PIOGLITAZONE: A NEW PARADIGM IN MANAGEMENT OF PCOS; AN OPEN LABELLED STUDY

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Abstract

Background: Metformin and spironolactone have been used extensively for treatment of polycystic ovary syndrome (PCOS) but pioglitazone still lacks sufficient evidence-based support to be recommended in standard guidelines. Aims We compared effects of pioglitazone in combination with either metformin or spironolactone in 50 PCOS females on clinical, metabolic and hormonal parameters.

Design: Prospective non-randomised open label study among women with PCOS at a tertiary care endocrine clinic.

Methods: Women (n=50) diagnosed with PCOS were categorised into two groups with group I treated with 15 mg pioglitazone and 50 mg spironolactone, group II treated with 15 mg pioglitazone and 1000 mg metformin. Various clinical, metabolic, hormonal parameters, insulin sensitivity markers like HOMA-IR, QUICKI and serum resistin, adiponectin were assessed at baseline and after six months.

Results: Both groups showed significant improvement in number of menstrual cycles per year and Ferrim an Gallwey (FG) score after six months. Serum total cholesterol, 2-hour plasma glucose post OGTT and HOMA-IR showed nonsignificant downward trend in both groups. Serum total testosterone also declined significantly in either group with nonsignificant superior trend observed in group II in comparison of group I (48% vs 31%). Serum adiponectin levels increased significantly in both groups despite increase in BMI and waist circumference. No major adverse events noted in either group. Conclusions: Thus addition of pioglitazone to spironolactone or metformin in PCOS women improves overall metabolic milieu and underlying insulin resistance despite increase in BMI. Randomized trials with longer duration of treatment are warranted to better evaluate effects of this combination therapy.

Introduction:

PCOS is one of the most common endocrine disorder in women, affecting about 5–15% women of reproductive age and characterised by triad of hyperandrogenism, chronic anovulation and polycystic ovaries. Insulin resistance is an important intrinsic aspect of PCOS seen in 50–70% of affected women and has been observed not only in obese but also in lean women with PCOS. Thus, insulin sensitizers (thiazolidinediones and metformin) can effectively decrease androgen synthesis in ovaries by ameliorating peripheral insulin resistance indirectly.

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Due to rise in the metabolic side effects secondary to use of oral contraceptives, administration of insulin sensitizers (metformin) either alone or in combination with antiandrogens (spironolactone) is emerging as a research area of interest for management of women with PCOS. Thiazolidinediones (TZD) are the peroxisome proliferator activated receptor (PPAR γ) synthetic ligands that regulate cellular functions to decrease insulin resistance. Although the treatment of PCOS with TZDs (pioglitazone) has been investigated in many small-scale clinical trials, it still lacks sufficient evidence-based supports due to non-availability of large-scale clinical trials to verify the efficacy and safety of TZD drugs for PCOS.

There are few head to head trials comparing the efficacy of pioglitazone in combination with metformin or spironolactone in decreasing hirsutism, body weight, insulin resistance and menstrual irregularity. We analysed effects of 6 months combination therapy of pioglitazone with either metformin or with spironolactone on metabolic, clinical and hormonal parameters of PCOS women. To the best of our knowledge none of the previous clinical trials have compared effects of pioglitazone when added to metformin or spironolactone in PCOS women.

**Methodology:**

**Subject selection:**
This was a prospective open label non-randomized trial in women attending the endocrine clinic of our tertiary care centre, between 2015 and 2018 for complaints suggestive of PCOS. A total of 80 women were screened for PCOS and those who qualified Rotterdam criteria (n=56) were invited to participate in the study. A written informed consent was obtained from each subject before participation. Pregnant, lactating women or those willing for conception in near future were excluded from trial. Beside this woman having thyroid dysfunction, hyperprolactinemia, non-classical CAH or on medications known to affect glucose or insulin metabolism were excluded. The study was approved and conducted according to the guidelines of the Institute’s ethics committee in accordance with the Helsinki Declaration Principles. The data was entered in pre-designed proforma for the PCOS clinic. Clinical details about the patients were written in proforma including menstrual history (age at menarche, number of cycles per year), features suggesting hyperandrogenism (hirsutism, androgenic alopecia, acne), weight gain, infertility and drug intake. All the women underwent anthropometric assessment (height, weight, waist and hip circumference), quantitation of hyperandrogenism (modified FG score), blood pressure measurement and detailed systemic examination. A single observer performed FG scoring and ultrasonography for documentation of polycystic ovarian morphology as per Rotterdam, 2003 criteria. The women were advised to observe barrier contraception during the study period.

