Author's response to reviews

Title: Association between erythrocyte Na+K+-ATPase activity and some blood lipids Type 1 diabetic patients from Lagos, Nigeria.

Authors:

Bamidele A Iwalokun (bamwal@yahoo.com)
Senapon O Iwalokun (senahoddy@yahoo.com)

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Author's response to reviews: see over
Dear Editor,
We thank you for a quick review of our manuscript titled “Association between Na⁺K⁺-ATPase activity and some blood lipid metabolites in insulin dependent patients from Lagos, Nigeria.”

Attached herewith is a modified manuscript addressing major and minor concerns raised by the reviewers. The areas corrected are also highlighted in a separate file. Thank you.

**Title**
The title of the manuscript has now been modified to read “Association between erythrocyte Na⁺K⁺-ATPase activity and some blood lipids in Type 1 diabetic patients from Lagos, Nigeria.” As suggested by reviewer 1

**Abstract**
The abstract was modified by correcting all the identified spelling errors (i.e. Friedewald instead of Friedwald and atherogenic instead of artherogenic). We have also included the percentage differences between diabetics with poor glycemic control, good glycemic control and all the patients compared to control Na⁺K⁺-ATPase activity (i.e. 52.5 – 64.4 -71.2%) in the result section.

The conclusion has also been modified to align with the modified title of the manuscript

**Introduction**
The first (lines 1, 6, 8, 15 – 16) in which diabetes mellitus and Na⁺K⁺-ATPase were described has been modified as suggested by the two reviewers. The diabetic patients have also been renamed as Type 1 and Type 2 diabetics in line with modern nomenclature. The word “microalbuminuria” has been corrected, while ‘derangement’ has been changed to ‘alteration’ as directed by reviewer 1 (page 4, lines 14 – 18).

**Materials and methods**

**Study design**
This has been modified with inclusion of criteria for identifying and selecting our patients as Type 1 diabetics. Both clinical judgment and laboratory results of fasting plasma glucose on two separate
occasions and glucose tolerance test were used by the endocrinologists on duty at the diabetic clinics for this selection.

**Na⁺K⁺-ATPase assay**

We agreed with the reviewers that ouabain at 0.1 – 1 mM is often used in the enzyme assay in imidazole buffer (pH 7.4). At the time of this study, it was digoxin that was available and accessible to us. However, we acknowledged the error of concentration of digoxin stated in the manuscript. The unit of concentration was wrongly typed as nM. It was actually in µM and the concentration was 200 µM (i.e 0.2 mM). Our inability to detect this typographical error was a gross oversight on our part. This has been rectified in the modified manuscript. Furthermore, the discrepancies observed by the reviewers regarding the calculation of Na⁺K⁺ATPase activity has also been corrected and re-written to improve clarity of the method used. The total volume of the assay was 500 µL instead of 500 mL and has been corrected accordingly.

The concern raised by reviewer 2 on the possibility of missing out Ca²⁺-ATPase activity in our assay is also noted. However, we doubt this possibility since calcium chloride was not included in the assay described by DeLuise and Flier (1982) that we used. This also applies to many other NaK-ATPase-assays. Examples are given below


However, we acknowledge the fact that to enhance CaATPase activity; corresponding assays may be supplemented with calcium chloride or calmodulin.

Although, the level of HbA₁c as a marker for assessing the extent of glycemic control is highly reliable, this parameter was not included in our protocol on cost ground and the fact that it was not done routinely in our laboratory. Meanwhile, only a few diabetic patients who could afford this test
provided results. But the small sample size together with the fact that our controls were not subjected to HbA1c test ruled out its use in this work.

However, in our future works, the relevance of Hba1c measurement in our selected diabetics and control cohorts will be taken into consideration.

The have expressed the blood lipids measured in this study in mg/dL in order to align with the definitions in which their aberrations (hypercholesterolemia, blood cholesterol > 200mg/dL, hypertriglyceridemia, blood TAG > 160mg/dL; elevated LDL-C, LDL-C > 150 mg/dL and reduced HDL, HDL < 35mg/dL) were based.

Results

The differences observed in the parameters such as percentage cases of hypercholesterolemia, hypertriglyceridemia and elevated LDL-C between Table 2 and result summary on Page 10 and Na+K+-ATPase activity and result summary on pages 10 and 11 have been corrected. Table 3 has further been modified by naming the parameters measured correctly and including Na+K+-ATPase in the diabetic patients as a percentage of control enzyme activity in parentheses as directed by reviewer 2.

