

Differences in Carotenoid Content of Normal Elderly and Alzheimer's Brains

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Background:

- Antioxidants or oxidative stress have been associated with increased risk of several diseases (cancer, heart disease, stroke, Eichholtzer et al 1992)
- Oxidative damage may contribute to age related eye disease (Hammond et al 1995)
- Oxidative stress may contribute to decreased cognition and possibly lead to AD (Berr et al 2000, Morris et al 2002).
- Levels of xanthophylls are proportionally higher in eye (neural tissue) relative to plasma levels (Bone et al 1988).
- Brain xanthophylls increased in response to dietary supplementation in Japanese Quail model (Toyoda et al 2002) and primate.
- Antioxidants and Carotenoids in blood have been associated with cognition (Perrig et al 1997, Wang et al 2008)

Background (cont)

- Levels of individual carotenoids, tocopherols, and retinol were recently reported in elderly human brain sections (Craft et al 2004).
- Matthews-Roth et al 1976 reported total carotenoids in human brain of two subjects receiving high β -carotene. This may be the first demonstration of human brain responding to carotenoids.
- Macular pigment optical density (MPOD) which measures xanthophylls in the eye has been correlated with cognition (presentations at this meeting).

Purpose:

- To identify and quantify the major Carotenoids, Tocopherols, and Retinol in elderly normal and AD human brain.
- To determine if analyte concentrations are different in normal vs AD brain.
- To determine if analyte concentrations are different in White vs Gray Matter.
- To determine if analyte concentrations are different in brain regions more vulnerable to AD.

Study Description

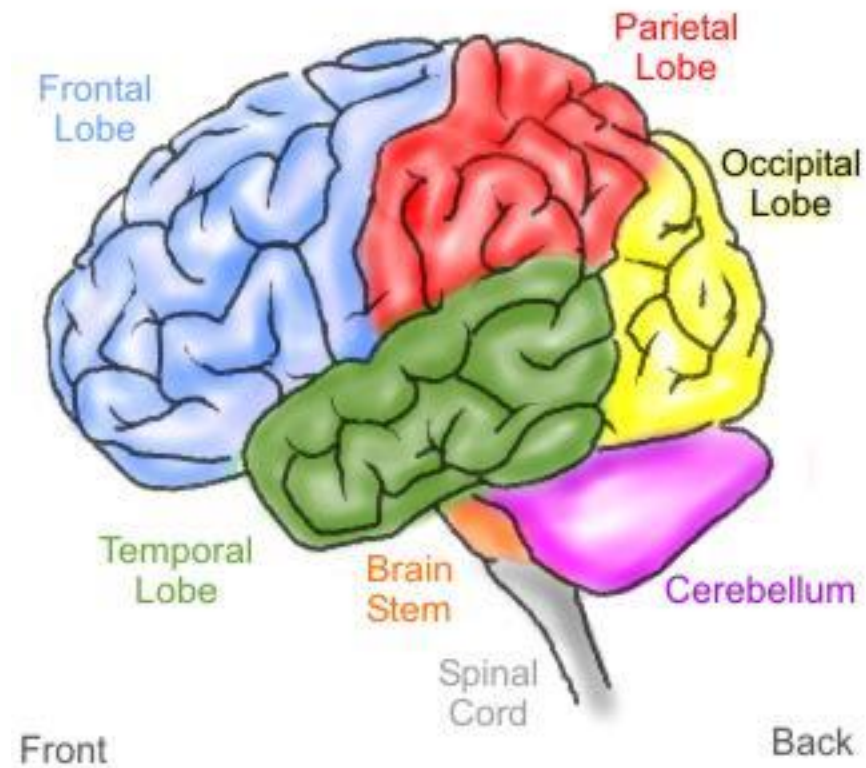
- 28 frozen sections of brains were obtained from the NIH-funded Harvard Brain Tissue Resource Center.
- Brains from 9 donors were diagnosed as AD, 5 donors were non-AD.
- One section from frontal region, representing AD-vulnerable area and one from occipital, representing AD-less vulnerable area.
- Upon partial thawing, sections were dissected into gray and white matter yielding 56 total samples.
- No matching blood samples or data on food consumption were available.

Sample Summary Table

	AD	C
Total Samples	36	20
%Male	67	60
%Female	33	40
Age	75.9	75.2

Brain Diagram

Regions of the Human Brain



Methods:

- Brain sections received frozen from Mass. General Hospital were dissected into gray and white matter.
- 1-3 g of tissue was extracted 3x with 90 hexane/10 ethyl acetate.
- A portion was saponified with KOH for 60 min in the presence of pyrogallol.
- The mix was diluted with water and extracted 3x with hexane/ethyl acetate.

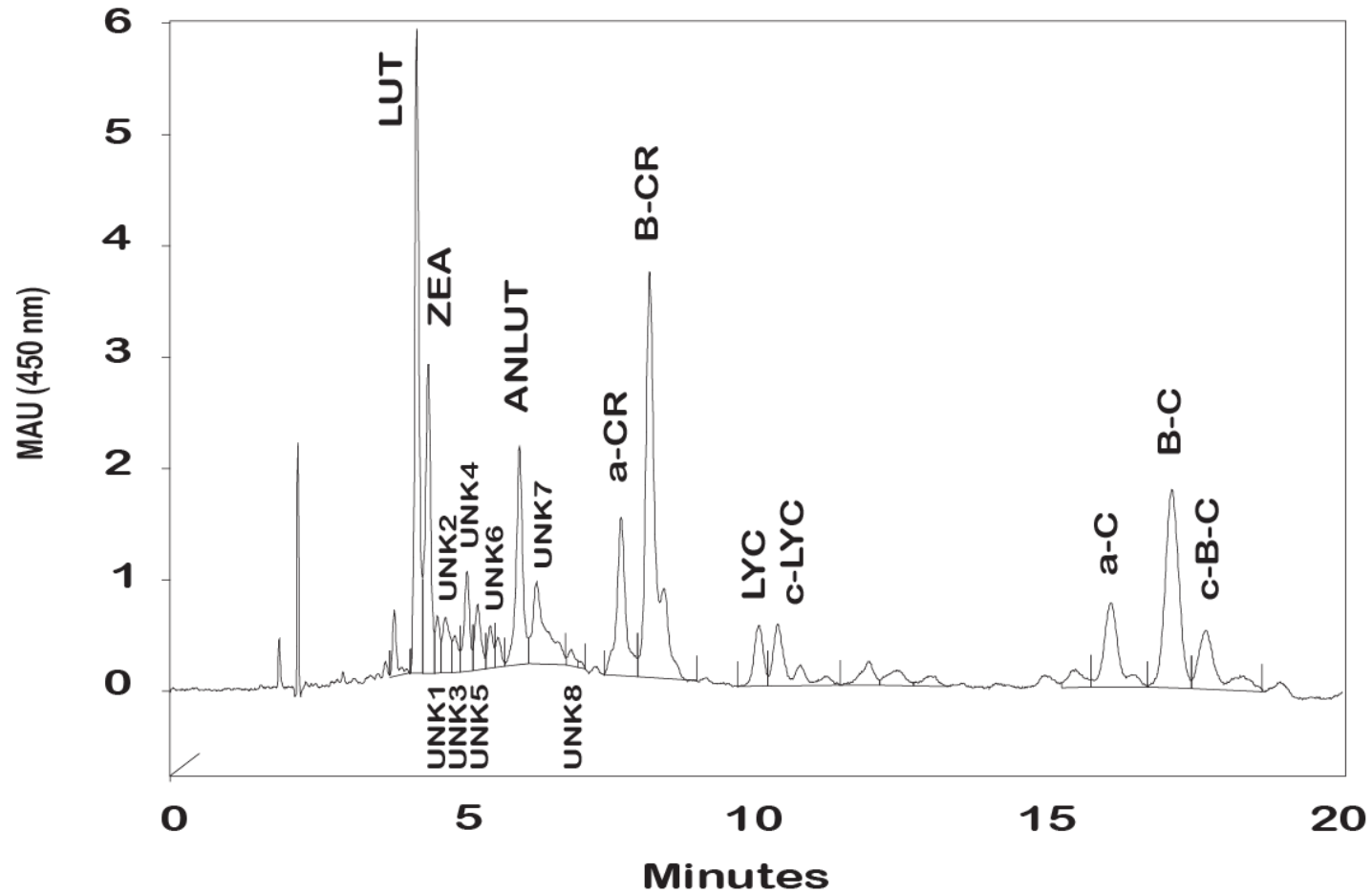
Methods cont.