**Laboratory evaluation:**
All the subjects reported underwent 75 grams OGTT after an overnight fast (10-12 hours). The blood sampling and USG pelvis were done on day 2nd or 3rd of spontaneous cycles or medroxyprogesterone induced withdrawal bleed in women with irregular cycles. After centrifugation, the samples were analysed for biochemical parameters on the same day, whereas those for hormone and inflammatory marker assays were stored at -80°C for further analysis.

**Assays:**
Blood glucose, liver functions, kidney functions, lipid profile were estimated using standard procedures on Cobas Integra (Roche Diagnostics, Germany). All the hormones and inflammatory markers, (17-hydroxy progesterone, LH, FSH, total testosterone, insulin, adiponectin, resistin) were estimated by electrochemiluminescence assay in (ECLIA) in the departmental laboratory (Cobas E 411, Roche Diagnostics, Germany). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (mIU/mL) × fasting glucose (mmol/L) /22.5 while Quantitative insulin sensitivity check index (QUICKI) was calculated as 1/ (log10 blood glucose 0hr + log 10 insulin 0hr). Biochemical hyperandrogenism was diagnosed if total testosterone levels were greater than 48 ng/dl.

**Intervention:**
All the patients underwent lifestyle counselling (increasing physical activity to minimum of 150 min/week) and healthy diet) at the beginning of study. The subjects were divided into two groups categorized on the basis of drug intake: group I (pioglitazone in combination with spironolactone, n=25), group II (pioglitazone in combination with metformin, n=25). Pioglitazone was administered in dose of 15 mg daily; metformin was administered as 500 mg twice daily and spironolactone 50 mg daily dose. These subjects were followed up for a period of six months and were reassessed at the end of trial for clinical, biochemical and hormonal parameters and any adverse events if experienced during study period were also noted.
Statistical analysis:
Data was analysed using programme SPSS version 22.0 (Statistical Package for Social Science (Chicago: SPSSS Inc., Illinois, IL, USA)). χ² test was used for comparison of dichotomous data. Continuous variables are presented as mean ± standard deviation, if they were normally distributed or as median and interquartile range if they are not normally distributed. The Kolmogorov-Smirnov test was used to test the normal distribution of continuous variables and an intention-to-treat analysis (ITT) was used to determine the robustness of the evidence. Analysis of variance (ANOVA) with Bonferroni corrections was used for comparing the differences between groups of continuous data in accordance with a normal distribution or Kruskal-Wallis test for non-normally distributed data when appropriate. A p value of less than 0.05 is considered as significant.

Results:
Out of a total of 80 subjects screened, 56 women were diagnosed as PCOS as per Rotterdam criterion and after excluding women who refused consent or had other exclusion criteria (n=6), 50 women were finally included in the study. At baseline women were divided in two equals (n=25) treatment groups (group I and group II) and were followed for six months. After excluding dropouts, those who stopped treatment because of adverse effects and those with incomplete data, we were left with a total of 42 women for final analysis as shown in figure 1.

Figure 1: Consort Chart Depicting The Flow Of The Patients Throughout The Study.
Baseline assessment
Comparison of baseline clinical, biochemical and hormonal parameters are presented in Table 1. All the baseline characteristics including mean age (years), age at menarche, number of menstrual cycles per year, weight, BMI, waist-hip ratio, FG score, fasting plasma insulin, fasting plasma glucose and serum total testosterone levels were comparable in both groups.

Follow up (6 months)
After six months of follow up, the number of menstrual cycles improved significantly in both groups (p<0.05), with a non-significant superior trend in group II compared to group I. A significant upward trend was observed in BMI and waist circumference in both groups at the end of study with a non-significant decline in waist hip ratio. There was significant decrease in FGscores and biochemical hyperandrogenism in either group post treatment. Mean FGscore was 9.33+2.15 in group I and 9.524+2.35 in group II (p>0.05) after treatment with no intergroup variation as shown in table 2. Systolic blood pressure declined in both groups post treatment thought declined significantly in group I only.

