UT Presidential Inauguration Week Event on the Health Science Campus
Proceedings of a Research Symposium
held in honor of the 17th President of
The University Of Toledo,
Dr. Sharon L. Gaber

Prince Ampem
PhD Trainee
Department of Physiology and Pharmacology

The University of Toledo College of Medicine & Life Sciences in conjunction with The Center for Hypertension & Personalized Medicine held a research symposium in honor of UT’s 17th President, Dr. Sharon L. Gaber on 22 September 2015. This memorable event, which took place on the Health Science Campus, brought to light the tremendous contribution that several years of hypertension research at UT has made in advancing cutting edge research. During the first session of the event, pre and post-doctoral trainees gave poster presentations focused on their research studies. A series of faculty oral presentations followed that was centered on the topic “Five Decades of Seminal Contributions of UT to Research in Hypertension.” The University of Toledo’s President, Dr. Gaber graced the event with her presence. The first talk entitled “Thought Leaders of UT in Hypertension Research: A Historical Perspective”, was given by Dr. Maurice Manning, Distinguished University Professor in the Department of Biochemistry and Cancer Biology who has served the University for over 45 years. He gave an impressive and thorough historical description of the people who have established and sustained hypertension research at UT, including the pioneers Dr. Murray Saffran, Dr. Amir Askari, Dr. Patrick Mulrow and Dr. John Paul Rapp. One of Dr. Rapp’s successors in the Department, Dr. Bina Joe has since taken on the mantle of leadership and made outstanding progress in the area of hypertension research. She is the founder and current Director of The Center for Hypertension & Personalized Medicine at UT.

The next two talks focused on highlights from recent research into the genetics of hypertension at UT. Dr. Bina Joe, chair of the Department of Physiology and Pharmacology discussed her laboratory’s work on the genetic causes of hypertension, including recent findings published in Nature Communications that targeted mutation of the transcription factor NR2F2 in rats validate genome-wide association studies showing that it is associated with hypertension in humans. In addition, she described her lab’s recent findings demonstrating a link between gut microbiota and blood pressure regulation. Dr. Ashok Kumar, Professor in the Department of Physiology and Pharmacology, talked about polymorphisms in the human angiotensinogen gene and showed that multiple transcription factors such as HNF1, C/EBP and glucocorticoid receptor bind more strongly to the promoter of this gene. This may lead to increased expression of this gene and increased blood pressure.
Several other talks that followed were focused on improving renal function, a critical area of current hypertension research at UT. This series started with Dr. Christopher Cooper, Dean of the College of Medicine who has a very strong passion for research. He described results from the Cardiovascular Outcomes in Renal Atherosclerotic Lesions (CORAL) clinical trial conducted at the University of Toledo with Dr. Cooper serving as the principal investigator. Results from the CORAL trial showed that stents used to relieve atherosclerotic renal-artery stenosis do not ultimately improve patient outcomes compared to standard medical therapy. These results were published in the New England Journal of Medicine in 2014. Dr. Steven Haller, Assistant Professor in the Department of Medicine reported related work showing that low levels of circulating CD40 are associated with loss of kidney function in patients with atherosclerotic renal artery stenosis, which led to studies using a custom-engineered rat model at the CD40 locus. He presented data to support the view that Cd40 could be a potential target to prevent kidney damage.

With supporting data from their extensive research, the next two speakers tied together kidney and cardiac health. Dr. Jiang Tian, Associate Professor in the Department of Medicine, described findings in support of his hypothesis that kidney disease can lead to cardiovascular disease through activation of Na+/K+ ATPase related signaling pathways. Dr. David J Kennedy, a former graduate student at the University of Toledo who recently returned from his Fellowship at the Cleveland Clinic and is now an Assistant Professor in the Department of Medicine, discussed his work on the regulation of cardiotonic steroids. In particular, he described his 2013 paper in the Journal of the American Heart Association and follow up studies showing that an HDL-associated enzyme called Paraoxonase protects against adverse cardiovascular events in humans and protects against cardiac injury in mice.

