Author's response to reviews

Title: Association of sleep duration and insulin resistance in Taiwanese vegetarians

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Author's response to reviews: see over
Dear BMC Public Health Editorial Team,

We would like to submit our revised manuscript entitled “Association of sleep duration and insulin resistance in Taiwanese vegetarians” for consideration of publication in the BMC Public Health. We are grateful for the invaluable suggestions provided by the reviewers and the editor. Below are our point-by-point responses to the comments and suggestions. The changes in the revised manuscript were marked with red text.

We hope that the editorial board and the reviewers will accept our revisions. We look forward to hearing from you.

Thank you and best regards.

Sincerely,

Jui-Kun Chiang, M.D., M.Sc.
Association of sleep duration and insulin resistance in Taiwanese vegetarians

**Editor, Comment #1**
There is a contradiction in the abstract. It is reported that "Insulin resistance was significantly associated with .. sleep duration of 8 hours or less per night". Then in the next sentence report a multiple regression model found an independent association "with sleep duration of more than 8 hours per night". This needs to be corrected.

Response to the Editor, Comment #1:
We have revised the whole paragraph of the Results section in the Abstract.

**Editor, Comment #2**
The appendix section refer to in the results section page 9 and provided at the end of the document before the reference should be provided as an additional file and uploaded rather than at the end of the manuscript.

Response to the Editor, Comment #2:
We have removed the appendix from the manuscript and uploaded it as an additional file.

**Editor, Comment #3**
It is not clear enough how the model in table 3 was decided.

Response to the Editor, Comment #3:
Table 3 presents the final model of the multivariate logistic regression. The final model was obtained using stepwise variable selection procedure based on the Akaike’s Information Criterion (AIC). The variables under evaluation during the model development were those listed in Table 1. We have added the list to the footnote of Table 3.

**Editor, Comment #4**
How were the tertiles of sleep duration divided they are not described in the methods. The analysis using tertiles of sleep duration is described briefly in the text but the data should be shown in an additional table. The analysis for this model should also be described in the text.

Response to the Editor, Comment #4:
Our main analysis treated sleep duration as a binary variable. In our initial analysis, we tested the association using tertiles of sleep duration but did not observe the presence of a U-shaped pattern. The choice of cutpoints for the tertiles was based on previous reports and a nonparametric smoothing from the Generalized Additive Model (GAM in R) of sleep duration after adjusting for waist circumference and ALT level. We have added a sentence on page 12.

**Editor, Comment #5**
The AUC not mentioned as an aim of the study, is described briefly in the results and then forms a major part of the conclusion.

**Response to the Editor, Comment #5:**
We have added the sentence “The area under the receiver operating characteristic curve was used to quantify prediction accuracy.” at the end of the last paragraph in the Background section.

**Editorial requirement #1**
Please mention in the manuscript the specific name of the Ethics committee that granted approval for your study.

**Response to the Editorial requirement #1**
The specific name of Ethics committee is “The Research Ethics Committee of the Buddhist Dalin Tzu Chi General Hospital”. The approved numbers are B09702038 and B09604003-1. We have added the information in the manuscript on page 8.

**Reviewer 1, Major Compulsory Revision #1**
How would socioeconomic characteristics of the study participants such as education and income compare to vegetarians and non-vegetarians in the general population?

**Response to Reviewer 1, Major Compulsory Revision #1**
In general, those who are adopting a vegetarian diet in the Western societies are more likely to be associated with higher socioeconomic status (SES). However, the main reason for adopting a vegetarian diet by individuals in Taiwan is religious beliefs rather than economic or health concerns. The existence of an association between vegetarianism and SES is not clear. Therefore, we did not ascertain income levels of the participants in our study. Nevertheless, we have collected data on education levels of 435 individuals who were non-vegetarians at the time of our study.
recruitment. Results showed that 30.1% of the vegetarians and 29.1% of the non-vegetarians had educational levels of primary school or below (p=0.815) (educational level were dichotomized to primary school or below vs. high school or above). No significant differences were observed in the educational levels between the two groups. We do not expect any major differences exist in SES between our study participants and the general population.

