PEER REVIEW HISTORY

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ARTICLE DETAILS

<table>
<thead>
<tr>
<th>TITLE (PROVISIONAL)</th>
<th>Association of obesity and overweight with the prevalence of insulin resistance, prediabetes, and clinical-biochemical characteristics among infertile Mexican women with polycystic ovary syndrome: a cross-sectional study.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUTHORS</td>
<td>Reyes-Muñoz, Enrique; Ortega-González, Carlos; Martínez-Cruz, Nayeli; Arce-Sánchez, Lidia; Estrada-Gutiérrez, Guadalupe; Moran, Carlos; Sánchez-Serrano, Ana; Higareda-Sánchez, Rodolfo; De la Jara-Díaz, Julio</td>
</tr>
</tbody>
</table>

VERSION 1 - REVIEW

<table>
<thead>
<tr>
<th>REVIEWER</th>
<th>Konstantinos Tziomalos</th>
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<tbody>
<tr>
<td></td>
<td>First Propedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, AHEPA Hospital, Thessaloniki, Greece</td>
</tr>
<tr>
<td>REVIEW RETURNED</td>
<td>31-Jan-2016</td>
</tr>
<tr>
<td>GENERAL COMMENTS</td>
<td>The present paper evaluates the effects of obesity on insulin resistance, prediabetes and biochemical characteristics in patients with polycystic ovary syndrome. The methods are adequate, the results are clearly presented and the discussion is comprehensive.</td>
</tr>
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<table>
<thead>
<tr>
<th>REVIEWER</th>
<th>Ken Sikaris</th>
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<tbody>
<tr>
<td></td>
<td>Sonic Healthcare; Melbourne Pathology</td>
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<tr>
<td>REVIEW RETURNED</td>
<td>04-Feb-2016</td>
</tr>
<tr>
<td>GENERAL COMMENTS</td>
<td>The finding of the association or insulin resistance with body weight is not new, even in within PCOS. Studies do exist on these questions of metabolic syndrome, insulin resistance, BMI and PCOS (e.g Hum Reprod. 2008 Sep;23(9):2113-21, Hum Reprod. 2014 Jul;29(7):1508-17, J Hum Reprod Sci. 2015 Oct-Dec;8(4):202-8, Hormones (Athens). 2015 Jan-Mar;14(1):101-8). What is different about the Mexican population to justify this publication? These same issues are also covered in a previous publication by some of the same authors (Int J Endocrinol. 2012;2012:317241). Abstract Page 2, Line 24 BMI equivalents in Mexico Page 2 Line 31 HOMA – depends on Insulin – consider QUICKI which has linear correlation with clamp studies. Provide SI units for glucose (mmol/L) in preference. Page 2 Line 54 DHEAS is age related – correct for age Method</td>
</tr>
</tbody>
</table>
The FAI is an unreliable measure of free testosterone, especially when SHBG concentrations are low and albumin binding becomes significant. Consider calculation based on Vermeulen equation. Furthermore the arbitrary definition of FAI>8.5% is not referenced or justified and leads to the discrepancy between biochemical hyperandrogenism being not significant between groups, but FAI being significantly different.

No indication of the quality of analyses is given such as internal QC for precision, External proficiency testing enrolment for accuracy or laboratory accreditation. For example why was biochemical hyperandrogenism (raised FAI) found to be not significant in table 1, between weight groups despite correlations with SHBG being significant as well as DHEAS which correlates with testosterone in women. This may simply be because inadequate precision of the Immulite testosterone assay at the low concentration found in women – what is the detection limit of the assay and how many women were below the detection limit? The difference in DHEAS, which is small in clinical terms, is probably because the measurement of DHEAS (in umol/L) is much easier than the measurement of testosterone (in nmol/L) or the estimation/calculation of free testosterone (in pmol/L).

17HOP4 typo
Oligo-amenorrhea typo

REVIEWS

The step wise increase on insulin resistance is no unknown in PCOS However, in the light of the Mexican American ethnic origin of the cohort studies a control sample of non PCOS age and BMI matched women is of great importance to arrive at a conclusion that PCOS and BMI cause the step increase in IR
Additionally there is no attempt on the part of the researchers to explore the occurrence and trends of the metabolic syndrome
The sampling method and time interval alongwith whether this was analysis of a retrospective data is not mentioned
Were the subjects treatment naive or had some been exposed to metformin and lifestyle modification
Although from a tertiary care institution with an emphasis on infertility this aspect had not been studied/reported
It is best that you score the degree of hirsutism based on modified Ferrmina Gallwey score please

> Reviewer #1
No comments.

