Bioactives

Poster Abstracts *1-19*

Poster Abstract 1

Design and development of a reporter plasmid for high-throughput screening of botanical extracts and small molecule libraries to discover prenylated flavonoid ligands of FXR

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The farnesoid X receptor (FXR) is a bile acid nuclear receptor and biological sensor for the regulation of bile acid biosynthesis. When a bile acid ligand binds FXR, it forms a heterodimer with the retinoid X receptor (RXR) and enters the cell's nucleus. The FXR/RXR heterodimer then binds to FXR response elements (FXREs) present in the promoters of its target genes. FXR activation leads to transcriptional repression of genes involved in de novo lipogenesis and gluconeogenesis and plays an important role in obesity, and metabolic syndrome. Interestingly, mice fed a high-fat diet supplemented with xanthohumol (XN), a prenylated flavonoid found in hops (Humulus lupulus), showed a dose-dependent decrease in body weight gain and glucose, leptin, and cholesterol levels. Using hydrogen deuterium exchange mass spectrometry (HDX-MS) combined with computational and fluorescence titration studies we indicated that XN binds within the canonical FXR ligand-binding pocket to modulate FXR target gene expression. We hypothesize that other prenylated flavonoids mediate their beneficial health effects by acting as ligands for FXR. To identify additional FXR ligands, we inserted three FXREs and a minimal thymidine kinase promoter into a two-step transcriptional activator (TSTA) reporter plasmid to drive expression of a Gal4 DNA binding domain-VP16 fusion protein. The fusion protein binds, in turn, to a GAL4 promoter in the TSTA reporter plasmid driving expression of β-Lactamase. This enables monitoring of promoter activity using a Förster resonance energy transfer (FRET)-based reporter system. We have shown that a natural ligand, chenodeoxycholic acid, and a synthetic ligand, GW4064, of FXR increase reporter gene expression. This plasmid will enable a robust, sensitive, and inexpensive high-throughput screening assay of small molecule libraries and botanical extracts for potential prenylated flavonoid ligands of FXR.

Poster Abstract 2

Effects of nitrate and nitrite exposure on zebrafish behavior and brain metabolic phenotyping

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The effects of nitrate and nitrite, present in the environment and our diet, on animal metabolism are controversial. We do not currently know the extent to which nitrate and nitrite exposure affects cognitive behavior and changes the abundance of downstream target molecules in the brain. In order to determine the physiological and cognitive effects derived from the exposure to different doses of nitrite and nitrate, we carried out a study with the aquatic model organism Danio Rerio (Zebrafish). Animals were exposed to sodium nitrite (9.75 and 19.5 mg/L), sodium nitrate (60.7, 303.5 and 606.9 mg/L), or control water. For the last three days of the experiment, two sets of fish were treated with 15N-nitrate or 15N-nitrite, in order to study the incorporation of these molecules into the brain metabolism. After four weeks, animals were euthanized and brains extracted. Samples were analyzed for untargeted metabolomics with a 5600 ABSciex TripleTOF in positive and negative mode. Pathway analysis showed significant differences in more than fifty identified compounds when comparing the metabolites of control and treated fish. The strongest variations were observed in the metabolism of aspartate, glutamate, leucine and taurine. Low incorporation of labeled nitrate and nitrite into the brain leads to the conclusion that the behavioral patterns observed in nitrate- and nitrite-treated fish, including anxiety and learning deficits, are due to indirect effects of those molecules on brain metabolism. Overall, we found a very significant depletion in many metabolites involved in the regulation of neuronal activity. For instance, the lower concentration of leucine in the brain of fish exposed to nitrite and nitrate may explain the reduction in glutamate metabolism and the observed behavioral patterns. Our results suggest that nitrate and nitrite treatment may be related to metabolic changes in zebrafish brain that result in behavioral changes associated with anxiety.

Poster Abstract 3

Metabolism of xanthohumol and 8-prenylnaringenin by intestinal microbiota

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Xanthohumol (XN), a flavonoid found in hops (*Humulus lupulus*), exerts mitigating effects on the metabolic syndrome. XN has several pharmacological targets in vitro but appears to be active at comparatively low concentrations in vivo, suggesting the involvement of bioactive metabolites. To fully understand XN's mechanism of action in vivo, it is necessary to gain more information on its fate in the body following ingestion, as a certain proportion of ingested secondary plant constituents undergo colonic microbial transformation. To study the metabolism of XN and related prenylated flavonoids, XN and 8-prenylnaringenin (8PN) were incubated with E. ramulus, a strictly anaerobic bacterium detectable in the gastrointestinal tract of most individuals. Evidence from our study shows that both XN and 8PN are extensively transformed by this gut microbe, producing metabolites with similar pharmacological properties. Moreover, co-cultures with E. limosum, another intestinal bacterium previously reported to be involved in XN gut metabolism prove that E. ramulus can utilize metabolites made readily available by another bacterium. Degradation pathways of XN are proposed based on the intermediates detected by high performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS).

Poster Abstract 4

Characterization and quantification of phytochemical constituents in the medicinal herb *Centella asiatica* by high-resolution mass spectrometry

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In Ayurvedic medicine, *Centella asiatica* (CA) has been used to prevent cognitive decline and for brain function improvement. The results of several studies in humans and rodent models have caused excitement in using CA preparations as a potential complementary medicine to improve memory in aging-related cognitive decline and maybe even Alzheimer's disease. CA has been reported to have also other biological activities beneficial for human health such as gastric ulcers, anti-inflammatory, wound healing, and immunostimulant properties. Geographical, genetic and post-harvest processing all affect the secondary metabolite composition of CA products, potentially influencing their biological effects and study reproducibility. In our laboratory, we favor the use of ultra-performance liquid chromatography (UPLC) in conjunction with accurate mass high-resolution tandem mass spectrometry for structural analysis and quantification, allowing detailed targeted and untargeted characterization of plant extracts.

Aqueous and ethanolic CA extracts were characterized using an ultra-performance liquid chromatograph (UPLC) connected to a Synapt G2 HDMS high-resolution accurate mass spectrometry system. For quantification of phytochemicals known to occur in CA, an AB Sciex Triple TOF 5600 mass spectrometer using a parallel reaction monitoring. The disclosed method allows quantification of a) seven flavonoids, b) three structural isomers of caffeoylquinic acids, c) five di-caffeoylquinic acids, d) six caffeic acids derivatives and e) the major saponins and sapogenins. Recovery experiments were carried out for CA extracts. CA samples were spiked with 24 available standards at two different concentration levels (0.25 ng and 5 ng on column for each compound). Recoveries of individual compounds were in range from 91 to 132 %. Three standard mixtures of known concentrations (low, medium and high) were analyzed. The analytical accuracy was in the range of 87-125 %, confirming the feasibility of the proposed procedure for quantitative analysis of CA samples. Differences were observed in the chemical profiles of the CA extracts, demonstrating that standardization and detailed characterization of CA extracts are pre-requisites to reliably and reproducibly study the biological activity of CA preparations.

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Poster Abstract 5

Profiling of specific metabolic signatures in deuterium-labeled and unlabeled broccoli sprouts with X^{13}CMS

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Cruciferous vegetables (crucifers) and their bioactive components in food, including indoles and isothiocyanates (ITCs) such as sulforaphane (SFN), appear to modulate cancer risk but observational data to date in humans are inconsistent. Such discrepancies arise in part owing to methodological limitations of accurately assessing dietary exposures on breast and prostate cancer risk. Despite widespread use, classical dietary-intake instruments including food frequency questionnaires and other dietary recall methods are subject to well-known limitations. To circumvent and address the unmet current need for reproducible measures of dietary intake and metabolic impacts of crucifers, we used deuterium-labeled broccoli sprouts in combination with mass spectrometry based metabolomics approaches to quantify and differentiate between broccoli-specific metabolites and their interactions with their molecular targets of action.
In methods, broccoli seeds were germinated for 5 days on H2O or 25% deuterium (D2O), refreshed twice per day, and the harvested sprouts were homogenized in methanol (500 µL) using a bullet blender with zirconium oxides beads. The extracts were centrifuged and the supernatants analyzed by UPLC-QToF mass spectrometry on a Sciex 5600 TripleToF instrument. The raw ToF-MS data were converted to mzXML format using MSConvert software. The converted mzXML data were processed with XCMS software for peak detection and retention-time alignment. The XCMS output was subsequently processed with X13CMS software to identify deuterium labeled compounds. XCMS and X13CMS were operated in R software.
We processed the raw LC-QToF MS data by XCMS yielding 1428 spectral features, which were re-analyzed using X13CMS software. This step yielded 152 isotopologue groups representing 152 deuterium-enriched metabolites covering glucosinolates derived from methionine (glucoraphanin) and tryptophan (glucobrassicin, neoglucobrassicin) as well as amino acids. The success of this approach does not depend on the level of deuterium incorporation as long as the enriched isotope pattern is clearly distinguishable from the natural isotope pattern.

