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By

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QUANTIFICATION OF LUTEIN + ZEAXANTHIN PRESENCE IN HUMAN PLACENTA AND CORRELATIONS WITH BLOOD LEVELS AND MATERNAL DIETARY INTAKE

Melissa K. Thoene, Ph.D.

University of Nebraska, 2018

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Lutein + zeaxanthin are carotenoids most recognized in eye health, but less is known about their status and transfer during pregnancy. While quantified in maternal and umbilical cord blood, they have never been analyzed in placenta tissue. Therefore, the purpose of this dissertation is to firstly quantify lutein + zeaxanthin concentrations in human placenta and to later correlate with levels in maternal dietary intake, maternal serum, and umbilical cord blood. Proportions of lutein + zeaxanthin will also be compared within diet, placenta, maternal serum, and umbilical cord blood among additionally analyzed carotenoids including lycopene, β-cryptoxanthin, α-carotene, and β-carotene. Lutein + zeaxanthin concentrations across all samples will be analyzed for relationships with maternal demographics and infant birth outcomes. An IRB-approved cross-sectional study enrolled 82 mother-infant pairs for infants born at Nebraska Medicine Hospital (Omaha, NE). Placenta, maternal serum, and umbilical cord blood samples were collected and analyzed for carotenoids concentrations. Mothers completed a food frequency questionnaire to identify usual intake. Demographic and birth outcome data were collected from the electronic medical record. Lutein + zeaxanthin were present in human placenta at median 0.105 micrograms/gram (mcg/g) and were significantly correlated with levels in maternal serum ($r=0.57$; $p<0.001$) and umbilical cord blood ($r=0.49$; $p=0.001$), but not maternal dietary intake ($p=0.110$). Lutein + zeaxanthin were the most prevalent carotenoids in human placenta (49.1%) and umbilical cord blood (37.0%), but not in maternal serum (18.6%) or dietary intake (19.4%). Median concentrations of lutein + zeaxanthin in placenta were lower in

Caucasian mothers (0.097 vs. 0.128 mcg/g; p=0.007), those with a smoking history (0.090 vs. 0.117 mcg/g; p=0.049), and approached significance in obese compared to normal weight mothers (0.098 vs. 0.125 mcg/g; p=0.088). Rate of lutein + zeaxanthin transfer from mother to infant was 16.0%, highest of all carotenoids. Conclusively from this study, lutein + zeaxanthin were identified as the two most prevalent in human placenta. Results highlight unique roles lutein + zeaxanthin may play in pregnancy, with potential benefits conferred to the developing infant.

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LIST OF ABBREVIATIONS

AMD	age-related macular degeneration
AREDS	Age-Related Eye Disease Study
BMI	body mass index
cm	centimeters
dL	deciliter
g	grams
HDL	high-density lipoprotein
kg	kilograms
L	liter
LDL	low-density lipoprotein
m	meter
mcg	micrograms
mcmol	micromole
mg	milligrams
mL	milliliter
mmol	millimole
mol	mole
MPOD	macular pigment optical density
ng	nanograms
NHANES	National Health And Nutrition Examination Survey
NICU	newborn intensive care unit
nmol	nanomole

ODU	optical density units
pg	picograms
pmol	picomole
RDS	respiratory distress syndrome

INTRODUCTION

It is well known the importance of adequate dietary intake of macro and micronutrients including protein, fat, carbohydrate, vitamins, and minerals. However, alongside these are nutrition-related components, not always deemed essential for life, but lending towards enhanced body functioning and overall health. Included in this category are carotenoids, which include over 600 types found in nature[1]. The most common carotenoids found in the human diet and resultantly in body tissues include α-carotene, β-carotene, lutein, zeaxanthin, lycopene, and β-cryptoxanthin[2]. Of these, lutein and its counterpart zeaxanthin are the focus of this dissertation.

Lutein and zeaxanthin are most well-recognized as protective against macular degeneration in older populations[3]. However, emerging evidence has identified associations between blood lutein and zeaxanthin levels and improved cognition in adults[4]. Though less is known about lutein and zeaxanthin in early life, they have been identified to consist of more than half of the carotenoids in brain tissue of young children[5]. Further research has also identified lutein and zeaxanthin as two top carotenoids in human milk[6,7], which suggests neonatal-specific benefits conferred during a period of rapid growth and brain development. Preliminary evidence to support this stems from research by Cheatham *et al.*, who reported a relationship between lutein (and choline) levels in maternal breast milk at several months postpartum and infant memory and cognitive outcomes[8]. With these considerations, special interest has been taken in pregnant and neonatal populations to firstly identify lutein and zeaxanthin status during these critical periods and to secondly observe dynamics of maternal to fetal carotenoid transfer. Lutein and zeaxanthin have been identified in maternal and fetal blood, but never in human placenta. Analysis of placental tissue to more

fully understand carotenoid transfer remains advantageous considering research by Palan *et al.,* who reported inconsistent carotenoid responses between placental tissue and maternal serum in preeclamptic mothers[9]. Furthermore, no study has simultaneously compared placenta, maternal, and fetal blood carotenoid levels in conjunction with maternal dietary carotenoid intake. Therefore, the primary purpose of this study is to quantify lutein + zeaxanthin presence in human placenta. Secondary aims will seek to identify if placenta levels are correlated with maternal dietary intake, maternal serum, and umbilical cord blood levels or if levels show relationships with birth outcomes.

CHAPTER 1: REVIEW OF THE LITERATURE

What are Lutein and Zeaxanthin?

Lutein and zeaxanthin are carotenoids, fat-soluble pigments which provide color to plants. Lutein and zeaxanthin commonly appear yellow, though high concentrations may appear orange-red in color. Lutein and zeaxanthin, along with β-cryptoxanthin, are more specifically known as xanthophyll carotenoids, defined as "any of several yellow to orange carotenoid pigments that are oxygen derivatives of carotenes"[10]. The most prevalent carotenoids in the American diet are segregated into two groups: provitamin A and non-provitamin A carotenoids. β-carotene, α-carotene, and β-cryptoxanthin are able to be cleaved and converted to retinol in the body, therefore being delegated as provitamin A carotenoids[1]. Lutein, zeaxanthin, and lycopene do not have similar Vitamin A activities, so are therefore listed as non-provitamin A carotenoids[1].

Informally, carotenoids are often called antioxidants. However, according to the Institute of Medicine, a dietary antioxidant is defined as "a substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological function in humans"[1]. They also provide three criteria for meeting this definition including "(1) the substance is found in human diets; (2) the content of the substance has been measured in foods commonly consumed; and (3) in humans, the substance decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species in vivo"[1]. By this definition and review in 2000, lutein and zeaxanthin, both non-provitamin A carotenoids, did not meet criterion for classification as dietary antioxidants[1]. While meeting the first two criteria, the third remained unmet. No unanimous dietary requirements have been set for these carotenoids, given no concrete and specific nutrient functions had previously been well-

identified. Similarly, while antioxidant activity had been demonstrated on an in vitro basis, more controversy existed on benefits of these carotenoids on an in vivo capacity[1].

Structure

Lutein and zeaxanthin are commonly analyzed simultaneously, as these isomers have a molecular structure of $C_{40}H_{56}O_2$ and a molecular weight of 568.886 grams/mole[11,12]. The difference in molecular structure is the location of the double hydrogen bond, therefore yielding three chiral centers in lutein and two in zeaxanthin[12]. Additionally, lutein contains both a β-ring and ε-ring end group, whereas zeaxanthin contains two β-ring end groups[13]. Though most naturally-occurring carotenoids are structured as *all-trans,* natural lutein sources are commonly present in *cis* form[1,14]. Meso-zeaxanthin is also analyzed in the literature, a diastereomer to zeaxanthin that structurally differs in the spatial orientation of one hydroxyl group[13]. Sources of meso-zeaxanthin are not well determined, with limited reports of presence in food while other sources hypothesize it as a derivative of lutein within the eye macula[13].

Lutein and zeaxanthin structure differently from other carotenoids, as evidenced in Figure 1. While α-carotene, β-carotene, β-cryptoxanthin, lutein, and zeaxanthin appear as similar polyene structures with two cyclic ends, only lutein and zeaxanthin have hydroxyl groups on both cyclic ends[1]. β-cryptoxanthin has one cyclic end with a hydroxyl group, allowing it to be deemed a xanthophyll[1]. The carotenes have no hydroxyl groups and lycopene has the most dissimilar structure of all with no cyclic ends, no hydroxyl groups, and a more linear polyene configuration[1]. Consideration of retinol's structure, as displayed in Figure 2, is useful in understanding the segregation of carotenoids into provitamin A and non-provitamin A categories. Simplistically, retinol is a polyene structure with one cyclic end containing no hydroxyl group[15]. Therefore, baseline structures of lycopene (no cyclic ends) and lutein +

zeaxanthin (contain hydroxyl groups on their cyclic ends) do not allow for conversion to retinol through cleavage, unlike the carotenes and β-cryptoxanthin.

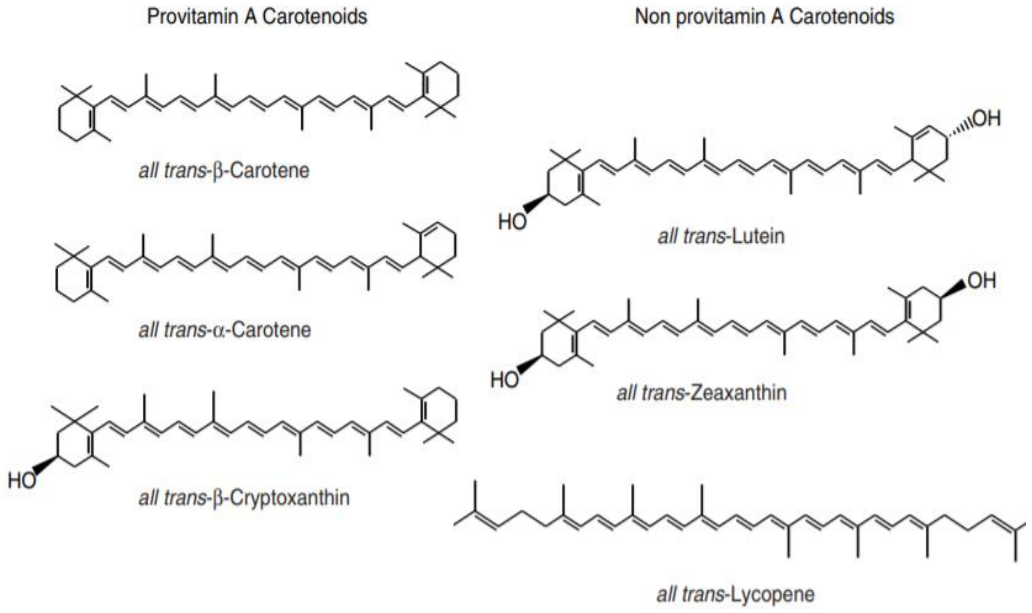

Figure 1: Structure of Provitamin A and Non-Provitamin A Carotenoids

*Reprinted with permission from *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*, 2000 the National Academy of Sciences, Courtesy of the National Academies Press, Washinton, D.C.[1]

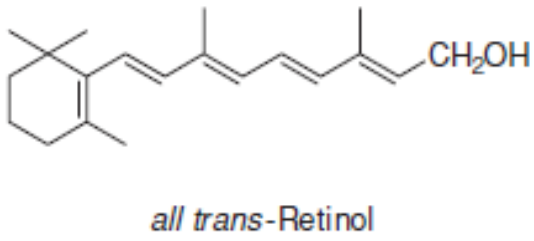

Figure 2: Structure of Retinol

*Reprinted with permission from *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc.*, 2001 the National Academy of Sciences, Courtesy of the National Academies Press, Washinton, D.C.[16]

Dietary Sources

Lutein and zeaxanthin can be synthesized by plants, but not humans[17]. Therefore, humans must rely on dietary consumption to increase body levels. Naturally-occurring sources of combined lutein + zeaxanthin primary include colored fruits and vegetables. Highest concentrated sources include dark green leafy vegetables, corn, and eggs[18]. Ratios of lutein to zeaxanthin content in natural foods have been assessed. Lutein is often the primary or only carotenoid source, with many foods containing no zeaxanthin. Foods containing more equivalent ratios of lutein: zeaxanthin are limited, with main examples as corn and eggs[19]. Combined lutein + zeaxanthin contents of food, as identified from the nutrient database from the United States Department of Agriculture, are exemplified in Table 1[18]. Combined content is listed in milligrams (mg) per 100 grams (g) and per one cup of food for comparison.

Table 1: Lutein and Zeaxanthin Content in Food

Food	Serving Preparation	Lutein + Zeaxanthin Content (mg) per 100 grams	Lutein + Zeaxanthin Content (mg) per 1 cup
Paprika	spice	18.94	20.93
Pepper, red, or cayenne	spice	13.16	11.38
Turnip greens	raw	12.83	7.05
Spinach	raw	12.20	3.66
Swiss chard	raw	11.00	3.96
Kale	raw	6.26	1.32
Watercress	raw	5.77	1.96
Basil	fresh	5.65	--
Parsley	fresh	5.56	3.34
Collards	raw	4.32	1.56
Mustard greens	raw	3.73	2.09
Arugula	raw	3.56	--
Pistachio nuts	raw	2.93	--
Green Peas	raw	2.48	3.59
Romaine lettuce	raw	2.31	1.09
Summer squash, all varieties	raw	2.13	2.40
Brussel sprouts	raw	1.59	1.40
Broccoli	raw	1.40	1.28
Yellow corn	Raw	0.64	0.93
Egg	Hard-boiled, chopped	0.50	0.48

Lutein and zeaxanthin are both available in supplement form, though are not routinely added to popular child, adult, or prenatal multivitamin formulations. Lutein may be offered singularly in supplement form, but zeaxanthin is often only included alongside lutein. Marigold flowers are often the ingredient for lutein supplements given its high natural concentration with an estimated content of 13.8-31.3 micrograms (mcg) lutein per gram of undried flower[20]. However in recent years, microalgae may be used commercially as it contains a higher free lutein content and has a faster growth rate than marigold flowers[21].

