NADH/FAD (redox) ratios in intact lungs. Early changes in these non-destructive measurements will provide the first data to predict later recovery or loss of lung viability. Preliminary data demonstrate pro-survival effects of cyclosporine A (CSA) and 20-hydroxyeicosatetraenoic acid (20-HETE); protection by either agent depends upon an action on mitochondria.

Dr. Jacobs and colleagues hypothesize that: a) ischemia-reperfusion results in complex I dysfunction and increased mtROS which lead to apoptosis and decreased cell survival, b) SPECT/CT and optical imaging can detect levels of apoptosis and altered mitochondrial bioenergetics in an IR survival model in a manner which predicts subsequent viability and c) targeting mitochondria with an agent that protects mitochondrial bioenergetics in lung IR will mitigate injury and preserve lung function as detected by SPECT/CT or optical imaging. Dr. Jacobs and colleagues will attempt to define early alterations in mitochondrial bioenergetics and mtROS generation in a rodent model of lung IR which predict the potential of lung to recover. They will also examine the role of mtROS in cell apoptosis/survival and lung function. The second aim is to assess apoptosis and redox ratios in left and right lungs of IR rats using SPECT/CT and optical imaging respectively, and compare these data to measurements, obtained before, to define a profile of imaging data which predict lung tissue survival. Finally, they will determine how and if agents including 20-HETE or the CSA-like immunosuppressive sanglifehrin A offer protection from IR and if this protection can be predicted by SPECT/CT or optical imaging. With their novel means to detect apoptosis and redox injury in vivo and in a survival model of IR injury, Dr. Jacobs and colleagues are poised to use SPECT/CT and optical imaging endpoints to define the first bedside tests that distinguish in vivo apoptosis or oxidoreductive state, and the potential of treatments to rescue “irreversible” lung injury. These studies will provide data to predict which lung injuries are destined to recover, and which will progress to non-viability days later.
Girija Ganesh Konduri, MD

Improving our understanding and treatment of newborn babies with respiratory failure is the overall goal of the research program in the neonatology laboratory of the CVC. Every year, newborn babies that fail to achieve normal respiratory and cardiac function at birth are referred to the Neonatal Intensive Care Unit at Children’s Hospital. These babies are cared for by the physicians in the Neonatology Division of the Medical College of WI. The Chief of the Neonatology Division, Dr. Girija Konduri, has established a research program to investigate the mechanisms of respiratory failure that can occur at birth. Dr. Konduri’s research is focused on the mechanism of a condition that affects newborn babies called Persistent Pulmonary Hypertension of the Newborn (PPHN). When babies are inside their mother’s womb, their lungs are filled with liquid and do not participate in getting oxygen into the blood. Since the fetal lungs are not respiring, they receive only a small fraction of the blood pumped by the heart. When the baby comes out and the umbilical cord is clamped, the lungs have to fill with air and take over the function of providing oxygen supply to the body. This requires establishing circulation of blood through the lungs to transport oxygen from the lungs to the baby’s organs. In babies with PPHN, the circulation of blood to the lungs does not get established and the baby becomes blue. The affected infants suffer from consequences of decreased oxygen supply to vital organs and have an increased risk of death or long term disabilities. Dr. Konduri’s research is focused on understanding the normal mechanisms by which circulation gets established in the lungs at birth and why this process does not occur in babies with PPHN. He found that constriction of an important blood vessel that connects heart and lungs in the fetus, called ductus arteriosus, leads to development of PPHN. He also found that blood vessels in the lung have increased amounts of oxygen free radicals in PPHN. This research is evaluating the reason for the narrowing of the ductus arteriosus in the fetus and a possible approach to clearing the oxygen free radical accumulation from the blood vessels in the lungs of babies with PPHN. These studies will lead to improved survival and decreased disability rates in affected infants.

