We are grateful to Ms. Claire Trias for her outstanding organization and professionalism, without which this event would not be possible.

We also thank Drs. Evan Hermel and Tami Hendriksz for serving as student presentation judges, and congratulate the participating students for their committment to research.

We are grateful to Touro University for support, Mr. Ralph Cuberos, Mr. Alex Perez, and the Facilies and Food Services Departments.

A. Gugliucci, MD, PhD
Professor of Biochemistry and Associate Dean for Research
Provost’s Welcome

Dear TUC Faculty, Students and Staff,

Welcome to what has become an annual campus tradition, the 9th Annual Research Day at Touro University California. As a newcomer to campus, I have eagerly awaited this event, so I could learn more about the scholarly interests of our learning community. A first class university is defined by many characteristics. Good teaching and students deeply engaged in the learning process is the foundation upon which a great institution is built. However, our university is heavily invested in graduate and professional education. It is essential that we create an environment which values and nurtures intellectual curiosity and the development of new knowledge which contributes to professional practice. Your presence here today demonstrates your understanding of this worthy goal and your support for faculty and students who are presenting their research. I look forward to working with you and other members of the campus community as we build a dynamic research environment at TUC.

Sincerely,

Marilyn Hopkins, D.N.Sc.

Provost and Chief Operating Officer
Dr. Qureshi received his MD from the College of Medicine, University of Dhaka, Bangladesh in 1986 and practiced as a physician until 1992. He received his MPH in Public Health from the National Institute of Preventive and Social Medicine, University of Dhaka, Bangladesh in 1992. He completed a MHSc in Epidemiology and a PhD in Immunology from the School of Medicine, University of the Ryukyus, Japan in 1995 and 1999, respectively. Dr. Qureshi held positions as post-doctoral research scholar and research associate in the departments of internal medicine, division of infectious diseases, and microbiology, immunology and molecular genetics at the University of Kentucky from 1999 through 2004.

His research has been funded by American Lung Association and American Society for Microbiologists. He has published extensively in many peer-reviewed journals as well as many abstracts and book chapters. His research interests include the role of dendritic cell in inadequate immune responses to P. carinii in neonates and RSV-induced susceptibility to asthma.

He joined the Touro University Nevada College of Osteopathic Medicine as an assistant professor of microbiology and immunology in 2004. Dr. Qureshi is also the director of research at Touro University Nevada.
Role of dendritic cell (DC) in inadequate immune responses to respiratory pathogens in neonates

Mahboob Qureshi, MD, PhD; Associate Professor of Microbiology and Immunology and Director of Research, Touro University Nevada, Henderson, NV

Neonates have a predominantly defective lung environment rather than inefficient immune effector cells accountable for inadequate immune responses to respiratory pathogens (Garvy & Qureshi, J. Immunol., 2000; Qureshi & Garvy, J. Immunol., 2001, Qureshi et. al., J. Immunol., 2003). However, it is not clearly understood as to whether other components of neonatal lung environment have adult-like functioning. Appropriate antigen presentation by professional antigen presenting cells (APCs) is a prerequisite for an effective immune response. DCs are the major APC in the respiratory tract. Inefficient DC function in the neonatal respiratory tract may be involved in defective neonatal host-responses to respiratory pathogens (Qureshi et al., J. Immunol., 2005). The current research studies in the lab intend to address the hypothesis that inefficient antigen uptake and migration of DCs to the draining lymph nodes as well as simultaneous maturation is inefficient in neonates resulting in inefficient proliferation and activation of effector T cells and delayed resolution of respiratory infections. A Pneumocystis carinii (PC) pneumonia (PCP) model has been used in these studies. The information regarding differential antigen uptake, migration ability, and the dynamics of chemokine receptor expression of neonatal and adult DCs have been utilized to better understand some important differences in the initiation of immune responses in the two age groups. A complete understanding of the role of DC in neonatal immune responses may lead to development of new form of immunotherapies, including DC-based vaccine strategies, which may be used as an adjunct to existing chemotherapy to protect the vulnerable neonates from respiratory pathogens.
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1. College of Pharmacy, Touro University, Mare Island-Vallejo, California
2. Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Mercer University, Atlanta, Georgia

B-2 Testing Ischemic Tolerance in 3xTg-AD Mice Using the Oxygen-Glucose Deprivation Model of Ischemia
R. c. Li and G. J. Klapstein
Department of Basic Sciences, Touro University-California, Vallejo, USA

B-3 RNA virus vaccines: Making cytokine-antigen fusion vaccines for cancer therapy
Hardeep Kaur, Kerrie Lee, Ronald Levy and Alison McCormick
1. Touro University California, College of Pharmacy, Vallejo CA
2. Stanford University School of Medicine, Department of Oncology, Stanford CA

B-4 Design, Synthesis and Evaluation of Indole Compounds as Novel Inhibitors targeting Gp41
Guangyan Zhou, Dong Wu, Evan Hermel, Edina Balogh and Miriam Gochin
1. Department of Basic Science, Touro University-California, Vallejo, CA 94592
2. Department of Pharmaceutical Chemistry, University of California San Francisco, CA 94143

B-5 Resveratrol restores Serum Paraoxonase Activity in the 3xTg-AD Mouse Model of Alzheimer's Disease
G. J. Klapstein, R. Caccavello and A. Gugliucci
1. Department of Basic Sciences, Touro University-California, College of Osteopathic Medicine, Vallejo, USA
2. Glycation, Oxidation, and Disease Laboratory, Touro-University-CA, Vallejo, CA. USA

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J. Revels, A. Carter, K. Vu, A. Pelzer, A. Shah and T. Elul
1. Touro University-California, Vallejo, CA
2. Merrit Microscopy Program, Merrit College, Oakland CA

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A. Gugliucci
1Glycation, Oxidation and Disease Laboratory, Department of Basic Sciences, Touro University - California, College of Osteopathic Medicine, Vallejo, USA

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S. Liu, and M. Louie
Department of Pharmaceutical Sciences, College of Pharmacy, Touro University - California, Vallejo, USA

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Russell Caccavello1, Artem Dyachenko2, Mike Wen3, Clive Pullinger3, Jean-Marc Schwarz2,3 and A. Gugliucci1
1Glycation, Oxidation and Disease Laboratory, Department of Basic Sciences, Touro University - California, College of Osteopathic Medicine, Vallejo, USA
2Touro University - California COM, Vallejo, USA
3University of California, San Francisco, USA

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3University of California Berkeley, Dept. of Letters and Science, Berkeley, CA
4University of California San Francisco, San Francisco, CA

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1Protease Inhibitor & Tumor Suppression Laboratory, Dept. of Biol. Sciences, College of Pharmacy
2Cancer & Aging Laboratory, Dept. of Basic Sciences, College of Osteopathic Medicine Touro University of California, Vallejo, CA.

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Alejandro Gugliucci1 and Russell Caccavello1
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Department of Basic Sciences, Touro University - California, College of Osteopathic Medicine, Vallejo, CA
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Teresita Menini\textsuperscript{1}, Alejandro Gugliucci\textsuperscript{1} Ricardo Hermo\textsuperscript{1}, and Satoshi Kimura\textsuperscript{2}
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\textsuperscript{2}Department of Laboratory Medicine and Central Clinical Laboratory Showa University Northern Yokohama Hospital Tsuzuki-Ku, Yokohama City, Japan

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Aging-Osteopathic Consortium, Touro University-California, Vallejo, CA; Gheens Center on Aging, University of Louisville, KY.

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Touro University-CA. Vallejo, CA.

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\textsuperscript{1}Touro University-California, College of Osteopathic Medicine, Vallejo, CA
\textsuperscript{2}University of California San Francisco, San Francisco, CA

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\textsuperscript{2}Department of Clinical Laboratory Medicine, Jichi Medical University, Tochigi 329-0498, Japan.
\textsuperscript{3}Clinical Pathology and Internal Medicine Departments, Showa University School of
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¹. College of Osteopathic Medicine, Touro University-California,
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¹. Touro University College of Osteopathic Medicine, 2. Shirati Hospital Medical
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California, Vallejo, CA
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General Hospital, CA
C-13 Serum ischemia modified albumin levels in obstructive sleep apnea patients: A pilot study
Alejandro Gugliucci1, Kazuhiko Kotani2, Ichiro Komada2, Naoki Sakane2 and Satoshi Kimura3
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C-14 Continuous positive air pressure treatment reduces serum advanced glycation endproducts in patients with obstructive sleep apnea syndrome: a pilot study
Kotani K, Sakane N, Sano Y, Tsuzaki K, Matsuoka Y, Egawa K, Yoshimura M, Horikawa C,
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Effect of penetration enhancers on transdermal delivery of penbutolol sulfate

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Background: Penbutolol, $[1-t$-butylamino-3-(2-cyclopentylphenoxy)propan-2-ol] is a non-cardioselective β-adrenoreceptor blocking agent and used for the treatment of hypertension. The conventional dosage form for penbutolol is tablet which has limitations including hepatic first-pass metabolism, high incidence of adverse effects due to variable absorption profile and poor patient compliance.

