The effects of Metformin on insulin resistance and cardiometabolic features in Malay women with PCOS

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Abstract

Objective: To compare the effects of metformin and lifestyle management to lifestyle management alone on the markers of insulin resistance, anthropometric, reproductive and metabolic parameters.

Design: Cross-sectional clinic based study of Malay women with PCOS and metabolic syndrome for 6 months.

Subjects: 43 Malay women with PCOS and metabolic syndrome were randomised into Metformin and Control Groups. PCOS was based on the Rotterdam criteria and the metabolic syndrome was diagnosed based on the Joint Interim Statement of the International Diabetes Federation criteria.

Measurements: Anthropometric, biochemical and endocrinological parameters were compared between the two groups of Malay PCOS women.

Results: Overall, both groups of women had very similar age and metabolic and endocrine characteristics. The metformin group became less insulin resistant (HOMA-IR, p =.016) with lower serum insulin (p =.021). Fasting plasma glucose had significantly risen among the control women, but only marginally (p =.042). The differences in serum SHBG, FAI and free testosterone between the groups were not significant, as were the other metabolic measures.

Conclusions: The addition of metformin to lifestyle modification over a period of 6 months reduced insulin resistance in Malay women with PCOS and metabolic syndrome. Metformin treatment may provide an effective early management of metabolic syndrome and related disorders.

Introduction

Polycystic ovary syndrome (PCOS) is a common heterogenous disorder which may affect up to 15% of reproductive age women depending on the population (Bozdog G 2016). It was initially thought of as a reproductive disorder but is now considered a metabolic condition associated with long term health risks.

It is remarkable that PCOS is associated with many of the commonly seen medical conditions. PCOS women may have many conditions similarly seen in the metabolic syndrome such as diabetes mellitus, hypertension, obesity and endothelial dysfunction (Grundy SM et al 2007 Moran LJ et al 2009). Elevated plasma levels of cholesterol, triglycerides and low density lipoprotein cholesterol (LDL-C) together with lowered high density lipoprotein cholesterol (HDL-C) are the atherogenic lipid abnormalities are also prevalent (Wild RA et al 1985). More recently, a large retrospective study comparing PCOS women with age matched controls has shown that PCOS women had increased risks of obesity, diabetes mellitus, hypertension, ischaemic heart disease and others (Hart et al 2015).

The metabolic syndrome is a collection of risk factors linked to the development of atherosclerotic cardiovascular diseases and type 2 diabetes mellitus (Moeller DE et al 2005). A recent subgroup metaanalysis among different-aged PCOS women showed that adult PCOS women had 2.6 fold odds of having metabolic syndrome compared to healthy controls (Behboudi-Gandevani S et al 2018).

Although the exact etiology of PCOS is still not known, it is established that hyperinsulinism and hyperandrogenism have a key role in its pathophysiology (Stepto NK et al 2013). Hyperinsulinemia is accepted as an early stage in the development of DM and the cardiovascular aspect of metabolic syndrome, independent of obesity. Insulin resistance is also associated with increased levels of LDL-C (Dejager S 2001). Furthermore, the increased insulin levels affect the ovary, liver and other organs to increase serum androgen levels (Teede et al 2007).

www.turkjphysiotherrehabil.org 25749
Insulin resistance is exacerbated further by obesity (Ng M et al 2014). Obesity is a common finding in PCOS and probably has a bidirectional relationship with it, that is, obesity increases PCOS prevalence and PCOS causes weight gain and obesity (Teede et al 2013).

Due to its strong strong association with obesity, weight loss is a key element in the management of overweight and obese PCOS women. The first line of therapy is lifestyle management which includes physical activity and dietary modification (Teede et al 2011). Lifestyle management can, however, be challenging and daunting and has limited efficacy (Moran LJ et al 2011). Pharmacotherapy is possibly the most effective form of therapy in the management of metabolic abnormalities.

Metformin is a commonly used type of insulin sensitiser in the management of diabetes mellitus and other conditions. It is a biguanide drug that has been demonstrated to improve insulin sensitivity in target tissues (Bailey C3 et al 1996). It acts by lowering blood glucose and improves insulin sensitivity mainly through its actions on the liver and peripheral tissues (Meyer C et al 2007). Since the 1990s, it has been shown that metformin improves insulin sensitivity and inhibits ovarian androgen production in women with PCOS (Nestler JE 2008, Diamanti-Kandarakis et al 2010).

Although many studies comparing the efficacy of lifestyle plus metformin to lifestyle alone in the management of weight loss, hyperandrogenism, insulin resistance and other clinical aspects of PCOS have been conducted, none have been carried out on Malay women. The primary aim of this study on Malay women with PCOS and metabolic syndrome was to examine the effects of metformin and lifestyle management on the surrogate markers of insulin resistance (fasting insulin, HOMA-IR). Secondary outcomes included anthropometric (waist circumference, BMI), reproductive [total and free testosterone, SHBG, free androgen index (FAI)] and metabolic [fasting glucose, HbA1c, lipids] parameters were also assessed.

Materials and methods

Subjects

In this case-control study, 58 Malay subjects with PCOS and metabolic syndrome were recruited from a specialist reproductive endocrine clinic. Using computer randomisation, 25 subjects were placed in the Metformin Group and 27 others into the Control Group. Subjects in both groups were taught lifestyle modification but the Metformin Group were also managed additionally with metformin. The ages of the women in the two groups were very similar (Mean 32.0 IQR 28.5-35.5 vs 31.5 27.8-38.0 for Metformin and Controls respectively).

