



Plasma homocysteine and macular thickness in older adults—the Rugao Longevity and Aging Study

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Received: 3 September 2020 / Revised: 8 March 2021 / Accepted: 9 April 2021 / Published online: 11 May 2021
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Abstract

Objectives To determine the association of plasma homocysteine levels with retinal layer thickness in a large community cohort of older adults.

Methods The Rugao Longevity and Ageing Study is an observational, prospective and community-based cohort study. A total of 989 older adults who underwent spectral-domain optical coherence tomography (SD-OCT) were included and analyzed. Foveal, macular retinal nerve fibre layer (mRNFL) and ganglion cell layer plus inner plexiform layer (GC-IPL) thicknesses were measured by SD-OCT. Plasma homocysteine levels were measured using chemiluminescence immunoassay. Linear regression analyses were performed to evaluate the relationship between plasma homocysteine and retinal layer thickness while controlling for confounding factors.

Results Of the 989 participants, 500 (50.56%) were men. The mean age was 78.26 (4.58) years, and the mean plasma homocysteine level was 16.38 (8.05) $\mu\text{mol/L}$. In multivariable analyses, each unit increase in plasma homocysteine was associated with an 8.84×10^{-2} (95% CI: -16.54×10^{-2} to -1.15×10^{-2} , $p = 0.032$) μm decrease in the average inner thickness of the GC-IPL after controlling for confounding factors. The association remained significant even in participants without major cardiovascular disease or diabetes ($\beta = -10.33 \times 10^{-2}$, 95% CI: -18.49×10^{-2} to -2.18×10^{-2} , $p = 0.013$). No significant associations of plasma homocysteine levels with macular thickness or mRNFL were found in primary and sensitivity analyses ($p > 0.05$).

Conclusions Increased plasma homocysteine levels are associated with a thinner GC-IPL. Plasma homocysteine may be a risk factor for thinner retinas in older adults.

Introduction

Spectral-domain optical coherence tomography (SD-OCT), a rapid and noninvasive imaging technique, is commonly used

to measure the retinal profile and has transformed our understanding of macular structure in health and disease [1]. Changes in retinal thickness have been increasingly recognized as potential diagnostic markers in hydroxychloroquine retinal toxicity [2, 3] and glaucoma [4–6]. Many cross-sectional and longitudinal studies have demonstrated the relationship between retinal thickness and Alzheimer’s disease [7–9]. In addition, the thinning of the retina has been associated with cerebral grey matter and white matter atrophy [10, 11], as well as worse white matter microstructure of the brain [11–13]. Retinal thickness was also recognized as a feasible biomarker for the early diagnosis of Alzheimer’s disease. Therefore, it is important to identify potential risk factors impacting retinal thickness and conduct interventions before clinical symptoms emerge.

Homocysteine is a sulfur-containing amino acid and acts as an intermediate product of methionine metabolism [14, 15]. It is a potential biomarker for predicting frailty

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Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41433-021-01549-3>.

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[16, 17] and cardiovascular and all-cause mortality [18, 19] in older adults. Previous studies have illustrated the deleterious effects of hyperhomocysteinemia (Hhcy) on retinal ganglion cells (RGCs), including decreased RGC viability [20], dysregulation of mitochondrial dynamics [21] and increased oxidative stress [22]. Hence, it is not surprising that increased plasma homocysteine levels are associated with the risk of retinal disease [23–25]. Previous studies have usually focused their attention on the association of homocysteine with retinal diseases, such as age-related macular degeneration [23], glaucoma [24] and diabetic retinopathy [25]. Moreover, those studies on plasma homocysteine with retinal thickness were conducted specifically in patients with diseases that directly influenced retinal thickness. There is still a lack of studies exploring the relationship in healthy older adults. Therefore, we conducted this study to explore the relationship between plasma homocysteine levels and retinal thickness measured by SD-OCT in older adults.

Methods

Participants

The Rugao Longevity and Ageing Study (RuLAS) is an observational, prospective and population-based cohort study [26]. The baseline survey was conducted between November 2014 and December 2014 (wave 1). A total of 1788 elderly adults aged 70–84 years were recruited. Then, follow-up surveys were conducted in April 2016 (wave 2), November 2017 (wave 3) and December 2019 (wave 4). This study was approved by the Human Ethics Committee of the School of Life Sciences of Fudan University, and informed consent was obtained from each participant.

In December 2019, a detailed ophthalmic examination was added to the protocol and performed at wave 4 of the cohort. A total of 2200 older adults were recruited in wave 4. Eye conditions were determined by an examining ophthalmologist (HS). Two experienced ophthalmologists (HS and WG) identified subjects with eye diseases based on the examination records and clinical history. Final adjudication was made by a senior ophthalmologist if a primary diagnosis was not definite during the initial assessment. SD-OCT was available for 1721 subjects (78.22%). Subjects who did not undergo SD-OCT ($n = 479$) or had ungradable OCT due to poor-quality scans ($n = 538$) were excluded, and 120 subjects were also excluded due to a lack of plasma homocysteine data. Furthermore, subjects with retinal-related diseases, including age-related macular degeneration ($n = 14$), diabetic retinopathy ($n = 6$), pathological myopia ($n = 39$) and other retinal diseases ($n = 15$), were

excluded. Finally, 989 participants were analyzed in this study. Age-related macular degeneration was defined according to the Wisconsin Age-related Maculopathy Grading System [27]. Diabetic retinopathy was graded using the Early Treatment Diabetic Retinopathy Study (ETDRS) criteria [28]. Pathological myopia was diagnosed as an eye with refractive error exceeding -6.0 dioptres with one or more of the following findings: tessellated fundus, diffuse chorioretinal atrophy, patchy chorioretinal atrophy, macular atrophy, or presence of lacquer cracks, choroidal neovascularization, Fuchs spot or posterior staphyloma [29]. In addition, the diagnoses of retinal detachment, epiretinal membranes and other diseases followed the clinical standard.

