Lecture 1

Targeted micronutrient supplementation with Carotenoids, omega-3 fatty acids and vitamin E and its effect on the symptoms and natural progression of Alzheimer’s disease

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Background: Alzheimer’s disease is a complex neurodegenerative disease with abnormalities in cognition, behaviour and day-to-day function. It is characterised by declines in memory, attention and language as well as behavioural and psychological changes. These changes have a negative impact on the patient’s quality of life. Our preliminary work has reported behaviour and functional benefits in patients with Alzheimer’s disease (AD) following targeted micronutritional supplementation.

Objective: To investigate the impact of these micronutrients on the progression of AD in a randomised controlled trial. Methods: Patients with mild-moderate AD consumed daily 1g fish oil (500mg DHA, 150mg EPA), carotenoids (10 mg lutein, 10 mg meso-zeaxanthin, 2 mg zeaxanthin) and 15mg vitamin E or placebo for 12 months in a double-blind, placebo-controlled trial. Carotenoids, ω-3FAs and vitamin E were quantified in blood and carotenoids in skin. AD severity was measured using the mini-mental state examination and dementia severity rating scale tools. Behaviour, mood and memory were measured using an informant-based questionnaire.

Results: After 12-months, the active group (n=50) compared to placebo (n=27), demonstrated statistically significant improvements in skin carotenoid, blood carotenoids, ω-3FAs and vitamin E concentrations (p<0.05, for all). The active group performed better in objective measures of AD severity with a statistically significant difference reported in the clinical collateral for memory (p < 0.001).

Conclusion: Exponential increases in the prevalence of AD is driving the need for interventions to ameliorate symptoms and improve quality of life in AD patients. This micronutrient dietary supplement should be considered in the holistic management of AD.

Lecture 2

Macular pigment supplementation in patients with chronic wet age-related macular degeneration: case series

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Purpose: To describe neovascular age-related macular degeneration (nAMD) patients that had poor response to antiangiogenic (antiVEGF) therapy and begun macular pigments (MP) supplementation.

Methods: Non-comparative retrospective case series. Intervention: Supplementation with Lutein (10mg), Meso-Zeaxanthin (10mg) and Zeaxanthin (2mg). Primary outcomes: Interval between treatments and number of antiVEGF intravitreal injections per year. Secondary outcomes: best corrected visual acuity (BCVA in LogMAR) and optical coherence tomography (OCT) findings. Descriptive statistics and Mann–Whitney U test was used for analysis.

Results: Eighteen eyes of 13 patients that failed to achieve antiVEGF treatment periods greater than 8 weeks in their first year. Nine (69.2%) females and 4 (30.8%) males. Mean age was 73.8 years. Bilateral disease was present in 5 of 13 (38.5%). First year vs
second year mean interval between treatments was = 5.87 weeks vs 8.16 [p = .00021] and mean number of injections were = 9.2 vs 6.7 [p = .00007]. OCT Central Subfield Thickness (CST) showed improvement from initial 280 µm vs 228 µm at month 24 [p = .02169]. No difference was found between initial and final BCVA = 0.83 vs 0.89 [p = .38974].

**Conclusions:** In this study, nAMD patients with poor response to antiVEGF therapy in the first year of treatment that initiated supplementation with MP, increased the period between treatments and decreased the number of injections per year. Although these results may be influenced by multiple factors, they may prelude a clinical trial to assess possible clinical roles of MP supplementation in active anti-VEGF therapy for nAMD.

**Lecture 3**

**The Xanthophyll Carotenoids: Bioavailability and Clinical Application**

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**Purpose:** This is an investigation into the bioavailability of the xanthophyll carotenoids (XCs), lutein (L), zeaxanthin (Z), and meso-zeaxanthin (MZ) in nutritional supplements, their measurement in human tissue, and their environmental and nutritional determinants in human beings.

**Methods:** Here we focus on published data from COAST, a 6-month randomised placebo-controlled trial involving 81 healthy volunteers that compared the bioavailability of different formulations of free L, Z, and MZ in sunflower or omega-3 oil versus L, Z, and MZ diacetates (Ld, Zd, and MZd) in a micromicellar formulation. Fasting serum carotenoids, macular pigment, and skin carotenoid score were analysed at baseline and 6 months. These results are compared to published data relating to other XCs interventions with focus on their clinical application.

**Results:** This novel formulation with acetate derivatives of L, Z, and MZ, was found to have better bioavailability by producing a higher response in serum for Z and MZ in comparison with classic microcrystals-in-oil formulations (p = 0.002 to 0.019).

**Conclusions:** Currently, these XCs have been proven to enhance visual performance and are potential preventive and therapeutic agents in retinal pathology. Understanding the complex interactions of the XCs would help improve the role of these antioxidants in human health. This research has progressed our scientific knowledge in a way that we can improve the delivery of these natural micronutrients to enhance human health and function with a positive impact of clinical application.

**Association between the gut microbiome and age-related macular degeneration (AMD) in the 14-year follow-up study to the Carotenoids in Age-related Eye Disease Study (CAREDS2)**

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**Purpose:** Little is known about how the composition and diversity of the gut microbiome differs by AMD severity level.

**Methods:** We conducted a cross-sectional study of the association between the gut microbiome and AMD in postmenopausal women who participated
in CAREDS2 (2016-2019). AMD status was determined primarily from grading of 30° stereoscopic fundus digital images and optical coherence tomography. Participants receiving injections for neovascular AMD (NVAMD) were coded as such. The V3-V4 region of the 16S ribosomal RNA gene from stool samples was sequenced to determine the relative abundance of taxa. PERMANOVA was used for β-diversity (between-sample diversity) by examining the Euclidean distance within and between AMD severity levels. ANOVA was used to examine α-diversity (within-sample diversity) across AMD severity levels. α-diversity measures included the Shannon entropy and Inverse Simpsons measures. Models were adjusted for age and body mass index.

Results: We recruited 609 women and 357 consented to participate. Participants returned 307 stool samples of good quality. The AMD severity levels included those with no apparent aging changes (n=124), normal aging changes (n=8), early AMD (n=59), intermediate AMD (n=97), geographic atrophy (n=3), and NVAMD (n=7). Nine women were missing outcome data. β-diversity did not differ across AMD severity levels (PERMANOVA p=0.80). Those with NVAMD compared to no apparent aging changes had lower mean α-diversity measures (pshannon=0.02; psimpons=0.03).

Conclusion: Preliminary analyses show that the α-diversity of the gut microbiome may be lower in those with NVAMD compared to those without AMD and no apparent aging changes.

Lecture 6
Calcification, vision loss and dementia
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Purpose: In health, calcification is required to form and maintain skeletal and dental tissues. But dysregulation of the mineralization process can lead to ectopic calcium phosphate mineral deposition. Our work explored how ectopic calcification contributes to age-related macular degeneration (AMD) and Alzheimer’s disease (AD).

Methods: Post-mortem eyes and brains were obtained with ethical permission from patients clinically diagnosed with AMD or AD and healthy
controls. In some experiments, whole eyes were flat-mounted onto glass slides. In other experiments, eye and brain tissues were embedded in paraffin and sectioned to a thickness of 7 µm. To visualize calcification, tissues were stained with OsteoSense680EX, a fluorescent dye specific for the calcium phosphate hydroxyapatite (HAP). To confirm the elemental composition of OsteoSense680EX positive structures, energy dispersive x-ray spectroscopy (EDX) and time of flight-secondary ion mass spectrometry (TOF-SIMS) were used.

Results: We found that both AMD and AD are associated with increased deposition of mineralized spherules in sub-RPE deposits as well as in the Bruch’s membrane. In AMD, large, calcified nodules heralded the rapid progression to end-stage disease. We also found that ectopic calcification of neurons and their nuclei are associated with p-Tau in the brain of AD patients.

Conclusions: Ectopic calcification is a potential new target for interfering with AMD and AD. Changes in the availability of calcium and phosphate through diet or supplementation or targeted intervention through proteins and lipids involved in mineralization should all be considered.

Lecture 7

Two-wavelength autofluorescence (2WAF) retinal imaging and serum carotenoids in aging and early-intermediate age-related macular degeneration (e-iAMD): ALSTAR2 baseline cohort

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Purpose: Comprehensive localization of lutein (L) and zeaxanthin (Z) at different stages of AMD, a central neurodegeneration, along with serum levels, could clarify the role of L and Z in retinal health and AMD progression, and the impact of supplementation. 2WAF imaging integrates a ratio of retinal autofluorescence emissions to yield macular pigment optical volume (MPOV).

