Center of Excellence in Environmental Toxicology

with

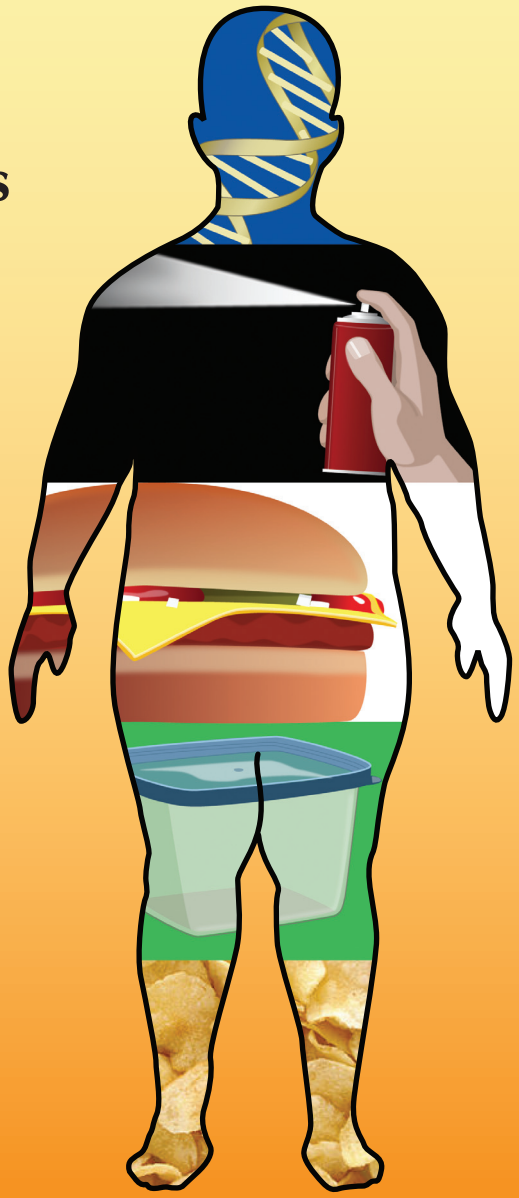
The Children's Hospital of Philadelphia

Sixth Annual CEET Symposium

Gene-Environment Interactions and Childhood Metabolic Disorders

June 1, 2012

Villanova Conference Center



Center of Excellence
in Environmental Toxicology (CEET)
with The Children's Hospital
of Philadelphia (CHOP)

SIXTH ANNUAL CEET SYMPOSIUM

Gene-Environment Interactions and Childhood Metabolic Disorders

Villanova Conference Center
June 1, 2012

Host Institution
CENTER OF EXCELLENCE IN ENVIRONMENTAL TOXICOLOGY
PERELMAN SCHOOL OF MEDICINE AT THE UNIVERSITY OF PENNSYLVANIA

Perelman School of Medicine University of Pennsylvania | CEET

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Sixth Annual CEET Symposium

Gene-Environment Interactions and Childhood Metabolic Disorders

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7:30 – 8:00 A.M. BREAKFAST AND REGISTRATION

SESSION 1: GENETIC AND ENVIRONMENTAL EFFECTS ON PEDIATRIC METABOLIC DISEASES

8:00 A.M. **Struan Grant, PhD**
Genome-wide Analyses of Childhood Obesity

8:30 A.M. **Rebecca Simmons, MD**
Epigenetics and Complex Metabolic Disease

9:00 A.M. **Nancy B. Spinner, PhD**
Towards Understanding the Complex Etiology of Biliary Atresia

9:30 A.M. **Virginia A. Stallings, MD**
Infant Feeding and Development Study: Safety of Soy Protein

10:00 – 10:30 A.M. BREAK AND POSTER SET UP

10:30 A.M. Keynote Lecture 1: **Bruce Blumberg, PhD**
Professor, Developmental & Cell Biology School of Biological Sciences, Professor, Biomedical Engineering, The Henry Samueli School of Engineering, University of California, Irvine

"Obesogens, Stem Cells and the Developmental Programming of Obesity"

11:30 – 12:30 P.M. LUNCH

SESSION 2: APPROACHES TO SOLVE PEDIATRIC DISEASE PUZZLES

12:30 P.M. **Jinbo Chen, PhD**
A Multi-Locus Likelihood Method for Assessing Parent-Of-Origin Effects Using Case-Control Mother-Child Pairs

1:00 P.M. **Hongzhe Li, PhD**
Statistical Methods for Analysis of Gut Microbiome Data

1:30 P.M. **Aalim Weljie, PhD**
Juvenile Dietary Programming and Obesity Risk: a Metabolomics Approach

2:00 – 2:15 P.M. BREAK

SESSION 3: COMMUNITY OUTREACH TO PEDIATRIC DISEASE

2:15 P.M. **Jennifer Culhane, PhD, MPH**
Update on the National Children's Study

2:45 P.M **Kevin C. Osterhoudt, MD, MS**
Risk Communication in Pediatric Environmental Health

3:15 – 4:15 P.M. BREAK AND POSTER VIEWING

4:15 P.M. Keynote Lecture 2: **Karen E. Peterson ScD**
Professor and Director, Human Nutrition Program, Director Formative Children's Environmental Health Center on: "Perinatal Exposures, Epigenetics, Child Obesity and Sexual Maturation"
Associate Director, Michigan Nutrition and Obesity Research Center, University of Michigan School of Public Health, Department of Environmental Health Sciences