Poster Abstract 6

Diet-gut microbiota interactions mediated by hop-derived xanthohumols in a mouse obesity model

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Xanthohumol (XN) is the principal prenylated flavonoid produced by hops (*Humulus lupulus L.*). Recent studies conducted by our group with rodents indicate that XN supplementation in food could treat or mitigate metabolic syndrome and obesity. The low bioavailability of XN and flavonoids in general suggests that at least part of the observed health benefits could come from XN biotransformed metabolites generated by the gut microbiome and the liver. We hypothesize that diet supplementation with XN mitigates gut dysbiosis and that the biotransformation products of XN are partly responsible for the observed health benefits. To test this hypothesis, we developed mass-spectrometry-driven workflows for characterizing XN biotransformation products along with metabolites of interest in biofluids. We collected feces from high-fat diet fed C57BL/6J male mice supplemented or not with 30 mg/kg of XN or the XN derivatives, dihydroxanthohumol (DXN) or tetrahydroxantohumol (TXN). We analyzed fecal extracts using a Synapt G2 HDMS mass spectrometry platform coupled to a Waters Acquity I-class UPLC system. We developed a method for the identification and quantification of XN, DXN and TXN along with their metabolites as well as endogenous metabolites, such as bile acids (BAs). We focused primarily on BAs because they are important cholesterol-derived metabolites involved in multiple physiological processes, including fat absorption. DXN and TXN supplemented mice excreted lower amounts of the parent flavonoid and their metabolites compared to XN, which is in agreement with the higher bioavailability of DXN and TXN. Moreover, we observed an overall increase in conjugated bile salts in feces of mice supplemented with DXN and TXN, but not in feces from XN-treated mice, compared to vehicle-treated animals. These results indicate that DXN and TXN treatment enhances BA excretion at the expense of hepatic cholesterol.

Poster Abstract 7

A novel nutritional formulation containing the prebiotic human milk oligosaccharide 2'-fucosyllactose reduces gastrointestinal symptoms and beneficially alters the gut microbiome in adults with gastrointestinal dysfunction

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Background: Gut dysbiosis, disruption in the homeostasis of the intestinal microbiota, contributes to the pathogenesis of many gastrointestinal disorders. Human milk oligosaccharides (HMOs), which are naturally occurring in human breast milk, are considered "bifidogenic" and "butyrogenic". In breast-fed infants, they serve as primary substrates for select *Bifidobacterium spp.* and are metabolized into butyrate by butyrate-producing gut microbiota. UGIR is a formulation that provides nutritional support for adults with gastrointestinal dysfunction; it contains a combination of essential macro and micronutrients and prebiotics, including 2'-fucosyllactose (2'FL), the most abundantly produced HMO. This study reports novel data on the effect of 2'FL, in the context of a comprehensive nutritional formulation, in adults with gastrointestinal dysfunction.

Methods: Adults with IBS, ulcerative colitis, Crohn's disease or celiac disease were recruited from four U.S. medical practices. Participants received one serving of UGIR twice daily for six weeks. Outcome measures included the Gastrointestinal Quality of Life Index (GIQLI) and a stool analysis panel.

Results: Twelve participants completed the study. GIQLI total score, gastrointestinal symptoms domain, and social function domain scores improved ($P<0.05$). Butyrate, acetate, and total SCFAs increased ($P<0.05$). Several commensal bacteria increased including *Bifidobacterium spp.*, *Bifidobacterium longum*, *Faecalibacterium prausnitzii*, *Aneurotruncus colihominis*, and *Pseudoflavonifractor spp.* ($P<0.05$).

Conclusions: UGIR consumption was associated with reduced gastrointestinal symptoms, increased fecal SCFAs, increases in several beneficial gut microbes (including species that have been shown to consume 2'FL as a substrate in vitro), increases in butyrate-producing species, and increases in species that have been previously shown to be low in patients with IBS and IBD. It is plausible that the improvements in butyrate levels and commensal gut microbiota contributed to the clinical benefits demonstrated on the questionnaire. These results suggest that UGIR is a promising novel nutritional formulation that could be used in the management of gastrointestinal dysfunction associated with gut dysbiosis.

Poster Abstract 8

Supplementation of mice fed a high fat diet with xanthohumol and its derivatives changes the composition of the fecal microbiota and is associated with observed metabolic improvements

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Despite our understanding of the underlying physiology and current prevention and treatment options, obesity still has reached epidemic proportions globally. The prenylated flavonoid xanthohumol (XN) found in hops and beer improves dysfunctional glucose and lipid metabolism in preclinical animal models of diet-induced obesity (DIO) and metabolic syndrome (MetS). We have shown that XN binds to the farnesoid X receptor (FXR) and regulates host genes involved in metabolism of cholesterol into bile acids and the cathelicidin antimicrobial peptide (CAMP) gene expression. In a 14-week study with C57BL/6J mice fed a high-fat diet containing XN, α,β-dihydro-XN (DXN) or tetrahydro-XN (TXN), the treatments resulted in abrogation of neuro-metabolic impairments. We hypothesize that consumption of XN or its hydrogenated derivatives by mice fed a high-fat diet induces CAMP and shapes the composition of the gut microbiota. Mice administered XN, DXN or TXN showed similar improvements of impaired glucose tolerance compared to the control; however, the derivatives decreased plasma insulin and leptin to a greater extent than XN. We sequenced the 16 rRNA genes of the microbes present in the feces of each animal. We observed statistically significant shifts in the structure and membership of the microbiota. The compounds significantly decreased the percentages of *Bacteroidetes* and *Tenernicutes*. In contrast, they significantly increased *Firmicutes*, *Proteobacteria* (DXN and TXN), and *Verrucomicrobia* (DXN and TXN). Specifically, *Oscillospira* (*Firmicutes*), an under-studied anaerobic bacterial genus, increased from 7% to 14% abundance after DXN and TXN supplementation. Moreover, we identified statistically significant correlations with weight gain, food intake, food efficiency, fasting glucose and levels of insulin and leptin with *Oscillospira*. We postulate that *Oscillospira*, along with other defined and undefined genera, may mediate some of the benefits from consumption of XN and its derivatives.

Poster Abstract 9

Hepatic and gut microbial metabolism of the hop hutraceutical, xanthohumol, in humans

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Xanthohumol, a prenylated flavonoid, is a yellow substance isolated from hops (*Humulus lupulus*) and found primarily in beer. Over the past years it has received a lot of attention due to its biological effects. This natural compound efficiently improves dysfunctional glucose and lipid metabolism in animal models of metabolic syndrome and could therefore have health protective actions against type 2 diabetes mellitus and cardiovascular diseases. In order to facilitate clinical studies in the future it is essential to establish a broad view of xanthohumol's metabolism. To determine xanthohumol and its metabolites simultaneously, our group has developed an extraction method using a paper strip for improved analyte detection and quantitation followed by a sensitive and selective high pressure liquid chromatography tandem mass spectrometry method that separates xanthohumol, isoxanthohumol, α,β-dihydroxanthohumol, 6-prenylnaringenin, and 8-prenylnaringenin. The present study focused on xanthohumol and its metabolites found in human urine in a crossover, double-blinded, placebo-controlled human intervention study at three dose levels of xanthohumol (OSU Institutional Review Board approval #6119). Healthy human subjects consumed a non-alcoholic beverage without and with xanthohumol for three weeks, with a washout period of three weeks. Findings show a spontaneous cyclization of xanthohumol into isoxanthohumol, which can be followed by a hepatic or gut microbial demethylation into 8-prenylnaringenin, an estrogenic natural compound. α,β-dihydroxanthohumol and 6-prenylnaringenin were also detectable in urine from subjects taking xanthohumol. The detection of α,β-dihydroxanthohumol, which lacks estrogenicity, suggests that human gut microbiota have the capability to hydrogenate the α,β-unsaturated bond of xanthohumol.

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Poster Abstract 10

Green tea extract improves microbiota composition and intestinal barrier function to prevent dietary fat-induced obesity by limiting gut-derived endotoxin translocation and adipose TLR4/ NFκB inflammation

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Gut-derived endotoxin translocation to the systemic circulation provokes obesity by inducing low-grade inflammation. We hypothesized that the antiinflammatory activities of green tea extract (GTE) would limit endotoxin-mediated inflammatory responses in obese mice by attenuating microbial dysbiosis and gut barrier permeability. C57BL6/J mice were fed a low-fat (LF) or high-fat (HF) diet containing GTE at 0% or 2% for 8 wk prior to assessing microbiota composition, gut permeability, and pro-inflammatory genes. Compared with LF mice, HF mice had increased ($P<0.05$) visceral adiposity, and adipose expression of TLR4/NFκB-dependent genes (TLR4, MD2, CD14, MyD88, TNF, iNOS, MCP1) and macrophage activation genes (F4/80, CD68). GTE in HF-fed mice mitigated adiposity and inflammatory responses. Compared with increased levels in HF controls, GTE attenuated FITC-dextran absorption following its oral gavage in association with lower portal vein endotoxin concentrations. Adipose mass, and TLR4 and TNFα mRNA levels were correlated with serum FITC-dextran and portal vein endotoxin ($P<0.03$; $r = 0.36$-0.62). GTE also increased intestinal microbial diversity and lowered the Firmicutes and Bacteroidetes ratio (F:B) in HF mice only. Intestinal inflammation was lower, and expression of hypoxia inducible factor-1α and tight junction proteins (claudin-1, occludin, zonula occludin-1) were higher in HF mice fed GTE. The F:B also correlated with serum FITC-dextran, portal vein endotoxin, and adiposity ($P<0.01$; $r = 0.40$-0.58). Adiposity and portal vein endotoxin were also inversely correlated with claudin-1 mRNA ($P<0.04$; $r = -0.36$ to -0.48). Collectively, GTE-mediated improvements in microbial dysbiosis and gut barrier function likely limit adiposity by attenuating pro-inflammatory TLR4/NFκB signaling otherwise induced by gut-derived endotoxin translocation.

