Physiology International 113 (2026) 3, 366–381
DOI: 10.1556/2060.2026.00843

1828
AKADÉMIAI KIADÓ

Effects of dehydration and hyperhydration on macular and peripapillary retinal thickness assessed by OCT

JELENA ĆURKOVIĆ[1], DEA SAMARDŽIĆ[1], IVANA BARUN[1], DONALD OKMAŽIĆ[1], TONI MATIJEVIĆ[2], MARKO KUMRIĆ[3,4,5], JOŠKO BOŽIĆ[3] and ANTE KREŠO[1]*

[1] Department of Ophthalmology, University Hospital of Split, Split, Croatia

[2] University of Split School of Medicine, Split, Croatia

[3] Department of Pathophysiology, University of Split School of Medicine, Split, Croatia

[4] Laboratory for Cardiometabolic Research, University of Split School of Medicine, Split, Croatia

[5] Department of Cardiovascular Diseases, University Hospital of Split, Split, Croatia

Received: February 13, 2026 • Revised manuscript received: May 8, 2026 • Accepted: June 24, 2026
Published online: August 7, 2026
© 2026 The Author(s)

ABSTRACT

Purpose: To determine whether short-term dehydration and hyperhydration affect OCT-derived retinal thickness in healthy young adults in a randomized cross-over design. *Methods:* Sixty volunteers aged 18–35 years completed two morning sessions ≥7 days apart: 12-h fluid restriction, representing relative dehydration, and water loading, defined as ≥2 L within 4 h. Spectral-domain OCT (Zeiss Cirrus HD-OCT 7000) was performed by a masked examiner. Primary outcomes were central subfield thickness (CST) and mean peripapillary retinal nerve fiber layer (RNFL) thickness; secondary outcomes were cube average thickness (CAT) and ganglion cell–inner plexiform layer (GCL–IPL) thickness. Hydration was documented using a self-reported thirst scale and recorded water intake. Within-subject comparisons used the Wilcoxon signed-rank test. *Results:* Median age was 28.5 years and 65% of participants were female. Thirst was higher during dehydration than hyperhydration ($P < 0.001$). After review of OCT outputs and exclusion of outlying paired observations for GCL–IPL and CAT, the direction and statistical interpretation of the findings remained unchanged. CAT was slightly lower during dehydration in both eyes. CST was lower during dehydration in the left eye, with a similar non-significant trend in the right eye. RNFL thickness did not differ between hydration states in either eye. GCL–IPL thickness showed only a small right-eye

* Corresponding author. E-mail: akreso345@gmail.com

AKJournals

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

difference and no significant difference in the left eye. *Conclusions:* Acute fluid restriction was associated with small reductions in macular OCT thickness measures, whereas RNFL thickness remained stable. The magnitude of these changes was modest and close to expected OCT test–retest variability, suggesting limited clinical relevance for routine individual interpretation but potential relevance for highly standardized research or longitudinal imaging settings.

KEYWORDS

optical coherence tomography, dehydration, hyperhydration, macular thickness, central subfield thickness, retinal nerve fiber layer, ganglion cell–inner plexiform layer, cross-over study, healthy volunteers, measurement variability

INTRODUCTION

Hydration status plays a fundamental role in systemic homeostasis and can influence the physiology of multiple organ systems, including the eye [1]. Ocular tissues are sensitive to changes in fluid balance due to their metabolic demands and precise structural composition. Previous studies have suggested that variations in hydration may affect intraocular pressure and potentially retinal morphology [1, 2].

Optical coherence tomography (OCT) enables highly reproducible, non-invasive measurements of retinal microstructure, including macular thickness and peripapillary retinal nerve fiber layer (RNFL) thickness [3, 4]. Because OCT is widely used for clinical decision-making and longitudinal monitoring, understanding sources of physiological variability is important when interpreting small changes over time [5]. The retina is among the most metabolically active tissues in the body and may be particularly sensitive to systemic fluid balance; therefore, acute dehydration or hyperhydration could plausibly introduce measurable variability in OCT-derived thickness parameters [2, 6, 7]. However, evidence on hydration-related OCT changes remains limited and inconsistent, particularly in healthy young adults, a population commonly used as controls in clinical trials and normative database studies [8]. Moreover, many prior studies did not directly compare dehydration and hyperhydration within the same individuals using a cross-over design, limiting the ability to isolate within-subject effects [8].

This study aims to evaluate the effects of controlled dehydration and hyperhydration on OCT-derived retinal parameters in healthy young adults using a cross-over design. The primary outcomes are changes in central subfield thickness (CST) and mean peripapillary RNFL thickness between hydration states. Secondary outcomes include cube average thickness (CAT) and macular ganglion cell–inner plexiform layer (GCL–IPL) thickness. These findings may help clarify the extent to which systemic hydration contributes to normal variability in OCT measurements and inform standardization of imaging conditions in research and clinical follow-up [3, 5].

MATERIALS AND METHODS

Study design

This prospective, randomized, cross-over study was conducted at the Department of Ophthalmology, University Hospital of Split, Croatia. The protocol adhered to the Declaration

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

of Helsinki and was approved by the Ethics Committee of University Hospital of Split (Split, Croatia), Class: 520-03/25-01/226; Registration No.: 2181-147/01-06/LJ.Z.-25-02; Date: 29 September 2025; Place: Split. Written informed consent was obtained from all participants prior to inclusion. The study was registered at ClinicalTrials.gov (Identifier: NCT07177131).

Participants

Healthy volunteers aged 18–35 years were recruited among hospital employees and medical students. Eligibility required normal ocular and systemic status on history and screening examination, and best-corrected visual acuity ≥1.0 (decimal). Exclusion criteria included active ocular disease that could affect retinal or optic nerve measurements (e.g., glaucoma/optic neuropathy, macular or retinal pathology), systemic conditions known to markedly affect fluid balance (e.g., renal disease, diabetes), pregnancy or breastfeeding, and contact lens wear within 24 h prior to OCT imaging.