"Can We Improve the Pediatric Obesity Research Paradigm? Environmental Health and Nutritional Science Perspectives"

5:15 P.M. DISCUSSION

5:45 – 6:30 P.M. WINE AND CHEESE RECEPTION

Keynote Speakers

Bruce Blumberg received the Ph.D. from the University of California, Los Angeles in 1987. His postdoctoral training was in the molecular embryology of vertebrate development at the Department of Biological Chemistry in the UCLA Medical School from 1988-1992. Dr. Blumberg was appointed as a Staff Scientist at The Salk Institute for Biological Studies, La Jolla, CA in 1992 where he focused on the molecular endocrinology of orphan nuclear receptors and their role in embryonic development and adult physiology. Dr. Blumberg joined the faculty at U.C., Irvine in 1998 where he is currently Professor of Developmental and Cell Biology, Pharmaceutical Sciences and Biomedical Engineering. His current research focuses on the role of nuclear hormone receptors in development, physiology and disease. Particular interests include patterning of the vertebrate nervous system, the differential effects of endocrine disrupting chemicals on laboratory model organisms compared with humans, interactions between xenobiotic metabolism, inflammation, and cancer, and the role of environmental endocrine disrupting chemicals on the development of obesity and diabetes. Dr. Blumberg and his colleagues originated the obesogen hypothesis which holds that developmental exposure to endocrine disrupting chemicals can induce permanent physiological changes. EDC exposure elicits epigenetic alterations in gene expression that reprogram stem cell fate to favor the development of fat cells. Exposed animals are predisposed to develop more and larger fat cells, despite normal diet and exercise which is likely to lead to weight gain and obesity over time.

Karen E. Peterson, ScD. Dr. Peterson is Professor and Director of the Human Nutrition Program, Department of Environmental Health Sciences at the University of Michigan School of Public Health and Adjunct Professor of Nutrition at the Harvard School of Public Health. Her research focuses on the influence of adverse exposures on child growth and maturation during sensitive developmental periods and the potential mediating influence of dietary quality and lifestyle behaviors on exposure-outcome associations in multi-ethnic, low income populations. She has conducted extensive research on the epidemiology and evaluation of population-based interventions addressing child obesity. Dr. Peterson is Principal Investigator of the P20 Formative Children's Environmental Health and Disease Prevention Center (P20 ES018171-01/ RD834800): "Perinatal exposures, epigenetics, child obesity & sexual maturation," serves as Associate Director of the University of Michigan Nutrition and Obesity Research Center (P30DK089503) and directs the Nutrition Assessment Laboratory of the Exposure Core of the UM Environmental Health Science Core Center (P30 ES017885): "Lifestage exposures and adult disease."

MISSION

The Center of Excellence in Environmental Toxicology (CEET) was launched in 2005 and receives grant support from the National Institute of Environmental Health Sciences. It is one of only seventeen designated Environmental Health Sciences Core Centers in the nation.

The mission of the CEET is to determine the mechanistic links between environmental exposures and diseases of environmental etiology. Understanding these processes can lead to early diagnosis, and prevention strategies. The overall goal is to improve environmental health and medicine in our urban region. Many of the solutions to the problems in this region will be translatable to other urban regions both nationally and globally.

The CEET is a flexible entity that marshals excellence in basic, translational, patient-oriented and population-based research in the School of Medicine and Children's Hospital of Philadelphia to facilitate an integrative approach to environmental health/medicine. Although primarily housed in the School of Medicine, the 49 CEET Investigators belong to 16 departments and 5 schools at the University of Pennsylvania.

The CEET marries its relevant research excellence to diseases of environmental etiology that affect our urban region. The CEET has an Affinity Group in Lung and Airway Disease (asthma, lung cancer, mesothelioma, and chronic obstructive pulmonary disease) because of the poor air quality and air pollution in our region (ozone, fine particulate matter, allergens, SO2, NO2 and CO emissions). The CEET also has an Affinity Group in Reproduction Endocrinology and Development because of the high incidence of adverse pregnancy outcomes that lead to low-weight birth and birth and developmental defects in our region. These organ-based themes are linked to our Affinity Groups in disease mechanism, which include Oxidative Stress and Oxidative Stress Injury and Gene-Environment Interactions.

The CEET enables its investigators to conduct modern predictive toxicology via its Molecular Profiling Core (MPC). The MPC employs Toxicogenomic and Toxicoproteomic approaches to identify the genomic and proteomic fingerprints that can be assigned to toxicant class, and to different stages of diseases of the environment. It is engaged in identifying and validating Biomarkers for these diseases.

The Integrated Health Sciences Facility Core (IHSFC) of the CEET provides assistance with a broad range of transdisciplinary services including study design, enrollment of research subjects, human and population exposures, data management, access to biological samples, biostatistical analyses, interpretation of results, and manuscript preparation.

The CEET conducts research relevant to the 45 Superfund Sites that permeate the region. Studies elucidate mechanisms of chemical toxicity; exposure levels, risk assessment and health hazard; bioremediation approaches; and effects on ecosystems and biodiversity.

The CEET works with and disseminates research findings to select local communities to empower them with new knowledge so that they are better informed to tackle issues of health disparities and environmental justice. To improve the environmental health of these and similar affected communities, the CEET is actively involved in the education of health care professionals (Residency Program in Occupational and Environmental Health, Nursing concentration in Occupational and Environmental Health, and Masters of Public Health Programs).