Poster Abstract 11

Green tea treatment in obese mice with nonalcoholic steatohepatitis lowers hepatic NFκB activation in association with altered relative abundance of phosphatidylcholine and bile acid metabolites

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Green tea extract (GTE) limits NFκB-mediated inflammation during nonalcoholic steatohepatitis (NASH). Our objective was to identify shifts in the global hepatic metabolome associated with GTE-mediated lowering of NFκB activation during NASH. Male C57BL/6J mice were fed a low-fat (LF) or high-fat (HF) diet for 12 wk to induce NASH. They then continued on these diets supplemented with 0 or 2% GTE (n = 10/ group) for an additional 8 wk prior to assessing metabolomics profiles. GTE attenuated histological evidence of NASH (hepatic steatosis, hepatocellular ballooning), hepatic NFκB activation, and lipid peroxidation that were otherwise increased in HF controls. Principal component analysis indicated that GTE in HF-fed mice restored the hepatic metabolome that was otherwise shifted away from LF-fed mice. Compared with HF controls, 129 metabolites were altered (≥2-fold; $P<0.01$) in response to GTE. GTE in HF mice decreased ($P<0.05$) the relative abundance of phosphatidylcholine catabolites (e.g. lysophosphatidylcholine, glycerophosphocholine) that were otherwise increased in HF controls. Compared with HF controls, GTE increased primary and secondary bile acid metabolites (e.g. chenodeoxycholic acid, cholic acid, sulfoglycolithocholate) and decreased hepatic cholesterol to levels not different from LF mice. Phosphorylated p65 and hepatic MDA were correlated with phosphatidylcholine metabolites ($P<0.05$; $r = 0.42$-0.63) whereas bile acid metabolites were inversely associated with hepatic cholesterol and phosphorylated p65 ($P<0.05$; $r = -0.39$ to -0.65). These findings suggest that metabolic shifts by GTE treatment function to lower NFκB activation in NASH by limiting lysophosphatidylcholine-mediated hepatic injury and increasing bile acid metabolites that promote FXR activation.

Poster Abstract 12

Effects of dietary resveratrol on the fecundity of a novel model, *Nothobranchius guentheri*

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The loss of female reproductive ability occurs midway through the lifespan in humans and is an early phenotype for human aging. Yet the number of women delaying pregnancy or postponing decisions about reproduction continues to rise in industrialized societies due to personal or socioeconomic circumstances, often resulting in subfertility or difficulty conceiving. There are few defined mechanisms associated with this etiology and equally few effective therapies. We used a novel emerging model, *Nothobranchius guentheri*, with an age-associated spectrum of changes analogous to that found in human fertility to test a possible solution to this problem. Our hypothesis is that resveratrol (RSV) will activate SirT1, an oxidative stress sensor and longevity assurance enzyme, and thus improve female fecundity in mid-life. RSV, a polyphenol found in grapes and red wine, has been presented both commercially and in studies as an anti-aging dietary supplement due to its ability to prolong both lifespan and health span. SirT1 is an NAD+ dependent histone deacetylase, whose activity is regulated by the nicotinamide to NAD+ salvage pathway, especially by the rate-limiting enzyme NAMPT. We found that female *N. guentheri* fed 600 µg RSV/g food into mid-life (~20 weeks), beginning at sexual maturity, showed increased fecundity compared to those on Control diet. Furthermore, ovarian NAMPT was found at higher concentrations in the RSV-fed fish which we expect leads to greater availability of NAD+ for sirtuin activity. This suggests that dietary RSV has a positive effect on female fertility and that it may become an effective therapy to regulate sirtuin activity and combat reproductive senescence.

Poster Abstract 13

Antiviral activity of scorpion venom peptides

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Arachnid venoms, utilized as a tool for defense and attack, by killing or immobilizing their predators, are composed by rich molecular diversity and complex mixture of proteins and peptides. More than 200 viruses are known to cause human diseases. Considered most common pathologies in humans, cardiovascular and infectious diseases and cancer are among the leading causes of deaths. The very few antiviral drugs commercially available can induce severe and considerable adverse effects, especially to those patients receiving lifelong treatment for diseases such as HIV. Furthermore, viruses possess rapid mutational capacity to trick and infect host cells. All these facts together have propelled the prospection for new antiviral drugs, particularly from natural products, as they constitute more than 25% of the new drug prototypes approved in the last decades. Among sources of natural products, scorpion venoms have revealed a great potential for drug discovery, and despite their harmful action mechanism most of them have components holding potential medicinal properties to cure diseases. It is widely reported that scorpion venoms are rich sources of antimicrobial substances, and contain a vast array of active biological compounds with distinct chemical structures. Scorpion venom is a complex mixture of hundreds of molecules, mostly peptides, which possesses a large array of biological activities betwixt: antiviral activity. In addition in scorpions the biologically active peptides are classified as disulfide-bridged peptides (DBPs) and non-disulfide-bridged peptides (NDBPs), with the former being the main components of scorpion venoms, responsible for the neurotoxic effects. Usually these DBPs target the ion channels of excitable and non-excitable cell membranes. These properties make these molecules interesting prototypes of drugs for the treatment of diverse diseases, particularly those affecting the neural system. In conclusion scorpion peptides exhibit direct virucidal activity. Because of the limited efficiency of commonly used drugs and emerging resistance of viruses, antiviral scorpion peptides may have the potential as putative therapeutic agents with antiviral activities.

Poster Abstract 14

To whom the side-effect of licorice, pseudoaldosteronism, will occur: A case-control study

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Background: Pseudoaldosteronism is a well-known side effect of licorice or glycyrrhizin (GL), one of major components in licorice. Pseudoaldosteronism mimics primary aldosteronism which causes hypokalemia, hypertension, and extremities' edema. GL can be used to treat chronic hepatitis, but also plays important role in pseudoaldosteronism. The importance of multidrug resistance protein 2 (Mrp2) in GL excretion has been reported recently. Dysfunction of Mrp2 causes elevated direct bilirubin (DB), GL, and its metabolites. Hence, elevated DB can be a risk predictor of pseudoaldosteronism. The relationship between pseudoaldosteronism and elevated DB, however, has not been studied as yet.

Objectives: To evaluate the relationship between elevated DB and hypokalemia, the most sensitive marker of pseudoaldosteronism.

Methods: This case-control study included patients with chronic hepatitis who visited the division of hepatology at Keio University Hospital between January 2009 and December 2015. Inclusion criterion was availability of laboratory data on DB and change in serum potassium.

Results: Data from 1392 patients (796 men) were used in the analysis, and the most common cause of chronic hepatitis was hepatitis type C virus infection. GL was used for 79 patients (GL+) and wasn't for 1313 (GL-). Mean age was 60.5 ± 14.2 years for GL+, and 58.3 ± 15.8 years for GL-. Hypokalemia tended to be noted more in GL+ patients (OR 1.69, 95%CI : 0.99-2.85, p=0.06). When we divided patients with or without elevated DB, hypokalemia was noted more in GL+ patients with elevated DB (OR 2.89, 95%CI : 1.15-8.00, p=0.02), but not in GL+ patients without elevated DB (OR 0.84, 95% CI : 0.31-2.20, p=1). From multi-variable logistic regression in GL+ patients, elevated DB and lower serum albumin level were related to hypokalemia, but age, sex, daily dose of GL, and duration of GL intake were not.

Conclusions: Elevated DB might be a predictor of pseudoaldosteronism due to GL.

Healthy Diets

Poster Abstracts 20-29

Poster Abstract 20

Optimizing gut flora for optimum health and disease prevention

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The gastrointestinal tract is considered one of the most complex microbial ecosystems on earth and the gut is the body site that is most densely populated with microbes which are known to affect digestion. By understanding the way foods interact with living organisms, it is certain to create diets that help people with disease control, as well as their health overall. The entire intestinal microflora is part of the immune system and about 80 percent of it originates in the gut. Moreover, studies have found that microbes of all kinds play instrumental roles in the functioning of the body. Research advances in the field of gastrointestinal health have noted that advanced DNA sequencing is now being used to shed light on the complex interactions of gut bacteria, and how such interactions affect health and the development of disease. This paper focuses on dietary patterns that provide positive control of gut microbiome that are associated with greater protective species and more health benefits. Fortunately, even if the beneficial bacteria are depleted, there are ways to increase them and help balance the bacteria in the digestive system. There are live microorganisms, probiotics, which, when consumed in adequate amounts, provide a health benefit while the prebiotics, including many dietary fibers, are food for the gut bacteria that stimulate the growth or activity of the intestinal bacteria. The current consensus is that gut bacteria have metabolic consequences for the host, with the understanding of what constitutes a healthy microbiota and how changes in its structure correlate with and affect health and disease.

Poster Abstract 21

Eat more plants! A community-based approach to early nutrition education

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Purpose: This cooking course was designed to teach children how to make tasty, nourishing food and foster an early love of healthy cooking.