Methods: Individuals >60 years of age were enrolled from primary ophthalmology clinics in an academic medical center (NCT04112667). Macular health and nutritional supplement use was assessed by color fundus photograph grading and self-report, respectively. 2WAF emissions were captured with a scanning laser ophthalmoscope. Non-fasting blood draws were assayed for L and Z (Eurofins Craft, Wilson NC USA).

Results: Of right eyes of 426 participants (72.0 ± 6.1 years; 61.3% female; 90.6% white, 7.8% black, 1.6% other), 217 (50.9%) were normal, 117 (27.5%) were e-AMD, and 71 (16.7%) were i-AMD. MPOV was higher in e-AMD than normal and higher still in i-AMD (p = 0.0003, ANOVA). Serum L similarly differed among groups (p<0.0001). Serum Z was highest in i-AMD (p <0.0001). Within each group, MPOV and serum L and Z were correlated (Spearman p<0.0001). MPOV and serum values were higher in 102 persons taking supplements than in 324 persons not taking supplements (p<0.0001). The association between MPOV and AMD status did not differ according to supplement use (p=0.1776).

Conclusion: In a cross-sectional analysis, MPOV was higher in e-iAMD eyes than in normal eyes and positively correlated with serum L-Z levels. These associations did not differ according to supplement use.

Lecture 8

Carotenoid Supplementation and Contrast Sensitivity: A New Report From CREST

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Purpose: High-performance visual function associated with central vision is mediated by the central retina (the macula), which accumulates three diet-derived xanthophyll carotenoids: lutein [L], zeaxanthin [Z], and meso-zeaxanthin [MZ]. This study investigates the relationship between change of mac-
ular pigment optical volume (MPOV) and area under the contrast sensitivity function (AUC) in individuals after supplementation with L, Z, and MZ and proposes AUC as a new method for quantifying spatial vision.

Methods: Active group subjects (n=52) consumed daily a formulation containing 10 mg L, 2 mg Z, and 10 mg MZ or placebo (n = 52) for a period of 12 months. Study visits occurred at baseline, 3, 6, and 12 months. AUC was the primary outcome measure (POM). Macular pigment optical volume (MPOV) was measured using dual-wavelength autofluorescence (Heidelberg Spectralis).

Results: For all participants, statistically significant correlations (r = 0.670; p < 0.001) between change in MPOV and change in AUC for the frequencies at or near the peak of the contrast sensitivity function were observed. Additionally, changes in CS at 6cpd were commensurate with improvements in AUC (r = 0.539; p < 0.001).

Conclusions: These results indicate that dietary fortification with macular carotenoids significantly increases the AUC for contrast sensitivity; this increase represents an effect on visual function far greater than that of a single frequency (e.g., 6 cpd), and should be considered in terms of clinically meaningful change in vision. The AUC for contrast sensitivity is a novel method for quantifying visual performance.

Lecture 10
The role of carotenoids and very-long-chain polyunsaturated fatty acids in retinal health and disease throughout the lifespan
Bernstein, PS

Purpose: The macular pigment (MP) carotenoids lutein, zeaxanthin, and meso-zeaxanthin and the very-long-chain polyunsaturated fatty acids (VLC-PUFAs) are nutrients that are specifically concentrated and/or synthesized in the retina, where they enhance normal visual function and protect against acquired and inherited retinal diseases. My laboratory is dedicated to understanding the molecular mechanisms underlying these effects and to developing evidence-based therapeutics.

Methods: We have developed novel models to study the roles of MP carotenoids and VLC-PUFAs in mice, zebrafish, and human retinal organoids, and we are conducting clinical trials in humans throughout the lifespan.
Results: Studies from my laboratory on BCO2 knockout “macular pigment mice” demonstrate protective effects of orally administered carotenoids that counteract oxygen-induced retinopathy in a mouse model of retinopathy of prematurity and that inhibit formation of bisretinoids A2E and iso-A2E in a mouse model of Stargardt-1 disease and AMD. Studies of potential beneficial effects of administration of synthetic VLC-PUFAs show very promising results in newly developed animal and organoid models of VLC-PUFA deficiency in which the ELOVL4 enzyme has been knocked out. Initial results of the Lutein and Zeaxanthin in Pregnancy (L-ZIP) study demonstrate improvements of ocular and systemic carotenoid status in the mother and child in subjects who received AREDS2 levels of lutein and zeaxanthin in prenatal vitamins.

Conclusions: MP carotenoids and VLC-PUFAs continue to show ocular benefits in health and disease throughout the lifespan and should be considered for next-generation supplements ranging from prenatal vitamins to “AREDS3” formulations.

Lecture 11
The Role of Long-Chain Omega-3 Polyunsaturated Fatty Acids in Ocular Health and Disease
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Purpose: Interest in long-chain omega-3 polyunsaturated fatty acids (PUFAs) was sparked in the 1970s by the findings of Bang and Dyerberg among the Greenland Inuit. Since then, these special nutrients have been the focus of more than 30,000 publications, and much has been learned about their holistic health benefits. However, although many healthcare providers recommend long-chain omega-3 PUFAs to select patients, further clarification is needed regarding their optimal use in various ocular conditions.

Methods: Research findings on the possible mechanisms of long-chain omega-3 PUFAs involved in the development and progression of dry eye disease, glaucoma, diabetic retinopathy (including cardiovascular disease implications), and age-related macular degeneration (AMD) were investigated. Both observational studies and randomized controlled trials that advance knowledge regarding use of long-chain omega-3 PUFAs in clinical practice for these ocular conditions were considered.

Results: There are multiple mechanisms of action that support the positive impact of long-chain omega-3 PUFAs in dry eye disease, to reduce glaucomatous oxidative stress, to lower retinal and cardiovascular disease risk in diabetes mellitus, and in AMD. Though not every study agrees, an overview of significant research findings adds valuable insight into the clinical use of these unique molecules for each condition.

Conclusions: Eyecare practitioners frequently manage patients with dry eye disease, glaucoma, diabetes mellitus, and AMD and have an opportunity to leverage the benefits of nutritional intervention to enhance care. Increased understanding of the role of long-chain omega-3 PUFAs in ocular health and disease along with proper implementation strategies can lead to improved patient outcomes.

Lecture 12
Omega-3 blood biomarkers and brain health
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The brain is a lipid-rich organ. There is experimental evidence that fatty acids play crucial roles in brain health. Much research has focused on the omega-3 docosahexaenoic acid (DHA), which is part of brain membranes. DHA can be obtained from diet (fatty fish and fish oils). This fostered interest in whether dietary DHA intake promotes brain health in later life. No randomized controlled trials (RCTs) on incident Alzheimer’s disease are available, while those on cognitive performance yielded mixed results, similar to those on incident stroke. Possible explanations for this apparent failure could be methodological. Was the omega-3 dose high enough and the duration long enough? Does the background omega-3 intake matter? And to what extent do non-modifiable factors modulate the response? In this new era of precision nutrition and personalized medicine, prospective cohort studies can help to identify which groups of people may be most likely to have a
beneficial response, helping to design future RCTs. However, a major limitation of most studies on nutritional epidemiology is the use of methods based on self-reporting, which generates inaccurate and imprecise dietary data. Given the marginal de novo synthesis of omega-3, direct chemical measurement of circulating omega-3 has emerged as an objective and valid surrogate of their dietary intake. We will discuss how lipidomic-assessed omega-3 blood biomarkers might help to better define future RCTs on omega-3 and brain health. In other words, trials involving supplementation with DHA should be designed so as to provide enough DHA to achieve a specific DHA target?

