Centre des Sciences du Goût et de l'Alimentation

AGRO SUP DIJON

INRA SCIENCE & IMPACT

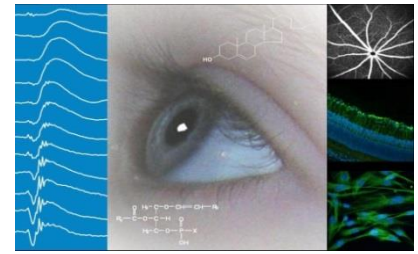
UNIVERSITÉ DE BOURGOGNE

cnrs

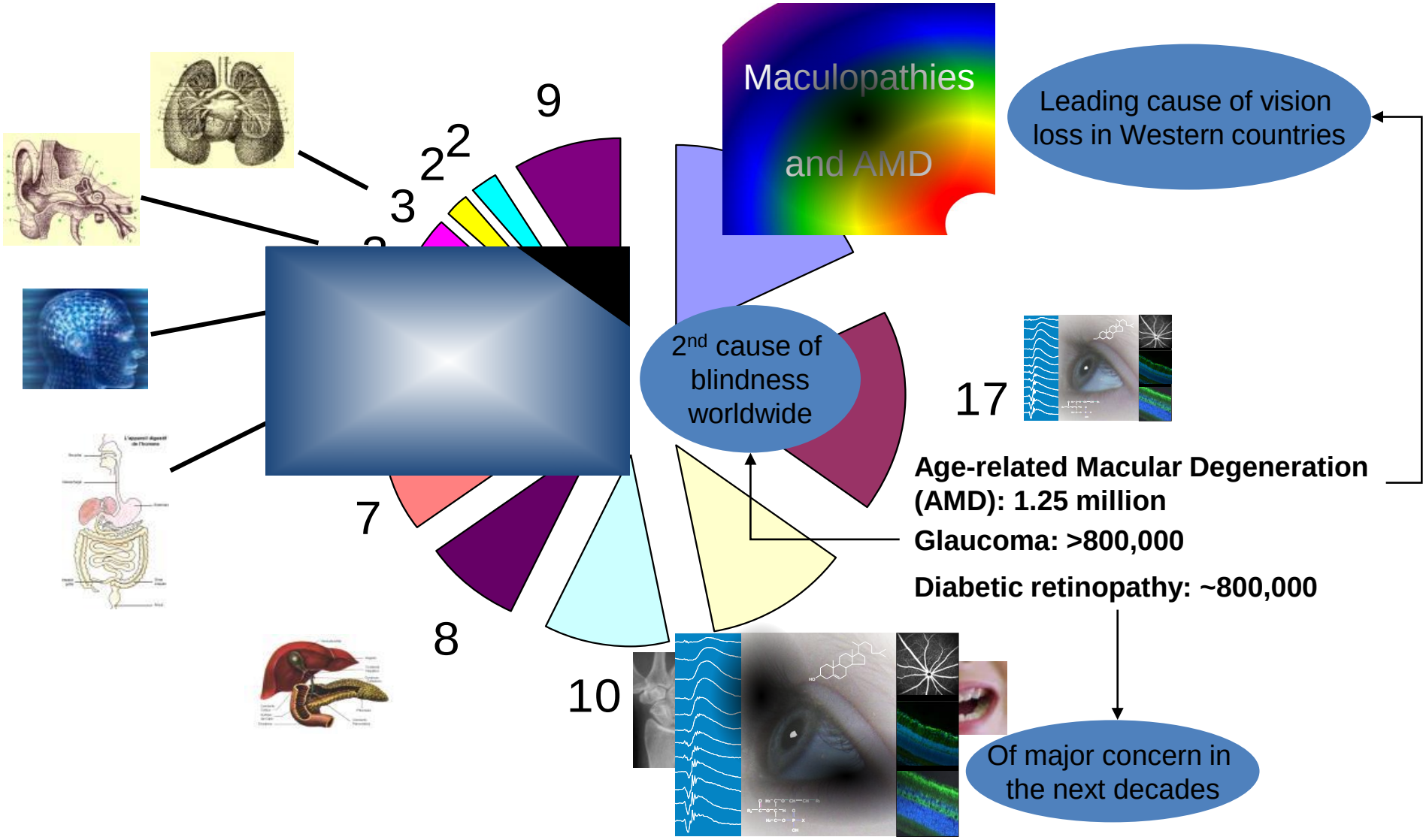
Lipides, santé et vieillissement
DMLA et pathologies oculaires

Lionel BRETILLON

Dijon

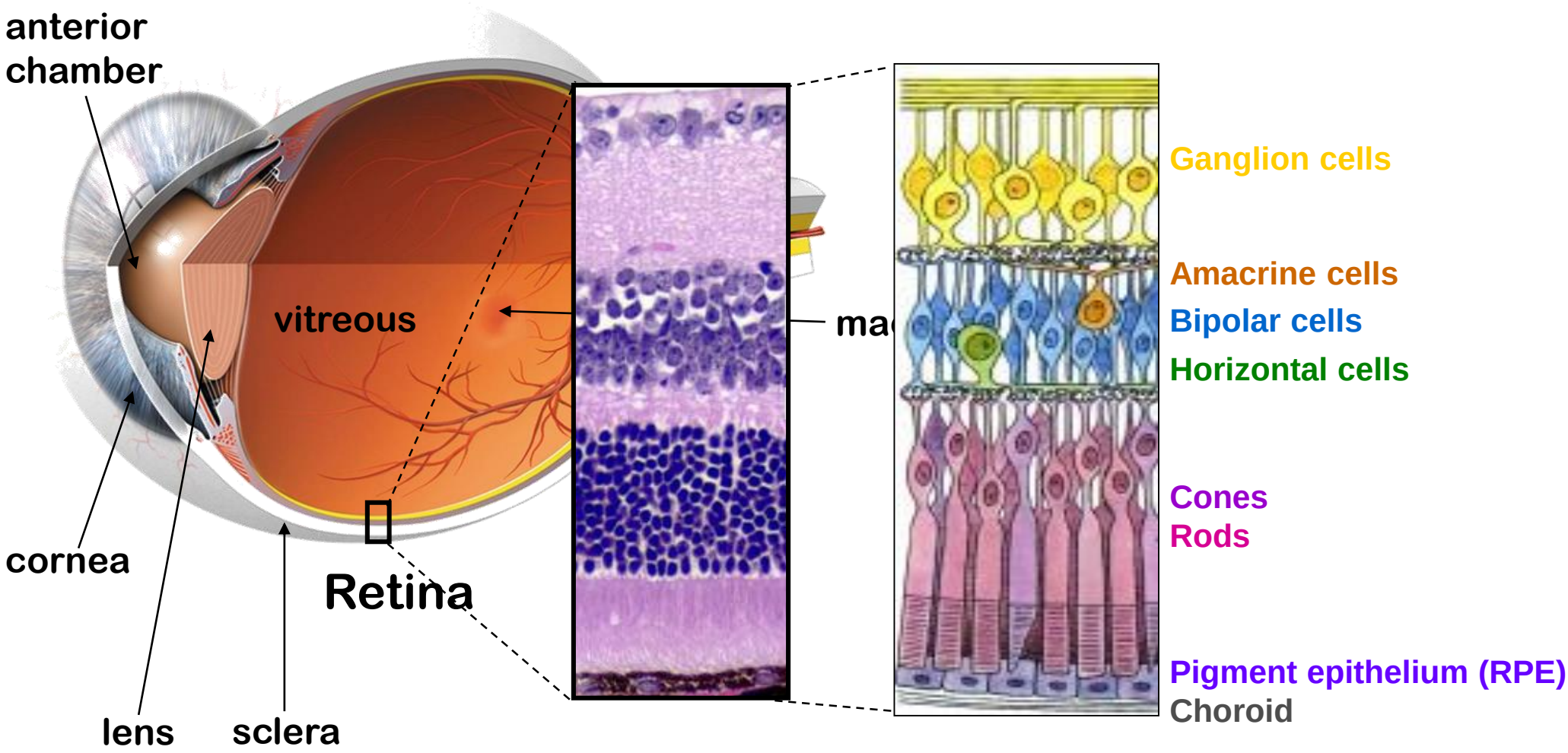


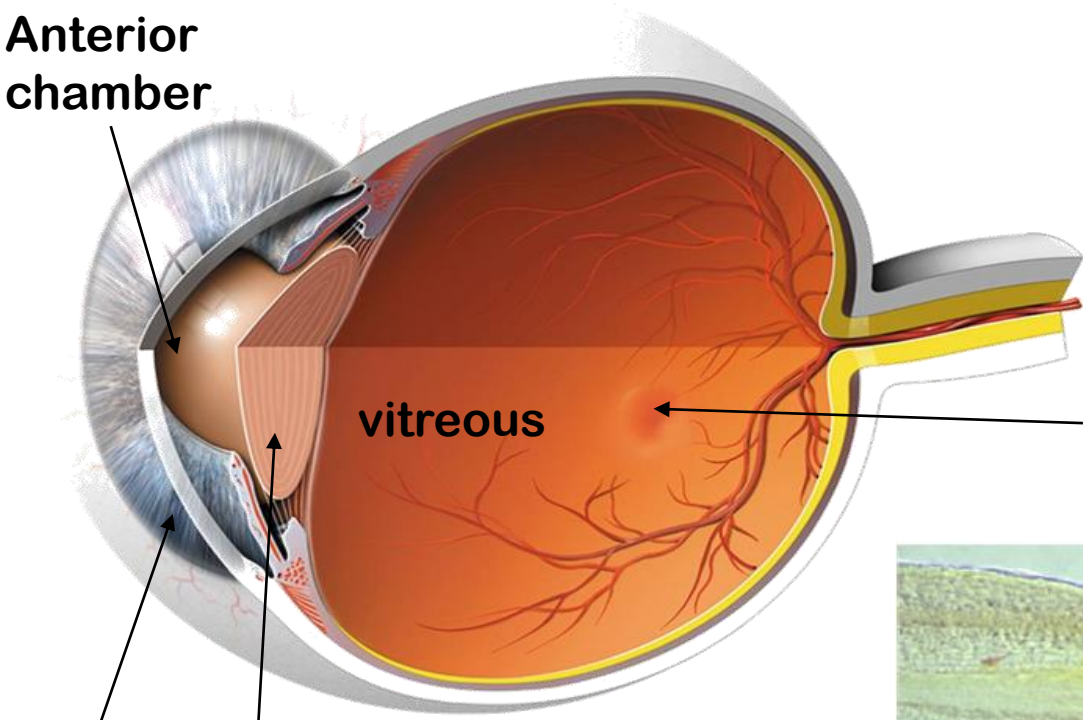
Prevalence of pathologies after the age of 65 years