Background: Childhood obesity is a growing nationwide epidemic (a). The standard American diet contains high amounts of fat, sodium, and sugar with deficiencies in fiber and vitamins. Numerous studies have shown that healthy eating can prevent and even reverse disease (b). Teaching children healthy cooking skills is a building block in the effort to prevent disease among families and decrease the burden of chronic disease.

Methods: A curriculum was designed using various online resources. The curriculum was designed for elementary students to participate in five one-hour classes over the course of one week at the Boys and Girls Club in Corvallis, OR. Each day highlighted different nutrition principles. The course also taught handwashing, knife safety, and oven safety. Approximately 40 minutes was used to prepare the food, and 20 minutes were used for discussions and educational activities. A survey was administered both before and after the class to assess changes in knowledge, behaviors, and attitudes about cooking and healthy foods.

Results: All the children who volunteered for the class already enjoyed cooking and believed that healthy eating is important. Although the number surveyed (5) was too small to complete meaningful statistical analysis, surveys showed an increased ability to identify plant-based foods and processed foods after the class was complete. Fruit consumption, veggie consumption, hand washing before cooking, and cooking at home also increased throughout the class. 4/5 of the children enjoyed the class and would take it again if offered.

Conclusion: Further research with a larger sample size is needed. However, surveys showed an increase in healthy eating behaviors such as eating at least one fruit and one veggie per day, helping with home cooking, and washing hands before cooking. The overall response to the class was positive, and it provided a fun and engaging environment for children to build their love of cooking and healthy foods.

Poster Abstract 22

Rural kid's cooking camp emphasizing a plant-based diet survey study

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Purpose: This week-long, three hours per day, cooking camp for kid's ages 7-13, emphasized a plant-based diet with nutrition and cooking lessons. The purpose was to increase participants' nutritional knowledge and their motivation to cook plant-based meals.

Background: Obesity has become an epidemic in the US. Plant-based diets (high in fruits, vegetables, whole grains, and legumes) have been associated with preventing and treating chronic diseases, including obesity. (a) Lack of nutritional knowledge and/or cooking skills are factors that can contribute to unhealthy dietary choices, resulting in obesity. (b) Community-based programs that offer culinary and nutrition instruction have been implicated as promising approaches to addressing unhealthy dietary habits of children. (b.c)

Methods: A curriculum was designed based on online nutrition resources and Lifestyle Medicine literature. Each day, the participants cooked various plant-based foods, attended nutrition lessons, and played games. The pre-camp survey, designed to examine knowledge and motivation for healthy eating and cooking, was offered at the beginning of the camp; 32 participants completed it. There were 27 participants who completed the post-camp survey on the last day.

Results: Chi-square analysis shows that five out of the thirteen questions significantly increased in favor of plant-based nutrition. The greatest improvements in nutritional knowledge pertained to the questions about meat and cow's milk, which increased by 63% and 50% respectively. Overall, interest in a plant-based diet increased by 33% (from 29% interested pre-camp to 62% interested post-camp.)

Conclusion: The participants of our study were enthusiastic and enjoyed the experience. The survey analysis shows increased nutritional knowledge and increased interest in plant-based diets. Culinary medicine provides a unique avenue to promote a plant-based diet for the purpose of preventing obesity and other chronic diseases. Offering cooking camps emphasizing plant-based diets, with nutrition lessons can be a successful mechanism of culinary medicine.

Poster Abstract 23

Assessment of gut microbiome role in diet-related changes in cognition

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Western diets can influence behavior and gut microbiome due to the excessive intake of high fat and sucrose. Altering the microbiome can also influence the brain and behavior. The hypothesis that was tested was that diet-induced changes in the microbiome cause changes in cognitive abilities. A previous study showed learning and cognitive flexibility deficits in mice fed a high sucrose diet. The present study was designed to investigate whether altering the microbiome, via antibiotic treatment, would change the behavioral results when animals were on a high sucrose diet. Eight week old, male mice were randomly assigned to either high-sucrose (12% Kcal fat, 18% protein, 70% CHO (primarily sucrose)) or control defined (13% Kcal fat, 25% protein, 62% CHO) diets and either water or a combination of 4 antibiotics (vancomycin, neomycin, metronidazole and ampicillin) in the water. The animals were tested during the study for memory, anxiety, impulsiveness, and cognitive flexibility. Step-down latency, novel object recognition and marble burying tasks were performed both pre- and 7 weeks post-diet change. The Morris water maze, which tested for long and short-term memory and cognitive flexibility, was conducted during week 8 post-diet change. We found a significant effect of the antibiotic treatment on long term memory. The mice on antibiotics performed better than those on water treatments, suggesting that animals with reduced gut bacteria were learning better than those with only water treatment. There were no effects of diet in this study, which may be due to the use of a defined control diet, rather than the chow used in the previous study. These results suggest that the microbiome does play a role in learning.

Poster Abstract 24

The cathelicidin antimicrobial peptide gene alters the gut microbiota of mice

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The gut microbiome influences human disease including Type 2 diabetes, obesity and metabolic syndrome and interacts with the innate immune system. The human cathelicidin antimicrobial peptide (hCAMP), an important component of the innate immune system, and vitamin D and xanthohumol, a natural compound found in beer hops, regulate its expression. The overall hypothesis of current research in the Gombart lab is that consumption of these micronutrients regulates expression of hCAMP in the gut and that this changes the composition of the bacteria in the gut. These changes may lead to reduced obesity while consuming a high fat diet due to changes in the metabolites produced by the bacteria that affect the metabolism of the host. The working hypothesis for this project is that the composition of the microbiota is different between the wild type (WT) C57BL/6J , humanized CAMP/wild type mice (Tg/WT), Camp knockout mice (KO) and humanized CAMP/Camp knockout mice (Tg/KO). 16s rRNA sequencing data were aligned to the Greengenes 13.8 16s rRNA database using QIIME 1.9 from which a high quality phylogenetic tree was created using FastTree 2.1.7. The phylogenetic distance between groups was measured using the weighted and unweighted versions UniFrac metric and alpha diversity was measured through the phylogenetic diversity metric. Permutational ANOVA showed significant differences in the composition of the microbiota between the different CAMP genotypes. Additionally, KO mice showed significantly lower measures of phylogenetic diversity than other CAMP genotypes. Several bacterial species and genera that varied significantly between genotypes were identified.

Poster Abstract 25

Dairy milk, regardless of fat content, protects against postprandial hyperglycemia-mediated oxidative stress, dysregulated arginine metabolism and increases in endothelin-1 in prediabetic adults

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Although epidemiological studies are equivocal regarding full-fat dairy consumption increasing cardiovascular disease (CVD) risk, controlled studies indicate that dairy milk, regardless of fat content, attenuates postprandial hyperglycemia (PPH), a predictor of CVD-related mortality. We hypothesized that dairy milk, independent of fat content, would protect against PPH-mediated oxidative stress that decreases nitric oxide (NO•) bioavailability by attenuating dysregulated arginine (ARG) metabolism and increases in endothelin-1. In a randomized, cross-over trial, prediabetic adults (n = 23) ingested iso-volumetric beverages (473 mL) of 75 g glucose alone (GLU) or with non-fat milk (NFM) or full-fat milk (FFM). Plasma glucose, malondialdehyde, ARG and metabolites [asymmetric dimethylarginine (ADMA); symmetric dimethylarginine (SDMA), homoarginine (hARG)], endothelin-1, and NO• metabolites (NOx) were measured at 30 min intervals for 180 min. Compared with the GLU trial, the NFM and FFM trials had similarly attenuated increases in glucose at 60-90 min, malondialdehyde at 30-60 min, and endothelin-1 at 30-60 min and 120 min ($P<0.05$). GlucoseAUC, malondialdehydeAUC, and endothelin-1AUC were lower in NFM and FFM compared with GLU. GlucoseAUC, malondialdehydeAUC, and endothelin-1AUC were positively correlated ($r = 0.34$-0.38, $P<0.05$). NFM and FFM trials, compared with GLU, also had similarly attenuated decreases in ARG at 30-120 min and 180 min, and NOx at 30 min and 90-180 min, and increases in ADMA/ARG, SDMA/ARG, hARG/ARG at 60-180 min. Compared with GLU, ADMA/ARGAUC, SDMA/ ARGAUC, and hARG/ARGAUC were lower, and NOxAUC higher, in NFM and FFM. GlucoseAUC and malondialdehydeAUC correlated with ADMA/ARGAUC, SDMA/ARGAUC, and hARG/ARGAUC ($r = 0.34$-0.46, $P<0.05$). Collectively, despite recommendations derived from epidemiological studies to limit full-fat dairy consumption, these findings support dairy milk, regardless of its fat content, to lower CVD risk by attenuating PPH-mediated oxidative stress responses that limit NO• bioavailability likely by competitively inhibiting NO• synthase and the availability and uptake of ARG, and its binding to NO• synthase.