Transdermal delivery offers an alternative mode of drug administration. A stable plasma drug concentration can be maintained over a prolonged period. However few drugs can penetrate the excellent barrier provided by the stratum corneum. Over the past few decades, chemical penetration enhancers have attracted considerable interest as a method of enhancing transdermal drug delivery.

In this project, we investigated the effect of chemical penetration enhancers on transdermal delivery of penbutolol sulfate across pig ear skin

Methods: In vitro permeation studies. The effect of 50% ethanol, 1% limonene and 2% isopropyl myristate on transcutaneous penetration of penbutolol sulfate was studied. An automated flow-through diffusion system (Permegear, Bethlehem, PA, USA) was used for in vitro permeation studies across pig ear skin dermatomed to 500 micrometers (Robbins Instruments, NJ, USA). The samples were collected with a Retriever IV fraction collector at 2 h intervals for 12 hours and analyzed by HPLC (UV detector at 271 nm; mobile phase-water: acetonitrile 70:30; 1ml/min).

Results: Penbutolol sulfate flux was calculated from the concentrations in the collecting tubes by using the equation; $J_p = F.C_a/A$ where $J_p$ is the flux through the skin, $C_a$ is the concentration of penbutolol in the acceptor phase, $A$ is the skin area and $F$ is PBS flow rate. Ethanol, isopropyl palmitate and limonene at the concentration of 50%, 1% and 2% respectively increased the steady-state flux of penbutolol sulfate 2.6 (19.40 μg/cm²), 2.2 (17.07 μg/cm²) and 3.4 times (26.38 μg/cm²) compared to passive delivery (7.76 μg/cm²).

Conclusion: Statistical analysis of permeation data was done using ANOVA. There was a statistically significant increase in transdermal steady-state flux of penbutolol sulfate following the use of chemical penetration enhancers compared to passive delivery.
Testing Ischemic Tolerance in 3xTg-AD Mice Using the Oxygen-Glucose Deprivation Model of Ischemia.

R-c. Li and G. J. Klapstein
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**Background:** Alzheimer’s disease (AD) shares many risk factors with vascular disease. Cerebral macrovascular pathology is a major cause of disability, dementia, and death. The pathology of large, macroscopically visible vessels is common in AD and some estimate that a third of AD patients have significant cerebrovascular disease (Kalaria, 2002), or that as many as 98% have amyloid angiopathy (Jellinger, 2002). Cerebral amyloid angiopathy (CAA), morphologically characterized by amyloid deposition in the vessel walls, which are altered to rigid tubes, is a chronic disease of the cortical and meningeal vessels that can cause intracerebral hemorrhages. Accumulating evidence has linked stroke and AD and shown that each exacerbates the severity of the other (Koistinaho 2002, Iadecola 1999, Jellinger 2000). In recent years it has been postulated that AD is primarily a vascular disorder with neurodegenerative consequences. In fact, reduced cerebral blood flow (Iadecola 1999) and a reduction in vasoreactivity (Jellinger 2000) have been found in AD patients. It is also known that A-beta has deleterious effect on cerebral vasculature, which compounds pathogenesis in AD as the reduction in cerebral blood flow hinders the clearance of A-beta from the brain (Iadecola 2004). These factors may predispose AD brain tissue to ischemic insults. For example, it has been shown that mice with a presenilin-1 mutation are more susceptible than WT mice to ischemic insult by vessel occlusion (Mattson 2000), but it is not known whether the neurons themselves develop increased ischemic sensitivity over the course of the disease. We will test whether the oxygen-glucose deprivation (OGD) model of ischemia can be used to investigate the development of ischemic sensitivity in hippocampal neurons in a triple transgenic mouse model of Alzheimer’s Disease.

**Methods:** Coronal brain slices (400μm) were placed in a recording chamber, and perfused constantly with oxygenated artificial cerebrospinal fluid (ACSF) at 32 ± 0.5°C in an atmosphere of warm, moist 95% O2-5% CO2 (carbogen). Constant current stimuli (100 μs duration) were delivered every 15 s. via a bipolar stimulating electrode placed in stratum radiatum of area CA1. Recording electrodes (3–4 MΩ) were filled with ACSF and extracellular field excitatory postsynaptic potentials (EPSPs) were recorded from CA1 pyramidal cell apical dendrites. OGD was induced for various durations by switching the atmosphere of the slices to 95% N2/5% CO2 instead of carbogen, and perfusing the slice with ACSF which had an equimolar substitution of sucrose for glucose, and which was also equilibrated with 95% N2/5% CO2. Control conditions were restored and EPSPs were monitored.

**Results:** Within a few minutes of OGD induction, synaptic responses were eliminated. A return to control conditions after 5-8 minutes of OGD often resulted in recovery of synaptic responses over a period of 10-60 minutes.
**Conclusions:** The OGD model of ischemia is appropriate for examining ischemic tolerance in the 3xTg-AD mouse model of Alzheimer's disease.
RNA virus vaccines: Making cytokine-antigen fusion vaccines for cancer therapy.

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Background: RNA virus vectors are attractive vaccine delivery agents, capable of directing high-level gene expression, and induce powerful anti-viral mechanisms of innate immunity. They are also attractive because they can be used to make customized vaccines for cancer therapy (Smith ML et al (07) Virology). We are testing a formulation of Semliki Forest Virus (SFV) RNA that can be used to express tumor antigens relevant to 38C13 and A20 mouse models of non-Hodgkin’s Lymphoma (NHL). These models are important, because the tumor antigens are weak self-antigens, similar to those expressed by human tumors. By using the SFV virus to express a weak tumor antigen, we hope to stimulate highly specific anti-tumor immunity that can break tolerance, and stimulate long lasting tumor eradication. We can also use the SFV system to produce fusions of tumor antigens to powerful cytokine immune stimulators, which should also improve tumor antigen vaccine potency.

Hypothesis: We will use the SFV expression system to test the hypothesis that SFV virus expression of a tumor-specific antigen alone, or fused to the mammalian cytokines GM-CSF or IL12, will improve anti-tumor immune responses, and generate tumor protective immunity.

Methods: SFV antigen and cytokine expression constructs were validated, singly or as fusions, by sequencing. SFV RNA constructs were used to synthesize synthetic RNA, and then tested by transfection into cells. ELISA and Western methods, using antigen and cytokine specific antibodies, were used to determine appropriate expression of protein products in transfection cell supernatants.

Results: SFV sequencing, and protein expression studies have verified that SFV antigen and cytokine expressing RNA’s produce protein products of the correct identity. We are currently testing particulate viral RNA for in vitro uptake into antigen presenting cells, in addition to in vivo vaccine potency testing in mice. We will select the vaccine candidates that stimulates the highest level of tumor antigen immunity for murine NHL tumor challenge studies.

Conclusions: Our experiments will test cytokine/antigen combinations that can improve SFV vaccine efficacy, and promote survival against a weakly immunogenic tumor antigen in mouse models of NHL. If successful, analogous formulations of human NLH vaccines will be developed for clinical trial applications.

Sponsored by an NIH R21 Grant (R21CA141094-01).
Design, Synthesis and Evaluation of Indole Compounds as Novel Inhibitors targeting Gp41

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**Background:** The HIV envelope glycoprotein gp41 plays a key role in the early stage of viral entry by introducing the viral genome into the target cell. The core crystal structure of gp41 shows that three helices of N-terminal heptad repeats form a central trimeric coiled-coil and three helices of C-terminal heptad repeats pack in an anti-parallel configuration into the highly conserved hydrophobic grooves on the surface of the coil. In each of the grooves, there is a deep hydrophobic pocket, which is critical for stability of the core structure and viral fusion.

**Hypothesis:** The hydrophobic pocket is an attractive drug target. We propose that any chemical entity binding to this cavity of gp41 will block six-helix bundle formation, and might have inhibitory activity against HIV-1 entry and prevent its replication.

**Methods:** Based on the structure of gp41, a series of indole ring containing compounds were designed, and synthesized using Suzuki Coupling reaction. The design strategy was to increase ligand hydrophobicity while maintaining solubility by introducing an indole ring. The indole ring on the ligand could possibly emulate the interaction of Trp residues which bind in the pocket of the 6HB structure, which includes a hydrogen bond between the indole NH of Trp631 and the backbone carbonyl of Leu568. Furthermore, it was anticipated that derivatives of indole would be synthetically feasible due to ease substitution at some positions. The binding models of these compounds with gp41 were predicted by AutoDock4.0.

**Results:** These compounds were evaluated using a fluorescence binding assay and cell-cell fusion assay. As expected, the observed binding constant of compound TU-11 was 2.1\(\mu\)M, and the IC\textsubscript{50} for cell-cell fusion inhibition was 1.1\(\mu\)M. There is reasonable but imperfect agreement between observed K\textsubscript{i} and K\textsubscript{i} predicted by AutoDock4.0; excellent correlation between the observed K\textsubscript{i} for binding and the IC\textsubscript{50} for cell-cell fusion.

**Conclusions:** From the initial hit to TU-11, we have obtained 260-fold improvement in the K\textsubscript{i} for binding and 510-fold increased activity against HIV-1 fusion in cell culture. Our studies demonstrate a series of novel chemical structures as inhibitors against gp41 and establish a solid foundation for future optimization.
Resveratrol restores Serum Paraoxonase Activity in the 3xTg-AD Mouse Model of Alzheimer’s Disease.

