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Vitamin D and macular thickness in the elderly: an Optical Coherence Tomography study

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ABSTRACT

Purpose. Vitamin D insufficiency is associated with age-related macular degeneration. Our objective was to determine whether low serum 25-hydroxyvitamin D (25OHD) concentration was associated with macular thickness among older adults with no signs of macular dysfunction.

Methods. Sixty-two French older community-dwellers with no patent macular dysfunction (mean±standard deviation, 71.2±5.0years; 45.2% female) included in the GAIT study (ClinicalTrials.gov number, NCT01315717) were separated into 2 groups according to serum 25OHD level (i.e., insufficient<50nmol/L or sufficient≥50nmol/L). The macular thickness was measured on 1000μm central macula with optical coherence tomography, and further binarized according to normal values of macular thickness (i.e., 267.74μm for males, and 255.60μm for females). Age, gender, number of comorbidities, cognitive disorders, body mass index, mean arterial pressure, visual acuity, intraocular pressure, serum calcium concentration and season of testing were considered as potential confounders.

Results. The mean serum 25OHD concentration was 61.2±26.3nmol/L. Patients with vitamin D insufficiency had a reduced macular thickness compared to those without (232.9±40.4μm versus 253.3±32.1 μm, P=0.042). After adjustment for potential confounders, vitamin D insufficiency was associated with a decreased macular thickness (ß=-59.4μm, P=0.001). Consistently, the participants with vitamin D insufficiency had a 3.7-fold higher risk of having abnormally low macular thickness compared to those with sufficient 25OHD level (P=0.042).

Conclusions. Vitamin D insufficiency was associated with reduced macular thickness among older patients with no patent macular dysfunction. This implies that vitamin D insufficiency may be involved in macular thinning, and provides a scientific base for vitamin D replacement trials in age-related macular degeneration.

Beyond its classical contribution to bone health, vitamin D is a secosteroid hormone involved in several target tissues expressing Vitamin D Receptors,[1,2] including the retina.[3] Epidemiological literature has recently reported an association between lower 25-hydroxyvitamin D (25OHD) concentrations and impaired visual acuity,[4] as well as an association between vitamin D insufficiency and age-related macular degeneration (AMD),[5-8] a clinical condition arising from progressive macular atrophy during aging. No previous epidemiological studies could determine whether AMD precipitated vitamin D insufficiency (due to its clinical expression with consequent loss of function and decreased sun exposure), or whether vitamin D insufficiency had a role in precipitating AMD. To date, no randomized controlled trial has explored yet the benefits of vitamin D supplementation to treat or prevent visual loss and/or AMD. Thus, to infer causality, and before conducting such a trial, it may be contributory to determine whether vitamin D insufficiency is associated with a reduced macular thickness (MT) among participants free of any known macular pathology, and thus independent of any clinical impact. The purpose of our study was to determine whether serum 25OHD was associated with MT measured with optical coherence tomography (OCT) retinal scanning in older adults with no clinical signs of macular dysfunction.

MATERIAL AND METHODS

Participants

We studied 73 community-dwellers (mean age 70.9±4.9 years; 42.9% female) followed in the Memory Clinic of Angers University Hospital, France, from November 2009 to June 2011 for a subjective memory complaint, and who were recruited into the Gait and Alzheimer Interactions Tracking (GAIT) study (ClinicalTrials.gov number, NCT01315717). The GAIT

study is an observational cross-sectional study designed to examine gait in older community-dwellers reporting subjective memory complaint. The sampling and data collection procedures have been described elsewhere in detail.[9] In summary, subjective memory complaint was documented using the Subjective Memory Complaints Questionnaire,[10] and the main exclusion criteria were age below 60 years, Mini-Mental State Examination (MMSE) score <10,[11] inability to walk independently, history of stroke, history of any acute medical illness within the past 3 months, current delirium, severe depression, and inability to understand or answer the study questionnaires. For the present analysis, participants were excluded when their refractive status was not fully determined (including the history of refractive status before cataract surgery, when applicable) or when a diagnosis of retinal or macular pathology was made, including advanced AMD (i.e., AREDS categories 3 and 4), diabetic retinopathy, vitreoretinal junction pathology, or macular detachment. Out of 73 participants included in the GAIT study, 11 participants were excluded; 3 due to a diagnosis of macular pathology (1 advanced AMD, 1 macular hole, 1 history of retinal detachment) and 8 because information on refractive status was not fully available. As a result, 62 participants were finally included in this analysis.

