

Anti-Müllerian hormone concentrations in androgen-suppressed women with polycystic ovary syndrome

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BACKGROUND: Current data suggest that excessive androgen exposure can lead to the development of polycystic ovaries and polycystic ovary syndrome (PCOS). Anti-Müllerian hormone (AMH) levels reflect the number of small antral follicles in the ovaries and are elevated in PCOS. We hypothesized that protracted reduction of circulating androgens and/or insulin resistance would reduce circulating AMH concentrations in women with PCOS.

METHODS: A prospective, randomized, double-blind 26 week long study was undertaken in 50 women with PCOS. They all received diet and lifestyle counselling, and metformin 850 mg three times daily. Concomitantly, they were randomized to either dexamethasone 0.25 mg daily ($n = 25$) or placebo ($n = 25$). Thirty-eight women completed the study. AMH (primary outcome) and other hormone levels were measured at inclusion and after 8 and 26 weeks of treatment.

RESULTS: At baseline in univariate regression analyses, AMH levels associated positively with testosterone levels ($P = 0.041$) and ovarian volume ($P = 0.002$). In multivariate regression analyses, AMH associated positively with testosterone ($P = 0.004$), and negatively with dehydroepiandrosterone sulphate (DHEAS) ($P = 0.001$) and C-peptide levels ($P = 0.020$). Circulating AMH concentrations were unaffected by 6 months of lifestyle counselling with metformin and placebo treatment. AMH levels were also unaffected by 6 months of androgen suppression with dexamethasone in addition.

CONCLUSIONS: AMH levels in untreated PCOS women associated positively with testosterone, and negatively with DHEAS and C-peptide levels. Six months of androgen suppression by either metformin or low-dose dexamethasone treatment failed to influence circulating AMH levels.

Key words: androgens / dexamethasone / anti-Müllerian hormone / polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS), the most common endocrine disorder in women of reproductive age, is characterized by polycystic ovaries (PCO), oligo-amenorrhea and hyperandrogenism (Norman *et al.*, 2007). In Caucasians, a PCOS prevalence of 5–7% has been reported (Diamanti-Kandarakis *et al.*, 1999; Asuncion *et al.*, 2000). The majority, i.e. at least 90%, of PCOS women have PCO (Welt *et al.*, 2006). Hyperandrogenism is the most prominent and constant diagnostic symptom of PCOS (Azziz *et al.*, 2006) and it derives from both ovarian and adrenal contributions (Martikainen *et al.*, 1996; Carmina, 1997).

PCOS is probably multifactorial in origin, and intrauterine androgen exposure, post-natal insulin resistance and hyperinsulinemia are all important pathogenic factors (Norman *et al.*, 2007). Insulin stimulates androgen synthesis directly in both the adrenals and the ovaries (Nestler, 1997; Franks *et al.*, 1999; la Marca *et al.*, 1999). Insulin may also be involved in the development of PCO (Norman *et al.*, 2007). However, androgens may also induce PCOS and the changes seen in PCO (Jonard and Dewailly, 2004). This is supported both by direct animal studies (Abbott *et al.*, 2002) and by the high prevalence of PCO seen in women with congenital adrenal hyperplasia as recently reviewed by Xita and Tsatsoulis (2006).

Circulating levels of anti-Müllerian hormone (AMH), secreted by the granulosa cells of early developing pre-antral and small antral follicles, are seen as a marker of ovarian follicular reserve in normal women (Visser *et al.*, 2006) and in PCOS (Pigny *et al.*, 2006; Chen *et al.*, 2008). However, in animal models, AMH acts as an inhibitor of primordial follicle recruitment and decreases the sensitivity of follicles for the FSH selection for dominance (Visser *et al.*, 2006). Women with PCOS show increased development of antral follicles compared with normal women (Pigny *et al.*, 2006). Accordingly, their circulating levels of AMH levels are two to three times increased (Pigny *et al.*, 2006; Somunkiran *et al.*, 2007). There is now a consensus that both in normally menstruating women and in PCOS women, circulating AMH represents the number of antral follicles as evaluated by transvaginal ultrasound estimation (Laven *et al.*, 2004; Eldar-Geva *et al.*, 2005; Fleming *et al.*, 2005, 2006; Pigny *et al.*, 2006; Chen *et al.*, 2008). The granulosa cells from small antral follicles of women with PCOS appear to secrete AMH in greater quantities than in normal women, so the total amount of AMH in the circulation may derive from a combination of increased follicle counts and increased specific secretion (Rice *et al.*, 2007).

