Effects of Metformin Treatment on Soluble Leptin Receptor Levels in Women with Polycystic Ovary Syndrome

Ru-biao LIU[1, 2], Yi LIU[1], Li-qun LV[1], Wei XIAO[1], Cheng GONG[1], Jian-xin YUE[3#]
[1]*Department of Obstetrics and Gynecology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China*
[2]*Department of Obstetrics and Gynecology, the Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430014, China*
[3]*Department of Otorhinolaryngology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China*

Summary: The effects of metformin treatment on soluble leptin receptor (sOB-R) levels in women with polycystic ovary syndrome (PCOS) were investigated. This prospective and open-label study was conducted by the Department of Obstetrics & Gynecology at Union Hospital, Tongji Medical College of Huazhong University of Science and Technology, China. Fifty-five women with PCOS and insulin resistance (IR) were treated with metformin for 6 months. According to body mass index (BMI), the patients were divided into two groups: lean PCOS group (BMI <23 kg/m^2, n=34) and overweight or obese PCOS group (BMI ≥23 kg/m^2, n=21). Before and after treatment, serum luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone (T), androstenedione (A), dehydroepiandrosterone sulfate (DHEAS), insulin and sOB-R levels were determined. Thirty-one BMI-matched ovulatory women served as controls. The results showed: (1) The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), androgen levels and hirsutism scores were higher, and sOB-R levels were lower in PCOS groups than in control group. A subgroup analysis of lean and overweight or obese PCOS patients revealed there was significant difference in sOB-R level between lean PCOS group and overweight or obese PCOS group. There were no significant differences in anthropometric parameters between lean PCOS patients and BMI-matched controls. However, sOB-R level was significantly lower in lean PCOS women than in controls. (2) There was no correlation between sOB-R level and BMI, waist and hip circumference, total testosterone, androstendione, DHEAS, LH or hirsutism scores in PCOS patients, but there was a significant negative correlation between sOB-R and HOMA-IR. (3) After treatment with metformin for 6 months, serum insulin levels decreased, and sOB-R levels increased significantly (P<0.01). It was suggested that considering low sOB-R levels supposedly compensate diminished leptin action, PCOS *per se* might cause leptin resistance. It is likely that reduction of hyperinsulinemia produced by metformin effectively improves the sOB-R levels in PCOS.
Key words: polycystic ovary syndrome; metformin; insulin resistance; leptin resistance; soluble leptin receptor

Polycystic ovary syndrome (PCOS) is a common endocrine disorder, affecting more than 5% of women of reproductive age. It is characterized by hyperandrogenism, chronic anovulation and infertility[1, 2]. At least 50% of women affected by PCOS are insulin resistance and overweight or obesity[3, 4]. Adipose tissue has been established as a major endocrine organ. Through the release of peptides, such as leptin[5], the adipose tissue is involved in the pathogenesis of several metabolic disorders. Leptin, the gene product of the ob gene, is primarily synthesized and secreted from adipose tissue and acts in the hypothalamus to regulate food intake and energy expenditure and also plays an influential role in reproduction[6]. Obese humans present with hyperleptinemia as an indicator of leptin resistance which, in turn, has been suggested to play a major role in the pathogenesis of obesity[7]. However, most of studies did not find a PCOS-specific influence on leptin, as no significant differences were found between body mass index (BMI)-matched PCOS women and healthy controls[8, 9]. The main determinants of leptin concentration in these studies were fat mass and the degree of obesity or insulin resistance.

Ru-biao LIU, E-mail: liurubiao@hotmail.com
#Corresponding author, E-mail: yuejianxin@live.cn

Both metabolic and endocrine parameters might correlate better with free leptin rather than with total leptin levels, as the former represents the biologically active fraction that is available for direct interaction with its receptor. The levels of free leptin can either be measured directly or assessed by determination of the soluble leptin receptor (sOB-R), which accounts for the majority of serum leptin-binding activity[10]. Several studies have shown that all PCOS women, regardless of their BMI, presented with lower sOB-R levels and higher free leptin indices than healthy controls[11, 12]. Lower sOB-R levels increase the free leptin index, a mechanism likely to compensate, at least in part, for leptin resistance in PCOS women.

