

LUTEINS IN LUPINS - AN EYE FOR HEALTH

Cathy Fryirs, Bronwyn Eisenhour and Sherry Duckworth

George Weston Technologies (A Division of George Weston Foods) 1 Braidwood Street, Enfield NSW 2136, Australia

Corresponding author's email sherry_duckworth@gwf.com.au

ABSTRACT

Research indicates that the degeneration of the central retina (macula) causes a gradual blurring of the central vision restricting the sufferer's ability to see fine details. As the symptoms develop central vision is lost leaving only peripheral vision. Such a condition affects approximately 30% of Caucasians aged 75 and over, with those over 55 at risk of developing age-related macular degeneration (AMD). Current extension of life expectancy is likely to lead to a corresponding increase in the incidence of AMD.

Studies have shown that lutein, which is highly concentrated in the macula, is thought to filter short or blue wavelengths of light. Work done in this area suggests that the blue light induces oxidative stress and leads to the formation of free-radicals, which damage the retina.

Although there are a number of causative agents such as smoking and family history, studies have found that people with lower blood levels of the carotenoids, particularly lutein and zeaxanthin, are more likely to develop AMD. The protection afforded by lutein and zeaxanthin is possibly through their ability to filter shorter wavelengths of light and their antioxidant activity. Lutein is not a cure for AMD, but can slow the progress of degeneration.

Lupins are known to be a rich source of lutein and this lutein can contribute to a dietary solution that helps to delay the onset as well as slow the development of the debilitating consequences of AMD.

This presentation provides a review of the literature on AMD, the role of lutein in the progression of the condition, and the potential for lupins to supply lutein in the diet.

KEYWORDS

lupin, lutein, AMD, macular degeneration

INTRODUCTION

Age-related macular degeneration (AMD) and the consequent loss of central vision is a concern for many Australians over 55 years of age. As life expectancy extends well into the 80s and 90s there is a

corresponding increase in the incidence of degenerative conditions. An understanding of the nature of the condition and the role of xanthophylls particularly lutein in the eye is the focus of this review. Lupins, as a valuable source of lutein, may contribute to delayed onset or slow the progress of the blurring of vision in responsive individuals.

Strategies that seek to reduce the physically debilitating impact of AMD on individuals and their families will have social and financial benefits as the cost to Australia of AMD is predicted to rise from the current level of \$2.6 billion to \$6.5 billion in 2025.¹

DESCRIPTION: AGE RELATED MACULAR DEGENERATION (AMD)

The macula is at the centre of the retina at the back of the eye. In this location there are many photoreceptors which enable us to see fine detail and colours. As we age the cells become less efficient, the membrane degenerates and waste products build up. Gradually central vision becomes blurred in the process termed macular degeneration (MD) or age-related macular degeneration (AMD).²

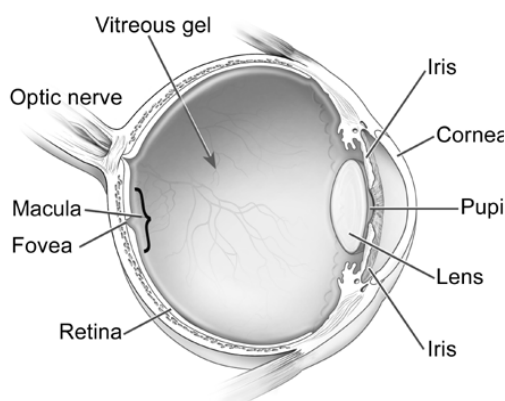


Diagram 1. Location of the macula.

Source: National Eye Institute

http://www.nei.nih.gov/health/maculardegen/armd_facts.asp

The early signs of age related macular degeneration include:

- blurred vision with close work;
- seeing straight lines as wavy (see Appendix 1);
- diminishing colour vision;
- holes or black spots (scotoma) in vision;

- extreme light sensitivity;
- poor light to dark adaptation;⁴

There are two types of age related macular degeneration:

The advanced form is termed wet AMD and vision loss can occur quickly. In wet AMD abnormal blood vessels grow under the retina. Since these blood vessels are fragile they leak blood and fluid causing damage to the macula. The first sign of wet AMD is that straight lines appear wavy.

