Adiponectin levels in women with polycystic ovary syndrome: impact of metformin treatment in a randomized controlled study

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INTRODUCTION

- Adipose tissue : as an endocrine organ → secretes a number of metabolically active proteins : adipokines
- Adiponectin:
  - produced by mature adipocytes in subcutaneous and visceral adipose tissue.
  - anti-inflammatory and insulin-sensitizing properties
  - hypoadiponectinemia → metabolic syndrome and with obesity (especially visceral obesity)
  - In animal studies: levels decline before the onset of obesity and type 2 diabetes →adiponectin treatment reverses these changes.

- adiponectin :
  - positively correlated to age and female sex
  - inversely correlated to insulin resistance, abdominal fat mass, fasting insulin and glucose levels.
- decreasing plasma adiponectin levels → increasing risk of metabolic syndrome
- increased adiponectin levels result in :
  - After weight reduction in obese humans with or without type 2 diabetes.
  - Improving insulin sensitivity
  - Reducing insulin levels with insulin-sensitizing glitazones
  - lifestyle intervention that produces a reduction in fat mass

- At least 50% of women with PCOS are obese (intra-abdominal fat mass, waist circumference, and waist-hip ratio) → higher than in healthy controls
- generally have higher insulin levels than weight matched controls → high risk of deteriorating glucose metabolism
- The high prevalence of insulin resistance among patients with PCOS make this group suitable for studying the relationship between adiponectin and insulin resistance.
- Some studies have found that in PCOS, adiponectin is lower than in BMI- and age-matched controls, while others do not find such a relationship.

- **Metformin** : effective in reducing insulin resistance in obese patients with PCOS, inducing ovulation and reducing T levels.
- A recent Cochrane review found no effect on weight or waist circumference but a small effect on waist-hip ratio.
- The aim of this study was to evaluate the effect of metformin in PCOS
- In this report
  - describe PCOS population with regard to correlations between adiponectin and anthropometric, hormonal, and metabolic factors.
  - evaluated the effect of metformin treatment on patients with PCOS with different levels of adiponectin.

MATERIALS AND METHODS

- Department of Obstetrics and Gynaecology, Holstebro Hospital, Holstebro, Denmark
- 2001 ~ 2005.
- 18–45 y/o with a serum T value above 52 mg/dL and oligo- or amenorrhea.
- Oligomenorrhoea : irregular bleeding periods with an interval varying between 5 weeks and 6 months
- amenorrhea : absent bleedings for at least 6 months.
- **Exclusion criteria:**
  - periclimacteric gonadotropin values, hyperprolactinemia, diabetes mellitus, impaired thyroid, renal or hepatic function, hormonal treatment, pregnancy, lactation, or a wish for fertility treatment.

Protocol

- randomized 6 months treatment: 850 mg of metformin VS. placebo twice daily → washout period (3 months) before crossing over to the alternate treatment for another 6 months.
- Participants were seen before and after each treatment period (after overnight fast of at least 8 hours) with light clothing.
- Waist circumference → umbilical level.
- Hip circumference → trochanter region.
- Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured
- Blood tests for *adiponectin, fasting venous plasma glucose (FPG), insulin, triglycerides (TGL), total and high-density and low-density lipoprotein (HDL and LDL) cholesterol, total T, and sex hormone–binding globulin (SHBG).*

- The tests used in this study have the following normal values:
  - FPG, <110 mg/dL;
  - fasting insulin, <5.8 mIU/mL;
  - total cholesterol, 131–270 mg/dL;
  - HDL cholesterol, 31–66 mg/dL;
  - LDL cholesterol, 58–189 mg/dL;
  - triglycerides, 44–204 mg/dL;
  - T, 17–52 ng/dL;
  - SHBG, 1–4 mg/dL.
- Insulin sensitivity was evaluated by the homeostasis model assessment (HOMA) index:

  fasting serum insulin (mIU/mL) X FPG (mg(dL) / 405.
- Obesity was defined as BMI $\geqq$30 kg/m2.

Plasma Adiponectin Analysis

- Plasma adiponectin was determined by an in-house time-resolved immunofluorometric assay based on two monoclonal antibodies and recombinant human adiponectin (R&D Systems, Abingdon, UK).
- All standards and unknown samples were analyzed in duplicate, with the exception of nonspecific binding, which was analyzed in quadruplicate.
- The intra-assay coefficient of variation was <5%, and the interassay coefficient of variation was <10%.

