Poster 1

Exploring cellular heterogeneity through single-cell proteomics

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Abstract

Single-cell proteomics provides a powerful approach to explore cellular heterogeneity and capture subtle biological changes often masked in bulk analyses. Here, we present two studies that highlight its critical importance. First, MDA-MB-231 breast cancer cell lines stably overexpressing Bcl-2 were generated through electroporation and clonal selection in G418-containing media. High Bcl-2 expression clones (MDA-MB-231/Bcl-2) were identified by Western blotting and subjected to chemical library screening alongside vector controls. Single-cell analysis revealed specific protein expression changes, including differential expression of BCL2L1, that were not detectable at the bulk population level. The second study investigates PC12 cell differentiation into sympathetic ganglion neurons induced by nerve growth factor (NGF). Using single-cell proteomic profiling, we not only monitor differentiation progression and identify protein and peptide markers but also unveil cellular heterogeneity within the differentiated population, which remains obscured in traditional bulk proteomics. Together, these studies underscore the vital role of single-cell analysis in revealing hidden molecular diversity.

Poster 2

Untargeted metabolomics and machine learning prediction of adulterated leaf contents in *Withania somnifera* extracts by liquid chromatography quadrupole time-of-flight mass spectrometry

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Abstract

Withania somnifera (WS), commonly known as Ashwagandha, is a natural plant product widely used in traditional medicine and as a botanical supplement due to its bioactive constituents. Different parts of the plant, including the leaves, root, stem, and mixtures of these parts, can be used for their therapeutic properties, but root is more commonly seen. This study investigated withanolide constituents and global plant metabolome of leaf-root mixtures in varying ratios using an AB Sciex ZenoTOF 7600 mass spectrometer. Using principal component analysis, untargeted metabolomics data was used to calibrate the untargeted contents resulting from leaf adulteration of pure root samples. The predictive capability of unsupervised principal component calibration was benchmarked with accurate quantitation of known phytochemicals. The method is applicable to investigative studies on botanical product adulteration cases where other parts of plants may be being used other than advertised, posing dangerous consequences. Additionally, the detection and quantification of phytochemicals in Ashwagandha was performed for targeted metabolites and the initial use of electron activated dissociation for withanolide structure elucidation was performed both de novo and using COSINE similarity networking of electron activated dissociation fragmentation data. The results of this study provide a valuable approach and a reliable method for detecting and quantifying phytochemicals in Ashwagandha, supporting quality control and standardization efforts.

Poster 3

Muscle protective effect of Alaska Pollock milt hydrolysate on dexamethasone induced C2C12 *in vitro* model

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Abstract

This study explored the protective effects of Alaska pollock (*Gadus chalcogrammus*) milt hydrolysates against dexamethasone (DEX)-induced muscle atrophy in C2C12 myotubes. Optimal conditions to induce atrophy were optimized using differentiated myotubes (7 days) with 50 μM DEX for 48 hours, which resulted in a 36.8% reduction in myotube diameter. Among enzymatic digestion, bromelain digested milt (MBD) exhibited highest protective efficacy compared to other tested samples. Further comparison with a free amino acid (FAA) mixture confirmed that the observed muscle-protective effects were attributed to peptides rather than amino acids. Further ultrafiltration showed that peptides under 1 kDa revealed maximum protection among fractions. Gene expression analysis revealed that MBD under 1 kDa fraction suppressed muscle protein degradation markers such as FoxO3, MuRF1, and Atrogin-1 which suggest inhibition of muscle protein breakdown through down regulating ubiquitin proteasome system. These findings indicate potential application of Alaska Pollock milt peptides as nutraceutical or additives for effectively preventing glucocorticoid-induced muscle atrophy.

Poster 4

Discovery of multifunctional peptides from Alaska Pollock milt using *in silico, in vitro,* and proteomic approaches

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Abstract

The increasing demand of multifunctional bioactive peptides has attracted attention to various protein sources, especially underutilized by-products. Among these, marine by-products, such as fish processing waste, have shown them to be highly promising sources due to their growing consumption rates, nutritional value and sustainability. This study focused on Alaska Pollock (*Gadus chalcogrammus*) milt protein as a novel source for potential bioactive peptides with anti-inflammatory, anti-hypertensive, and anti-diabetic properties. Protein profile was identified and characterized through optimized LC-MS proteomic analysis and revealed 1,411 proteins and 7,359 peptide residues. Sequential *in silico* gastrointestinal digestion with abundant proteins and bioactivity screening narrowed down 22 novel, non-toxic, and stable peptides with promising bioactivity potential scores. Molecular docking analysis with peptides targeting key proteins, including DPP-IV, alpha-glucosidase, ACE, GLP-1 receptor, COX-2, MuRF1, and the 20S proteasome has performed to simulate peptide-protein interaction to predict inhibitor or enhancer. One of the peptides CLPPH exhibited favorable binding affinities, pharmacokinetics, and was synthesized for further validation. *In vitro* assays confirmed CLPPH's inhibitory effects on anti-inflammation (nitric oxide production, IC50 = 1.91 mg/mL), anti-diabetes (DPP-IV, IC50 = 1.25 mg/mL), anti-hypertension (ACE, IC50 = 0.33 mg/mL), and alpha-glucosidase (IC50 = 2.65 mg/mL). These findings elucidate the potential of CLPPH as a multifunctional therapeutic peptide and support the sustainable valorization of Alaska Pollock milt as a source of functional foods and nutraceuticals for application.

Poster 5

Revealing bioactive cannabinoids beyond CBD from hemp extract using machine learning

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Abstract

Hemp (Cannabis sativa L.) refers to the plants that contain less than 0.3% (dw) of psychoactive ∆9-THC (tetrahydrocannabinol). Hemp is rich in non-psychoactive CBD (cannabidiol) and contains more than 100 minor cannabinoids. CBD is one of the few cannabinoids that have been extensively studied and used for pharmaceutical and medicinal purposes. However, there is growing interest in the therapeutic potential of non-psychoactive minor cannabinoids, particularly for low-grade inflammation and pain. Thus, this study aims to reveal bioactive minor cannabinoids from the CBD-depleted hemp extract by investigating their anti-inflammatory effects *in vitro* and integrating a machine learning approach. CBD-mother liquor (CBD-ML), the byproduct of crystallizing CBD from hemp extract, was fractionated into 47 fractions using a Sephadex LH-20 column. CBD-ML and fractions were analyzed using UPLC-QToF-MS/MS and LI-ESI-ToF (loop-injection combined with electrospray ionization and high-resolution accurate mass spectrometry), respectively. Murine macrophage RAW264.7 cells were treated with fractions (1 µg/ml) and lipopolysaccharide (LPS, 100 ng/ml) for 24 h to evaluate their effects on low-grade inflammation. Random forests analysis was conducted to predict the bioactive cannabinoids from CBD-ML using mass peak intensities of fractions and bioassay results as input. In CBD-ML, 25 cannabinoids were identified by comparing with our in-house spectral database. LPS significantly elevated iNOS-mediated nitric oxide (NO) production compared to the control. Fractions between #20-45 significantly suppressed this LPS-induced NO production ($p<0.05$). Based on the random forests analysis, 8 out of 63 peaks were predicted to exhibit anti-inflammatory activity, with cannabinoid acids showing the highest predictive importance for anti-inflammatory effect. This machine learning application demonstrates its effectiveness in expediting the discovery of bioactive minor cannabinoids. Exploring bioactive minor cannabinoids from the waste product of the CBD industry will support the development of novel hemp-derived nutraceuticals and keep the agricultural industry sustainable.

Poster 6

Comparing apples to apples: Evaluating foodomics in precision nutrition research featuring the influence of polyphenols on the gut microbiome

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Abstract

The relationship between diet and health has been recognized for centuries, from the medicinal use of herbs in ancient civilizations to contemporary public health campaigns. Today, this connection is reflected in colloquial advice like "an apple a day keeps the doctor away" and formalized through initiatives such as the U.S. Department of Health and Human Services' "Food Is Medicine" program, which aims to integrate nutrition into healthcare to prevent and manage chronic diseases. To support such initiatives, we need a rigorous understanding of the full spectrum of food composition – including both essential nutrients and bioactive non-nutritive compounds such as polyphenols. Food composition is highly dynamic and influenced by factors such as plant genotype, growing conditions, harvest timing, and processing methods. These variations impact not only nutritional value but also the presence of phytochemicals with potential health effects. Advanced omics technologies have revealed a vast and largely unexplored food metabolome, yet these tools remain underutilized in clinical nutrition research.

In this review, we surveyed clinical trials in the Cochrane Library from 2014-2024 using the keywords "polyphenols" and "gut microbiome." Of the 38 studies identified, none applied untargeted omics to analyze food composition, and only five used such approaches for clinical biospecimens. Targeted analyses of known compounds were more common – used in 18 studies for food and 24 for clinical samples. These findings reveal a significant gap in how food complexity is integrated into human intervention trials. By embracing untargeted omics and foodomics approaches, researchers can generate more comprehensive datasets and ask more precise questions – not just whether "an apple a day keeps the doctor away," but which apple, grown under what conditions, and how it interacts with the human gut microbiome to influence health.

Poster 7

Proteomic stable isotope probing as a precision tool for identifying gut microbial fiber utilization in a complex community

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Abstract

Inulin, a prebiotic fiber found in foods such as wheat and onions, is increasingly consumed as a supplement to support gut health, with the global market projected to reach $2.2 billion by 2030. While prebiotic-rich diets are broadly associated with improved health outcomes, individual responses vary-likely due to competitive interactions among microbial taxa occupying distinct gastrointestinal (GI) niches. A key knowledge gap remains in identifying the primary microbes responsible for inulin metabolism within spatially structured gut communities, as most microbiome studies rely on methods that cannot directly track metabolic activity and stool samples that do not capture spatial dynamics.

This study investigated inulin-degradation mechanisms using a multi-compartmental gastrointestinal simulator that models both luminal and mucosal environments. A healthy human microbiome was subjected to two diet treatments (high fiber and high protein), each supplemented with isotopically labeled inulin. Over 24 hours, samples were collected from colonic compartments for 16S rRNA sequencing and proteomic stable isotope probing. Microbial communities differed significantly between mucosal and luminal fractions and across colonic regions. Following 24 hours of incubation, taxonomic families displayed diet-specific labeling patterns. *Peptostreptococcaceae* and *Pseudomonadaceae* had higher labelling frequency under the high-protein diet, whereas *Cellulosilyticaceae* and *Ruminococcaceae* demonstrated the highest labelling frequency in the high-fiber conditions, suggesting that dietary composition may influence which taxa preferentially utilize prebiotics.

These findings highlight the importance of understanding the relationship between spatial organization within the GI tract and direct assessment of microbial metabolism by moving beyond stool-based sampling in microbiome research. By identifying active inulin degraders and their localization within the gut environment, this study contributes to a more nuanced understanding of prebiotic metabolism by the gut microbiome. Ultimately, this work supports precision nutrition therapies for managing gut-related disorders such as irritable bowel disease, metabolic syndrome, and conditions linked to gut-brain axis dysregulation.