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Background: Resveratrol, a phytoalexin found in red wine, peanuts, soybeans, and pomegranates, is currently the focus of intense research both in the cardiovascular system and the brain. Current research suggests resveratrol may enhance prognosis of neurological disorders such as Parkinson’s, Huntington’s, Alzheimer’s diseases and stroke (Raval, 2008, Curr Medicinal Chem). Resveratrol has also been shown to induces paraoxonase 1 expression in human cell lines (Curtin, 2008, J Cell Biochem). Serum paraoxonase 1 (PON1) is an HDL-associated lipo-lactonase, which is synthesized and secreted by the liver (Deakin, 2002, J Biol Chem). PON1 has anti-oxidative properties, and reduces the susceptibility of LDL to lipid peroxidation in vitro (Mackness, 1991, FEBS Lett). It also functions to protect the cholinergic system against nerve gases and the organophosphate family of pesticides, and it has been suggested that PON-1 genetic variants might affect individual susceptibility to environmental events, such as exposure to cholinesterase inhibitors (Leduc, 2009, Eur J Neurosci). Furthermore, mutations of the PON1 gene were found to be significantly increased in AD patients relative to age-matched controls (Leduc, 2008, Neurodegener Dis.).

Hypothesis: We hypothesize that dietary supplementation with Resveratrol will increase serum paraoxonase activity in the 3xTg-AD mouse model of AD.

Methods: Age matched mutant and WT mice (269-273 days old at end of study) were placed into one of four treatment arms: WT control diet, WT resveratrol diet, Mutant control diet, Mutant resveratrol diet. Control diet consisted of standard mouse chow (Harlan 2018 rodent diet). For the resveratrol diet, resveratrol (Whole Health, Inc.) was incorporated into mouse chow (325mg/540g) to give an approximate daily dose of 200 mg/kg. All mice were fed ad libitum with free access to food and water for a period of 1 month. Blood was collected ex vivo and serum was kept at -80°C until analysis. Serum PON1 triesterase activity was determined from the initial velocity of paraoxon hydrolysis to p-nitrophenol, its production followed kinetically at 37°C and recorded at 405 nm in a Versa Max microplate reader (Molecular Devices).

Results: Results are reported as ave ± sem, in units of paraoxonase activity per litre of serum. As reported previously, we found that mutant mice on control diet have lower PON activity than WT on control diet (mutant = 50.0 ± 2.5; WT = 77.5±3.9, N=2, p=0.001). In WT mice, Resveratrol diet had no significant effect on PON activity (Resv. diet = 67.1 ± 13.1; control diet = 77.5±3.9, N=2, p=.473), however Resveratrol diet had a significant effect on raising PON activity in mutants (Resveratrol diet = 71.5 ± 8.1, control diet = 50.0 ± 2.5, N=2, p<0.045), such that Resv-fed mutants were no longer significantly different from WT control diet (mutant Resv. diet = 71.5 ± 8.1, WT control diet = 77.5±3.9, N=2, p=.524)
**Conclusions:** Resveratrol is effective at correcting deficits in serum paraoxonase activity found in a mouse model of AD, but does not increase PON activity in WT mice.

*Supported by Touro University-CA*
β-catenin regulates diverse pathfinding behaviors of optic axons in the developing visual projection of an in vivo vertebrate system.

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Background: Visual function is a critical human sense that is reduced in many diseases, including genetic disorders, amblyopia, glaucoma, diabetic neuropathy and neurodegenerative diseases. Understanding the mechanisms that first establish visual function will help treat these diseases. To generate the visual projection, optic neurons must extend their axons from the retinal layer and grow to the brain along a specific path. β-catenin is a highly-conserved target of Cadherin and Wnt signals, regulators of pathfinding of optic (and many other) axons in the developing brain. However, the functions of β-catenin have not been studied in pathfinding of optic (or any other) axons in a vertebrate model system whose visual projection is similar to that of humans.

Hypothesis: Our primary hypothesis is that β-catenin regulates diverse behaviors of optic axons pathfinding in the developing visual system of Xenopus tadpoles, an in vivo vertebrate model system.

Methods: Our approach is to induce loss-and gain-of-function (LGOF) for β-catenin, and associated factors, in individual optic neurons from Xenopus tadpoles. We will then perform imaging and quantitative analysis of optic axons and their growth cones pathfinding in vivo, in their native environment.

Results: β-catenin LGOF mutants, NTERM, and β-cat107 oppositely regulated several conserved behaviors of optic axons pathfinding to their target in whole brains. Specifically, β-catenin LGOF mutants oppositely regulated defasciculation and fine-grade directionality of optic axons and selectively inhibited lamellipodial and filopodial protrusions in their growth cones in vivo.

Conclusions: These results suggest that β-catenin, acting downstream of two major axon guidance ligands is required for several pathfinding behaviors that establish the visual projection in a vertebrate animal whose visual pathway is similar to that of humans. This work provides fundamental data that will lead to the development of treatments aimed at regenerating the visual projections in humans suffering from sight-threatening diseases.
Dissecting Cellular Pathways of Oncogenic Ras Isoforms
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Background: Cancer is the second leading cause of death in the United States. It was estimated that 1,437,180 people were diagnosed with and 565,650 people died of cancer in 2008. In 2005, cancer care cost taxpayers $209.9 billion and the costs are rising every year. A better understanding of the cancer biology is therefore needed to help the development of effective cancer therapeutic intervention.

Cancer cells are known to harbor multiple genetic changes, including activations of oncogenes and inactivations of tumor suppressor genes. For instance, ras gene encodes a small GTP-bound protein that, upon activation, mediates various important cellular responses through cascades of protein kinase signaling pathways. A single nucleotide mutation in codon 12, 13 or 61 of the ras gene results in constitutive activation of the Ras proteins, which exhibit oncogenic potential. Importantly, activated mutations in the ras gene are found in 30% of all human cancers.

Four ras genes have been identified in mammalian cells: H-ras, K-ras (4A, 4B), N-ras. All Ras proteins share highly homologous sequences in their N-termini, but each has a unique C-terminus. Studies have suggested that activated mutations in each ras gene are associated with specific types of cancer. It is, however, unclear whether activation of these Ras isoforms triggers different biological activities in human cells. The goal of this research proposal is aimed at dissecting the cell signaling pathways mediated by oncogenic Ras isoforms.

Hypotheses: We hypothesize that activation of each Ras isoform is linked to a specific signaling pathway, leading to differential cellular responses. We also hypothesize that specific codon mutations in the ras gene may trigger differential cellular outcomes.

Methods: Human normal fibroblasts as well as tumor cell lines were retrovirally transduced with H-RasV12, N-RasV12, K-RasV12, K-RasL61 or an empty vector control. Cells were selected and monitored for cell growth and changes in the expression of various signaling proteins.

Conclusion: Expression of each of the Ras isoform trigger differential cellular outcomes likely through distinct signaling pathways.

Significance: Specific oncogenic Ras proteins are associated with distinct types of cancers. Understanding the differential signaling pathways mediated by specific oncogenic Ras isoforms may impact future development of pharmacological agents that will be more specific in targeting and treating the underlying cause of cancer.
Differential Effect of HOCL on the lactonase versus the triesterase activity of paraoxonase 1 in human LDL

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Background: Chlorination of tyrosine, has been previously shown to inactivate paraoxonase-1 (PON-1) in HDL. PON-1 could be implicated in mechanism by which HDL inhibits LDL oxidation. PON-1 is a promiscuous esterase, its active site has residues which govern substrate selectivity. PON-1 triesterase activity towards paraoxon was employed in former studies. However, the lactonase activity is considered the physiological, cardioprotective function of PON-1.

Hypothesis: Due to the differences in the residues controlling substrate selectivity, we hypothesized that there might be differential inactivation of the two activities (triesterase vs lactonase) when HDL is exposed to HOCl.

Methods: Human HDL (1 mg/ml) was incubated in PBS containing 2 mmol/L CaCl2, pH 7.4, at 37 °C for up to 3 h with 1-100 μmol/L of freshly prepared HOCl/OCl– or buffer as control. After incubation, PON-1 (triesterase) activity was determined using paraoxon as a substrate, from the initial velocity of p-nitrophenol production at 37 °C and recorded at 405 nm. For lactonase activity, we employed 2 and 4 μl respectively. PON-1 lactonase activity was kinetically determined at 37 °C using 5 (thiobutyl)butyrolactone (TBBL) and recorded at 405 nm.

Results: As reported previously, HOCl incubation produces a dose-dependent inactivation of PON-1 triesterase activity in HDL (IC50: 25 micromol/L). No inactivation of the lactonase activity was found under the same incubation conditions.

Conclusions: We show that HOCl at physiological concentrations does not affect PON-1 lactonase activity while it decreases its triesterase activity. Our data suggest that HOCl does not act on the catalytic residues and acts differentially in the substrate binding domains. They also underscore the importance of using more natural substrates for PON-1 to make better predictions for human disease.