PCOS was diagnosed based on the Rotterdam criteria, requiring the presence of 2 of 3 features of androgen excess (clinical or biochemical hyperandrogenism), ovulatory dysfunction and polycystic ovarian morphology (PCOM) (REA-SPCW group, 2004). Ovulatory dysfunction was defined as oligomenorrhea (spontaneous intermenstrual cycles of ≥ 45 days or ≤ 8 menstrual cycles per year), or amenorrhea (absence of menstruation for > 182 days). Based on Southeast Asian cut-offs, clinical hyperandrogenism was defined as hirsutism diagnosed when the subjects scored ≥ 2 on the mFG score, acne or androgenic alopecia (Afifi L et al 2017). Biochemical hyperandrogenism was diagnosed if FAI and/or cFT were more than 7.1% and 0.035 nmol/L, respectively (based on ROC curve analysis of Malaysian women) (Dineshinee RN et al 2018). PCOM was at least one ovary with ≥12 antral follicles measuring 2-9 mm in diameter, or an ovarian volume of >10 cm³ on either ovary, measured in 3 dimensions using the formula for a prolate ellipsoid (length x width x height x 0.52) and excluding any cysts, dominant follicles or corpora lutea (Balen AH 2003).

Polycystic ovaries were diagnosed via high resolution abdominal or vaginal ultrasound scanning (TAS or TVS, respectively) using a Mindray DC6 ultrasound machine (Mindray Medical International Limited). TVS was the method of choice unless the subject objected, in which case TAS was carried out. TAS was focused on establishing ovarian volume due to the inability to accurately estimate the number of follicles. The endovaginal probe had a frequency of 5-9 MHz. Apart from the ovaries, the uterine dimensions, presence of growths, ovarian cysts and follicles were also noted.

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25750
Abdominal obesity was defined as waist circumference $\geq 88$ cm based on the cut-off point for the South East Asian population (Alberti KG et al 2009). BMI was calculated as weight (kg) divided by height $\times$ height (m$^2$) and overweight and obesity were defined as BMI 25.0-29.9 and $\geq 30.0$ kg/m$^2$ respectively.

Dyslipidemia in Malaysian women has been defined as either TG $> 1.7$ mmol/L, HDL-C $< 1.2$ mmol/L, LDL-C $> 2.6$ mmol/L and TC $> 5.2$ mmol/L (Ministry of Health Malaysia Management of Dyslipidemias 2017).

Hypertension was diagnosed by either a previous diagnosis of hypertension, use of antihypertensive medication or the persistent elevation of systolic BP $\geq 130$ mmHg or diastolic BP $\geq 80$ mmHg.

Metabolic syndrome was based on the Joint Interim Statement of the International Diabetes Federation (IDF) (Alberti KGMM 2009). This required at least 3 out of 5 components to diagnose the metabolic syndrome. The 5 components are waist circumference $> 80$ cm (country specific for South Asian population), HDL-C $< 1.2$ mmol/L, TG $> 1.7$ mmol/L, BP $\geq 130/85$ mmHg and fasting glucose level of $\geq 5.6$ mmol/L.

Lifestyle modification was defined as diet, exercise, behavioural change such as education and counselling or a combination of these for any duration.

Subjects were excluded from the study if they were found to be pregnant, had any chronic medical conditions, specifically hypertension, DM, thyroid disorders, and where on any medications known to affect reproductive function, metabolism or liver protein production. All women underwent testing for serum FSH, LH, TSH, 17-hydroxprogesterone and prolactin to rule out hypo- and hypergonadotropic hypogonadism, thyroid disease, non-classical congenital adrenal hyperplasia and hyperprolactinemia respectively. Cushing’s syndrome was ruled out clinically. Undiagnosed vaginal bleeding and other significant genital tract pathology were also excluded. None of the study subjects smoked or had any systemic diseases. Measures of weight and fat distribution were not part of the recruitment criteria.

Informed consent was obtained from all subjects according to the protocol prior to involvement in the research. Study approval was obtained from the National Heart Institute of Malaysia Ethics Committee (IJNEC) and the Institutional Review Board of the University of Cyberjaya.

Insulin sensitivity

Insulin resistance was estimated by homeostasis model assessment (HOMA-IR) and glycosylated haemoglobin A1c (HbA1c). The HOMA-IR score was calculated using the formula [fasting serum insulin (mIU/L) x fasting plasma glucose (mmol/L)]/22.5. A HOMA-IR of $< 0.99$ in Malay women indicated normal insulin sensitivity (Al-Mahmood AK et al 2006). Area under the ROC curve glucose and insulin were calculated using the trapezoidal rule (Atabek ME 2007, Sakaguchi K et al 2015).

Normal levels of HbA1c were set at $< 5.6$% (38.0 mmol/mol), prediabetes between 5.6-6.2% (38.0-44.0 mmol/mol) and diabetes $> 6.3$% (45.0 mmol/mol), with normal fasting plasma glucose levels being $< 6.0$ mmol/L (Ministry of Health Malaysia Management of T2DM 2015).

Procedures

Measurements were taken on recruitment (basal measurements) and repeated at the end of the trial at 6 months (end measurements).