- The combined extract was washed with water then evaporated under nitrogen.
- The residue was dissolved in 25 uL ethyl acetate and diluted with 75 uL mobile phase and centrifuged prior to injection.
- Data were analysed by ANOVA. P-values <0.05 were considered significant.

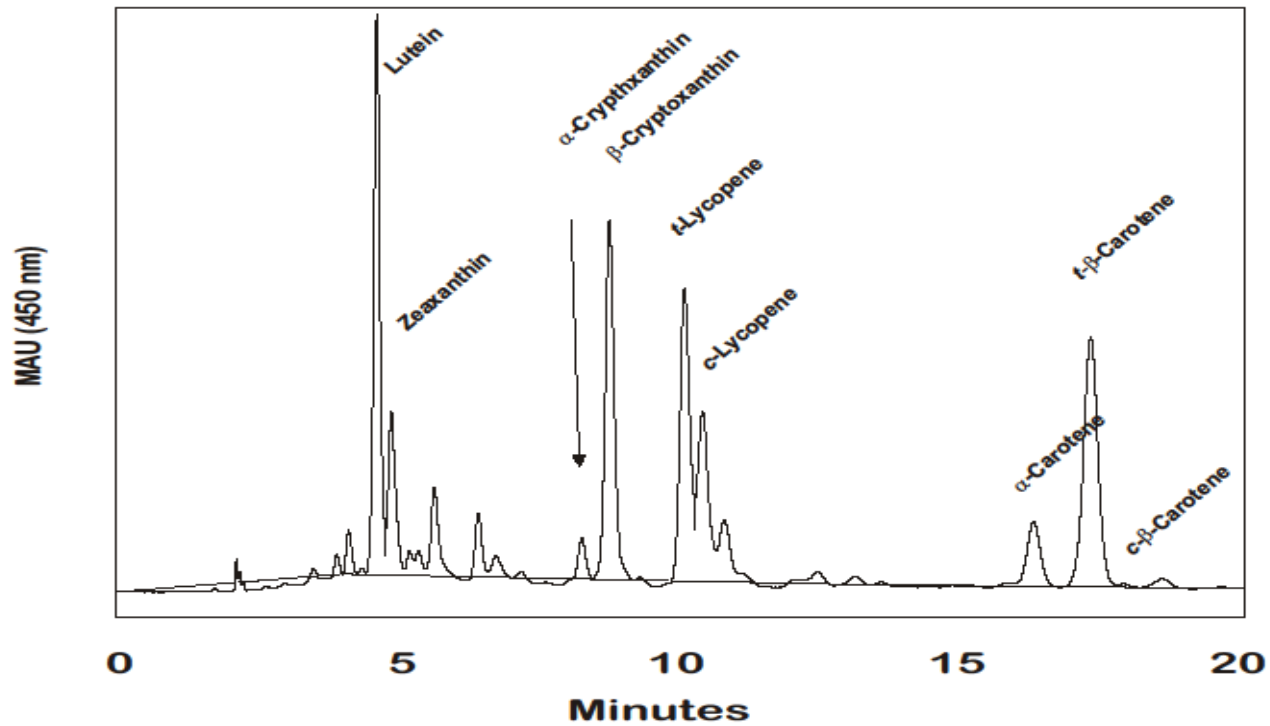
HPLC Conditions:

- Column: Spherisob ODS2, 3um, 250 x 4.0 mm with Ti frits
- Mobile Phase: 80 ACN/15 Dioxane/2.5 MeOH/2.5 IPA/0.1 TEA (150 mM AmOAc in alcohol components)
- Temp: 31°C
- Flow: 1.2 mL/min
- Detect: Visible 450 nm; Fluor 330nm Ex/460nm Em, 296nm Ex/340nm Em
- Calib: Ext. Std. using peak area