Dietary Recommendations and Intakes

No unanimous consensus recommendations for daily consumption of lutein and zeaxanthin exist. There are no dietary reference intakes or tolerable upper level intakes for carotenoids set for by the Institute of Medicine, unlike most vitamins and minerals[2]. Lutein intakes of 6 mg/day are generally encouraged to promote eye health[22]. Recommended intakes to promote slower progression of age-related macular degeneration stems from one large trial, the Age-Related Eye Disease Study (AREDS)[3]. Results, now promoted by both the American Optometric Association[23], encourage 10 mg/day of lutein and 2 mg/day of zeaxanthin[3]. Pharmacological references report that dosing of 5 mg/day is commonly used with some trials even providing 10-20 mg/day for minimum of 3-6 months[24]. One popular-brand prenatal supplement provides 6 mg of lutein per daily supplement, but no zeaxanthin[25]. Of further interest, in 2010 the 'Panel on Food Additives and Nutrient Sources added to Food' by the European Food Safety Authority discussed the safety of the addition of lutein from *Tagetes erecta* (marigold flower) in food. *Tagetes erecta* is a marigold flower containing at least 80% of carotenoids as lutein and zeaxanthin. They concluded an acceptable daily intake to be 1 mg/kilogram(kg) of body weight per day[26]. Similarly, they raised no safety concerns for adding 250 mcg of lutein per liter of infant formula[27].

There appears to be limited toxicology information available. Currently, there is "strong safety evidence" with lutein doses of 20 mg/day[24]. Only one case report is available that describes an adverse outcome[28]. It was identified that a woman in her sixties took a 20 mg/day lutein supplement for eight years alongside high daily dietary intake of lutein from broccoli, kale, and spinach[28]. Her serum lutein level was 519 nanograms(ng)/milliliter(mL) when she presented with fovial crystals in each eye, yet no visual changes. After seven months of discontinuing the lutein supplement, the fovial crystals resolved only in one eye and her serum level decreased to 191 ng/mL[28]. Beyond this case report, the only other identified result of high dietary carotenoid intake is benign skin yellowing[2]. Comparatively, Ravikrishnan *et al.* provided rats with up to 400 mg/kg/day of combined lutein + zeaxanthin for 90 days and reported no significant adverse outcomes in body, organ, ophthalmic, or biochemical measures[29].

Despite safe higher end dosing, most research in the United States report intakes of <5 mg/day. In fact, literature suggests average intakes closer to 1-3 mg/day for most Americans[14]. Large cohort data is from the National Health and Nutrition Examination Survey (NHANES). Combined lutein + zeaxanthin intake in women of child bearing age (categories 12-49 years; N=6,600) was estimated at mean 1.14 to 1.77 mg/day[30]. Comparatively, men of the same age range consumed mean of 1.40 to 2.05 mg/day[30]. Another large cohort from the Nurses' Health Study and Health Professionals Follow-Up Study (N=52,740) reported average estimated intakes of 1.66 to 4.78 mg/day across quintiles[31]. Interestingly, acculturation in the United States is associated with decreasing serum carotenoid levels, including that of lutein + zeaxanthin, most attributable to a lower intake of fruits and vegetables[32]. This is consistent with NHANES data which reported among adults age 19 years or older (N=16,338) that only 12.9% met the minimum recommendations for vegetable intake based on their usual dietary intake[33]. Furthermore, while dark green vegetables contribute most to a higher lutein intake, only 5.9% of

adults met minimum recommend intakes[33]. Current recommendations by the United Stated Department of Agriculture for women within child-bearing age (included categories of age 14-50 years) is 1 ½ - 2 cups/day fruit and 2 ½ cups/day of vegetables (with 1 ½ cups/week coming from dark green vegetables)[34,35]. It has been estimated that lutein + zeaxanthin intake would range from 4-7 mg/day if consuming the recommended daily amount of fruits and vegetables[36].

Differences in dietary intake of lutein + zeaxanthin may vary depending on age, education level, race, and socioeconomic levels. For example, Johnson et al. reported increasing lutein + zeaxanthin intake across increasing age categories[37]. Likewise, Rock *et al.* predicted in a multivariate regression model every decade of age to increase dietary lutein + zeaxanthin intake by 3.9% (N=2,786)[38]. Rock *et al.* also predicted individuals with graduate degrees to consume 23.8% more lutein + zeaxanthin compared to those receiving a high school education or less[38]. In regard to race, data primarily identifies Caucasians as consuming the lower amounts than African-Americans and Hispanic groups.[30,39]. Similarly, Rock *et al.* reported that African-American, Hispanic, and "other" race categories were predicted to consume between 6.2-15.7% more lutein + zeaxanthin compared to Caucasians[38]. Lastly, NHANES III data (N=17,002) conducted multivariate linear regression for serum carotenoid levels across quartiles of neighborhood deprivation, reporting significant negative associations between serum lutein + zeaxanthin and worsening deprivation[40]. Though no data on dietary intake was reported in this study, author conclusions were that limited food access and security reduce diet quality. Furthermore, reduced diet quality caused by socioeconomic disparities likely limit fruit and vegetable intake, which ultimately decrease carotenoid consumption.

Digestion and Metabolism

There are many steps in the process of carotenoid absorption, including that of lutein and zeaxanthin which are both lipophilic. Firstly, the bioavailability of lutein and zeaxanthin is determined by the structure of the food matrix, which allows for their release and subsequent ability to be absorbed. The bioavailability varies dependent on dietary form, co-ingested food, and cooking techniques. For example, singular dietary supplements may have high absorption up to 70%, as these are often emulsified and protected[2]. In contrast, bioavailability from natural food may be as low as 2-5% for carotenoids[2]. Cooking, steaming, or processing of vegetables can weaken the food matrix structures, therefore allowing increased carotenoid release[2]. Released carotenoids have lower ability to disperse throughout digestive fluid due to their hydrophobicity[41]. Consequently, carotenoids cling to lipids, providing rationale for increased absorption when simultaneously consumed with dietary fat[1,2,41]. Fat consumption also stimulates bile acid and enzyme secretion, which allows carotenoids to solubilize with bile acid micelles[2]. Primary structures of complete micelles are made of bile acids, fatty acids, phospholipids, cholesterol, and monoacylglycerols[41]. Once incorporated into micelles, carotenoids have increased absorption by intestinal cells[41]. Given lutein and zeaxanthin are categorized as non-provitamin A and are unable to be cleaved, they are absorbed by the intestinal cells intact[2]. They are subsequently integrated into chylomicrons, enter the lymphatic system, and later enter the bloodstream[2]. Lipoprotein lipase degrades the chylomicrons, and remnants are later taken up by the liver[41]. The liver either stores the released carotenoids or secretes them back into the circulatory system exclusively by lipoprotein transporters[41]. While many carotenoids are transported by low-density lipoprotein (LDL) cholesterol (i.e. α-carotene, β-carotene, and lycopene), the xanthophyll carotenoids (including lutein and zeaxanthin) tend to be more polar and are more commonly carried and received by high-density lipoprotein (HDL)

cholesterol[41]. However, lutein and zeaxanthin may also be carried by very low-density lipoproteins[1]. It has been theorized that the polar carotenoids are carried toward the surface of the lipoproteins, whereas the remaining carotenoids are more internally located[1]. Lipoproteins transport lutein and zeaxanthin via the blood to various tissues of the body, the most common areas being the eyes, liver, and adipose tissue[1]. Carotenoids may later be excreted with intestinal bile or through the urine[2].

While lipoproteins transport carotenoids in the human body, transfer of carotenoids from breast milk to infants is more highly influenced by milk fat globules once they enter human milk[6]. It is also reported that overall carotenoid concentrations are increased in hind milk compared to fore milk, hypothesized to be due to the higher fat content[42]. Notably, research has questioned the bioavailability of lutein in infant formula compared to human milk. Bettler *et al.* demonstrated that similar amounts of lutein in breastmilk compared to formula lead to four times higher lutein levels in infant serum[43]. Similarly, breastmilk-fed infants had baseline serum lutein levels that were approximately six times higher than that those of infants receiving formula without lutein[43]. Relevant however, is that popular-brand infant formulas in North America are now adding milk fat globule membrane to their products[44]. This leads to question if added milk fat globule membranes would enhance lutein absorption in formula-fed infants, given previous research correlating human milk carotenoid content with milk fat globule content[6]. Additionally, lutein and zeaxanthin are more easily solubilized into lipid bilayer membranes than non-xanthophyll carotenoids, so incorporation into milk fat globule membrane may improve absorption opportunities[45].

Only limited research has assessed carotenoid presence in placenta, but never of lutein or zeaxanthin[9]. As combined lutein + zeaxanthin has been quantified in umbilical cord blood[46], there is potential that lutein + zeaxanthin is present in placental tissue. Review of basic placenta function allows for greater comprehension of how carotenoids may transfer from mother to fetus during pregnancy. The placenta remains a unique organ in pregnancy that serves many functions to the fetus comprising similar roles as a lung, kidney, digestive, and immune system[47]. Ingested maternal nutrients are absorbed in the intestine and transported to the maternal circulatory system, as previously reviewed. This then allows transfer to various tissues throughout the body, such as the placenta. Highly vascularized, the placenta contains two unique circulatory systems: the uteroplacenta and the fetoplacenta[47]. Unique is that maternal and fetal blood do not intermix in the placenta, yet maternal blood flow to the placenta is estimated at 600-700 mL per minute at time of birth[47]. Instead, maternal blood enters the placenta via spiral arteries into an intervillous space where it flows over villi[47]. The villi include a continuous layer of special epithelial cells called syncytiotrophoblasts, which create two membranes: one facing maternal circulation (microvillous membrane) and one facing the fetal capillaries (plasma membrane)[48]. These two layers dictate which nutrients are able to cross into the fetal blood circulation. The first layer of syncytiotrophoblasts are continuously bathed in maternal blood and allow for absorption of nutrients (like carotenoids), whereas the second layer near fetal circulation is selective in the molecule permeability, generally restrictive towards large molecule diffusion[48]. It is important to note that the surface area of syncytiotrophoblasts increases across the trimesters, which allows for increasing fetal growth. In example, surface area increases from 5 to 11-12 meters$(m)^2$ from 28 weeks gestation to term[47]. Previous research has reported increasing maternal carotenoid blood levels throughout pregnancy[46], which may be hypothesized to be associated with increasing maternal circulating fatty acids,

triglycerides, and cholesterol as pregnancy progresses[49]. Ultimately, the placenta contains lipoprotein receptors, allowing transfer into fetal circulation which allows bound nutrients, like carotenoids, to simultaneously be transported. Primary lipoprotein receptors among placental trophoblasts include low-density lipoprotein receptor for up uptake of LDL cholesterol and scavenger receptor class B type I for uptake of HDL cholesterol[50].

It is reported that blood levels of carotenoids peak within 24-48 hours following singular dosing[1]. This is consistent with Romagnoli *et al.* who supplemented ten preterm infants with lutein (0.5 mg/kg) and assessed plasma lutein levels at baseline, followed by 6, 24, 48, and 120 hours after one time dosing[51]. Supplementation increased plasma levels by 13.5% at 24 hours, 16.7% at 48 hours, with a return to baseline by 120 hours. The half-life of lutein within blood has been suggested to be approximately 5.6 days with dosing of 18 days to reach >90% steady state concentration[52]. Likewise, the half-life of zeaxanthin has been suggested at 5 days, with 17 days to reach steady state[53].

Factors influencing normal digestion and absorption include certain medications such as lipid-lowering drugs or pectin supplements, as they limited absorptive capabilities[2]. Carotenoids compete with one another for absorption, so high supplementation of one may negative influence blood concentrations of another. For instance, high supplementation of β-carotene has previously demonstrated an inverse relationships with blood lutein concentrations[2]. However, this effect is likely more pronounced with supplement use as opposed to regular dietary intake. Lastly, intestinal or hepatic diseases may hinder normal digestion as both systems are integral to normal carotenoid metabolism. In example, Schupp *et al.* demonstrated significantly lower plasma lutein (87 vs. 190 nanomoles/Liter (nmol/L)) and zeaxanthin (27 vs. 75 nmol/L) levels in cystic fibrosis adults (N=10) compared to healthy controls[54]. There also remains emerging evidence indicating differences in carotenoid metabolism based on genetics.

For instance, Mohn *et al.* also reported differences in brain levels of lutein and zeaxanthin, despite similar dietary intakes, in primates of Indian compared to Chinese origin[55]. Likewise, Meyers *et al.* reported differences in lutein + zeaxanthin eye tissue levels based on various carotenoid metabolism polymorphisms, after adjustment for dietary intake of lutein + zeaxanthin (N=1,585)[56]. Multiple polymorphisms for protein have also been identified that influence lutein and zeaxanthin metabolism, uptake, transport, and stabilization within body tissues[57].

Presence in Tissue, Blood, and Human Milk

Current literature reports quantitative lutein and zeaxanthin tissue concentrations primarily within the blood, liver, kidney, adipose tissue, and brain[1]. However, levels have also been detected in multiple other areas of the body including the skin, breast, uterus, ovary, testes, adrenals, pancreas, spleen, heart, and thyroid[58-60]. Of these areas, carotenoids are most concentrated within adipose tissue and the liver, as these are the primary storage sites[1]. Uniquely however, the eye most selectively uptakes lutein and zeaxanthin levels over remaining carotenoids[61]. For comparison of quantitative values and abbreviations in the following text, please reference the following conversions: 1 gram (g) = 1,000 milligram (mg) = 1,000,000 micrograms (mcg) = 1 x 10^9 nanograms (ng) = 1 x 10^{12} picograms (pg). Conversion are similar for mole (mol), millimole (mmol), micromole (mcmol), nanomole (nmol), and picomole (pmol). Additionally, conversion factor for lutein and zeaxanthin from mcg/deciliter (dL) to mcmol/Liter (L) is 0.01758[1].

Lutein and zeaxanthin are present in all ocular regions except the vitreous, sclera, and cornea[62]. Tissue lutein + zeaxanthin concentrations within the eye have been reported at 0.1-1.0 pmol/m^2 in the inner retinal layer, 44.1 ng/g epithelial layer, 15.1 ng/g for nuclear layer[63,64].

Ultimately however, Lutein and zeaxanthin remain the two most prominent carotenoids within the eye macula, though meso-zeaxanthin is also present[61]. The *macula lutea*, or macula, is located within the posterior center of the retina near the foveal region, an area with dense number of cone receptors to allow optimal vision[65]. It has been identified that zeaxanthin is most highly concentrated within the central fovea with lutein more so in the periphery[65]. The density of these carotenoids account for the yellow coloring seen within this region and are standardly referred to as macular pigment[61,65]. Macular pigment is also termed interchangeably in the literature with macular pigment optical density, or MPOD. Macular pigment is measured in optical density units (ODU) by heterochromatic flicker photometry[65], which commonly ranges from 0-1[66]. As background, heterochromatic flicker photometry is able to measure MPOD, based on the ratio of blue light wavelength in the central retina compared to that in the peripheral retina where there is minimal wavelength. The flicker occurs when the central wavelength moves outward where there is limited wavelength, with the two measurement points becoming unequal, and therefore reading a density level[67]. As macular pigment consists of lutein and zeaxanthin, it remains of interest to compare MPOD values. Continued research is also reporting positive correlations between MPOD values, dietary intake, and blood lutein and zeaxanthin levels[68,69]. Suggested ranges for MPOD vary slightly, but are estimated to be <0.20 as low, 0.2-0.5 as mid-range, and >0.5 as high[66]. Newborn MPOD in term-born infants is much lower than adults with range reports of 0.04-0.16 ODU (average of 0.087; N=16)[70]. MPOD reportedly increases with age, with Bernstein *et al.* finding undetectable values in preterm infants, but a range of less than 0.05 to almost 0.40 ODU in children age 0-7 (N=51)[71]. Contrasting data by Bone *et al.* detected macular pigment in the eyes of fetuses at 17-22 weeks gestational age, though a yellow spot could not be visualized[72]. Lutein was reported as the primary macular pigment in subjects under two years of age (N=7), but zeaxanthin was the

primary macular pigment in the older subjects (n>30)[72]. Comparison of data by Sasano *et al.* reported detection of measurable MPOD in low birth weight infants (N=45) as low as a gestational age of 33 weeks, 2 days[73]. Mean MPOD was 0.076 ODU in infants at 36 weeks and regression analysis demonstrated a strong positive linear trend between infant gestational age and MPOD ($R^2 = 0.91$; $p<0.001$)[73].

