Poster #1

Metabolomic Profiling of Phytochemicals in Deuterium-labeled Collard Greens by HPLC-MS/MS

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ABSTRACT

Phytochemicals derived from cruciferous vegetables, like collard greens and broccoli, have been associated with health benefits including prevention and suppression of cancer in pre-clinical models. In contrast, there are inconsistent associations between cruciferous vegetable intake and disease prevention in humans, which may stem from methodological limitations in accurately assessing dietary exposure, and creating a major barrier in the field. Our goal was to explore use of metabolomics to develop a biomarker of cruciferous vegetable consumption. Collard greens were grown hydroponically with 31% $^{2}H_2O$ for 6 weeks to create intrinsically-labeled vegetables. Our hypothesis is that deuterium-labeled collard greens can be used as a tool to differentiate between plant-derived and host-derived metabolites following cruciferous vegetable consumption. High-pressure liquid chromatography Triple Q-ToF mass spectrometry based untargeted metabolomics was performed in information-dependent MS/MS acquisition mode in both positive and negative ion mode.

Among the metabolites extracted from deuterium-labeled collard greens, twenty were identified using PeakView including glucose, chlorophyll A, glucosinolates (glucobrassicin, sinigrin), flavonols (quercetin), and their glycosides. Identified metabolites showed partial deuteration from $^{2}H_0$ (M_0) to $^{2}H_{27}$ (M_{27}). The maximum abundance of stable isotopomers was $^{2}H_4$ (M_4) for glucobrassicin and $^{2}H_{12}$ (M_{12}) for 1,2,2'-trisinapoylgentiobiose. A more automated approach was tested, and 90% of metabolites in labeled collard greens were successfully quantified with El Maven and PollyIsoCorrect software. Work using R-based packages XCMS and X^{13}CMS is ongoing to complete an untargeted analysis of the vegetable and preliminary results show 13,776 metabolic features detected, 9 which tentatively matched the metabolites identified in labeled collard greens.

Future work will profile the plasma and urine metabolome from 21 healthy adults who have consumed these deuterium-labeled collard greens. This work will assist in differentiating plant-derived vs host-derived metabolites following cruciferous vegetable intake, and assist in the identification of more sensitive and specific biological biomarkers of human cruciferous vegetable consumption.

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Poster #2

After School Cooking Courses Empower Elementary and Middle School Aged Children to Make Lasting Healthy Food Choices

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ABSTRACT

BACKGROUND: Fruit and vegetable consumption, as part of a healthy diet, plays an important role in optimal growth, weight management, and chronic disease prevention. Formal nutrition education alone has limited effect on nutrition and dietary behavior of youth, whereas cooking programs can have a positive influence on children's food preferences, attitudes, and behaviors.

OBJECTIVE: The objective was to evaluate the efficacy of two 6-week after school cooking courses designed for elementary and middle school aged students, respectively. Both courses combined food/nutrition knowledge, kitchen safety, and cooking/food preparation and tasting.

METHODS: Participants and their parents completed surveys before and after taking the course that evaluated food and nutrition knowledge and cooking self-efficacy, attitudes, and activities.

RESULTS: The after-school cooking courses successfully introduced elementary and middle-school aged students to elementary food and nutrition knowledge and kitchen safety skills. The courses successfully taught participants the importance of healthy nutrition and increased self-reported healthy activities. The courses helped participants make healthful, complete, and balanced meal choices.

CONCLUSIONS: After school cooking courses, empower elementary and middle school aged students to make lasting healthy food choices.

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Poster #3

Harvest Box Program Improves Fruit and Vegetable Consumptions in Low-income, High-obesity Risk Hispanic Families

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ABSTRACT

BACKGROUND: Overweight and obesity are associated with increased risk for multiple types of cancer, many of which have a high incidence rate in Benton County, specifically among low-income Hispanic families. To increase consumption of fruits and vegetable among low-income, high-obesity risk, Hispanic families we developed a 12-week long weekly farm stand program, which provides not only fresh fruits and vegetables but also hands-on food and nutrition information on how to prepare healthy meals from them, including taste testing.

OBJECTIVE: The objective was to evaluate the efficacy of the *Harvest Box Program (HBP*) to improve fruit and vegetable consumption in low-income, high-obesity risk Hispanic families.

METHODS: Families were referred to the program by physicians and community health navigators at a local school. Students orchestrated weekly Farm Stand events for families to choose fruits and vegetables for their boxes, taste recipe samples and take home recipes and informational sheets about foods provided each week. Participants completed weekly surveys prior to choosing their fruits and vegetables.

RESULTS: Most participants (88%) consumed most or all of their fruits and vegetables in their boxes. Over the 12-week program, nearly all participants (94%) tried new recipes, and 75% reported that they either greatly increased (42%) or somewhat increased (33%) their fruit and vegetable consumption as a result of the program.

CONCLUSIONS: *Harvest Box Program* improves fruit and vegetable consumption in low-income, high-obesity risk Hispanic families.

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Poster #4

Are We Eating Enough Legumes? Evidence from the National Health and Nutrition Examination Survey 2011-2014 and Beans, Lentils, Peas 2017 Survey

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ABSTRACT

Background: Ample scientific evidence suggests the disease prevention benefits of regular legume consumption, however, the most recent data on legume consumption patterns in the U.S. adults remain unknown.
Objective: Evaluate legume consumption patterns in U.S. adults by using cross-sectional data from the 2011-12 and 2013-14-year cycles of NHANES and a cross-sectional, on-line survey conducted using families around Corvallis, Oregon named "Beans, Lentils, Peas (BLP) Survey".
Study Design, Settings, Participants: Participants were grouped into non-legume consumers and consumers, and further grouped into low mature legume consumers (<37.5 g/d, which are the dietary recommendations for mature legume consumption), marginal mature legume consumption (37.5-87.49 g/d, the latter being the cut-off point demonstrating nutritional and disease prevention benefits), and disease prevention mature legume consumers (≥87.5 g/d legume consumption).
Measurable Outcome/Analysis: Groups were compared using t-tests (for comparison of legume consumers vs. non-consumers) or generalized least-squared means (for comparison among legume consumer groups) for continuous data and a chi-square test (NHANES) or Fisher's exact test (BLP) for categorical data. All tests were two-sided. Significance of group differences was determined at $P \leq 0.05$.
Results: Legume consumption remained low in U.S. adults with a declining trend from 2011 to 2014 (mature legumes: 12.8 to 8.3%; dry beans: 10.0 to 6.5%). Less than 5% of the population consumed legumes on a daily basis; approximately 1/3 of the population did not consume legumes during the last month. Low consumers ate a limited variety of legumes (dry beans and green legumes) on a weekly to monthly basis. Disease prevention consumers (16% of the population) ate legumes daily or every other day and included chickpeas, lentils and dry peas to their legume mix.
Conclusion: Legume consumption declined rather than increased in U.S. adults, warranting improved communication about the disease prevention benefits of regular legume consumption.

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Poster #5

A Cross-Sectional Analysis to Identify Reasons for the Disconnect Between Dietary Recommendations and Legume Consumption Patterns in U.S. Adults

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ABSTRACT

BACKGROUND: Although legumes are recognized as a food group with dietary recommendations, less than 15% of U.S. adults consume legumes on a given day.
OBJECTIVE: The objective was to identify reasons for the disconnect between dietary recommendations and legume consumption patterns in U.S. adults.
METHODS: In an online survey, we identified benefits, barriers, and preferences for legume consumption. The selected response group were adults in the Corvallis, Oregon, area that are interested in healthy nutrition. To better understand their food choices, we subdivided respondents based on their recent legume consumption pattern: none, below current dietary recommendations (<37.5 g/d; low), above current dietary recommendation but below levels demonstrating nutritional and disease prevention benefits (37.5-87.49 g/d/; marginal) and disease prevention legume consumers (≥87.5 g/d legume consumption).
RESULTS: Based on their perceptions, we identified three legume consumer groups corresponding to their recent legume consumption: 'skeptics' (non-legume consumers), 'starters' (low or marginal legume consumers), and 'experienced' (disease prevention legume consumers). Independent of their legume consumption patterns, respondents were not aware of the disease prevention benefits of regular legume consumption. The differences between those who consume sufficient amounts of legumes and those who do not centered around, differences in perceptions of taste and texture and of gastro-intestinal health. Those who consume sufficient amounts of legumes enjoy their taste and their digestive benefits, whereas, non-consumers dislike their taste and texture and experience gastro-intestinal discomfort after consuming them.
CONCLUSIONS: Reasons for these differences are that regular legume consumers are experienced in using a variety of legume types, dishes, and preparation techniques, whereas non-consumers have limited interest or knowledge about legumes' disease prevention benefits, type and dish variety, and preparation techniques, which should be the focus of future legume information materials.

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Poster #6; Lightning Talk Speaker

Brain α-Tocopherol Stereoisomer Profile is Differentially Impacted by Mode of Feeding and Source of α-Tocopherol in Infant Rhesus Macaques

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ABSTRACT

RRR-α-tocopherol (*RRR*-α-T) is the naturally occurring stereoisomer of α-T and is more bioactive than *all-rac*-α-tocopherol (*all-rac*-αT), a synthetic mixture of eight α-T stereoisomers. Infant formulas often contain *all-rac*-α-T to meet infant vitamin E requirements, but its impact on neonate α-T tissue status has been studied to a limited extent. Our objective was to determine α-T concentrations in tissues from infant rhesus macaques that were breastfed (BF) or fed an infant formula with *RRR*-α-T or *all-rac*-α-T. From birth to 6-mo of age, infant rhesus macaques (*Macaca mulatta*; n=7-8/group) were BF or fed infant formula supplemented with *RRR*-α-T (22 µmol α-T/L; RRR-IF), or with *all-rac*-α-T (37 µmol α-T/L; AR-IF). We measured total α-T and α-T stereoisomers in milk, plasma, liver and six brain regions (cerebellum, striatum, and occipital, temporal, motor and prefrontal cortices), as well as urinary levels of α-carboxyethyl-hydroxychroman (α-CEHC), an α-T catabolite and biomarker of α-T adequacy. Although total α-T concentrations in RRR-IF and AR-IF were higher than that of dam milk, total α-T concentrations in plasma, lipoprotein fractions, and brain did not differ among groups. The proportion of non-*RRR*-α-T stereoisomers in the feedings was higher in AR-IF (87.5%) and breast milk (68%) than in RRR-IF (0%). Surprisingly, non-*RRR*-α-T stereoisomers accumulated more in BF plasma and all brain regions compared with AR-IF; non-*RRR*-α-T in RRR-IF was nearly absent. α-CEHC concentration in terminal urine samples was higher in RRR-IF compared with the BF group. Thus, although α-T form and mode of feeding did not affect total α-T in plasma or brain, urinary α-CEHC was highest in monkeys fed RRR-IF. That BF brain and plasma more greatly accumulated non-*RRR*-α-T stereoisomers than *RRR*-α-T compared with formula-feeding suggests differential mechanisms of α-T trafficking and warrants study of specific α-T stereoisomers on infant development.

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Poster #7; Young Investigator Applicant

The Role of Thioredoxin Reductase 1 in Redox Regulation of Melanocyte Homeostasis

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ABSTRACT

Melanocytes (MCs) undergo a significant degree of oxidative stress in undertaking their physiological role to protect the skin from ultraviolet (UV) irradiation. One of the primary harmful effects of UV irradiation of the skin is the disruption of the physiological redox state via the generation of reactive oxygen species (ROS). These ROS are implicated in a host of dermatological pathologies including skin cancer, skin aging, and vitiligo. Part of the skin's response to UV insult is to induce MCs to produce melanin, a process itself responsible for ROS production. Accordingly, the body has developed numerous endogenous mechanisms to protect against the harmful effects of elevated ROS. One such mechanism is the thioredoxin 1 system comprised of NADPH, thioredoxin reductase 1 (TR1), and thioredoxin (TRX). This system is crucial for the maintenance of peroxiredoxins, enzymes responsible for eliminating H_2O_2. To address the role of TR1 in redox regulation of MC homeostasis, we have developed a transgenic mouse line (*Tyr-Cre*$^{tg/+}$*/ Tr1*$^{L2/L2}$) harboring Tyrosinase:Cre and floxed alleles of *Txnrd1*, the mouse gene for TR1. This will lead to the generation of *Tr1*$^{mel-/-}$ mice with constitutive, selective ablation of melanocytic TR1 and enable further study of its role in hair follicle stem cell and differentiated MC homeostasis. This mouse model will make it possible to observe how the absence of the TR1 system impacts MCs over the course of development, through the aging process, and following insults like UV irradiation. These mice have apparent pigmentation defects displaying a white belly spot as well as reduced pigmentation in the ears, paws, and tail. Additionally, our preliminary data shows that pharmacological induction of melanogenesis via forskolin is inhibited. We also have *in vitro* data in PIG1 immortalized human MCs that shows siRNA knockdown of TR1 results in reduced pigmentation, tyrosinase activity, and TYRP1 expression.