Lecture 14
Lessons from the oldest old: What have we learned about nutrition and cognition in centenarians across the world?
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Dementia is a major public health problem in aging societies that causes significant healthcare cost and burden. To accentuate the seriousness of this epidemic, currently there are no established preventive and therapeutic strategies for Alzheimer’s disease (AD), the most common form of age-related dementia. Systematic reviews of cross-sectional and longitudinal studies, trials, and meta-analyses have concluded that better adherence to healthy dietary patterns is related to delayed onset of cognitive decline and lower risk of AD or dementia in older adults, but limited evidence exists for the oldest old. Existing clinical and epidemiological evidence suggests that the pathophysiology of dementia among the oldest old is distinct from lesser-aged individuals. With centenarians being the fastest growing demographic group in many developed countries, we need a better understanding of the role of nutrition on cognitive health in this unique demographic group and its implication in lesser-aged older adults. This lecture aims to summarize the dietary intake and nutrition data, the neuropathology of AD and other dementia, the cognitive profile, and their correlations in different centenarian populations across geography and cultures. Data are drawn mainly from population-based cohorts such as the Okinawa Centenarian Study, the China Hainan Centenarian Cohort Study, the 100-Plus Study, the Medical Research Council Cognitive Function and Ageing Study, the New England Centenarian Study, and the Georgia Centenarian Study. Current research gaps are also discussed to push the field toward established dietary recommendations for healthy cognitive aging.

Lecture 15
Personalized Nutrition: Innovations in Nutritional Cognitive Neuroscience
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Purpose: Nutritional cognitive neuroscience is an emerging interdisciplinary field of research that seeks to understand nutrition’s impact on cognition and brain health across the life span. Research in this burgeoning field demonstrates that many aspects of nutrition—from entire diets to specific nutrients—affect brain structure and function, and therefore have profound implications for understanding the nature of healthy brain development and aging. The aim of this talk is to examine recent advances in nutritional cognitive neuroscience, with an emphasis on methods that enable discovery of nutrient biomarkers that predict individual differences in healthy brain aging.

Methods: We propose an integrative framework that calls for the synthesis of research in nutritional epidemiology and cognitive neuroscience, incorporating: (i) methods for the precise characterization of nutritional health based on the analysis of nutrient biomarker patterns (NBPs), along with (ii) modern indices of brain health derived from high-resolution magnetic resonance imaging (MRI).

Conclusions: By integrating cutting-edge techniques from nutritional epidemiology and cognitive neuroscience, nutritional cognitive neuroscience will continue to advance our understanding of the beneficial effects of nutrition on brain development and aging. Ultimately, the development of predictive nutrient patterns to enhance brain health will provide an empirically
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Methods: Revision of bibliography.

Results: Carotenoids play key roles in Nature. They are essential in photosynthesis, the engine of life and food production on Earth. They can also be converted into important derivatives. Examples are vitamin A, an essential nutrient in humans, or the phytohormones abscisic acid or strigolactones. These intervene in the resistance to stresses or the establishment of symbiotic relationships, which are important for plant development and resilience and therefore for food security. Through the colour they impart to flowers and fruits as well as the aroma imparted by some derivatives, carotenoids are important for pollination and seed dispersal, which are in turn key for the production of foods. Beyond their roles as colorants and vitamin A precursors, carotenoids are also important in foods as lipophilic antioxidants that can protect them against oxidation under certain conditions. Moreover, ample evidence has accumulated from studies of diverse nature indicating that carotenoids could intervene in health-promoting biological actions that can contribute to reduce the risk of developing certain diseases. With the exception of very high doses of \( \beta \)-carotene in susceptible individuals, the consumption of carotenoids is very safe.

Conclusions: Considering the climate change scenario and the large impact that the agrofood industry has on the use of resources (water, fertilizers, energy) current research should eventually converge into the sustainable use of resources and technologies for the production of carotenoid-containing products.

Lecture 16

Diet for protection against cognitive decline and Alzheimer’s disease

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Modifying dietary behaviour could be a promising way to enhance cognition and delay or prevent dementia in later life. Several dietary factors influence dementia risk in humans, for example, vitamin E, B vitamins, omega-3 fatty acids and healthy dietary patterns, particularly the Mediterranean Diet, have been shown to be neuroprotective, while high intake of saturated fat accelerates cognitive decline. It is not entirely clear how diet offers neuroprotection, but several putative mechanisms include beneficial effects on neuronal cell signalling, vascular, antioxidant and anti-inflammatory biological pathways. Given that the pathophysiological changes of dementia accumulate years before cognitive impairment becomes evident, understanding the influence of diet on brain health across the life-course is important to inform prevention strategies. Further research is needed to investigate diet-associated neurological change from the earliest through to latest stages of cognitive decline. Furthermore, intervention strategies require insight into mechanisms involved in diet-induced cognitive change and an understanding of how to support dietary behaviour change, particularly in high risk populations.

Lecture 17

Carotenoids in the era of sustainable health-promoting diets

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Purpose: To summarize some aspects to be considered for the sustainable production and rational use of carotenoid-products intended for humans.

Methods: Revision of bibliography.

Results: Carotenoids play key roles in Nature. They are essential in photosynthesis, the engine of life and food production on Earth. They can also be converted into important derivatives. Examples are vitamin A, an essential nutrient in humans, or the phytohormones abscisic acid or strigolactones. These intervene in the resistance to stresses or the establishment of symbiotic relationships, which are important for plant development and resilience and therefore for food security. Through the colour they impart to flowers and fruits as well as the aroma imparted by some derivatives, carotenoids are important for pollination and seed dispersal, which are in turn key for the production of foods. Beyond their roles as colorants and vitamin A precursors, carotenoids are also important in foods as lipophilic antioxidants that can protect them against oxidation under certain conditions. Moreover, ample evidence has accumulated from studies of diverse nature indicating that carotenoids could intervene in health-promoting biological actions that can contribute to reduce the risk of developing certain diseases. With the exception of very high doses of \( \beta \)-carotene in susceptible individuals, the consumption of carotenoids is very safe.

Conclusions: Considering the climate change scenario and the large impact that the agrofood industry has on the use of resources (water, fertilizers, energy) current research should eventually converge into the sustainable use of resources and technologies for the production of carotenoid-containing products.

Lecture 18

Carotenoid food supplements: The role of stability testing and regulation

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Purpose: The nutraceutical market is largely unregulated. Dietary supplements aimed at eye health with the carotenoids lutein, zeaxanthin and \( \text{meso}-\text{zeaxanthin} \) are not an exception. Some coun-
tries require the achievement of only 80% of their label claim, adducing difficulties in the assessment of these molecules. Furthermore, a significant number of products in the market are below their label claim, or do not accurately name the carotenoids contained.

**Methods:** We have established the start-up ‘Supplement Certified’ to test food supplements containing carotenoids (and omega-3 fatty acids) to support nutraceutical companies aiming to meet regulations and provide to the public products compliant with their label claim.

**Results:** Data have improved and validated the U. S. Pharmacopeia method describing the assessment of these molecules, and we are working with a number of companies to test the strength and stability of their products over shelf life.

**Conclusions:** Further efforts from nutraceutical companies are desirable to offer compliant and stable products to the public, which commits significant amounts of money expecting to acquire products of the highest quality.

**Lecture 19**

**Dynamic Visual Skills & Peripheral Awareness: Why we need to go far beyond “20/20” in academics and concussion / mTBI**

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2College of Optometrists of Ontario (Regulatory Body, Ontario, Canada)
3Board Director, Optometry Examining Board of Canada (OEBC)

**Purpose:** Primary purpose is to review literature on oculomotor and dynamic visual skills deficiencies both in vision related learning difficulties (VRLD) and in adult / pediatric post-concussion syndrome (PCS) cases, including peer reviewed journals published both by Dr. Quaid himself and other. The secondary purpose of this presentation is to show footage from actual cases from clinics which have critical associated learning points that connect to the aforementioned literature review.

**Methods:** Review via oral presentation of Pubmed listed research and presentation of data from several papers reviewing how visual skills are related to reading and concussion based symptomatology will be presented. Dr. Quaid will also present data collected from Ontario (Canada) clinics with Federal funding pertaining to both reading function (pediatric VRLD, n = 143) and also recovery from PCS (adult data, n=110), which has also been presented in poster format at other conferences and is currently being prepared for publication.

**Results:** Data will be presented showing that neuro-optometric visual rehabilitation is of value in both vision based learning difficulty cases in addition to post-concussion syndrome (PCS) cases with significant visual symptomatology.

**Conclusions:** Visual rehabilitation strategies employed by neuro-optometrists with appropriate advanced training, with the help of lenses, prisms and therapy techniques, are of value in cases of VRLD and PCS. Advanced training in the area of Neuro-Optometry is as a result of this being considered in Canada for formal speciality status within Optometry as a profession.

