Effect of Metformin-sustained Release Therapy on Low-density Lipoprotein Size and Adiponectin in the South Indian Women with Polycystic Ovary Syndrome

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Abstract

Objectives: The aim of the study is to compare surrogate markers of cardiovascular disease (CVD) risk, such as adiponectin (APN) levels and low-density lipoprotein (LDL) size, before and after sustained release metformin (Met-SR) therapy in women with polycystic ovarian syndrome (PCOS). Methods: Sixty women with PCOS and sixty age-matched controls in the age group 18–45 years were recruited after obtaining informed consent. Women with PCOS were initiated on Met-SR 1 g orally, which was increased to 1.5 g after 2 weeks and continued up to 24 weeks. Demographic data along with family history of type 2 diabetes mellitus, PCOS, and CVD were collected. Lipid profile plasma APN levels and LDL size were measured before and after therapy in the PCOS group. Data analysis was performed using the GraphPad Prism-5 software. Results: Women with PCOS had greater dyslipidemia, lower APN level and LDL size, and increased lipid accumulating product index as compared to controls. After 6 months of Met-SR therapy, women with PCOS demonstrated significant increase in plasma APN levels and LDL size and significant decrease in weight, waist-hip ratio (WHR), waist circumference (WC), and blood pressure (BP). A significant decrease was observed in body mass index (BMI) in the overweight and obese PCOS subgroups. Conclusion: Met-SR increases LDL size, APN concentration and decreases weight, WC, WHR, and BP in patients with PCOS. Met-SR may have salutary effects on LDL particle size through effects on APN levels in women with PCOS.

Keywords: Adiponectin, cardiovascular disease, low-density lipoprotein size, metformin, polycystic ovarian syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disorder, affecting 5%–10% of women in the reproductive age. It is characterized by chronic anovulation, infertility, signs and symptoms of hyperandrogenism, acanthosis nigricans, insulin resistance (IR), type 2 diabetes mellitus (T2DM), dyslipidemia, and atherosclerosis.⁶⁻⁷ Approximately 50%–70% of women with PCOS have central obesity and dyslipidemia, represented by increased triglycerides (TGLs) and low-density lipoprotein cholesterol (LDL-C) and decreased levels of high-density lipoprotein cholesterol (HDL-C) which may increase cardiovascular risk.⁸ The prevalence of dyslipidemia is not the same in all populations with PCOS; ethnicity appears to play a major role.

LDL is an atherogenic type of lipoprotein; its density varies from 1.006 to 1.063 g/ml. When the LDL particle size is <25.5 nm, it is known as small dense-LDL (sd-LDL). sd-LDL appears to confer a three-fold increase in the risk of cardiovascular disease (CVD).⁹⁻¹⁰ Adiponectin (APN) is an adipokine that is expressed exclusively in white adipose tissue and demonstrates insulin-sensitizing properties. It is hypothesized to play a protective role in the development and progression of obesity, T2DM, and CVD. APN has demonstrated anti-inflammatory, anti-thrombotic, anti-atherogenic, and cardioprotective properties both in vitro and in vivo models. Low levels of APN may be associated with obesity, IR, metabolic syndrome, T2DM, and CVD.¹¹

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Polymorphism of T45G in APN gene has been associated with PCOS.\[^6\]

Metformin (Met) is an oral biguanide insulin-sensitizing agent used to treat T2DM and has been reported to restore normal ovulatory cycles and improve fertility rate and dyslipidemia in PCOS. It has been reported to improve endothelial function, vascular inflammation, lipid profile, and other risk factors of CVD.\[^7\] We studied the association of lipid parameters in a South Indian population with PCOS and in the influence of Met-sustained release (Met-SR) on these parameters.

**Methods**

**Subjects**

Sixty women who were newly diagnosed for PCOS and sixty controls between the age group of 18–45 years seeking advice at the Department of Endocrine and Reproductive Medicine at Sri Ramachandra University, Chennai, India, were recruited based on the Rotterdam diagnostic criteria.\[^8\] Women were excluded from the study if they had T2DM, impaired fasting glucose, impaired glucose tolerance, thyroid dysfunction, were planning pregnancy or pregnant, and taking oral contraceptive pills or Met. The study protocol was approved by the Institutional Ethics Committee of Sri Ramachandra University. All women were given the same advice regarding benefits of lifestyle modifications through diet and exercise; no additional advice was given for weight reduction during the study period.