Poster 8

Olive leaves as source material for novel products containing polyphenols

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Abstract

We developed a processing method for freshly picked olive leaves that retains high levels of polyphenols. Oven drying (90°C) reduced moisture content from 54% to <3%. Next we evaluated cleaning methods. A water rinse (1 minute) was compared to a bleach (200 ppm) rinse (1 minute) followed by a water rinse (1 minute); both steps were followed by patting the leaves dry with toweling, then oven drying. Leaf samples (~30 g fresh or ~15 g dried leaves) were pulverized to a powder in liquid nitrogen in a Waring metal blender and stored fewer than 2 weeks at -80°C until analysis. There were no differences in total polyphenols between untreated and water rinsed leaves; however, oven drying (90°C) following rinsing with bleach preserved polyphenols better compared to just oven drying ($P<0.001$), all results are reported per dry weight. By contrast, both alpha- and gamma-tocopherols were present in the fresh leaves, but both decreased by ~50% following drying; bleach had no further effect. To confirm the beneficial effect of the bleach rinse on polyphenols prior to drying, individual polyphenols (hydroxytyrosol, oleuropein and rutin) were quantitated using targeted LC/MS-MS. All tested were found to increase significantly ($P <0.01$) compared to fresh leaves. In summary, the bleach water pre-treatment step increased the total polyphenols compared to a water only rinse, preserving the fresh leaf amounts. The observed increase was confirmed in analyses of selected individual compounds. Further work is necessary to ascertain whether conjugates of polyphenols present in the leaves are released during bleach processing. Overall, the addition of a bleach water rinse step further improved polyphenol preservation and can be done to make a product safe for human consumption.

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Poster 9

Enhanced structural elucidation of conjugated xanthohumol metabolites with electron activated dissociation

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Abstract

Xanthohumol (XN), the major prenylflavonoid found in hops (Humulus lupulus L.), possesses diverse bioactive properties, including anti-obesity, anti-inflammatory, antioxidant, and antiplatelet effects. *In vivo*, XN is metabolically converted to isoxanthohumol (IX), which can undergo further biotransformation into 6-prenylnaringenin (6PN) and 8-prenylnaringenin (8PN), along with various phase I and II modifications such as hydroxylation, sulfation, hydrogenation, oxidation, and glucuronidation. However, the structural characterization of regiospecific glucuronidated and sulfated XN metabolites remains a challenge when relying solely on collision-induced dissociation (CID) MS/MS, especially in the absence of authentic standards. In this study, we assess the utility of the SCIEX ZenoTOF 7600 system equipped with electron-activated dissociation (EAD) MS/MS, which generates unique fragmentation patterns distinct from those of CID. Our findings demonstrate the improved capability of EAD in profiling XN and its conjugated metabolites, highlighting its potential as a powerful tool for structural elucidation in metabolite analysis.

Poster 10

Characterizing the immunomodulatory effects of peptides from human donor milk between different processing methods

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Abstract

Objectives: Human milk is recognized for its rich composition of bioactive components, needed for infant development and immune system support. Alterations in composition and bioactivity have been observed in frozen and holder pasteurized (HoP) human donor milks. Additionally, investigations have revealed that peptide fractions from human milk protein digests exhibit diverse impacts on proinflammatory cytokine secretion in macrophages, suggesting their potential for immunomodulation. Alterations in bioactivity due to milk handling may influence the efficacy of donor human milk in modulating infant immune responses. To address this, we measured the macrophage-immunomodulatory activity of peptides released after simulated preterm infant digestion from freeze/thaw (FT) and HoP treated donor milk.

Methods: Human donor milk samples underwent simulated digestion utilizing a static *in vitro* digestion model modified to mimic the conditions of the preterm infant gastrointestinal tract. *In vitro* digestion samples were collected at 60-min of intestinal digestion (120-min post digestion initiation). Peptides from the digesta samples were isolated via ethanol precipitation and C18 solid phase extraction. PMA-differentiated THP-1 cells (ATCC#: TIB-202) were used to probe the immunomodulatory activity of the extracted peptides by pretreating with each individual peptide sample followed by LPS from *E. coli* (O55:B5) as an inflammatory challenge. Proinflammatory cytokines TNF-α and IL-8 were quantified post-incubation using ELISA.

Results: The cytokine measurements for both TNF-α and IL-8 in the FT and HoP samples displayed a dose-dependent reduction when compared to the control samples. Both TNF-α and IL-8 were measured as significantly different between treatments ($p < 0.001$).

Conclusions: This suggests potential differences in the immunomodulatory peptide profiles between the FT and HoP milk handling techniques. The current work expands understanding of the potential of human-milk protein-derived peptides to alter infant health. This foundational research will inform the incorporation of bioactive peptides into both human milk and formula feedings to enhance clinical outcomes in preterm infants.

Poster 11

In vitro evaluation of different capsule polymer combinations for targeted delivery of acid-sensitive enzyme

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Abstract

Oral delivery of nutraceuticals such as enzymes and probiotics requires protective systems to preserve bioactivity and absorption, as gastrointestinal conditions can rapidly degrade these ingredients. This study evaluated the performance of DUOCAP® capsule-in-capsule technology in protecting pancrelipase, a gastric acid-sensitive enzyme, during upper gastrointestinal transit. Using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®), we assessed enzyme activity and capsule dissolution under fasted and fed conditions representative of a healthy adult. Three DUOCAP® capsule-in-capsule configurations were tested: DRcaps® inside DRcaps® (DR-in-DR), DRcaps® inside Vcaps® Plus (DR-in-VCP), and Vcaps® Plus inside DRcaps® (VCP-in-DR), alongside single DRcaps® and Vcaps® Plus capsules as controls. Each capsule contained pancrelipase (150 mg) and caffeine (50 mg), with caffeine serving as a dissolution marker. Enzymatic activity was quantified via tributyrin-to-butyrate conversion. Results showed that the DR-in-DR configuration provided the most delayed release, evidenced by slower caffeine release and reduced butyrate formation, indicating enhanced protection of pancrelipase in acidic gastric conditions. VCP-in-DR and DR-in-VCP provided protection with releasing the pancrealipase in the upper small intestine, which is the target site of this enzyme. In contrast, single DR® and single Vcaps® Plus capsules exhibited rapid release of both caffeine and enzyme in stomach, suggesting gastric degradation of unprotected enzyme. These findings highlight the potential of DUOCAP® capsule-in-capsule technology for delivering gastric acid sensitive enzymes like pancrelipase to the small intestine. This approach may support improved oral enzyme therapies and targeted nutrient delivery.

Poster 12

A characterization of the isopropyl alcohol extraction and concentration of 2,5-dihydroxybenzoic acid matrix effects on MALDI mass spectrometry imaging of mouse brain tissues

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Abstract

Matrix assisted laser desorption mass spectrometry imaging (MALDI-MSI) is a powerful technique that allows for the characterization and identification of chemical signals, and how signals are spatially distributed throughout a tissue, allowing for detailed spatial omics analysis of tissue samples. In MALDI-MSI, tissues must first be thinly sliced and placed on a conductive slide. The tissue and slide are then coated uniformly in an energetically excitable crystalline chemical matrix. The slide is then placed in the instrument where an ultraviolet laser repeatedly strikes the matrix in a fixed pattern. Energy from the laser is transferred from the laser to the tissue, which is simultaneously ionized and vaporized for mass spectrometry analysis. While MALDI-MSI is a widely used technique, publications on matrix effects are sparsely published and the impacts of the matrix on imaging experiments remains not fully characterized. We hypothesize that using a mixture of isopropanol, methanol, and water instead of the standard methanol and water mixture could potentially result in further extraction of lipids and result in more robust imaging results. Additionally, we are interested in identifying the relationship between matrix concentration and signal number and strength. To accomplish this, we sprayed three mouse brain tissues and three lipidomics internal standard spots with three different 2,5-dihydroxybenzoic acid (DHB) concentrations, and three additional brain slices and lipidomics spots with varying ratios of isopropanol, methanal, and water, using a standard DHB concentration. These slides were then subsequently imaged using MALDI-MSI. The signal intensities and overall number of signals of bisecting K-means segments in these slides were then compared between tissue slices to quantify the quality of the imaging and assess if isopropanol extraction results in improvements to MALDI-MSI experiments and observe the relationship between DHB concentration and MALDI-MSI results.

Poster 13

Improving the identification of cannabinoids with chronic pain therapeutic potential through electron-activated dissociation (EAD) MS/MS

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Abstract

Chronic pain effects more than 50 million U.S. adults due to inadequate pain management resources. Minor cannabinoids show promising bioactive properties for managing chronic pain. We conducted an *in vivo* study that showed robust antinociceptive and sleep promoting effects of CBD-mother liquor in mice, indicating the presence of minor cannabinoids with analgesic potential. Therefore, their isolation and characterization are essential for identifying therapeutic compounds. Collision-induced dissociation (CID) MS/MS is commonly used for structural identification of cannabinoids by fragmenting molecules through controlled collisions with N2. However, CID provides fragmentation spectra with limited information, particularly in its inability to differentiate closely related cannabinoid isomers. Therefore, additional structural studies are needed. Electron-activated dissociation (EAD) MS/MS produces unique fragments compared to CID MS/MS by exposing precursor ions to a beam of electrons with tunable energy. Here, we compare positive ion mode EAD and CID MS/MS spectra for cannabinoids to evaluate whether EAD is a more efficient and diagnostic technology for identifying cannabinoids in cannabis. A SCIEX ZenoTOF 7600 system collected CID and EAD MS/MS data from thirty-five cannabinoid standards (Caymen Chemical). A collision energy of 40 V was used for CID. For EAD, a beam current of 7500 nA and a kinetic energy of 12 eV were used. EAD displayed unique fragment ions for cannabinoids that were not observed in CID spectra. For example, the EAD MS/MS spectrum of CBD revealed multiple fragment ions, including m/z 65, 115, 128, 145, 174, and 273 that were not observed in the CID MS/MS spectrum. EAD MS/MS also gave us the ability to differentiate bicyclic (CBCA), tricyclic (Δ^9-THCA-A), and tetracyclic (CBLA) isobaric cannabinoids without additional confirmation studies, such as NMR. Additionally, we have developed fragmentation pathways of minor cannabinoids using EAD data. EAD MS/MS assists us in identifying novel minor cannabinoids with pain therapeutic potential.

Poster 14

From ocean to therapy: Exploring the bioactive potential of peptides from the red seaweed, *Devaleraea mollis*

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Abstract

Seaweed is a nutrient-rich food with many applications in the food, cosmetic and pharmaceutical industries. Its rich protein content and abundance of essential nutrients position it to be a valuable contributor to human health and balanced nutrition. Bioactive protein extracts from different seaweed varieties have demonstrated therapeutic potential with anti-inflammatory, antihypertensive, and antidiabetic effects. This investigation focuses on extracting, characterizing, and processing proteins from the red seaweed *Devaleraea mollis* (Pacific dulse). The nutritional profile of this species, native to the Pacific coast, remains largely unexplored. Our current experimental approach focuses on extracting phycoerythrin under aqueous conditions and digesting it to produce bioactive peptides. To make the digests more relevant to nutritional applications, we use enzymatic cleavage methods that mimic those of the gastrointestinal tract. We are employing SDS-PAGE and liquid chromatography-mass spectrometry (LC-MS) to investigate the sizes and sequences of the digests. In parallel, we are running antimicrobial and antioxidant assays to assess their bioactivity. Our next step includes leveraging machine learning tools to predict within the mixtures of peptide fragments the sequences that are responsible for bioactivity. Overall, this work furthers our understanding of how widely consumed foods contribute to human health. It also reveals opportunities to use natural food sources for the production of bioactive peptides with antimicrobial and anti-aging properties.

