Center for Diabetes & Obesity Research
Roseman University of Health Sciences
[private non-profit health-professional university with 501(c)(3) status]
Las Vegas/Henderson, Nevada

Mary Johls using advanced nano-proteomics (NanoPro 1000) to study pancreatic α- & β-cells, diabetic neuropathies, lung cancer, lymphoma & novel c-Src phosphorylation (pp. 3 & 7)

Janica Wong studying signaling proteins [CREB, cIAP-1, livin, Mcl-1, PKG-Iα & survinin] mediating cell survival in bone marrow stem cells, vascular cells & pancreatic α- & β-cells & the exaggerated cell proliferation & resistance to chemotherapy in lung / ovarian cancer cells (pp. 4 & 7)

To help support our fight against diseases, contact: rjohls@roseman.edu or mjohls@roseman.edu;
Ronald Fiscus (702-427-3139) or Mary Johls (702-802-2823), Roseman Research Fac., 10530 Discovery Dr, Las Vegas, NV 89135.

Awards:
Sept. 20, 2012: Dr. Fiscus was awarded the “2012 Nevada Researcher of the Year Award” and Roseman University the “2012 Nevada Research Organization of the Year Award” by the Nevada Biotechnology & Sciences Consortium (NevBio).

Nov. 2, 2012: Dr. Fiscus was awarded the “3rd Best Oral Presentation” (out of >100 abstracts) and Mary Johls, Janica Wong & Ben Costantino “Best Posters” at the 10th World Congress of Insulin Resistance, Diabetes & Cardiovascular Disease (Los Angeles, CA) (details, pp. 6 & 7).

July 2013: Dr. Fiscus was awarded the “2013 Healthcare Hero / Technology & Research Award” by Anthem-Blue Cross & Nevada Business mag.

Pathological Conditions Studied in Our Center:
- Type 1 & Type 2 diabetes mellitus (T1D & T2D):
  Developing better ways of preventing & treating T1D & T2D; Nutra- & pharmaceuticals activating patient’s own stem-cell-recovery.
- Obesity (How to reverse fat accumulation & prevent T2D).
- Alzheimer’s disease, ED & other neurological complications of diabetes.
- Cardiovascular (CV) diseases associated with T1D & T2D/obesity:
  Hypertension, Coronary Artery Disease (CAD) & Stroke.

Our Mission:
Our research team is dedicated to discovering new (previously unrecognized) molecular mechanisms mediating the pathogenesis of diabetes (both T1D & T2D) & associated pathological complications [ peripheral neuropathies, ED, Alzheimer’s, cardiovascular (CV) diseases and diabetes-associated cancers]. Our team is using the most advanced technologies, including TIRF microscopy (p. 5) and ultrasensitive capillary electrophoresis (CE)-based nano-fluidic protein analysis systems [NanoPro 100 & NanoPro 1000 (nano-proteomics), p. 3, the first of their kind in the Nevada / Utah / Arizona & LA-CA regions of the U.S. ], to find new, more effective ways of preventing & treating diabetes & pathological complications (Alzheimer’s, neuropathies, CV diseases & cancers).

Pathological Complications of Diabetes Mellitus

<table>
<thead>
<tr>
<th>Type 1 &amp; 2 Diabetes Mellitus</th>
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<tbody>
<tr>
<td>High glucose levels in blood (Hyperglycemia)</td>
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<tr>
<td>Advanced Glycation End Products (AGEs)</td>
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<tr>
<td>Oxygen free radicals &amp; damage of nitric oxide (NO) / protein kinase G (PKG) signaling pathway</td>
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<td>Chronic Inflammation</td>
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<tr>
<th>Cardiovascular diseases (Hypertension, Coronary artery disease, Stroke)</th>
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<tr>
<td>Diabetic retinopathy &amp; blindness</td>
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<tr>
<td>Diabetic foot (Foot ulcerations &amp; amputations)</td>
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<tr>
<td>Increased risk of certain cancers [e.g. breast, colon, liver, lung, ovarian, pancreatic &amp; prostate cancers &amp; blood cancers (leukemia, lymphoma &amp; myeloma)]</td>
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Publications during our first 2 ½ Years (details p. 7):
- 10 Peer-reviewed Research Articles (2010-2013)
- 3 Book Chapters (2012-2013)
- 5 Poster Presentations / Abstracts (2010-2013)