Baseline characteristics collected included age, sex, and body mass index (BMI). Arterial hypertension status and history of ocular surgery were recorded. For the purpose of this study, "healthy volunteers" were defined as participants without ocular pathology affecting OCT measures and without systemic diseases expected to significantly influence fluid balance; well-controlled arterial hypertension and a remote history of uncomplicated ocular surgery were not exclusionary provided screening was unremarkable and OCT image quality criteria were met.

Pre-visit questionnaire and hydration assessment

Before each visit, participants completed a standardized questionnaire documenting potential confounders, including habitual caffeine consumption and contact lens use. Hydration status was assessed subjectively via:

1. A self-reported thirst rating scale (0–10; 0 = no thirst, 10 = maximum thirst).
2. Self-reported total water intake (liters) in the preceding 4 h.

No objective biochemical markers (e.g., serum electrolytes, osmolality) were collected.

Hydration protocol and randomization

Each participant completed two study sessions (relative dehydration and hyperhydration) with a minimum 7-day washout period. The order of sessions was randomized using a computer-generated random sequence, allocating participants 1:1 to one of two sequences (dehydration-first or hyperhydration-first). Randomization was performed by an investigator not involved in OCT acquisition using a computer-generated sequence, with allocation stored in sealed envelopes or a protected file.

1. **Relative dehydration:** abstaining from water intake for 12 h before measurement (food permitted; no beverages).
2. **Hyperhydration:** participants were instructed to consume at least 2 L of water within 4 h prior to measurement; actual intake was recorded.

Compliance was assessed via self-reported intake and the recorded "water intake in the last 4 h" variable.

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

OCT imaging

All assessments were performed in the morning (8:00–10:00 a.m.) to minimize diurnal variation. Spectral-domain OCT imaging was performed using Zeiss Cirrus HD-OCT 7000 (Carl Zeiss Meditec, Dublin, CA, USA) by the same experienced examiner. The examiner was masked to hydration condition during OCT acquisition; participants completed the hydration/thirst questionnaire after imaging. Both right and left eyes were imaged to assess inter-eye consistency of hydration-related OCT changes; however, to avoid inter-eye dependency bias, right and left eyes were analyzed separately and were not pooled into a single-eye dataset.

Macular and optic nerve head scans were acquired using standard Cirrus protocols (e.g., Macular Cube 512 × 218 and Optic Disc Cube 200 × 200). Optical coherence tomography angiography (OCTA) scans were also acquired using a 3 × 3 mm scan pattern. OCTA-derived vascular parameters were explored in an exploratory manner; however, because no statistically significant differences were observed between dehydration and hyperhydration states, these data were not included among the main outcomes of the present report.

Outcomes

Primary outcomes were:

- Central Subfield Thickness (CST) (μm)
- Mean (average) peripapillary RNFL thickness (μm)

Secondary outcomes included:

- Cube Average Thickness (CAT) (μm), defined as the device-reported mean thickness across the macular cube scan

Mean ganglion cell–inner plexiform layer (GCL-IPL) thickness (μm), as reported by the Cirrus Ganglion Cell Analysis algorithm.

CST was selected as a primary macular outcome because it is a routinely reported and clinically relevant measure of central macular structure, whereas CAT was included as a secondary outcome because it reflects average thickness across the broader macular cube. Together, these parameters allowed assessment of both central and more global macular thickness changes. Mean peripapillary RNFL thickness was selected as a primary optic nerve–related outcome because it is a standard OCT parameter used in neuroretinal assessment and longitudinal monitoring. Mean GCL–IPL thickness was included as a secondary macular neuroretinal parameter to explore whether inner retinal layers show hydration-related variability.

For each OCT parameter, the within-subject effect of hydration status was evaluated by comparing dehydration vs. hyperhydration sessions. For regression modeling, change scores were defined as:

- ΔCST = CST(hyperhydration) − CST(dehydration)
- ΔCAT = CAT(hyperhydration) − CAT(dehydration)

Definition of “more pronounced dehydration” subgroups

To explore whether the magnitude of macular thickness change depended on dehydration intensity, participants were categorized into two dehydration-severity strata based on the

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

subjective thirst score recorded during the dehydration session (thirst >7 vs. ≤ 7). This subgroup analysis was based on median of subjective thirst level during dehydration period.

Image quality control

All OCT scans were reviewed for adequate image quality and checked for motion artifacts and segmentation errors. Scan acceptance followed manufacturer-recommended quality criteria; specifically, Cirrus signal strength (0–10) was required to be ≥ 6 and scans had to be free of significant motion artifacts or segmentation errors. Scans not meeting criteria were repeated when feasible; scans with persistent artifacts or segmentation failure were excluded.

Statistical analysis

Statistical analysis and visual rendering was done in GraphPad Prism (version 10.5.0.; GraphPad Software, San Diego California, USA). Continuous variables were summarized as median (interquartile range, IQR) and categorical variables as *n* (%). Within-subject comparisons between dehydrated and hyperhydrated states were performed using the Wilcoxon signed-rank test. For Wilcoxon signed-rank tests, signed ranks were calculated from paired within-subject differences defined as hyperhydration minus dehydration. Positive signed ranks therefore indicated higher values during hyperhydration, whereas negative signed ranks indicated higher values during dehydration. Accordingly, a predominance of positive signed ranks was interpreted as indicating lower values in the dehydrated state.

A two-tailed *P*-value <0.05 was considered statistically significant.

