Author’s response to reviews

Title: Polycystic ovary syndrome is a risk factor for sarcopenic obesity: A case control study

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Author’s response to reviews:

Thanks to the reviewers again for their comments on our manuscript. We have indicated the responses to the second review as “Response2” below. Revisions in our manuscript are highlighted in yellow.

Mohd Ashraf Ganie, MBBS MD DM (Reviewer 3): Reviewer comments. The manuscript submitted by Mc Breairty E et al is a trying to address an important area of PCOS suggesting that women with PCOS have a high prevalence of sarcopenic obesity, that can be correlated to insulin resistance and inflammation.

However, there are several serious concerns to be addressed before considering it for publication. My views are as follows:

a) Abstract

a. The PCOS subjects and controls have been taken from heterogeneous population which will have impact on the results as different ethnic groups have different built which will in turn effect appendicular muscle mass.

Response: All participants for the PCOS and control group were recruited from the same geographic area, and therefore from a very homogenous population (i.e. Saskatoon SK, Canada). This has been clarified in the abstract (line 11) and the text of the manuscript (line 62).
Comment: As per 2016 census Europeans are 69.6%, total minority of 19.8%, Aboriginal group of 11.6%, we can consider it as relative homogenous not homogenous population, but heterogeneous in genes, which will give erroneous results. Please add it this as limitation.

Response: We have included this as a limitation (lines 261-262)

b. It is also not clear as to how the sample size was calculated?. Sample size of 68 appears small to reach a definite conclusion.

Response: We have added a sample size calculation based on clinically-significant differences for sarcopenic obesity (lines 116-119):

“The minimal sample size was determined using the mean and SD of our control group for %ASM (30.2±3.0%) and a predicted mean for sarcopenic obesity that was 2 SD lower (i.e. 24.1%, as a clinical cut-off for sarcopenic obesity [20-22]), a power of 90% and alpha of 0.05. This required a sample size of n=7 per group.”

Comment: OK

c. Age range of 18-35 could have been categorized as they have evaluated the group together which will give erroneous results as the muscle mass of 18-year old PCOS subject will be different when compared to 35 year old PCOS subject, appendicular muscle mass is negatively correlated with age.

Response: We have added a figure with a plot of % appendicular muscle mass versus age for PCOS and control participants (figure 2) to address this comment. The figure shows that across age groups, the women with PCOS had consistently lower % appendicular muscle mass compared to controls.

Comment: OK

d. Result in abstract mentions that skeletal muscle mass was correlated positively with vitamin D, where as vitamin D levels were not done in control group of subjects, we cannot assume vitamin D levels in controls and correlate it without assessing the Vitamin D levels.

Response: This has been clarified as only in women with PCOS on line 21 of the

Abstract:
Furthermore, % appendicular skeletal muscle mass correlated positively with vitamin D ($r = 0.398; p < 0.0001$) in women with PCOS.

Comment: Please add this as limitation

Response2: We have added this as a limitation (lines 256-259)

b) Introduction:

a. Line 2 prevalence of PCOD is given for the pre menopausal women. Why was age group limited between 18-35 as it may limit generalization of results.

Response: The age was limited to 18-35 as menstrual cycles can become irregular after age 35. We have highlighted that our results are not generalizable to women outside this age range at the end of the discussion section (lines 258-260).

Comment: OK

b. Appendicular muscle mass correlates negatively with markers of inflammation. Why in the present study analysis of the inflammatory markers was not done in control group, so difference of inflammatory markers between PCOS and controls and its effect on the appendicular muscle mass cannot be assessed.

Response: The control group was primarily used to determine the prevalence of sarcopenic obesity in women with PCOS. Throughout the manuscript we have clarified correlations are specific to women with PCOS and not as a general finding. Although inflammatory markers were not assessed in the control participants, we did compare the level of inflammatory markers in participants with PCOS to the normative ranges (Table 2); this would approximate what would be seen in a control group.

Comment: Appendicular mass correlates negatively with Vitamin D levels, in controls we cannot overlook the effect of Vitamin D which is mandatory for the analyzing the difference between PCOD cases and controls. It should be added as limitation.

Response2: We have added this as a limitation (lines 256-259)

c) Methods:
Controls were not fully evaluated, testosterone and Ultrasound was not done to rule out hyper androgenic state.

Response: The control group was used to determine prevalence of sarcopenic obesity in women with PCOS and we note in our study limitations that we did not carry out these assessments on our control group (lines 250-252)

Comment: This is a major limitation which cannot be overlooked.
Response2: We clarified this as a limitation (lines 250-252)

In contrast, PCOS was not absolutely ruled out in our control population which may be a limitation of this study, as examination for hyperandrogenicity and polycystic ovaries was not completed.

Response: Our recruitment specified healthy women with regular menstrual cycles. Women were excluded if they had any chronic conditions or irregular menstrual cycles; this has been clarified on lines 64-67:

Control participants were recruited via postings on the University of Saskatchewan website requesting participation from healthy females between 18 and 35 years old with regular menstrual cycles. Controls were excluded if they had any self-identified chronic conditions or irregular menstrual cycles.

Comment: In control group PCOS was not fully ruled out which is a confounding factor. Please add this as limitation.
Response2: This is included as a limitation (lines 250-252)

a. Details of control recruitment has not been mentioned as to how it was done (Line 60)
Response: We have added further details of control recruitment on lines 64-67: Control participants were recruited via postings on the University of Saskatchewan website requesting participation from healthy females between 18 and 35 years old with regular menstrual cycles. Controls were excluded if they had any self-identified chronic conditions or irregular menstrual cycles.