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Poster #8

Loss of Aryl Hydrocarbon Receptor Potentiates Self-renewal of Colonic Stem and Progenitor Cells

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Background: The aryl hydrocarbon receptor (AhR) is a ligand-activated basic helix-loop-helix transcription factor, and is activated by xenobiotics, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) and structurally related compounds, as well as diet and gut microbial-derived metabolites. Emerging studies indicate that AhR signaling plays a crucial role in regulating intestinal cancer. Since transformation of adult stem cells plays a pivotal role in initiating intestinal cancer, we determined how AhR signaling affects the dynamics and functionality of colonic $Lgr5^+$ stem cells and progenitor (transit amplifying) cells.
Methods: Inducible and constitutive intestine-specific AhR knock-out mouse models were used to assess colonic stem and progenitor cell cytokinetics. Mouse and human colonic organoids were utilized to determine the effects of AhR on the clonogenicity of colonocytes. Stem and progenitor cell gene expression was evaluated by qPCR and RNA sequencing. The interaction between AhR and the FoxM1 promoter was probed using chromatin immunoprecipitation. Colon cancer was induced by AOM (carcinogen) and DSS (inflammation-inducing agent) co-exposure.
Results: The inducible deletion of AhR targeted to $Lgr5^+$ stem cells increased the percentage of colonic stem cells and enhanced organoid initiating capacity and growth of both sorted stem cells and progenitor cells. In contrast, TCDD (AhR agonist) exposure inhibited organoid initiating capacity and growth in AhR-wildtype mice. The loss of AhR significantly increased basal stem cell proliferation, the number of proliferating cells per crypt, and promoted colonocyte cell proliferation in response to crypt injury. Consistent with these observations, RNA sequencing data from sorted stem and progenitor cells revealed that AhR knockout increased the expression level of FoxM1 signaling pathway genes involved in cell cycle progression and cell proliferation. Consequently, suppression of FoxM1 abrogated the increased clonogenicity of AhR KO stem/progenitor cells. Moreover, analysis of a constitutive intestine-specific AhR KO mouse model of colitis-associated colon cancer revealed an enhanced tumor burden.
Conclusions: These findings indicate that AhR KO promotes colitis-associated colon tumorigenesis by increasing colonic stem/progenitor cell proliferation and self-renewal, principally by upregulating FoxM1-dependent signaling, and suggest that diet and microbial-derived AhR-ligand dependent signaling suppresses neoplastic colon growth.

Poster #9

Clinical Evaluation of Red Clover-drug Interactions Using Probe Substrates of Cytochrome P450 Enzymes and a Red Clover Dietary Supplement

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ABSTRACT

Due to health concerns related to use of hormone therapy at menopause, many women are using botanical dietary supplements as potentially safer alternatives. Extract of red clover (*Trifolium pratense* L.), containing estrogenic isoflavones, is one of popular botanical dietary supplements marketed for the management of menopausal symptoms. But its constituents were reported to *in vitro* inhibit some cytochrome P450 enzymes (CYPs) involved in drug metabolism, such as CYP1A1, 1A2, 1B1, 2C9, 2D6, and 3A4. Previous Phase I pharmacokinetics study indicated that the half-lives of these isoflavones were 13-23 hours. To evaluate the potential for drug- and biologically standardized red clover dietary supplement caused pharmacokinetic alterations with four FDA-approved drugs each serving as a probe substrate for CYP in 16 peri- and post-menopausal women.

Low doses of an oral cocktail of caffeine, tolbutamide, dextromethorphan, and alprazolam (probes for metabolism by CYP1A2, CYP2C9, CYP2D6, and CYP3A4, respectively) were administered to peri- and post-menopausal women (ages 40-79) at baseline and then again after consuming a red clover dietary supplement twice daily for 14 days. Serial blood samples were drawn and analyzed for concentrations of each probe substrate drug over time, and concentration-time curve values were compared. A Shimadzu Nexera UHPLC system with a Shimadzu LCMS-8060 triple quadrupole mass spectrometer was used for the quantitative analysis of all four probe substrates in serum. Data acquisition was performed using Shimadzu Labsolution software and concentration-time curves were calculated using WinNonlin pharmacokinetics software.

The measurement for all 16 subjects has been completed and the data suggest no significant changes in drug metabolism after red clover supplementation although *in vitro* assays predicted *in vivo* drug-botanical interactions.

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Poster #10

Zinc Status Elicits Age-Dependent Effects in the Gut Microbiome

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Background: Aging is associated with progressive immune dysfunction, including impaired adaptive response and increased susceptibility to infection. Aging is also associated with chronic inflammation that correlated with the promotion of many age-related diseases. Zinc is an essential micronutrient critical for immune function. In US, 12% of the population do not consume the EAR for zinc. in older populations the prevalence of inadequate zinc intake increases to ~40%. Moreover, zinc levels are also often depressed in aged individuals, even when consuming a zinc-adequate diet. Increasing evidence indicates that the interaction among gut microbiota, the immune system, and diet contributes to age-related inflammation. **Hypothesis/study design:** We hypothesize that age-related decline in zinc status contributes to immune dysregulation and chronic inflammation and is correlated with specific taxa in the gut microbiome. We studied the effects of dietary zinc supplementation and marginal zinc deficiency on changes in microbial communities in young and old mice. Young (2 mo) and old (24 mo) C57Bl/6 mice were fed a zinc adequate (ZA, 30ppm Zn), zinc supplemented (ZS, 300ppm Zn), or marginal zinc deficient (MZD, 6 ppm Zn) diets for 6 wks. 16S rRNA amplicon sequencing was performed on fecal samples at study start and end; cecal and colon samples at study end. **Results:** Age correlated with overall microbial composition in the gut, according to a PERMANOVA test and a permutation test, regardless of zinc status. Generalized linear models developed for each genus were used to identify significant correlations for zinc status, age and specific taxa. A significant interaction between age and MZD diets was found; no similar interactions were found with ZS diets. **Conclusion:** Zinc deficiency elicits a varied effect on the microbiome that is dependent upon host age. Conversely, zinc supplementation elicits smaller changes on the microbiome, and the changes identified are comparable regardless of age.

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Poster #11; Lightning Talk Speaker

Harnessing Phytochemicals to Protect Neuronal and Glial Cells from Oxidative Stress

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ABSTRACT

Oxidative stress and amyloid beta toxicity are involved in the pathogenesis of Alzheimer's diseases. We have previously demonstrated that an extract prepared of the plant *Achillea fragrantissima (Af)* protected cultured brain astrocytes from oxidative stress-induced cell death and down regulated microglial activation. Using activity guided fractionation, we have purified from *Af* an active flavonoid named 3,5,4'- trihydroxy-6,7,3'-trimethoxyflavone (TTF). TTF protected cultured astrocytes from H_2O_2 –induced cell death via interference with cell signaling (inhibition of SAPK/JNK, ERK 1/2, and MEK1 phosphorylation) and by reducing the levels of oxidative stress-induced intracellular reactive oxygen species (ROS). The mechanism of the protective effect of TTF against H_2O_2-cytotoxicity could not be attributed to a direct H_2O_2 scavenging but rather to the scavenging of free radicals as was shown in cell free systems. In addition, TTF protected cultured neuronal cells from amyloid beta cytotoxicity via interference with cell signaling events and by reducing the amyloid beta - induced levels of intracellular ROS. Moreover, TTF exhibited anti-inflammatory activities and inhibited the LPS-elicited secretion of the proinflammatory cytokines Interleukin 6 (IL-6) and IL-1beta from microglial cells. Our results suggest that TTF might be a therapeutic candidate for the treatment of Alzheimer's disease as well as other neurodegenerative diseases where oxidative stress, neuroinflammation and amyloid beta toxicity are part of the pathophysiology.

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Poster #12; Young Investigator Applicant

Exploring the Anticancer Action of Novel AhR Ligands in Triple-negative Breast Cancer

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that controls diverse biological processes including development, differentiation, cell-cycle progression and cell death. The AhR is capable of sensing a wide array of both endogenous and exogenous molecules, and agonism of the receptor by select ligands can direct specific transcriptional outcomes. The AhR is highly up-regulated in treatment-resistant breast cancers, and activation of the receptor by small-molecules screened and developed in our lab has been shown to arrest the cell-cycle and induce apoptosis. Positioning the AhR as an anticancer target in breast cancer represents a promising therapeutic avenue.

Poster #13; Lightning Talk Speaker; Young Investigator Applicant

Kaempferol Increases Levels of Coenzyme Q in Kidney Cells and Serves as a Biosynthetic Ring Precursor

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ABSTRACT

Coenzyme Q (Q) is a lipid-soluble antioxidant essential in cellular physiology. Patients with Q deficiencies, with few exceptions, seldom respond to treatment. Current therapies rely on dietary supplementation with Q_{10}, but due to its highly lipophilic nature, Q_{10} is difficult to absorb by tissues and cells. Plant polyphenols, present in the human diet, are redox active and modulate numerous cellular pathways. In the present study, we tested whether treatment with polyphenols affected the content or biosynthesis of Q. Mouse kidney proximal tubule epithelial (Tkpts) cells and human embryonic kidney cells 293 (HEK 293) were treated with several types of polyphenols, and kaempferol produced the largest increase in Q levels. Experiments with stable isotope ^{13}C-labeled kaempferol demonstrated a previously unrecognized role of kaempferol as an aromatic ring precursor in Q biosynthesis. The metabolism of kaempferol responsible for its incorporation into the Q biosynthetic pathway remains to be established, although two possibilities can be proposed: (1) kaempferol could act directly as a Q precursor being itself a substrate for the Coq2 transferase and would be subsequently metabolized and modified by different Coq proteins until it reaches the final structure of Q; or alternatively (2) kaempferol could be cleaved in the cell to yield potential ring precursors which would be then integrated into this pathway. Investigations of the structure-function relationship of related flavonols showed the importance of two hydroxyl groups, located at C3 of the C ring and C4' of the B ring, both present in kaempferol, as important determinants of kaempferol as a Q biosynthetic precursor. The role of kaempferol as a precursor that increases Q levels identify this flavonol as a potential candidate in the design of interventions aimed on increasing endogenous Q biosynthesis, particularly in kidney.

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Poster #14

Validation of an *in vitro-in vivo* Hybrid Approach for Studying Modulation of NRF2 in Bovine Mammary Cells

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ABSTRACT

High-producing dairy cows experience increases in oxidative stress as they transition from pregnancy to lactation. Nuclear factor erythroid 2-related factor 2 (NRF2) represents a potential target to mitigate oxidative stress and improve animal productivity and health. Several polyphenols present in pasture plants act as NRF2 agonists. The aim of the present study was to establish an *in vitro-in vivo* hybrid system to investigate activation of NRF2 in bovine cell models. To accomplish the aim, we used a combination of a gene reporter assay with synthetic NRF2 modulators, in association with blood serum collected from periparturient cows and immortalized bovine mammary cells. Cell viability was assessed using fluorescent dyes. Using this model to perform a dose-effect study, we were able to identify differences in NRF2 activation or inhibition by four different synthetic NRF2 modulators. It was found that sulforaphane (SFN) at 10 µM and tBHQ at 10 µM were effective activators, while brusatol (BRU) at 100 nM and ML385 at 50 µM were effective inhibitors. H_2O_2 failed to activate NRF2, a result that was confirmed by the lack of difference in NRF2 activation between pre- and postpartum serum, despite a significantly higher reactive oxygen species concentration in the latter (32.8 vs. 48.9 µM). SFN and BRU significantly reduced cell viability (<80%) but this was prevented when the treatment was performed in serum. Modulation of NRF2 was also observed with SFN and BRU when added to serum; however, BRU was more effective in inhibiting NRF2 when combined with blood serum collected from pre- vs. post-partum cows. Overall, we successfully established an *in vitro-in vivo* model to study NRF2 in bovine mammary cells that will continue in helping achieve the long-term goal of modulating NRF2 with secondary metabolites in pasture plants.