Our Center studies molecular mechanisms mediating pathogenesis of types 1 & 2 diabetes & their pathological complications (see Model above), with special emphasis on developing new therapies for preventing & treating diabetes & the associated pathologies, Alzheimer’s, ED, peripheral neuropathies, CV diseases & cancers.
Management Team:
Ronald R. Fiscus, Ph.D., Vice President for Research, Director of the Center for Diabetes & Obesity Research, Co-Director of Program for Novel Therapeutics in Neurological & Psychiatric Disorders, Head of the Alzheimer’s & Parkinson’s Disease Research Group, Senior Scientist in the Cancer Research Center, Professor of Pharmaceutical Sciences in the College of Pharmacy

Background:
- Research training in Analytical Chemistry / Biochemistry at Iowa State University & Molecular Pharmacology at University of California San Diego (UCSD) Medical School & Stanford University Medical School, which included the early discovery of nitric oxide (NO) & atrial natriuretic peptide (ANP) as stimulators of cyclic GMP (cGMP) elevation & protein kinase G (PKG) activation within intact living cells. Dr. Fiscus’ data provided early evidence of cGMP / PKG signaling pathway as key mediator of NO- & ANP-induced vasodilation & antihypertensive effects (contributing to 1998 Nobel Prize / Medicine, see below).
- Identified cGMP & the PKG-1α splice variant as key mediators in cell survival & DNA synthesis / growth-promoting effects of NO and ANP/BNP at physiological levels [picomolar (pM) to low-nanomolar (nM)], e.g. preventing neural damage caused by toxic levels (>100 nM) of NO & reactive oxygen species (ROSs). NO (pM – low-nM) selectively activates PKG-1α, protecting against neurotoxicity & promoting prolif./chemoresistance in cancer & stem cells.
- Significant contribution to research in Dr. F. Murad’s Lab at Stanford University Medical School, awarded 1998 Nobel Prize in Physiology or Medicine (see publications below).
- Research on early type-5 phosphodiesterase (PDE5) inhibitors (the prototypes of Viagra™, Cialis™ & Levitra™), showing synergistic enhancement of NO’s ability to activate cGMP / PKG signaling pathway & cause subsequent biological effects, now used as highly-successful therapeutic agents for treating erectile dysfunction (ED) & pulmonary hypertension.
- 21 years as Professor at Medical Schools, developing new Med. Curriculums & Molecular Biology/Pharmacology Research.

Publications contributing to the 1998 Nobel Prize in Physiology or Medicine (awarded to Dr. Fiscus’ supervisor at Stanford Medical School, Dr. Ferid Murad, Assoc. Chair, Dept. of Medicine, Stanford Univ., & Chief of Medicine, Palo Alto VA Med. Ctr.) (selected from 10 peer-reviewed research articles published with Dr. Murad) - Establishing cyclic GMP-dependent protein kinase (protein kinase G, PKG) as key mediator of NO-, EDRF (endogenous NO)- & ANP-induced vasodilation / antihypertensive effects.


Mary G. Johlfs, M.S, Director of Research Operations / Scientist

Background:
Mary Johlfs received her Masters degree in Biological Sciences from Northern Illinois University before starting a research position in the biotechnology industry at PDL Biopharma. Mary Johlfs has also held a research and managerial position at the University of Illinois at Chicago and the Nevada Cancer Institute (NVCI), Las Vegas, NV. At the NVCI, Mary Johlfs was the Lab Manager and Senior Research Associate in Dr. Fiscus’ lab, while Dr. Fiscus served as Director of the Cancer Molecular Biology Section at NVCI from mid-2006 to the end of 2009.

Mary Johlfs has over 14 years of research experience in the fields of cellular and molecular biology. She also has vast experience with state-of-the-art research instrumentation (e.g. NanoPro 100 and NanoPro 1000 systems for ultrasensitive quantification of protein expression and protein phosphorylation, details on p. 3). Mary serves as the Director of Research Operations for all Roseman Research Centers & Programs and is a top-ranked / extensively-published Scientist at Roseman University of Health Sciences (pp. 3 & 7).
Discovery of novel, previously unrecognized proteins involved in neuroprotection & improved survival of pancreatic α & β-cells (preventing Alzheimer’s disease, Parkinson’s disease, diabetic neuropathies & damage of α & β-cells during diabetes) and in abnormal proliferation & chemoresistance in cancer cells.