In dry AMD there is a gradual breakdown of the photosensitive cells. As the condition progresses a blurring of the central vision occurs.⁵

Macular degeneration is the leading cause of blindness in Australia⁶ and nearly two out of every three people will eventually develop age related macular degeneration and one in four will lose their vision.⁷ For Australians over 50 years one in seven is affected by macular generation.⁸

RISK FACTORS FOR AMD

Age: People over the age of 60 are at greater risk of getting AMD (30% risk for those over 75 years).

Smoking: Smokers have 3 times the risk of a non-smoker and may develop AMD 10 years earlier.⁹

Obesity: Research studies suggest a link between obesity and the progression of early and intermediate stage AMD to advanced AMD.¹⁰

Race: Caucasians particularly those with blue, grey or green eyes have less pigment in the retina.

Family history: Those with immediate family members who have AMD are at a higher risk of developing the disease.¹¹

Gender: Women appear to be at greater risk than men.¹²

Low dietary or plasma levels of carotenoids, particularly lutein and zeaxanthin.¹³

The carotenoid pigments Lutein and Zeaxanthin are found at relatively high concentration levels in the macular region of a healthy eye. The protection they give to the retina may be as a result of their antioxidant activity in protecting against oxidative stress and by filtering the shorter wavelengths of light. In this way they provide protection against the oxidative effect of blue light.¹⁴

TREATMENT

There is no cure for the condition. However lifestyle changes and dietary intervention may reduce the risk and slow down the rate of vision loss.

The treatment for wet AMD includes the use of laser surgery, photodynamic therapy and injections of drugs into the eye. These treatments are designed to reduce the

impact of leaking blood vessels but may not slow the loss of vision.¹⁵

A large number of studies have evaluated the benefits of lutein in the diet. Some recent clinical trials are summarised in Appendix 2. These studies indicate that maintaining the level of Macular Pigment Optical Density (MPOD) in the retina and in the sera are important in delaying onset and slowing the progress of dry ADM. There is evidence that Lutein plays an important role. Some of the studies in which the effect of lutein intake is unclear indicate the importance of monitoring other factors that may reduce the effectiveness of lutein intake. In addition, increased intake of lutein is not able to reverse damage that has already occurred so the timing of dietary intervention is relevant.

SOURCES OF LUTEIN

Appendix 3 lists the lutein and zeaxanthin contents of a variety of foods. Cognis and Kemin have produced supplements containing lutein extracted from marigolds. Kale (40 mg/100 g) and spinach (11 mg/100 g) are the richest food sources of lutein and zeaxanthin with full fat lupin flour a complementary source (4.4 mg/100 g).

BIOAVAILABILITY

There is a difference in the bioavailability of lutein from different sources. In a study in men Lutein bioavailability was higher from lutein enriched eggs than spinach and from supplements.¹⁶ Lutein in cooked egg yolk for instance, is highly available, presumably because the lipid matrix is optimal for absorption.¹⁷ Bioavailability of lutein esters (linkage of lutein with fat) or lutein solubilised in oil may be higher than that of the unattached lutein as measured by serum concentration.¹⁸ Although, there is currently no studies providing evidence, the presence of lutein in esters associated with the lipid may make lupin a valuable source of bioavailable lutein.

Lutein esters are hydrolysed and transported as lutein after digestion¹⁹ to the macula area of the eye so all forms of lutein are important.