Statistics

- The statistical software program Stata, version 9.2 (StataCorp 2005), was used for the statistical evaluation.
- Analysis of variance (ANOVA) was performed to identify differences between groups, and the differences were subsequently tested with t-test.
- To identify independent determinants of adiponectin, a multivariate linear regression analysis was performed.
- Some controls were cancelled because the women failed to turn up or wanted to skip a control for personal reasons
- At some occasions, blood tests were not done because the woman had not been fasting for at least 8 hours.

RESULTS
Baseline

- 52 women were included in the study.
- 40 (71%) were obese (BMI $\geqq$30).
- The study group was divided according to their adiponectin levels at baseline:
  - group 1 with adiponectin levels >7.6 mg/L;
  - group 2 with adiponectin levels $\leqq$7.6 and >5 mg/L;
  - group 3 with adiponectin levels$\leqq$5 mg/L.

TABLE 1

Values of studied variables according to adiponectin levels.

Variable	Group 1 (n = 17)	Group 2 (n = 17)	Group 3 (n = 18)	*P* value
Age, y	31 (19, 44)	32 (25, 38)	33 (24, 42)	NS
BMI, kg/m^{2}*	32 (22, 48)[a]	33 (23, 42)	37 (23, 55)	NS
WHR*	0.83 (0.63, 1.00)[b]	0.86 (0.73, 0.95)[a]	0.90 (0.81, 1.00)	< .01
SBP, mmHg*	131 (110, 170)	133 (115, 190)	137 (100, 180)	NS
DBP, mmHg*	82 (60, 110)	85 (65, 120)	88 (60, 115)	NS
T, ng/dL*	79.8 (57.9, 132)	81.8 (51.3, 152.2)	86.7 (48.1, 151.3)	NS
SHBG, μg/dL*	0.98 (0.35, 1.78)	0.8 (0.28, 1.55)	0.78 (0.4, 1.38)	NS
Cholesterol, mg/dL*	193 (154, 248)	189 (85, 290)	197 (147, 263)	NS
HDL, mg/dL	54 (34, 77)[b]	45 (37, 57)	42 (36, 56)	< .01
LDL, mg/dL*	110 (81, 167)	115 (77, 185)	124 (85, 181)	NS
TGL, mg/dL	121 (50, 193)	120 (4, 256)	124 (64, 394)	NS
FPG, mg/dL*	93 (79, 117)	96 (77, 110)	97 (79, 126)	NS
Insulin, μIU/mL	6.74 (2.19, 7.5)[b]	6.84 (2.94, 40.75)[a]	18.14 (3.70, 50.25)	< .01
HOMA index, mg/dL × μIU/mL	1.56 (0.43, 6.72)[b]	1.61 (0.61, 9.60)[a]	4.23 (0.72, 12.28)	< .01

Note: Group 1: adiponectin >7.6 mg/L; group 2: adiponectin ≤7.6 and >5 mg/L; group 3: adiponectin ≤5 mg/L. Mean (5th–95th percentiles) for normally distributed (*) data, otherwise median (5th–95th percentiles). *P*-value for difference across means evaluated by one-way ANOVA. Differences between means tested by unpaired *t*-test.

[a] $P < .05$ compared with group 3.

[b] $P < .005$ compared with group 3.

Trolle. Effect of metformin on adiponectin in PCOS. Fertil Steril 2010.

After 6 months of metformin treatment

TABLE 2

Differences between values after 6 months of metformin and 6 months of placebo, paired *t*-test.

Variable	Group 1	Group 2	Group 3
Weight, kg	−2.29 (−5.83, 1.24)	−4.26 (−7.65, −.87)[a]	−3.41 (−6.71, −0.10)[a]
Adiponectin, mg/L	−0.73 (−2.87, 1.40)	−0.77 (−2.25, 0.70)	0.26 (−0.22, 0.73)
WHR	0 (−0.02, 0.02)	−0.02 (−0.05, 0.01)	0.01 (−0.01, 0.03)
SBP, mmHg	0 (−8, 7)	−5 (−14, 3)	−1 (−10, 7)
DBP, mmHg	−1 (−5, 4)	−2 (−7, 3)	−5 (−12, 2)
T, ng/dL	−7.2 (−19.3, 4.9)	−7.5 (−19.6, 4.6)	1.4 (−17.3, 19.9)
SHBG, μg/dL	−0.03 (−0.18, 0.13)	0.05 (−0.08, 0.2)	−0.13 (−0.63, 0.4)
Cholesterol, mg/dL	9.3 (−6.9, 25.5)	−4.6 (−23.6, 14.7)	−11.2 (−27.0, 4.6)
HDL, mg/dL	0 (−4.6, 4.2)	−1.5 (−7.3, 3.9)	3.1 (−0.4, 6.6)
LDL, mg/dL	0.8 (−15.4, 17.0)	−1.2 (−25.9, 23.2)	−0.8 (−16.6, 15.1)
TGL, mg/dL	0.9 (−26.6, 27.4)	0.9 (−24.8, 27.4)	−16.8 (−31.9, −1.8)[a]
FPG, mg/dL	−4.1 (−10.6, 2.3)	−3.1 (−6.5, 0.5)	−3.6 (−9.9, 2.7)
Insulin, μIU/mL	−2.40 (−6.96, 2.16)	−3.66 (−8.30, 0.99)	1.45 (−11.19, 14.10)
HOMA index, mmol/L × μIU/mL	−0.87 (−2.08, 0.33)	−0.99 (−2.47, 0.49)	0.15 (−5.27, 5.57)