**Lecture 20**

**Antioxidant supplementation and genetics: What’s the story?**

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Free radicals commonly form during normal cellular activities although excess production can lead to oxidation and inflammation, characteristics typically associated with ageing and progression of multiple chronic diseases. Antioxidants limit free radical-induced tissue damage by preventing their formation through a scavenging process that promotes their breakdown. Observational studies have reported associations between circulating vitamin E and carotenoid levels with improved brain and vision health outcomes, including antioxidant, anti-inflammatory and cholesterol-lowering activities reducing morbidity and mortality, although support from randomised clinical trials has not been convincing. Despite reported associations between multiple genetic variants and antioxidant levels, their potential influence on antioxidant status and subsequent health outcomes is generally insufficiently considered in supplementation trial approaches.

The effects of polymorphisms in genes related to absorption, bioavailability and post-ingestion mechanistic effects on antioxidant levels can be poten-
Abstracts

Considered and weighted with other models already reported to create the resulting model for clinical guidance for recommendations for nutrition and use of supplements.

**Results:** 526 publications were found in a search with keywords of AMD, progression and risk factors. Variables consistently found as risk factors were selected and grouped based on their corresponding risk category. The risks were ranked by risk level in a model of high, low and medium risks according to the interaction between them. Among the factors found are: smoking history, age, diet, BMI, drusen and choroid characteristics, etc. This model will be used in a prospective clinical study for modifiable risk modification and supplementation recommendations.

**Conclusions:** This integrative model may be useful for carrying out nutritional and supplementation interventions in patients at higher risk. This potential guide can be especially beneficial in low-income populations where nutritional and supplementation expense in old age is high and must be made efficiently.

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**Lecture 22**

**Blue light: the good, the bad, and the misunderstood**

Temple, S1,2,3,4

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**Purpose:** Blue light (380-500 nm) represents one third of the spectral range reaching the retina. And while it does good, by setting our circadian sleep-wake cycle and contributing to colour vision, it can also be bad due to its high energy per photon, enabling it to cause photochemical damage that accumulates through life. There is a body of research and widespread belief that blue light from sunlight, LED’s and digital devices poses a hazard to the retina, but simultaneously there is resistance to the value of blue light-filtering lenses and macular pigments in protecting against blue light, suggesting that the potentially confounded by environmental, dietary and gene-gene interactions. Furthermore, the allele frequency for many polymorphisms differs significantly by ethnicity, meaning the significance of intervention response assessed across populations may not be consistent. Reported associations between ophthalmic disease-specific genetic risk and response to antioxidant supplementation recommendations remain largely unsubstantiated due to a lack of independent verification and validation, leading to potential misinterpretation.

Studies investigating the effects of antioxidants on inflammation and oxidative stress and subsequent health outcomes have been inconsistent. Future well-designed studies that consider genetic variation with respect to population-specific allele frequencies will better inform our understanding of antioxidant effects on inflammatory and oxidative stress biomarkers and health outcomes, particularly in response to different supplementation types and dosing.

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**Lecture 21**

**Targeted nutrition for the retina. Experience in Mexico**

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**Purpose:** To present the development and initial use of an eclectic, individual risk analysis model based on macular and choroid phenotypic clinical characteristics determined by OCT, individual epidemiological factors, and population nutritional background, to identify patients and first-degree relatives at increased risk of macular degeneration appearance or progression in order to make targeted changes to the nutrition and supplementation based on risks.

**Methods:** Through a systematic review of literature, reported risk factors for the onset or progression of age-related macular degeneration were identified. These risks were classified into individual epidemiological and anatomical factors. These were considered and weighted with other models already reported to create the resulting model for clinical guidance for recommendations for nutrition and use of supplements.

**Results:** 526 publications were found in a search with keywords of AMD, progression and risk factors. Variables consistently found as risk factors were selected and grouped based on their corresponding risk category. The risks were ranked by risk level in a model of high, low and medium risks according to the interaction between them. Among the factors found are: smoking history, age, diet, BMI, drusen and choroid characteristics, etc. This model will be used in a prospective clinical study for modifiable risk modification and supplementation recommendations.

**Conclusions:** This integrative model may be useful for carrying out nutritional and supplementation interventions in patients at higher risk. This potential guide can be especially beneficial in low-income populations where nutritional and supplementation expense in old age is high and must be made efficiently.
Abstracts

Lecture 23

Nutritional Breeding of Orange Corn: Selection for Higher Lutein and Zeaxanthin for Brain and Ocular Health

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Purpose: World populations suffer from increased rates of age-related macular degeneration (AMD), a leading cause of blindness. Dietary deficiencies in macular carotenoids lutein and zeaxanthin appear related to increased rates of AMD.

Methodology: We are breeding orange corn for higher levels of lutein and zeaxanthin, which accumulate in the macula of the eye and can provide protection. We use visual, colorimetric, and HPLC-based selection for higher levels of macular carotenoids.

Results: Orange corn is milled into consumer products orange grits, cornmeal, and flour available in USA. We have fed orange corn to laying hens which significantly increased levels of lutein and zeaxanthin in egg yolks. This makes the yolks a consumer desirable, visually identifiable deep orange color, and an excellent source of highly bioavailable macular carotenoids. We have developed orange popcorn hybrids with higher levels of lutein and zeaxanthin after popping. Popcorn is considered a healthy snack food. Initial proof of concept work in new experimental breeding lines of orange sweet corn showed unexpected high levels of macular carotenoids at the fresh eating stage. We estimate the macular carotenoids provided from a medium-sized ear or 100g of orange sweet corn to potentially be 3-6 mg, similar to raw or cooked peas and kale, or raw spinach. Notably, orange sweet corn may be more desirable than spinach or kale to many consumers and thus more likely eaten.

Conclusion: Orange corn based products are available and more products are forthcoming, and all may help address dietary Lutein / Zeaxanthin deficiencies.

dangers/benefits of blue light are generally misunderstood.

Methods: Literature review, spectral radiance measurements of urban verses natural environments and spectral transmission of lenses.

Results: Research shows that blue light has greater than 2.3 electron volts per photon, giving it enough energy to cause the formation of free radicals, which contribute to long term accumulation of oxidative damage. My measurements show that there is more blue light in urban than natural environments and that blue light filtering lenses can decrease blue light reaching the retina.

Conclusions: Blue light from all sources will contribute to cumulative photochemical retinal damage much like smoking does for lung cancer: it is all about exposure not intensity. Blue light-filtering lenses offer a way to reduce lifelong exposure, as do sunglasses and photochromic lenses, but nature’s solution, our macular pigments, provide a mechanism that does not interfere with blue light sensors that entrain our circadian rhythm.
BON 2022 01

Designing high zeaxanthin capsicums

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**Purpose:** Orange capsicums (Capsicum annuum) are one of the richest natural sources of dietary zeaxanthin. Despite this potential benefit to eye health, orange capsicums are still not as popular as red capsicums. This is partly attributable to the lack of research on orange capsicums, and a general lack of knowledge about them by nutritionists. In the current study, we focused on investigating the reasons behind high levels of zeaxanthin production in orange capsicums.

**Methods:** Eight orange and three red capsicum varieties were investigated for their carotenoid profiles and genetic variations in two key carotenoid biosynthesis genes, capsanthin capsorubin synthase (CCS) and zeaxanthin epoxidase (ZEP). Cleaved Amplified Polymorphic Sequences (CAPS) primers were used to identify the ZEP allele in the varieties, while PCR was used to identify the presence/absence of a putative CCS deletion.

**Results:** In seven of eight orange varieties, CCS was found to contain a large deletion, while ZEP was found to contain an A/G single nucleotide polymorphism. In the eighth variety, carotenoid profiles suggested some level of ZEP and CCS activity giving the fruit a darker orange colour; however, while ZEP was confirmed as the functional A variant, the CCS deletion was detected. In red varieties both CCS and ZEP were found to be respectively present and functional.

**Conclusion:** High zeaxanthin is suggested to be achieved in capsicums by a combination of a non-functioning CCS and reduced function ZEP. Further study needs to be conducted on the dark-orange variety to better understand the produced carotenoids in this variety.