The assessment of lutein bioavailability is difficult because there are differences in the way that individuals respond to lutein in the diet.²⁰ In the analysis researchers need to account for the lutein stored in the tissues, the lutein present in the diet and the persistence of lutein in the retina after supplementation has been stopped.²¹

SAFETY OF LUTEIN

The Joint Expert Committee on Food Additives (JECFA) has determined that the acceptable daily intake (ADI) level for Lutein is up to 2 mg per kg of body weight.²² For a 58 kg woman and a 70 kg man this would equate to 116 mg and 140 mg respectively. The Observed Safe Level (OSL), based on human supplementation studies, is suggested as 20 mg lutein per day.²³ The recommended amount in the literature

considered to be of value in increasing macular pigment optical density (MPOD) is from 6-10 mg per day.²⁴

Supplement manufacturers Kemin and Cognis have completed the notification processes for US FDA GRAS (Generally Regarded As Safe) for their products.^{25,26}

However, in the 2006 review of intervention and observational studies the FDA did not approve a health claim linking the intake of lutein and the risk of age-related macular degeneration in America.²⁷ The opposition to acceptance of the health claim included comments that questioned whether esterified lutein was more effective than free lutein, indicated that the claim should mention lutein and lutein containing foods, noted that a brand name (Xangold®) should not be in the claim and that stated that zeaxanthin should also be included.²⁸

STABILITY OF LUTEIN

Research suggests that the free lutein is not stable to heat and UV light. However when the lutein hydroxyl groups are attached to fatty acids the resultant ester is

much more stable. Free Lutein and Lutein esters do not differ significantly in their ability to act as antioxidants.²⁹

USING LUPINS AS A SOURCE OF LUTEIN

Dietary modelling demonstrates that the recommended 6 mg lutein and zeaxanthin / day³⁰ can be achieved with the inclusion of lupin bread in a meal. The food combinations are set out in Appendix 4.

CONCLUSION

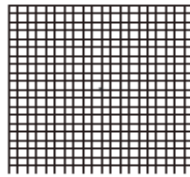
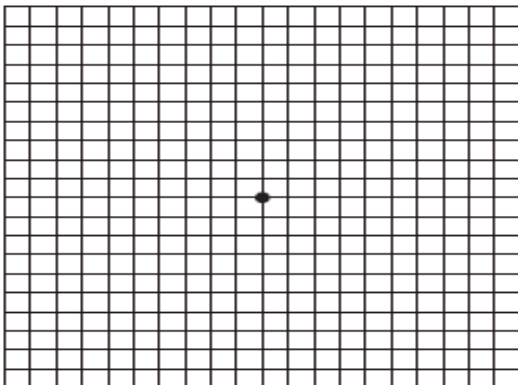
Since there is no cure for AMD, delaying the onset and lessening the severity of the damage is crucial. Clinical studies support the role of the xanthophylls, lutein and zeaxanthin in the macular pigment in sera and in the retina. The supply of xanthophylls needs to come from a dietary source and full-fat Lupin flour containing significant quantities of lutein in both free and esterified forms can be used to increase the intake of lutein.

Further work is needed to provide assurance that the lutein content of lupin flour remains bioavailable in a range of food products exposed to different processing and storage conditions.

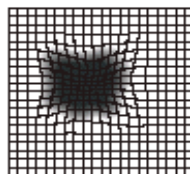
Appendix 1.

Amsler Grid eye examination

This is an Amsler Grid, which can be used to test for symptoms of MD



Normal vision



Consult your eye practitioner immediately

Directions

1. Do not remove glasses or contact lenses you normally wear for reading.
2. Hold the grid approximately 35cm from your face in a well-lit room.
3. Cover one eye with your hand and focus on the centre dot with your uncovered eye. Repeat with the other eye.
4. If you see wavy, broken or distorted lines, or blurred or missing areas of vision, you may be displaying symptoms of MD and should contact your optometrist or ophthalmologist immediately.

Appendix 2. Review of recent clinical studies with lutein.