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Thio-cyclohexane ring modified SHetA2 analogs as cancer prevention agents
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Background: Previously, we synthesized SHetA2 (compound NSC 726189) and its analogs, which have been shown to be a novel class of cancer prevention agents. Preclinical studies have demonstrated that SHetA2 analogs can induce the apoptosis of cancer cells while sparing normal cells, and exhibit anticancer activity in vivo. SHetA2 has received considerable scientific attention in recent years. The thio-cyclohexane ring modified analogs of SHetA2 was not reported.

Hypothesis: Since substituted thio-cyclohexane ring is lipophilic, we hypothesized that other SHetA2 analogs with similar lipophilic substituents inhibit cancer cell growth.

Methods: Commercial reagents and solvents were obtained from Aldrich Inc. and used as received. Compounds were prepared by known methods. Purification beyond recrystalization was done by flash chromatography (Merck flash chromatography silica gel 40 μm particle size. All 1H and 13C NMR spectra were performed using CDCl₃ or DMSO-d₆ as solvent and obtained on a Broker 400 MHz NMR spectrometer. Signals were referenced to TMS. Compounds 1-6 were evaluated for their ability to inhibit the growth of ER positive breast cancer cell line MCF-7. Cells were plated in 96 well plates and treated with varying concentrations (10⁻¹² M to 10⁻⁴ M) of each compound. Cell growth was analyzed using an MTT assay 2, 4 and 6 days after treatment against vehicle control.

Results: We have developed a novel procedure to obtain a variety of SHeA2 analogs. As expected, MCF-7 cell was growth inhibited by all of SHeA2 analogs synthesized. The growth inhibition at 10⁻⁵ M of compound 1 and 6 are 32%, 50%, 60%, 89%, 75% and 39% compared to vehicle control. The most potent compound is compound 4 (89%).

Conclusion: Six SHeA2 analogs were designed, synthesized and evaluated in vitro. These compounds represent a novel class of cancer prevention agents. These data can be used for systemic study of structure activity relationship of SHeA2 analogs, which would benefit the design of more potent and less toxic cancer prevention agents from this class. It can be used to study the mechanism of action and aid in the understanding of the molecular pathways associated with cancer pathology.
A method to isolate pure apoB48-containing lipoproteins by affinity chromatography to study human enteric de novo lipogenesis

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Background: Recent animal studies have demonstrated that de novo lipogenesis (DNL) is an active pathway in the intestine of hamsters and that chylomicron triglyceride (TG) production is also increased with insulin resistance. Traditionally, enteric production of TG is known as the result of re-esterification of absorbed mono-glycerides and fatty acids packed with apoB48.

Hypothesis: We hypothesize that DNL may be active in the human intestine and is an important contributor to chylomicron TGs.

Methods: To test the hypothesis pure chylomicron fractions are needed. Ultracentrifugation yields chylomicron fractions that are contaminated with VLDL (containing apoB100), potentially flawing the results. In this methodological paper we describe a method to yield pure chylomicrons from ultracentrifuged sera fractions - chylomicrons and triglyceride-rich lipoprotein (TRL). We developed an ApoB-100 affinity matrix, using an antibody specific to ApoB-100 epitopes. The first step was to purify the antibody from sera using classical antibody purification techniques. An anti-ApoB100 affinity matrix was made using purified human LDL (Low Density Lipoprotein, ApoB-100) as a ligand, using AminoLink Plus (Thermo). The anti-ApoB-100 was pooled and concentrated using 10kDa MWCO centrifugal filters (Amicon), for binding to Protein G UltraLink (Thermo). Ultrapel Protein G was used to bind the antibody in the correct orientation, by the Fc (heavy chain) portion, and cross linked with Disuccinimidyl μSuberate (DSS, Thermo) to provide binding stability during use. The separation of ApoB-100 from lipoprotein fractionated serum was done by incubating the fractions (Chylomicron and TRL) overnight on fresh equilibrated resin, overnight at 4°C. The volumes used 200ul equilibrated resin (anti-ApoB-100) to 800ul sample. The resin/sample mixture was pipeted into a spin column (Thermo PD# 69725) and the sample was centrifuged out, (Flow Through, FT). The FT was reapplied to fresh resin and the process reapeted for three consecutive passes. The columns were then washed (high salt) and eluted (low pH), all steps were followed by centrifugation for 5 minutes at 200 rpm. Samples were taken at each step for evaluation by Silver Stain, and ApoB specific ELISA's ApoB-total Elisa Kit, AlerChek,inc., all species of ApoB and, Human ApoB-48 ELISA Kit, Shibayagi Co. Inc., ApoB-48 specific.
**Results:** As expected the data showed that ApoB-100 had been removed sequentially after three passes over fresh resin. Samples were taken at each step and analyzed by Silver Stain and by ApoB specific ELISA. The Silver Stain gels showed a depletion of ApoB-100 in the sequential FT’s 1 & 2 with little or no ApoB-100 in FT 3. The elute gel showed the specific elution of ApoB-100, again with little or no ApoB-100 in the last pass. The ApoB-48 specific ELISA showed that the elution fractions contain no ApoB-48. The apparent capacity of the ApoB-100 affinity resin was approximately 100-140µg/ml, calculated from the combined elute of passes 1 and 2.

**Conclusions:** We validate a method to yield pure apoB48 particles (chylomicrons) from human serum samples. The elimination of ApoB-100 particles (VLDL) was complete, removing, and specifically eluting ApoB-100 by affinity chromatography. We applied this method to our ongoing study on human enteric DNL shown in poster #11.

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**Does de novo lipogenesis occur in the gut? A pilot human study**

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**Background:** *De novo* lipogenesis (DNL), the conversion of sugar to fat, is thought to be an active pathway in hepatic and adipose tissue. It has been shown that insulin resistance leads to higher hepatic DNL, which is associated with increased very low density lipoprotein triglyceride (VLDL-TG) levels, a factor linked to higher risk of cardiovascular disease. In the postprandial state most of the plasma TGs are found in the chylomicron lipoprotein produced in the intestine and released into the lymph. Recent animal studies have demonstrated that DNL is an active pathway in the intestine of hamsters¹ and that chylomicron TG production is also increased with insulin resistance².

**Hypothesis:** We hypothesize that DNL may be active in the human intestine and is an important contributor to chylomicron TGs.

**Methods:** One non-obese non-diabetic male subject was admitted to the Clinical Research Center at San Francisco General Hospital for a one-day inpatient stay during which a high carbohydrate shake (50% carbohydrate, 35% fat and 15% protein energy) was consumed every 30 min for 8 hours with periodic blood draws. Plasma samples were ultracentrifuged to separate chylomicron, VLDL and triglyceride rich lipoprotein (TRL) fractions. Lipids were chemically extracted, TGs were isolated by TLC, and subsequently derivatized and analyzed by gas chromatography/mass spectrometry (GC/MS) to measure DNL.

**Results:** The data from this pilot study show the presence of intestinal DNL on the order of 5-10% while hepatic DNL peaked at 10-15%. The total DNL contribution to (TRL) was 11%, suggesting a higher contribution of intestinal DNL to TRL in the postprandial state. Because ultracentrifugation alone may lead to incomplete separation of VLDLs and chylomicrons, further separation of ApoB100 and ApoB48 particles is necessary to confirm these preliminary data. A method to further isolate the chylomicron fraction by using ApoB100 antibodies has been developed and it confirmed the validity of these results. (See poster #10)

**Conclusion:** This is the first report showing an active DNL pathway in the intestine of humans. This pathway may be critical in the modulation of postprandial TG levels.  

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Study of the Mechanism of Interaction of Cystatins with Tumor Cells

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Background: Cystatins are endogenous protease inhibitors of lysosomal cysteine proteases, such as cathepsin B. We have recently coined cystatin M (CST6) as a novel tumor suppressor gene for breast cancer. Forced overexpression of CST6 in tumor cells inhibits their proliferation both \textit{in vitro} and \textit{in vivo}. Overexpression of another cystatin, cystatin C (CST3), however, stimulates the proliferation of several normal and tumor cell types. CST3 is expressed in most cells and tissues and acts like a growth factor. As both CST3 and CST6 are secreted proteins, we aim at elucidating their molecular pathways of action. The long-term objective of our studies is to understand the role of cystatins in tumor growth and metastasis.

Hypothesis: CST3 and CST6 bind to specific cell surface receptors involved in regulating cell proliferation.

Methods: We developed a cellular ELISA to measure and characterize the cystatin-binding activity of various cell types. Briefly, cells were seeded at equal densities in black 96-well microtiter plates with flat and clear bottoms and grown until they reached semi-confluency. The cells were then washed in ice-cold PBS and incubated for 30 min on ice with various amounts of recombinant human CST3 (0 to 800 nM) in the absence or presence of an excess of other recombinant cystatins (CST5, CST6 or CSTA/STFA) or the synthetic broad-spectrum cysteine protease inhibitor E64. Cell monolayers were washed to remove unbound material and fixed in 2\% paraformaldehyde. Free binding sites, unreacted paraformaldehyde and endogenous peroxidase were blocked. Cell surface-bound CST3 was subsequently assessed using ELISA.