Poster 15

Medium-chain triglycerides in the ketogenic diet shift gut microbiota composition in the small intestine

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Abstract

The spatial organization of the gut microbiome is important for microbial lipid metabolism and diet-driven microbe-host interactions. Yet, how gut microbial lipolytic activity and lipid-driven metabolism in the small intestine respond to different dietary fats remains poorly understood. The ketogenic diet-a high-fat, low-carbohydrate regimen-has been effective in seizure reduction and is increasingly explored as an alternative therapy for other neurological disorders. In this study, we examined how the microbiota composition and function differ in response to different lipid sources in ketogenic diet. Using samples from mice fed a control diet (CD), a classical ketogenic diet (KD), and a medium chain triglyceride-enriched ketogenic diet (MCT-KD), we analyzed microbial shifts and lipid metabolite profiles. We found that MCT-KD increased α- diversity in the small intestine and altered β-diversity compared to CD and KD. Relative abundances of *Muribaculaceae* and *Coriobacteriaceae* UCG002 were higher in the small intestine with MCT-KD whereas *Faecalibaculum* was enriched under both KD types. Our findings provide insights into how specific microbes process dietary fats and how their metabolic activity is modulated by lipid type, offering the potential to develop precision nutrition strategies targeting gut microbiome.

Poster 16

Gut microbiome and pre-intervention diet dictate response to broccoli sprout consumption

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Abstract

Broccoli sprouts have been extensively studied for their production of anti-cancer and anti-inflammatory compounds, notably sulforaphane (SFN). However, human intervention trials observe large variation in the physiological effect of broccoli sprout consumption across individuals, possibly due to the presence of personalized factors that impact SNF bioactivity. The gut microbiome is also highly personalized and known to contribute to SFN metabolism. Emergent work has also linked other dietary components, notably fiber, to SFN bioactivity. Given that the diet has a profound influence over which bacteria reside in the gut, we hypothesized that diet and the gut microbiome interact to define, at least in part, an individual's response to broccoli sprout consumption. To test this hypothesis, we conducted a broccoli sprout human feeding trial with 38 participants. Participants recorded 7-day food diaries before the intervention and fecal samples collected at baseline yielded gut microbiome profiles. We quantified urinary SFN metabolites over a 72-hour period after consumption. Mediation analysis tested two complementary pathways: (1) whether gut microbes link pre-intervention diet to SFN metabolism, and (2) whether diet links microbiome composition to SFN metabolism. Our analysis identified seven bacterial genera that significantly mediated associations between dietary components and SFN metabolites. Moreover, unsupervised clustering identified three distinct microbiome community types in the study population that were driven by distinct dietary patterns. Community types differentially predicted the excretion of SFN-Nitrile, a biologically inert metabolite, indicating that diet-structured microbiomes modulate SFN biotransformation. Together, the results suggest that diet, especially the types of carbohydrates and fiber, structures the gut microbiome in ways that affect how humans respond to feeding trials. Our findings show that interaction between the diet and gut microbiome influences SFN bioactivity. This implies that pre-intervention diet should be considered when designing cruciferous-vegetable interventions, and short-term dietary "priming" of the gut microbiome may optimize individual responses.

Poster 17

Healthy plant-based diet is associated with adiposity indicators in Costa Rican adults

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Abstract

Background: Plant-based diets have been associated with favorable health outcomes; however, not all plant-based diets are of the same quality.
Objective: This study aimed to investigate the associations between three plant-based diet indices, including an overall plant-based diet index (PDI), a healthy plant-based diet index (hPDI) and an unhealthy plant-based diet index (uPDI), and different measures of adiposity including wrist circumference, waist circumference, waist-to-hip ratio, and body mass index (BMI).

Methods: This study used data from 2,027 controls enrolled in the Costa Rica Heart Study. A validated food frequency questionnaire was used to create the plant-based diet indices. Multivariable linear regression was used to examine the associations between the quintiles of the plant-based diet indices and adiposity indicators, adjusting for potential confounders.

Results: Higher adherence to the hPDI is associated with lower adiposity across all indicators with strongest effect detected with waist-circumference. For instance, participants in the highest hPDI quintile had 2.11 cm (-3.53, -0.69) reduction in waist circumference and 0.71 kg/m2 (-1.33, -0.90) reduction in the BMI compared to participants in lowest quintile. Significant linear trends were observed with wrist circumference (p-trend= 0.0283), waist circumference (p-trend=0.0027), waist-to-hip ratio (p-trend=0.0339), and BMI (p-trend=0.020). Moreover, higher overall PDI adherence was inversely associated with waist circumference (-1.15 (-1.89, -0.41); p=0.0012) and BMI (-0.43 (-0.75, -0.10); p=0.0047). In contrast, uPDI showed no significant associations with adiposity indicators.

Conclusions: Adherence to hPDI is associated with reduced adiposity whereas uPDI did not show the same benefits in Costa Rican Adults, suggesting that quality of the plant-based diet can affect the body composition.

Poster 18

Non-negative matrix factorization for dietary pattern analysis

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Abstract

Background. Traditional dietary pattern analysis relies on clustering techniques that assume a Gaussian data distribution and the existence of "average" dietary patterns. Further, clustering is typically done on pre-grouped foods rather than raw intake data. Non-negative matrix factorization (NMF) offers advantages for dietary pattern analysis by handling sparse, non-negative data and learning parts-based representations to identify both global patterns and individual preferences. This study aimed to identify dietary patterns in European adults using raw food intake data.

Methods. We analyzed UK Biobank participants who completed at least two 24-hour dietary recalls, excluding those with unrealistic energy intake. Data included raw food intake (servings/day), food group intake (grams/day), and nutrient intake (grams/day). After discretizing continuous variables through histogram binning, we applied term frequency-inverse document frequency (TF-IDF) weighting and used NMF to generate patient and food loadings across 30 latent factors. K-means clustering was used to derive population-level dietary patterns.

Results. 103,647 individuals were included in the cohort (55% women; age: 56 +- 7.8 years; BMI: 26.6 +- 4.6 kg/m2). The W (patient) matrix was 103,647 x 30 with ~40% missingness and the H (feature) matrix was 30 x 2479 with ~5% missingness. Six dietary patterns with specific food intake behaviors were identified: 'Low energy intake', 'Traditional tea/coffee with light meals', 'High milk/dairy consumers', 'High energy, nutrient dense', 'High energy, high added sugars' and "General/varied intake".

Conclusion. This study demonstrates the utility of TF-IDF and NMF in identifying global dietary patterns from raw food intake data, without the need for dimension reduction prior to analysis. We identified 6 main dietary patterns with distinct food consumption behaviors. Ongoing efforts include characterizing socioeconomic, clinical, and nutritional characteristics across patterns and using multi-layer NMF to capture hierarchical relationships between foods, food groups, and nutrients.

Poster 19

Changes in self-reported dietary patterns during weight loss maintenance vs weight regain: A secondary analysis of the Look AHEAD Trial

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Abstract

Background: Weight loss is known to mitigate the cardiometabolic risks associated with obesity, however, weight regain is common after weight loss. Limited information exists on how dietary patterns change in adults who regain weight after weight loss vs. those who maintain the weight loss after lifestyle interventions.

Methods: Five hundred fifty-two adults (60±1.0 years, 33.8±0.4 kg/m^2) with type 2 diabetes mellitus from the Look AHEAD Trial achieved ≥7% weight loss after Year 1, completed follow-up visits through Year 4, and provided three 134-item food frequency questionnaires (FFQ). Weight "regain" was defined as regaining ≥50% of initial weight lost. Twenty-three food groups derived from the FFQ were expressed as servings*day^{-1}*1000 kcal^{-1}. Dietary patterns were determined at baseline and follow-up using established DASH diet scores (scale:0-9) and principal components analysis (PCA; higher scores = more adherent). Repeated measures linear mixed models assessed differences in PCA dietary pattern scores, and DASH scores, adjusted for randomization site, age, and sex.

Results: DASH scores changed similarly over time, but the regain group's scores decreased more from Year 1 to Year 4 compared to the maintain group (regain: baseline:3.27±1.39, Y1:5.66±1.65, Y4: 4.60±1.60; maintain: baseline:3.41±1.47, Y1:5.58±1.62, Y4:5.01±1.66; difference $p<0.01$). Of the two PCA-derived dietary patterns assessed, Pattern 1 (vegetable, fruit, and fish intake) decreased more in the regain group by Year 4 (regain: baseline:-0.03±1.92, Y1:-0.07±1.69, Y4:-0.31±1.70; maintain: baseline:0.03±1.70, Y1:0.06±1.70, Y4:0.26±1.81; $p<0.01$), while Pattern 2 (low-fiber grains and high-fat animal proteins) increased in the regain group by Year 4 (regain: baseline:0.10±1.41, Y1:0.01±1.14, Y4:0.18±1.33; maintain: baseline:-0.08±1.24, Y1:-0.01±1.21, Y4:-0.15±1.15; $p=0.03$).

Conclusion: These data demonstrate that differences in diet pattern trajectories between "weight regainers" and "weight loss maintainers" emerge at Year 4 post-intervention among adults in this cohort and suggest that the shift in dietary patterns may influence weight loss maintenance following a lifestyle intervention.

Poster 20

Metabolic predictors of responsiveness to calorie restriction in obese diversity outbred mice

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Abstract

Calorie restriction (CR) is a cost-effective, non-pharmacological weight loss strategy, but achieving and maintaining significant weight loss through CR may not be successful for some. Signatures predictive of who will (or will not) favorably respond to CR are needed to develop more precise and sustainable anti-obesity treatment approaches. We used the genetically heterogenous Diversity Outbred (DO) mice to identify metabolic predictors of responsiveness to CR-mediated weight loss. Male and female DO mice (n=300) were fed a high-fat diet for 12 weeks to induce obesity, followed by an 8-week CR regimen. After the study, we assessed body weight, composition, blood glucose, and plasma metabolic hormones in response to CR. Mice were then ranked by percent body weight change, and extreme quartiles were classified as CR responders (n=67), sensitive to CR-mediated weight loss, and nonresponders (n=67), resistant to CR-mediated weight loss. Responsiveness to CR-induced weight loss was substantially heterogeneous, with sex-specific differences. Logistic regression models indicated that male CR resistance was associated with lower glucose levels, while female CR resistance was associated with lower insulin, resistin, and HOMA-IR levels, as well as higher ghrelin. Moreover, lower leptin levels predicted CR resistance in both sexes. In parallel, targeted semi-quantitative metabolomics revealed key urinary metabolites that distinguish CR responders from nonresponders, independent of sex. Notably, glutamic acid and hydroxyproline were lower in nonresponders, with specific metabolites such as putrescine distinguishing male nonresponders and dopamine, histamine, lysine, and spermine distinguishing female nonresponders. Multivariate receiver operating characteristic analyses revealed moderate-to-robust prediction models of CR-mediated weight loss. Pathway analysis identified metabolic pathways such as arginine and proline metabolism and glutamate biosynthesis that were indicative of metabolic reprogramming. Together, these findings build a signature for CR-mediated weight loss that should be validated and extended in humans to further personalize weight loss regimens.