Healthspan and Aging

Poster Abstracts 30-39

Poster Abstract 30

Age-related loss of hepatic Nrf2 protein homeostasis is attributed to heightened expression of miR-146a

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Nrf2 regulates the expression of numerous anti-oxidant, anti-inflammatory, and metabolic genes. Paradoxically, we observed that Nrf2 protein levels decline in the livers of aged rats despite the inflammatory environment evident in that organ. To investigate the cause(s) of this loss, we examined the age-related changes in Nrf2 protein homeostasis and activation in cultured hepatocytes from young (4-6 months) and old (24-28 months) Fischer 344 rats. While no age-dependent change in Nrf2 mRNA levels was observed ($p>0.05$), Nrf2 protein content, as well as the basal and inducible expression of Nrf2-dependent genes were attenuated with age. Conversely, overexpression of Nrf2 in cells from old animals reinstated gene induction. Treatment with Nrf2-inducer, anetholetrithione (A3T), along with bortezomib to inhibit degradation of existing protein, caused Nrf2 to accumulate significantly in cells from young animals ($p<0.05$), but not old. This indicated a lack of new Nrf2 synthesis. We hypothesized that the loss of synthesis with age may partly stem from an increase in microRNA inhibition of Nrf2 translation. Microarray analysis revealed that six microRNAs significantly increased with age (>2-fold, $p<0.05$). One of these, miRNA-146a, is predicted to bind with high complementarity to Nrf2 mRNA. Transfection of hepatocytes from young rats with a miRNA-146a mimic caused a 55% attenuation of Nrf2 translation ($p<0.05$) that paralleled the age-related loss of Nrf2. Overall, these results provide novel insights for the age-related decline in Nrf2 and identify new targets to maintain Nrf2-dependent detoxification with age.

Poster Abstract 31

The role of proteostasis in protein aggregation in long-lived species

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Research over the past decade supports the hypothesis that proteotoxicity, the accumulation of misfolded proteins and aberrant protein aggregates, plays a major role in aging and age-related diseases. This view is based on observations such as: 1) mechanisms that maintain protein structure (proteostasis) decline with age in several animal models and in senescent human fibroblasts, 2) increasing proteostasis leads to a decrease in proteotoxicity and an increased lifespan and healthspan in *C. elegans.* However, almost all of the data on the relationship between longevity and proteotoxicity comes from studies done in short-lived animals, e.g., primarily *C. elegans* and mice. It is not clear that these observations will translate to species with a long lifespan, such as humans, and because of the difference in time scales, it is possible that the mechanisms used by long-lived species to increase lifespan might differ from mechanisms used by invertebrates or even mice. In this work we are using a comparative biology approach to determine whether enhanced proteostasis reduce proteotoxicity in long-livedspecies. Our previous data showed that fibroblasts from long-lived species have higher activity of proteostatic mechanisms when compared to short-lived ones. This led us to investigate the handling of proteotoxicity and protein aggregation by these species. Based on our data we hypothesized that the enhanced proteostasis mechanisms in long-lived species will confer more protection against toxic misfolded proteins. To test our hypothesis we used a fluorescence based aggregation model (polyQ82-YFP) in skin fibroblasts from long-lived and short-lived species.

The aims of this study are to: 1) Determine if naked mole rat (NMR) fibroblasts handle proteotoxicity induced by polyQ82-YFP better than mouse fibroblasts, and 2) Delineate the mechanism(s) through which NMR fibroblasts manage polyQ82-YFP induced proteotoxicity.

Poster Abstract 32

Dietary restriction improves cognitive performance and maintains hepatic Sirt7 levels in *Nothobranchius guentheri*, a short-lived model of aging

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Human life expectancy has grown over the last century, but the number of years lived relatively healthfully and independently with minimal medical needs, or health span, has not kept pace. This is detrimental not only for the individual, but it places additional strain on an already overburdened health care system. It is essential that we identify strategies for improving human health span. One such strategy, evaluated in laboratory animals, is dietary restriction (DR) of 20-40% of caloric intake. DR is known to lengthen lifespan and delay age-associated disease onset. This strategy is difficult to test in humans due to compliance, and challenging to test in many model species because evaluating age-related effects over the lifetime is resource and labor intensive. To overcome this hurdle, we have developed a novel animal model, *Nothobranchius guentheri*, a short-lived (< 1 year) fish that experiences rapid aging and can withstand up to 50% DR with no ill effects. Using male *N. guentheri*, we evaluated the ability of DR to prevent age-related decline in cognitive function. Our results demonstrated that fish whose diet was restricted by 50% performed better in an active avoidance test, and middle aged DR fish showed no decline in performance versus young control fish. We also measured hepatic levels of the nuclear sirtuins, histone deacetylases that regulate the cellular response to nutrient availability. Loss of sirtuin activity is strongly correlated with aging. We found that the expression of SirT7 protein, which is mainly nucleolar, was preserved by DR from early to middle age. SirT7 plays a role in maintaining the rate of protein synthesis, a process that declines with age. We have thus identified a potential DR-regulated molecular target for improving health span.

Poster Abstract 33

Long-term supplementation with a novel nutrient blend mimics calorie restriction transcriptomics in multiple tissues of old mice

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Identification of caloric restriction mimetics (CRMs), compounds that mimic the beneficial effects of caloric restriction (CR) without restriction of dietary energy would be an advancement in anti-aging science. The present study investigated whether the transcriptomic profiles of a putative CRM nutrient blend could mimic that of CR in diverse tissues following long-term feeding. B6C3F1 male mice; n=7 per group. Young Controls (YC; 5 months) and 3 groups treated from 14-30 months of age: Old Controls (OC), Old CR (OCR; 25% CR) and Old Supplemented (OS). Gene expression profiling in cerebral cortex tissue (CCT), skeletal muscle (gastrocnemius) (SKL), heart (HRT) and liver (LVR) was performed using Affymetrix Mouse 2.0ST arrays. Principal component analysis revealed that gene expression profiles of YC and OC were distinct from one another in all tissues. Using differential analysis, genes commonly expressed in OCR and OS groups compared to the OC group were identified in CCT (3,468), SKL (2,386), HRT (3,523) and LVR (1,276). The OS mimicked OCR transcriptomics most dramatically in tissues most relevant to aging and age-associated diseases, CCT, HRT and SKL. These CRM effects, elicited by a mid-life intervention, may have positive implications for healthy human aging or 'youthspan' and warrants further investigation.

Poster Abstract 34

Rapamycin inhibits the secretory phenotype of senescent cells by an Nrf2-independent mechanism

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Senescent cells contribute to age-related pathology and loss of function, and their selective removal improves physiological function and extends longevity. Rapamycin, an inhibitor of mTOR, inhibits cell senescence in vitro and increases longevity in several species. Nrf2 levels have been shown to decrease with aging and silencing Nrf2 gene induces premature senescence. Therefore, we explored whether Nrf2 is involved in the mechanism by which rapamycin delays cell senescence. In wild-type (WT) mouse fibroblasts, rapamycin increased the levels of Nrf2, and this correlates with the activation of autophagy and a reduction in the induction of cell senescence, as measured by SA-β-galactosidase (β-gal) staining, senescence-associated secretory phenotype (SASP), and p16 and p21 molecular markers. In Nrf2KO fibroblasts, however, rapamycin still decreased β-gal staining and the SASP, but rapamycin did not activate the autophagy pathway or decrease p16 and p21 levels. These observations were further confirmed in vivo using Nrf2KO mice, where rapamycin treatment led to a decrease in β-gal staining and pro-inflammatory cytokines in serum and fat tissue; however, p16 levels were not significantly decreased in fat tissue. Consistent with literature demonstrating that the Stat3 pathway is linked to the production of SASP, we found that rapamycin decreased activation of the Stat3 pathway in cells or tissue samples from both WT and Nrf2KO mice. Our data thus suggest that cell senescence is a complex process that involves at least two arms, and rapamycin uses Nrf2 to regulate cell cycle arrest, but not the production of SASP.

Poster Abstract 35

Safe and proven daily actions to improve your healthspan

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There is vast confusion and much disagreement about nutrition and human aging research in the American people. Within the community of geroscientists, however, there is optimism about some new discoveries. This poster documents disagreements even among LPI attendees and proposes possible resolutions.

All decisions in this presentation are based on peer-reviewed research supporting choices that are likely to extend most people’s healthspan.

Choices for diet, supplements, and various activities are listed. Some of these are organized into a morning and evening routine. There are also a few recipes. The objective is to promote awareness and discussion of choices to improve your own and others’ healthspans.

Comments are invited to: ordman@beloit.edu

Poster Abstract 36

Cellular Senescence Contributes to Hepatic "Inflamm-aging"

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Our lab has recently shown that old (24 months) Fischer 344 (F344) rat livers have increased expression of genes related to inflammation and tissue remodeling. These results indicate that livers from old animals are in a state of chronic inflammation, which increases the risk of hepatocarcinogenesis and metabolic dysfunction. The exact cause(s) of this inflammatory phenotype are unknown, other tissues, such as skin fibroblasts, are known to accumulate senescent cells. Senescent cells are identified by cell cycle arrest, lysosome expansion, and the secretion of pro-inflammatory factors (senescence-associated secretory phenotype or SASP) that ultimately result in the perpetuation of senescence. As of now, there is minimal evidence that the liver, a regenerative organ, accumulates senescent cells with age. Thus, the purpose of this study is to examine the amount of cellular senescence in the aging rat liver to gain an understanding of its contribution towards the pro-inflammatory phenotype that our previous work has shown.