Before final analysis, paired OCT measurements were reviewed for extreme within-subject differences between the dehydration and hyperhydration sessions. Outliers were defined at the level of paired within-subject change, calculated as hyperhydration minus dehydration. As in the previous revised analysis, for GCL–IPL and CAT, paired observations with an absolute within-subject difference greater than 9 μm were excluded from the final analysis. This threshold was applied to identify paired differences considered implausibly large relative to the expected short-term variability of these OCT-derived parameters. No additional exclusions were applied for GCL–IPL or CAT.

In response to the reviewer's concern regarding large CST changes, CST analyses were additionally repeated after exclusion of paired observations with an absolute within-subject CST difference greater than 25 μm. These observations were considered outlying paired CST changes because their magnitude was substantially larger than the expected hydration-related effect observed in the remaining cohort and could not be confidently attributed to hydration status alone. The revised Fig. 3 presents the CST paired comparison after exclusion of these CST outliers. The direction and statistical interpretation of the CST results remained unchanged after this sensitivity analysis.

To evaluate factors associated with OCT change measures, multivariable linear regression models were fitted separately for each eye with ΔCST and ΔCAT as dependent variables. Prespecified covariates included age, sex, and subjective thirst level (0–10). Regression coefficients were reported as unstandardized $\beta \pm$ standard error with corresponding *P*-values. Period and carryover effects were not formally tested.

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

RESULTS

Baseline characteristics of the studied population are presented in Table 1. No participants were excluded during screening. The study included 60 participants with a median age of 28.5 years (IQR 25.0–34.3), of whom 39 (65.0%) were female. Median body mass index was 23.8 kg m^{-2} (IQR 21.1–27.1). Arterial hypertension and a history of ocular surgery were each present in 2 participants (3.3%). After review of the original OCT outputs and exclusion of paired observations with absolute within-subject change >9 µm for GCL–IPL and CAT, analyses were repeated using the cleaned dataset. The number of included paired observations was 59 and 58 for GCL–IPL in the right and left eye, respectively, and 57 and 56 for CAT in the right and left eye, respectively. Exclusion of these outlying observations did not materially alter the direction, statistical significance, or interpretation of the findings.

No significant difference in mean RNFL thickness was observed between dehydrated and hyperhydrated states in either eye (right eye: 96.5 [IQR 91.8–104.0] µm vs. 97.5 [IQR 91.0–104.0] µm, $P = 0.658$; left eye: 95.5 [IQR 90.4–104.0] µm vs. 95.5 [IQR 90.2–103.8] µm, $P = 0.221$) (Fig. 1).

After exclusion of outlying paired observations with absolute within-subject change >9 µm, mean GCL–IPL thickness was analyzed in 59 right eyes and 58 left eyes. In the right eye, median GCL–IPL thickness was identical between dehydration and hyperhydration states (83.0 [IQR 79.0–86.0] µm vs. 83.0 [IQR 79.0–86.0] µm), although paired analysis showed a small shift toward lower values during dehydration [$P = 0.013$]. In the left eye, no significant difference in mean GCL–IPL thickness was observed between dehydration and hyperhydration states (83.0 [IQR 79.0–86.0] µm vs. 83.0 [IQR 79.0–86.0] µm, [$P = 0.351$]) (Fig. 2).

After review of paired CST data, observations with an absolute within-subject CST change greater than 25 µm were excluded from the sensitivity analysis presented in Fig. 3. After exclusion of these paired CST outliers, CST remained lower during dehydration than hyperhydration, and the statistical interpretation of the finding was unchanged.

Table 1. Baseline characteristics of the study population

Parameter	Total ($n = 60$)
Age, years	28.5 (25.0–34.3)
Female sex, *n* (%)	39 (65.0)
Body mass index, kg m^{-2}	23.8 (21.1–27.1)
Arterial hypertension, *n* (%)	2 (3.3)
History of ocular surgery, *n* (%)	2 (3.3)
Habitual caffeine consumer, *n* (%)	49 (81.7)
Contact lenses, *n* (%)	7 (11.7)
Subjective thirst level (10-point scale)*	
Dehydration period	7 (5–8)
Hyperhydration period	1 (0–2)
Total water intake in the last 4 h, L	
Dehydration period	0 (0)
Hyperhydration period	2.5 (2–3)

Data presented as *n* (%) or median (IQR). *$P < 0.001$, Wilcoxon signed-rank test.