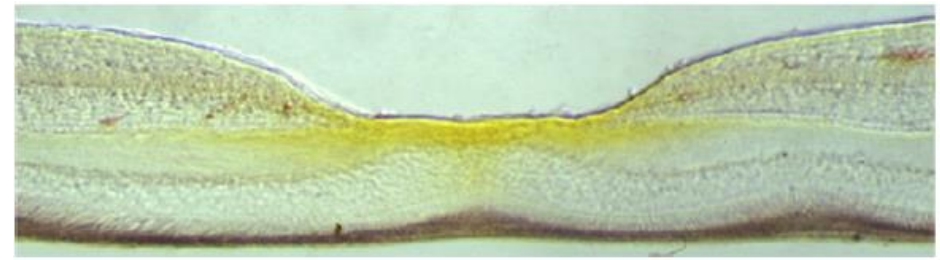
France

Organization of the retina

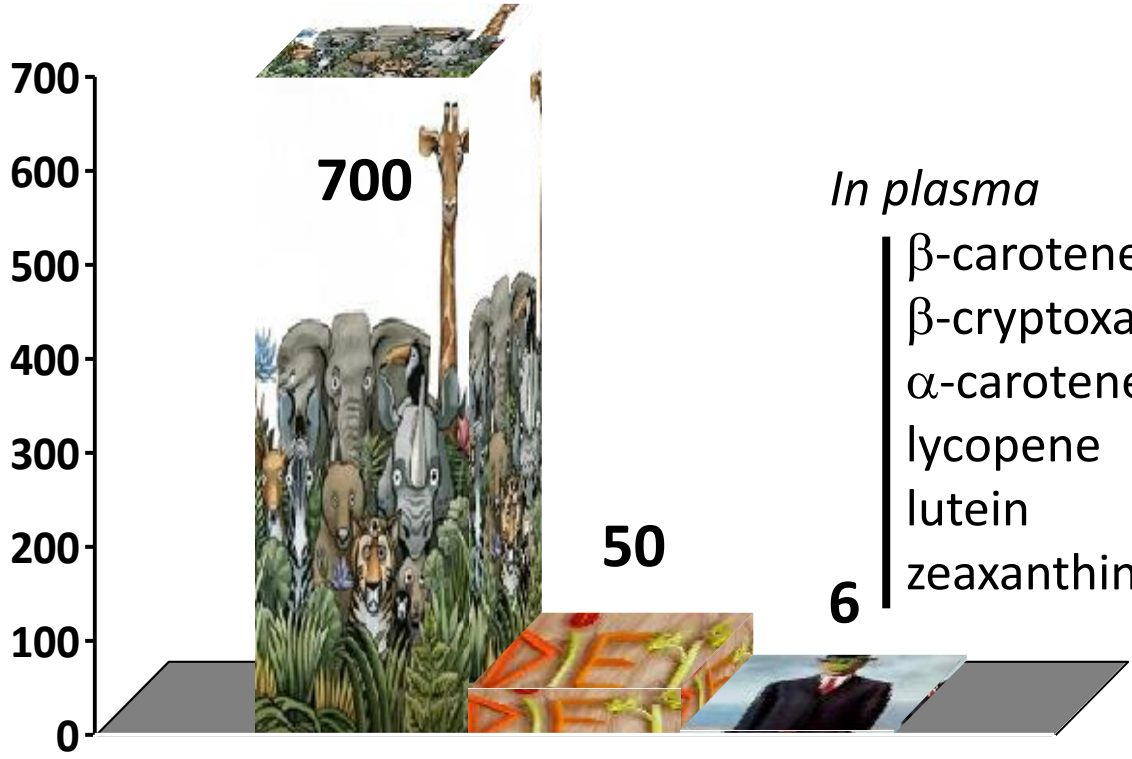




Retina

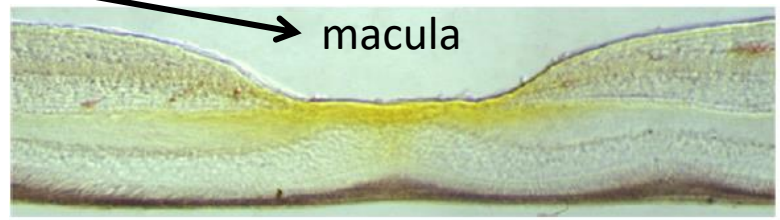


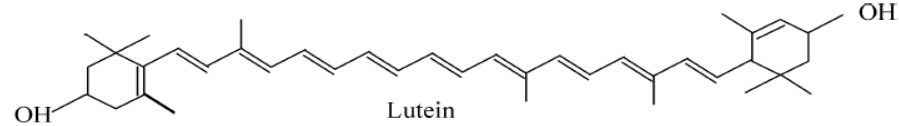
Carotenoids

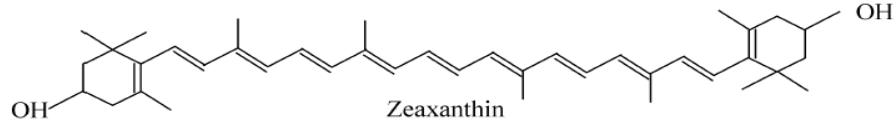


In plasma

- β-carotene
- β-cryptoxanthin
- α-carotene
- lycopene
- lutein
- zeaxanthin

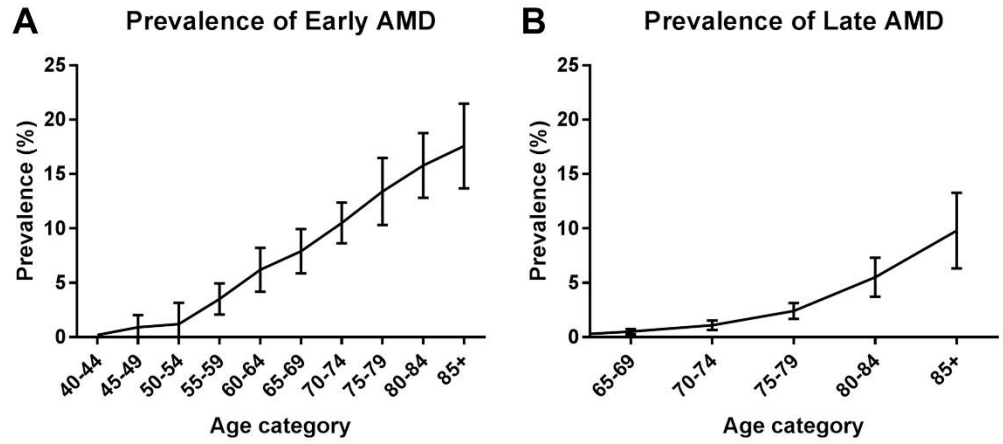


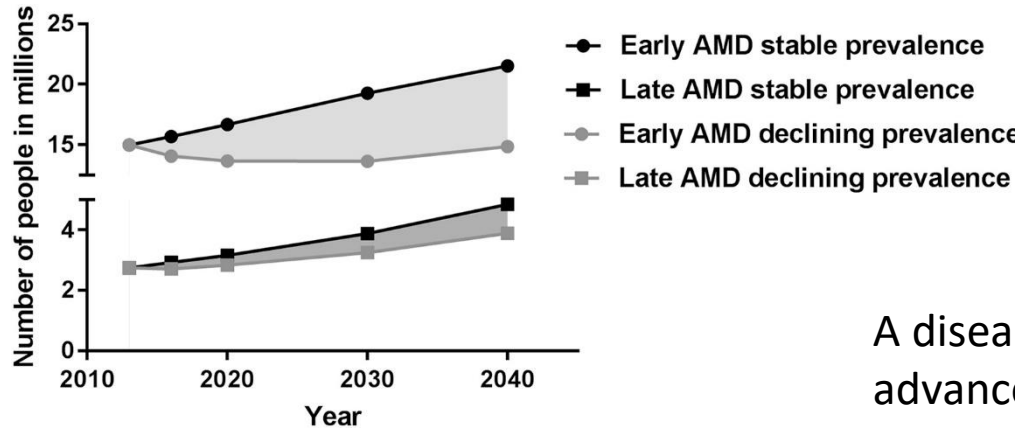




Prevalence and projections of AMD in Europe

Data from the European Eye Epidemiology consortium (n=42080 patients, 10 countries)





A disease which prominent risk factor is advanced age

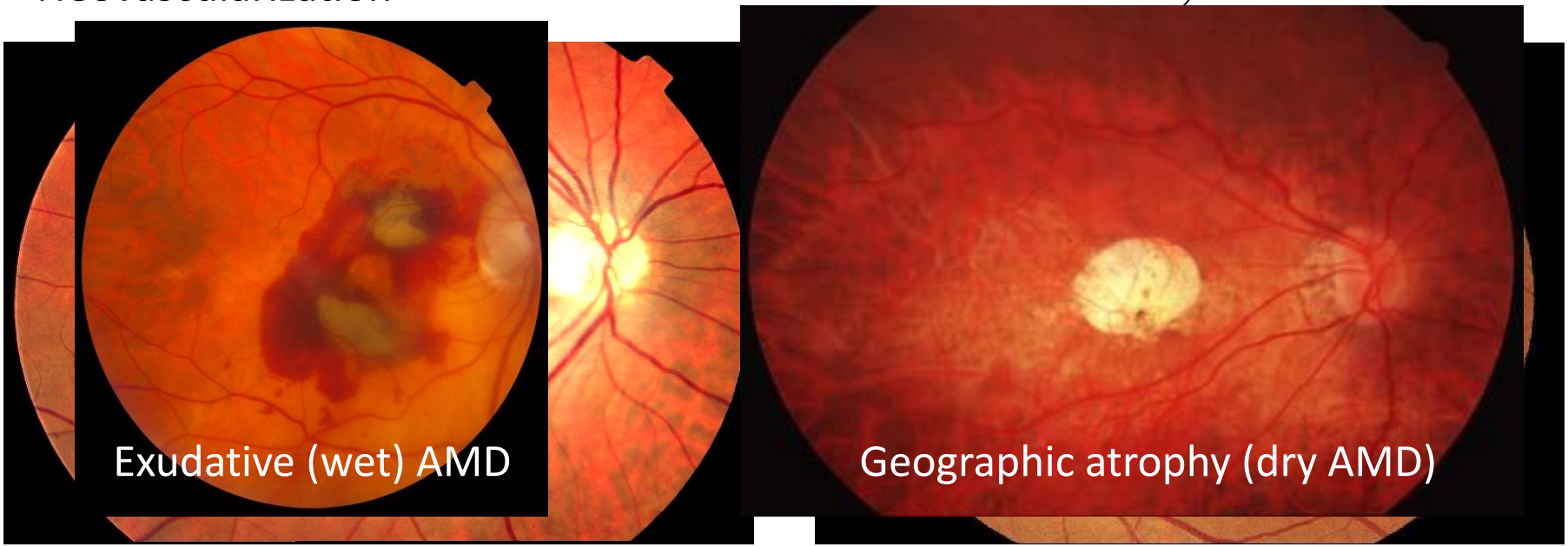
Colijn 2017 Ophthalmology

From maculopathies to Age-related Macular Degeneration

Criteria:

- Drusen
  - Size: <63µm – 63-125µm – >125µm
  - Area covered
  - In 1 or 2 eyes
- Pigment abnormalities in 1 or 2 eyes
- Geographic atrophy
- Neovascularization