Author	Demographics	Diet Change	Measure	Conclusion
Mares <i>et al.</i> ³² 2006	Women 53-68 Adjusted for fibre and PUFA intake Waist circumference and diabetes	Lutein zeaxanthin	Macular pigment optical density (MPOD) in blood	MPOD directly related to intake lutein and zeaxanthin.
Khachik <i>et al.</i> ³³ 2006	Elderly human subjects with and without AMD	Lutein zeaxanthin	Lutein, zeaxanthin, carotenoids, retinol, tocopherols metabolites	Increase in serum lutein and zeaxanthin levels No apparent toxicity for lutein supplements 10 mg/d 6 mths.
Wei Wang <i>et al.</i> ³⁴ 2007	Patients with AMD	High and low lutein and zeaxanthin diet	Plasma and lipoprotein carotenoids	2 x as much lutein and zeaxanthin were transported by HDL compared to LDL.
San Giovanni, J. <i>et al.</i> ³⁵ 2007	AREDS Age related eye Disease Study 4519 participants 60-80 year olds	Lutein zeaxanthin	Stereoscopic colour fundus photographs Self-administrated questionnaire	Higher dietary intake of lutein/zeaxanthin independently associated with decreased likelihood of AMD.
Robman, L. <i>et al.</i> ³⁶ 2007	254 with early AMD	Lutein Zeaxanthin and fats	AMD progression (3 measures)	Increased intakes Lutein, Zeaxanthin and omega 3 fatty acids associated with progression of AMD. Could be caused by changes to healthier diet during the trial.
Parisi, V. <i>et al.</i> ³⁷ 2008	27 non-advanced AMD	Carotenoid and antioxidant supplementation	Multifocal electroretinograms	In non advanced cases, AMD can be improved by the supplementation with carotenoids and antioxidants.
Bartlett, H. <i>et al.</i> ³⁸ 2008	45 healthy	Lutein and vitamins and minerals	Distance and near visual acuity, contrast sensitivity, photostress recovery time	No evidence of improvement on visual acuity for images illuminated by white light.
Rosenthal, J. <i>et al.</i> ³⁹ 2006	45 persons 60 years and older	Lutein (2.5, 5 or 10 mg)	Serum lutein concentration	Increasing doses of lutein significantly increased serum lutein.
Wenzel, A. <i>et al.</i> ⁴⁰ 2007	25 Married couples	Dietary questionnaire	MPOD	More factors than just similar dietary intakes influence MPOD.
Bone, R. <i>et al.</i> ⁴¹ 2007	10 persons	Meso-zeaxanthin	MPOD	Meso zeaxanthin is generally effective at raising MPOD.
Aleman, T. <i>et al.</i> ⁴² 2007	11 persons with retinal degenerations	Lutein	MPOD	Lutein increased MP in some patients. No change in central vision over 6 months.
Schlach, W <i>et al.</i> ⁴³ 2007	LUXEA (LUtein Xanthophyll Eye Accumulation) study	Lutein Zeaxanthin	MPOD Blue light sensitivity	Increases in MPOD.
Reboul <i>et al.</i> ⁴⁴ 2007	8 healthy men	Lutein with a combinations of antioxidants	Postprandial lutein response in the chylomicron-rich fraction	Lutein absorption is not affected by conc of vitamins C and E but was impaired by carotenoids naringenin (polyphenol).
Kopsell, D. <i>et al.</i> ⁴⁵ 2006	10 persons	13 spinach cultigens (low and high lutein)	MPOD	Lutein concentration in spinach determined MPOD but not in all individuals.
Wenzel, A. <i>et al.</i> ⁴⁶ 2006	24 females 24-59 years	6 eggs/week (good source of lutein and zeaxanthin)	MPOD	Increase in MPOD.
Tan <i>et al.</i> ⁴⁷ 2008	3654 participants > 49 yrs old 2454 re-examined after 5-10 yrs	Dietary questionnaire on antioxidant intakes	Graded stereoscopic retinal photographs	Higher dietary intake lutein and zeaxanthin reduced the risk of AMD. Higher beta-carotene intake increased the risk.