Demographic, physiological and clinical data were collected from the RuLAS in wave 4. Demographic data included age, sex, marital status, education, smoking, and alcohol consumption. Physiological variables, including body mass index (BMI), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride and creatinine, were also measured. Clinical variables, including high-sensitivity C-reactive protein (Hs-CRP) levels and self-reported major cardiovascular disease (CVD) and diabetes mellitus, were collected. Major CVDs, including cerebral infarction, stroke, cerebral haemorrhage, coronary heart disease, myocardial infarction and heart failure, were recorded. Participants who self-reported a diagnosis of diabetes mellitus, used medication for diabetes mellitus, or had fasting glucose greater than 125 mg/dL were coded as having diabetes mellitus. In addition, mobility (measured by the timed up and go test), grip strength, depressive status (assessed by the 15-item Geriatric Depression Scale) and cognitive function (assessed by revised Hasegama's dementia scale) were assessed. Plasma homocysteine levels were measured using a chemiluminescence immunoassay.

SD-OCT scan

SD-OCT scanning was performed without pupil dilation using the spectral domain OCT-HS100 (Canon Inc, Tokyo, Japan) with macular 3D scans over a 10×10 mm area (1024 A-scan \times 128 B-scan). The measurement of the right eye was used if the signal strength index was ≥ 5 . Otherwise, the left eye was used. The ETDRS grid focused on the macula was used for OCT measurements. The ETDRS grid consisted of three concentric circles of 1-, 3-, and 6-mm diameters. The 3- and 6-mm circles were each divided into superior, inferior, nasal, and temporal quadrants. Thickness measurements were conducted by an experienced examiner (HS). To determine the intra- and interobserver repeatability of the measurements, the first 20 images were obtained twice by the same examiner (HS). Another examiner (WG) independently

repeated the measurements with the same 20 measurements to determine the interobserver repeatability [30]. The intraobserver (intraclass correlation coefficient [ICC]: 0.993; 0.991–0.994) and interobserver (ICC: 0.991; 0.989–0.993) reproducibility were excellent for all measurements. The thickness was automatically segmented and manually corrected by two masked examiners (HS and WG) if necessary. The following segmented intraretinal layers were analyzed: full-thickness, macular retinal nerve fibre layer (mRNFL), ganglion cell layer plus inner plexiform layer (GC-IPL). The mean thickness was calculated as follows: average inner thickness = (superior inner + inferior inner + nasal inner + temporal inner)/4, and average outer thickness = (superior outer + inferior outer + nasal outer + temporal outer)/4.

Statistical analyses

First, we describe the characteristics of the participants in our study. Continuous and categorical variables are presented as the mean with standard deviation (SD) and frequency (%), respectively. Group differences were analyzed by chi-square or *t* tests. Second, we evaluated the relationship between plasma homocysteine levels and retinal thickness using linear regression in four models. Model 1 was unadjusted; model 2 was adjusted for age, BMI, sex, smoking, alcohol consumption, marital status, and illiteracy; model 3 was adjusted for model 2 + fasting glucose, triglyceride, HDL, LDL, creatinine and Hs-CRP levels; and model 4 was adjusted for model 3 + CVD, hypertension, diabetes, mobility, grip strength, depressive status and cognitive function. Finally, sensitivity analyses using linear regression models were introduced to validate the relationship in participants without CVD and/or diabetes. A *p* value (two-tailed) less than 0.05 was considered statistically significant. All analyses were conducted by SPSS 22.0 or R (Version 3.6.1: www.r-project.org/).

Results

Characteristics of included participants

Between December 2019 and January 2020, 2200 older adults were recruited. Among them, 989 (44.958%) participants were analyzed (Supplemental Table 1). Tables 1 and 2 summarize demographic, physiological and clinical data for the participants with SD-OCT measurements. In this cross-sectional study, the mean (SD) age of the participants included in this study was 78.26 (4.58) years. The proportion of participants who were men was 50.56%, 114 (9.83%) had self-reported major CVD events, 140 (14.16%) had diabetes, 563 (56.93%) had self-reported hypertension

99(10.01%). The mean plasma homocysteine level was 16.38 (8.05) $\mu\text{mol/L}$. Table 2 shows the retinal layer thickness of the participants in the RuLAS. The detailed thicknesses of the sectors were also shown (Table 2).

Associations of plasma homocysteine with overall macular and mRNFL thicknesses

In primary (Table 3) and sensitivity (Table 4) analyses, no significant association was found between full retinal thickness and plasma homocysteine levels in either unadjusted or adjusted models ($p > 0.05$).

In primary analyses (Table 3), the association of plasma homocysteine levels with mRNFL thickness was significant for the average outer thickness ($\beta = -4.32 \times 10^{-2}$, 95% CI: -8.35×10^{-2} to -0.29×10^{-2} , $p = 0.036$) but not for the average inner thickness ($\beta = -1.43 \times 10^{-2}$, 95% CI: -3.68×10^{-2} to -0.82×10^{-2} , $p = 0.213$) in unadjusted models. The relationships between the three sectors of the mRNFL and homocysteine levels were significant (superior outer, nasal outer). However, these associations of homocysteine with the average and sector thicknesses were not significant after adjusting for age, body mass index, sex, smoking, alcohol consumption, marital status, illiteracy, self-reported CVD, hypertension, diabetes, mobility, grip strength, fasting glucose, triglyceride, HDL, LDL, and creatinine levels, depressive status and cognitive function. In sensitivity analyses (Table 4), similar results showed that there was no significant relationship between plasma homocysteine levels and mRNFL thickness ($p > 0.05$).