The CEET also disseminates its mission and its research findings to all stakeholders including community organizations, local, state and federal officials and agencies (Pennsylvania Department of Health, Pennsylvania Department of Environmental Protection, Environmental Protection Agency) to affect change in environmental health and public health policies.

CENTER OF EXCELLENCE IN ENVIRONMENTAL TOXICOLOGY

University of Pennsylvania School of Medicine

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AND DEVELOPMENT

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Rey Panettieri, MD
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Anil Vachani, MD

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GENE-ENVIRONMENT INTERACTIONS

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Co-Leader: Alexander S. Whitehead, DPhil
Marisa Bartolomei, PhD
Shelley Berger, PhD
Ian Blair, PhD
Jinbo Chen, PhD
Jason Christie, MD, MSCE
Hakon Hakonarson, MD, PhD
John Hogenesch, PhD
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Reproduction, Endocrinology, and Development (READ)

R1 Sweet Surprise: The Impact of High Fructose Corn Syrup Ingestion during Childhood on the Development of Adult Obesity

Shazia Bhat, MD[1], Alexa Vitins PhD[2], Isaac Sasson MD, PhD[2], Rebecca Simmons, MD[2]

[1]Children's Hospital Philadelphia, Philadelphia, PA; [2]University of Pennsylvania

Background: Since 1970 the prevalence of obesity has risen at an alarming rate. High sugar intake is a compelling potential etiology for this increase, especially given that the rise in obesity parallels a surge in the consumption of added sugar, particularly in the form of high fructose corn syrup (HFCS). Determining whether sugar in any form, or HFCS in particular, causes increased adiposity is critical in understanding and preventing obesity.

Objective: To determine whether HFCS ingestion starting in early childhood results in increased obesity and metabolic derangement in adulthood compared to other types of sugar.

Design/Methods: Starting at weaning, C57/Bl 6 mice were exposed to water containing either sucrose, glucose (20% solution each), HFCS (50% solution), or no added sugar. All mice had ad lib access to a standard chow. The mice were sacrificed at 100 days of age.

Results: In the males, over the study period, total caloric intake did not differ between HFCS and control animals, however a mild increase was seen in sucrose and glucose exposed animals (20% and 13% over controls respectively). Caloric intake from sugar did not differ between the sucrose and HFCS groups; there was a slight, but statistically significant increase in caloric intake from sugar in the glucose group. At the end of the study period, the HFCS exposed mice weighed significantly more than the other groups. Body fat content (measured by NMR) in all three sugar exposed groups was more than double that of controls. Glucose tolerance was impaired in HFCS animals compared to controls, but not in sucrose or glucose exposed animals. Non-esterified fatty acids (NEFA), triglycerides (TG), fasting glucose, and cholesterol levels did not differ between the 4 groups. Leptin levels were significantly higher in the HFCS and glucose exposed groups compared to controls; however, adiponectin levels were significantly higher in the HFCS group only. In the females, the total caloric intake was similarly higher in the sucrose and glucose groups, with caloric intake from sugar being higher in the HFCS group. However, at the end of the study period, there was no difference in weight, glucose tolerance, NEFA, TG, cholesterol, insulin, leptin, or adiponectin levels. Body fat content was higher in the glucose exposed females alone.

Conclusions: Despite lower total caloric intake compared to sucrose and glucose exposed animals, males exposed to HFCS were heavier, had increased adiposity and glucose intolerance, and elevated leptin and adiponectin levels indicative of abnormal fat metabolism. These findings raise a serious concern that HFCS intake during childhood may be contributing to the rise in adult obesity and its associated health problems.

R2 Oocyte from Obese Dams Programs Offspring

Isaac Sasson MD/PhD, Alexa Vitins PhD, Monica Mainigi, MD, Rebecca Simmons MD

University of Pennsylvania

Numerous studies in humans and animal models have demonstrated that maternal morbid obesity is associated with subfertility, intrauterine growth retardation, and an increased risk of metabolic abnormalities in the offspring. However, it is not known if the critical window of exposure occurs prior to (oocyte) or during in-utero development. We hypothesized that the phenotypes observed in the offspring of obese dams were due to the effects of maternal obesity on the oocyte prior to pregnancy. As previously reported by others, oocyte quality was significantly poorer in diet induced obese (DIO) dams compared to controls. The number of oocytes that

Reproduction, Endocrinology, and Development (READ)

failed to undergo maturation (GVBD) was 25% in DIO dams vs. 4% in controls ($p<0.05$). In addition, 10% of DIO oocytes were degenerated vs. 0% in controls ($p<0.05$). Expression arrays in DIO oocytes showed differential expression of genes regulating pathways related to chromatin remodeling, RNA processing, mitochondrial metabolism, and embryonic growth. Two-cell embryos were generated by mating DIO dams (35.9g) to wild-type males (GFP-) and control dams (20.6g) to males expressing GFP under a ubiquitous promoter (GFP+). Similar numbers of each type of embryo were transferred together into either a DIO or control pseudo-pregnant recipient. Surprisingly, when delivered from a control recipient, DIO-derived pups had lower fetal (e12.5) and neonatal weights than siblings derived from a control-dam ($p<0.001$). Pre-implantation exposure to a high fat diet had no effect on adult weight in female progeny; however, animals born to a DIO carrier were significantly larger then animals delivered to a control carrier regardless of their pre-implantation exposure ($p<0.01$). Progeny of the DIO carrier displayed impaired glucose tolerance (IGT) when compared to progeny of the control carrier ($p<0.05$). Surprisingly, when carried by a DIO recipient, the control-derived progeny displayed a significantly greater IGT then the DIO-derived siblings ($p<0.05$). Exposure of the oocyte to obesity affects the resulting embryo such that progeny display decreased fetal and neonatal weight. By contrast, adult weight in female progeny is impacted by maternal weight during pregnancy rather than pre-implantation embryonic exposure. Exposure of the post-implantation embryo to maternal obesity results in IGT. This IGT is exacerbated when there is a mismatch between the pre-implantation and post-implantation exposures. These data suggest that pre-implantation exposure to a maternal HFD programs how the resulting progeny utilize nutrient resources.