Poster 21

Meta analysis reveals that prebiotic dietary fiber interventions lead to more consistent responses in the microbiome than non-fermentable placebo

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Abstract

The gut microbiome comprises hundreds of bacterial taxa and the abundance of each taxon exhibits variability between individuals. However, certain taxa confer health benefits to the host and are targets for selective promotion. This has led to a need for an increased understanding of how prebiotic fiber interventions affect the abundances of bacterial taxa in the context of inter-individual variability. In this study we conducted meta-analysis on 13 studies comprising 1,405 biological samples from 433 participants, testing the effect of 39 different dietary fibers. For each study, we obtained data consisting of the abundances of bacterial taxa in three sets of fecal samples: 1) before intervention, 2) after dietary fiber intervention, and 3) after placebo intervention (controls) with a non-fermentable carbohydrate (ex. maltodextrin). For each of the three sets of samples, we calculated the average abundance of each bacterial taxon across the study participants as well as its variability between participants. We explored the relationship between these quantities using Taylor analysis, which yields the slope of the relationship between the abundance of a taxon and its variability between individuals. For all three sets of samples, results showed that as the average abundance of a taxon increased, its variability between individuals also increased. However, the slope of this relationship was greatest in the initial samples (2.27±.02), followed by its value in controls (2.24±02) with both values significantly ($p<0.05$) greater than in samples after fiber intervention (2.16±.01), showing that prebiotic fibers have a unifying (variance dampening) effect despite inter-individual variability. Overall, results show that prebiotic dietary fiber interventions lead to more consistency in the abundance distributions of gut microbes than non-fermentable carbohydrates, suggesting the potential for prebiotic fibers to be used to selectively promote beneficial gut microbes for improved human health.

Poster 22

Long-term consumption of Oregon blackberries improved cognition in healthy older humans

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Abstract

Preclinical and clinical trials have shown that flavonoid-rich fruits, such as berries, can improve markers of cognitive function. Further studies have shown berries to be rich in phenolic compounds that can potentially modulate inflammatory processes in the brain and body-especially in healthy older humans to improve memory and executive function. However, cognitive studies on blackberries have yet to be administered to humans. Our goal for the current study is to determine if short- or long-term consumption of Oregon blackberries can influence markers of cognitive function in older people, and if these are influenced by changes in the resident gut microbiota.

Adults (55 years or older) were prescreened for age-related cognitive deficits, but not dementia. Twenty-nine participants underwent cognitive testing (testers were blinded to the treatment), with the use of the NIH Toolbox Cognitive Battery and our virtual version of the Morris water maze, at baseline and following an acute dose (20 gm) and then 20 gm twice daily freeze-dried blackberry powder mixed in water for 12 weeks. The control group consumed only water. Our results indicated that there were improvements in the test scores for the participants consuming blackberries for 12 weeks in composite scores for fluid cognition (memory, speed of processing, flexibility; $p=.04$), crystallized cognition (vocabulary; $p=.02$), and total cognition ($p=.0009$). There was also an increase in depression scores ($p=.047$). There were no significant effects of treatment on virtual water maze tasks for memory and cognitive flexibility. The findings therefore conclude that long-term consumption of blackberries in older adults can buffer their age-related cognitive decline in fluid and crystallized intelligence.

Poster 23

Almond consumption causes compositional shifts in gut microbiota associated with cardiometabolic improvements in participants with metabolic syndrome

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Abstract

Objective: Intestinal dysbiosis can contribute to the progression of metabolic syndrome and affect the development of cardiometabolic diseases, especially in people consuming poor diets with limited bioactive compounds. Almonds are a rich dietary source of bioactive food components, such as fiber, polyphenols and various vitamins and minerals. Previous work has shown that almond snacking improves gut inflammation and cardiovascular health in persons with metabolic syndrome. Here we test the hypothesis that almond-related improvements in health are driven by changes in the gut microbiome in individuals with metabolic syndrome.

Methods: Adults with metabolic syndrome (n=77) were recruited and consumed either almonds (2 oz whole dry roasted) or crackers (equal caloric content) as a daily snack for 12 weeks. Gut microbiome profiling was completed on stool samples collected at 0, 4, and 12 weeks using 16S rRNA gene sequencing. Effects of almond consumption on gut microbiota alpha and beta diversity, and differential abundance of bacterial taxa was analyzed and Spearman's correlations were calculated to investigate the relationship between bacterial taxa and health biomarkers.

Results: Beta diversity of gut microbiome, but not alpha diversity, was significantly different between almond and cracker consumers ($P = 0.02$). Almond consumption was associated with increased abundance of several beneficial microbial taxa, including Monoglobus, Lachnospiraceae, and Bacteroides. Lachnospiraceae abundance showed a significant negative correlation with waist circumference ($P < 0.01$), indicating a possible beneficial relationship. Similarly, Bacteroides was negatively correlated with a biomarker of gut inflammation, calprotectin ($P< 0.05$). Analysis is underway to see if the effects of almond consumption on cardiometabolic biomarkers are mediated by shifts in bacterial taxa.

Conclusion: Almond snacking was associated with significant shifts in the taxonomic diversity of the gut microbiome and correlated with health biomarkers in participants. This work suggests that a daily snack of almonds could benefit people with metabolic syndrome.

Poster 24

Intestinal-level prebiotic and anti-inflammatory activities of whole wheat improve glucose tolerance in individuals with prediabetes: A randomized crossover trial

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Abstract

While whole grain consumption is associated with lower cardiometabolic disease risk, findings from randomized clinical trials (RCTs) are equivocal regarding their glucose-lowering benefits. These disparities may be due to insufficient dietary control that limits the ability to assess the benefits of whole grains independently. We hypothesized that whole wheat bread would improve glycemia by alleviating inflammation and improving microbiota composition and function. Adults with prediabetes (n=39, 40.5±2.0 y, 34.7±0.9 kg/m^2) completed a crossover RCT where they received either 160 g/d of whole wheat bread (WHEAT) or refined bread (WHITE) as part of a two-week fully controlled diet. Fasted plasma samples and fecal samples were collected on days 1 and 14 of each treatment arm. On day 14, participants completed a 3-h glucose tolerance test. Compliance to test bread was < 98%. Fasting plasma glucose and insulin decreased ($P_{time} < 0.005$) during both study arms, regardless of treatment. $AUC_{0\text{-}180min}$ of postprandial Δglucose concentrations were lower ($p = 0.03$) and Δinsulin concentrations tended to be higher ($p = 0.06$) after WHEAT. Plasma inflammatory proteins (IL-6, CRP, TNFα), fecal calprotectin and myeloperoxidase (MPO), and fecal short-chain fatty acids were unaffected in WHEAT. Decreases in plasma CRP (day 14 - day 1) were associated with improved glucose tolerance ($r = 0.32$, $p = 0.04$). Microbiota analysis indicated that α- or β-diversity metrics were unaffected, but differential abundance of butyrate-producing Butyricicoccus increased in WHEAT ($p = 0.04$). Higher relative abundance of Butyricicoccus at day 14 tended to correlate with improved glucose tolerance ($r = -0.28$, $p = 0.08$) and was associated with reduced fecal MPO ($r = -0.32$, $p = 0.05$) and increases in fecal butyrate ($r = 0.34$, $p = 0.03$). Findings from this controlled clinical trial demonstrate that WHEAT improves glucose tolerance in prediabetes, potentially through a prebiotic butyrogenic activity that alleviates intestinal inflammation.

Poster 25

Integration of the urine metabolome with gut microbiota following broccoli sprout consumption

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Abstract

Objectives: While the benefits of eating broccoli sprouts are typically attributed to sulforaphane, other phytochemicals from broccoli sprouts could play a synergistic role in health. Microbial metabolism of compounds from broccoli may also play a role in generating novel bioactive compounds, however, the relationship between specific microbes and the abundance of metabolites from broccoli sprouts remains unclear. The goals of this study were to characterize the urine metabolome following broccoli sprout consumption to; 1) identify metabolites that may contribute to the health benefits of eating broccoli, and 2) characterize relationships between broccoli-associated metabolites with gut microbes.

Methods: Healthy adult participants (n = 32) consumed a single serving of broccoli or alfalfa sprouts (control food). Urine was collected at 3, 6, 24, 48 and 72 h post sprout consumption and analyzed by untargeted metabolomics (LC-MS/MS). Stool samples were collected at baseline and gut microbiome was profiled using 16S rRNA gene sequencing.

Results: 276 metabolites were differentially abundant between broccoli sprout and alfalfa sprout consumers urine (compared at matched time points 3, 6, 24, 48 and 72 h post sprout consumption, $q < 0.05$). 75% of significant metabolites increased with broccoli sprout consumption, relative to metabolite abundance in alfalfa controls. Some anti-cancer compounds of interest that increased with broccoli sprout consumption include sinapic acid, chlorogenic acid, 5-caffeoylquinic acid, iberin-N-acetyl-cysteine, and sulforaphane-N-acetyl-cysteine. PLS-DA identified sulforaphane-nitrile and 3 additional metabolites which discriminate between broccoli- and alfalfa-sprout consumers, and persisted in urine 48 h post broccoli consumption. Thirty-one relationships were identified between broccoli-associated metabolites and microbiota including members of the genus *Fusicatenibacter*, *Roseburia*, and *Ruminococcus*.

Conclusion: Members of the gut microbiota may affect the metabolism of some phytochemicals in broccoli sprouts and broccoli sprouts contain a diverse set of phytochemicals that are likely to contribute to the health benefits associated with cruciferous vegetables.

Poster 26

The ketogenic diet increases memory in female ApoE4 mice

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Abstract

Inheritance of ApoE4 is the single largest genetic determinant for Alzheimer's disease (AD). There are ~34 million human females in the US bearing ApoE4, who bear a 4-fold increased AD risk at age 60y as a result. We've shown previously that the Keto Diet (KD) extends both lifespan and healthspan in aged C57Bl6 mice. KD and BHB itself raise synaptic plasticity in the PS1/APP mouse model that models less than 1% of human AD. BHB alone increases synaptic plasticity in 5XFAD mice. We now extend these studies to mice that model ApoE4, the single most impactful genetic driver of AD. We observe that while KD confers an overall cognitive benefit to both male and female ApoE4 mice in aggregate, this benefit is much stronger in female ApoE4s. Female Apoe4s experienced significantly increased synaptic plasticity as measured by LTP (Long-Term Potentiation) and spatial memory, and significantly increased biochemical pathways underlying synaptic plasticity, p-Erk and p-CREB in hippocampi. Transcriptomic analysis of ApoE4 mouse brains registered a greater impact of KD on synaptic plasticity pathway CREB in females as well. Thus the lipid-rich KD diet rescued age-dependent cognitive and memory losses more in female than male ApoE4 mice. If these precision health results in ApoE4 mice were to translate to humans, they suggest that Keto diets may have especial benefit in human females bearing ApoE4. Our overall hypothesis is that brain inflammation rises with age, inhibiting synaptic plasticity required for memory formation. We propose that BHB produced during KD dose-dependently mitigates this rise in inflammation that constrains synaptic plasticity.

Poster 27

Small molecule Bcl-2 functional converters

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Abstract

Bcl-2, an anti-cell death protein, is overexpressed in many human cancers and contributes to tumor development, progression, and resistance to chemotherapy. High Bcl-2 expression is associated with poor prognosis and treatment outcomes. We have discovered a pathway to convert Bcl-2 from a cytoprotective to cytodestructive protein through binding by the orphan nuclear receptor Nur77, which exposes a hidden "killer BH3 domain" of Bcl-2 (Science 289: 1159-64, 2000, PMID: 10947977; and Cell 116, 527-540, 2004, PMID: 14980220). Building on this mechanism, we developed Nur77-derived Bcl-2 Converting Peptides (NuBCPs) that bind Bcl-2 and convert Bcl-2 from a protector to a killer protein (Cancer Cell 14, 285-98, 2008, PMID: 18835031; Oncotarget 9, 26072-26085, 2018, PMID: 29899843; Apoptosis 10.1007, 2019, PMID: 30612317). More recently, we have identified small molecules, termed as 'Bcl-2 functional converters', that mimic NuBCP activity and selectively induce apoptosis in Bcl-2-overexpressing cancer cells (Cancer Research Communications 4(3):634-644, 2024, PMID: 38329389; ACS Pharmacol Transl Sci.;7(5):1302-1309, 2024, PMID: 38751629). This targeted conversion of Bcl-2 opens new avenues for treating cancers that rely on Bcl-2 for survival.

