Abstract Index

BEHAVIORAL

Early and Slow Weight Loss Responders' Perceptions of a Worksite Diabetes Prevention Trial Featuring an Adaptive Intervention: A Qualitative Perspective

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Background: Behavioral weight loss interventions demonstrate success on average; yet, some participants respond more slowly and may benefit from an augmented, tailored approach. The current study implemented an adaptive intervention for slow weight loss responders in a worksite Diabetes Prevention Program.

Objective: The purpose of this qualitative analysis was to understand participants' perceptions of the 12-month behavioral weight loss program, experiences implementing behavioral intervention strategies, and recommendations for future intervention implementation.

Methods: Adults ≥ 21 years old with overweight or obesity and prediabetes (n=39) at a Midwestern university were recruited and volunteered for a semi-structured interview. Individual interviews were conducted post-treatment. In-person interviews were offered prior to the SARS CoV-2 pandemic; videoconferencing interviews occurred following pandemic onset.

Results: Data were thematically analyzed to identify recurring themes among early and slow weight loss responders. Social support received from weekly group sessions was a critical intervention component. Some early slow responders expressed greater difficulty establishing specific yet achievable goals and requested additional study contact prior to the 6-month maintenance phase. Randomization at month five disrupted group dynamic. Slow responders who received the intervention via videoconference believed the format interfered with their ability to establish friendships with other participants and gain insight and support from others' lived experiences.

Conclusions: Multifaceted, structured, behavioral weight loss interventions should consider participant heterogeneity when implementing behavioral strategies. Findings suggest slow weight loss responders may benefit from greater emphasis on goal setting and striving, agency thinking, and social support. Future research is needed to examine the implications of these insights on intervention outcomes.

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Human milk feeding mode and duration among preterm toddlers

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Background: Infants born preterm encounter barriers to human milk feeding, especially at the breast. Infants fed exclusively pumped milk have been shown to receive human milk for fewer total months than infants who are also fed at the breast, yet research is needed to establish this phenomenon within preterm populations.

Objective/Hypothesis: In a secondary analysis of a randomized controlled trial among toddlers born <35 weeks' gestation, we explored the association between human milk feeding mode and duration.

Methods: Children were enrolled at 10-17 months of age (adjusted for prematurity) and caregivers reported children's feeding history. Children were classified as having been fed exclusively by pumping or by a combination of at the breast and pumping, during the first year of life. Linear regression was used to estimate the association between mode and total duration of milk feeding, adjusted for confounders. To explore whether severity of prematurity was an effect modifier, we tested an interaction with gestational age (<32 vs. ≥ 32 weeks).

Results: Mean (std) feeding duration of toddlers fed exclusively pumped milk (n=164) was 3.48 (3.68) months, whereas that of toddlers fed at the breast and pumped milk (n=155) was 6.78 (4.69) months (p<0.0001). Toddlers fed exclusively pumped milk had a shorter milk feeding duration, compared to those fed at the breast and pumped milk (β_{adj} = -2.46, 95% CI= -3.64, -1.27). The association between mode and duration differed for toddlers born <32 vs. ≥ 32 weeks' gestation, with β_{adj} = -4.23 (95% CI= -6.10, -2.37) and β_{adj} = -1.55 (95% CI= -3.17, 0.06), respectively (*P*-for-interaction =0.17).

Conclusions: Exclusive pumping was negatively associated with human milk feeding duration among infants born preterm. Severe prematurity exacerbated this association, underscoring the need to focus interventions to enhance initiation and maintenance of feeding at the breast among this high-risk group.

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Barriers to and Facilitators of Farmers' Markets Use Among Low-Income U.S. Households: A Qualitative Systematic Review

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Background: Food insecurity increases the risk of diet-related chronic disease among low-income populations due to persistent systemic health inequities. Short value chain (SVC) models of healthy food access—i.e., programs that connect consumers more directly to producers—may be one solution for addressing food and nutrition insecurity. Farmers' markets (FM) in particular have emerged with exponential growth as an alternative to conventional food retail, although systematic syntheses of literature on their utilization among low-income households in the United States (US) is limited.

Objective/Hypothesis: To characterize qualitatively-reported barriers to and facilitators of low-income households' participation in FM interventions.

Methods: As part of a larger systematic review analyzing quantitative outcomes of and qualitative engagement with SVC models of healthy food access, this presentation focuses on qualitative studies of FM engagement. Nine electronic databases were searched in June 2021 for full-text articles published from 2000-2020. Covidence and Excel were used for full-text screening and data extraction, respectively. Studies met the following inclusion criteria: data collected via focus groups or in-depth interviews and participants were low-income, based in the US, and had participated in a FM program. Risk of bias was assessed independently by two reviewers using the Standards for Reviewing Qualitative Research checklist. Results of included studies were uploaded to NVivo for thematic analysis.

Results: Of the 37 studies included in the parent systematic review, 17 met the inclusion criteria for analysis. Most studies were of "good" quality (11 good, 4 fair, 2 poor). Common barriers included 1) lack of awareness, 2) perceived cost, and 3) lack of convenience. Common facilitators included 1) supporting the local community, 2) produce quality, 3) financial incentives, and 4) values-based motivations.

Conclusions: To maximize engagement with and impact of FM interventions, current and future programs need to enhance convenience and engage in strategic marketing that leverages common facilitators and motivators of engagement.

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Rising Rates of Kidney Cancer in the United States: Variation Among States and the Relationship to Obesity, Hypertension, and Smoking

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Background: In the United States (US), renal cell carcinoma (RCC) is the 6th and 9th most common malignancy in men and women, respectively, and represents a compendium of several histopathologic subtypes.

Objective/Hypothesis: This study was to describe the variation in the incidence of RCC subtypes among states and its relationship to demographics and the prevalence of obesity, hypertension, and smoking between states.

Methods Age adjusted incidence rates (AAIR) for RCC in individual states during 2001-2019 were acquired from the Surveillance, Epidemiology and End Results Program (SEER) Database. Prevalence of obesity and current smoking (1991-2019), and hypertension (1991-2019) was obtained from the Behavioral Risk Factor Surveillance System (BRFSS). All data were aggregated at the state level. The correlation between AAIR for RCC subtypes and the prevalence of risk factors in individual states was determined by linear regression.

Results During 2001-2019 AAIR for RCC increased by 31% with a male:female rate ratio of 2.0. Black males experienced a higher incidence than whites, while Asian/Pacific Islanders showed a significantly lower risk. In 2019 the AAIR among states varied by 1.6-fold from a low of 11.8 (Hawaii) to a high of 19.3 (Louisiana). During this interval, the prevalence of obesity and hypertension continuously increased, while current smoking displayed a decreasing trend in both males and females. Linear regression analysis shows that the prevalence of obesity, current smoking, and hypertension in 2007-2009 in individual states are each significantly associated with the future incidence of RCC in 2017-2019.

Conclusions: RCC incidence varies significantly among states in the US, and is strongly associated with the prevalence of obesity, hypertension, and smoking. Efforts to control the critical risk factors of obesity and smoking while improving the management of hypertension are key to reducing the growing burden of RCC.

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CASE STUDY

Refractory Vitamin A Deficiency in Decompensated Cirrhosis: Assessment, Treatment, and Monitoring

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Background: Identifying and treating micronutrient deficiencies requires biochemical assessment, a nutrition-focused physical exam, and correlation with clinical picture. Once identified, few guidelines exist for treatment strategies. This case presents severe micronutrient deficiencies identified and treated by an interprofessional team in a hepatobiliary clinic.

Diagnosis: This 31-year-old female patient presents with a history of congenital biliary atresia and decompensated cirrhosis. The patient underwent a hepatoportoenterostomy at age 6 weeks to reconstruct bile ducts and restore bile flow. Biochemical assessment reveals severe vitamin A deficiency and suboptimal zinc status. Physical assessment reveals telogen effluvium and transient blurry vision. Past medical history includes alcoholism in remission, obesity class II, and major depressive disorder.