Standard anthropometric measurements for all subjects were height, weight, waist circumference, body mass index (BMI) and blood pressure (BP). Height and weight were measured with light clothing on and without shoes. Weight was measured on a calibrated beam scale to the nearest 0.5 kg. Height and waist circumference were measured to the nearest 0.5 cm with a measuring tape. Waist measurements were taken halfway between the lower rib margin and the iliac crest at the end of a gentle expiration. BP was measured with a random-zero sphygmomanometer using an appropriate sized cuff applied to the left arm in a sitting position after at least 5 minutes of rest. Patients with persistently elevated BP were rechecked on another occasion prior to confirmation of hypertension. The modified Ferriman-Gallwey (mFG) visual score was used to assess and quantify hirsutism (Ferriman D 1961). The trained investigator assessed the 9 specified androgen-dependent

www.turkjphysiotherrehabil.org 25751
areas of the body (upper lip, chin, upper and lower back, upper arms, chest, upper and lower abdomen and thighs). Each site was rated on a 5-point Likert scale ranging between 0–4 (from absent to extensive terminal hair). A complete assessment included facial and body hair inspection, oily skin, acne, androgenic alopecia and acanthosis nigricans.

**Laboratory Investigations**

All blood samples were drawn at one sitting as a measure of convenience to the subjects between 0800–1000 after an overnight fast of at least 8 hours. Sampling was carried out during the early follicular phase of the menstrual cycle (days 1-3) in those with eumenorrhea or mild-to-moderate oligomenorrhea. In amenorrheic or severe oligomenorheic subjects, samples were taken during the early phase of a withdrawal bleed induced by a 7-day course of oral norethisterone. Sera planned for periodic shipment to the core laboratory for eventual assay was aliquoted into 1.5 cc microfuge tubes and stored at −20 to −70 degrees C. Aliquoting was done to preserve the basal levels of potential analytes in a frozen state, since thawing and refreezing of samples has a deleterious effect. For all measurements, the inter-assay coefficient of variation was less than 10% while the intra-assay variation was less than 15%. For the biochemical assays, venous blood was collected into serum gel tubes (BD Biosciences, Mountain View, CA) and fluoride oxalate tubes. Samples were separated by centrifugation at 2000 x g for 15 minutes and serum aliquots were stored at -20°C within 1 hour of collection. Fasting plasma glucose was measured using an enzymatic colorimetric method with hexokinase, whilst HbA1c was assayed using the HPLC method. Serum insulin was assayed using a competitive chemiluminescent immunoassay performed on the DPC Immulite 2000 analyser (Euro/DPC, Llanberis, UK). The analytical sensitivity of the insulin assay was 2µU/ml, the coefficient of variation was 6% and there was no stated cross reactivity with proinsulin.

Medical assay kits were used for serum total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) assessment using Modular Analytics SWA (Roche), TG was assayed with Lipase/glycerol kinase/GPO-PAP and HDL-C and TC both with cholesterol esterase and cholesterol oxidase. Low-density lipoprotein cholesterol (LDL-C) was calculated using an online calculator (merckmanuals.com) based on Friedwald’s equation \[ \text{LDL-C} = \text{TC} - \text{HDL-C} - (\text{TG}/2.2) \] (Knopfholz I et al 2014). This equation, however, was not applicable with high TG values (TG > 4.5 mmol/L).

Steroid hormones were tested in batches within 2-4 weeks of being frozen. All assays were performed in the same laboratories and assays did not change from 2012 to the end of sampling, 2017. Both total testosterone (TT) and SHBG were stored at 4°C until analysis. Dehydroepiandrosterone sulphate (DHEAS) was stored at −40°C until analysis which was done on a monthly basis. TT, SHBG, prolactin, TSH, FSH, LH, insulin, DHEAS and 17α-hydroxyprogesterone were measured by enzyme-linked immunoabsorbent assay (ELISA) using kits supplied by IBL International, Germany on a multiplate reader (Multiskan GO, Thermo Scientific). Free androgen index (FAI) was calculated using the formula \[ \text{TT (nmol/L) x 100/SHBG (nmol/L)} \]. Calculated free testosterone (cFT) was obtained from an online calculator at issam.ch/freetesto.htm based on the Vermeulan formula.

**Treatment**

All subjects received standard written dietary advice aimed at reducing the daily intake by 500 kcal. This balanced weight reducing diet generally involved 50% carbohydrates and was low in fats (only 10%). The subjects were encouraged to increase daily exercise by more than 15 minutes, in the form of walking, running or other. However, the adherence to the exercise regimen was not formally assessed. Nevertheless, the subjects were active encouraged to exercise as well as comply with the dietary protocol at every contact.

The Metformin Group received generic metformin 850 mg 12 hourly for 6 months. and the Control Group were prescribed placebo 12 hourly for 6 months. All tablets were provided free to the study subjects. In order to decrease the risk of gastrointestinal side effects, the dose was built up during the first 2 weeks up to the maintenance dose and the subjects were instructed to take the medication together with their meals.

All subjects on metformin were followed without any experimental intervention as metformin is conventional treatment for obesity and PCOS and includes basic instructions on diet and physical
activity. No pharmaceutical company participated in or influenced study design, collection, analysis or interpretation of data, in the writing of the report or the decision to submit the paper for publication. 

Follow up visits were conducted every 2 months for the duration of the study. Side effects of metformin treatment were recorded, but apart from mild gastrointestinal effects, no severe effects were reported during the study.

