Ocufolin®

The New Way in DR and AMD Supplementation

Riskfactor Homocysteine

Elevated Hcy levels along with oxidative stress have been associated in the etiology of several vascular diseases that can lead to the development of choroidal neovascularization (CNV) in AMD and DR (Singh et al. 2017, Huang et al. 2015). Hcy may be a potential useful biomarker in assessing the microvascular risk in diabetes, and hyperhomocysteinemia is a potential risk factor for DR, especially PDR (Xu et al. 2014). Hyperhomocysteinemia (HHcy) is associated with several human visual disorders, such as diabetic retinopathy (DR) and age-related macular degeneration (AMD). Breakdown of the blood-retinal barrier (BRB) is linked to vision loss in DR and AMD (Mohamed et al. 2017).

Correlation Homocysteine and Folate

Elevated serum tHcy and folate and vitamin B12 deficiencies predicted increased risk of incident AMD, which suggests a potential role for vitamin B12 and folate in reducing AMD risk (Gopinath et al. 2013, BMES).

Folic acid versus L-Methylfolate

Naturally occurring 5-MTHF has important advantages over synthetic folic acid – it is well absorbed even when gastrointestinal pH is altered and its bioavailability is not affected by metabolic defects (Scaglione et al. 2013). Not only is L 5-MTHF more effective in raising blood folate concentrations after 12 weeks than folic acid, but it is also unlikely to mask vitamin B-12 deficiency (Henderson et al. 2018). Folic acid is a synthetic form of the vitamin, which is only found in fortified foods, supplements and pharmaceuticals. It lacks coenzyme activity and must be reduced to the metabolically active tetrahydrofolate form within the cell. (Pietrzik et al., 49, (2010), 535).

Nutrition versus Supplementation

Disease-induced nutritional deficiencies often cannot be addressed by nutrient intakes derived from a whole food-based diet alone (Stover et al. 2017).

Ocufolin®

currently the only ocular diagnose based supplement under medical supervision containing L-Methylfolate, the particularly active form of folic acid

Using Ocufolin® forte as a supplement to reduce the risk of Age-related Macular Degeneration (AMD)

The ARED Studies showed reduced progression to advanced stages of the disease in AMD patients with morphological AMD impairment. However, the effect evidenced in the ARED Studies was restricted to the combination of antioxidants, zinc and Lutein/Zeaxanthin. After AREDS, efforts to develop appropriate formulations ceased. The impact of folates on the complex retinal pathology of AMD was hardly considered.

Folate deficiency has been identified as an important pathogenic factor in eye diseases such as AMD. Insufficient folate levels lead to increased homocysteine. Homocysteine levels >15 µmol/l are linked to increased risk of AMD incident in patients under 75. Additionally, hyperhomocysteinemia combined with low Vitamin B12 levels represents a major risk factor (Blue Mountains Eye Study, 2013).

Using Ocufolin® forte as a supplement to reduce the risk of Diabetic Retinopathy (DR)

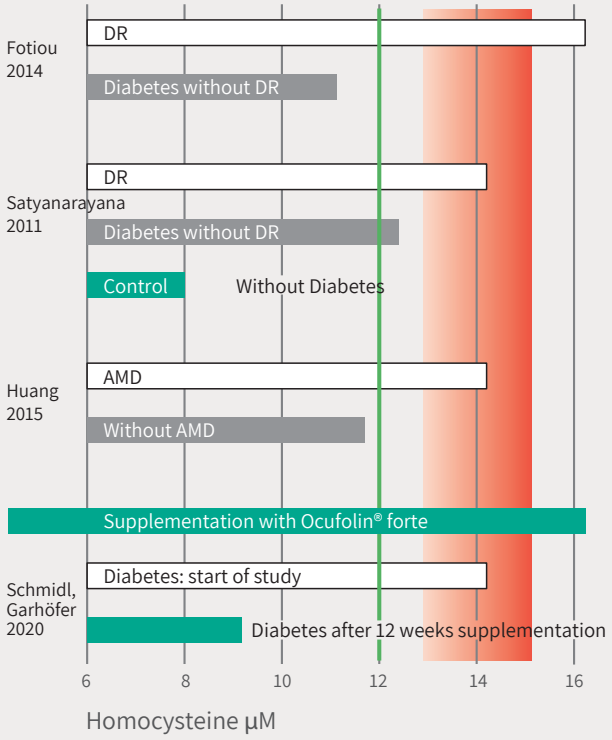
In a systematic review and meta analysis high homocysteine levels have been shown to be a risk factor for DR (Xu et al. 2014). The status of vitamins B was identified to be a major cofounding factor for DR (Satyanarayana et al. 2011). Patients with elevated homocysteine showed coincidently low folate and vitamin B12 levels (Fotiou et al. 2014). Above a threshold level for homocysteine of >13 µmol/l the risk of DR increases significantly.

Vitamin D is a multi functional fat soluble ingredient identified as a potent inhibitor of retinal neovascularization in ischemic retinopathy (Qin et al. 2017).

In response to these results, two ophthalmologists in the USA developed Ocufolin®, a formulation designed specifically for use in ophthalmology. It is currently the only ocular supplement containing the particularly active form of folic acid: L-Methylfolate Calcium.

An important criterion in developing the formulation of Ocufolin® was the description of the existence of the folate receptor alpha (FRα) and the reduced folate transporter (RFT-1) at the retinal pigment epithelium (RPE). This evidenced the important role of folate in retinal metabolism (Smith et al. 2000).

Homocysteine Impact on DR and AMD



Fotiou 2014: Hcy extremely elevated in 65 T2D-patients with DR compared to 75 T2D controls without DR.

Satyanarayana 2011: Hcy elevated in 200 Typ-2 Diabetes (T2D) patients with DR compared to 100 T2D-patients without DR.

Huang 2015: Hcy elevated in 1'072 AMD-cases compared to 1'202 controls, (Meta-Analysis of 11 studies).

Schmidl, Garhöfer 2020: Hcy-level efficiently reduced in 24 diabetic patients (T1DM, T2DM) with and without DR.