It is established that metformin treatment of women with PCOS usually results in reduction of circulating androgens and a modest improvement of ovulation frequency (Costello *et al.*, 2007). Short-term (1 week) treatment with metformin in women with PCOS does not change AMH while the number of antral follicles may be reduced (Bayrak *et al.*, 2007). There are two reports that suggest that after protracted (several months) metformin treatment AMH is reduced (Fleming *et al.*, 2005; Piltonen *et al.*, 2005), while ovarian volume is unchanged or reduced (Fleming *et al.*, 2005).

The present study was performed to explore the possible roles of altered circulating androgens and insulin on the development of small antral follicles in PCOS women. In this prospective, randomized, placebo controlled study, we primarily explored the roles of suppression of androgens. Further, in the placebo-treated control group, the effect of reduction of insulin resistance by metformin was examined. This was performed in PCOS women treated with a single session program of diet and lifestyle recommendations. At the same time, they were started on metformin, and randomized to placebo or dexamethasone in a double-blind design over 26 weeks. The effects on circulating androgens were measured directly, and the effects on small antral follicle changes were evaluated indirectly, by way of circulating levels of AMH.

Materials and Methods

Fifty women with PCOS were recruited from either our University Hospital (Trondheim), gynecological outpatient clinic or by advertisement in the local newspaper, and results from the primary study have been published previously (Vanky *et al.*, 2004). Inclusion criteria were age between 18 and 40 years and PCO (≥ 9 sub-capsular follicles visualized in one plane with a diameter of 3–8 mm), verified by transvaginal ultrasonography. Ultrasonographic examinations were performed at inclusion, and week 26. Patients were examined in the supine position with a 6 MHz probe. All recordings were performed using a MultiSync M500 Synergy, Diasonic Ultrasound instrument (Israel). Patients with serum progesterone levels >4.0 nmol/l, indicating the luteal phase, were reexamined 2 weeks later. Both ovaries were measured in three dimensions (i.e. length, depth and height) three times. A mean value was calculated for each of the three

dimensions. The ovarian volume was calculated using the following equation: length \times depth \times height $\times 0.5$.

In addition, at least one of the following criteria had to be fulfilled: testosterone >2.5 nmol/l, sex hormone-binding globulin (SHBG) <30 nmol/l, fasting C-peptide >1.0 nmol/l, oligo-amenorrhea (length of menstrual cycle >35 days or <10 periods per year) or hirsutism, judged clinically as male pattern growth of body hair. In retrospect, evaluating ovarian volume (right and/or left) >10 ml, free testosterone index (total testosterone $\times 10/\text{SHBG}$) >0.6 and menstrual pattern, we could conclude that all the participants also fulfilled the 'Rotterdam 2003' criteria for PCOS (The Rotterdam European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine -sponsored PCOS consensus workshop group, 2004).

Exclusion criteria included pregnancy, breastfeeding, known liver disease or alanine aminotransferase >60 IU/l, creatinine >130 $\mu\text{mol/l}$, known alcohol abuse, diabetes mellitus and treatment with oral glucocorticoids or hormonal contraceptives. Patients could be included if hormonal contraception had been discontinued at least 1 month previously. Only 4 of the 38 participants who completed the study used hormonal contraceptives and had a 1 month of wash out before inclusion.

Congenital adrenal hyperplasia was excluded by 17-hydroxprogesterone measurements and all participants had normal prolactin levels (<784 mIU/l).

Of the original 50 patients, 4 patients became pregnant, despite being instructed to use non-hormonal contraception during the study period (three in the dexamethasone group and one in the placebo group). Three patients withdrew due to gastrointestinal side effects of metformin (nausea or frequent diarrheal lasting more than 3 weeks). Two patients withdrew due to motivation failure, two withdrew without giving any reason and in one woman early ovarian failure had been overlooked. Accordingly, 38 women completed the whole study, 18 in the dexamethasone group and 20 in the placebo group.

Venous blood samples were drawn from an antecubital vein, between 8:00 and 10:00 a.m. after an overnight fast at randomization and at the end of the study (26 weeks after inclusion). Blood samples were centrifuged at room temperature within 30 min and stored at -70°C until analysis (1–9 months for androgens and C-peptide, 3–4 years for AMH) as described below.