In the past, therapeutic approaches to PCOS have focused on suppressing ovarian androgen production or ovulation induction. Recently, insulin sensitizers such as metformin[13, 14] have been used to reduce the level of hyperinsulinemia and negative impact on ovarian function and possibly to prevent long-term consequences of hyperinsulinemia. In this study, we evaluated the effect of metformin treatment on sOB-R levels in women with PCOS.

1 MATERIALS AND METHODS

1.1 Subjects

Fifty-five women with PCOS, ranging in age from 21 to 30 years, were enrolled in this study. The study was approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology, and informed consent was obtained from each patient. PCOS was diagnosed based on Rotterdam diagnosis standard, typical ovarian morphology assessed by transvaginal ultrasonography, hyperandrogenism (i.e. elevated total or free testosterone or androstenedione concentrations), and chronic anovulation[15]. Patients with theca cells hyperplasia, hyperprolactinemia, adrenal cortical hyperplasia, ovary androgenization tumor or acanthosis nigrans were excluded from the study. Hirsutism was evaluated by the modified Ferriman-Gallwey method, and the mean score was 4.2±2.5 (mean±SD). Menstrual bleeding occurred every 45–60 days. Insulin resistance was diagnosed by oral glucose tolerance test (OGTT). The criteria for hyperinsulinemia were as follows: insulin basal levels >15 mol/L or insulin level (1-h after 75 g oral glucose test) >109.8 mIU/L and insulin level (2-h after 75 g oral glucose test) >89.0 mIU/L. According to BMI, the patients with PCOS were divided into two subgroups: lean PCOS (BMI <23 kg/m^2, n=34) and obese PCOS (BMI ≥23 kg/m^2, n=21). All recruited women were required to be otherwise healthy.

Healthy controls (n=31) were matched according to BMI (<23 kg/m^2) with a subgroup of lean PCOS women (n=34). Controls were recruited from a screening program for employees instituted at Union Hospital, Tongji Medical College of Huazhong University of Science and Technology. In control women, all Rotterdam criteria of PCOS were excluded. PCOS as well as control subjects had not taken any medication known to affect carbohydrate metabolism or endocrine parameters for at least 3 months before entering the study. Women taking contraceptive pills were also excluded from the study.

1.2 Study Protocol

The patients entered the study on days 3–5 of induced menstrual bleeding. All received 1500 mg/day metformin (Metforal; China) as tablets uninterruptedly for 6 months. Hirsutism was evaluated by a modified form of the Ferriman–Gallwey method that has been used in our clinic for many years[16]. A score was assigned to patients before and after 6 months of metformin therapy. A score >5 is indicative of hirsutism. Patients were followed up for 6 months with plasma progesterone determinations on day 22 of the menstrual cycle.

1.3 Data Collection

In PCOS subjects and control women, clinical parameters were assessed by physical examination, including the degree of hirsutism by evaluating the FG score and anthropometric measurements including body weight in kg (BW) and waist (W) and hip (H) circumference in cm. BMI was calculated as weight/(height)^2 (kg/m^2). Parameters of insulin resistance and β-cell function were evaluated using a 3-h OGTT. After an overnight fast of 12 h, patients ingested 75 g glucose and had their glucose and insulin levels determined at baseline and at 30, 60, 120 and 180 min. Insulin resistance and β-cell function were defined by the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)[17]. Except for women with amenorrhea, all laboratory determinations were performed in the early follicular phase of the cycle.

