PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Effect of Vitreomacular Adhesion and Vitreous Gel on Age-related Reduction of Macular Thickness: A retrospective observational study
AUTHORS	Kumagai, Kazuyuki; Hangai, Masanori; Furukawa, Mariko; Suetsugu, Tetsuyuki; Ogino, Nobuchika

VERSION 1 - REVIEW

REVIEWER	Timothy L Jackson King's College London
REVIEW RETURNED	23-Jun-2016

GENERAL COMMENTS	The authors describe a longitudinal study of 337 patients, looking at the effect of vitreomacular attachment on the age-related change in retinal thickness. They compare three groups: normal eyes with vitreomacular adhesion (VMA), normal eyes with vitreomacular separation (VMS) and eye post-vitrectomy. Follow up ranged from 24 to 60 months Whilst the paper will not have a direct effect on clinical care, the question posed by the authors is of clinical interest, as there is growing recognition that the vitreomacular interface may influence the macular disease. The study design is reasonable, but he paper might present the results using more standard conventions. Having said that, the changes required are relatively simple. The key issue is that the vitrectomy group has concomitant disease that is treated during surgery and this may influence the change in macular thickness independent of vitreous separation. Please state the hypothesis. ABSTRACT The authors describe two primary outcomes – there can, by definition, be only one. Whilst a co-primary is possible that is statistically more complicated than is needed and I suggest choosing one primary and demoting the other to secondary. It is difficult to understand from the abstract what is being compared. The abstract appears to report significant changes over time within the VMA group and within both the VMS and vitrectomised group, but also some comparison across groups. I suggest presenting mean (SD) annual change in the three groups (eg +2.5 +/- 2.4, -2.5+/-3.1, and 2.6 +/- 1.2) and then statistically compare both VMS and vitrectomy (separately) with VMA.

KEYWORDS

I suggest adding vitreomacular adhesion which is more commonly used term than vitreomacular attachment. Add posterior vitreous detachment and vitreomacular interface.

ARTICLE SUMMARY

The article summary describes the methodology but it should also describe the result.

STRENGTHS AND LIMITATIONS

There were not a large number of patients eg 54 VMA eyes is not especially large
Surgery being performed by one surgeon is not a particular strength – the article is not describing a surgical technique as such, and even if it were, having only one surgery may make the results less generalizable ,

There may well be confounding due to concomitant disease, eg closure of a macular hole may alter thickness.

There were differences in age across groups although that need not appear in the summary of weaknesses (suggest it appears in the discussion) as the age gap was small and probably of little biological significance.

METHODS

Please confirm the healthy volunteers provided written informed consent to undergo longitudinal OCT observations. Assuming this was done per protocol why was there such a range of follow up intervals? Was this due to loss to follow up, protocol deviations, or some other issue?

Was the trial registered somewhere? Is the protocol in the public domain eg institutional repository

It would help readers to provide specific inclusion and exclusion criteria. Much of this inforomation is included in the report but a specific inclusion/exclusion section makes it easier to find. Also, what happened to patients whose VMA status was unclear (often quite a sizeable proportion have no vitreous face evident on OCT, which can be due to full attachment or full detachment)? Were these eyes excluded? Is so that should be a specific exclusion criteria.

What was done about segmentation errors? It appears images with centering or segmentation errors were simply excluded, but was there the facility to correct centering/segmentation errors as there can often be consistent/repeated errors. Note different OCT machines use different anatomic layers for their segmentation lines. Please provide more information since this is the primary outcome.

In table 1 please clarify what the p values relate to – is it VMS vs VMA and also a comparison of absent gel vs VMA (in which chase shouldn't there be two p values or were both <0.0001?). Thanks for clarifying.