R3 Filtration of Bisphenol A Using Activated Charcoal

Claire Song[1], Jeffrey Field[2]

[1]TREES Program, Department of Pharmacology, University of Pennsylvania

[2]Center of Excellence in Environmental Toxicology, Perelman School of Medicine, University of Pennsylvania

Endocrine-disrupting compounds, or hormone disruptors, are a major health concern for both humans and animals. In particular, the compound bisphenol A (BPA) has garnered great controversy in its widespread use and detrimental health effects. Because BPA coats the insides of water pipes, it is impossible for humans to avoid. Additionally, since BPA is a component of many plastics, BPA runoff from landfills often ends up in local bodies of water, affecting aquatic life. One solution that has surfaced to solve this problem is using activated charcoal to filter BPA out of water. In this experiment, this method was tested using varying concentrations of charcoal, from 1% to 15% (increasing in increments of 2.5%) to attempt to filter out BPA from a 2.5E -4 M BPA solution. A standard curve for BPA wavelength absorption was developed and a spectrophotometer was used to determine the amounts of BPA before and after filtration. Ultimately, it was revealed that all concentrations of charcoal from 1% to 15% could filter out nearly 100% of the BPA from the solution, as the absorbance of the supernatant after centrifugation of the charcoal-treated solution was near 0 in all cases. From this experiment, it appears that activated charcoal is a promising method of removing BPA from water. However, further experiments must be performed to determine the ubiquity of charcoal in filtering other hormone disruptors, as well as to optimize the current process for widespread use.

Reproduction, Endocrinology, and Development (READ)

R4 Establishment of Normal Sperm Nuclear Architecture Requires Poly(ADP-ribose) Metabolism during Spermatogenesis

Mirella Meyer-Ficca, Jessica Bader, Ralph Meyer
University of Pennsylvania School of Veterinary Medicine

In contrast to somatic cells, DNA in sperm is highly condensed through complexation with protamines, and only a minor portion of the genome remains nucleosomal. In addition, the sperm nuclear architecture is unique and highly ordered. Pericentromeric regions of individual chromosomes form a common centrally positioned heterochromatin block ("chromocenter"), and telomeres localize in pairs at the nuclear periphery. Thus chromosomes stretch from the chromocenter to the nuclear envelope. The mechanisms that establish and maintain the specific sperm nuclear architecture during spermiogenesis are not yet well understood.

The exchange of histones to protamines requires concurrent DNA relaxation involving the coordinated activities of topoisomerase 2 beta and poly(ADP-ribose) polymerase (PARP). PARP inhibition during spermiogenesis leads to reduced sperm chromatin condensation and increased residual histones. Using fluorescence in situ hybridization (FISH) of pericentric heterochromatin regions,we show here that reduced PAR metabolism resulted in significantly smaller volumes occupied by the heterochromatic chromocenter and in fragmentation of the chromocenter into several clusters. The specialized nuclear structure of spermatozoa may be functionally relevant for the paternal genome activation after fertilization, and for early embryonic gene expression. Because levels of cellular PAR formation depend directly on environmental factors (e.g. genotoxic stressors and nutrition), we propose that the PAR pathway may therefore be capable of altering sperm epigenetic information in response to environmental factors.

R5 Crystal Structures of Human 17beta-hydroxysteroid dehydrogenase type 5 (AKR1C3) in Complex with N-phenylanthranilic Acid and Indomethacin Based Selective Inhibitors

Mo Chen[1], Adegoke Adeniji1, Barry Twenter[2], Andrew Liedtke3, Jeffrey Winkler[2], Lawrence Marnett[3], David Christianson[2], Trevor Penning[1]

[1]Center of Excellence in Environmental Toxicology, Perelman School of Medicine, University of Pennsylvania; [2]Department of Chemistry, University of Pennsylvania, [3]Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN

AKR1C3 is among the most up-regulated genes in castrate resistant prostate cancer (CRPC). The enzyme functions downstream in the androgen biosynthetic pathway by converting delta4-androstene-3,17-dione to testosterone and 5alpha-androstane-3,17-dione to 5alpha-dihydrotestosterone. Inhibition of AKR1C3 may selectively prevent androgen biosynthesis in CRPC without perturbing adrenal steroidogenesis. We have developed two series of analogs based on nonsteroidal anti-inflammatory drugs N-phenylanthranilic acid and indomethacin as AKR1C3 inhibitors. Our lead compounds show nanomolar potency and greater than 100-fold selectivity for AKR1C3 over the other related AKR1C isoforms involved in androgen metabolism. Here we present the crystal structures of AKR1C3•NADP+ in complex with 3-((4-nitronaphthalen-1-yl)amino)benzoic acid (2) and 2'-desmethyl-indomethacin (3). Both inhibitors are anchored to the oxyanion site via their carboxylate groups. The naphthyl ring of 2 and the p-chlorobenzoyl ring of 3 both extend into AKR1C3 subpocket 1 deeply. This pen etration cannot be accommodated by the other AKR1C isoforms that have smaller subpockets and is likely responsible for the inhibitory selectivity for AKR1C3. Cell-based assays of compound 2 indicate that in addition to acting as an AKR1C3 inhibitor, it also functions as an androgen receptor antagonist, suggesting that the

compound can be optimized as a bifunctional agent. The 2'-desmethyl-indomethacin complex structure shows that this analog binds differently to indomethacin and that this new binding pose can be used to improve analog synthesis. The biological properties of indomethacin analogs are presented in a separate abstract submitted by Adeniji, AO, et al.

