Author’s response to reviews

Title: Circulating CD36 and oxLDL levels are associated with cardiovascular risk factors in young subjects: a case-control study.

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Author’s response to reviews: see over
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BMC Cardiovascular Disorders Editorial Team.

Thanks for your comments and suggestions to improve our paper “Circulating CD36 and oxLDL levels are associated with cardiovascular risk factors in young subjects: a case-control study”, by the authors: Luz Elena Ramos-Arellano, José Francisco Muñoz-Valle, Ulises De la Cruz-Mosso, Aralia Berenice Salgado-Bernabé, Natividad Castro-Alarcón and Isela Parra-Rojas. We have read the comments from Referees and answered point by point each of them.

We think the manuscript has improved and hope it be now ready for publication if your think so.

We appreciate your fine attentions.

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Reviewer 1: Lisa Lincz

Reviewer's report:

This is a small case-control study investigating the possible association of sCD36 and oxLDL levels with cardiovascular risk factors in young subjects (18-25 years old). Apart from knowing that 55 of the 188 participants are obese, it is unclear how many actually have any other cardiovascular risk factors and therefore difficult to determine how valid the results are (ie., statistically powered). From the OR confidence intervals in tables 5 and 6, I suspect the study is underpowered and some variables may have too few cases to be included in this style of analysis. Nonetheless, the results are mainly confirmatory of other studies and as the authors concede, are limited by lack of prospective outcome data.

Minor essential revisions:

1. Some grammatical errors throughout manuscript. For instance ‘Endocytosis’ is used improperly on two occasions: …‘capacity or bind and endocytosis oxLDL’….Would be more appropriate as...’capacity to bind and promote endocytosis of oxLDL’…

Answer: The correction was made in the paper.

The page 2, lines 5-7.
CD36 has been shown to play a critical role in the development of atherosclerotic lesions by its capacity to bind and promote endocytosis of oxidized low-density lipoprotein (oxLDL) …..

The page 4, lines 1-3.
Monocyte/macrophage CD36 has been shown to play a critical role in the development of atherosclerotic lesions by its capacity to bind and promote endocytosis of oxLDL, and it is implicated in the formation of foam cells [18,19].

2. The last paragraph of the introduction contains an unfinished sentence at ref 24.

Answer: The correction was made in the paper:

The page 4, lines 11-13.
Due to the wide-spread tissue expression of CD36 and its broad range of functions it is difficult to foresee which specific pathological processes may reflect alterations in sCD36 [24].

3. The captions for some tables refer to differences between genders although this is not indicated in the table. I think the authors meant groups or cohorts?

Answer: The data in Tables 1, 2 and 3 are reported comparing the results obtained in the two study groups (normal weight and obese subjects).
4. Full terms for biochemical test abbreviations should be included in the methods section.

**Answer:** The correction was made in the paper:

*The page 5, lines 6-9.*

Abnormal biochemical levels were identified when total-cholesterol (TC) $\geq 200$ mg/dL, triglycerides (TG) $\geq 150$ mg/dL, low-density lipoprotein cholesterol (LDL-C) $>100$ mg/dL, high-density lipoprotein cholesterol (HDL-C) $< 40$ mg/dL and glucose $>100$ mg/dL, based on the criteria of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) [27].

5. Results. Paragraph 1/ Table 1: Total leukocyte count should be included for completeness in light of reference to this in other studies of obesity in the discussion.

**Answer:** The correction was made in the discussion:

*The page 7, lines 23-26.*

The leukocyte count is considered as an indicator of inflammatory status in obesity [31]. In addition, research has shown that adults and children with obesity have higher levels of leukocytes, mainly monocytes, compared with adults and children of normal weight [32-34].

6. Chi square p-values should be included for hypertension and gender.

**Answer:** The correction was made in the results:

*The page 6, lines 3-7.*

General and biochemical characteristics of the study subjects are shown in table 1. The measurements of body weight, height, systolic and diastolic blood pressure, prevalence of hypertension, and TC, LDL-C, TG, sCD36 and oxLDL levels were higher in obese subjects than in normal-weight controls, as well as, monocyte and platelet count ($P<0.05$). There were no significant differences by gender ($P= 0.162$).

7. Paragraph 2/Tables 2 & 3: The % fat mass and monocyte count were not statistically significantly correlated with sCD36 in normal-weight controls ($P>0.05$). Similarly the p-value for correlation of oxLDL with TC in the normal weight group was not significant ($p=0.053$). The corresponding incorrect statements should be removed from the text.

**Answer:** The correction was made in the paper:

*The page 6, lines 9-16.*

In all subjects studied, sCD36 was significantly correlated with weight, BMI, waist, hip, waist-to-hip ratio, % fat, % fat mass, LDL-C, oxLDL and monocyte count. The obese
subjects showed a high correlation among BMI and sCD36 \((r=0.50, \ P=0.028)\) (table 2). The oxLDL levels were correlated positively with weight, BMI, waist, hip, waist-to-hip ratio, % fat, % fat mass, and TC, TG, LDL-C, sCD36 and monocyte count, in all subjects. In normal-weight and obese groups, oxLDL levels showed a high correlation with weight, BMI, waist, waist-to-hip ratio, % fat mass, and TG, LDL-C levels and monocyte count (table 3).

8. Table 4 introduces new terms that should be more clearly defined. Indicating cut-off values on the table for each yes/no variable would be useful. The number \((n)\) of subjects in each yes and no stratification should be indicated.

