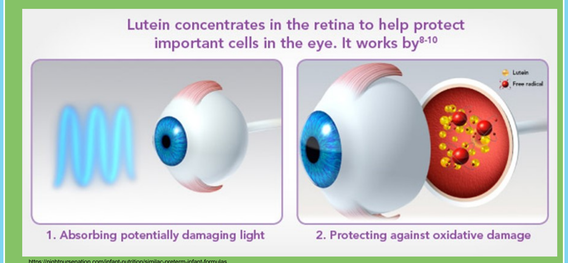
The Effect of Seasonal Variations on Concentrations of Lutein and Zeaxanthin in the Diet, Blood, and Retinal Tissue

Introduction

Lutein and Zeaxanthin

- Naturally occurring carotenoids
  - Green, leafy vegetables
  - Brightly colored fruits
- Major components of the human retina (Bone et al., 1985)
  - Measured via macular pigment density (Snodderly et al., 2004)
- Blue light filters
  - Protect retina from high-energy photons of blue light (Snodderly, 1995)
- Antioxidants
  - Inhibit free radical damage and decreases oxidative stress (DiMascio, 1991)

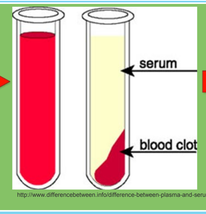


https://nightnursenation.com/infant-nutrition/similac-preterm-infant-formulas

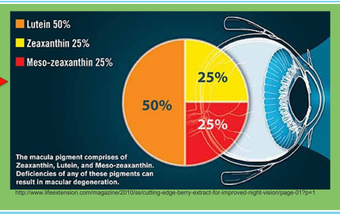
Review of Literature

Progression Through The Body

http://www.eyepromise.com/natural-eye-vitamins/eyepromise/zeaxanthin



http://www.differencebetween.info/difference-between-plasma-and-serum



http://www.lifeextension.com/magazine/2010/ts/cutting-edge-berry-extract-for-improved-night-vision/page-01?pr=1

Effects of Lutein and Zeaxanthin on Visual Function

- Lutein may influence early maturation of the retina (Hammod, 2008)
- Carotenoid supplementation may improve vision/ visual performance
- May help further reduce the risk of late Age-Related Macular Degeneration (Age-Related Eye Disease Study Research Group, 2013)

TABLE. EFFECT OF SUPPLEMENTATION IN AREDS2[a]

	Kaplan-Meier Probability of Progression		Comparison With Placebo Reduction in Progression to Advanced AMD		
	Rate	n	Hazard ratio	CI (range)	P value
Placebo	31%	493 eyes of 406 participants			
Lutein and zeaxanthin	29%	468 eyes of 399 participants	0.90	98.7% (0.76-1.07)	.12
DHA and EPA	31%	507 eyes of 416 participants	0.97	98.7% (0.82-1.16)	.70
Lutein and zeaxanthin and DHA and EPA	30%	472 eyes of 387 participants	0.89	98.7% (0.75-1.06)	.10

Abbreviations: AREDS, Age-Related Eye Disease Study; AMD, age-related macular degeneration; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.
[a]Adapted from the AREDS2 Study Group. *JAMA*. 2013;309(19):2005-2015.

http://eyetubeod.com/2013/05/areds2-substituting-lutein-and-zeaxanthin-for-beta-carotene-may-be-warranted

Seasonal Variations in Blood Lipid Levels

- L and Z are found exclusively on lipoproteins
- Mean serum lipid values peak in the winter and trough in the summer
  - Light exposure
  - Plasma volumes (Ockene et al., 2004)
  - Hormones, lipoprotein lipase activity (Donahoo et al., 2004)
- Seasonal variations in certain chronic diseases associated with high cholesterol and triglyceride levels (Marti-Solder et al., 2014; Hopstock et al., 2013)

Purpose

- Evaluate seasonal variations in MPD while accounting for:
  1) Confounding variables (age, sex, BMI, blood lipids, dietary intakes)
  2) Possible effects of sunlight on MPD
- Are increased dietary intakes of L/ Z needed during certain times of the year?
- Should seasonal timing be accounted for when designing L/Z studies?
- Most effective method of measuring L/Z intake?

Research Questions

1-3) How do concentrations of (1) dietary L/Z intake, (2) serum L/Z, (3) MPD change with seasonal variations?