	RESULTS Were all the macular holes closed or did thickness measurements include persisting macular holes. DISCUSSION Please cite the supporting refs for the first sentence. It is important to stress that the differences across groups, whilst statistically significant, are very small (mostly <1 micron). The biological significance of such a small change is uncertain and this should be discusssed. The authors mention that the eyes were at least 1 year post vitrectomy before having their first study OCT. This is very helpful. The information should be added to methods eg eye with less than 1 year post vitrectomy were excluded. Indeed, it might help readers to provide the full list of inclusion/exclusion as on online-only supplement. The authors note the ILM peeling tends to speed up the rate of macular thickness reduction in the inner temporal and inferior sectors but I note this comment comes immediately after one noting that they didn't have sufficient numbers to evaluate the effect of ILM peeling and so this comment is probably best removed. Also, I would avoid introducing results in the discussion, rather than in the results section (where I didn't see this information). Alternatively are they citing another study (in which case please cite)? The discussion of weaknesses needs to further consider confounding from eye disease and surgery. I suggest adding that patients were at least a year after vitrectomy, which is a strength that somewhat mitigates the effect of confounding from surgical benefit. FIGURE Please explain in the legend what the error bars are (SD/SEM)

REVIEWER	Akinori Uemura Ophthalmology, Kagoshima City Hospital Japan
REVIEW RETURNED	04-Jul-2016

GENERAL COMMENTS	1) It would not be appropriate to make mention to the association between avitreous eyes and retinal thickness changes because surgical intervention including ILM peeling is likely to be responsible for the change of macular thickness even if the measurement is done at least 1 year after surgery. Therefore, comparison of two groups, VMA group and VMS group, would be appropriate for the purpose of this study. 2) In this paper, the main outcome measures were the rates of macular thickness change, analyzed with the macular change analysis program. However, the authors had also better determine

	the significance of the changes between two points (before and after) of measures of macular thickness in each group. 3) Usually absolute values of macular thickness cannot be adapted if the data have a wide range of values. Does the statistical significance of the results change when using the percentages of the thickness? 4) The authors described that "The status of posterior vitreous attachment was confirmed to be the same in each of the SD-OCT image in all of the healthy subjects", however it has largely been a clinical experience that the status of posterior vitreous attachment can change according to the observational period, rather in the average of 36 months. If the subjects with any changes of the status of posterior vitreous attachment were excluded in this study, please so state in the method section. 5) The authors should state how did the healthy subjects be recruited in this study? 6) In each group, the subject's age is 56 or over. If the authors exclude subjects under 56 years of age, add the statement in the method section. 7) Looking at figure 1(box-and-whisker plots), there appears to be an outlier. Does excluding the subject change the statistical significance of the results? 8) The first paragraph of discussion should be moved to introduction. It's easy to understand why the authors classified the subjects into several groups. 9) As the authors said, previous reports mentioned that the central foveal thickness (CFT) increased with age. However, CFT in the VMS group decreased in the study. It is essential for the authors to discuss the reason. 10) The significant difference of subject's mean age between VMA and VMS may influence the result of this study.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Timothy L Jackson
Institution and Country
King's College London

Please state any competing interests or state 'None declared':
None

Please leave your comments for the authors below

The authors describe a longitudinal study of 337 patients, looking at the effect of vitreomacular attachment on the age-related change in retinal thickness. They compare three groups: normal eyes with vitreomacular adhesion (VMA), normal eyes with vitreomacular separation (VMS) and eye post-vitrectomy. Follow up ranged from 24 to 60 months

Whilst the paper will not have a direct effect on clinical care, the question posed by the authors is of clinical interest, as there is growing recognition that the vitreomacular interface may influence the macular disease.

The study design is reasonable, but the paper might present the results using more standard conventions. Having said that, the changes required are relatively simple.

The key issue is that the vitrectomy group has concomitant disease that is treated during surgery and this may influence the change in macular thickness independent of vitreous separation.

Please state the hypothesis.
Answer: Thank you for your comment. This study was conducted because we were interested in determining how the vitreous gel is involved in the changes in the macular thickness. Our hypothesis has been added to the third paragraph of the Introduction section as follows:

Page 6, Introduction, 3rd paragraph, 1st sentence
“We hypothesize that the presence of vitreous gel prevents a decrease in macular thickness.”