Associations of plasma homocysteine with GC-IPL thickness

In primary analyses (Table 3), the association of plasma homocysteine levels with average inner thickness was significant in unadjusted ($\beta = -9.56 \times 10^{-2}$, 95% CI: -16.28×10^{-2} to -2.83×10^{-2} , $p = 0.005$) and adjusted models ($\beta = -8.84 \times 10^{-2}$, 95% CI: -16.54×10^{-2} to -1.15×10^{-2} , $p = 0.024$). The association of plasma homocysteine levels with GC-IPL sectors was also significant in the superior inner, inferior inner, nasal inner, temporal inner and temporal outer sectors. In addition, the associations of plasma homocysteine levels with the superior inner layer and temporal inner layer were still significant after controlling for confounding factors.

In sensitivity analyses (Table 4), the relationship between plasma homocysteine levels and average inner thickness was significant in unadjusted ($\beta = -11.12 \times 10^{-2}$, 95% CI: -18.46×10^{-2} to -3.78×10^{-2} , $p = 0.003$) and adjusted models ($\beta = -10.33 \times 10^{-2}$, 95% CI: -18.49×10^{-2} to -2.18×10^{-2} , $p = 0.013$) after excluding participants with

Table 1 Characteristics of included study population.

Characteristics	Total (N = 989)	Males (N = 499)	Females (N = 489)
Age, Mean (SD), years	78.26 (4.58)	78.33 (4.27)	78.27 (4.91)
Homocysteine [‡] , mean (SD), $\mu\text{mol/L}$	16.38 (8.05)	18.19 (10.24)	14.54 (4.17)
≤ 75 years [‡] , Mean (SD), $\mu\text{mol/L}$	14.49 (4.29)	17.05 (8.64)	13.04 (2.94)
76–80 years [‡] , Mean (SD), $\mu\text{mol/L}$	15.89 (5.44)	17.37 (8.52)	14.64 (4.27)
81–85 years [‡] , Mean (SD), $\mu\text{mol/L}$	16.83 (5.04)	20.13 (13.74)	15.42 (3.92)
≥ 86 years [‡] , Mean (SD), $\mu\text{mol/L}$	19.90 (6.97)	21.90 (12.07)	17.42 (5.98)
Body mass index, Mean (SD), kg/m^2	24.02 (3.74)	23.76 (8.94)	23.90 (5.32)
Smoking [‡] , No. (%)	243 (24.57%)	223 (44.69%)	20 (4.09%)
Alcohol accumulation [‡] , No. (%)	348 (35.19%)	242 (48.50%)	106 (21.68%)
Married and living together [‡] , No. (%)	615 (62.18%)	349 (69.94%)	266 (54.40%)
Illiteracy [‡] , No. (%)	373 (37.71%)	68 (13.63%)	305 (62.37%)
Major CVD events, No. (%)	99 (10.01%)	47 (9.42%)	52 (10.63%)
Hypertension [‡] , No. (%)	563 (56.93%)	266 (53.31%)	297 (60.74%)
Anti-hypertension drugs, No. (%)	207 (20.93%)	104 (20.84%)	103 (21.06%)
Diabetes, No. (%)	140 (14.16%)	65 (13.03%)	75 (15.34%)
Time up and go test [‡] , mean (SD), m/s	9.92 (3.07)	9.49 (2.79)	10.35 (3.28)
Grip strength [‡] , mean (SD), kg	21.98 (7.96)	26.74 (7.51)	17.11 (4.87)
FBG, Mean (SD), mg/dL	104.95 (29.31)	104.50 (31.94)	105.40 (26.39)
Triglyceride [‡] , mean (SD), mmol/L	1.49 (1.27)	1.32 (1.36)	1.66 (1.14)
HDL, mean (SD), mmol/L	1.67 (0.37)	1.65 (0.35)	1.68 (0.39)
LDL [‡] , mean (SD), mmol/L	3.16 (0.71)	3.03 (0.68)	3.29 (0.72)
Creatinine [‡] , mean (SD), $\mu\text{mol/L}$	72.25 (21.80)	81.10 (24.47)	63.25 (13.75)
Hs-CRP [‡] , median (IQR), mg/L	1.40 (0.80, 3.10)	1.40 (0.80, 3.30)	1.50 (0.80, 2.80)
GDS-15 [‡] , mean (SD)	2.36 (2.05)	2.12 (1.90)	2.61 (2.18)
HDS-R [‡] , mean (SD)	22.53 (6.27)	25.80 (5.25)	19.19 (5.42)

Major CVD including cerebral infarction, stroke, cerebral haemorrhage, coronary heart disease, myocardial infarction and heart failure. Continuous and categorical variables were present as M (SD) and frequency (%). Group difference between sex were analyzed by *t*-test or chi-square.

Hs-CRP high-sensitivity C-reactive protein, *GDS-15* the 15-item Geriatric Depression Scale, *HDS-R* revised Hasegama's dementia scale, *FBG* fasting blood glucose, *HDL* high-density lipoprotein, *LDL* low density lipoprotein, *SD* standard deviation, *IQR* interquartile range, *CVD* cardiovascular disease.

[‡]*p* value < 0.05; [‡]*p* value < 0.01; [‡]*p* value < 0.001.

major CVD and/or diabetes. The associations of plasma homocysteine levels with particular GC-IPL sectors (superior inner, inferior inner, nasal inner, temporal inner, nasal outer, temporal outer layers) were significant in unadjusted models, with the exception of the inferior outer layer. Novelty, the association of plasma homocysteine levels with GC-IPL thickness remained significant after adjusting for confounding factors in participants without CVD and/or diabetes in the superior inner layer, temporal inner layer and temporal outer layer.