Over the last 4 years, novel observations made in Dr. Konduri’s laboratory found that the cells lining the blood vessels have dysfunction of the mitochondria that normally consume oxygen to provide ATP, the cellular source of energy. New studies proposed by the investigators in the laboratory lead to the award of 4 new grants from NIH over the last 18 months to study alteration in lung biology in pulmonary hypertension. In addition to Dr. Konduri’s, two other neonatology faculty members are actively investigating lung biology. Dr. Ru-Jeng Teng studies oxidative stress and its effects on angiogenesis in the lung as well as tetrahydrobiopterin and the function of nitric oxide synthase in the lung. Dr. Venkatesh Sampath’s work is described in another section. In addition to these 3 faculty members, there are currently 4 neonatology trainees that are conducting research in the CVC neonatology laboratory. The support of the CVC with its collection of scientists and core facilities and resources is critical to the success of the overall mission of the Neonatology section.
**Meetha Medhora, PhD**

Effects of ionizing radiation on the lungs. Dr. Medhora’s group is identifying agents to mitigate injury to the lungs that may occur after radiotherapy, a nuclear accident or radioterrorism. Using a rat model, they have observed radiation to the thorax resulting in lung injury after 6-8 weeks. This recovers and is followed by fibrotic remodeling of lung tissue starting 7 months after exposure. They measure acute and chronic injuries to the pulmonary vasculature. Currently they are testing a number of pharmaceutical reagents in an effort to identify mitigators and treatment for the radiation lung injury. In the past year they have been funded to study the effect of combined injuries by radiation to the lung and skin. This is a collaborative project between the Departments of Radiation Oncology, Dermatology and Medicine at MCW and the Radiation Center at the University of Rochester.

The Figure below demonstrates the effect of radiation on the lung and the mitigating effect of drug treatment with captopril on the injury. Sections of rat lungs stained with hematoxylin and eosin show radiation induced increase in cellularity and loss of alveolar structure (middle panel). This injury is mitigated by treatment with captopril started soon after irradiation.

Vasoreactive and antiapoptotic actions of EETs and 20-HETE (products of the essential fatty acid arachidonic acid). The endogenous fatty acids EETs and 20-HETE have potent vasoreactive and other properties. In collaboration with the laboratory of Dr. Elizabeth Jacobs they have demonstrated that EETs protect pulmonary arteries ex vivo using vessels from three species, human, mouse and rat. 20-HETE also protects rat pulmonary and cerebral arteries from hypoxia and reoxygenation injury ex vivo. Dr. Medhora and her group are currently studying the intracellular mechanisms for protection by these fatty acids.

**Daisy Sahoo, PhD**

Dr. Sahoo and her group are measuring HDL function and developing a clinical assay that will revolutionize assessment of cardiovascular risk.

Despite the years of epidemiological evidence that supports an inverse relationship between HDL-cholesterol levels and cardiovascular disease (CVD), the antiatherogenic benefits of raising HDL levels are currently controversial. For example, genetically-modified mice that have high HDL levels are more prone to atherosclerosis. Further, recent large-scale clinical studies revealed that patients who had normal levels of HDL-cholesterol still suffered cardiovascular events. Moreover, use of a drug that nearly doubled HDL levels in a phase III human clinical trial came to an abrupt end due to high patient mortality. For these reasons, there is now a greater emphasis on measuring “HDL function” as a better predictor of CVD than low levels of HDL-cholesterol.

Basic scientists of the Atherosclerosis Affinity Group of the Cardiovascular Center, Drs. Kirkwood Pritchard, Hao Zhang and Daisy Sahoo, are currently developing novel *in vitro* assays of HDL function that have the potential to revolutionize the way physicians diagnose and treat patients with cardiovascular risk. Recent studies suggest that the oxidation status of HDL plays an important role in determining how this lipoprotein prevents or promotes atherosclerosis. As such, Drs. Pritchard, Zhang and Sahoo hypothesize
that oxidation impairs HDL function by altering its ability to bind biomolecules (i.e. cargo) that are involved in inflammation and HDL-dependent cholesterol metabolism, thus making it atherogenic and unable to efficiently participate in reverse cholesterol transport.

Using biolayer interferometry (BLI), a new label-free technique for measuring biomolecular protein-protein interactions, these investigators are now able to measure the rates at which functional and non-functional (i.e. oxidized) HDL binds key proteins that are critical for cholesterol removal from the body. BLI analyzes the interference pattern of light reflected from two surfaces. The first surface is the layer of immobilized protein that serves as an internal reference. As the number of biomolecules binding to the biosensor increases, the aqueous protein layer increases, causing a shift in interference that can be measured in real-time. The Octet Red is a fully automated, 8 channel BLI instrument. It requires extremely small sample sizes and is highly reproducible. The design of the Octet Red is equivalent to having 8 BiaCore instruments, which use a similar technology (surface plasmon resonance) to analyze protein-protein interactions. The Octet Red can measure protein concentration (via determining rates of antigen-antibody binding) in 96 samples at once, in less than 20 min and at a cost of ~30¢/sample. Furthermore, HDL binding affinity for different proteins at the same time can be determined quickly and in real time.