At the end of 6 months, all subjects in both groups were checked for the same clinical, hormonal and metabolic parameters conducted at baseline. Side effects of treatment and withdrawals were assessed and recorded. All assessments were made by the same researcher and clinical nurse.

**Measures**
The primary outcome measures were fasting insulin, HOMA-IR and insulin area under the curve (AUC). The secondary outcome measures were:

i. fasting blood glucose, HbA1c and lipid profile (triglycerides, cholesterol, HDL-C and LDL-C)
ii. total and free testosterone, SHBG and FAI
iii. waist circumference and BMI

**Statistical analysis**
All statistical analyses was performed with SPSS 22.0 (Statistical Packages for Social Sciences (SPSS for Mac, version 22.0 SPSS, Chicago, IL, USA).

A 2-sided p < .05 denoted statistical significance.

Power calculations were performed based on the degree of change in IR and insulin assay sensitivity in a similar past study (Meyer et al 2007). Twenty one subjects were required for each group, but allowing for a dropout rate of 20%, the number of subjects was set at 25 per group. Including 25 subjects per group also powered the study for the secondary outcome measures.

Normality testing of the data was carried out and most of the data was found to be not normally distributed.

The Mann-Whitney U test was used for significance testing of distribution variables between the Metformin and Control groups, and the Wilcoxon-signed rank test was used to compare the basal and end values within groups. Continuous variables presented as median and interquartile range (IQR). Categorical variables are expressed as percentage and 95% confidence interval (CI), and analysed by the chi-squared test. The Spearman correlation was used to explore correlations between the variables.

Analysis was carried out on an intention to treat basis whereby data from all the subjects was analysed according to their group. Subjects who withdrew within the first 4 months of the study were classified as non responders. No interim analysis was conducted. No adjustments were made for multiple hypothesis testing as all the outcomes were considered exploratory.

**Results**
Because of dropouts, the total number of subjects in the Metformin and Control Groups were 21 and 22 respectively. The reasons for dropping out were personal, severe gastrointestinal symptoms from metformin and exclusion due to pregnancy (See Figure 1).

Baseline clinical and endocrine parameters are shown in Table 1. The measurements taken after 6 months of treatment in the Metformin and Control Groups are shown in Tables 2 and 3 respectively.
At the beginning of the study, the metformin women were compared to the women in the control group. They had significantly elevated blood sugar and insulin levels and were more insulin resistant ($p = .001$).

Table 1. Baseline clinical, metabolic and endocrine parameters in subjects in the metformin and control groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Metformin ($n = 21$)</th>
<th>Controls ($n = 22$)</th>
<th>Mann-Whitney $U$</th>
<th>Significance $p$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.0 (28.5-35.5)</td>
<td>31.5 (27.8-38.0)</td>
<td>214.0</td>
<td>.679</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>29.2 (27.8-35.4)</td>
<td>24.4 (21.9-25.3)</td>
<td>404.0</td>
<td>&lt; .001</td>
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<tr>
<td>Waist circumference (cm)</td>
<td>90.0 (88.0-99.5)</td>
<td>74.5 (70.0-83.2)</td>
<td>429.5</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>
Table 1. Baseline clinical, metabolic and endocrine parameters in subjects in the metformin and control groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Weight (kg)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>HOMA-IR</th>
<th>Fasting Glucose (mmol/L)</th>
<th>HbA1c</th>
<th>Serum Insulin (U/mL)</th>
<th>Total Cholesterol (mmol/L)</th>
<th>LDL-C (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>SHBG (nmol/L)</th>
<th>DHEAS (µg/dL)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>HOMA-IR</th>
<th>Fasting Glucose (mmol/L)</th>
<th>HbA1c</th>
<th>Serum Insulin (U/mL)</th>
<th>Total Cholesterol (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>70.0 (65.5-87.0)</td>
<td>60.0 (50.8-63.0)</td>
<td>422.5</td>
<td>.&lt; .001</td>
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<tr>
<td>SBP (mm Hg)</td>
<td>119.0 (110.0-124.0)</td>
<td>110.0 (110.0-120.0)</td>
<td>298.5</td>
<td>.082</td>
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<tr>
<td>DBP (mm Hg)</td>
<td>76.0 (70.0-80.0)</td>
<td>70.0 (60.0-76.3)</td>
<td>333.0</td>
<td>.010</td>
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<tr>
<td>HOMA-IR</td>
<td>6.0 (3.4-13.9)</td>
<td>1.3 (0.9-3.3)</td>
<td>364.0</td>
<td>.001</td>
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<tr>
<td>Fasting Glucose (mmol/L)</td>
<td>5.6 (5.1-8.0)</td>
<td>4.7 (4.5-5.1)</td>
<td>372.5</td>
<td>.001</td>
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<tr>
<td>HbA1c</td>
<td>5.6 (5.4-6.8)</td>
<td>5.2 (5.0-5.4)</td>
<td>373.0</td>
<td>.001</td>
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<tr>
<td>Serum Insulin (U/mL)</td>
<td>19.8 (14.2-39.6)</td>
<td>17.9 (11.7-23.2)</td>
<td>-3.49</td>
<td>&lt; .001</td>
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<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>5.6 (4.5-5.8)</td>
<td>5.3 (5.0-6.0)</td>
<td>0.44</td>
<td>.664</td>
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</table>

Note: Values presented as median (interquartile range). HOMA-IR = homeostatic model assessment of insulin resistance; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; TG = triglycerides; DHEAS = dehydroepiandrosterone sulphate; SHBG = sex hormone binding globulin; HbA1c = Haemoglobin A1c; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure. Statistically Significant values are highlighted.