4) Does the correlation between measures of L/Z status change with season?

5) Does the variability of data differ among measures of L/Z status?

Hypotheses

H1) Dietary L/Z will be lowest in winter and highest in the summer.

H2) Serum L/Z concentrations will be highest in the winter (high blood lipids) and lowest in the summer (low blood lipids).

H3) MPD concentrations will be highest in the winter (high blood lipids) and lowest in the summer (low blood lipids).

H4) The correlation between measures of L/Z status will differ among seasons due to seasonal differences in intakes and the times required for dietary L/Z to be taken up into the serum and macula.

H5) Dietary L/Z data, followed by serum L/Z data, and MPD data.

Methods

Overview

- Three measures of L/Z intake: diet, serum, eye
- Data grouped into four seasons: winter, spring, summer, fall
- Analysis via one-way ANOVA or ANCOVA

Subject Characteristics

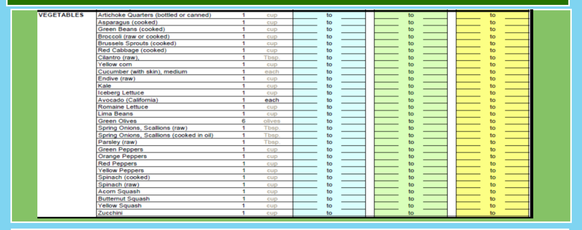
- Baseline data from 162 subjects from four different nutritional interventions
- Recruited from general population in/ around Boston, MA

Table 1. Study Population Characteristics

	Age (years)		BMI[1] (kg/m²)		LZQ[2] (mg/d)		Serum LZ[3] (pmol/L)		MPD[4]	
	n	mean ± SD	n	mean ± SD	n	mean ± SD	n	mean ± SD	n	mean ± SD
M	68	59.71 ± 14.16	68	25.82 ± 2.99	48	5.83 ± 6.98	31	103.10 ± 23.79[a]	63	0.40 ± 0.16
F	94	61.22 ± 13.83	94	24.46 ± 3.37	70	7.12 ± 6.70	47	117.42 ± 49.47[a]	87	0.39 ± 0.15
All	162	60.59 ± 13.95	162	25.03 ± 3.28	118	6.59 ± 6.82	78	111.73 ± 41.62	150	0.39 ± 0.15

[1]BMI: body mass index; [2]LZQ: lutein and zeaxanthin questionnaire; [3]Serum LZ: serum lutein and zeaxanthin; [4]MPD: macular pigment optical density; [a,b] trend for a difference between males and females (p <0.07).

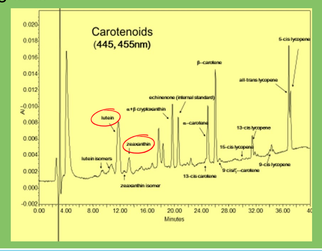
Dietary L/Z Questionnaire (LZQ)



VEGETABLES	Artichoke Quarters (bottled or canned)	1	cup	____	to	____	to	____	to	____	to	____	to
	Asparagus (cooked)	1	cup	____	to	____	to	____	to	____	to	____	to
	Green Beans (cooked)	1	cup	____	to	____	to	____	to	____	to	____	to
	Broccoli (raw or cooked)	1	cup	____	to	____	to	____	to	____	to	____	to
	Brussels Sprouts (cooked)	1	cup	____	to	____	to	____	to	____	to	____	to
	Red Cabbage (cooked)	1	cup	____	to	____	to	____	to	____	to	____	to
	Cilantro (raw)	1	Tbsp.	____	to	____	to	____	to	____	to	____	to
	Yellow corn	1	cup	____	to	____	to	____	to	____	to	____	to
	Cucumber (with skin), medium	1	each	____	to	____	to	____	to	____	to	____	to
	Endive (raw)	1	cup	____	to	____	to	____	to	____	to	____	to
	Kale	1	cup	____	to	____	to	____	to	____	to	____	to
	Iceberg Lettuce	1	cup	____	to	____	to	____	to	____	to	____	to
	Avocado (California)	1	each	____	to	____	to	____	to	____	to	____	to
	Romaine Lettuce	1	cup	____	to	____	to	____	to	____	to	____	to
	Lima Beans	1	cup	____	to	____	to	____	to	____	to	____	to
	Green Olives	6	olives	____	to	____	to	____	to	____	to	____	to
	Spring Onions, Scallions (raw)	1	Tbsp.	____	to	____	to	____	to	____	to	____	to
	Spring Onions, Scallions (cooked in oil)	1	Tbsp.	____	to	____	to	____	to	____	to	____	to
	Parsley (raw)	1	Tbsp.	____	to	____	to	____	to	____	to	____	to
	Green Peppers	1	cup	____	to	____	to	____	to	____	to	____	to
	Orange Peppers	1	cup	____	to	____	to	____	to	____	to	____	to
	Red Peppers	1	cup	____	to	____	to	____	to	____	to	____	to
	Yellow Peppers	1	cup	____	to	____	to	____	to	____	to	____	to
	Spinach (cooked)	1	cup	____	to	____	to	____	to	____	to	____	to
	Spinach (raw)	1	cup	____	to	____	to	____	to	____	to	____	to
	Acorn Squash	1	cup	____	to	____	to	____	to	____	to	____	to
	Butternut Squash	1	cup	____	to	____	to	____	to	____	to	____	to
	Yellow Squash	1	cup	____	to	____	to	____	to	____	to	____	to
	Zucchini	1	cup	____	to	____	to	____	to	____	to	____	to

