PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Association of endotoxemia with serum free fatty acids in metabolically healthy and unhealthy abdominally obese individuals: a case-control study in northwest of Iran
AUTHORS	Saghafi-Asl, Maryam; Amiri, Parichehr; Naghizadeh, Mahsa; Ghavami, Seyed Mostafa; Karamzad, Nahid

VERSION 1 - REVIEW

REVIEWER	Eun-Jung Rhee Kangbuk Samsung Hospital
REVIEW RETURNED	16-Jan-2017

GENERAL COMMENTS	This study compared seum FFA and LBP between MHAO and MUAO subjects. However, they did not find any differences of these surrogate markers between the two groupsl they only found the correlations between those two markers. I think the results failed to show positive conclusion, regarding the main problem of this paper.

REVIEWER	Amir Almasi-Hashiani Royan Institute, I.R.Iran
REVIEW RETURNED	29-Jan-2017

GENERAL COMMENTS	Abstract 1- What kind of matching the authors done. (Paired or frequency) 2- Please add statistical software to the method. 3- In the last sentence of results, p value should be report. Introduction 4- The introduction is too long. If it is possible, please delete some less important issues. Methods: 5- What kind of matching the authors done (Paired or frequency)? 6- What are your inclusion criteria? The authors just report exclusion criteria. 7- This study was done on volunteer participants; please add this issue as one of your limitation. 8- Sample size determination needs more information such as standard deviation or percent. 9- If the paired matching was done, so the analysis should be paired. 10- In sample size section, what are the numbers 31 and 32 in parenthesis?

	Results: 11- How the authors control the confounding effect?

REVIEWER	Pietro VAJRO UNIVERSITY OF SALERNO - ITALY
REVIEW RETURNED	11-Feb-2017

GENERAL COMMENTS	The MS by Maryam Saghafi-Asl and colleagues reports an association between endotoxemia and serum free fatty acids in individuals with metabolically healthy and unhealthy abdominally obesity. Apart from a number of typos, the MS is well written. However there are several issues that might be improved. ABSTRACT: 1. Authors please explain "all" the abbreviations used in the Abstract (FFA, FBS, etc, ..) INTRODUCTION 1. This Reviewer believes that this section might be considerably shortened METHODS 1. page 8: Authors please give the reference for the exclusion of pts with FBS≥126 mg/dl and give details if this was verified in at least 2 occasions RESULTS 1. Page 11: a. Was the same cut-off point of 95 cm used for males and females ? b. Please fully comment in the Methods and Discussion sections on the IDF/International Diabetes Federation definition of MetS as the presence of central obesity (WC ≥ 90 cm in men, WC ≥ 80 cm in women) and/or other references with gender and age related norms. c. Authors should try to include into the statistics also the "cm in excess of the 95cm (and gender related 90 and 80 cm norms as well) cut-off points. 2. Page 11: Authors explain abbreviations of MUFA, PUFA, TG, BP when they are first used DISCUSSION 1. Page 14 : Authors explain abbreviation of PA. 2. Limitations of the study (e.g. insulin resistance was not studied) should be included and discussed also in the body of the discussion . TABLE 1: Authors have you got information on fruit and vegetables intake ? If yes, data should be shown and included into statistics. A comment on this issue is worth also in the Discussion section. MINOR TYPOS 1. page 5 check grammar : plasma FFAs are increased among the

	obese as it is released… = should be : ….. are released
	2. page 8 : FBS≥126 mg/d …. should be : mg/dl
	3. page 8: 5cc blood was obtained should be : were
	4. and serum FFAs and LBP which were analyzed, should delete: which
	5. page 12 Neither FFAs nor LBP was significantly…., should be: were significantly ..
	6. page 15 .. found that the association of LBP with MetS was only significant in.. should be : … was significant only in ..
	7. Table 1. and 2 … between MUAO and MHAO patients.. should be: in MUAO and MHAO

VERSION 1 – AUTHOR RESPONSE

Responses to Reviewer 1
Eun-Jung Rhee
Kangbuk Samsung Hospital
Please state any competing interests or state 'None declared': None declared

It was already written at the end of the manuscript; however, it was clearly written as below:The authors have none to declare.