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Poster #16; Lightning Talk Speaker

Lipidomic and Metabolomic Analysis of Western Diet-Induced Nonalcoholic Steatohepatitis (NASH) in Ldlr$^{-/-}$ Mice

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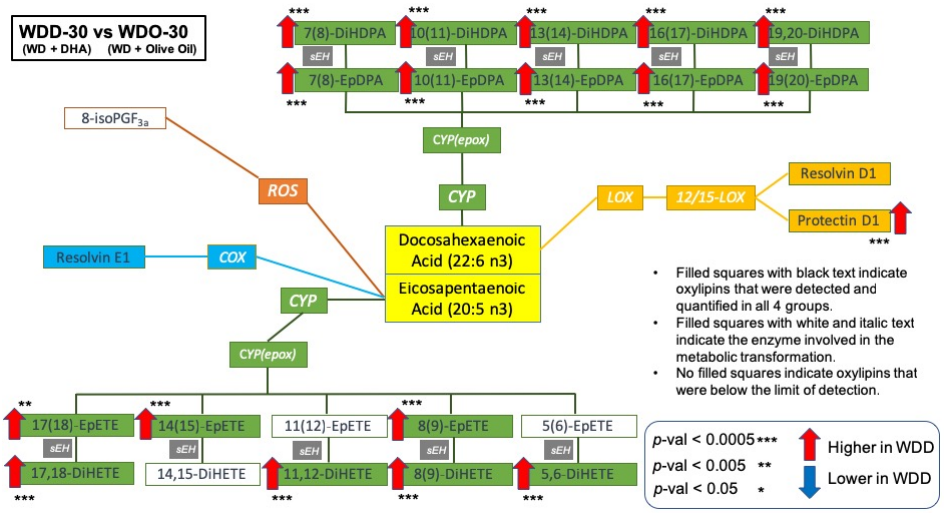
ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. NAFLD ranges from benign steatosis to nonalcoholic steatohepatitis (NASH). Using *Ldlr* $^{-/-}$ mice as a preclinical model of western diet (WD)-induced NASH, we previously established that dietary supplementation with docosahexaenoic acid (DHA) attenuated WD-induced NASH. In order to better understand the changes responsible for the blockade of NASH progression, we performed LC-MS/MS un-targeted and targeted lipidomic and metabolomic analysis in mouse liver samples. *Ldlr* $^{-/-}$ mice fed the WD for 22 weeks developed metabolic syndrome and a severe NASH phenotype. These mice were randomized to: a baseline group (WDB, sacrificed at 22 wks) and 2 treatments: 1) WD + olive oil (WDO); 2) WD + DHA (WDD). The 2 treatment groups were maintained on their respective diets for 8 wks. An additional group was maintained on standard laboratory chow (Reference Diet, RD) for the 30-wk duration of the study. Over 800 compounds were annotated by time-of-flight accurate mass detection, MS/MS fragment characterization and retention time. The principal component analysis for the annotated lipids showed a strong clustering for each treatment, with a clear separation between the RD and WD groups and overlapping between the mice fed with WD with and without olive oil supplementation. The WDD group exhibited a lipidomic profile more similar to the RD group than to the WD and WDO groups. Quantification of hepatic oxylipins by targeted LC-MS/MS established that DHA supplementation re-established the relative oxylipin abundance found in the RD group. Among the most significant oxylipin changes, we observed higher concentrations of protectin D1 ($p < 0.0005$) in the WDD group when compare with the WDO group, confirming the strong anti-inflammatory and anti-apoptotic effects derived from DHA supplementation, which may be in part responsible for the observed blockade of DHA on NASH progression.



Poster #17; Lightning Talk Speaker; Young Investigator Applicant

Vitamin E Deficiency Depletes Redox-active Thiols to Disrupt Zebrafish Embryo Patterning and Early Neurogenesis Events

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ABSTRACT

Vitamin E (VitE) is essential in embryo development and its deficiency results in severe neurological defects and embryonic mortality. Nearly a century of data from experimental animals show that VitE deficiency causes neurologic development failures and fetal death. To obtain embryos for study, zebrafish were fed defined diets with (500 mg/kg; E+) or without (E-) VitE. Adult zebrafish were spawned to produce E+ and E– embryos, collected at 12, 24, 36 and 48 hours post-fertilization (hpf). E- embryos underwent two mortality events with 25% dead at 12 hpf and another 40% between 36- and 48 hpf. E+ embryos over this same time period lost only 25% of the developmentally-staged cohort. Although the mechanism that defines VitE essentiality is unknown, deficiency results in lipid peroxidation (LPO) and lipid hydroperoxide (LOOH) generation. Glutathione (GSH), a redox-sensitive thiol, is used to detoxify LOOH. Thus, we hypothesized that VitE deficiency depletes critical thiols and increases LPO-dependent neurologic patterning defects. Redox-sensitive thiols [GSH, cysteine, GSH disulfide (GSSG), homocysteine and cystine] were quantitated by UPLC-MS/MS using a derivatization step with N-ethylmaleimide in the presence of an internal standard (IS, GSH-$^{13}C_2$-^{15}N). *In situ* hybridization using RNA probes was used to evaluate gastrulation and cell fate specification events (gsc, pax2a), notochord development (col2a1a, col9a2), brain barrier formation (sox10, foxd3) and VitE trafficking (abca1a, ttpa). At 12 hpf, E+ and E- embryos have similar thiol status, but by 48 hpf GSH, cysteine, homocysteine and cystine are lower in E- embryos (F-test, p<0.001). At 12 hpf, E- relative to E+ embryos have (1) diminished notochord formation (decreased col2a1a and col9a2) and (2) abnormal neural crest cell migration (sox10) suggesting large-scale failures in neurogenesis. Overall, VitE's essential mechanism is clarified through VitE deficiency that impedes zebrafish embryogenesis by altering thiol-redox status and key embryonic patterning steps.

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Poster #18; Young Investigator Applicant

Modeling Total Dietary Nitrate from Foods, Beverages, and Supplements

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ABSTRACT

Background: Dietary nitrate is an inorganic anion associated with health risks and benefits depending on the source and level of exposure. Dietary nitrate is reduced to nitrite by sublingual bacteria and further reduced to the bioactive compound, nitric oxide. National survey data provide estimates of dietary nitrate and nitrite consumption for average persons, but do not represent populations that select high nitrate foods on a regular basis. With the aim of depicting potential total nitrate consumption, we quantified nitrate and nitrite in samples of food patterns, foods, beverages and supplements to gain a more complete understanding of nitrate consumption. **Methods:** Nitrate and nitrite was extracted with the Seeley hot water method and quantified using ozone chemiluminescence in 42 dishes from different cultures, 80 beverages, and 19 supplements. Nitrate and nitrite values were calculated and expressed relative to the World Health Organization's Acceptable Daily Intake (ADI) limit of 222 mg nitrate / day for a 60 kg adult. **Results:** Diets rich in leafy green and root vegetables such as a DASH dietary pattern offer the highest nitrate exposure from foods. Nitrate in dietary patterns were: DASH (1222 mg/day), Chinese (231.1 mg/day), Japanese (218.6 mg/day), American (109.8 mg/day), Indian (100.8 mg/day). Leafy green vegetable and/or beet juices and concentrated beet-based supplements have the potential to deliver > 500 mg of nitrate per serving. For illustrative purposes, we combined nitrate content of diet, beverage choice, water and supplements to estimate low, medium and high nitrate exposures. Potential nitrate exposure ranged from 130.29 mg nitrate / day to the highest extreme of 2.73 g nitrate / day, demonstrating the inherent variability of nitrate consumption from different food sources. Nevertheless, these data allow us to consider how food choices may be compared relative to the ADI and the potential health effects of nitrate consumption.

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Poster #19

Astaxanthin Inhibits Interleukin-8 Expression in *Helicobacter pylori*-infected Gastric Epithelial Cells

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ABSTRACT

Helicobacter pylori (H. pylori) infection leads to gastric inflammation, peptic ulcer and gastric carcinoma. *H. pylori* activates NADPH oxidase and increases reactive oxygen species (ROS), which induce NF-κB activation and IL-8 expression in gastric epithelial cells. Dysfunctional mitochondria trigger inflammatory cytokine production. Peroxisome proliferator-activated receptors-γ (PPAR-γ) regulate inflammatory response. Astaxanthin is a powerful antioxidant that protects cells against oxidative stress. The present study was aimed at determining whether astaxanthin inhibits *H. pylori*-induced mitochondrial dysfunction, NF-κB activation, and IL-8 expression via PPAR-γ activation in gastric epithelial cells. Gastric epithelial AGS cells were treated with astaxanthin, NADPH oxidase inhibitor apocynin and PPAR-γ antagonist GW9662, and infected with *H. pylori.* As a result, *H. pylori* caused an increase in intracellular and mitochondrial ROS, NF-κB activation and IL-8 expression, but decreased mitochondrial membrane potential and ATP level. Astaxanthin inhibited *H. pylori*-induced alterations (increased ROS, mitochondrial dysfunction, NF-κB activation, and IL-8 expression). Astaxanthin activated PPAR-γ and its target gene catalase in *H. pylori*-infected cells. Apocynin reduced ROS and inhibited IL-8 expression while astaxanthin did not affect NADPH oxidase activity. Inhibitory effects of astaxanthin on ROS levels and IL-8 expression were suppressed by addition of GW9662. In conclusion, astaxanthin inhibits *H. pylori*-induced mitochondrial dysfunction and ROS-mediated IL-8 expression by activating PPAR-γ and catalase in gastric epithelial cells.

Poster #20

Astaxanthin Induces Expression on Antioxidant Enzymes in Expression in *Helicobacter pylori*-infected Gastric Epithelial Cells

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ABSTRACT

Helicobacter pylori (H. pylori) infection leads to gastric diseases such as gastritis and gastric carcinoma. *H. pylori* increases reactive oxygen species (ROS), which induce and inflammatory cytokine expression in gastric epithelial cells. Peroxisome proliferator-activated receptors-γ (PPAR-γ) regulate inflammatory responses. Astaxanthin is a keto-carotenoid. It belongs to a larger class of chemical compounds known as terpenes. Astaxanthin shows antioxidant activity and protects the cells against oxidative stress. The present study was aimed at determining whether astaxanthin activates PPAR-γ and expression of its target antioxidant genes and whether astaxanthin inhibits cytokine expression in *H. pylori*-infected gastric epithelial cells. Gastric epithelial AGS cells were treated with astaxanthin and/or PPAR-γ antagonist, and infected with *H. pylori.* As a result, *H. pylori* caused an increase in intracellular ROS and cytokine expression, in gastric epithelial cells. Astaxanthin inhibited *H. pylori*-induced cytokine expression and activated PPAR-γ and its target gene superoxide dismutase and catalase in *H. pylori*-infected cells. Inhibitory effects of astaxanthin on ROS levels and cytokine expression were suppressed by addition of a PPAR-γ antagonist. In conclusion, astaxanthin inhibits *H. pylori*-induced cytokine expression by activating PPAR-γ and inducing its target genes superoxide dismutase and catalase in gastric epithelial cells.

Poster #21

Mutagenicity and Toxicity Testing of Anti-cancer Bcl-2 Functional Converters

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ABSTRACT

Breast cancer is a heterogenous disease with prognosis and treatment varying dependent on the subtype. There are 3 breast cancer molecular sub-type identifiers: human epidermal growth factor 2 (HER2) receptors, estrogen receptors (ER), and progesterone receptors (PR). Triple Negative Breast Cancer (TNBC) encompasses all breast cancers that test negative for estrogen receptors, progesterone receptors, and HER2. TBNC makes up roughly 10-20% of breast cancers, and among all breast cancer subtypes, is associated with the worst prognosis, high recurrence, and limited treatment options. Bcl-2 is expressed in roughly 40% of all TNBC cases, the Bcl-2 protein family plays a crucial role in the regulation of apoptosis by modulating the integrity of the mitochondrial outer membrane (MOM). The Bcl-2 protein family includes both anti and pro-apoptotic members, each sharing one or more of the four characteristic Bcl-2 Homology (BH) domains. More specifically the BH3 domain, which, when bound with other proteins within the Bcl-2 family, not only inhibits anti-apoptotic proteins but simultaneously activates pro-apoptotic proteins. The current installment for Bcl-2 target specific treatments was the discovery of Bcl-2 Functional Convertors (BFCs), small molecules with the capacity to convert anti-apoptotic proteins to pro-apoptotic proteins. This research will investigate the efficacy of Bcl-2 functional convertors (BFCs) and BFCs in combination with chemotherapy in Triple Negative Breast Cancers (TNBC) in both 2D and 3D assays. 3D Assays such as spheroid culturing with hypoxic conditions will provide treatment data more representative of 3D tumor morphology and internal human conditions. Standard safety assays such as the Ames test and Micronucleus Assy will help determine any mutagenic and toxic properties of our BFCs in cancerous/non-cancerous cell lines. This data will be used to identify a safe lead compound to use in future preclinical testing.

Poster #22

Balance Method for Estimation of Deuterium-Labeled Alpha-Tocopherol Absorption in Healthy Women

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ABSTRACT

Objective: We hypothesized that fractional intestinal d3-alpha-tocopherol (d3-α-T) absorption could be measured using the balance method to quantify the difference between intake and excretion.
Methods: Both oral d3-α-T with a breakfast shake containing 40% fat and intravenous d6-α-T (30 mg each) were administered simultaneously. Participants (n=6 women) collected all feces up to 96 h. The pooled, frozen (-80 C) fecal collection for each 24 h was weighed and a 2:1 ratio of homogenizing solution (ascorbic acid, delta-T, penta-methyl chromanol and diethylene triamine penta-acetic acid) was added. After defrosting overnight, the sample was homogenized and aliquoted for analysis by liquid chromatography/mass spectrometry of labeled and unlabeled α-T. The amounts were quantitated by comparison to authentic standards and corrected for internal standard recoveries which were more than 55%.
Results: The time course of excretion demonstrated that 72 h was sufficient to collect >90% of the total d3-α-T excreted. Fractional d3-α-T absorption was calculated from the difference in the dose administered and the sum of the d3-α-T excreted over the 96 h. Fractional absorption averaged approximately 66%. About 6% of the IV administered d6-α-T dose was excreted over the 96-h study period, with daily excretion about 0.5 mg. These data confirm that the IV-administered d6-α-T was not excreted in large amounts via the bile and feces over the 96 h. Thus, the excreted d3-α-T from the oral dosing is not extensively contaminated with d3-α-T, which has undergone enterohepatic circulation. About 6 mg unlabeled-α-T was also excreted daily, representing both unabsorbed dietary α-T and that excreted from the body.
Conclusions: In conclusion, fractional α-T absorption is about 66% when the α-T is consumed with a 40% fat meal given as a breakfast shake. By judicious use of protective antioxidants and metal-chelating reagents, fecal vitamin E could be protected from loss and accurately quantitated.