Appendix 3. Lutein and zeaxanthin content of foods and supplements.⁴⁸

Source	Lutein and zeaxanthin (micrograms per 100 g)
Cognis Xangold Lutein 30% (Marigold) ⁴⁹	30,000,000
Kemin FloraGlo Lutein 20% in safflower oil (Marigold) ⁵⁰	20,000,000
Kale, raw	40,000
Kale, cooked	16,000
Spinach, raw	11,000
Spinach, cooked	7,000
Full-fat Lupin Flour ⁵¹	4,400
Broccoli	2,500
Cos lettuce	2,500
Lupins ⁵²	2,000
Sweetcorn	1,800
Brussel sprouts	1,500
Peas	1,400
Persimmon	800
Green beans	600
Okra	400
Iceberg lettuce	350
Cabbage	300
Carrots	300
Tangerine	250
Celery	200
Orange	200
Tomato	150
Orange juice	100
Papaya	75
Green peppers	75
Peaches	60
Egg	55
Cantaloupe melon	40
Watermelon	20
Grapefruit	13

Appendix 4. Food combinations ≥ 6 mg (lutein + zeaxanthin) per day

Food combination 1	
4 slices Lupin Bread	1.4
40 g Baby Spinach (eg, in a mixed salad)	4.4
1 X 50 g egg (boiled)	0.2
Total Lutein (+ Zeaxanthin)	6.0
Food Combination 2	
1 Slice Potato and Spinach Frittata	2.4
2 x lupin bread	0.7
Garden Salad with Kale (10 g) and Spinach leaves (20 g)	6.2
Total Lutein (+ Zeaxanthin)	9.3
Food Combination 3	
1 Serve Kale and Pesto Pasta	10.0
1 Slice Lupin Bread	0.7
Garden Salad with Kale (10 g) and Spinach leaves (20 g)	6.2
Total Lutein (+ Zeaxanthin)	16.9
Food Combination 4	
Kelloggs Corn Flakes (45 g)	0.2
4 slices Lupin Bread	1.4
Avocado (30 g), Iceberg Lettuce (20 g), Sundried Tomato (30 g)	0.6
Scrambled Eggs + Parsley (16 g)	1.2
Vege Stirfry with Corn (30 g), Peas (30 g), Beans (30 g), Broccoli (40 g), Carrot (30 g) and coriander (15 g)	2.4
Pistachio Nuts (unsalted) (30 g)	0.4
Total Lutein (+ Zeaxanthin)	6.0

Foods used for dietary modelling	Lutein content ⁵³ (mg)	Amount
Lupin Bread (made with 40% lupin flour)	0.7	2 slices
Spinach	11	100 g
Egg	0.17	1 egg (50 g)
Kale	40	100 g
Corn	1.8	100 g
Peas	1.35	100 g
Broccoli	2.2	100 g
Pistachio Nuts	1.2	100 g
Parsley	5.5	100 g
Beans (Green)	0.71	100 g
Coriander	0.7	100 g
Sundried Tomato	1.4	100 g
Carrot	0.67	100 g
Iceberg Lettuce	0.28	100 g
Avocado	0.27	100 g
Kelloggs Corn Flakes	0.34	100 g
Meals		
Potato and Spinach Frittata ⁵⁴	2.37	per serve
Kale and Pesto Pasta ⁵⁵	10	per serve
Scrambled Eggs	0.28	per serve (2 eggs)