Comment: OK
b. A diagnosis of PCOS was excluded, 'can be rephrased as 'PCOS with following conditions were excluded from the study'. Line 70

Response: This phrase has been replaced as recommended.

Comment: OK

c. PCOS were diagnosed on the basis of irregular cycles and radiological evidence of more than 25 follicles per ovary. Which criteria were fitted? Why testosterone levels were not done for the PCOS subjects (Line 65). Besides androgens especially testosterone have significant relevance to the muscle mass.

Response: We have clarified the PCOS diagnoses which included assessment of

A diagnosis of PCOS required 1) either oligo-amenorrhea and/or polycystic ovaries defined as >25 follicles visualized by transvaginal ultrasonography to reflect the newest guidelines for polycystic ovaries (PCO) recommended by the Androgen Excess and Polycystic Ovary Syndrome Society, and 2) hyperandrogenism as defined by a Ferriman and Gallwey score of >6 and/or biochemical hyperandrogenemia [2, 18, 19].

Comment: Testosterone levels are very important for diagnosis of PCOS, there are several reasons for oligomenorrhoea which were not ruled out biochemically in the cases, some cases have only polycystic ovarian morphology where rest of the biochemical and clinical parameters are normal. Please add this as limitation.

Response2: As per our method section, for a diagnosis of PCOS, participants were required to have either clinical or biochemical hyperandrogenemia (line 75) so there would be no cases in our study without this parameter.

Results:

a. Correlations of appendicular skeletal mass was done in both sarcopenic and non sarcopenic obese, however there is no mention of any such correlation of appendicular skeletal mass in control group (Line 115) So we cannot correlate the results between PCOS subjects and controls.

Response: We did not assess any biochemical measures in the control group so throughout the manuscript we have indicated that all correlation results are specific to the PCOS population.
Comment: It is mandatory to do the correlation in order to come to conclusion to compare results sarcopenic obesity in PCOS Vs controls. In the study correlation is done between obese and non obese cases, where obesity itself an important factor in decrease of appendicular muscle mass. Please add this as limitation

Response2: The lack of determination of correlations in the control group is included as a limitation (lines 258-259)

b. Again HOMA and hsCPR was only done only in PCOS subjects whereas it was not done in control group, so we cannot assess the effect of inflammatory markers on sarcopenic obesity when we do not have the inflammatory markers controls for comparison (Line 131).

Response: We have indicated that all correlation results are specific to the PCOS population. We have also compared HOMA and hsCPR results for women with PCOS to normative values (Table 2) which would approximate what would be seen in a control population.

Comment:

c. Vitamin D levels were insufficient in the PCOS sarcopenic obese group which will have negative effect on appendicular skeletal mass calculation (Line 134). It is confounder.

Response: There was a positive correlation between vitamin D levels and % appendicular muscle mass in women with PCOS; therefore, the reviewer brings up a good point that this could affect appendicular muscle mass. Although there was a large proportion of vitamin D deficiency in our women with PCOS, it is typical of women in our country, which has a high rate of vitamin D deficiency because of long winters and lower sun exposure due to northern latitude. When we compared the % of women with PCOS who had vitamin D levels less than 50 nmol/L, it was identical to national data for the same age group (i.e. 41%, Statistics Canada). We therefore consider that this was not a confounder specific to the women with PCOS in our study, as they are representative of the typical Canadian. We have added this point to the manuscript (lines 229-233):

“A large proportion of the women with PCOS had low vitamin D levels (Table 2); however, this is typical of young Canadian women because of the limited sun exposure due to northern latitude. The proportion of women with PCOS with vitamin D levels below 50 nmol/L in our study (41%), is identical to the proportion of Canadians in the same age group from the general Canadian population [40].”

e) Discussion:
a. As already mentioned (Line 159) healthy female control group was not evaluated as expected.

Response: The control group was used to assess the prevalence of sarcopenic obesity in PCOS; we have included the lack of biochemical assessments in this group as a limitation to the study (line 255). We also feel the comparison of biochemical measures in the PCOS group can be compared to normative values (Table 2) to derive an indication of how they compare to a control population.

Comment: As mentioned earlier in the comments, we cannot assume the results. Please add this as limitation.

Response2: We have added more detail on this limitation (lines 254-259)

b. In the study it is assumed that sarcopenic obesity is associated with PCOS because of high levels of inflammatory markers, while in the present article there is no mention of inflammatory markers in control group, which could have given us a better outlook to how inflammatory markers have effect on sarcopenic obesity in PCOS subjects (Line 171).

Response: We acknowledge this is a limitation of the study (line 255) and compared the values for women with PCOS to normative values (i.e. for C-reactive protein) in Table 2.

Comment: As accepted there are limitations in the study done, we need to evaluate the controls as well to come to conclusion. Please add this as limitation.

Response2: The lack of metabolic markers in the control group is listed as a limitation (lines 254-256).

c. Similarly the conclusion that higher waste circumference is associated with sarcopenic obesity can not be drawn as there is no mention of waist circumference in control group for comparison of the same (Line 204).

Response: We have recognized this as a limitation to the study (end of discussion section) (line 255).

d. PCOS was not ruled out in control group (Line 219) as required baseline investigations were not done.
Response: We have addressed this in our limitations on line 251. In contrast, PCOS was not absolutely ruled out in our control population which may be a limitation of this study, as examination for hyperandrogenicity and polycystic ovaries was not completed.

General comments

1. There are many typos errors and language needs to be improved.

2. References can be updated.

Response: We have included several additional recent references in the manuscript.

The manuscript has been thoroughly proof-read for any typos.