BON 2022 02

Generation and characterization of Elovl4b knockout zebrafish as a model for juvenile-onset macular degeneration

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**Purpose:** Very-long-chain polyunsaturated fatty acids (VLC-PUFAs) are a distinct class of retinal lipids with chain lengths greater than 24 carbons. ELOVL4 is the protein responsible for VLC-PUFA biosynthesis. However, in patients with autosomal dominant Stargardt 3 disease, the gene encoding ELOVL4 is mutated, and as a result, a dysfunctional, truncated protein is produced instead, resulting in low retinal VLC-PUFAs. Low VLC-PUFAs are also characteristic of the more common disease, age-related macular degeneration. Since homozygous Elovl4 ablation is neonatal lethal in mice due to catastrophic drying due to the loss of their protective skin barrier, we examined a zebrafish model of Elovl4 deficiency.

**Methods:** Elovl4b knockout zebrafish were generated by creating a deletion mutation in exon 2 of the Elovl4b gene using CRISPR-Cas9. Mutant zebrafish were screened for non-specific mutations by outcrossing mutant adults with wild-type fish. Their eyes were isolated, total fatty acids extracted, and the quantity of VLC-PUFAs determined through gas chromatography and mass spectrometry.

**Results:** We found that homozygous Elovl4b mutant zebrafish eyes had altered lipid profiles and a significantly lower abundance of C30 to C34 VLC-PUFAs compared to age-matched wild-type controls.

**Conclusion:** Our data indicate that the loss of Elovl4b in zebrafish can cause an ocular phenotype comparable to macular degeneration. We anticipate diminished ERG amplitudes, indicative of a loss of photoreceptor function and a thinner outer nuclear layer than controls. We plan to correct such abnormalities by feeding synthetic VLC-PUFAs.
BON 2022 03

The Lutein and Zeaxanthin in Pregnancy (L-ZIP) Trial – Initial Results
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Purpose: We investigated whether prenatal carotenoid supplementation counteracts maternal carotenoid depletion, particularly in the third trimester, and if it can enhance maternal and infants’ systemic and ocular carotenoid status.

Methods: In this controlled prospective trial (ClinicalTrials.gov identifier: NCT03750968; still ongoing), 47 participants were randomized to receive standard-of-care prenatal vitamins with or without added lutein/zeaxanthin for 6 to 8 months. The primary outcome was maternal carotenoid status in the skin, serum, and eye measured at the end of each trimester and postpartum with HPLC, resonance Raman spectroscopy, and dual-wavelength autofluorescence, respectively. The secondary outcome assessed infants’ carotenoid levels in the umbilical cord blood, skin, and eyes using similar techniques as above but optimized for infants.

Results: Masked study analysis shows an increase in maternal serum lutein and zeaxanthin (L+Z), skin carotenoids, and macular pigment optical density and volume at 90 eccentricities from the first trimester, peak at the third trimester, and decline postpartum. Postpartum maternal serum L+Z levels significantly associate with umbilical cord blood L+Z levels (r=0.80, p=0.002). Infants’ skin carotenoids significantly correlate with umbilical cord blood L+Z levels (r=0.62, p=0.024). Postpartum maternal skin carotenoids correlate with serum L+Z levels (r=0.63, p=0.020).

Conclusion: Unmasked study results will be available in early February 2022, and we will then know whether prenatal carotenoid supplementation prevents maternal carotenoid depletion and enhances infants’ fovea development and function. Also, our results may serve as a basis for future large-scale research in normal and high-risk pregnancies and could guide policy decisions about prenatal carotenoid recommendations.

BON 2022 04

The impact of carotenoids on visual function and shooting sport performance
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Purpose: A shooter’s visual system is always under intense pressure to achieve stability of hold, timing of triggering, aiming accuracy and cleanliness of trigger, which influences shooting performance and are partly dependent on visual stimuli. Current data on dietary or supplemented lutein, zeaxanthin, and meso-zeaxanthin (L,Z,MZ) and visual performance from healthy populations has yet to be translated for athletes. Here, we present our project design that will examine the effects of these nutrients on visual function (VF) and sport performance (SP) amongst Singapore shooting athletes.

Methods: We will perform a L,Z,MZ interventional placebo-controlled study in Singapore shooting athletes with duration of 9 months, and assess the following: demographic, medical, lifestyle status and vision history (via questionnaire); dietary intake of lutein and zeaxanthin (DI), anthropometry, VF and macular pigment ocular density (MPOD). Main outcome measurements will include L,Z,MZ serum concentrations, MPOD, VF and SP; (total centre aiming time [TA], perceived target size [PT] and shooting accuracy [SA]).

Results: Comparison and association on serum concentration, VF (visual acuity, contrast sensitivity, glare discomfort, photostress recovery) and MPOD between groups will be analysed to understand the effects of L,Z,MZ nutrients on VF. Relationship between VF and SP will be determined via VF, MPOD, TA, PT and SA data.

Conclusion: This study may provide a novel and significant impact within the shooting sporting community via evidence-based nutrition and vision guidelines playing a fundamental role in shooting performance. Further understanding and utilisation of carotenoid nutrition in athletes may offer a competitive advantage by improving performance.
**Abstracts**

**BON 2022 05**

The Development of Lutein-enriched Yoghurt to improve Human Health

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**Purpose:** The consumption of lutein is essential for human vision and cognition. However, the consumption of this carotenoid by the population is deficient, and lutein supplements are hard to ingest, especially for children and the elderly. Yoghurt is a wide-consumed daily food product with a high loading capacity for lipophilic substances. To contribute to the extra delivery of lutein to the population, we developed a lutein-enriched yoghurt product at lab scale and pilot scale and investigated several flavour additives to improve the flavour of this product.

**Methods:** Lutein micro-crystal, lutein micro-micelle and lutein extract were used to prepare lutein-enriched yoghurt with the lutein content from 0 to 20 mg/100 g. The content and lutein stability in yoghurt were evaluated by HPLC, and the flavour improvement of this yoghurt product was achieved by sensory evaluation.

**Results:** The lutein stability in yoghurt was excellent at a concentration of 15 mg/100 g for all three types of lutein sources during 35 days’ analysis. However, the sensory evaluation results showed that lutein extract had the lowest effect on the original flavour of the yoghurt, followed by lutein micro-micelle and lutein crystal. Lutein micro-crystal and lutein micro-micelle precipitated after the high shear homogenisation process, but no sediment was found for lutein extract. In addition, a combination of flavour additives was screened by sensory evaluation and the thermal stability of the combination was also verified.

**Conclusions:** Overall, yoghurt is a very effective carrier for lutein, and lutein extract has the highest solubility and lowest flavour impact among the three different lutein sources, making it an excellent ingredient for the preparation of lutein-enriched yoghurt.

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**BON 2022 06**


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**Purpose:** The aim of this study was to examine the influence of ApoE genotype on known relationships between poly- and mono- unsaturated fatty acids (PUFAs and MUFAs), organization of functional brain networks, and cognitive control in older adults.

**Methods:** We analyzed cross-sectional data from 51 participants (mean age 68.5). We applied a moderated moderation model with ApoE genotype as the moderating moderator (z), PUFA + MUFA nutrient intake as the moderator (w), small world propensity of the dorsal attention network (DAN) as the predictor (x), and D-KEFS Trail composite score as the outcome (y).

**Results:** The overall moderated moderation model was significant F (7, 43) = 2.26, p = 0.047, R2 = 0.27. We observed a significant 2-way interaction (p = 0.025) between the PUFA + MUFA nutrient pattern and ApoE genotype and a significant 3-way interaction (p=0.031) between functional organization, nutrient group, and ApoE genotype.

**Conclusion:** Building on previous findings (Zwilling et al., 2019) we observed that individuals with high, long-term dietary intake of PUFAs and MUFAs (measured via diet history questionnaire) were more likely to have small world organization of the DAN and score higher on assessments of cognitive control than individuals with low dietary intake.
In addition, we found that the magnitude of this effect increased with genotype risk, where the effect was highest for individuals with one or more e4 allele. This work highlights the impact of gene-environment interactions and can be readily applied to nutritional interventions aimed at improving brain health in aging.

**BON 2022 07**

**Macular Pigment Optical Density in a Healthy Ghanaian Sample**

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**Purpose:** Macular pigment (MP) measurements are indicators of fruit and vegetable intake, and these measurements are known to serve as nutritional biomarkers in ocular disease, brain health, and cognition. There is a paucity of research on the determinants of MP in sub-Saharan Africa, particularly Ghana. Moreover, expertise and equipment in this field have been lacking on the African continent. Previous research into the determinants of MP has been largely conducted in the western world. This evidence gap necessitated this study investigating the level of MP and its associated factors in a healthy Ghanaian sample.