Results: Recombinant human CST3 bound to the surface of breast tumor cells in a dose-dependent and saturating manner, which suggests the presence of a cell surface receptor for this cystatin. A CST3 variant lacking cysteine protease inhibitory function bound equally well to cells as did wild-type CST3 and so did a preformed complex between CST3 and the plant cysteine protease papain. This suggests that CST3 does not bind to a putative cell surface cysteine protease. An excess of E64 had no effect on the binding of CST3 to breast epithelial cells, while an excess of closely related cystatins partially competed with binding.

Conclusions: Our results suggest that there exists a putative receptor that is able to bind several related cystatins. These data provide a sound basis for studying the potential of this
putative receptor in transducing the cystatin signal and modulating tumor growth and metastasis. Ultimately, this research may provide novel therapeutic avenues for treatment of malignant growth.
Aspirin inhibits serum paraoxonase 1 physiological activity: preliminary report

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Background: The antioxidant activity of high-density lipoprotein resides largely on its paraoxonase 1 content. Experiments with transgenic knock-out mice indicate the potential for this enzyme to protect against atherogenesis. This relationship has been further strengthened by the publication of the first prospective study showing that low serum PON-1 activity is an independent predictor of new CHD events. Paraoxonase-1 (PON1) is a promiscuous esterase and lactonase associated with HDL and linked to atheroprotection.

Hypothesis: Given the ester nature of aspirin and its similarity with some of the PON-1 substrates we hypothesized that aspirin inhibits PON1.

Materials and Methods: We set up dose response curves for PON1 activities in the presence of 0-2 mmol/l aspirin. We run the experiments both in purified HDL and in 3 human volunteer serum samples. We isolated HDL by sequential flotation ultracentrifugation (d=1.21g/ml). PON1 monoesterase activity was measured using phenylacetate as substrate and following product formation at 270 nm. PON-1 triesterase activity was determined using paraoxon as a substrate, from the initial velocity of p-nitrophenol production at 37 °C and recorded at 405 nm. PON-1 lactonase activity was kinetically determined at 37 °C using 5 (thiobutyl)butyrolactone (TBBL) and recorded at 405 nm.

Results: We found a dose dependent inhibition by aspirin of both lactonase and arylesterase activities of PON-1 that reaches 30% at 2 mmol/l (p < 0.001). Results are consistent for pure HDL and total serum in all samples studied. The effect on PON-1 triesterase activity (paraoxon) is minimal.

Conclusion: This is the first report to show aspirin inhibits paraoxonase activity. Results also show the complexity of PON-1 active site, since one of the activities changes little. The lactonase activity, which is considered to be the physiologically relevant, is impacted. After a 500 mg dose, aspirin levels in plasma peak at 0.15 mmol/l, and we show inhibition of 8-10% of PON-1 activity at those concentration, thus these results may be physiologically relevant. Low dose (preventive) aspirin would have no deleterious effect on PON-1, while anti-inflammatory full doses may well have. Lineweaver kinetic studies are ongoing in our lab.

Sponsored by Touro University.
Activation of the cardiac potassium channel HERG may be a determinant of the extracellular potassium dependency of block of HERG by terfenadine and bepridil.

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**Background:** One form of Long QT syndrome, referred to as acquired Long QT syndrome, has been shown to primarily result from a reduction in the cardiac potassium channel HERG (human ether-a-go-go related gene) by a large number of pharmaceutical compounds. In some instances Long QT syndrome will degenerate into the potentially lethal arrhythmia torsade de pointes, characterized by a rapid heart rate and severely compromised cardiac output. Many patients requiring medication also present with abnormal serum electrolyte levels due to a variety of conditions including gastrointestinal dysfunction, renal and endocrine disorders, diuretic use, alcoholism, and aging. Extracellular electrolytes, in particular extracellular potassium, have significant influence on HERG channel behavior and have been shown to alter drug block of HERG. However, the mechanisms by which drug block is altered in different extracellular solutions are not well understood.

**Hypothesis:** Block of HERG by drugs with different mechanisms of action on the HERG channel will show different dependencies on extracellular potassium. In particular drugs that can be trapped inside the channel after the channel closes will show a different dependency on extracellular potassium compared to drugs that are not trapped inside the channel after the channel closes.

**Methods:** We used two-electrode voltage clamping of Xenopus oocytes to measure blockade of both the wild-type HERG channel (WT) and a mutant HERG channel with a single amino acid substitution (D540K). cRNA of either WT HERG or the D540K mutant was injected into enzymatically defolliculated oocytes and currents recorded 1-5 days after injection.

**Results:** Block of WT HERG by terfenadine and bepridil was unchanged with increasing extracellular potassium. Block of HERG by 1 mM terfenadine was 78%±1% in 0 mM K+ and 80%±1% in 20 mM K+. Block by 1 mM bepridil was 71%±1% in 0 mM K+ and 65%±3% in 20 mM K+. The mutant D540K displays an unusual gating property in that it opens upon hyperpolarization as well as depolarization. This is in contrast to WT HERG channels which only open with depolarization and then close with hyperpolarization. Block of D540K by 1 mM terfenadine and 1 mM bepridil decreased with increasing extracellular potassium. Block of D540K by 1 mM terfenadine was 90%±1% in 0 mM K+ and 83%±0% in 20 mM K+. Block of D540K by 1 mM bepridil was 58%±3% in 0 mM K+ and 31%±4% in 20 mM K+. In addition block of D540K by 1 mM bepridil in 20 mM Cs (a less permeant ion than K+) was 44%±4%.
**Conclusions:** Recent experiments indicate that terfenadine and bepridil can be trapped inside the channel after the channel closes and that the D540K mutant channel is unable to trap these drugs. In addition we have reported that block of WT HERG by quinidine and cisapride, two drugs that are not trapped inside the channel after the channel closes, show a strong correlation with the permeant ion: increasing extracellular potassium decreases block of WT HERG by both quinidine and cisapride. Together these results suggest that the permeant ion is not able to destabilize a trapped drug (terfenadine and bepridil) but is able to destabilize a drug that is not trapped (quinidine and cisapride) and indicate a possible role for the activation gate in determining the extracellular potassium dependency of block of HERG.
The antioxidant enzyme serum paraoxonase 1 is not a serum acetyl-salicylic esterase
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Background: Serum aspirin esterase (AE) activity may be one of the factors responsible for the aspirin resistance syndrome which complicates prevention of stroke and myocardial infarction. This activity has been linked to pseudocholinesterase and albumin, however, recent work has shown correlations indicating that more than 50% of the AE activity cannot be accounted for by the former two proteins. Paraoxonase-1 (PON1) is a promiscuos esterase and lactonase associated with HDL and linked to atheroprotection.

Hypothesis: We hypothesized that PON1 displays AE activity.

Materials and Methods: We determined AE and PON1 activities in 20 human volunteer serum samples. Serum AE activity was evaluated at 37°C with 1 mM aspirin as substrate, kinetically following salicylate absorption at 300 nm and PON1 activity was measured using phenylacetate as substrate and following product formation at 270 nm. We isolated HDL by sequential flotation ultracentrifugation (d=1.21g/ml) and determined its aspirin esterase and PON1 activities.

Results: Serum AE activity in our volunteers was 101.2 +/- 23.3 nmol/ml/min which is in agreement with previous reports. No significant aspirin esterase activity was found in purified HDL, which displayed high arylesterase activity. No correlation was found between serum aspirin esterase and PON1 activities.

Conclusion: In spite of the typical promiscuity of PON1 in HDL our work failed to demonstrate any significant role in aspirin metabolism. The identity of all the enzymes responsible for hydrolysis of aspirin in serum, which may explain, in part, the aspirin resistance syndrome, remains an open field.

Sponsored by Touro University and Showa University.
A screening for the life-extending genes that can suppress age-related oxidative stress in C. elegans
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Background: Oxidative stress is a major risk factor for a wide variety of age-related diseases, including age-related memory impairment (AMI), neurodegenerative diseases and cardiovascular diseases. However, it remains to be determined how aging and increased oxidative stress can be prevented.

Hypothesis: The life-extending genes that can confer resistance to oxidative stress show low levels of ROS (reactive oxygen species). They include the insulin/IGF-1 pathway genes (age-1, PI3 Kinase gene), and the serotonin pathway gene (ser-1, serotonin/octopamine receptor gene).

Methods: ROS was visualized by using ROS marker dyes, called DCF, and examined by fluorescent microscope. The images were used to measure ROS levels in vivo. We examined cellular compartmentalization of age-related ROS in living C. elegans.

Results: We are currently setting up and optimizing the experiments. Preliminary results suggest that mutations in age-1 and ser-1 significantly reduced levels of ROS. Other genes remain to be tested.

Conclusions: The results suggest the age-1 and ser-1 mutations suppress age-related oxidative stress. It remains to be tested: (1) whether the mutations can delay the occurrence of oxidative stress; and (2) whether they completely suppress oxidative stress.
CLINICAL SCIENCES
Women and Anabolics: A Unique Comparison between Female and Male Steroid Users
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Background and Hypothesis: Abuse of anabolic androgenic steroids (AAS) by strength-trained males has been well documented over the past two decades. Despite reports of admitted AAS use among females, little is known about this unique subset of users. The objective of this study was to provide an in-depth analysis of female self-reported AAS users and to compare this cohort to male self-reported AAS users.