In addition to a full medical examination and blood tests for vitamin D, calcium and albumin concentrations, all included participants underwent a comprehensive ophthalmic clinical examination, evaluating visual acuity, intraocular pressure, fundoscopy, and retinal fundus color imaging.

Macular thickness measurement with OCT

Central MT was determined automatically and analyzed using spectral-domain HD-OCT Cirrus (Carl Zeiss Meditech, Dublin, CA). The pupil was not dilated. In every OCT map, MT detection was performed automatically without manual operator adjustment. Cirrus HD-OCT

images were generated using the Macular Cube 512 × 128 scans. Each image had 5 μm axial and 10 μm transverse resolutions in tissue and consisted of 512 × 128 volume cube. The scanning area was 6 × 6 mm. The cube was composed of 128 horizontal examination lines of 512 A-scans each. A measure of MT of 1000 μm central retina with cirrus HD-OCT was performed by an experienced orthoptist for each eye. The MT was estimated based on the 1 mm central retinal thickness area, as described in the Early Treatment Diabetics Retinopathy Study (ETDRS). The average value of two eyes in the same participant was used in our analysis. Abnormally low MT was defined using normal values provided in previous literature (i.e., MT = 267.74 μm for males, and MT = 255.60 μm for females).[12]

Serum vitamin D insufficiency

Venous blood was collected from resting participants. Serum 25OHD concentration, an effective indicator of vitamin D status,[13] was measured by radioimmunoassay (DiaSorin corp., Stillwater, MN). Intra- and interassay precisions were respectively 5.2% and 11.3%. Vitamin D insufficiency was defined for 25OHD concentrations <50 nmol/L according to the definition of the World Health Organization[14] and the US Institute of Medicine[15] (to convert to ng/mL, divide by 2.496). All measurements were performed locally at the University Hospital of Angers, France.

Covariables

The best corrected visual acuity was measured with Monoyer charts and converted into logMAR for statistical analysis purposes. The intraocular pressure in mmHg was measured by noncontact tonometry (Nidek, Nidek Co., Ltd., Aichi, Japan). Average values of two eyes in the same participant were used in our analysis. After fundoscopy, images of the retinal fundus were systematically taken via non-mydriatic fundus photography and reexamined post-hoc by

an experienced ophthalmologist. Evaluation of comorbidities (i.e., diseases lasting at least 3 months or running a course with minimal change, whatever the etiology) was based on self-report and medical record. All participants in the study had a cognitive assessment at the time of inclusion. Cognitive disorders were defined as either mild cognitive impairment or dementia, and were diagnosed using the consensus Winblad et al. criteria[16] and the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition,[17] as appropriate. The body mass index (BMI) was calculated as: [weight (kg) / height^2 (m^2)]. Weight was measured with a beam balance scale, and height with a height gauge. The supine mean arterial pressure (MAP; i.e., the average blood pressure that occurs over the entire course of the blood pressure cycle) was calculated from the systolic (SBP) and diastolic blood pressures (DBP) using the following formula: [MAP = (SBP + 2 x DBP) / 3].[18] The season of evaluation was recorded as follows: spring from March 21 to June 20, summer from June 21 to September 20, fall from September 21 to December 20, winter from December 21 to March 20. Finally, the serum concentration of calcium was measured using automated standard laboratory methods at the University Hospital of Angers, France. Because of the high prevalence of hypoalbuminemia in older adults, calcium values were corrected according to the formula: [corrected calcium value = Ca + 0.02 (46-albumin)].

Age, gender, number of comorbidities, cognitive disorders, BMI, MAP, mean visual acuity, intraocular pressure, serum calcium and season of testing were considered as potential confounders in our analysis.

