Author's response to reviews

Title: Macular Thickness Measurements in Healthy Norwegian Volunteers: An Optical Coherence Tomography Study.

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Version: 3 Date: 4 March 2010

Author's response to reviews: see over
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Title: Macular Thickness Measurements in Healthy Norwegian Volunteers: An Optical Coherence Tomography Study.

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Version: 3 Date: 3 March 2010

Author's response to reviews: see over
Dear BioMed Central Editorial Team,

Thank you for your comments and instructions which have undoubtedly helped us to improve our manuscript.

As we addressed the very relevant concern about the low cut cut-off for signal quality, which necessitated a re-inspection of the OCT scans, we noticed that we by accident had included measurements from three subjects twice in the analysis. In addition, the age-variable had to be recalculated from the date of examination, as we by error had used age to the date of analysis in the first version. We therefore reanalyzed the data, this time also including the subject who fell out of the original statistics (as described in methods section in the original version of the manuscript), and another subject who had been measured after the original inclusion date. All tables and figures are therefore mended accordingly.

In methods section we excluded the justification discussion for pupil dilatation, as reviewer number 2 kindly suggested. We also extended our description of inclusion criteria for laterality randomization.

The discussion has been extended providing potential physiological reasons behind our observations, as requested by reviewer 3, which included providing a broad evidence background for our hypothesis. This has resulted in substantial changes to our manuscript’s content.

Table’s titles were adjusted to conform to BMC style (shortened) and legends mended as requested.

Point- to point amendments:

Reviewer number three’s report

Major compulsory revisions

Methods, page 7. Signal strength (SS) of 3 has been defined as the cut-off. This is very low signal strength. Usually SS of 6 or higher are used for inclusion criteria, considering the fact that the participants of this study were healthy with good BCVA. Please provide the number of subjects/scans with SS of 3-5 and the number of subjects/scans with SS of 6 and above in the manuscript.

SS is included in the manuscript as requested: Methods section “Signal strength was 4 or 5 in 38 scans, 6 or 7 in 43 scans and 8 to 10 in 26 included scans”. Mean SS(SD) is also included in Table 1.

The reason why low cut-off for SS was chosen is that we wished to set same inclusion criteria for patients (in parallel studies) as for healthy subjects in this normative study.
Comparing normative data with very high SS with data from patients with lower SS could bias the results.

Lower SS are achieved in measurements through undilated pupils (Smith M et al: Effect of pupillary dilatation on glaucoma assessments using optical coherence tomography. Br J Ophthalmol 2007, 91:1686-1690), as conducted in our study, while most other reports with higher SS were conducted on dilated pupils. However, low SS does not seem to influence the MMT measurements as reported by Barkana et al. (Inter-device Variability of the Stratus Optical Coherence Tomography. Am J Ophthalmol 2009, 147:260-266.) and Muscat et al. (Repeatability and reproducibility of macular thickness measurements with the Humphrey OCT system. Invest Ophthalmol Vis Sci 2002, 43:490-495.) These matters are pointed out in the Methods section and are somewhat extended in the mended manuscript: “Eye movements, fixation losses and blinks prolonged the procedure, while undilated pupils[38] and soft contact lenses [39] deteriorated the signal strength. Barkana et al. [40] found that signal strength did not affect any measured MMT parameters when scans with signal strength ≥4 (out of maximum ten) were included (using the fast macular protocol) [40]. Moreover, Muscat et al. [41] showed that even considerably degraded signal strength produced accurate, precise and reproducible MMT measurements”. However, when SS was assessed in the present study sample (Table 1) it turned to be good after all.

Methods, page 8, last paragraph. These data belong to Results section. Please provide these data in a table and a summary of the table in the Results section.

Table 1 is inserted as requested.

Methods, page 10. What posthoc analysis did you use with ANOVA to address the issue of multiple comparisons?

Each ANOVA was carried out assessing two dependent variables at a time. Moreover, categorical variables were dichotomous. For this reason, we did not perform post-hoc comparisons within the ANOVA analysis. Data reduction was achieved by using regional variables, obviating the need for correction for multiple comparisons, in line with the view expressed by several statistical authorities (e.g. Perneger TV. What’s wrong with Bonferroni adjustments BMJ 1998;316:1236-8).

Discussion, page 12. Please provide the reference numbers after Huang’s and Guedes’ names.