In addition to no unanimous recommendations for dietary lutein + zeaxanthin intake, there are no reference ranges for blood levels. Concentration examples reported by the Institute of Medicine include 0.10-1.23 mcmol/L or 4.8-69.8 mcg/dL for adult serum[1]. It has been proposed that plasma lutein + zeaxanthin ≥0.67 mcmol/L (or $\geq$38 mcg/dL) are associated with the lowest risk of age-related macular degeneration(AMD)[1]. In example during pregnancy, Horton *et al.* reported increasing serum concentrations of lutein + zeaxanthin across each trimester (mean of 0.41 mcmol/L 1st trimester, 0.58 2nd trimester, 0.61 3rd trimester)[46]. Mother and infant blood levels of lutein and zeaxanthin are correlated, with newborn levels at roughly 18-29% of maternal levels[46,70,74,75]. Newborn serum levels of lutein have been reported at averages of 17.1 ng/mL (range 4.9–33.8) and average serum zeaxanthin of 7.14 ng/mL (range 0.9–15.9), respectively[70]. Umbilical levels have been reported at 0.10 mcmol/L (range 0.065-0.28)[46] and 0.13 mcmol/L[76].

Reported concentrations of lutein + zeaxanthin in alternative tissues are as follows: 0.10-3.0 mcmol/g or 0.06-6.9 mcg/g in liver, 0.037-2.1 mcmol/g or 0.05-5.9 mcg/g in kidney, and 0.1-2.3 mcmol/g or 0.05-1.3 mcg/g in lung[1]. Adipose tissue concentrations of lutein + zeaxanthin have been reported at 0.92 $\pm$ 0.53 mcg/g in men and 1.21 + 0.73 mcg/g in women, though women had higher dietary intake of lutein + zeaxanthin than men in this study[77]. Of interest, Chung *et al.* reported higher carotenoid concentrations in abdominal adipose tissue compared to less centrally-located adipose like the thigh or buttocks[78]. Examples of combined

lutein + zeaxanthin concentrations in adipose tissue are 456.3 pmol/mg (abdomen) compared to 268.5 (thigh) and 277.0 (buttock)[78].

Lutein and zeaxanthin have also been identified as two of the four of the primary carotenoids in the brain tissue of deceased children less than 1.5 years of age[5]. Lutein itself accounted for 59% of overall brain carotenoids, being detected at a concentration range of 0–181.7 pmol/g and zeaxanthin at range 0–33.94 pmol/g[5]. Furthermore, the mean lutein concentration was >40 pmol/g whereas the mean combined concentration of three other carotenoids (zeaxanthin, cryptoxanthin, and β-carotene) was $\leq$40 pmol/g[5]. While there were cases in which infants had no detectable levels of at least one of these carotenoids, only preterm infants had undetectable levels of lutein or zeaxanthin[5]. Preterm infants had statistically lower concentrations of brain lutein compared to term-born infants, but there were no statistical differences for zeaxanthin[5]. Comparatively, Tanprasertsuk *et al.* reported increasing brain concentrations of lutein with increased age, including levels of 43.95 ± 9.53 pmol/g in infants (1-4 months, N=10), 62.61 ± 5.18 in older adults (age 55-86 years, N=8), and 97.70 ± 19.59 in centenarians (age 98-105 years, N=10)[79]. A larger scale study of 80-100 year old adults (N=47) also detected significantly higher lutein presence in all brain tissue compared to other carotenoids of zeaxanthin, carotene, lycopene, and cryptoxanthin[80]. Total lutein and zeaxanthin were most highly detected in the cerebellum at concentrations of 176.4 ± 16.6 pmol/g and 52.9 ± 4.3 pmol/g[80]. It is estimated that xanthophyll carotenoids account for 66-77% of all brain carotenoids[81], suggesting selective uptake by the body.

Infants not taking table food only consume lutein and zeaxanthin available in breast milk or commercial infant formula. Research reports lutein and zeaxanthin content of maternal breast milk is directly correlated with mother's dietary intake and blood levels[6,82]. In example, Lipkie *et al.* reported that 45% of the variability of lutein content in breast milk is due to

maternal plasma levels[6]. Similarly, 40% of the variability in neonatal plasma lutein levels are due to the carotenoid content in breast milk[6], demonstrating infant dependence on maternal supply. Analysis of breast milk samples at 2, 4, 13, and 26 weeks postpartum from mothers (N=60) with infants born >2.5 kg and >37 weeks gestational age in the United States, Mexico, and China demonstrated an interquartile range of lutein at 70-179.5 nmol/L and zeaxanthin at 20.5-48.1 nmol/L[6]. Lutein and zeaxanthin consisted of approximately 42% and 11% of milk carotenoids when compared with β-cryptoxanthin, lycopene, α-carotene, and β-carotene[6]. Canfield *et al.* also reported variability in lutein and zeaxanthin breast milk content across nine different countries[7]. There was limited assessment of dietary carotenoid intake of included mothers, but lutein and zeaxanthin concentrations in the United States were the lowest than any other analyzed country at concentrations of 0.888±0.096 nmol/g of lipid and 0.026±0.001 mcmol/L [7]. Lutein remained the top carotenoid in breast milk samples for 4 out of 9 countries, but the highest in milk samples from the United States was β-carotene with lutein in second[7]. In contrast, Khachik *et al.* reported in a sample of American women that while the top three carotenoids in maternal serum were firstly lycopene, β-carotene second, and thirdly lutein, the most prevalent carotenoids in their breast milk were lutein and zeaxanthin[83].

Local data from 12 samples of breast milk from mothers in the Midwest demonstrated average lutein + zeaxanthin levels at 40.1 (±42.5) mcg/L[84]. Similarly, comparison of pasteurized donor human milk to these 12 maternal milk samples demonstrated significantly lower concentrations of lutein and zeaxanthin at 21.4 mcg/L[84]. Donor human milk from this study was exposed to the Holder method of pasteurization, which heats the milk to 62.5 °Celsius for 30 minutes[85]. Lutein and zeaxanthin should be able to withstand this temperature, as their melting points are 196 and 215.5 °Celsius, respectively[11,12]. Alternative rationale for lower levels in donor human milk compared to early mother's own milk is demonstrated by a decrease in

maternal carotenoid levels during the first 2-4 weeks of lactation, with stabilization of levels between 4-16 weeks of lactation[6,86]. For example, Gossage *et al.* reported lutein concentrations in milk to consist of 25% of total analyzed carotenoids at four days post-partum compared to 50% at 32 days post-partum, showing a less significant decline in concentration compared to other carotenoids[86]. Likewise, Hanson *et al.* reported lutein + zeaxanthin to consist of 21.7% of Midwest early maternal milk samples (in comparison with α-carotene, β-carotene, β-cryptoxanthin, and lycopene)[84]. Donor milk analysis demonstrated lutein + zeaxanthin to consist of 39.3% of analyzed carotenoids[84]. While not all commercially-available infant formulas in North American contain lutein, Hanson *et al.* reported the combined lutein + zeaxanthin content in a sample of term infant formula at 58.4 mcg/L[84].

Mechanism of Action

In plants, the primary purposes of carotenoids are to absorb light for photosynthesis and to provide photoprotection for chlorophylls[87]. Carotenoids also add color to plants and may enhance their flavor and aroma[87,88]. In humans, the primary established function of carotenoids is the pro-vitamin A activity for select carotenoids, such as α-carotene, β-carotene, and β-cryptoxanthin[2]. Additional functions of non-provitamin A carotenoid have been suggested in the literature, but complete mechanisms of action have not been fully identified.

As reported previously, lutein and zeaxanthin are located within the macula of the eye, in the layer covering the photoreceptors[65]. Lutein and zeaxanthin contain conjugated double bonds within their molecular structure, allowing them to absorb visible light[89]. Due to these capabilities, lutein and zeaxanthin filter short-wave blue-light (400-500 nanometers) before entering the photoreceptors[61,65]. Blue-light at <400 nanometers may be partially filtered by the cornea and lens[89]. However, filtering blue-light of greater intensities is thought to reduce retinal

oxidative stress, subsequently reducing progression of macular degeneration[65]. Furthermore, blue-light is thought to "scatter" more than other wavelengths and cause visual "glare", which results in reduced image clarity[61]. Conclusively, appropriate filtering of blue-light by lutein and zeaxanthin enhances acute vision while simultaneously preserving long-term vision[61].

Lutein and zeaxanthin in eye tissue are also to quench singlet oxygen, including singlet oxygen photosensitizers. Lutein and zeaxanthin have lower energy levels than singlet oxygen, but are able to excite towards singlet oxygen levels and subsequently reduce both molecules back to ground state[45]. The excess energy is then released as heat[45]. Most oxygen quenching by carotenoids occurs through this physical process, allowing the integrity of the carotenoid to be maintained[90]. Less than 0.05% occurs by chemical quenching, which ultimately destroys the carotenoid[90].

Lutein and zeaxanthin are also theorized to confer further benefits to membrane layers, most commonly reported in the eye, due to their molecular structure[45]. Firstly, lutein and zeaxanthin reportedly demonstrate higher membrane solubility compared to less polar carotenoids as a result of their end hydroxyl groups. In an example from review by Widomska and Subczynski, solubility thresholds are estimated at 10 mole percent for zeaxanthin and 15 mole percent for lutein in fluid-phase model membranes[45]. Comparatively, this threshold is around 0.5 mole percent for β-carotene[45]. In addition, the hydroxyl groups on lutein and zeaxanthin's cyclic ends allow them to be oriented perpendicular within the membrane bilayer, whereas non-polar carotenoids are more randomly oriented[45]. Transmembrane orientation of lutein and zeaxanthin is anticipated to improve membrane stability, decrease membrane fluidity, and alter membrane permeability[45]. Furthermore, as the lipid-rich membrane bilayer is susceptible to lipid peroxidation, lutein and zeaxanthin provide defense against oxidative damage by scavenging free radicals and oxygen singlets[11,45]. Also due to transmembrane

orientation with exposed hydroxyl groups, lutein and zeaxanthin are capable of scavenging both within and around the membrane, unlike non-polar carotenoids[45]. Lastly, lutein and zeaxanthin may reduce accumulation of lipofuscin, the intracellular buildup of oxidized cross-linked proteins as a result of lipid peroxidation[11].

Additional mechanisms of action for lutein and zeaxanthin are less well-defined. It is suggested that carotenoids in general may provide cancer protection, as they may increase levels of specific proteins which thwart unrestrained cell proliferation[11]. However, of greater interest is recent research, which attempts to identify additional roles of lutein and zeaxanthin within the brain. In example, Lieblein-Boff *et al.* assessed brain tissues of post-mortem infants (N=30), comparing lutein concentrations with metabolites[91]. Results identified correlations between lutein concentrations and fatty acids, lysophospholipids, amino acids, and homocarnosine[91]. These metabolites serve as brain osmolytes, neurotransmitters, or antioxidants, suggesting improved brain development and function with increased presence of lutein[91]. A review by Perrone *et al.* also reported lutein supplementation to downregulate oxidative stress and inflammation in rats with ischemia injury, subsequently offering neuroprotection[17].

Beneficial and Adverse Effects

To date, primary research on lutein and zeaxanthin in human health focuses on benefits of vision, cognition, and cancer, with emerging research during pregnancy and infancy. Of more limited studies published, no strong associations have been linked between lutein and zeaxanthin levels and/or dietary intake with cardiometabolic function, respiratory function, or the development of Type 2 diabetes[92-97]. Similarly, only one case report identified potential adverse effects of high dietary intake and body levels[28].

Eye Health

The role of lutein and zeaxanthin has been more widely studied in eye health, more specifically in AMD. AMD is a result of the deterioration of the retina, which contains the macula that aids in central vision and is the leading cause of vision loss in the United States[98,99]. As background, AMD has three stages 1) early AMD, characterized by no vision loss but presence of small yellow deposits beneath the retina called drusen, 2) intermediate AMD, which may include some vision loss alongside larger drusen, and 3) late AMD, consistent with noticeable vision loss and drusen[98]. Most data comes from a second cohort of AREDS, which described the main effects of lutein + zeaxanthin supplementation over five years in subjects $\geq$50 years with existing AMD to have a 10% reduction in progression to advanced AMD compared to un-supplemented subjects[100]. No benefit of lutein + zeaxanthin was found on the development of cataracts[101]. Remarkably, supplementation of lutein + zeaxanthin compared to β-carotene (15 mg/day) demonstrated a hazard ratio of 0.82 for progression to late AMD (p=0.02) and 0.78 for the development of neovascular AMD (p=0.01)[102]. Similarly in patients with large bilateral drusen at start of supplementation, lutein + zeaxanthin demonstrated a hazard ratio of 0.76 (p=0.02) for progression to late AMD compared to β-carotene[102]. Conversely, a 2017 Cochrane analysis concluded lutein and zeaxanthin supplementation to have only similar or slightly reduced progression to late AMD (RR 0.94, 95% CI 0.87 to 1.01), neovascular AMD (RR 0.92, 95% CI 0.84 to 1.02), and geographic atrophy (RR 0.92, 95% CI 0.80 to 1.05), all of low certainty evidence[103]. It was also reported that lutein supplementation decreases the risk of progression to a visual loss of $\geq$15 letters compared to no supplementation[103].