The final speaker, Dr. Guillermo Vazquez, Associate Professor in the department of Physiology and Pharmacology described his laboratory’s work showing that TRPC3, a Ca2+ - permeable channel, contributes to development and progression of atherosclerosis. Some of his laboratory’s recent findings using a transgenic mouse model with endothelial overexpression of the human TRPC3 channel were published in Proceedings of the National Academy of Sciences (PNAS). He described that his work on macrophage and endothelium has revealed TRPC3 as a molecule with potential to be exploited as a therapeutic target in preventing atherosclerotic plaque progression and rupture.

Throughout the symposium and across all the different research areas, several themes emerged: (1) Teamwork is key to success in the current research climate; both internal and external collaboration is critical. (2) With hard work and relentless dedication, unfunded research can lead to exciting findings that provide the basis for future funded work. (3) Clinical observations can be explored fruitfully in animal models and the new knowledge gained can translate to new therapeutic options in the clinic. (4) In the research setting, a tolerance for failure is required for ultimate success. (5) Research in both males and females is important for clinical relevance. In summary, this research symposium not only provided an opportunity to discuss about ongoing research in hypertension at UT, but also created a suitable platform to ignite collaborative work in an effort to advance translational research at UT.
University of Toledo Integrated Core Facilities

Andrea Kalinoski, Ph.D.
Technical Director, Integrated Core Facilities

The Core Research Laboratories at The University of Toledo Health Science Campus have been consolidated into one program called The University of Toledo Integrated Core Facilities (ICF) under the direction of David Allison, M.D., Ph.D. and Andrea Kalinoski, Ph.D., Technical Director. This integration of the previous Advanced Microscopy & Imaging Center, Flow Cytometry and the Genomics Core Facilities will help carry out UT’s strategic mission to improve the human condition by providing state of the art technological support to the UT research community. The ICF in the College of Medicine acquires, maintains and supports high-end instrumentation that facilitates cutting-edge research to be performed by helping researchers economically and efficiently take advantage of innovative technology and collaborate with experts in the field. The ICF serves the Research Enterprise of the University of Toledo Health Science Campus, Main Campus, nearby universities as well as a number of industrial users in the surrounding communities. The ICF also provides individual investigator consultation, collaboration and/or discussion of project design, expenses for preparation of grant applications, letters of support, publications and faculty recruitment. We provide the expertise, training and supervision for faculty, staff, students and outside users on the operation of all systems. Individual or group training sessions are conducted several times a week and we are available to assist as needed when users have problems or questions on the individual systems.

The ICF provides laser-based flow cytometry and cell sorting, fluorescence and light microscopy, advanced laser scanning confocal microscopy, electron microscopy, whole animal imaging, non-invasive ultrasound technology, histology services and gene expression analysis on the Affymetrix GeneChip platform, of the highest quality at an economical price. The ICF also offers comprehensive training on all instruments, online scheduling calendars and provides letters of support for grant applications. The ICF also asks for faculty advice on strategic investments to be made in future state-of-the-art technologies and/or technical expertise to best support our research mission. If you would like to tour the facilities, set-up an imaging appointment or discuss a new project/feasibility of studies please contact:

Andrea Kalinoski, Ph.D.
Block Health Science Building
Room 048
419-383-4205
andrea.kalinoski@utoledo.edu

David Weaver, D.D.S., Ph.D.
Block Health Science Building
Room 053
419-383-6105
david.weaver@utoledo.edu

All University of Toledo Integrated Core facilities can also be found online: http://www.utoledo.edu/corelabs/

Instruments available in the UT Flow Cytometry Core include:

BD Biosciences FACSCalibur™ system is an automated benchtop flow cytometry system that offers four-color capability and can perform cellular analysis. Designed specifically to support a wide range of applications, with easy to use software, multi-sample loading options, and intuitive instrument and fluidics control, to improve laboratory productivity. Specifically, the FACSCalibur system is used for routine cell analysis, assay development, verification, and isolation of cellular populations of interest. Sample analysis is easy and quick due to the alignment-free optical design, interbeam compensation, and dual-laser technology.
**FACSAria IIu High-Speed Cell Sorter** features a gel-coupled cuvette flow cell with interchangeable nozzles to accommodate a range of particle sizes for high-performance analysis and sorting. There are 3 lasers (Violet- 407nm, Argon Blue- 488nm and a Helium Neon Red- 633nm) and 12 detectors available for analysis and/or sorting. Advantages include: a high-speed sort rate with up to ~25,000 events(cells)/second with aggregate discrimination by BD doublet discrimination module. Multi-well plate accessories are available with temperature controlled sample and sorting receptacles and an aerosol management system.