Our Center studies the neurological complications of diabetes & obesity, including Alzheimer’s diseases & peripheral nerve damage caused by high-fat / high-glucose levels and insulin resistance. Previously, our lab identified the 1α isof orm of PKG (PKG-1α) as a key mediator of cell survival & neuroprotection, preventing cellular damage caused by high / toxic levels (>100 nM) of NO, oxygen free radicals & other reactive oxygen species (ROSs).


At Roseman University, we use state-of-the-art instrumentation [e.g. NanoPro 100 & 1000 systems (this page) and TIRF microscopy (details, p. 5)] as ultrasensitive technologies for determining the role of previously-unrecognized proteins in the cyto-protection of neurons / β-cells / stem cells against toxicity of high-level NO & ROSs, preventing pathogenesis of diabetes, neuropathies & CV diseases, & the exacerbated proliferation & chemoresistance in cancers.

Illustration of the exquisite sensitivity & separation capabilities of the NanoPro 100 system, able to quantify proteins with a sensitivity >100 times better than conventional Western blot analysis (used by other labs). Our newer, more advanced NanoPro 1000 system has an additional 5 times higher sensitivity (i.e. >500 times better than conventional Western blot analysis), thus allowing discovery of previously unrecognized proteins in cells.

Neuronal cell line NG108-15 expresses exclusively the PKG-Iα splice variant of PKG-I, determined by NanoPro 1000 system. This instrument also detects phosphorylated forms of PKG-Iα. Data from our book chapter in “Protein Kinase Technology” in NEUROMETHODS (Fiscus & Johls, 2012) (details p. 7). PKG-1α is essential for protecting the survival & functions of neural cells.
Janica has also found a key role of low-level NO & downstream PKG-Iα activation in promoting DNA synthesis / cell proliferation and protecting against apoptosis in OP9 bone marrow-derived mesenchymal (stromal) stem cells, human lung & ovarian cancer cells and mouse pancreatic β-cells. The role of PKG-Iα was defined by using both pharmacological inhibition of kinase activity and genetic modulation using gene knockdown with siRNA targeting PKG-Iα.


Cellular model illustrating our data identifying the role of nitric oxide (NO) and downstream activation of PKG-Iα in cancer cells of epithelial origin (including breast, lung, ovarian and prostate cancers as well as mesothelioma). The NO / cGMP / PKG-Iα signaling pathway plays a central role in promoting c-Src activation & the exaggerated DNA synthesis / cell proliferation of cancer cells as well as resistance to chemotherapeutic agents like cisplatin (chemoresistance) in certain resistant populations of cancer cells. The exaggerated proliferation & chemoresistance can result in a relapse of tumor growth following chemotherapy. Also illustrated above is the key role of the NO / cGMP / PKG-Iα signaling pathway in vascular endothelial cells, promoting angiogenesis (new blood vessel growth).

Janica’s data has shown that NO at low physiological levels (0.01 – 10 nM NO) promotes DNA synthesis / cell proliferation and cell survival in vascular smooth muscle cells (VSMCs) via a mechanism involving stimulation of PKG-Iα kinase activity. Higher levels of NO, which additionally activate the other splice variant of PKG-1 (i.e. PKG-Iβ) and other NO-mediated mechanisms (e.g. S-nitrosylation and tyrosine-nitration of proteins), have the opposite effect, inhibiting cell proliferation & inducing apoptotic cell death. Interestingly, prior activation of the cytoprotective PKG-Iα by natriuretic peptides (ANP / BNP) dramatically protects VSMCs against toxic / pro-apoptotic effects of high-level (>100 nM) NO (Wong & Fiscus, 2010).

NCI-H23 cells [non-small cell lung cancer (NSCLC) cells]. NSCLC is the most common form of lung cancer.

TIRF microscopy image of NCI-H23 lung cancer cells, with fluorescent staining for PKG-Iα (green).

The above image shows subcellular localization of PKG-Iα at the plasma membrane (as illustrated in the cellular model on page 4).

Our current experiments are determining the co-localization of PKG-Iα with other oncogenic proteins, e.g. c-Src (a non-receptor tyrosine kinase) and mutated EGFR, common targets of newly-developed anti-cancer therapies.