Methods: A web-based survey was posted on 38 discussion boards of various fitness, bodybuilding, weightlifting, and steroid websites between February and June 2009. Among 2,380 survey attempts, 1,519 subjects fully completed and submitted a valid survey. Among 518 reported AAS users, 12 were female and 506 were male.

Results: The female AAS users reported using an average of 8.8 performance enhancing agents in their routine. Both female and male users were motivated to utilize AAS to increase muscle mass, increase strength, and improve physical appearance. Over half (58.3%) of the female AAS users met criteria for substance dependence disorder compared to 23.4% of male users ($p=0.011$). Half of the female AAS users had been professionally diagnosed with a psychiatric illness compared to 18.2% of male users ($p=0.014$). Reported history of sexual abuse (41.7% vs. 6.1%; $p<0.001$) and physical abuse (33.3% vs. 10.0%; $p=0.029$) were both more prevalent among female AAS users relative to male AAS users.

Conclusions: Both female and male AAS users practice polypharmacy and are motivated to utilize AAS to increase muscle mass, increase strength, and improve appearance. Female AAS users are more likely to have qualified for substance dependence disorder, have been diagnosed with a psychiatric illness, and have a history of sexual or physical abuse than male AAS users.
An Association between Schistosomiasis Prevalence and Preventive Education in Minigo, Tanzania

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Background: Minigo is a fishing village (pop. 3635), located within the Lake Victoria Region. Infested waters put villagers at risk for contracting the trematodes, *Schistosoma haematobium* and *Schistosoma mansoni*, parasitic determinants for the chronic disease schistosomiasis. The eggs are the primary cause of damage to the bladder, liver, and intestines. Annual screening and provisional treatment has been performed in Minigo since 2005.

Hypothesis: Residents of Minigo, Tanzania who displayed preventive behavior should have a decreased prevalence of Schistosomiasis.

Methods: Discussion with Minigo’s leaders resulted in a pre-announced two-day screening, including an educational session on the first day concerning risks of infection, symptoms, and prevention. Light microscopy confirmed schistosomes in stool and urine samples taken following registration and an oral questionnaire. Test positive subjects were given a physical exam and treated with Praziquantel. Fishermen received prophylactic treatment.

Results: Of the 229 participants (m=138, f=91), the overall schistosomiasis prevalence was 12.2%. In participants that returned from 2008, the prevalence was 13.6%. Of the 52.4% question-responsive (n=109), the prevalence was 10.4% in the 44% who reported preventive behavior and 14.8% in the 55.9% who reported non-preventive behavior.

Conclusions: While screening and treatment of schistosomiasis are important components of a preventive treatment plan, educational sessions instruct participants on prevention. Results display an effective decrease in prevalence when education that encourages preventive behavior is provided to a community. Extension of screening services from point-of-care facilities to communities would facilitate access to education and supervise compliance to preventive intervention.
Patients with non-alcoholic fatty liver disease have increased gluconeogenic flux towards hepatic glycogen

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Background and Hypothesis: The liver plays a critical role for glucose homeostasis by modulating hepatic glucose production and uptake. Hypoglycemia is prevented by hepatic glycogenolysis and gluconeogenesis (GNG). During hyperglycemia, glucose production is suppressed and hepatic glycogen stores are replenished by direct glucose uptake (direct pathway) or indirectly by taking up gluconeogenic substrates to make hepatic glucose stored as glycogen (indirect pathway)¹. Non-alcoholic fatty liver disease (NAFLD) is becoming more prevalent in our society affecting about one-third of Americans². Insulin resistance (IR) may predispose one to develop NAFLD³. Patients with IR have an increased supply of gluconeogenic precursors without significant increases in glucose production⁴. We propose that in the transition from fasting to hyperinsulinemia, subjects with NAFLD have an increased flux of gluconeogenic precursors to hepatic glycogen when compared to control subjects.

Methods: Six test subjects with evidence of fatty liver (high lipid to water ratios as measured by proton magnetic resonance spectroscopy) and seven controls were admitted to the Clinical Research Center at San Francisco General Hospital. Following an overnight fast, subjects underwent intensive tracer studies to measure hepatic uridine diphosphate (UDP)-glucose flux in the fasted state and during a hyperinsulinemic-euglycemic clamp. D-galactose-1d was used to label UDP-glucose, which in turn was sampled by acetaminophen, serving as a “pharmacological probe”. Acetaminophen was conjugated with UDP-glucose in the liver to form acetaminophen glucuronide (GlcUA), which was subsequently excreted in the urine. Labeled urinary GlcUA was isolated by HPLC, and derivatized for GC/MS analysis. The flux of UDP-glucose was calculated by the tracer dilution method⁵.

Results: Comparing the change of UDP-Glucose flux from fasting to hyperinsulinemic state (i.e. during the clamp), a significant increase (p<0.016) of the indirect pathway was observed in NAFLD subjects (0.16±0.02 mg/kg*min or a 68% increase) compared to controls (0.05±0.03 mg/kg*min or a 21% increase). Notably, average fasting insulin in subjects with NAFLD was nearly twice that of controls.

Conclusion: The results suggest that more GNG substrates are taken up by the liver and channelled by the indirect pathway to hepatic glycogen in subjects with NAFLD. This increased flux may impact hepatic glycogen stores but also hepatic de novo lipogenesis.


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Serum aspirin esterase activity increases after hemodialysis in patients with end stage renal disease

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Background: Research on variations of serum aspirin esterase (AE) in specific disease entities is scarce. End stage renal disease patients undergoing hemodialysis have a cycle of clearance and accumulation of uremic toxins.

Aims: We evaluated AE status in renal failure in a case–control study with 39 ESRD patients on hemodialysis who were recruited from the Nephrology Unit, Dept. of Internal Medicine, Showa University Northern Yokohama Hospital, Japan. Age and gender-matched control subjects (n =30) were selected from hospital personnel with no history of renal disease. We also evaluated the changes in AE after dialysis.

Materials and Methods: Creatinine, urea; glycemia, cholesterol; triglycerides; HDL cholesterol, LDL cholesterol, albumin were measured by standard methods. Aspirin esterase activity was measured kinetically at 300 nm, 37°C for 15 min. Data were expressed in nmol of acetylsalicylic acid hydrolyzed per minute and per milliliter (nmol/mL/min). The runs were blanked against reagent (to control for spontaneous hydrolysis of acetylsalicylic acid). The intra-assay CV is 4% and the inter-assay 5%, respectively.

Results: AE levels are lower in our series of ESRD patients 30.9 +/- 8.9 nmol/ml/min vs 39.9 +/- 9.1 for control subjects (p<0.0001). AE levels increases dramatically by 33% to control levels after each dialysis (p<0.0001).

Conclusions: In conclusion, our data suggest that AE activity is 33% lower in ESRD patients, hence, their serum metabolism of aspirin is slower and the doses more efficient. However, after dialysis, the situation reverses, they have more activity and then less efficiency for each dose. Changes in AE activity in these patients are shown here for the first time and suggest mechanisms such as removal of inhibitors by dialysis or modulation of lipid parameters as significant pathways to explore in further studies.

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* MPH Student, COP 2014
Effects of Osteopathic Manipulation on Acute Mountain Sickness
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Background: Acute Mountain Sickness (AMS) is a common illness seen in unacclimatized persons shortly after ascent to high altitude. Altitude illness is the result of an inadequate acclimatization to high altitude caused by alterations in the normal compensatory responses to low oxygen environments; however, the exact mechanisms accountable for AMS symptoms are not well understood.

Hypothesis: Since increased oxygen saturation levels have been shown to alleviate AMS symptoms, we hypothesized that osteopathic manipulative techniques (OMT) aimed at mobilizing the respiratory apparatus and correcting related dysfunction will decrease the severity of AMS symptoms.

Methods: Thirteen healthy volunteers (9 males, 4 females, age: 23-54), all residents from near sea level, were exposed to an altitude of 12,500ft for a total duration of 3 nights and 3 days. A predefined set of OMT was performed by a licensed osteopathic physician on the treated group (n=7) immediately upon arrival at altitude then twice a day. The control group had no treatments of any kind (n=6). Prior to and following OMT all subjects filled out a standardized Lake Louise Self-report Questionnaire (LLQ) rating their symptoms for fatigue, dizziness, sleep quality, gastrointestinal status and headache severity. The subjects’ oxygenation status (SaO\textsubscript{2}) was monitored during each completion of the LLQ by pulse oximetry.

Results: While both groups showed a gradual improvement in SaO\textsubscript{2} over time only the treatment group showed a significant positive correlation between SaO\textsubscript{2} and exposure time (p<0.01). Due to variable starting SaO\textsubscript{2} values in the subject pool, the same analysis was performed with data normalized to each subject’s starting value to better reflect individual improvement. Again, only the treatment group yielded a significant positive correlation between SaO\textsubscript{2} improvement and hours at altitude (p<0.01). Both groups experienced AMS, but only the treatment group displayed a significant correlation between the decrease in AMS symptoms and the increase in SaO\textsubscript{2} (p<0.01).