Note: Group 1: adiponectin >7.6 mg/L; group 2: adiponectin ≤ 7.6 and >5 mg/L; group 3: adiponectin ≤ 5 mg/L

[a] $P < .05$.

Trolle. Effect of metformin on adiponectin in PCOS. Fertil Steril 2010.

there was **no significant difference** in adiponectin levels!

Effect of Metformin

- As group size after treatment was small because of dropout, the groups were combined for further study of all subjects completing both treatment periods.
- mean weight, FPG, and HOMA index → significantly lower after metformin than after placebo, adiponectin did not change (Table 3).
- 6 women lost at least 10% in weight on metformin, but their increase in adiponectin did not reach statistical significance (data not shown, P=.075, Wilcoxon sign-rank test).

TABLE 3

Values after treatment with metformin or placebo.

Variable (N)	Metformin	Placebo	*P* value
Weight, kg* (41)	94.1 (87.4–100.7)	97.3 (90.6–104.1)	.0007
WHR* (37)	0.86 (0.84–0.89)	0.86 (0.84–0.89)	.8109
SBP, mmHg* (36)	129 (123–135)	131 (125–136)	.3204
DBP, mmHg* (36)	83 (79–86)	85 (81–89)	.1173
T, ng/dL* (37)	66.6 (58.2–74.6)	70.9 (64.6–77.2)	.2733
SHBG, μg/dL* (36)	0.84 (0.72–0.95)	0.87 (0.71–1.03)	.7082
Cholesterol, mg/dL* (36)	188 (179–198)	190 (180–201)	.6147
HDL, mg/dL (36)	49 (37–77)	49 (36–74)	.6373
LDL, mg/dL* (36)	119 (110–127)	119 (108–130)	.9271
TGL, mg/dL (36)	103 (53–276)	121 (58–242)	.3705
FPG, mg/dL* (29)	94 (91–97)	98 (95–99)	.0166
Insulin, μIU/mL (30)	58.2 (10.8–208.4)	86.0 (21.3–250.0)	.1306
HOMA, mmol/L × μIU/mL (24)	1.65 (0.29–7.02)	2.86 (0.65–9.23)	.0345
Adiponectin, mg/L (30)	6.22 (3.22–13.83)	6.29 (3.30–14.51)	.2942

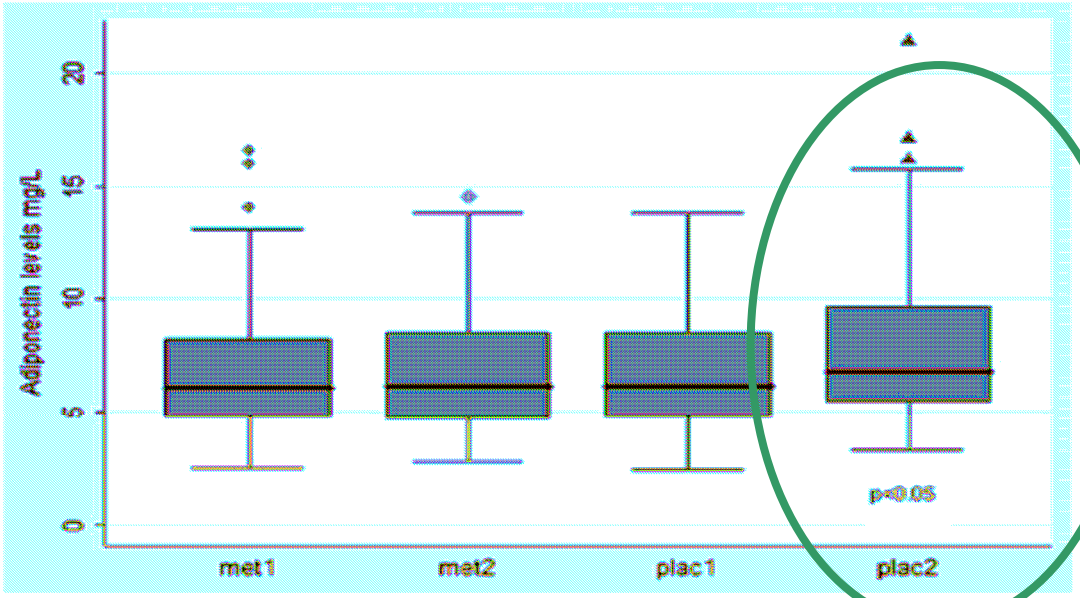
Note: Mean (5th–95th percentiles) for normally distributed (*) data, otherwise median (5th–95th percentiles). Only subjects completing both treatment periods are included. Normally distributed variables were tested by paired *t*-test; otherwise the Wilcoxon signed rank-sum test was used. N is the number of pairs tested.