Study protocol

All participants received individual, written and verbal diet and lifestyle counselling at inclusion (Week 0). Thereafter no such advice was given. Concomitant with diet and lifestyle counselling, all the participants were started on metformin 850 mg (metformin hydrochloride, Metformin[®], Weifa A/S, Oslo, Norway). All the patients used metformin once daily during the first week, twice daily during the second week and thereafter three times daily for the rest of the study period. At inclusion (Week 0), the participants were also randomized to additional treatment with either dexamethasone 0.25 mg (dexamethasone natriumphosphate, Decadron[®], MSD, Drammen, Norway) or identical placebo, daily at bedtime. Accordingly, our study has two arms; one with women treated with diet and lifestyle advice, metformin and placebo, and the other with women treated with diet and lifestyle advice, metformin and dexamethasone. Both groups were treated for 26 weeks. Dexamethasone and identical placebo capsules were produced and packed by our local hospital pharmacy.

The primary outcome measure was circulating AMH levels, while testosterone, androstenedione, dehydroepiandrosterone sulphate (DHEAS), C-peptide and ovarian volume (mean of right + left ovary, or only the contralateral ovary if a follicle >10 mm was seen) were secondary outcome measures for correlation analyses.

The Committee for Medical Research Ethics of Health Region IV, Norway, and The Norwegian Medicines Agency approved the study. A written informed consent was obtained from each patient before inclusion, and the declaration of Helsinki was followed throughout the study.

Assays

AMH was estimated by single measurements by an enzyme-linked immunosorbent assay provided by Diagnostic Systems Laboratories (Webster, TX, USA) using the reagents and calibrators supplied by the manufacturer. Values are presented as nanograms per millilitre (conversion factor to pmol/l = ng/ml \times 7.1). AMH estimation was done in one single run with one kit. Reference values for the normal population were not available from the manufacturer as the kit was for research purposes only.

Testosterone and androstenedione were measured by a double-antibody technique on an Elecsys 2010 analyser (Roche Diagnostics GmbH, Mannheim, Germany) using reagents and calibrators supplied by the manufacturer. DHEAS concentrations were measured using a competitive immunoassay on an Immulite 2000 analyser using the reagents and calibrators supplied by the manufacturer (Diagnostic Products Corporation, Los Angeles, CA, USA). C-peptide was analysed on an Immulite 2000 analyser using reagents, methods and calibrator obtained from the instrument supplier (Diagnostic Products Corporation). For AMH the intra-assay coefficient of variation was 8.9%, for testosterone 5.5%, for androstenedione 8.4%, for DHEAS 4.4% and for C-peptide 4.5%.

Statistical analysis

All statistical procedures were performed using the Statistical Package for the Social Sciences (SPSS) version 15.01 for Windows SPSS Inc. (Chicago, IL, USA). Sample size calculations were performed for the original study. We achieved 23–23 patients in each group at 90% of power to detect 1.0 nmol/l change in testosterone between groups. SDs were estimated to 0.6 nmol/l. We anticipated a 5–10% possible 'drop out' and included 25 patients in each group.

Mann–Whitney test for independent samples and Wilcoxon signed ranks test for paired samples were used for comparisons as appropriate. Treatment effects were investigated with analysis of covariance adjusted for patient age. Values are reported as means and SD. Uni- and multivariate linear regression analyses were used for correlation analyses. In regression analyses, values are reported as mean and 95% confidence intervals (CI). *P*-values <0.05 were considered significant. No adjustments for multiple comparisons were performed.

Results

The study population

The mean age of the participants who completed the study was 30.6 ± 5.9 and 26.4 ± 3.8 years in the placebo and dexamethasone groups, accordingly ($P = 0.019$) (Table I). BMI, age at menarche and bleedings per year were equal between groups. All biochemical variables, except DHEAS, were equal between study groups at inclusion (Table II). The apparent difference in DHEAS levels disappeared after adjustment for age.

At inclusion the patients who withdrew from the study were equal to those who completed the study with respect to age, body weight, BMI, DHEAS, androstenedione, testosterone, C-peptide, age at menarche and bleeding per year (data not shown). However, AMH was lower in those who withdrew (7.55 ± 3.28 versus 14.01 ± 9.95 ng/ml; $P = 0.043$).