Poster 28

Mass spectrometry-based proteomic of therapy-induced senescence in triple-negative breast cancer

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Abstract

Triple-negative breast cancer (TNBC) is an aggressive subtype with high recurrence, poor prognosis, and no targeted therapies. One important therapy response in TNBC is cellular senescence, a stable cell cycle arrest triggered by stresses such as DNA damage. While senescence initially stops proliferation of damaged cells, senescent cells remain metabolically active and influence their surroundings through the senescence-associated secretory phenotype (SASP), a heterogeneous mix of cytokines, chemokines, proteases, growth factors, an extracellular vesicles. The SASP plays a pivotal role in shaping the tumor microenvironment by influencing immune surveillance, sustaining chronic inflammation, and contributing to therapy resistance.

In this study, our first aim is to induce senescence in the TNBC cell line *MDA-MB-231* using two distinct methods: a chemotherapy reagent (doxorubicin) and ionizing radiation. We will then characterize intracellular proteomic changes using high-resolution mass spectrometry. Senescence induction will be confirmed by morphological assessment, β-galactosidase staining followed by comprehensive proteomic analysis.

Our second aim is to profile the extracellular compartment of senescent cells with the goal of identifying candidate biomarkers of senescence. By integrating intracellular and extracellular proteomic data, we will generate a comprehensive molecular map of senescence in TNBC and reveal how therapeutic stress reshapes the secretome and its signaling crosstalk with the tumor microenvironment.

Poster 29

Aggregation of the nuclear envelope protein lamin in aging and disease

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Abstract

Despite extensive research, the mechanisms which drive the natural aging process remain elusive. One gene associated with aging is LMNA, which codes for lamin proteins which help form the inner nuclear membrane, providing structure to the nucleus and influencing chromatin organization, transcriptional regulation, and cell-cycle progression. Mutations in LMNA have been shown to cause a variety of age-dependent diseases including the accelerated aging disease Hutchinson-Guilford progeria as well as tissue specific diseases such as Charcot-Marie-Tooth disease (CMT), Emery-Dreifuss muscular dystrophy (EDMD), and dilated cardiomyopathy (DCM). Since lamin is expressed in all cell types, the mechanism which governs tissue specificity in these diseases is of particular interest. Using the fruit fly *Drosophila melanogaster* as a model system we tested what mechanisms mediate the degradation of lamin during aging. We find that lamin is targeted for degradation by autophagy through an interaction with the p38 MAPK (p38Kb), a regulator of lifespan and aging in flies, and the co-chaperone starvin (stv, BAG3 in mammalian systems). Loss of p38Kb leads to the accumulation of lamin and abnormally shaped nuclei with chromosomal leakage. We also find that expressing mutant lamins which cause DCM, CMT, or both in fly muscle tissue effect lamin aggregation and association with p38Kb and stv. In addition, expression of these mutant lamins also impair locomotor functions. As AMPK signaling interacts with p38Kb in mammalian systems and has been shown to target a mutant form of LaminC (a homologue of lamin) for degradation in the adult fly muscle, we tested if AMPK also plays a role in mutant Lamin degradation. We find that AMPK can target the degradation of one Lamin mutant protein but not wild type or other disease mutants, suggesting that different mutant forms of Lamin can be recognized by specific degradation pathways.

Poster 30

Characterizing the lipidomic profile of the 5xFAD Alzheimer's disease mouse model: A comparative study using MALDI mass spectrometry imaging

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Abstract

Alzheimer's disease (AD), a major cause of death in Americans over 65, exists in two forms: sporadic (SAD) and familial (FAD). The 5xFAD mouse model, which carries five FAD mutations in the presenilin 1 gene, promotes excess beta-amyloid production and plaque buildup, leading to neurodegeneration. This makes the 5xFAD mouse a valuable model for studying AD pathology and treatment efficacy. One treatment we want to evaluate is mitoQ: a common mitochondria-targeted antioxidant supplement shown to mitigate cognitive decline.

In collaboration with Dr. Kathy Magnusson's research, 5xFAD mice have displayed neurophysiological changes as young as 15 days of age. To further investigate these changes from a lipidomics perspective, we compared the brain lipidomic profile of 1 and 8-9 month old 5xFAD mice to their wild type (WT) counterparts.

Utilizing MALDI Mass Spectrometry Imaging (MALDI-MSI) technology, we developed a workflow for on-tissue lipid profiling at near cellular resolution. When paired with traditional in-solution mass spectrometry, a broad class of lipids were identified while preserving the visualization of these lipids across complex and heterogeneous brain regions. For drug detection, MSI can visualize mitoQ on-tissue, quantifying uptake and distribution across the blood-brain barrier.

Together, the in-solution and imaging data sets revealed lipid dysregulation in the 5xFAD mouse brain as young as 1 month of age. Comparison across young and old 5xFAD mice illustrated age related lipid alterations as beta-amyloid build-up worsens. In both young and old 5xFAD mice, we identified potential lipid biomarkers for disease diagnosis, prognosis, or treatment. For future work with MSI technology, we want to evaluate mitoQ dose and response by targeting these lipid biomarkers. Not only does our research shed light on early AD development and treatment, it highlights the advantages of spatial lipidomics in health and neuroscience.

Poster 31

Fasting and postprandial triacylglycerols characterize obesity and associate with SNPs for SULF2

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Abstract

We aim to identify metabolites that characterize obesity at fasting and after meal consumption and determine whether these metabolites are under genetic regulation to gain insight into links between inherited risk and metabolic dysfunction with obesity.

Fasting metabolites were analyzed from 231 healthy adults from the USDA Nutritional Phenotyping Study (ClinicalTrials.gov: NCT02367287). Participants had a body mass index (BMI) of 18.5-24.9 kg/m2 (normal-weight (NW), n=139) or <30.0 kg/m2 (obese (Ob), n=92). Plasma samples were collected at 0h, 0.5h, 3h, and 6h following consumption of a high-fat, high-carbohydrate meal challenge for postprandial metabolomics in a subset: NW (n=59) and Ob (n=47). Metabolomics data was collected with mass spectrometry-based MxP Quant 500 kits (Biocrates Life Sciences). Metabolites with variable importance in projection (VIP) scores <1.0 in body weight group partial least squares discriminant analysis (PLS-DA) were considered discriminators. Genotype data from 228 participants (BMI 18.5-44 kg/m2) were imputed and restricted to biallelic variants with minor allele frequency <1%. Lead variants were identified from genome-wide association study results using PLINK v1.9 (http://pngu.mgh.harvard.edu/purcell/plink/).

A 4- and 3-component PLS-DA model achieved classification accuracy of 78.6% (fasting model) and 78.1% (postprandial model), respectively, indicating good group separation. Twenty-three metabolites - including glycerophospholipids and triacylglycerols (TG) contributed strongly to NW and Ob group separation in both fasting and postprandial states. Of these 23 metabolites, TG(16:0_32:3) and TG(16:1_34:2), significantly associated with lead SNP rs914869 located on chr20:47751375 and mapped to the SULF2 gene (beta=0.57, P=4.03x10-8 and 2.04x10-8, respectively). SULF2 has been associated with increased HDL lipid levels, BMI, body fat, and metabolic syndrome. Plasma concentrations of TG(16:0_32:3) and TG(16:1_34:2) were higher in Ob (padj<0.01).

Plasma metabolite profiles differentiate individuals with obesity with some triglycerides associated with strong genetic effects. These results support the involvement of SULF2 in lipid metabolism, which may contribute to metabolic disorder development.

Poster 32

Examining the effectiveness of AhR ligand combinations as novel therapeutics in triple-negative breast cancer

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Abstract

The Aryl Hydrocarbon Receptor (AhR) is a ligand-activated transcription factor with biological roles in regulating development, xenobiotic metabolism, cell cycle progression, and cell death. Previous work in the Kolluri lab (Elson et al., 2023) has demonstrated that AhR activation by specific ligands can promote tumor suppression and growth inhibition in multiple cancer types. A screening of compounds was performed to identify potential synergies with previously identified AhR ligands. Several promising compounds were identified, with Compound 1 and Compound 2 being identified as the primary hits. Triple Negative Breast Cancer cells were treated with Compound 1 and 2, along with others, in combination with previously identified AhR ligands to examine their effectiveness and identify potential synergies. These combination treatments highlighted several promising synergies between compounds as well as several combinations that demonstrated additive effects. To examine the effect of combination treatment with Compound 1 on healthy cells, primary breast epithelial cells were treated with Compound 1 combinations. These combination treatments with Compound 1 were observed to have no detrimental effect on the primary breast epithelial cells, indicating that the effects of combination treatment are targeted and specific to cancerous cells.

Poster 33

Early sleep fragmentation in heterozygous 5xFAD mice is primarily seen in females

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Abstract

Alzheimer's disease (AD) is an incurable brain disease that is the most common form of dementia. Sleep alterations have long been associated with Alzheimer's disease (AD), but whether it is an early symptom or develops later remains unknown. The 5xFAD mouse model has five mutations, linked to familial (inherited) AD, which leads to amyloid overexpression. Amyloid beta protein is present by 1 month of age, but plaques do not form until 2-4 months. Based on early mitochondrial and electrophysiology changes, we hypothesized that 5xFAD heterozygotes (HET) would show behavioral alterations early in development. Het and wild-type (WT) littermates at 1, 2, 3, 4 and 6 months of age (n=5-7) were measured via previously validated non-invasive sensors for 12 hours (6pm-6am) to score 3-stage sleep/wake: wake, non-rapid eye movement sleep (NREM), and REM sleep. Mice were pair-housed by sex in home cages with a temporary insert enabling sight, smell, and sound interactions. Sleep was scored manually in 10-second epochs in Spike2 software. ANOVAs were performed between genotypes, sex and age. Alterations in sleep architecture were identified in Het animals as early as 1 month of age. Compared to WT littermates, Het mice exhibited increased sleep fragmentation and more microarousals. Effects were more pronounced in females than in males. Across ages, Het mice showed increased time awake and decreased sleep time, latency to rapid eye movement (REM) and total time in REM. Most genotype differences widened by 6 months of age. Given that transgene expression was evidenced by day 15 in 5xFADHets and escalated with age, the observed sleep dysregulation is likely reflective of the accumulation of soluble amyloid-beta oligomers. These results highlight early, sex-specific disruptions in sleep that may serve as preclinical markers of AD-related pathology.

Poster 34

Potential of sulforaphane supplementation for Alzheimer's disease and traumatic brain injury in *Drosophila melanogaster*

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Abstract

Objectives: Sulforaphane (SFN), a bioactive isothiocyanate derived from cruciferous vegetables, exhibits potent antioxidant and anti-inflammatory properties. As oxidative stress, neuroinflammation, and mitochondrial dysfunction are central to the pathology of neurodegenerative diseases and traumatic brain injury (TBI), we hypothesize that SFN mitigates these pathological processes and improves functional outcomes in *Drosophila melanogaster* models of Alzheimer's disease (AD) and TBI.