**BD BACSAria I1U 488nm, 633 and 405nm lasers**
- 13 color, 15 parameter sorting and analysis
- Aerosol Management system for containment of pathogens
- 1, 2, 3, and 4 population sorting at one time
- direct cell sorting into 6, 12, 24, 48, 96, and 384 well plates
- High viability and purity of sorted populations

Flow Cytometry Facility  
Health Science Campus  
Health Education Building  
Room 233a  
Phone: 419-383-3402

**Purification of Cell Populations**

**Instruments available within the UT AMIC Core Facility:**

**Leica TCS SP5 Laser Scanning Confocal Microscope** equipped with both conventional and high-speed resonance scanners. This includes 5 conventional lasers plus multi-photon excitation, producing the following laser excitation lines: 458, 488, 514, 561, 633, and a tunable Ti-Sapphire MP laser 710-990nm. This system is capable of collecting up to 5 colors simultaneously for quantitative confocal image analysis in both live cell and animal imaging, fixed tissue and includes the capabilities for 3D reconstruction, FRAP and FRET, animation, stereo imaging, single layer projection, time lapse collection, and co-localization analysis.
Olympus IX81 Inverted Microscope equipped for TIRFM (Total Internal Reflection Fluorescence Microscopy) with 3 solid state laser lines (488, 543 and 633). This system is run on MetaMorph software and is equipped with an environmental chamber for live cell imaging using either UV or lasers as an excitation source. This system is also equipped with an automated turret for long term live capture using multiple fluorescent markers that allows the visualization of fluorescent molecules either in wide-field (conventional) or exclusively at the cell-glass interface. This latter capability allows selective, real-time tracking of single molecule or particle dynamics at the surface of living cells.

Zeiss Axioplan upright light microscope for brightfield and differential interference contrast (DIC) microscopy. Image capture is performed electronically with a Zeiss Axiocam 105 Color - 5 Megapixel digital camera. This is a small, fast (up to 47 fps) camera system for all routine imaging at high resolution.

IVIS | Spectrum

**IVIS Spectra**n whole animal fluorescence imaging system developed by Xenogen/Caliper Life Science. The IVIS Spectrum is a multimodal bioluminescent and fluorescent imaging system specifically designed for noninvasive imaging of cells and tissues in small animals. This instrument facilitates the study of biological processes via fluorescence in small animals, including tumor growth, cancer metastasis, bacterial infections, immune responses and inflammation, and regulation of tissue-specific gene expression.

Acuson Sequoia™ C512 cardiac ultrasound imaging system by Siemens Medical Solutions. This echocardiography system is widely accepted as a valuable research tool for studying a broad range of cardiovascular disease processes in small animals, including ischemic heart disease, heart failure, cardiac hypertrophy and remodeling, hypertension and diabetic cardiomyopathy. The Sequoia system provides a full range of echocardiographic capabilities including high-resolution imaging, tissue harmonic imaging, differential echo amplification, spectral Doppler (Pulsed and Continuous Wave), color Doppler (for measurements of velocity energy and tissue Doppler imaging), and M-mode and color-Doppler M-mode imaging. This system is extremely versatile and ideal for noninvasive imaging of research animal models including rats and mice.
Left Ventricular function analysis in a rat shock model. A) Baseline B) Shock and C) Resuscitation

Advanced Microscopy & Imaging Center
Health Science Campus
Block Health Science Building
Room 057
Phone: 419-383-4205

The Genomics Core Laboratory performs gene expression analyses with the Affymetrix GeneChip system. Standard Affymetrix protocols are used by the investigator to prepare biotin-labeled cRNA starting from either total RNA or mRNA. The GCL accepts only prepared biotin-labeled, fragmented cRNA samples. The hybridization is then performed, post-hybridization washing and staining, and scanning of fluorescence levels utilizing a GeneArray™ 3000 (6G) scanner.