**Methods:** This cross-sectional study enrolled 301 healthy participants (aged 21.11±1.91 years) at the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Participants completed comprehensive ophthalmic, anthropometric, dietary, and cognitive assessments. MP was assessed using the Macular DensitometerTM.

**Results:** The mean MP at 0.5° and 1.0° eccentricities were 0.37±0.16 and 0.34±0.15 optical density units, respectively. Dietary intake of lutein ranged from 0.00 to 73.60 mg/day, and dietary intake of zeaxanthin was 0.00 to 39.00 mg/day. After statistical adjustment, MP was unrelated (p>0.05) with visual function. However, a statistically significant association was found between MP at 0.5° and the FAS phonemic fluency score (r = 0.002, p = 0.016).

**Conclusion:** This study provides novel data on the environmental and nutritional determinants of MP in the Ghanaian population.

**BON 2022 08**

**Planned human trial to test the bioavailability of a lutein-enriched yogurt (Vistamilk-Fortixan)**

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**Purpose:** Unhealthy diets are one of the main risk factors for non-communicable diseases globally. Research shows that increased intake of xanthophyll carotenoids, via ingestion of commercially available supplement capsules, can positively impact visual and cognitive performance. However, taking capsules does not suit everyone, either due to personal choice or medical reasons. To address this, we propose to develop a lutein-fortified dairy product to positively impact on human health and function. We will assess the bioavailability of lutein from this yogurt through conducting a human intervention trial.

**Methods:** This initial response study will be a randomised trial lasting four days, and will be carried out on a group of ~10 participants aged 30 to 50 years. Participants will consume a single 100 g dose of either one of two formulations of lutein-fortified yogurt. Blood samples will be taken at scheduled times over the 12 hours post-dose. There will be three planned study visits at 24, 48 and 72 hours post-dose, where one blood sample will be collected from each participant at each visit. Serum lutein concentration will be analysed using high performance liquid chromatography-diode array detector (HPLC-DAD). The data will be analysed using the trapezoidal AUC method.

**Conclusion:** We hypothesize that this investigation will provide robust evidence of the bioavailability of both lutein formulations, and will therefore
demonstrate the successful delivery of lutein to humans by using dairy products as a vehicle.

**BON 2022 09**

**To p or not to p – and what is the effect?**

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‘A statistically significantly change in weather is expected in the coming weeks (p < 0.001)’. In a weather report, a comment like this would be inconceivable. However, similar statements are common throughout scientific articles. The statement contains no information that a recipient is practically interested in: will it become warmer or colder? How much change in weather is expected? The p value, derived statistically, does not answer these questions. It leaves but a vague feeling that the weather may not stay the same in the coming weeks.

In clinical studies, most research includes statistical analysis of observed data, where authors solely rely on p-values to answer research questions. Typically, p<0.05 is used to declare “statistical significance” and hence, “proves” that, for example, an intervention has an effect on the outcome measure. As such, the p-value has long been the figurehead of statistical analysis – but its position is under threat. Many researchers are aware of p’s frailties, and its susceptibility for misinterpretation, but are less clear about how they might analyse data to ensure study results are not only significant, but meaningful. We propose that different estimates of the observed size of the effect (such as risk difference, relative risk or hazard ratio) be considered when analysing data. These effect size measures can be accompanied by an estimate of their precision, such as a 95% confidence interval. Such a duo can then be used to answer the important question: Are the results clinically meaningful?

**BON 2022 10**

**Association of skin carotenoid levels with cognitive impairment screened by Mini-Cog in patients with glaucoma**

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**Purpose:** The aim of this study was to explore the association between skin carotenoid (SC) levels and cognitive impairment screened by Mini-Cog in patients with glaucoma.

**Methods:** 812 eyes of 406 participants (mean age, 79.5 ± 7.6 years; 228 men, 178 women) with primary open-angle glaucoma (57.6 %), exfoliation glaucoma (18.6 %), and other glaucoma types (23.8 %), whose SC measurements were available, were included retrospectively. Clinical data including SC levels, ophthalmologic measurements, and cognitive function screened by Mini-Cog were collected by chart review. Comparisons of clinical parameters between the two groups divided by Mini-Cog results were performed with G test or Fisher’s exact probability test followed by mixed-effects regression models.

**Results:** Twenty-eight of 406 participants (6.9%) were Mini-Cog positive. The mean SC level of the Mini-Cog positive group was lower than that of Mini-Cog negative group (269.5 ± 86.4 and 329.2 ± 120.4, respectively) (p=0.0104). By mixed-effects regression analysis, Mini-Cog positive was significantly associated with lower SC levels (p=0.0006). However, SC level was not significantly associated with the mean visual field mean deviation (p=0.3035).

**Conclusions:** Cognitive impairment assessed by Mini-Cog was associated with lower SC levels in patients with glaucoma.

**BON 2022 11**

**Nutrition in Healthy Brain Aging: A Comprehensive Investigation of Nutrient Biomarkers, Multimodal Brain Imaging, and Cognitive Measures of Brain Age**

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Abstracts

Purpose: Brain age is an indicator of brain integrity and accurately predicts cognitive function. Brain age is influenced by lifestyle factors, including diet and nutrition. The study sought to identify specific dietary nutrients that promote a brain age younger than chronological age.

Methods: We assessed brain metrics, cognition, and dietary intake of 111 older adults using comprehensive brain imaging scans of volumetrics, white matter (DTI), functional connectivity and magnetic resonance spectroscopy (MRS); neuropsychological tests of memory, intelligence, and executive function were conducted; blood-based nutrient biomarkers reflecting dietary intake were collected. From this, brain age was determined for all study participants. Hierarchical clustering identified brain structure and function differences for brain age groups. Brain age was associated with cognitive outcomes and nutrients.

Results: Individual brain age determined assignment into either the delayed (average brain age 59.7 years) or accelerated (average brain age 65.1 years) brain age group. The delayed brain age group had larger brain volumes, more robust white matter tracts, better functional connectivity and higher concentrations of brain metabolites compared to the accelerated brain age group. Delayed brain age resulted in better scores on all 15 measures of cognitive performance. Moreover, individuals in the delayed brain age group had greater concentrations of several important classes of nutrients, including lutein, zeaxanthin, MUFAs, PUFAs, long chain SFAs, and Vitamin E.

Conclusion: Brain integrity, as measured by brain age, is sensitive to diet and nutrition. The current study identified nutrients that can decrease brain age. Lower brain age resulted in better brain function, brain structure, and cognitive outcomes.

BON 2022 12

B vitamins and incidence of advanced AMD: the Alienor Study

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Purpose: B vitamins have been proposed to protect against age-related macular degeneration (AMD), but epidemiological studies are scarce and their results are conflicting. We investigated associations of dietary intake and serum status of B vitamins with incidence of advanced AMD in older adults.

Methods: The Alienor study is a prospective population-based cohort of 963 residents of Bordeaux, France, who were 73 years or older at baseline (2006-2008). The present study included 861 participants with complete ophthalmologic and vitamin B data. Incidence of advanced AMD was based on retinal fundus photographs and spectral domain optical coherence tomography examinations. Diet data were assessed with a 24h dietary recall. Associations between B vitamins and AMD were estimated using Cox proportional hazard models.

Results: Participants with normal serum folate (≥10 nmol/L) had a 51% reduced risk for advanced AMD in the fully-adjusted Cox model (HR, 0.49 [0.25-0.95]). Participants with higher dietary intake of B5 and B6 vitamins had an up to 28% lower risk for developing advanced AMD (HR, 0.72 for 1-SD increase [0.53-0.99]; HR, 0.90 [0.81-0.99], respectively).

Conclusion: This cohort study of French older adults suggests a strong association between a normal serum folate status, a high dietary intake of B5 and B6 and a lower risk for developing advanced AMD. Eating a healthy diet rich in B vitamins, particularly folate (leafy vegetables, fruits, whole grains), B5 (meat products, bread, milk-based products, vegetables) and B6 (liver, fish, leafy vegetables) may help to reduce vision loss due to the advanced forms of AMD.
BON 2022 13

Analysis of lutein, zeaxanthin and meso-zeaxanthin in dietary supplements by chiral HPLC-DAD: Single-laboratory method validation

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Purpose: In the US in 2021 alone, over 600 suppliers marketed supplements containing lutein (L) and different combinations of 3R,3'R-zeaxanthin (RZ) and meso-zeaxanthin (MZ). At Supplement Certified Ltd, we aim to achieve ISO/IEC 17025:2017 accreditation to help these companies comply with their label claims. To do this, we have validated our analysis method as part of our application.