Difference between folic acid and L-Methylfolate

Studies by the US ophthalmologists showed many of their patients had a genotype which inhibits the metabolism of folic acid to L-methylfolate (MTHFR polymorphism). For patients with this genotype, even increased intake of folic acid does not guarantee that the retina is supplied with sufficient metabolized active folate.

The New Way in DR and AMD Supplementation

Ocufolin®: State of the art

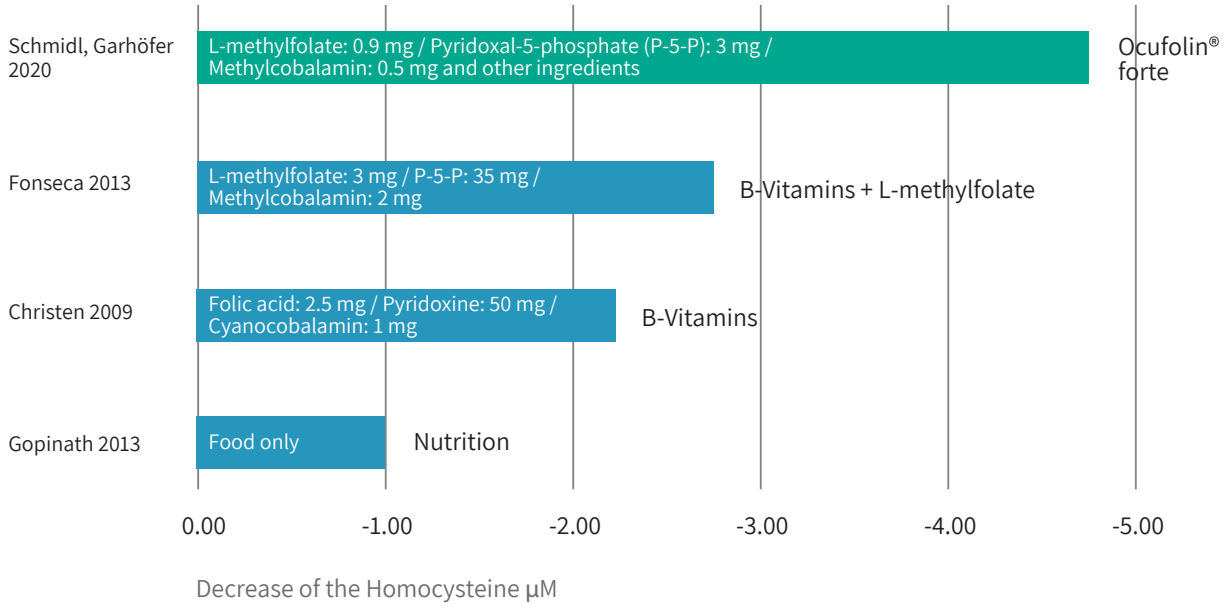
Calcium L-Methylfolate, is a highly bioavailable and active ingredient. This is the new active agent in Ocufolin®. Using calcium-L-methylfolate in a supplement provides the retina with active folate to reduce or reverse deficiencies resulting from genetic, dietary causes or diseases.

- The AREDS1 formula (2001): the combination of antioxidants, β-carotene and zinc can reduce the progress to more advanced stages of AMD by 25 % over a period of 6.3 years.
- The AREDS2 formula (2013): patients with Lutein deficiency benefit most from Lutein/Zeaxanthin (25 % reduction in the likelihood of further deterioration, as in AREDS1 with β-carotene). Unsaturated fatty acids provided no additional benefit in the ARED Study.
- Ocufolin®-formula: the essential components are L-methylfolate, B-vitamins, vitamin D, NAC plus ingredients from the ARED2 Study galenically optimized.

	AREDS1	AREDS2	Ocufolin®
Antioxidants	Yes	Yes	Yes
Zinc	Yes	Yes	Yes
Lutein/Zeaxanthin	No	Yes	Yes
L-Methylfolate	No	No	Yes
Vitamin D3, B2, B6, B12	No	No	Yes

AREDS1: Report No 8, Arch Ophthalmol, 119, (2001)1417. | AREDS2: JAMA, 309, (2013), 2005.

Reduction Homocysteine (Effect of Nutrition or Dietary Supplements)



Schmidl, Garhöfer, Mol. Vis., 26, (2020), 326: Hcy-level most efficiently reduced in DM patients after intake of Ocufolin® forte containing significantly lower quantities of each ingredient.
Fonseca et al., Am J Med, 126, (2013), 141: Hcy-level significantly reduced in T2D patients after intake of Metanx (T2D patients with peripheral neuropathy), "Medical Food"
Christen 2009: Hcy-level reduced in treated patients compared to placebo. AMD-incidents correlated with Hcy. (WAFACS)
Gopinath 2013: Hcy level reduced in patients with intake of folate and B12 from food. (BMES)

Pilot Study Ocufolin® forte

At the University of Vienna a pilot study assessed the effect of a 3-month folate supplementation with Ocufolin® forte on systemic homocysteine plasma concentration and ocular blood flow in patients with diabetes. Results show that 3 months intake of Ocufolin® forte is capable of significantly reducing blood homocysteine level in patients with diabetes. In addition, a tendency towards an increase in total retinal blood flow was observed. In diabetic retinopathy, it has been shown that the disease is associated with early retinal vascular dysregulation. “In the later states of the disease, retinal tissue hypoxia is a major trigger of sight-threatening neovascularization. In age-related macular degeneration, there is increasing evidence that reduced blood flow in the choroid is associated with the development and progression of the disease” (Pemp. Schmetterer, Can J Ophthalmol, 43, (2008), 295).

In case studies improvements in diabetic and hypertensive retinopathy have been reported (Wang et al., Eye and Vision, 2019; 6:21).

www.pubmed.ncbi.nlm.nih.gov/31363484

Availability of Ocufolin®

Ocufolin® is actually manufactured in Switzerland, presented in Blister Packs with 90 Capsules, in two formulations, Ocufolin® forte and Ocufolin® prevent.