Treatment: A comprehensive review of the electronic medical record indicates this patient was continuously prescribed varying doses of oral and intramuscular vitamin A supplementation in inpatient and ambulatory settings. This includes history of high-dose oral vitamin A ranging from 10,000-50,000 units daily and a 3-day course of 100,000 units via intramuscular injection and co-treatment of zinc deficiency to ensure adequate circulating retinol-binding protein.

Uniqueness: Despite aggressive and consistent supplementation, normalization of serum retinol was not achieved. The pharmacokinetics of the administered vitamin A is unknown. Decompensated cirrhosis increases risk of fat maldigestion through altered bile synthesis and excretion through bile canaliculi. Maldigestion increases the risk of vitamin and mineral deficiencies, which when untreated contribute to consequential health issues such as metabolic bone disease, xerophthalmia, neurological complications, and hyperkeratosis.

Conclusion: In decompensated cirrhosis, there lacks sufficient guidelines for micronutrient dosing when traditional strategies are unsuccessful, as well as for strategies of prevention and treatment of deficiencies. Collaborations with pharmacy and medicine allowed for thorough assessment and establishment of a safe treatment and monitoring plan. Clinical research is needed to understand strategies for acceptable and safe dosing strategies for patients with chronic, unresponsive fat-soluble vitamin deficiencies.

CLINICAL TRANSLATIONAL

Mitigation of Salmonella Typhimurium in Nutrient Film Technique (NFT) hydroponic system for improved food safety and nutrition

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Background: Hydroponic farming systems play an increasingly vital role in the sustainable production of nutrient-rich food. Although there are food safety risks in hydroponic production, validated sanitation food safety protocols are lacking. The effectiveness of sanitizers in eliminating human pathogens from hydroponic surfaces, and their effect on crop quality and nutrient composition are unknown.

Objective/Hypothesis: This study aimed to evaluate the effectiveness of SaniDate (previously found sanitizer efficient against human pathogens) and pH-manipulated nutrient solution in eliminating the human pathogen *Salmonella* Typhimurium while conserving lettuce quality and nutritional attributes.

Methods: Using a randomized completed block design, *Salmonella* Typhimurium (LT2JSG626) was inoculated into NFT system with lettuce and treated with SaniDate12.0 (200ppm) or reduced pH (pH 4, 5). Crop health (yield, color) and nutrient concentrations (Provitamin A, lutein) were examined by weighing and using HPLC-DAD, respectively.

Results: SaniDate treatment significantly reduced lettuce yield (P=0.03). Compared with the non-treated control (193.2±15.2g/plant), the yield of treated crops was reduced by 57.2%. The weight did not significantly differ for pH treatments (P= 0.11). Compared to the non-treated control, bacterial reduction was observed across all treatment groups. SaniDate 12.0 was most effective in reducing the pathogen concentration on lettuce roots, while the pH 4 of nutrient solution resulted in the highest reduction in rockwool and on edible leaves ($p<0.004$). The lettuce quality measured Carotenoid levels were in the range of 18.4 mg/100g of dry lettuce, consistent with expected levels for the lettuce type.

Conclusions: The study provided information for producers and stakeholders to use in developing standards and protocols for hydroponic system sanitation and crop contamination prevention with human pathogens.

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Pharmacokinetics of tomato steroidal alkaloids in healthy adults following consumption of two doses of tomato juice

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Background: Steroidal alkaloids derived from tomatoes are gaining traction as a potentially health beneficial class of phytochemicals based on a growing number of *in vitro* and *in vivo* studies. Prior to investigating the bioactivity of tomato steroidal alkaloids (TSAs) in humans, understanding their pharmacokinetic behavior after absorption is important.

Objective/Hypothesis: The objective of this study is to elucidate the pharmacokinetics, that is to define the bioavailability and metabolism, of TSAs following a single tomato juice containing meal. I hypothesize that TSAs will be absorbed at similar levels compared to structurally related compounds with known absorption (e.g. ~5% for phytostrols) and that they will undergo extensive phase I and phase II metabolism.

Methods: Healthy subjects (n = 11, 6M/5F) participated in a randomized crossover trial where they consumed 94 g juice (low dose) and 505 g juice (high dose) with a two-week washout period in between doses. Blood samples were collected at 11 time points over 12-hours following test meal consumption, and plasma was isolated to be analyzed using UHPLC-QTOF-MS. Resulting chromatograms and MS/MS spectra were used for compound identification and quantification.

Results: Significant difference ($p < 0.05$) was observed in fractional absorption between the high (10% ± 5%) and low (5% ± 2%) dose of tomato juice. More than 90% of the quantified TSAs found in plasma have undergone either a phase I or phase II biotransformation in both interventions. Baseline-corrected area under the curve (AUC) values were calculated to estimate relative exposure of each metabolite, and it was found that dihydroxytomatidine, a phase I metabolite, was most abundant at 812.20 ± 157.44 nmol*h/L for the high dose and 60.97 ± 7.87 nmol*h/L for the low dose.

Conclusion: This study reports the first pharmacokinetic data for TSAs and provides context for future studies investigating the potential role that these compounds may play in human health.
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Ultraprocessed food intake and diet quality in preterm toddlers

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Background: The 2020-25 Dietary Guidelines for Americans (DGA) were the first to include recommendations for children aged 0-24 months. These guidelines do not include any recommendations on ultraprocessed foods (UPFs), which are ubiquitous in contemporary children's diets.

Objective/Hypothesis: Using baseline diet data from a dietary supplement trial of toddlers born preterm, we evaluated the correlation between toddlers' UPF intake and their diet quality as recommended by the DGA.

Methods: Children were enrolled at 10-17 months (adjusted for prematurity). At baseline, caregivers reported toddlers' past month diet using a food frequency questionnaire (FFQ). Items in the FFQ were classified as UPF using the NOVA classification system and % energy from UPFs was calculated. The Toddler Dietary Quality Index (TDQI), a diet quality score reflecting adherence to DGA recommendations, was also calculated. The TDQI has 14 components, and its total score ranges 0-100 with higher scores indicating better diet quality. We assessed correlations of UPF % energy with the TDQI and its components using Pearson correlation coefficients (r).

Results: Among the 333 toddlers included, the mean (SD) percent UPF intake was 37.6% (13.2) and the mean (SD) TDQI was 50.5 (9.9). There was a moderate and statistically significant negative correlation between the total TDQI score and UPF intake (r=-0.43, p<0.0001). The TDQI components with the strongest negative correlations were the added sugars (r =-0.42, p<0.0001), dairy (r =-0.39, p<0.0001), vegetables (r =-0.32, p<0.0001), and sodium (r =-0.24, p<0.0001) scores. In contrast, UPF intake was positively correlated with the TDQI's linoleic acid component (r=0.30, p<0.0001).

Conclusions: There is a moderate and statistically significant correlation between UPF intake and toddler diet quality. While the DGA do not provide specific recommendations on UPF intake, these results show that it may be difficult for toddlers to meet recommendations with a diet high in UPFs.

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Dietary Naringenin and Cancer Cachexia in Mice: Effects on Neuro-regulation of Mobility

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Background: Cancer cachexia, a progressive wasting of adipose and skeletal muscle, frequently occurs in cancer patients and is responsible for up to 20% of cancer related deaths. Many cancer therapies neglect treating reductions in physical activity, which are associated with increased risk of death. Naringenin is a phytochemical found in citrus fruits that has anti-inflammatory and anticancer activities and may promote physical activity.

Objective: This study will investigate whether naringenin can preserve or increase locomotor activity in mice with cancer cachexia and determine the relationship between hypothalamic and serum IL-1β levels and locomotor activity.