1.4 Biochemical Assays

Serum leptin and sOB-R were measured using ELISA kits [Diagnostic Systems Laboratories (DSL), USA]. The DSL ACTIVE Human Leptin ELISA is an enzymatically amplified "two-step" sandwich-type immunoassay. The DSL ACTIVE Leptin Soluble Receptor ELISA is a "one-step" sandwich-type immunoassay. The theoretical sensitivity or minimum detection limit as calculated by interpolation of the mean plus two standard deviations of 12 replicants of the zero standard was 0.05 ng/mL for leptin and 0.14 ng/mL for sOB-R respectively. Serum glucose concentrations were measured by a commercial glucose dehydrogenase technique. Serum insulin concentrations were measured by a double antibody radioimmunoassay (Phadeseph Insulin RIA; Pharmacia, Sweden). Serum concentrations of LH and FSH were measured by solid phase, two-site

time-resolved immunofluorometric assays (DELFIA; Pharmacia Wallac, Finland). The intra- and inter-assay coefficients of variation were 5.2% and 9.4% for LH, and 4.7% and 4.3% for FSH, respectively. Total testosterone was determined using a commercial radioimmunoassay kit (Farmos Diagnostica, Finland). The intra- and inter-assay coefficients of variation were 13.0% and 15.6% respectively. Androstenedione and dehydroepiandrosterone sulfate (DHEAS) were determined using radioimmunoassay kits (Diagnostic Products Corporation, USA). The intra- and inter-assay coefficients of variation were 8.3% and 9.2% for androstenedione, and 4.7% and 4.6% for DHEAS, respectively.

1.5 Statistical Analysis

Data are presented as median plus range for parametric data. For better comparison, means±SD is also shown. Correlations between variables were examined by Pearson correlation coefficient (r_s) because analyzed data were not normally distributed. Differences between the groups were evaluated with the Mann–Whitney *U* test. *P* values <0.05 were considered significant. The box-plot graph was computed using GraphPad Prism 5 (GraphPad Software, Inc., USA).

2 RESULTS

2.1 Clinical, Hormonal and Metabolic Characteristics of Women in Controls, Lean and Overweight or Obese PCOS Patients

The HOMA-IR, androgen levels and hirsutism scores were higher, and sOB-R levels were lower in PCOS patients than in controls. In a subgroup analysis of lean and obese PCOS patients, significant differences were found in sOB-R levels (fig. 1). There was no significant difference in anthropometric parameters between lean PCOS patients (*n*=34) and BMI-matched controls (*n*=31). However, sOB-R levels were significantly lower in lean PCOS women than in controls (table 1).

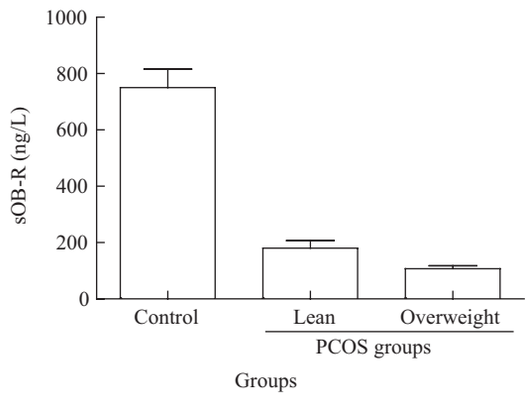

Fig. 1 sOB-R levels in three groups
The sOB-R levels were significantly lower in lean or overweight PCOS women than in controls.

In PCOS patients, no correlation was found between sOB-R and BMI, waist and hip circumference, total testosterone, androstendione, DHEA, LH or hirsutism scores. However, there was significantly negative correlation between sOB-R and HOMA-IR (table 2).

2.2 Changes of Serum Glucose and Insulin Levels in PCOS Patients after Metformin Treatment for 6 Months

After oral administration of metformin for 6 months, the insulin level in PCOS groups in every phase was decreased significantly (*P*<0.01, fig. 2), and there was no significant difference in serum glucose level before and after treatment (*P*>0.05, fig. 3).