Reference numbers are inserted (number 32 and 55): “Normal aging seems to affect macular cone function [46] but not foveal cone density [47]. Most [44, 45, 48-54] but not all [32, 55] studies on the OCT did not report an association between foveal thickness and age in mixed gender group, which is in agreement with our study”

Tables. Please provide the definitions of abbreviations clearly, separately and in order of appearance, in the captions of all tables.
Minor essential revisions

Discussion. Why a significant interaction between region and parity was observed? Why parity has an impact but contraceptive does not? What is the possible physiology behind these findings? Please discuss properly.

Parous women had thicker retinas mostly in the central region (Table 6) but a significant interaction between region and parity was not observed (Table 3). We have discussed the association between parity and MMT in a new paragraph in the discussion:

“Reproductive experience is also associated with long term alterations in sex hormone metabolism [80, 81]. Parity seems to lower estrogen levels in parous premenopausal compared with nulliparous women [81], partly by altering the sensitivity to sex hormones [82, 83]. Reproductive history factors like long and/or irregular menstrual cycles, early menopause, pregnancy losses and multiparity are associated with higher risk for cardiovascular disease [25]. Biomarkers of cardiovascular disease (CRP and homocysteine) are also elevated in AMD, suggesting a common pathogenesis of low grade chronic inflammation and atherosclerosis for both conditions [28]. Hence, the association between MMT and parity (Table 6) can possibly be related to a combination of hormonal and cardivascular risk factors. It is also reassuring to note that the intermittent use of contraceptives did not seem to affect retinal thickness.”

Why did we not find any effects of contraceptives and MMT? In the present study contraception use was defined by as a regular oral intake for at least three months prior to inclusion. Neither type and dosage of various gestagens and estrogens in the contraceptive pills nor the cumulative time of use were registered. It is accordingly possible that a biologically relevant effect of oral contraceptives could have been demonstrated in a larger study. Anyway, it should be noted that rather short-lasting current use of contraceptives did not seem to affect retinal thickness. This interpretation has been added to the discussion.

Discussion, page 13. Overall the Discussion is weak. It mainly restates the results; whereas it can provide great insight toward gender, hormones and macular thickness in Norwegians. You need to discuss the physiology behind aging, macular thickness and AMD risk factors. How and why it is more frequent in Norwegians? What do supporting literatures say? Why gender differences existed in all areas? Is it a hormonal effect, a chromosome dependent factor or something else? There is no proper discussion for why the mean foveal thickness was higher in parous women.

We believe that hormones, estrogens in particular, are the most probable factors to explain gender differences in macular thickness, although differences in body size
between the genders may also have an impact. The discussion was extended and rewritten to include these gender- and parity-related themes.

We do not know why early age-related maculopathy is more frequent in Norwegians. However, a large proportion of Norwegian women are current or former smokers, many choose not to have children and surgically induced menopause is not uncommon. Ultraviolet radiation is high during summers, but low during winters. However, mean macular thickness in our sample did not appear to differ from values previously reported in whites. This issue can be addressed by investigating differences between older Norwegians and the elderly in other populations, which is beyond the scope of the current study and is therefore not included in our revised discussion.

Discussion, page 13, last paragraph. There are publications regarding estrogen and increased risk of macular hole; hormones and retinal thickness; contraceptive and macula; hormones and macular thickness and estrogen and AMD in PubMed. You may gain insight by reviewing these publications to enrich your discussion.

We focused the discussion mainly on normal aging and hormones, as abnormal mechanisms may be involved in the pathogenesis of macular holes and neovascular AMD. However, the Background section was extended to include some of these matters:

“Macular aging involves alterations in its function, structure [7] and blood supply [8], which are partly induced by chronic low-grade inflammation [9]. Complex multifactorial genetic and environmental factors may accelerate the aging process or trigger a progressive and irreversible loss of central vision [10], as in age-related macular degeneration [11, 12]. Some of these factors seem to be modulated by gonadal sex hormones [13-15].”

Introduction, page 4: It has been stated that hormonal factors seem to be associated with ARMD and that the prevalence of early ARMD seems to be higher in the unban Norwegians. Is there any data/publication indicating earlier age of menopause in Norwegian women? Please include in Introduction.

Am J Epidemiol 2003, 157:923-929). This aspect has been briefly mentioned in the Background (Introduction) paragraph. However, since our MMT values were similar to those reported from other whites and since we had not asked for surgical/natural menopause, hormone replacement therapy, smoking habits, time at first or last delivery, we did not include this topic in the Discussion.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interest.