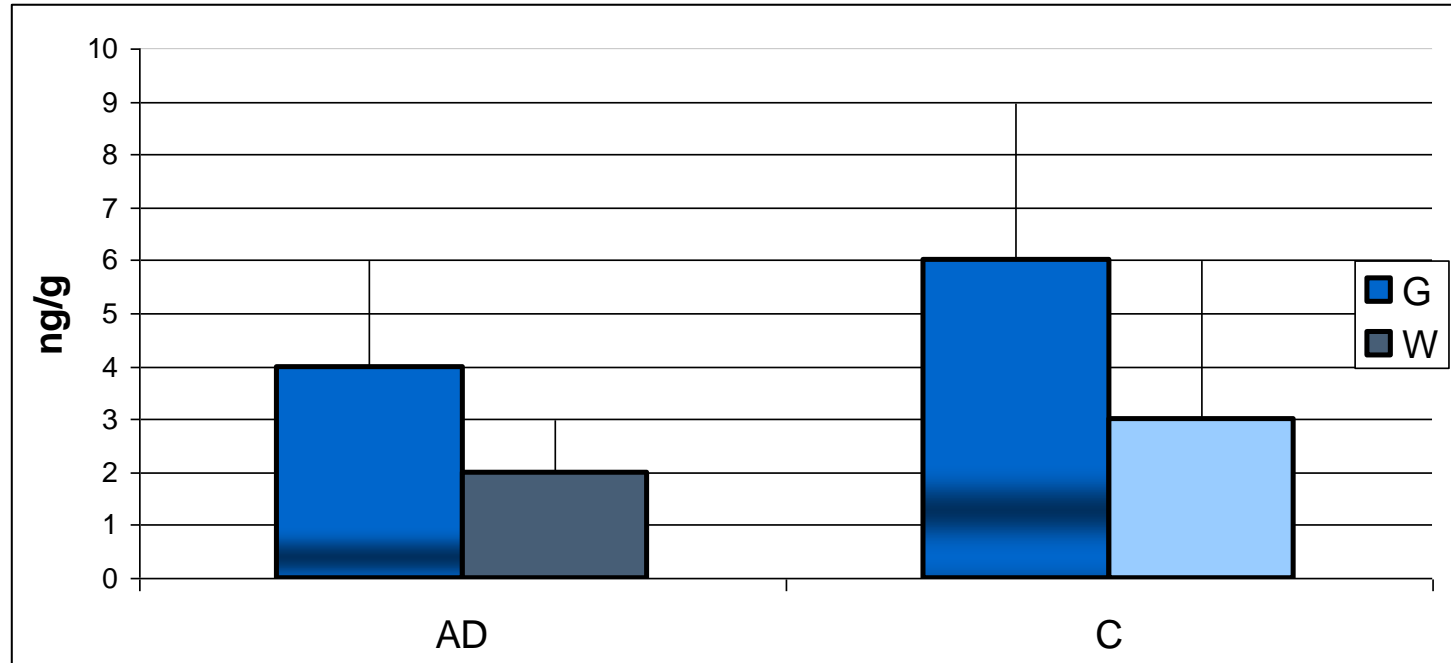
Carotenoids in Human Brain



Serum Carotenoids

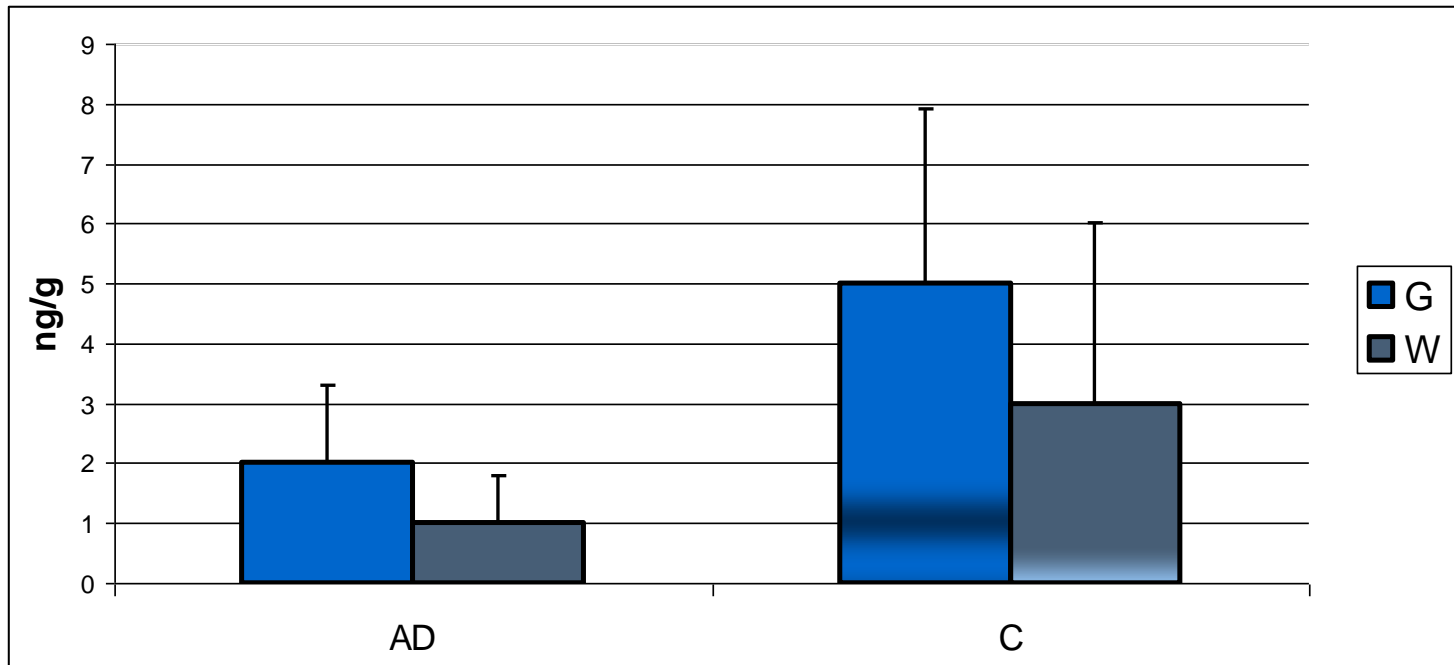


Disease Status*Brain Matter Lutein



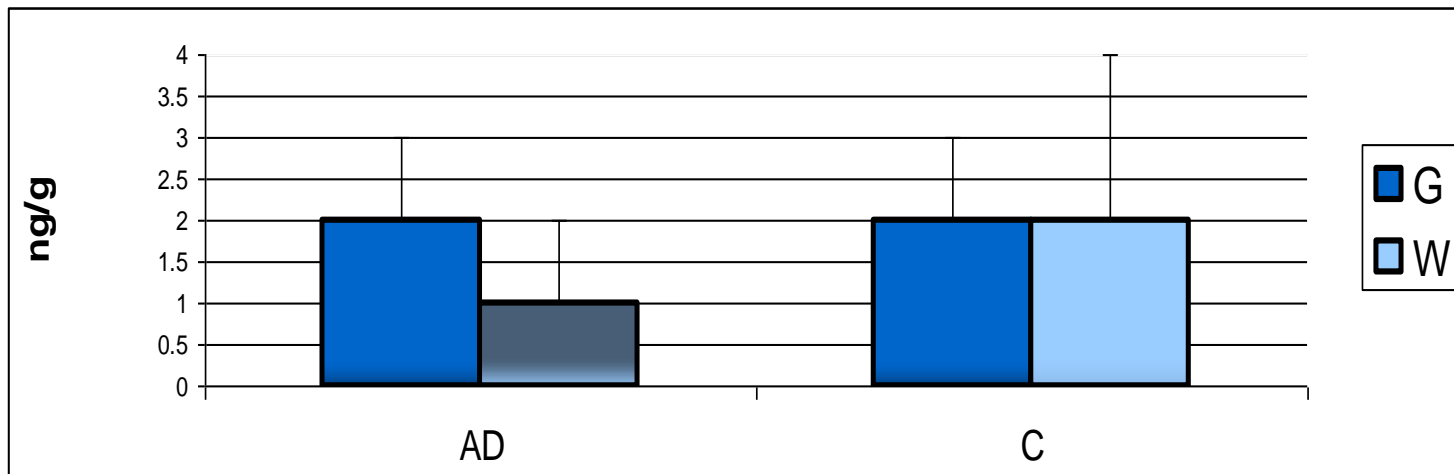
	p-Value
AD, C	0.046
G, W	0.0012

Disease Status*Brain Matter Zeaxanthin



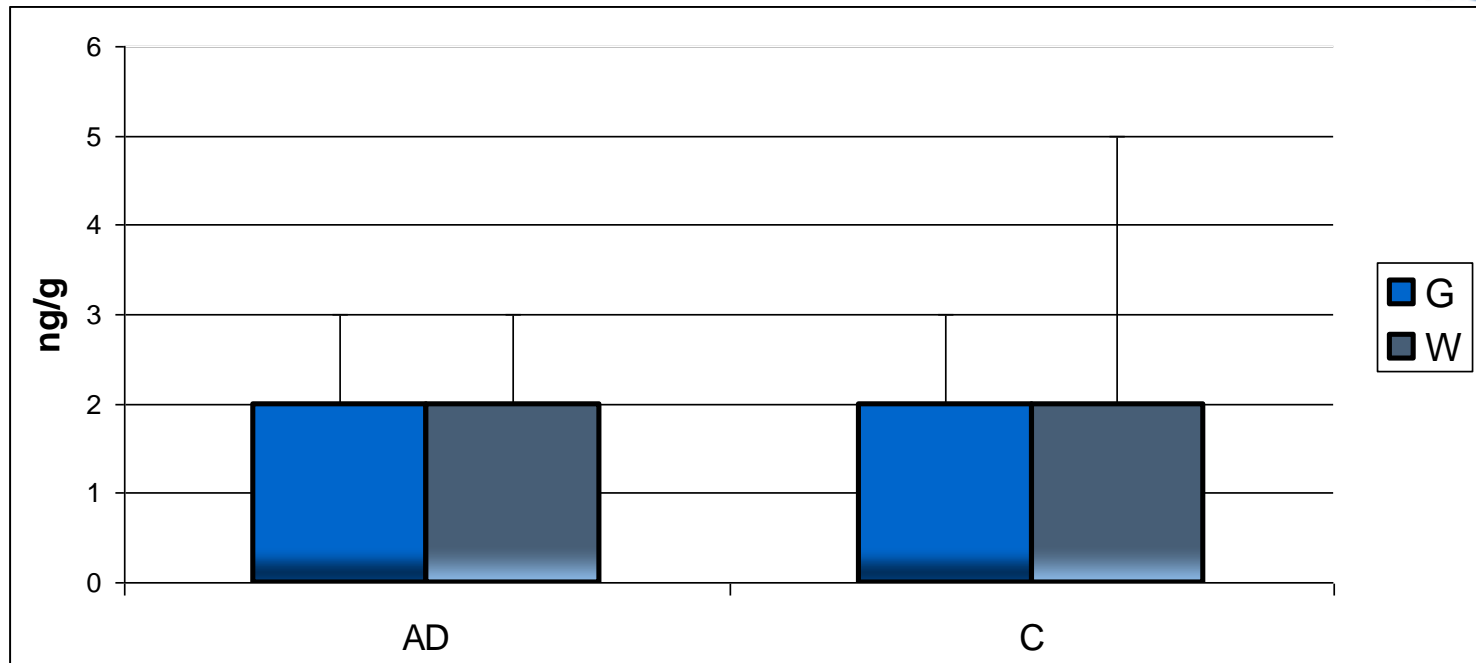
	P-Value
AD, C	0.0014
G, W	0.0096