Discussion

To our knowledge, this is the first study to identify that increased plasma homocysteine levels were associated with thinner GC-IPL after adjustment for confounding factors in

a large, healthy community cohort of older adults (Fig. 1). This association remained significant in the sensitivity analyses that excluded participants with major CVD and/or diabetes. In contrast, no significant associations of plasma homocysteine level with mRNFL thickness were observed. Our findings suggested that increased homocysteine might be a risk factor for decreased retinal thickness.

There is still a lack of research exploring the precise measurement of retinal thickness and plasma homocysteine levels in older adults. Previous studies focused their attention on the association of homocysteine with age-related macular degeneration [23, 31], diabetic retinopathy [32–34] and glaucoma [24, 35]. Our results added complementary knowledge; we found a significant association between plasma homocysteine levels and GC-IPL thickness in healthy older adults, who were specifically not patients with a disease that directly influences retinal thickness. Notably,

Table 2 Characteristics of macular thickness in included study individuals.

Characteristics	Findings ($N = 989$)
Full thickness	
Foveal, mean (SD), μm	222.69 (23.37)
Central subfield, mean (SD), μm	264.89 (23.39)
Average inner, mean (SD), μm	327.86 (17.50)
Average outer, mean (SD), μm	290.42 (15.10)
Superior inner, mean (SD), μm	328.96 (19.07)
Inferior inner, mean (SD), μm	327.97 (19.40)
Nasal inner, mean (SD), μm	334.18 (19.69)
Temporal inner, mean (SD), μm	320.35 (18.01)
Superior outer, mean (SD), μm	297.40 (19.24)
Inferior outer, mean (SD), μm	277.64 (18.57)
Nasal outer, mean (SD), μm	307.07 (17.14)
Temporal outer, mean (SD), μm	279.57 (15.89)
RNFL sectors thickness	
Average inner, mean (SD), μm	20.28 (2.90)
Average outer, mean (SD), μm	34.31 (5.20)
Superior inner, mean (SD), μm	20.76 (4.31)
Inferior inner, mean (SD), μm	23.37 (4.69)
Nasal inner, mean (SD), μm	19.41 (3.55)
Temporal inner, mean (SD), μm	17.58 (2.69)
Superior outer, mean (SD), μm	35.52 (7.01)
Inferior outer, mean (SD), μm	38.24 (7.16)
Nasal outer, mean (SD), μm	44.27 (7.59)
Temporal outer, mean (SD), μm	19.21 (2.71)
GC-IPL sectors thickness	
Average inner, mean (SD), μm	84.23 (8.69)
Average outer, mean (SD), μm	66.78 (6.06)
Superior inner, mean (SD), μm	85.33 (9.96)
Inferior inner, mean (SD), μm	85.07 (10.06)
Nasal inner, mean (SD), μm	86.18 (9.63)
Temporal inner, mean (SD), μm	80.32 (9.17)
Superior outer, mean (SD), μm	67.80 (8.42)
Inferior outer, mean (SD), μm	59.88 (7.50)
Nasal outer, mean (SD), μm	70.87 (7.01)
Temporal outer, mean (SD), μm	68.57 (7.13)

GC-IPL ganglion cell layer plus inner plexiform layer, RNFL retinal nerve fibre layer.

we used precise measurements of retinal thickness by SD-OCT to evaluate the associations. Our results provide direct evidence that increased plasma homocysteine concentrations were associated with a thinner GC-IPL.

Interestingly, clinical studies have illustrated the potential benefit of Hcy-lowering interventions (such as vitamin B and folic acid supplementation). A pilot study found that intervention with Metanx[®] vitamin tablets appeared to reduce retinal oedema and increase light sensitivity in

subjects with nonproliferative diabetic retinopathy [36]. In addition, high vitamin B6 intake was associated with a lower incidence of diabetic retinopathy in Japanese patients with type 2 diabetes [37]. Similarly, participants with serum vitamin B-12 deficiency or folate deficiency had a higher risk of incident early and late AMD [31, 38], while participants who reported supplementary vitamin B-12 intake had a reduced risk of incident AMD (OR = 0.53; 95% CI: 0.33, 0.85) [31]. Therefore, homocysteine-lowering interventions may be beneficial for the retina. Conversely, similar to the unclear association of Hcy with CVD [39], the refined impact of Hcy-lowering therapy regarding the benefits of macular thickness needs to be validated in intervention studies in the future.

The most likely mechanism for the increased plasma homocysteine levels being associated with thinner macular GC-IPL is that Hcy induced disorder in the structure and function of RGCs. It has been demonstrated that Hhcy could induce death of RGCs in vitro. The potential mechanisms of death include dysregulation of mitochondrial dynamics [21], elevated intracellular calcium and increased oxidative stress in the form of increased superoxide and nitric oxide levels [20, 34, 40]. In in vivo studies, homozygous and heterozygous deletion of the cystathionine- β -synthase (*cbs*) gene in mice (resulting in increased plasma homocysteine and representing a model for severe homocysteinemia [41]) also had elevated retinal homocysteine [22]. The retinas of *cbs*^{+/-} mice maintained on a high-methionine diet (*cbs*^{+/-} HM) demonstrated an approximate 20% decrease in cells of the ganglion cell layer and increased expression of *Aven*, *Egr1*, and *Bat3* in the retina [42]. *Mthfr*^{+/-} mice with Hhcy also demonstrated reduced RGC function and a thinner ganglion cell layer [43, 44]. The lesion may be due to the death of RGCs caused by stimulation of n-methyl-D-aspartate receptors [45], reduced levels of NRF2 and the capacity of Müller cells to maintain RGCs in mice [20].