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

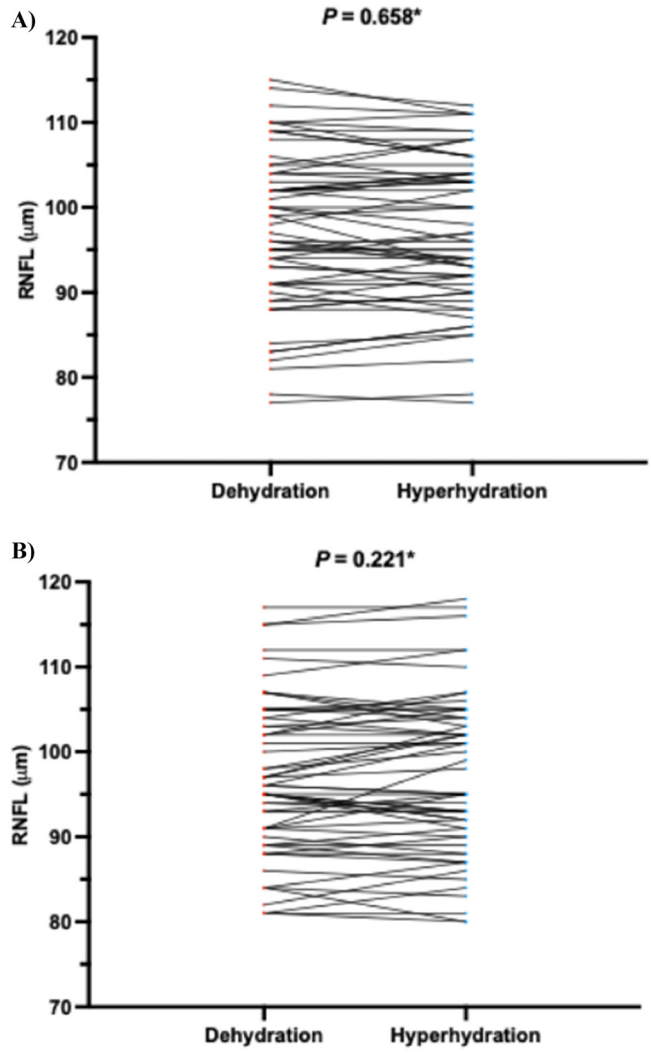


Fig. 1. Comparison of mean RNFL thickness between dehydrated and hyperhydrated states. A) Right eye; B) Left eye. Abbreviations: RNFL: Retinal Nerve Fiber Layer. *Wilcoxon signed-rank test

CST was lower during dehydration than hyperhydration in the left eye, with a similar non-significant trend in the right eye. Although median CST values were similar (254.0 [237.0–265.0] μm vs. 253.5 [241.0–270.3] μm), paired analysis demonstrated lower CST in the dehydrated state in the majority of participants, as reflected by the predominance of positive signed ranks (hyperhydrated − dehydrated: 1,538 vs. 115.5, $P < 0.001$). Accordingly, in the left eye, CST was lower in the dehydrated state (254.5 [IQR 236.0–266.0] μm) compared with the hyperhydrated state (255.5 [IQR 241.0–270.5] μm, $P < 0.001$) (Fig. 3). CST was lower during dehydration than hyperhydration in both eyes (both $P < 0.001$) (Fig. 3). In multivariable linear

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

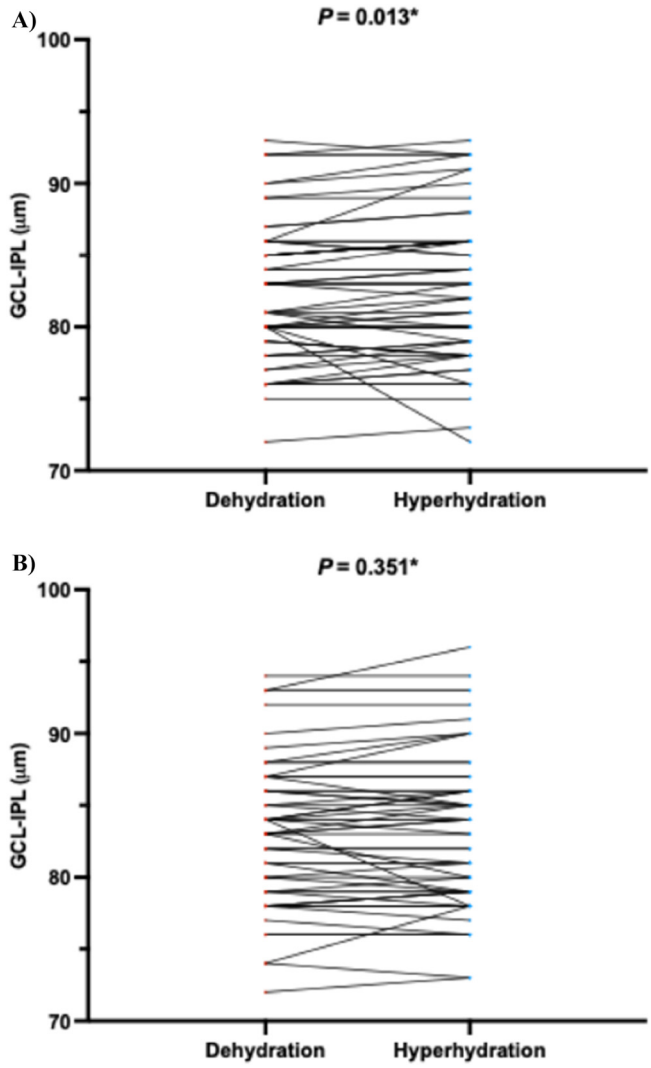


Fig. 2. Comparison of mean GCL-IPL thickness between dehydrated and hyperhydrated states. A) Right eye; B) Left eye. Abbreviations: GCL-IPL: Ganglion Cell-Inner Plexiform Layer. Wilcoxon signed-rank test

regression analysis, ΔCST was not independently associated with age, sex, or subjective thirst level for either eye (Table 2).

Paired observations with an absolute within-subject CST difference greater than 25 µm were excluded from this sensitivity analysis. A) Right eye; B) Left eye. Abbreviation: CST, central subfield thickness. Wilcoxon signed-rank test.

After exclusion of outlying paired observations with absolute within-subject change >9 µm, CAT remained slightly lower in the dehydrated state in both eyes. In the right eye, CAT was