Methods: 24 male CD2F1 mice will be implanted with electronic telemetry transmitters prior to starting diets to continuously monitor locomotor activity. Mice will be fed a standardized diet supplemented with (NAR) or without (CON) 2% naringenin starting two weeks before being inoculated with 1x10^6 C26 adenocarcinoma cells (+) or PBS (-). Final groups will be 8 (NAR+), 4 (NAR-), 8 (CON+), 4 (CON-). IL-1β levels will be measured from hypothalamic tissue and serum using specific anti-mouse ELISAs.

Results: Our hypothesis is that tumor bearing mice fed naringenin will have significantly higher levels of locomotor activity compared to tumor bearing mice fed a control diet at the onset of cancer cachexia. We expect reductions in hypothalamic and serum IL-1β levels will be predictive of the preservation of locomotor activity.

Conclusions: If our hypothesis is correct, naringenin may be a promising adjuvant therapy that would warrant further investigation in future clinical trials involving patients with cancer cachexia.

Acknowledgements: This project will be supported by the Center for Advanced Functional Foods Research and Enterprise and the Ohio Agriculture Research and Development Center.

Cancer Activity and Lifestyle Measurement (CALM) Study: A Feasibility Study to Evaluate Lipidome Changes After Anthracycline Treatment in Women with Breast Cancer

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Background: Although anthracycline chemotherapy (AC) is highly effective in treating breast cancer, AC often has toxic effects in non-tumor, mitochondrial rich tissues including cardiac and skeletal muscle. AC inhibits topoisomerase 2 to impair the cell cycle and damages the inner mitochondrial membrane that is rich in cardiolipin (CL) phospholipid. Disruption of CL leads to heart failure and skeletal muscle weakness. The primary fatty acyl in CL is linoleic acid (LA), an essential fatty acid in the human diet.

Hypothesis/Objective: Hypothesis: LA-rich CL will be reduced in women after AC treatment. Objective: Measure CL species before and after AC treatment in peripheral blood lymphocytes (PBMCs) isolated from women with breast cancer. Both total and tetralinoleoyl-CL levels of PBMCs decreased after one cycle of AC. Dietary LA influences the effects of AC on CL and cardiac muscle physiology.

Methods: Women with stage I-III breast cancer who were planning with their oncology team to undergo AC treatment were recruited. Body anthropometrics were measured, and blood samples were taken before and after one cycle of AC treatment. Participants were asked to complete questionnaires regarding diet, physical activity, and quality of life at each visit.

Results: Using a spaghetti plot analysis, breast cancer patients' CL 1448 PBMC decreased following treatment of AC, while CL 1450 PBMC increased. The ratio of 1448:1456 PBMC also appears to decrease following treatment as well.

Conclusions: Using a fully powered cohort, a future study should evaluate whether higher LA is cardioprotective and supports cardiac and skeletal muscle function. These findings could also support testing the effect of including seed oils high in LA to improve outcomes for breast cancer patients undergoing AC.

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Effects of dietary linoleic acid on insulin sensitivity and preservation of adipose tissue and skeletal muscle in mouse model of cancer cachexia

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Background: Cancer is the second leading cause of death in the United States. 30-80% of cancer patients develop cachexia, a condition characterized by depleted adipose tissue and muscle wasting. Prior studies indicate that cancer cachexia is preceded by insulin resistance, and that linoleic acid (LA) improves insulin sensitivity in mice with cancer cachexia. However, the effect of improved insulin sensitivity on cancer cachexia has yet to be investigated.

Objective: This study will evaluate the effect of dietary LA on the development of cancer cachexia by measuring insulin sensitivity, body composition, mitochondrial function, and gene transcription changes in a mouse model.

Methods: In this 2x2 study design, 36 CD2F1 mice will be randomized into 4 groups, fed a control or a LA rich diet, and inoculated with 1x10^6 C26 adenocarcinoma cells or phosphate-buffered saline. Mice will be euthanized at onset of cachexia, defined here as a loss of more than 20% of the initial body weight. Body composition (echoMRI), insulin tolerance test, *in vitro* muscle strength, mitochondrial function in skeletal muscle, and gene regulation (RNA-sequencing) will be measured. Pre- and post-necropsy data on these measurements will be analyzed to identify the effects of LA using a 2-way ANOVA.

Results: It is hypothesized that tumor bearing mice fed on the an LA rich diet will have improved insulin sensitivity, resulting in preservation of adipose tissue, muscle strength, and higher muscle mitochondrial function, and delayed onset of cancer cachexia compared to their control peers. It is also expected that RNA-sequence data will reveal novel information on how LA modulates metabolic pathways, contributing a protective effect against cachexia.

Conclusions: If successful, study findings will contribute to future research on preventing cancer progression and improving the quality of life of cancer patients.

Acknowledgements: This project will be supported by the Ohio Agriculture Research and Development Center.

Associations of Inflammatory and Insulinemic Dietary Patterns with Colorectal Adenomas in the Prostate, Lung, Colorectal and Ovarian Cancer (PLCO) Cohort

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Background: Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the second leading cause of cancer deaths in the U.S**.** It is a multi-stage process, and most CRCs develop through the adenoma-carcinoma sequence. Though modifiable exposures like diet may play a major role in CRC etiology, the underlying mechanisms are not well understood. This study examined the association between dietary patterns called empirical dietary index for hyperinsulinemia (EDIH) score that affects circulating C-peptide, a marker of insulin resistance and empirical dietary inflammatory pattern (EDIP), a marker of inflammation with colorectal adenomas.

Objective/hypothesis: While these novel EDIH/EDIP dietary patterns have been strongly associated with CRC risk, how they may influence earlier stages of colorectal carcinogenesis has not yet been elucidated. Therefore, the objective of our study was to calculate the EDIP and EDIH scores at PLCO baseline and assess their associations with risk of colorectal adenomas. Additionally, we compared our results with the Healthy Eating Index (HEI-2015) that access overall dietary quality.

Methods: Utilizing 21,192 participants in (PLCO) Cancer screening cohort, we calculated EDIH/EDIP scores, and overall dietary quality measured via the healthy eating index (HEI-2015), from food frequency questionnaires (FFQ). In multivariable-adjusted logistic regression, we investigated associations of these dietary indices with adenoma, advanced adenoma (n=19,493), and recurrent adenoma (n=1,699).

Results: EDIH was not associated with adenoma or advanced adenoma but was marginally associated with recurrent adenoma. The OR (95%CI) comparing highest (lowest insulinemic) versus lowest (most hyper-insulinemic) quintiles was 0.74 (0.54, 1.02) after multivariable adjustment including BMI. In contrast, EDIP and HEI-2015 were not associated with any of the three outcomes.

Conclusions: In the PLCO cohort, we did not observe substantial associations between dietary patterns and risk of colorectal adenomas.

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Role of the Registered Dietitian Nutritionist in Supporting Pregnancy Outcomes in Women with a History of Bariatric Surgery: A Literature Review

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Background: Due to its restrictive and malabsorptive processes, metabolic and bariatric surgery (MBS) is associated with several nutritional deficiencies that must be closely monitored and treated. Due to increased nutritional requirements to support fetal development, pregnancy after MBS may heighten this risk for deficiency and lead to adverse birth outcomes. Registered Dietitian Nutritionists (RDN) can provide medical nutrition therapy that optimizes nutritional intake while reducing MBS-related gastrointestinal symptoms and manage micronutrient deficiencies that support maternal and fetal health. Though recommendations have been developed for the care of pregnant patients with a history of MBS, the role of the RDN is not described within these guidelines.

Objectives: This literature review will highlight consensus recommendations that can be applied by the RDN when caring for this population. Examples of how this may be implemented in clinical practice based on available evidence will also be presented.