Conclusions: The improvement in SaO\textsubscript{2} over time and the decrease in AMS symptoms both indicate that acclimatization is taking place. In our study these improvements were significant only in the treatment group and therefore support our hypothesis that a standardized OMM regiment can be beneficial to the acclimatization process. These results warrant further research using a larger double-blinded study in the efficacy of OMT as treatment for AMS.
Leprosy: Fading or Forgotten? Evaluation and Intervention for the Improvement of Physical and Psychosocial Disabilities amongst the Leprosy Patients in Shirati, Tanzania.

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Purpose: The purpose of this project is to conduct a primary needs assessment and to evaluate the facility for long standing care of leprosy patients. The facility was evaluated, needs were identified, and an intervention plan was implemented. Phase II to be conducted in 2010 will evaluate the efficacy of the intervention plan.

Background: Though the prevalence of leprosy has declined in Tanzania, the physical and psychosocial manifestations of this disease still affect each patient’s quality of living, social status and family life. Previously conducted studies demonstrate that long-term community based rehabilitation may prevent the worsening of leprosy-associated disabilities.

Methods: A questionnaire was developed and interviews were conducted amongst the leprosy residents where the quality of physical rehabilitation, living accommodations, and social support was assessed.

Results: The results indicated that the majority of leprosy patients were not satisfied with the charity food provision, the lack of income generation and the lack of assistance with mobility (i.e. canes, wheel chairs, prosthetics). In order to begin addressing these concerns, a safe clean water supply was provided, resources for food self sufficiency and income generation were provided, and the pre-existing food resources were improved.

Conclusions: It was concluded that long term facilities with charity resources are insufficient and that long term care should be based on the provision of income generating and self-sustaining projects. Phase II of this study will evaluate the efficacy of the interventions implemented, and identify areas for improving mobility and integration within the community.
Osteopathic Survey of Somatic Dysfunction and Zink Compensatory Patterns in Sololá, Guatemala

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Background: There is a paucity of research done in developing countries on J. Gordon Zink DO’s model of compensatory patterns of fascial restriction and ease. The Compensatory Pattern is assessed in a supine patient by noting ease of rotation at the following four junctions: Cranio-cervical, Cervico-thoracic, Thoraco-lumbar, Lumbo-sacral. The Common Compensatory Pattern has been Left/Right/Left/Right (LRLR). Is the “common” compensatory pattern truly common to all humans or is it an artifact of the lifestyle in industrialized countries? In Sololá, Guatemala, the pre-existing cultural category for a group of healers known as sobadores (bone-setters) made Sololá an ideal place for this study, where Osteopathic Manipulative Medicine (OMM) was culturally well-received by the community.

Objective: To survey Zink Compensatory Patterns among the Maya men and women in Sololá, Guatemala.

Materials & Methods: Patients were screened for musculoskeletal and visceral problems and referred to the OMM clinic by the physician at a local primary care clinic. 40 patients (30 female, 10 male) participated in the study. Verbal and/or written consent was obtained from all participants. Translation was provided as needed. Four TUCOM students, who had completed their first year of osteopathic medical education and had received additional training in Zink screening by faculty, performed an osteopathic structural exam using standard OMM tables. Results were noted on a Zink Fascial Screening Exam form developed by John C. Glover, DO, FAAO.

Results: Zink fascial screening data demonstrated a wide range of fascial strain patterns. 26% of our 40 patients had a Left Right Left Right (LRLR) compensatory pattern (the “Common” pattern seen in prior research). 6% had a RLRL compensatory pattern. Furthermore, 69% of all patients showed Right Rotational fascial preference at the Cervico-thoracic junction, 46% of whom had a left rotated compensatory pattern at their Occipito-atlantal and Thoraco-lumbar junctions.

Conclusion: The Common Compensatory Pattern of LRLR was the most consistently noted pattern among the Maya in Sololá, Guatemala. The wide variation noted in Zink patterns may actually represent the population studied, or be an artifact related to small n and intra-examiner reliability. Documentation of examiner reliability during the study, as well as prior to, should be included in future research.

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Mogollon, Drs. Celia and William VanWisse for their valuable teachings, and our osteopathic medical student volunteers for their dedication.

NOTE: This study has been approved in its entirety by the TUCOM IRB Committee.
Somatic Dysfunction among the Homeless Clients of Suitcase Clinic in Berkeley, California – A Retrospective Chart Review
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Background: In 1989, University of California, Berkeley students established the all-volunteer Suitcase Clinic (SC) to provide medical, chiropractic, optometry, legal and dental services to the homeless. Since 2008, OMS I & II and faculty from Touro University College of Osteopathic Medicine (TUCOM) have contributed osteopathic manipulative treatment (OMT) to the SC. The SC clients were challenging. They were found to have multiple body regions with somatic dysfunction (SD) whether they had single or multiple chief complaints (CC). We wondered if there were CC and/or patterns of SD unique to the homeless and if so, could these help us better resolve musculoskeletal dysfunctions in the homeless at SC.

Hypotheses: There are CC and/or patterns of SD unique to the homeless population. Body regions with SD are not limited to the area of the CC.

Objective: To assess the frequency distribution of CC and SD from OMT encounters at SC in Berkeley, CA.

Methods: We performed a retrospective chart review of all OMT encounters from the SC since 2008 (n=203). All data were analyzed using Epi Info™ 3.5.1.

Results: The majority of SC clients were returning clients with new, multiple CC (n=203). For encounters with new CC (n=132), the most prevalent CC in descending order were pain in: lower extremities, low back, neck, and shoulder. The greatest prevalence of SD by body region in descending order was: pelvis (sacrum + innominate), thoracic, and cervical.

For encounters with single CC (n=70), the following areas of SD were noted in descending order of prevalence: lower extremity - pelvis (80%), low back – lumbar (85.7%) and pelvis (85.7%), neck - head (100%) and cervical (100%), shoulder - upper extremity (84.6%), thoracic (69.2%), and cervical (53.8%).

From all encounters, regardless of CC (new or recurring, single or multiple), the most prevalent SD was in the pelvis.

Conclusion: Prevalent CC among SC clients involved lower extremities, low back, neck, and shoulder. Distinct patterns of SD were present. Based on these CC and SD patterns, tailored self-care exercise pamphlets were developed for distribution to clients after OMT visits. Suitcase training sessions will also be revised to ensure that students are proficient in diagnosis and treatment of, as well as exercises for, these hi-yield regions. These simple, self-care exercises, which require no gym or equipment, can help clients decrease pain, maintain OMT effects between visits, and better withstand the repetitive stresses of
homeless life. Empowering clients to take part in their own healthcare can decrease their sense of helplessness. These interventions will allow TUCOM students and faculty to provide more effective osteopathic care for the SC clients: mind, body, and spirit.
Free serum paroxonase 1 activity is much lower in neonates than in adults
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Background: Paraoxonase 1 (PON-1, EC 3.1.8.1) could be implicated in the mechanism by which HDL inhibits LDL oxidation. PON-1 activity largely depends on its association with apolipoprotein AI (apoAI) and phospholipids in HDL, although a free form can be found. Free PON-1 is inactive against lipid peroxides, it increases in diabetes patients and this mechanism may contribute to the lower activity found in these patients and to a dysfunctional more atherogenic HDL particle. Neonates have low serum lipoproteins, which are mainly HDL and lower PON-1 activity than adults. There are no reports on free PON-1 in neonates.

Aims of the study: To compare free PON-1 activity in neonates and adults.

Methods: We performed a case-control study with 40 newborns (normal term deliveries), Apgar 8-9; and 30 healthy adults. PON-1 activity was measured in total serum and in lipoprotein deficient serum (LPDS). Briefly 100 μl serum was diluted with 100 μl NaBr to achieve a d = 1.220 g/ml). A 10 μl aliquot was taken (for total enzyme activities), and the rest of the samples were centrifuged at 150,000 g in a Beckman 42.2 Ti-rotor, for 24 h at 10 °C. Serum PON-1 (triesterase) activity was determined using paraoxon as a substrate, from the initial velocity of p-nitrophenol production at 37 °C and recorded at 405 nm. After correction for dilution, activities are expressed in U/l and in ratio free/total.

Results: As expected, total PON-1 activity was lower in neonates than in adults: 30 +/- 11 vs 144 +/- 20 U/l, p<0.0001. However, percentage of free PON-1 was almost 3-fold lower in neonates than adults: 10 +/- 3 vs 40 +/- 11 %, p<0.001.

Conclusions: This is the first report addressing free PON-1 in early life. Our data suggest that HDL-PON-1 interaction in neonates favors the association of the enzyme to the particle, rendering it more active. An alternate explanation is that there is low dissociation of PON-1 from neonate HDL due to low exposure to oxidants and LDL.

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Assessing Student Knowledge and Interest in the Implementation of Pharmacogenomics

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Rationale: Pharmacogenomics is an emerging area of study that will greatly impact the practice of pharmacy as well as the direction of patient care. According to the third edition of the competency guidelines set forth by the National Coalition for Health Professional Education in Genetics, improved aptitude in genetics is imperative for proper disease management and diagnosis.