In Europe, it has to date already been registered and has been available in Switzerland, Austria and Germany as a supplement since June 2016. Additional European Countries to follow in the near future. Ocufolin® is recommended to be used under the supervision of an ophthalmologist.

Importance of Formulation

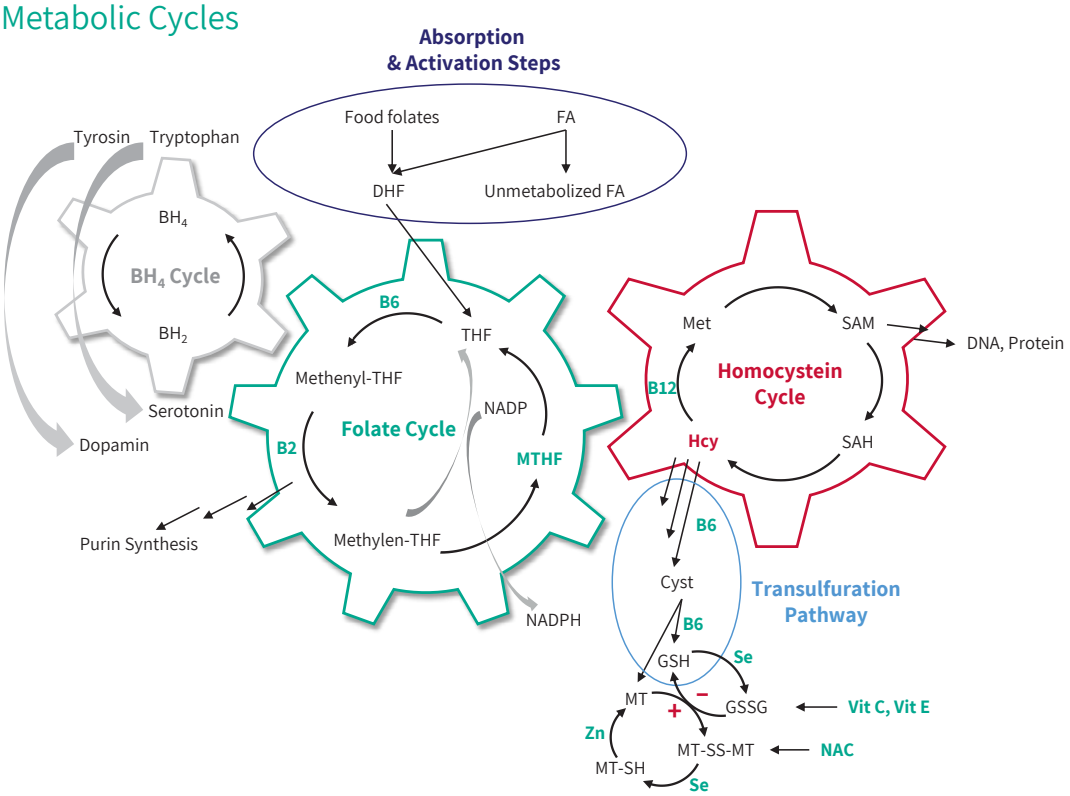
Ocufolin® in its lipophilic galenic preparation ensures proper absorption.

Encapsulating or solubilizing the ingredient in lipid excipients can lead to increased solubilization and absorption, resulting in enhanced bioavailability. (Kalepu et al., Acta Pharmaceutica Sinica B, 2013; 3(6): 361–372).

The importance of “nutritional and medical food therapies for diabetic retinopathy” has been reviewed (Shi et al., Eye and Vision, 2020; 7:33).

www.pubmed.ncbi.nlm.nih.gov/32582807

Composition in Metabolic Cycles



Sources: Lee et al., J Clin Org, 127, (2017), 1856; Ruttkay-Nedecky et al., Int J Mol Sci, 14, (2013), 6044 ; Dahlhoff et al., PLOS One, 8, (2013), e57387; Schmidt et al., Clin Sci, 113, (2007), 47; Ueland et al., Annu. Rev. Nutr. 35, (2015), 33; Fan et al., Nature, 510, (2014), 298; Fernandez-Arroyo et al., Oncoscience, 2, (2016), 958.

Abstracts: AMD und Folate

Hyperhomocysteinemia and Age-related Macular Degeneration: Role of Inflammatory Mediators and Pyroptosis; A Proposal

Mahavir Singh, Suresh C. Tyagi, Medical Hypotheses 105 (2017) 17–21

Age-related macular degeneration (AMD) and pyroptosis cause irreversible vascular changes in the eyes leading to central vision loss in patients. It is the most common eye disease affecting millions of people aged 50 years or older, and is slowly becoming a major health problem worldwide. The disease mainly affects macula lutea, an oval-shaped pigmented area surrounding fovea near the center of retina, a region responsible for visual acuity. It is fairly a complex disease as genetics of patients, environmental triggers as well as risk factors such as age, family history of CVDs, diabetes, gender, obesity, race, hyperopia, iris color, smoking, diabetes, exposure to sun light and pyroptosis have all been clubbed together as probable causes of macular degeneration. Among genes that are known to play a role include variant polymorphisms in the complement cascade components such as CFH, C2, C3, and CFB as potential genetic risk factors. So far, AMD disease hypothesized theories have not resulted into the anticipated impact towards the development of effective or preventive therapies in order to help alleviate patients' suffering because, as of today, it is still unclear what actually initiates or leads to this dreaded eye condition. Based upon our extensive work on the metabolism of homocysteine (Hcy) in various disease conditions we, therefore, are proposing a novel hypothesis for AMD pathogenesis as we strongly believe that Hcy and events such as pyroptosis make a greater contribution to the overall etiology of AMD disease in a target population of susceptible hosts by inciting and accelerating the inherent inflammatory changes in the retina of these patients (Fig. 2). In this context, we further state that Hcy and pyroptosis should be considered as legitimate and valuable markers of retinal dysfunction as they not only aid and abet in the development but also in the progression of AMD in older people as discussed in this paper. This discussion should open up new avenues in tackling inflammatory and pyroptosis centered pathways that are up-regulated or solely promoted by Hcy interaction within the ocular compartment of AMD susceptible hosts.

www.ncbi.nlm.nih.gov/pubmed/28735646

Homocysteine, folate, vitamin B12, and 10-y incidence of age-related macular degeneration (BMES).