Table 1 Clinical, hormonal and metabolic characteristics of controls, lean and overweight or obese PCOS patients (means±SD)

Parameters	Control group	Lean PCOS group	Overweight PCOS group
n	31	34	21
Age (years)	25.2±5.2	25.5±4.4	26.4±3.2
BMI (kg/m^2)	21.4±2.3	21.3±2.4	25.03±3.1$^{*\#}$
Waist circumference (cm)	71.8±8.1	72.1±8.6	80.4±6.9$^{*\#}$
Hip circumference (cm)	89.8±5.2	90.5±6.2	100.8±6.4$^{*\#}$
Waist:hip ratio	0.7±0.1	0.7±0.05	1.0±0.05$^{*\#}$
Hirsutism scores	2.4±2.1	4.9±3.2*	5.2±3.7*
FIN (mIU/L)	6.9±3.1	17.9±6.9*	19.8±7.6*
HOMA-IR	0.6±0.4	1.7±0.8*	1.8±0.6*
FGP (mmol/L)	4.5±0.8	4.5±0.4	4.6±0.6
sOB-R (ng/L)	750.2±112.8	180.4±46.5*	106.9±18.5$^{*\#}$
LH (IU/L)	5.8±2.5	13.6±2.2*	11.2±2.6*
LH:FSH ratio	0.8±0.3	2.8±0.4*	2.3±0.4*
Testosterone (mmol/L)	0.8±0.5	1.5±1.0*	1.6±0.9*
Androstendione (μmol/L)	6.2±2.3	10.6±5.5*	10.7±2.3*
DHEAS (μmol/L)	37.5±23.1	75.3±43.4*	77.5±30.1*

FIN: fasting insulin; FGP: fasting serum glucose. $^{*}P$<0.01 *vs.* controls, $^{\#}P$<0.01 *vs.* lean PCOS patients

Table 2 Correlation of sOB-R levels with anthropometric, hormonal and metabolic parameters in PCOS women

Variables	r_s	P
BMI	–0.19	NS
Waist circumference	–0.30	NS
Hip circumference	–0.30	NS
Hirsutism scores	–0.20	NS
HOMA-IR	–0.82	0.029
LH	0.09	NS
Testosterone	–0.15	NS
Androstendione	–0.10	NS
DHEAS	–0.23	NS

NS: not significant

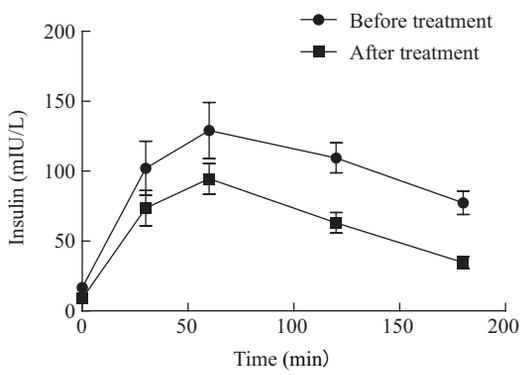

Fig. 2 Changes of insulin levels in PCOS after metformin treatment
After oral administration of metformin for 6 months, the insulin level in PCOS groups in every phase was decreased significantly.

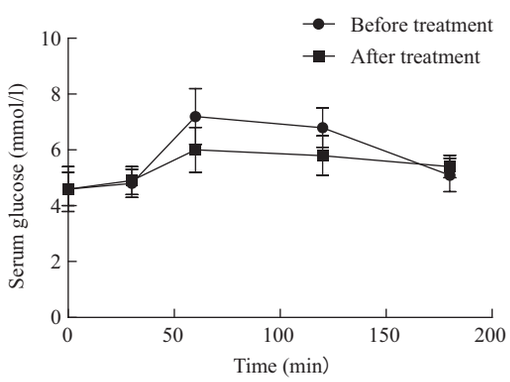

Fig. 3 Changes of serum glucose levels in PCOS after metformin treatment
After oral administration of metformin for 6 months, the serum glucose level in PCOS groups in every phase was decreased, but there was no significant difference.