The Figure below is a diagram of BLI biosensor loaded with anti-apoA-I antibody to capture HDL. Immunocaptured HDL is then probed with specific antibodies, pro- and anti-oxidant enzymes, cholesterol metabolizing enzymes or the extracellular domains of different receptors and transporters.

Already, preliminary data demonstrate that oxidized HDL (generated in vitro) directly impacts its ability to bind proteins associated with inflammation and cholesterol transfer as compared to HDL that was not oxidized. More importantly, HDL isolated from hypercholesterolemic mice displayed increased binding affinities for pro-oxidant enzymes compared to HDL isolated from healthy mice. Therefore, these data provide strong support for the concept that BLI assays are able to distinguish between control HDL and HDL from established murine models of atherosclerosis.

Drs. Pritchard, Zhang and Sahoo have recently submitted an R01 grant proposal to the National Institutes of Health to support the continuation of these highly innovative studies. The objectives of this proposal are to determine how oxidized lipid and protein composition of reconstituted HDL influences HDL’s interactions with key biomolecules as cholesterol is transported from the arteries to the liver for disposal. These studies will be performed in parallel using cell culture assays and BLI methodologies. Critically, BLI assays will be used to analyze samples from healthy patients, as well as those with clinically-documented atherosclerosis, to determine if and the extent to which atherosclerosis impairs HDL interactions with biomolecules that mediate HDL metabolism. Together, these exciting studies will, for the first time, test the hypothesis that HDL binding affinity can be used to distinguish between functional and dysfunctional HDL.

Successful completion of the above studies will lay the foundation for the development of new clinical assays for determining HDL “function”. The BLI assays will allow us to perform large clinical studies to fully test the idea that dysfunctional HDL is a better indicator of atherosclerotic risk. Further, these standardized, validated, accurate and robust assays have high diagnostic potential as they can be performed quickly, and in a cost-effective manner in a clinical laboratory setting. As large-scale application of our assays holds greater promise for predicting CVD than HDL-cholesterol levels, physicians may be able to prevent cardiovascular events in patients with normal HDL-cholesterol because, for the first time, they will be able to identify which patients have dysfunctional HDL.
Venkatesh Sampath, MD, MRCPCh

Our laboratory seeks to understand the role of innate immune signaling in the pathogenesis of diseases of premature infants. Our research focuses on how lipopolysaccharide (LPS) mediated endothelial injury contributes to vascular remodeling in bronchopulmonary dysplasia (a chronic form of inflammatory lung disease in premature infants). We have identified NADPH oxidase (Nox) as a critical regulator of LPS mediated endothelial injury using in vitro techniques. We propose to expand on this research by identifying the specific isoforms of Nox that mediate LPS-dependent endothelial toxicity and examining the biological mechanisms underlying Nox-dependent endothelial injury and microvascular remodeling. This work is supported by a R03 grant from the National Institutes of Child Health and Development (NICHD) entitled “LPS-mediated oxidative stress and pulmonary vascular injury in bronchopulmonary dysplasia”.

On the translational aspect, we are investigating whether functional genetic variation in the Toll-like receptors pathway genes (innate immune genes) modulate susceptibility to bronchopulmonary dysplasia and other diseases of the preterm infants. Our preliminary data suggest that loss of function polymorphisms in this pathway does increase susceptibility to lung disease in premature infants. This research is funded by a NIEHS Children’s Environmental Health Sciences pilot award entitled “TLRs-modulators of environmental lung injury in premature infants”, which will determine whether TLR/Nrf2 genetic variation alters susceptibility to oxidative stress and susceptibility to bronchopulmonary dysplasia in preterm infants.

Jennifer Strande, MD, PhD

Dr. Strande researches the role of thrombin and its receptors in the inflammation and fibrosis that occurs in the heart after ischemic events. While reducing risks using preventive strategies is important in combatting heart disease, we still need new treatment strategies for prevention of the adverse effects of heart disease.