Please leave your comments for the authors below.
This study compared serum FFA and LBP between MHAO and MUAO subjects. However, they did not find any differences of these surrogate markers between the two groups.They only found the correlations between those two markers. I think the results failed to show positive conclusion, regarding the main problem of this paper.
I would like to bring to attention the following text that already appears in the manuscript which addresses non-significant differences between the two groups.
In the discussion:
It is assumed that the association between serum LBP level and MetS observed in previous studies 19, 24-25 is mediated by BMI or WC, and finding no association between serum LBP level and incidence of MetS in our study, in which the WC-matched controls were included, is not unexpected. Overall, what makes our research different from most of previous ones is that in our study we matched the two groups based on WC, rarely observed in prior reports. Most of the previous studies have examined either MetS patients vs. those without the syndrome or metabolically healthy vs. metabolically unhealthy, regardless of their BMI or WC status and based on different metabolic health criteria 3, 8, 28. A few have examined inflammatory markers between metabolically healthy and unhealthy persons, considering WC or abdominal obesity 36, 38, 39.

In the conclusion:
Our study indicated that WC could be a strong mediator of the association between serum LBP, FFAs, and metabolic alterations. In fact, the levels of LBP and FFAs seem to be more related to abdominal obesity than to the presence or absence of metabolic health. The results also suggested a significant correlation between serum FFAs and LBP in abdominally obese population, which seems to be independent of metabolic aberrations.

Responses to Reviewer 2
Amir Almasi-Hashiani

Royan Institute, I.R.Iran
Please state any competing interests or state 'None declared': I have not any competing interests.
It was already written at the end of the manuscript; however, it was clearly written as below: The authors have none to declare.

Please leave your comments for the authors below
Abstract
1- What kind of matching the authors done. (Paired or frequency)?
We did frequency matching in the present study. It was added in page "7 in methods section" as the below sentence: Frequency matching was carried out for the present study.

2- Please add statistical software to the method.
Agree. It was added to the abstract as below: Data were analyzed using SPSS ver. 17.0.

3- In the last sentence of results, p value should be reported.
Agree. P-value was added to the end of the last sentence of the abstract as below: "Neither FFAs nor LBP were significantly correlated with dietary intake or metabolic parameters (p>0.05)".As dietary intake or metabolic parameters were many and there were no significant correlations, therefore, the overall p-value was reported as p>0.05.

Introduction
4-The introduction is too long. If it is possible, please delete some less important issues.
Agree. The introduction was summarized as far as possible. The removed parts were highlighted by track changes observed in the manuscript.

Methods:
5- What kind of matching the authors done (Paired or frequency)?
We did frequency matching in the present study. It was added in page "7 in methods section" as the below sentence: "Frequency matching was carried out for the present study".

6- What are your inclusion criteria? The authors just report exclusion criteria.
It was previously reported in the methods that "Individuals aged 18-60 years with abdominal obesity were included in the study". However, the sentence was completed as below: Apparently healthy individuals aged 18-60 years with abdominal obesity were included in the study".

7- This study was done on volunteer participants; please add this issue as one of your limitation.
Agree. The below sentence was added to the limitations: "4) The present work was carried out on volunteer participants. Though all volunteers were randomly recruited from general population after public announcement and based on the eligible criteria".

8- Sample size determination needs more information such as standard deviation or percent.
Thank you for pointing out this issue. The following text was added to completeinformation on sample size: "The higher sample size was calculated for serum FFAs than LBP, using literature-derived data 34 for nonalcoholic fatty liver disease (NAFLD) patients; the effect size for serum FFAs was 0.20 nmol/l (SD1=0.34 nmol/l and SD2=0.53 nmol/l). Therefore, sample size estimation was based upon this parameter with 80% power and α-error of 5%and a case to control ratio of 1:1. It was predicted that 79 persons in each group would detect changes in serum FFAs as well as serum LBP level, using the two-means formula."

9- If the paired matching was done, so the analysis should be paired.
As paired matching was not done for the present study (please refer to the answer given in question 5 above), then it was not considered in the analysis.

10- In sample size section, what are the numbers 31 and 32 in parenthesis?
Thank you for carefully commenting on our manuscript. References 31 and 32 were mistakenly brought in the manuscript and therefore, were removed from it.