Table 1:- Comparative analysis of clinical parameters before and after treatment in Group I and Group II.

|                          | Group I                        | Group I                        | Group II                       | Group II                       |
|--------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
|                          | 0 months                       | 6 months                       | 0 months                       | 6 months                       |
| Mean Age (years)         | 19.048+3.840                   | 21.431+4.30                    | 22.67+2.94                     | 24.30+4.29                     |
| Weight (kg)              | 53.81+9.03                     | 57.76+8.7a                     | 54.66+7.01                     | 60.42+9.6a                     |
| BMI (kg/m²)              | 22.67+3.87                     | 24.39+4.12a                    | 22.47+2.94                     | 24.30+4.29a                    |
| Waist (cm)               | 73.66+5.76                     | 79.42+6.07a                    | 77.23+8.06                     | 84.00+9.6a                     |
| W/H ratio                | 0.8863+0.043                   | 0.8690+0.035                   | 0.9053+0.055                   | 0.8878+0.577                   |
| Ferriman-Gallwey score   | 13.00+3.6                      | 9.33+2.15a                     | 12.38+3.6                      | 9.524+2.35a                    |
| score (FGS core)         |                                |                                |                                |                                |
| Number of cycles /years  | 11.095+5.7                     | 16.57+7.2a                     | 8.23+3.40                      | 13.57+4.83a                    |
| Systolic blood pressure  | 111.43+11.95                   | 109.21+9.16                    | 116.19+10.713                  | 109.52+11.60a                  |
| (mmHg)                   |                                |                                |                                |                                |
| Diastolic blood pressure | 75.95+8.89                     | 76.31+9.55                     | 77.38+9.168                    | 74.52+8.35                     |
| (mmHg)                   |                                |                                |                                |                                |

Results are given as mean+ SD
\( a \) P < 0.05 for comparison within the group, by paired Wilcoxon test:
\( b \) P < 0.05 for comparison between Pioglitazone and metformin and pioglitazone with spironolactone groups

Among various metabolic parameters assessed post treatment, group II showed significant decline in fasting insulin level from an average of 10.46+4.01 to 6.95+2.51U/ml (p<0.05) whereas the decline was nonsignificant in group I. HOMA-IR and QUICKI showed improvement in both group post treatment indicating decline in insulin resistance though it was nonsignificant in either group. Other metabolic parameters like serum total cholesterol and serum triglycerides also declined post treatment non-significantly in group II as shown in table 2.

Serum adiponectin an insulin sensitivity marker increased almost 4 times in both groups from mean basal value of 6.86+4.49 to 23.80+13.36U/ml (p<0.05) in group I and from mean value of 6.25+3.5 to 23.79+12.62U/ml (p<0.05) in group II after 6 months of treatment. On the other hand, serum resistin increased from basal levels in both groups contrary to the decline expected post treatment.

Serum total testosterone also declined significantly in either group post treatment (48.06+37.99 to 33.65+17.51ng/dl; p<0.05 in group I) and (60.66+45.49 to 31.83+14.00ng/dl; p<0.05 in group II) with nonsignificant superior trend observed in insulin sensitizer group in comparison of pioglitazone and spironolactone (48% vs 31%) respectively.
Table 2: Comparative analysis of metabolic and hormonal parameters in Group I and Group II before and after treatment.