Assessment of MPOD in conjunction with AMD remains of interest as lower levels may result in worsened outcomes[104]. Meta-analyses by Ma *et al.* on the MPOD changes with lutein, zeaxanthin, and/or meso-zeaxanthin supplementation demonstrated an increase for both AMD

patients (weighted mean difference, 0.07 ODU; 95% CI, 0.03 to 0.11) and healthy adult subjects (weight mean difference, 0.09 ODU; 95% CI, 0.05 to 0.14)[68]. Further meta-analyses on dose-response estimated a 1 mg/day increase in supplementation to increase MPOD by 0.005 ODU in AMD patients and 0.04 ODU in health subjects throughout the study period[68]. A greater increase in MPOD was reported with longer supplementation in AMD subjects and with >10 mg/day of combined xanthophyll supplementation in healthy subjects[68]. Of interest, meso-zeaxanthin supplementation improved MPOD, but the addition of zeaxanthin combined with lutein did not increase levels compared to lutein supplementation alone[68]. In contrast, Henricksen *et al.* reported opposing results when assessing MPOD levels in newborn infants. Results demonstrated that both infant and maternal serum zeaxanthin levels correlated with infant MPOD, but not lutein levels or combined lutein + zeaxanthin levels[70]. Infant serum zeaxanthin levels were more strongly correlated with infant MPOD than maternal serum levels ($r= 0.63$ vs. 0.59)[70]. Two supplementation trials (i.e. 0.14 mg/day lutein + 0.06 or 0.006 mg/day zeaxanthin) in preterm infants born $\leq$32 weeks gestational age have demonstrated no influence on the development of retinopathy of prematurity[92,105,106]. Alternatively, Rubin *et al.* reported improved rod photoreceptor sensitivity and a lower progression to severe retinopathy in preterm infants born <33 weeks who received a lutein-containing formula vs. unsupplemented formula until 50 weeks gestational age[107]. A recent study in young healthy non-smokers (N=32) identified correlations between MPOD values and auditory function, a novel finding in lutein and zeaxanthin research[108].

Pregnancy and Infancy

Analysis of lutein levels during pregnancy or around time of birth is sparser than research in eye health. Multiple studies have attempted to compare lutein supplementation with resulting biomarkers of oxidative stress. To start, Lorenzoni *et al.* provided a multinutrient

supplement (containing 10 mg/day lutein and 2 mg/day zeaxanthin) to diabetic mothers throughout the third trimester of pregnancy compared to untreated controls (total N=24)[109]. Blood analysis of total hydroperoxides, or "oxidative stress" at time of birth in mothers revealed no significance between groups. However, infants born to supplemented mothers demonstrated significantly lower levels total hydroperoxide levels at 2 hours of life compared to control infants, but no difference at 48 hours of life[109]. Similarly, Perrone *et al.* conducted a randomized controlled trial that supplemented healthy breastfed newborns with 0.14 mg lutein + 0.0006 mg zeaxanthin at 6 and 36 hours of age and compared to control infants (N=75)[110]. Blood analysis at 48 hours of life compared to umbilical cord blood showed supplemented infants to have lower total hydroperoxides and increased biological antioxidant potential than controls[110]. Costa *et al.* supplemented preterm infants (mean gestational age of 30.4 weeks) with 0.5 mg/kg/day lutein and 0.02 mg/kg/day zeaxanthin for six weeks and found no statistical differences in total antioxidant status between groups at each weekly checkpoint[111]. Clinical outcomes were not assessed in any of these studies, which limit clinical application. Of relevance, however, Hanson *et al.* reported a trend towards higher Apgar scores at five minutes of life in American and Nigerian infants born with higher plasma lutein levels[75].

Of further interest, a case-control study of pregnant women (N=5,337) reported significantly lower lutein levels at 24-26 weeks of pregnancy in preeclamptic women compared to healthy controls (median 96 vs. 119 mcg/mL). Likewise, adjusted regression analysis in this study demonstrated higher maternal lutein levels in mid-pregnancy resulted in lower relative risk ratios for developing early (RRR 0.53; 95% CI 0.35-0.80) and late-onset preeclampsia (RRR 0.62; 95% CI 0.47-0.82)[112]. Likewise, results in a population of Zimbabwean women (N=261) by Williams *et al.* reported statistically lower maternal plasma concentrations of both lutein and zeaxanthin in preeclampsia cases compared to healthy controls[113]. While placenta levels of

lutein and zeaxanthin have not yet been quantified, analysis remains of interest in light of research by Palan *et al.* who compared placenta, maternal serum, and umbilical cord blood levels of four carotenoids (α-carotene, β-carotene, lycopene, and canthaxanthin) in pre-eclamptic versus healthy pregnant women[9]. Results demonstrated that levels of canthaxanthin, a xanthophyll like lutein and zeaxanthin, were significantly lower in the placenta of pre-eclamptic women[9]. Despite lower placenta levels, no differences were identified in maternal serum or umbilical cord blood levels between groups[9].

Cancer

Multiple systemic reviews and meta-analyses have been conducted to compare the dietary intake of multiple carotenoids on the risk of developing various cancers. It must first be noted that some results determine lower cancer risk with higher dietary intake of combined carotenoids[114]. However, multiple analyses have revealed no significance of dietary lutein + zeaxanthin intake alone on risk of developing pancreatic[115], lung[114], breast[116], or gastric cancer[117]. Alternatively, dietary intake has been associated with lower risk of developing non-Hodgkin lymphoma (RR 0.87, 95% CI 0.78-0.97)[118]. While dietary intake of lutein has not been associated with breast cancer, blood lutein concentrations at 25 mcg/dL has been associated with lower risk (RR 0.68; 95% CI 0.52-0.89)[116].

Additional singular studies have provided mixed results of lutein and zeaxanthin in association with cancer risk. Blood concentrations of lutein + zeaxanthin, irrespective of dietary intake, have been associated with lower risk of developing ovarian cancer (OR 0.21; 95% CI 0.09-0.52)[119], but not bladder[120] or colorectal cancer[121]. Dietary intakes of lutein and zeaxanthin have identified no associated with prostate cancer[122] or renal cell carcinoma[123,124]. However, contrasting data by Hu *et al.* reported lower odds of developing renal cell carcinoma (OR 0.77;

95% CI 0.62-0.95) with higher dietary lutein + zeaxanthin intake, with this effect being more pronounced in overweight subjects or those with a smoking history[125]. Dietary intakes of lutein have been associated with lower risk of developing melanoma in one study[126], but no associations have been identified with intake and risk of squamous or basal cell carcinoma[127,128].

Cognitive Performance

As lutein and zeaxanthin are present in brain tissue, studies have analyzed blood levels in correlation with cognition throughout the lifespan. Findings remain novel, as most available data has only been published within the past several years. To start, an Irish study of adults ≥50 years (N=4,000) reported higher plasma lutein and plasma zeaxanthin levels to be associated with improved composite scores of memory, executive function, and global cognition[4]. High plasma zeaxanthin, though not lutein, was associated with improved processing speed[4]. Furthermore, Mullan *et al.* demonstrated significantly lower plasma lutein levels in patients with Alzheimer's Disease compared to unaffected controls, but no difference in zeaxanthin levels[129]. Nolan *et al.* reported Alzheimer's patients to have lower serum lutein + zeaxanthin levels and MPOD values compared to non-diseased controls[130]. MPOD scores in older adults with mild cognitive impairment have also been related to scoring for the mini-mental state examination as well as visual-spatial, constructional, language, and attention performances[131]. Additionally, higher serum lutein, serum zeaxanthin, and lutein brain tissue concentrations were associated with improved cognitive testing scores prior to demise in adults age 80-100 years[80].

Effects of lutein and zeaxanthin supplementation has so far demonstrated unchanged or improved cognitive outcomes. For instance, Power *et al.* conducted a randomized controlled trial of healthy subjects (N=91) with low MPOD levels, showing improved pair-associated learning and memory after one year of supplementation with lutein (10 mg/day), zeaxanthin (2

mg/day), and meso-zeaxanthin (10 mg/day)[132]. Similarly, Lindbergh *et al.* supplemented older adults (mean age 72 years; N=44) for one year with lutein + zeaxanthin (combined 12 mg/day) and later conducted cognitive testing and functional magnetic resonance[133,134]. Results identified supplementation to buffer a decline in performance on a verbal learning task, but notably enhanced brain perfusion and neural activity compared to the placebo group[133,134]. Even in younger adults age 18-30 years, supplementation with lutein (10 mg/day) and zeaxanthin (2 mg/day) over a one year period resulted in higher MPOD values, but also improved reasoning, attention, and spatial memory[135]. In contrast, data from the AREDS study found no significant differences in yearly cognition scores between supplemented vs. placebo groups[136].

Lutein and zeaxanthin levels in associated with cognition is less studied in the pediatric population. Limited studies include that by Cheatham *et al.* who analyzed breast milk samples at age 3-5 months in comparison with cognitive testing at 6 months of age, demonstrating improved memory recognition in infant's receiving breast milk with combined higher lutein and choline levels[8]. Two additional studies of pre-pubescent children with higher MPOD values demonstrated weak to moderate associations with more accurate performances during a cognitive control task as well as executive processing and brief intellectual ability[137,138].

Modifiable Lifestyle Factors Influencing Body Levels

Smoking

Smoking status has been associated with blood lutein + zeaxanthin levels. For example, a review by Alberg reported the average active smoker experiences decreases in circulating lutein + zeaxanthin levels by 14% compared to a 5% decrease for lycopene and a 25% decrease for α-carotene, β-carotene, and cryptoxanthin[139]. Similarly in an Australian study of subjects >25

years of age (N=1,597), serum lutein + zeaxanthin was statistically associated with smoking status, with a serum level of 0.43 vs. 0.32 mcmol/L in never vs. current smokers (was 0.40 in former smokers, defined as smoking "less than daily for the past 3 months")[140]. Similarly, from NHANES III 1988-1994 data (N=17,002), Stimpson *et al.* analyzed tobacco use by serum cotinine level[40]. Subjects with a cotinine levels >14.1 ng/mL had a mean serum lutein + zeaxanthin level of 20.58 mcg/dL compared to 23.72 mcg/dL in subjects with cotinine levels <14.1 ng/mL, roughly a 15% difference[40]. A cross-sectional study (N=2,786) conducted a multivariate analysis of predictors of serum lutein and zeaxanthin concentrations, predicting smoking to decrease serum levels by 13.6% and 9.5%[38]. However, it must be noted that this multivariate analysis also further predicted smokers to have a 12.1% decrease in dietary lutein + zeaxanthin intake compared to non-smokers[38]. In continued analysis, they predicted a 10% increase in dietary lutein + zeaxanthin intake would increase serum levels by 2.4%, indicating a nearly 5 or 6-fold increase in dietary intake to counteract negative smoking effects[38]. Only few studies have demonstrated minimal effects of smoking on serum lutein + zeaxanthin levels[141,142]. Limited data has analyzed differences in lutein and zeaxanthin in tissue based on smoking status.

Alcohol Intake

Research had identified a trend towards lower serum lutein + zeaxanthin levels with increased alcohol intake, though results are more variable and not as strongly supported as smoking. Stimpson *et al*. reported from large NHANES data (N=17,002) no difference in mean serum lutein + zeaxanthin levels between current or former/never alcohol users[40]. Notably however, there was no reported information on dietary intake or amount of alcohol intake for comparison. Alternative evidence primarily suggests that high intakes ($\geq$2 drinks per day in women) are of significance. For better evaluation, one "drink" consists of 14 grams of alcohol, equivalent to 12 ounces beer, 5 ounces wine, or 1.5 ounces hard liquor[143]. Research by Coyne *et*

al. demonstrated no difference in serum lutein + zeaxanthin level at limited alcohol intake vs. ≤60 drinks/month (0.42 mcmol/L for both groups)[140]. However, the notable difference was at >60 drinks/month with a geometric mean serum lutein + zeaxanthin level of 0.34 mcmol/L ($p<0.01$), approximately a 19% decrease[140]. A small, dated study (N=18 women) compared plasma lutein + zeaxanthin levels during a period of no alcohol intake vs. a period of drinking 30 g/day[144]. Plasma levels were 17% lower with consistent high alcohol intake compared to none[144]. Ultimately, the World Health Organization has reported that individuals who smoke or abuse alcohol have increased exposure to free radicals which results in lower body levels of "antioxidants"[145]. While lutein + zeaxanthin have not been deemed antioxidants by definition, many argue they have antioxidant-like properties.

Body Mass Composition

Studies have demonstrated alterations in serum carotenoid levels based on body mass composition and body mass index (BMI). As reference, BMI (in kg/m^2) classifications for adults are categorized as follows: <18.5 (underweight), 18.5-24.9 (normal), 25.0-29.9 (overweight), and >30.0 (obese)[146]. According to these classifications in an Australian study of subjects >25 years (N=1,597), BMI was statistically associated with serum lutein level with a decreasing trend across increasing BMI categories (i.e. 0.43 vs. 0.41 vs. 0.37 mcmol/L in normal, overweight, and obese subjects respectively)[140]. Similarly, Andersen *et al.* reported in subjects age 18-30 years (N=3,071; 55% female) over a 7-year time period that those with BMI levels >30 kg/m^2 had serum concentrations that were approximately 22% lower (α-carotene, β-carotene, zeaxanthin/lutein, and β-cryptoxanthin) compared to those with BMI <22 kg/m^2 [147]. Mean serum levels of combined lutein + zeaxanthin also demonstrated a downward trend with increasing BMI categories (p-value of <0.0001 for trend), even after adjustment for dietary intake of fruit and vegetables[147]. Similar trends have been identified in children age 6-16

(N=4,231), reporting downward trending serum lutein + zeaxanthin levels with upward trending BMI growth percentiles (most significant if BMI plotted >85th% on the growth charts from the Centers of Disease Control and Prevention), though no details on dietary intake were reported[148]. Notably, Gruber *et al.* reported from NHANES III data that participants (N=7,059) had lower serum lutein + zeaxanthin levels with higher fat-free mass, in addition to both higher body fat percentage[39]. When analyzing subjects in the top quartile for dietary lutein intake but comparing those in the highest vs. lowest quartile for serum lutein + zeaxanthin level, fat free mass remained a statistically significant factor[39]. In contrasting review, Bovier et al. reported that adiposity was not associated with serum lutein + zeaxanthin levels, but instead was associated with MPOD[149]. However, limitations to this study include that all subjects were young and of normal weight (mean age 22.5 years with mean BMI of 23.4 kg/m^2 [149]. Few studies have specifically trended differences in lutein + zeaxanthin levels in tissues across BMI categories.