LITERATURE CITED

- ¹Van Newkirk, M., M. Nanjan, J. Wang, P. Mitchell, M. Taylor and C. McCarty. 2000. The prevalence of age-related maculopathy: the visual impairment project in Ophthalmology 107: 1593-1600.
- ²Centre for Eye Research Australia. 2006. Centrally Focussed The impact of age-related macular degeneration Access Economics.
- ³National Eye Institute
http://www.nei.nih.gov/health/maculardegen/armd_facts.asp (accessed 2008).
- ⁴Kondrot E. 2002. Initial results of microcurrent stimulation in the treatment of age related macular degeneration Townsend Letter for Doctors and Patients, October 2002.
- ⁵National Eye Institute. 2003. Age-related macular degeneration. What you should know. US Department Health and Human Resources.
- ⁶Chopdar, A., U. Chakravarthy and D. Verma. 2003. Age-related macular degeneration. British Medical Journal. 326: 485-488.
- ⁷Centre for Eye Research Australia. 2006. Centrally Focussed The impact of age-related macular degeneration Access Economics.
- ⁸Mitchell, P., H. Taylor, J. Keeffe, H. Vu, J. Wang, E. Rochtchina and L. Pezzullo. 2005. Vision loss in Australia. Medical Journal of Australia 12(11): 565-568.
- ⁹Smith, W. and P. Mitchell. 1998. Family history and age-related maculopathy: The Blue Mountains eye study Australia and New Zealand Journal of Ophthalmology August 26(3): 203-206.
- ¹⁰Mares, J.A., T.L. LaRowe, D.M. Snodderly, S.M. Moeller, M.J. Gruber, M.L. Klein, B.R. Wooten, E.J. Johnson and R.J. Chappell. 2006. Predictors of optical density of lutein and zeaxanthin in retinas of older women in the Carotenoids in Age-Related Eye Disease Study, an ancillary study of the Women's Health Initiative. American Journal of Clinical Nutrition 84(5): 1107-1122.
- ¹¹National Eye Institute. 2003. Age-related macular degeneration What you should know US Department Health and Human Resources.
- ¹²National Eye Institute. 2003. Age-related macular degeneration What you should know US Department Health and Human Resources.
- ¹³Wei Wang, S.L. Connor, E.J. Johnson, M.L. Klein, S. Hughes and W.E. Connor. 2007. American Journal of Clinical Nutrition 85(3): 762-769.
- ¹⁴Junghans, A., H. Sies and W. Stahl. 2001. Macular pigments lutein and zeaxanthin as blue light filters studied in liposomes. Archives of biochemistry and biophysics 15 July; 391(2): 160-4.
- ¹⁵National Eye Institute. 2003. Age-related macular degeneration What you should know US Department Health and Human Resources.
- ¹⁶Chung, H., H. Raemussen and E. Johnson. 2004. Lutein bioavailability is higher from lutein-enriched eggs than from supplements in spinach in men. Journal Nutrition 132: 3668-3673.
- ¹⁷Handelman, G., Z. Nightingale, A. Lichtenstein, E. Schaefer and J. Blumberg. 1999. Lutein and zeaxanthin concentrations in plasma after dietary supplementation with egg yolk. American Journal of Clinical Nutrition, August 70: 2 247-251.
- ¹⁸Bowen P., S. Herbst-Espinosa, E. Hussain and M. Stacewicz-Sapuntzakis. 2002. Esterification does not impair lutein bioavailability in humans Journal Nutrition December 132: 3668-3673.
- ¹⁹Furr, H. and R. Clark. 1997. Intestinal absorption and tissue distribution of carotenoids. J. Nutr. Biochem. 8: 364-377.
- ²⁰Hammond Jr, B., E. Johnson, R. Russell, N. Krinsky, K. Yeum, R. Edwards and D. Snodderly. 1997. Dietary modification of human macular pigment density. Investigative Ophthalmology and Visual Science 38: 1795-1801.
- ²¹Bowen P., S. Herbst-Espinosa, E. Hussain and M. Stacewicz-Sapuntzakis. 2002. Esterification does not impair lutein bioavailability in humans Journal Nutrition December 132: 3668-3673.
- ²²World Health Organisation and United Nations. 2004. Joint Expert Committee on Food Additives report 63rd meeting.
- ²³Shao, A. and J. Hathcock. 2006. Risk assessment for the carotenoids lutein and lycopene. Regul Toxicol Pharmacol August 45(3): 289-298.
- ²⁴Trumbo, P.R. and K.C. Ellwood. 2006. Lutein and zeaxanthin intakes and risk of age-related macular degeneration and cataracts: an evaluation using the Food and Drug Administration's evidence-based review system for health claims. Am. J. Clin. Nutr. November 84(5): 971-4.
- ²⁵<http://www.floraglolutein.com/what-sets-us-apart.cfm> (accessed 2008).
- ²⁶<http://www.cognis.com/products/Business+Units/Nutrition+and+Health/Dietary+Supplements/Xangold/> (accessed 2008).
- ²⁷Trumbo, P.R. and K.C. Ellwood. 2006. Lutein and zeaxanthin intakes and risk of age-related macular degeneration and cataracts: an evaluation using the Food and Drug Administration's evidence-based review system for health claims. Am. J. Clin. Nutr. November 84(5): 971-4.
- ²⁸US Food and Drug Administration. 2005. Qualified Health Claims: Letter of Denial – Xangold® Lutein Esters, Lutein, or Zeaxanthin and Reduced Risk of Age-related Macular Degeneration or Cataract Formation (Docket No. 2004Q-0180) December 19 downloaded from <http://www.cfsan.fda.gov/~dms/qhclutei.html> 07.07.08.
- ²⁹Subiago, A., H. Wakaki and N. Morita. 1999. Stability of Lutein and its myristate esters. Biosci. Biotechnol, Biochem 63: 10 1784-1786.
- ³⁰Trumbo, P.R. and K.C. Ellwood. 2006. Lutein and zeaxanthin intakes and risk of age-related macular degeneration and cataracts: an evaluation using the Food and Drug Administration's evidence-based review system for health claims. Am. J. Clin. Nutr. November 84(5): 971-4.