| Parameter                        | Group I (n=21) 0 months | Group I (n=21) 6 months | Group II (n=21) 0 months | Group II (n=21) 6 months |
|----------------------------------|-------------------------|-------------------------|--------------------------|--------------------------|
| Plasma glucose fasting post OGTT (mg/dl) | 86.29±11.72            | 88.52±11.40            | 85.60±9.32               | 88.4±10.65               |
| Plasma glucose post OGTT 2HR (mg/dl) | 107.24±25.44           | 100.40±21.14           | 111.33±30.42             | 109.5±15.96              |
| Fasting Insulin (uIU/ml)         | 10.78±5.85             | 7.93±3.90              | 10.46±4.01               | 6.95±2.51                |
| Serum total cholesterol (mg/dl)  | 168.28±36.79           | 164.95±37.25           | 161.47±25.88             | 159.73±25.41             |
| Serum triglycerides (mg/dl)      | 112.19±47.05           | 92.90±32.02            | 111.33±30.42             | 116±38.41b               |
| SGPT (IU/L)                      | 22.33±8.616            | 19.33±5.61             | 28.94±20.93              | 18.68±7.7a               |
| QUICKI                           | 0.347±0.034            | 0.360±0.028            | 0.343±0.021              | 0.363±0.019              |
| HOMA-IR                          | 2.30±1.34              | 1.71±0.83              | 2.21±0.94                | 1.50±0.52                |
| LH(IU/L)                         | 5.468±4.61             | 4.818±3.98             | 6.422±4.72               | 5.08±2.57                |
| FSH(IU/L)                        | 6.678±1.844            | 6.373±1.605            | 5.346±1.32               | 5.93±1.45                |
| Serum total testosterone (ng/dl) | 48.058±37.99           | 33.65±17.51a           | 60.66±45.49              | 31.83±14.00a             |
| Serum resistin (ng/ml)           | 3.32±2.08              | 5.87±3.17a             | 3.01±2.022               | 7.23±4.28a               |
| Serum adiponectin (ug/ml)        | 6.86±4.49              | 23.80±13.36a           | 6.25±3.5                 | 23.79±12.62              |

Results are given as mean± SD
a P < 0.05 for comparison within the group by paired Wilcoxon test:
b P < 0.05 for comparison between Pioglitazone and metformin and pioglitazone with spironolactone groups

Adverse events:
Overall, only 8 patients had adverse events. In group I four subjects withdrew their medication because of polymenorrhoea and in group II only 4 women had abdominal pain and facial puffiness necessitating drug withdrawal. During the study period there were no major change in liver enzymes as reported in some previous studies as an adverse effect of pioglitazones.

Discussion: -
Insulin resistance is considered to play a central role in pathogenesis of PCOS in both obese and lean women. 8,9,10 Basic understanding of this mechanism has laid down importance of insulin sensitizers (metformin and thiazolidinediones) in management of PCOS in recent years. They are replacing the well-established and frequently used oral contraceptives, which aggravate the insulin resistance and glucose intolerance.11 Metformin has been used since 1960s for the treatment of PCOS subjects however all women with PCOS, do not respond to metformin.12,13 Metformin fails to promote resumption of normal menses in up to 23% of women with PCOS as it often fails to normalize androgens.14,15,16

TZDscan decrease the insulin resistance, modify the adipocyte differentiation, inhibit the VEGF-induced angiogenesis, decrease leptin levels (perhaps leading to an increased appetite) and have anti-inflammatory effects.17,18 Thus, in this open label study we compared combination of pioglitazone to spironolactone or metformin regarding overall improvement in clinical, hormonal and metabolic parameters after 6 months of treatment. There have been no large clinical trials comparing the efficacy of pioglitazone in combination with metformin or spironolactone in decreasing hirsutism, body weight, insulin resistance and menstrual irregularity.
Charles J. Glueck in 2003 in a pioneer study prospectively observed additive effect of pioglitazone to poor responders of metformin. There were significant incremental benefits beyond metformin and diet. Insulin resistance fell, HDL cholesterol and SHBG rose (p< 0.01) despite nonsignificant change in weight, DHEAS and serum testosterone (p> 0.05).

Yuanyuan Wu in 2017 experimented combination therapy of metformin and pioglitazone on rat model of PCOS and concluded that metformin and pioglitazone combination therapy demonstrated greater efficacy in ameliorating PCOS through regulating the AMPK/PI3K/JNK pathway. Thus, combination of both drugs is expected to result in better outcome.

After 6 months of treatment in our study 80% subjects resumed normal cycles in pioglitazone and metformin group in comparison of pioglitazone and spironolactone group where 50% subjects complained poly-menorrhoea. Spironolactone is known to cause such irregularity in menstrual cycles as a result of which four subjects on pioglitazone and spironolactone stopped drug in between the study period. There was significant increase in BMI and waist circumference after treatment in either group which can be attributed to fluid retention and increase in subcutaneous fat secondary to thiazolidinediones. Despite weight gain various metabolic and hormonal parameters improved variably in both groups which indicates decrease in visceral fat considered to be metabolically unhealthy. Similar results were obtained by Yifeng Xu et al in 2017 who conducted a meta-analysis on differential effect of metformin and pioglitazone in PCOS patients and found that pioglitazone improved menstrual cycle regularity and ovulation better than metformin but significantly increased BMI compared with metformin.