R6 Development of Potent and Selective Indomethacin Analogs for the Inhibition of AKR1C3 in Castrate Resistant Prostate Cancer

Andy Liedtke[1], Michael Byrns[2], Mo Chen[2], Yi Jin[2], Larry Marnett[1], Trevor Penning[2]

[1]A.B. Hancock Jr. Memorial Laboratory for Cancer Research, Departments of Biochemistry, Chemistry, and Pharmacology, Vanderbilt Institute of Chemical Biology,Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, TN; [2]Center of Excellence in Environmental Toxicology, Perelman School of Medicine, University of Pennsylvania

Castrate resistant prostate cancer (CRPC), a fatal metastatic form of prostate cancer often develops in prostate cancer patients that were initially responsive to androgen deprivation therapy. CRPC is characterized by a reactivation of androgen receptor (AR) signaling often driven by elevated expression of enzymes involved in local androgen biosynthesis and consequently increased intratumoral androgen production, despite the existence of castrate levels of circulating androgens. AKR1C3 also known as (Type 5 17β-hydroxysteroid dehydrogenase) is among the most upregulated genes in CRPC and converts androgen precursors 4-androstene-3,17-dione and 5α-androstandione to the potent androgens, testosterone and 5α-dihydrotestosterone, respectively. The involvement of AKR1C3 in the pre-receptor regulation of AR action and its intratumoral localization makes it an important target in treatment of CRPC. Inhib itors of AKR1C3 should not inhibit the related isoforms, AKR1C1 and AKR1C2 since the latter enzymes are involved in the inactivation of 5α-dihydrotestosterone within the prostate.

Indomethacin used clinically to inhibit cyclooxygenase enzymes inhibits AKR1C3 potently and displays high selectivity over AKR1C1 and AKR1C2. We report the discovery of three classes of Indomethacin-based nanomolar inhibitors of AKR1C3 with over 100 fold selectivity over AKR1C1 and AKR1C2. The lead compounds are also devoid of inhibitory activity on cyclooxygenase enzymes and display robust inhibition of testosterone formation in a LNCaP-AKR1C3 prostate cancer cell line. Indomethacin has been shown to block PSA, ERG expression, and cell proliferation in a VCaP xenograft model of CRPC in an AKR1C3 dependent manner providing in vivo efficacy data (Cai at al., Cancer Res. 71: 6503, 2011). An X-ray crystal structure of a lead compound (2'-des-methyl-indomethacin) bound to the AKR1C3.NADP+ complex is presented as a separate abstract (Chen et. al, Crystal structures of human 17β-hydroxysteroid dehydrogenase type 5 (AKR1C3) in complex with N-phenylanthranilic acid and indomet hacin based selective inhibitors). These inhibitors are predicted to have comparable pharmacokinetic profiles to Indomethacin and have the potential to be therapeutic agents for the treatment of CRPC *[Supported by R01-CA97044, P30-ES013508, and a Prostate Cancer Foundation Challenge Award to TMP and by R01-CA889450 to LJM]*

Reproduction, Endocrinology, and Development (READ)

R7 Functional Basis of Cytochrome P450 Oxidoreductase (POR) Deficiency Caused by the Q153R and A287P Mutations in POR: Transient Kinetic Dissection of Electron Transfer

Yi Jin[1], Walter Miller[2]

[1]Department of Pharmacology, University of Pennsylvania Perelman School of Medicine, [2]Department of Pediatrics, University of California

Cytochrome P450 oxidoreductase deficiency (PORD) is a genetic disorder, majority of which characterized by severe birth defects such as skeletal malformations and deformities that resemble the Antley-Bixler syndrome, genital anomalies and disordered steroidogenesis. PORD is caused by mutations in the POR gene that result in defective POR, which in turn result in impaired functions of various microsomal cytochrome P450 (CYP) enzymes. POR is the obligate electron donor for all 50 human microsomal CYP enzymes, including those involved in the synthesis of cholesterol, steroids, vitamin D and bile acids as well as those involved in drug metabolism. Using transient kinetic (stopped-flow) techniques, we examined the effect of two PORD mutations, Q153R (associated with both loss-of-function and gain-of-function of CYP activity in vitro) and A287P (found in 40% of patients of European ancestry), on the ability o f POR to accept electrons from NADPH, to transfer electrons between its flavin centers, and to donate electrons to its classic test recipient, cytochrome c. Dissection of the reaction of POR with NADPH and cytochrome c revealed different molecular defects on individual steps of POR electron transfer chain caused by the Q153R and A287P mutations in the POR gene.