**Study design**

Demographic data were collected, and lipid profile was measured for both controls and patients with PCOS. Patients with PCOS were initiated on 1 g of Met-SR orally daily for 1 week which was then increased to 1.5 g per day from the second week and continued for 24 weeks. Blood samples were collected before and after Met therapy in all women with PCOS. An intravenous blood sample of 6 ml was collected in ethylenediamine tetra acetic acid-coated vacutainer. Samples were allowed to clot adequately and were centrifuged at 2000 rpm for 10 min to separate the plasma, and the plasma samples were stored at −20°C until analysis.

**Clinical and biochemical measurements**

Height and weight were measured by standard procedures and recorded in centimeters to the nearest 0.1 cm and kilograms to the nearest 1 kg, respectively. Blood pressure (BP) was measured twice in all women after a 5-min rest in a sitting position with a digital manometer. The average of the two measurements was utilized in the study. Body mass index (BMI) was calculated as body weight (kg) divided by height (m\(^2\)).\[^9\] Waist-hip ratio (WHR) was measured in centimeters at the narrowest part between the lower border of the rib cage and the iliac crest and the hip circumference at the highest extension of the buttocks. All measurements were recorded with the women in a standing position with the arms at rest at their sides and feet joined as described earlier.\[^10\] Lipid estimation, including TGL, total cholesterol (TC), and HDL-C, were done by Siemens ADVIA 1800 fully automated analyzer. LDL-C and very LDL (VLDL) cholesterol were estimated by Friedewald equation. The lipid accumulating product (LAP) index was estimated using the standard formula (waist circumference [WC, cm] − 58 × TG [mmol/l]).\[^11\] Plasma APN was measured by standard ELISA technique using the Ray Bio Human Adiponectin ELISA kit (Cat-ELH-ADIPONECTIN-001).\[^12\] LDL fraction was isolated from fresh plasma by single vertical discontinuous density gradient ultracentrifugation by modifying the protocol of Ani et al.,\[^13\] including use of NVT-65.2 rotor, adapter no: 362198, and 4.9 ml tubes. The density of the plasma was adjusted to 1.21 g/ml by the addition of solid potassium bromide (0.365 g/ml). Centrifuge tubes were loaded by layering 1.5 ml of density adjusted plasma under 3.4 ml of 0.154 mol/L sodium chloride and centrifuged in a Beckman L7-55 ultracentrifuge at 40,000 rpm at 10°C for 2.5 h with maximum acceleration. The yellow LDL band which settled in the upper middle portion of the tube was collected into a Hamilton syringe by puncturing the tube. The extracted LDL was confirmed by doing gel check by radial immunodiffusion technique.\[^14\] All the samples were kept at −20°C until measurement for size. The LDL particle size was measured by Malvern Zetasizer-Nano-S (Zetasizer Ver. 6.20 Serial Number: MAL1049897) at Center for Nanoscience and Technology, Anna University, Chennai. All the measurements were performed at 25°C in triplicate with automatic duration using distilled water as a solvent. The data were analyzed by the Zetasizer software (DTS nano-services, version 5.02, Malvern, England).\[^15\]

**Statistical analysis**

The difference between control and PCOS groups were analyzed by Student’s unpaired t-test. Women with PCOS were divided into three groups (obese, overweight, and normal) based on recommendations for South Asian populations. PCOS subgroups before and after treatment were analyzed by using the paired t-test. All results were expressed as means ± standard deviation; the P < 0.05 was considered statistically significant using GraphPad Prism-5 (San Diego California).

**Results**

Baseline characteristics of the control and PCOS subjects are summarized in Table 1. The effects of Met-SR on menstrual function, testosterone, free androgen index, and IR have been published elsewhere.\[^16\] Women with PCOS had a higher (but nonsignificant) prevalence of a positive family history of PCOS, CVD, and T2DM. PCOS women had significantly higher levels of weight, BMI, TC, TGL, LDL, and LAP Index (P < 0.05). Significant lower levels of LDL particle size (P < 0.05) were observed but not of APN. A nonsignificant lower HDL-C (P = 0.08) was also observed in the PCOS group. Among subgroups with PCOS, LDL particle size was lower in obese and overweight patients when compared with normal weight individuals. APN levels were correspondingly higher.

A statistically significant decrease in WHR and BMI was seen in patients with PCOS after 6 months of Met-SR.
therapy [Table 2]. LDL particle size was increased from 26.04 ± 1.03 nm to 28.10 ± 1.02, P < 0.05 (95% confidence interval [CI] -2.42–1.68). APN levels increased from 6.83 ± 1.74 to 8.52 ± 1.72 mg/L (95% CI 1.06–2.31). The significance persisted even after adjusting for BMI (r = −0.68; P < 0.05). A significant correlation between APN levels and LDL particle size was seen before (r = 0.4; P < 0.05) and after (r = 0.32; P < 0.05) Met-SR therapy. When adjusted for BMI – significance was lost prior therapy (r = 0.3; P = 0.318) but preserved after therapy with Met-SR (r = 0.22; P < 0.05).