4 stages: from maculopathy to AMD



Normal fundus **Drusen**

Age-adjusted associations between potential risk factors and AMD

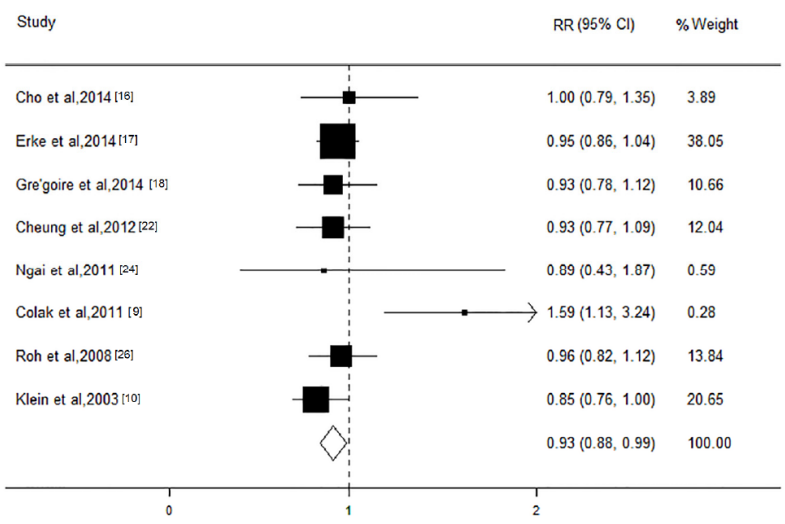
Pooled findings from the Rotterdam, Blue Mountains and Beaver Dam Eye Studies (n=14752 participants)

Potential Risk Factor	All 3 Sites	n/N
Age (index = 55–69)	1.00	25/7393
70–79	6.02 (3.82–9.47)	76/3839
80–86	25.3 (16.2–39.5)	94/1237
Gender (index = female)		195/12469
Male	0.95 (0.70–1.28)	
Smoking		194/12298
Never (index)	1.00	
Ex-smoker	1.36 (0.97–1.90)	
Current smoker	3.12 (2.10–4.64)	
Body mass index		191/12300
Underweight (BMI < 20)	1.75 (0.95–3.22)	
Normal (index)	1.00	
Overweight (30 > BMI > 25)	1.19 (0.85–1.98)	
Obese (BMI ≥ 30)	1.30 (0.85–1.98)	
Cardiovascular and other disease (index = no disease, normal range)		
AMI	0.81 (0.51–1.30)	192/12310
Angina	0.78 (0.50–1.23)	193/12326
Stroke	0.94 (0.55–1.60)	193/12354
Hypertension	1.09 (0.79–1.50)	194/12390
Cholesterol (per mmol/L)	1.00 (0.88–1.14)	173/10862
HDL-cholesterol (per mmol/L)	1.27 (0.90–1.80)	173/10859
Iris color		193/12333
Gray or blue (index)	1.00	
Hazel or green	0.79 (0.51–1.22)	
Tan or brown	0.88 (0.61–1.22)	
Age at menopause		115/6811
Age ≥45 yrs (index)	1.00	
Age <45 yrs	1.13 (0.73–1.76)	
Time from menarche to menopause		114/6752
<30 yrs (index)	1.00	
30–35 yrs	0.76 (0.45–1.31)	
≥35 yrs	0.83 (0.51–1.34)	
Hormone replacement therapy (index = never)		120/6954
	1.00	
Ever	0.77 (0.43–1.39)	

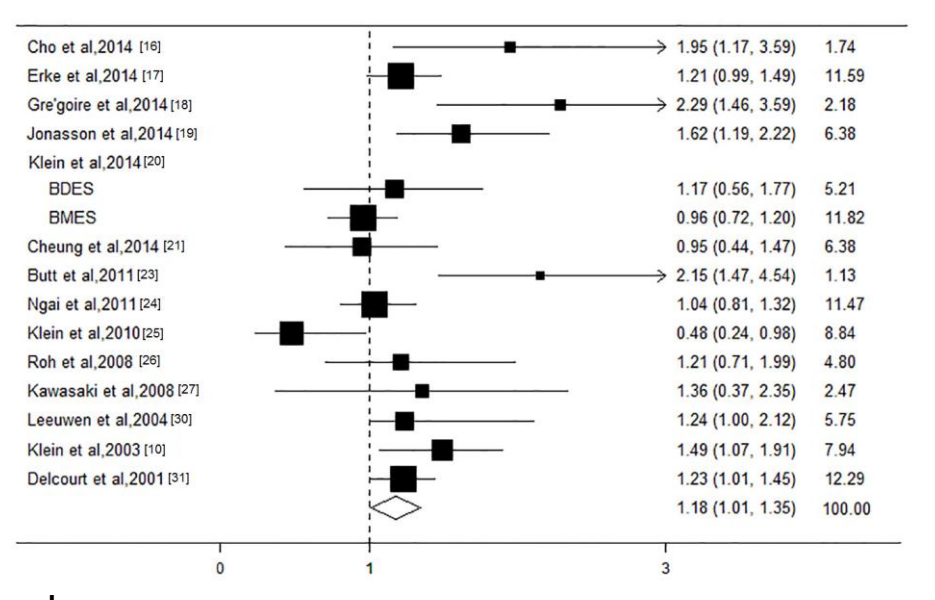
Smith 2001 Ophthalmology

Plasma cholesterol and AMD risk

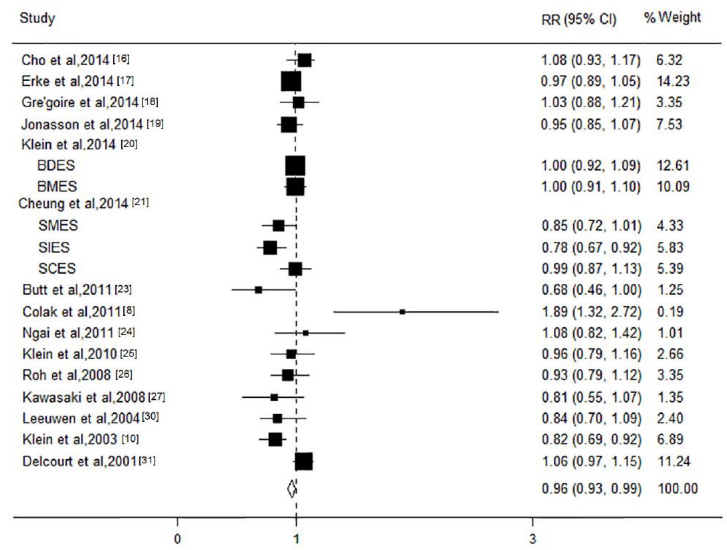
LDL-cholesterol



HDL-cholesterol

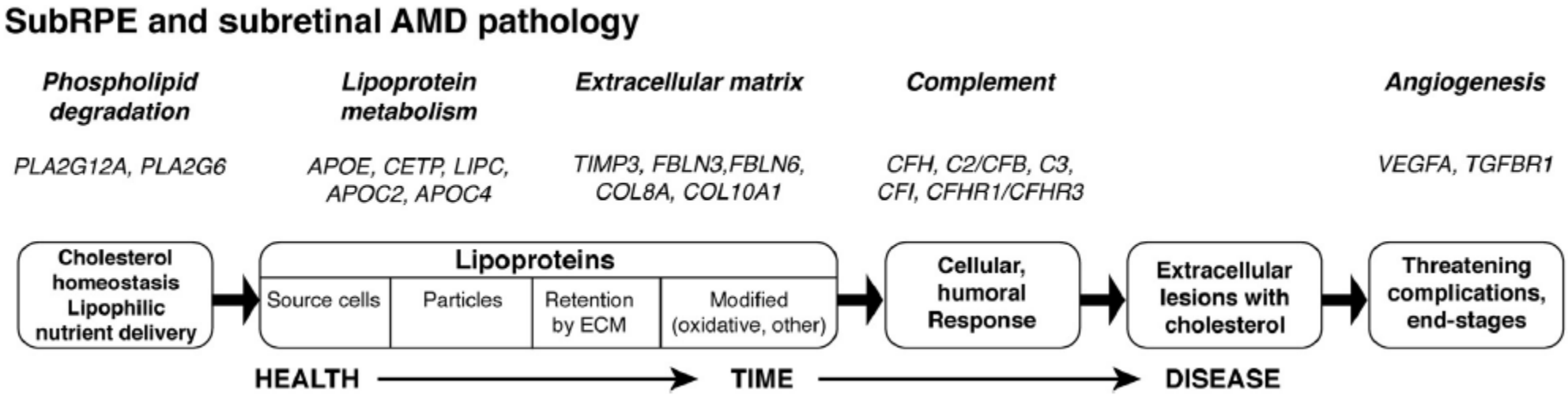


Total-cholesterol



Wang 2016 Nutrients

From aging retina to AMD



Pikuleva 2014 Prog Retin Eye Res

Polymorphisms in genes encoding lipid components and AMD

Table 1
Variants in or near genes encoding components of the lipid metabolism, reported to be associated with AMD at genome wide level ($p < 5 \times 10^{-8}$) (Fritsche et al., 2016).

Gene	Variant	Position in gene	Minor allele	Minor allele frequency		Association results	
				Cases	Controls	OR	P
ABCA1	rs2740488	intronic	C	0.255	0.275	0.89	6.0×10^{-7}
LIPC	rs2043085	21 kb upstream	C	0.350	0.381	1.15	7.7×10^{-13}
LIPC	rs2070895	intronic	A	0.195	0.217	0.86	1.8×10^{-10}
CETP	rs5817082	intronic	CA	0.232	0.264	0.87	2.7×10^{-8}
CETP	rs17231506	1 kb upstream	T	0.348	0.315	1.11	1.2×10^{-6}
APOE	rs429358	coding; Cys156Arg	C	0.099	0.135	0.67	3.9×10^{-39}
APOE (EXOC3L2/MARK4)	rs73036519	distant; 335 kb downstream	C	0.284	0.302	0.91	2.4×10^{-5}

Van Leeuwen 2018 Prog Retin Eye Res

Gene factors and blood lipid associations in AMD

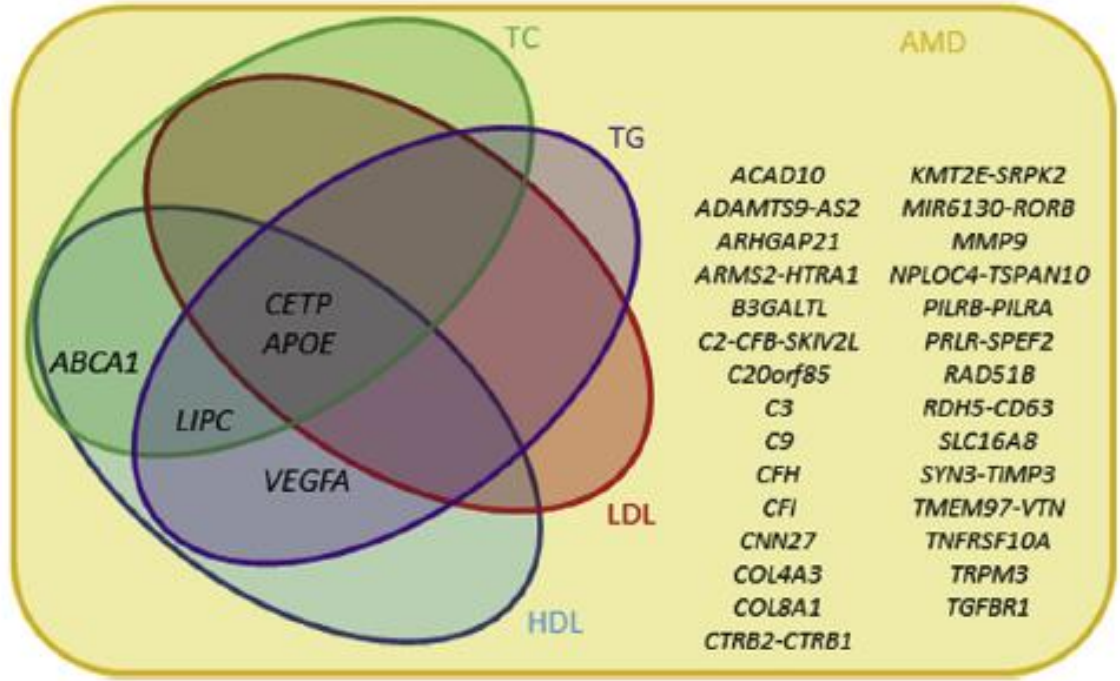


Fig. 7. *The overlap between genes identified by GWAS of blood lipid levels and the genes identified by GWAS of AMD.*
Five of the genes identified by the largest GWAS to be associated with AMD (Fritsche et al., 2016) are also associated by GWAS with blood lipid levels. Only loci that overlap between the variants found for AMD and the variant found blood lipid levels are considered overlapping loci. GWAS, genome-wide association study; AMD, age-related macular degeneration.