Reviewer 1, Major Compulsory Revision #2
In the methods section it is mentioned that the study participants were recruited from a regional hospital in south Taiwan at the time of their physical examination between May 2007 and April 2008. Was this part of a regular, routine physical examination or were the subjects being examined because of some specific ailment or follow up procedure?

Response to Reviewer 1, Major Compulsory Revision #2
It was part of a regular routine physical examination. We have added the information on page 7.

Reviewer 1, Major Compulsory Revision #3
The authors report that sleep duration was assessed by responses from separate questions on hours of sleep... Could the authors clarify the procedure and describe more in detail the questions used for this assessment.

Response to Reviewer 1, Major Compulsory Revision #3
Information regarding sleep duration was assessed by sleep hours of week day and weekend seperately. The mean sleep duration was calculated by the formula (weekday sleep duration x 5 + weekend sleep duration x 2) divided by 7. The assessment was conducted face-to-face by a research assistant. We have added a description in the Methods section.

Reviewer 1, Major Compulsory Revision #4
Considering the low numbers of subjects that slept >8 hrs (n=62) how much statistical power did the adjusted logistic regression model have?

Response to Reviewer 1, Major Compulsory Revision #4
Based on a calculation using a 2 x 2 table of Sleep > 8 hours (Yes/No) versus Insulin Resistance (Yes/No), the post hoc study power was estimated to be 0.91.
Reviewer 1, Major Compulsory Revision #5
In the statistical analysis section the complete model with all the covariates of the final logistic regression analysis should be given.

Response to Reviewer 1, Major Compulsory Revision #5
We have listed the covariates that were under evaluation during the model development in the Statistical Analysis section.

Reviewer 1, Major Compulsory Revision #6
The authors chose to exclude diabetic subjects. What was the rationale for that exclusion? What was the total number and the distribution in regards to sleep duration of those excluded for being diabetic? How would the results have been modified if diabetic subjects had been included?

Response to Reviewer 1, Major Compulsory Revision #6
Since the aims of the study were to investigate the association between sleep duration and insulin resistance, and also to develop a simple model to predict insulin resistance in clinical settings. Therefore, individuals with known diabetes were considered as patients with insulin resistance in this study. In addition, medications used by patients with diabetes might influence insulin resistance. Therefore, we excluded diabetic subjects in this study.

Reviewer 1, Major Compulsory Revision #7
Did the authors gather any information on medications that would have altered the blood chemistry?

Response to Reviewer 1, Major Compulsory Revision #7
Yes, medications used for the treatment of blood lipid disorders, gout, and endocrine disorders were collected and evaluated in the statistical analysis. None of those factors were significant factors in the final model.

Reviewer 1, Major Compulsory Revision #8
The authors conclude that sleep duration of more than 8 hours per night is an independent risk factor associated with increased insulin resistance in vegetarians. Considering the low numbers of participants that slept #8 hrs/day, missing information on sleep quality, sleep apnea, depression and other relevant factors this statement should be revised and be more cautiously formulated.
Response to Reviewer 1, Major Compulsory Revision #8
We agree with the Reviewer’s advice and have mentioned these factors in the limitation of the Discussion section with two new references.

Reviewer 1, Minor Essential Revision #1

Response to Reviewer 1, Minor Essential Revision #1
We have changed the reference according to the reviewer’s advice.

Reviewer 1, Minor Essential Revision #2
The authors use HOMA (Homeostatic model assessment) as a surrogate measure of insulin resistance. In their article on Use and Abuse of HOMA Modeling Wallace et al. report that “HOMA estimates of insulin sensitivity are usually not normally distributed. The data should be tested for normality, and if they are found to not be normal, they should be logarithmically transformed and reported as geometric means with appropriate measures of dispersion.” How did the authors conform with this recommendation?

Response to Reviewer 1, Minor Essential Revision #2
We agree with the reviewer that HOMA-IR estimates should be tested for normality when it is modelled as a continuous variable. Since we have dichotomized HOMA-IR data into two categories and subsequently analyzed its association using logistic regression, it is not necessary to evaluate the normality assumption.

Reviewer 1, Minor Essential Revision #3
Tables 1 and 2 should include dietary energy intake.