Table I Baseline characteristics of the study participants with PCOS who completed the study

Variable	Placebo (<i>n</i> = 20) (mean \pm SD)	Dexamethasone (<i>n</i> = 18) (mean \pm SD)	<i>P</i> -value *
Age (years)	30.6 ± 5.9	26.4 ± 3.8	0.019
BMI (kg/m ²)	33.4 ± 7.5	32.8 ± 6.7	0.81
Age at menarche (years)	12.9 ± 1.4	12.5 ± 1.6	0.22
Bleedings per year (no.)	4.4 ± 3.9	4.9 ± 3.2	0.44

*The study groups were compared with Mann–Whitney test for independent samples.

Changes in androgens during the study

Table II shows that treatment with metformin and lifestyle advice (placebo group) resulted in increases in DHEAS and decreases in androstenedione, testosterone and C-peptide. In the dexamethasone group, DHEAS, androstenedione and testosterone decreased during the study period. Comparisons at Week 26 showed that testosterone was significantly lower in the dexamethasone group than placebo ($P = 0.033$), while DHEAS tended to be lower ($P = 0.082$).

Metformin and dexamethasone effects on AMH concentrations and ovarian volume

Table III shows that baseline AMH levels in the patients who completed the study were in the upper normal range, and that there were no differences between the treatment groups. Our normal data are derived from women aged 30 to 37 years undergoing IVF, and whose egg yields were the average \pm 1SD. The range so calculated is 3.8 to 18 pmol/L. After 26 weeks of treatment, AMH concentrations showed no indications of change in either treatment group. Baseline and 26 weeks AMH levels were equal both in the dexamethasone ($P = 0.98$) and in the placebo ($P = 0.60$) groups. Ovarian volumes were also unchanged over the 26 week study period.

Separate uni- and multivariate analyses in the dexamethasone and placebo groups revealed no significant association between individual changes in AMH levels from inclusion to study Week 26 and changes in DHEAS, androstenedione, testosterone, C-peptide or ovarian volume (data not shown).

AMH and relationship with other factors prior to and during the study period

At baseline, univariate regression analyses in untreated PCOS women (at inclusion, before diet and lifestyle advice and any medication), AMH associated positively with circulating testosterone ($P = 0.041$) and ovarian volume ($P = 0.002$) (Table IV). However, in multivariate regression analyses, AMH associated negatively with DHEAS ($P = 0.001$) and C-peptide ($P = 0.020$) and positively with testosterone ($P = 0.004$).

In univariate regression analyses in the placebo group after 26 weeks of treatment AMH levels associated positively with androstenedione ($P = 0.013$) and ovarian volume ($P = 0.001$). In multivariate regression analyses, AMH levels associated positively with

Table II Body weight and endocrine outcome variables during the study

Variable	Study group	Week 0		Week 26		P-value*	P-value [#]
		Mean ± SD		Mean ± SD			
Body weight (kg)	Placebo	95.3 ± 23.0		91.2 ± 22.0		<0.0005	
	Dexamethasone	94.1 ± 19.7		91.6 ± 20.1		0.035	0.34
DHEAS (μmol/l)	Placebo	6.2 ± 3.4		7.2 ± 3.6		0.005	
	Dexamethasone	7.8 ± 3.1 [§]		5.4 ± 3.1		0.002	<0.0005
Androstendione (nmol/l)	Placebo	18.3 ± 6.8		16.9 ± 6.9		0.048	
	Dexamethasone	20.4 ± 6.7		14.8 ± 4.3		0.001	0.004
Testosterone (nmol/l)	Placebo	3.12 ± 1.57		2.79 ± 1.28		0.038	
	Dexamethasone	3.26 ± 1.62		2.01 ± 1.01 [§]		0.001	0.002
C-peptide (nmol/l)	Placebo	1.6 ± 0.6		1.3 ± 0.5		0.002	
	Dexamethasone	1.7 ± 1.0		1.5 ± 0.8		0.082	0.023

DHEAS: dehydroepiandrosterone sulphate.

*Each study group was investigated with Wilcoxon signed ranks test for paired samples for possible difference between week 0 and week 26.

[#]Treatment effect by intervention investigated by analysis of covariance (ANCOVA) adjusted for patient age.

[§]Difference between groups at each time point investigated with Mann–Whitney test for independent samples. Only P-values <0.05 indicated.

