One Carbon Metabolism and Aging Eye Disease: Exploring Preventive Therapeutic Targets

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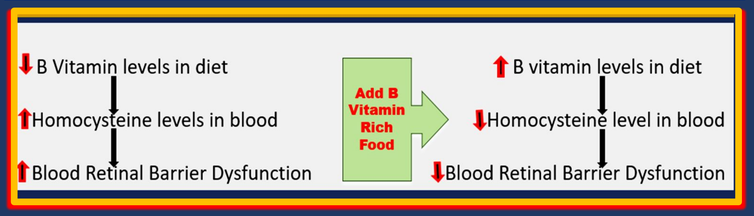
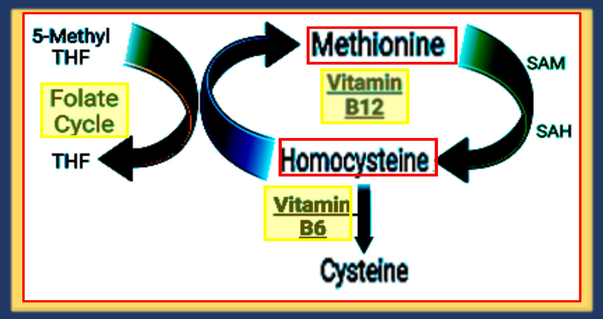
HYPOTHESIS





BACKGROUND

Homocysteine (Hcy) is an amino acid intermediate in the methionine and folate cycles that is dependent on B vitamins such as pyridoxine (B6), folate (B9) and cobalamin (B12). Deficiency in these vitamins leads to elevated levels of plasma Hcy known as hyperhomocysteinemia (HHcy) which is a major risk factor for neurodegenerative and cardiovascular disorders. B12 deficiency has been reported to have harmful effects on ocular structure and retinal vasculature in humans[1]. Previous research has demonstrated a strong correlation between elevated levels of homocysteine (HHcy) and the onset of age-related macular degeneration (AMD), which is linked to reduced levels of vitamin B12[2].

In a prior investigation conducted by our laboratory, demonstrated unreported impacts of elevated homocysteine levels on the structure and function of retinal pigment epithelium (RPE), concluding in the emergence of characteristics resembling age-related macular degeneration[3]. Moreover, we reported the involvement of HHcy in development of DR and proposed Hcy as a biomarker for DR. Furthermore, our studies reported HHcy induced dysfunction of the BRB, activates oxidative and endoplasmic reticulum stresses, and induces retinal neovascularization and epigenetic modifications suggesting the involvement of Hcy in the pathogenesis of diabetes-induced retinal damage[4]. Additionally, a correlation was found between elevated serum homocysteine levels and the severity of retinopathy[3].

To assess the effectiveness of B12 treatment, we investigated whether supplementation with B vitamins (B6, B9, B12) would expedite homocysteine metabolism via the remethylation pathway. This was done to clear elevated homocysteine levels in the blood stream and alleviate retinal dysfunction using an experimental mouse model

RESULTS

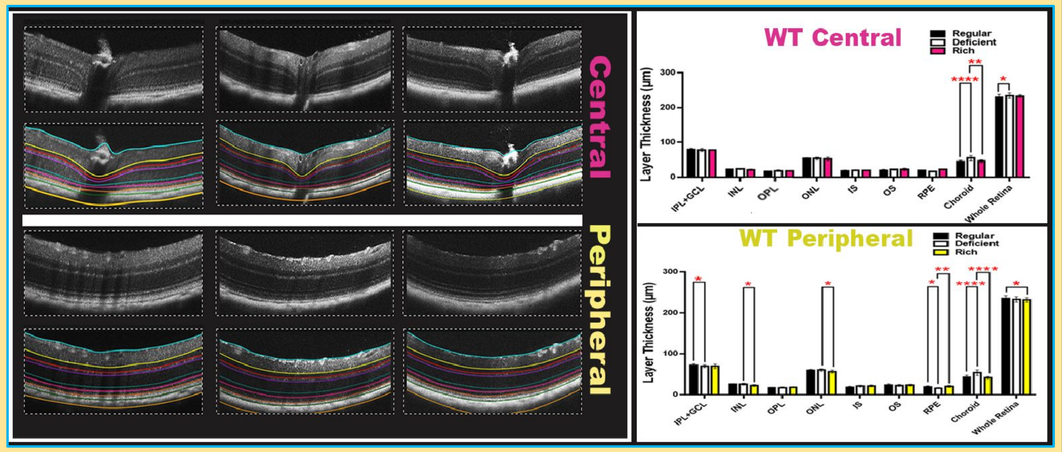


Fig.1.A Optical Coherence Tomography (OCT) images for wild-type, C57BL6 (WT) mice retina on customized B vitamin diet regimen showing morphological changes in the retina centrally and peripherally (Regular diet □ deficient diet □ rich diet). **1.B.** Analysis for the OCT measurements that was conducted using Phoenix Insight 2D software. The analysis is showing statistically significant changes mainly in the RPE/choroid areas of the retinas. B vitamin deficiency significantly increased choroidal thinness indicating neovascularization compared to regular diet group and this was reduced to normal by vitamin B supplementation both in the peripheral and central retina. Also, B vitamin deficiency significantly decreased RPE thickness (indicating death) compared to regular diet, that was restored to normal level by the B vitamin supplementation diet as noticed in the retina peripherally. B vitamin supplementation protected the retina from the effect of HHcy (induced by B vitamin deficiency) by restoring RPE and reducing choroidal neovascularization.
P<0.5. (IPL/GCL, inner plexiform layer/ganglion cell layer, INL inner nuclear layer, OPL outer plexiform layer, ONL outer nuclear layer, IS inner segment, OS outer segment, RPE retinal pigment epithelium),n=24