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

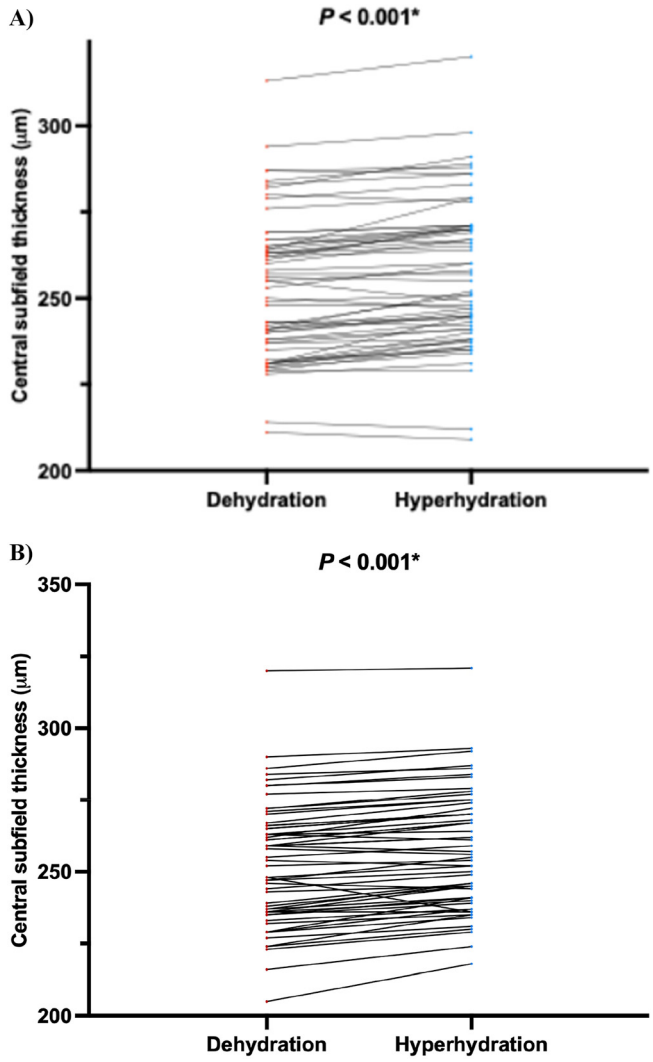


Fig. 3. Comparison of central subfield thickness between dehydrated and hyperhydrated states after exclusion of paired CST outliers

286.0 [IQR 276.0–292.0] μm during dehydration and 285.0 [IQR 276.5–294.0] μm during hyperhydration, with a median paired change of 1.0 [IQR −1.0 to 4.0] μm, [$P = 0.042$]. In the left eye, CAT was 282.0 [IQR 275.3–289.0] μm during dehydration and 283.0 [IQR 275.0–289.8] μm during hyperhydration, with a median paired change of 2.0 [IQR −0.75 to 4.0] μm, [$P = 0.005$] (Fig. 4). In multivariable linear regression analysis, ΔCAT in the right eye was not independently associated with age, sex, or subjective thirst level. Conversely, in the left eye, both age and subjective thirst level were independently associated with ΔCAT (Table 3).

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

Table 2. Multivariable linear regression analysis of factors associated with ΔCST

Eye	Parameter	$\beta \pm$ standard error*	*P*
Right eye	Age, years	−0.05 ± 0.04	0.493
	Sex	0.23 ± 0.21	0.834
	Subjective level of thirst (0–10)	0.25 ± 0.24	0.317
Left eye	Age, years	−0.06 ± 0.15	0.271
	Sex	−1.87 ± 1.51	0.221
	Subjective level of thirst (0–10)	0.23 ± 0.09	0.495

*unstandardized beta coefficient. Abbreviations: CST: Central Subfield Thickness.

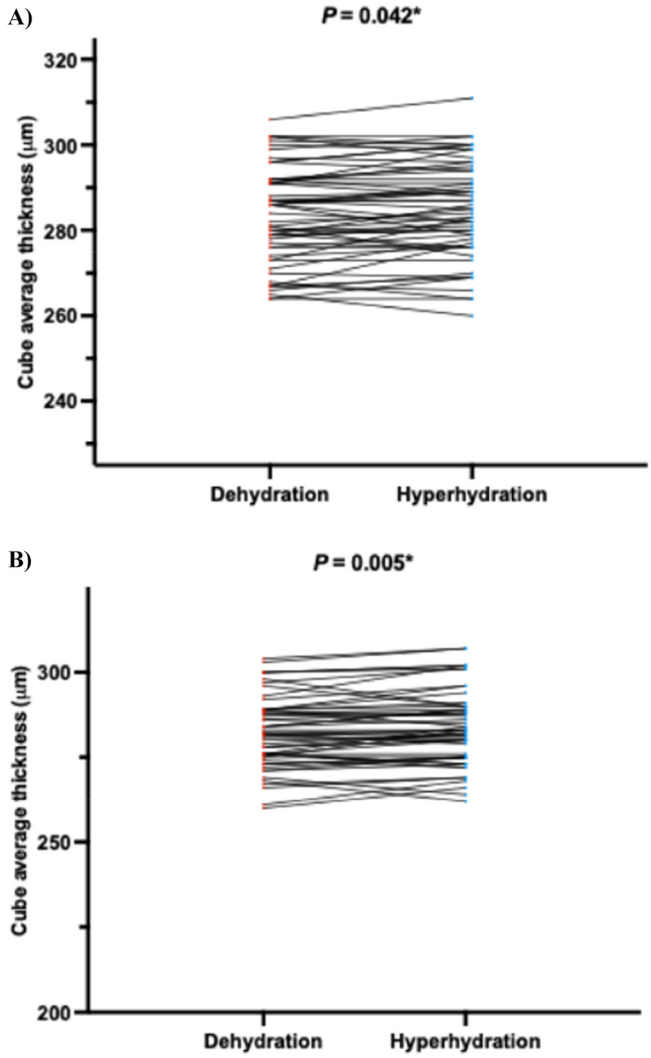


Fig. 4. Comparison of Cube Average Thickness between dehydrated and hyperhydrated states. A) Right eye; B) Left eye. Wilcoxon signed-rank test

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

Table 3. Multivariable linear regression analysis of factors associated with ΔCAT

Eye	Parameter	$\beta \pm$ standard error*	*P*
Right eye	Age, years	0.05 ± 0.07	0.521
	Sex	2.19 ± 1.90	0.253
	Subjective level of thirst (0–10)	−0.69 ± 0.42	0.106
Left eye	Age, years	−0.26 ± 0.11	0.023
	Sex	1.70 ± 2.93	0.565
	Subjective level of thirst (0–10)	1.51 ± 0.65	0.025

*unstandardized beta coefficient. Abbreviations: CAT: Cube Average Thickness.