Table III AMH levels and ovarian volume according to study groups through treatment

	Study group	Week 0		Week 26		P-value [#]
		Mean ± SD	P-value*	Mean ± SD	P-value*	
AMH (ng/ml)	Placebo	15.3 ± 11.5		15.2 ± 12.0		
	Dexamethasone	12.6 ± 7.8	0.53	14.1 ± 13.6	0.61	0.69
Bleedings per year (no.)	Placebo	4.4 ± 3.9		7.5 ± 3.4 [§]		
	Dexamethasone	4.9 ± 3.2	0.44	8.6 ± 3.5 [§]	0.26	0.20
Ovarian volume (ml)	Placebo	15.2 ± 6.9		14.9 ± 6.7		
	Dexamethasone	13.2 ± 3.6	0.81	12.7 ± 4.6	0.42	0.69

*The study groups were compared with Mann–Whitney test for independent samples.

[#]Treatment effect by intervention investigated by ANCOVA analyses adjusted for patient age.

[§]Each study group was investigated with Wilcoxon signed ranks test for paired samples for possible difference between week 0 and week 26. Only P-values <0.05 are indicated.

testosterone ($P = 0.020$), and negatively with DHEAS ($P = 0.013$) and C-peptide ($P = 0.031$). In the dexamethasone group, neither univariate nor multivariate regression analyses at week 26 revealed any associations between AMH and the other variables studied.

In separate uni- and multivariate regression analyses, the change in AMH during the study did not associate with baseline AMH values or baseline values of any of the other variables given in Table IV (data not shown).

Discussion

These results demonstrate that, contrary to our hypothesis, reducing biosynthesis and circulating concentrations of androgens using dexamethasone in women with PCOS failed to modify circulating AMH concentrations. Furthermore, protracted treatment with either metformin or a combination of metformin and dexamethasone did not influence ovarian volumes.

Our findings are perhaps surprising given both direct and circumstantial evidence supporting a role for androgens in follicular development

and survival although other mechanisms have also been proposed (Visser *et al.*, 2006). Androgens play a role in stimulating early (FSH independent) stages of follicular growth (Vendola *et al.*, 1998; Weil *et al.*, 1999), and occupation of the androgen receptor appears to be a critical step in growth factor mechanisms (Hickey *et al.*, 2005). In female-to-male transsexuals, testosterone administration results in PCOS-like changes (Futterweit and Deligdisch, 1986; Spinder *et al.*, 1989; Pache *et al.*, 1991). During this high-dose androgen treatment in women, gonadotrophins become suppressed, and ovaries enlarged with morphological changes meeting the criteria of PCO. Under these circumstances, androgens appear to induce PCO-like ovarian changes in humans, independent of the effect of gonadotrophins. These studies suggest that testosterone (androgens) contributes directly to the pathogenesis of PCO and PCOS, and the accompanying elevation of AMH concentrations, not only by intrauterine androgen exposure as recently reviewed (Xita and Tsatsoulis, 2006), but also in adult women when exposed to hyperandrogenism.

The present observation that a reduction of androgens over a protracted period, sufficient to explore effects from the earliest stages of

Table IV Linear regression analyses of AMH (ng/ml) in women with PCOS

	Univariate			Multivariate		
	B	95% CI	P-value	B	95% CI	P-value
At baseline (de novo PCOS women; n = 38)						
DHEAS ($\mu\text{mol/l}$)	-0.61	-1.60 to 0.39	0.23	-2.07	-3.21 to -0.93	0.001
Androstenedione (nmol/l)	0.43	-0.05 to 0.91	0.079	0.24	-0.32 to 0.81	0.39
Testosterone (nmol/l)	2.11	0.10 to 4.13	0.041	4.47	1.52 to 7.43	0.004
C-peptide (nmol/l)	-1.67	-5.95 to 2.61	0.43	-4.84	-8.86 to -0.82	0.020
Ovarian volume (cm^3)	0.88	0.35 to 1.40	0.002	0.33	-0.18 to 0.84	0.20
After 26 weeks of treatment (placebo group; n = 20)						
DHEAS ($\mu\text{mol/l}$)	-0.87	-2.67 to 0.93	0.32	-3.42	-5.97 to -0.87	0.013
Androstenedione (nmol/l)	1.05	0.25 to 1.43	0.013	0.06	-0.99 to 1.12	0.90
Testosterone (nmol/l)	4.47	-0.14 to 9.07	0.056	11.0	2.00 to 20.1	0.020
C-peptide (nmol/l)	3.08	-9.35 to 15.52	0.61	-11.9	-22.5 to -1.3	0.031
Ovarian volume (cm^3)	1.19	0.54 to 1.84	0.001	0.20	-0.59 to 0.99	0.60
After 26 weeks of treatment (dexamethasone group; n = 18)						
DHEAS ($\mu\text{mol/l}$)	0.53	-1.91 to 2.96	0.65	-0.13	-4.43 to 4.18	0.95
Androstenedione (nmol/l)	-0.31	-2.04 to 1.43	0.71	-1.50	-5.12 to 2.13	0.37
Testosterone (nmol/l)	1.70	-5.86 to 9.27	0.64	0.74	-16.38 to 17.86	0.92
C-peptide (nmol/l)	-4.52	-13.45 to 4.42	0.30	0.55	-16.69 to 17.80	0.94
Ovarian volume (cm^3)	1.45	-0.26 to 3.18	0.089	1.98	-0.58 to 4.53	0.11