In our study there was significant decline in hirsutism and serum testosterone in both groups post treatment but the trend of decline in serum testosterone was nonsignificantly higher in pioglitazone and metformin group (47%) in comparison of pioglitazone and spironolactone group (33%). This can be attributed to additive effect of metformin and pioglitazone in decreasing insulin resistance and LH mediated androgen secretion from ovarian theca cells. This was further confirmed when we compared decline in fasting insulin levels after 6 months treatment in either group. Combination of pioglitazone and metformin significantly decreased fasting insulin in comparison of pioglitazone and spironolactone. However, insulin sensitivity markers improved in either group in our study post treatment.

Among adipokines adiponectin which is considered to be indicator of metabolic milieu of PCOS subjects, markedly improved after treatment in both groups however intergroup analysis revealed no significant difference. Serum adiponectin level are negatively correlated with obesity, insulin resistance and metabolic syndrome. High adiponectin levels may reduce the risk of insulin resistance and type 2 diabetes. Thus, its significant increase in either group indicates overall improvement in inflammation and metabolic derangements with pioglitazone.

Though serum resistin levels increased after treatment in either group but previous studies have reported weaker association of resistin with insulin resistance. A recent cohort study by Hivert et al provided evidence that plasma levels of resistin are positively correlated with IR, but such relationship becomes weaker when compared with that of other adipokines, such as adiponectin.

In conclusion combination of pioglitazone with metformin and spironolactone resulted in marked improvement in clinical and biochemical hyperandrogenism along with overall improvement in metabolic milieu as indicated by significant rise in serum adiponectin levels in either group. Intergroup analysis revealed better efficacy of pioglitazone and metformin in achieving menstrual regularity, decline in serum total testosterone and fasting insulin levels. Though there was gain in BMI in either group secondarily to TZD but overall metabolic profile, insulin resistance markers, hyperandrogenism declined significantly. Pioglitazone may open up as a possible new treatment in women with PCOS since it can restore menstrual cyclicity, achieve a better ovulatory rate, improve clinical signs of hyperandrogenism, though more prospective studies with large sample size and longer follow up are needed to assess efficacy of pioglitazone in combination with metformin and spironolactone in PCOS patients.

Main drawbacks of our study are small sample size and short follow up to assess traditional adverse effects associated with pioglitazone. We could not measure subcutaneous and visceral fat separately which could better explain metabolically favourable weight gain in either group because of use of TZD.
Conflict of interest:
The authors declare that there is no conflict of interest regarding the publication of this paper.

Funding:
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements:-
We are highly grateful to the study subjects for the compliance to drugs and department of biostatistics, SKIMS, Srinagar, Jammu and Kashmir for assisting in data analysis

