Dr. Lian-Sheng Ma, 03-03-2022
Company Editor-in-Chief,
Baishideng Publishing Group Inc
California, USA

Subject: Submission of the revised manuscript (ref.: 74162)

Dear Dr. Ma,

I am submitting the revised version of the manuscript entitled, "Disrupted leptin-fatty acid biosynthesis is an early manifestation of metabolic abnormalities in schizophrenia (ref.: 74162)".

I am highly thankful to the reviewers for taking their precious time to review the above manuscript and proving their valuable comments.

I have revised the above manuscript step by step in light of the comments raised by the reviewers. The responses to their comments are attached below and corrections/modifications in the revised manuscript have been highlighted yellow.

Please note that I performed this study long back (2001-2003) in the laboratory of Dr. Sahebarao P. Mahadik, Department of Psychiatry and Health Behavior, Medical College of Georgia, Augusta, GA, USA. Since it has been very long time, the IRB approval and consent records could not be retrieved as all the records have been destroyed 7-8 years ago. I contacted Dr. Mahadik in this regard; he verified that this study was approved by the IRB of both, Medical College of Georgia, Augusta, GA and Dwight David Eisenhower Army Medical Center, Fort Gordon, GA, USA. Most of the patients and controls used in this study have also been used before in our other publications (Schizophr Res, 2002, 58, 1-10, Schizophr Res, 2002, 58, 55-62, and Schizophr Res, 2003, 60, 117-123). An email response from Dr. Mahadik is attached below for confirmation.

I hope that the revised manuscript has been considerably improved and it will be suitable for publication in the World J Psychiatry.

With sincere regards,

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ANSWERS TO REVIEWER'S COMMENTS (manuscript no.: 74162)

1. Reviewer's code: 05794765

1. It is not clear how the researchers arrived at these numbers for the study. It would have been ideal for the researchers to do a power calculation or it would be ideal to explain in discussion why they did not do a power calculation in this study.

Response - I sincerely thanks the reviewer for raising the power calculation issue. As mentioned in the text, FEP patients were enrolled from consecutive admissions over a period of 2-3 years and each patient was followed up to six months after hospitalization to establish the presence of schizophrenia psychosis. Since we had limited number of patients diagnosed and hospitalized at different time points over the designated period, power calculation was not performed. Control subjects were recruited new from the surrounding communities and were similar to the FEP patients in age and socioeconomic background. Medicated chronic schizophrenia (CSZ) patients were also new and had the same socioeconomic background as the patients with FEP, but they were on treatment with antipsychotic drugs for several years when the blood was drawn and clinical symptoms were evaluated. However, CSZ patients had first episode of psychosis and diagnosis at about the same age as FEP patients.

2. I would suggest making inclusion ad exclusion criteria more explicit in the study.

Response - As suggested by the reviewer, inclusion and exclusion criteria has been mentioned in the methods section of the revised manuscript.

3. Paragraph beginning with "3. Literature search and inclusion criteria" did not show any inclusion criteria. Inclusion and exclusion criteria may be clearly mentioned in the study. Referral to their older study also did not give clear inclusion exclusion criteria (Ref 19 in the paper)

Response - I apology for the inconvenience, this section is not appropriate to the study; therefore, it has been omitted from the revised manuscript. For clarification, literature related to the role of leptin and fatty acids in the development of insulin resistance and other metabolic comorbidities was searched only for deciphering the underlying mechanism(s). However, the inclusion and exclusion criteria of patients have been mentioned in the revised manuscript.

4. It was not clear when this study was done; period referring to original study dates back to early 2000's. It is of interest to know that medicated CSZ patients group were new and the control group were new. If they have done a historical comparison, that may be made clear in methods and in discussion. It is also interesting to know over what period participants were recruited.

Response - This study was conducted during the years 2001-2003 and was partially supported by an NIH R01 grant (1R01AT000147-01, grant period 1999-2003) funded to Dr. Sahebarao P. Mahadik.

This study has not been published yet. It was designed to analyze the association between plasma leptin, fatty acids, and clinical symptoms primarily in patients with FEP, which were enrolled from consecutive admissions and followed up to six months after hospitalization to establish the presence of schizophrenia psychosis. Patients with CSZ and control subjects were included in this study for comparison purpose only; this has been mentioned in the revised manuscript. Control subjects were recruited from the surrounding communities and were similar in age and socioeconomic background to the patients with FEP. Patients with CSZ had the same socioeconomic background as the patients with FEP, but they were on treatment with antipsychotic drugs for several years when the clinical symptoms were analyzed and blood samples were taken. Both groups of patients and control subjects have been used in our earlier publications (Schizophr Res, 2002, 58, 1-10, Schizophr Res, 2002, 58, 55-62, and Schizophr Res, 2003, 60, 117-123).

5. It may be appropriate to mention who evaluated patients for schizophrenia and what criteria were used.

Response - Although reviewer has raised a very crucial point, the criteria for evaluating schizophrenia patients was mentioned in the methods section of the manuscript. However, the role of the author who helped in evaluating the patients has been acknowledged in the revised manuscript.

6. Readers would like to know the socio demographic features of patients in two groups and the controls and this may be included as a table in results.

Response - As suggested by the reviewer, a table containing demographic features of both patients groups and controls has been included in the revised manuscript.