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Poster #23; Lightning Talk Speaker

The Steady State Concentrations of Antioxidants and Peroxidation Products can be used to Classify the Biomarkers

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ABSTRACT

"Oxidative stress" is an ill-defined term, being dependent on the method of its evaluation. Quantitation in terms of a universal criterion is impossible. The most commonly used biomarkers are lipid peroxidation products, particularly hydroperoxides and aldehydes. In the MARK-AGE study of more than two thousand individuals, the OS, as evaluated on the basis of the steady state concentration of a given peroxidation product either does not correlate with the OS determined on the basis of another biomarker or when the results based on two biomarkers correlate significantly with each other, the correlations were weak. We think that the possibility that the different biomarkers reflect different manifestations of one factor (one type of OS) is unlikely. The results of our recent study agree with the hypothesis that different biomarkers reflect different, thus far unidentified, types of OS. The aim of our current research is to identify the difference between the alleged sub-groups (types) of OS.

Our working hypothesis is that the different types of OS (i.e. the different biomarkers) correlate differently with different antioxidants. We studied the correlations between the steady state concentrations of eight peroxidation products and 11 low molecular weight antioxidants. In fact, increase of concentration of MDA is associated with decrease of GSH concentration, indicating that MDA and GSH reflect the same type of OS. By contrast protein carbonyls and nitrotyrosine are apparently markers of another type (or types) of OS.

It appears that both the different biomarkers reflect different types of OS. We propose that the OS, as determined on the basis of the MDA (and GSH), reflects the same type of OS, which we denote OS_a and the other biomarkers be denoted OS_b.

Poster #24

Absorption and Metabolism of Irilone in the Caco-2 Cell Model

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Red clover (*Trifolium pratense* L.) is a popular isoflavone-containing botanical dietary supplement conventionally used in hormone replacement therapy for menopause, irilone is an important isoflavone from red clover. The intestinal absorption and metabolism of irilone were investigated *in vitro* using the human intestinal Caco-2 cell culture model (1) to understand the mechanism of its high oral bioavailability. The effects of irilone on the permeabilities of other isoflavones in red clover (daidzein, formononetin, biochanin A, and genistein) and their metabolites were also studied. In addition, how other constituents in the red clover extract affect the absorption and metabolism of irilone was investigated to explain bioavailabilities observed in clinical trials.

Poster #25; Young Investigator Applicant

Antiproliferative and Cytotoxic Activity of Xanthohumol and Its Non-Estrogenic Derivatives in Colon and Hepatocellular Carcinoma Cell Lines

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ABSTRACT

Xanthohumol (XN), a prenylated flavonoid found in hops, inhibits growth in a variety of cancer cell lines; however, its use raises concerns as gut microbiota and the host's hepatic cytochrome P450 enzymes metabolize it into the most potent phytoestrogen known, 8-prenylnaringenin (8-PN). The XN derivatives dihydroxanthohumol (DXN) and tetrahydroxanthohumol (TXN) are not metabolized into 8-PN and they show higher tissue concentrations in vivo compared with XN when orally administered to mice at the same dose. Here we show that DXN and TXN possess improved anti-proliferative activity compared with XN in two colon (HCT116, HT29) and two hepatocellular (HepG2, Huh7) carcinoma cell lines, as indicated by their respective IC50 values. Furthermore, XN, DXN, and TXN induce extensive apoptosis in all these carcinoma cell lines. Finally, TXN induces G0/G1 cell cycle arrest in the colon carcinoma cell line HT29. Our findings suggest that DXN and TXN could show promise as therapeutic agents against colorectal and liver cancer in preclinical studies without the drawback of metabolism into a phytoestrogen. In future experiments, we will identify the molecular mechanisms by which these derivatives mediate their effects and perform in vivo preclinical studies with mouse models of inflammatory bowel disease and colorectal carcinoma.

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Poster #26; Young Investigator Applicant

The Role of Intestinal Microbiota in High-Fat Diet Induced Obesity

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ABSTRACT

A calorie-dense diet is a well-established risk factor for obesity, hepatic steatosis, and subsequently metabolic syndrome (MetS), whereas the absence of an intestinal microbiota can prevent it. To better understand the role of intestinal microbiota in diet-induced obesity prevention, we used a 2 x 2 statistical design and fed conventional (CV) and germ-free (GF) 8-to-10-week-old Swiss Webster mice for 10 weeks a diet containing either 10% (Control) or 60% (HFD) fat derived calories (10-11 mice/group). The HFD increased food conversion and body weight independently of the increase associated with the presence of intestinal microbiota. Furthermore, the HFD decreased feed intake in CV but not in GF-mice, which resulted in larger fat pads in GF mice fed HFD. With respect to glucose regulation, the HFD increased basal plasma glucose and insulin concentrations independently of the increase associated with the presence of intestinal microbiota. The HFD changed lipid regulation, as indicated by increased LDL-cholesterol concentrations in plasma, and triacylglycerol and ceramide levels in liver. The HFD increased plasma triacylglycerol and HDL-cholesterol concentrations in CV but not in GF mice. In conclusion, in Swiss Webster mice, the removal of intestinal microbiota cannot prevent most of the detrimental effects of a high-fat, calorie dense diet in the development of obesity and MetS. In future experiments, using Swiss-Webster mice as a model, we will show that the gut microbiota is required to mediate the benefits of the botanical xanthohumol, in ameliorating symptoms associated with diet-induced obesity.

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Poster #27; Young Investigator Applicant

Integration of Untargeted and Targeted Mass Spectrometry for the Characterization of Botanical Extracts: Application to Extracts of *Centella asiatica*, an Ayurvedic Herb with Potential Neuroprotective Effects

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ABSTRACT

All of us are exposed to a vast diversity of phytochemicals through our diet. Herbal products are recognized for treating diseases and promoting health and resilience, nevertheless, the inconsistent phytochemical composition of botanical products dictates the potential benefits or toxicity as well as the results of pre-clinical or clinical trials. Consequently, difficulties in establishing the effects of botanical products reside in obtaining batch-to-batch reproducibility regarding their phytochemical composition. In this study, we developed an effective method for the in-depth characterization of plant extracts and quantification of marker compounds in the same chromatographic run using a quadrupole time-of-flight analyzer in the data-dependent acquisition (DDA) mode. This procedure not only acquires the spectral fingerprint of the extracts but also combines a post-acquisition precursor ion quantification procedure for determining levels of distinct phytochemicals in various *Centella asiatica* (*C. asiatica*) extracts, an ayurvedic herb with traditional applications in improving mental health and cognitive function. This integrated workflow allowed the tentative identification of 117 compounds, chemically interconnected based on Tanimoto 2D structure similarity. In addition to this, the data acquired, can be mined in the future in case of new compounds are identified in the plant.

We validate the accurate quantification of 24 phytochemicals commonly found in CA extracts, and this methodological approach is generally applicable to other botanical products. The differences in the composition of phytochemicals across different *C. asiatica* accessions was substantial, confirming that detailed characterization of plant extracts is crucial for reproducible application in laboratory studies, clinical trials, and safe ingestion.

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Poster #28

Dietary Antioxidants Attenuate Hyperoxia-induced Lung Injury Immune Function

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ABSTRACT

Mechanical ventilation with hyperoxia is commonly used to treat patients with respiratory distress and those undergoing surgery. However, prolonged exposure to hyperoxia can induce hyperoxia-induced acute lung injury (HALI) and cause dysfunction, injury and even death of lung cells. The aim of this study was to determine whether dietary antioxidants can attenuate HALI and hyperoxia-induced cellular dysfunction. Here we show that ascorbic acid can ameliorate HALI via reducing leukocyte infiltration and accumulation of HMGB1 in the airways. Ascorbic acid and sulforaphane can attenuate hyperoxia-induced macrophage dysfunction through an HMGB1-mediated pathway and by reducing oxidative stress.

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Poster #29

Gender Differences in Behavioral Deficits, and Response to *Centella asiatica* Water Extract and Constituent Compounds, in the 5XFAD Mouse Model of Alzheimer's Disease

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ABSTRACT

Centella asiatica water extract (CAW) administered in drinking water improves cognitive function in aged wild-type (WT) and Alzheimer's disease (AD) model 5XFAD mice. Eight-month old male and female 5XFAD mice and WT littermates were fed AIN-93 purified diet or AIN-93M containing CAW (1%) or postulated active compounds – triterpenes (TT; 0.045%), caffeoylquinic acids (CQA; 0.016%), or both (TT+CQA; 0.061%). Contextual fear response (CFR) was tested at 4 weeks treatment to assess memory impairment. Hippocampal synaptic and antioxidant response element (ARE) gene expression was analyzed after 5 weeks treatment. In males, 5XFAD genotype caused decreased CFR freezing behavior (-39%; p=0.003) vs WT littermates, which was mitigated completely by CAW, TT+CQA, TT, or CQA treatment ($p<0.05$). 5XFAD pathology reduced synaptophysin (-39%; p=0.021) and increased ARE gene *Ho-1* (+269%; p=0.014) expression compared to WT males. CQA treatment increased synaptophysin (+246%; p=0.015) expression in 5XFAD males vs untreated controls. For ARE genes, TT treatment decreased *Nqo1* (-65%; p=0.018) and TT+CQA treatment increased *Nrf2* and *Gclc* (+75%; p=0.010, +82%; p=0.003) in 5XFAD males. In females, neither AD pathology nor treatment resulted in significant differences in CFR freezing. However, AD pathology increased *Ho-1* (+76%; p=0.043) and decreased *Gclc* (-32%; p=0.010). Also, synaptophysin decreased in a non-significant trend with pathology (-35%; p=0.056) but increased with TT (+202%; p=0.002) and CQA (+204%; p=0.008) treatments to 5XFAD females. CAW increased *Nrf2* (+70%; p=0.028) and *Nqo1* (+74%; p=0.020) but reduced *Gclc* (-40%; p=0.045). TT+CQA increased *Nrf2* (+69%; p=0.007), *Ho-1* (+84%; p=0.004), and *Gclc* (+98%; p=0.002), while TT increased *Ho-1* (+153%; p=0.015), and CQA increased *Nrf2* (+58%; p=0.001) and *Ho-1* (+236%; p=0.007). These data demonstrate gender-dependent differences in pathology and sensitivity to CAW and selected components in 5XFAD mice. While TT and CQA recapitulate some of CAW's effects, the data suggests complex interactions of CAW components which require further exploration.

Poster #30

GNPS Analysis Integrated with New Model-predicted Bioactives in Hops Extract

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ABSTRACT

Despite advances during the last two decades of combinatorial chemistry, natural products continue to play a key role in drug discovery. However, the isolation and identification of the bioactive molecules from complex mixtures of natural compounds is still challenging.
In this scenario, we developed a new strategy to accelerate natural products discovery. As a proof-of-principle, we performed a chemical fingerprinting of the acetone crude hops extract by LC-HRMS/MS, collecting accurate mass and structural information for hundreds of compounds. Before the bioactivity evaluation, we fractionated the hops extract by LH20 chromatography into 40 fractions. This resulted in a fluctuation in relative abundance of natural products across the fractions, which we individually tested for iNOS inhibitory activity by the Griess assay and then we characterized them for their chemical composition by LC-HRMS/MS and NMR. The MS/MS data from the hops fractions were organized by the Global Natural Products Social Molecular Networking (GNPS) algorithm in 5122 nodes, grouped in 393 clusters, which were very difficult to interpret. Therefore, we applied the ElasticNet approach, a statistical model that allows prediction of bioactives in extracts by correlating bioactivity with the chemical features of the compounds in the different fractions. We obtained a "primed GNPS" network, predicting that only 11 features and 4 clusters are related to the observed bioactivity. Moreover, since the GNPS platform gives access to a growing online database of known compounds, the predicted peaks could be annotated readily or could be linked to a nearby known node with an interpretable mass difference (carbon atom, methyl group, hydrogens, etc), allowing the identification of the predicted bioactive molecules, i.e., prenylflavonoids in our example.
This approach can be applied to different natural product extracts to accelerate dereplication, lead prioritation and annotation of bioactive natural products.

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Poster #31

In-vitro Cellular Bioenergetic Effects of Melatonin and Hydrocortisone

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ABSTRACT

Oxidative stress induced mitochondrial dysfunction has been implicated in conditions as wide ranging as aging, bone mass regulation, ischemia/reperfusion injury, male infertility, neurodegenerative diseases, sepsis, and various cancers. Melatonin is synthesized mainly in the pineal gland and is thought to systematically manage sleep-wake cycle, but it has now been shown to also be produced in the mitochondria for localized use. Hydrocortisone (or the hormone cortisol when supplied as a medication), is an adrenal gland product associated with glucose regulation and anti-inflammatory effects. These hormones have been identified as potent mitochondria protectors, preventing free radical stress and regenerating mitochondrial/glycolytic function. Despite these promising findings, a study is yet to be published directly linking mitochondrial response to melatonin and hydrocortisone. This study directly observed cellular oxygen consumption rate (OCR) as a measure of mitochondrial activity in lung fibroblasts (WTHBF-6) and in prostate cancer cells (PC3). Additionally, performed glycolysis stress tests (based on extracellular acidification rate - ECAR) measured three key parameters of glycolytic function including glycolysis, glycolytic capacity and glycolytic reserve. The results of this cellular bioenergetics study reveal time- and dose-dependent reduction in maximum respiration and glycolytic activity in response to melatonin and hydrocortisone, indicating that the mechanism of the anti- or pro-oxidative properties of these hormones is still not fully understood and needs further studies.