Objective: To evaluate the education and interest of pharmacy students in pursuing a career in pharmacogenomics.

Methods: First and third year students from eight pharmacy schools in California were invited to participate in a survey developed by the Touro University CAPSLEAD team. Students completed either a hard copy or electronic version of the survey indicating their attitudes towards pursuing a career in pharmacogenomics as well as feelings of preparedness for such a career path.

Results: A total of 714 students attempted the survey, of which 644 were fully completed and included in the final cohort. Statistical analyses showed that if pharmacogenomics was incorporated into the pharmacy curriculum, students were more likely to view pharmacogenomics as important to the future practice of pharmacy. First-year students were also more open to the inclusion of pharmacogenomics in their preference of practice than third-year students.

Conclusion: While students agree that pharmacogenomics is important to the future practice of pharmacy, it appears that there is limited interest in the subject. Further investigation is necessary to evaluate the desire of pharmacy students to incorporate pharmacogenomics into their future practice.
4-year Assessment of Providing Access to Healthcare for Villagers in Shirati, Tanzania

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Background: A need assessment study was conducted in Shirati, Tanzania in 2005, to identify the major barrier for women to safe hospital delivery. Reasons such as lack of awareness, poverty, or cultural beliefs ranked the least whereas lack of transportation was the main obstacle. In addition to pregnant women, the study showed that children with malaria or other illnesses were unable to receive proper treatment due to lack of access to the hospital.

Hypothesis: We hypothesize that providing physical access to hospital via bicycle will increase in access to health care for pregnant women and children in rural parts of Tanzania.

Method: Appropriate intervention plan was initiated by providing modified bicycles to five villages annually in the beginning of 2006. Details including reasons for bike usage and patient information were recorded in a notebook kept by a bike guardian in each village. Barriers to adequate use were identified as well as frequency of the utilization of the bike was evaluated annually for 4 years.

Results: The four year assessment of the project from 2006-2009 confirmed that with more reliable transportation, there was an increase in access to health care including emergencies, prenatal/postnatal care, and delivery at the hospital. Furthermore, some women changed their preference for using the services of a traditional birthing attendant to delivery at the hospital. Use of the bicycles was not solely restricted for transportation to the hospital, but also for occasional personal matters.

Conclusion: Access to health care in remote rural areas can be achieved by providing local means of sustainable transportation, minimizing restrictions and regulations on its use, and provision of autonomous control by villagers.
Use of a Continuous Glucose Monitoring System in Clinical Research
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Background: The DEXCOM continuous glucose monitoring system (CGMS), which was developed for individuals with diabetes, also has great potential as a new source of research data, allowing continuous glucose measurements in clinical research settings. The system includes a sensor inserted into the abdominal subcutaneous tissue by an introducer needle applicator, which relays glucose values every 5min to the receiver by radiofrequency¹ and allows real time glucose trends to be measured for up to seven days and nights.

Hypothesis: This methodology can be used to monitor glucose in subjects with and without Non-Alcoholic Liver Disease (NAFLD) to assess the effect of metabolic abnormalities associated with excess liver fat on glucose control.

Methods: Participants with NAFLD and healthy controls were studied in the General Clinical Research Center at San Francisco General Hospital. The sensor was inserted and CGMS units were calibrated under the supervision of a nurse. A frequently sampled 75-g oral glucose tolerance test was performed, and values from the CGMS were compared to those measured in real time by the glucose oxidase assay (YSI STAT 2300 glucose analyzer, Yellow Scientific OH). Subsequently, the CGMS units were worn by participants as outpatients for five days to assess glucose excursions when following their typical habits and diets.

Results: During the OGTT the CGMS measurement was consistent with measurements on the YSI instrument (Fig 1.). Data were consistent with previous studies showing a delay in interstitial glucose levels. Values attained during the OGTT validated the use of DEXCOM CGMS as a reliable tool for measuring blood glucose levels. During the outpatient monitoring, when subjects were divided in two groups based on fasting insulin levels, four of five subjects (80%) with fasting insulin levels ≥10 μU/mL had at least one glucose excursion above 200 mg/dL, whereas such excursions were seen in only two out of six subjects (33%) with fasting insulin levels <10 μU/mL.

Figure 1: Comparison of glucose excursions measured in real time by DEXCOM and YSI during an Oral Glucose Tolerance Test (OGTT).
Conclusions: In our hands, the continuous glucose monitoring system provided accurate measurements of blood glucose measures during an OGTT. Preliminary results using this system on an outpatient basis suggest that persons with elevated fasting insulin levels are more likely to have glucose excursions >200 mg/dL. Taken together, these results demonstrate the utility of continuous glucose monitoring in clinical research.


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Serum ischemia modified albumin levels in obstructive sleep apnea patients before and after treatment: A pilot study

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Background. Obstructive sleep apnea syndrome (OSAS) is associated with insulin resistance and the metabolic syndrome. It increases the rate of cardiovascular morbidity, which has been suggested to be partly related to increases in oxidative stress and a state of inflammatory cell activation. Ischemia-modified albumin (IMA) is proposed as a novel marker sensitive to cardiac ischemia with the potential to become a triage tool in suspected acute coronary syndrome patients. IMA is produced by oxidative attack on albumin by reactive oxygen species (ROS) at sites of ischemia.

Hypothesis. We hypothesized that the cycles of hypoxia/reoxygenation in OSAS, may increase IMA levels. IMA levels measurement is inexpensive, quick and easily automated. If proven true, the biomarker could help monitor the effectiveness of the treatment of these patients.

Methods. Ten patients (5 male, 5 female) with OSAS (more than 15 events of apnea-hypopnea/hour) and 20 age-matched controls were studied. The patients were studied at diagnosis and after 6 months of continuous positive airway pressure treatment (CPAP). All subjects were non smokers. Subjects with a history of diabetes, myocardial ischemia, renal, thyroid or systemic diseases were excluded. IMA was measured using a test based on the ischemia-induced decrease in cobalt 2+ binding as previously described. All serum samples were kept at -80°C before analysis. We introduced minor modifications in the method to adapt it to a 96 well plate reader (Versa Max from Molecular Devices). All samples were run in duplicates on the same plate, 2 times. The intra-assay CV was 5%. Other parameters were measured using standard clinical laboratory methods.

Results: During the observation period, no subjects experiences CV events. In OSAS patients under treatment the lowest saturation oxygen concentration during sleep measured by infrared methods, significantly recovered from 79.0 +/- 7.5 to 90.0 +/- 5.5 % (p = 0.027). IMA levels were 0.69 +/- 0.08 in controls and 0.65 +/- 0.1 in sleep apnea patients. The treatment of continuous positive airway pressure treatment had no significant effect on the marker (0.64 +/- 0.1), although a trend to decrease was noted.

Conclusions: Even within the limitations of small sample numbers, this is the first study measuring IMA in OSAS patients, a frequent co-morbidity in insulin resistance. Our data show that IMA levels do not change in OSAS patients as compared to control subjects nor it changes with the CPAP treatment. IMA is produced by free radical attack on a terminal peptide of albumin, associated with focal ischemia. This pilot study seems to indicate that
hypoxia/reoxygenation does not induced localized high fluxes of reactive oxygen species that could modify the molecule.

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Continuous positive air pressure treatment reduces serum advanced glycation endproducts in patients with obstructive sleep apnea syndrome: a pilot study
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Background: Low caloric diet (LCD) is used for weight loss. Paraoxonase 1 (PON-1) is associated with the antioxidant functions of high-density lipoprotein (HDL). Among limited data on the relationships between obesity and PON-1, there has been no study on the effects of a stand-alone LCD on the physiological lactonase activity of PON-1.

Aims and methods: We investigated the prospective effects of LCD intervention (2 months) for weight loss on serum PON-1 activities (lactonase, arylesterase [mono-esterase] and tri-esterase) and HDL cholesterol (HDL-C), and their association with low-density lipoprotein cholesterol (LDL-C) in overweight and non-morbidly obese but otherwise healthy women (n = 30; mean age, 50.3 years; mean body mass index [BMI], 28.5 kg/m(2)). In addition to the data such as BMI, blood pressure, blood glucose and lipids, PON-1 activities were examined between pre- and post-intervention.

Results: The intervention reduced all metabolic outcomes, and PON-1 lactonase activity (determined with 5-[thiobutyl]butyrolactone) significantly decreased by 6.1%, paralleled by arylesterase (by 7.3%) and tri-esterase (by 7.8%). In multiple regression analysis, the percent change of PON-1 lactonase was significantly, positively and independently correlated to that of LDL-C (beta = 0.51), HDL-C (beta = 0.40), and BMI (beta = 0.37).

Conclusion: Our results showed that the solo diet treatment on weight loss might reduce serum PON-1 lactonase activity with reduced HDL-C and LDL-C. The relationship between the lactonase and LDL-C may be adaptive, plausibly hypothesizing less need for PON-1 activity as an antioxidant property to protect lipoproteins. Further research is needed to confirm this prediction.

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