Statistical analysis

The participants' characteristics were summarized using means and standard deviations (SD) or frequencies and percentages, as appropriate. As the number of observations was higher than 40, comparisons were not affected by the shape of the error distribution and no transform

was applied.[19] Firstly, comparisons between participants separated into two groups based on serum 25OHD (i.e., <50 nmol/L or ≥50 nmol/L) were performed using the Chi-square test or Student's t-test, as appropriate. Secondly, univariate and multiple linear regressions (i.e., fully adjusted model and backward model) were used to examine the association between vitamin D insufficiency (independent variable) and the MT (dependent variable), while adjusting for potential confounders. Correlation between MT and serum 25OHD concentration used as a quantitative variable was also performed. Finally, logistic regressions were used to examine the association between having abnormally low MT (dependent variable) and participants' characteristics (independent variables). P-values < 0.05 were considered significant. All statistics were performed using SPSS (v19.0, IBM Corporation, Chicago, IL) and Review Manager (v 5.1, The Nordic Cochrane Centre, Copenhagen, Denmark).

Ethics

Participants participating in the study were included after having given their written informed consent for research. The study was conducted in accordance with the ethical standards set forth in the Helsinki Declaration (1983) and the protocol was approved by the University of Angers Ethical Review Committee (CPP Ouest II - 2009-12).

RESULTS

Among 62 older participants included in this analysis (mean age, 71.2±5.0 years; 45.2% female; 100% Caucasian), the mean serum 25OHD concentration was 61.19±26.34 nmol/L. Seventeen participants (27.4%) had vitamin D insufficiency. As indicated in Table 1, the participants with vitamin D insufficiency had lower MT than those with 25OHD≥50nmol/L (232.88±40.41 µm versus 253.27±32.09 µm, P=0.042). There were no significant differences for the other clinical characteristics (Table 1). In particular, the mean logMAR visual acuity

was 0.07±0.10, with no difference between those with vitamin D insufficiency and those without. Only 14 patients were pseudophakic, with a similar distribution in the group with vitamin D insufficiency and in the group without (P=0.913). In all, 3 participants had a history of high myopia of at least –5.0 dpt; including 2 before cataract surgery, and 1 at the time of assessment. The mean binocular intraocular pressure was 15.96±2.84 mmHg, with no between-group difference (P=0.523). Lastly, 9 participants had asymptomatic macular drusen, without advanced AMD (P=0.667 for between-group comparison).

Figure 1 shows representative examples of central MT obtained from OCT retinal scanning in a participant with normal (A) and insufficient (B) vitamin D status respectively (P<0.05).

As illustrated in Table 2, univariate linear regression showed a significant association between vitamin D insufficiency and MT. This association remained significant even after adjustment for all potential confounders (ß=-51.74, P=0.014), and was retained in the backward model (Table 2). Using serum 25OHD concentration as a quantitative variable, we found no significant correlation with MT (r=0.11, P=0.410).

Lastly, the logistic regression model showed that the participants with vitamin D insufficiency had a risk multiplied by 3.7 to have abnormally low MT compared to those with sufficient level of 25OHD (P=0.042) (Figure 2).

DISCUSSION

The main finding of this OCT study is that, irrespective of all measured potential confounders, vitamin D insufficiency was associated with a thinner central macular thickness among older adults with no patent macular dysfunction.

To the best of our knowledge, this study is the first to assess and report such an association.