follicular development, does not result in ovarian changes as represented by AMH output, suggesting that numerous mechanisms are responsible for the induction and maintenance of the morphological changes observed in the ovaries of women with PCOS. It also indicates that androgen-mediated support for follicular growth, development and survival is not a simple direct cause and effect relationship.

Our observations add support to the concept that circulating AMH does not hold a simple linear relationship with follicle number, as previously suggested by the observation that AMH is more elevated in hyperandrogenic compared with normoandrogenic women with PCO despite comparable numbers of small follicles (Eldar-Geva et al., 2005). The markedly decreased AMH levels seen in PCOS women with diabetes type I compared with non-diabetic PCOS women, despite comparable androgen levels, is also compatible with this view (Codner et al., 2007).

It is also possible that the partial suppression of androgens seen in the present study is insufficient to reduce the biological effect on ovarian androgen receptors. Thus, the underlying pathology may relate to androgen receptor sensitivity as much as circulating androgen concentrations (Shah et al., 2008). Blocking the androgen receptor, as in treatment with the anti-androgen flutamide, induces a much larger reduction in the biological androgen effect at the cellular level, and further exploration of effects upon follicular dynamics represented by AMH output is awaited. Accordingly, our study indicates that suppression of adrenal androgens, either with or without modification of insulin sensitivity using metformin, is not a clinically valid approach to normalization of ovarian morphology or follicular dynamics in women with PCOS.

Two previous reports of protracted treatment with the insulin sensitizer metformin resulted in improved insulin sensitivity, reduced

circulating androgen levels and also reductions in AMH levels (Fleming et al., 2005; Piltonen et al., 2005). The present study, with its 6 month treatment period, does not confirm these observations, as AMH levels remained unchanged in the control group despite reduced insulin levels as evaluated by reduced C-peptide levels. This might indicate that the effect of metformin on AMH emerges only after 6 months treatment. However, it may also indicate that the modest changes seen in those two reports are related to normal time-dependent evolution of AMH rather than an effect of treatment *per se* (Visser et al., 2006). However, it should be noted that the age of the participants in the two previous studies is comparable to the age of the participants in the present study.

The baseline associations between AMH and other factors in untreated women with PCOS, support data reported previously. Univariate analyses revealed that AMH associated positively with testosterone and ovarian volume. In multivariate analyses, both C-peptide and DHEAS became negatively associated with AMH while the positive association with testosterone was strengthened. The association between AMH and ovarian volume, testosterone and insulin has been reported previously (Fleming et al., 2005; Chen et al., 2008). However, the possible association with the adrenal androgen precursor DHEAS has not been described before.

In the control group, i.e. PCOS women treated with diet and lifestyle advice, and metformin, the associations between AMH and other factors both in univariate and multivariate analyses were essentially unchanged during the study. However, in the dexamethasone group (metformin + dexamethasone-treated women), all associations were abolished by treatment. This effect applied to ovarian volume and DHEAS as well as the circulating androgens, which may be

confused by assay sensitivity limitations. An obvious explanation would be that the modification of adrenal androgens by dexamethasone actively disrupts the usual mechanisms regulating the latter stages of follicular growth and development in PCOS. This, in turn, suggests possible effective inter-organ relationships. However, why or how adrenal androgen suppression should disrupt relationships while maintaining follicular profiles is difficult to explain. It is interesting to note that in PCOS women treated with GnRH-agonists for down-regulation during IVF treatment, levels of DHEAS and DHEA decrease, further supporting an ovarian—adrenal interplay (Kjøtrød *et al.*, 2008). In this context, further exploration of the mineralocorticoid receptor activity in normal and PCOS ovaries may prove rewarding.