Table 2. Outcomes in the metformin group (n = 21).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (b)</th>
<th>After 6 months (a)</th>
<th>Difference (a-b)</th>
<th>Test statistic</th>
<th>p value*</th>
<th>Mdn, IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>29.2 (27.8-35.4)</td>
<td>30.0 (27.4-34.7)</td>
<td>-0.44</td>
<td>.664</td>
<td>-0.30, -1.45, 1.04</td>
<td></td>
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<tr>
<td>Waist circumference (cm)</td>
<td>90.0 (88.0-99.5)</td>
<td>90.7 (87.2-97.7)</td>
<td>-0.67</td>
<td>.503</td>
<td>-1.80, -6.50, 2.35</td>
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<tr>
<td>Weight (kg)**</td>
<td>70.0 (65.5-87.0)</td>
<td>71.0 (66.6-85.7)</td>
<td>-1.01</td>
<td>.313</td>
<td>-0.50, -2.95, 1.60</td>
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<tr>
<td>SBP (mm Hg)</td>
<td>119.0 (110.0-124.0)</td>
<td>113.0 (105.0-120.0)</td>
<td>-1.38</td>
<td>.167</td>
<td>-5.0, -9.50, 1.50</td>
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<tr>
<td>DBP (mm Hg)</td>
<td>76.0 (70.0-80.0)</td>
<td>75.0 (72.0-80.0)</td>
<td>0.24</td>
<td>.815</td>
<td>0.00, -5.00, 4.50</td>
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<tr>
<td>HOMA-IR</td>
<td>6.0 (3.4-13.9)</td>
<td>5.3 (2.7-6.2)</td>
<td>-2.62</td>
<td>.097</td>
<td>-2.58, -4.42, -0.38</td>
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<tr>
<td>Fasting Glucose (mmol/L)</td>
<td>5.6 (5.1-8.0)</td>
<td>6.4 (5.1-6.8)</td>
<td>-0.44</td>
<td>.664</td>
<td>-2.70, -0.91, 0.53</td>
<td></td>
</tr>
<tr>
<td>HbA1c</td>
<td>5.6 (5.4-6.8)</td>
<td>5.7 (5.2-6.4)</td>
<td>0.00</td>
<td>1.000</td>
<td>-0.13, -0.33, 0.47</td>
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<tr>
<td>Serum Insulin (U/mL)</td>
<td>19.8 (14.2-39.6)</td>
<td>17.9 (11.7-23.2)</td>
<td>-3.49</td>
<td>&lt; .001</td>
<td>-0.13, -0.33, 0.47</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>5.6 (4.5-5.8)</td>
<td>5.3 (5.0-6.0)</td>
<td>0.44</td>
<td>.664</td>
<td>0.28, -0.32, 0.64</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Outcomes in the metformin group (n = 21).

<table>
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<tr>
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<th>Baseline (b)</th>
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<th>Test statistic</th>
<th>p value*</th>
<th>Mdn, IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C (mmol/L)**</td>
<td>0.9 (0.7-1.1)</td>
<td>1.0 (0.9-1.3)</td>
<td>3.49</td>
<td>&lt;.001</td>
<td>0.13, 0.07,0.24</td>
</tr>
<tr>
<td>LDL-C (mmol/L)**</td>
<td>3.0 (2.4-4.1)</td>
<td>3.3 (2.7-3.9)</td>
<td>28.77</td>
<td>.689</td>
<td>0.07, 0.17,0.38</td>
</tr>
<tr>
<td>Serum TG (mmol/L)</td>
<td>2.4 (1.9-3.3)</td>
<td>2.2 (1.7-3.0)</td>
<td>-1.75</td>
<td>.078</td>
<td>-0.18, 0.06,0.29</td>
</tr>
<tr>
<td>DHEAS (µg/dL)**</td>
<td>109.5 (70.5-128.0)</td>
<td>98.5 (72.8-129.9)</td>
<td>-0.87</td>
<td>.383</td>
<td>-0.16, -0.32,0.23</td>
</tr>
<tr>
<td>SHBG (nmol/L)**</td>
<td>46.3 (35.1-57.5)</td>
<td>50.7 (37.9-87.2)</td>
<td>28.77</td>
<td>.017</td>
<td>4.72, 4.98,18.13</td>
</tr>
<tr>
<td>Total Testosterone (nmol/L)</td>
<td>1.8 (1.3-2.3)</td>
<td>1.9 (1.3-2.5)</td>
<td>-1.31</td>
<td>.189</td>
<td>-0.05, -0.19,0.31</td>
</tr>
<tr>
<td>Free androgen index (FAI)</td>
<td>3.9 (2.9-5.1)</td>
<td>3.4 (2.3-5.6)</td>
<td>-2.18</td>
<td>.027</td>
<td>-0.48, -0.42,0.02</td>
</tr>
<tr>
<td>Free testosterone (pmol/L)</td>
<td>26.0 (19.2-35.5)</td>
<td>23.2 (17.6-32.5)</td>
<td>-2.18</td>
<td>.027</td>
<td>-2.10, -5.30,0.35</td>
</tr>
</tbody>
</table>

Note: Values presented as median (interquartile range). HOMA-IR = homeostatic model assessment of insulin resistance; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; TG = triglycerides; DHEAS = dehydroepiandrosterone sulphate; SHBG = sex hormone binding globulin; HbA1c = Haemoglobin A1c; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure. Statistically Significant values are highlighted.