Disease Status*Brain Matter Anhydrolutein



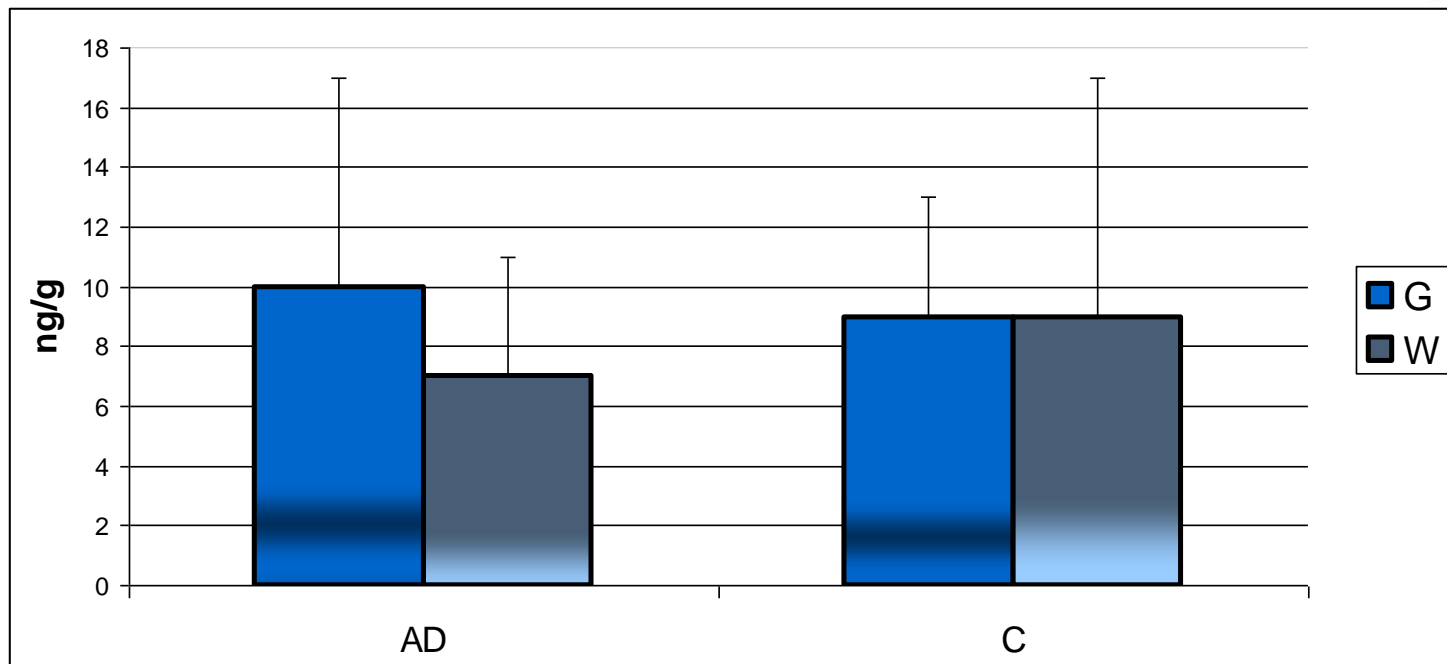
	P-Value
AD, C	0.0568
G, W	0.7737

Disease Status*Brain Matter α Crytoxanthin



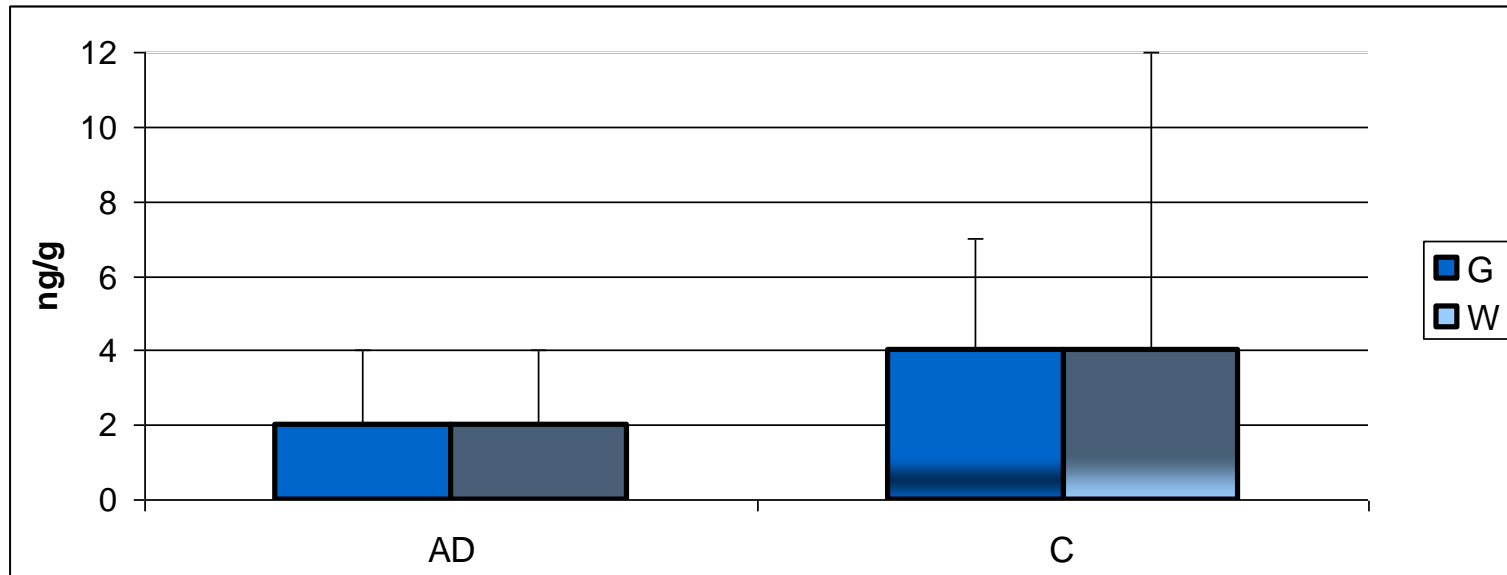
	P-Value
AD, C	0.5047
G, W	0.885

Disease Status*Brain Matter β Cryptoxanthin



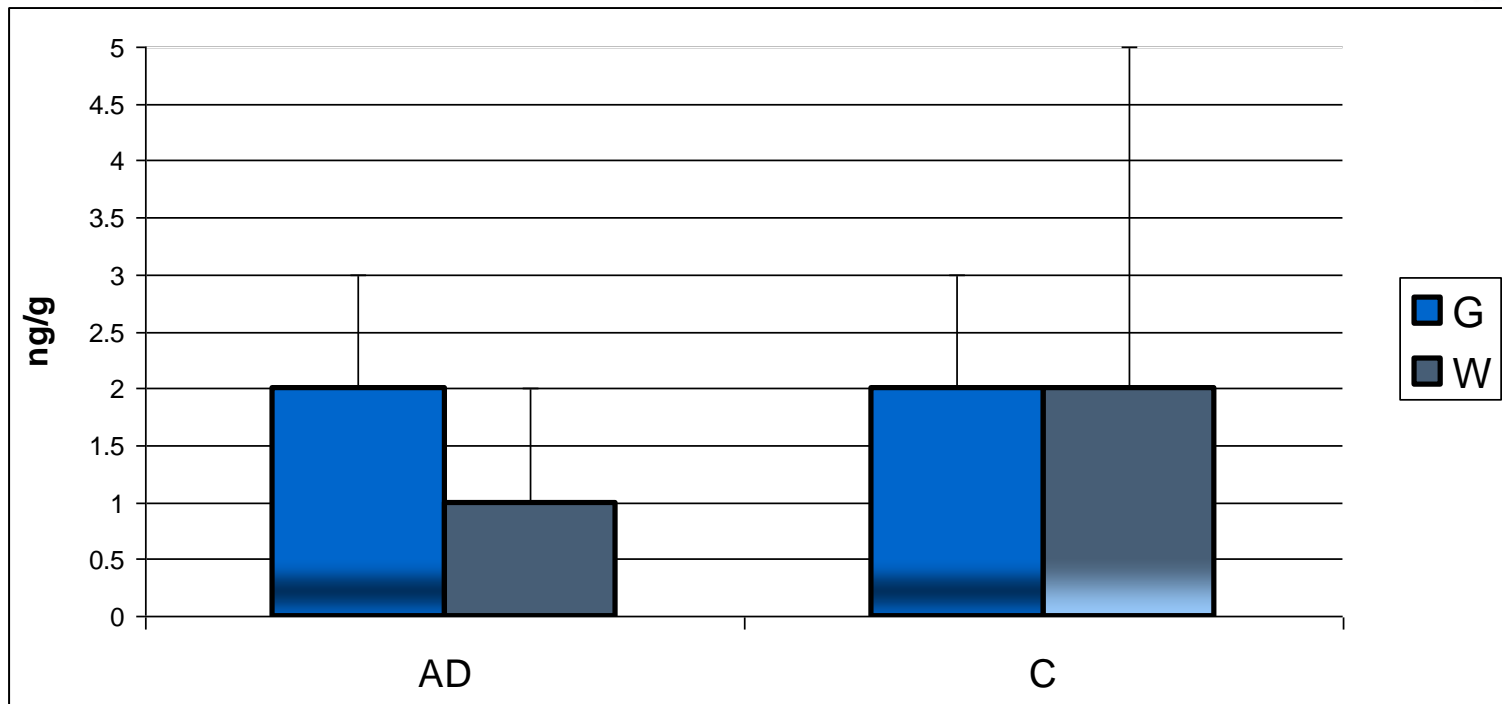
	P-Value
AD, C	0.9418
G, W	0.2317

Disease Status*Brain Matter Lycopene