7. Result section 4 paragraph 2 - Conclusion made on CSZ patients that they have a higher plasma leptin levels and no correlation to PANSS. This may require to be evaluated carefully as BMI in these patients were significantly higher compared to control group. This may be a treatment effect and researchers have pointed out this. But it is possible that there number of other confounders for higher BMI and plasma leptin levels which includes food habits, type of food they consume, even though previous studies has shown that clozapine increases weight gain. Unless researchers clarify their BMI prior to initiation of Clozapine in CSZ group this may be prone to errors concluding that they have a higher BMI and plasma leptin levels due to Clozapine.

Response - I am thankful to the reviewer for raising a very valid point. As mentioned above, CSZ patients have higher plasma leptin levels but no correlation to PANSS. Indeed, in both CSZ and FEP patients, there was a negative correlation between plasma leptin and PANSS, but it did not return significance in CSZ patients (please see Figure 2C and 2D), whereas in FEP patients it was significant (Figure 2A and 2B). Numerous studies have shown that atypical antipsychotic drugs including clozapine, olanzapine, and others increase leptin production and body weight/body mass index (BMI). Therefore, higher plasma leptin and BMI that was observed in the medicated CSZ patients compared to the CNT subjects and FEP patients could be a treatment effect. Moreover, CSZ patients were from the Veterans Affair Medical center with similar army background as the FEP

patients, other confounders such as food habits, and type of food they consumed may have had negligible effect as army follows strict diet rules.

8. Relevance of section 3 is not clear in this manuscript. Conventionally literature search and inclusion criteria for literature search are done in systematic reviews. This study being a case control design, need of such an approach may be reviewed

Response - As suggested by the reviewer, this section does not have much relevance to the study; therefore, it has been removed from the revised manuscript.

9. ARRIVE guideline is for reporting animal guideline. It would have been more acceptable if they would have gone through STROBE.

Response - I highly appreciate the reviewer for pointing out this error, it has been corrected in the manuscript.

I would suggest including a paragraph on strengths and limitations of the study. External validity remains limited to low numbers and type of population they chose.

Response - As suggested by the reviewer, a paragraph (last paragraph in the discussion section) on the strength and limitations of the study has been included in the revised manuscript.

10. In the methods and material section 2.1 N=14 for CNT group was mentioned initially (Line 2). But in line 22 controls were mentioned as 12 showing a disparity in numbers.

Response - This has been corrected in the revised manuscript.

11. Typing error noted in its heading and in manuscript 2.1 a) last line “form” ; 5 Discussion, b)paragraph 2 line 4 “playa”

Response - These typing errors have been corrected in the revised manuscript.

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2. Reviewer’s code: 02445242

I think that the word "correlation" should be replaced by the word "association" in certain parts of the manuscript such as the Abstract-Background, Introduction & Discussion.

Response - As suggested by the reviewer, the word 'correlation' has been replaced with the word 'association' in most parts of the text.

The conclusions of the Abstract do not seem to be based on the results of the study. This has to be modified.

Response - The conclusion of the abstract has been modified accordingly, please see the abstract of the revised manuscript.

Person first language should be used throughout the manuscript. Thus, for example, phrases such as "drug-naive first-episode psychosis (FEP) patients, medicated chronic schizophrenia (CSZ) patients, and healthy control (CNT) subjects." (Introduction) - should be changed to patients/persons with drug-naive first-episode psychosis and patients/persons with medicated chronic schizophrenia

Response - As suggested by the reviewer, person first language has been used for patients and CNT subjects wherever appropriate.

Certain aspects of the patient sample are relevant to the results of the study. Patients with FEP were recruited from an inpatient sample. They are thus probably not truly representative of community-based patients with FEP who attend early intervention services. This would explain the rather late onset (22 years) in this sample.

Response - I am highly thankful to the reviewer for raising community based early intervention issue, which is vital for the long-term remission/recovery and preventing the relapse in schizophrenia. However, patient with FEP included in the present study were from an army medical center, and had very similar socioeconomic backgrounds as the patients with CSZ and control subjects since they come from surrounding communities. Moreover, as the army follows a strict protocol with a routine medical checkup, patients with FEP were screened immediately for psychotic behavior and admitted to inpatients hospital for follow up. Therefore, patients with FEP included in this study were intervened very early (had the shortest reported duration of psychosis $\leq$5 days). The blood was drawn on the second visit (1-2 weeks after the first visit) before starting treatment. Each patient was followed for up to six months for establishing the presence of schizophrenia. The late onset (22 years), which the reviewer had mentioned, is actually the time from the first-episode diagnosis for patients with CSZ.

Moreover, it has not been clarified whether all patients with FEP were diagnosed with schizophrenia, because a proportion of them can turn out to have bipolar disorder later.

Response - All the patients with FEP had confirmed diagnosis of schizophrenia or schizofriniform disorders as they were followed up for six months from the date of first visit. During the follow up period patients with primarily bipolar symptoms or depression were excluded from the study.

The choice of only patients being treated with clozapine for the chronic group was not clear. Was there any particular reason for this?

Response - Regarding clozapine treated patients, there is some correction. Actually, these patients were treated with different atypical antipsychotic drug; although, mostly with clozapine. This has been corrected in the revised manuscript.

More details of the literature search are required in the Methods and particularly in the Results section. Was this a systematic review? Where guidelines followed? How many articles were selected? Was a quality analysis or risk of bias estimation done? The limitations of the study and of the extant literature (derived from the review) must be mentioned.

Response - This section is not required as it is not relevant to the study; therefore, it has been omitted from the revised manuscript. Literature related to the role of leptin and fatty acids in the development of metabolic comorbidities was searched only to decipher the underlying mechanism(s).