Methods: Transgenic flies expressing human Amyloid beta peptides (Aβ40 or Aβ42) were used to model AD pathology. Adult females were fed diets containing SFN (3.125 mg/mL) or control diets. Cognitive performance was assessed using a choice-based memory assay. In the TBI model, wild-type flies were pre-treated with or without SFN prior to closed head injury. Innate immune and inflammatory marker expression was determined using quantitative PCR (qPCR). SFN uptake and metabolism in flies were quantified via mass spectrometry.

Results: Mass spectrometry confirmed bioavailability of SFN in *Drosophila*, with metabolic profiles similar to those observed in humans. In the AD model, SFN treatment significantly improved memory performance in Aβ40-expressing flies but had no effect in the more pathogenic Aβ42-expressing line. qPCR analysis revealed that SFN suppressed the expression of inflammation-related genes in AD brains. In the TBI model, injury induced robust upregulation of these genes. The effects of SFN on post-injury phenotypes and inflammatory gene expression is ongoing.

Conclusions: SFN improved cognitive performance and attenuated neuroinflammatory gene expression in Aβ40-expressing flies, suggesting its therapeutic potential in early or less severe AD pathology. Ongoing analysis in the TBI model will determine whether SFN similarly mitigates injury-induced inflammation. These findings support further investigation of SFN as a dietary intervention targeting neuroinflammatory pathways in both chronic and acute neurodegenerative conditions.

Poster 35

Aryl hydrocarbon receptor inhibits liver cancer cell growth *in vivo* via global suppression of protein synthesis

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Abstract

The aryl hydrocarbon receptor (AhR) is an environment-sensing transcription factor that translocates from cytosol to nucleus upon binding to its ligand. The AhR has emerged as a promising drug target in cancer therapy because of its tumor-suppressive functions. Here, we report an anti-tumor effect of a novel AhR ligand 4,11-dichloro-7H-benzimidazo[2,1-a]benzo[de]iso-quinolin-7-one (DiCl-BBQ) in multiple hepatocellular carcinoma cells. DiCl-BBQ significantly inhibited cell proliferation at submicromolar concentrations and induced sustained growth arrest even after the compound was removed. Using inducible shRNA and CRISPR-Cas9 systems, we confirmed that DiCl-BBQ's antiproliferative effects were AhR-dependent. Mechanistically, DiCl-BBQ induced G1 cell cycle arrest without triggering apoptosis and suppressed cell division as evidenced by CM-DiI and BrdU assays. An AhR antagonist CH223191 abrogated the inhibitory effect of DiCl-BBQ further confirming that the agonistic ligand requires the AhR for tumor suppression. Proteomic profiling revealed marked downregulation of translation-related proteins, including ribosomal components and initiation factors, corroborated by reduced puromycin incorporation in SUnSET assays. Functional enrichment analysis identified RNA metabolism, translation, and ribonucleoprotein biogenesis as key affected pathways. Additionally, DiCl-BBQ modulated p53-associated proteins and epigenetic regulators, suggesting a role in irreversible growth arrest. Importantly, DiCl-BBQ-treated HepG2 cells barely proliferated *in vivo* in zebrafish xenograft model. Taken together, AhR agonist DiCl-BBQ is a promising lead for targeted cancer therapeutics with a sustained effect through its capability of suppressing cellular protein synthesis in AhR-high hepatocellular carcinoma.

Poster 36

The rationale for "MitoG" a mitochondrial glutathione analog to slow or prevent neurodegenerative diseases

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Abstract

Mitochondrial dysfunction and oxidative stress are common early features of virtually all neurodegenerative diseases. A key consequence of this dysfunction is the depletion of glutathione (GSH), a crucial mitochondrial antioxidant and detoxifying agent, which further accelerates disease progression.

Efforts to restore mitochondrial GSH levels have long been explored as a potential strategy to prevent or slow neurodegenerative diseases. However, replenishing mitochondrial GSH remains a challenge. Since GSH is synthesized in the cytoplasm and transported into mitochondria via non-specific carriers – which often become impaired – current approaches, such as administering GSH precursors, fail to effectively restore mitochondrial GSH levels.

To address this issue, we propose a novel approach to maintaining mitochondrial GSH in neurological disorders. We hypothesized that targeting GSH delivery directly to mitochondria could overcome existing replenishment barriers. To achieve this, we developed MitoG – a GSH analog conjugated to triphenylphosphonium (TPP), designed to accumulate in mitochondria. Testing MitoG in 5xFAD Alzheimer's mice revealed promising results: improved mitochondrial morphology, enhanced cristae integrity, and increased hippocampal neuronal connectivity. These findings suggest that MitoG represents a potential therapeutic strategy for neurodegenerative diseases.

Poster 37

Subcellular changes at 1 month of age primarily localized to the CA1 region of the hippocampus in 5xFAD heterozygote

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder involving gradual loss of memory and executive function, marked by degeneration of brain regions critical for memory such as the hippocampus. A hallmark of AD is the accumulation of amyloid plaques, aggregates of beta-amyloid protein, that define the disease pathology. AD places a substantial burden on individuals, families, and healthcare systems. With an ageing population, AD's impact is expected to increase significantly. The 5xFAD mouse model carries five familial AD mutations, develops amyloid plaques around 2-4 months, and exhibits cognitive decline by 4-6 months. Most studies focus on stages after plaque deposition, leaving pre-plaque changes prior to 2 months largely unexplored.

We examined the hippocampus of 1-month-old 5xFAD heterozygous (Het) mice to identify early functional and subcellular changes before amyloid plaque formation. Quantitative PCR confirmed significantly higher 5xFAD transgene expression in CA1 enriched tissue at 1 and 3 months compared to wild type and 0.5-month-old Hets. Electrophysiological recordings from the Schaffer collateral CA1 pathway revealed hyperexcitability, with female 5xFAD Hets showing increased AMPA receptor and GluN2B responses compared to wild type. Golgi-Cox staining combined with Sholl analysis showed reduced dendritic complexity and length in CA1 pyramidal neurons of 5xFAD Hets, without significant changes in CA3 or DG. Electron microscopy revealed altered mitochondrial morphology within CA1 synaptic compartments. Spatial transcriptomics showed CA1-specific gene expression changes related to mitochondrial function, oxidative phosphorylation, and synaptic signaling, while changes in CA3 and DG were not significant.

These findings demonstrate synaptic, dendritic, and mitochondrial alterations in the CA1 region before amyloid deposition at 2-4 months, and the CA1 was more affected than other hippocampal areas. This refined timeline offers opportunities to explore mechanisms and targets for early intervention. Understanding these changes may guide approaches to prevent or slow disease progression and improve cognitive outcomes.

Poster 38

Toll-like receptor-mediated repression of vitamin D-induced cathelicidin antimicrobial peptide gene expression may involve NF-κB

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Abstract

Cathelicidin antimicrobial peptide gene, CAMP, defends against a range of bacterial diseases. Vitamin D receptor (VDR) binding to a vitamin D response element in the CAMP gene promoter in the presence of 1,25-dihydroxyvitamin D3 [$1,25(OH)_2D_3$] induces expression of the CAMP gene in both humans and primates, particularly in macrophages. To evade immune responses, numerous pathogens have devised ways to decrease the production of CAMP. The activation of toll-like receptors (TLRs) 2, 3, and 4 can suppress $1,25(OH)_2D_3$-induced expression of CAMP in human peripheral blood-derived macrophages by an unknown mechanism. Since macrophages are critical for innate immune responses against a variety of pathogens, we propose that CAMP suppression could affect infectious disease pathology in patients.

To identify the mechanism by which TLR signaling suppresses CAMP expression, we implemented a methodical CRISPR-Cas9 gene knockout approach using PMA-differentiated THP1 macrophages, beginning at the cell surface and progressing toward key intracellular mediators. We first targeted TLR4, which responds to LPS and strongly suppresses vitamin D-induced CAMP expression in both primary and PMA-differentiated THP1 macrophages. We then targeted the two primary adaptor proteins, MyD88 and TRIF, of TLR4 and IRF3, a transcription factor activated by TRIF.

Disruption of TLR4, TRIF, and MyD88 alleviated the suppression, but IRF3 knockouts still displayed suppression of CAMP gene expression in the presence of $1,25(OH)_2D_3$. Taken together, these data suggest that NF-κB may mediate the suppression of vitamin D-induced CAMP expression, because NF-κB is a central transcription factor activated by both MyD88- and TRIF-dependent pathways. Currently, we are generating NFKB1 knockouts to determine its role in suppression. Future work will focus on determining the mechanism by which this occurs and extending this to primary macrophages. Our findings will further our understanding of host-pathogen interactions which could inform clinical evaluation and improve outcomes for infectious disease patients.

Poster 39

Sex hormones and micronutrient absorption: A review of the influence of estrogen and progesterone across the menopausal transition

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Abstract

Micronutrient absorption and retention are tightly regulated by hormonal signals, yet the relationship between sex hormones – particularly estrogen and progesterone – and nutrient absorption impact remains underrecognized in clinical nutrition. This narrative review explores the current evidence on how estrogen and progesterone modulate micronutrient absorption and metabolism, and how these processes are altered during menopause. Literature was sourced through PubMed, Google Scholar, and Medline, focusing on studies that examined hormonal influence on intestinal absorption of select nutrients.

Estrogen has demonstrated a key role in enhancing calcium and magnesium absorption, supporting vitamin D metabolism, and maintaining gut integrity – all of which contribute to optimal micronutrient utilization. Progesterone has shown complementary roles, including effects on protein metabolism and gut motility that may indirectly impact nutrient assimilation. However, less is known about other integral micronutrients during the menopausal transition. Concurrent age-related changes, such as reduced gastric acid production and altered gut microbiota, further exacerbate micronutrient malabsorption, even in the presence of adequate intake. Calcium, magnesium, iron, and vitamin B_{12} micronutrient dynamics are explored in this review.

Understanding these mechanisms is clinically valuable for anticipating nutrient insufficiencies and designing proactive dietary strategies for the perimenopause and menopause transition. Functional signs of nutrient decline – such as bone loss, fatigue, cognitive change, and mood disturbance – may often reflect underlying hormonal shifts rather than dietary inadequacy alone. Highlighting the link between hormonal status and micronutrient dynamics reinforces the importance of dietary quality across the lifespan and suggests a preventive strategy.

Poster 40

L-Ascorbic acid enhances catecholamine synthesis in differentiated SH-SY5Y cells

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Abstract

L-ascorbic acid (ASC, vitamin C) is a redox-active micronutrient essential for monooxygenase function and maintenance of oxidative balance. Subclinical ASC depletion, affecting up to 30% of the population, may disrupt neurotransmission by impairing monoamine biosynthesis and elevating oxidative stress. In rodent models, ASC deficiency reduces cortical and striatal dopamine (DA) and its metabolites, correlating with behavioral phenotypes indicative of dopaminergic dysfunction. Clinically, ASC supplementation demonstrates antidepressant properties, while also potentiating selective serotonin and norepinephrine reuptake inhibitors. We hypothesized that ASC enhances catecholamine synthesis through transcriptional regulation of biosynthetic enzymes. We employed SH-SY5Y neuroblastoma cells differentiated with 10 0x000B5M all-*trans*-retinoic acid over 7 days as a model of human catecholaminergic neurons. Catecholaminergic identity was confirmed with RNA sequencing. Following 24-hour exposure to 0, 50, 100, 200, or 500 0x000B5M ASC, intracellular monoamines and their metabolites were quantified via high-performance liquid chromatography. ASC induced a concentration-dependent increase in intracellular DA and norepinephrine. Elevated levels of 3-methoxytyramine and dihydroxyphenylacetic acid further support enhanced synthesis. No significant effects were observed in serotonin or its metabolite 5-hydroxyindoleacetic acid, underscoring catecholamine selective enhancement. Quantitative PCR revealed ASC-dependent upregulation of phenylalanine, tyrosine, and tryptophan hydroxylases, but not of catabolic genes including monoamine oxidase A, aldehyde dehydrogenase 2, catechol-O-methyltransferase. No effects were observed in the dopamine synthetic enzyme, aromatic L-amino acid decarboxylase or the norepinephrine synthetic enzyme dopamine-beta-hydroxylase. This data supports ASC enhancement of catecholamine biosynthesis via transcriptional up-regulation of rate-limiting synthetic enzymes, rather than suppression of degradation pathways. This mechanistic insight provides a cellular basis for ASC's neurochemical effects and supports its potential utility as an adjunctive therapy in catecholaminergic disorders.