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Poster #32

Automation and Application of Magnetic Based Affinity Selection Screening for Targets of Retinoid X Receptor alpha (RXRα)

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ABSTRACT

Natural products have historically been excellent leads for the discovery of biologically active compounds including therapeutics. However, there has been a decline in the natural product research mainly in large pharmaceutical firms due to the complexity of natural product extracts. In order for natural product drug discovery to continue generating more interest, new and innovative approaches are required. Recently, screening combinatorial libraries and natural product extracts has been enhanced by newer affinity selection based screening assays including pulsed ultrafiltration mass spectrometry (PUF-MS) and Magnetic Microbead Affinity Selection Screening (MagMASS). The later method was automated in this study, which enhanced the screening speed desired in screening assays. Compared to other screening methods, like PUF-MS, the automated MagMASS is rapid and requires about 1.2 hr to run the assays including the washes and elution per 96-well microtiter plate. The automated MagMASS is 2-fold faster compared to manual version, and is more consistent. For example, the binding assay using natural ligand of RXRα, LG100268, had 10-fold lower standard error (0.965 ± 0.008 and 0.898 ± 0.08 respectively). The automated MagMASS was thus applied in screening extracts prepared from 22 botanical plants for ligands of RXRα. Various compounds from *Glycyrrhiza uralensis* (GU) methanol extract were identified as hits during the screening that was done in 4 replicates. Due to unavailability of standards, an in-house library was set up using references and FooDB database. In total, 60 compounds in the database were established and 44 compounds in GU extract were tentatively characterized by matching the in-house library. All compounds were determined by MS/MS fragmentation, isotope ratio, and mass accuracy within 3 ppm in PeakView software (Sciex, Ontario, Canada). The XIC of all detected compounds and a summary of all detected peaks from the MagMASS assay with the extract from GU will be described.

Poster #33

Aryl Hydrocarbon Receptor (AhR) Mediated Anticancer Effects of 11BBQ in Lung Cancer

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ABSTRACT

Lung cancer is the leading cause of cancer death—more than colon, breast, and prostate cancers combined. Much progress has been made in personalized medicine, but most lung-cancer patients are not eligible for current targeted therapies. AhR has a promiscuous binding pocket for small molecules and is highly expressed in lung cancers, thereby making it a promising therapeutic target to develop anti-lung cancer drugs and expand the range of patients who can benefit from targeted therapy. AhR is a ligand-activated transcription factor that regulates many cellular processes, including cell cycle and cell death, and these effects are ligand specific. We identified a small molecule, high affinity AhR agonist, named 11BBQ, that exhibit anti-cancer properties in lung cancers. Data from AhR knockout cell models showed that the ligand 11BBQ exerts its effect by modulating AhR activities to induce cell cycle arrest. The growth-inhibition effect of 11BBQ on cancer cells is persistent in zebrafish xenograph models. Gene expression study using RNA-seq showed the enrichment of genes involve in negative cell cycle regulation, including the cyclin inhibitor $p27^{kip1}$. Knockout of $p27^{kip1}$ in lung-cancer cells revealed that the $p27^{kip1}$ plays an important role in 11BBQ-induced growth arrest. In addition, RNA-seq revealed an unanticipated regulation of a new identified tumor suppressor gene by AhR. Current efforts focus on understanding how AhR regulate this new tumor suppressor gene and the role of this gene in the effect of 11BBQ on lung cancers.

Poster #34; Young Investigator Applicant

Quantification of Superwarfarin Rodenticides in Plasma using High-Performance Liquid Chromatography-Tandem Mass Spectrometry

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ABSTRACT

Superwarfarins are long-acting anticoagulant rodenticides (LAARs), that are 100 times more potent than warfarin, and function by inhibiting the vitamin K cycle, preventing the function of vital clotting factors and other vitamin K dependent proteins. Prolonged exposure to LAARs can lead to various coagulopathy disorders, neurological damage and in some cases death. The standard of care for LAAR poisoning is treatment with fresh-frozen plasma and vitamin K_1 supplementation, which is adjusted based on prothrombin time (PT). Due to the long LAAR half-lives of up to a month, poisoning victims require continuous PT monitoring and vitamin K_1 supplementation. PT provides information on vitamin K_1 levels but not on superwarfarin levels. It is vital to know the extent of LAAR exposure to guide coagulopathy therapy and to understand the pharmacokinetics of diastereoisomer pairs of LAARs. Therefore, we developed a rapid, sensitive, and selective UHPLC-MS/MS-based method to separate and quantitate diastereoisomeric pairs of the LAARs brodifacoum (BDF), difenacoum (DFC), bromadiolone (BDL), difethialone (DFT) and flocoumafen (FCF) in human blood. Analyses of clinical specimens facilitates the determination of LAARs exposure and pharmacokinetics of LAARs diastereoisomers to refine vitamin K_1 therapy.

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Poster #35; Sponsor

A Supplement Containing Specialized Pro-resolving Mediators (SPM) Improved Physical Functions in Subjects with Fibromyalgia: An n-of-1 Type Clinical Evaluation

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ABSTRACT

Background: For patients with fibromyalgia (FM), symptoms including pain, fatigue, and sleep and mood disturbances lead to marked mental and physical quality of life (QOL) burden. Potential contributing factors to FM include altered pain processing, stress, immune dysfunction and systemic inflammation.

Rationale: Inflammation resolution and tissue healing are actively coordinated by a group of lipid mediators known as SPM. Administration of a specific SPM (resolvin D2) in a mouse model of FM counter-acted brain neurotransmitter imbalance and improved the mood and behavior. The current study aimed to evaluate the impact of an SPM supplement on the well-being and QOL in patients with FM in a real-life clinical setting.

Methods: Subjects with FM (n=7) were recruited. After a phase-in period, subjects consumed 2 SPM supplement softgels (delivering 1g of Active Fractionated Marine Lipid Concentrate with proprietary, standardized SPM content) daily for 12 weeks. At each clinic visit (including a post-study follow-up that took place 12 weeks after the end of supplementation) subjects completed clinical exam and questionnaires including Revised Fibromyalgia Impact Questionnaire (FIQR) to assess the impact to QOL and severity of FM.

Results: All subjects completed the study. Scores on FIQR indicated an improvement in fibromyalgia severity and in physical function and activities of daily living. These improvements were seen without corresponding increases in reported pain. Six of the 7 subjects were considered to be responders to the supplement. The average reduction in FIQR total score in these 6 subjects was 18.7 out of 100 points. Greater response was seen in subjects without Chronic Inflammatory Response Syndrome (CIRS).

Conclusions: Improvements in physical function have the power to improve overall QOL in patients with FM. This study provide initial proof-of-concept data suggesting that supporting inflammation resolution through SPM supplementation may have a potential role in the management of FM.

Poster #36

A Product for Health Span Based on the Synergy Between Vitamins C and K

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ABSTRACT

The Federal law DSHEA prohibits manufacturers from making medical claims for nutritional supplements without FDA approval. This prevents people learning of the benefits of nutritional supplements like vitamins C & K1 (VC, VK1), despite peer-reviewed scientific reports of their benefits. In addition, it is difficult to obtain funding for methods to prevent cancer: "Despite more than 2.4 million papers published on cancer research to date, conventional medicine has largely failed to identify safe, low-cost, effective methods of cancer intervention and prevention."

Here I report on a safe, low-cost, scientifically justified method likely to have multiple health benefits, including preventing cancer and reducing risk for heart disease. Both VC and VK1 are GRAS substances with individual and synergistic health benefits. I developed a tablet that combines VC and VK1. It is likely to produce the benefits which are reported in the literature, justified by research, but cannot be claimed on the product.

VC at high dosages becomes a pro-oxidant generating free radicals that kill cancer cells. A dosage of 2g VC twice a day for 2 consecutive days produces a bladder [VC] able to kill cancer tissues *in vitro*. VK1 at high dosages activates enzymes that remove calcium from arteries, reducing risk for heart disease. VK1 is converted *in vivo* to generate VK3, which enhances free radical production from VC up to 40x, so that serum [VC] may kill cancer cells.

Three years of clinical trials for patients who have had bladder cancer have been planned to determine the health benefits, including monitoring cancer, heart disease, Alzheimer's, and injuries from falls. We are planning a trial and would like input into the study design. Until funded, we are not recruiting subjects or physicians. The supplements have not been manufactured yet. Contact me to receive information about the supplement or trial as it becomes available.

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Poster #37; Lightning Talk Speaker; Young Investigator Applicant

Intestinal FXR Signaling Mediates the Anti-Obesity Benefits of Xanthohumol

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ABSTRACT

Xanthohumol (XN), a bioactive prenylated flavonoid found in hops, reduces manifestations of obesity and metabolic syndrome by improving dysfunctional glucose and lipid metabolism in animal fed a high-fat diet (HFD). We hypothesize the anti-obesity effects of XN are mediated through FXR signaling, as XN binds to this nuclear receptor with a pivoting role in the regulation of glucose and lipid metabolism, and in the maintenance of bile salt homeostasis. To test our hypothesis, we used liver- and intestine-specific FXR-knockout mice. We find that while the knockout of FXR in the liver worsens symptoms of the metabolic syndrome under HFD conditions, intestine-specific FXR-null mice are resistant to HFD-induced metabolic impairments. Treatment with XN improves weight gain and metabolic parameters in wild-type and liver-specific knockout mice. These effects are associated with changes in FXR targets gene expression and modifications of the bile acid pool. Thus, *in vivo*, XN ligand-receptor interaction with FXR plays a minor role in its properties, which are largely regulated by XN-induced modification of bile acids profiles and the resulting modulation of FXR in the intestine.

Poster #38; Young Investigator Applicant

Targeting Therapy Resistant Cancer Through Bcl-2 Functional Conversion

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ABSTRACT

Emergence of tumor resistance to cancer therapeutics is a major cause of treatment failure, which results in poor overall survival. Identification of novel molecular targets present in resistant cell populations, especially targets that develop as resistance is acquired, are essential for addressing therapeutic resistance and prolonging patient survival. The B-cell lymphoma 2 (Bcl-2) family of proteins is associated with therapy resistance mechanisms. In this study, we investigated mechanisms of resistance to chemotherapeutics and found upregulation of anti-apoptotic members of the Bcl-2 family in response to several chemotherapeutics. Cell lines that developed acquired resistance to chemotherapeutics were susceptible to small molecule Bcl-2 functional converters, which convert Bcl-2 from a protector to a killer of cancer cells. The combination of chemotherapeutics and Bcl-2 functional converters leads to synergistic induction of apoptosis in therapy resistant cancer cells. Thus, this study identifies a potential strategy to treat patients who have developed acquired resistance and are no longer responsive to current chemotherapeutics. This highlights the potential of Bcl-2 expression as a biomarker of resistance and the novel strategy of Bcl-2 functional conversion to prevent and treat resistant cancer.

Poster #39; Young Investigator Applicant

Total Synthesis of Isotopologues of Xanthohumol and its Congeners for Biological Studies

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ABSTRACT

Xanthohumol (XN) is the primary prenylated chalcone found in the inflorescence of hops plants. It has been shown to have various biological functions, including potential broad-spectrum anticancer properties and hormonal effects applicable to treatment of hot flashes and osteoporosis. Previously, total syntheses of $[^{13}C]_2$-XN, $[^{13}C]_3$-XN, and $[^{13}C]_5$-XN were developed (J. Label Compd. Radiopharm. 2017, 60, 639) to assist studies to better understand the complex bioactivity of xanthohumol and its metabolites, and the role they may play in the remediation of metabolic syndrome. Notably, dihydroxanthohumol (DXN) and related XN derivatives lacking the α-β-unsaturated ketone functionality are unable to cyclize into 8-prenylnaringenin, allowing some of the biological effects to be accessed in the absence of potentially undesired estrogenic activity (Scientific Reports 2018, 8, 613). Current investigation of synthetic routes to DXN and related derivatives will be described, including a modification of the previously developed XN synthesis, and a route via functionalization of the natural product phloretin.

Poster #40

Tyrosine Nitration Induces a Metabolic Reprogramming in Neurofibromatosis Type 2-Associated Schwannoma Cells

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ABSTRACT

BACKGROUND: Neurofibromatosis type 2 (NF2) is a genetic tumor disorder of the nervous system caused by inactivation of the merlin tumor suppressor gene. NF2 patients develop multiple tumors throughout their life for which there is no effective treatment. The hallmark of the disease is the development of vestibular schwannomas (VS) along the acoustic nerve. Production of the oxidant peroxynitrite and tyrosine (Tyr) nitration of proteins occurs in multiple tumor types. However, the role of Tyr nitration in tumorigenesis is unknown. We showed that VS and merlin-deficient (MD) Schwann cells have significantly increased levels of peroxynitrite and Tyr nitration compared to wild-type (WT) Schwann cells. Further, prevention of tyrosine nitration using urate significantly and selectively decreases MD-Schwann cell survival. We also found that Tyr nitration decreases the activity of the oxidative phosphorylation in these cells. **OBJECTIVE:** Our goal was to elucidate the role of peroxynitrite and tyrosine nitration in the regulation of tumor cell metabolic reprogramming, survival and proliferation.