R8 Growth Retarded Rats Have Increased Total Body Fat Mass and Increased Hepatic Triglyceride Content at 2 Weeks

Julie Dobkin[1,2], Alexa Vitins[2], Rebecca Simmons[2], Sara Pinney[2]

[1] Division of Endocrinology and Diabetes, The Children's Hospital of Philadelphia, Philadelphia, PA, [2]Department of Pediatrics, Perelman School of Medicine of the University of Pennsylvania

Intrauterine growth retardation (IUGR) has been linked to the development of type 2 diabetes and obesity in adulthood. Neonatal treatment of IUGR animals with Exendin-4 (Ex4), a long acting GLP-1 analog used to treat humans with type 2 diabetes, prevents the development of glucose intolerance, insulin resistance and obesity in the IUGR rat. Our objective was to characterize development of obesity in the IUGR rat and to investigate the mechanism by which neonatal treatment with Ex-4 prevents obesity.

Methods: IUGR was induced by bilateral uterine artery ligation in fetal life. Ex-4 or vehicle (PBS) was given by subcutaneous injections on postnatal days 1-6 of life. NMR was performed to determine lean and total fat mass at 2 weeks of age. Hepatic triglyceride content was measured by ELISA.

Results: As previously reported, IUGR pups weighed less than control animals at 2 weeks of age. IUGR pups treated with vehicle or Ex-4 had 50-62% greater fat mass per gram of body weight compared to control pups ($p<0.05$). There was no difference in lean body mass among IUGR and control animals. Hepatic triglyceride content in IUGR vehicle treated pups was 78% greater than control ($p<0.05$) and only 30% greater in the IUGR pups treated with Ex-4.

Conclusion: IUGR animals already have increased fat mass per total body weight at 2 weeks of age despite a decreased total body weight and similar lean mass proportion compared to control animals. The increase in hepatic triglyceride content in IUGR at 2 weeks suggests that changes in hepatic metabolism may be driving this increase in adiposity.

Gene-Environment Interactions

G1 Obesity Susceptibility Loci and Associations across the Pediatric Body Mass Index Distribution

Jonathan Mitchell[1], Hakon Hakonarson[2], Timothy Rebbeck[3], Struan Grant[2]

[1]University of Pennsylvania, [2]Children's Hospital of Philadelphia, Philadelphia, PA, [3]Center of Excellence in Environmental Toxicology, Perelman School of Medicine, University of Pennsylvania

Most studies investigating obesity susceptibility loci studied the mean BMI z-score in children. We aimed to determine if previously identified obesity susceptibility loci are associated uniformly with pediatric BMI across the BMI distribution. Children and adolescents were recruited from the Children's Hospital of Philadelphia (n=7,225). In this population FTO (rs3751812), MC4R (rs12970134), TMEM18 (rs2867125), BDNF (rs6265), TNNI3K (rs1514175), NRXN3 (rs10146997), SEC16B (rs10913469), and GNPDA2 (rs13130484) have been associated with BMI using linear and logistic regression. In the present study these associations were re-assessed using quantile regression. BMI z-score was modeled as the dependent variable, and genotype risk score (sum of risk alleles carried at the 8 loci) was modeled as the independent variable. Each additional increase in genotype risk score was associated with an increase i n BMI z-score at the 5th, 15th, 25th, 50th, 75th, 85th and 95th BMI z-score percentiles by 0.04 (±0.02, p=0.08), 0.07 (±0.01, p=9.58 x 10-7), 0.07 (±0.01, p=1.10 x 10-8), 0.09 (±0.01, p=3.13 x 10-22), 0.11 (±0.01, p=1.35 x 10-25), 0.11 (±0.01, p=1.98 x 10-20), and 0.06 (±0.01, p=2.44 x 10-6), respectively. Each additional increase in genotype risk score was associated with an increase in mean BMI z-score by 0.08 (±0.01, p=4.27 x 10-20). In conclusion, obesity risk alleles were more strongly associated with increases in BMI z-score at the upper tail compared to the lower tail of the distribution. Previous studies may have underestimated the strength of the association between these obesity-susceptibility loci and pediatric BMI in the context of obesity.

G2 Investigating the Role of cis-acting Elements in Epigenetic Change due to Environmental Perturbation

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As mounting evidence demonstrates a significant involvement of environmental factors in human disease, there is a specific need to understand how our genetic/epigenetic makeup affects our response to environmental perturbation and potentially leads to disease. Our approach utilizes the epigenetic effects of vinclozolin observed at imprinted loci as a system for dissecting potentially interacting genetic/epigenetic/environmental factors. Vinclozolin is a fungicide used on both edible (e.g. canola and wine grapes) and non-edible vegetation (e.g. turf grass). It is well characterized as an antiandrogenic compound and recently has been shown by Stouder and colleagues 2010, to cause transgenerational epigenetic effects at imprinted genes. Genomic imprinting is an essential part of mammalian development. "Imprinted" alleles are epigenetically marked (established) in the germ line based on parent of origin resulting in monoallelic expression of the associated genes later in development. Imprinted gene expression is tightly regulated by both genetic and epigenetic factors (DNA methylation at CpG dinucleotides). Loss of imprinting results in defects in development and subsequent disease. Here, we are characterizing the effects of intrauterine exposure to vinclozolin on DNA methylation at the H19/Igf2 imprinted locus in mature sperm carrying either wild-type or CpG depleted mutant alleles. These studies aim to determine whether mutations in cis exacerbate the effects of vinclozolin on the establishment of epigenetic states at imprinted loci. Thus far, our preliminary investigation of sperm from wild-type males exposed to

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vinclozolin in utero, either by feed or by intraperitoneal (IP) injection, shows no change in methylation at the H19/Igf2 locus. However, sperm from male progeny of mutant dams (carrying CpG depleted alleles at H19/Igf2) exposed to vinclozolin by IP show signifi cant changes in DNA methylation. Studies are ongoing to screen additional mutants in order to fully characterize the nature of the defects in DNA methylation observed and to specifically identify the cis-acting factors responsible. Additional loci are also being examined to determine if the effect is localized to the H19/Igf2 locus. These ongoing studies are an exciting and unique opportunity to study the environmental component of potentially disease-causative changes in DNA methylation during germ cell development.