DISCUSSION

This randomized cross-over study examined the effects of controlled dehydration and hyperhydration on OCT-derived retinal thickness parameters in healthy young adults. The main finding was that 12 h of fluid restriction was associated with small but statistically significant differences in macular thickness measures, although the direction of change was not uniform across all parameters. CAT was slightly lower in the dehydrated state in both eyes. CST was also lower in the dehydrated state in the left eye and showed a similar, although non-significant, trend in the right eye. Thus, macular thickness changes were generally small and directionally consistent, rather than showing opposite directionality between eyes. In contrast, mean RNFL thickness did not differ between hydration states in either eye, and changes in GCL–IPL thickness were small and not fully consistent between eyes. Mean GCL–IPL thickness was slightly lower in the dehydrated state in the right eye, while no significant difference was observed in the left eye. Given the small effect size, its proximity to expected GCIPL test–retest variability, and the multiple endpoints analyzed without correction, this isolated finding should be interpreted cautiously and considered exploratory pending confirmation in studies with objective hydration markers and prespecified GCL–IPL analyses. The absence of significant RNFL change in either eye suggests that peripapillary RNFL thickness is relatively stable under short-term hydration manipulation, possibly because of differences in tissue composition, anatomical location, and lower susceptibility to acute systemic fluid shifts compared with macular thickness measures. The inter-eye inconsistency in GCL–IPL findings may reflect physiological asymmetry, segmentation variability, or statistical noise related to multiple OCT endpoints. Overall, the results suggest that acute fluid restriction can produce measurable, but very modest, shifts in macular thickness on OCT, while peripapillary RNFL thickness appears relatively stable under these conditions. The incomplete parallelism between CST and CAT may reflect both anatomical and methodological factors. CST represents the central subfield and may therefore be more sensitive to small localized changes or segmentation variability, whereas CAT reflects the average thickness across the broader macular cube and may better capture diffuse, low-amplitude changes. Regional differences in vascular density, tissue composition, and susceptibility to measurement noise may therefore explain why these parameters did not change identically across eyes and hydration states.

Although statistically significant, the absolute magnitude of macular changes was modest, approximately 1–5 μm. This scale is close to the test–retest variability reported for contemporary spectral-domain OCT devices, which implies that the observed differences are unlikely

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

to be clinically meaningful for routine patient-level interpretation [3–5]. The statistical significance likely reflects the within-subject cross-over design and the consistency of the direction of change across participants, rather than a large physiological effect size. Therefore, these differences should be interpreted primarily as a minor source of measurement variability rather than as clinically relevant retinal structural changes. This may be most relevant in research settings or in highly standardized longitudinal assessments designed to detect very small changes [5].

Earlier DRCR.net reproducibility data showed that OCT-derived retinal thickness measurements may vary substantially in some cases, with changes exceeding expected physiological variation requiring cautious interpretation [9]. In the context of the present study, this is relevant for interpreting isolated large within-subject OCT changes, which may reflect measurement variability, segmentation-related issues, or physiological diurnal variation rather than hydration status alone [9]. Therefore, extreme values were carefully rechecked against the original OCT scans, and sensitivity analyses after exclusion of outlying GCL–IPL and CAT paired observations were performed to assess the robustness of the findings.

Comparison with previous research shows heterogeneous findings. Balk et al. reported minimal or no effect of oral hydration on OCT-derived retinal layer thickness, suggesting that hydration accounts for only a small proportion of normal OCT variability [10]. In contrast, studies of acute physical exercise, including intense exertion and endurance events, have reported transient changes in retinal and choroidal parameters that may reflect hemodynamic stress and fluid shifts, with thinning of total retinal thickness shortly after exercise in some protocols [11, 12]. Studies using fasting-related dehydration paradigms have also reported changes in posterior segment parameters, including choroidal thickness and, in some reports, retinal or vascular metrics, further supporting heterogeneity across dehydration models [13–15]. Differences in dehydration induction, fluid restriction compared with diuretics, fasting, or heat and exercise, likely contribute to the inconsistent results across studies. Protocol duration, achieved dehydration severity, accompanying hormonal responses, and how hydration status was assessed also vary substantially and may influence retinal and choroidal perfusion and tissue hydration in different ways.

The mechanisms underlying the observed changes cannot be established from this study, but several pathways are plausible. The retina is among the most metabolically active tissues in the body, and therefore potentially sensitive to systemic volume status and microcirculatory changes [6, 7]. Dehydration can cause mild hemoconcentration and reduced plasma volume, which may alter retinal perfusion and transcapillary fluid exchange, leading to subtle changes in intracellular and extracellular water distribution within retinal tissue. Hyperhydration may partially reverse these effects. Evidence that acute water loading can induce short-term changes in posterior segment structures comes from water drinking test studies, which have demonstrated measurable changes in choroidal thickness and volume after acute fluid intake [2, 16, 17]. In addition, OCTA study in healthy subjects performed by Soydan et al., suggest that acute water loading can influence vascular or perfusion-related metrics, even when thickness changes are small or inconsistent [8]. The greater sensitivity of macular metrics, CST and CAT, compared with RNFL thickness may reflect differences in tissue composition and vascular architecture, as macular tissue has very high metabolic demand and tightly regulated microcirculation. The inconsistent GCL–IPL findings between eyes may reflect the small effect size, physiologic asymmetry, and measurement variability related to segmentation [5]. Additional parameters,

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

including OCTA-derived vascular metrics and choroidal thickness, may provide complementary information because they may be more directly influenced by short-term changes in ocular perfusion, vascular filling, or systemic fluid balance.