Ben Costantino operating Roseman’s TIRF (total internal reflection fluorescence) microscope, possessing a unique laser system that generates an evanescent wave of light that illuminates only the closest 100 nanometers (i.e. 0.1 microns), only at the cell’s plasma membrane, thus improving the signal-to-noise-ratio by ~100 fold. This allows imaging of proteins and other signaling molecules near the cell membrane with a sensitivity much better than conventional microscopy or confocal microscopy.

Ben is also developing a novel technique for quantifying protein kinase activity in biological samples & recombinant proteins, using a new near-infrared fluorescence (NIRF)-based methodology, rather than the older technique requiring use of radioactive material. Our new NIRF-kinase-assay methodology provides sensitivity and accuracy similar to the older 32p-based assays [the ‘gold standard’ of kinase assays, used by Dr. Fiscus for quantifying PKA and PKG kinase activity while a postdoc / scientist at UCSD and Stanford University Schools of Medicine (see publications listed on p. 2)]. Our new methodology has the unique feature of being able to quantify the intracellular kinase activities of both PKG & protein kinase C (PKC) simultaneously & selectively.

Bone marrow-derived mesenchymal (stromal) stem cells (BM-MSCs), multipotent cells capable of differentiating into fat cells, bone cells or cartilage cells, used at Roseman University as a model of obesity & type-2 diabetes (with differentiation of BM-MSCs into fat cells). Shown above are TIRF microscope images of these cells, featured on the cover of the Spring 2012 issue of Roseman’s Remedy magazine.

Undifferentiated BM-MSC (OP9 cell), used as a “pre-adipocyte”. Green staining represents PKG-Iα localized at the plasma membrane (by TIRF microscopy) and blue staining represents the nucleus (by wide-field fluorescent microscopy). Merged image.

These BM-MSCs are partially differentiated into fat cells. Note that some cells contain lipid droplets, whereas others do not. Interestingly, PKG-Iα is pushed out of the way by the growing lipid droplets.

Differentiated adipocyte (fully-developed fat cell) that has originated from a bone marrow-derived mesenchymal stem cell (our cellular model used for developing new therapies for preventing & treating obesity / Type-2 diabetes).
Nov. 29, 2010. Novel role of nitric oxide / cyclic GMP / protein kinase G type-Iα (PKG-Iα) signaling pathway in promoting cell survival and DNA synthesis / proliferation in various types of cancer cells and stem / progenitor cells. Presented to the NIH Program Project Grant (NIH PO1) group studying molecular mechanisms of diabetic retinopathy, directed by Dr. Maria Grant, M.D., Professor, Department of Pharmacology & Therapeutics, College of Medicine, University of Florida, Gainesville, FL.

June 26, 2011. Protein kinase G type-I phosphorylates c-Src at serine-17 and promotes cell survival, proliferation and attachment in human mesothelioma and non-small cell lung cancer cells. 5th International Conference on Cyclic GMP (cGMP): Generators, Effectors and Therapeutic Implications, Halle, Germany, June 24-26, 2011.

Sept. 26, 2011. PKG-Iα activation by picomolar-to-low-nanomolar NO or ANP promotes DNA synthesis / cell proliferation & protects neural and vascular cells against the toxic / pro-apoptotic effects of high-level NO (model of cytoprotection during inflammation). 2nd International Conference of Inflammation & Translational Medicine, Shanghai, China, Sept. 25-26, 2011.


Nov. 2, 2012. Involvement of physiological-level nitric oxide (NO)/protein kinase G type-Iα (PKG-Iα) signaling in protecting against diabetic neuropathies: Glucose effects on PKG-Iα expression and downstream signaling quantified by ultrasensitive capillary-electrophoresis (CE)-based NanoPro 1000 protein analysis system. 10th World Congress of Insulin Resistance, Diabetes & Cardiovascular Disease, Los Angeles, CA, Nov. 1-3, 2012. (Awarded 3rd Best out of over 100 abstracts.)

Collaborations with 9 Top-rated Scientists from Around the World:

Ferid Murad, MD, PhD (1998 Nobel Prize in Physiology or Medicine), Professor of Biochemistry & Molecular Biology, George Washington University, Washington, D.C. & Director, Murad Research Institute, Shanghai, China.

(Former supervisor of Dr. Fiscus at Stanford University Medical School). Collaborative project: “PKG Isoforms in Glioma Cells”.