Specific Aims and Hypotheses

While literature review has identified benefits and quantitative blood and tissue analysis of lutein and zeaxanthin in humans, most research has been conducted in older aged adults. Less complete information is known about lutein + zeaxanthin status during pregnancy and infancy, with detailed specifics of dietary to maternal to fetal transfer. Similarly, while levels of lutein and zeaxanthin have been quantified in maternal and infant blood, no data is available on placental concentrations to better understand nutrient transfer. Considering these literature gaps, specific aims for this study are as follows:

1. To quantify lutein + zeaxanthin tissue levels in human placenta.
2. To determine the relative proportions of lutein + zeaxanthin levels in placenta, umbilical cord blood, maternal serum, and maternal dietary intake compared to other carotenoids (lycopene, β-cryptoxanthin, α-carotene, and β-carotene).
3. To determine correlations between lutein + zeaxanthin levels in placenta and levels in maternal serum, umbilical cord lutein, and maternal dietary intake.
4. To determine if there are relationships between maternal demographic or infant birth outcomes and lutein + zeaxanthin levels in placenta, maternal serum, and umbilical cord blood. Further aim will be to identify predictors of maternal serum, placenta, and umbilical cord blood levels of lutein + zeaxanthin.

Based on information obtained from literature review, result hypotheses of listed specific aims are as follows. 1) Lutein + zeaxanthin will be detectable in human placenta. Placenta is an intermediary tissue between maternal and umbilical cord blood and lutein + zeaxanthin have previously been quantified in both blood samples. 2) Lutein + zeaxanthin will not be of the highest proportion of analyzed carotenoids in maternal dietary intake. Though American adults generally consume inadequate amounts of fruits and vegetables, less than 10% eat adequate amounts of recommended orange or dark green vegetables[33] (both primary sources of lutein + zeaxanthin), compared to 38.3% for "starchy" and 48.9% for "other" vegetables[33]. Lower anticipated proportions of lutein + zeaxanthin within maternal dietary intake will relay to subsequent areas of transfer including maternal blood, placenta, and umbilical cord blood. 3) Placenta lutein + zeaxanthin levels will demonstrate significant positive correlations with maternal and umbilical cord blood levels. Furthermore, significant positive correlations will exist between maternal dietary intake of lutein + zeaxanthin and all three blood and tissue levels. Humans are unable to synthesize these carotenoids, so tissue concentrations can only be

achieved with dietary intake. 4) Placental levels of lutein + zeaxanthin will be lower in instances of maternal obesity and smoking, with similar effects existing for maternal serum and umbilical cord blood. Placenta, maternal serum, and umbilical cord blood lutein + zeaxanthin levels will also be lower in cases of infant respiratory distress syndrome (RDS) and subsequent admission to the newborn intensive care unit (NICU) as past literature has identified a negative relationship between lutein supplementation and oxidative stress markers in infants[109,110]. Lastly, maternal dietary lutein + zeaxanthin intake will remain a significant predictor of levels in both blood and placenta, given humans cannot synthesize lutein + zeaxanthin.

CHAPTER 2: METHODS

Study Design

After Institutional Review Board approval through the University of Nebraska Medical Center, mother-infant pairs were screened and approached for informed written consent. Eligibility requirements included mothers at least 19 years of age who were free of liver, kidney, or gastrointestinal disease that influences nutrient absorption. Included mothers delivered at least one live-born infant at Nebraska Medicine hospital (Omaha, Nebraska, USA) in 2017 who did not have congenital abnormalities or an inborn error of metabolism. No infants deemed ward of state were approached for consent to participate, per state law[150]. Mothers with multiple gestation were eligible for inclusion. Both term-born and preterm infants were included. No incentives were offered so as not to influence decision to participate.

A placenta, maternal blood, and infant umbilical cord blood sample were taken at time of delivery in enrolled mother-infant pairs to send for carotenoid analysis. Analyzed carotenoids were combined lutein + zeaxanthin, lycopene, β-cryptoxanthin, α-carotene, and β-carotene. Placenta samples were fixed in a 10% formalin solution. Maternal blood samples were collected as part of a routine blood draw. Umbilical cord blood is routinely collected at all deliveries and stored in the hospital laboratory for use as needed. Both maternal and umbilical cord blood samples were light protected and frozen immediately once attained by the research team to preserve samples. Goal volumes for samples were 10 g placenta, 1 mL maternal blood, and 5 mL cord blood. The Biomarker Research Institute at Harvard University conducted analysis of carotenoids using high-performance liquid chromatography. Internal quality control is compared among four control samples analyzed within each analysis, including two identical high-level and two low-level samples. Internal analysis is also monitored by participation in the

standardization program for carotenoid analysis through the National Institute of Standards and Technology, United States of America. Placenta carotenoid levels were reported in mcg/g and blood carotenoid levels were reported in mcg/L.

The validated Harvard Willett food frequency questionnaire[151], which includes four pages questioning usual dietary intake and dietary supplement use over the past one year, was completed by mothers at time of hospitalization for delivery. Questionnaire results provided data for this study on average daily calorie, carbohydrate (g), protein (g), fat (g), and carotenoid (mg) intake. Mothers also provided self-reported pre-pregnancy height and weight which were used to calculate pre-pregnancy BMI. Calculations of BMI were used to identify BMI categories for underweight ($\leq$18.5 kg/m^2), normal (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese ($\geq$30 kg/m^2)[146]. Mothers also completed the 18-item United States Household Food Security Survey from the United States Department of Agriculture, which identifies high, marginal, low, and very low food security[152].

Additional demographic and clinical outcome information was collected for mothers and infants at time of delivery from the online electronic health system. Data collected for mothers included years of age, race, mode of delivery (vaginal vs. Cesarean), smoking history (yes/no), smoking category (never, current, or former smoker), diagnosis of diabetes during pregnancy (yes/no), and diagnosis of preeclampsia (yes/no). Data collected for infants included gender, gestational age at birth (in weeks and days), preterm birth (yes/no; defined as gestational age <37 weeks at birth[153]), birth weight (g), birth head circumference (cm), birth length (cm), APGAR score at 1 and 5 minutes of life, RDS (yes/no), and NICU admission (yes/no).

Statistical Analysis

All statistical analyses were conducted via SPSS software. As quantification and comparison of lutein + zeaxanthin in placenta, maternal serum, umbilical cord blood, and

maternal dietary intake are the primary aims of this study, histograms were developed for each to compare the distribution of data. As histograms revealed right-skewed data that was not normally distributed, descriptive statistics for numerical values of all carotenoids (lutein + zeaxanthin, lycopene, β-cryptoxanthin, α-carotene, and β-carotene) are reported with median, interquartile range, minimum, and maximum. To maintain consistency, all other continuous variables are reported in similar descriptive statistics, regardless of data distribution. The median value for each carotenoid was divided by the sum of all carotenoid medians for a specific sample (placenta, maternal serum, umbilical cord blood, maternal dietary intake), then multiple by 100 to obtain the proportion. Frequencies and proportions for categorical variables were calculated.

The Mann-Whitney U test was used to compare median values for continuous variables. The Kruskal-Wallis test compared variance of continuous data between more than two categorical groups. In addition to skewed data, non-parametric tests were justified for analysis when the sample size for categorical groups was small ($n<30$). Spearman's correlation coefficients were used to assess correlations between two continuous variables. A p-value of <0.05 was considered statistically significant for all analyses.

Multivariate linear regression modeling with backwards elimination was used to identify significant predictors of lutein + zeaxanthin levels in placenta. Included variables within the model were those identified to have a significant relationship with placental lutein + zeaxanthin levels by correlation or by comparison of medians between groups. Umbilical cord blood lutein + zeaxanthin levels, despite being significantly correlated with placenta, was not included as a variable in the model as umbilical cord blood receives nutrients from maternal serum through placenta. Significant variables included were maternal serum lutein + zeaxanthin levels

(continuous), race category (categorical, Caucasian vs. non-Caucasian), maternal smoking history (categorical, yes/no), and gestational age at birth (continuous).

Similar multivariate linear regression modeling was conducted to identify significant predictors of maternal serum lutein + zeaxanthin levels. Included variables within the model were those identified to have a significant relationship with maternal serum lutein + zeaxanthin levels by correlation or by comparison of medians between groups. Final included variables used for regression modeling were weeks of gestation at birth (continuous), maternal pre-pregnancy BMI (continuous), maternal obesity (categorical, yes/no, non-obese included underweight, normal, and overweight categories), maternal smoking history (categorical, yes/no), and maternal dietary intake of lutein + zeaxanthin (continuous). While gestational age at birth is reported as weeks and days, this variable was expressed in the regression model as weeks only. This was completed by converting additional days of gestation to the percent of one week (i.e. 40 weeks and 5 days = 40.7 weeks). Though significantly correlated, levels of lutein + zeaxanthin levels for umbilical cord blood and placenta were not included as variables in the model as maternal blood is the supplier of nutrients to these areas.

Lastly, a multivariate regression model with backwards elimination was conducted to predict lutein + zeaxanthin levels in umbilical cord blood. Included variables within the model were those identified to be significantly correlated with umbilical cord blood. Significant variables included maternal lutein + zeaxanthin serum levels, placenta lutein + zeaxanthin levels, and gestational age at birth. Before regression analysis, it was recognized that maternal serum and placental lutein + zeaxanthin levels are correlated and expected to be significant predictors umbilical cord blood levels. Though collinearity is present, regression analyses were still performed to identify if gestational age at birth was a significant predictor. Coefficients of determination were reported from SPSS for all final multivariate linear regression models.

CHAPTER 3: RESULTS

Demographic Characteristics and Outcomes Data

There were 82 mother-infant pairs included in this analysis. There were no multiple births in this sample. As the primary aim of this study was to analyze lutein + zeaxanthin concentrations in placenta, all mother-infant pairs were included if placenta analysis was available. Inclusion of all placenta data resulted in incomplete data reported for remaining continuous and categorical variables if they were not available. Analysis of carotenoids in mothers' milk was not analyzed as only two mothers had milk samples available. Continuous response demographic and outcome data for mother-infant pairs are displayed in Table 2. Categorical response demographic, outcome data and frequencies are displayed in Table 3.

Table 2: Continuous Responses of Maternal and Infant Demographics and Outcomes

	N	Median	Interquartile Range	Minimum	Maximum
Gestational Age at Birth[a]	82	39^5	1^4	32^1	42^1
Birth Weight (g)	82	3,512	535	1,580	4,617
Birth HC (cm)	81	34.9	1.3	24.5	38.1
Birth Length (cm)	81	50.8	3.8	34.3	55.2
Apgar Score 1 minute	80	8.0	0.3	1.0	9.0
Apgar Score 5 minute	80	9.0	0.0	5.0	9.0
Maternal Age	81	29	9	19	43
Maternal Pre-Pregnancy BMI (kg/m^2)	75	26.5	9.7	16.0	46.3
Maternal Calorie/day Intake	80	2,052	968	712	5,758
Maternal Protein/day Intake (g)	80	79	37	22	205
Maternal Fat/day Intake (g)	80	78	41	16	228
Maternal Carbohydrate/day Intake (g)	80	261	139	114	772

[a]Reported in “weeks^days”.

Table 3: Categorical Responses of Maternal and Infant Demographics and Outcomes

	N	Frequency (%)
Infant Gender	82	30 female (36.6%) 52 male (63.4%)
NICU Admit	82	7 (8.5%)
RDS	82	3 (3.7%)
Preterm Birth	82	2 (2.4%)
Delivery Mode	81	66 vaginal (81.5%) 15 Cesarean (18.5%)
Maternal Diabetes	81	6 (7.4%)
Preeclampsia	81	0%
Maternal Race	82	56 Caucasian (68.3%) 12 African American (14.6%) 5 Hispanic (6.1%) 2 Asian/Pacific Islander (2.4%) 7 Other/Unknown (8.5%)
Smoking Status at Delivery	81	56 never smoker (69.1%) 8 current smoker (9.9%) 17 former smoker (21.0%)
Maternal Pre-pregnancy BMI Category	75	Underweight 2 (2.7%) Normal weight 28 (37.3%) Overweight 21 (28.0%) Obese 24 (32.0%)
U.S. Household Food Security	78	56 high food security (71.8%) 12 marginal food security (15.4%) 10 low food security (12.8%) 0 very low food security (0%)

Maternal Dietary Intake, Placenta, and Blood Levels of Carotenoids

Histograms for results of lutein + zeaxanthin levels within maternal diet, placenta, maternal serum, and umbilical cord blood are displayed in Figures 3-6. Maternal dietary intake of carotenoids is listed in Table 4. Placenta carotenoid levels are displayed in Table 5. Maternal serum and umbilical cord blood carotenoid levels are listed in Table 6 and Table 7. Figures 7-10 shows spread of carotenoid levels in maternal diet, placenta, and blood samples via boxplots.

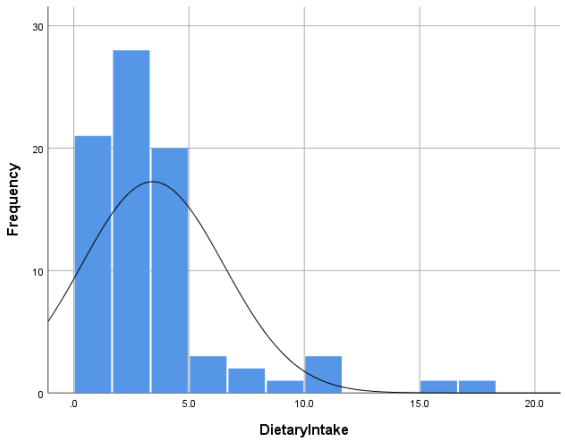

Figure 3: Histogram of Maternal Dietary Intake of Lutein + Zeaxanthin (mcg/day).

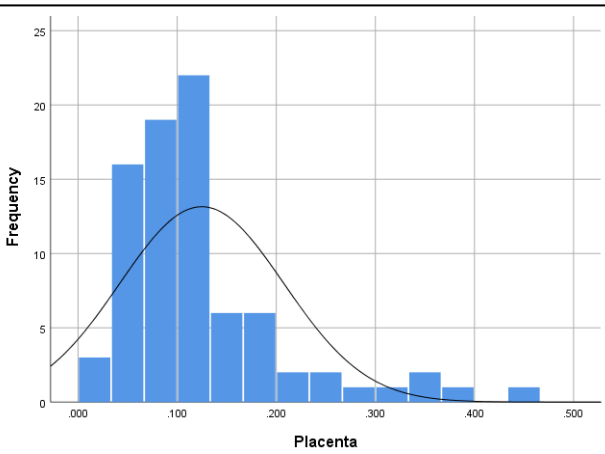

Figure 4: Histogram of Lutein + Zeaxanthin Levels in Placenta (mcg/g).

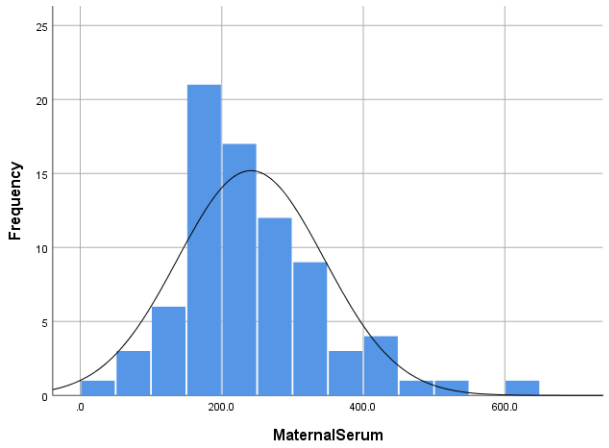

Figure 5: Histogram of Lutein + Zeaxanthin Levels in Maternal Serum (mcg/L).