VERSION 1 – AUTHOR RESPONSE
1) C: The finding of the association or insulin resistance with body weight is not new, even in within PCOS. Studies do exist on these questions of metabolic syndrome, insulin resistance, BMI and PCOS (e.g. Hum Reprod. 2008 Sep;23(9):2113-21, Hum Reprod. 2014 Jul;29(7):1508-17. J Hum Reprod Sci. 2015 Oct-Dec;8(4):202-8 Hormones (Athens). 2015 Jan-Mar;14(1):101-8). What is different about the Mexican population to justify this publication?

R: This is the first study among infertile Mexican women that use cut-off point for IR applicable to our population; in addition, we are showing the prevalence of IR, prediabetes and clinical-biochemical characteristics according to body mass index. Few studies have explored these topics comparing normal weight, overweight and obese women, and we believe that this study will contribute to make decisions for the physician in daily clinical practice in front of the patient. Physician should consider certain clinical and biochemical parameters that must be corrected before attempting pregnancy among infertile women with PCOS based on BMI.


R: In Mexico, for clinical studies we use the World Health Organization classification for BMI.

3) C: Page 2 Line 31 HOMA – depends on Insulin – consider QUICKI which has linear correlation with clamp studies. Provide SI units for glucose (mmol/L) in preference.

R: Even though some studies had showed that QUICKI and HOMA have a similar correlation with clamp studies, in a recent meta-analysis QUICKI showed a better correlation than HOMA (r= 0.61 CI 0.55 to 0.65 vs -0.53 CI -0.60 to -0.46, for QUICKI and HOMA respectively). However, we decided to use HOMA because we have a cut-off to define insulin resistance (IR) for Mexican population and we have not a cut-off to define IR by QUICKI. This statement was added in the discussion section of the revised manuscript.

We changed glucose (mg/dL) to SI units (mmol/L).

4) C: Page 2 Line 54 DHEAS is age related – correct for age

R: We agree with the reviewer, but because age in the three study groups is similar, we consider that correction for age it is not necessary.

5) C: The FAI is an unreliable measure of free testosterone, especially when SHBG concentrations are low and albumin binding becomes significant.

R: We consider that this is a topic of controversy. Most routinely available methods for measuring serum androgens suffer from major limitations. Studies performed in normal and PCOS women showed a good correlation of FAI with freeT measured by liquid chromatography-tandem mass spectrometry vs immunoassay, for example:

Bui et al., reported reference intervals and biologic variation for T, freeT, and FAI in women with accurate methods and to test the discriminative value of these parameters in a PCOS population. T was measured by liquid chromatography–tandem mass spectrometry (LC–MS/MS) and by Architect® 2nd generation T Immunoassay. The areas under the curve of receiver operator characteristic plots were not different for T, freeT, or FAI when T was measured by LC–MS/MS versus immunoassay based on prediction of PCOS. FAI and freeT were the strongest predictors of PCOS. (Bui HN, Sluss PM, Hayes FJ, et al. Testosterone, free testosterone, and free androgen index in women: Reference intervals, biological variation, and diagnostic value in polycystic ovary syndrome. Clin Chim Acta. 2015 Oct 23;450:227-32).
Barth et al, reported using LC–MS/MS method for analysing testosterone and androstenedione (Ad) to study the reference ranges and diagnostic utility in PCOS, that diagnostic performance using receiver operator characteristic plots showed area under the curve (AUC) for FAI 0.81, testosterone 0.75 and Ad 0.66. (Barth JH, Field HP, Yasmin E, Balen AH. Defining hyperandrogenism in polycystic ovary syndrome: measurement of testosterone and androstenedione by liquid chromatography-tandem mass spectrometry and analysis by receiver operator characteristic plots. Eur J Endocrinol. 2010 Mar;162(3):611-5).

We now addressed this point in the discussion section and these references were added to support our measures.

6) C: Consider calculation based on Vermeulen equation.

R: Unfortunately, we are not able to calculate based on Vermeulen equation in this study because albumin is not systematically measured in our clinic in these patients.

7) C: Furthermore the arbitrary definition of FAI>8.5% is not referenced or justified and leads to the discrepancy between biochemical hyperandrogenism being not significant between groups, but FAI being significantly different.

R: We have changed the definition of FAI to >4.5% based on the Fox et al study that reports that FAI >4.5% gives the best results in terms of overall diagnostic accuracy of PCO with 94% of sensitivity and 88% of specificity, compared with total testosterone >3nmol/L, progestin challenge and serum LH. This reference was added: Fox R. et al. The diagnosis of polycystic ovaries in women with oligoamenorrhoea: predictive power of endocrine test. Clin Endocrinol 1991; 34:127-31.