Oxidative Stress and Oxidative Stress Injury

O1 Evidence for Mitochondrial Adaptation to Chronic Glucose Exposure in Beta Cells

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Pancreatic beta cells increase in number, size, and insulin synthesis/secretion to compensate for higher endocrine demands driven by increased circulating glucose. We have found that the expression of PIF1, encoding a DNA helicase, is glucose responsive and encodes two translation products that target either the nucleus or mitochondria by an alternative translation start mechanism. The overexpression of mitochondrial PIF1 in mouse embryonic fibroblasts increases mitochondrial DNA (mtDNA) levels, suggesting a mechanism to elevate production of the mtDNA-encoded proteins required for the ATP synthesis that fuels increased insulin production. In islets, PIF1 transcript levels correlate with markers of cell cycle progression and are elevated in genetic models of compensation (Keller et al., 2008). PIF1 expression also correlates with, and is dependent on, expression levels of PDX1, a major glucose responsive pancreatic beta cell transcription factor, during chronic exposure to high glucose or PDX1-knockdown in cell culture. We hypothesize that elevation of PIF1 transcripts in response to increasing glucose acts to coordinate beta cell replication, ATP production, and increased insulin synthesis/secretion during beta cell compensation.

Oxidative Stress and Oxidative Stress Injury

O2 p53 Mutagenesis by Benzo[*a*]Pyrene derived Radical Cations

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Benzo[*a*]pyrene (B[*a*]P), a prevalent human carcinogen and mutagen, is metabolically activated into DNA-reactive metabolites via three different enzymatic pathways the anti-(+)-benzo[*a*]pyrene 7,8-diol 1,2-epoxide pathway (cytochrome P450/ epoxide hydrolase catalyzed) (B[a]PDE), the benzo[*a*]pyrene o-quinone pathway (aldo ketose reductase (AKR) catalyzed) and the B[*a*]P radical cation pathway (CYP peroxidase catalyzed). We used a yeast p53 mutagenesis system to assess mutagenesis by B[*a*]P radical cations. Because radical cations are short-lived, they were generated in situ by reacting B[*a*]P with Cumene hydroperoxide (CuOOH) and horse radish peroxidase (HRP) and then monitoring the generation of the more stable downstream products, B[*a*] P-1,6-dione and B[*a*]P-3,6-dione. Typically 4 µM radical cation was formed from 50 µM of B[*a*]P. In the mutagenesis assays, the radical cations showed a dose-dependent increa se in mutagenicity from 0.25 uM to 10 uM B[*a*]P with no significant increase seen with further escalation to 50uM B[*a*]P. However, mutagenesis was 200-fold less than with the AKR pathway derived B[*a*]P, 7-8 dione (BPQ). Mutant p53 plasmids, which yield red colonies, were recovered from the yeast to study the pattern and spectrum of mutations. The mutation pattern observed was G to T (31%) > G to C (29%) > G to A (14%). The frequency of hotspots mutated by the B[*a*]P radical cations was essentially random and not enriched for cancer hotspots. The quinone products of radical cations, B[*a*]P-1,6-dione and B[*a*]P-3,6-dione were more mutagenic than the radical cation reactions, but still less mutagenic than AKR derived B[*a*]P-7,8-dione (BPQ). We conclude that B[*a*]P radical cations are weakly mutagenic compared to redox cycling PAH o-quinones such as BPQ. Our results suggests that mutations caused due to smoking in p53 are less likely to be attributed to B[*a*]P radical cation s compared to the metabolites B[*a*]PDE and BPQ.

Biomarker

B1 Application of Stable Isotope Dilution Liquid Chromatography Tandem Mass Spectrometry (LS-MS/MS) Methods for the Determination of the Androgen Metabolome in Serum in a Total Androgen Pathway Suppression Clinical Trial

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Androgen deprivation therapy (ADT) is a cornerstone of prostate cancer treatment. However, after initial success with ADT, a subset of patients can develop a more aggressive, castration-resistant prostate cancer (CRPC). The vast majority of prostate cancer deaths arise from CRPC. The clinical efficacy of the CYP17A1 inhibitor, abiraterone acetate, in the treatment of CRPC confirms that the disease remains hormonally driven. Quantification of the androgen metabolome in serum and tumor biopsies can inform one as to the metabolic pathway

Biomarker

that has been activated in CRPC tumors. This information can assist the clinician to custom tailor a therapeutic approach for maximum efficacy. To this end, a stable isotope dilution liquid chromatography electrospray ionization tandem mass spectrometry (SID-LC-ESI-MS/MS) method has been developed to detect ketosteroids as Girard T oximes and hydroxysteroids as picol inic esters. Accuracy and precision values have been obtained for ketosteroid quantification. Glucuronate and sulfate conjugates were quantitated using titered enzymatic hydrolysis. Limits of quantitation vary from metabolite to metabolite and range between 1-10 pg on column. We have applied our method to quantify serum androgen levels from patients enrolled in the Total Androgen Pathway Suppression (TAPS) Trial. In arm 1 of this trial, patients were given a combination of Goserelin and Bicalutamide; in arm 2, patients were given Goserelin and Dutasteride; in arm 3, patients were administered Goserelin, Dutasteride and Bicalutamide; and in the TAPS arm, arm 4, patients were given Goserelin, Dutasteride, Bicalutamide and Ketoconazole. In all four arms of the trial, we observed dramatic suppression of testosterone levels when comparing serum samples before and after treatment. In arms 2-4, we found statistically significant drops in androsterone and dihydrotestosterone l evels. Finally, in the TAPS arm, we observed statistically significant suppression in DHEA-sulfate and DHEA-glucuronide levels in addition to all three of the aforementioned metabolites. This data was strongly concordant with quantification done using an alternative SID-LC-ESI-MS/MS assay conducted by an independent analyst.