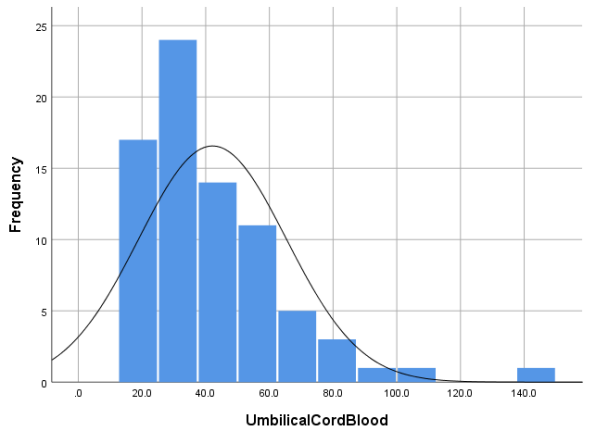

Figure 6: Histogram of Lutein + Zeaxanthin Levels in Umbilical Cord Blood (mcg/L).

Table 4: Maternal Dietary Intake of Carotenoids (mg/day)

N=80	Median	Interquartile Range	Minimum	Maximum
Lutein + Zeaxanthin	2.48	2.25	0.27	17.78
Lycopene	4.94	3.69	1.27	21.29
β-cryptoxanthin	0.12	0.16	0.02	0.62
α-carotene	0.49	0.56	0.01	4.61
β-Carotene	4.72	3.34	0.94	27.45

Table 5: Carotenoid Levels in Placental Tissue (mcg/g)

N=82	Median	Interquartile Range	Minimum	Maximum
Lutein + Zeaxanthin	0.105	0.082	0.030	0.460
Lycopene	0.057	0.050	0.010	0.160
β-cryptoxanthin	0.016	0.010	0.000	0.090
α-carotene	0.008	0.010	0.000	0.080
β-Carotene	0.028	0.040	0.002	0.230

Table 6: Carotenoid Levels in Maternal Serum (mcg/L)

N=79	Median	Interquartile Range	Minimum	Maximum
Lutein + Zeaxanthin	220.8	122.3	25.7	605.5
Lycopene	599.1	349.4	25.8	1,109.4
β-cryptoxanthin	140.7	138.4	20.9	530.1
α-carotene	42.6	55.4	2.4	1,022.3
β-Carotene	186.0	287.4	7.1	3,003.1

Table 7: Carotenoid Levels in Umbilical Cord Blood (mcg/L)

N=77	Median	Interquartile Range	Minimum	Maximum
Lutein + Zeaxanthin	34.8	29.5	14.6	144.7
Lycopene	26.6	20.1	6.5	454.9
β-cryptoxanthin	17.2	15.7	4.6	683.4
α-carotene	3.5	7.2	0.0	939.4
β-Carotene	11.9	17.8	0.0	286.6

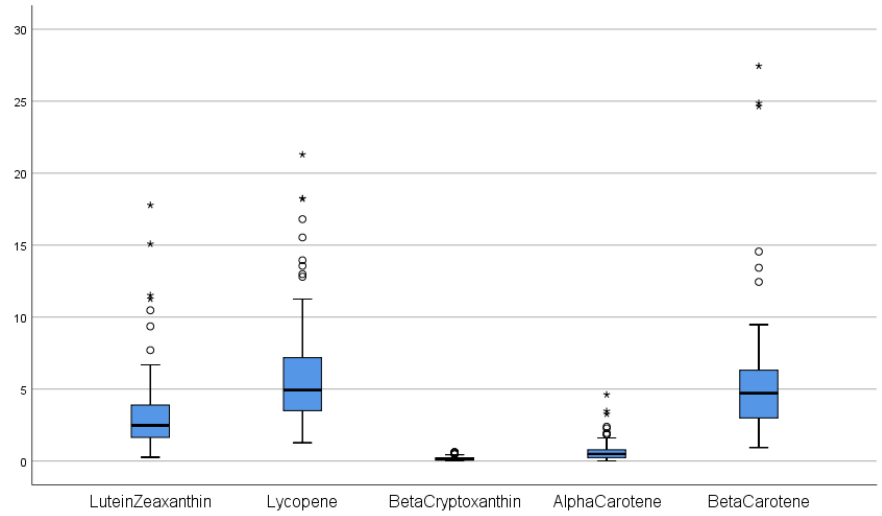

Figure 7: Boxplots of Maternal Dietary Intake of Carotenoids (mg/day).

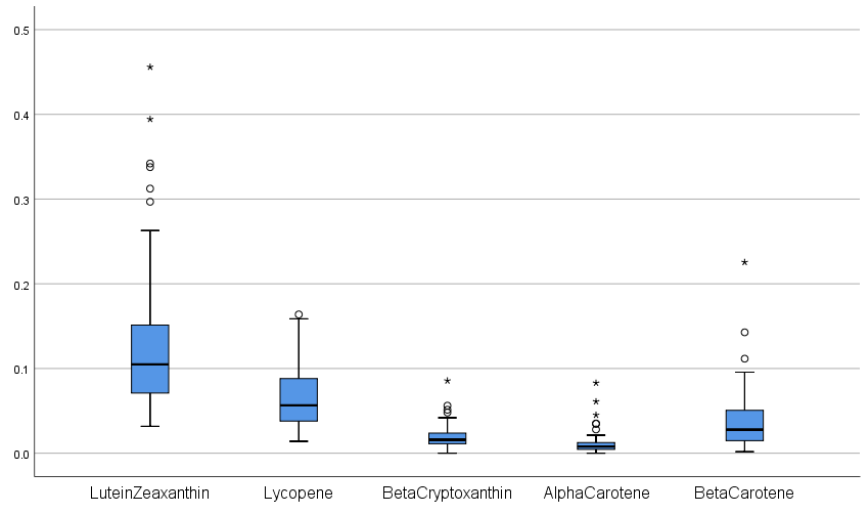

Figure 8: Boxplots of Carotenoid Levels in Placental Tissue (mcg/g).

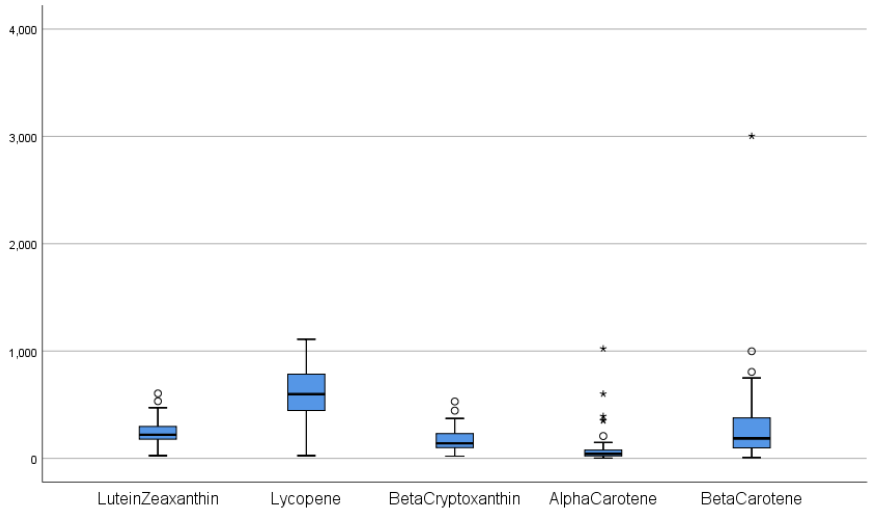

Figure 9: Boxplots of Carotenoid Levels in Maternal Serum (mcg/L).

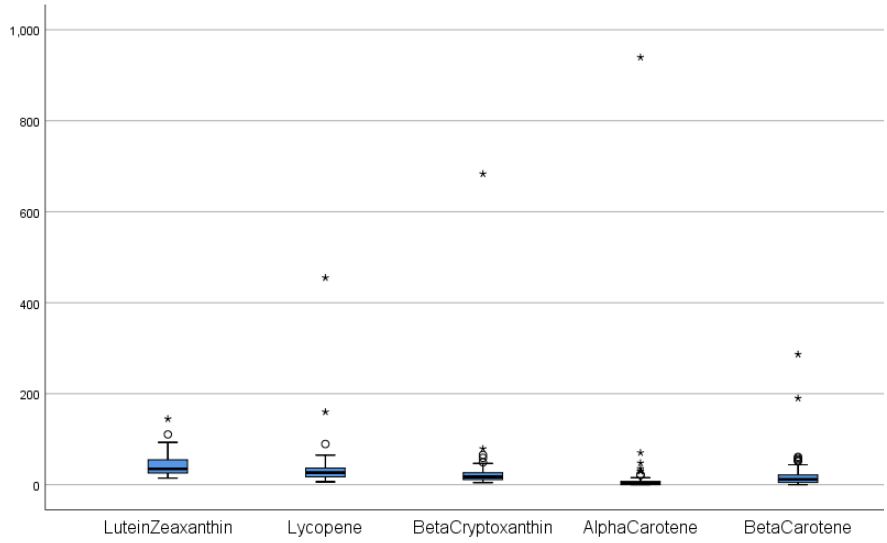

Figure 10: Boxplots of Carotenoid Levels in Umbilical Cord Blood (mcg/L).

Proportions of Carotenoids in Maternal Dietary Intake, Placenta, and Blood

Proportions of carotenoids in dietary intake, placenta, and blood levels are listed in Table 8 for comparison. Median percent of umbilical cord blood carotenoid levels compared to maternal levels are also displayed in Table 8. Non-provitamin A carotenoids (lutein + zeaxanthin and lycopene) consisted of 58.1% of maternal dietary intake, followed by 69.0% maternal serum, 75.7% placenta, and 65.3% umbilical cord blood. Xanthophyll carotenoids (lutein + zeaxanthin and β-cryptoxanthin) consisted of 20.4% maternal dietary intake, 30.4% maternal serum, 56.6% placenta, and 55.3% umbilical cord blood.

Table 8: Proportions of Median Carotenoid Levels within Maternal Diet, Placenta, Maternal Serum, and Umbilical Cord Blood

	Maternal Dietary Intake	Placenta	Maternal Serum	Umbilical Cord Blood	Percent Cord: Maternal Serum
Lutein + Zeaxanthin	19.4%	49.1%	18.6%	37.0%	16.0%
Lycopene	38.7%	26.6%	50.4%	28.3%	5.0%
β-cryptoxanthin	1.0%	7.5%	11.8%	18.3%	12.0%
α-carotene	3.9%	3.7%	3.6%	3.7%	6.0%
β-Carotene	37.0%	13.1%	15.6%	12.7%	5.4%

Correlations

All placental and blood lutein + zeaxanthin levels were significantly correlated with one another. Placental levels were more strongly correlated with maternal serum ($r=0.57$; $P<0.001$) than umbilical cord blood ($r=0.49$; $p<0.001$). The strongest correlation between all levels was between maternal serum and umbilical cord blood levels ($r=0.65$; $p=<0.001$).

Dietary intake of lutein + zeaxanthin was not correlated with placental concentrations ($r=0.18$; $p=0.110$), but correlated significantly with maternal lutein + zeaxanthin serum levels ($r=0.30$; $p=0.007$) and approached significance with umbilical cord blood ($r=0.20$; $p=0.086$). Dietary intake of lutein + zeaxanthin was correlated with increased calorie ($r=0.40$; $p<0.001$), carbohydrate ($r=0.37$; $p=0.001$), fat ($r=0.42$; $p<0.001$), and protein ($r=0.39$; $p<0.001$) intake. However, intake of any specific macronutrient was not correlated with placental or blood lutein + zeaxanthin levels.

Gestational age at birth correlated with lutein + zeaxanthin levels in placenta ($r=0.26$; $p=0.019$), maternal serum ($r=0.29$; $p=0.010$), and umbilical cord blood ($r=0.41$; $p<0.001$), but not maternal dietary intake ($p=0.160$). After excluding preterm infants ($N=2$), correlations still remained significant. Gestational age at birth also correlated with the percent of lutein + zeaxanthin levels in umbilical cord blood compared to maternal serum ($r=0.31$; $p=0.008$).

Maternal self-reported pre-pregnancy BMI as a continuous response was significantly correlated with maternal lutein + zeaxanthin serum levels ($r= -0.25$; $p=0.034$), but not maternal dietary intake, placenta, or umbilical cord blood levels. There were no correlations between dietary intake or placental or blood levels with maternal age, infant APGAR score at 1 and 5 minutes of life, and infant birth anthropometric measurements (for weight, length, and head circumference).

Maternal age and pre-pregnancy BMI as continuous variables were not correlated with rate of lutein + zeaxanthin transfer from maternal serum to umbilical cord blood.

Comparison of Medians

There was no statistical difference across categories of responses for the United States Household Food Security Survey (high, marginal, or low food security) and median lutein + zeaxanthin levels in placenta, blood, or maternal dietary intake. There was no difference in median lutein + zeaxanthin levels for dietary intake, placenta, maternal serum, umbilical cord blood between categorical responses for NICU admit, RDS, delivery mode, maternal diabetes, preterm birth, or infant gender.

Transfer rates of carotenoids from maternal serum to umbilical cord blood were quantitatively higher for xanthophyll carotenoids (lutein + zeaxanthin and β-cryptoxanthin at 16.0% and 12.0%) compared to non-xanthophyll carotenoids (lycopene, α-carotene and β-Carotene at 5.0%, 6.0%, and 5.4%). Rate of lutein + zeaxanthin transfer from maternal to umbilical cord blood did not differ according to maternal smoking history. Median transfer rates of lutein + zeaxanthin remained consistent between highest and lowest quartiles for maternal serum levels (17.0% vs. 16.0%; $p=0.333$), despite higher dietary intake of lutein + zeaxanthin (2.05 vs 3.63 mg/day; $p=0.015$) and obvious higher maternal serum levels (153.3 vs 327.1 mcg/L; $p<0.001$).

Differences across all race categories in median lutein + zeaxanthin level in placenta approached significance ($p=0.077$), but not for maternal serum, umbilical cord blood, or maternal dietary intake. In comparison of Caucasian vs. African American groups, only median placenta levels remained statistically different (0.097 vs 0.129 mcg/g; $p=0.022$). There were no median differences for any value between Caucasian and Hispanic groups or between Hispanic

and African-American groups. In comparison of Caucasian vs. non-Caucasian (eliminating "unknown" subjects, N=7), there were significant differences in lutein + zeaxanthin levels in placenta (0.097 vs 0.128 mcg/g; p=0.007), but only approached significance in maternal serum (208.3 vs. 253.7 mcg/L; p=0.071) and umbilical cord blood (33.4 vs. 48.2 mcg/L; p=0.069). Dietary intake was statistically similar between groups (2.31 vs. 3.31 mg/day; p=0.436).