*The exact p-value (2-sided test) is computed based on the binomial distribution because there are 25 or fewer cases.
**The distribution of the differences data was symmetrical in shape - Wilcoxon signed-ranks test used.

---

Table 3. Outcomes in the Control Group (n = 22).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (b)</th>
<th>After 6 months (a)</th>
<th>Test statistic</th>
<th>p value*</th>
<th>Mdn, IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 (21.9-25.3)</td>
<td>24.8 (24.8-26.5)</td>
<td>1.066</td>
<td>.286</td>
<td>0.28, 0.29,1.18</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>74.5 (70.1-83.2)</td>
<td>76.5 (66.6-81.7)</td>
<td>0.436</td>
<td>.664</td>
<td>0.50, 1.86,4.62</td>
</tr>
<tr>
<td>Weight (kg)**</td>
<td>60.0 (50.8-63.0)</td>
<td>58.5 (52.8-65.3)</td>
<td>30.80</td>
<td>.230</td>
<td>1.00, 1.28,2.20</td>
</tr>
<tr>
<td>SBP (mm Hg)**</td>
<td>110.0 (110.0-120.0)</td>
<td>110.0 (101.5-117.8)</td>
<td>22.83</td>
<td>.125</td>
<td>2.00, -5.75,2.25</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>70.0 (60.0-76.3)</td>
<td>70.0 (68.0-72.8)</td>
<td>0.00</td>
<td>1.00</td>
<td>0.00, 5.00,0.00</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.3 (0.9-3.3)</td>
<td>1.4 (1.0-2.4)</td>
<td>-0.21</td>
<td>.832</td>
<td>-0.69, -0.84,0.56</td>
</tr>
<tr>
<td>Fasting Glucose (mmol/L)**</td>
<td>4.7 (4.5-5.1)</td>
<td>5.0 (4.9-5.5)</td>
<td>30.80</td>
<td>.001</td>
<td>0.36, 0.78,0.55</td>
</tr>
<tr>
<td>HbA1c</td>
<td>5.2 (5.0-5.4)</td>
<td>5.2 (4.9-5.5)</td>
<td>-0.34</td>
<td>.733</td>
<td>-0.12, -0.32,0.23</td>
</tr>
<tr>
<td>Serum Insulin (µU/mL)**</td>
<td>6.9 (4.3-15.0)</td>
<td>6.0 (4.2-10.2)</td>
<td>-1.07</td>
<td>.286</td>
<td>-0.50, -3.64,1.28</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>5.4 (4.4-5.8)</td>
<td>5.1 (4.9-5.7)</td>
<td>-0.21</td>
<td>.832</td>
<td>-0.12, -0.33,0.65</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.3 (1.1-1.5)</td>
<td>1.3 (1.2-1.5)</td>
<td>0.64</td>
<td>.523</td>
<td>0.09, 0.11,0.23</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.8 (2.3-3.4)</td>
<td>2.9 (2.5-3.6)</td>
<td>1.49</td>
<td>.134</td>
<td>0.11, -0.13,0.39</td>
</tr>
<tr>
<td>Serum TG (mmol/L)</td>
<td>1.4 (0.7-1.6)</td>
<td>1.6 (1.2-2.0)</td>
<td>1.49</td>
<td>.134</td>
<td>0.21, 0.05,0.51</td>
</tr>
<tr>
<td>DHEAS (µg/dL)**</td>
<td>87.4 (60.8-143.1)</td>
<td>101.8 (88.2-130.7)</td>
<td>0.21</td>
<td>.832</td>
<td>0.54, -16.78,38.31</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>70.7 (60.0-98.6)</td>
<td>85.2 (55.5-102.7)</td>
<td>30.80</td>
<td>.095</td>
<td>14.16, -10.72,26.16</td>
</tr>
<tr>
<td>Total Testosterone (nmol/L)</td>
<td>1.7 (1.0-2.3)</td>
<td>1.9 (1.2-2.4)</td>
<td>0.44</td>
<td>.664</td>
<td>0.08, 0.28,0.35</td>
</tr>
<tr>
<td>Free androgen index (FAI)</td>
<td>2.7 (1.5-3.4)</td>
<td>2.3 (1.6-3.6)</td>
<td>0.00</td>
<td>1.00</td>
<td>-0.13, -0.78,0.53</td>
</tr>
</tbody>
</table>

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They also had significantly higher triglyceride and lower HDL-C levels. However, total cholesterol and LDL-C levels were almost identical between the groups. The metformin subjects had overall higher FAI and free testosterone and lower SHBG levels, but total testosterone values were similar. After treatment six months later, the metformin subjects had become less insulin resistant, with HOMA-IR and serum insulin levels decreasing significantly, representing a 9.5% decline in insulin levels from the baseline ($p = .007$). No significant difference in the area under the ROC curve insulin was observed (Table 6). A slight increase in HDL-C levels was noted ($p < .001$). FAI and free testosterone levels had decreased ($p = .027$) and SHBG increased ($p = .017$). This drop in FAI was due to a significant increase in SHBG concentrations while the total testosterone remained the same. Fasting plasma, HbA1c, BMI and waist circumference remained the same throughout.

In the control women managed solely by lifestyle modification, all primary and secondary measures were similar throughout the study period.