Discrepancy between biochemical hyperandrogenism persist even after FAI definition was changed. This discrepancy may be because FAI is compared among groups has a quantitative variable and biochemical hyperandrogenism as a dichotomy variable.

8) C: Page 7 Line 16-35. No indication of the quality of analyses is given such as internal QC for precision, External proficiency testing enrolment for accuracy or laboratory accreditation. For example, why was biochemical hyperandrogenism (raised FAI) found to be not significant in table 1, between weight groups despite correlations with SHBG being significant as well as DHEAS which correlates with testosterone in women. This may simply be because inadequate precision of the Immulite testosterone assay at the low concentration found in women – what is the detection limit of the assay and how many women were below the detection limit? The difference in DHEAS, which is small in clinical terms, is probably because the measurement of DHEAS (in umol/L) is much easier than the measurement of testosterone (in nmol/L) or the estimation/calculation of free testosterone (in pmol/L).

R: We agree. We clarify the quality of total testosterone and SHBG analyses and internal control. This statement was added to the manuscript "We assessed serum testosterone and SHBG by solid-phase competitive chemiluminescent enzyme immunoassay (IMMULITE 2000 Immunoassay System, Siemens). The analytical sensitivity is 0.5 and 0.02 nmol/L and CV is (7.2-24.3%) and (4.2-6.6%) for TT and SHBG respectively. There were two women below the detection limit of TT and none for SHBG.

9) C: Page 9 Line 11: 17HOP4 typo

R: Thanks, this was corrected by 17-OHP4

10) C: Page 10 Line 49 Oligo-menorrhea typo

R: Thanks, this was corrected by oligomenorrhea.
11) C: The step wise increase on insulin resistance is no unknown in PCOS. However, in the light of the Mexican American ethnic origin of the cohort studies a control sample of non PCOS age and BMI matched women is of great importance to arrive at a conclusion that PCOS and BMI cause the step increase in IR.

R: This is a good suggestion, but this was not the objective of our study. We want to report the association of BMI with the prevalence of IR among infertile Mexican PCOS women. Our aim was not to show if BMI is the cause of IR among Mexican women with PCOS.

12) C: Additionally there is no attempt on the part of the researchers to explore the occurrence and trends of the metabolic syndrome.

R: Again, this was not the aim of the study. We did not explore the occurrence and trends of the metabolic syndrome because in our clinical practice are selective indication to perform lipids determinations like BMI > 27 kg/m2 and/or HOMA > 2.5%. Women enrolled in this study had not systematically determination of lipids.

13) C: The sampling method and time interval along with whether this was analysis of a retrospective data is not mentioned

R: In the original manuscript, page 6 we mentioned: “In this cross-sectional study, participants were women treated at the infertility clinic of the National Institute of Perinatology in Mexico City from 2009 to 2013” and in page 7 we mentioned: “Clinical and ultrasound data were obtained from clinical records, and biochemical data were obtained from the database of Endocrinology department”. We have added in the first paragraph of the Method section that this is a retrospective study.

14) C: Were the subjects treatment naive or had some been exposed to metformin and lifestyle modification

R: Women enrolled were naive, so this statement was added to the participants section: All women were recruited at the first visit, and they were not exposed to metformin or lifestyle modification intervention three months previous to this study.

15) C: Although from a tertiary care institution with an emphasis on infertility this aspect had not been studied/reported.

R: That is right. This aspect had not been reported in any tertiary care institution of Latin America.

16) C: It is best that you score the degree of hirsutism based on modified Ferrmina Gallwey score please

R: We agree. This is a limitation of the study that was highlighted in the discussion, in the revised version of the manuscript, and this statement was added: “the severity of hirsutism is not comparable among groups, because in our institution the total Ferriman Gallwey score is not documented, only a Ferriman Gallwey score > 8 is considered as hirsutism”.
GENERAL COMMENTS

With regard to determining correlations between variables like age, BMI and hyperandrogenism with the outcome variables of IT prediabetes diabetes are best added.

In the conclusion the authors need to specify Mexican American women with PCOS presenting to an infertility clinic.