ABSTRACT
The authors describe two primary outcomes – there can, by definition, be only one. Whilst a co-primary is possible that is statistically more complicated than is needed and I suggest choosing one primary and demoting the other to secondary.
Answer: We have selected the rates of change in the central sector as the primary outcome, and placed the rates of macular thickness change in the inner four sectors as the secondary outcomes. The sentences on the relationships between macular thickness and other factors (multiple regression analyses) were deleted. We revised the sentence as follows:

Page 2 Abstract
“Primary and secondary outcome measures:The primary outcome measure was the rate of change of the macular thickness in the central sector. The secondary outcomes were the rates of change in the macular thickness in the inner four sectors.”

It is difficult to understand from the abstract what is being compared. The abstract appears to report significant changes over time within the VMA group and within both the VMS and vitrectomised group, but also some comparison across groups. I suggest presenting mean (SD) annual change in the three groups (eg +2.5 +/- 2.4, -2.5+/-3.1, and 2.6 +/- 1.2) and then statistically compare both VMS and vitrectomy (separately) with VMA.
Answer: As suggested, we have added the means ± SDs of the annual changes of each group. We have separated the vitrectomized group into the ILM-on and the ILM-off groups. We also changed the Results and Conclusion sections as follows:

Page 2 Abstract
“Results: The annual rate of change in the macular thickness of the central sector was 0.76±1.8 μm/year in the VMA group, -0.58±2.3 μm/year in the VMS group, -1.57 ± 1.9 μm/year in ILM-on group, and -0.86±3.1 μm/year in the ILM-off group. There was a significant difference between the rate of central sector thickness change in the VMA and VMS groups (P=0.0001). The presence of VMA was a significant factor associated with an increase in the central sector thickness (P=0.0055). When the healthy and ILM-on groups were compared, the rate of a decrease in central sector thickness was faster in the ILM-on group (P=0.0043). Multiple regression analyses showed that not peeling the ILM during the vitrectomy was a significant factor associated with a decrease in the central sector thickness (P=0.044).
Conclusion: The presence of a VMA and a vitreous gel may restrain the macular thickness reduction.”

KEYWORDS
I suggest adding vitreomacular adhesion which is more commonly used term than vitreomacular attachment. Add posterior vitreous detachment and vitreomacular interface.
Answer: We have added vitreomacular adhesion to the keywords:
“macular thickness, vitrectomy, vitreomacular adhesion, vitreomacular separation, posterior vitreous detachment, vitreomacular interface”

ARTICLE SUMMARY
The article summary describes the methodology but it should also describe the result.
Answer: We have revised the Summary as follows:

Page 5 Article Summary
“The rate of macular thickness change was determined in eyes with vitreomacular adhesion, with vitreomacular separation, and without vitreous gel due to vitrectomy in the spectral-domain optical coherence tomographic images. The macular thickness increased in eyes with vitreomacular adhesion in all sectors, while it decreased in eyes with vitreomacular separation and in vitrectomized eyes.”

STRENGTHS AND LIMITATIONS
There were not a large number of patients eg 54 VMA eyes is not especially large
Surgery being performed by one surgeon is not a particular strength – the article is not describing a surgical technique as such, and even if it were, having only one surgery may make the results less generalizable,

There may well be confounding due to concomitant disease, eg closure of a macular hole may alter thickness.

There were differences in age across groups although that need not appear in the summary of weaknesses (suggest it appears in the discussion) as the age gap was small and probably of little biological significance.
Answer: We have removed the sentences on a large number of subjects, a same surgeon, and subject ≥56-years-of-age. We have added a sentence on concomitant diseases treated during the surgery. As for the strength, we added a sentence about vitrectomized group examined at least 1 year after the vitrectomy. We have revised this sections follows:

Page 5 Strengths and limitations
λ The patients in vitrectomized groups were examined at least 1 year after the vitrectomy when the confounding effects of the surgery were somewhat mitigated.
λ The vitrectomized group had concomitant diseases that were treated during the surgery and this may have influenced the changes in the macular thickness independent of the vitreous separation
λ The sample size was different in each group with the ILM-on group being the fewest with 26 eyes.