Results:
11- How the authors control the confounding effect?

In the present study, waist circumference, age, and gender were the most confounding factors. As we matched them before the study, and as energy intake and body mass index of the subjects were not significantly different between the two groups, readjusting (controlling) them while performing logistic regression seems to be unnecessary.

Responses to Reviewer 3
• Pietro VAJRO

UNIVERSITY OF SALERNO – ITALY
Please state any competing interests or state 'None declared': NONE
It was already written at the end of the manuscript; however, it was clearly written as below: The authors have none to declare.

Please leave your comments for the authors below:
The MS by Maryam Saghafi-Asl and colleagues reports an association between endotoxemia and serum free fatty acids in individuals with metabolically healthy and unhealthy abdominally obesity.

Apart from a number of typos, the MS is well written.
However there are several issues that might be improved.

ABSTRACT:
1. Authors please explain "all" the abbreviations used in the Abstract (FFA, FBS, etc, ..)
Agree. All the abbreviations were explained in the abstract.

INTRODUCTION
1. This Reviewer believes that this section might be considerably shortened
Agree. The introduction was summarized as far as possible, with track changes observed in the manuscript.

METHODS
1. page 8: Authors please give the reference for the exclusion of pts with FBS≥126 mg/dl and give details if this was verified in at least 2 occasions.
Agree. The reference was added for FBS>126mg/dl 29 and as FBS was measured in two occasions it was also mentioned in page 8.

RESULTS
1. Page 11:
a. Was the same cut-off point of 95 cm used for males and females?
Yes. This was exactly according to theIranian National Committee of Obesity in which waist circumference≥95 cm was introduced as abdominal obesity for both genders, based on a valid study 27.

b. Please fully comment in the Methods and Discussion sections on the IDF/International Diabetes Federation definition of MetS as the presence of central obesity (WC ≥ 90 cm in men, WC ≥ 80 cm in women) and/or other references with gender and age related norms.

In the present work, we studied on metabolically healthy or unhealthy abdominally obese persons, not patients with MetS. Therefore, we used the criteria proposed by Meigs et al. 28 in which metabolic health was defined as the presence of <3 of the following metabolic abnormalities including abdominal obesity (WC≥95cm for both genders according to the Iranian National Committee of Obesity)27; high serum triglyceride (TG) concentration (≥150 mg/dL); low serum high density lipoprotein cholesterol (HDL-C) (<40 mg/dL for men and <50 mg/dL for women); elevated blood pressure (BP) (≥130/85 mmHg); and fasting blood sugar (FBS) (≥100 mg/dL). Though these criteria look similar to IDF criteria for MetS, we used the one proposed by Meigs et al. 28 for defining metabolic health. And, as suggested in many reports, we used Population- andcountry-specific definitionsfor waist circumference in our study**. This was more highlighted in methods section.

c. Authors should try to include into the statistics also the "cm in excess of the 95cm (and gender related 90 and 80 cm norms as well) cut-off points.
As mentioned above, we used WC≥95cm for both genders according to the Iranian National Committee of Obesity 27 (methods, page 6, reference 27) and entered only those with abdominal obesity into the study. Therefore, we did not rank WC and we did not use gender related 90 and 80 cm norms as well) cut-off points.

2. Page 11: Authors explain abbreviations of MUFA, PUFA, TG, BP when they are first used.
Agree. All the abbreviations were checked and explained in the whole manuscript.

DISCUSSION
1. Page 14: Authors explain abbreviation of PA.
Agree. PA which stands for physical activity (PA) was explained in Discussion section, page 14.

2. Limitations of the study (e.g. insulin resistance was not studied) should be included and discussed also in the body of the discussion.
Thank you for your close attention to the manuscript. The below sentence was added to the Discussion, page 14: "Several studies have demonstrated a strong association of IR with obesity, low HDL-C, hypertriglyceridemia, and hypertension 10, 43 as well as inflammatory factors 44. However, in our study, we could not assess IR, due to some financial deficits."

TABLE 1: Authors have you got information on fruit and vegetables intake?
If yes, data should be shown and included into statistics.
A comment on this issue is worth also in the Discussion section.