Methods: Our in-house method is based on the US Pharmacopeia method “Aztec Marigold Zeaxanthin Extract”, with improvements. We have performed a single-laboratory validation of our method following Eurachem Guidelines, and we have compared our results with AOAC SMPR® 2016.004.

Results: Our method improvements consist of eliminating the use of chlorinated solvents, reducing solvent use, reducing analysis time and improving work conditions for the technician. Reproducibility for MZ, L and Z was 4.71 ± 2.00, 4.32 ± 1.55 and 7.10 ± 2.08, respectively (n = 63). Repeatability was 0.68 ± 0.51 % (n = 65). Linearity of our regression lines was typically 2.5 – 90 ng mL⁻¹, with the lower linearity limit set as experimental LOQ. Recovery of lutein was 101.58 ± 0.90 % (n = 3). Robustness of our method was demonstrated to achieve >99 % of analyte concentration tested when the extraction solvent, drying conditions and light conditions were changed.

Conclusions: Our in-house method improves upon the US Pharmacopeia method to analyse zeaxanthin stereoisomers, while complying with AOAC SMPR® 2016.004. We aim to submit this validated method as part of our application to achieve ISO/IEC 17025:2017 accreditation for Supplement Certified Ltd.

BON 2022 14

Correction of the influence of cataract on macular pigment measurement by autofluorescence technique using deep learning. The second report

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Purpose: Measurements of macular pigment optical density by the autofluorescence technique yield underestimations of actual values in eyes with cataract. We developed a correction algorithm using deep learning (DL) (Obana A et al. TVST 2021). In the present study, we improved this algorithm using a larger sample.

Methods: Macular pigment optical density (MPOD) was measured with Spectralis (Heidelberg Engineering, GmbH) before and after cataract surgery for 253 patients under Ethics Committee approval. The correction factor (CF) to estimate a true MPOD value (= postoperative value) from the preoperative value based on the preoperative image was obtained. Three types of images, autofluorescence images by blue and green light, and subtraction images of these two, were input to VGG16, a type of convolutional neural network (CNN). In order to compensate for the limited amount of data, the data were augmented by random cropping and left / right inversion. The accuracy of estimation was improved by fine tuning using a model pre-trained in the ImageNet database. This method was evaluated by 10-fold cross-validation. The CNN parameters were optimized by back propagation + Adam using the mean square error as a loss function.

Results: The error between corrected value and true value of MPOD volume in the area within 9° eccentricities was 9.3% with the previous algorithm and 7.3% with the present algorithm.

Conclusion: The present algorithm resulted in smaller error than our previous algorithm. The AI
correction method was considered useful for the measurement of MPOD in aging eyes with cataracts.

**BON 2022 15**

**Prediction of WASI-II Scores from Volumetric Brain Data and Nutrients**

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**Purpose:** Measuring and modeling nutrition status using biological variables is important to create dietary recommendations to promote brain health. We characterize nutrient biomarker patterns using nutrition and brain volumetric data that are linked to individual differences in cognitive performance (WASI-II scores) by answering two research questions. First, we aim to identify the latent associations among the variables that are most predictive of WASI-II. Second, using the latent association we identify the brain-nutrient biomarkers that are main predictors of WASI-II.

**Methods:** We collected data from 58 older adults (116 total), including cognitive testing (WASI-II; Stano J 1999), resting-state fMRI, and blood-samples. Features correspond to volumes of 17 brain ROI and 25 nutrients, including: carotenoids, vitamins, saturated and trans fatty acids, fatty acids. Further details appear in Zwilling, C. et al. (2019).

We studied the relation of nutrients and volumetric-features using several factorization methods: linear (PCA), independent (ICA), non-linear (Kernel embedding with RBF; K-RBF), third-order tensor (CMTF), and inner-point-preservation (Sammon). We build linear and non-linear regression models (Random Forest) using the factorization method with better identification of WASI-II, and analyze the models.

**Results:** Feature-relevance was measured by use of p-values (linear) and mean decrease-in-impurity (non-linear). 10-fold cross-validation is applied to identify models and features. Some relevant ROI: rh_middletemporal, lh_supramarginal, rh_caudalanteriorcingulate, lh_superiorfrontal. Some relevant nutrients: C15_0, C18_1n_9, C18_2n_6, C20_2n_6, cis_lutein, trans_lutein.

**Conclusion:** Our framework reveals non-parametric patterns of associations among nutrients and cortical volumes. We identified a few examples of relations involved. These patterns could be useful for advancing the theory of nutritional cognitive neuroscience.

**BON 2022 16**

**Supplementation with a marine derived Very Long Chain fatty acid concentrate can increase VLCFA in the eye and brain**

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**Purpose:** Endogenous Very Long Chain Fatty Acids (VLCFA) (C>24) are mainly found in tissues expressing the enzyme ELOVL4, required for their synthesis from shorter chain precursors. These tissues include the eye, meibomian glands, skin, testis, and spermatozoa, with the highest amounts of VLCFA found in the eye. Several associations have been drawn between ELOVL4 mutations and human pathologies. In the eye, heterozygous mutation in the ELOVL4 gene is associated with reduced VLCFA levels and development of Stargardt macular dystrophy type 3 (STGD3), a condition characterised by juvenile, progressive vision loss.

The development of a method for extracting and concentrating marine derived VLCFAs with a scalable production using processes approved for human consumption has made feeding studies easier to conduct, which have previously been hampered by lack of available VLCFAs.

**Methods:** Our team conducted a feeding study to determine uptake of VLCFA in mice after supplementation with a marine-derived VLC-concentrate. Thirty male C57/b16 mice were randomised to control plant oil, control fish oil, or VLC-concentrate diet groups. After 4 weeks of feeding, several organs were sampled and analysed for FA composition. VLCFAs were converted into methyl esters and quantitative measurements were made by GC-MS.
**Results:** There was a significant uptake of VLC-CFAs in both brain and eye tissue of mice fed the VLC-concentrate, demonstrating uptake of VLC-CFAs in target organs.

**Conclusion:** It is therefore of interest to investigate in future studies whether a VLC-concentrate supplement can have beneficial effects in VLC-deficient states, such as STGD3.

**BON 2022 17**

**The antioxidant capacity of the adult human vitreous body**

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**Purpose:** Oxidative stress and depleted intravitreal antioxidant capacity are two mechanisms that underlie vitreous degeneration and vitreous floaters, the most common visual symptom reported by patients who attend eye clinic appointments. This study was, therefore, conducted to evaluate the antioxidant capacity of the adult human vitreous.

**Methods:** Vitreous samples from 28 patients, who underwent vitrectomy for macular hole and epiretinal membrane at the Warsaw Medical University, were assessed for intravitreal antioxidant capacity using 2 different colorimetric methods: ferric ion reducing antioxidant parameter (FRAP) and 2,2’-diphenyl-1-picrylhydrazyl (DPPH). Body mass index (BMI) was computed from weight and height measurements.

**Results:** Of the 28 patients (mean age = 74 years), 16 were females and 12 were males. The mean FRAP values were 0.4505µmol/ml and 0.3713µmol/ml for females and males, respectively. Mean DPPH values were 0.2316mg/ml and 0.2237mg/ml for females and males, respectively. The highest FRAP and DPPH values were observed in the youngest age cohort (63-67 years) and the lowest values, in the older age cohort (78-82 years). FRAP correlated negatively with age, weight and BMI (p<0.05 for all; r for age, weight and BMI = -0.2487, -0.0313 and -0.0036, respectively, for females and -0.2255, -0.4196 and -0.3759, respectively, for males).

**Conclusion:** The findings of this study indicate that vitreous antioxidant capacity is dependent on various factors including age, sex and BMI. In addition, intravitreal antioxidant capacity decreases with age. Body weight may play an important role in the oxidative stress of the vitreous body.