Methods: Peer-reviewed articles using the following key words were located using the PubMed database: “bariatric surgery, “pregnancy,” “dietitian,” “micronutrient deficiency,” “vitamin D deficiency,” “zinc deficiency,” folate deficiency,” “thiamine deficiency,” and “cobalamin deficiency.” Systematic review articles, randomized trials, observational studies, and case reports were included.

Results: The consensus recommendations that fall within the RDN scope of practice include nutrition advice, nutrition monitoring, supplementation, and weight monitoring in the MBS population. Available evidence shows the value of the RDN in pregnant and MBS cohorts separately, but studies involving pregnant women with a history of MBS are limited.

Conclusion: Further research is needed on the influence of dietary-based interventions delivered by RDNs on maternal health and birth outcomes.

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Cross-sectional associations of serum fatty acids and self-reported cognitive outcomes in women with breast cancer undergoing chemotherapy

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Background: Chemotherapy-induced neuroinflammation may contribute to cognitive dysfunction. Dietary and serum polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) have been associated with inflammation and cognitive performance in elders. **Objective/Hypothesis**: The objective of this project was to determine if serum MUFAs and PUFAs were correlated with cognitive and mental health outcomes in women undergoing chemotherapy for breast cancer.

Methods: Serum samples and data on self-reported cognitive function were collected from 60 women undergoing adjuvant chemotherapy for breast cancer at baseline, just prior to the fourth cycle of chemotherapy (C4), and 6 months after chemotherapy (6M). Serum fatty acids (FAs) were measured using gas chromatography. Spearman's rank correlation was used to quantify the association between 7 serum fatty acids of interest and scores from the Multifactorial Memory Questionnaire (MMQ), Behavior Rating Inventory of Executive Function–Adult Version (BRIEF-A), UCLA loneliness scale (Loneliness), State and Trait Anxiety Inventory (STAI), and Center for Epidemiologic Studies Depression Scale (CES-D) at all 3 timepoints.

Results: Higher adrenic acid (C22:4n-6) was significantly associated with better MMQ scores (rho= -0.52, p=0.0027 for strategies subscale; rho=0.45, p=0.0092 for feelings subscale) and with a non-significant trend for better BRIEF-A scores (rho= -0.33, p=0.068) at 6M. A significant association was found between higher gondoic acid (C20:1n-9) and better MMQ feelings subscale (rho=0.38, p=0.028) at 6M. Non-significant trends were discovered between higher C20:1n-9 and better MMQ mistakes subscale (rho=0.32, p=0.073) and CES-D scores (rho= -0.34, p=0.051) at 6M

Conclusions: Overall, higher serum C20:1n-9 and C22:4n-6 were associated with better self-reported cognitive assessments in 60 women with breast cancer 6 months post-chemotherapy treatment. Future research will examine associations among serum fatty acids, inflammation, and cognitive function, adjusting for possible covariates.

Acknowledgments: Ohio State University Stephanie Spielman Breast Cancer Center Kroger Fund; The Ohio State University Center for Clinical and Translational Science

Diet and Nutritional Compromise During Multimodality Head and Neck Cancer Curative Treatment and its Relationship with Cognitive Dysfunction

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Background: Rigorous multimodality combinations of surgery, chemotherapy, and radiotherapy are often required to cure head and neck cancers (HNC). Therapy has significant acute toxicities impacting intake and nutritional status. In addition, long term survivorship issues are many and include the syndrome of cancer related cognitive impairment (CRCI). As such, we hypothesize that nutritional compromise during therapy is a factor potentiating neurological injury and compromising repair leading to CRCI. There are multiple nutrients and bioactive compounds in food such as carotenoids (lutein) and polyphenols in fruits and vegetables, among others, which are hypothesized to impact risk of neurological injury and repair. The intake of these food components has not been fully quantified during modern therapy for HNC.

Objective/Hypothesis: We will test the feasibility of implementing dietary assessments combined with cognitive testing into multimodality treatment plans for 30 HNC patients. We will obtain preliminary data regarding dietary patterns, specific foods, bioactive compounds, and nutrients associated with CRCI.

Methods: Dietary assessment and cognitive function will be obtained before therapy, at the completion of therapy, and 6 months after. Diet is assessed by 3-day diet record and food frequency questionnaires. The NIH Toolbox Cognition Battery is a series of questionnaires assessing specific domains of cognition. Biomarkers of nutritional status will include carotenoid assessment via skin scan, macular pigment scan, and HPLC analysis of blood. Signatures of malnutrition will be defined by metabolomic analysis.

Results: Recruitment and data collection is underway with a goal of 30 patients. **Conclusions**: Data collection will be completed by fall 2023 and analytics by spring of 2024. This study will demonstrate feasibility of nutritional and cognitive assessment in this cohort of patients undergoing complex multimodality therapy and provide preliminary data regarding dietary components impacting CRCI for testing in large studies with NIH funding.

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Daily Ingestion of Green Tea Extract Confection Effectively Increases Plasma Catechins without Affecting Systemic Inflammation in Healthy and Metabolic Syndrome Adults

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Background: Catechin-rich green tea extract (GTE) in obese rodents improve cardiometabolic health by decreasing systemic inflammation.

Objectives: We hypothesized that the anti-inflammatory activities of GTE would lower systemic inflammation in persons with MetS.

Methods: In a randomized, double-blind, placebo-controlled crossover study, MetS and healthy persons completed two 28-d interventions in which they received GTE confections (890 mg/d total catechins) or matched-placebo confections while following a low-polyphenol diet. On days 0 (PRE), 14 (MID), and 28 (POST), anthropometrics, 3-d food records, and fasting blood samples were obtained. Plasma catechins and valerolactones were measured by LC/MS. RNA extracted from whole blood at POST was used to assess the expression of pro-inflammatory genes using RT-qPCR. Data were analyzed by repeated measures ANOVA.

Results: MetS (n = 21; 40 ± 3 y) and healthy (n = 19; 34 ± 2 y) adults completed the study with high compliance (>95% of confections consumed) and no changes in body mass, no adverse effects, or changes in aminotransferases. Diet polyphenol intakes were lower ($P<0.0001$) at MID and POST regardless of health status and treatment. Plasma catechins and valerolactones were marginally detectable at PRE, but increased ($P\leq0.0001$) at MID and POST only in the GTE arm regardless of health status as expected. However, whole blood mRNA expression of CD14, MCP1, MD2, MyD88, MPO, IL6, IL8, TNFα, TLR4, p65 were unaffected by GTE.

Conclusions: Findings of this 1-mo intervention support that catechin-rich GTE confection supplementation safely increases circulating catechins and their microbial metabolites. Even though no changes were observed regarding the inflammatory responses at the mRNA level, underway measurement of these biomarkers at the protein level may provide more insights.

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Associations of specific fruits and vegetables with circulating biomarkers of inflammation, insulin response and lipids

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Background: Biomarkers in blood offer the opportunity to identify individuals at risk of disease processes related to specific causes of morbidity and mortality. Indeed, circulating biomarkers indicative of systemic inflammation are of particular interest. Understanding the relationships between the intake of bioactive rich fruits and vegetables and relevant inflammatory biomarkers related to disease in humans will help design interventions to improve health.

Objective: We tested the hypothesis that specific fruits and vegetables may uniquely influence health related biomarkers associated with inflammation.

Methods: We conducted a cross-sectional analysis of 36,643 postmenopausal women (Women's Health Initiative) with baseline (1993–1998). We calculated intakes of 39 fruits, vegetables (FV) and FV groups from food frequency questionnaire data and tested associations with 25 circulating biomarkers of inflammation. Multiple linear regression was used to estimate the percent difference in natural log-transformed biomarker concentrations per 1 standard deviation increment in individual fruits, vegetables or FV groups.