Title: Macular Thickness Measurements in Healthy Norwegian Volunteers: An Optical Coherence Tomography Study.
Version: 1 Date: 28 January 2010
Reviewer: Pradeep Venkatesh
Reviewer's report:

Minor Revisions

1. Page 3, conclusions: ‘……… women should to be studied further’. Delete ‘to’

   ok

2. Page 4, 2nd para: ‘There is inconsistency to whether MMT…..’ Insert ‘as’ before to.

   ok

3. Page 5, 1st para: Subjects with diabetes or systemic inflammation conditions were not included. Change ‘inflammation’ to ‘inflammatory’.

   ok

4. Page 6, 2nd para: ‘Anamnesis was taken during the session…….’ Not clear about what Anamnesis implies. Consider using a more common word.

   Replaced with “Medical history “

5. Page 7, 2nd line: …..dilatation with tropicamid… Correct to tropicamide.
Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Reviewer's report
Title: Macular Thickness Measurements in Healthy Norwegian Volunteers: An Optical Coherence Tomography Study.
Version: 1 Date: 29 January 2010
Reviewer: Rong-Kung Tsai

Reviewer's report:
This manuscript is a well-designed study to investigate the normal database of macular thickness in normal Norwegians, the results showed that there is no difference with previous study in white. Authors further tried to explore the gender, parity and age factors with results of macular thickness measurement. The normal database is not new; however, this study has addressed some interesting issues about hormone factors and macular thickness. Unfortunately, the case number enrolled is limited to answer these interesting questions and the conclusion is vague.

Major Compulsory Revisions:

1. Case number is limited to investigate the hormone effect and MMT in woman. This limitation should be addressed in discussion

The issue has been addressed in the discussion:

... “We did not ask for the use of hormone replacement therapy, but the sample was nevertheless too small to test for the additive effects of a greater number of factors on the MMT. These issues should be addressed in a larger study.”

2. Abstract Conclusion; ” whereas age-matching of MMT data is not necessary,” is not applied to the result” Mean foveal thickness (1 mm in diameter) was positively associated with age in females”. These descriptions are confusing, the conclusion should be revised.

The conclusion has been revised:
“Age and gender should be taken into consideration when establishing normal ranges for MMT in younger subjects. The gender difference in retinal thickness in young, but not older adults suggests a gonadal hormonal influence. The possible association between parity and retinal structure, and its clinical relevance, should be studied further.”

3. Methods. Pharmacological dilatation was not routinely applied because neither Koozekanani [1] nor Paunescu [2] found a significant effect of dilatation on any of macular thickness measurements. A recent paper for this issue (not cited; J Glaucoma 2008;17:30–35) demonstrated that macular thickness measurements performed using OCT showed no significant differences before and after pupillary dilation. Pupil dilatation is not a necessary procedure for Stratus OCT.

Thank you very much for the comment. We changed our justification discussion and added the recommended reference in the manuscript:

“Pharmacological dilatation was not routinely applied, as it is not a necessary procedure for the Stratus OCT [3, 4].” ...

4. Discussion “Since normal macular aging could represent a transition towards an EAMD, we speculate if increase in MMT could represent an independent risk factor for EAMD. Changes in MMT with age could occur earlier, to a larger degree, or more frequently in Norwegians.” This is too speculative and beyond the evidences provided by authors, take an example; it was not true for men in this study. These sentences should be revised or deleted.

This sentence has been deleted, and normal aging has been discussed in depth (see above).

5. Needs some language corrections before being published.

Minor Essential Revisions:

P4L4: There is inconsistency to whether MMT varies with age and gender in published papers. mean macular thickness (MMT)

Corrected to “However, there is inconsistency as to whether macular thickness (MMT) varies with age and gender in published papers.” ....

2. P13 “supporting the a possible association between EAMD and hormonal factors”—check English writing.

Sentence has been deleted.
3. Table 3 Redundant sentence “For definition of macular regions see Fig.1.”, please check

*Thank you, this sentence has been deleted.*

Reviewer's report
Title: Macular Thickness Measurements in Healthy Norwegian Volunteers: An Optical Coherence Tomography Study.
Version: 1 Date: 4 February 2010
Reviewer: Mitra Sehi

*Thank you again for your comments*

*Sincerely yours*
Alexandra Wexler