Genomics Core Facility
Health Science Campus
Health Education Building
Room 200
Phone: 419-383-6105

The Histology Core Lab is offering services to researchers which include processing, embedding, sectioning and staining (H&E or special stains) of formalin fixed paraffin embedded tissues. Special stains include H&E, trichrome, PAS, Oil Red O, etc. Immunohistochemistry and immunofluorescence staining is also be provided.

The state-of-the-art Electron Microscopy (EM) Laboratory is part of the Advanced Microscopy & Imaging Center. The EM facility is directed by Dr. William Gunning who specializes in ultrastructural diagnosis of human disease and also provides research support to the University of Toledo. The EM lab is equipped with two transmission electron microscopes, one being used for clinical diagnostic purposes and the other available for use by researchers.

William Gunning, Ph.D.
Block Health Science Building
Room 029
419-383-3484
william.gunning@utoledo.edu

All University of Toledo Integrated Core facilities can also be found online:
http://www.utoledo.edu/corelabs/

New Faculty
The human body’s ability to regulate volume through the control of salt and water balance is one of the most fundamental physiologic processes which requires intricate integration of multiple body systems – including cardiovascular, nervous, endocrine, and urinary – and has enormous influence on both health and disease. Central to this process is an elegant regulatory system composed of effector steroid hormones – known as cardiotonic steroids (CTS) – and their receptor complex, the sodium-potassium adenosine triphosphatase (Na/K ATPase). CTS are ligands of the Na/K-ATPase and production of these hormones are a compensatory mechanism for natriuresis (excretion of sodium in the urine via action on the kidney proximal tubular Na/K-ATPase) and vascular tone in volume-expanded states such as salt-sensitive hypertension and chronic kidney disease (CKD), as well as edematous states like heart failure (HF) and pre-eclampsia.

However, CTS also exert “off-target” signal transduction effects beyond their direct effects on the Na/K-ATPase. Hence, chronic stimulation of Na/K-ATPase signaling by CTS has important implications for not only for the natriuretic response to increased salt and water load but also has been implicated in a “trade-off” pathological adaptation to volume expansion including hypertension, hypertrophy, and fibrosis. One of our lab’s major contributions to this field is the clinical and experimental evidence demonstrating the pro-inflammatory and pro-fibrotic pathways initiated by these steroid hormones in both cardiac and renal tissue in the setting of HF and CKD which make them attractive therapeutic targets for intervention in cardiac and renal disease (Figure 1, see article 1 and article 2).

Cardiac and renal diseases such as HF and CKD disproportionately affect minorities, especially African Americans and Hispanics, have a more malignant course in these populations, and levy an enormous and growing global socioeconomic and health burden on both individuals and family units across every continent. Patients with HF and CKD experience progressive cardiac and renal compromise (referred to as “cardio-renal syndrome”) leading to recurrent hospitalizations and clinical deterioration which contemporary therapies of neurohormonal blockade have failed to adequately address. As the synthesis and regulation of CTS in volume-expanded states such as HF and CKD is unknown, developing a fundamental, integrated, and mechanistic understanding of the CTS-Na/K ATPase effector/receptor complex is of critical importance. Thus, the overall goal of my laboratory’s ongoing and planned research program is to address this critical unmet need as it relates to the synthesis, regulation, translational significance, and therapeutic targets of the CTS-Na/K ATPase axis.

As it relates to the counter-regulatory mechanisms which help govern the CTS-Na/K ATPase axis, we have identified novel mechanistic interactions between the lactonase activity of an enzyme made in the liver (called Paraoxonase-3 or PON-3) and the lactone ring in CTS which is responsible for its interaction with the Na/K ATPase. PON-3 is a hydrolytic lactonase enzymes that is generated in the liver and may also circulate bound to high-density lipoprotein (HDL). Our preliminary experimental and clinical data demonstrate an association between diminished lactonase activities of PON and cardiac and renal disease severity and progression (see article 3), yet the underlying mechanism(s) are unknown. We have recently discovered that cardiac and renal fibrosis is significantly increased following insult in mice in which PON-3 is knocked-out. Together, these findings imply that PON-3 exhibits a protective role by modulating pro-fibrotic pathways.