Results: Higher total fruit was associated with lower concentrations of CRP, TNFα and leptin. Several commonly consumed fruits such as apple, banana, dried fruits, and avocado have strong associations with these markers. Vegetables had greater impact on biomarkers than fruits, with higher total vegetables associated with lower levels of CRP, TNFα, TNFα-R1, TNFα-R2, and leptin. Associations ranged from -2.30 (TNFα) for dark-yellow vegetables to +53.9 (leptin) for French fries. Higher tofu intake was strongly associated with lower levels of CRP and leptin. While higher sweet potatoes and yams intake was associated with lower concentrations of CRP, IL6 and leptin, higher intake of French fries was associated with higher concentrations of these markers.

Conclusion: We observe that diets rich in fruits and vegetables are associated with reduced biomarkers of inflammation that are associated with chronic disease processes. In addition, specific fruits and vegetables have unique patterns of impact on specific biomarkers.

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Milk Fat Globule Membrane-Enriched Dairy Milk Does Not Adversely Affect Fasting or Postprandial Cardiometabolic and Intestinal Biomarkers in Adults with Metabolic Syndrome

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Background: Dairy milk-derived milk fat globule membrane (MFGM) has anti-inflammatory activities in preclinical models and infants but has received limited study in adults. We hypothesized that an MFGM-enriched dairy milk beverage (MEB) would alleviate endotoxemia-associated inflammation in adults with metabolic syndrome (MetS).

Methods: In a 2-arm, randomized, crossover trial, persons with MetS (n = 24; 37.3 ± 2.0 y) were provided 3 daily servings of MEB formulated with 2.3 g/d of milk-phospholipids and butter fat or a matched comparator beverage (COMP) formulated with soy lecithin and palm/coconut oils (75:25) for 2-wk while following a prescribed diet; para-aminobenzoic acid (PABA) was added to each beverage. Circulating endotoxin, glucose, insulin, incretins, and triglycerides were measured spectrophotometrically pre- and post-intervention and for 3-h postprandially at 2-wk after consuming a high-fat/high-carbohydrate meal that contained non-digestible sugar probes. mRNA expression of pro-inflammatory genes were assessed from whole blood. Fecal calprotectin and myeloperoxidase were evaluated by ELISA. Urine was collected for 24-h post-intervention to assess gut permeability by LC-MS.

Results: Participants completed the study with no adverse effects and with high compliance based on spot urine PABA increasing by 6-times, on average, throughout each arm compared with pre-study concentrations. Between-treatment energy and macronutrient intakes did not differ. Fasting concentrations of endotoxin, glucose, incretins, and triglyceride were unaffected at post-intervention, and meal-induced increases in these markers did not differ between treatments. Expression levels of 6 different pro-inflammatory genes from whole blood were unaffected by test beverages. Markers of gut health, including fecal calprotectin and myeloperoxidase, as well as urinary excretion of gut sugar probes, did not differ between treatments.

Conclusions: MEB did not alleviate endotoxemia or improve gut barrier function, metabolic health, or inflammation in persons with MetS. This suggests that MFGM-enriched dairy milk does not adversely impact cardiometabolic health compared with a beverage formulated with plant-derived phospholipids and oils.

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Elucidating the Role of Provitamin A and Vitamin A in influencing Metabolic Disease Risk

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Background: Mendelian randomization approach indicated evidence of causality between genetically regulated plasma concentrations vitamin A-related biomarkers (β-carotene, retinol, and retinol binding protein 4 (RBP4)), and metabolic syndrome (MetS). However, some of these effects may be more directly mediated via other vitamin-A related metabolites and proteins, not measured previously. It is unclear if provitamin A supplementation can influence concentrations of vitamin-A and MetS pathology related metabolites and/or concentrations of proteins.

Objective: To determine which additional vitamin-A related metabolites and proteins may be associated with incidence of MetS and if long-term provitamin A supplementation can alter their concentrations.

Methods: Pilot samples from the Physician's Health Study (PHS) and Women's Health Genome Study (WGHS) were selected from subjects who are healthy (n = 8 men, 8 women) and subjects who developed MetS (n = 8 men, n = 8 women). PHS subjects provided samples at enrollment and follow-up, and WGHS subjects samples are from baseline only. Blood plasma samples will be extracted using a biphasic method, with the nonpolar extract analyzed using a targeted Ultra Performance Liquid Chromatography- Mass Spectrometry (UPLC-MS/MS) method to quantitate beta-carotene, retinal, retinoic acid, retinol, phase I metabolites of retinoic acid. The non-polar and polar extracts will be analyzed using UPLC-HRMS lipidomics and metabolomics approaches, respectively. Blood plasma proteomics, providing relative blood concentrations of 1500 proteins, will be performed by SomaLogic, Inc. Logistic regression will be used to evaluate the influence of the totality of the "omics" data collected on the diagnosis of MetS.

Expected Results: Increase in blood concentrations of retinol and retinoic acid and/or decreases in phase I metabolites, in MetS subjects relative to controls. The participants supplemented with pro-vitamin A will have higher blood concentrations of retinol and retinoic acid as compared to the placebo.

Acknowledgements: We thank Chris Pfeffer and his team for sample selection.

MOLECULAR/BASIC

Carotenoid encapsulation increases astaxanthin ester and lutein ester bioaccessibility and Caco-2 cell uptake

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Background: Fat-soluble nutrient encapsulation with a mixture of lecithin and medium chain triglycerides can improve solubility in aqueous food products. However, it is uncertain if such an encapsulation approach will improve bioaccessibility and intestinal cell uptake of carotenoid esters. It is also not known spray-drying of this product (producing a powder) influences bioaccessibility and cell uptake of carotenoid esters, relative to the aqueous-based product.

Objective: We sought to determine if novel encapsulation of carotenoid esters improves bioaccessibility and Caco-2 cell uptake, relative to a non-encapsulated carotenoid ester oleoresin control.

Methods: Lutein and astaxanthin ester-rich oleoresins were tested separately in these experiments and served as our positive controls. They were tested with an aqueous encapsulated product(VitaSperse) and powdered encapsulated product(VitaDry) formulation by digesting with 100 mg soybean oil. *In vitro* gastric, duodenal, and jejunal digestion were mimicked for 1 hour each, with cholesterol ester lipase added during the intestinal phase. Following digestion, the micellular fraction was isolated, and mixed with media, and then incubated with differentiated Caco-2 cells for 4 hours at 37 °C. Chyme, micelle, and cell extracts were made using nonpolar solvents, and analyzed using high performance liquid chromatography-diode-array detection equipped with a C30 column. External calibration curves were used for quantitation. ANOVA followed by Tukey's post-hoc test was used to evaluate differences(P<0.05 considered statistically significant).

Results: Vita products increased astaxanthin bioaccessibility 2-2.5x, relative to control. VitaDry and VitaSperse improved astaxanthin cell uptake 2.2x and 1.9x, respectively, compared to control. The Vita products also increased lutein bioaccessibility by 2.4-3x, compared to control. The Vita products also improved lutein cell uptake by ~1.9x as compared to control(with no difference between VitaDry and VitaSperse).

Conclusions: The novel encapsulation approach not only functions to improve lutein- and astaxanthin-ester solubility, but also to increase bioaccessibility and Caco-2 cell uptake in an *in vitro* model of digestion.

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Effects of encapsulation on Iron Chlorophyllin (IC) stability during in-vitro digestion, and iron uptake by Caco-2 cells

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Background: Iron fortification of staple crops is a strategy employed to combat iron deficiency in developing countries. Iron chlorophyllin (IC), which has a structure like heme, may provide a more bioavailable source of iron compared to organic iron. Our group observed that the solubility of IC under gastric and intestinal digestive conditions remains limited in the absence of protein. IC has a green color, which may be unappealing as a fortificant in staple crops beige in color. Our collaborators have proposed to encapsulate IC to mask its color and taste, and this encapsulation may influence IC solubility during digestion and iron uptake by Caco-2 cells.