Response to Reviewer 1, Minor Essential Revision #3
No dietary information was collected in our study and therefore dietary energy intake could not be estimated. Nevertheless, results from a nutritional study on 109 Taiwanese vegetarians and 107 age- and sex-matched omnivores found that energy intake was significantly lower in female vegetarians but not in male vegetarians when compared with the omnivore counterparts. Vegetarians also consumed significantly less protein, fat, and cholesterol than the omnivores (Lu SC, Wu WH, Lee CA, Chou HF,
Lee HR, Huang PC. LDL of Taiwanese vegetarians are less oxidizable than those of omnivores. J Nutr 2000; 130: 1591–1596.) Another study on 49 healthy Buddhist lactovegetarians and 49 omnivores in Taiwan also reported that vegetarians consumed less energy, fat, and protein, but more dietary fiber than the omnivores (Hung CJ, Huang PC, Li YH, Lu SC, Ho LT, Chou HF. Taiwanese vegetarians have higher insulin sensitivity than omnivores. Br J Nutr 2006;95:129-135.). This information was added to the Methods section on page 7.

**Reviewer 1, Minor Essential Revision #4**

It would be helpful to include the variables of the complete model in Table 3.

**Response to Reviewer 1, Minor Essential Revision #4**

We have listed the variables that were eliminated during the stepwise regression process in the footnote of Table 3.

**Reviewer 2, Major Point #1**

Authors partition sleep duration into two groups (≤8, >8). According to the previous studies, which evaluated relationship between sleep duration and metabolism, obesity, or diseases, such as hypertension, short sleep duration is also risk factor. Moreover, these kinds of study partition sleep duration into 4 or 5 groups (6<, 6-7, 7-8, >8, etc). Please explain why authors decided to do so.

**Response to Reviewer 2, Major Point #1**

We are grateful for this suggestion. The choice of cutpoints in our study was guided by a plot of nonparametric smoothing obtained from the Generalized Additive Model (GAM in R) and cutpoints used in previous reports (6-8 hours was considered as normal sleep duration). We tested the association using sleep duration divided into tertiles. The results showed that the effect of sleep time duration of 6 to 8 hours on HOMA-IR was similar to that of less than 6 hours. Therefore, we combined the two categories in our final analysis.

**Reviewer 2, Major Point #2**

The AUC with multi-variate logistic regression analysis is low. There may be more important factors to predict insulin resistance.

**Response to Reviewer 2, Major Point #2**

According to Hosmer and Lemshow (2000), as a general rule, AUC of 0.7 and above is considered acceptable discrimination. The AUC of our final model was 0.69 and is
close to the acceptable value. We agree with the reviewer that a larger AUC is desirable. There may be factors that can improve our prediction model. Nevertheless, those factors will be other than those that have been evaluated in our study (sex, age, waist circumference, white blood cell count, total cholesterol, triglyceride, HDL-C, LDL-C, creatinine, blood urea nitrogen, ALT, education level, energy expenditure, and sleep duration) and may not be readily available or routinely collected in clinical situations. Therefore, despite an AUC of 0.69, we believe our model can be of use in clinical practice as a simple adjunctive method for early detection of insulin resistance.

Reviewer 2, Minor Point #1
How different are the dietary nutrition between people with normal food intake and vegetarians? Because this study deals with metabolism (insulin resistance), nutrition such as carbonate are important. Please describe these differences from normal people.

Response to Reviewer 2, Minor Point #1
Unlike Western vegetarian diets, which consist mainly of vegetables, fruits, nuts, eggs, and dairy products, a typical Chinese Buddhist vegetarian diet includes mainly rice, grains, vegetables, fruits, and significant amount of soya products. Two studies on Taiwanese reported no significant differences in the consumption of carbohydrates between vegetarians and omnivores. The two studies were briefly described in the Methods section on page 7.

Reviewer 2, Minor Point #2
I don’t think that univariate logistic regression analysis is necessary, because authors had already performed univariate analysis with t-tests or Wilcoxon rank-sum tests, and multi-variate logistic regression analysis. Too much analysis may induce # error.