The association of plasma homocysteine with mRNFL thickness was not significant after adjusting for potential confounding factors. The RNFL is composed of RGC axons, while the GC-IPL is composed of RGC bodies and dendrites [35, 46]. We speculated that Hhcy-induced RGC death occurs over a gradual time course and that RNFL thickness may gradually decrease over long periods of time. A similar condition was found in previous studies on the association of retinal thickness with dementia [7] and obstructive sleep apnoea syndrome [47]. Thinner GC-IPL thickness rather than RNFL thickness was correlated with prevalent dementia and obstructive sleep apnoea syndrome in those studies. Due to the cross-sectional nature of our study in community-based older adults and the lack of published studies to compare and explore the reasons for the

Table 3 Association of homocysteine with retinal thickness parameters in all participants.

	Model 1		Model 2		Model 3		Model 4	
	Beta (95% CI), ×10 ⁻²	p value	Beta (95% CI), ×10 ⁻²	p value	Beta (95% CI), ×10 ⁻²	p value	Beta (95% CI), ×10 ⁻²	p value
Full thickness								
Foveal	4.54 (-13.60, 22.68)	0.623	-7.96 (-27.85, 11.93)	0.432	-4.75 (-25.46, 15.95)	0.652	-4.32 (-27.84, 19.20)	0.718
Central subfield	6.02 (-12.13, 24.17)	0.515	-13.16 (-32.83, 6.51)	0.189	-9.01 (-29.44, 11.43)	0.387	-4.08 (-27.22, 19.06)	0.729
Average inner	-11.92 (-25.48, 1.65)	0.085	-15.66 (-30.39, -0.92)	0.037	-11.49 (-26.77, 3.79)	0.140	-10.69 (-26.52, 5.13)	0.185
Superior inner	-8.95 (-23.74, 5.84)	0.235	-11.03 (-27.24, 5.19)	0.182	-8.52 (-25.39, 8.34)	0.321	-7.16 (-24.75, 10.44)	0.425
Inferior inner	-15.65 (-30.68, -0.06)	0.041	-16.77 (-32.96, -0.59)	0.042	-10.50 (-27.25, 6.25)	0.219	-7.86 (-25.18, 9.45)	0.373
Nasal inner	-11.84 (-26.33, 2.65)	0.109	-17.10 (-33.05, -1.16)	0.036	-11.59 (-28.11, 4.93)	0.169	-5.42 (-14.12, 3.28)	0.221
Temporal inner	-11.28 (-25.24, 2.68)	0.113	-17.80 (-32.84, -2.75)	0.020	-15.43 (-31.12, 0.26)	0.054	-14.25 (-30.62, 2.11)	0.088
Average outer	-11.10 (-22.81, 0.06)	0.063	-10.52 (-23.38, 2.34)	0.109	-7.17 (-20.53, 6.49)	0.292	-6.55 (-20.25, -8.99)	0.349
Superior outer	-9.05 (-23.97, 5.88)	0.235	-6.10 (-22.43, 10.23)	0.464	-3.28 (-20.35, 13.78)	0.706	-1.28 (-18.96, 16.40)	0.887
Inferior outer	-14.18 (-28.57, 0.02)	0.053	-13.66 (-29.60, 2.27)	0.093	-9.83 (-26.31, 3.65)	0.242	-8.91 (-25.99, 8.18)	0.307
Nasal outer	-13.06 (-26.33, 0.02)	0.054	-13.91 (-28.57, 0.75)	0.063	-10.43 (-25.67, 4.81)	0.180	-9.29 (-25.07, 6.49)	0.248
Temporal outer	-8.14 (-20.46, 4.19)	0.195	-8.40 (-21.94, 5.13)	0.223	-5.15 (-19.25, 5.96)	0.474	-3.89 (-18.47, 10.68)	0.600
RNFL sectors								
Average inner	-1.43 (-3.68, 0.82)	0.213	-2.55 (-5.01, -0.09)	0.043	-2.12 (-4.68, 0.43)	0.103	-1.84 (-4.45, 0.76)	0.165
Superior inner	-3.03 (-6.47, 0.42)	0.085	-2.47 (-6.15, 1.21)	0.188	-2.05 (-5.91, 1.81)	0.298	-1.37 (-5.36, 2.61)	0.500
Inferior inner	-3.32 (-7.03, 0.39)	0.079	-4.57 (-8.52, -0.63)	0.023	-3.87 (-7.96, 0.20)	0.063	-3.08 (-7.28, 2.12)	0.151
Nasal inner	-2.04 (-4.90, 0.82)	0.161	-3.05 (-6.09, -0.01)	0.049	-3.04 (-6.22, 0.14)	0.061	-2.67 (-5.86, 0.53)	0.101
Temporal inner	1.14 (-0.97, 3.25)	0.291	-0.10 (-2.34, 2.15)	0.933	0.46 (-1.85, 2.79)	0.692	0.56 (-1.83, 0.29)	0.647
Average outer	-4.32 (-8.35, -0.29)	0.036	-4.66 (-9.08, -0.25)	0.038	-3.61 (-8.18, 0.95)	0.121	-3.16 (-7.82, 1.51)	0.184
Superior outer	-6.33 (-11.80, -0.85)	0.024	-5.60 (-11.51, 0.31)	0.063	-4.30 (-10.45, 1.85)	0.171	-3.78 (-10.01, 2.46)	0.235
Inferior outer	-5.77 (-11.59, 0.05)	0.052	-6.32 (-12.42, -0.21)	0.043	-5.41 (-11.69, 0.87)	0.091	-4.58 (-11.08, 1.91)	0.166
Nasal outer	-6.33 (-12.45, -0.20)	0.043	-6.12 (-12.57, 0.33)	0.063	-5.37 (-12.10, 1.35)	0.117	-4.94 (-11.87, 1.99)	0.162
Temporal outer	0.46 (-1.74, 2.67)	0.678	-0.62 (-2.92, 1.68)	0.597	0.63 (-1.75, 3.01)	0.602	0.87 (-1.60, 3.34)	0.489
GC-IPL sectors								
Average inner	-9.56 (-16.28, -2.83)	0.005	-11.28 (-18.59, -3.97)	0.003	-9.72 (-17.30, -2.15)	0.012	-8.84 (-16.54, -1.15)	0.024
Superior inner	-10.37 (-18.85, -1.89)	0.017	-11.50 (-19.98, -3.01)	0.008	-10.45 (-19.23, -1.66)	0.020	-9.73 (-18.65, -0.81)	0.033
Inferior inner	-9.43 (-18.13, -0.74)	0.034	-10.56 (-18.98, -2.13)	0.014	-16.61 (-16.39, -1.00)	0.083	-6.06 (-14.91, 2.79)	0.179
Nasal inner	-9.19 (-17.35, -1.04)	0.027	-8.50 (-16.66, -0.33)	0.041	-6.77 (-15.25, 1.72)	0.118	-5.42 (-14.12, 3.28)	0.221
Temporal inner	-13.39 (-20.81, -5.97)	< 0.001	-14.56 (-22.21, -6.91)	< 0.001	-13.99 (-21.98, -5.99)	< 0.001	-13.28 (-21.47, -5.09)	0.002
Average outer	-6.79 (-11.48, -2.10)	0.005	-5.08 (-10.18, 0.03)	0.051	-4.28 (-9.53, 0.98)	0.111	-4.04 (-9.42, 1.33)	0.140
Superior outer	-5.61 (-12.81, 1.59)	0.127	-3.39 (-10.60, 3.82)	0.357	-3.18 (-10.68, 4.32)	0.405	-2.91 (-10.64, 4.83)	0.461
Inferior outer	-3.90 (-10.11, 2.30)	0.217	-4.21 (-10.53, 2.11)	0.191	-3.28 (-9.77, 3.21)	0.321	-3.56 (-10.14, 3.03)	0.289
Nasal outer	-5.83 (-11.68, 0.15)	0.051	-5.01 (-10.93, 0.91)	0.097	-3.92 (-10.01, 2.18)	0.207	-3.74 (-10.01, 2.53)	0.242
Temporal outer	-6.28 (-12.39, -0.17)	0.044	-7.70 (-13.73, -1.67)	0.012	-6.72 (-12.98, -0.46)	0.035	-5.96 (-12.43, 0.51)	0.071