Among the subgroups of patients with PCOS, there was significant reduction in weight and BMI in both the overweight and obese subgroups (P < 0.05) after 6 months of Met-SR. A nonsignificant reduction in weight (P = 0.84) and BMI (P = 0.38) was seen in the normal-weight subgroup. A significant increase in APN levels and LDL particle size (P < 0.05) and significant reduction of WHR, WC, and systolic and diastolic BP (P < 0.05) were observed in all the three subgroups [Table 3].

**DISCUSSION**

To our knowledge, this is the first study from South India to examine the effects of Met-SR on LDL size and other surrogate markers of CVD in PCOS. Similar to others,[17] we observed elevated TC: HDL-C in PCOS women when compared to the controls. A nonsignificant positive family history of CVD, T2DM, and PCOS was seen in our study, the trend similar to previous studies.[18,19]

Weight and BMI reductions were seen with MF-SR in all the three groups and significantly in the overweight and obese groups [Table 2]. WHR, a measure of body fat distribution, showed a significant reduction in all subgroups (P < 0.05) in line with others.[20] Systolic and diastolic BP also decreased significantly in all the three subgroups after 6 months of therapy. However, the difference in the mean was higher in obese subgroup of women. LDL size has been associated with progression of CVD.[21] There are few studies that examined LDL size in PCOS. Treatment of children and adolescents aged 4–18 years for 6–7 months with Met in combination with therapeutic lifestyle change increased LDL size by 5% (20.5–21.4 nm with P < 0.05).[22]

The effect of Met on APN levels in PCOS in various studies differs from no effect[23,24] to increase[25,26] and even decrease.[27,28] In our study, baseline APN levels were only nonsignificantly lower in patients with PCOS when compared with controls. However, among the subgroups, APN levels were highest in normal weight PCOS and lowest in the obese subgroup. Met-SR therapy increased APN levels significantly from baseline in each subgroup as well as in the PCOS group as a whole. This is consistent with an effect of Met on APN receptor-1 and 2 (AdipoR-1 and AdipoR-2).[29] It is possible that some of the salutary effects of Met may indeed be mediated through APN.[30,31] This is suggested by the observation in our study after adjustment for BMI APN levels correlated with LDL particle size after Met-SR therapy. Met may also increase lipoprotein lipase mass leading to increased catabolism of

**Table 1: Comparison of baseline characters of cardiovascular disease risk factors in control and polycystic ovarian syndrome women groups**

| Parameter                  | Control (n=60) | PCOS (n=60) | P     |
|----------------------------|---------------|-------------|-------|
| Age (years)                | 26.08±3.78    | 24.75±3.64  | 0.05  |
| Weight (kg)                | 55.45±7.02    | 61.95±11.98 | <0.05*|
| BMI (kg/m²)                | 23.77±3.28    | 25.76±4.93  | <0.05*|
| WC (cm)                    | 77.03±13.86   | 83.92±17.59 | <0.05*|
| WHR                       | 0.78±0.04     | 0.81±0.05   | <0.05*|
| Systolic BP (mm of Hg)     | 108.60±4.01   | 120.20±4.87 | <0.05*|
| Diastolic BP (mm of Hg)    | 77.08±2.17    | 78.50±3.89  | <0.05*|
| TC (mg/dL)                 | 142.20±13.69  | 162.10±18.30| <0.05*|
| TGL (mg/dL)                | 74.70±7.82    | 116.70±23.22| <0.05*|
| LDL-C (mg/dL)              | 75.55±12.52   | 89.72±21.90 | <0.05*|
| HDL-C (mg/dL)              | 51.55±4.11    | 49.45±8.46  | 0.08 (NS)|
| VLDL-C (mg/dL)             | 15.07±1.55    | 23.37±4.67  | <0.05*|
| TC: HDL-C                  | 2.76±0.29     | 3.44±1.00   | <0.05*|
| APN (mg/l)                 | 6.98±0.72     | 6.87±1.74   | 0.53 (NS)|
| LDL size (nm)              | 28.64±0.85    | 26.04±1.03  | <0.05*|
| LAP index                  | 16.16±11.93   | 36.48±29.22 | <0.05*|
| Family history of CVD, %   | 20 (n=12)     | 33.3 (n=20) | 0.10 (NS)|
| Family history of T2DM, %  | 25 (n=15)     | 35 (n=21)   | 0.23 (NS)|
| Family history of PCOS, %  | 15 (n=9)      | 26.6 (n=16) | 0.11 (NS)|

| Using unpaired Student’s t-test; values are expressed in mean±SD. |
| *Statistically significant (P<0.05). NS: Statistically nonsignificant (P>0.05). |
| WC: Waist circumference, WHR: Waist-hip ratio, BMI: Body mass index, BP: Blood pressure, LAP: Lipid accumulation product, CVD: Cardiovascular disease, TC: Total cholesterol, TGL: Triglycerides, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, APN: Adiponectin, T2DM: Type 2 diabetes mellitus, PCOS: Polycystic ovarian syndrome, SD: Standard deviation |