B2 Identification of Covalent Benzo[*a*]pyrene-7,8-dione-DNA Adducts in Human Lung Cells

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Metabolic activation of the proximate carcinogen B[*a*]P-7,8-trans-dihydrodiol by aldo-keto reductases (AKRs) leads to benzo[*a*]pyrene-7,8-dione (B[*a*]P-7,8-dione) that is both electrophilic and redox-active. B[*a*]P-7,8-dione generates reactive oxygen species resulting in oxidative DNA damage in human lung cells. However, information on the formation of covalent B[*a*]P-7,8-dione-DNA adducts is lacking. We studied covalent DNA adduct formation of B[*a*]P-7,8-dione in human lung adenocarcinoma A549 cells, human bronchoalveolar H358 cells, and immortalized human bronchial epithelial HBEC-KT cells. After treatment with 2 μM B[*a*]P-7,8-dione, the cellular DNA was extracted from the cell pellets subjected to enzyme
hydrolysis and subsequent analysis by LC-MS/MS. Several covalent DNA adducts of B[*a*]P-7,8-dione were only detected in A549 and HBEC-KT cells. In A549 cells the structures of B[*a*]P-7,8-dione DNA adducts wer e identified as hydrated N2-2'-deoxyguanosine-B[*a*]P-7,8-dione and hydrated N1-2'-deoxyguanosine-B[a]P-7,8-dione. In HBEC-KT cells the structures of B[*a*]P-7,8-dione DNA adducts were identified as hydrated 2'-deoxyadenosine-B[*a*]P-7,8-dione, hydrated N3-2'-deoxyadenosine-B[*a*]P-7,8-dione and either unhydrated N1 or N3-2'-deoxyadenosine-B[*a*] P-7,8-dione. In each case adduct structures were characterized by MSn spectra. Adduct structures were also compared to those synthesized from reactions of B[*a*]P-7,8-dione with either deoxyribonucleosides or salmon testis DNA but were found to be different. *(Supported by 1R01-CA39504 and P30ES-013508 to TMP).*

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B3 Formation and Structural Characterization of a Catechol-O-sulfate Metabolite of benzo[a]pyrene-7,8-dione in Three Human Lung Cells

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Benzo[*a*]pyrene (B[*a*]P), a representative polycyclic aromatic hydrocarbon, is a ubiquitous environmental pollutant which occurs in tobacco smoke and residues of fossil fuel combustion. Metabolic activation of the proximate carcinogen B[*a*]P-7,8-trans-dihydrodiol by aldo-keto reductases (AKRs) leads to B[*a*]P-7,8-dione that is redox-active and generates reactive oxygen species that leads to oxidative DNA damage in human lung cells. Sulfation of the B[*a*]P-7,8-catechol by human sulfotransferases (SULTs) is one Phase II metabolic pathway that may detoxify B[*a*]P-7,8-dione. We investigated the occurrence of this pathway in human bronchoalveolar H358 cells, human lung adenocarcinoma A549 cells, and immortalized human bronchial epithelial HBEC-KT cells following treatment of B[*a*]P-7,8-dione for 24 h. A monosulfate conjugate of B[*a*]P-7,8-catechol was detected in the medium from each cell line using HPLC-UV and L C-MS/MS. An authentic metabolite standard was synthesized with human homogeneous recombinant SULT1A3, subsequently purified by HPLC and characterized by 1D, 2D [1H]- NMR and [13C] NMR. The chemical structure of the major cellular metabolite was identified to be 8-hydroxy-B[*a*]P-7O-sulfate. It is concluded that human SULTs may play a critical role in the detoxification of B[*a*]P-7,8-dione in lung cells *[Supported by P30-ES013508 and 1R01-CA-39504 awarded to TMP].*

Community Outreach and Education Core

C1 Applications of Activated Charcoal Solutions in Endocrine-disrupting Chemical Filtration: A Study of Estradiol and Bisphenol A Contaminant Remediation

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The presence and effects of endocrine-disrupting chemicals (EDCs) like natural and synthetic estrogens on animal and human populations has become a cause for concern. EDCs present in the environment have caused increased incidences of intersex marine vertebrates as well as various malformations and adverse health effects in humans. It is imperative that methods of filtering or otherwise removing endocrine disruptors be created and implemented. One such promising method is that of activated charcoal filtration. The bioluminescent yeast estrogen screen (BLYES) developed by Sanseverino et. al was used to determine the estrogenicity of standard and experimental solutions. Experiments using a 10% activated charcoal solution achieved 99.11% and 79.26% reductions, respectively in the concentration of two known natural and synthetic estrogens, 17β-estradiol and bisphenol A. Activated charcoal holds great potential for reusable and highly effective EDC filtration, but more experiments must still be performed to optimize this method and to enact widespread application.

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