This novel finding is consistent with the result of a recent study highlighting an association between vitamin D insufficiency and impaired visual acuity in older adults.[4] The authors found among 311 older adults (mean age, 71.7±5.5 years; 39.9% female) that low serum 25OHD concentrations were associated with reduced vision (P=0.001).[4] Beyond the possible onset of optic neuropathy in the case of low 25OHD status,[20] vitamin D insufficiency-related impaired vision has tentatively been explained by AMD.[5-8] The firsts to report this association were Parekh and colleagues who showed among 7752 adults (mean age, 56.6 years; 56.6% female; 11% with AMD) that the OR for early AMD was 0.64 for participants in the highest versus lowest quintile of serum 25OHD (P-trend<0.001).[5] In the second study by Millen and colleagues,[6] increased serum 25OHD concentrations were associated with decreased odds of early AMD among 968 women aged <75 years (OR for highest quintile versus lowest quintile=0.52; P-trend=0.02). However, this result was not confirmed in a population of women aged 75 and older.[6] Recently, Seddon and colleagues also reported that higher dietary intakes of vitamin D were found in the twins with less severe AMD compared to monozygotic co-twins with more severe AMD (P=0.01).[21] Although dietary intakes of vitamin D are only an approximate measure of the actual serum vitamin D status,[22] this study suggested a protective effect of vitamin D against the development of AMD. Finally, a case-control study comparing 31 patients with AMD and 34 controls, reported an association between vitamin D insufficiency <50nmol/L and late stages of AMD (OR=3.10, P=0.031).[8] However, because of the cross-sectional design of studies showing an association between vitamin D insufficiency and AMD, and because of two inconclusive studies,[23,24] it remains thus far impossible to determine whether vitamin D insufficiency had a role in precipitating AMD or whether AMD precipitated vitamin D insufficiency. Importantly, our study, despite its cross-sectional design, highlights an association between vitamin D insufficiency and subclinical macular changes, and thus reinforces the hypothesis of an adverse impact of vitamin D insufficiency on the

retina. Consistent is the finding in aged mice that vitamin D administration for 6 weeks significantly reduced aging processes.[25] Treated mice showed significant reductions in retinal inflammation and levels of amyloid-beta accumulation, together with an improvement of the visual function. This implies that vitamin D insufficiency may be involved in MT thinning, in particular in AMD. Other possible mechanisms have been proposed, including the anti-inflammatory properties of vitamin D. Indeed, several studies have shown epidemiological associations between vitamin D insufficiency and a number of inflammatory diseases including multiple sclerosis or rheumatoid arthritis.[1] Moreover calcitriol experimentally suppresses antiretinal autoimmunity in experimental autoimmune uveitis induced in mice, through inhibitory effects on the Th17 effector response.[26] Finally, vitamin D may protect against wet AMD with its anti-angiogenic properties by inhibiting the proliferation of endothelial cells that express VDRs.[27] Albert and colleagues have shown, in mice with oxygeno-induced ischemic retinopathy and choroidal neovascularization, that a significant reduction in retinal neovascularization was obtained within the calcitriol-treated group compared to control animals.[28]

The finding that vitamin D insufficiency is associated with reduced MT has interesting potential clinical implications. Indeed, even if there was no correlation in our study between MT and serum 25OHD concentration used as a quantitative variable, it is of note that providing a result in terms of linear correlation —in other words, reporting a change in MT related to a change of 1 nmol/L of serum 25OHD— has only poor significance for clinical practice compared to showing an association between vitamin D insufficiency and thinner MT. To the best of our knowledge, there are no clear reference values for a 'clinically relevant change' in MT. Of note, the generally accepted and clinically relevant reference value for serum 25OHD concentration is considered to be around 50 nmol/L.[14,15] In our study, we found a significant decrease of 20.4 μm (8.1%) in MT when comparing vitamin D

insufficiency with vitamin D sufficiency (Table 2). Such estimates may help to justify, plan, evaluate, and compare the effectiveness of interventions aiming at preventing macular pathology with vitamin D supplements that would utilize MT change as an outcome measure. Some potential limitations of our study should be considered. First, the study cohort was restricted to relatively healthy community-dwelling older participants who might be unrepresentative of the population of all seniors. In particular, only 27.4% of participants had vitamin D insufficiency here, although 40-70% of seniors are generally thought to have vitamin D insufficiency in Europe.[2] Even if multiple conditions contribute to serum 25OHD status,[13] this small prevalence of vitamin D insufficiency was likely explained by the satisfactory nutritional status of participants, as indicated by the mean BMI above 25 kg/m^2, and by the relatively low morbidity burden (Table 1). Second, the cohort was limited to 62 participants, which may have exposed to lack of statistical power. Third, although we excluded participants with advanced macular pathology that could modify the association, a small proportion of participants with other ocular conditions, such as a history of high myopia or asymptomatic drusen, was still included. Despite these limitations, we were able to show a 3.7-fold higher risk of having abnormally low MT in the case of vitamin D insufficiency among older adults free of clinical retinal diseases. Further well-conducted multicentric and preferably longitudinal observational cohort studies are needed to corroborate these results on larger samples of participants before recommending vitamin D replacement trials.