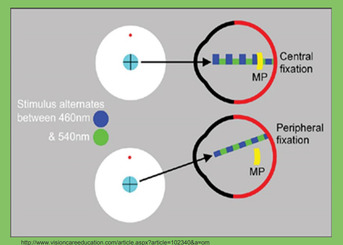
Serum L/Z

- Measure of L/Z in blood tissues
- Standard lipid extraction measures
  - Blood samples centrifuged to obtain serum
- Reverse-Phase High Performance Lipid Chromatography (HPLC) Analysis
  - L and Z quantified by determining peak areas in HLPC chromatograms



Macular Pigment Density (MPD)

- Non-invasive measure of L and Z in retinal tissue
- Heterochromatic flicker photometry (HFP) (Snodderly et al., 2004)
  - Small test field superimposed on blue background
  - Alternates between wavelength absorbed by MP (blue) and reference wavelength (green) —> flickering light
  - Amount bluish light required to produce flicker null = measure of MPD



http://www.visioncareeducation.com/article.aspx?article=102340&a=om

Results

Relationships Between Confounding Variables and Measures of L/Z Status

- Information on what factors to correct for in statistical analysis
- LZQ: HDL
- Serum L/Z: BMI, Total Chol
- MPD: -

Table 4. Age/BMI/Blood Lipids/MPD

	LZQ[2](mg/d)			SerumLZ[3] (pmol/L)			MPD		
	n	correl	p	n	correl	p	n	correl	p
Age (years)	118	0.099	0.29	78	0.018	0.88	162	-0.049	0.538
BMI[1] (kg/m²)	118	0.089	0.34	78	-0.265	0.02*	162	-0.008	0.920
Total Chol[2] (mg/dL)	118	0.074	0.43	78	0.539	<.0001*	127	-0.080	0.374
TG[3] (mg/dL)	118	-0.028	0.76	78	-0.022	0.85	126	-0.036	0.692
HDL[4] (mg/dL)	109	0.196	0.04*	78	0.280	0.01*	109	-0.030	0.756
LDL[5] (mg/dL)	109	0.066	0.50	78	0.599	<.0001*	109	-0.098	0.311

[1]BMI: body mass index; [2]Total Chol: total cholesterol; [3]TG: triglycerides; [4]HDL: high-density lipoproteins; [5]LDL: low-density lipoproteins; [6]LZQ: lutein and zeaxanthin questionnaire; [7]Serum LZ: serum lutein and zeaxanthin); [8]MPD: macular pigment optical density *Significant correlation (p<0.05)

Effect of Daylength/ Seasonal Variations on Diet (Q1), Serum (Q2), and MPD (Q3)

- No significant differences

Table 5. Effect of Daylength Differences on Diet, Serum, and MPD

Group	0	1	2	3
Hours of daylight	9.12 - 9.60	9.84 - 11.04	11.28 - 13.44	13.68 - 15.36
n	37	38	41	44
LZQ*	7.77 ± 8.29	8.47 ± 8.28	5.45 ± 5.05	4.77 ± 4.31
Serum LZ*	317.22 ± 152.80	289.38 ± 157.97	365.33 ± 166.56	327.12 ± 139.82
MPD	0.39 ± 0.15	0.38 ± 0.19	0.39 ± 0.15	0.40 ± 0.14

*log transformed [1]LZQ: lutein and zeaxanthin questionnaire (mg/d); [2]Serum LZ: serum lutein and zeaxanthin (umol/L); [3]MPD: macular pigment optical density **Day lengths were collected based on date. Four categories were created, with group 0 having the shortest day lengths and group 3 have the longest day lengths.