Chikao Morimoto, MD, PhD, Professor & Chair, Department of Clinical Immunology, & Director, Advanced Clinical Research Center, University of Tokyo, Tokyo, Japan, & Visiting Professor, College of Pharmacy, Roseman University of Health Sciences.

Dr. Morimoto is a world expert on CD26 / dipeptidyl peptidase type-IV (DPP-IV) protein and its involvement in immunity, diabetes & cancer. Collab. project: “Molecular Mechanism of CD26 / DPP-IV-Targeted Therapies in Cancer & Diabetes”.

Maria Grant, MD, Professor, Dept. of Pharmacology & Therapeutics, College of Medicine, University of Florida, Gainesville, FL.

Dr. Grant is a leader in Diabetic Retinopathy, with special interest in the role of NO and its stimulation of the phosphorylation of VASP (a PKG substrate & regulator of actin filament / focal adhesion) in endothelial cells and endothelial progenitor cells (EPCs).

Dr. Fiscus serves on the Scientific Advisory Board for the NIH Program Project Grant (NIH PO1 grant) on “Diabetic Retinopathy” at the University of Florida, headed by Dr. Grant. Collaborative project: “NanoPro analysis of cytoprotective PKG isoforms in EPCs”.

Elaine L. Leung, PhD, Assistant Professor, State Key Laboratory of Chinese Medicines, Macau University of Science & Technology (MUST), Macau Special Admin. Region, China.

Dr. Leung is a former Ph.D. student & Postdoc supervised by Dr. Fiscus. Collab. projects: “Cancer Stem Cells (CSCs) as the ‘Seeds’ of Lung Cancer” & “New Treatments for Diabetes: Improved Survival of Isolated Pancreatic β-cells for Transplantation Therapy”.

Maria P. Wong, MD, Associate Professor, Department of Pathology, The University of Hong Kong, Faculty of Medicine, Hong Kong Special Admin. Region, China. Collaborative project: “Identifying Cancer Stem Cells as the Origin of Lung Cancer in Patients”.

Thuc (Tim) Le, PhD, Assistant Professor, Nevada Cancer Institute (NVCI)/Desert Research Institute (DRI), Las Vegas, NV.


Oscar B. Goodman, Jr., MD, PhD, Comprehensive Cancer Centers of Nevada, Las Vegas, NV, & Adjunct Professor, Roseman University of Health Sciences. Dr. Goodman is a leading clinician/scientist studying the isolation & characterization of circulating tumor cells (CTCs).

Collab. Project: “NanoPro Analysis of CTCs – Ultrasensitive/Quantitative Tool for Personalized Medicine”.

Aurelio Lorico, MD, PhD & Germana Rappa, MD, PhD, Co-Directors of Roseman’s Cancer Research Program, Associate Professors, College of Pharmacy, Roseman University of Health Sciences, Henderson/Las Vegas, NV.

Dr. Lorico & Dr. Rappa are leaders in the field of CD133 protein in stem cell biology and the interdependence between cancer cells and bone marrow-derived stem cells. Collab. Project: “NanoPro Analysis of CD133 & β-catenin in Cancer & Stem Cells”.
Research Articles (peer-reviewed):


Book Chapters:


Poster Presentations (last two presented):

Johlfs, M.G., Coffman, R., Rosenberg, H. and R.R. Fiscus. (2012) Involvement of physiological-level nitric oxide (NO)/protein kinase G type-1α (PKG-1α) signaling in protecting against diabetic neuropathies: Glucose effects on PKG-1α expression and downstream signaling quantified by ultrasensitive capillary-electrophoresis (CE)-based NanoPro 1000 protein analysis system. 10th World Congress of Insulin Resistance, Diabetes & Cardiovascular Disease, Los Angeles, CA, Nov. 1-3, 2012. (Special Award—Top Poster Presentations out of >100)

Wong, J.C., Coffman, R., Rosenberg, H. and R.R. Fiscus. (2012) Essential role of the nitric oxide (NO) / protein kinase G type-1α (PKG-1α) signaling pathway in promoting the survival and insulin secretion of pancreatic β-cells. 10th World Congress of Insulin Resistance, Diabetes & Cardiovascular Disease, Los Angeles, CA, Nov. 1-3, 2012. (Special Award—Top Poster Presentations out of >100)

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