Table 4 shows a comparison of the significant median differences between the study groups at the end of the trial. The metformin group was significantly less insulin resistant (HOMA-IR, $p = .016$) with lower serum insulin ($p = .021$). Fasting plasma glucose had risen among the control women, but only marginally ($p = .042$). The differences in serum SHBG, FAI and free testosterone between the groups were not significant, as were the other metabolic measures.

Table 4. Comparison of the significant median differences between both study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median difference</th>
<th>$U$</th>
<th>Standardised Test statistic</th>
<th>$p$ value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Metformin (n=21)</td>
<td>Controls (n=22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>-2.58 (-4.42,-0.38)</td>
<td>-0.69 (-0.84, 0.56)</td>
<td>132.00</td>
<td>-2.41</td>
</tr>
<tr>
<td>Fasting Glucose (mmol/L)</td>
<td>-2.70 (-0.91,0.53)</td>
<td>0.36 (0.78,0.55)</td>
<td>147.50</td>
<td>-2.03</td>
</tr>
<tr>
<td>Serum Insulin (U/mL)</td>
<td>-0.13 (-0.33,0.47)</td>
<td>-0.50 (-3.64,1.28)</td>
<td>136.00</td>
<td>-2.31</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>0.9 (0.7-1.1)</td>
<td>1.0 (0-9.1-3)</td>
<td>291.5</td>
<td>1.52</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>46.3 (35.1-37.5)</td>
<td>50.7 (37.9-87.2)</td>
<td>223.00</td>
<td>-1.94</td>
</tr>
<tr>
<td>Free androgen index (FAI)</td>
<td>3.9 (2.9-5.1)</td>
<td>3.4 (2-3.5-6)</td>
<td>180.00</td>
<td>-1.24</td>
</tr>
<tr>
<td>Free testosterone (pmol/L)</td>
<td>26.0 (19.2-35.5)</td>
<td>23.2 (17.6-32.5)</td>
<td>177.5</td>
<td>-1.30</td>
</tr>
</tbody>
</table>

Note: Values presented as median (interquartile range). HOMA-IR = homeostatic model assessment of insulin resistance; HDL-C = high density lipoprotein cholesterol; SHBG = sex hormone binding globulin.

*The asymptotic $p$-value (2-sided test) is computed based on the binomial distribution because there are 25 or fewer cases.

Three subjects on metformin dropped out of the study due to diarrhoea and headaches. Mild gastrointestinal disturbances and loss of appetite were common occurrences in the subjects taking metformin, but mostly disappeared after an initial short period.

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Discussion
We report for the first time a case-control analysis of 43 Malay women with PCOS where lifestyle modification and metformin therapy is associated with a decrease in insulin resistance compared to lifestyle alone. Other metabolic, reproductive and anthropometric outcomes did not differ significantly across the 2 groups.

Metaanalyses of randomised controlled trials (RCTs) have revealed a significant heterogeneity in the ability of metformin to reduce insulin and testosterone levels and affect body weight even after accounting for confounding factors (Tang T et al 2012). This suggests that the response to metformin therapy in PCOS is likely to be mediated by other factors that probably include genetics. Indirect evidence for this comes from the heritability of glycemic response to metformin seen in patients with T2DM (Zhou K et al 2014). However, no direct evidence of the ethnic or genetic influence of metformin responsiveness in PCOS women is as yet available and can only be provided by large scale genome-wide studies. Our study on Malay women, although having some limitations, may add to the ethnic variability of metformin response in PCOS women.

One of the keys to the management of PCOS is to address the insulin resistance underlying it. Many of the metabolic features of PCOS are a consequence of insulin resistance and hyperinsulinemia (Dunaif A et al 1989). Although PCOS women are mostly overweight and obese, adiposity alone cannot account for the magnitude of insulin resistance present (Dunaif A et al 1992). The International Diabetes Federation (IDF) has identified PCOS as an independent risk factor for Type 2 Diabetes Mellitus (T2DM) (Alberti KM et al 2007).

Evidence-based guidelines recommend lifestyle modification as the first line of management in PCOS (Teede HJ et al 2011). There are numerous challenges in compliance and sustainability in this mode of treatment. Weight loss may also induce adaptive responses that lead to poor sustainability of the regimen. This may render lifestyle changes inadequate especially in obese women, in which case, pharmacotherapy may provide an effective and important adjunct.

Metformin addition to lifestyle has been shown to be a highly effective medical treatment for insulin resistance. In our subjects, this combination significantly decreased the level of insulin resistance as measured by HOMA compared to lifestyle alone. This finding has been reported in other individual studies.

In the long term, the improvement in insulin sensitivity may be able to decrease the occurrence of T2DM in our Malay patients. The Indian and US Diabetes Prevention studies which likely included many women with PCOS reported that metformin reduced progression to T2DM (Knowler WC et al 2002, Ramachandran A et al 2006).

We have noted the decrease in insulin resistance effected by metformin in our study subjects. This finding does not seem to be universal and there exists significant heterogeneity in the effects of metformin in lowering insulin levels. Metaanalyses of RCTs provide varying outcomes of the effects of metformin on serum insulin, from a positive effect to no effect at all (Pau CT et al 2014, Naderpoor N et al 2015). An important reason for this discordance in findings is the methods used in determining insulin resistance. As in the majority of clinical studies, ours used HOMA as a surrogate marker for insulin resistance. This methodology has failed to demonstrate an improvement in insulin sensitivity in some cases (Naderpoor N et al 2015). The gold standard for methods of measuring insulin resistance are the clamp studies. Trials that used euglycemic clamps have consistently shown a reduction of insulin resistance in adults and adolescents with PCOS (Moghetti P et al 2000, Meyer C et al 2007). Although HOMA-IR is decidedly inferior to the clamp techniques for the investigation of IR in women with PCOS, it simplifies the study of a large number of subjects by using just a single pair of fasting glucose and insulin samples per person when previously it required a large number of samples from each subject.