Gopinath B, Flood VM, Rochtchina E, Wang JJ, Mitchell P. Am J Clin Nutr. 2013 Jul; 98 (1): 129-35.

BACKGROUND: Epidemiologic evidence of a relation between serum total homocysteine (tHcy), vitamin B12, and folate and age-related macular degeneration (AMD) is inconsistent and unresolved.

OBJECTIVE: In this cohort study, we aimed to investigate associations between intakes and serum concentrations of folate and vitamin B12 or serum tHcy and 10-y AMD incidence.

DESIGN: Serum folate, vitamin B12, and tHcy were determined from blood samples drawn in 1997-1999 from cohort members aged ≥ 55 y. AMD was assessed in 1760 survivors from retinal photographs taken in 2002-2004 and 2007-2009. Total intakes of folate and vitamin B12 were assessed by using a food-frequency questionnaire.

RESULTS: After adjustment for age, sex, current smoking, white blood cell count, and fish consumption, each 1-SD increase in serum tHcy was associated with increased risk of incident early and any AMD [ORs (95 % CIs): 1.33 (1.09, 1.63) and 1.33 (1.11, 1.60), respectively]. Participants with a serum vitamin B12 deficiency (<185 pmol/L) had higher risk of incident early and late AMD [ORs (95 % CIs): 1.58 (1.06, 2.36) and 2.56 (1.38, 4.73), respectively]. Folate deficiency (<11 nmol/L) was associated with 75 % and 89 % increased risk of incident early and any AMD, respectively, 10 y later. Participants who reported supplementary vitamin B12 intake had 47 % reduced risk of incident any AMD (OR: 0.53; 95 % CI: 0.33, 0.85).

CONCLUSION: Elevated serum tHcy and folate and vitamin B12 deficiencies predicted increased risk of incident AMD, which suggests a potential role for vitamin B12 and folate in reducing AMD risk.

www.ncbi.nlm.nih.gov/pubmed/23636242

Dietary folate, B vitamins, genetic susceptibility and progression to advanced nonexudative age-related macular degeneration with geographic atrophy: a prospective cohort study.

Merle BM, Silver RE, Rosner B, Seddon JM., Am J Clin Nutr. 2016 Apr;103(4):1135-44.

BACKGROUND: There is growing evidence of the importance of nutrition in age-related macular degeneration (AMD), but few studies have explored associations with folate and B vitamins. No effective therapeutic strategy for geographic atrophy (GA) is available, and prevention could be of great value.

OBJECTIVE: We investigated associations between dietary folate, B vitamins, and progression to GA and whether these associations might be modified by genetic susceptibility.

DESIGN: Among 2525 subjects (4663 eyes) in the Age-Related Eye Disease Study, 405 subjects (528 eyes) progressed to GA over 13 y. Folate and B vitamins were log transformed and calorie adjusted separately for men and women. Ten loci in 7 AMD genes [complement factor H, age-related maculopathy susceptibility 2/high-temperature requirement A serine peptidase 1, complement component 2, complement component 3, complement factor B, collagen type VIII 1, and RAD51 paralog B] were examined. Survival analysis was used to assess associations between incident GA and dietary intake of folate and B vitamins. Interaction effects between these nutrients and genetic variation on AMD risk were also evaluated. Subjects with at least one eye free of advanced AMD at baseline were included in these analyses.

RESULTS: There was a reduced risk of progression to GA with increasing intake of thiamin, riboflavin, and folate after adjusting for age, sex, and total energy intake (P-trend = 0.01, 0.03, and 0.001, respectively). After adjustment for demographic, behavioral, ocular, and genetic covariates, trends remained statistically significant for folate (P-trend = 0.007) and were borderline for thiamin (P-trend = 0.05). Riboflavin did not retain statistical significance (P-trend = 0.20). Folate was significantly associated with lower risk of incident GA among subjects homozygous for the complement component 3 (C3) R102G rs2230199 nonrisk genotype (CC) (HR = 0.43; 95 % CI: 0.27, 0.70; P = 0.0005) but not subjects carrying the risk allele (G) (P = 0.76). Neither folate nor any B vitamin was significantly associated with neovascular AMD.

CONCLUSIONS: High folate intake was associated with a reduced risk of progression to GA. This relation could be modified by genetic susceptibility, particularly related to the C3 genotype.

www.ncbi.nlm.nih.gov/pubmed/26961928

Folic acid, pyridoxine, and cyanocobalamin combination treatment and age-related macular degeneration in women: the Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS).

Christen WG, Glynn RJ, Chew EY, Albert CM, Manson JE. Arch Intern Med. 2009 Feb 23;169(4):335-41

BACKGROUND: Observational epidemiologic studies indicate a direct association between homocysteine concentration in the blood and the risk of age-related macular degeneration (AMD), but randomized trial data to examine the effect of therapy to lower homocysteine levels in AMD are lacking. Our objective was to examine the incidence of AMD in a trial of combined folic acid, pyridoxine hydrochloride (vitamin B6), and cyanocobalamin (vitamin B12) therapy.

METHODS: We conducted a randomized, double-blind, placebo-controlled trial including 5442 female health care professionals 40 years or older with preexisting cardiovascular disease or 3 or more cardiovascular disease risk factors. A total of 5205 of these women did not have a diagnosis of AMD at baseline and were included in this analysis. Participants were randomly assigned to receive a combination of folic acid (2.5 mg/d), pyridoxine hydrochloride (50 mg/d), and cyanocobalamin (1 mg/d) or placebo. Our main outcome measures included total AMD, defined as a self-report documented by medical record evidence of an initial diagnosis after randomization, and visually significant AMD, defined as confirmed incident AMD with visual acuity of 20/30 or worse attributable to this condition.