2.3 Changes of Serum Sex Hormone Levels in PCOS after Metformin Treatment for 6 Months

As shown in table 3, after metformin treatment for 6 months, the levels of LH, LH/FSH ratio, testosterone, androstendione and dehydroepiandrosterone (DHEA) in PCOS patients decreased significantly as compared with controls (P<0.01).

Table 3 Changes of serum sex hormone levels in PCOS after metformin treatment

Parameters	Before treatment	After treatment
LH (IU/L)	12.4±2.2	4.2±1.7*
LH:FSH ratio	2.8±1.5	0.8±0.3*
Testosterone (mmol/L)	2.4±1.7	0.8±0.6*
Androstendione (μmol/L)	10.6±4.1	5.6±2.2*
DHEA (μmol/L)	76.5±46.4	56.8±22.1*

*P<0.01 *vs.* before treatment

2.4 Changes of sOB-R Levels in PCOS after Metformin Treatment for 6 Months

After metformin treatment for 6 months, the sOB-R levels in PCOS patients increased significantly as compared with those before metformin treatment (126.71±29.6 *vs.* 609.89±156.7, P<0.01).

3 DISCUSSION

Leptin regulates food intake and energy expenditure[18] and also plays an influential role in reproduction, as indicated by leptin deficiency. Ob/ob mice from the C57BL/6J strain, which have a spontaneous mutation in the ob gene and synthesize a truncated, inactive leptin molecule, develop profound obesity and become infertile[19]. Fertility can be restored by treatment with human recombinant leptin[20]. Women undergoing IVF therapy who tend to obesity show a reduced ovarian response and increased serum leptin concentrations, but unchanged leptin concentrations in follicular fluid[21]. For women with PCOS, whether or not high leptin levels participate in this disturbed gynecological event is still a matter of debate[22, 23].

Previous studies in normal subjects indicate that insulin may have an effect on free leptin. Free leptin levels increased in response to insulin infusion during hyperinsulinemic euglycemic clamp studies[24]. In a group of subjects with impaired glucose tolerance, free leptin concentrations were found to correlate with fasting insulin concentrations, an association that remained significant after adjustment for BMI[25]. Other studies have shown adiposity and fat mass to be the main predictors of leptin secretions[26]. However, conflicting results were reported on the relation of sOB-R to insulin and adiposity in normal or diabetic subjects. Ogawa *et al*[27] observed that sOB-R has a negative correlation with fasting insulin and HOMA-IR in a group of 419 healthy and lean subjects. On the other hand, Chan *et al*[24] found that adiposity, rather than insulin, was a major determinant of sOB-R in a group of young subjects with normal BMI. In the present study, a significantly negative correlation was found between sOB-R and HOMA-IR. Lean PCOS patients and BMI-matched controls did not differ in anthropometric parameters. However, sOB-R levels were significantly lower in lean PCOS women than

in these controls. The reduction of sOB-R could not be explained by differences in body fat content or other metabolic parameters in PCOS. The discrepant findings from the present study to previous reports could be attributed to differences in patient populations. Previous reports used subjects that had low or normal weights, and it is possible that sOB-R is regulated differently in obese individuals.

The interplay of sOB-R and the reproductive system is quite unclear. While some studies have found an association of sOB-R with estradiol and free testosterone[24], sex hormones did not affect sOB-R levels in a Japanese population[28]. In analogy to leptin, sOB-R did not correlate with total testosterone, androstendione, DHEAS, LH or hirsutism scores in Chinese PCOS cohort.

In the last 10 years, insulin-lowering drugs have become widely used in the treatment of PCOS, particularly for the induction of ovulation. Many studies have demonstrated the efficacy of metformin, a biguanide normally used to treat non-insulin-dependent diabetes, in inducing ovulation in PCOS patients with insulin resistance[29, 30]. Metformin is the most thoroughly investigated insulin-lowering agent used to treat PCOS; it enhances insulin sensitivity in the liver, where it inhibits hepatic glucose production, and in muscle, where it improves glucose uptake and use[31]. To gain further insight into the effects of insulin resistance and chronic hyperinsulinemia on the leptin system, we ameliorated the degree of insulin resistance by treating women with PCOS with the insulin-sensitizing agent Metformin and studied the changes in sOB-R levels. After treatment with metformin for 6 months, serum insulin levels decreased, while sOB-R levels increased significantly in PCOS patients. This further substantiates that insulin resistance and chronic hyperinsulinemia play roles in the leptin system in women with PCOS.