The clotting pathway culminates in the formation of thrombin, which is best known for acting on its cellular receptors to cause platelet aggregation and thrombosis. One of the primary treatments strategies for patients with coronary artery disease is to acutely stop this process, in the condition of acute myocardial infarction, or to chronically inhibit this process to prevent recurrent heart attacks. Thrombin also acts through its receptors on other cell types and organs such as liver, lung and kidney to cause effects such as inflammation and fibrosis. Inflammation and fibrosis contribute to left ventricular remodeling and eventually cardiomyopathy.

Dr. Strande has shown that in rats treated with either direct thrombin inhibitors or thrombin receptor antagonists, the heart is protected against ischemia by a reduction of the damaging effects of free radicals and inflammation. Furthermore, she has shown that in long term studies, a thrombin receptor antagonist also limits cardiac fibrosis and protects the heart from adverse remodeling after myocardial infarction. As a physician, Dr. Strande sees first-hand how heart damage resulting from heart attacks affects the mental and physical well-being of patients and their families. By recognizing the gaps in treatments for people affected by heart attacks and heart failure, she can identify new targets for potential therapies. She also has the basic research training to perform the research studies needed to advance these new potential therapies which may have a major impact on keeping patients with cardiovascular disease feeling better.

The Figure below shows typical photographs of myocardial slices from three control (top row) and three
parstatin-treated hearts (bottom row). Infarcted areas are pale grey, whereas viable myocardium is dark red. There is a pronounced protecting effect of parstatin on the area of the myocardium infarcted.

Michael Widlansky, MD, MPH

While poor blood sugar control has been shown to be related to worse outcomes in patients with diabetes, recent large studies trying to determine if tight blood sugar control reduces cardiovascular risk have been disappointing. The reasons behind these disappointing results remain unclear. However, all of these studies have shown patients who use medications to try to tightly control their blood sugar levels have much higher rates of low blood sugar levels. Low blood sugar levels have been associated with poorer outcomes as well, but the mechanism of this remains unclear. Our laboratory has been using both cell culture studies as well as studies in human blood vessels to determine if short exposures to low glucose, as are common in tight blood sugar control regimens, cause blood vessels to lose their ability to dilate and develop inflammation. Inhibition of the ability to dilate and the development of inflammation in blood vessels is known to increase the risk of heart attacks and strokes. Our current findings suggest that short exposure to low glucose does lead to this abnormal blood vessel function.

Our future plans are to study this effect in humans using a model that confines the low glucose levels to the blood vessels in a forearm. Data from these studies may reveal new targets and directions for therapy to reduce cardiovascular risk in diabetic patients.
Carol Williams, PhD

Atherosclerosis and its complications cause almost one million deaths per year in the United States. Atherosclerosis results from excessive proliferation and migration of vascular smooth muscle cells, which promotes plaque formation and blockage of blood flow in major vessels. The Williams’ laboratory is investigating novel ways to inhibit the proliferation and migration of vascular smooth muscle cells, which will help define new ways to prevent and treat atherosclerosis.

Dr. Williams and her team are focusing their research on a group of proteins called “small GTPases”. Small GTPases are emerging as therapeutic targets in atherosclerosis, because these proteins promote the migration and proliferation of vascular smooth muscle cells. There are many different types of small GTPases, including the proteins known as Rho, Rac, Ras, and Rap. Since there are so many different types of small GTPases, inhibiting the activity of only one type of small GTPase might not be enough to stop the excessive proliferation and migration of vascular smooth muscle cells during atherosclerosis. Instead, simultaneously blocking the activity of multiple small GTPases at one time might be a better way to inhibit atherosclerosis. The Williams’ laboratory is investigating this new strategy of simultaneously inhibiting multiple small GTPases at one time, by conducting research on the unique protein known as SmgGDS. SmgGDS can interact with multiple small GTPases, including Rho, Rac, Ras, and Rap. This unique feature makes SmgGDS a master regulator of these small GTPases. The Williams’ laboratory is testing the hypothesis that blocking the activity of SmgGDS in vascular smooth muscle cells will block the activity of all small GTPases in these cells, and diminish the migration and proliferation of vascular smooth muscle cells.