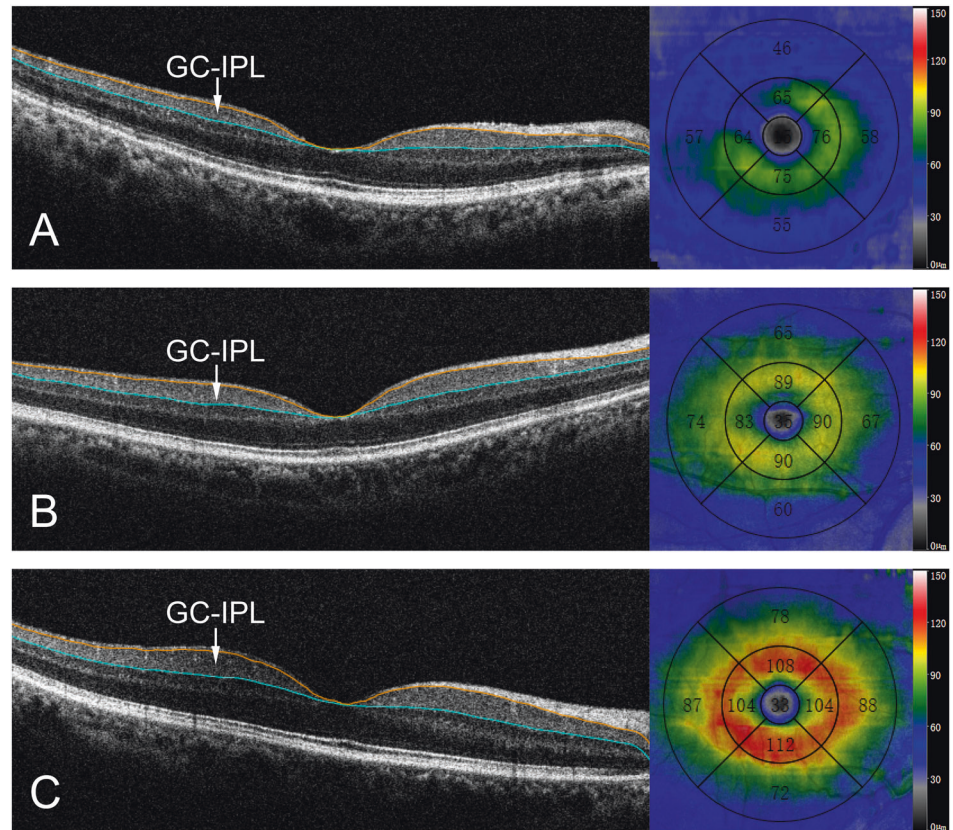
Model 1: unadjusted; Model 2: adjusted for age, body mass index, sex, smoking, alcohol accumulation, married status, illiteracy; Model 3 adjusted for model 2 + fasting glucose, triglyceride, high-density lipoprotein, low-density lipoprotein, creatinine and Hs-CRP. Model 4 adjusted for model 3 + self-report major CVD events, hypertension, diabetes, mobility, grip strength, depressive status and cognitive function.

Table 4 Association of homocysteine with retinal thickness parameters in older adults without CVD or diabetes.