Poster 41

Defining the link between per- and polyfluoroalkyl substances (PFAS), developmental neurotoxicity, and gut microbiome assembly in zebrafish

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are a large class of synthetic chemicals extensively used in modern consumer products and increasingly detected in the environment. Mounting evidence suggests PFAS exposure may contribute to neurodevelopmental pathologies, yet the underlying mechanisms remain incompletely understood. We hypothesize that PFAS-induced dysbiosis of the gut microbiome may alter gut-microbe assembly, potentially influencing neurodevelopment and behavior. In previous work, 58 unique PFAS were tested for effects on zebrafish embryos. Zebrafish behavior was observed at 24 hours post-fertilization (hpf) and 120 hpf using a photomotor tracking system. We found 20 PFAS caused abnormal larval zebrafish behavior suggesting changes in their developmental neuropathologies. In the present study, we focused on these 20 PFAS to investigate whether PFAS exposure alters gut microbiota assembly and, in turn, contributes to behavioral changes. We then applied 16S rRNA gene sequencing to fecal samples from the same exposures to profile gut microbial communities. Notably, 11 of these 20 PFAS significantly disrupted the gut microbiome by reshaping community assembly. Our findings underscore the potential role of the gut microbiome in mediating the adverse effects of PFAS on neurodevelopment. Ongoing work seeks to clarify the different connections that link PFAS-induced gut dysbiosis and host behaviors, ultimately guiding the development of more sophisticated screening pipelines to identify key factors that contribute to neurodevelopmental outcomes.

Poster 42

LC-MRM-MS profiling of oxylipins and fatty acids in human milk after hydrolysis

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Abstract

Human milk is a primary source of nutrition for newborns, especially during the first six months of life. Polyunsaturated fatty acids (PUFAs) in human milk can be oxidized to generate oxylipins, which play key roles in regulating various biological processes, including inflammation, pain response, and vascular permeability. Human milk also contains fatty acids, which are energy sources and membrane constituents that influence cell and tissue metabolism, function, and responsiveness to hormonal and other signals. Here, we describe a well-established sample preparation method for the simultaneous extraction of oxylipins and fatty acids from human milk via basic hydrolysis. Two LC-MRM-MS platforms were applied to characterize oxylipins and fatty acids in human milk. Our results showed that the total number of oxylipins and fatty acids detected in human milk was 58 and 15, respectively, with good reproducibility (RSD < 15%). Except for TXB2-d4 = 20%, PGE2-d4 = 0 % and PGD2-d4 = 0.04%, the recoveries of each internal standard were greater than 80% with RSD < 10%. In order to investigate whether hydrolysis caused the low recoveries of the above three oxylipins, we spiked internal standards to the samples before and after hydrolysis. The results showed that TXB2 was extensively degraded during basic hydrolysis while PGE2 and PGD2 were completely degraded. Our data also indicate that the most abundant saturated, monounsaturated, and polyunsaturated fatty acids in human milk is palmitic acid (C16:0), oleic acid (C18:1 n9), and linoleic acid (C18:2), accounting for approximately 80% of total fatty acids. About 87% of the detected oxylipins were derived from linoleic acid, including 9-HODE, 13-HODE, 13-KODE, 9-KODE, 12(13)-EpOME, 9(10)-EpOME, 9(10)-DiHOME, and 12(13)-DiHOME. The sample preparation protocol described here has been successfully applied to the simultaneous extraction of oxylipins and fatty acids in human milk and may also be applicable to other biological samples.

Poster 43

LC-QTOF and LC-TIMS-qQTOF MS analysis of fecal inoculum biotransformation products and LC-MRM-MS analysis of human withanolide pharmacokinetics of an ashwagandha supplement

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Abstract

To analyze *in vitro* metabolism by the human gut microbiome and *in vivo* human plasma pharmacokinetics of constituents derived from an ashwagandha (*Withania somnifera*) supplement, liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (LC-QTOF), trapped ion mobility quadrupole time-of-flight mass spectrometry (LC-TIMS-QTOF-MS), and multiple-reaction-monitoring mass spectrometry (LC-MRM-MS) were employed. Phytochemical markers in an ashwagandha supplement were quantified by LC-MRM-MS (Waters Xevo TQXS). The supplement was extracted and subjected to *in vitro* anaerobic digestion (24 h) with fecal slurry from two donors in a medium (PBS supplemented with BHI broth, hemin, and vitamin K) simulating the gut environment. The same ashwagandha supplement was orally administered to a 72-year-old male. Selected withanolides were quantified in plasma samples collected over 12h post-ingestion using LC-MRM-MS. Preliminary results indicated a variety of *in vitro* transformations of withanolides following incubation with gut microbiota from human fecal inocula. Unsaturated carbocycles formed from conversion of epoxide functionalities, in addition to the α,β-unsaturated keto-functionalities of the A-ring and lactone ring, can serve as potential substrates for bacterial enoate reductases which results in saturated ring systems that could modulate bioactivity. Analysis also confirmed hydrolysis of glycosidic constituents, and sulfonation. In the human pharmacokinetic study, withaferin A, which is abundant in the ashwagandha supplement, was detected early in the plasma samples, attaining peak levels relatively soon after supplement administration. By contrast, sominone was only detected in plasma samples collected much later. The delayed time course suggests potential generation of sominone as a transformation product of other constituents in the supplement. Sominone has attracted attention due to its ability to enhance neurite outgrowth in cultured neurons and ameliorate cognitive decline in a mouse model of Alzheimer's disease. Taken together, our data suggest that sominone may be generated *in vivo* from precursor

molecules found in ashwagandha, potentially through transformation in the gastrointestinal tract.

Poster 44

Identification and quantitation of bioactive alkaloids in *Withania somnifera* using high-resolution mass spectrometry and multiple reaction monitoring

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Abstract

Withania somnifera (WS; ashwagandha) is a widely used botanical dietary supplement in the U.S., traditionally linked in Ayurvedic medicine to support relief of anxiety, stress, insomnia and age-related neurological changes. While withanolides are often credited for the biological effects of WS, the diversity and bioactivity of alkaloids in WS remain underexplored. This study uses high-resolution tandem mass spectrometry and multiple reaction monitoring for identification and quantitation of WS root alkaloids, separated by ultra-high performance liquid chromatography. The workflow addresses key analytical challenges such as low-abundance compounds, isobaric interferences, and limited standards. Fragmentation patterns aided in identifying structurally related alkaloids, and a rapid extraction protocol enabled reproducible quantitation. Acetyltropine and other previously unreported tropane alkaloids were confirmed, supporting their future discovery in WS and similar botanicals.

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Understanding glial neuropeptides: Processing, release, and function

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Abstract

The nervous system is primarily comprised of two cell types, neurons and glia. Together, these cells are responsible for proper nervous system function, including sensory processing and cognition. Although much is known how neurons communicate, less is known about the molecular mechanisms of glial cell communication and their contribution to neuronal functions. This project aims to identify the role of glial neuropeptides and the mechanisms that glial cells use to process and release neuropeptides. We use *Caenorhabditis elegans (C. elegans)* as a model organism to study glia-neuron interactions because of its simplified nervous system (302 neurons and 56 glia), conserved cell functions, and genetic tractability. We have previously identified a glial-specific neuropeptide, *nlp-16*, whose function is currently unknown. To determine the function of *nlp-16*, we are using an *nlp-16* null line, which will be tested with stress reporter lines and in a variety of behavioral assays (lifespan, chemotaxis, brood size, dietary and environmental changes, etc.). Additionally, previous work has shown that *C. elegans* glia cells do not express the canonical neuronal neuropeptide processing and release genes. By snRNA-seq, we identified two proprotein convertases, kpc-1/FURIN and bli-4/PCSK-6 in our dataset and confirmed bli-4 expression in a subset of glia, suggesting a role in neuropeptide processing. Additionally, *in vivo* analyses show that UNC-31/CAPS, a calcium-dependent dense-core vesicle secretion factor essential for neuropeptide release in neurons, is not present in glia. This finding suggests that glia rely on a fundamentally distinct, non-canonical mechanism for neuropeptide secretion. Building on candidates identified in our snRNA-seq dataset and leveraging the expression of glial *nlp-16* as a tool, we are investigating how glial neuropeptides are processed and released through an alternative macromolecular pathway. Determining the mechanisms through which glia process and release neuropeptides may provide clues about how glial cells contribute to nervous system communication and function.

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Spatial metabolomics reveals tissue-specific metabolic reprogramming during *Drosophila*-parasitoid interactions

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Abstract

To understand how infections reshape host metabolism at the tissue level, we developed an integrated liquid chromatography-trapped ion mobility-time-of-flight mass spectrometry (LC timsTOF MS) and matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) pipeline to map whole-organism metabolic responses in parasitoid-infected *Drosophila melanogaster* larvae. Unlike conventional homogenization approaches, our method preserves spatial complexity to pinpoint where immune-relevant metabolic changes occur.

We first identified infection-induced metabolic changes through untargeted LC timsTOF MS by comparing naive larvae to those at 6- and 18-hours post-infection (hpi) with *Ganaspis hookeri.* These temporal profiles guided our selection of candidate metabolites (lipids) for spatial analysis. After optimizing cryosectioning protocols, we validated tissue architecture using hematoxylin and eosin (H&E) staining, confirming intact fat body, gut, and hemocyte structures. Initial MALDI-MSI runs on the Bruker timsTOF fleX system now detect spatially resolved metabolites, though we continue refining sectioning consistency.

Our spatial data allow us to directly link metabolic shifts to specific tissues: our data show alterations in lipids and other metabolites in immune cell-rich regions during infection. By correlating LC timsTOF MS profiles with MALDI-MSI maps and eventually incorporating genetic perturbations, we will determine how localized metabolic changes contribute to defense. This work not only establishes *D. melanogaster* as a premier model for spatial metabolomics but also creates an adaptable platform for studying metabolic signaling in development, disease, and stress responses.

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How does amyloid beta contribute to healthy immune function in *Drosophila melanogaster*?

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Abstract

Alzheimer's disease (AD) is one of the leading causes of death in the United States; as the population continues expanding, the number of people diagnosed with AD will continue to rise. Amyloid beta (Aβ), a key protein in AD pathogenesis, is thought to naturally function as an immunopeptide released in response to infection or injury. The immune hypothesis of AD posits that the failure to clear Aβ may result in AD. In a *Drosophila* model of infection, we hypothesize that Aβ acts as an opsonin to direct immune activity to sites of infection or injury.

To test the contribution of Aβ to the immune response, we used *Drosophila melanogaster* to genetically manipulate levels of *Drosophila* amyloid beta (dAβ) and the amyloid precursor protein-like (APPL) and evaluated their ability to respond to parasitoid wasp infection. Parasitoid wasps infect the developing *Drosophila* larvae, and we can dissect infected larvae to track immune activity against wasp eggs. We tested the ability of APPL and dAβ mutant or overexpression flies to resist infection and found that APPL mutant flies exhibit an impaired immune response and capsule formation in response to infection by parasitoid wasps. Notably, the expression of dAβ rescues this response.