Table 6. Effect of Seasonal Variations on Diet, Serum, and MPD

Season	Winter	Spring	Summer	Fall
Range	Dec 1 - Feb 28	Mar 1 - May 31	Jun 1 - Aug 31	Sep 1 - Nov 30
n	49	35	31	47
LZQ*	9.38 ± 9.0	5.15 ± 4.5[a]	5.50 ± 5.36[a]	5.20 ± 5.29
Serum LZ*	317.22 ± 152.80	289.38 ± 157.97	365.33 ± 166.56	327.12 ± 139.82
MPD	0.38 ± 0.15	0.39 ± 0.12	0.40 ± 0.16	0.39 ± 0.17

*log transformed [1]LZQ: lutein and zeaxanthin questionnaire (mg/d); [2]Serum LZ: serum lutein and zeaxanthin (umol/L); [3]MPD: macular pigment optical density Unshared superscripts have a trend for being different (p <0.07)

Relationship Among Measures of L/Z Status By Season (Q4)

- LZQ significantly correlated with serum L/Z during the spring and summer
- MPD significantly correlated with serum L/Z during the winter and fall
- Spring and summer: strong diet/ serum relationship due to more bioavailable food choices being made
- Fall and winter: subsequent improved serum/ MPD relationship reflects the time required for the uptake of dietary L/Z into the circulation for eventual incorporation into the macula

Table 7. Seasonal Differences in Correlations between Diet, Serum, and Macular Pigment

	Winter (12- 2)			Spring (3-5)			Summer (6-8)			Fall (9-11)		
	n	correl	p	n	correl	p	n	correl	p	n	correl	p
LZQ[1] vs Serum[2]	15	0.29	0.2944	16	0.53	0.0347*	19	0.52	0.0225*	28	-0.0842	0.6701
LZQ[1] vs MPD[3]	37	0.24	0.1525	23	0.26	0.2309	24	0.07	0.7452	34	-0.07909	0.6566
Serum[2] vs MPD[3]	15	0.63	0.0118*	16	0.02	0.9414	19	0.28	0.2456	28	0.392357	0.0389*

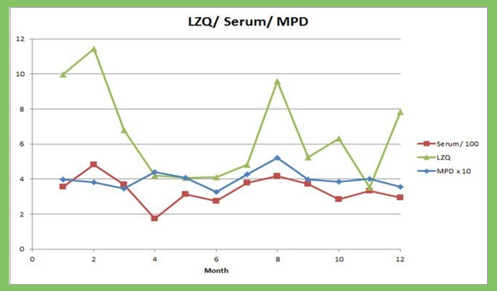
[1]LZQ: lutein and zeaxanthin questionnaire (ug/d); [2]Serum LZ: serum lutein and zeaxanthin (umol/L); [3]MPD: macular pigment
*Significant correlation (p<0.05)

Variability of Measures of L/Z Status (Q5)

- LZQ had the highest variability (day to day)
- Serum L/Z was less varied (week to week)
- MPD had the lowest variability (month to month)

Table 8. Variability of LZQ, Serum LZ, and MPD

	mean	SD	coefficient of variance
LZQ	6.59	6.82	1.03
Serum LZ	326.17	152.51	0.47
MPD	0.39	0.15	0.38



Conclusions

- Time of the year in which studies are conducted should not be a concern for study design (Nolan et al., 2006)
- Proposed possible mechanisms for spring and summer diet/ serum relationship and fall and winter serum./ MPD relationship
- MPD should be used in as primary measure of L/Z in studies (Hammond BR et al., 1997)

Limitations

- Results may not be translatable to other geographical areas or populations
- Study consisted of a relatively small group (n = 162)

Future Research

- Cross-sectional design does not allow us to demonstrate a cause and effect
- Longitudinal study with measures of LZQ, serum L/Z, and MPD in the same population

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