Van Leeuwen 2018 Prog Retin Eye Res

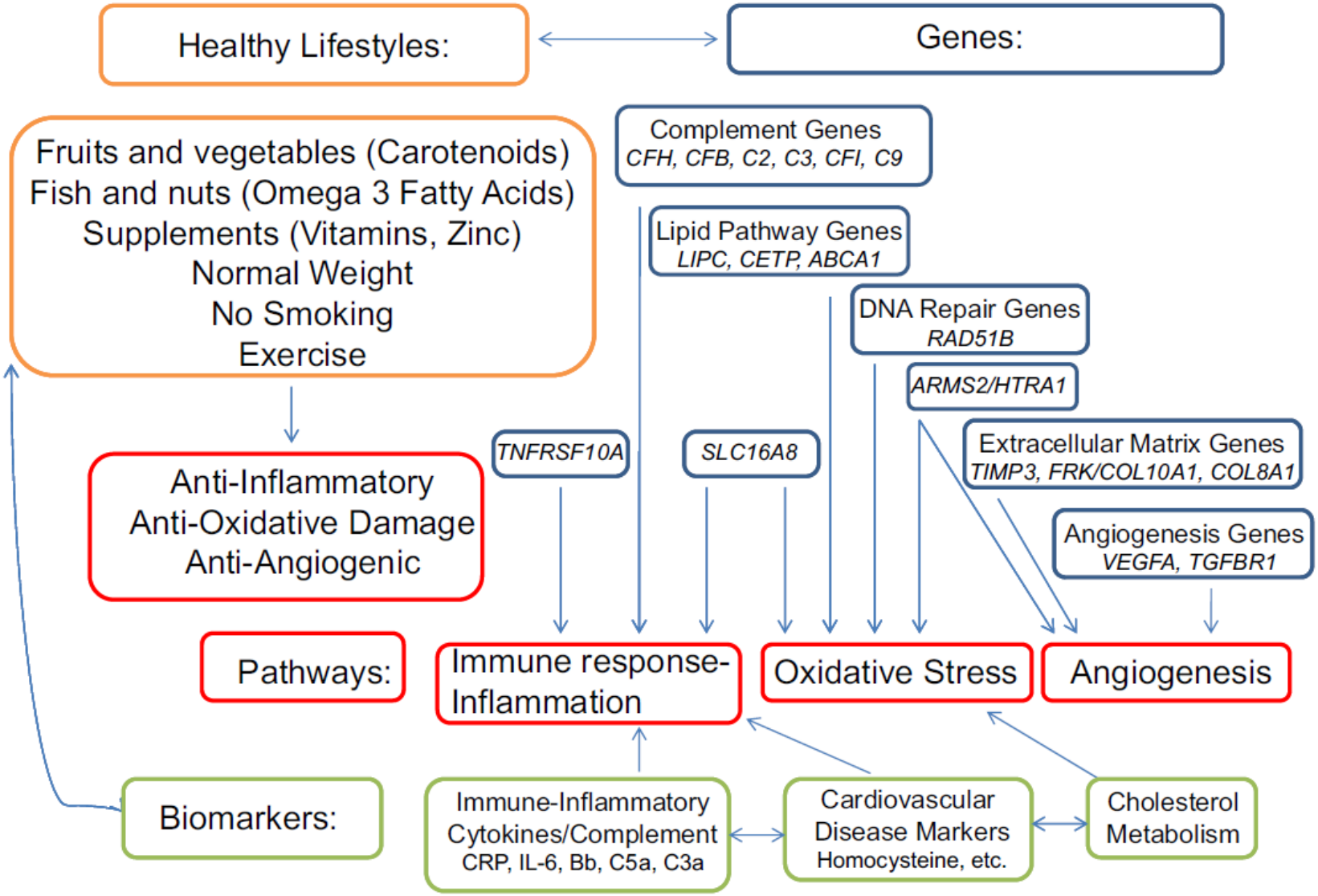


Fig. 1. Diagram of interplay between environmental and genetic risk factors that mediate AMD risk.

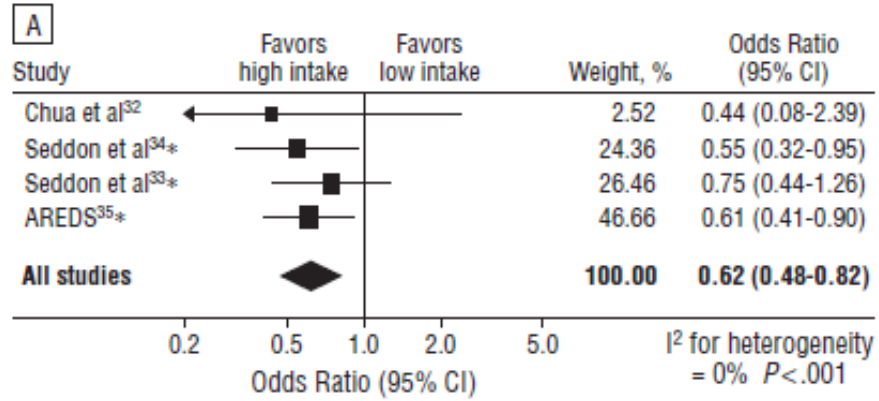
Sobrin 2014 Progr Retin Eye Res

Fish intake, ω3 fatty acids and AMD

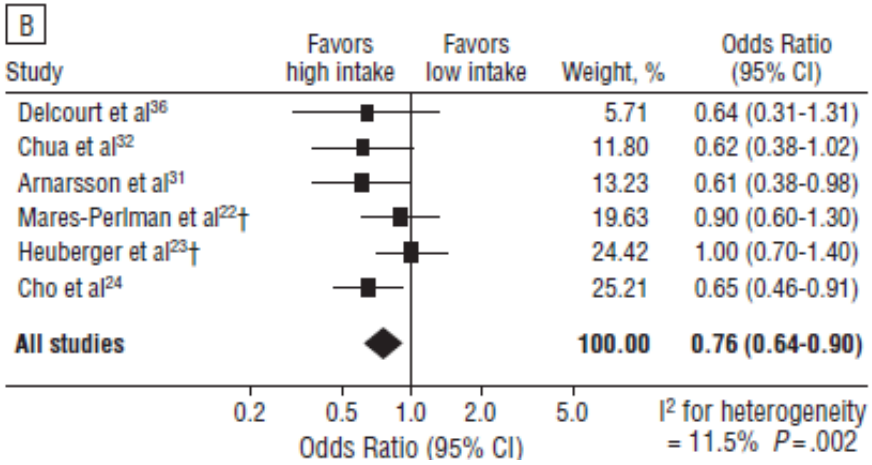
Meta-analysis from 7 databases

(88974 people including 3204 AMD)

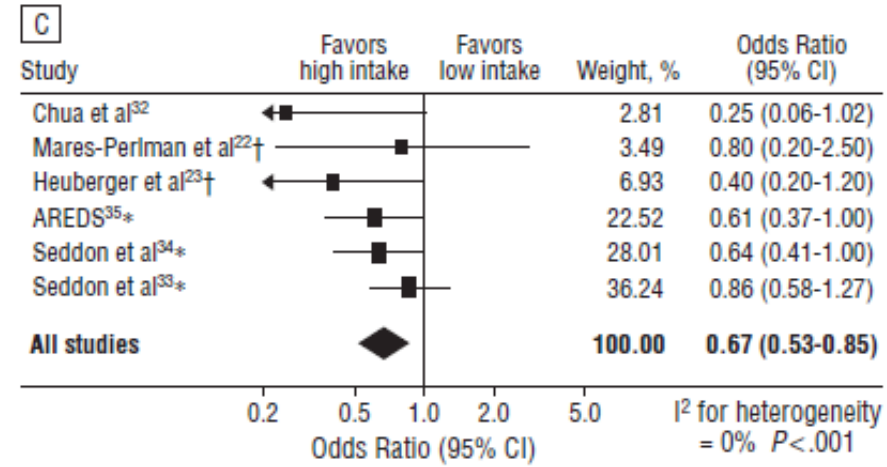
ω3 and late AMD



Fish intake and early AMD



Fish intake and late AMD



Chong 2008 Arch Ophthalmol

Protection from AMD is associated with fish consumption above 2 servings of fish per week

Table 2. Odds Ratios for AMD According to Fish Intake

	<1 Serving/wk	1 Serving/wk	≥2 Servings/wk	*P* Trend
Cases/controls, No.	74/131	75/144	73/184	
Median intake (servings per day)	0.080	0.18	0.36	
Adjusted OR*	1.0	0.97	0.68	.07
Multivariate OR1 (95% CI)†	1.0	0.94 (0.64-1.38)	0.63 (0.41-0.97)	.03
Multivariate OR2 (95% CI)‡	1.0	1.0 (0.67-1.48)	0.64 (0.41-1.00)	.04

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; OR, odds ratio.

*Adjusted for age (60-69, 70-79, and 80+ years), log calories (continuous), and protein intake (quartiles).

†Adjusted for education (≥high school vs <high school); smoking (current/past/never in the multivariate fish models); age (60-69, 70-79, and 80+ years); body mass index, calculated as weight in kilograms divided by the square of height in meters (<25, 25-29.9, and 30+); systolic blood pressure; cardiovascular disease; log calories (continuous); protein intake (quartile); log calorie-adjusted beta-carotene intake (continuous); alcohol intake (continuous); and physical activity (continuous, times per week vigorous).

‡Adjusted for variables in model 1 plus total intake of zinc, vitamin C, and vitamin E (log scale for all 3).