In conclusion, 6 months of androgen suppression by low-dose dexamethasone failed to modify follicular dynamics as represented by the changes in circulating AMH levels in women with PCOS treated with diet, lifestyle advice and metformin.

Author's role

S.M.C. had the original idea, performed the statistical analyses and was responsible for preparing the manuscript. E.V. performed the initial study, and took part in the hypothesis, discussion of results and preparation of the manuscript. R.F. took part in developing the hypothesis, interpretation of results and preparation of the manuscript.

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References

- Abbott DH, Dumesic DA, Franks S. Developmental origin of polycystic ovary syndrome—a hypothesis. *J Endocrinol* 2002;**174**:1–5.
- Asuncion M, Calvo RM, San Millan JL, Sancho J, Avila S, Escobar-Morreale HF. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. *J Clin Endocrinol Metab* 2000;**85**:2434–2438.
- Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE *et al.* Position statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. *J Clin Endocrinol Metab* 2006;**91**:4237–4245.
- Bayrak A, Terbell H, Urwitz-Lane R, Mor E, Stanczyk FZ, Paulson RJ. Acute effects of metformin therapy include improvement of insulin resistance and ovarian morphology. *Fertil Steril* 2007;**87**:870–875.
- Carmina E. Prevalence of adrenal androgen excess in PCOS. In: Azziz R, Nestler E, Dewailly D (eds). Chapter 37, Lippincott-Raven Publishers, Philadelphia, 1997, pp. 385–394.
- Chen MJ, Yang WS, Chen CL, Wu MY, Yang YS, Ho HN. The relationship between anti-Müllerian hormone, androgen and insulin resistance on the number of antral follicles in women with polycystic ovary syndrome. *Hum Reprod* 2008;**23**:952–957.
- Codner E, Iñiguez G, Villarreal C, Lopez P, Soto N, Sir-Petermann T, Cassorla F, Rey RA. Hormonal profile in women with polycystic ovarian syndrome with or without type I diabetes mellitus. *J Clin Endocrinol Metab* 2007;**92**:4742–4746.
- Costello MF, Shrestha B, Eden J, Johnson NP, Sjoblom P. Metformin versus oral contraceptive pill in polycystic ovary syndrome: a Cochrane review. *Hum Reprod* 2007;**22**:1200–1209.
- Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG, Zapanti ED, Bartzis MI. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. *J Clin Endocrinol Metab* 1999;**84**:4006–4011.
- Eldar-Geva T, Margalioth EJ, Gal M, Ben-Chetrit A, Algur N, Zylber-Haran E, Brooks B, Huerta M, Spitz IM. Serum anti-Müllerian hormone levels during controlled ovarian hyperstimulation in women with polycystic ovaries with and without hyperandrogenism. *Hum Reprod* 2005;**20**:1814–1819.
- Fleming R, Harborne L, MacLaughlin DT, Ling D, Norman J, Sattar N, Seifer DB. Metformin reduces serum mullerian-inhibiting substance levels in women with polycystic ovary syndrome after protracted treatment. *Fertil Steril* 2005;**83**:130–136.
- Fleming R, Deshpande N, Traynor I, Yates RW. Dynamics of FSH-induced follicular growth in subfertile women: relationship with age, insulin resistance, oocyte yield and anti-Müllerian hormone. *Hum Reprod* 2006;**21**:1436–1441.
- Franks S, Gilling-Smith C, Watson H, Willis D. Insulin action in the normal and polycystic ovary. *Endocrinol Metab Clin North Am* 1999;**28**:361–378.
- Futterweit W, Deligdisch L. Histopathological effects of exogenously administered testosterone in 19 female to male transsexuals. *J Clin Endocrinol Metab* 1986;**62**:16–21.
- Hickey TE, Marrocco DL, Amato F, Ritter LJ, Norman RJ, Gilchrist RB, Armstrong DT. Androgens augment the mitogenic effects of oocyte-secreted factors and growth differentiation factor 9 on porcine granulosa cells. *Biol Reprod* 2005;**73**:825–832.
- Jonard S, Dewailly D. The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, may be the main culprit for the follicular arrest. *Hum Reprod Update* 2004;**10**:107–117.
- Kjøtrød SB, Sunde A, von Düring V, Carlsen SM. Possible metformin effect on adrenal androgens during pretreatment and IVF cycle in women with polycystic ovary syndrome. *Fertil Steril* 2009;**91**:500–508.
- la Marca A, Morgante G, Paglia T, Ciotta L, Cianci A, De Leo V. Effects of metformin on adrenal steroidogenesis in women with polycystic ovary syndrome. *Fertil Steril* 1999;**72**:985–989.
- Laven JS, Mulders AG, Visser JA, Themmen AP, De Jong FH, Fauser BC. Anti-Müllerian hormone serum concentrations in normoovulatory and anovulatory women of reproductive age. *J Clin Endocrinol Metab* 2004;**89**:318–323.
- Martikainen H, Salmela P, Nuojua-Huttunen S, Perala J, Leinonen S, Knip M, Ruokonen A. Adrenal steroidogenesis is related to insulin in hyperandrogenic women. *Fertil Steril* 1996;**66**:564–570.
- Nestler JE. Insulin regulation of human ovarian androgens. *Hum Reprod* 1997;**12**:53–62.
- Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. *Lancet* 2007;**370**:685–697.
- Pache TD, Chadha S, Gooren LJ, Hop WC, Jaarsma KW, Dommerholt HB, Fauser BC. Ovarian morphology in long-term androgen-treated female to male transsexuals. A human model for the study of polycystic ovarian syndrome? *Histopathology* 1991;**19**:445–452.
- Pigny P, Jonard S, Robert Y, Dewailly D. Serum anti-Müllerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006;**91**:941–945.