Hypothesis: Encapsulating IC increases its solubility during *in vitro* digestion and increases Caco-2 cell uptake and iron delivery.

Methods: Encapsulated IC, non-encapsulated IC and $FeSO_4$ will be subjected to in vitro digestion. Gastric and small intestinal digestion will be done at a pH of 2 and 6.5 at 37 °C for 60 and 120 min, respectively. Each product digesta will be diluted with media and incubated for 4 h at 37 °C with differentiated Caco-2 cells, in addition to a set cell treated with media alone (negative control). The digesta will be removed and the cells incubated with media alone for additional 20 h to facilitate ferritin synthesis. Total iron and ferritin concentrations will be evaluated using Atomic Absorption Spectrometry and a ferritin ELISA assay, respectively. One-way ANOVA followed by Tukey post-hoc test will be used to determine significant differences among the treatments ($P< 0.05$).

Expected Results: We expect IC encapsulation to produce a product which is more water soluble than non-encapsulated IC after digestion, deliver more cellular iron compared to non-encapsulated IC and $FeSO_4$. These first tests are an important step to establish if IC can be used as a successful fortificant to combat iron deficiency in developing countries.

The Role of High Phosphate Diet on Muscle Metabolism

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Background: Inorganic phosphate (Pi) is commonly used as a preservative and flavor enhancer in the Western diet. Physical inactivity, a common feature of Western societies, is associated with increased cardiovascular morbidity and mortality. It is unknown if excess dietary Pi contributes to exercise intolerance and physical inactivity.

Objective/Hypothesis: We hypothesize that a high Pi diet negatively affects muscle function and metabolism, resulting in decreased exercise capacity.

Methods: We used clinical data and human blood samples from Dallas Heart Study-2 (DHS-2) participants to study the relationship between serum Pi levels and physical activity. To determine direct effects of dietary Pi on exercise capacity, we measured oxygen uptake, serum non-esterified fatty acid (NEFA), and glucose during exercise treadmill test in mice fed either a high Pi or normal Pi diet for 12 weeks. To study the effects of Pi on muscle metabolism and expression of genes involved in fatty acid metabolism, we cultured differentiated C2C12 myotubes in media containing 1-3mM Pi to simulate in vivo Pi conditions.

Results: We found that higher serum Pi levels were independently associated with reduced moderate-to-vigorous physical activity and increased sedentary time in DHS-2 participants. In mice, high Pi diet reduced oxygen uptake, treadmill duration, spontaneous locomotor activity, and fat oxidation. In skeletal muscle, high Pi diet led to downregulation of genes involved in fatty acid synthesis, release, and oxidation, and upregulation of genes involved in glucose metabolism. Similar results were recapitulated in C2C12 myotubes with high Pi media.

Conclusions: Our findings demonstrate a detrimental effect of excess dietary Pi on skeletal muscle fatty acid metabolism and exercise capacity. In the future, we plan to investigate the mechanisms by which Pi regulates gene expression, and the potential reversibility of these effects. Dietary Pi may represent a novel and modifiable target to reduce physical inactivity associated with the Western diet.

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Dietary milk fat globule membrane improves neonatal piglet intestinal architecture and enhances mucosal diamine oxidase activity following lipopolysaccharide challenge

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Background: Early life stressors alter the gut development trajectories and increase disease risk throughout the life span. Enteric diseases increase morbidity and mortality in livestock and human infant. Dietary milk fat globule membrane (MFGM) has beneficial effects on promoting gut health. However, mechanisms of actions are still being defined and the role of MFGM in mitigating gut function during physiological stressors needs further investigation.

Objective: The objective is to define the effect of MFGM supplementation on neonatal piglets growth performance and intestinal barrier function during acute systemic lipopolysaccharide (LPS) challenge.

Methods: Thirty-two one-day-old piglets were assigned to either soy or MFGM phospholipid supplemented diet (0.75% w/w) ± LPS challenge in a 2 × 2 factorial design (n = 8/trt). Piglets were fed for 21 d and 8 animals/dietary treatment received either saline or LPS injections 4 h prior to euthanasia. Growth performance was recorded daily and gain to feed intake ratios were calculated. Intestinal villus height (VH) and crypt depth (CD) were evaluated by haematoxylin and eosin staining. Diamine oxidase (DAO) activity, a biomarker of intestinal integrity, was measured in the jejunal, ileal, and colonic mucosa.

Results: Supplementation of MFGM had no adverse impacts on growth performance ($P > 0.05$). Dietary MFGM increased VH (soy: 348.8 μm and MFGM: 427.0 μm) and decreased CD (soy: 166.3 μm and MFGM: 147.4 μm) in ileum, thereby increasing the VH to CD ratio (soy: 2.2 and MFGM: 3.1) ($P < 0.05$). Further, there was a 2.9-fold increase in colon DAO activity with MFGM diet compared to soy-fed pigs, regardless of LPS challenge ($P < 0.05$). Additionally, LPS treatment decreased jejunal mucosal DAO activity by 46.7% compared to saline treatment ($P < 0.05$).

Conclusions: Dietary MFGM improved neonatal piglet ileum architecture and increased colon DAO activity, potentially attenuating the intestinal barrier disruption following LPS challenge.

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The Effects of Myostatin Mutation on the Tibia Bone Quality in Male and Female Japanese Quail

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Background: Using the CRISPR/Cas9 system, myostatin (MSTN) mutant quail was generated in our laboratory to demonstrate increased muscle mass and decreased fat deposition by MSTN mutation in the avian species. Although improved bone quality had also been reported in MSTN mutant mice, the effect of MSTN mutation on avian bone quality had not been investigated, yet.

Objective/Hypothesis: It is hypothesized that the bone quality of MSTN mutant birds may be improved compared to wild-type (WT) controls.

Methods: Tibia bones were collected from MSTN mutant and WT male quail at 4 months old, and females at 5 weeks and 4 months old. Using micro-Computed Tomography (micro-CT) scanning, bone sizes and diaphyseal and metaphyseal quality parameters were compared between MSTN mutant and WT groups. In addition, bone breaking strength (BBS) were measured to further confirm the quality measured by Micro-CT scanning.

Results: MSTN mutant groups showed longer, wider, and thicker tibia bones compared to WT groups regardless of sex and age. In addition, bone quality of males at 4 months old and females at 5 weeks old were significantly improved by MSTN mutation as shown in higher BBS, bone mineral density, bone mineral contents (BMC), and bone volume in diaphyseal and metaphyseal regions of tibia bones. In females at 4 months old, higher total volume (TV) and surface in metaphysis and higher BMC and TV in diaphysis of MSTN mutant group compared to those of WT group indicated remaining positive effect of MSTN mutation on bone quality, despite the physiological change by egg production after sexual maturation.

Conclusions: The MSTN mutant quail model provided new insights into positive regulation of bone size and quality by genetic mutation in male and female avian species.

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Retinol Binding Protein 7 Promotes Adipogenesis *in vitro* and Regulates Expression of Genes Involved in Retinol Metabolism

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Background: Retinol is an essential nutrient in animals. Its metabolites, specifically retinoic acid (RA), are crucial for cell differentiation, including adipogenesis. Retinol binding protein 7 (Rbp7) is under the control of PPARγ, the master regulator of adipogenesis. However, the role of RBP7 in adipogenesis is unclear.

Objective/Hypothesis: All RBP proteins, that are responsible for retinol delivery and intracellular transport, demonstrated their effects on lipid metabolism in knockout models. However, until now, it is unclear whether RBP proteins can influence RA production and functions in the cytosolic or nuclear compartment of adipocytes. Therefore, our study aimed to understand cellular functions of Rbp7 in adipocytes.