	Model 1		Model 2		Model 3		Model 4	
	Beta (95% CI), $\times 10^{-2}$	p value	Beta (95% CI), $\times 10^{-2}$	p value	Beta (95% CI), $\times 10^{-2}$	p value	Beta (95% CI), $\times 10^{-2}$	p value
Full thickness								
Foveal	0.07 (-19.17, 20.56)	0.945	-9.37 (-31.17, 12.42)	0.399	-6.89 (-29.30, 15.51)	0.546	-8.77 (-34.85, 17.31)	0.509
Central subfield	1.65 (-17.95, 21.26)	0.868	-14.37 (-35.57, 6.83)	0.184	-12.30 (-34.04, 9.44)	0.267	-10.25 (-35.41, 14.90)	0.424
Average inner	-11.88 (-26.74, 2.98)	0.117	-15.83 (-31.84, 0.17)	0.052	-12.34 (-28.70, 4.02)	0.139	-10.33 (-27.29, 3.63)	0.232
Superior inner	-9.50 (-25.44, 6.45)	0.243	-11.90 (-29.27, 5.47)	0.179	-10.62 (-28.42, 7.18)	0.242	-8.61 (-27.13, 9.91)	0.362
Inferior inner	-14.83 (-31.47, 1.82)	0.081	-16.21 (-33.97, 1.55)	0.074	-9.97 (-28.08, 8.14)	0.280	-7.29 (-26.08, 11.49)	0.446
Nasal inner	-13.23 (-29.03, 2.56)	0.100	-18.79 (-36.07, -1.51)	0.033	-14.23 (-31.90, 3.44)	0.114	-7.59 (-16.95, 1.77)	0.112
Temporal inner	-9.98 (-25.17, 5.21)	0.198	-16.44 (-32.63, -0.24)	0.047	-14.53 (-31.20, 2.15)	0.088	-12.47 (-29.79, 4.84)	0.158
Average outer	-11.34 (-24.31, 1.63)	0.086	-12.20 (-26.43, 2.02)	0.093	-10.03 (-24.66, 4.61)	0.179	-9.09 (-24.07, 5.89)	0.234
Superior outer	-12.88 (-29.41, 3.64)	0.126	-11.87 (-29.92, 6.18)	0.197	-9.06 (-27.72, 9.61)	0.341	-7.39 (-26.72, 11.95)	0.453
Inferior outer	-13.16 (-28.99, 2.67)	0.103	-13.61 (-31.08, 3.85)	0.126	-12.55 (-30.48, 5.37)	0.169	-11.71 (-30.30, 6.88)	0.216
Nasal outer	-14.01 (-28.78, 0.08)	0.063	-16.52 (-32.79, -0.02)	0.047	-14.01 (-30.76, 2.74)	0.101	-13.19 (-30.63, 4.25)	0.138
Temporal outer	-5.31 (-19.04, 8.42)	0.448	-6.82 (-21.90, 8.26)	0.375	-4.48 (-20.04, 11.08)	0.572	-3.51 (-19.60, 12.58)	0.669
RNFL sectors								
Average inner	-4.73 (-9.12, -0.34)	0.035	-2.03 (-4.67, 0.62)	0.133	-2.00 (-4.72, 0.71)	0.148	-1.59 (-4.35, 1.18)	0.260
Superior inner	-1.59 (-5.25, 2.06)	0.393	-2.22 (-6.24, 1.80)	0.278	-2.45 (-6.62, 1.71)	0.248	-1.65 (-5.95, 2.65)	0.452
Inferior inner	-2.30 (-6.21, 1.61)	0.248	-3.93 (-8.15, 0.28)	0.067	-3.43 (-7.75, 0.87)	0.118	-2.52 (-6.95, 1.91)	0.264
Nasal inner	-1.55 (-4.58, 1.49)	0.317	-2.46 (-5.81, 0.89)	0.149	-2.70 (-6.16, 0.75)	0.125	-2.33 (-5.79, 1.13)	0.187
Temporal inner	1.34 (-0.79, 3.48)	0.217	0.52 (-1.74, 2.78)	0.653	0.58 (-1.73, 2.90)	0.621	0.85 (-1.50, 3.21)	0.477
Average outer	-1.59 (-5.25, 2.06)	0.393	-5.58 (-10.33, -0.83)	0.021	-5.09 (-9.95, -0.02)	0.040	-4.32 (-9.28, 0.64)	0.087
Superior outer	-5.40 (-11.27, 0.48)	0.072	-4.60 (-10.97, 1.78)	0.158	-3.84 (-10.40, 2.72)	0.250	-2.99 (-9.63, 3.65)	0.377
Inferior outer	-8.19 (-14.15, -2.22)	0.007	-10.00 (-16.52, -3.48)	0.003	-9.68 (-16.31, -3.06)	0.004	-9.60 (-15.44, -1.76)	0.014
Nasal outer	-5.86 (-12.31, 0.59)	0.075	-6.99 (-14.02, 0.04)	0.051	-6.67 (-13.90, 0.56)	0.071	-5.90 (-13.40, 1.61)	0.123
Temporal outer	0.52 (-1.75, 2.79)	0.654	-0.73 (-3.19, 1.72)	0.559	-0.18 (-2.71, 2.35)	0.887	0.19 (-2.42, 2.79)	0.887
GC-IPL sectors								
Average inner	-11.12 (-18.46, -3.78)	0.003	-13.13 (-20.99, -5.28)	0.001	-11.91 (-19.93, -3.88)	0.004	-10.33 (-18.49, -2.18)	0.013
Superior inner	-11.94 (-20.31, -3.57)	0.005	-13.65 (-22.79, -4.51)	0.003	-13.45 (-22.76, -4.14)	0.005	-11.95 (-21.39, -2.51)	0.013
Inferior inner	-12.00 (-20.57, -3.43)	0.006	-11.80 (-20.97, -2.63)	0.012	-8.80 (-18.11, 0.52)	0.064	-6.88 (-16.32, 2.55)	0.152
Nasal inner	-9.03 (-17.24, -0.83)	0.031	-10.98 (-19.85, -2.11)	0.015	-9.50 (-18.60, -0.41)	0.041	-7.95 (-16.95, 1.77)	0.112
Temporal inner	-11.51 (-19.26, 3.76)	0.004	-16.10 (-24.35, -7.86)	<0.001	-15.87 (-24.39, -7.36)	<0.001	-14.28 (-22.96, -5.60)	0.001
Average outer	-7.12 (-12.28, -1.97)	0.007	-6.22 (-11.80, -0.65)	0.029	-5.42 (-11.09, 0.26)	0.061	-5.07 (-10.86, 0.72)	0.086
Superior outer	-7.45 (-14.69, -0.20)	0.044	-4.67 (-12.69, 3.36)	0.257	-3.91 (-12.17, 4.37)	0.355	-3.62 (-12.13, 4.90)	0.404
Inferior outer	-5.87 (-12.29, 0.55)	0.073	-5.02 (-11.92, 1.88)	0.154	-4.52 (-11.57, 2.53)	0.208	-4.83 (-11.98, 2.32)	0.185
Nasal outer	-6.94 (-12.93, -0.96)	0.023	-6.03 (-12.53, 0.46)	0.068	-5.10 (-11.69, 1.50)	0.130	-4.73 (-11.47, 2.02)	0.169
Temporal outer	-8.23 (-14.14, -2.32)	0.006	-9.16 (-15.55, -2.77)	0.005	-8.15 (-14.69, -1.60)	0.015	-7.28 (-14.02, -0.55)	0.034