METHODS
Please confirm the healthy volunteers provided written informed consent to undergo longitudinal OCT observations. Assuming this was done per protocol why was there such a range of follow up intervals? Was this due to loss to follow up, protocol deviations, or some other issue?
Answer: A signed informed consent was obtained from all the patients including the healthy group. The healthy subjects were the patients who were being followed because of a cataract and did not have any retinal diseases. There is a large range in follow-up period because we selected only the latest OCT scan for the retinal thickness measurements. We collected OCT scans at every visit for the patients after the vitrectomy, so the range of 24 to 60 months is simply due to the difference in the continuation periods in the outpatient visits. There was no loss during the follow-up period because of the retrospective nature of this study, i.e., we searched for the medical records of the patients after the vitrectomy. We have added the descriptions on the healthy group and the selection of OCT scan as follows:

Page 7, Materials and Methods, Participants section, 1st paragraph
“This was a longitudinal retrospective study of subjects who were examined at the Shinjo

Ophthalmologic Institute, Kami-iida Daiichi General Hospital, and the Nishigaki Eye Clinic from October 2007 through December 2015. The subjects consisted of healthy controls and the patients who had undergone vitrectomy. The healthy controls (healthy group) were the patients who were being followed because of a cataract and did not have any retinal diseases. The healthy group was classified into a VMA group consisting of 54 eyes and a VMS group consisting of 164 eyes. The VMA status was confirmed to be the same in each of the SD-OCT image in all subjects of the healthy group."

Page 10, Materials and Methods, HD-OCT Recordings section, 2nd paragraph
"The average increase or decrease in the retinal thickness/year was determined using the Cirrus macular change analysis program. The macular change analysis program compares the OCT scans from consecutive examinations. The retinal alignment was based on the vessel landmarks to the initial OCT images, which was done automatically. The analyses were made on the central and the inner four ETDRS sectors with a mean interval of 36 months (range, 24-60 months) between the two observational scans. For the vitrectomized group, a first OCT scan was recorded at least 1 year after the vitrectomy. Then, a second OCT scan was recorded at least 2 years after the first scan. Therefore, all patients from this group were examined at least 3 years after the vitrectomy. When several OCT scans were available from the same patient, only the latest scan was selected for the analysis."

Was the trial registered somewhere? Is the protocol in the public domain eg institutional repository.

Answer: This trial was not registered in public domain or institutional repository. However, the medical records are available.

It would help readers to provide specific inclusion and exclusion criteria. Much of this information is included in the report but a specific inclusion/exclusion section makes it easier to find. Also, what happened to patients whose VMA status was unclear (often quite a sizeable proportion have no vitreous face evident on OCT, which can be due to full attachment or full detachment)? Were these eyes excluded? Is so that should be a specific exclusion criteria.
Answer: We have added a subheading, "Inclusion and exclusion criteria" in the Methods section and included the following paragraphs:

Page 8, Materials and Methods, Inclusion and Exclusion Criteria section
"The inclusion criteria for both groups were OCT image signal intensity ≥7, axial length <28.00 mm, and age ≥56 years at the time of the first OCT measurement. The inclusion criteria only for the healthy group were BCVA ≥20/20, no significant cataract, and normal foveal contour. The inclusion criteria only for the vitrectomized group were outpatient visit continued >3 year after the vitrectomy with OCT images available at each visit and the VMA status remain unchanged at these visits.

The exclusion criteria for both groups were persistent centering and segmentation errors. In addition, subjects with any ocular or systemic disorders that could affect the retinal thickness, e.g., glaucoma, optic nerve diseases, and diabetes mellitus, were excluded. The exclusion criteria only for the healthy group were unclear VMA status, i.e., cases in which full adhesion or detachment of posterior vitreous could not be determined, vitreomacular traction, foveal deformation, and the change in VMA status at second OCT scan. The exclusion criteria only for the vitrectomized group was outpatient visit continued <3 year after the vitrectomy."

What was done about segmentation errors? It appears images with centering or segmentation errors were simply excluded, but was there the facility to correct centering/segmentation errors as there can often be consistent/repeated errors. Note different OCT machines use different anatomic layers for their segmentation lines. Please provide more information since this is the primary outcome.