Tolle. Effect of metformin on adiponectin in PCOS. Fertil Steril 2010.

- adip… …the plac… …nd 3 mon…
- The … …eriod was sign… …ds (Fig. 1).

FIGURE 1

Boxplot of all adiponectin measurements in the treatment periods. The mean value during the second placebo period is significantly higher than during any other period (*t*-test, $P < .05$). Met1: Values of patients receiving metformin during the first 6 months' period (n = 89). Met2: Metformin during second 6 months' period (n = 100). Plac1: Placebo during first 6 months' period (n = 72). Plac2: Placebo during second 6 months' period (n = 64).



Trolle. Effect of metformin on adiponectin in PCOS. Fertil Steril 2010.

DISCUSSION

- In this study of women with PCOS, adiponectin levels were closely linked to visceral obesity as estimated by *WHR, insulin, insulin resistance, and HDL cholesterol*.

- Longitudinal studies of women with PCOS:
  - compared with BMI and age-matched controls, the PCOS population shows an increased coronary artery calcium deposit and an increased intima-media thickness ➔ risk markers for cardiovascular disease

- In postmenopausal women suspected for coronary artery ischemia ➔ women who had symptoms of PCOS earlier in life
  - cardiovascular event–free survival was shorter
  - coronary artery disease more frequent

- The risk of developing cardiovascular disease and type 2 diabetes is closely linked to the metabolic syndrome, which is present in 40%–50% of women with PCOS

- Our study was not designed to study adiponectin levels in women with PCOS compared with controls. *(This has been done by other researchers with different results)*
  - a lower adiponectin level has been reported in PCOS women than in weight matched controls, while other studies have been unable to confirm this.
  - An elevated T level is a key feature of PCOS → in postmenopausal women ➔ high T levels increase the risk of multivessel coronary artery disease.
  - In some studies, T is found to correlate positively with adiponectin levels, and in others negatively or not at all
- **We observed no association between adiponectin and T.**

- In spite of significant weight loss and improvement in insulin sensitivity during metformin treatment ➔ **adiponectin levels did not change.**
- Spranger et al. : treated a small group of adipose women with PCOS with metformin for 6 months without obtaining any change in adiponectin.
- In contrast: a significant weight loss and increase in adiponectin were obtained in diabetes patients after 12 months of treatment, when metformin was added to pioglitazone.
- In our study population of 52 women, only 6 lost 10% in weight and adiponectin did not increase significantly, but a larger number of patients losing more weight may have produced another result.

- The means of all adiponectin measurements performed during the second placebo period after 6 months of metformin and 3 months of washout were higher than during the other periods.
- This period thus followed a metformin-induced weight loss and an improvement in insulin sensitivity approximately 6 months earlier.
- Hypothesized: the increase in adiponectin takes sometime to develop after weight loss?

Summary

- in our PCOS population, low adiponectin levels are closely linked to insulin resistance, HDL cholesterol, and abdominal adiposity expressed as WHR.
- Despite a significant weight loss and improvement in insulin sensitivity after 6 months of metformin treatment, adiponectin levels did not increase.
- metformin treatment is not a way of increasing adiponectin to improve the metabolic profile and lower the risk of long-term metabolic disease as type 2 diabetes in PCOS.

- There are indications in this study and the literature that weight loss is crucial and that the change in adiponectin may appear later than the change in insulin sensitivity.
- This again spotlights **weight reduction and persistent lifestyle change** as interventions of vital importance in PCOS.

Thanks for your listening!