Methods: To analyze tissue specific expression of Rbp7, databases were obtained from the NCBI and confirmed by western blot analysis and qPCR. In this study, 3T3-L1 cells were used as an *in vitro* model to overexpress or knock-down Rbp7 and induced adipogenic differentiation. Differentiated adipocytes were stained by Oil-Red-O and the levels of triglycerides were quantified. In addition, luciferase assay was applied to measure RARE activity.

Results: Rbp7 overexpression promoted 3T3-L1 preadipocyte differentiation with increased triglyceride accumulation and up-regulation of pro-adipogenic genes. Rbp7 deficient adipocytes had opposite effects of the overexpression, which were rescued by RA supplementation. Indirect assessment of relative nuclear RA levels using RAR response element (RARE)-Luc reporter assay demonstrated that Rbp7 overexpression significantly increased RARE-Luc reporter activity. Rbp7 overexpression significantly increased expression of Raldh1, responsible for RA production, and up-regulation of Lrat and Cyp26a1, involved in retinol storage and RA catabolism, respectively, in 3T3-L1 cells. Rbp7 deficient adipocytes had opposite effects of the overexpression of those genes involved in retinol metabolism.

Conclusions: RBP7 increases transcriptional activity of RARE that may induce negative feedback responses via regulation of the gene expression for retinol homeostasis.

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Voluntary Exercise Does Not Prevent High-Phosphate Diet-Induced Mitochondrial Dysfunction in Mice

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Background: Inorganic phosphate (Pi) is used extensively as a preservative and flavor enhancer in the western diet. We previously reported that excess dietary Pi has detrimental effects on skeletal muscle function and metabolism that is independent of obesity and cardiac contractile function. We demonstrated that mice fed high-Pi diet (HP) for 12 weeks displayed decreased maximal exercise capacity and spontaneous locomotor activity. In skeletal muscle, HP diet led to downregulation of genes involved in fatty acid synthesis, release, and oxidation, and upregulation of genes involved in glucose metabolism. Consequently, mitochondrial function was significantly impaired in skeletal muscle from mice fed HP diet.

Objective/Hypothesis: Voluntary exercise prevents the negative consequences of HP diet.

Methods: We fed mice a normal-Pi (NP) or HP diet and housed them in voluntary wheel running cages for 12 weeks. Physiological parameters, exercise capacity, mitochondrial respiration, and gene expression (qPCR) were measured throughout the study.

Results: HP diet did not alter body weight or composition, and NP- and HP-fed mice voluntarily ran the same distance throughout the study. We observed no differences in medium-intensity exercise capacity between NP- and HP-fed mice subjected to voluntary wheel running. Despite preserved exercise capacity, we found that mitochondrial function in muscle from HP-fed mice was significantly decreased after 5 weeks, and mitochondrial function continued to decline after 12 weeks of HP diet and voluntary exercise. To investigate mechanisms of HP-induced mitochondrial dysfunction we measured expression of key metabolic genes and discovered that fatty acid metabolism genes were significantly decreased in muscle from mice fed HP diet and subjected to voluntary exercise.

Conclusions: Exercise does not prevent the negative metabolic consequences of consuming a high-phosphate diet. Future studies will investigate the mechanisms by which Pi regulates gene expression. Dietary Pi may represent a novel and modifiable target to reduce physical inactivity associated with the Western diet.

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Identification of Novel Genes Involved in Zinc Homeostasis Using a Fission Yeast Model System

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Background: Zinc is an essential nutrient and both deficiencies and toxicities are implicated in chronic diseases. In humans, the abundance of many mRNA transcripts is dependent upon zinc, but the mechanisms of these changes are largely unknown. To gain insight into how gene expression is altered by cellular zinc status, here we use yeast as a model organism to identify genes essential for zinc homeostasis.

Objective/Hypothesis: In the fission yeast *Schizosaccharomyces* pombe, we have found that *sod1* mRNA and protein levels are regulated by zinc and that this regulation is independent of transcription factors known to regulate *sod1*, and of *loz1*, the major known regulator of zinc homeostasis in *S. pombe*. My goal is to identify regulatory regions and genes which are necessary and sufficient for zinc-dependent regulation of *sod1*.

Methods: To identify *sod1* regulatory regions, I generated reporter constructs and analyzed their activity in cells grown in varying zinc levels. I also used RNA blot and immunoblot analysis to measure *sod1* mRNA and protein abundance in *sod1* truncation strains grown in varying zinc levels. To identify genes required for zinc-dependent *sod1* regulation, *sod1* mRNA and protein abundance were examined in deletion strains grown in varying zinc levels.

Results: *sod1* mRNA and protein abundance is lower in zinc-deficient cells compared to zinc-replete cells. These zinc-dependent differences were dependent upon the *sod1* 3' UTR and were lost in deletion strains lacking the RNA-binding protein Zfs1 or components of the Ccr4-Not complex. A reporter construct driven by a 1.8 kb *sod1* promoter did not show significantly different activity in cells grown in low and high zinc.

Conclusions: *sod1* mRNA and protein levels are dependent upon zinc. Full zinc-dependent regulation is dependent upon the *sod1* 3' UTR, the RNA- binding protein Zfs1, and the Ccr4-Not complex.

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Lipocalin-2 Expression Changes Gut Microbiome Composition and Diversity, and Promotes Tumor Growth in a Mouse Model of Pancreatic Ductal Adenocarcinoma

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Background: Pancreatic Ductal Adenocarcinoma (PDAC) is a deadly disease. Lipocalin-2 (Lcn2) is a pro-tumorigenic, bacteriostatic molecule, increased in the serum and tumor of PDAC patients. PDAC patients experience microbial dysbiosis which can worsen tumor outcomes. Understanding whether Lcn2 modulates bacteria and PDAC outcomes could generate novel therapies for patients.

Hypothesis: Lack of systemic and tumor derived Lcn2 will decrease PDAC growth and improve microbiome composition and diversity.

Methods: *Lcn2* expression was deleted via CRISPR from PDAC cells ($KRas^{G12D}$/$Trp53^{-/-}$/PDX-1-CRE; mKPC) to generate $Lcn2^{+/+}$ and $Lcn2^{-/-}$ mKPC cells that were injected orthotopically into WT and Lcn2 KO mice. Serum Lcn2 was measured via ELISA and *Lcn2* gene expression via RT-qPCR. Stool was collected before and after PDAC. Microbial DNA extraction and shallow shotgun genome sequencing were performed using the Qiagen DNeasy 96 PowerSoil Pro kit and the Illumina NovaSeq instrument. Data was analyzed using One Codex.

Results: Mice that lack of either tumor or host Lcn2 had less tumor growth compared to control, however tumor weights at endpoint were similar among all groups. Serum and tumor Lcn2 were highest in WT mice with $Lcn2^{+/+}$ mKPC cells followed by WT mice with $Lcn2^{-/-}$ mKPC cells. Serum Lcn2 levels correlated positively with tumor growth. Tumor *Lcn2* expression modulated microbiome composition in WT mice. After PDAC, beta-diversity differentially clustered WT mice by their tumor *Lcn2* expression, and Gene Ontology analyses showed an upregulation in iron binding processes, glutamate and fatty acid synthesis in mice injected with mKPC $Lcn2^{+/+}$ cells.

Conclusions: Presence of both host and tumor Lcn2 promotes PDAC growth. Lcn2-induced microbiome modulation indicates crosstalk between tumor *Lcn2* and the gut. Additionally, overexpression of LCN2 in PDAC could explain some of the microbial dysbiosis seen in patients.

