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PART 1:

Journal Name:	<u>Ophthalmology Research: An international Journal</u>
Manuscript Number:	2013_OR_4795
Title of the Manuscript:	Vitamin D deficiency may be a risk factor for ophthalmopathy in patients with Graves' hyperthyroidism but not Hashimoto's thyroiditis
Type of the Article	Research paper

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(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)

- This form has total 7 parts. Kindly note that you should use all the parts of this review form.



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PART 2: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	1. It has been reported that patients with autoimmune thyroid diseases including Graves' disease have lower vitamin D status, but this manuscript reports that vitamin D deficiency is not related with the onset of autoimmune thyroid diseases. Please discuss this point compared with other previous reports. 2. Please discuss the mechanism in which low vitamin D values may influence the development of ophthalmopathy in patients with Graves' disease.	1. The focus of our study was to determine whether the development of ophthalmopathy was linked to lower vitamin D levels in patients with thyroid autoimmunity. As pointed out, We did not show any overall difference between patients with Graves' disease, Hashimoto's thyroiditis and multi nodular goitre - included as a control group - when tested at the first visit, only. In our area, which includes the Blue Mountains region west of Sydney, the prevalence of vitamin deficiency is very high. The apparent discrepancy in respect to other reports showing the correlation between low vitamin D and an increased prevalence of thyroid autoimmunity, in particular Graves' disease, is probably explained by the fact that the prevalence is very high in all groups of patients, thus masking a possible relationship between the autoimmune reaction itself and Vitamin D deficiency. We have included a short section concerning this apparent discrepancy in the Discussion section of the revised manuscript It is well known that vitamin D has effects on the immune system that may be implicated in the observed increased



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	3. Is the incidence of ophthalmopathy in patients with Graves' disease higher in high latitude countries?	prevalence of thyroid and other autoimmune disorders, as well as various cancers, in areas where there is low vitamin D. Many immune cells, including T lymphocytes and dendritic cells, express the Vitamin D receptor (VDR), stimulation of which has been shown to enhance tolerogenicity. Tolerogenic dendritic cells then promote the development of the regulatory T cells or Tregs, which induce peripheral tolerance. Thus one can postulate that modulation of the VDR, which would be dependent on existing levels of Vitamin D in the environment, alters the function of the T regulatory cells and thus promotes the development of autoimmune disorders including ophthalmopathy. We have added a short paragraph to the Discussion section of the paper in which we address possible effects of Vitamin D deficiency on the development of thyroid autoimmunity, in particular the ophthalmopathy In the present study we have not reported TSH receptor antibody (TSHr Ab) levels, which were not measured routinely as a
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		clinical tool. Several patients were tested for these antibodies and there was no relationship between TSHr Ab positivity, or their serum titres, and the eye disorder. However, we are now measuring these antibodies, in a new TSI Thyregain reporter bioassay, in an ongoing prospective study in which we are correlating Vitamin D levels with several risk factors for ophthalmopathy including TSH receptor antibodies. The latter antibodies are closely linked to ophthalmopathy and it will also be of interest to determine their relationship with Vitamin D levels in patients with ophthalmopathy. In another study we are performing a meta-analysis of the relationship between TSHr Abs and ophthalmopathy and we do show that there is a relationship between the two, although there are many discrepancies and exceptions. A role of TSHr Abs and the development of ophthalmopathy has long been postulated but this is certainly not conclusive and ongoing studies are necessary, in particular using the new TSI assay mentioned above. We are also examining other potential risk factors including the severity of the thyroid
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	4. Is there the association of TSH-receptor antibodies, especially thyroid stimulating antibody (TSA_{Ab}), with ophthalmopathy or vitamin D status in patients with Graves' disease examined?	autoimmune process, smoking and thyroid antibody levels and their inter relationships with implications in patients with Graves' disease in whom ophthalmopathy may be prevented by smoking cessation and Vitamin D repletion. In the discussion section of the revision we now go into more detail concerning the mechanism for the development of ophthalmopathy in respect to the various risk factors, including Vitamin D deficiency. This is an interesting question and as far as we know no such studies have been carried out. Indeed, in high latitude countries, such as the Scandinavian countries and Canada, there are higher rates of autoimmune disease in particular rheumatoid arthritis, type 1 diabetes and multiple sclerosis, whereas in tropical countries autoimmune disorders are less common; for example Graves' disease and Hashimoto's thyroiditis are uncommon in Africa. Of course, there can be other factors including iodine availability which may impact on the prevalence in equatorial regions and Vitamin D levels may be important. In Australia, sunscreen use to combat the effects of ultraviolet light in development
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		of melanomas and other skin cancers led to a sharp reduction in Vitamin D levels in the 1980s. On the other hand, milk is supplemented with Vitamin D in Canada and the relationship between the prevalence of ophthalmopathy and Vitamin D levels in these and other high latitude countries would be influenced by the amount of Vitamin D that patients receive, apart from sun exposure. It would be worthwhile to compare otherwise similar groups of patients who were not receiving vitamin D supplements in single centres in Canada and Sydney. In the present manuscript we mention this interesting point and discuss how this may be studied in the future. As mentioned above, although TSHr antibodies are thought by most workers to be the cause of the initial inflammation in the orbital fibroblasts and pre-adipocyte this has not been proven. Recent studies using a new sensitive bioassay for TSI suggest that there is a strong relationship between ophthalmopathy and TSI levels. However, patients with so called “euthyroid” Graves’ disease who have the same ophthalmopathy but no evidence of
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		thyroid autoimmunity have not yet been studied. These studies do raise the possibility that these antibodies may be present in all patients with euthyroid Graves' disease, but only for a short time at the beginning of the disease course. Low Vitamin D become a further risk factor for the development of ophthalmopathy in these patients. In other studies we have shown that Vitamin D deficiency is a risk factor in Graves' disease but not Hashimoto's thyroiditis in whom the eye disease is generally less severe and less frequent While Vitamin D deficiency is a risk factor, it does not appear to enhance the probability of developing ophthalmopathy in patients with higher levels of TSHrAbs although we need more data to confirm this.
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Minor REVISION comments	1. Introduction. The reference fails to document the statement attributed to it. For example, Reference [4] reports the association of vitamin D status with type 1 diabetes, and Reference [5] shows the link of vitamin D insufficiency and Graves' disease. 2. Page 5, line 130. "< 0-.05" should be changed to "< 0.05". 3. Page 6, line 145. Please mention odds ratio 0.328 (confidence interval 0.125-0.863) p = 0.020. 4. Page 6, line 146. Although the authors describe that p value is "0.679" in the text, p value is "0.678" in Table 1. 5. Page 6, line 147. "Graves' patients" should be changed to "patients with Graves' disease". 6. Table 3. "Ophth." Should be changed to "Ophthal". 7. Reference [6] lacks the names of the authors. 8. References [20] and [21] are identical.	Indeed, several of the references were incorrectly assigned statements in the text and these have all been corrected. We thank the reviewer for pointing out this important error in our manuscript. This has been corrected as requested The above line 145 in the initial version of the manuscript has been inserted, as page 5 line 149 in revised manuscript. This has been corrected in page 5 line 150 in revised manuscript Corrected as suggested by the reviewer in page 5 line 152 in revised manuscript Accordingly corrected. The authors names have been inserted Reference 21 has been omitted.
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<u>Optional/General</u> comments	The manuscript by Lahooti et al. suggested that vitamin D deficiency is a risk factor for ophthalmopathy in patients with Graves' disease. The findings reported here are significant. Several concerns with the manuscript are listed in the above text.	
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