In summary, our results indicate that in PCOS patients there was a significantly negative correlation between sOB-R and insulin resistance, rather than BMI, anthropometric parameters and androgen levels. Considering low sOB-R levels supposedly compensate diminished leptin action, PCOS *per se* might cause leptin resistance. It is likely that reduction of hyperinsulinemia that is induced by metformin effectively improves the sOB-R levels in patients with PCOS.

Conflict of Interest Statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1 Rothenberg SS, Beverley R, Barnard E, *et al*. Polycystic ovary syndrome in adolescents. Best Pract Res Clin Obstet Gynaecol, 2018,48:103-114

2 Pasquali R, Gambineri A. New perspectives on the definition and management of polycystic ovary syndrome. J Endocrinol Invest, 2018,41(10):1123-1135

3 Behboudi-Gandevani S, Ramezani Tehrani F, Rostami Dovom M, *et al*. Insulin resistance in obesity and polycystic ovary syndrome: systematic review and meta-analysis of observational studies. Gynecol Endocrinol, 2016,32(5):343-353

4 Jeanes YM, Reeves S. Metabolic consequences of obesity and insulin resistance in polycystic ovary syndrome: diagnostic and methodological challenges. Nutr Res Rev, 2017,30(1): 97-105

5 Davis C, Mudd J, Hawkins M. Neuroprotective effects of leptin in the context of obesity and metabolic disorders. Neurobiol Dis, 2014,72 Pt A:61-71

6 Zhang Y, Chua S Jr. Leptin Function and Regulation. Compr Physiol, 2017,8(1):351-369

7 Cui H, Lopez M, Rahmouni K. The cellular and molecular bases of leptin and ghrelin resistance in obesity. Nat Rev Endocrinol, 2017,13(6):338-351

8 Dallel M, Sghaier I, Finan RR, *et al*. Circulating leptin concentration, LEP gene variants and haplotypes, and polycystic ovary syndrome in Bahraini and Tunisian Arab women. Gene, 2019,694:19-25

9 Daghestani MH, Daghestani M, Daghistani M, *et al*. A study of ghrelin and leptin levels and their relationship to metabolic profiles in obese and lean Saudi women with polycystic ovary syndrome (PCOS). Lipids Health Dis, 2018,17(1):195

10 Schaab M, Kratzsch J. The soluble leptin receptor. Best Pract Res Clin Endocrinol Metab, 2015,29(5):661-670

11 Sepilian VP, Crochet JR, Nagamani M. Serum soluble leptin receptor levels and free leptin index in women with polycystic ovary syndrome: relationship to insulin resistance and androgens. Fertil Steril, 2006,85(5):1441-1447

12 Tu X, Yu C, Gao M, *et al*. LEPR gene polymorphism and plasma soluble leptin receptor levels are associated with polycystic ovary syndrome in Han Chinese women. Per Med, 2017,14(4): 299-307

13 Stagi S, Ricci F, Bianconi M, *et al*. Retrospective Evaluation of Metformin and/or Metformin Plus a New Polysaccharide Complex in Treating Severe Hyperinsulinism and Insulin Resistance in Obese Children and Adolescents with Metabolic Syndrome. Nutrients, 2017,9(5): pii E524

14 Moro F, Morciano A, Tropea A, *et al*. Effects of drospirenone-ethinylestradiol and/or metformin on CD4(+)CD28(null) T lymphocytes frequency in women with hyperinsulinemia having polycystic ovary syndrome: a randomized clinical trial. Reprod Sci, 2013,20(12): 1508-1517