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Effect of Novel Mild Electric Processing Technologies on Orange Juice Micronutrient Content

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Background: Traditional food processing approaches usually employ high heat which reduces the concentration of heat-labile nutrients and can alter the organo-leptic properties of the finished product. It is hypothesized that novel processing approaches, like moderate electric field (MEF) may inactivate microorganisms and enzymes in fruit juice while also retaining nutrients and flavor molecules. However, impacts of MEF treatment on nutrients, especially heat-sensitive compounds like ascorbic acid and carotenoids, in orange juice has not yet been tested.

Objective: To study the effect of shear stress(SS) alone and MEF+SS treatments on carotenoids including beta-carotene and vitamin C (ascorbic acid) concentrations in orange juice.

Methods: Orange juice was treated with either SS or SS+MEF at 3 temperatures, 27, 40 and 50°C, over 10 min, with samples taken at 0, 2.5, 5, 7.5, 10 min. Each condition was tested in triplicate. Samples were vortexed and extracted using a bi-phasic liquid-liquid approach. Methanol was used to denature proteins and metaphosphoric acid was added to acidify the polar phase, while a mixture of hexane/acetone to extract out the carotenoids in nonpolar phase. A C18 column will be used to quantitate the ascorbic acid, and a C30 column used to quantitate the carotenoids, in conjunction with high-performance liquid chromatography-diode array detection. External calibration curves will be used for quantitation.

Anticipated Results: Decreases in vitamin C are expected to result with increasing temperature applied to the juices regardless of SS or MEF + SS treatment. No change overall in carotenoids is expected regardless of treatment SS or MEF + SS treatment.

Anticipated Conclusions: We anticipate that MEF will demonstrate better at conserving heat sensitive nutrients relative to more traditional thermal processing parameters. This would indicate it has a potential application in food industrial to reduce nutrients loss during food processing, and thus contribute to human health.

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Estimation of brewers' spent grain (BSG) composition by mid-infrared spectroscopy

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Background: Brewers' spent grain (BSG), a major by-product that accounts for 85% breweries waste, is an excellent source of dietary fibers and proteins. There is a growing interest in exploring BSG as an ingredient in foods. It is essential to determine the composition of BSG to get desired nutritional profile. However, the conventional wet chemistry methods are time consuming, labor intensive, and costly. In this study, we investigated the application of mid-infrared spectroscopy for quantifying nutrients in BSG.

Objective/Hypothesis: Mid-infrared spectroscopy can be an alternative to conventional wet chemistry methods for quantification of BSG components.

Methods: The proteins, carbohydrates, and lipids in BSG samples were also determined using reference wet chemistry methods. Fourier Transform Infrared (FTIR) spectrometer was used to scan the BSG samples at a wavenumber range of 4000 to 700 cm^{-1}. The absorbance spectra from the scans were analyzed using supervised classification technique like soft independent modeling of class analogy followed by partial least squares regression for developing the calibration model.

Results: The amount of components in BSG samples obtained from reference methods agreed with published values. A calibration model was developed for each nutrient. The developed models had a standard error of validation less than 0.5%. The correlation coefficient of validation and calibration were above 0.90. These results suggest that the spectroscopy can provide fast and reliable results for estimating BSG composition within few seconds.

Conclusions: An accurate measurement of composition is essential for nutrition labels. The studied FTIR spectroscopic method was easy to use for rapid quantification of nutrients without any need of sample preparation or training. The quality of developed prediction models needs to be periodically validated to check the accuracy of values. A cost economics also need to be considered for application of FTIR spectrometers in the analytical labs due to the high initial investment involved.

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Consumption of a Ketogenic Diet enhanced Vitamin B6 Metabolism pathway but did not Inhibit Tumor Growth in an Obesity-Associated PDAC Mouse Model

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Background: Pancreatic ductal adenocarcinoma (PDAC) has a dismal 5-year survival rate due to ineffective treatments. Obesity, which can be the result of western dietary patterns, is a recognized risk factor for PDAC. Thus, developing effective weight management strategies is essential to decrease obesity, and potentially mitigate PDAC risk. The high-fat, low-carbohydrate ketogenic diet (KD) promotes weight loss and is a promising therapeutic strategy in some cancer preclinical models by altering tumor metabolism.

Hypothesis: The KD will disrupt cancer metabolism, promote weight loss, and delay tumor growth in obesity-associated PDAC.

Methods: C57BL/6 mice were fed an obesogenic high-fat diet (HFD) for 15 weeks to induce obesity, after which were randomized to a control diet (CD), a KD, or continued in the HFD for 7 weeks. After, mice were orthotopically injected with mouse KRas^{G12D}/Trp53$^{-/-}$/PDX-1-CRE (mKPC) cells containing the firefly luciferase gene. Tumor growth was monitored through In Vivo Imaging System. Body weight, glucose tolerance tests, and ketone body (KB) measurements were performed at different timepoints. Tumors metabolomics were analyzed by Liquid Chromatography Mass Spectrometry (LC-MS).

Results: Mice gained weight after 15 weeks of an obesogenic diet. Mice fed a KD and a CD significantly lost weight after the diet interventions. Mice fed a KD maintained increased KB concentrations and increased glucose tolerance after tumors. However, none of the dietary interventions decreased tumor growth. Tumors showed significant enrichment in metabolic processes, such as, vitamin B6 metabolism in mice fed a KD compared to HFD group.

Conclusions: Although the KD induced weight loss and altered tumor metabolism, it was insufficient to decrease tumor growth in an obesogenic PDAC mouse model. However, vitamin B6 has shown antitumor properties in other mouse models. Understanding the availability and functions of vitamin B6 metabolism induced by a KD, could indicate its potential as an intervention for obesity-associated genetically engineered mouse models of PDAC.

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Evaluating the Toxin Binding Capacity of Iron Chlorophyllin (IC)

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Background: Humans are often exposed to low concentrations of toxins in improperly stored or prepared food (i.e. aflatoxin B1 (AFLB1), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), carbaryl) or water (i.e. bisphenol A (BPA), carbaryl). One approach to reduce toxin exposure is the co-consumption of structure which binds the toxin during digestion and facilitates excretion. The porphyrin backbone of chlorophyll, the green pigment in vegetables, can be modified with heat and acid to chelate copper, zinc, or iron instead. These metallo-chlorophyll derivatives are robust to digestion, and the ability of copper and zinc derivatives to bind a variety of toxins to reduce DNA damage and facilitate excretion has been reported before in multiple models (i.e. in-vitro systems, in trout, and in humans). However, a limited number of toxins have been tested with iron chlorophyll derivatives, and thus its toxin binding potential is largely unknown.

Objective/Hypothesis: To determine the ability of iron chlorophyllin (IC) to bind to AFB1, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), carbaryl in an aqueous medium.

Methods: Increasing IC concentrations (0, 10, 20, 30, 40, 50, 75, and 100 μM) were tested in an aqueous medium containing AFB1 (10 μM), MeIQx (563 nM), BPA (11 μM), and carbaryl (248 nM). Doses of toxin were chosen to reflect the upper bound of concentrations previously reported in food and water sources. The titrated solutions were incubated at 37 °C for 2 h, and the change in fluorescence emission intensity and UV-Vis λ_{max}, indicative of toxin binding, were recorded. Dissociating constants (K_d) were calculated based on the Langmuir equation.

Results: IC fluorescence quantum yield increase following the reactions with MeIQx, BPA, carbaryl, and AFB1 suggested IC binding to these structures. AFB1 binding was further confirmed due to the fact that it acts as a quencher, and thus the Stern–Volmer constant K=0.42 was also taken into account. The Kd for AFB1, MeIQx, BPA, and carbaryl were estimated as 33.86, 9.39, 124.28, and 40.67 μM, respectively.

Conclusions: These results demonstrate that IC has the capacity to bind a number of toxins found in food and water sources, and may serve as one strategy to facilitate detoxification and reduce exposure.

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