Answer: We did not try to correct the segmentation errors because the manual corrections cause subjective bias due to differences in boundary lines drawn by the delineators. In addition, it was not possible to correct the centering errors because the Cirrus macular cube scans is a 6 × 6-mm square. For example, if the center drifted temporally from the actual fovea and it is manually shifted nasally for correction, the nasal edge of an image would not be present. Thus, the scans with centering errors or segmentation errors were excluded during the measurements by the examiners. We have revised the sentences as follows:

Page 9, Materials and Methods, HD-OCT Recordings section, 1st paragraph
"The Cirrus HD-OCT (Carl Zeiss Meditec Inc, Dublin, California, USA) was used to obtain the axial images of the vitreoretinal interface. At least 5 B-scan images of 6 mm in length passing through the fovea along the horizontal and vertical axes were recorded with 4096 A-scan resolution and 0.25-mm intervals between neighboring B-scans. The average regional thicknesses of the Early Treatment Diabetic Retinopathy Study (ETDRS) sectors and 6×6-mm macular cube scans were composed of 200 B-scans and 200 A-scans centered on the fovea. Experienced OCT examiners scanned the retina at least 3 times to obtain images with signal intensities of ≥7. The images with centering or segmentation errors were excluded. The retinal thickness was automatically measured with the vitreoretinal interface as the inner border of the retina and the anterior border of the RPE as the outer border of the retina."

In table 1 please clarify what the p values relate to – is it VMS vs VMA and also a comparison of absent gel vs VMA (in which chase shouldn't there be two p values or were both <0.0001?). Thanks for clarifying.
Answer: In the revised manuscript, we decided to change Table 1 so that we could include the information on whether the ILM was or was not peeled during the vitrectomy. Thus, the vitrectomy group was separated into the ILM-on and ILM-off groups and comparisons were made between these two subgroups. The healthy group was divided into VMA and VMS groups for comparison. To clarify what the P values relate to, we added the statistical tests used to compare between each subgroup in the footnote to the tables.

RESULTS
Were all the macular holes closed or did thickness measurements include persisting macular holes.

Answer: All macular holes were closed after the surgery. We added the following sentence:

Page 11, Results, 1st paragraph, 1st ~2nd sentence
"The baseline demographics of the subjects and the mean rates of macular thickness change in the VMA, VMS, ILM-on, and ILM-off groups are shown in Table 1. The MHs were closed in all 110 eyes after the vitrectomy, and no surgical complications were observed in the vitrectomized group."

DISCUSSION
Please cite the supporting refs for the first sentence.
Answer: The second reviewer suggested that this sentence should be moved to the Introduction section. However, when we did this, we noticed there it was similar to what we wrote in the second sentence of the first paragraph of the Introduction section starting from, "The results of…". Therefore, we decided to delete this sentence to avoid redundancy.

It is important to stress that the differences across groups, whilst statistically significant, are very small (mostly <1 micron). The biological significance of such a small change is uncertain and this should be discusssed.
Answer: We have made major revisions in the Discussion section, and the biological/physiological significance of our research was discussed. Originally, we were interested in examining whether the

increased oxygen level after vitreous gel removal could cause a decrease in the macular thickness. Also, we examined whether such changes were common in diseased eyes and healthy eyes, e.g., diabetic macular edema and healthy eyes without fundus abnormalities. We added the following paragraph in the Discussion section:

.

Page 17, Discussion, 7th paragraph

"Although our study showed that the annual change in macular thicknesses for each group was significant, the amount of change was very small (mostly <1 μm). Our hypothesis was based on the idea that the retinal oxygen distribution change occurring after the vitreous gel removal will eventually lead to retinal thinning. Vitrectomy leads to an increase in the retinal oxygen concentration.17 Elevated intraocular oxygen levels tend to be beneficial for the resolution of macular edema, but may contribute to the development of nuclear cataract and primary open-angle glaucoma.23-25 An intact vitreous gel may play an active role in preventing retinal tissue damage from free oxygen radicals.24-25 As the vitreous gel undergoes liquefaction by aging or surgical removal, the fluid that replaces the vitreous gel promotes a rapid distribution of oxygen within the eye through fluid mixing23-25 leading to a wider area of retinal tissue damages and thinning by exposure to free radicals. Thus, we hypothesized that the presence of vitreous gel may help restrain the age-related reduction of the macular thickness. Still, the reason for the faster rate of macular thickness reduction in the vitrectomized group compared to the VMS group was not definitively determined."