Seddon 2007 Arch Ophthalmol

…primarily in patients with low intake in linoleic acid

Table 4. Odds Ratios for AMD by Quartile of Omega-3 Intake, Linoleic Acid Intake, and Omega-3 Intake Within Strata of Linoleic Acid Intake

Fatty Acid Intake	Quartile of Omega-3 Intake 1	2	3	4	*P* Trend
Omega-3 intake					
Cases/controls, No.	64/102	61/120	49/114	48/123	
Median intake, g	0.06	0.12	0.20	0.35	
Adjusted OR*	1.0	0.82	0.62	0.60	.02
Multivariate OR1 (95% CI)†	1.0	0.79 (0.52-1.21)	0.60 (0.36-0.97)	0.56 (0.33-0.94)	.01
Multivariate OR2 (95% CI)‡	1.0	0.80 (0.53-1.21)	0.60 (0.36-0.99)	0.55 (0.32-0.95)	.02
Linoleic acid intake					
Cases/controls, No.	43/127	60/110	65/107	54/115	
Median intake, g	7.12	10.45	13.34	18.46	
Adjusted OR*	1.0	1.72	1.81	1.37	.42
Multivariate OR1 (95% CI)†	1.0	1.89 (1.15-3.11)	2.07 (1.17-3.63)	1.56 (0.79-3.08)	.26
Multivariate OR2 (95% CI)‡	1.0	1.85 (1.12-3.08)	1.99 (1.12-3.54)	1.46 (0.72-2.96)	.32
Linoleic acid intake, quartiles 1 and 2 (≤11.79 g)					
Cases/controls, No.	41/66	35/65	17/54	10/52	
Median intake of omega-3, g	0.06	0.12	0.20	0.35	
Adjusted OR*	1.0	0.79	0.90	0.92	.001
Multivariate OR1 (95% CI)†	1.0	0.97 (0.54-1.76)	0.48 (0.22-1.04)	0.30 (0.12-0.74)	.002
Multivariate OR2 (95% CI)‡	1.0	0.94 (0.52-1.72)	0.39 (0.18-0.88)	0.23 (0.09-0.57)	<.001
Linoleic acid intake, quartiles 3 and 4 (≥11.80 g)					
Cases/controls, No.	23/36	26/55	32/60	38/71	
Median intake of omega-3, g	0.06	0.12	0.20	0.36	
Adjusted OR*	1.0	0.79	0.90	0.92	.98
Multivariate OR1 (95% CI)†	1.0	0.74 (0.37-1.47)	0.82 (0.40-1.69)	0.85 (0.41-1.77)	.93
Multivariate OR2 (95% CI)‡	1.0	0.73 (0.35-1.55)	0.84 (0.37-1.89)	1.07 (0.46-2.50)	.66

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; OR, odds ratio.

*Adjusted for log calories (continuous) and protein intake (quartile).

†Adjusted for education (≥high school vs <high school); smoking (current/past/never); age (60-69, 70-79, and 80+ years); body mass index, calculated as weight in kilograms divided by the square of height in meters (<25, 25-29.9, and 30+); systolic blood pressure; cardiovascular disease; log calories (continuous); protein intake (quartile); log calorie-adjusted beta-carotene intake (continuous); alcohol intake (continuous); and physical activity (continuous, times per week vigorous).

‡Adjusted for variables in model 1 plus total intake of zinc, vitamin C, and vitamin E (log scale for all 3).

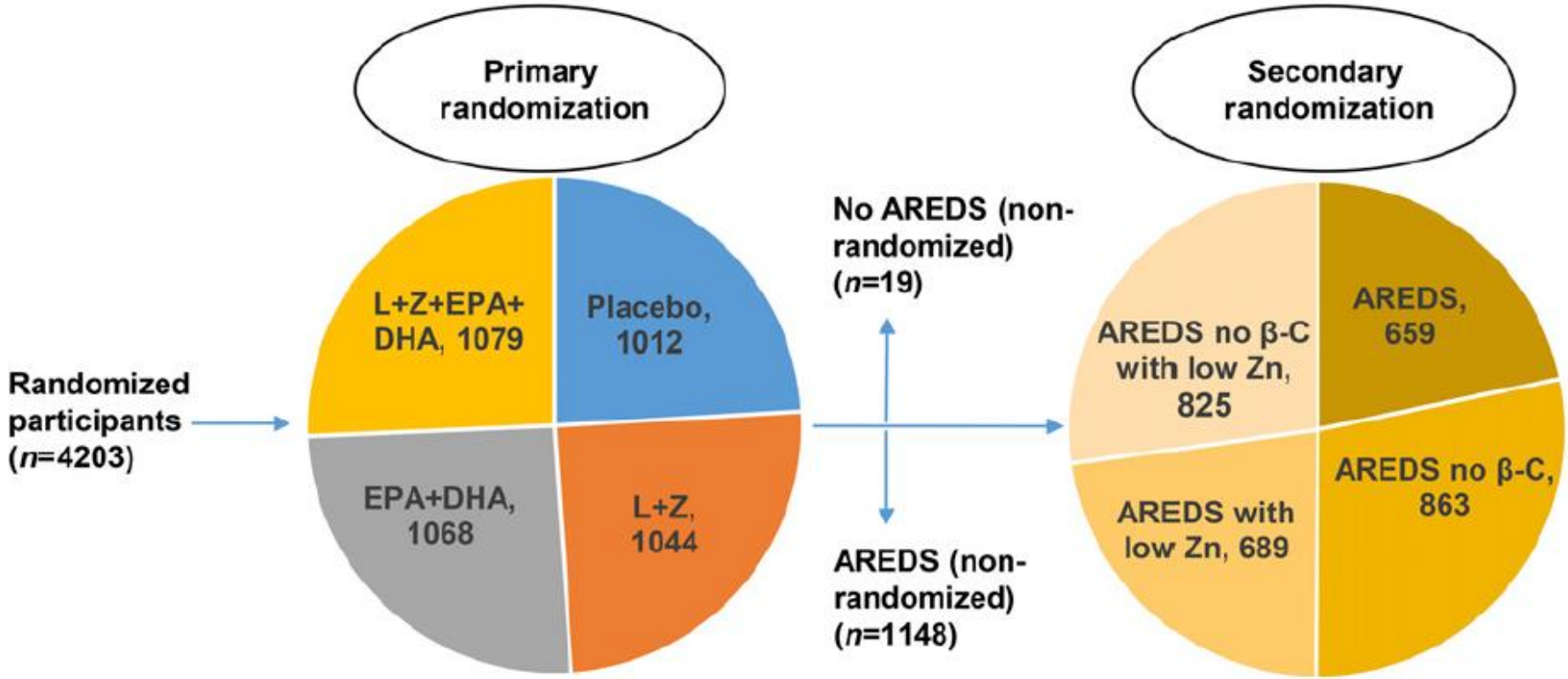
Seddon 2007 Arch Ophthalmol

Age-Related Eye Disease Study 2

- 4203 participants, 50-85 years
- Inclusion criteria: drusen in both eyes or drusen in 1 eye+AMD in the other eye
- Follow-up: 5 years
- Primary outcome: occurrence of CNV or GA in 1 eye

AREDS 2013 JAMA

Design



Supplements

Lutein: 10mg/day

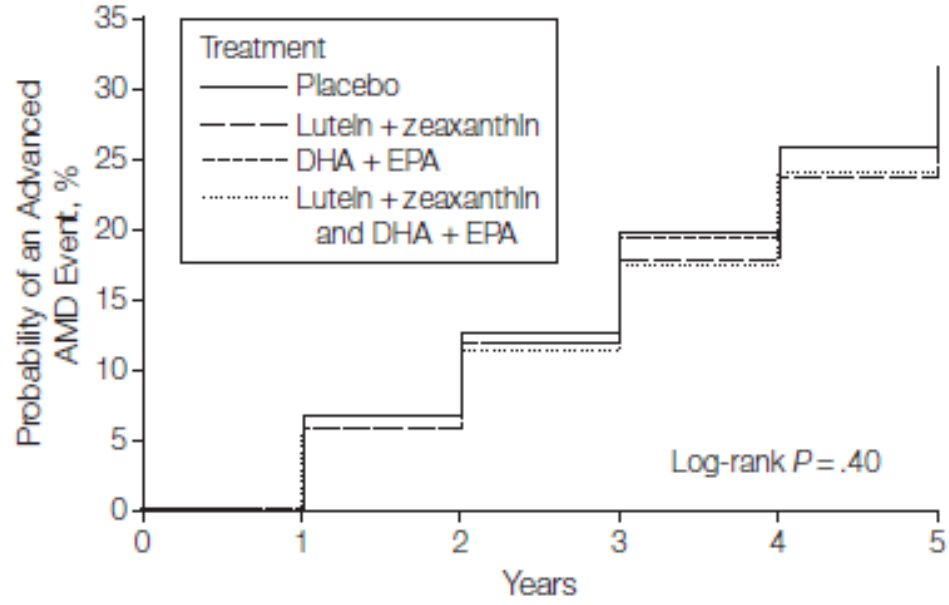
Zeaxanthin: 2mg/day

EPA: 650mg/day

DHA: 350mg/day

Age-Related Eye Disease Study 2 Results

Figure 2. Probability of Eyes of AREDS2 Participants Developing Advanced Age-Related Macular Degeneration in the Primary Analyses

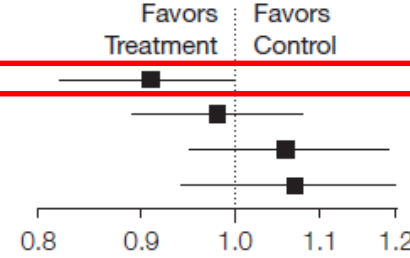


No. of eyes at risk						
Placebo	1691	1647	1509	1377	1243	900
Lutein + zeaxanthin	1709	1673	1535	1405	1284	938
DHA + EPA	1749	1702	1549	1420	1260	897
Lutein + zeaxanthin and DHA + EPA	1742	1683	1542	1415	1281	923

AREDS 2013 JAMA

Figure 4. Main Effects of Lutein + Zeaxanthin, Omega-3 Long-Chain Polyunsaturated Fatty Acids, Zinc, and Beta Carotene on Progression to Advanced Age-Related Macular Degeneration (AMD)

