Original Article

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

D. Rama Nagendra Kumar, Krishna G. Seshadri[1], Monna Pandurangi[2]

Department of Endocrinology, Diabetes and Metabolism, Sri Ramachandra Medical College, Porur, Chennai, Tamil Nadu, [1]Department of Endocrinology and Medical Education, Sri Balaji Vidyapeeth, Puducherry, [2]Department of Reproductive Medicine, Sri Ramachandra Medical College, Porur, Chennai, Tamil Nadu, India

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.[1] 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.[2] 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).[3,4] 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.[5]

Access this article online

Quick Response Code:

Website: www.ijem.in

DOI: 10.4103/ijem.IJEM_154_17

Address for correspondence: Dr. Krishna G. Seshadri, Sri Balaji Vidyapeeth, Puducherry, India.
E-mail: krishnagseshadri@gmail.com

How to cite this article: Kumar DR, Seshadri KG, Pandurangi M. Effect of metformin-sustained release therapy on low-density lipoprotein size and adiponectin in the South Indian women with polycystic ovary syndrome. Indian J Endocr Metab 2017;21:679-83.

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].

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/m^2)	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

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

Parameter	PCOS		*P*	95% CI
	Baseline ($n=60$)	After therapy ($n=60$)		
Regular menstrual cycle	31.7% (19/60)	61.7% (37/60)	<0.05***	1.25-1.51
BMI (kg/m^2)	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/m^2)	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

TGL-rich lipoprotein with resultant LDL particle size increase. Met may also suppress VLDL synthesis in liver by improving effect of insulin action.[32]

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.

Acknowledgments

We would like to thank the Bristol-Myers Squibb for providing partial fund to do this project. And special thanks to Dr. Usha Rani, Dr. Sanjeeva Reddy, Reproductive Medicine Department, SRU, for supporting in sample collection, Dr. Ravi Shankar, Community Medicine Department, SRU, for helping in statistics, Dr. Sriram Mahadevan, Department of Endocrinology, Diabetes and Metabolism, SRU, and Dr. Maddaly Ravi, Department of Human Genetics, SRU, for supporting in manuscript preparation.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Singh B, Panda S, Nanda R, Pati S, Mangaraj M, Sahu PK, *et al.* Effect of metformin on hormonal and biochemical profile in PCOS before and after therapy. Indian J Clin Biochem 2010;25:367-70.
2. Peigné M, Dewailly D. Long term complications of polycystic ovary syndrome (PCOS). Ann Endocrinol (Paris) 2014;75:194-9.
3. Hirayama S, Miida T. Small dense LDL: An emerging risk factor for cardiovascular disease. Clin Chim Acta 2012;414:215-24.
4. Jungner I, Sniderman AD, Furberg C, Aastveit AH, Holme I, Walldius G. Does low-density lipoprotein size add to atherogenic particle number in predicting the risk of fatal myocardial infarction? Am J Cardiol 2006;97:943-6.
5. Ai M, Otokozawa S, Asztalos BF, White CC, Cupples LA, Nakajima K, *et al.* Adiponectin: An independent risk factor for coronary heart disease in men in the Framingham offspring Study. Atherosclerosis 2011;217:543-8.
6. Baldani DP, Skrgatic L, Ougouag R. Polycystic Ovary Syndrome: Important Underrecognised Cardiometabolic Risk Factor in Reproductive-Age Women. Int J Endocrinol 2015;2015:17. doi:10.1155/2015/786362.
7. Milewicz A. Metformin for polycystic ovary syndrome. Endokrynol Pol 2013;64:409-14.
8. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41-7.
9. Aziz N, Kallur SD, Nirmalan PK. Implications of the revised consensus body mass indices for Asian Indians on clinical obstetric practice. J Clin Diagn Res 2014;8:OC01-3.
10. Palomba S, Orio F Jr., Falbo A, Russo T, Tolino A, Zullo F. Clomiphene citrate versus metformin as first-line approach for the treatment of anovulation in infertile patients with polycystic ovary syndrome. J Clin Endocrinol Metab 2007;92:3498-503.
11. Hosseinpanah F, Barzin M, Erfani H, Serahati S, Ramezani Tehrani F, Azizi F. Lipid accumulation product and insulin resistance in Iranian PCOS prevalence study. Clin Endocrinol (Oxf) 2014;81:52-7.
12. Tsao TS, Lodish HF, Fruebis J. ACRP30, a new hormone controlling fat and glucose metabolism. Eur J Pharmacol 2002;440:213-21.
13. Ani M, Moshtaghie AA, Ahmadvand H. Comparative effects of copper, iron, vanadium and titanium on low density lipoprotein oxidation *in vitro*. Iran Biomed J 2007;11:113-8.
14. Dudman NP, Blades BL, Wilcken DE, Aitken JM. Radial immunodiffusion assay of apolipoprotein B in blood dried on filter paper – A potential screening method for familial type II hypercholesterolaemia. Clin Chim Acta 1985;149:117-27.
15. O'Neal D, Harrip P, Dragicevic G, Rae D, Best JD. A comparison of LDL size determination using gradient gel electrophoresis and light-scattering methods. J Lipid Res 1998;39:2086-90.
16. Kumar RN, Seshadri KG, Pandurangi M. Effect of sustained released metformin therapy on phenotypic and biochemical markers of insulin resistance in polycystic ovary syndrome in South Indian women. Int J Reprod Contracept Obstet Gynecol 2016;5:1026-30.
17. Lemieux I, Lamarche B, Couillard C, Pascot A, Cantin B, Bergeron J, *et al.* Total cholesterol/HDL cholesterol ratio vs. LDL cholesterol/HDL cholesterol ratio as indices of ischemic heart disease risk in men: The Quebec Cardiovascular Study. Arch Intern Med 2001;161:2685-92.
18. Lerchbaum E, Schwetz V, Giuliani A, Obermayer-Pietsch B. Influence of a positive family history of both type 2 diabetes and PCOS on metabolic and endocrine parameters in a large cohort of PCOS women. Eur J Endocrinol 2014;170:727-39.
19. Kulshreshtha B, Singh S, Arora A. Family background of Diabetes Mellitus, obesity and hypertension affects the phenotype and first symptom of patients with PCOS. Gynecol Endocrinol 2013;29:1040-4.
20. Aruna J, Mittal S, Kumar S, Misra R, Dadhwal V, Vimala N. Metformin therapy in women with polycystic ovary syndrome. Int J Gynecol Obstet 2004;87:237-41.
21. National Cholesterol Education Program (NCEP). Expert Panel on

Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143-421.
22. Dejager S, Pichard C, Giral P, Bruckert E, Federspield MC, Beucler I, *et al.* Smaller LDL particle size in women with polycystic ovary syndrome compared to controls. Clin Endocrinol (Oxf) 2001;54:455-62.
23. Ahmed AJ, Ali SH, Hassan NF. Impact of metformin treatment on serum adiponectin levels in obese women with polycystic ovary syndrome in relation to insulin resistance. IJSN 2012;3:847-52.
24. Trolle B, Lauszus FF, Frystyk J, Flyvbjerg A. Adiponectin levels in women with polycystic ovary syndrome: Impact of metformin treatment in a randomized controlled study. Fertil Steril 2010;94:2234-8.
25. Hamed HO. Role of adiponectin and its receptor in prediction of reproductive outcome of metformin treatment in patients with polycystic ovarian syndrome. J Obstet Gynaecol Res 2013;39:1596-603.
26. Agarwal N, Rice SP, Bolusani H, Luzio SD, Dunseath G, Ludgate M, *et al.* Metformin reduces arterial stiffness and improves endothelial function in young women with polycystic ovary syndrome: A randomized, placebo-controlled, crossover trial. J Clin Endocrinol Metab 2010;95:722-30.
27. Huypens P, Quartier E, Pipeleers D, Van de Casteele M. Metformin reduces adiponectin protein expression and release in 3T3-L1 adipocytes involving activation of AMP activated protein kinase. Eur J Pharmacol 2005;518:90-5.
28. Schmid PM, Resch M, Schach C, Birner C, Riegger GA, Luchner A, *et al.* Antidiabetic treatment restores adiponectin serum levels and APPL1 expression, but does not improve adiponectin-induced vasodilation and endothelial dysfunction in Zucker diabetic fatty rats. Cardiovasc Diabetol 2013;12:46.
29. Ismail TA, Soliman MM, Ismail SA. Adiponectin regulation in type 2 diabetic rats: Effects of insulin, metformin and dexamethasone. Am J Pharmacol Toxicol 2013;8:197-208.
30. Sivalingam VN, Myers J, Nicholas S, Balen AH, Crosbie EJ. Metformin in reproductive health, pregnancy and gynaecological cancer: Established and emerging indications. Hum Reprod Update 2014;20:853-68.
31. Perez A, Jacks R, Arora V, Spanheimer R. Effects of pioglitazone and metformin fixed-dose combination therapy on cardiovascular risk markers of inflammation and lipid profile compared with pioglitazone and metformin monotherapy in patients with type 2 diabetes. J Clin Hypertens (Greenwich) 2010;12:973-82.
32. Ohira M, Miyashita Y, Ebisuno M, Saiki A, Endo K, Koide N, *et al.* Effect of metformin on serum lipoprotein lipase mass levels and LDL particle size in type 2 diabetes mellitus patients. Diabetes Res Clin Pract 2007;78:34-41.