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CONFLICT OF INTEREST

- **Disclosures:**
  - No conflicting relationship exists for any author.
- **Funding:**

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  - The sponsor had no role in the design and conduct of the study, in the collection, management, analysis, and interpretation of the data, or in the preparation, review, or approval of the manuscript.

AUTHORS CONTRIBUTIONS

– AG has full access to all of the data in the study, takes responsibility for the data, the analyses and interpretation, and the conduct of the research, and has the right to publish any and all data, separate and apart from the attitudes of the sponsor.
– Study concept and design: CA and OB.
– Acquisition of data: AG, DM, CA and OB.
– Analysis and interpretation of data: CA and AG.
– Drafting of the manuscript: CA and AG.
– Critical revision of the manuscript for important intellectual content: OB and DM.
– Obtained funding: OB.
– Statistical expertise: CA.
– Administrative, technical, or material support: OB.
– Study supervision: CA.

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Table 1. Characteristics and comparison of the participants (n=62) separated into two groups based on serum 25-hydroxyvitamin D concentration.

	Total cohort (n = 62)	Serum 25-hydroxyvitamin concentration (nmol/L)		P-Value*
		<50 (n = 17)	≥50 (n = 45)	
Clinical measures				
Age (years), mean ± SD	71.23 ± 4.97	71.88 ± 5.17	70.98 ± 4.92	0.527
Female, n (%)	28 (45.2)	8 (47.1)	20 (44.4)	0.854
Number of comorbidities †, mean ± SD	2.18 ± 1.64	2.82 ± 2.07	1.93 ± 1.39	0.055
Cognitive disorders ‡, n (%)	35 (56.5)	12 (70.6)	23 (51.1)	0.168
Body mass index (kg/m^2), mean ± SD	25.76 ± 3.72	26.80 ± 4.69	25.36 ± 3.26	0.176
Mean arterial pressure (mmHg), mean ± SD	97.07 ± 11.98	94.91 ± 14.88	97.90 ± 10.77	0.417
Ophthalmic examination				
Visual acuity (logMAR) ‖, mean ± SD	0.07 ± 0.10	0.08 ± 0.11	0.06 ± 0.10	0.630
History of high myopia §, n (%)	3 (4.8)	0 (0.0)	3 (6.7)	0.275
Macular thickness (μm) ‖, mean ± SD	247.68 ± 35.43	232.88 ± 40.41	253.27 ± 32.09	**0.042**
Intraocular pressure (mmHg) ‖, mean ± SD	15.96 ± 2.84	15.50 ± 2.11	16.12 ± 3.08	0.523
Drusen detection, n (%)	9 (14.5)	3 (17.6)	6 (13.3)	0.667
Pseudophaky, n (%)	14 (22.6)	4 (23.5)	10 (22.2)	0.913
Serum measures				
25OHD concentration (nmol/L), mean ± SD	61.19 ± 26.34	29.00 ± 9.53	73.36 ± 19.42	**< 0.001**
Calcium (mmol/L), mean ± SD	2.37 ± 0.10	2.33 ± 0.08	2.39 ± 0.10	**0.032**
Season of blood collection				0.221
Spring, n (%)	17 (27.4)	2 (11.8)	15 (33.3)	
Summer, n (%)	10 (16.1)	2 (11.8)	8 (17.8)	
Autumn, n (%)	31 (50.0)	11 (64.7)	20 (44.4)	
Winter, n (%)	4 (6.5)	2 (11.8)	2 (4.4)	

25OHD: 25-hydroxyvitamin D; SD: standard deviation; *: comparisons of participants with normal vitamin D concentrations (i.e., ≥50 nmol/L) with participants with vitamin D deficiency (i.e., <50 nmol/L) based on Chi-square test or Mann-Whitney U-test, as appropriate; †: diseases lasting at least three months or running a course with minimal changes; ‡: mild cognitive impairment or dementia; ||: average value of two eyes in the same participant used; §: spherical equivalent refraction of at least –5.0 dpt; P-value significant (i.e. P<0.05) indicated in bold.