Interestingly, the lactonase activities of PON-3 hydrolyze CTS to their open-ring forms which, unlike native CTS, are incapable of stimulating the collagen formation involved in heart and kidney fibrosis. As we have observed in our preliminary studies that circulating CTS levels are raised in the setting of HF and CKD, that CTS levels are inversely proportional to PON lactonase activity, that cardiac and renal fibrosis after CTS infusion or cardio-renal insult is increased in PON-3 knock-out mice, and that PON-3 gene therapy is capable of attenuating these effects, we hypothesize that CTS-induced adverse cardiac and renal fibrosis following cardio-renal insult is regulated by PON-3.
Hence, our approach is to test the hypotheses that cardiac and renal protection by PON-3 can deter a) progressive cardiac fibrosis and diastolic dysfunction in CKD; and b) progressive renal fibrosis and tubular dysfunction in HF and c) that the mechanism occurs via modulation of pathogenetic pathways induced by CTS signaling through the Na/K ATPase.

Kuali Coeus and Online Research Administration at UT

Jamie Van Natta
Research Systems Developer

William S. Messer, Jr., Ph.D.
Vice President of Research

Historically, The University of Toledo (UT) has a strong research community consisting of faculty, students and staff. The University strives to maintain a solid support system for that research community. As federal regulations change and research evolves, research administration and technical needs also change. To meet new challenges, UT chose to adopt a third party research administration system that would better facilitate both researchers and administrators in the UT research enterprise. Kuali Coeus (KC) was chosen for its breadth of functionality as well as its system-to-system submission capabilities.

The original Coeus software was developed at the Massachusetts Institute of Technology (MIT) as a client-server application. It was designed to be open-source, which meant that it was free to anyone in the university community who wanted to implement it. As more schools got on board, the product requests and development needs became too large for a single institution to handle and so a consortium was formed. The consortium developed the product together so that it not only handled proposal development and award tracking, but also human and animal research, conflict of interest and negotiation modules. Eventually that consortium joined an existing community, Kuali, to become the research administration piece of the Kuali suite of higher education products, which lead to it becoming a web-based open source solution.

Today, the Kuali Coeus product is still free and open source, but many institutions have taken the base code and customized it to meet their institutions’ need and business practices. This is the process in which we find ourselves at the University of Toledo. The decision was made to begin with the IRB module, as that seemed to provide the most opportunity to offer more services and value to the research community. We’ve moved deliberately and carefully as it was also a more immature module in the product and needed some customizations to accommodate the business practices at UT. We began using the product in the back office of the Department of Human Research Protections (DHRP) in December of 2013. As of January 2015, all new application submissions to the Social, Behavioral and Educational IRB have been submitted via KC. We are now happy to say that, with recent customizations, the Biomedical IRB will be requiring application submissions via KC for all Exempt and Expedited applications starting September 28th. Beginning in October, all application submissions including Convened research will be done via KC. See the IRB Corner of this newsletter for a detailed description of upcoming deadlines.

What’s up next for KC at UT? To complete the IRB functionality there are a few final customizations to get in place. Additionally we will be importing into KC all legacy human subjects research applications from our old research administration system so that all active human subjects research will be in one system and be maintained, renewed and amended in a consistent manner.

Currently in progress is the investigation and implementation of the next modules. The Proposal Development module is where funded research will be proposed, developed, budgeted, routed and electronically submitted. The Conflict of Interest module allows for electronic disclosure and maintenance of potential and actual financial and fiduciary conflicts of interest. Additionally, the Award module tracks and maintains funded research. Each of these modules will have an individual roll out plan and go-live date that will be published on the Research page of the UT website. Each module will also have in-person training available as well as online documentation as we progress.