METHODS: We used complementary biochemistry, molecular and cell biology methodologies together with extracellular flux analysis to determine the metabolic phenotype and cell survival of WT- and MD-Schwann cells in the presence and absence of urate, a natural scavenger of peroxynitrite-derived radicals. **RESULTS:** Here we show that tyrosine nitration regulates a metabolic reprogramming in human MD-Schwann cells characterized by decreased activity of mitochondrial complex IV, increased glycolysis and almost complete glutamine dependency. Prevention of Tyr nitration reversed the metabolic phenotype of MD-Schwann cells back to that of WT-Schwann cells. We showed that nitrated Hsp90 inhibits mitochondrial activity in tumor cells. We found Hsp90 selectively nitrated in MD-Schwann cells and associated with mitochondria. Further, the intracellular delivery of nitrated Hsp90 in WT-Schwann cells significantly decreased mitochondrial oxygen consumption, as observed in MD-Schwann cells containing endogenous nitrated Hsp90, without affecting glycolysis.

CONCLUSIONS: Our observations suggest that nitrated Hsp90 plays a role in the metabolic reprogramming of schwannoma cells associated with NF2, and could be an exceptional tumor-directed therapeutic target in the short term. The identification of additional nitrated proteins that promote schwannoma growth could provide novel targets for the treatment of NF2 and possibly other tumor types as well.

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Poster #41

Say NO to Disease: Effect of Nitric Oxide on Disease Prevention and Reversal

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ABSTRACT

Nutrition recommendations that prevent chronic disease should include whole foods that provide the body with nutrients that allow the body to produce Nitric Oxide (NO). Studies show that consuming NO-producing foods on a regular basis can improve risk factors for chronic disease, like enhancing blood circulation and oxygenation of tissues. NO-producing foods may be even more important for children whose healthy eating habits in youth may prevent disease in adulthood. Although Nitric Oxide is just one micronutrient, it works synergistically with other compounds in whole foods that can improve health. Even educators who are not trained in nutrition can encourage the intake of whole foods, including those that contain the precursors to Nitric Oxide. Translating this research for practical application is equally as important as the original research.

Poster #42

Induction of $p27^{Kip1}$ by Select AhR Ligands for Cancer Therapy

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ABSTRACT

The cell cycle regulator $p27^{Kip1}$ is a cyclin dependent kinase inhibitor that plays a pivotal role in controlling cell proliferation. Fluctuation in levels of $p27^{Kip1}$ protein dictates the progression of the cell cycle and its misregulation directly affects cell proliferation. Down-regulation of $p27^{Kip1}$ causes activation of effectors responsible for cell cycle division and its loss has been shown to correlate to poor prognosis in breast cancer patients. In the contrary, high levels of $p27^{Kip1}$ cause cell cycle arrest and growth inhibition which can lead to programmed cell death. Importantly, $p27^{Kip1}$ has been shown to induce apoptosis of cancer cells. Although its role as cell cycle inhibitor has been widely studied, its apoptotic facet is unclear. It is assumed that $p27^{Kip1}$ protein levels are regulated post-translational although mechanisms that modulate the transcription of the gene are poorly understood. Our laboratory has identified a compound that activates $p27^{Kip1}$ promoter and induces its RNA and protein expression in triple negative breast cancer (TNBC) cells causing inhibition in cell growth. Interestingly, we have discovered the mechanism that mediates the activation of $p27^{Kip1}$ gene. We have determined that up-regulation of $p27^{Kip1}$ occurs through activation of the aryl hydrocarbon receptor (AhR). We have also observed that treatment with our compound caused truncation and cytosolic localization of $p27^{Kip1}$ which are characteristic of apoptotic cells. Our data suggests that cell growth inhibition of treated TNBC cells is due to cell cycle arrest and apoptosis.

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Poster #43

Mitochondrial Heterogeneity Corresponds with Altered Cardiolipin Content in an Aging Model but not in a β-amyloid Expressing Mouse Model

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ABSTRACT

Mitochondria ultimately donate electrons from catabolism of organic compounds to terminal O_2 through the electron transport chain (ETC) complexes. However, this process may produce reactive oxygen species (ROS) as a by-product. In part, ROS appearance is modulated by the dynamic balance between "supercomplexes" (SC) of the ETC and that of individual complexes, where SC's purportedly limit ROS by increased efficiency of electron transfer and energy production. In part, SC formation/disassembly is regulated by both the levels and subtypes of a key mitochondrial phospholipid, cardiolipin.

Because increased ROS appearance is considered a characteristic of both aging and age-related diseases (e.g. Alzheimer's and Parkinson's diseases, heart failure, and kidney diseases), we previously examined the extent of age-related differences in mitochondrial SC composition and levels in cardiac interfibrillar mitochondria from young (3 mo) and old (24-26 mo) Fisher 344 rats. Results showed that with age, the most complex SCs (i.e. "respirasomes" consisting of ETC complexes I, III, and IV) declined with core SCs remaining (Gómez 2009, Arch Biochem Biophys). These changes coincided with a 50% decline in 18:2 cardiolipin, the main cardiolipin species in cardiac mitochondria.

Because of the profound age-related loss of SC levels and complexity, we further hypothesized and expected a similar attenuation of SCs in a β-amyloid overexpressing mouse model of Alzheimer's disease (5xFAD). Additionally, we anticipated that SC alterations would coincide with changes in cardiolipin content. Examination of hippocampal mitochondria in 5xFAD mice vs. age-matched controls, however, revealed marked increases in SC size heterogeneity in 1-month-old mice. This size heterogeneity was not the result of cardiolipin content in 5xFAD mice, as it was unchanged.

We conclude that while mitochondrial SC heterogeneity is part of both the etiology of aging and in early pre-symptomatic events of β-amyloid accumulation in 5xFAD mice, the mechanism(s) associated with supercomplex alterations are not necessarily derived from changes in cardiolipin.

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Poster #44; Young Investigator Award

Aggresome-like Formation Promotes Resistance to Proteotoxicity in Cells from Long-lived Species

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ABSTRACT
Protein homeostasis, or proteostasis, is a hallmark of aging which involves multiple protein quality control processes that work together to ensure the health of the cellular proteome. The capacity of cells to maintain proteostasis declines with age, causing rapid accumulation of damaged proteins and protein aggregates, and is considered to play an important role in age-related disease etiology. While our group and others have identified that proteostasis is enhanced in long-lived species, to the best of our knowledge there is no data on whether this leads to better resistance to proteotoxicity. We compared the sensitivity of cells from long- (Naked Mole Rat, MR) and short- (Mouse) lived species to proteotoxicity, by measuring the survival of fibroblasts to polyQ toxicity, a well-established polyglutamine (PolyQ) model of protein aggregation. Additionally, to evaluate the contribution of proteostatic mechanisms to proteotoxicity resistance, we down-regulated a key protein of each mechanism (autophagy - ATG5; ubiquitin-proteasome – PSMD14; and chaperones – HSP27) in MR fibroblasts. Furthermore, we analyzed the formation and sub-cellular localization of inclusions in long- and short-lived species. Here we show that fibroblasts from long-lived species are more resistant to proteotoxicity than their short-lived counterparts. Contrary to our expectations, this does not occur because the MR cells have less polyQ82 protein aggregates, but rather they have an enhanced capacity to handle misfolded proteins and form protective juxtanuclear and aggresome-like inclusions. All three proteostatic mechanisms contribute to this resistance to polyQ toxicity, but autophagy has the greatest effect. Overall our data suggest that the resistance to proteotoxicity observed in long-lived species is not due to a lower level of protein aggregates but rather to enhanced handling of the protein aggregates through the formation of aggresome-like inclusions, a well-recognized protective mechanism against proteotoxicty. It would seem that the enhanced resistance to proteotoxicity of long-lived species is due to the way they manage protein aggregation, rather than how they eliminate protein aggregation.

Funding: NIA/NIH R03AG05239

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Poster #45; Lightning Talk Speaker

The Role of Nrf2 in Brain Cellular Senescence

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ABSTRACT

Aging is a risk factor for the deterioration of biochemical pathways that lead to many neurodegenerative diseases. Levels of the transcriptional factor, Nuclear factor erythroid 2–related factor 2 (Nrf2) decrease in mammalian aging. Previous data from our lab has demonstrated an increase in cellular senescence in several tissues, including the brain of Nrf2 knockout mice (Nrf2KO). Senescent cells promote inflammation via the secretory associated senescence phenotype (SASP). Inflammation is a characteristic of many neurodegenerative diseases, and it is thought that cellular senescence contributes via the SASP. It is then hypothesized that the absence of Nrf2 will lead to an increase in cellular senescence in the hippocampus of mice, and be a driver for age-associated cognitive decline and neurodegeneration. The goal of this study was to identify an increase in cellular senescence in Nrf2KO mice compared to the WT. The hippocampus was primarily used to identify senescent cells and their characteristics, due to its association with memory loss and neurodegeneration. Via β-galactosidase staining, qPCR, Western Blotting and Immunohistochemistry, the levels of senescent cells and their molecular markers (p16, p21 and SASP members) were experimentally determined for the hippocampus of WT and Nrf2KO mice. The results demonstrated that Nrf2KO mice have increased levels of cellular senescence. We found that p16 levels were significantly elevated and a higher amount of proinflammatory cytokines, (i.e., Interleukin 6 and TNFα), were present in the hippocampus of Nrf2 KO mice when compared to the WT mice. Currently, we want to determine whether Rapamycin treatment will have a beneficial effect on senescence in this tissue, to test this, Nrf2 KO mice have been treated with Rapamycin (4 mg/kg) every other day for 14 weeks via IP injection. Similar experimental techniques to those aforementioned will be used to analyze hippocampus for senescence markers post-trial.

Poster #46; Young Investigator Applicant

Isomeric Identification of Procyanidins Using Ultrahigh Pressure Liquid Chromatography-Tandem Mass Spectrometry

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ABSTRACT

Procyanidins are composed of isomeric catechin and epicatechin with different interflavan linkages between monomers which build complex polymeric compounds. Procyanidins have been demonstrated to exhibit a wide array of therapeutic effects where different isomeric forms were more prominent in specific biological activities. It is important to properly identify procyanidins by the specific interflavan bond rather than the broadly stated degree of polymerization and linkage type (A, B, or mixed). High-resolution product ion tandem mass spectra have the potential to facilitate differentiation of isomeric procyanidins differing only by C4→ C6 versus C4 →C8 interflavan bonds between catechin and epicatechin subunits. Isomeric procyanidin standards consisting of dimers, trimers and tetramers containing different C4→ C6 and C4→ C8 linkages were analyzed utilizing UHPLC-MS/MS on a high-resolution Shimadzu 9030 QToF mass spectrometer. The fragmentation pattern of each pair of isomeric procyanidins were examined to identify critical fragment ions, which facilitate the differentiation of procyanidins containing C4→ C6 or C4→ C8 bonds.

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Poster #47

Early Sex Differences in AMPA and NMDA Receptor Responses in 5xFAD Mice

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ABSTRACT

Alzheimer's disease (AD) is the most common form of dementia. Mutations in presenillin or amyloid precursor protein (APP) in familial AD lead to overproduction of amyloid. Changes in amyloid occur early in AD, before the onset of behavioral signs. Based on this, and early changes seen in N-methyl-D-aspartate receptors (NMDARs) in presenillin models with single mutations, we aimed to study the difference in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and NMDARs in a more complex model.

In 15-day or 4 month old 5xFAD (two presenillin and 3 APP mutations) mice, the field excitatory post synaptic potentials (fEPSPs) in hippocampal slices were analyzed using a multielectrode array. AMPAR and NMDAR antagonists were applied and input (fiber volley amplitude) / output (fEPSP amplitude) curves were analyzed. Inhibition curves were used to analyze the effect of magnesium on non-AMPAR responses in 15 day, 1 and 2 month old 5xFAD mice.

Fifteen-day old hemizygous 5xFAD male mice showed increased fEPSP amplitudes for AMPARs, NMDARs, GluN2A NMDAR subunits and the remaining NMDARs. By 4 months, their amplitudes were at wild type (WT) levels. Female hemizygous mice showed no genotypic differences at 15 days but were hyporesponsive at 4 months for AMPAR fEPSPs. GluN2A fEPSPs for the female hemizygotes were reduced from WT at both 15-days and 4 months of age and for the remaining NMDARs there were no differences between genotypes at 15-days or 4 months of age. The data suggest that disease progression is different between sexes; glutamate receptor changes may begin earlier than 15-days old of age in female mice or the early enhancements in male glutamate receptors are not related to the later reductions seen in both sexes. Magnesium inhibited non-AMPAR responses in 15 day and 1 month old mice only, suggesting that magnesium supplements could prevent excitotoxicity at younger ages.