The Figure below illustrates how vascular smooth muscle cells make many types of small GTPases, including Rho, Rac, Ras, and Rap. These small GTPases can promote the proliferation and migration of vascular smooth muscle cells, which can promote atherosclerosis. SmgGDS is a unique protein that regulates all of these small GTPases. Stopping the interaction of SmgGDS with small GTPases might offer a new therapeutic approach to prevent and treat atherosclerosis. ♥
Normal blood flow in the heart is essential. When coronary blood flow is reduced or blocked as occurs in coronary artery disease, the supply of oxygen and nutrients to the heart is impeded, which can lead to a heart attack. Dr. Zhang lab’s current focus is to study the signaling mechanisms by which shear stress, a mechanical force generated by blood flow, causes blood vessels to dilate. Flow-mediated dilation is one of the most important regulators of blood vessel tone and regional blood flow. This lab has recently received a five-year grant from the National Institutes of Health’s National Heart, Lung, and Blood Institute to investigate whether a calcium ion channel (TRPV4) located on the cell surface membrane of vascular endothelial cells serves an essential and conserved signaling component for shear-induced dilation in mice and humans. These studies will contribute to a better understanding of how blood flow is regulated in the heart, and may lead to new therapeutic targets for coronary heart disease and other cardiovascular disorders.

Ashraf El-Meanawy, MD

Fetal programming of kidney development can have a significant impact on the development of hypertension and chronic kidney disease (CKD) in adult life. Reduced nephron number at birth is a widely identified risk factor for hypertension in humans. The most common causes of reduced nephron number in man are prematurity and intrauterine growth retardation (low birth weight). In 2008 12.3% of life birth in the United States occurs prematurely with significant racial predominence. The molecular pathways leading to low nephron number are not known; moreover the mechanisms by which low nephron number lead to hypertension and CKD is not well studied and mainly speculative. Dr. El-Meanawy’s laboratory is studying kidney development, specifically in relation to low nephron number and its effect on adult blood pressure and renal disease susceptibility. Genetic control of kidney development: Dr El-Meanawy’s laboratory has been investigating the genetic causes of reduced nephron number in the oligosyndactyly (Os) mouse model. The mutant mice are born with half the nephrons of the wild type and develop progressive proteinuria, renal dysfunction and histopathology picture of focal and segmental glomerulosclerosis.

We used fluorescent in situ hybridization and identified the Os mutation to be an inversion affecting chromosome 8 in the Os mouse. There is no significant genetic material loss from the affected chromosome, but gene rearrangement. We identified a finite number of candidate genes in the Os locus to be deregulated in the developing Os kidney. We have established the role of the identified candidate genes in nephrogenesis using an in vitro metanephric organ culture system in combination with lentivirus technology. We currently are investigating the mechanism of candidate genes control of renal development.

Prematurity, kidney disease and hypertension: We developed and characterized a mouse model of prematurity induced nephron number reduction. The premature mice displayed >20% reduction in nephron number. The mice developed significant proteinuria
and the glomeruli showed the expected compensatory hypertrophy. The premature mice showed elevated blood pressure compared to full term animals. The study of this mouse model will improve our understanding of fetal programming of kidneys and will help the development of therapies to support normal organ development in premature infants.

The Figure 1 below presents the images of kidney sections from Os and WT (wild type) mice stained with Masson’s trichrome showing the increased collagen deposition in the Os glomeruli (20X magnification).

The Figure 2 shows that the uteric bud branching is reduced when the culture is infected with the lentivirus carrying test gene (right), compared to a control lentivirus.

Frank Park, PhD and Kevin Regner, MD

Renal epithelial cells play a fundamental role in kidney health and disease. During normal development, the renal epithelium proliferates in an orchestrated manner ultimately leading to a mature, functioning kidney. Interestingly, in mature kidneys the relatively dormant renal epithelial cells can revert to a state of active proliferation due to defects in specific genes, such as in polycystic kidney disease (PKD), or following acute kidney injury. The mechanisms involved in regulating this change in epithelial cell number are poorly understood.