- ³¹Royal New Zealand Institute for the blind (visited 2008) <http://www.rnzfb.org.nz/learnaboutblindness/eyecondition/s/amd/amslrpdf>.
- ³²Mares, J.A., T.L. LaRowe, D.M. Snodderly, S.M. Moeller, M.J. Gruber, M.L. Klein, B.R. Wooten, E.J. Johnson and R.J. Chappell. 2006. Predictors of optical density of lutein and zeaxanthin in retinas of older women in the Carotenoids in Age-Related Eye Disease Study, an ancillary study of the Women's Health Initiative. *American Journal of Clinical Nutrition* 84(5): 1107-1122.
- ³³Khachik, F., F.F. de Moura, E.Y. Chew, L.W. Douglass, F.L. Ferris III, J.H. Kim and D.J.S. Thompson. 2006. The effect of lutein and zeaxanthin supplementation on metabolites of these carotenoids in the serum of persons aged 60 or older. *Investigative Ophthalmology and Visual Science* 47(12): 5234-5242.
- ³⁴Wei Wang, S.L. Connor, E.J. Johnson, M.L. Klein, S. Hughes and W.E. Connor. 2007. *American Journal of Clinical Nutrition* 85(3): 762-769.
- ³⁵SanGiovanni, J., E. Chew, T. Clemons, F. Ferris, G. Gensler, A. Lindblad, R. Milton, J. Seddon and R. Sperduto. 2007. The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS Report No. 22. *Archives of Ophthalmology (US)* September 125(9): 1225-1232.
- ³⁶Robman, L., H. Vu, A. Hodge, G. Tikellis, P. Dimitrov, C. McCarty and R. Guymer. 2007. Dietary lutein, zeaxanthin, and fats and the progression of age-related macular degeneration. *Canadian Journal of Ophthalmology* October 42(5): 720-726.
- ³⁷Parisi, V., M. Tedeschi, G. Gallinaro, M. Varano, S. Saviano and S. Piermarocchi. 2008. Carotenoids and antioxidants in age-related maculopathy italian study: multifocal electroretinogram modifications after 1 year. *Ophthalmology (US)* February 115(2): 24-333.
- ³⁸Bartlett, H. and F. Eperjesi. 2008. A randomised controlled trial investigating the effect of lutein and antioxidant dietary supplementation on visual function in healthy eyes. *Clinical Nutrition (Edinburgh, Scotland)*. April 27(2): 218-227.
- ³⁹Rosenthal, J.M., J.H. Kim, F. de Monastario, D.J.S. Thompson, R.A. Bone, J.T. Landrum, F.F. de Moura, F. Khachik, H.P. hen, R.L. Schleicher, F.L. Ferris III and E.Y. Chew. 2006. Dose-ranging study of lutein supplementation in persons aged 60 years or older *Investigative Ophthalmology and Visual Science* 47(12): 5227-5233.
- ⁴⁰Wenzel, A.J., J.P. Sheehan, J.D. Burke, M.G. Lefsrud and J. Curran-Celentano. 2007. Dietary intake and serum concentrations of lutein and zeaxanthin, but not macular pigment optical density, are related in spouses. *Nutrition Research* 27(8): 462-469.
- ⁴¹Bone, R.A., J.T. Landrum, Y. Cao, A.N. Howard and F. Alvarez-Calderon. 2007. Macular pigment response to a supplement containing meso-zeaxanthin, lutein and zeaxanthin. *Nutrition and Metabolism* 4 (12) May.