The authors mention that the eyes were at least 1 year post vitrectomy before having their first study OCT. This is very helpful. The information should be added to methods eg eye with less than 1 year post vitrectomy were excluded. Indeed, it might help readers to provide the full list of inclusion/exclusion as on online-only supplement.

Answer: As mentioned, we added a subheading "Inclusion and exclusion criteria" in the Methods section. We were able to refer to full inclusion/exclusion in this section, so we did not provide online-only supplemental materials.

The authors note the ILM peeling tends to speed up the rate of macular thickness reduction in the inner temporal and inferior sectors but I note this comment comes immediately after one noting that they didn't have sufficient numbers to evaluate the effect of ILM peeling and so this comment is probably best removed. Also, I would avoid introducing results in the discussion, rather than in the results section (where I didn't see this information). Alternatively are they citing another study (in which case please cite)?

Answer: We decided to include these data in the Results section and to add the comments in the Discussion section. There are no citations because all the data are from the current study. We added following paragraphs in the Results and the Discussion sections:

Page 12, Results, 3rd paragraph

"The results of the multiple regression analyses for macular thickness changes in the vitrectomized group are shown in Table 3. The ILM peeling was a factor significantly associated with a decrease in macular thicknesses in the inner temporal and inferior sectors (P=0.0096 and P=0.0047, respectively). The central sector thickness was not affected by the ILM peeling (P=0.58)."

Page 16, Discussion, 5th paragraph

"In the vitrectomized group, the macular thickness decreased in all of the sectors. The rate of macular thickness decrease was faster in the central sector of the ILM-on group at -1.57 μm/year. In contrast, the rate of decrease was faster in the inner four sectors of the ILM-off group at -1.72 to -2.47μm/year. ILM peeling tended to increase the rate of macular thickness reduction in the inner temporal and inferior sectors (Table 3). However, the ILM-on group consisted of only 26 eyes (21.8% of all vitrectomized eyes), so the results may show significant differences in other sectors if more data were

available in this group."

The discussion of weaknesses needs to further consider confounding from eye disease and surgery. I suggest adding that patients were at least a year after vitrectomy, which is a strength that somewhat mitigates the effect of confounding from surgical benefit.
Answer: We have revised the strengths and weaknesses of our study in the Discussion section as follows:

Page 18, Discussion, 9th paragraph
"The strength of this study is that the patients in the vitrectomized groups were examined at least 1 year after the vitrectomy when the confounding effects of the surgery were somewhat mitigated. There are several limitations to this study. First, the vitrectomized group had concomitant diseases, mostly MHs, that were treated during the surgery and this may have influenced the changes in the macular thickness independent of the vitreous separation. Second, we examined subjects who were ≥56-years, and the rates of macular thickness reduction may not apply to younger individuals. Third, the results need to be confirmed by a larger sample size because the number of eyes in the VMA group as well as in the ILM-on group were small. Fourth, we measured the thickness of the entire retina, and did not measure the thickness of each layer. Some authors have reported that individual retinal layers were selectively affected by age.12,13 Future studies should be designed to determine whether the reduction in the macular thickness is related to a specific retinal layer."

FIGURE
Please explain in the legend what the error bars are (SD/SEM)
Answer: We added a following comment in our figure legend:

Page 24, Figure legend, 3rd sentence
"The error bars represent the standard deviations."

Reviewer 2:
Reviewer Name
Akinori Uemura

Institution and Country
Ophthalmology, Kagoshima City Hospital
Japan
1) It would not be appropriate to make mention to the association between avitreous eyes and retinal thickness changes because surgical intervention including ILM peeling is likely to be responsible for the change of macular thickness even if the measurement is done at least 1 year after surgery. Therefore, comparison of two groups, VMA group and VMS group, would be appropriate for the purpose of this study.
Answer: The main purpose of our study was to compare the differences in the annual changes of macular thickness in eyes with and without vitreous gel. I agree with your comment that ILM peeling is likely to be responsible for the change of the macular thickness, so we decided to separate vitrectomized group into the ILM-off group and ILM-on group. We compared the healthy group (VMA+VMS groups) with the ILM-on group, so that the comparisons of annual macular thickness changes between the groups with and without a vitreous gel will be more meaningful.