**BON 2022 18**

**Characterization of carotenoid raw materials used as natural food colorants in the European Union**

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**Purpose:** Consumers are increasingly aware of the importance of using natural additives in food. Cybercolors (National Food Innovation Hub, Moorepark, Fermoy, Ireland) prepares and markets natural colors in the EU, including the carotenoids beta-carotene (*Dunaliella*), lutein (calendula), capsorubin (paprika), and the apocarotenoids bixin and nor-bixin (Annatto). It is well known that carotenoids have remarkable health benefits. As these carotenoids can be applied in a wide range of concentrations to foods, detailed knowledge of the composition of these raw materials is important for the development of new functional foods.

**Methods:** We are characterizing the carotenoid composition of the raw materials mentioned by establishing the appropriate saponification conditions and analysis by HPLC-DAD.

**Results:** Calendula and paprika extracts consist of carotenoids esterified with fatty acids; saponification conditions and carotenoid composition of these materials will be presented at the conference. The composition of the extract of the microalgae *Dunaliella* and of Annatto will also be presented.
Conclusions: Analysis of these raw materials and future analysis of other raw materials prepared by Cybercolors, including anthocyanins, chlorophyll, turmeric, beetroot and carmine, will strengthen our knowledge to support the possibility of food fortification with these natural colours.

BON 2022 19

Assessment of vitamin D3 levels in tear, saliva and plasma

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Purpose: The metabolic importance of vitamin D3 has been increasingly observed, and its deficiency is related to several diseases, including eye diseases (glaucoma, AMD, keratoconus, uveitis) and neurodegenerative diseases (Parkinson, Alzheimer, multiple sclerosis, dementia). Its level is almost always measured only in plasma, and little importance has been due to tear and salivary levels. The aim of this study is to simultaneously assess, for the first time in the medical literature, the levels of vitamin D3 in plasma, saliva and tears in healthy subjects.

Methods: Blood, saliva and tear samples were collected from 12 healthy subjects and the vitamin D3 levels of these samples were measured by the electrochemiluminescence method.

Results: Plasma, tear and salivary levels of vitamin D3 were evaluated in 12 individuals, mean age 45.33 years, 6 men and 6 women, and the mean results found were: 90.71 ng/ml (sd +/- 11.62) in the tear, 86.46 ng/ml (sd +/- 13.95) in saliva, and 25.77 ng/ml (sd +/- 9.17) in plasma. No statistically significant difference was found between tear and salivary vitamin D3 values, but a statistically significant difference was found between tear and plasma (p<0.00001) and between salivary and plasma vitamin D3 levels (p<0.00001).

Conclusions: No statistical difference between tear and salivary vitamin D3 levels was observed, but, there was a statistically significant difference (p<0.00001) between tear and plasma levels, and between salivary and plasma levels of vitamin D3. More research will be needed to understand these results.

BON 2022 20

Xanthophyll Carotenoids and Cardiometabolic Risk

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Purpose: Xanthophyll carotenoids may have a role in preventing cardiometabolic diseases (CMD), perhaps due to their antioxidant and anti-inflammatory properties. Here we examine the relationship between xanthophyll carotenoid status and risk of CMD development in participants at high and low risk of CMD.

Methods: Seventy-eight participants without medically diagnosed health conditions were recruited into this study. We divided participants into two groups: Group 1 (n = 38) = no CMD risk factors (no-risk, apparently healthy) and Group 2 (n = 40) = two or more CMD risk factors (high-risk). Serum lutein was assessed by HPLC and skin carotenoid score was assessed via carotenoid skin scanner.

Results: Mean (±SD) serum lutein in the no-risk group was 0.224 (±0.103) μmol/L and in the high-risk group was 0.169 (±0.091) μmol/L (p=0.015). Mean (±SD) skin carotenoid score in the no-risk group was 40,434 (±12,670) and in the high-risk group was 31,182 (±10,641) (p=0.003).

Conclusion: Our finding that apparently healthy individuals at high risk of developing CMD exhibit low levels of carotenoids in blood and skin suggests that targeted carotenoid interventions should be considered as a part of a preventive strategy for CMD.

BON 2022 21

Prevalence of Ocular Pathologies and Visual Impairment among Elderly Residents in Irish Nursing Homes

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Purpose: Ocular conditions can lead to visual impairment which ultimately affects quality of life. Vision loss is more common in institutionalised populations and among the elderly in residential care. The aim of this study is to investigate the prevalence of ocular pathologies and visual impairment in elderly residents of nursing homes (NHs) in Ireland.

Methods: This cross-sectional study will be conducted in a representative sample of elderly residents of NHs in the South East of Ireland. Standard ophthalmic eye examination will be performed from history taking, unaided monocular and binocular visual acuities using a digital acuity system LogMar chart, pinhole visual acuity, intraocular pressure using rebound contact tonometer, external eye examination using handheld slit lamp biomicroscope or penlight to non-mydriatic retinal camera.

Results: It is expected that this study will identify the prevalence of diagnosed and undiagnosed ocular pathologies and visual impairment among elderly residents in NHs. In addition, it will investigate related factors between ocular pathologies and visual impairment, such as ethnicity, race, sex, education status (literacy level), location, nutritional status and age.

Conclusion: This study will provide an understanding of the prevalence of ocular pathologies and vision impairment, including their related factors, among elderly residents in NHs. These findings will inform preventive strategies for timely diagnosis and management policies to improve quality of eyecare in this population.

BON 2022 22

Oxocarotenoids reduce neuronal cell oxidised phospholipids

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Oxidised phosphatidylcholines (oxPCs) are increased in neurodegenerative diseases such as Alzheimer’s disease. Oxocarotenoids, oxygen-containing carotenoid pigments found to be favourably accumulated in the human brain. These known to have potent antioxidant activity and has been reported to promote mitochondrial health. Therefore, this project aimed to investigate the hypothesis that lower concentrations of mitochondrial zeaxanthin and lutein may result in greater phospholipid oxidation and predispose to neurodegeneration.

Neuroblastoma SHSY-5Y cells were differentiated into neuronal-like cells by exposure to 10µM all-trans retinoic acid and growth factors in neurobasal media. After 10 days differentiation, dSH-SY5Y cells were treated with carotenoids (0.1, 1, and 10µM lutein and 0.05, 0.5, and 5µM zeaxanthin) for 24h at 37°C. The metabolic activity of cells in the presence of carotenoids were analysed using the CellTiter-Blue® assay (Promega, UK) and cellular glutathione (GSH) levels were measured by GSH recycling assay. Cell lysates were collected, and lipid extracts were prepared as described before. A panel of 12 oxidised phospholipids were measured by liquid chromatography -mass spectrometry methods developed in our lab. Lutein and zeaxanthin reduced mitochondrial ROS and increased cellular GSH levels compared to the untreated control. Pro-inflammatory oxPCs (POVPC, PONPC, SOVPC, SGPC SAzPC and HOOAPC) were reduced in lutein (1µM) and zeaxanthin (5µM) treated cells. Mitochondrial oxygen consumption rate was significantly increased in the presence of 5µM zeaxanthin and 1 µM lutein. In summary, oxocarotenoids could rescue oxidative phenotype of neuronal cells by increasing GSH levels and reducing truncated oxPCs.

BON 2022 23

Can a lutein and zeaxanthin intake screener provide useful data?
Preliminary results of a validation study

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Purpose: Habitual dietary lutein (L) and zeaxanthin (Z) intake is a significant confounding variable when investigating change in L/Z macular concentrations in prescriptive L/Z intervention studies (sup-
The differences in L/Z intake between the 24-hour recalls and screener reported by participants were normally distributed, suggesting no systematic bias. The mean difference in L/Z intake reported between the recalls and screener was 0.17 mg (95% limits of agreement from -0.77 mg to 1.12 mg).

**Conclusions:** The mean difference demonstrated a bias of 0.17 mg/day greater L/Z intake reported through 24-hour recalls compared to the weekly screener. Younger Australian females are known to underreport more than males or older adults. The 95% limits of agreement indicate the weekly screener may be valid for observing changes to habitual intake greater than 1.9 mg/day of L/Z. Further data will assist in elucidating the value of the screener.

Methods: Healthy adults were recruited to complete two 24-hour diet recalls and one weekly screener per week for 4 consecutive weeks. The degree of agreement between the L/Z intake captured by the 24-hour recalls and weekly screener (expressed as mean daily intake in milligrams) was tested by Bland-Altman plot analysis.

Results: Preliminary analysis of 16 participants (81% female, median age 28 years) was performed.
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