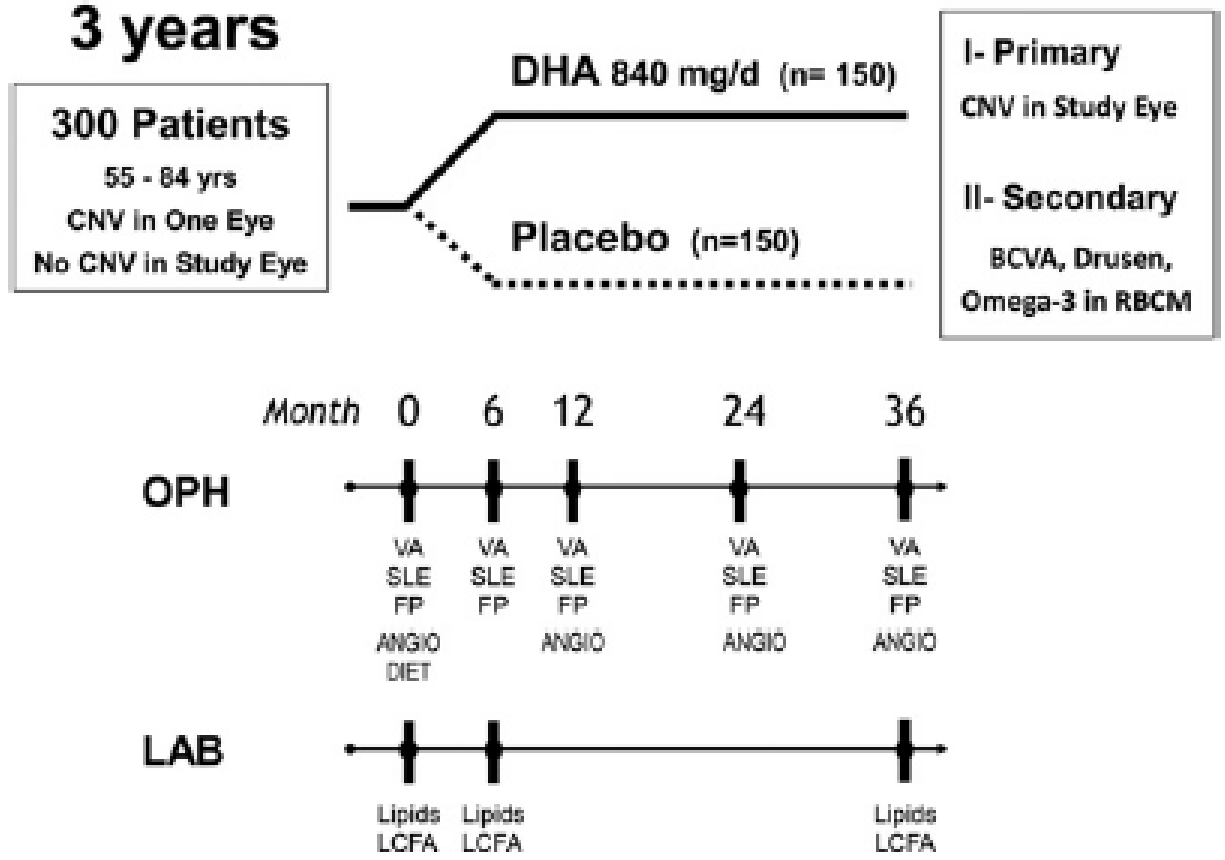
	Treatment		Control				
Treatment Main Effect	Eyes	Advanced AMD Events	Eyes	Advanced AMD Events	Hazard Ratio (95% CI)	Favors Treatment / Favors Control	*P* Value
Lutein + zeaxanthin	3451	940	3440	1000	0.91 (0.82-1.00)		.05
DHA + EPA	3491	979	3400	961	0.98 (0.89-1.08)		.74
Low-dose zinc	2468	726	2501	704	1.06 (0.95-1.19)		.32
Beta carotene	2221	647	2212	622	1.07 (0.94-1.20)		.31



Participants assigned to the control group were also given Age-Related Eye Disease Study (AREDS) supplement either within or outside of the secondary randomization for the 4 variations of the AREDS supplements; thus, there is no true placebo group. DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid.

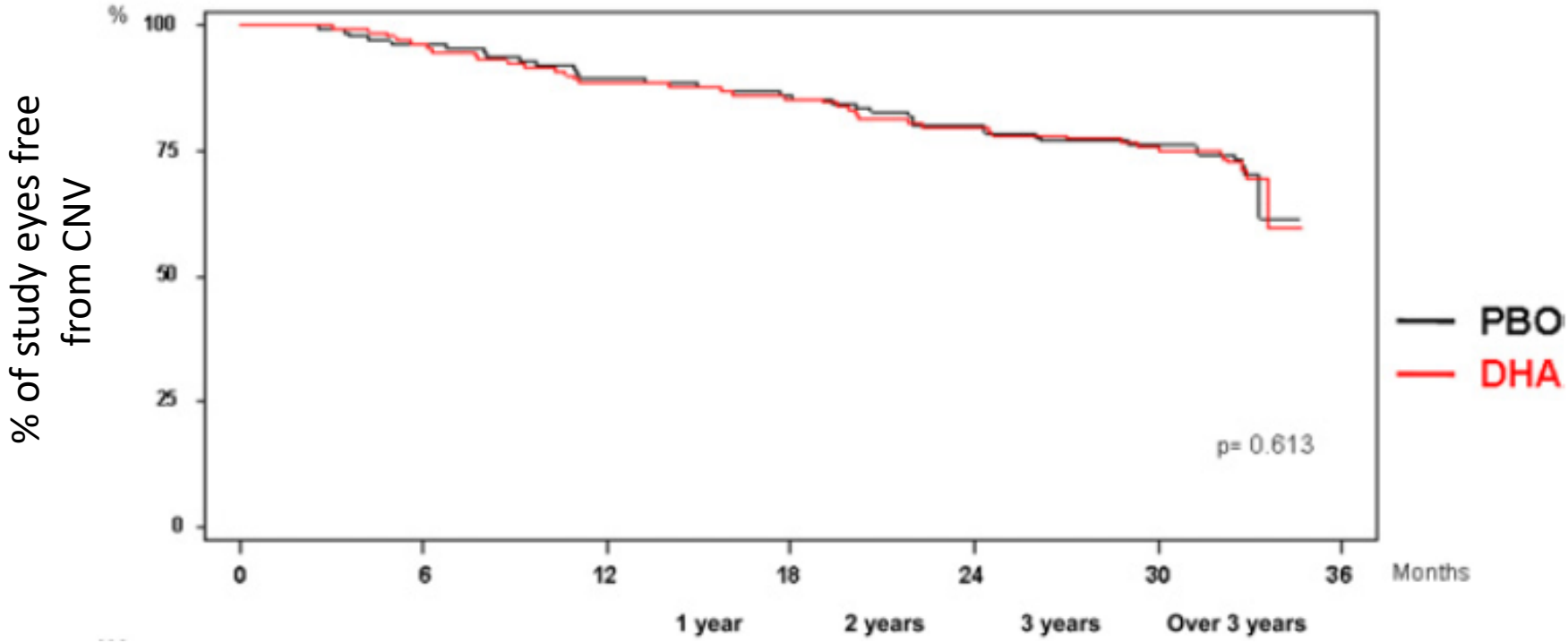
AREDS 2013 JAMA

« Nutritional AMD Treatment 2 » (NAT2) Study



Souied 2013 Ophthalmology

Incidence of neovascular complications in the fellow eye



Souied 2013 Ophthalmology

Fatty acid enrichment in NAT2 patients

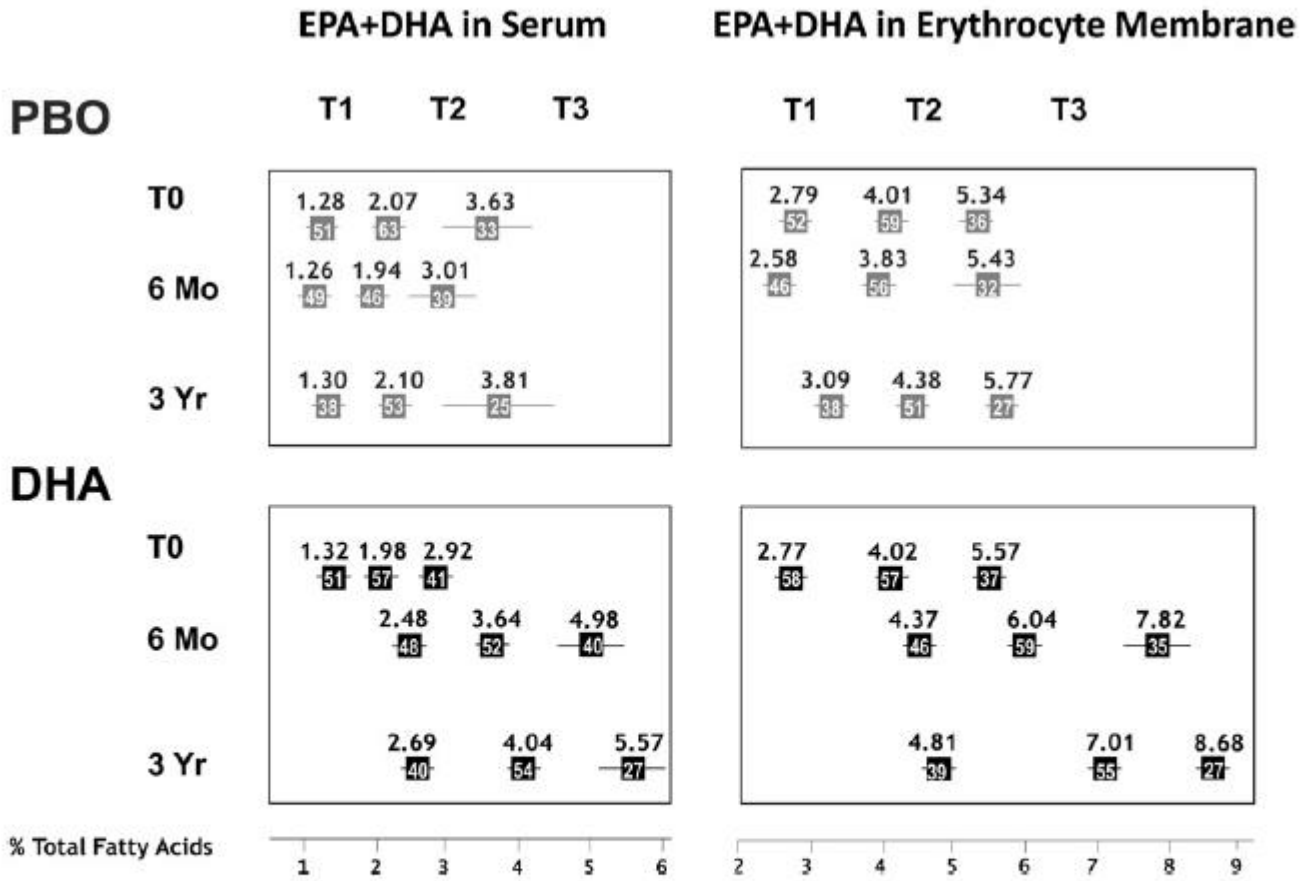


Figure 4. Graphs showing the distribution by tertiles of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) levels in placebo (PBO) and DHA groups at each time point of the study. T1, T2, and T3 = first, second, and third tertiles of EPA plus DHA levels, respectively, measured at baseline (T0), 6 months (6 Mo) and 3 years (3 Yr). Numbers above symbols represent the mean for each tertile of levels. Lines across symbols represent the standard deviation. Numbers within symbols represent the number of individuals in each group. **Top**, Distribution of EPA plus DHA levels observed in (**Left**) serum and (**Right**) red blood cell membrane (RBCM) in the PBO group. **Bottom**, Distribution of EPA plus DHA levels observed in (**Left**) serum and (**Right**) RBCM in the DHA group.