RESULTS: After an average of 7.3 years of treatment and follow-up, there were 55 cases of AMD in the combination treatment group and 82 in the placebo group (relative risk, 0.66; 95 % confidence interval, 0.47–0.93 [P = 0.02]). For visually significant AMD, there were 26 cases in the combination treatment group and 44 in the placebo group (relative risk, 0.59; 95 % confidence interval, 0.36–0.95 [P = 0.03]).

CONCLUSIONS: These randomized trial data from a large cohort of women at high risk of cardiovascular disease indicate that daily supplementation with folic acid, pyridoxine, and cyanocobalamin may reduce the risk of AMD.

www.ncbi.nlm.nih.gov/pubmed/19237716

Homocysteine and the risk of age-related macular degeneration: a systematic review and meta-analysis.

Huang P, Wang F, Sah BK, Jiang J, Ni Z, Wang J, Sun X. Sci Rep. 2015 Jul 21;5:10585.

Contrasting results have been reported regarding the associations between plasma total homocysteine (tHcy) and B vitamin levels and age-related macular degeneration (AMD) risk. Thus, we aimed to systematically evaluate these associations. Relevant case control studies in English were identified via a thorough search of the PubMed, Medline, and Embase databases from inception to June 2014. The results were pooled using Review Manager 5.2.1. Eleven studies (including 1072 cases and 1202 controls) were eligible for analysis of tHcy levels; additionally, 3 studies (including 152 cases and 98 controls) were eligible for analysis of folic acid and vitamin B12 levels. The

cumulative results demonstrated that the plasma tHcy level among the AMD cases was 2.67 µmol/L (95 % confidence interval [CI], 1.60–3.74) higher than that among the controls. In contrast, the vitamin B12 level among the AMD cases was 64.16 pg/mL (95 % CI, 19.32–109.00) lower than that among the controls. Subgroup analyses showed that the folic acid level was 1.66 ng/mL (95 % CI, 0.10–3.21) lower for the wet type. Together, the results demonstrated that AMD is associated with elevated tHcy levels and decreased vitamin B12 levels. Plasma tHcy may act as a modulator of the risk for AMD based on the current evidence.

www.ncbi.nlm.nih.gov/pubmed/23636242

Abstracts: AMD and Vitamin D

Association between vitamin D status and age-related macular degeneration by genetic risk

Amy E. Millen, Kristin J Meyers, Zhe Liu, Corinne D Engelman, Robert B Wallace, Erin S LeBlanc, Lesley F. Tinker, Sudha K Iyengar, Jennifer Robinson, Gloria E. Sarto and Julie A Mares, JAMA Ophthalmol. 2015 Oct; 133(10): 1171–117

IMPORTANCE: Deficient 25-hydroxyvitamin D [25(OH)D] concentrations have been associated with increased odds of age-related macular degeneration (AMD).

OBJECTIVE: We examined 1) whether this association is modified by genetic risk for AMD and 2) if there is an association between AMD and single nucleotide polymorphisms (SNPs) of genes involved in vitamin D transport, metabolism and genomic function.

DESIGN, SETTING, PARTICIPANTS: Women were postmenopausal and participants of the Carotenoids in Age-Related Eye Disease Study (CAREDS) (54 to <75 years) with available serum 25(OH)D concentrations (assessed from 1994–1998), genetic data, and measures of AMD (n=142) assessed at CAREDS baseline from 2001–2004 (n=913).

MAIN OUTCOMES AND MEASURES: Prevalent early or late AMD was determined from graded, stereoscopic fundus photographs. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for AMD by the joint effects of 25(OH)D (<30, ≥30 to <50, ≥50 to <75, and ≥75 nmol/L) and risk genotype (noncarrier, one, or two risk alleles). The referent group was noncarriers with adequate vitamin D status (≥75 nmol/L). Joint effect ORs were adjusted for age, smoking, iris pigmentation, self-reported cardiovascular disease, self-reported diabetes status, and hormone use. Additive and multiplicative interactions were assessed using the Synergy Index (SI) and an interaction term, respectively.

RESULTS: We observed a 6.7-fold increased odds of AMD (95% CI=1.6, 28.2) among women with deficient vitamin D status (25(OH)D <30 nmol/L) and two risk alleles for complement factor H (CFH) Y402H (SI for additive interaction=1.4, 95% CI=1.1, 1.7; p for multiplicative interaction=0.25. A significant additive (SI=1.4, 95% CI=1.1, 1.7) and multiplicative interaction (p=0.02) was observed for deficient women with two high risk complement factor I (CFI) (rs10033900) alleles (OR=6.3, 95% CI=1.6, 24.2). The odds of AMD did not differ by genotype of candidate vitamin D genes.

CONCLUSIONS AND RELEVANCE: In this study, the odds of AMD was highest in those with deficient vitamin D status and two risk alleles for CFH and CFI genotype suggesting a synergistic effect between vitamin D status and complement cascade protein function. Limited sample size led to wide confidence intervals. Findings may be due to chance or explained by residual confounding.

www.ncbi.nlm.nih.gov/pubmed/26312598

Abstracts: L-Methylfolate vs folic acid

L-5-Methyltetrahydrofolate Supplementation Increases Blood Folate Concentrations to a Greater Extent than Folic Acid Supplementation in Malaysian Women

Amanda M Henderson, Rika E Aleliunas, Su Peng Loh, Geok Lin Khor, Sarah Harvey-Leeson, Melissa B Glier, David D Kitts, Tim J Green, and Angela M Devlin, J Nutr. 2018 Jun 1; 148(6): 885–890.

BACKGROUND: Folic acid fortification of grains is mandated in many countries to prevent neural tube defects. Concerns regarding excessive intakes of folic acid have been raised. A synthetic analog of the circulating form of folate, L-5-methyltetrahydrofolate (L-5-MTHF), may be a potential alternative.