There likely will be challenges to overcome as the new KC system is implemented, so faculty and student patience will be appreciated as we work through any difficulties. Also we welcome feedback from the research community as we look for ways to improve processes. It’s an exciting and challenging time for the research community at UT as we focus on growing the research enterprise and we look forward to a more streamlined research administration experience as we complete the implementation of the remaining modules.
Clinical Research Snippets

Anand B. Mutgi, MD
Sadik A. Khuder, PhD

Breast Cancer is the most common type of cancer in women with an incidence of 1.6 million cases per year worldwide. Despite early detection and improved treatments, breast cancer mortality remains high. Several preventable risk factors (genetics, hormones, nutrition, and life style) and treatments (use of aspirin, estrogen antagonists and prophylactic mastectomies) have been identified. Of these approaches, diet and nutrition have been studied extensively. This month we review a randomized controlled study that showed significant benefit of Extra Virgin Olive oil (EVOO) added to a Mediterranean diet.


This was a well-designed study (PREDIMED- Prevencion Con Dieta Mediterrania) comparing the effects of low fat diet vs. Mediterranean diet (rich in fruits vegetables, fish and olive oil) vs. Mediterranean diet combined with EVOO. The main outcome of benefits in cardiovascular disease were established within 5 years.

The study was conducted in primary care health centers in Spain and involved 4282 women and 3165 men between 60-80 years of age. The present article addressed a secondary outcome of prevention of “invasive breast cancer” in the 4282 women. Patients were randomly assigned to either a low fat diet control group (n=1391) or Mediterranean diet with 30 gm of mixed nuts (n=1285) or Mediterranean diet with EVOO supplement (n=1476).

There were 35 confirmed new cases of invasive breast cancer detected, (17 in control, 10 in Mediterranean diet and 8 in Mediterranean diet with EVOO). Breast cancer incidence was significantly lower in the group taking Mediterranean diet with EVOO and the risk reduction was 68% compared to the control and was statistically significant. Mediterranean diet with nuts did not reduce the risk significantly.

In discussing the limitations, the authors acknowledge the small number of cases of breast cancer and difficulty in assessing genetic and reproductive factors. The detection of cases was not standardized. However due to randomization these effects were minimized.

In explaining the benefits, EVOO is felt to provide a large amount of monounsaturated fatty acids including Oleic acid and other biologically active compounds that have anti-proliferative effects, reduce intracellular oxidative stress and DNA damage. Oleocanthin and Oleoropein inhibit tumor growth and cause apoptosis of breast cancer cells. Several other mechanisms have been proposed.

Considering the dual benefit of EVOO on prevention of cardiac events and reduction in invasive breast cancer with no major adverse effects, we feel adding EVOO to a Mediterranean diet goes a long way in preventing two high frequency and high impact diseases.

Note: study used 1 liter/week of EVOO per family but did not clarify the amount per individual. We estimate it to be 30 ml or 1 oz. per day per person.

New Clinical Trials

A Prospective, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of Preoperative Antithrombin Supplementation in Patients Undergoing High-Risk Cardiac Surgery with Cardiopulmonary Bypass.
Dr. Schwann - Surgery
A Phase 3, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study with an Open-Label Phase to Determine the Efficacy and Safety of Tozadenant as Adjunctive Therapy in Levodopa-Treated Patients with Parkinson’s Disease Experiencing End-of-Dose “Wearing-Off”.

Dr. Elmer - Neurology

Publication Update of Online Journal "Translation"

Keith Crist, Ph.D.
Editor-in-Chief, Translation

The Journal continues to solicit manuscripts, providing an opportunity for UT medical students, graduate students, residents, fellows and faculty to publish relatively modest but important observations in a timely manner.

Journal home page: http://utdr.utoledo.edu/translation

Aims and Scope: The journal will publish original articles of basic or clinical research, case reports, and reviews. The manuscript will be evaluated based on the results presented in its original submission, without reviewer recommendation for additional datasets in student projects completed under the Medical Student Summer Research Program. To date, the average time from manuscript receipt to completion of first review is 32 days. Average time from submission to publication is 139 days. There are no publication charges.

Manuscripts are published as they complete review and final approval.

IRB Corner

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Contact Us

Health Science Campus • Center for Creative Education Bldg.
2920 Transverse Drive, Floor 3 • Toledo, OH 43614
Phone: 419.383.6919 • ClinicalResearch@utoledo.edu