Model 1: unadjusted; Model 2: adjusted for age, body mass index, sex, smoking, alcohol accumulation, married status, illiteracy; Model 3 adjusted for model 2 + fasting glucose, triglyceride, high-density lipoprotein, low-density lipoprotein, creatinine and Hs-CRP. Model 4 adjusted for model 3 + self-report major CVD events, hypertension, diabetes, mobility, grip strength, depressive status and cognitive function.

Fig. 1 Individual examples of horizontal B-scans (left) and thickness map (right) showing macular ganglion cell layer plus inner plexiform layer (GC-IPL) segmentations (arrows). **A** A 80-year-old male with plasma homocysteine of 13.5 $\mu\text{mol/L}$. **B** A 87-year-old male with plasma homocysteine of 17.6 $\mu\text{mol/L}$. **C** A 86-year-old male with plasma homocysteine of 21.8 $\mu\text{mol/L}$. These examples are representative of results observed in this study that increased plasma homocysteine was associated with thinner GC-IPL.



different roles of the GC-IPL and mRNFL in retinal thickness, it remains unclear whether the RNFL is associated with plasma homocysteine. Our study was the first to explore the relationship between plasma homocysteine levels and retinal thickness. More importantly, it illustrated the consistent and robust relationship between them.

Limitations

Several limitations were presented in this study. First, we conducted a cross-sectional study to evaluate the association of plasma homocysteine levels with retinal thickness. Our findings only suggested an association but not a causative relationship. Therefore, longitudinal studies should be conducted to validate our findings in the future. Second, the mean age of the participants was relatively high at 78.37 (4.53) years, and the mean plasma Hcy was as high as 16.38 (8.05) $\mu\text{mol/L}$ in our study, which limits the generalizability of our results. Considering this, we performed sensitivity analyses after excluding participants with CVD and/or diabetes. Further studies should be conducted in younger populations to verify our conclusions. Third, a very large number of participants were excluded due to our strict inclusion criteria. Therefore, many uncontrolled factors

might have affected our findings. Fourth, vitamin B and folic acid affect plasma Hcy *in vivo*. However, we could not eliminate the influence of these factors because of the lack of data in our study. Fifth, we measured and explored the relationship between macular thickness and plasma Hcy, but choroidal thickness was not measured in our study, which could be an interesting area of study in the future. Last, the multiple testing was not analyzed and it may increase false-positive results.

The strengths of our study included a community-based setting in older adults, a large sample size and extensive data on covariates.

Conclusions

In this cross-sectional study of 1060 older adults with SD-OCT measurements in the RuALS, increased plasma homocysteine levels were associated with a thinner GC-IPL after adjustment for demographic, physiological and clinical confounding factors. We provided direct evidence that increased plasma homocysteine levels are associated with a thinner GC-IPL. Plasma homocysteine may be a risk factor for a thinner GC-IPL.

Summary

What was known before

- Plasma homocysteine levels are associated with macular thickness in patients with age-related macular degeneration, diabetic retinopathy and glaucoma.

What this study adds

- Thinner ganglion cell layer plus inner plexiform layer (GC-IPL) rather than macular retinal nerve fibre layer (mRNFL) thickness was associated with increased plasma homocysteine levels in healthy older adults, who were specifically not patients with disease that directly influence the macular thickness.

Acknowledgements We acknowledge all participants involved in the present study from the RuLAS. We acknowledge the support from the government of Rugao, the Public Health Bureau of Jiang'an township and Rugao city, the Bureau of Civil Affairs, the Rugao People's Hospital and all the local village physicians.

Funding This work was financially supported by grants from the National Key R&D Program of China (2018YFC2000400), the National Natural Science Foundation of China (81571372), the National Key R&D Program of China (2016YFC0904800, 2019YFC0840607), the National Science and Technology Major Project of China (2017ZX09304010), and the Natural Science Foundation of China (81970810). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contributions HZ, HS, XX, DL and XW: study design, interpretation of results, preparation and editing manuscript. HZ: data analysis. JW and XJ: study design. HZ, HS, XS, XX, DL, GW and XW: data collection. All authors were involved in the preparation of the manuscript and final approval of the submitted and published versions.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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