Reviewer's report

Title: Strong association between non alcoholic fatty liver disease (NAFLD) and low 25(OH) vitamin D levels in an adult population with normal serum liver enzymes.

Version: 3 Date: 21 March 2011

Reviewer: Jane Lynch

Reviewer's report:

1. Is the question posed by the authors new and well defined?

This question is of interest because it addresses adults at risk for fatty liver who do not present with elevated liver enzymes as the risk factor for NAFLD. It is estimated that at least one third of patients with fatty liver do not have elevated ALT.

Although the hypothesis is well defined, the introduction includes a description of the current theories of both NASH and NAFLD which could be summarized to allude to the role of NAFLD to possible early mechanisms of insulin resistance. This rationale would then explain why a better understanding low vitamin D’s role in fatty liver would be relevant to metabolic syndrome research and for potential prevention of cirrhotic liver disease. Because low vitamin D is known to be more prevalent in obesity, one would expect a positive correlation with metabolic syndrome and this is not the focus of this paper.

2. Are the methods appropriate and well described, and are sufficient details provided to replicate the work?

The study hypothesis was that there was a direct association between hypovitaminosis D and the presence of NAFLD in subjects with various degrees of insulin-resistance and related metabolic disorders. The Surrogate measure to estimate the degree of fatty liver was abdominal ultrasonaography to quantify fat in the liver as well as a Fatty-Liver-Index correlate of NAFLD( reference 25). The use of the ultrasound surrogates is not ideal, especially in obese patients, however it is a practical approach commonly used in practice.

The remaining assessment for other causes of liver disease was certainly extensive however this would more typically be done for patients with findings of an elevated AST,ALT or other evidence of liver disease in the initial evaluation of a patient with elevated liver function tests.

The studies for HOMA IR to provide measures of Insulin resistance are appropriate and interesting in this paper. No measure of insulin secretion or sensitivity testing is available because these require OGTT derived data for the equations.
3. Are the data sound and well controlled?

Of the 262 consecutive subjects, 52 had T2DM. Of the patients with T2DM, there was a 3:1 ratio of positive NAFLD. The data regarding the subgroup with T2DM was addressed in table 5 where the study sample was stratified according to serum 25(OH) vitamin D quartiles. The prevalence of NAFLD, MS and its components was increased in the lowest 25(OH) vitamin D quartile whereas the prevalence of T2D was similar in the bottom and top quartiles with a p value of .001. The clinical significance of this data on only a total of 52 T2DM individuals who all had relatively low vitamin D levels was based on the mean overall group differences of 14.8 and 20.5 which had a SD of 9 in each. I think this study would be stronger with this group treated as a different cohort or analyzed separately from the non DM patients.

If there is an independent association between MS and low vitamin D, it is not surprising that the cohort with NAFLD (BMI 31.3 and waist circumference 105.9) had lower vitamin D than those without NAFLD (BMI 25.8 and waist circumference 90.2). The data probably needs to be analyzed with a multivariate analysis to control for the role of the BMI and/or waist circumference to be more compelling that this relationship described is not one related to body size and MS.

However, it is interesting that the association between NAFLD and low 25(OH) vitamin D levels was independent from age, sex, triglycerides, HDL and FBG in a multiple regression analysis model. The subgroup of normal weight subjects (n=70) with a BMI< 25 are interesting and would reinforce the findings of less correlation with lipid profiles since these were normal weight individuals referred to the clinics for suspected MS.

It is surprising to see the very high correlation between ultrasound and FLI index in this study knowing the issues with surrogate ultrasound measures for fatty liver in obese patients.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?

Yes.

5. Are the discussion and conclusions well balanced and adequately supported by the data?

Similar to my comments regarding unfocused introductory information, the final discussion should likewise focus on the topic of this specific paper. For example, the following discussion content is not one addressed in this paper and could be summarized to a sentence with reference alluding to the concerns that vitamin D may play a role in both NAFLD and cirrhosis outcomes.

“Since insulin-resistance is known to promote fibrosis progression [37,38], it can be speculated that vitamin D may ameliorate hepatic outcomes not only though
an anti-inflammatory action but also by an insulin-sensitizing effect. Thus, it was largely demonstrated that calcitriol exerts an insulin-sensitizing action by increasing the expression of insulin receptors in peripheral tissues and facilitating the insulin-mediated glucose transport.”

6. Do the title and abstract accurately convey what has been found? Yes.

7. Is the writing acceptable?

There are many vocabulary errors and typos which could easily be corrected with minor editing by authors familiar with scientific English language.

**Quality of written English:** Needs some language corrections before being published

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

**Declaration of competing interests:**

- I am involved in a study of adolescents with vitamin D deficiency and abnormal liver findings consistent with NAFLD. This study has been funded as a pilot with outcomes not yet assessed. The funding and private organization would not gain or lose financially from the publication of this paper, either now or in the future.

I do not hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this paper, either now or in the future. I do not hold and am not applying for any patents relating to the content of the manuscript, nor have I received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript. I have no other financial or non-financial competing interests.