- ⁴²Aleman, T.S., A.V. Cideciyan, E.A.M. Windsor, S.B. Schwartz, M. Swider, J.D. Chico, A. Sumaroka, A.Y. Pantelyat, K.G. Duncan, L.M. Gardner, J.M. Emmons, J.D. Steinberg, E.M. Stone and S.G. Jacobson. 2007. Macular pigment and lutein supplementation in ABCA4 - associated retinal degenerations. *Investigative Ophthalmology and Visual Science* 48(3): 1319-1329.
- ⁴³Schalch, W., W. Cohn, F.M. Barker, W. Kopcke, J. Mellerio, A.C. Bird, A.G. Robson, F.F. Fitzke and F.J. van Kuijk. 2007. Xanthophyll accumulation in the human retina during supplementation with lutein or zeaxanthin – the LUXEA (LUtein Xanthophyll Eye Accumulation) study. *Archives of Biochemistry and Biophysics* 458(2): 128-135.
- ⁴⁴Reboul, E., S. Thap, F. Tournaire, M. Andre, C. Juhel, S. Morange, M.J. Amiot, D. Lairon and P. Borel. 2007. Differential effect of dietary antioxidant classes (carotenoids, polyphenols, vitamins C and E) on lutein absorption. *British Journal of Nutrition* 97(3): 440-446.
- ⁴⁵Kopsell, D.A., M.G. Lefsrud, D.E. Kopsell, A.J. Wenzel, C. Gerweck and J. Curran-Celentano. 2006. Spinach cultigen variation for tissue carotenoid concentrations influences human serum carotenoid levels and macular pigment optical density following a 12-week dietary intervention. *Journal of Agricultural and Food Chemistry* 54(21): 7998-8005.
- ⁴⁶Wenzel, A.J., C. Gerweck, D. Barbato, R.J. Nicolosi, G.J. Handelman and J. Curran-Celentano. 2006. A 12-wk egg intervention increases serum zeaxanthin and macular pigment optical density in women. *Journal of Nutrition* 136(10): 2568-2573.
- ⁴⁷Tan, J.S.L., J.J. Wang, V. Flood, E. Rohtchina, W. Smith and P. Mitchell. 2008. Dietary Antioxidants and the Long-term Incidence of Age-Related Macular Degeneration: The Blue Mountains Eye Study *Ophthalmology* 115(2): 334-341.
- ⁴⁸www.nal.usda.gov/fnic/foodcomp/Data/car98/car98.html (accessed 2008).
- ⁴⁹<http://www.cognis.com/products/Business+Units/Nutrition+and+Health/Our+Products/Product+Catalog/> (accessed 2008).
- ⁵⁰http://www.floraglolutein.com/product_forms/KHMKTC-022-000124_20_Safflower_Oil_Sellsheet.pdf (accessed 2008)
- ⁵¹Adelaide University (2006) Certificate of analysis Lupin Flour.
- ⁵²Sweetingham, M. <http://www.agric.wa.gov.au/content/amt/agb/lupins4foodhealth.pdf> (accessed 2008).
- ⁵³USDA nutrient database <http://www.nal.usda.gov/fnic/foodcomp/search/>.
- ⁵⁴Charlton, J. 2005. Potato and Spinach Fritata Australian Good Taste July 59 downloaded from <http://www.taste.com.au/recipes/2439/potato+spinach+fritata> 7.07.08.
- ⁵⁵Kale Pesto Pasta Downloaded from <http://www.vegbox-recipes.co.uk/recipes/kale-recipe-1.php> 7.07.08.