Souied 2013 Ophthalmology

Incidence of CNV as a function of fatty acid enrichment

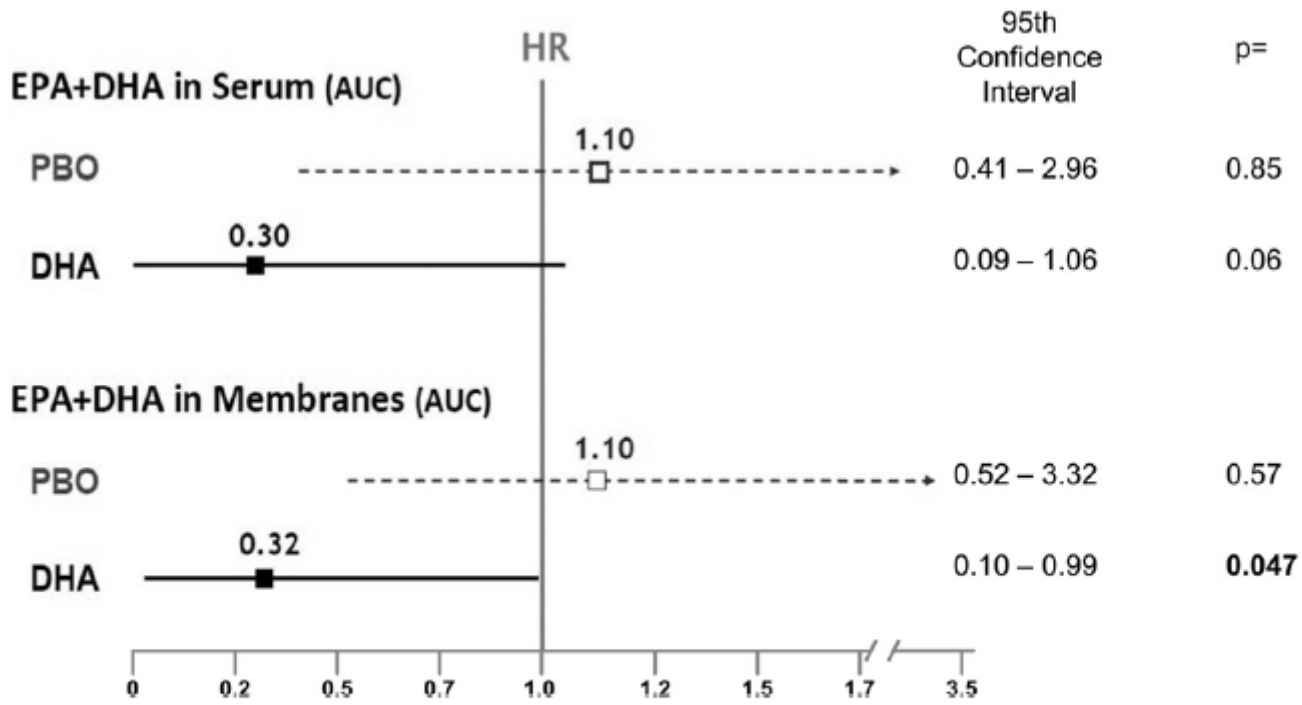


Figure 5. Graph showing the hazard ratio (HR) for 3-year choroidal neovascularization (CNV) incidence as a function of the area under the receiver operating characteristic curve (AUC) of polyunsaturated fatty acid levels measured in serum and red blood cell membranes over the study period. Numbers above symbols represent the HR for CNV incidence computed from the Cox model of the higher versus the lower tertile of the AUC of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) levels over 3 years, in (**Top**) serum and (**Bottom**) red blood cell membranes. Placebo (PBO) group = open symbols; DHA group = filled symbols; lines across symbols = 95% confidence interval; dashed lines = PBO group; solid lines = DHA group.

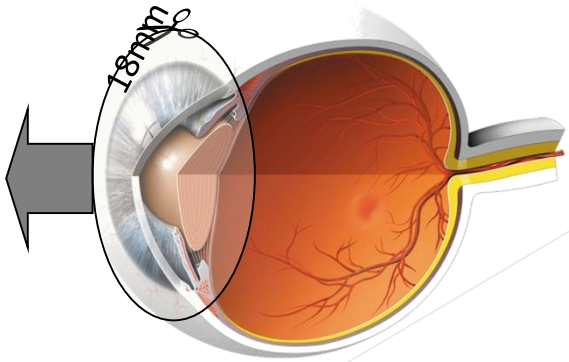
Souied 2013 Ophthalmology

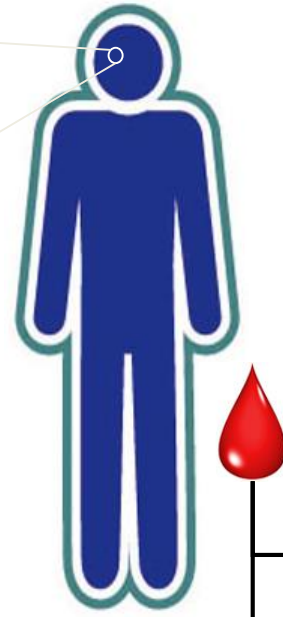
Effect of DHA treatment according to CFH polymorphism in NAT2 patients

CFH Y402H	Placebo group		DHA group		*P-value*
	% of CNV	Hazard risk	% of CNV	Hazard risk (95% CI)	
Model 1 (adjusted for age and gender) (n=250)					
TT (non-risk)	*38.2*	1.0	16.7	0.25 (0.08-0.79)	*0.02*
CT	26.0	1.0	29.2	1.11 (0.53-2.30)	*0.79*
CC	23.1	1.0	39.5	2.09 (0.90-4.89)	*0.09*
Model 2 (adjusted for age, gender, BMI, smoking status, HDL-chol, TG, ARMS2 A69S polymorphism) (n=250)					
TT (non-risk)		1.0		0.14 (0.03-0.59)	*0.008*
CT		1.0		1.19 (0.56-2.51)	*0.65*
CC		1.0		2.33 (0.98-5.55)	*0.06*

Merle 2015 PLoS ONE

Would retinal DHA be associated to dietary intakes ?





12 women and men

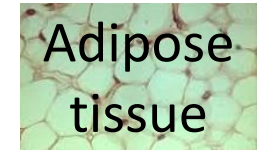
Fundus free from retinal pigment abnormalities and large drusen

Mean age 83±9yr (range 59-95yr)

Plasma (short history intake)

Red blood cells (middle)

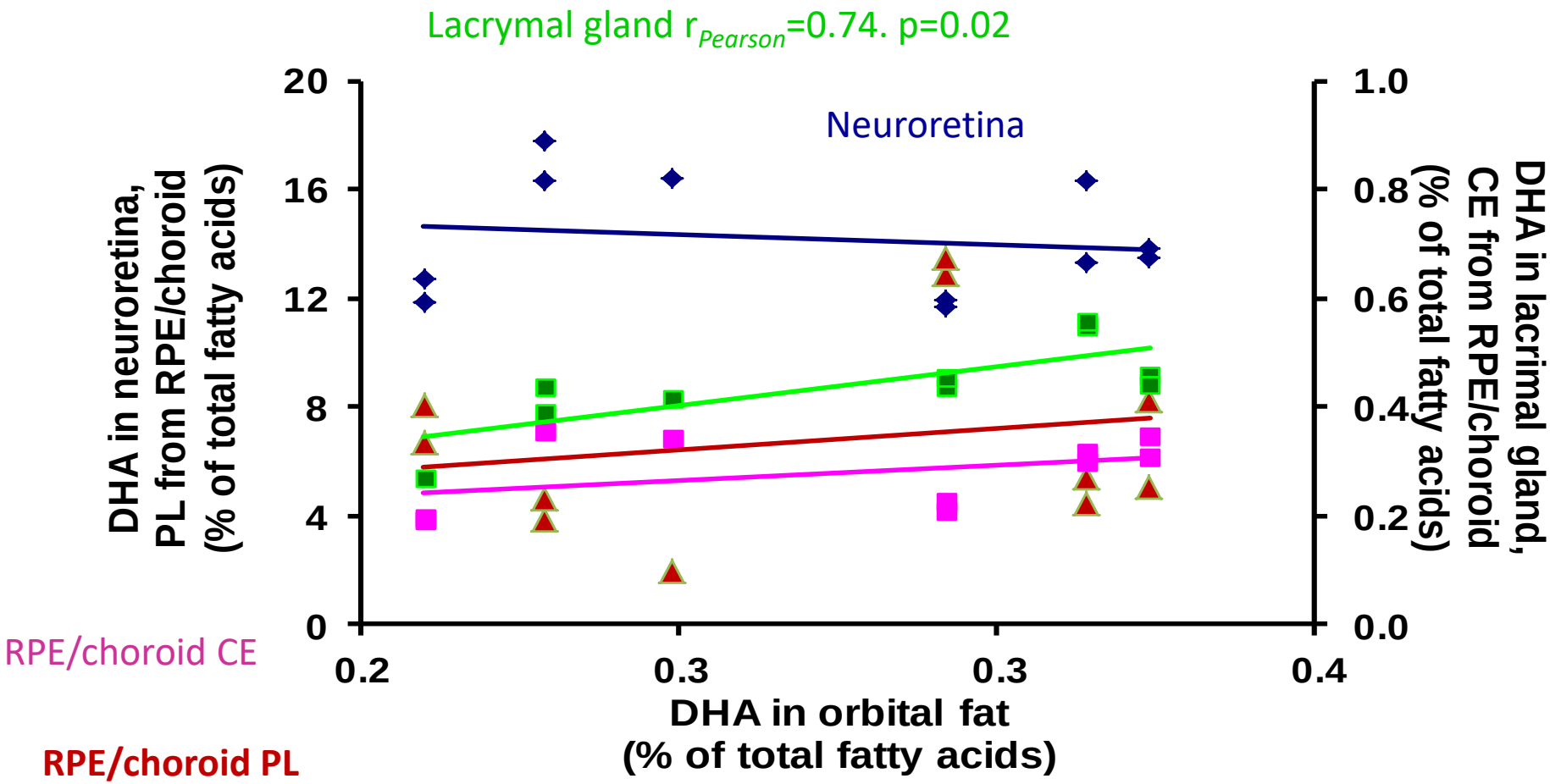
RPE/choroid



(long)