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Poster #48

Detection of Antioxidant Compounds in *Moringa oleifera* Flowers by HPLC-FLD/ECD and TLC and Phytochemical Analysis

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ABSTRACT

Moringa oleifera is a tree distributed in Mexican semiarid and coastal regions. *M. oleifera* flowers is a rich reservoir of bioactive phytochemicals and crude flower extract showed medical properties. A simple, and sensitive method based on HPLC with fluorescence and electrochemical detection was developed for the determination of antioxidants in different fractions. In addition, a thin-layer chromatography separation on TLC silica gel 60 plates and UV detection was used. Phytochemical screening showed the presence of flavonoids, alkaloids and saponins in all extracts. Our results indicate that ethyl acetate extract seem to be a richer source of antioxidants than other fractions.

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Poster #49

Modulation of AhR activity by 11BBQ in Cancer Cells

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ABSTRACT

11-chloro-BBQ (11BBQ) was discovered as a small molecule, high affinity agonist of the aryl hydrocarbon receptor (AhR). The AhR is a transcription factor that recognizes specific DNA motifs, typically referred to as xenobiotic responsive element (XRE). 11BBQ binding promotes translocation of the AhR from cytosol to nucleus and enables its ability to function as a transcription factor. Several newly designed analogs of 11BBQ are being tested on AhR-induced transcriptional responses. In addition, we are currently investigating activity of 11BBQ in lung and breast cancer cells along with the newly designed analogs. The goal of this study is to test and establish AhR-mediated responses by 11BBQ and its analogs in cancer cells.

Poster #50

Antioxidant Activity of the Extracts from Pericarp and Seed of *Zanthoxylum limonella* (Dennst.) Alston

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ABSTRACT

Zanthoxylum limonella (Dennst.) Alston is a spicy plant in the family Rutaceae. It is widely distributed in Southeast Asia such as India, Sri Lanka, Myanmar, Philippines, and Thailand. Local people in Thailand often use the fruits of *Z. limonella* to add flavor and aroma for many favorite dishes such as curry, spicy salad and fried chicken. This study aimed to investigate total phenolic content and antioxidant activity of the pericarp and seed extracts from *Z. limonella*. The antioxidant activity of the extracts was tested by DPPH, FRAP and ORAC assays. It was found that the pericarp extract contained more phenolic compounds than that of the seed extract. The total phenolic contents, expressed as mg GAE/g DW, of the extracts from pericarp and seed were 10.29 ± 0.56 and 1.23 ± 0.19, respectively. The results from DPPH assay showed higher TEAC values of the pericarp extract than that of the seed extract (0.547 ± 0.024 vs 0.204 ± 0.050 mg trolox/g DW) indicating higher antioxidant activity of the pericarp extract. Similar findings on antioxidant activity were also found with FRAP and ORAC assays. In summary, this study clearly demonstrated that the pericarp extract of *Z. limonella* exhibited a higher antioxidant activity than that of the seed extract.

Poster #51

Triphenylphosphonium-Glutathione as a Protectant Against Mitochondrial Decay

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ABSTRACT

Mitochondrial decay is an intrinsic part of Alzheimer's Disease (AD), as well as other age-associated neurodegenerative pathologies. In particular, mitochondrial *matrix glutathione* (**mGSH**), which is essential for normal mitochondrial function, declines in AD; mGSH loss is one of the earliest hallmarks associated with its onset. Mitochondria do not synthesize GSH; rather, mGSH is supplied from the cytosol via slow facilitated transport. This lack of equilibrium with the cytosol severely challenges the cell's ability to maintain sufficient mGSH levels when its rate of oxidation exceeds supply. Such constraints to maintaining mGSH constitute a key *barrier* to creating an effective mGSH-restoring treatment. There is no current clinical therapy that remediates mGSH. Compounds that increase cellular GSH do not sustainably improve the mitochondrial GSH pool. The lack of mitochondrial GSH-remediating therapeutics is a serious impediment to preventing mitochondrial dysfunction during the pathophysiologies that occur in neurodegeneration, and specifically, AD. We created a novel compound, ***trip**henyl**p**hosphonium-GSH* (**TPP-GSH**) to overcome the obstacles for mGSH therapy. We show that water soluble TPP-GSH elevates mGSH levels in rat hepatocytes, and restores mitochondrial structure and function in transgenic mice that develop AD-like pathology. We propose that TPP-GSH will supply GSH to mitochondria and serve as an *mGSH-restoring and -augmenting therapy*.

Poster #52; Lightning Talk Speaker

Kefir Probiotic Protocol Significantly Reduces Hospital Acquired *C. difficile* Infections

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ABSTRACT

C. difficile infections (CDI) are a major healthcare-associated infection (HAI). Cases in the United States are tracked by the Centers for Disease Control and Prevention (CDC), which reports that antibiotics increase CDI risk 7- to 10-fold. Some probiotics, such as *L.casei* and *L. rhamnosis*, reduce this risk and other probiotics have no effect.
METHODS: To reduce HAI CDI rates, we implemented a protocol using a commercial, mixed probiotic kefir drink (Nancy's® Kefir), having at least 10 billion probiotics per 60 mL dose, taken once or twice daily. HAI *C. difficile* rates were reported as cases per 10,000 patient days, using CDC statistical reports, on actual vs. expected cases over a five-year period, for 177,287 patient days in total.
RESULTS: During the first year, 2012, rate was 6.5 vs. 7.7 expected (p=0.456), but had limited orders-only implementation. In 2013, the protocol implementation was expanded for nurses using flavored kefir as a floor stock item freely available to administer per protocol. This implementation significantly reduced HAI rates, as evidenced by a year-over-year 44% increase in purchased kefir, associated with 57% lower actual vs. CDC-expected rates (3.0/ vs. 6.9/10,000, respectively, p=0.0021). The hospital's stable background in standard infection prevention measures further increased confidence in the observed impact, since those measures had not improved more than 5% in 2013, and actual rates continued to exceed CDC expectations, in 2014 (3.9 vs. 6.7, respectfully, p=0.0282), 2015 (3.5 vs. 6.7, p=0.0096), and 2016 (3.9 vs. 7.6, p=0.0046).
CONCLUSION: Over 5 years and 177,287 patient days, implementation of a protocol to administer probiotics from Nancy's® kefir reduced hospital-acquired *C. difficile* rates to <4.0 cases per 10,000 patient days (p<0.03); estimated to have prevented over 53 cases and 4 deaths, with net cost savings of over half a million dollars, and an estimated return-on-investment of $31:$1.

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Poster #53

Medical Food Grade Cranberry Extract Eliminates *E. coli* Catheter Associated Urinary Tract Infections (CAUTIs) in the Hospital

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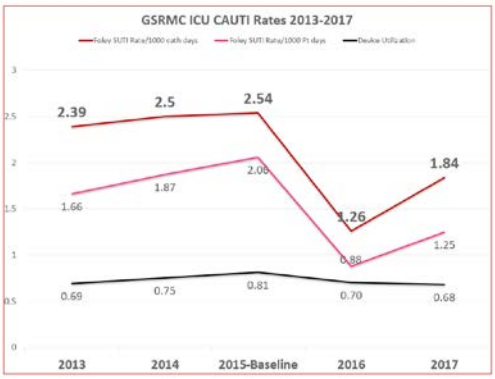
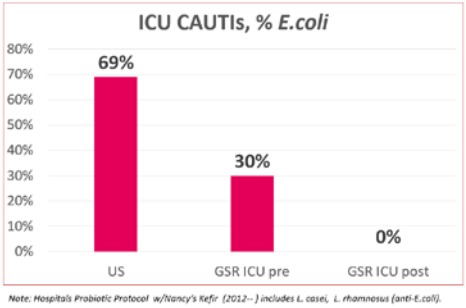
ABSTRACT

Urinary Catheters are placed in 45-79% of ICU patients, 12-16% other inpatients. Catheter Associated Urinary Tract Infections (CAUTIs) are the 4th most common Healthcare Associated infection (HAI) in the U.S. They are most common cause of secondary bacteraemia in hospitals. Risk for CAUTIs increases 3-7% per catheter day. CAUTIs increase mortality, morbidity and prolong length of stay. Antibiotics are used in 60-80% of CAUTI patients, this increases risk of antibiotic resistant organisms and antibiotic associated diarrhea, including *C.difficile*, a high mortality HAI. *E. coli* accounts for 69% of CAUTIs in the US. Quality cranberry products help prevent *E.coli* CAUTIs with adequate doses (36-72 mg) and timing (every 8-12 hrs) of A-type proanthocyanidins (A-PAC). *E. coli* fimbriae will attach to A-PACS instead of the urinary tract wall. When E. *coli* can't attach, it can't invade and cause infections. Once *E.coli* attaches, cranberry can't help. CDC tracks CAUTI rates. ICU rates are 1.2-4.1 per 1000 catheter days.

INTERVENTION: Joined new ICU CAUTI Prevention Initiative efforts by Infection Prevention and ICU RNs in improved catheter insertion, care, and removal with the addition of a low volume Medical Food quality cranberry extract, UTI-STAT® 30 ml twice a day; oral or enteral, equivalent to ~8 oz (240ml) 100% cranberry juice twice daily. Medical foods are FDA regulated, approved and inspected. They are not dietary supplements.



Hospital had zero *E.coli* CAUTIs in 2017



RESULTS: Overall reduction of 28-50% in ICU CAUTIs (2.54 to 1.26-1.84/1000 catheter days) , 27% fewer catheter days in 2017 vs. 2015; Elimination of E.coli CAUTIs in ICU. Year 2: expand project to PCU/hospital. PCU 66.7% reduction in CAUTIs pre/post. Complete elimination of *E.coli* CAUTIs hospital wide in 2017.

CONCLUSION: Multi-disciplinary CAUTI prevention initiative was effective at reducing CAUTIs in ICU and PCU and the UTI-STAT® cranberry product was effective at eliminating *E.coli* associated CAUTIs hospital wide.

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Poster #54; Young Investigator Applicant

GNPS Enables Metabolite Discovery Associated with GABA Neurotransmitters in the Hippocampus of Mice Brain

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ABSTRACT

We develop approaches that allow the discovery of bioactive principles in complex natural product extracts. Towards this goal, we have focused on the discovery of bioactive compounds in hop extracts. We have used GNPS molecular networks in conjunction with computational methods to associate bioactivity with distinct natural products and scaffold. Here we report our initial efforts of using GNPS bioactivity-based molecular networking analysis for the discovery of neuroactivity. Methanol extract of hops suppresses synaptic transmission when field excitatory post-synaptic potential (fEPSP) is applied in mouse hippocampus. Six prenylflavones were quantified from methanol extracted hops such as xanthohumol, isoxanthohumol, desmethylxanthohumol, 6- and 8-prenylnaringenins and 6-geranylnaringenin. Prenylflavonoids from hops are polyphenols class of compound which has been studied extensively for their bioactive property including positive allosteric modulation of Gamma-aminobutyric acid (GABA) receptor. GABA A receptors are ligand-gated ion channels responsible for the mediation of fast inhibitory action of GABA in the brain. A study showed that xanthohumol, isoxanthohumol, and 8-prenylnaringenin potentiated GABA-induced displacement of ^{3}H-ethynylbicycloorthobenzoate radioligand binding in a concentration-dependent manner. Our electrophysiology study on mice brain slices also supported these findings. The electrophysiology results were incorporated into molecular networking analysis in order to identify the molecular diversity associated with the shift in baseline potential. Global Natural Product Social Molecular Networking (GNPS) was used to construct the molecular networks by aligning MS/MS spectra of the parent ions in mass spectrometry analysis. GNPS performed spectral matching using MS2 fragmentation pattern against the spectral libraries which enabled us to annotate the compounds present in the hops extract. Similar fragmentation patterns led us to a problem of annotation of isomers as these compounds could not be separated by usual LC-MS techniques. Traveling-wave ion mobility mass spectrometry technique was used to annotate isomers using a quadrupole time-of-flight mass spectrometer. IMS drift times were converted to collision cross section (CCS) values. For instance, the isomers xanthohumol and isoxanthohumol that are associated with GABA neurotransmitter could be resolved by IMS because they have different CCS values but are not distinguishable by mass spectrometry alone.

Poster #55; Lightning Talk Speaker

Upregulation of Thioredoxin-Interacting Protein in Brain of Amyloid Precursor Protein/Presenilin 1 Transgenic Mice and Beta-amyloid Treated Neuronal Cells

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ABSTRACT

Alzheimer's disease (AD) is characterized by progressive neurodegeneration in brain with the aging as the major risk factor. Beta-amyloid (Aβ) is a feature characteristic of this disease. Although Aβ contributes to AD pathological development, eventually causing neurodegeneration and cognitive impairment, the precise mechanisms of this contribution remain unclear. Many studies indicate that Aβ causes oxidative stress. Previously we found that nitrosylated protein levels were increased in the brain of amyloid precursor protein (APP)/presenilin 1 (PS1) double transgenic mice, an animal model for AD, suggesting cysteine oxidation may contribute to AD. Thioredoxin (Trx) is a major oxidoreductase that reverses cysteine oxidation such as sulfenylation and nitrosylation, and protects the cells against oxidative stress. Trx-interacting protein (Txnip) is an endogenous Trx inhibitor. To understand the involvement of Trx and Txnip in AD development, we investigated Trx and Txnip in the brain of APP/PS1 mice.