Livers were harvested from young (3-6 month) and old (24-27 month) F344 rats throughout the study. Histological analysis of immune cell infiltration was used to assess inflammation and tissue remodeling. Using hemotoxylin and eosin staining, histological analysis revealed that livers from old rats had increased bile duct hyperplasia and extensive fibrosis in comparison to young animals. In addition, livers from old animals showed increased immune cell infiltration as assessed by CD45 staining (young = 37.50 ± 5.500; old = 137.500 ± 11.50). Additionally, immune cell infiltration appeared to be highest near bile ducts in old animals.

Furthermore, there was an age-related increase in staining for beta-galactosidase, an established marker of cellular senescence. While it is not clear which cells express increased levels of beta-galactosidase in cryo-sectioned tissue, isolated liver parenchymal cells (i.e. hepatocytes) show increased expression of beta-galactosidase. Hepatocytes are integral in detoxification, metabolism of nutrients, and bile acid secretion. Thus, these results suggest that cellular senescence may play a role in the compromised liver function observed in older animals.

To quantify the SASP observed in the aging rat liver, whole liver tissue was used for qPCR analysis. Our results show that interleukin-6, an established marker of general inflammation and the SASP, increases over 3-fold with age. Using the ratio of interleukin-6 to interleukin-10, known pro-inflammatory and anti-inflammatory interleukins, respectively, there is a shift towards a pro-inflammatory state. A marker of more advanced cellular senescence, microRNA-146a, increases 2.70-fold in old liver tissue. Additionally, we have found that there is increased secretion of microRNA-146a in exosomes from hepatocytes in old animals, indicating that hepato-senescence may contribute to systemic inflammation in old animals. Overall, our work shows that the aging rat liver has a pro-inflammatory phenotype characteristic of "inflamm-aging", and we have preliminary evidence that cellular senescence possibly plays a key role in its cause.

Vitamins and Minerals

Poster Abstracts 40-49

Poster Abstract 40

Adverse effects of arsenic exposure and zinc deficiency on pancreatic beta cells and insulin production

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Insulin is a vital hormone secreted by beta cells in the pancreas to lower glucose levels in the blood. Zinc is an essential micronutrient that plays an important role in insulin storage, signaling, and secretion. Arsenic is an environmental toxicant that millions of people are chronically exposed to, and this exposure can co-exist with zinc deficiency. Zinc deficiency and inorganic arsenic exposure have both been independently associated with an increased risk of developing diabetes, but it is not known whether they interact to promote diabetes. Here, we tested the hypothesis that arsenic can deplete zinc levels in pancreatic beta cells, and co-exposure to arsenic and zinc deficiency alters insulin regulation and production. Rat pancreatic beta cells were cultured in zinc adequate or zinc deficient media and exposed to arsenic at 0, 50, or 500 ppb concentrations. The combination of zinc deficiency and arsenic exposure decreased cell proliferation and increased cell death more than either zinc deficiency or arsenic exposure alone. Arsenic dose-dependently altered the expression of the transcription factors (Neurod1, Pdx1) that regulate insulin production at the mRNA level, while zinc deficiency did not significantly affect their expression. There were no significant effects of zinc deficiency, or arsenic exposure, on the mRNA levels of the pancreatic-specific zinc transporter Znt8. Arsenic significantly downregulated Glut2 mRNA levels at the 500 ppb dose. Arsenic also decreased insulin levels secreted by the beta cells, and suppressed the expression of the gene that produces insulin (Ins1) at the 500 ppb dose. In contrast, zinc deficiency upregulated expression of Ins1, and increased insulin release. This project has enhanced the understanding of the relationships between zinc, arsenic, and insulin and further investigation of this topic can influence the prevention and/or treatment of diabetes.

Poster Abstract 41

Adverse consequences of zinc deficiency and arsenic exposure to zebrafish development

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Zinc deficiency and chronic low level exposures to inorganic arsenic in drinking water are both significant public health concerns that affect millions of people including pregnant women. These two conditions often co-exist in the human population, but little is known about their interaction, and in particular whether zinc deficiency sensitizes individuals to arsenic exposure and toxicity, especially during critical windows of development. To address this, we utilized the *Danio rerio* (zebrafish) model to test the hypothesis that parental zinc deficiency sensitizes the developing embryo to low-concentration arsenic toxicity, leading to altered developmental outcomes. Adult zebrafish were fed a defined diet that contained either 33.8 ug/g zinc (zinc adequate) or 14.5 ug/g zinc (zinc deficient) and subsequently spawned to produce zinc adequate or zinc deficient embryos respectively. The zinc adequate and zinc deficient embryos were then treated with environmentally relevant concentrations of 0, 50, and 500 ppb arsenic. Arsenic exposure significantly reduced the amount of zinc in the developing embryo by ~7%. The combination of zinc deficiency and low-level arsenic exposures did not sensitize the developing embryo to increased developmental malformations or mortality. The combination did cause a 40% decline in physical activity of the embryos, and this decline was significantly greater than what was observed with zinc deficiency or arsenic exposure alone. Significant changes in RNA expression of genes that regulate zinc homeostasis, response to oxidative stress and insulin production (including zip1, znt7, nrf2, ogg1, pax4, and insa) were found in zinc deficient, or zinc deficiency and arsenic exposed embryos. Overall the data suggests that the combination of zinc deficiency and arsenic exposure has harmful effects on the developing embryo and may increase the risk for developing chronic diseases like diabetes.

Poster Abstract 42

Interactions between arsenic exposure and zinc deficiency on zinc status, oxidative stress, and inflammatory response

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Zinc is an essential micronutrient important in many biological processes. Zinc has antioxidant and anti-inflammatory properties, and zinc deficiency results in increased oxidative stress and inflammatory response. It is estimated that 12% of Americans do not consume adequate zinc, and are at risk for marginal zinc deficiency. Arsenic is a naturally occurring element found in the environment. Arsenic contamination is a major public health concern in the United States and worldwide. Arsenic exposure shares many hallmarks of zinc deficiency, including increased inflammation and oxidative stress, and both can contribute to the development of chronic diseases including cardiovascular disease, diabetes, and cancer. Notably, populations at risk for arsenic exposure also have co-existing signs of zinc deficiency. To date, very little is known regarding the interactions between zinc status and arsenic exposure, and whether zinc status impacts the susceptibility of arsenic toxicity and vice versa. The goal of this study was to examine the combined effects of zinc deficiency and arsenic exposure on oxidative stress and inflammatory response. Our hypothesis was that zinc deficiency sensitizes cells to arsenic-induced toxicity by amplifying oxidative stress and inflammatory response. In cell culture, zinc deficient THP1 monocytes had reduced intracellular zinc and increased proinflammatory (IL8) and oxidative stress (HMOX-1) response. Exposure to arsenic (0.1-10uM) resulted in further reduction in intracellular zinc, and enhanced IL8 and HMOX-1 expression. In animal models, marginally zinc deficient mice had further decrease in zinc status when exposed to arsenic (50-500ppb) in the drinking water. Zinc deficient mice had increased baseline expression of inflammatory markers in the liver. Upon LPS challenge to elicit an acute inflammatory response, arsenic-exposed mice had a further increase in proinflammatory response. Our data suggest that arsenic exposure interacts with zinc deficiency to further reduce overall zinc status, and amplified oxidative stress and proinflammatory response.

Poster Abstract 43

Ascorbate deficiency and mitochondrial dysfunction in the APP/PSEN1 mouse models of Alzheimer's disease

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Mitochondrial dysfunction is elevated in very early stages of Alzheimer's disease and exacerbates oxidative stress, which contributes to disease pathology. We show that ascorbate is critical in supporting mitochondrial function, including oxygen consumption and energy production. Mitochondria were isolated from 4-month-old wild-type mice, transgenic mice carrying the APPSWE and PSEN1dE9 mutations, mice with decreased brain and mitochondrial ascorbate (vitamin C) via heterozygous knockout of the sodium dependent vitamin C transporter, type 2 (SVCT2+/-) and the combined mutant (SVCT2+/-;APP/PSEN1). In closed high-resolution respirometry chambers, mitochondrial isolates from SVCT2+/- mice consumed less oxygen and exhibited decreased mitochondrial membrane potential compared to wild-type isolates. Conversely, isolates from APP/PSEN1 mice consumed more oxygen, and exhibited an increase in mitochondrial membrane potential, but had a significantly lower ATP/ADP ratio compared to wild type isolates. Greater levels of reactive oxygen species were produced in mitochondria isolated from both APP/PSEN1 and SVCT2+/- mice compared to wild-type isolates. Acute administration of ascorbate to isolated wild-type mitochondria increased oxygen consumption compared with untreated mitochondria suggesting ascorbate may support energy production. Additionally, mitochondria isolated from mice with SVCT2 over-expression (SVCT2Tg) show a decrease in reactive oxygen species with no change in mitochondrial function compared to wild type isolates. This study provides further evidence that mitochondrial dysfunction can occur at an early, prodromal stage of Alzheimer's disease, prior to the presence of amyloid β aggregates and cognitive deficits, and that ascorbate deficiency leaves mitochondria vulnerable to oxidative damage, thereby contributing to Alzheimer's disease progression. Adequate ascorbate intake is an effective and inexpensive mitochondrial-targeted antioxidant that supports brain health. It can provide a potent preventative strategy against neurodegenerative disease, particularly in the populations most at risk for Alzheimer's disease in which stores are often depleted through mitochondrial dysfunction and elevated oxidative stress.