Because of the hierarchical nature of G proteins within the signal transduction cascade, our lab postulates that heterotrimeric G proteins are critical molecular switches that activate pathways involved in epithelial cell proliferation in PKD and following acute kidney injury. The classic mechanism by which G proteins transmit information from the outside of the cell is through the stimulation of G protein coupled receptors (GPCRs). Emerging evidence suggests that heterotrimeric G protein activity and signaling can be regulated independently of cell surface receptors through the action of a number of accessory proteins. Recent studies in our lab have identified abnormal expression of one particular accessory protein, activator of G protein signaling 3 (AGS3), in kidneys from multiple models of autosomeal recessive (ARPKD) and autosomal dominant polycystic kidney disease (ADPKD), as shown in the Figure below. The Figure illustrates the increased protein production of AGS3 in ADPKD kidneys. (A) Gross morphology of kidneys harvested from normal and ADPKD mice. (B) Protein expression of AGS3 is shown to be markedly elevated in ADPKD kidneys compared to normal kidneys. (C) GAPDH is a loading control and did not differ between normal and ADPKD samples. This demonstrates that the same amount of protein was used and any differences in AGS3 levels is attributed to real changes in protein abundance.

This unexpected finding led us to postulate whether normal renal epithelial cells can be induced to express AGS3. To address this question, our lab collaborated with a clinical investigator, Kevin R. Regner M.D.,
whose research focuses on acute kidney injury. We found that acute kidney injury is a potent inducer of AGS3 in renal epithelial cells. Conversely, genetic loss of AGS3 markedly impairs the regeneration of the kidney following injury, and may have the potential to reduce the progression of PKD.

In all, our scientific contribution provides the first step in a continuum of research that is expected to establish a fundamental role for AGS3 in promoting epithelial cell proliferation following biological perturbations. These new studies may open the door to the development of novel therapeutic interventions targeted to AGS3 and its associated G-protein subunits to either promote renal regeneration following acute kidney injury, or inhibit cystogenesis in proliferative genetic diseases.

**Andrey Sorokin, PhD**

A recent study of Dr. Sorokin provides evidence for a novel mechanism by which COX-2 activity is regulated and was just published (Yang C. and Sorokin A., Cell Signal. 2011; 23: 99-104). The study first showed that COX-2 induced the expression of fibronectin and that this involved the cyclooxygenase activity of COX-2, which was underscored by the fact that prostaglandin E2 also stimulated the activity of the fibronectin promoter. The study then demonstrated that COX-2 binds to adaptor protein ELMO-1 and that ELMO-1 enhances the COX-2-mediated induction of the fibronectin promoter. Thus, we demonstrated that COX-2 cyclooxygenase activity was increased as a result of protein-protein interaction between ELMO-1 and COX-2. These results, together with co-precipitation and colocalization data, suggested a novel mechanism of regulation of COX-2 activity and identified ELMO-1 as a protein which regulates COX-2 through direct protein-protein interaction. The significance of our findings is based on the fact that regulation of cyclooxygenases through protein-protein interactions is a novel concept and our data set the basis for design of new type of COX-2 inhibitors capable to interfere with protein-protein interactions important for increase of cyclooxygenase activity in human pathologies. Our study uncovers a novel type of cellular regulation of COX-2 activity by protein-protein interaction. This is an original concept which was never proposed for either cyclooxygenases or for other enzymes which catalyze arachidonic acid.

The Figure below shows that ELMO-1 forms a complex with COX-2 in mesangial cells. Expression of the endogenous and overexpressed proteins was determined by immunoblotting of total cell lysates (TCL).

Increase of COX-2 activity is an important factor in a wide variety of pathologies including inflammation, multiple types of cancers and renal diseases. Even though selective inhibitors of COX-2 activity are generally accepted as efficient drugs to combat these pathologies, there are important issues with side effects of these drugs. Our study uncovers a novel type of cellular regulation of COX-2 activity by protein-protein interaction. This is an original concept which was never proposed for either cyclooxygenases or for other enzymes which catalyze arachidonic acid.
According to the National Institutes of Health, more than 25 million people, or 8.3% of the population, have diabetes in the United States. This incidence is expected to increase in coming years. Based on the National Diabetes Information, the cost of diagnosed diabetes in 2007 was 174 billion dollars. Numerous complications have been associated with diabetes and include heart disease, kidney disease, high blood pressure, and stroke. Diabetic kidney injury can lead to end-stage renal disease and mortality. In diabetic patients, increased salt reabsorption in the kidney may play a role in the development of high blood pressure; however, the mechanism is not known.