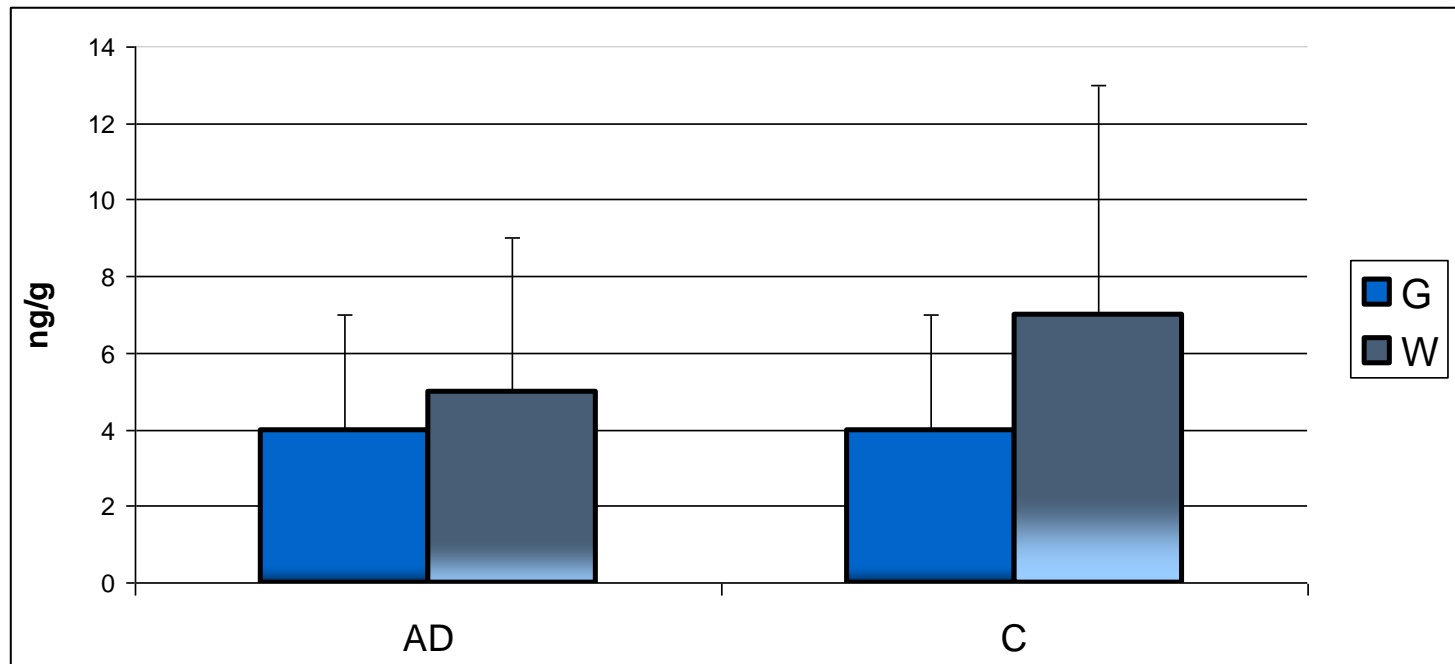
	P-Value
AD, C	0.0567
G, W	0.8643

Disease Status*Brain Matter α Carotene



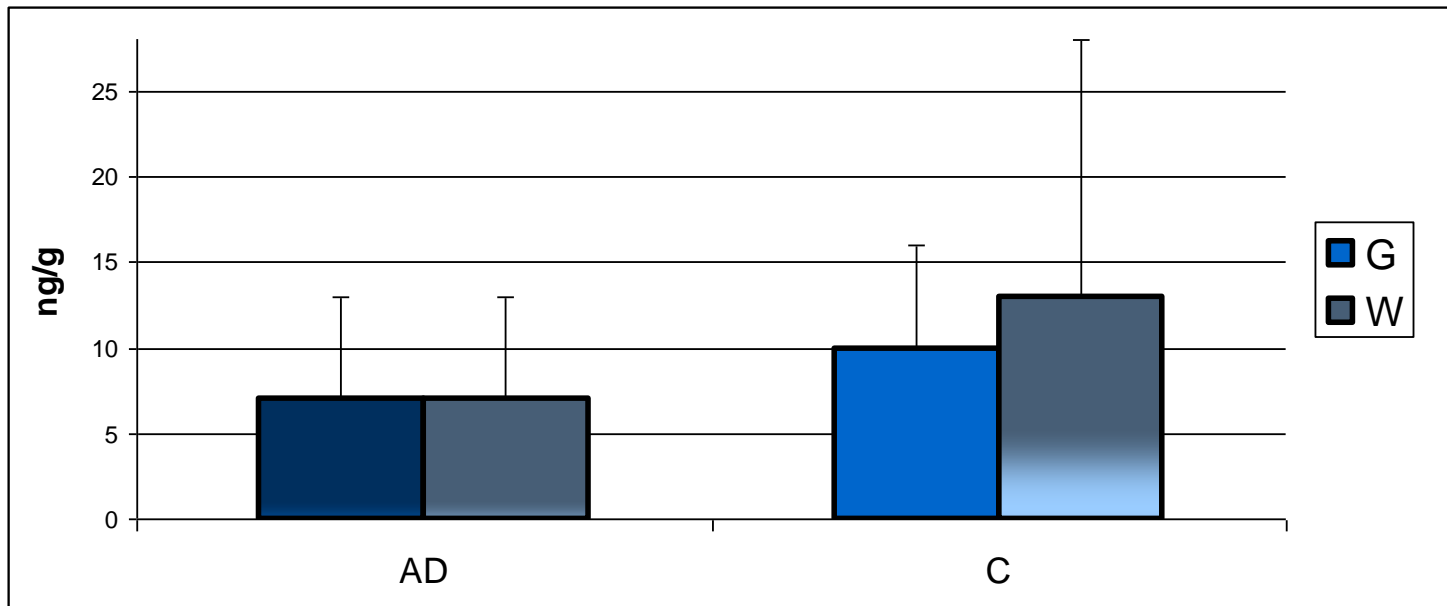
	P-Value
AD, C	0.2617
G, W	0.7326

Disease Status*Brain Matter β Carotene



	P-Value
AD, C	0.2681
G, W	0.1299

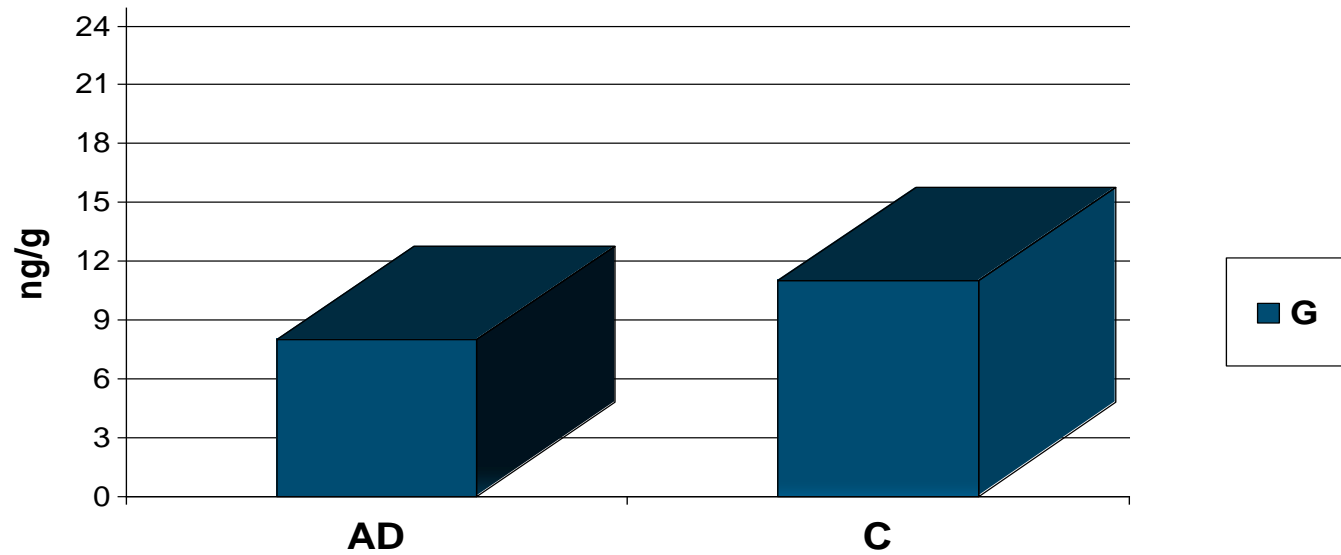
Disease Status*Brain Matter Total Carotenes