OBJECTIVE: The objective of this study was to determine the effects of folic acid or L-5-MTHF supplementation on blood folate concentrations, methyl nutrient metabolites, and DNA methylation in women living in Malaysia, where there is no mandatory fortification policy.

METHODS: In a 12-wk, randomized, placebo-controlled intervention trial, healthy Malaysian women (n = 142, aged 20–45 y) were randomly assigned to receive 1 of the following supplements daily: 1 mg (2.27 μmol) folic acid, 1.13 mg (2.27 μmol) L-5-MTHF, or a placebo. The primary outcomes were plasma and RBC folate and vitamin B-12 concentrations. Secondary outcomes included plasma total homocysteine, total cysteine, methionine, betaine, and choline concentrations and monocyte long interspersed nuclear element-1 (LINE-1) methylation.

RESULTS: The folic acid and L-5-MTHF groups had higher (P < 0.001) RBC folate (mean ± SD: 1498 ± 580 and 1951 ± 496 nmol/L, respectively) and plasma folate [median (25th, 75th percentiles): 40.1 nmol/L (24.9, 52.7 nmol/L) and 52.0 nmol/L (42.7, 73.1 nmol/L), respectively] concentrations compared with RBC folate (958 ± 345 nmol/L) and plasma folate [12.6 nmol/L (8.80, 17.0 nmol/L)] concentrations in the placebo group at 12 wk. The L-5-MTHF group had higher RBC folate (1951 ± 496 nmol/L; P = 0.003) and plasma folate [52.0 nmol/L (42.7, 73.1 nmol/L); P = 0.023] at 12 wk than did the folic acid group [RBC folate, 1498 ± 580 nmol/L; plasma folate, 40.1 nmol/L (24.9, 52.7 nmol/L)]. The folic acid and L-5-MTHF groups had 17% and 15%, respectively, lower (P < 0.001) plasma total homocysteine concentrations than did the placebo group at 12 wk; there were no differences between the folic acid and L-5-MTHF groups. No differences in plasma vitamin B-12, total cysteine, methionine, betaine, and choline and monocyte LINE-1 methylation were observed.

CONCLUSIONS: These findings suggest differential effects of L-5-MTHF compared with folic acid supplementation on blood folate concentrations but no differences on plasma total homocysteine lowering in Malaysian women. This trial was registered at clinicaltrials.gov as NCT01584050.

https://www.ncbi.nlm.nih.gov/pubmed/29878267

Folate, folic acid and 5-methyltetrahydrofolate are not the same thing

Francesco Scaglione, Giscardo Panzavolta, Xenobiotica, 2014; 44(5): 480–488

1. Folate, an essential micronutrient, is a critical cofactor in one-carbon metabolism. Mammals cannot synthesize folate and depend on supplementation to maintain normal levels. Low folate status may be caused by low dietary intake, poor absorption of ingested folate and alteration of folate metabolism due to genetic defects or drug interactions.
2. Folate deficiency has been linked with an increased risk of neural tube defects, cardiovascular disease, cancer and cognitive dysfunction. Most countries have established recommended intakes of folate through folic acid supplements or fortified foods. External supplementation of folate may occur as folic acid, folinic acid or 5-methyltetrahydrofolate (5-MTHF).
3. Naturally occurring 5-MTHF has important advantages over synthetic folic acid – it is well absorbed even when gastrointestinal pH is altered and its bioavailability is not affected by metabolic defects. Using 5-MTHF instead of folic acid reduces the potential for masking haematological symptoms of vitamin B12 deficiency, reduces interactions with drugs that inhibit dihydrofolate reductase and overcomes metabolic defects caused by methylenetetrahydrofolate reductase polymorphism. Use of 5-MTHF also prevents the potential negative effects of unconverted folic acid in the peripheral circulation.
4. We review the evidence for the use of 5-MTHF in preventing folate deficiency.

https://www.ncbi.nlm.nih.gov/pubmed/24494987

Abstracts: DR and homocysteine

Relationship between homocysteine level and diabetic retinopathy: a systematic review and meta-analysis

Xu et al. Diagnostic Pathology 2014, 13:167

BACKGROUND: The relationship between homocysteine (Hcy) and diabetic retinopathy (DR) remains unclear to date. Therefore, a systematic review and meta-analysis was performed on the relationship between Hcy level and DR.

METHODS: Studies were identified by searching PubMed, Embase, and Web of Science databases until 5 May, 2014.

RESULTS: A total of 31 studies involving 6,394 participants were included in the meta-analysis. After pooling the data from each included study, the blood Hcy concentration in the DR group was observed to be higher than that in the control group [WMD = 2.55; 95 % confidence interval (CI), 1.70–3.40], and diabetes mellitus (DM) patients with hyperhomocysteinemia were at a risk for DR [odds ratio (OR) = 1.93; 95 % CI, 1.46–2.53]. Considering the different DM types, hyperhomocysteinemia in T1DM (OR = 1.83, 95 % CI, 1.28–2.62) was associated with DR rather than in T2DM (OR = 1.59, 95 % CI, 0.72–3.51). Considerable statistical heterogeneity in the overall summary estimates was partly explained by the geographical differences.

CONCLUSIONS: Results from this current meta-analysis indicate that hyperhomocysteinemia is a risk factor for DR, especially proliferative DR. Differences between geographical regions were observed in the relationship between hyperhomocysteinemia with T1DM risk. Given the heterogeneous results, the relationship between high Hcy and DR needs further investigation.

https://doi.org/10.1186/s13000-014-0167-y

Vitamin Status as a Determinant of Serum Homocysteine Concentration in Type 2 Diabetic Retinopathy