Our research has provided evidence that a novel kidney transporter known as SGLT3 may play a role in development of high blood pressure in diabetes by mediating increased kidney absorption of salt in response to increasing levels of glucose. The long-term goal of our research study is to find means to inhibit increased salt absorption in the kidney and prevent the development of hypertension in diabetic patients.

The Figures below depict the role of renal SGLT in the pathogenesis of diabetes. We use cultured kidney cells from human (A), mouse (C), and pig (D), to measure changes in gene and protein expression levels of SGLTs. We also examine SGLTs function by performing assays either on the individual over-expressed protein (B) or on SGLT expressed at the cellular level (A, C, D). Fluorescence techniques are used to measure transports of glucose (C) and sodium (D) by SGLTs in kidney cells.
The general mission of the CVC Advisory Board is to serve as advocates in the community, thereby supporting the primary objectives of the CVC. These objectives are to contribute to the eventual elimination of heart disease, stroke and other cardiovascular diseases as human health problems through coordinated research, clinical treatment, control and education, and to provide the citizens of Southeastern Wisconsin with state-of-the-art therapy, research, control and educational programs for all aspects of cardiovascular disease. The specific purpose of the CVC Advisory Board is to provide financial support for the research and clinical programs carried on by the CVC and to educate the community in cooperation with the Board of Trustees of The Medical College of Wisconsin on the causes, prevention, diagnosis and treatment of cardiovascular disease. Bruce E. Jacobs, a lifetime member of the CVC Advisory Board, was the Founding Chair. Other lifetime members include Byron Foster, Gordon Gunnlaugsson and William H. Levit, Jr.

Current composition of the Board

| Chair: | Nancy J. Sennett |
| Vice-chair: | Johan C. R. Segerdahl |
| Emeritus Members: | James D. Bell, William D. Browne, John J. Burke, Jr. |
It is anticipated that the Medical College of Wisconsin will be investing nearly $100,000 generated from the event into heart research that will help the Cardiovascular Center find new ways to save lives. The CVC Golf Challenge is a high profile event which has benefitted from the support of business and community. It is truly unique, intimate, focused on golf and known as one of the most enjoyable charitable outings in the greater Metropolitan Milwaukee area. A strong 12-year run of the tournament has raised nearly $1 million to advance breakthrough heart research. The philanthropic support generated by the golf tournament enables scientists to start and continue promising research.

“Have a heart ” Motorcycle Ride

This ride took place on June 11, 2011. It is a 70-mile scenic ride through the hills and countryside of Southeastern Wisconsin to benefit heart research.

It is organized and sponsored by Harley-Davidson® Powertrain Operations, W156 N9000 Pilgrim Rd, Menomonee Falls, WI. After the ride, there is complimentary lunch and live music by “Maple Road”, “Jasper Oats” and “October Soul”, raffle and door prize drawing at Suburban Motors. A commemorative pin is given with pre-registration. The money raised benefits the Cardiovascular Center research.
Cullen Run/Walk

This event supports heart research at the Cardiovascular Center. To date, Cullen Run/Walk proceeds have helped several research programs, including studies to identify genetic risk factors of heart disease, to investigate the Female Athlete Triad and its potentially life-threatening link to heart disease, and to better understand the correlation between plaque buildup and coronary artery disease. The Cullen Run/Walk is an 8-kilometer USA Track and Field competitive run and a 2-mile fun run/walk following a scenic path along Underwood Parkway in Wauwatosa. Sponsored by the Cullen family and the Badgerland Striders, the event includes famous Cullen Family & Friends Chili, refreshments, food, door prizes, an awards ceremony and live music for the entire family. The event is held in memory of Steve Cullen, a former Milwaukee alderman, who died in 1995 at age 40 of sudden cardiac arrhythmia. His father, at age 41, and two brothers, ages 53 and 51, also died of heart disease. The Cullen Run/Walk has grown in attendance by over 500% since its inception, encouraging heart healthy lifestyles for all participants, their families and friends and contributing over $120,000 to cardiovascular research and awareness.
Geurts’ lab (from back to front): Jason Klotz, Bradley Endres, Molly Corbett, Sheila Reagles, Michael Grzybowski, Sheng Yang, PhD