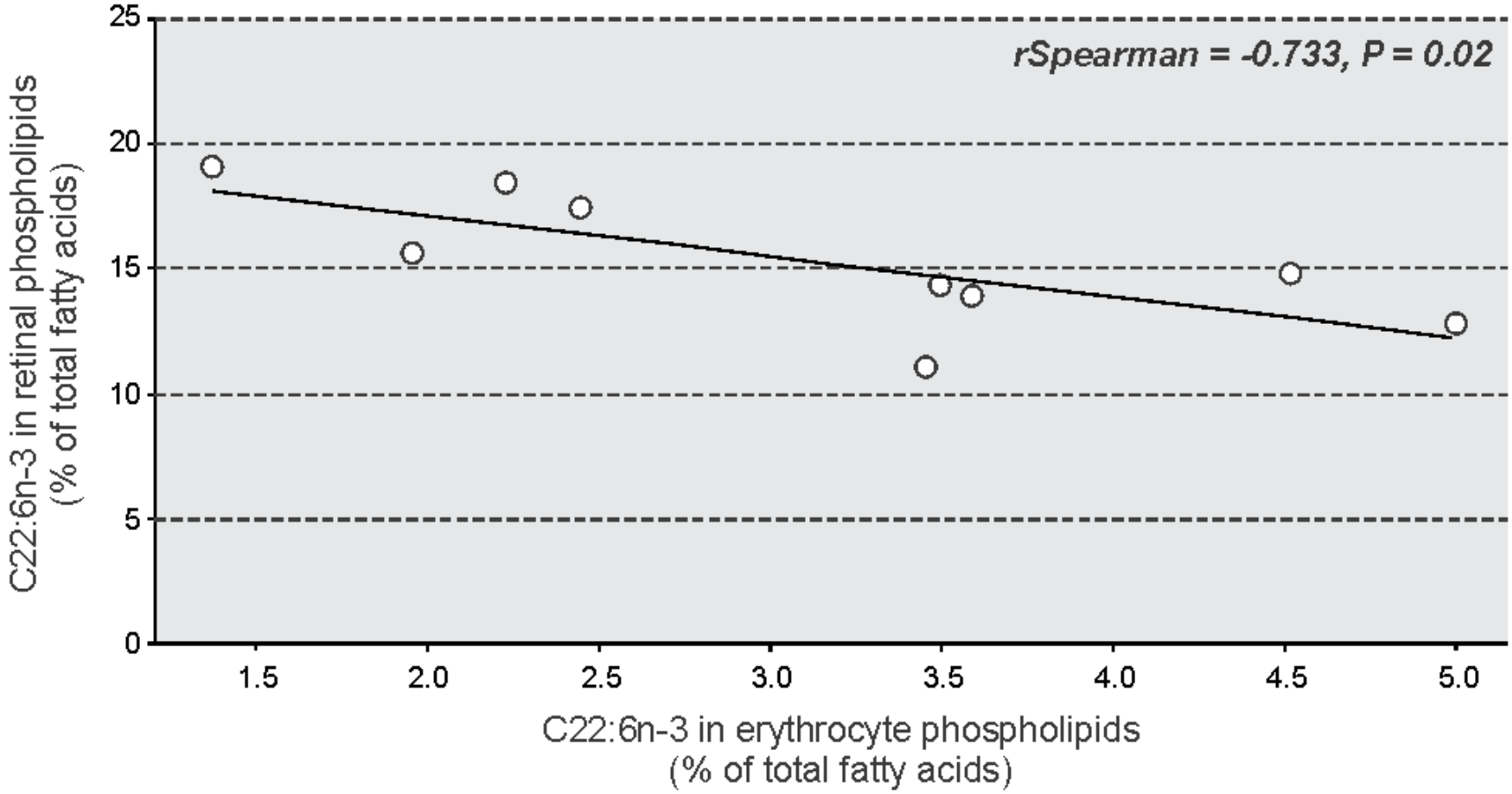
Bretillon et al. 2008 Exp Eye Res
Acar et al. 2012 PLoS ONE

Neither adipose nor circulating DHA was associated with retinal DHA



Bretillon *et al*. 2008. *Exp Eye Res*

Neither adipose nor circulating DHA was associated with retinal DHA



Acar *et al. 2012.* PLoS ONE

Plasma metabolomics in AMD patients
(UPLC-MS analysis)

Table 2. Multivariate Logistic Regression Analysis for Age-Related Macular Degeneration Patients versus Controls

	Metabolites in AMD Groups Compared with Controls					
	Decreased in AMD Patients (Odds Ratio <1)		*Increased in AMD Patients (Odds Ratio >1)*		Total	
Significance Level	*No.*	*%*	*No.*	*%*	*No.*	*%*
$P < 0.05$	59	8.5	28	4.0	87	12.5
$P < 0.01$	24	3.4	9	1.3	33	4.7
$P < 0.001$	6	0.9	1	0.1	7	1.0

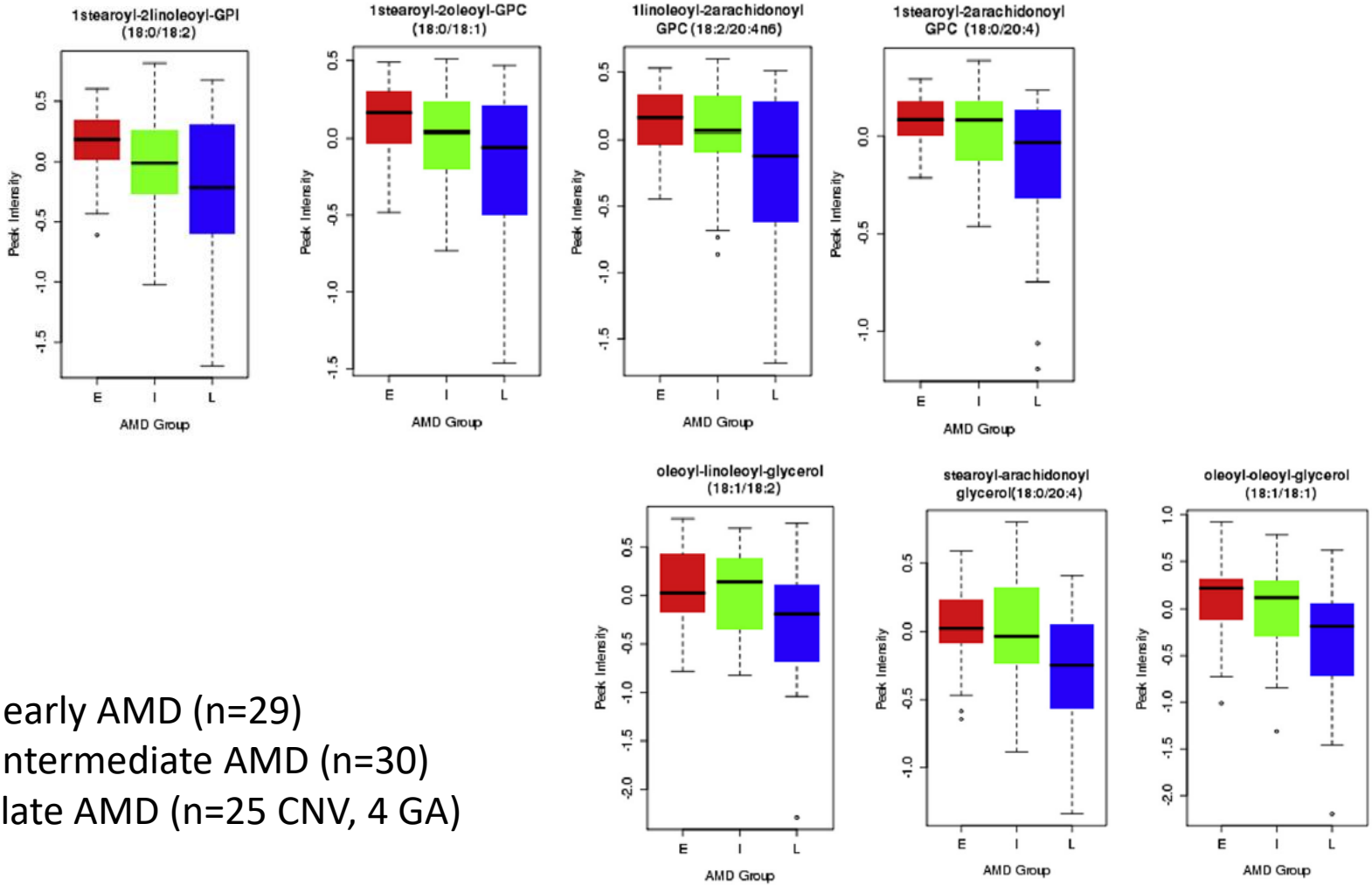
Among the 87 metabolites identified, 72 were relevant to lipid pathways

Table 3. Significantly Different Metabolites ($P < 0.001$) between Patients with Age-Related Macular Degeneration and Controls

Biochemical	Superpathway	Subpathway	Metabolites in AMD Patients vs. Controls	Odds Ratio*	P Value
Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2]*	Lipid	Diacylglycerol	Decreased	0.0961	0.0008
Stearoyl-arachidonoyl-glycerol (18:0/20:4) [1]*	Lipid	Diacylglycerol	Decreased	0.0411	0.0009
Oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]*	Lipid	Diacylglycerol	Decreased	0.0463	0.0002
Oleoyl-arachidonoyl-glycerol (18:1/20:4) [1]*	Lipid	Diacylglycerol	Decreased	0.111	0.0007
1-Palmitoyl-2-arachidonoyl-GPC (16:0/20:4n6)	Lipid	Phosphatidylcholine	Decreased	0.0004	0.0006
1-Stearoyl-2-arachidonoyl-GPC (18:0/20:4)	Lipid	Phosphatidylcholine	Decreased	0.0002	0.0005
Adenosine	Nucleotide	Purine metabolism, adenine containing	Increased	3.7422	0.0009

Lains 2018 Ophthalmology

Plasma metabolomics in AMD patients
(UPLC-MS analysis)



E: early AMD (n=29)
I: intermediate AMD (n=30)
L: late AMD (n=25 CNV, 4 GA)

Lains 2018 Ophthalmology

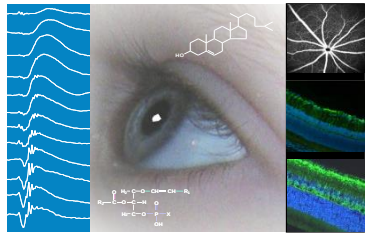
Unresolved questions

- Need for relevant biomarkers to identify:
  - susceptible patients (inter-individual differences in fatty acid or carotenoid incorporation)
  - the efficacy of dietary approaches (ω3 and carotenoids)
- What consequences of dietary interventions in the retina
  - lipid mediators (cell-specific)
  - local vs systemic effect (inflammation, lipid homeostasis)
- Should we consider diet instead of nutrients:
  - in primary, in secondary prevention (AMD prevention with ω3 in low ω6 intake)
  - in high genetic risk patients (ω3 in non genetic risk patients)

Acknowledgments

Eye & Nutrition Research Group, *Dijon*

Niyazi Acar, *PhD*
Marie Agnès Bringer, *PhD*
Alain Bron, *MD*
Catherine Creuzot-Garcher, *MD, PhD*
Elodie Masson, *PhD*
Bénédicte Buteau
Stéphane Grégoire
Laurent Leclère
Marie Annick Maire
Lucy Martine
Cynthia Fourgeux, Emilie Simon, Sarah Saab, Magalie Thierry, Elisa Vidal, Elise Léger-Charnay, Maissa Albouery, Rémi Karadayi, *PhD students*



Analytical Platform, *Dijon*

Olivier Berdeaux, *PhD*
Stéphanie Cabaret

Ophthalmology Department, *Saint Etienne*

Gilles Thuret, *MD, PhD*
Philippe Gain, *MD, PhD*
Zhiguo HE, *PhD*

Inserm "Bordeaux Population Health Research Center" (BPH) UMR1219, *Bordeaux*

Cécile Delcourt, *PhD*
Bénédicte Merle, *PhD*

Funding agencies and programs