- Piltonen T, Morin-Papunen L, Koivunen R, Perheentupa A, Ruokonen A, Tapanainen JS. Serum anti-Müllerian hormone levels remain high until late reproductive age and decrease during metformin therapy in women with polycystic ovary syndrome. *Hum Reprod* 2005; **20**:1820–1826.
- Rice S, Ojha K, Whitehead S, Mason H. Stage-specific expression of androgen receptor, follicle-stimulating hormone receptor, and anti-Müllerian hormone type II receptor in single, isolated, human preantral follicles: relevance to polycystic ovaries. *J Clin Endocrinol Metab* 2007; **92**:1034–1040.
- Shah NA, Antoine HJ, Pall M, Taylor KD, Azziz R, Goodarzi MO. Association of androgen receptor CAG repeat polymorphism and polycystic ovary syndrome. *J Clin Endocrinol Metab* 2008; **93**:1939–1945.
- Somunkiran A, Yavuz T, Yucel O, Ozdemir I. Anti-Müllerian hormone levels during hormonal contraception in women with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol* 2007; **134**:196–201.
- Spinder T, Spijkstra JJ, van den Tweel JG, Burger CW, van Kessel H, Hompes PG, Gooren LJ. The effects of long-term testosterone administration on pulsatile luteinizing hormone secretion and on ovarian histology in eugonadal female to male transsexual subjects. *J Clin Endocrinol Metab* 1989; **69**:151–157.
- The 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**:411–417.
- Vanky E, Salvesen KA, Carlsen SM. Six-month treatment with low-dose dexamethasone further reduces androgen levels in PCOS women treated with diet and lifestyle advice, and metformin. *Hum Reprod* 2004; **19**:529–533.
- Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. *J Clin Invest* 1998; **101**:2622–2629.
- Visser JA, de Jong FH, Laven JS, Themmen AP. Anti-Müllerian hormone: a new marker for ovarian function. *Reproduction* 2006; **131**:1–9.
- Weil S, Vendola K, Zhou J, Bondy CA. Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. *J Clin Endocrinol Metab* 1999; **84**:2951–2956.
- Welt CK, Gudmundsson JA, Arason G, Adams J, Palsdottir H, Gudlaugsdottir G, Ingadottir G, Crowley WF. Characterizing discrete subsets of polycystic ovary syndrome as defined by the Rotterdam criteria: the impact of weight on phenotype and metabolic features. *J Clin Endocrinol Metab* 2006; **91**:4842–4848.
- Xita N, Tsatsoulis A. Review: fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. *J Clin Endocrinol Metab* 2006; **91**:1660–1666.

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