Poster Abstract 44

Local sustained delivery of 1α,25(OH)2D3 by nanofiber wound dressings induces human cathelicidin antimicrobial peptide expression both *in vitro* and i*n vivo*

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Multi-drug resistant bacteria are a major challenge for treatment of surgical site and traumatic wound infections. Human cathelicidin antimicrobial peptide (CAMP) is critical for skin barrier defense, wound healing and can effectively combat drug resistant bacterial infections. Recently, we demonstrated that 25(OH)D_3 encapsulated in poly(e-caprolactone) (PCL) nanofibers was released in a sustained manner and induced CAMP gene expression in keratinocyte and monocyte cell lines for up to five days in vitro. We propose using this novel strategy to achieve effective and constant local delivery of 1α,25$(OH)_2D_3$ to enhance wound healing and minimize the potential risk of infections by augmenting innate immune responses. Treatment of human myeloid leukemia cell line U937 with 1α,25$(OH)_2D_3$ loaded PCL (PCL-D) fibers significantly induced sustained cathelicidin mRNA and protein expression for five days. In vivo studies with a human CAMP transgenic mouse model showed a statistically significant 1.5-fold increase in human CAMP mRNA expression after three days in the wounds containing PCL-D fibers versus PCL only. Furthermore, increased expression of Cyp24a1 was observed in the kidney of PCL-D treated animals indicating systemic release of 1α,25$(OH)_2D_3$ from the PCL nanofibers. Also, PCL-D induced CAMP protein expression in human skin explants in vitro. In conclusion, 1α,25$(OH)_2D_3$ loaded PCL nanofiber dressings significantly induced both CAMP mRNA and protein levels in cell lines, a transgenic mouse model and in human skin. These preliminary findings suggest that we could enhance innate immunity by inducing antimicrobial peptide production and possibly mitigate the selection for multidrug resistance and improve wound healing. Future work will focus on developing these biocompatible and biodegradable wound dressings for this purpose using the model systems described above. Furthermore, we will identify the molecular mechanisms by which vitamin D improves barrier defenses and enhances wound healing.

Poster Abstract 45

Chronic vitamin E deficiency in adult zebrafish dysregulates brain lipids, energy metabolism, and cognitive function

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Zebrafish (*Danio rerio*) are a recognized model for studying consequences of increased oxidative stress within the brain. The lipophilic antioxidant vitamin E (α-tocopherol; VitE) has an established role in neurological health and cognitive function, but the mechanisms involved remain unknown. We hypothesized that VitE deficient zebrafish would display cognitive impairments associated with elevated lipid peroxidation and metabolic disruptions in the brain. In the present study, we investigated behavioral perturbations due to chronic VitE deficiency in adult zebrafish fed from 45 days to 18-months of age diets that were either VitE-deficient (E–) or VitE-sufficient (E+). Quantified α-tocopherol concentrations at 18-months in E– brains (5.7 ± 0.1 nmol/g tissue) were ~20-times lower than in E+ (122.8 ± 1.1; *n*= 10/group). We found that chronic VitE deficiency impairs both associative (avoidance conditioning) and non-associative (habituation) learning in adult zebrafish. Further, inadequate brain VitE led to altered brain PL and lysoPL compositions, and perturbed choline and methyl-donor metabolites. The depletion of choline and the dysregulation of PC composition suggests that methyl donor availability became limiting as the animal attempted to correct the loss of phosphatidyl choline with docosahexanoic acid (DHA-PC). VitE deficiency also resulted in decreases in brain metabolite concentrations in the glycolytic pathway, the pentose phosphate pathway and the citric acid cycle; these outcomes suggest that the energy demand increased in order to repair the damage caused by increased lipid peroxidation and thus altered steady-state energy metabolism. Increases in ß-hydroxybutyrate and ketogenic substrates further emphasize that the ketone availability is increased in the E– brain. Taken together, our findings suggest that VitE plays a major role in preventing the dysregulation of brain energy metabolism by protecting lipids from increased peroxidation, and that its deficiency induces a variety of metabolic alterations that have largely been under appreciated. Although VitE deficiency leads to major alterations in lipid and energy metabolites, apparently resulting from metabolic responses that allow the animal to function in the face of increased lipid peroxidation and oxidative damage, these changes are insufficient to prevent major deficits in cognitive function in the aging fish.

Poster Abstract 46

Optimizing vitamin C impact in treating infection

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Vitamin C has been documented in the scientific literature to be a powerful therapy for resolving and helping to resolve a wide variety of infections, often as a monotherapy. Viral infections have been proven to be especially susceptible. As vitamin C has been shown to support and stimulate immune function by many different mechanisms, clinical research is now clearly showing that it can be expected to augment, sometimes additively and often synergistically, multiple other effective traditional and non-traditional protocols for the treatment of infectious diseases.

To date, no viruses have been reported that are not effectively treated/resolved by vitamin C, whether in the test tube, tissue preparations, the laboratory animal, or the human. This includes Dengue, Chikungunya, Zika, and Ebola infections. It now appears that using sufficient doses of different forms of vitamin C, such as oral liposome-encapsulated forms, can achieve comparable results to the more traditional intravenous forms of administration. The addition of ozone, hydrocortisone, zinc, antibiotics, and other anti-infection measures to vitamin C all appear to have often profound clinical impacts on infections still felt to be effectively untreatable by traditional medicine.

The Multi-C Protocol will also be briefly discussed, as the high dosing of multiple forms of vitamin C appears to sometimes have effects not achievable with one form alone.

Cancer

Poster Abstracts 50-59

Poster Abstract 50

Application of *quantitative redox biology* to basic research on the use of pharmacological ascorbate in cancer therapy

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Ascorbate functions as a versatile reducing agent in biology. At healthy, physiological concentrations (40 - 80 μM) it exhibits antioxidant properties and is essential in maintaining the function of many enzymes. However, when used at pharmacological doses (P-AscH- plasmalevels≈2 mM,achievedv iaIV),its oxidation can deliver a high flux of H_2O_2. This unique feature of P-AscH- is currently being investigated for use as an adjuvant to standard of care cancer therapy.

A great deal of information on the potential mechanism of the action of P-AscH- is gathered in pre-clinical studies that employ cell culture. In these studies, we have found that expression of dose/ exposure to P-AscH- is better specified as mole of ascorbate per cell. We have also developed a kinetic assay to determine the rate constant for removal of extracellular hydroperoxide by cells (kcell) as well as a quantitative assay for catalase.

We have demonstrated quantitatively that catalase is the major enzyme contributing to kcell. Rate constants for removal of H_2O_2 (kcell) and catalase activities were determined for 15 tumor and 10 normal cell lines of various tissue types. A differential in the capacity of cells to remove H_2O_2 was revealed, with the average kcell for normal cells being twice that of tumor cells. The ED50 (50 %clonogenic survival) of P-AscH- correlated directly with kcell and catalase activity. The response to pharmacological ascorbate in murine-models of pancreatic cancer paralleled the in vitro results, e.g. kcell, when these same cells were exposed to P-AscH-. Quantitative approaches increase efficiency of the research effort, increase rigor and reproducibility, and importantly yield more information from data. (Supported by NIH grants R01 CA169046, R01 CA184051, P30 CA086862, and P42 ES013661.)

Poster Abstract 51

4D quantitative image analysis of cancer cell invasion in a brain microenvironment using ImageJ software

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Acquisition of quantitative data of individual tumor cell invasion within live brain environment is currently limited. Individual cell monitoring is needed to understand cellular mechanisms for aggressive cancer behavior so that these mechanisms can be targeted or exploited for therapies. While numerous in vitro systems are well adapted to elucidate these mechanisms, they lack a dynamic environment that present numerous cell signals, mechanical forces and host cell interactions that combine to provide complex conditions that influence cell behavior. It is this complex microenvironment that cancer is found in humans and therefore it provides the optimal conditions for studying tumor cell invasion. To study glioblastoma (GBM), a very aggressive and deadly brain cancer, our lab has developed an in vivo model where fluorescently dyed human cancer cells are transplanted into the brain of larval zebrafish to monitor invasion of the tumor cells through time-lapse confocal imaging. Detailed 4D images of the entire tumor cell population in the brain microenvironment were produced. Using ImageJ software (Fiji), 4D images of the population of individual tumor cells in larva brains can be automatically tracked to provide a litany of quantifiable data. The resulting data contains information on the invasiveness of individual tumor cells in brain tissue. Data from this method can generate many different quantifiable tumor cell invasion statistics that can help in elucidating specific mechanisms used by cells to maneuver or invade through a brain microenvironment. These statistics can be used to help determine in what ways invasion is affected for tumor cells exposed to potential therapies or alterations in protein expression. Parameters for invasiveness can be set to create subpopulations of tumor cells where the subpopulations are seen to have different responses. All this leading to reliable data that can be more easily compared across studies.