**Table 2: Comparison of subjects with polycystic ovarian syndrome before and after therapy**

| Parameter     | Baseline (n=60) | PCOS After therapy (n=60) | P     | 95% CI       |
|---------------|----------------|---------------------------|-------|---------------|
| Regular menstrual cycle | 31.7% (19/60) | 61.7% (37/60)             | <0.05*** | 1.25-1.51     |
| BMI (kg/m²)   | 25.76±4.93    | 24.67±4.12                | <0.05*** | −1.09-6.89    |
| WHR           | 0.81±0.05     | 0.79±0.05                 | <0.05*** | 0.01-0.02     |
| Adiponectin (mg/L) | 6.83±1.74    | 8.52±1.72                 | <0.05*** | 1.06-2.31     |
| LDL-size (nm) | 26.04±1.03    | 28.10±1.02                | <0.05*** | −2.42–1.68    |

| BMI: Body mass index, WHR: Waist-hip ratio, LDL: Low-density lipoprotein, PCOS: Polycystic ovarian syndrome, CI: Confidence interval, ***: Significant |
Table 3: Effect of Metformin (sustained release) on CV risk markers in three subgroups with PCOS

| Parameter                  | Normal weight (n=15) | Overweight (n=26) | Obese (n=19) |
|----------------------------|----------------------|-------------------|--------------|
|                            | Before therapy       | After therapy     | P            | Before therapy | After therapy | P            | Before therapy | After therapy | P            |
| Weight (kg)                | 49.33±5.40           | 49.13±3.87        | 0.84 (NS)    | 59.77±4.76    | 57.27±4.43    | <0.05*       | 74.89±9.97    | 69.32±9.56    | <0.05*       |
| BMI (kg/m²)                | 19.61±1.47           | 20.01±1.80        | 0.38 (NS)    | 25.27±1.53    | 24.22±1.97    | <0.05*       | 31.29±3.25    | 28.98±3.12    | <0.05*       |
| WC (cm)                    | 68.07±8.31           | 62.07±7.61        | <0.05*       | 80.88±9.03    | 72.85±9.26    | <0.05*       | 100.10±18.49  | 91.84±18.22  | <0.05*       |
| WHR                        | 0.76±0.03            | 0.74±0.03         | <0.05*       | 0.81±0.03     | 0.78±0.04     | <0.05*       | 0.85±0.04     | 0.83±0.04     | <0.05*       |
| Systolic BP (mm of Hg)     | 116.40±4.62          | 114.30±4.30       | <0.05*       | 119.5±3.96    | 116.7±3.56    | <0.05*       | 124.1±3.32    | 120.9±3.31    | <0.05*       |
| Diastolic BP (mm of Hg)    | 76.47±3.83           | 75.53±3.92        | <0.05*       | 78.35±3.83    | 75.73±3.28    | <0.05*       | 80.32±3.30    | 78.05±2.59    | <0.05*       |
| APN (mg/L)                 | 8.57±1.13            | 10.43±1.17        | <0.05*       | 6.80±1.69     | 8.58±1.15     | <0.05*       | 5.51±0.96     | 6.92±1.03     | <0.05*       |
| LDL-particle size (nm)     | 26.82±1.11           | 28.79±0.91        | <0.05*       | 26.04±0.78    | 28.14±0.83    | <0.05*       | 25.44±0.88    | 27.50±1.01    | <0.05*       |

Using paired Student’s t-test; values are expressed in mean±SD. *Statistically significant (P<0.05). NS: Statistically nonsignificant (P>0.05). WC: Waist circumference, WHR: Waist-hip ratio, BMI: Body mass index, BP: Blood pressure, SD: Standard deviation, APN: Adiponectin, LDL: Low-density lipoprotein

Conclusion

Met-SR therapy increases LDL particle size and APN levels and provides favorable reduction in weight, BMI, and BP in South Indian women with PCOS.

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Conflicts of interest

There are no conflicts of interest.

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