To better understand the role of dAβ in infection, we dissected eggs from fly larvae at specific time points following infection and stained them with Congo red to visualize amyloids. Under polarized light, the stain reacts via birefringence when amyloids are present. We detected birefringence on the surface of the developing wasp egg from the dAβ lines prior to the encapsulation, indicating that dAβ and immune cell activity co-localize. Our data suggests a natural function of Aβ is to act as an opsonin and that its aggregation may activate immune cells responsible for inflammation seen in AD.

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Characterization of parasitoid wasp neprilysin-like proteins for amyloid-beta degradation

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Abstract

Neprilysin (NEP), a transmembrane metallopeptidase, has garnered attention for its potential pharmacological application in the treatment of Alzheimer's Disease (AD) and cardiovascular disease (CVD). Neprilysin possesses a large ectodomain present within the M13 family of metallopeptidases. NEP's active site coordinates a zinc ion, facilitating peptide cleavage. NEP targets a variety of substrates, including Amyloid-beta (Aβ), a protein associated with AD. Targeted treatment of CVD involves inhibition of NEPs to prevent excess degradation of natriuretic peptides, while proposed AD treatment would involve increased Aβ-targeted protease activity. This dichotomy presents the need for specialized NEPs that can selectively target and degrade Aβ. Our lab has identified neprilysin-like proteins in 3 parasitoid wasp species, some of which are predicted to possess altered function based upon preliminary comparative genomics data. These parasitoids reproduce by infecting the fruit fly *Drosophila melanogaster*. Upon infection, *Drosophila* employs a cellular immune response to melanize and encapsulate the wasp egg. In turn, the wasp counteracts this by delivering virulence factors during infection to prevent encapsulation. Several of these wasp NEP-like proteins (wNEPs) are present in the wasp venom, which are found to degrade host-expressed Aβ. This project aims to characterize the proteolytic activity of these wNEPs, which could alleviate the pre-existing dichotomy in the NEP-targeted treatment of AD. Here, we have begun cloning 5 wNEP genes found in the parasitoid *Ganaspis hookeri* into expression vectors using Gibson assembly. Upon transformation, they will be transfected into *Drosophila* S2 cells for expression and purification. Once consistent, high-yield expression of wNEP in S2 cells is obtained, we will begin investigating the activity of these proteins as it pertains to their substrate specificity, ability to degrade Aβ peptides, as well as immune signaling peptides in *Drosophila melanogaster*.

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FDA - Not allowed!

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Abstract

The Food and Drug Administration (FDA) was set up in 1906 for consumer protection from unsafe and mislabeled food and drugs. The Dietary Supplement Health and Education Act (DSHEA) was passed on 1994. It amended the Federal Food, Drug, and Cosmetic Act, establishing a specific regulatory framework for dietary supplements, distinguishing them from drugs. DSHEA aimed to ensure consumer access to a wide variety of dietary supplements while providing consumers with more information about their intended use. In the current regulatory environment, the health benefits of food and supplements are substantially neglected while the products of the pharmaceutical industry corral the attention of both doctors and patients. I am writing a book about the many problems because of these regulations which increase medical costs and human suffering. This poster lists the topics of the book, and summarizes the challenges we face in educating the public about the benefits of diet, exercise, and environment while trying to avoid legal challenges. Chapters describe my initial FDA experience in 1994 when I found the optimum dosage of vitamin C, 500mg twice a day. Then I discuss FDA challenges when educating people to reduce the risk for strokes, cancer, arthritis, ulcers, baldness, muscle injury, acupuncture, herbal remedies, protein misfolding, weight loss, sunscreen, depression, and nutritional supplements. Reducing medical expense and human suffering through nutrition education is a goal I have shared with the Linus Pauling Institute since 1984.

Poster 50

Reimagining nutrition education: The information nutrition framework

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Abstract

BACKGROUND: The healthcare landscape has shifted from information scarcity to overabundance, creating a critical paradox: dietetic students can have "a head full of data but lack the cognitive frameworks to metabolize it." Just as consuming nutrients without proper digestion leads to malnutrition, acquiring information without processing frameworks leads to professional inadequacy. Current graduates suffer from information "indigestion"-able to recite guidelines and calculate macros but struggling to make adaptive decisions when faced with complex, real-world clients. With generative AI making information recall obsolete, this educational model becomes unsustainable.

PROPOSED SOLUTION: Transform education from an Information Economy to a Digestion Economy, shifting focus from content delivery to information utilization. This metacognitive approach teaches problem-solving through five iterative steps: (1) organize information, (2) prioritize through discernment, (3) make decisions under uncertainty, (4) reflect on outcomes, and (5) refine approaches.

INFORMATION NUTRITION FRAMEWORK: This framework addresses "What information do you actually need?" through five integrated layers that build professional competence:

- Technical Knowledge: Understanding numbers as tools, not rules (e.g., "1800 kcal/day" is a starting point, not a prescription)
- Lived Experience: Recognizing practical realities (a meal plan may be nutritionally perfect but culturally inappropriate or financially unfeasible)
- Situational Analysis: Probing for context-specific factors (food access, cooking skills, family dynamics, work schedules)
- Pattern Recognition: Drawing on previous cases to inform current counseling approaches
- Social Understanding: Delivering expertise with appropriate uncertainty, empathy, inquiry, and collaborative spirit

Each layer reveals how seemingly sufficient information (lab values, diet recalls, BMI) requires multiple dimensions of understanding to become clinically actionable.

PROPOSED TEACHING TOOLS: Implementation uses case-based scenarios where students develop actual counseling scripts for real-world situations. Example: "A newly diagnosed diabetic patient shows you their glucose log and asks 'Why are my numbers still high when I'm following the diet?'" Students must navigate incomplete information, probe for hidden factors

(medication timing, stress, sleep, food literacy), recognize patterns, and craft therapeutic responses-moving from "diet technician" thinking to "nutrition therapist" practice.

TEACHING GOAL: Develop professionals who function as "nutrition detectives" capable of information digestion, not just information recall. Success is measured by patient outcomes and behavior change, not knowledge scores. This approach creates dietitians who can adapt to emerging evidence, navigate ambiguity, and deliver value in an AI-augmented world where problem-solving trumps information retrieval.

CONCLUSION: The Information Nutrition Framework represents a fundamental shift from treating dietetic education as information provision to developing cognitive digestive systems. By teaching students that "guidelines are tools, not rules," we prepare professionals who can metabolize complex information into personalized interventions. This framework becomes increasingly critical as generative AI handles information retrieval, leaving dietitians to excel at the uniquely human skills of synthesis, cultural sensitivity, and adaptive counseling in complex, ambiguous clinical situations where the "right" answer depends entirely on the human being sitting across from them.

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Reimagining nutrition education with generative AI: The information microbiotic framework

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Abstract

BACKGROUND: Current nutrition education utilizes AI as sophisticated calculator, an efficiency tool for faster information retrieval. This mirrors our historic misunderstanding of gut microbiota as mere digestive aids rather than essential partners. Just as we cannot properly digest food without our microbiome, students cannot digest information in our oversaturated environment without symbiotic support. Humans simultaneously process food, information, and emotions-all requiring collaborative digestion, not isolated consumption.

CURRENT APPROACH: Universities position AI as an answer machine, accelerating student "indigestion": more information, delivered faster. Students use AI to eliminate struggle, replacing thinking with retrieval. This "antibiotic approach" weakens cognitive digestion, just as antibiotics impair gut health. We create professionals who can recall but cannot understand, apply, or synthesize.

PROPOSED SOLUTION: Reimagine AI as cognitive microbiome-a symbiotic partner in information digestion. Calculators provide solutions where one exists. AI provides options where multiple exist. Humans understand what each partner can/cannot provide, choose appropriate solutions for specific cases, and remain accountable for decisions. No single partner has the solution-solutions emerge from collaboration.

INFORMATION MICROBIOTIC FRAMEWORK: Four pillars build professional symbiosis:

- Digestive Partnership: Humans provide frameworks for mechanical digestion; AI processes continuously (like bacteria 24/7); humans synthesize wisdom from digested material
- Mutual Dependency: Humans provide nutritional frameworks; AI helps digest overload using these frameworks
- Cognitive Stability: AI maintains consistency when humans become overwhelmed
- Emotional Resilience: AI offers infinite patience without judgment; humans provide purpose and ethics

IMPLEMENTATION: Case-based scenarios position AI as stable partner. Example: "Diabetic patient becomes irate during consultation-how could AI support you?" AI serves as: emotional regulation partner with difficult clients, cognitive support for perplexing symptoms, time management ally under pressure, and judgment-free practice partner.

CONCLUSION: The formula: Human+AI symbiosis < Human+AI-as-tool. Like our microbiome, AI becomes essential for digesting complexity into practical solutions, creating professionals who thrive in information abundance.

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OSU Mass Spectrometry Center: Capabilities for nutrition, dietary supplement and biomedical research

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From discovery to public health impact: Research support services and educational outreach at the Linus Pauling Institute

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SimpleStep technology enables rapid measurement of proteins, PTMs, small molecules, and transcription factor activity

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Abstract

Accurate quantitation and characterization of proteins and small molecules is essential in our pursuit of optimal human health. Enzyme-linked immunosorbent assays (ELISAs) are an important tool for the quantification of proteins and small molecules in a biological sample; however, typical ELISAs suffer from lengthy protocols, high variability and limited validation. SimpleStep ELISA technology addresses these limitations by offering researchers 90-minute, one-wash, sandwich ELISA assays in both 96 and 384-well formats, compatible with automated liquid handling systems. Each assay is rigorously validated to pass linearity, recovery, and precision experiments to ensure accuracy and reproducibility. Batch-to-batch consistency is ensured through our recombinant monoclonal antibodies. SimpleStep ELISAs have evolved beyond protein quantitation and are now available for 1.) quantitation of protein post-translational modifications (PTMs) 2.) competitive SimpleStep (cSSE) ELISAs for small molecule and peptide quantification 3.) semi-quantitative measurement of transcription factor activity. PTM SimpleStep ELISAs allow researchers to quantify modified proteins against a purified standard. Recent releases include Tau phosphorylated at T217, T231, S396, and S199 and p53 phosphorylated at S15 and S392, and acetylated at K384. Additional targets are currently in development. In addition to PTMs we now support researchers in the quantification of small molecules. The competitive SimpleStep ELISA applies SimpleStep technology to the competitive ELISA format, allowing for a single-wash, 90-minute competitive ELISA for the quantitation of small molecules. Current kits include Prostaglandin E2, kanamycin, Mouse IgG1, gentamicin, 3-nitrotyrosine, cortisol, HIS-tag, cAMP, and cGMP with more targets currently in development. Lastly, we have developed a semi-quantitative transcription factor activity ELISA platform. This platform uses oligonucleotide-coated plates containing consensus sequences for transcription factor binding. Activated transcription factors bind the oligo and are detected via HRP-conjugated monoclonal antibodies, enabling a 90-minute, single-wash readout of transcription factor activation. To date, over 1,700 SimpleStep ELISA kits are available for protein quantitation, alongside 16 PTM kits, 12 competitive kits, and one transcription factor activity kit. New assays are released monthly, expanding the toolkit for researchers seeking fast, reliable, and scalable biomolecular quantitation.