Median lutein + zeaxanthin levels in placenta (0.090 vs. 0.117 mcg/g; p=0.049) and maternal serum (187.4 vs. 249.3; p=0.008) were statistically lower in mothers with a smoking history (current or former smoker) compared to never-smokers, though dietary intake was not statistically different (2.65 vs. 2.41 mg/day; p=0.181). Comparison of median lutein + zeaxanthin levels in current vs. former smokers was not significantly different for placenta (p=0.932) or maternal serum (p=0.428). Though not statistically significant, quantitative median values of umbilical cord blood were lower in infants born to mothers with a smoking history compared to never smokers (28.7 vs 37.8 mcg/L; p=0.227). Median lutein + zeaxanthin dietary intakes, placenta, and blood levels across smoking categories are displayed in Table 9.

Table 9: Median Maternal Dietary Intake, Placenta, Maternal Serum, and Umbilical Cord Lutein +Zeaxanthin Levels Across Reported Smoking Categories

	N	Maternal Dietary Intake (mg/day)	Placenta (mcg/g)	Maternal Serum (mcg/L)	Umbilical Cord Blood (mcg/L)
Never Smoker	54	2.65	0.117	249.3	37.8
Current Smoker	8	2.89	0.099	205.7	28.2
Former Smoker	17	2.20	0.088	186.4	29.5
p-value[a]		0.146	0.144	0.022	0.482

[a]Kruskal-Wallis test to compare across all smoking categories

There were no differences in lutein + zeaxanthin levels across all BMI categories for placenta (p=0.268), maternal serum (p=0.169), umbilical cord blood (p=0.565), or maternal dietary intake (p=0.199). However, comparison of median values in obese vs. non-obese (combination of underweight, normal, and overweight categories) showed significant differences in maternal serum levels (192.6 vs. 233.7 mcg/L; p=0.044), but not for placenta, umbilical cord blood, or maternal dietary intake. In further comparison of median values in normal weight vs. obese mothers, significant differences were identified for maternal dietary intake (p=0.042) and approached significance for placenta (p=0.088) and maternal serum (p=0.058), but not umbilical cord blood (p=0.439). Median lutein + zeaxanthin dietary intakes, placenta, and blood levels across BMI categories are displayed in Table 10.

Table 10: Median Maternal Dietary Intake, Placenta, Maternal Serum, and Umbilical Cord Blood Lutein +Zeaxanthin Levels Across Reported BMI Categories

	N	Maternal Dietary Intake (mg/day)	Placenta (mcg/g)	Maternal Serum (mcg/L)	Umbilical Cord Blood (mcg/L)
Underweight	2	5.38	0.089	305.3	52.2
Normal	28	3.57	237.3	237.3	36.5
Overweight	21	2.18	0.114	223.8	38.7
Obese	24	2.38	0.098	192.6	33.0
p-value[a]		0.199	0.268	0.169	0.565

[a]Kruskal-Wallis test to compare across all BMI categories

Significant Predictors of Lutein + Zeaxanthin Levels in Placenta and Blood

In backwards multivariate regression analysis to predict placental lutein + zeaxanthin concentrations, eliminated non-significant variables included gestational age at birth and smoking history. After backwards model elimination of variables, the only significant predictors of placental lutein + zeaxanthin levels were maternal lutein + zeaxanthin serum levels ($p<0.001$) and race category Caucasian vs. non-Caucasian ($p=0.037$), explaining 43.4% of the variation in placental lutein + zeaxanthin levels. After adjusting for maternal serum levels, the model predicts placental levels of lutein + zeaxanthin to be 0.033 mcg/g higher in non-Caucasians than any other race category.In similar multivariate linear regression modeling to identify predictors of lutein + zeaxanthin in maternal serum, eliminated non-significant variables included maternal obesity, followed by weeks of gestation at birth ($p=0.054$) and lastly maternal pre-pregnancy BMI ($p=0.072$). In final modeling, only maternal smoking history ($p<0.032$) and maternal dietary intake ($p<0.001$) were significant predictors, explaining 24.7% of the variation of maternal serum lutein + zeaxanthin levels. After adjusting for maternal dietary intake of lutein + zeaxanthin, maternal serum levels are predicted to be 51.1 mcg/L lower in mothers with a smoking history compared to never smokers. After adjusting for smoking history, the model predicts a 14.3 mcg/L increase in maternal serum levels for every 1 mg increase in dietary lutein + zeaxanthin intake. Of note, weeks of gestation at birth approached significance before elimination ($p=0.054$). When included in the model with smoking history and maternal dietary intake of lutein + zeaxanthin (both remained significant), pre-pregnancy BMI had remained significant at $p=0.049$. Had both variables of gestation at birth and pre-pregnancy BMI remained within the model, a 1 kg/m^2 increase in maternal pre-pregnancy BMI would predict maternal serum levels to decrease by 3.1 mcg/L. Also, with weeks of gestation at birth and maternal pre-pregnancy

BMI included, the effect of mothers with a smoking history becomes more significant with a predicted decrease in serum lutein + zeaxanthin levels of 64.8 mcg/L.

In final multivariate regression analysis to predict lutein + zeaxanthin levels in umbilical cord blood, significant predictors included lutein + zeaxanthin levels in maternal serum ($p<0.001$) and placenta ($p=0.003$), and weeks of gestational age at birth ($p=0.006$). This model explained 62.9% of the variation in lutein + zeaxanthin levels in umbilical cord blood. After adjustment for maternal serum and placental lutein + zeaxanthin levels, every week of birth after 37 weeks gestational age is predicted to increase umbilical cord blood levels by 3.2 mcg/L.

CHAPTER 4: DISCUSSION

Placenta Levels

As hypothesized for specific aim one of this study, lutein + zeaxanthin was present in placenta tissue and able to be quantified. Prior to this study, placental carotenoid levels have been studied only to a limited degree. Lycopene, α-carotene, β-carotene, and canthaxanthin (a xanthophyll) have been analyzed previously, but never lutein + zeaxanthin[9]. Lutein + zeaxanthin levels in placenta are within previous reported ranges in liver, kidney, and lung[1]. However, upper end placenta ranges were quantified at 0.46 mcg/g compared to these other tissues that exceeded >1 mcg/g. Comparatively, conversions of placenta lutein + zeaxanthin concentrations to ng/g (range 30-460) remained similar or higher than previous reports of lutein levels within the epithelial (44.1 ng/g) and nuclear eye layers (15.1 ng/g)[63]. Comparisons to retinal tissue cannot be made as concentrations are reported per m^2. In conversion, placental concentrations would range from 56-802 pmol/g (median 220 pmol/g). Placenta levels in this study are primarily higher than reported adipose tissue (268.5-456.3 pmol/g)[78] and brain concentrations (0-176.4 pmol/g lutein and 52.9 pmol/g zeaxanthin)[5,79,80]. Notably, all non-provitamin A carotenoids were detected in each placenta sample. Alternatively, a few placenta samples detected no quantitative values of β-cryptoxanthin or α-carotene, both provitamin A carotenoids.

Contrary to hypothesis for aim two of this study, results identify combined lutein + zeaxanthin to be the most prevalent analyzed carotenoids in placental tissue at 49.1%, nearly double the second highest carotenoid at 26.6%. Comparatively, lutein + zeaxanthin were also the most prevalent in umbilical cord blood at 37.0%, but not in maternal serum or dietary

intake. However, comparison of sample is not direct, as samples include that of tissue, blood, and the food matrix.

Increased concentrations of lutein + zeaxanthin in placental tissue, compared to other analyzed carotenoids may be justified by their structural properties. Firstly, placental tissue contains two membrane layers[48] and lutein and zeaxanthin are more highly soluble within membrane tissue than other carotenoids[45]. Secondly, presence of hydroxyl groups in xanthophyll carotenoids yields them more polar than non-xanthophyll carotenoids. As lutein and zeaxanthin contain hydroxyl groups on both ends, it has been theorized that they are affixed more firmly within the a lipid bilayer membrane, by having their hydroxyl ends located on opposite polar sides of the lipid bilayer[154]. In contrast, nonpolar non-xanthophyll carotenoids are oriented more randomly within the hydrophobic core of the lipid bilayer[45]. In retinal tissue, Widomska and Subczynksi concluded a transmembrane orientation to improve stability of lutein and zeaxanthin within tissue and to slow removal, though this concept may be applied to alternative membranous tissue like placenta[45].

While structural properties of lutein and zeaxanthin increase potential for uptake, it remains uncertain if there is an element of selective uptake by placenta. For example, presence and orientation of lutein + zeaxanthin within the placenta membrane may improve its physical strength and stability[45,154]. Similarly, placental damage by lipid perioxidation may be decreased by lutein and zeaxanthin scavenging free radicals[11,45]. Likewise, transmembrane orientation of lutein + zeaxanthin increases hydrophobicity of the inner bilayer, which influences membrane permeability[45]. It is hypothesized that improved placental structure and selective permability of substrate from maternal blood is favorable in the protection and nourishment of the developing fetus. Multiple research has demonstrated that disruptions in placenta function negatively impact pregnancy outcomes, including fetal growth restriction and increased risk of stillbirth[155].

Later consequences to the offspring include increased risk of developmental delay or autism and fetal programming that may result in chronic disease such a hypertension, coronary artery disease, or Type 2 diabetes[156-158]. If these adverse outcomes can be prevented or diminished by improved placental composition, uptake of lutein and zeaxanthin may be a mechanism of biological adaptation.

As hypothesized for specific aim three of this study, there were statistically significant positive correlations between placenta tissue concentrations of lutein + zeaxanthin and maternal serum and umbilical cord blood levels. Placenta concentrations are more strongly correlated with maternal serum than umbilical cord blood levels. This finding is justified by the route of lutein + zeaxanthin transfer from food to mother to infant, as maternal blood flows to placenta providing a source of lutein + zeaxanthin for uptake. Uniquely, umbilical cord blood lutein + zeaxanthin levels were more strongly correlated with maternal serum levels than placental concentrations, despite never mixing. However, while the placenta directly passes nutrients into the fetal blood circulation, the original supply of lutein and zeaxanthin sources from maternal blood. Correlations with umbilical cord blood identify how many components must function properly for the fetus to receive adequate nutrition from mother.

Maternal and Umbilical Cord Blood Levels

Lutein + zeaxanthin were detected in all maternal and umbilical cord blood samples. Maternal serum levels of lutein + zeaxanthin in this study (range 25.7-605.5 mcg/L) were within range of previous reports (48-698 mcg/L), with the exception of a lesser low end value[1]. Umbilical cord blood lutein + zeaxanthin levels in this study (converted to median 0.06 mcmol/L or 34.8 ng/mL) were lower than previous reports of 0.10 mcmol/L[46] in umbilical cord blood, but higher than levels of 24.2 ng/mL in newborn serum[70]. Results indicate a maternal to fetal

transfer rate of lutein + zeaxanthin at 16.0%, higher than any other carotenoid in this study but still on lower than most past literature reports of 15.1- 29.4%[46,70,74,75]. Notably however, lutein + zeaxanthin consisted of 18.6% of carotenoids in maternal blood, but 37.0% in umbilical cord blood.

One theory for higher proportions in umbilical cord blood is based on transportation of carotenoids in blood by lipoproteins. It has been reported that most hydrocarbon carotenoids are preferentially carried by LDL cholesterol, whereas polar xanthophylls like lutein + zeaxanthin have more ability to be carried by HDL cholesterol[1,159,160]. In example, a small study by Wang *et al*. (N=12) reported HDL to carry approximately 52% of lutein and 44% of zeaxanthin, whereas only 20-25% of lycopene, α-carotene, and β-carotene[159]. Additionally, it must be noted that adults and neonates have different ratios of blood lipoproteins. In example, large cohort data from American adults (N=424,201) show higher blood LDL levels compared to HDL, often at a ratio of 2:1[161]. Alternatively, neonates have more circulating HDL, with summary data from Woollett and Heubi reporting ratios closer to 1:1[162]. Consequently, a higher blood ratio of HDL compared to LDL in neonates, with bound lutein + zeaxanthin, would explain higher presence in umbilical cord blood compared to LDL-bound carotenoids. Past literature also demonstrates significant correlations between blood lutein + zeaxanthin levels and circulating HDL cholesterol. Many studies have identified significant correlations between blood HDL cholesterol levels and serum or plasma lutein (r=0.32-0.36), zeaxanthin (r=0.26), and combined lutein + zeaxanthin (r=0.30)[163-165]. Serum LDL cholesterol levels have been weakly correlated (r=0.18) with plasma lutein + zeaxanthin levels in one study[165], but more often demonstrate no correlation at all[163,164]. In further support, Connor *et al.* compared plasma and tissue concentrations of lutein in normal control vs. study chicks (N=48) with a genetic mutation causing HDL cholesterol deficiency. Results showed study chicks had higher LDL, but significantly lower HDL levels compared to

control chicks. Study chicks had significantly lower plasma lutein levels compared to controls (45.6 mcg/dL vs. 504 mcg/dL) at time of hatching[166]. After 28 days of being fed a high or low lutein diet, plasma and retinal lutein concentrations had increased in all study chicks, however not as significantly as the control chicks with higher HDL levels[166]. Final plasma levels at the end of the study period were 651 vs. 54.4 mcg/dL and 1,148 vs. 238 mcg/dL (study vs. control) in the low and high lutein diets[166]. These results ultimately highlight the unique role HDL cholesterol plays in lutein + zeaxanthin transportation and provides rationale for why bound carotenoid proportions may be altered between infant and maternal blood. This theory is further supported by results in this study in which all xanthophyll carotenoids increased proportions from maternal to umbilical cord blood, whereas non-xanthophyll carotenoids either maintained or decreased in proportions.

In multivariate regression modeling, maternal serum remained a significant predictor of placental lutein + zeaxanthin levels. Similarly, both maternal serum and placental lutein + zeaxanthin levels remained significant predictors of umbilical cord blood levels, further illustrating the route of nutrient transfer from mother to infant. Results of regression modeling also indicate increasing explanation of the variation in lutein + zeaxanthin levels as route of transfer progresses from maternal serum at 24.7%, to placenta at 43.4%, to lastly umbilical cord blood at 62.9%. This signifies that maternal serum levels of lutein + zeaxanthin are more acutely sensitive to external influencers compared to placenta or umbilical cord blood. However, prolonged altered levels from maternal serum are subsequently relayed to placenta and umbilical cord blood.