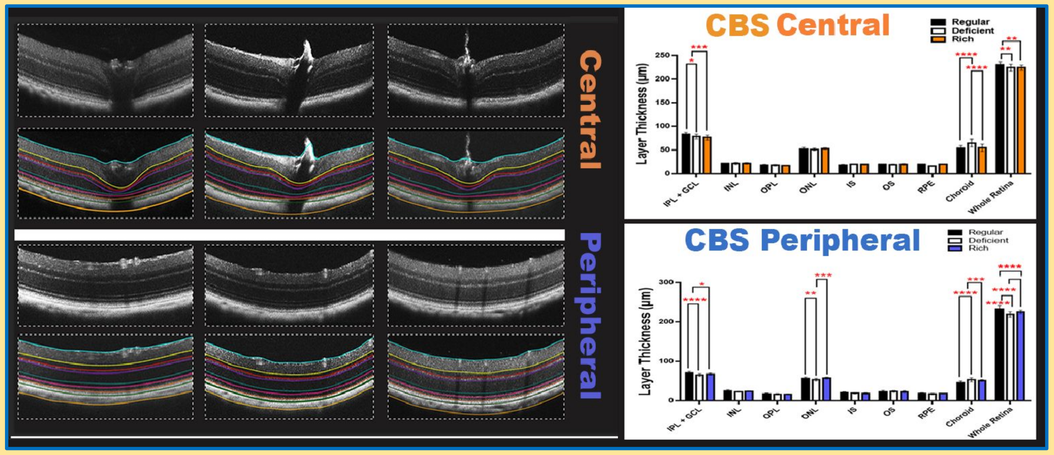
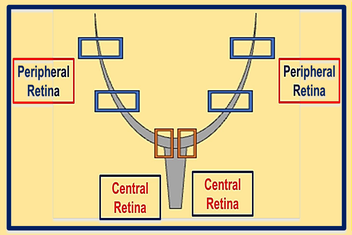
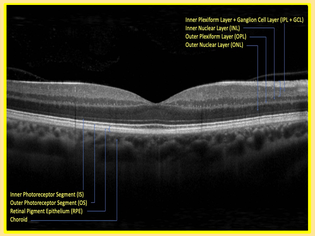


Fig.2.A Optical Coherence Tomography (OCT) images for CBS (mice with HHcy), showing morphological changes in the retina centrally and peripherally (Regular B vitamin diet □ deficient B vitamin diet □ rich B vitamin diet). **2.B.** Analysis for the CBS mice retina on customized diet regimen showing statistically significant changes in the IPL+GCL, choroid and retina centrally, and choroid, ONL, and IPL+GCL peripherally. Mice with B vitamin deficient diet had an increase in choroidal thickness and reduction in retinal thickness both centrally and peripherally compared to those that were on regular diet and this was corrected by B vitamin supplementation. B vitamin supplementation corrected the decrease in the IPL+GCL and ONL thickness in the B vitamin deficient diet group peripherally**.** Moreover, B vitamin supplementation helped to reduce the effect of HHcy induced reduction in the neuronal part of the retina (GCL and ONL), RPE, choroidal neovascularization, and increased over all retinal thickness.
P<0.5. (IPL/GCL, inner plexiform layer/ganglion cell layer, INL inner nuclear layer, OPL outer plexiform layer, ONL outer nuclear layer, IS inner segment, OS outer segment, RPE retinal pigment epithelium), n=24

METHODS





Schematic Of Retina With Images Taken Peripherally, Centrally, and OCT retinal layers

- Mice with HHcy due to Cystathionine Beta-Synthase enzyme deficiency (CBS) mice (24 mice) and wild type, C56-black-6 (WT) mice (24 mice) were placed on a commercialized diet (regular, vitamin B deficient and B vitamin supplemented diet) for nine months.
- **CBS mice** were divided into three groups (8 mice/group), feeding on regular, vitamin B deficient, and B vitamin supplemented diet.
- **WT mice** were initially divided into two groups:
  - One group of 8 mice were placed on a normal diet
  - 16 were put on a vitamin B deficient diet
  - Once vitamin B levels were low, 8 WT mice were placed on a vitamin supplemented diet while the remaining 8 mice stayed on the vitamin deficient diet.
- Mice were evaluated every 8 weeks with Optical Coherence Tomography (OCT) imaging to evaluate retinal structures and layers in the 3 test groups for both the CBS and WT mice with 6 readings/retina
- Images were captured with Phoenix Micron IV retinal imaging system and analyzed with Phoenix Insight 2D software.

CONCLUSIONS

B vitamin supplementation improved metabolism of Hcy and decreased the retinal morphologic changes caused by HHcy, prompting discussion that B vitamins can be a potential therapeutic target and preventive method for aging diseases with HHcy such as diabetic retinopathy and age-related macular degeneration.

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