

**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 \geq 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 ≥ 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 ≤ 7.6 and >5 mg/L;
 - 🌀 group 3 with adiponectin levels ≤ 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, μ g/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, μ IU/mL	-2.40 (-6.96, 2.16)	-3.66 (-8.30, 0.99)	1.45 (-11.19, 14.10)
HOMA index, mmol/L \times μ IU/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 \leq 7.6 and >5 mg/L; group 3: adiponectin \leq 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

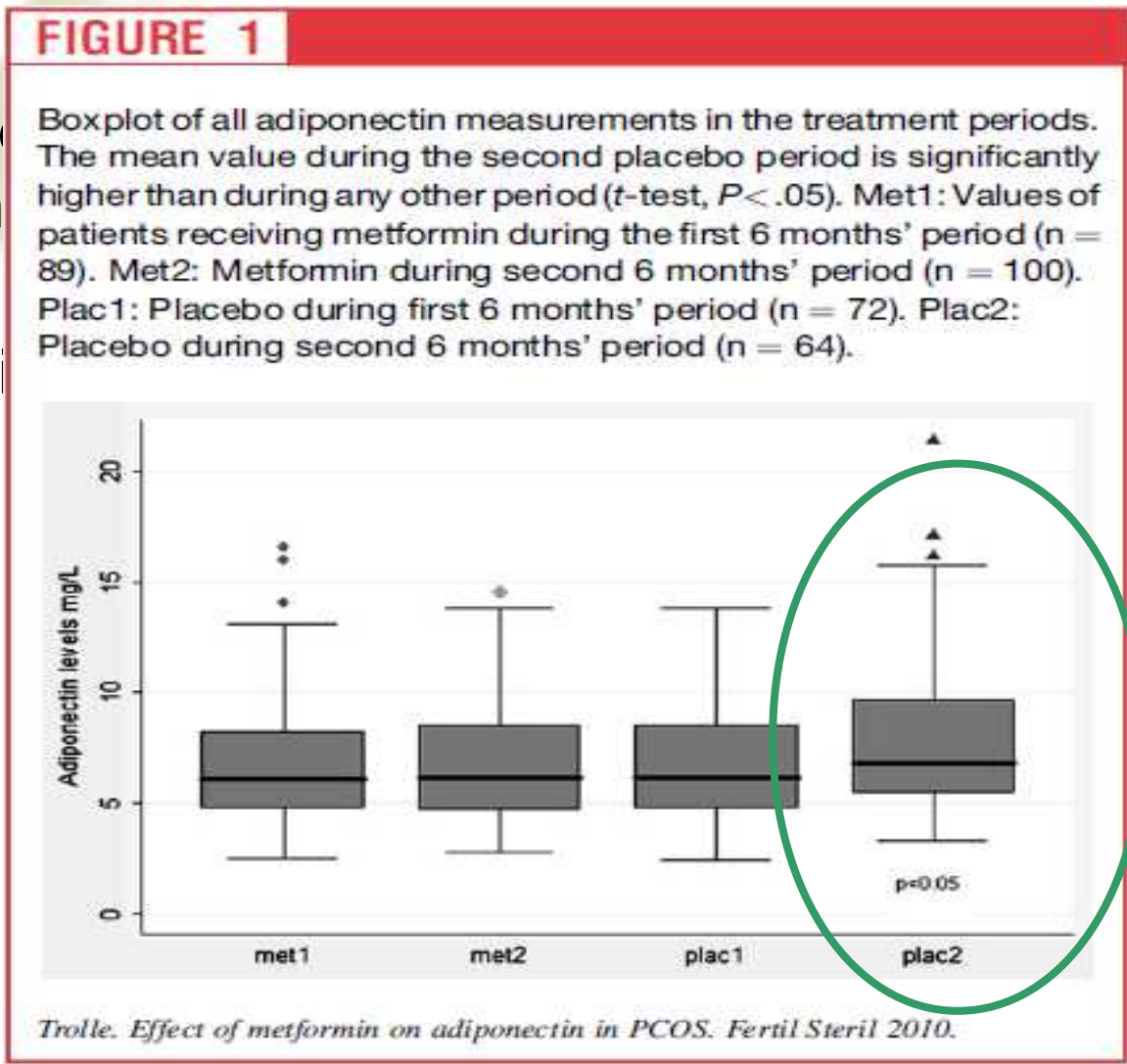
Values after treatment with metformin or placebo.

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, μ g/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, μ IU/mL (30)	58.2 (10.8–208.4)	86.0 (21.3–250.0)	.1306
HOMA, mmol/L \times μ IU/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.

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

- ❖ adiponectin levels during placebo period were significantly higher than during metformin periods (Fig. 1).



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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!