	P-Value
AD, C	0.1028
G, W	0.556

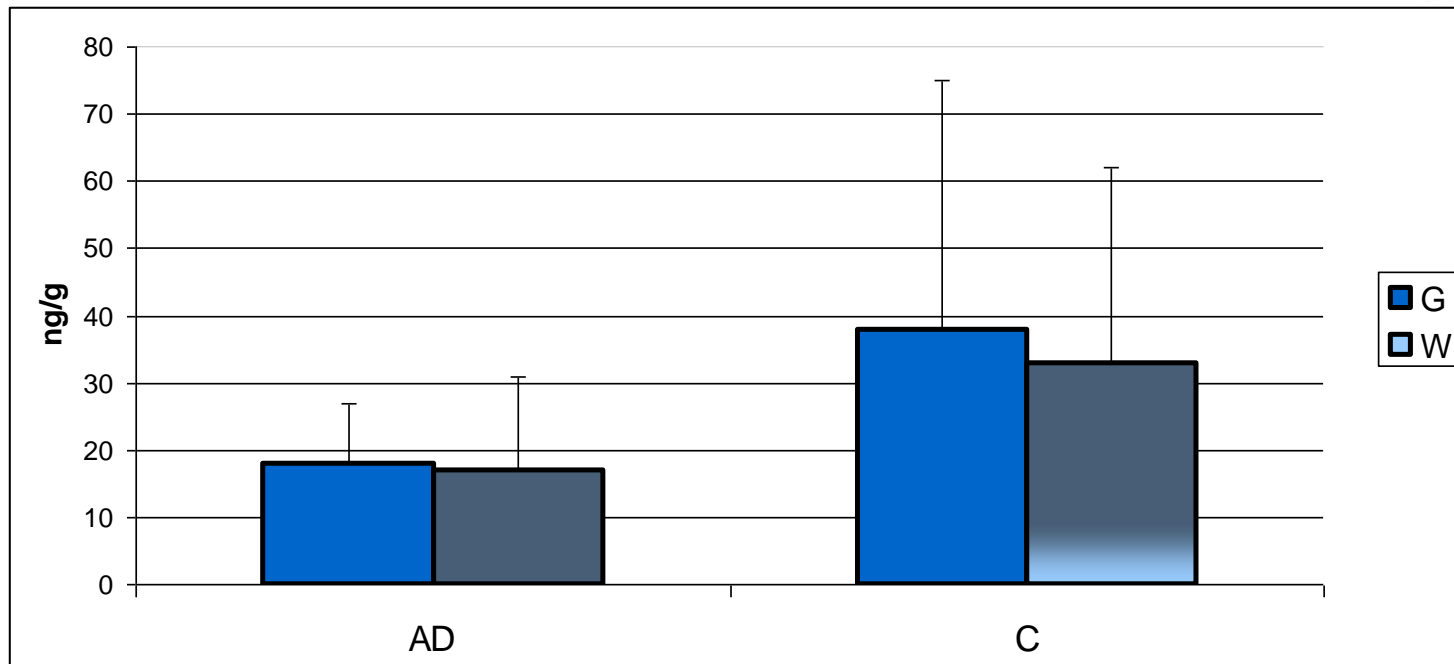
Disease Status

Total Carotenes



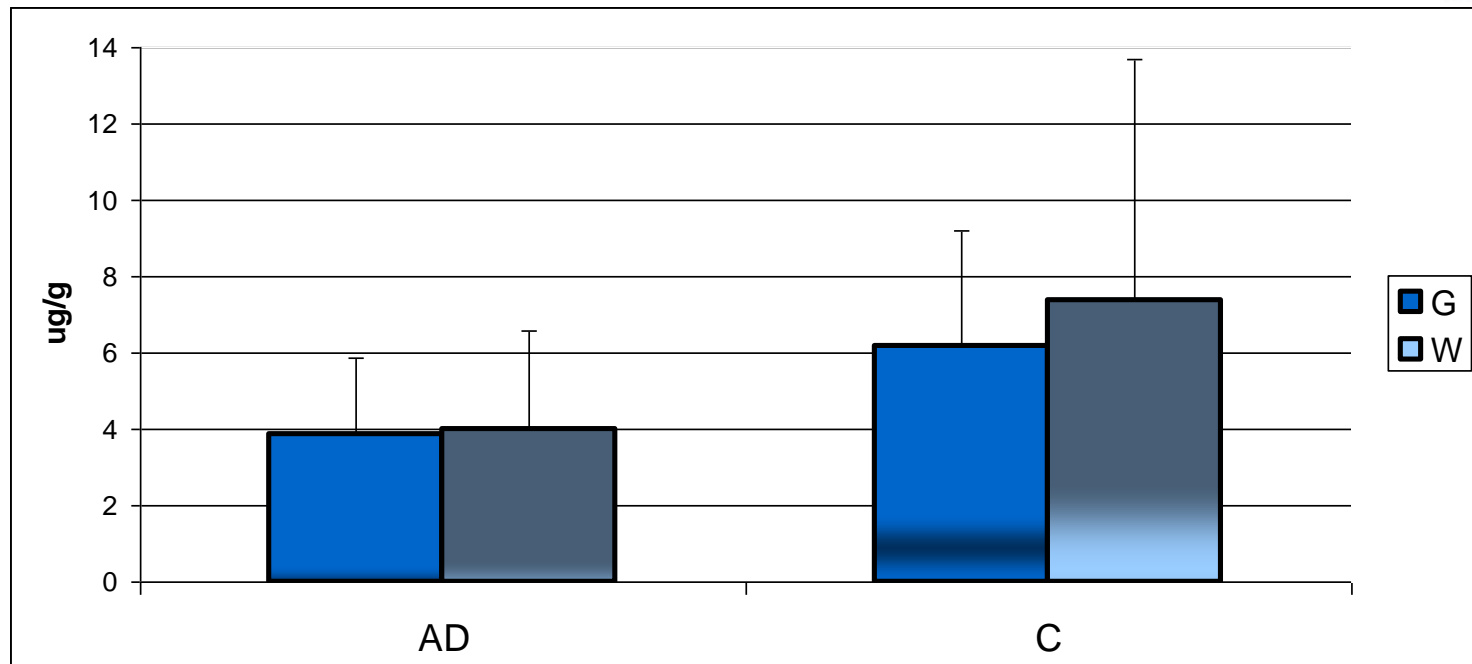
	P-Value
AD, C	0.1993

Disease Status*Brain Matter Retinol



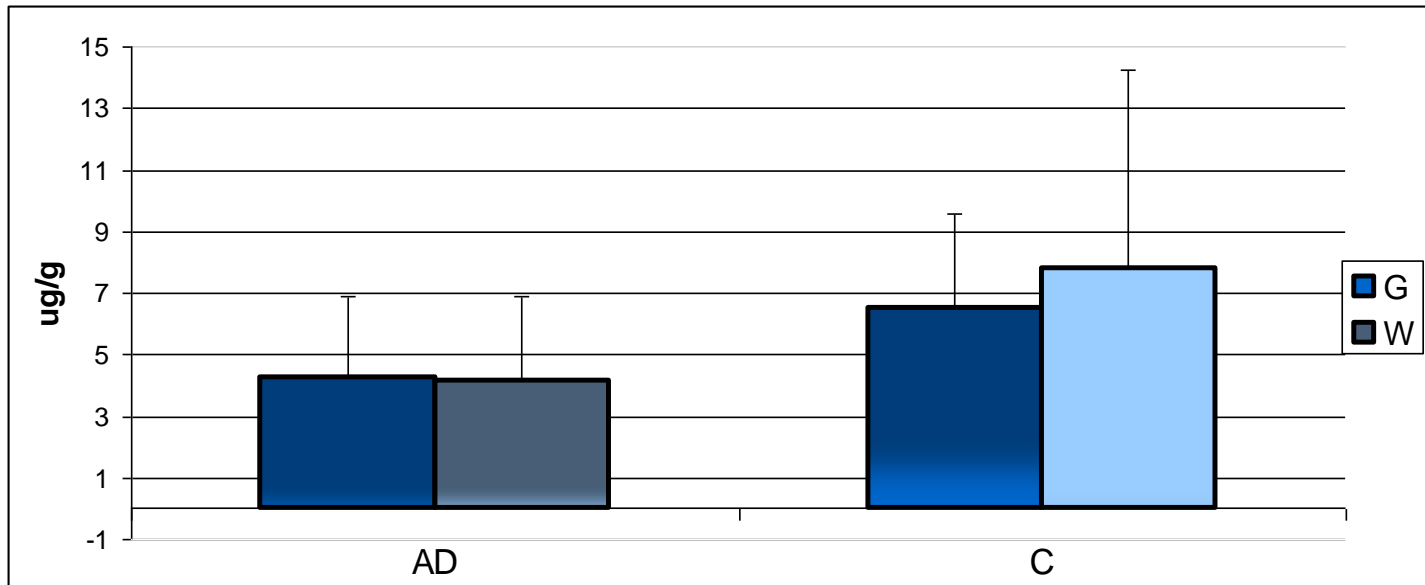
	P-Value
AD, C	0.0053
G, W	0.7103

Disease Status*Brain Matter α Tocopherol



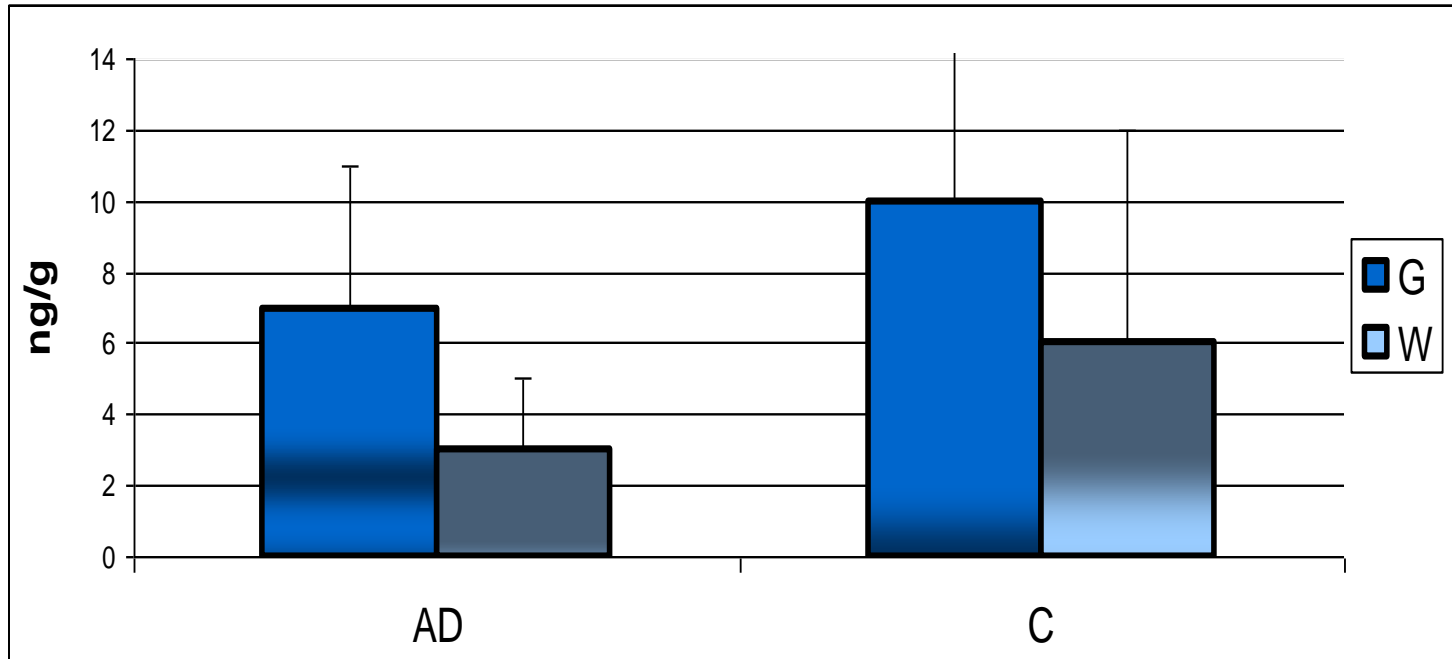
	P-Value
AD, C	0.0045
G, W	0.5905

Disease Status*Brain Matter Total Tocopherols



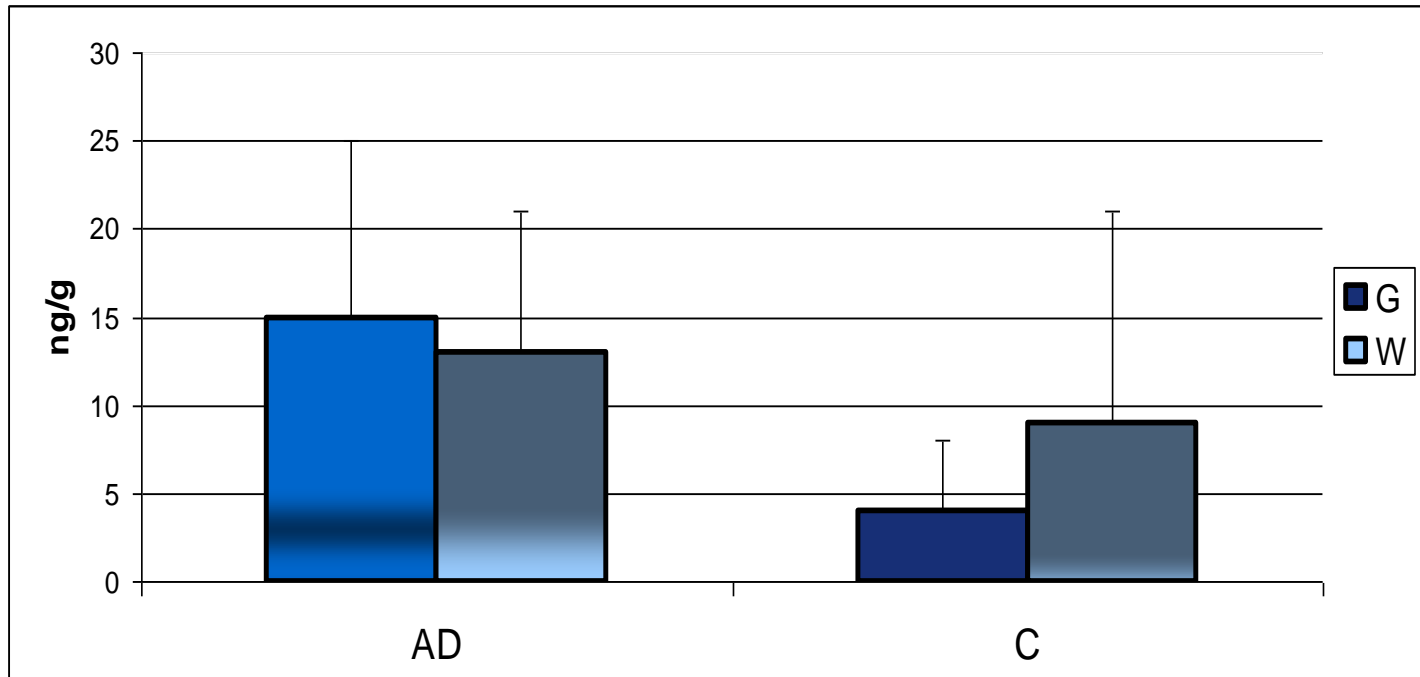
	P-Value
AD, C	0.006
G, W	0.6946

Disease Status*Brain Matter Lutein + Zeaxanthin



	P-Value
AD, C	0.0088
G, W	0.0023

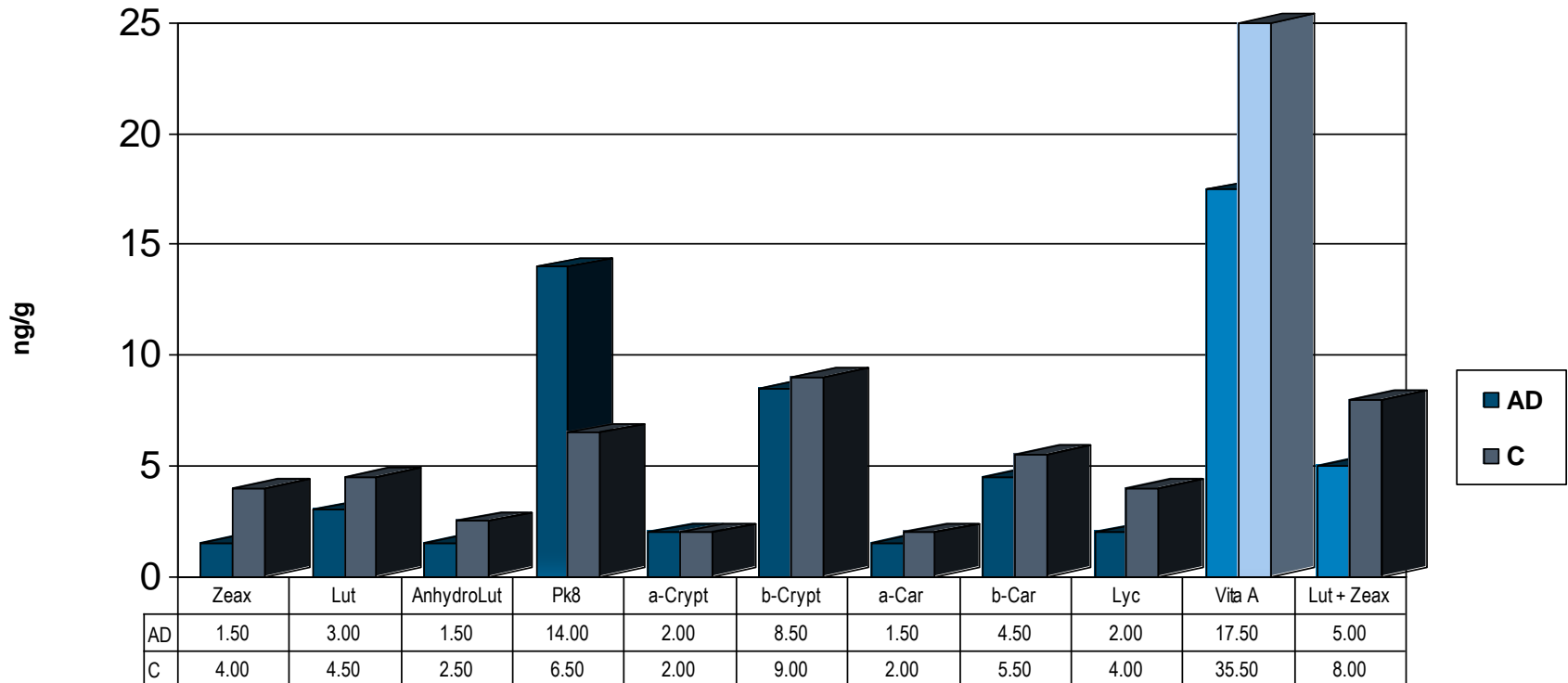
Disease Status*Brain Matter Pk8



	P-Value
AD, C	0.0026
G, W	0.9022

Summary Table

Disease Status



Carotenoids, Retinol, and Tocopherols in Human Brain

AD vs C and Gray vs White

Analyte	ANOVA	
	p-value	