We did not aim to examine dietary habits of the subjects like fruit and vegetable consumption in this manuscript. Since it was not aimedto be examined in our study. It was important for us to analyze the eaten food by 3-day food record in order to obtain energy and macronutrients intake for possible confounding effect. However, their intakes were not significantly different between the two groups and therefore had no confounding effect.

MINOR TYPOS
1. page 5 check grammar : plasma FFAs are increased among the obese as it is released… = should be : ….. are released
2. page 8 : FBS≥126 mg/d …. should be : mg/dl
3. page 8: 5cc blood was obtained should be : were
4. and serum FFAs and LBP which were analyzed, should delete: which

5. page 12 Neither FFAs nor LBP was significantly…., should be: were significantly ..

6. page 15 .. found that the association of LBP with MetS was only significant in.. should be : … was significant only in ..

7. Table 1. and 2 … between MUAO and MHAO patients.. should be: in MUAO and MHAO

Agree. All of these grammatical mistakes were corrected in the manuscript, as suggested.

References: (Reference numbers are matched with manuscript references)

3. Karelis A and Rabasa-Lhoret R. Inclusion of C-reactive protein in the identification of metabolically healthy but obese (MHO) individuals. Diabetes & metabolism. 2008; 34: 183-4.

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10. Moro E, Gallina P, Pais M, Cazzolato G, Alessandrini P and Bittolo-Bon G. Hypertriglyceridemia is associated with increased insulin resistance in subjects with normal glucose tolerance: evaluation in a large cohort of subjects assessed with the 1999 World Health Organization criteria for the classification of diabetes. Metabolism. 2003; 52: 616-9.

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28. Meigs JB, Wilson PW, Fox CS, et al. Body mass index, metabolic syndrome, and risk of type 2 diabetes or cardiovascular disease. The Journal of Clinical Endocrinology & Metabolism. 2006; 91: 2906-12.

34. Zhang J, Zhao Y, Xu C, et al. Association between serum free fatty acid levels and nonalcoholic fatty liver disease: a cross-sectional study. Sci Rep. 2014; 4: 5832.

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44. Schmidt MI and Duncan BB. Diabesity: an inflammatory metabolic condition. Clinical Chemistry and Laboratory Medicine. 2003; 41: 1120-30.

**. Alberti K, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome. Circulation. 2009; 120: 1640-5.

REVIEWER	Amir Almasi-Hashiani Royan institute Iran
REVIEW RETURNED	12-Mar-2017

GENERAL COMMENTS	Overall, authors addressed all my previous comments and suggestions. Minor revision: In the sample size section, it is clear that SD is Standard deviation but it would be better to specify the abbreviation in the first appearance in the text. Also, in this part, please mention that SD1 and SD2 relate to which groups.

REVIEWER	Pietro VAJRO University of Salerno – ITALY
REVIEW RETURNED	28-Feb-2017

GENERAL COMMENTS	The Authors have adequately modified the MS following this Reviewer's requests. The MS appears much improved.

VERSION 2 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 3
Pietro VAJRO
UNIVERSITY OF SALERNO – ITALY
Please state any competing interests or state 'None declared': NONE
It was already written at the end of the manuscript; however, it was clearly written as below: The authors have none to declare.

Competing interest was reported as "none declared".

Please leave your comments for the authors below
The Authors have adequately modified the MS following this Reviewer's requests.
The MS appears much improved.

Reviewer: 2
Amir Almasi-Hashiani
Royan Institute, I.R.Iran
Please state any competing interests or state 'None declared': I have not any competing interests.
It was already written at the end of the manuscript; however, it was clearly written as below: The authors have none to declare.

Competing interest was reported as "none declared".

Please leave your comments for the authors below
Overall, authors addressed all my previous comments and suggestions.

Minor revision:
In the sample size section, it is clear that SD is Standard deviation but it would be better to specify the abbreviation in the first appearance in the text.
Also, in this part, please mention that SD1 and SD2 relate to which groups.

The abbreviations of SD was specified in the first appearance, as track-changed in the text.
SD1 and SD2 were specified; SD1 was for contorls and SD2 was for NAFLD patients.