Several limitations should be considered. First, a 12-h restriction may not produce uniform or maximal dehydration across participants, stronger or longer protocols could yield different effects but may be less feasible. Second, hydration status was assessed using subjective thirst ratings and self-reported fluid intake, without objective biomarkers such as serum or urine osmolality, electrolytes, or urine specific gravity, which limits quantification of dehydration severity and mechanistic interpretation. In addition, although OCTA-derived vascular parameters were explored, no statistically significant differences between dehydration and hyperhydration states were observed. Because these analyses were exploratory and not among the predefined main outcomes, they were not presented in detail in the current report. Although all included OCT scans met the predefined manufacturer-recommended signal strength threshold and were reviewed for motion artifacts and segmentation errors, complete SSI values were not available for all scans. Therefore, a formal paired comparison of signal strength between dehydration and hyperhydration states could not be performed, and subtle differences in image quality cannot be fully excluded as a contributor to the very small observed thickness differences. Third, the cohort consisted of healthy young adults, limiting generalizability to older individuals and patients with systemic disease where autoregulation and ocular tissue properties may differ. Finally, period and carryover effects were not formally tested, so subtle cross-over related biases cannot be fully excluded.

In terms of clinical implications, typical day-to-day variation in hydration is unlikely to materially affect OCT interpretation in most patients, particularly for RNFL monitoring. Nevertheless, in research contexts where small group differences are targeted, or when evaluating minimal macular thickness change near a decision threshold, standardizing imaging conditions, including time of day and pre-imaging fluid intake instructions, may reduce measurement noise. Water drinking test literature in glaucoma highlights that a standardized fluid challenge can produce measurable ocular responses and has been used as a provocative test, which supports the broader concept that short-term systemic fluid shifts can affect ocular measurements [18–20]. Future studies should incorporate objective hydration measures, examine dose–response relationships, include older and clinical populations, and evaluate additional OCT parameters, including choroidal thickness and OCTA derived vascular metrics, to better define the link between systemic hydration and retinal structure.

CONCLUSION

Acute hydration manipulation was associated with small but statistically significant reductions in macular thickness, CST and CAT, in healthy young adults, while RNFL thickness remained stable and GCL–IPL effects were minimal or inconsistent. In our data, CAT was lower in the dehydrated state in both eyes, and CST was lower in the dehydrated state in the left eye with a similar, non-significant trend in the right eye. Although these changes are likely clinically insignificant for routine OCT follow-up, they indicate that hydration status can contribute to subtle variability in macular OCT measures and may warrant standardization in studies designed to detect very small retinal thickness differences.

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

Disclosure of potential conflicts of interest: The authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval: The study was approved by the Ethics Committee of the University Hospital of Split (Split, Croatia) (Class: 520-03/25-01/226; Registration No.: 2181-147/01-06/LJ.Z.-25-02; Date: 29 September 2025) and was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Standards of reporting: We report this randomized cross-over trial in accordance with CONSORT reporting guidelines.

Consent to participate: Written informed consent was obtained from all participants prior to study participation and data collection.

Author contributions: All authors meet the ICMJE authorship criteria. Jelena Ćurković, Dea Samardžić, Ivana Barun, Donald Okmažić, and Toni Matijević contributed to data acquisition and data processing, and participated in the interpretation of the results and critical revision of the manuscript. Jelena Ćurković wrote the first draft of the manuscript. Ante Krešo conceived the study idea and contributed to study design and methodology. Marko Kumrić performed the statistical analysis. Ante Krešo and Marko Kumrić revised and edited the manuscript. All authors approved the final version and agree to be accountable for all aspects of the work.

ACKNOWLEDGEMENTS

The authors received no specific funding for this work and have no technical assistance to acknowledge. The study was registered at ClinicalTrials.gov (Identifier: NCT07177131).

LIST OF ABBREVIATIONS

- **BMI** Body mass index
- **CAT** Cube Average Thickness
- **CST** Central Subfield Thickness
- **GCL–IPL** Ganglion Cell Layer–Inner Plexiform Layer
- **IQR** Interquartile range
- **OCT** Optical Coherence Tomography
- **OCTA** Optical Coherence Tomography Angiography
- **RNFL** Retinal Nerve Fiber Layer
- **SD** Standard deviation
- **SSI** Signal strength index (Cirrus signal strength)