Fotiou et al., Journal of Diabetes Research, Volume 2014, Article ID 807209, 7

We investigated the association of serum homocysteine levels and vitamin status with type 2 diabetic retinopathy. This study included 65 patients with and 75 patients without diabetic retinopathy. Patients with diabetic retinopathy had significantly higher serum homocysteine levels ($P < 0.001$), higher prevalence of hyperhomocysteinemia ($P < 0.001$), lower serum folic acid ($P < 0.001$), and vitamin B12 ($P= 0.014$) levels than those without diabetic retinopathy. Regression analysis revealed that homocysteine was an independent risk factor for diabetic retinopathy and there was a threshold in its serum level (13.7 μmol/L), above which the risk of diabetic retinopathy greatly increases (OR = 1.66, $P = 0.001$). Folic acid was associated with decreased odds for diabetic retinopathy (OR = 0.73, $P < 0.001$). There was a threshold in serum vitamin B12 level (248.4 pg/mL), below which serum homocysteine concentration significantly increases with decreasing serum vitamin B12 ($P = 0.003$). Our findings suggest that hyperhomocysteinemia is an independent risk factor for the development and progression of diabetic retinopathy. Decreased serum levels of folic acid and vitamin B12, through raising serum homocysteine concentrations, may also affect the diabetic retinopathy risk.

http://dx.doi.org/10.1155/2014/807209

Status of B-Vitamins and Homocysteine in Diabetic Retinopathy: Association with Vitamin-B12 Deficiency and Hyperhomocysteinemia

Satyanarayana A, Balakrishna N, Pitla S, Reddy PY, Mudili S, et al. (2011) Status of B-Vitamins and Homocysteine in Diabetic Retinopathy: Association with Vitamin-B12 Deficiency and Hyperhomocysteinemia. PLos one, 6(2011)1

Diabetic retinopathy (DR) is a common cause of blindness. Although many studies have indicated an association between homocysteine and DR, the results so far have been equivocal. Amongst the many determinants of homocysteine, B-vitamin status was shown to be a major confounding factor, yet very little is known about its relationship to DR. In the present study, we, therefore, investigated the status of B-vitamins and homocysteine in DR. A cross-sectional case-control study was conducted with 100 normal control (CN) subjects and 300 subjects with type-2 diabetes (T2D). Of the 300 subjects with T2D, 200 had retinopathy (DR) and 100 did not (DNR). After a complete ophthalmic examination including fundus fluorescein angiography, the clinical profile and the blood levels of all B-vitamins and homocysteine were analyzed. While mean plasma homocysteine levels were found to be higher in T2D patients compared with CN subjects, homocysteine levels were particularly high in the DR group. There were no group differences in the blood levels of vitamins B1 and B2. Although the plasma vitamin-B6 and folic acid levels were significantly lower in the DNR and DR groups compared with the CN group, there were no significant differences between the diabetes groups. Interestingly, plasma vitamin-B12 levels were found to be significantly lower in the diabetes groups compared with the CN group; further, the levels were significantly lower in the DR group compared with the DNR group. Higher homocysteine levels were significantly associated with lower vitamin-B12 and folic acid but not with other B-vitamins. Additionally, hyperhomocysteinemia and vitamin-B12 deficiency did not seem to be related to subjects' age, body mass index, or duration of diabetes. These results thus suggest a possible association between vitamin-B12 deficiency and hyperhomocysteinemia in DR. Further, the data indicate that vitamin-B12 deficiency could be an independent risk factor for DR.

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Hyperhomocysteinemia Alters Retinal Endothelial Cells Barrier Function and Angiogenic Potential via Activation of Oxidative Stress

Riyaz Mohamed, Isha Sharma, Ahmed S. Ibrahim, Heba Saleh, Nehal M. Elsherbiny, Sadanand Fulzele, Khaled Elmasry, Sylvia B. Smith, Mohamed Al-Shabrawey, Amany Tawfik, Nature Scientific Reports (2017), 7: 11952

Hyperhomocysteinemia (HHcy) is associated with several human visual disorders, such as diabetic retinopathy (DR) and age-related macular degeneration (AMD). Breakdown of the blood-retinal barrier (BRB) is linked to vision loss in DR and AMD. Our previous work revealed that HHcy altered BRB in retinal endothelial cells in vivo. Here we hypothesize that homocysteine (Hcy) alters retinal endothelial cell barrier function and angiogenic potential via activation of oxidative stress. Human retinal endothelial cells (HRECs) treated with and with-

out different concentrations of Hcy showed a reduction of tight junction protein expression, increased FITC dextran leakage, decreased transcellular electrical resistance and increased angiogenic potential. In addition, HRECs treated with Hcy showed increased production of reactive oxygen species (ROS). The anti-oxidant N-acetyl-cysteine (NAC) reduced ROS formation and decreased FITC-dextran leakage in Hcy treated HRECs. A mouse model of HHcy, in which cystathionine-β-synthase is deficient (cbs−/−), was evaluated for oxidative stress by dichlolorofluorescein (DCF), dihydroethidium (DHE) staining. There was a marked increase in ROS production and augmented GSH reductase and antioxidant regulator NRF2 activity, but decreased antioxidant gene expression in retinas of hyperhomocysteinemic mice. Our results suggest activation of oxidative stress as a possible mechanism of HHcy induced retinal endothelial cell dysfunction.

https://www.nature.com/articles/s41598-017-09731-y

Abstract: DR and vitamin D

The Association between Vitamin D Deficiency and Diabetic Retinopathy in Type 2 Diabetes: A Meta-Analysis of Observational Studies

Qin et al., Nutrients 2017, 9, 307

Emerging evidence from in vivo and in vitro studies have shown that vitamin D may play an important role in the development of diabetic retinopathy (DR), but individually published studies showed inconclusive results. The aim of this study was to quantitatively summarize the association between vitamin D and the risk of diabetic retinopathy. We conducted a systematic literature search of Pubmed, Medline, and EMBASE updated in September 2016 with the following keywords: “vitamin D” or “cholecalciferol” or “25-hydroxyvitamin D” or “25(OH)D” in combination with “diabetic retinopathy” or “DR”.

Fifteen observational studies involving 17,664 subjects were included. In this meta-analysis, type 2 diabetes patients with vitamin D deficiency (serum 25(OH)D levels <20 ng/mL) experienced a significantly increased risk of DR (odds ratio (OR) = 2.03, 95% confidence intervals (CI): 1.07, 3.86), and an obvious decrease of 1.7 ng/mL (95% CI: −2.72, −0.66) in serum vitamin D was demonstrated in the patients with diabetic retinopathy. Sensitivity analysis showed that exclusion of any single study did not materially alter the overall combined effect.