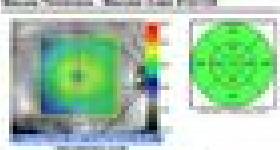
Table 2. Univariate and multiple linear regressions showing the cross-sectional association between macular thickness * (dependent variable) and vitamin D insufficiency † (independent variable), adjusted for potential confounders ‡ (n=62)

	Central macular thickness *								
	Unadjusted Model			Fully adjusted Model			Backward Model		
	ß	[95%CI]	P-Value	ß	[95%CI]	P-Value	ß	[95%CI]	P-Value
Vitamin D insufficiency †	**-20.38**	**[-40.03;-0.74]**	**0.042**	**-51.74**	**[-91.61;-11.86]**	**0.014**	**-59.44**	**[-90.46;-28.42]**	**0.001**
Age	-0.91	[-2.73;0.92]	0.326	0.63	[-2.44;3.71]	0.667	-	-	-
Female gender	**-21.69**	**[-39.04;-4.33]**	**0.015**	-23.16	[-54.19;7.88]	0.133	-24.35	[-49.47;0.77]	0.057
Number of comorbidities ‖	-4.91	[-10.36;0.54]	0.076	-1.64	[-10.06;6.78]	0.685	-	-	-
Cognitive disorders §	-11.99	[-30.03;6.05]	0.189	-8.65	[-46.32;29.02]	0.632	-	-	-
Body mass index	-2.15	[-4.54;0.25]	0.078	-0.69	[-5.99;4.60]	0.784	-	-	-
Mean arterial pressure	-0.02	[-0.89;0.84]	0.960	1.12	[-0.67;2.91]	0.203	1.23	[-0.10;2.57]	0.068
Visual acuity *	21.32	[-90.59;133.22]	0.703	52.57	[-131.28;236.43]	0.551	-	-	-
Intraocular pressure *	-3.12	[-7.20;0.96]	0.131	-5.58	[-11.82;0.65]	0.073	-4.66	[-9.71;0.39]	0.068
Serum calcium concentration	42.51	[-48.59;133.60]	0.354	55.42	[-152.63;263.47]	0.579	-	-	-

β: Coefficient of regression corresponding to a change in macular thickness; CI: confidence interval; *: average binocular measure; †: serum 25-hydroxyvitamin D <50 nmol/L; ‡: including the influence of seasons; ||: diseases lasting at least three months or running a course with minimal changes; §: mild cognitive impairment or dementia; β significant (i.e., $P < 0.05$) indicated in bold.

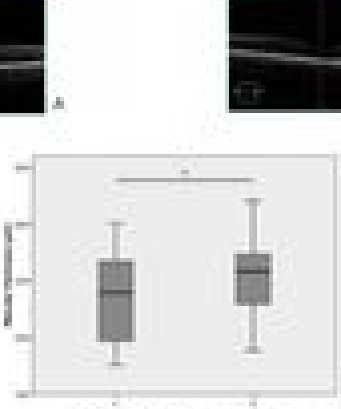
Figure 1. Representative examples of macular thickness measured with optical coherence tomography (OCT) in a participant with sufficient (A) and insufficient (B) vitamin D status. To facilitate the comparison, a box plot of each group point is shown (C). *: macular thickness in the group with vitamin D insufficiency significantly thinner than that in the group with vitamin D sufficiency ($P<0.05$).

Figure 2. Odds ratio [95% confidence interval (CI)] of having abnormally low macular thickness (i.e., MT<267.74μm for males, and MT<255.60μm for females) according to participants' characteristics (n=62).



A

B



C