Lastly, and in contrast to hypothesis for specific aim four, placental levels of lutein + zeaxanthin were not altered in cases of infant RDS or NICU admission. Maternal and umbilical cord blood levels were not altered either. Notably however, samples sizes were small.

Dietary Intake

Increasing macronutrient intake in this study was correlated with increased lutein + zeaxanthin intake, illustrating higher carotenoid intake from increased food consumption. As hypothesized in study aim three and four, maternal dietary intake of lutein + zeaxanthin was significantly positively correlated with maternal serum lutein + zeaxanthin levels and was also remained a significant predictor of maternal serum levels. However contrary to hypotheses, maternal dietary lutein + zeaxanthin intake was not correlated with placenta or umbilical cord blood levels, nor was it a predictor of either. These results only add to our understanding of nutrient transfer from food to mother to infant. While placenta and umbilical cord blood lutein + zeaxanthin levels rely significantly on maternal blood levels, maternal serum levels rely significantly on dietary intake of lutein + zeaxanthin as it cannot be synthesized. This correlated chain of carotenoid transfer highlights how heavily dependent infants are on their mothers for receiving nutrients.

Maternal dietary intake of lutein + zeaxanthin averaged 2.48 mg/day, which is higher than previous Midwestern data reporting intakes at 1.1 mg/day (N=280)[167]. Still, median intakes remained inadequate to meet varying recommendations that promote eye health (minimum 6-10 mg/day lutein + 2 mg/day zeaxanthin)[3,22]. In fact, only 6.3% (5/80) of mothers consumed $\geq$10 mg/day of combined lutein + zeaxanthin, but 11.3% (9/80) mothers consumed <1 mg/day. While there are no unanimous recommendations for daily lutein + zeaxanthin consumption, average maternal intake in this population of Midwestern mothers is expected to be low. The Centers for Disease Control and Prevention reported in 2015 only 11.4% of Nebraskans (N=13,771) consumed recommend fruit intake with only 7.9% for recommended vegetable intake[168]. Additionally, it remains uncertain if maternal dietary intake was not high enough in

this sample to significantly influence placenta or umbilical cord blood levels, as lutein + zeaxanthin levels fractionate as maternal blood distributes them to various body tissues.

Thirteen percent of mothers in this sample reported low food security, similar to 11.8% of United States households reporting low or very low food security at any point in 2017[169]. Though past data has identified decreased lutein levels based on lower socioeconomic status[40], food insecurity in this study was not associated with lower dietary lutein + zeaxanthin intakes or levels in blood or placenta. One explanation for no significant differences are that median maternal dietary lutein + zeaxanthin intakes were low at baseline. Therefore, it cannot be extrapolated if intake is low as result of limited access to fruit and vegetables or if due to maternal choice.

Race

After adjustments for maternal serum levels, maternal race (Caucasian vs. non-Caucasian) remained a statistically significant predictor of placental lutein + zeaxanthin concentrations. This remains a unique finding, given maternal lutein + zeaxanthin serum levels are significant predictors of placental levels. However, no analyses between race and maternal serum levels reached significance. Comparison to literature remains complex, as not all studies report both blood levels and dietary intake. Also, available research primarily analyzes blood levels, not tissue. Nonetheless, past studies have identified potential racial differences in blood levels irrespective of dietary intake. For example, Rock *et al.* predicted in a multivariate analysis that African-American individuals would consume 9.9% more lutein + zeaxanthin than Caucasians, but this race category was predicted to have serum lutein and zeaxanthin levels that were roughly 20% higher than Caucasians, after adjusting for many variables including smoking and BMI[38]. In further relevance, NHANES 2003-2006 data reported higher concentrations of xanthophyll carotenoids (N=4,416) in Mexican Americans and non-Hispanic blacks compared to

non-Hispanic whites after adjustment for multiple factors including smoking, BMI, dietary supplement use, blood fatty acid profile, lifestyle, and socioeconomic factors[170]. While dietary intake of xanthophylls (lutein + zeaxanthin and β-cryptoxanthin) from this data was not reported, author conclusions were made that genetic differences may alter fatty acid metabolism and subsequent fat-soluble nutrients[170]. While placental differences cannot be fully explained at present, this study has identified racial disparities which atypically favor non-Caucasian populations.

Smoking

As hypothesized in study aim four, mothers with a smoking history had serum and placental levels that were 24.8% and 23.1% lower than mothers who had never smoked despite similar dietary intakes of lutein + zeaxanthin. Though not statistically significant, lutein + zeaxanthin levels in umbilical cord blood were 24.1% lower in infants born to mothers with a smoking history, which may questionably be clinically significant. A decline in lutein + zeaxanthin levels by roughly 24% is similar to one other study reporting a decline of 25% in smokers[140]. Multiple other studies only report differences around 13-15%[38,139]. Lutein + zeaxanthin levels likely decrease by scavenging free radicals and oxygen singlets induced by smoking. Novel in this study, however, is that decreased tissue lutein + zeaxanthin levels are reported in addition to blood levels. Even more original, is that this may be one of the first studies illustrating lower carotenoid levels in placenta based on smoking status.

In a regression analyses to predict lutein + zeaxanthin levels in maternal serum, placenta, and umbilical cord blood levels, smoking history only remained a significant predictor for maternal serum levels. Smoking did not alter rate of lutein + zeaxanthin transfer from mother to infant. These results highlight that maternal smoking most directly decreases maternal serum levels. Subsequently, smoking more indirectly decreases lutein + zeaxanthin in

placenta and umbilical cord as result of lower concentrations initially present in maternal blood. This theory also remains consistent, as lutein + zeaxanthin levels decreased similarly by 23.1-24.8% across all samples.

In regression analyses, mothers with a smoking history were predicted to have serum lutein + zeaxanthin levels that were 51.1 mcg/L lower than mothers who had never smoked. In comparison, it was predicted that maternal serum lutein + zeaxanthin levels would increase by 14.3 mcg/L for every 1 mg increase in dietary lutein + zeaxanthin. Therefore, it can be concluded that mothers with a smoking history in this sample need to increase their daily lutein + zeaxanthin intake by approximately 3.6 mg/day in order to combat negative effects of smoking. This yields an increase in dietary lutein + zeaxanthin by 2.5 times as their median baseline intakes were 2.41 mg/day.

Obesity

Rates of obesity by pre-pregnancy maternal BMI (32.0%) are reasonably consistent with national rates at 39.8%[171]. In a regression analysis to identify predictors of maternal lutein + zeaxanthin in serum, maternal pre-pregnancy BMI approached significance after adjustment for maternal dietary intake, close to meeting hypothesis for study aim four. While mothers of normal weight statistically consumed 1.5 times more lutein + zeaxanthin, there were no significant differences between overweight and obese mothers. Quantitatively however, obese mothers had decreased lutein + zeaxanthin levels by 13.9% in serum, 14.0% in placenta, and 14.7% in umbilical cord blood compared to overweight mothers. Results are parallel to past literature reporting down trending lutein levels with increasing adiposity[38,39,140,147,148]. While comparison of all lutein + zeaxanthin levels were not significantly different by statistical comparison, quantitative differences may still be clinically impactful. Example is by *Panagos et al.*, who identified obese mothers to produce breast milk with lower lutein content compared to

normal weight mothers, regardless of dietary intake[172]. It is unknown how this impacts infant development, as lutein and zeaxanthin are highly prevalent carotenoids in human milk and brain tissue[5-7].

Placenta lutein + zeaxanthin levels approached significance in normal weight vs. obese mothers (p=0.088), but maternal obesity was not identified as a significant predictor of placenta levels. Conclusively, like smoking, maternal obesity most directly causes decreased levels in maternal serum. Decreased lutein + zeaxanthin levels in placenta are more indirectly due to lower supply in maternal serum. Reasons for decreased lutein + zeaxanthin levels in the blood of obese individuals stem from two ideas. Firstly, adipose tissue remains a primary storage site for carotenoids, so more adipose tissue requires higher amount carotenoids pulled from blood to equate to similar concentrations found in leaner individuals. Secondly, being overweight or obese promotes inflammation and more oxidative stress in the body, with more carotenoids being used to combat negative effects[173,174]. One limitation of this study is that inflammatory markers were not analyzed.

Gestation Age at Birth

Gestational age at birth correlated with maternal serum and placenta, but most strongly with umbilical cord blood levels of lutein + zeaxanthin. Gestational age at birth also correlated with the percent of lutein + zeaxanthin levels in umbilical cord blood compared to maternal serum. In regression modeling, gestational age at birth only remained a significant predictor of umbilical cord blood levels. Considerations of increased transfer align with pregancy pathology, were placental surface area of syncytiotrophoblasts increase with advancing gestation to allow for higher nutrient transfer and fetal growth[47]. Blood lutein and circulating cholesterol have also been reported in mothers across increasing trimesters[46,49]. So, combined higher substrate in blood and increased absorptive capacity of the placenta positively increase umbilical cord blood

levels. Most infants in this study were born at term (97.6%), so results indicate increased lutein + zeaxanthin transfer and umbilical cord blood levels, even after 37 weeks gestation. However, it would be expected for placenta to continue augmenting transfer up until point of birth, as it will be decease function once the infant is delivered.

Application to Clinical Practice

As lutein + zeaxanthin have more potential for beneficial than adverse outcomes, lifestyle factors should focus on optimizing blood and tissue levels. Results of this study, along with literature review, clearly indicate a need for overall higher dietary intake of fruits and vegetables within the United States population. Current recommendations for women are 1 ½ - 2 cups/day fruit and 2 ½ cups/day of vegetables (with 1 ½ cups/week coming from dark green vegetables)[34,35]. This research additionally supports the growing need to identify concrete dietary recommendations for daily lutein + zeaxanthin intake that promotes improved health outcomes. Though favorable across all stages of the lifespan, emphasis in pregnant and neonatal populations may transpose the greatest benefits during a period of rapid growth and development.

Beyond dietary intake, pregnant women or those considering pregnancy should be encouraged to avoid or quit smoking. Likewise, in current or former smokers, consuming higher intakes of fruits and vegetables than minimum recommendations should be employed to counteract negative effects. Women considering pregnancy should be encouraged to maintain healthy weights. Otherwise, pregnant women should be encouraged to gain appropriate weight during pregnancy, as excessive gain is likely due to adiposity only. In pregnant women who are overweight or obese, it may be beneficial to consume higher dietary intake as lutein + zeaxantin levels my clinically be lower than lower weight mothers.

Future Research

This study provides a comprehensive database for enhancing our knowledge of how lutein + zeaxanthin transfer from food to mother to infant during pregnancy. However, additional information to supplement this study include breast milk samples from each mother to analyze carotenoid content. Data on maternal cholesterol levels, including HDL and LDL, may explain further variances in maternal to fetal carotenoid transfer. Maternal inflammation would also be assessed by varying markers, such as C-reactive protein or cytokines. Similarly, identification of additional placental receptors and transporters would allow more comprehensive understanding of carotenoid transfer through placenta. Full combined data would provide sound baseline for future prospective studies.

As lutein + zeaxanthin have demonstrated visual and cognitive benefits in elderly populations but to a limited degree in children, prospective studies would analyze these outcomes in the infants of this study. For example, developmental outcomes of infants could be assessed at one or two years of life to identify if lutein + zeaxanthin levels in any samples at birth are associated with neurodevelopmental or cognitive outcomes. If correlations or multivariate analyses identified significance, follow-up studies would continue to be assessed at varying time points, including elementary school age or into young adulthood. Similar assessments could be conducted for vision. The purpose of this type of prospective analysis is to identify if lutein + zeaxanthin status during pregnancy and early infancy impact long-term or life-long outcomes. Furthermore, if maternal dietary remained significant to later outcomes of the offspring, it may allow more concrete recommendations to be made for daily intake of lutein + zeaxanthin.

With results of this studying identifying lutein + zeaxanthin as the most prevalent carotenoids in human placenta, future studies may take interest in cases of placental

dysfunction. Similar to lutein and zeaxanthin being selectively up taken by retinal tissue, there still remains consideration if placental tissue purposefully uptakes these carotenoids to aid with oxidative defenses, structure maintenance, and strengthened permeability. Given placental function has already been linked to infant outcomes, future studies would compare lutein + zeaxanthin concentrations in cases of placental insufficiency or fetal growth restriction. More novel studies would identify if higher lutein + zeaxanthin dietary intake, and subsequent levels in placental tissue, could prevent or diminish dysfunction.

Strengths and Limitations

The primary strength of this study is that placenta carotenoid levels have never been quantified for lutein + zeaxanthin presence. Furthermore, no study has assessed placental levels concurrent with maternal dietary intake, maternal serum, and umbilical cord blood levels to observe full maternal-fetal transfer dynamics. Furthermore, jointly comparing the most six prevalent carotenoids within the American diet allows comparisons of proportions and transfer rates.

Limitations of this study include that some outcomes are self-reported by mothers, such as pre-pregnancy height and weight used to calculate BMI, smoking status, and usual dietary intake. Furthermore, smoking status did not distinguish between frequency or various forms of use including cigarette, electronic cigarette, cannabis, vaping, or second-hand smoke inhalation. Similarly, there is no time frame since quitting in self-reported former smokers. Though alcohol intake may alter lutein + zeaxanthin levels, intake by mothers was not analyzed in this study.

Infant head circumference and linear measurements may vary dependent on measuring tape placement. Ultimately, the largest limitations of this study include that no recommend dietary intakes or serum references levels have been identified for most carotenoids, including

lutein + zeaxanthin, which makes comparisons more subjective. It also remains difficult to compare previous studies as lutein and zeaxanthin may be analyzed separately or conjointly. Lastly, there remain additional factors that were not analyzed (i.e. inflammatory markers) but have potential to explain more variance in blood or placenta carotenoid levels. Similarly, full identification of placenta receptor sites and placenta function is not complete.

CONCLUSION

Carotenoids lutein + zeaxanthin were quantified in human placenta at median 0.105 mcg/g and were significantly correlated with lutein + zeaxanthin levels in maternal serum and umbilical cord blood, but not maternal dietary intake. Only maternal serum levels were significantly correlated with maternal dietary intake. Lutein + zeaxanthin were also the most prevalent carotenoids in placenta and umbilical cord blood, when compared alongside α-carotene, β-carotene, lycopene, and β-cryptoxanthin. Lutein + zeaxanthin rate of transfer from maternal to fetal blood was 16.0%, highest of all carotenoids. Caucasian mothers had lower placental levels than non-Caucasian mothers. Maternal smoking history and obesity are modifiable factors that negatively impact placental levels. Conclusively, lutein + zeaxanthin consisted of 49.1% of placental carotenoids, highlighting unique roles lutein + zeaxanthin may play during pregnancy. Future research is needed to identify specific benefits conferred to the developing infant.

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