The title of Figure 1 as “Lineweaver-Burk plot of erythrocyte membrane Na+K+-ATPase in control and type 1 diabetic patients” is now given with normal replaced by control as suggested by reviewer 2.

The values in the inset were not vmax and km. They indicated percentage reduction in Vmax and Km values of type 1 diabetics poor glycemic control (A1) and all diabetic patients compared with patients with good glycemic control (A2). This was indicated as a legend in Figure 1

Explanation for Table 4: title and interpretation

In our Table 4, where relationships between Na+K+-ATPase and other parameters analyzed in this study were summarized P < 0.05 was found for FBS and TC in all diabetic patients and for FBS, TC,
LDL-C, TAG for our diabetic subgroup with poor glycemic control. We considered these relationships significant based on the P value and direct (positive) or inverse (negative) based on the magnitude of the correlation coefficient (r). In the statistical analyses summarized in Table 4, the multiple variables were individually regressed with NaKATPase activity of the diabetic subgroups (A1, n = 20; and A2, n = 14) and all the patients (n = 34) producing their characteristics regression equation and correlation values. This informed the title of Table 4 as multivariate regression analysis instead of Pearson correlation analysis.

Our observation above consequently informed our conclusion that in Type 1 Nigerian diabetics with poor glycemic control, the relationship between Na+K+-ATPase and atherogenic blood lipids is promoted coupled with a greater reduction in erythrocyte pump activity

We also acknowledge the suggestion of reviewer 1 on the relevance of blood pressure of our diabetic patients. Yes, many studies have reported the association between elevated blood pressure and atherogenic blood lipids measured in this study. We have also previously reported the association between elevated blood pressure and risk of microalbuminuria in both type 1 and type 2 diabetic Nigeria as stated in the introduction section of this manuscript. Hypercholesterolemia, hypertriglyceridemia and elevated LDL-C as independent markers of hypertension have also been severally reported in the literature. However, since blood pressure measurement did not feature in the present work, its involvement in our assertions and discussion of this work cannot be justified.

Discussion

We have also modified the discussion with respect to our explanation for the observed greater reduction in Na+K+ATPase activity in our type 1 diabetic A1 subgroup (poor glycemic control). Our explanation is now based on the report by Yagihashi et al [(2005) [now reference No. 43] that this protein together with PKC is highly prone to glycation resulting in greater reduction of their activity and a recent study by Sampathkumar et al [2006] [now reference No. 44] in which
hypogluthathionemia and lipid peroxidation were associated with greater reduction in Na+K+ATPase activity. The latter was also suggested to us by reviewer 1.

Meanwhile, all the typographical errors (e.g. susceptible instead of susceptibility, atherogenic instead of artherogenic, alteration instead of derangement, microalbuminuria instead of microbuminuria) sited by the reviewers in the previous discussion have also been corrected.

References

We have removed 2 references: Abidoye et al (formerly reference 43) and Vague et al (2004) (formerly reference No. 45) and replaced them by the references of Yagihashi et al (2005) now reference No. 43 and Sampathkumar et al (2006) now reference No. 44. The former reference No. 44 has been moved to No. 45 and so on to 50.

The two typographical errors sited by the reviewer 2, Friedewald instead of Friedwald and ultracentrifuge instead of ultracentrifugation have also been corrected.

The above explains how we have corrected the returned manuscript.

We thank you for your editorial work and quick review of our manuscript. All the technical and typographical errors sited by the reviewers are highly appreciated.

Iwalokun, BA and Iwalokun, SO

(Authors).

Declaration of Competing Interest

We have no competing interest. This section has also been included in the revised manuscript.

Acknowledgement

The authors which to thank the laboratory staff and attending endocrinologists at General Hospital, Lagos for their technical assistance. The secretarial and statistical assistance rendered by Mrs. Adefehinti, CO and Mr. Osho, F are highly appreciated. This statements have also been included in the Acknowledgement section of the manuscript.
Our roles are as follows:

Iwalokun, B.A – Conceived the idea of the study, wrote the proposal for ethical approval (with clinical inputs from Iwalokun, SO), provided the literature, did the laboratory analyses and wrote the manuscript.

Iwalokun, S.O is in charge of patient recruitment, drafting of the consent form and administration of the questionnaire, manuscript editing and input.