	AD vs C	G vs W
Lutein	0.046	0.0012
Zeaxanthin	0.0014	0.0096
Anhydrolutein	0.0568	0.7737
α -Cryptoxanthin	0.5047	0.885
β -Cryptoxanthin	0.9418	0.2317
Lycopene	0.0567	0.8643
α -Carotene	0.2617	0.7326
β -Carotene	0.2681	0.1299
Total Carotenes	0.1028	0.556
Retinol	0.0053	0.7103
α -Tocopherol	0.0045	0.5905
γ -Tocopherol	0.3743	0.3518
δ -Tocopherol	0.9421	0.2664
Total Tocopherols	0.006	0.6946
Lutein + Zeaxanthin	0.0088	0.0023
Total Antioxidants	0.0061	0.7006

Unknown Peaks in Human Brain

AD vs C and Gray vs White

Analyte	ANOVA	
	P-value	
	AD vs C	G vs W
Peak 1	0.0604	0.2628
Peak 2	0.0259	0.934
Peak 3	0.046	0.8491
Peak 4	0.0048	0.9808
Peak 5	0.1389	0.8587
Peak 6	0.073	0.8239
Peak 7	0.1738	0.196
Peak 8	0.0026	0.9022

Observations:

- Measurable and variable amounts of Carotenoids, Tocopherols, and Retinol occur in Human Brain.
- Lutein, Zeaxanthin, Retinol and α -Tocopherol were significantly lower in AD vs C brain.