In conclusion, the evidence from this meta-analysis indicates an association between vitamin D deficiency and an increased risk of diabetic retinopathy in type 2 diabetes patients.

http://www.mdpi.com/2072-6643/9/3/307

Abstract: Folate transport and RPE

Expression and Differential Polarization of the Reduced-folate Transporter-1 and the Folate Receptor alpha in Mammalian Retinal Pigment Epithelium

Smith, Chancy et al., THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 275, No. 27, Issue of July 7, pp. 20676–20684, 2000

The differential polarized distribution of the reduced folate transporter (RFT-1) and folate receptor a (FR), the two proteins involved in the transport of folate, has been characterized in normal mouse retinal pigment epithelium (RPE) and in cultured human RPE cells. RPE cells mediate the vectorial transfer of nutrients from choroidal blood to neural retina. Whereas FR is known to be present in many cell types of the neural retina, in situ hybridization analysis in the present study demonstrated that RFT-1 is present only in RPE. Laser-scanning confocal microscopy using antibodies specific for RFT-1 demonstrated an apical distribution of this protein in cultured human and intact mouse RPE, which contrasts with the basolateral distribution of FR in these cells. The expression of RFT-1 in the RPE cell apical membrane was confirmed by functional studies with purified apical membrane vesicles from bovine RPE. These studies, done with N5-methyltetrahydrofolate (the predominant folate derivative in blood) and folate as substrates, have shown that RFT-1 functions in a Na- and C12-independent manner. The transporter is specific for folate and its analogs. A transmembrane H1 gradient influences the transport function of this protein markedly; the transport mechanism is likely to be either folate/H1 co-transport or folate/OH2 exchange. Based on the differential polarization of FR and RFT-1 in RPE, we suggest that these two proteins work in a concerted manner to bring about the vectorial transfer of folate across the RPE cell layer from the choroidal blood to the neural retina. This constitutes the first report of the differential polarization of the two folate transport proteins in any polarized epithelium.

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Folate nutrition and blood–brain barrier dysfunction

Patrick J Stover, Jane Durga and Martha S Field, Current Opinion in Biotechnology 2017, 44:146–152

Mammals require essential nutrients from dietary sources to support normal metabolic, physiological and neuronal functions, to prevent diseases of nutritional deficiency as well as to prevent chronic disease. Disease and/or its treatment can modify fundamental biological processes including cellular nutrient accretion, stability and function in cells. These effects can be isolated to a specific diseased organ in the absence of whole-body alterations in nutrient status or biochemistry. Loss of blood-brain barrier function, which occurs in inborn errors of metabolism and in chronic disease, can cause brain-specific folate deficiency and contribute to disease co-morbidity. The role of brain folate deficiency in neuropsychiatric disorders is reviewed, as well as emerging diagnostic and nutritional strategies to identify and address brain folate deficiency in blood–brain barrier dysfunction.

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Abstract: Ocufolin® Pilot Study

A pilot study to assess the effect of a 3-month folate supplementation on systemic homocysteine plasma concentration and ocular blood flow in patients with diabetes

Clinical investigator: Assoc. Prof. PD Dr. Gerhard Garhöfer, Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria. Schmidl, Garhöfer, et al., Mol. Vis., 2020; 26:326

SCOPE: There is evidence that a folate deficiency – and as the biological consequence of the latter – higher homocysteine plasma levels are associated with an increased risk of vascular associated diseases. For the eye, it has been shown that higher intake of folate reduces the risk of vascular related diseases such as age related macular degeneration. Further studies suggest that decreased serum levels of folate and vitamin B12 may be an independent risk factor for diabetic retinopathy. The reason for the association of low folate levels and the increased risk for vascular-associated ocular diseases is not entirely clear but may be at least partially related to an impairment of local blood flow regulation in these patients.

Whether supplementation with folate may improve vascular regulation has not yet been sufficiently investigated. However, given that the potential effect size of a folate substitution on blood flow and systemic blood parameters is unclear, a proper statistical design for a large, controlled, randomized study is difficult. Thus, the present pilot study should (1) provide information about the homocysteine lowering potential of the formulation under study and (2) identify potential vascular related outcome parameters for further, larger, placebo-controlled studies and provide sufficient data to allow for a proper statistical planning for such a study.

Consequently, the current study seeks to investigate the effect of a 3-month supplementation with folate on systemic homocysteine plasma levels. Further, ocular blood flow and endothelial function in the ocular microcirculation will be assessed. For this purpose, a group of 25 patients with diabetes mellitus will be included in the study. Outcome parameters will be assessed at baseline and after a 3-month supplementation with folate.

RESULTS: After three months, plasma homocysteine concentration significantly decreased from 14.2 ± 9.3 to 9.6 ± 6.6 µmol/L ($p < 0.001$). In addition, a tendency towards an increase in total retinal blood flow from 36.8 ± 12.9 to 39.2 ± 10.8 µl/min was observed, but this effect did not reach the level of significance ($p = 0.11$). Supplementation had no effect on retinal vessel diameters, retinal blood flow in single vessels, retinal oxygen saturation or flicker-induced vasodilatation or the response of retinal blood flow to flickering light. IOP significantly decreased from 14.8 ± 3.0 to 13.4 ± 2.2 mmHg ($p = 0.02$).

SUMMARY/CONCLUSION: The present data show that a 3-months intake of a dietary supplement containing a moderate dose of folate is safe and capable of significantly reducing blood homocysteine levels in patients with diabetes. This is of special importance, since higher homocysteine plasma levels have been found to be associated with an increased risk of vascular associated diseases. Systemic folate supplementation also had a slight effect on retinal blood flow and a significant effect on IOP. Further studies on these parameters in larger cohorts should be performed.

www.molvis.org/molvis/v26/326

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