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

REFERENCES

1. Stewart IB, Dias B, Borg DN, Bach AJE, Feigl B, Costello JT. Intraocular pressure is a poor predictor of hydration status following intermittent exercise in the heat. Front Physiol 2017 Feb 1; 8: 36. PubMed PMID: 28203205; PubMed Central PMCID: PMC5285388. https://doi.org/10.3389/fphys.2017.00036
2. Mansouri K, Medeiros FA, Marchase N, Tatham AJ, Auerbach D, Weinreb RN. Assessment of choroidal thickness and volume during the water drinking test by swept-source optical coherence tomography. Ophthalmology 2013 Dec; 120(12). PubMed PMID: 24021895; PubMed Central PMCID: PMC3833954. https://doi.org/10.1016/j.ophtha.2013.07.040
3. Paunescu LA, Schuman JS, Price LL, Stark PC, Beaton S, Ishikawa H, et al. Reproducibility of nerve fiber thickness, macular thickness, and optic nerve head measurements using StratusOCT. Invest Ophthalmol Vis Sci 2004 Jun; 45(6): 1716–24. PubMed PMID: 15161831; PubMed Central PMCID: PMC1993821. https://doi.org/10.1167/iovs.03-0514
4. Parravano M, Oddone F, Boccassini B, Menchini F, Chiaravalloti A, Schiavone M, et al. Reproducibility of macular thickness measurements using cirrus SD-OCT in neovascular age-related macular degeneration. Invest Ophthalmol Vis Sci 2010 Sep; 51(9): 4788–91. PubMed PMID: 20435585. https://doi.org/10.1167/iovs.09-4976
5. Wadhwani M, Bali SJ, Satyapal R, Angmo D, Sharma R, Pandey V, et al. Test-retest variability of retinal nerve fiber layer thickness and macular ganglion cell-inner plexiform layer thickness measurements using spectral-domain optical coherence tomography. J Glaucoma 2015; 24(5): e109–115. PubMed PMID: 25517254. https://doi.org/10.1097/IJG.0000000000000203
6. Wangsa-Wirawan ND, Linsenmeier RA. Retinal oxygen: fundamental and clinical aspects. Arch Ophthalmol Chic Ill 1960 2003 Apr; 121(4): 547–57. PubMed PMID: 12695252. https://doi.org/10.1001/archopht.121.4.547
7. Eshaq RS, Wright WS, Harris NR. Oxygen delivery, consumption, and conversion to reactive oxygen species in experimental models of diabetic retinopathy. Redox Biol 2014 Apr 18; 2: 661–6. PubMed PMID: 24936440; PubMed Central PMCID: PMC4052533. https://doi.org/10.1016/j.redox.2014.04.006
8. Soydan A, Ulaş F, Kaymaz A, Toprak G, Uyar E, Çelebi S. Investigation of the short-term effects of water drinking test on the eye using optical coherence tomography angiography in young healthy male subjects. Cutan Ocul Toxicol 2022 Dec; 41(4): 291–5. PubMed PMID: 36170464. https://doi.org/10.1080/15569527.2022.2126493
9. Diabetic Retinopathy Clinical Research Network, Krzystolik MG, Strauber SF, Aiello LP, Beck RW, Berger BB. Reproducibility of macular thickness and volume using zeiss optical coherence tomography in patients with diabetic macular edema. Ophthalmology 2007 Aug; 114(8): 1520–5. PubMed PMID: 17353052; PubMed Central PMCID: PMC2253719. https://doi.org/10.1016/j.ophtha.2006.10.055
10. Balk LJ, Oberwahrenbrock T, Uitdehaag BMJ, Petzold A. Physiological variation of retinal layer thickness is not caused by hydration: a randomised trial. J Neurol Sci 2014 Sep 15; 344(1–2): 88–93. PubMed PMID: 25005893. https://doi.org/10.1016/j.jns.2014.06.031
11. Szalai I, Csorba A, Pálya F, Jing T, Horváth E, Bosnyák E, et al. The assessment of acute chorioretinal changes due to intensive physical exercise in young adults. PloS One 2022; 17(5): e0268770. PubMed PMID: 35613112; PubMed Central PMCID: PMC9132279. https://doi.org/10.1371/journal.pone.0268770
12. Mauget-Faÿsse M, Arej N, Paternoster M, Zuber K, Derrien S, Thevenin S, et al. Retinal and choroidal blood flow variations after an endurance exercise: a real-life pilot study at the Paris marathon. J Sci Med Sport 2021 Nov 1; 24(11): 1100–4. https://doi.org/10.1016/j.jsams.2021.03.013

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC

13. Uyar E, Dogan U, Ulas F, Celebi S. Effect of fasting on choroidal thickness and its diurnal variation. Curr Eye Res 2019 Jul; 44(7): 695–700. PubMed PMID: 30777786. https://doi.org/10.1080/02713683.2019.1584677
14. Gokmen O, Ozgur G. The effect of religious fasting and dehydration at Ramadan on choroidal thickness and retinal vessel densities, measured with optical coherence tomography angiography. Eur J Ophthalmol 2021 Mar; 31(2): 497–504. PubMed PMID: 32981332. https://doi.org/10.1177/1120672120958297
15. Shokoohi-Rad S, Shoeibi N, Ghasemi Nour M, Bakhtiari E. Evaluation of the effect of religious fasting on retinal vessel density and retinal thickness using optical coherence tomography angiography. Neuro-Ophthalmol Aeolus Press 2022; 46(3): 147–53. PubMed PMID: 35574164; PubMed Central PMCID: PMC9103594. https://doi.org/10.1080/01658107.2021.1995442
16. Kandarakis SA, Katsimpris A, Kourti P, Psinakis F, Karmiris E, Papakonstantinou E, et al. The effect of the water drinking test on ocular parameters and choroidal thickness in glaucoma suspects. Medicina (Mex) 2023 Feb 16; 59(2). https://doi.org/10.3390/medicina59020381
17. Arora KS, Jefferys JL, Maul EA, Quigley HA. Choroidal thickness change after water drinking is greater in angle closure than in open angle eyes. Invest Ophthalmol Vis Sci 2012 Sep 21; 53(10): 6393–402. https://doi.org/10.1167/iovs.12-10224
18. Susanna R, Clement C, Goldberg I, Hatanaka M. Applications of the water drinking test in glaucoma management. Clin Exp Ophthalmol 2017 Aug; 45(6): 625–31. PubMed PMID: 28164419. https://doi.org/10.1111/ceo.12925
19. Razeghinejad MR, Tajbakhsh Z, Nowroozzadeh MH, Havens SJ, Ghate D, Gulati V. The water-drinking test revisited: an analysis of test results in subjects with glaucoma. Semin Ophthalmol 2018; 33(4): 517–24. PubMed PMID: 28537521; PubMed Central PMCID: PMC5701879. https://doi.org/10.1080/08820538.2017.1324039
20. Kerr NM, Danesh-Meyer HV. Understanding the mechanism of the water drinking test: the role of fluid challenge volume in patients with medically controlled primary open angle glaucoma. Clin Exp Ophthalmol 2010 Jan; 38(1): 4–9. PubMed PMID: 20447094. https://doi.org/10.1111/j.1442-9071.2009.02183.x

Open Access statement. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted use, distribution, and reproduction in any medium for non-commercial purposes, provided the original author and source are credited, a link to the CC License is provided, and changes – if any – are indicated.

Brought to you by MTA Könyvtár és Információs Központ olvasók | Unauthenticated | Downloaded 11/11/26 10:44 AM UTC