- Significant differences occur between gray and white matter.

Observations:

- In AD, white matter was consistently and significantly (for Lut, Zx, and Unk's) lower in analytes.
- The concentration of carotenoids are much lower in the brain (ng/g) than in plasma and other tissues (mg/g).
- Peak 8, which is yet unknown, has a significant positive relationship with AD. This is the reverse of the majority of the other analytes.

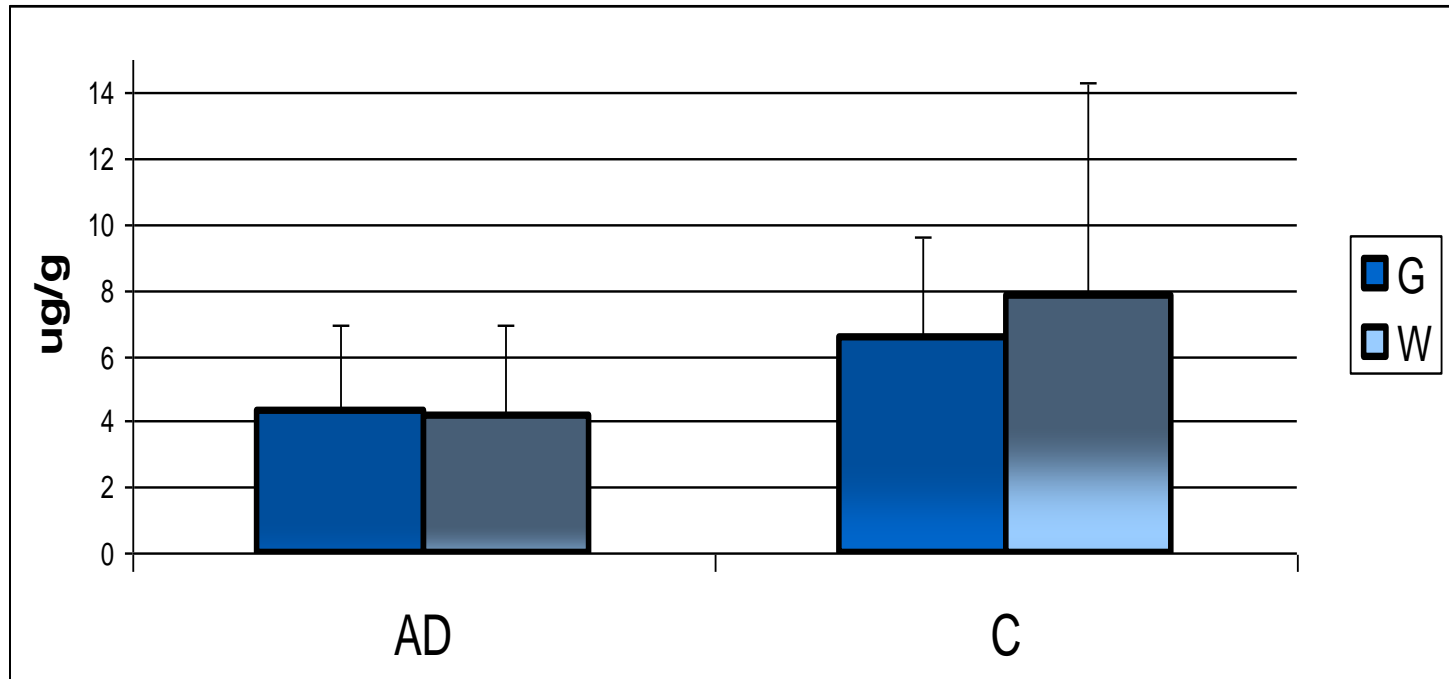
Acknowledgments

- Applied Food Biotechnology, Inc.
- NIH-funded Massachusetts's Alzheimer's Disease Research Center

Acknowledgments

- Thomas B. Haitema
- Kevin M. Garnett, Applied Food Biotechnology
- E. Tessa Hedley-Whyte and Karlotta Fitch, Massachusetts's General Hospital

Disease Status*Brain Matter Total Antioxidants



	P-Value
AD, C	0.0061
G, W	0.7006