References:-
1. Carmina E, Lobo RA. (1999) Polycystic ovary syndrome (PCOS), arguably the most common endocrinopathy is associated with significant morbidity in women. J Clin Endocrinol Metab 84:1897–1899.
2. OE, Canbay A, Fuhrer D, Reger-Tan S. (2017) Metabolic and androgen profile in underweight women with polycystic ovary syndrome. Arch GynecolObstet 296:363–371.
3. Doganay M, Ozyer SS, Var T, Tonguc E, Gun Eryilmaz O, Ozer I et al. (2015) Associations between adipocyte fatty acid-binding protein and clinical parameters in polycystic ovary syndrome. Arch GynecolObstet 291(2):447–450.
4. Schoppee PD, Garney JC, Veldhuis JD. (2002) Putative activation of the peroxisome proliferator-activated receptorgamma impairs androgen and enhances progesterone biosynthesis in primary cultures of porcine theca cells. BioReprod66(1):190–198.
5. González-Ortiz M, Hernández-Salazar E, Kam-Ramos AM and Martínez-AbundisE. (2007) Effect of pioglitazone on insulin secretion in patients with both impaired fasting glucose and impaired glucose tolerance. Diabetes Res Clin Pract 75: 115-118.
6. Díaz-Delfín J, Morales M and CuellesC. (2007) Hypoglycemic action of thiazolidinediones/peroxisome proliferator-activated receptor gamma by inhibition of the c-Jun NH2-terminal kinase pathway. Diabetes 56: 1865-1871.
7. Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH. (2010) Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone,d-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. Cochrane Database Syst Rev 1:CD003053
8. Ibáñez L, Vallus C, Ferrer A, Marcos MV, Rodríguez-Hierro, de Zegher F. (2001) Sensitization of insulin induces ovulation in nonadolescents with anovulatory hyperandrogenism. J Clin Endocrinol Metab 86:3595–3598
9. RL, Makris A, Randall RW, Daniels G, Kistner RW, Ryan KJ. (1986) Insulin stimulates androgen accumulation in incubations of ovarian stroma obtained from women with hyperandrogenism. J Clin Endocrinol Metab 62: 904–910
10. De Leo V, la Marca A, Petraglia F.(2003) Insulin-lowering agents in the management of polycystic ovary syndrome. Endocr Rev 24:633–667
11. Jose´ Gomes Batista, Jose´ Maria Soares, Carla Cristina Maganhin. (2012) Assessing the benefits of rosiglitazone in women with polycystic ovary syndrome through its effects on insulin-like growth factor 1, insulin-like growth factor-binding protein-3 and insulin resistance: a pilot study. CLINICS 67(3):283-287
12. Lord JM, Flight IH, Norman RJ.(2003) Metformin in polycystic ovary syndrome: systemic review and meta-analysis. BMJ 327:951–955
13. Castello MF, Eden JA.(2003) A systemic review of the reproductive system effects of metformin in patients with polycystic ovary syndrome. FertilSteril 79:1–13
14. Velazquez, E., Mendoza, S., Hamer, T., Sosa, F. and Glueck, C. (1994) Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia,insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism, 43, 647±654.
15. Glueck, C., Wang, P., Fontaine, R., Tracy, T. and Sieve-Smith, L. (1999b) Metformin-induced resumption of normal menses in 39 of 43 (91%) previously amenorrheic women with the polycystic ovary syndrome. Metabolism, 48, 511-519
16. Fleming, R., Hopkinson, Z. and Wallace, R. (2002) Ovarian function and metabolic factors in women with oligomenorrhea treated with metformin in a randomized double blind, placebo-controlled trial. J. Clin. Endocrinol. Metab., 87, 569-574.
17. Waki HY, Yamauchi T, Kadowaki T. (2010) Regulation of differentiation and hypertrophy of adipocytes and adipokine network by PPARgamma. Nihon Rinsho 68(2):210–216
18. Panigrahy D, Singer S, Shen LQ et al. (2002) PPARγ ligands inhibit primary tumor growth and metastasis by inhibiting angiogenesis. J Clin Invest 110(7):923–932
19. Charles J.Glueck, Andrew Moreira, Naila Goldenberg, Luann Sieve and Ping Wang. (2003) Pioglitazone and metformin in obese women with polycystic ovary syndrome not optimally responsive to metformin. Human Reproduction 18(8):1618-1625
20. Yanyuan Wu, Pengfen Li, Dan Zhang and Yingpu Sun. (2018) Metformin and pioglitazone combination therapy ameliorate polycystic ovary syndrome through AMPK/PI3K/JNK pathway. Experimental and Therapeutic medicine 15: 2120-2127
21. Yifeng Xu, Yanxiang Wu, Qin Huang. (2017) Comparison of the effect between pioglitazone and metformin in treating patients with PCOS: a meta-analysis. Arch Gynecol Obstet 296:661–677
22. Berg AH, Combs TP, Du X. (2001) The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001; 7:947–53.
23. Steppan CM, Bailey ST, Bhat S, et al. (2001) The hormone resistin links obesity to diabetes. Nature 409:307–12.
24. McTernan PG, McTernan CL, Chetty R, et al. (2002) Increased resistin gene and protein expression in human abdominal adipose tissue. J Clin Endocrinol Metab 87:2407
25. Hivert MF, Sullivan LM, Fox CS, et al. (2008) Associations of adiponectin, resistin, and tumor necrosis factor-alpha with insulin resistance. J Clin Endocrinol Metab 93:3165–72.