Using immunoblotting analysis, we found that although Trx protein levels were not changed, Txnip protein levels were significantly increased in hippocampus and frontal cortex of 9 and 12-month old APP/PS1 mice when compared to wild-type mice. Txnip protein levels were also increased by Aβ treatment in primary cultured mouse cerebral cortical neurons and HT22 mouse hippocampal cells. Using biotin switch and dimedone conjugation methods we found that Aβ treatment increased protein nitrosylation and sulfenylation in HT22 cells. Down regulation of Txnip using CRISPR/Cas9 method in HT22 cells attenuated Aβ-induced protein nitrosylation and sulfenylation. Our findings suggest that Aβ may increase Txnip levels, subsequently inhibiting Trx reducing capability and enhancing protein cysteine oxidation, which contributes to oxidative stress in AD. Our findings also indicate that Txnip may be a potential target for the treatment of AD. This research is supported by a Research Grant from the Alzheimer Society Research Program of Canada (JFW).

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Poster #56

Vitamin E Deficiency Causes Increased Lipid Peroxidation and Alteration of Sphingolipid Species and Long Chain Polyunsaturated Fatty Acids in Zebrafish Eyes

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ABSTRACT

Vitamin E (VitE) provides protective effects for cell membranes subjected to oxidative stress. Ceramide, a lipid species which induces apoptosis caused by oxidative damage, is implicated in photoreceptor death, and is a hallmark of the genetic disorders called Retinitis Pigmentosa (RP). These disorders are characterized by the breakdown or loss of photoreceptor cells in the eye; photoreceptor membrane damage is prevented by VitE. Although the mechanisms by which VitE deficiency causes photoreceptor loss is unclear, we hypothesized that VitE depletion would result in an accumulation of certain sphingolipids such as ceramide, subsequently leading to increased cell death.

Eyes from adult zebrafish, fed diets sufficient (E+) or deficient (E-) in VitE for one year, were studied. Relative abundance of identified lipid species was determined by UPLC-TOF-MS/MS. Of the sphingolipids identified, ceramide 28:1 and sphingosine 18:0 were elevated in E- eyes relative to E+ ($p<0.05$), whereas sphinganine 16:0 and 18:0, and sphingomyelin 16:1, were reduced in E- eyes relative to E+ ($p<0.05$). In response to oxidative damage caused by VitE deficiency, long chain polyunsaturated fatty acids (LC PUFA) levels decrease as they are oxidized into other products, including reactive aldehydes. Therefore, we hypothesized that VitE deficiency would also cause depletion of LC PUFA's. Our results showed multiple free fatty acids [22:6, 22:5, 22:4, 22:1, 20:4, and 18:1] were significantly depleted in E- relative to E+ ($p<0.05$). Thus, we propose that VitE deficiency in zebrafish eyes causes lipid peroxidation and may alter levels of certain lipid species linked to cell death.

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Poster #57; Young Investigator Applicant

Urinary Estrogen Derivatization and High-Resolution LC-MS Analysis to Determine Modulation of Estrogen Metabolism in Women Resulting from Use of Botanical Dietary Supplements

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ABSTRACT

Menopause is the natural decline of estrogen levels for women in their late 40s to early 50s. It can cause long-lasting health effects, including loss of bone density and increased risk of cardiovascular disease. Hormone therapy is the conventional treatment used to manage symptoms such as hot flashes and vaginal dryness; however, prolonged use of hormone therapy might increase risks of heart disease, stroke, blood clots, and breast cancer. In particular, the increased breast cancer risk has been associated with 4-hydroxyestradiol, an estrogen metabolite formed by P450 1B1, which can form an ortho-quinone and cause oxidative DNA damage through redox cycling. Approximately 70% of American peri- and post-menopausal women have reported the consumption of botanical dietary supplements (BDS) to manage menopausal symptoms. We hypothesize that most BDS used by women to manage menopausal symptoms are safe, but some may pose risk through the modulation of estrogen biosynthesis and metabolism. We carried out post-analysis of urine from our completed phase I and phase II clinical trials of hops (*Humulus lupulus*) and red clover (*Trifolium pratense*) in peri- and post-menopausal women to measure the trace levels of estrone, estradiol, and their metabolites. This requires development of a highly-sensitive liquid chromatography-high resolution mass spectrometry method to identify changes in estrogen levels after consumption of the BDS for 14-days. This analysis utilized dansyl chloride derivatization and differential isotope labeling to enhance sensitivity and improve quantitation. For 16 women in a phase II clinical trial receiving a hop supplement, we measured a reduction in the levels of 4-hydroxyestradiol, a genotoxic metabolite formed via 4-hydroxylation of estradiol by P450 1B1. No changes were observed for other analytes, including 2-hydroxyestradiol, another estrogen metabolite which is formed from estradiol by P450 1A1. These data support our hypothesis that some BDS can selectively affect the modulation of estrogen metabolism.

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Poster #58

Metabolism of Xanthohumol by Intestinal Bacterium in Humans

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ABSTRACT

Xanthohumol (XN), a prenylated flavonoid from hops, improves dysfunctional glucose and lipid metabolism in animals. However, its metabolic transformation into the estrogenic metabolite, 8-prenylnaringenin (8-PN), poses a potential health concern for its use in humans. A reduction product of XN, α,β-dihydroxanthohumol (DXN), showed negligible affinity for estrogen receptors, and cannot be metabolically converted into 8-prenylnaringenin. In previous work, we demonstrated that oral treatment of mice with DXN ameliorates the neurocognitive-metabolic impairments associated with HFD-induced obesity without risk of liver injury and adverse estrogenic effects. Moreover, we recently found that certain gut microbes have the ability to convert XN into DXN and IXN into 8-PN. The aim of the present study was to investigate the conversion of XN into DXN in humans by LC-MS/MS. The human study design is a randomized, double-blinded, placebo-controlled cross over study with twenty healthy male and female subjects (18 – 50 yr). The study consisted of a 3-week treatment phase (24 mg XN per day), a 3-week washout phase, and a 3-week placebo phase. Urine samples (24 hours) were collected at the beginning and end of either treatment phase or placebo phase. Levels of XN, DXN, and other metabolites were determined in urine samples by HPLC (Shimadzu; Columbia, MD, USA) coupled to a hybrid triple quadrupole-linear ion trap mass spectrometer (4000 QTRAP; AB Sciex; Concord, canada) equipped with an electrospray ionization source. XN and IXN were dominant among all subjects with treatment. The level of IXN was much higher than XN in these samples. This observation indicated most of XN converts to IXN in the human body. DXN, 6-PN, and 8-PN were also found in urine samples treatment with XN, indicating that hydrogenation of XN and O-demethylation represent gut microbe-mediated metabolic pathways.

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Poster #59

Effects of Zinc Status and Aging on Age-related Immune Dysfunction and Chronic Inflammation

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Backgound: Aging is associated with progressive immune dysfunction, including impaired adaptive response, increased susceptibility to infection, and reduced vaccination efficacy. Aging is also associated with chronic inflammation that correlated with the promotion of many age-related diseases. Zinc is an essential micronutrient critical for immune function. In US, 12% of the population do not consume the estimated average requirement for zinc. The prevalence of inadequate zinc intake is even higher among older populations, and are at increased risk for marginal zinc deficiency. Effects of zinc deficiency share similarities to age-related immune dysfunction, including impaired adaptive immunity and increased in proinflammatory response.

Hypothesis/study design: We hypothesize that age-related decline in zinc status contributes to immune dysregulation and chronic inflammation in the elderly. We studied the effects of dietary zinc supplementation and marginal zinc deficiency on changes in mucosal immunity and inflammatory response in young and old mice. Young (2 mo) and old (24 mo) C57Bl/6 mice were fed a zinc adequate (ZA, 30ppm Zn), zinc supplemented (ZS, 300ppm Zn), or marginal zinc deficient (MZD, 6 ppm Zn) diets for 6 wks. Serum zinc status, cytokines, and naïve/memory T-cell phenotypes, were determined at the end of the study.

Results: Old mice had reduced zinc and increased proinflammatory cytokines MCP1 and IL6 in the serum, increased Th1/Th17/inflammatory cytokines (IFNγ, IL17, TNFα, respectively) and decreased naïve CD4 T-cells in the mesenteric lymph nodes (MLN). ZS significantly increased serum zinc levels, decreased TNFα, IFNγ, IL17 in MLN, and increased naïve T-cell populations in aged mice. MZD further reduced serum zinc and increased serum IL6 levels in aged mice.

Conclusion: ZS improved the immune function of aged mice and reduced inflammatory response, and MZD further increased age-related inflammation. Our data suggest that zinc status is an important contributing factor in age-related immune dysfunction and chronic inflammation.

Poster #60

Pseudoaldosteronism as an Adverse Effect of *Glycyrrhiza* Intake: Pathophysiology and Detection of the Causative Metabolite

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ABSTRACT

Glycyrrhizin (GL) is the active ingredient of licorice root (Glycyrrhiza root), which is commonly used as a sweetener in foods and medicinal herbal remedies worldwide. GL is responsible for the sweetness and various pharmacological activities of licorice. Occasionally, liquorice consumption causes pseudoaldosteronism as a side effect, causing edema, hypokalemia, and hypertension by inhibiting 11*β*-hydroxysteroid dehydrogenase type 2 (11*β*HSD2) in the renal tubular epithelium. 11*β*HSD2, the type 2 isoenzyme of 11*β*HSD, converts glucocorticoid cortisol into its inactive metabolite, cortisone. Impaired 11*β*HSD2 activity via competitive inhibition or downregulation by GL metabolites leads to increased cortisol concentration, sodium accumulation, and potassium and hydrogen exchange via excretion, even though renin and aldosterone activities are suppressed, ultimately leading to symptoms of pseudoaldosteronism.

Several candidate GL metabolites have been proposed as the causative agents of liquorice-induced pseudoaldosteronism. Recently, our team first detected high-level of 18*β*-glycyrrhetyl-3-*O*-sulfate (GA-3-sulf) in human blood samples using LC-MS/MS. Plasma GA-3-sulf concentration was negatively correlated with plasma renin and aldosterone activity and potassium level. GA-3-sulf was transported into renal tubular cells via organic anion transporter 1 and 3, and it inhibited 11*β*HSD2 activity. Sulfotransferase 2A1 in hepatocytes metabolized 18*β*-glycyrrhetinic acid into GA-3-sulf. Moreover, low serum albumin level and impaired biliary excretion of conjugated bilirubin were clinical risk factors of hypokalemia following licorice intake.
Our results showed that GA-3-sulf was a possible causative agent of liquorice-induced pseudoaldosteronism, and that several clinical conditions, especially liver dysfunction, were risk factors for this syndrome. We are currently working to establish an ELISA system for easy detection of high blood GA-3-sulf concentration for detecting and predicting liquorice-induced pseudoaldosteronism in daily clinical settings.

Poster #61; Young Investigator Applicant

TXN – a Bioactive Xanthohumol Derivative – Prevents Diet-Induced Dyslipidemia Possibly by Promoting Cholesterol Efflux: an Alternative to Statins?

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ABSTRACT

In a recent study, we showed for the first time that tetrahydro-xanthohumol (TXN), a potent, non-estrogenic bioactive xanthohumol derivative, decreased hyperlipidaemia in diet-induced obese (DIO) male mice. In this current study, we fed C57BL/6J male mice a low-fat diet (LFD control), a lard-rich high fat diet (HFD control) or HFD diet containing TXN at 30 mg/kg body weight, or xanthohumol at 30 mg/kg or 60 mg/kg body weight for 16 weeks. We observed a significant delay in the development of DIO and metabolic syndrome in TXN-treated mice. Specifically, total plasma cholesterol levels of TXN-treated mice at week 0, 6 and 16 were maintained at the same level seen in the LFD control mice, while the plasma cholesterol levels in the HFD control mice significantly increased over time. Moreover, a significant 43% increase per gram of fecal excretion of cholesterol was also observed in TXN-treated mice as compared to HFD control mice. No significant difference in the amount of fecal triglycerides were noted between these two groups. In the previous study, we demonstrated using lipidomics that TXN reduces hyperlipidaemia and lipid accumulation in the liver of mice fed the same high-fat diet and that oxidized phosphocholines in the liver were significantly decreased (unpublished data). We hypothesize that TXN prevents diet-induced dyslipidemia and hypercholesterolemia by inhibiting lipid and cholesterol accumulation in the peripheral tissues and by promoting cholesterol excretion in the feces. This could be achieved by improving complete fatty acid β oxidation in the mitochondria and by promoting cholesterol mobilization from peripheral tissues back to the liver and small intestines for bile excretion through the reverse cholesterol transport (RCT) pathway. Further analyses are underway to test this hypothesis. This project is funded by National Institutes of Health Grant #1R01AT009168; Marion T. Tsefalas Graduate Fellowship, Linus Pauling Institute, Oregon State University; Hopsteiner Inc., New York; and the Buhler-Wang Research Fund.

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