GENERAL COMMENTS

The investigators have studied the prevalence of insulin resistance in a large population of patients diagnosed with PCOS according to the Rotterdam 2003 criteria. This reviewer has the following comments:

1) As the investigators note, this manuscript has the strength that it addresses an issue seldom reported in Latin American populations.
2) It has a number of limitation, although many can be addressed.
3) As noted HOMA-IR is a poor measure of insulin resistance, although useful in popualtional studies.
4) The study is retrospective.
5) The investigators describe the phenotype of the patients. However, they do not present the effect of the phenotypes on the results of IR, etc., the primary measures of the manuscript.
6) The quality of the TT and A4 assay is very poor (Immulite), as acknowledged by the investigators. Do they not have sampled stored that could not be sent for analysis, elsewhere even for part of the population? They also need to establish normal ranges in their own populations.

GENERAL COMMENTS

This cross sectional study on 538 patients with PCOS examines the metabolic associations with respect to weight. They conclude that obese and over weight patients with PCOS have a higher prevalence of insulin resistance and prediabetes compared to normal weight women. These data are in accord with other studies in PCOS.

Comments

The strength of this study is the number of subjects that were
The main weakness of the study is that weight matched non-PCOS women were not compared. It is therefore not possible to determine the PCOS features independent of weight. It is becoming increasingly evident that when weight matched subjects are used then many of the features of PCOS then become non significant. Therefore the conclusion that the prevalence of IR and prediabetes increases with weight is true but we do not know if this is specific to those with PCOS or more a generally to weight per se.

It is not clear if the 613 patients initially identified were the entire cohort presenting sequentially.

I am unclear why the number of patients was not 100% for oligo/anovulaion – did the authors include patients with polycystic ovaries plus hyperandrogenism but with a regular menstrual cycle? If so then these often have the mildest metabolic abnormalities and some would consider that this is not PCOS.

Clinical assessment of hirsutism can be subjective. Does the analysis hold true or indeed strengthened if only those with biochemical hyperandrogenism are included in the analysis? Do those women with biochemical hyperandrogenism, oligo/anovulation and polycystic ovaries have a more severe phenotype as documented in the literature?

The authors are correct in identifying that as not all patients received an OGTT that it is possible to miss both prediabetes and diabetes in these patients

SHBG levels may reflect insulin resistance – was this correlated and assessed when obesity was accounted for?

### VERSION 2 – AUTHOR RESPONSE

Reviewer #1
C: With regard to determining correlations between variables like age, BMI and hyperandrogenism with the outcome variables of IR pre diabetes diabetes are best added.
R: We add the correlations in the results section of the revised manuscript.

C: In the conclusion the authors need to specify Mexican American women with PCOS presenting to an infertility clinic.
R: We did not study Mexican American population, since Mexican American define Mexican women who live in U.S.A. So, we consider that Mexican women is a more appropriate term. We added this statement in the conclusion: Mexican women with PCOS attending to an infertility clinic.

Reviewer: # 2
The investigators have studied the prevalence of insulin resistance in a large population of patients diagnosed with PCOS according to the Rotterdam 2003 criteria. This reviewer has the following comments:
C) As the investigators note, this manuscript has the strength that it addresses an issue seldom reported in Latin American populations. It has a number of limitation, although many can be addressed. As noted HOMA-IR is a poor measure of insulin resistance, although useful in populational studies. The study is retrospective.
R) We agree; these topics had been addressed along the manuscript.
C) The investigators describe the phenotype of the patients. However, they do not present the effect of the phenotypes on the results of IR, etc., the primary measures of the manuscript.
R) We add the effect of phenotypes on the primary results: HOMA-IR, IR prediabetes and diabetes.
C) The quality of the TT and A4 assay is very poor (Immulate), as acknowledged by the investigators.
Do they not have sampled stored that could not be sent for analysis, elsewhere even for part of the population? They also need to establish normal ranges in their own populations.

R) We agree, most routinely available methods for measuring serum androgens have major limitations. Unfortunately, we did not store samples to send for new analysis. On going research in our lab is conducted to establish normal ranges for TT and A4 in our population, using standard techniques.

Reviewer: #3

This cross sectional study on 538 patients with PCOS examines the metabolic associations with respect to weight. They conclude that obese and over weight patients with PCOS have a higher prevalence of insulin resistance and prediabetes compared to normal weight women. These data are in accord with other studies in PCOS

Comments

C) The strength of this study is the number of subjects that were studied. The main weakness of the study is that weight matched non-PCOS women were not compared. It is therefore not possible to determine the PCOS features independent of weight. It is becoming increasingly evident that when weight matched subjects are used then many of the features of PCOS then become non significant. Therefore the conclusion that the prevalence of IR and prediabetes increases with weight is true but we do not know if this is specific to those with PCOS or more a generally to weight per se.