Metformin impact on body weight and composition has conflicting results. Most metaanalyses of metformin alone report no evidence (Tang T et al 2010). Metformin and lifestyle was more effective in reducing BMI compared to lifestyle alone (Naderpoor N et al 2015). This analysis
compared nine RCTs with 493 subjects which revealed no effects although other metaanalyses have shown different results.

We report no differential impact of lifestyle and metformin on hyperandrogenism as measured in our subjects. We did not use the gold standard method for detecting the various androgen levels, that is liquid chromatography tandem mass spectrometry. This may be the cause of the differing outcome in our study compared to others where weight loss and medications significantly reduce androgen levels in PCOS (Nestler JE et al 1989). It is estimated that metformin reduces testosterone levels by up to 25% (McCartney CR et al 2016). This effect may be more pronounced in non-obese women (Tang T et al 2010).

The testosterone lowering mechanism of metformin is likely multiple. The most prominent action is believed to be from the reduction of hyperinsulinism (Nestler JE et al 1997). However, in vitro studies have also demonstrated lowering of testosterone levels in the absence of any improvement in insulin sensitivity (Pau CT et al 2014).

Our study was only designed to detect changes in serum androgen indices and not clinical hyperandrogenism. This is because metformin has not been shown to be an effective therapy for hyperandrogenic symptoms such as acne, hirsutism and menstrual irregularity (Cosma M et al 2008).

We only noted a slight increase in HDL-C levels in our study subjects on metformin and lifestyle ($p < .001$). However, when compared to the control subjects, this rise was not significant. Previously, the use of metformin in PCOS women has also shown no effects on serum cholesterol and triglycerides (Naderpoor N et al 2015).

LDL-C is an important clinical treatment target emphasised in worldwide guidelines as the primary cholesterol target. This is because it is the major lipoprotein associated with the formation of atherogenic plaque in coronary artery disease. Low-density lipoprotein cholesterol (LDL-C) was calculated using an online calculator (merckmanuals.com) based on Friedwald’s equation \[ \text{LDL-C} = \text{TC} - \text{HDL-C} - (\text{TG}/2.2) \] (Knopholz J et al 2014). This was originally developed in 1972 for research purposes but has been widely adopted in clinical practice since due to its economy and simplicity. However, it is claimed that this formula is prone to inaccuracies at low LDL-C or high triglycerides levels which results in marked underestimation of LDL-C. In this era where lower LDL-C levels can be achieved through the use statins and other therapies, these inaccuracies may not be acceptable as they can cause the deferment or withdrawal of lipid lowering agents and increasing patients’ risks.

The gold standard of LDL-C measurement are direct assays such as preparative ultracentrifugation but these are time consuming, complicated and expensive. Even direct assays have limitations in identifying small and dense LDL-C. Later studies and other studies have shown that the Friedwald formula is a reliable method to estimate LDL-C in patients with metabolic syndrome (Knopholz J et al 2014).

The long term safety and tolerability of metformin has been confirmed in many studies (Diabetes Prevention Program Research Group 2012). Lactic acidosis is exceptionally rare and was previously reported in women with renal insufficiency taking metformin (Bailey CJ 1996, DeFronzo RA 2000). The common side effects of metformin as seen in our study are generally well known and include appetite loss, nausea, vomiting, diarrhoea, abdominal bloating. These are usually transient and mild.

This study contributed to the knowledge of PCOS in Malay women. There is a dearth of knowledge on this topic and filling in the gaps, the management of the local women with PCOS can be further advanced. There was general clinical homogeneity as the age, baseline weight, BMI and geographical limit of the study groups was similar.

Our study was suffered from certain limitations. As in many other studies of metformin in PCOS, ours had a small sample size and brief duration (McCartney CR et al 2016). Furthermore, PCOS phenotypic subtypes were not identified and all subjects were grouped as one. To further assess its effects in PCOS women, a trial of metformin in lean PCOS women would be informative. This would obviate the confounding effects of obesity and assess the direct effects of metformin on hyperinsulinaemia and IR. This was also not a randomised placebo controlled trial and there may have been bias in the measurements and reporting of the results.

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This study evaluates the effectiveness of metformin in a cohort of mostly obese Malay women with PCOS. As a significant percentage of PCOS women are lean (about 10%) and have normal BMI and waist circumference, it would be important to note the effects of metformin on such a population. This would add further to the knowledge of the inherent metabolic characteristics of PCOS women irrespective of the effects of obesity.

While lifestyle modification is the most important and initial step for the management, the addition of metformin appears to provide additional advantages in managing some metabolic effects seen in PCOS.

In our study of Malay PCOS women with metabolic syndrome, a group of Malay women with PCOS and metabolic syndrome managed with metformin and lifestyle modification for 6 months had reduced levels of insulin resistance and hyperandrogenism compared to those managed with lifestyle only. Metformin treatment may provide an effective early management of metabolic syndrome and related disorders in women with PCOS.

References

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