Response to Reviewer 2, Minor Point #2
The univariate analysis with t-tests or Wilcoxon rank-sum tests in Table 1 was used to evaluate the characteristics of the participants based on their sleep duration whereas the univariate logistic regression used in Table 2 presents the unadjusted odds ratios of the associations between insulin resistance and various independent variables. The two univariate analyses showed different aspects of the study results. Nevertheless, we will accept the suggestion if the reviewer and the editor decide that Table 2 have to be removed. Meanwhile, we have kept it in this version of the revised manuscript.
**Reviewer 2, Minor Point #3**
This kind of study sometimes mentions SAS (Sleep apnea syndrome). Authors should refer this disease, even if there is no data about SAS.

**Response to Reviewer 2, Minor Point #3**
We are grateful for this suggestion. We have added information on SAS in the limitation section with two new references.

**Reviewer 3, Major Compulsory Revision #1**
‘Sleep duration’ needs to be analysed as a continuous variable. This is particularly important given that a) this is the key variable under consideration in the article and b) a non-linear relationship between sleep and insulin resistance has been found before (author’s ref 9). Ideally one could do a multiple logistic regression with spline terms for modelling the non-linear relationship of insulin resistance with sleep duration; at the very least though one should try the regression with linear, quadratic and possibly higher order terms for sleep duration. An appropriate graphical summary of the relationship should then be produced. See, for example, ‘loess’ function family in R.

**Response to Reviewer 3, Major Compulsory Revision #1**
We are grateful for this suggestion. We have added a new figure (Figure 2) in the manuscript to show the plot of nonparametric smoothing obtained from the generalized additive model of sleep duration (in continuous scale) after adjusting for waist circumference and ALT levels. We have conducted analysis using sleep duration categorized as tertiles but it was dichotomized with a cutpoint of 8 hours in the final analysis because the effect of sleep time duration of 6 to 8 hours on HOMA-IR was similar to that of less than 6 hours. In addition, the use of a cutpoint of 8 hours per night is simpler to ascertain from patients in clinical situations than the use of exact sleep duration.
Reviewer 3, Major Compulsory Revision #2
The research question could be clarified a little: are we interested in whether there is the same relationship between sleep duration and insulin resistance in vegetarians as in the general population, or whether it is different (i.e. there is some interaction between vegetarian status and sleep duration variables in the prediction of insulin deprivation). And why should one think to look in this subgroup in particular – if I show a result for a population, I could then posit infinite hypotheses about whether the same effect is found in any subgroups within that population. Some of this is hinted at in the background and discussion but could be made clearer.

Response to Reviewer 3, Major Compulsory Revision #2
In the Background section, we have indicated that previous studies have demonstrated an association (direct association or U-shaped association) between short sleep duration and increased insulin resistance in the general population. It has been postulated that decreased sleep duration could increase body weight, affect glucose metabolism, and increase blood pressure. It has also been reported that long-term Buddhist vegetarians had significantly lower blood pressure, cholesterol, LDL-cholesterol, triglycerides, and fasting blood sugar compared with omnivores. Therefore, we want to investigate whether the beneficial effects of vegetarianism could be offset any negative effects caused by a decrease in sleep duration. We have revised the Background section accordingly on page 6.

Reviewer 3, Minor Essential Revision #1
I have a couple of questions about the recruitment:
- Participants are recruited following a physical examination: what causes people to
report for this examination? Is it routine (in which case what proportion of the population report?) or is it triggered by something?
b. What proportion of eligible participants were actually recruited?

Response to Reviewer 3, Minor Essential Revision #1
The physical examinations were regular routine visits. They were not triggered by any diseases. Since our hospital is not the only hospital providing physical examinations in the region, we are unable to estimate the proportion of the population reported. We have added descriptions of recruitment on page 10. A total of 1,290 individuals were approached at their physical examination. Of those, 515 were identified as vegetarians and 485 (94.2%) agreed to participate in the study. A further 35 individuals were excluded because of their diseases and 17 individuals had missing data.

Reviewer 3, Minor Essential Revision #2
The methods section of the abstract needs rewording to clarify that there are 433 participants AFTER restriction to Buddhist vegetarians.

Response to Reviewer 3, Minor Essential Revision #2
We have revised the Method and Results section of the Abstract to describe the number of participants.

Reviewer 3, Discretionary Revision #1
Connected to points 3b and 4, one could include a flow chart describing the recruitment process.

Response to Reviewer 3, Discretionary Revision #1
We have revised the Results section and included additional descriptions of the recruitment process (page 10).