R) We agree with this comment, but we consider that this was not the aim of our study. We want to report the association of BMI with the prevalence of IR and prediabetes among infertile Mexican PCOS women. Our aim was not to show if BMI is the cause of IR and prediabetes among Mexican women with PCOS. We add in the discussion section that further studies are needed to know if the increased prevalence of IR and prediabetes is effect of weight or an effect of PCOS.

C) It is not clear if the 613 patients initially identified were the entire cohort presenting sequentially.

R) Yes, the 613 patients were presented sequentially during the study period. We clarify this topic in methods and results section.

C) I am unclear why the number of patients was not 100% for oligo/anovulaion – did the authors include patients with polycystic ovaries plus hyperandrogenism but with a regular menstrual cycle? If so then these often have the mildest metabolic abnormalities and some would consider that this is not PCOS.

R) We use the Rotterdam criteria for the diagnosis of PCOS, and 5.6% (30 women) of the entire cohort had regular menstrual cycle. We added in table 5 the prevalence of IR, prediabetes and diabetes according with phenotype.

C). Clinical assessment of hirsutism can be subjective. Does the analysis hold true or indeed strengthened if only those with biochemical hyperandrogenism are included in the analysis?

R) We agree with this comment and this is part of the limitations of the study. We have added a correlation between biochemical hyperandrogenism and HOMA IR, however an analysis considering only biochemical hyperandrogenism is not the objective of the study, and according to the Rotterdam criteria we decided to include women with clinical and/or biochemical hyperandrogenism.

C) Do those women with biochemical hyperandrogenism, oligo/anovulation and polycystic ovaries have a more severe phenotype as documented in the literature?

R) Yes, there was a positive correlation between biochemical hyperandrogenism and HOMA IR, but when we considered clinical and/or biochemical hyperandrogenism there was not significant correlation with HOMA IR. There was higher concentration of insulin and HOMA IR value in women with phenotypes that included hyperandrogenism + oligo-anovulation. Prevalence of IR, prediabetes and diabetes, show a higher tendency in women with phenotypes that included hyperandrogenism + oligo-anovulation, but were not statistically different. We have added this finding in the results section.

C) The authors are correct in identifying that as not all patients received an OGTT that it is possible to miss both prediabetes and diabetes in these patients.

R) We agree. This is a limitation of the study assessed in the discussion section.
C) SHBG levels may reflect insulin resistance – was this correlated and assessed when obesity was accounted for?

R) We agree, SHBG levels were significantly lower among obese women compared to normal weight women, we added a correlation between SHBG and HOMA IR in the results section.

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**GENERAL COMMENTS**

The main issue that i have is regarding the population and my comment about the need for a weight matched cohort of non PCOS to answer the question. The authors answered " We want to report the association of BMI with the prevalence of IR and prediabetes among infertile Mexican PCOS women." Indeed this study does answer that question but what we do not know is does it matter if the diagnosis is of PCOS or not

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**AUTHOR RESPONSE**

Reviewer Name: Stephen Atkin
Institution and Country: Weill Cornell Medicine - Qatar
Competing Interests: none

C: The main issue that i have is regarding the population and my comment about the need for a weight matched cohort of non PCOS to answer the question. The authors answered " We want to report the association of BMI with the prevalence of IR and prediabetes among infertile Mexican PCOS women." Indeed this study does answer that question but what we do not know is does it matter if the diagnosis is of PCOS or not

R: In this study we found that overweight and obesity are associated with higher prevalence of IR and prediabetes than normal weight women, only in Mexican women with PCOS and infertility, that was the primary aim of our study. We are not able to show if the increased prevalence of IR and prediabetes is due to PCOS, and we consider that this is a different research question that was not addressed in our study. A matched normal weight group without PCOS was not included because in our Institution we systematically measure insulin and androgens only to women that have one or more of the following characteristics: irregular menses, hirsutism, polycystic ovary morphology or BMI > 27 kg/m2. We are conducted now a prospective study to address if diagnosis of PCOS increases the prevalence of IR and prediabetes, or if they are only directly consequence of BMI.
Association of obesity and overweight with the prevalence of insulin resistance, pre-diabetes and clinical–biochemical characteristics among infertile Mexican women with polycystic ovary syndrome: a cross-sectional study

Enrique Reyes-Muñoz, Carlos Ortega-González, Nayeli Martínez-Cruz, Lidia Arce-Sánchez, Guadalupe Estrada-Gutierrez, Carlos Moran, Ana Paola Sánchez-Serrano, Rodolfo Higareda-Sánchez and Julio Francisco de la Jara-Díaz

BMJ Open 2016 6:
doi: 10.1136/bmjopen-2016-012107

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