15 Broekmans FJ, Knauff EA, Valkenburg O, *et al*. PCOS according to the Rotterdam consensus criteria: Change in prevalence among WHO-II anovulation and association with metabolic factors. BJOG, 2006,113(10):1210-1217

16 Cook H, Brennan K, Azziz R. Reanalyzing the modified Ferriman-Gallwey score: is there a simpler method for assessing the extent of hirsutism? Fertil Steril, 2011,96(5):1266-1270.e1

17 Majid H, Masood Q, Khan AH. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR): A

Better Marker for Evaluating Insulin Resistance Than Fasting Insulin in Women with Polycystic Ovarian Syndrome. J Coll Physicians Surg Pak, 2017,27(3):123-126
18 Liu W, Zeng Q, Chen Y, *et al*. Role of Leptin/Osteopontin Axis in the Function of Eosinophils in Allergic Rhinitis with Obesity. Mediators Inflamm, 2018,2018:9138 904
19 Friedman JM. Leptin, leptin receptors, and the control of body weight. Nutr Rev, 1998,56(2 Pt 2):s38-46; discussion s54-75
20 Egan OK, Inglis MA, Anderson GM. Leptin Signaling in AgRP Neurons Modulates Puberty Onset and Adult Fertility in Mice. J Neurosci, 2017,37(14):3875-3886
21 Almog B, Azem F, Kapustiansky R, *et al*. Intrafollicular and serum levels of leptin during *in vitro* fertilization cycles: comparison between the effects of recombinant follicle-stimulating hormones and human menopausal gonadotrophin. Gynecol Endocrinol, 2011,27(9):666-668
22 Goumenou AG, Matalliotakis IM, Koumantakis GE, *et al*. The role of leptin in fertility. Eur J Obstet Gynecol Reprod Biol, 2003,106(2):118-124
23 Vazquez MJ, Romero-Ruiz A, Tena-Sempere M. Roles of leptin in reproduction, pregnancy and polycystic ovary syndrome: consensus knowledge and recent developments. Metabolism, 2015,64(1):79-91
24 Chan JL, Bluher S, Yiannakouris N, *et al*. Regulation of circulating soluble leptin receptor levels by gender, adiposity, sex steroids, and leptin: observational and interventional studies in humans. Diabetes, 2002,51(7):2105-2112
25 Leonhardt W, Horn R, Brabant G, *et al*. Relation of free and specifically bound leptin to insulin secretion in patients with impaired glucose tolerance (IGT). Exp Clin Endocrinol Diabetes, 1999,107(1):46-52
26 Raffaelli M, Iaconelli A, Nanni G, *et al*. Effects of biliopancreatic diversion on diurnal leptin, insulin and free fatty acid levels. Br J Surg, 2015,102(6):682-690
27 Ogawa T, Hirose H, Yamamoto Y, *et al*. Relationships between serum soluble leptin receptor level and serum leptin and adiponectin levels, insulin resistance index, lipid profile, and leptin receptor gene polymorphisms in the Japanese population. Metabolism, 2004,53(7):879-885
28 Shimizu H, Shimomura K, Negishi M, *et al*. Circulating concentrations of soluble leptin receptor: influence of menstrual cycle and diet therapy. Nutrition, 2002,18(4):309-312
29 Morley LC, Tang T, Yasmin E, *et al*. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. Cochrane Database Syst Rev, 2017,11:Cd003053
30 Sivalingam VN, Myers J, Nicholas S, *et al*. Metformin in reproductive health, pregnancy and gynaecological cancer: established and emerging indications. Hum Reprod Update, 2014,20(6):853-868
31 Logie L, Lees Z, Allwood JW, *et al*. Regulation of hepatic glucose production and AMPK by AICAR but not by metformin depends on drug uptake through the equilibrative nucleoside transporter 1 (ENT1). Diabetes Obes Metab, 2018,20(12):2748-2758

(Received Sept. 6, 2018; revised June 12, 2019)