**Answer:** The correction was made in table 4.

9. Tables 5 & 6. It is unclear what is meant by ‘upper to third quartile’. Is this simply the third quartile?

**Answer:** The correction was made in the paper.

The page 6, lines 23-27.
For the analysis of association with some cardiovascular risk factors, sCD36 was classified into tertiles (first tertile <23.3 ng/mL, second tertile 23.3 to 97.8 ng/mL and third tertile >97.8 ng/mL), because there are no reference values established. It was observed that obese subjects had 5.8 times higher risk of sCD36 in third tertile than normal weight controls, adjusted for age and gender \((P=0.014)\) (table 5).

The page 6, lines 27-29 and page 7, lines 1-6.
The oxLDL levels were also classified into tertiles (first tertile <31.9 U/L, second tertile 31.9 to 48.0 U/L and third tertile >48.0 U/L) to analyze its association with cardiovascular risk factors. The table 5 shows that subjects with hypertriglyceridemia had 7.5 times higher risk of oxLDL levels in third tertile than subjects without these abnormalities, individuals with fasting impaired LDL-C had 4.5 times higher risk of oxLDL levels in third tertile and subjects with hypertriglyceridemia had 17.9 times higher risk of oxLDL levels in third tertile, adjusted for age, gender and BMI. While obese individuals had 7.4 times higher risk of oxLDL levels in third tertile than controls, adjusted for age and gender.

10. Also unclear why each table does not list the same cardiovascular risk factors?

**Answer:** The correction was made in table 5.
11. Although not labelled, I am assuming the first column of OR, R2 and p-values in each table is for the univariate analysis and the second column is the adjusted values. If so, the first column is not contributing any more information than already shown in tables 2&3. I would suggest eliminating the first column of OR, R2 and p-values and then combining tables 5 and 6 into 1 table showing only the adjusted values for oxLDL and CD36 in side by side columns for ALL cardiovascular risk factors.

**Answer:** The correction was made in the paper in table 5.

12. Discussion. 3rd paragraph: Have others shown increased platelet counts in obesity? Some mention and reference should be made here.

**Answer:** The correction was made in the discussion:

The page 7, lines 26-30.

Regarding increased platelet count in subjects with obesity, similar findings have been shown in other studies, where the platelet count is higher in adolescents and adults with obesity than normal weight subjects [35,36]. It has been shown that interleukin-6 (IL-6) induces differentiation of megakaryocytes into platelets, IL-6 is produced by adipose tissue [37,38].
Reviewer 2: Yasushi Ishigaki.

Reviewer's report:

The authors examined the significances of plasma oxidized LDL and CD36 as a marker of metabolic disorders in young subjects in this study. They found significant associations of these parameters with both dyslipidemia and obesity-related values. The important aspect of this report was its focus on young subjects and the novel finding was the association between plasma oxLDL and the count of monocytes. However, this study had several major problems.

Major

1. The authors found significant associations of plasma oxidized LDL or CD36 with both dyslipidemia and obesity-related values. However, it was already reported that plasma concentration of oxLDL and CD36 were associated with obesity-related values. While this is first report in young subjects, the authors should discuss the novel aspects of this study. In addition, the influence of adiposity should be more addressed.

Answer: The discussion of this point was included in page 9, lines 2-6.

Considering the associations shown in this study sCD36 and oxLDL levels with traditional cardiovascular risk factors such as obesity, hypercholesterolemia, hypertriglyceridemia and fasting impaired LDL-C, both sCD36 and oxLDL levels can be incorporated into cardiovascular risk factors in young subjects for diagnosis early of cardiovascular disease.

2. The results concerned with monocytes were interesting. Thus, the authors should present the data of differential leukocyte count and high sensitive CRP, if possible.

Answer: In this study, hsCRP levels were not measured and only considered the monocyte and platelet counts because they are the main cells where CD36 receptor is expressed, therefore, a greater increase in the monocyte and platelet counts in obese subjects than normal weight subjects may help explain the increase of sCD36 in subjects with obesity. As mentioned in the discussion (page 8, lines 3-8):

In this study, we observed that sCD36 was higher in obese subjects than in normal weight subjects (143.3 ng/mL vs. 32.3 ng/mL, \( P=0.002 \)), these results are congruent to the reported in previous studies [21,44]. This may be due to obese subjects showed an increased number of platelets and monocytes, as was recently reported that the circulating form of the CD36 receptor is associated with microparticles mainly
originated of platelets, leukocytes and endothelial cells as a result of stimuli or apoptosis [8,25].

3. The sample size was rather small, especially in obese subjects.

**Answer:** The sample size in this study was small and is also one of the limitations of the study, however, we regard that achieved observe interesting findings that can be used for future research in this topic.

4. In the part “Discussion”, both first and second paragraph, which is discussed about hypertension and dyslipidemia, were not important issues in this study. Thus, these paragraphs would be shortened or eliminated.

**Answer:** The paragraphs were shortened.

**Minor**

5. The statistical significance of DBP in Table 1 should be re-examined.

**Answer:** The statistical of DBP in table 1 was re-examined and statistical significance was maintained ($P=0.003$).

6. In table 1, the value of TC, 160 mg/dL, was thought to be low, since the value of HDL-C was 44 and LDL-C was 117, respectively.

**Answer:** The data presented in Table 1 were re-examined and all retained the same values.