Poster Abstract 52

Primary T-cell Lymphoblastic Leukemia (T-ALL) xenotransplant in zebrafish as a model for chemoprevention

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Acute Lymphoblastic Leukemia (ALL) is the most frequently diagnosed cancer in children and about 15% of ALL cases have origins in T-cells (T-ALL). Zebrafish develop most tumor types found in humans with comparable signaling pathways and morphology, making them a promising model for high throughput chemoprevention and therapy screening. The acid condensation products (RXM) of indole-3-carbinol (I3C), notably 3,3'-diindolylmethane (DIM), have shown chemopreventative and chemotherapeutic properties employing human T-ALL cells in vitro and mouse xenografts of human T-ALL as well as maternal diet supplementation with I3C in a mouse transplacental model. Current efforts aim to recapitulate this response with a T-ALL xenotransplant model in zebrafish. We have established a method for T-ALL xenotransplantation in zebrafish using commercially available T-ALL cells (HSB-2) from ATCC and then applied the same method using primary cells from pediatric patients at Oregon Health and Sciences University (OHSU). Casper strain (transparent) embryos at 6 hour post- fertilization (hpf) were dechorionated and exposed to 1 µM DIM, the predetermined NOAEL, until 48 hpf. At 48 hpf, embryos were injected with fluorescently labelled pediatric T-ALL cells, and xenotransplants were maintained at 33°C while being monitored and imaged over Rhodamine and Brightfield at 4 days post-injection (dpi) and 6 dpi. Fish that were treated with DIM in both DMSO vehicle and gel vehicle showed less T-ALL cells migrating in their vascular system compared to control groups. Next in model development is to expose the zebrafish to an I3C reaction mixture before injection and monitor cell migration and proliferation. This pilot project grant is supported by the Horizon Initiative, an OSU-OHSU Cancer Prevention Consortium.

Poster Abstract 53

The aryl hydrocarbon receptor mediates growth-inhibitory effects of SU5416 in hepatoma cells

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The aryl hydrocarbon receptor (AhR) is a potential clinical target for cancer and autoimmune dysfunction. Identifying selective AhR modulators that produce desirable clinical outcomes represents an opportunity for developing new anti-cancer agents. Repurposing clinically-used drugs with established safety profiles that activate the AhR represents a good starting place to pursue this goal. In this study, we characterized the AhR-dependent effects of SU5416 (Semaxanib) following its identification in a small-molecule library screen. SU5416 potently activated AhR-dependent reporter genes, induced AhR nuclear localization, facilitated AhR-DNA binding and increased expression of its endogenous target genes. SU5416 significantly inhibited proliferation of Hepa1 hepatoma cells in an AhR-dependent manner, but did not induce apoptosis. SU5416 also inhibited the growth of human HepG2 liver cancer cells. The effects of SU5416 correlated with an increased G1 population and increased expression of cell cycle inhibitor p21cip1/waf1 at both the mRNA and protein level. Increased expression of p21cip1/waf1 by SU5416 required expression of both AhR and Arnt. In addition, evidence for long-term activation of the AhR in vivo by a single dose of SU5416 was identified by analyzing published microarray data. Our results provide support for continued investigation of the AhR as therapeutic for cancers such as hepatoma. In addition, our findings raise the possibility that some of the previously observed anti-proliferative effects of SU5416 may be due to activation of the AhR.

Poster Abstract 54

Targeting therapy resistant cancer through Bcl-2 functional conversion

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Emergence of tumor resistance to cancer therapeutics is a major cause of treatment failure which results in poor overall survival. Patients who have stopped responding to current treatments due to acquired resistance have a very poor prognosis and few alternative treatment options. Tackling this therapy resistance will not only improve prognosis for many but increase the impact of current therapies. Identifying molecular targets that are present in the therapy resistant cell population is essential to prevent resistance and treat patients that have developed acquired resistance to therapy. The B-cell lymphoma 2 (Bcl-2) family of proteins is associated with 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 resistant to chemotherapeutics were susceptible to 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.

Poster Abstract 55

AhR-dependent induction of $p27^{Kip1}$ by select AhR ligands

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that was firstly described as a mediator of toxicity of environmental pollutants such as dioxins. Upon ligand-binding, latent AhR localized in the cytosol translocates to the nucleus and heterodimerizes with AhR nuclear translocator (ARNT). The active transcription factor complex regulates the expression of responsive genes. Nonetheless, a novel anticancer role has recently been attributed to AhR due to its involvement in cell proliferation inhibition. Previous data showed that activation of AhR induces $p27^{Kip1}$, a cell cycle regulatory gene that controls cell fate1. $p27^{Kip1}$ inhibits cyclin-dependent kinases (CDKs) and regulates the entry of cells into the S phase. Recent evidence from Takahashi's research group revealed an essential role for the AhR-induced $p27^{Kip1}$ upon dioxin exposure on neuronal toxicity. In their study, up-regulation of $p27^{Kip1}$ led to quiescence of neural progenitor cells and reduction in the number of neurons. Herein we hypothesize that activation of $p27^{Kip1}$ by the AhR has therapeutic utility by suppressing cancer cell proliferation.

Our laboratory has identified AhR modulators that activate AhR, induce up-regulation of $p27^{Kip1}$ mRNA and protein levels and inhibit cancer cell viability. These compounds are termed as Selective Modulators of Ah Receptor Transcription (SMAhRTs) and are structurally distinct from dioxins. Our results demonstrate that SMAhRT or their analogs cause rapid up-regulation of $p27^{Kip1}$ in an AhR-dependent manner. In addition, SMAhRTs selectively inhibit cell viability of cancer cells through activation of the AhR. Future experiments aim to study the signaling pathways initiated after activation of AhR by SMAhRTs, and $p27^{Kip1}$-mediated cell fate decisions.

Poster Abstract 56

Grain legume consumption may provide chemo-preventive effects on incident and prevalent colorectal adenoma and colorectal cancer: A meta-analysis of human studies

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Background: Grain legumes are plants that are harvested solely for their dry grains. The American Institute for Cancer Research made no conclusions regarding the relation between grain legume consumption and colorectal cancer (CRC) in 2007 because of limited data available. As more data have been published since then, we re-evaluated the relation between grain legume consumption and colorectal adenoma (CRA) and CRC risk.

Methods: We conducted a meta-analysis of human studies to evaluate the association between grain legume consumption and risk of CRA and CRC. Eleven prospective cohorts (1,533,527 participants including 12,274 cases) and 12 retrospective studies (42,473 controls and 12,408 cases) were included in the meta-analysis. The pooled risk ratios (RR) and 95% confidence interval (CI) for the highest (~45 g/d) comparing with the lowest (0 g/d) grain legume intake group were evaluated through a random effects model. Also, heterogeneity of estimates (I^2), influential risk estimates, and publication bias were examined using funnel plots and Egger's method.

Results: In cohort studies, high grain legume consumption was inversely associated with risk of incident CRA (RR=0.72; 95% CI: 0.60-0.87; I^2=0%), prevalent CRA (RR=0.87; 95% CI: 0.75-1.01; I^2=0%), and CRC (RR=0.89; 95% CI: 0.83-0.96; I^2=8.9%) and no significant publication bias (P=0.13). In retrospective studies, similar associations were observed between grain legume intake and risk of prevalent CRA (RR=0.93; 95% CI: 0.84-1.03; I^2=0%) and CRC (RR=0.77; 95% CI: 0.66-0.89; I^2=53.3%), the heterogeneity of the latter can be explained by differences in CRC estimates between men (RR=0.79; 95% CI: 0.60-1.05; I^2=0%) and women (RR=0.48; 95% CI: 0.34-0.69; I^2=0%). When both study types were combined, high grain legume consumption was inversely associated with risk of CRA (RR=0.87; 95% CI: 0.81-0.94) and CRC (RR=0.82; 95% CI: 0.74-0.91).

Conclusions: Regular grain legume consumption (~45 g/d) may provide a chemo-preventive effect against colorectal neoplasia.

Poster Abstract 57

Chemopreventive response markers to navy bean extract in a mouse model of inflammation-induced colorectal cancer

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Background: Consumption of dry beans and their fractions decrease colorectal neoplasia; however, the underlying molecular mechanisms are unclear. Aim of the study was to identify response indicators of dietary attenuation of colorectal tumorigenesis using metabolic, cytokine, and genomic profiling. **Methods:** After azoxymethane/dextran sodium sulfate (AOM/DSS) induction, mice were fed an AIN93G diet containing either 0 (Control) or 10% navy bean ethanol extract (BE) for 4, 41, or 85 days. Magnetic resonance imaging colonography of life mice was done to observe diet-induced differences in AOM/DSS-induced colorectal inflammation and tumorigenesis. Serum, colon tissue, and fecal samples were collected and analyzed for metabolite (serum, feces), cytokine (serum), and gene profiles (colon tissue).

Results: Dietary BE attenuated AOM/DSS-induced chronic colitis, aberrant epithelial cell proliferation, fecal blood (heme), and tumorigenesis in the colon. Response indicators were serum markers of fatty acid oxidation (carnitine, hexanoylcarnitine), and the pentose phosphate pathway (sedophetulose-7-phosphate) and fecal medium-chain fatty acids (hexanoate, octonoate) and plant phenolics (vanillate), the AOM/DSS-induced increase was attenuated by dietary BE. Moreover, dietary BE increased fecal markers of apoptosis (ribose, uracil, pseudouridine, xanthine, hypoxanthine) and attenuated the AOM/DSS-induced decrease in serum glycerophosphocholine and cytochrome P450 2c55 mRNA.

Conclusions: Dietary BE may inhibit survival and proliferation of premalignant colorectal epithelial cells by limiting their nutrient supply and resolving inflammation.