2) In this paper, the main outcome measures were the rates of macular thickness change, analyzed with the macular change analysis program. However, the authors had also better determine the significance of the changes between two points (before and after) of measures of macular thickness in each group.
Answer: Because of the retrospective nature of this study, we could not collect any OCT scans before

the vitrectomy, so there are no means to compare the macular thickness change before and after the surgery.

3) Usually absolute values of macular thickness cannot be adapted if the data have a wide range of values. Does the statistical significance of the results change when using the percentages of the thickness?
Answer: We found that the statistical significance did not change by adopting the data using the percentages of the macular thickness change. We provided online only supplementary Tables 1-5 showing the data from each table in percentage of the thickness change.

4) The authors described that "The status of posterior vitreous attachment was confirmed to be the same in each of the SD-OCT image in all of the healthy subjects", however it has largely been a clinical experience that the status of posterior vitreous attachment can change according to the observational period, rather in the average of 36 months. If the subjects with any changes of the status of posterior vitreous attachment were excluded in this study, please so state in the method section.
5) The authors should state how did the healthy subjects be recruited in this study?
6) In each group, the subject's age is 56 or over. If the authors exclude subjects under 56 years of age, add the statement in the method section.
Answers to comments #4, #5, and #6:
We agree and have included the inclusion and exclusion criteria for the healthy subjects including the age. We have added a subheading section, "Inclusion and exclusion criteria" in the Methods section and included the following paragraphs:

Page 8, Materials and Methods, Inclusion and Exclusion Criteria section
"The inclusion criteria for both groups were OCT image signal intensity ≥7, axial length <28.00 mm, and age ≥56 years at the time of the first OCT measurement. The inclusion criteria only for the healthy group were BCVA ≥20/20, no significant cataract, and normal foveal contour. The inclusion criteria only for the vitrectomized group were outpatient visit continued >3 year after the vitrectomy with OCT images available at each visit and the VMA status remain unchanged at these visits.

The exclusion criteria for both groups were persistent centering and segmentation errors. In addition, subjects with any ocular or systemic disorders that could affect the retinal thickness, e.g., glaucoma, optic nerve diseases, and diabetes mellitus, were excluded. The exclusion criteria only for the healthy group were unclear VMA status, i.e., cases in which full adhesion or detachment of posterior vitreous could not be determined, vitreomacular traction, foveal deformation, and the change in VMA status at second OCT scan. The exclusion criteria only for the vitrectomized group was outpatient visit continued <3 year after the vitrectomy."

7) Looking at figure 1 (box-and-whisker plots), there appears to be an outlier. Does excluding the subject change the statistical significance of the results?
Answer: We did not detect any outliers in our results. We revised Figure 1 so that the vitrectomized group is separated into the ILM-on and the ILM-off groups.

8. The first paragraph of discussion should be moved to introduction. It's easy to understand why the authors classified the subjects into several groups.
Answer: We tried to move it to the Introduction section, but then it was similar to what we wrote in the second sentence of the first paragraph of Introduction section starting from "The results of…". Therefore, we decided to delete this sentence to avoid redundancy.

9. As the authors said, previous reports mentioned that the central foveal thickness (CFT) increased with age. However, CFT in the VMS group decreased in the study. It is essential for the authors to

discuss the reason.
10. The significant difference of subject's mean age between VMA and VMS may influence the result of this study.
Answers to comments #9 and #10:
We have revised this paragraph in the Discussion section as follows:

Page 16, Discussion, 4th paragraph
"Other authors have reported that the central foveal thickness increases with increasing age.5,10,11 The significant difference in the mean ages of the VMA and VMS groups may have affected our results. However, the multiple regression analyses of the healthy group showed that the age was not significantly associated with the macular thickness. Instead, the presence of VMA was a significant factor associated with the macular thickness increase of each sector. The results of the earlier studies showed that the central foveal thickness increased with increasing age in both VMA and VMS groups. Thus, the proportion of the individuals with VMA may be higher in these reports leading to the observed increase in central sector thickness."