Title: Weight loss and vascular inflammatory markers in overweight women with and without polycystic ovary syndrome

Short title: Weight loss and inflammation in PCOS

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Abstract

Polycystic ovary syndrome (PCOS) is associated with increased cardiovascular disease risk. The effect of weight loss on the vascular inflammatory markers plasminogen activator inhibitor-1 (PAI-1), asymmetric dimethylarginine (ADMA) and soluble vascular cell adhesion molecule-1 (sVCAM-1) and intracellular adhesion molecule-1 (sICAM-1) is unknown. Overweight women with (n=14) and without (n=13) PCOS of comparable age and BMI undertook an 8-week weight loss program. Women with PCOS had elevated PAI-1, sVCAM-1 and sICAM-1 cross-sectionally before or after weight loss compared to controls. For all women, sVCAM-1 (p=0.026) and sICAM-1 (p=0.040) equivalently decreased with weight loss. Women with PCOS have elevated inflammatory markers which weight loss partially improves.

Key words: Polycystic ovary syndrome, weight loss, cardiovascular disease, inflammation

Introduction

Polycystic ovary syndrome (PCOS) affects up to 18% of reproductive-aged women. It is associated with elevated risk factors for cardiovascular disease (CVD) including endothelial dysfunction, dyslipidaemia and markers of systematic inflammation such as C-reactive protein (Moran et al., 2009, Orio et al., 2005, Orio et al., 2006). While obesity worsens and weight loss improves its presentation (Andersen et al., 1995, Moran et al., 2007), the effect of weight loss on CVD risk is poorly studied. Vascular inflammatory markers associated with CVD include asymmetric dimethylarginine (ADMA) (nitric oxide synthase inhibitor), plasminogen activator inhibitor-1 (PAI-1) (fibrinolysis inhibitor) and soluble vascular cell adhesion molecule-1 (sVCAM) or intercellular adhesion molecule-1 (sICAM-1) (leukocyte recruitment and adhesion molecules). These markers have all been reported to be associated with the development of cardiovascular disease (Valkonen et al., 2001, Thogersen et al., 1998, Blankenberg et al., 2001), are elevated in PCOS (Moran et al., 2009, Diamanti-Kandarakis et al., 2006, Orio et al., 2004) and reduced by weight loss or insulin-sensitising agents in the general population or PCOS (Andersen et al., 1995, Diamanti-Kandarakis et al., 2006, Rissanen et al., 2001). There is limited research assessing weight loss and these markers in PCOS. One small study reported decreases in PAI-1 activity (Andersen et al., 1995) but no studies have assessed ADMA, sVCAM-1 or sICAM-1 or compared the effect of weight loss between women with and without PCOS. The aim of this study was to examine the effect of weight loss on vascular inflammatory markers in overweight women with and without PCOS.

Methods

This is a secondary analysis of a larger study (Moran et al., 2007) of overweight (BMI>25 kg/m^2) women with (n=14) and without (n=13) PCOS (ESHRE/ASRM criteria). This subset was selected due to the groups of PCOS and control women having comparable age and BMI's and frozen samples for analysis. There were no differences between women included and excluded from this sub-study for anthropometric or biochemical characteristics either at baseline or over the

intervention. Exclusion criteria were pregnancy, breastfeeding and use of oral contraceptives, endocrine hormonal or insulin-sensitising treatment unless ceased 2 weeks (hormonal or insulin sensitising treatment) or 4 weeks (oral contraceptives) pre-study. All subjects followed an energy-restricted diet for 8 weeks with 2 meals/day replaced with commercially available meal replacements (5208.4±740.3 kJ/day) (Slimfast; Unilever Australasia, Australia) (Moran et al., 2007). Dietary intake and physical activity were assessed from fortnightly 3-day consecutive food records and 7-day 24-hour physical activity records. Institutional Review Board approval was obtained. All subjects gave informed written consent.

Fasting weight, height, BMI, fat and fat free mass (bioelectrical impedance), waist circumference, insulin, testosterone, sex hormone-binding globulin (SHBG) and ADMA were measured as previously described (Moran et al., 2007, Moran et al., 2009). PAI-1, sVCAM-1 and sICAM-1 were analysed on a Multiplex (Luminex Corporation, USA) using commercially available kits (Linco Research, USA). Insulin resistance and hyperandrogenism were assessed using homeostasis model assessment (HOMA) (fasting insulin (mU/L) x glucose (mmol/L)/22.5) and free androgen index (FAI) (testosterone/SHBG x 100).

Data are expressed as mean±SD or mean difference and 95% confidence interval and log-transformed where skewed. Data were analysed by one-way ANOVA (baseline data), repeated-measured ANOVA (comparison between time points) or correlations. Two-tailed analysis was performed using SPSS 10.0 (SPSS Inc, Chicago) with statistical significance at $p<0.05$. On post-hoc calculations, we were powered to detect the observed difference of 73% in sICAM-1 and 92% in PAI-1 with weight loss between women with and without PCOS.

Results

Of n=37 participants originally recruited (n=18 PCOS, n=19 controls), n=5 discontinued (lost to follow-up n=3 PCOS, n=2 controls) and n=32 completed the intervention (n=15 PCOS, n=17 controls) and n=27 were analysed for this sub-study (n=14 PCOS, n=13 controls). At baseline, the women with and without PCOS had comparable ages (p=0.06), weight (p=0.92) and BMI (p=0.86) (Table 1). n=3 PCOS and n=1 controls were smokers. There were no differences in energy, macronutrient intake or physical activity for women and without PCOS (Moran et al., 2007). Compared to controls, women with PCOS had elevated FAI (week 0 and 8 p=0.002), insulin (week 0 p=0.03, week 8 p=0.028) and HOMA (week 0 p=0.032, week 8 p=0.035). Similar decreases in weight, waist circumference, body composition, FAI ($P \leq 0.001$), insulin (p=0.028) and HOMA (p=0.035) occurred for all women (Table 1).

Women with PCOS had elevated PAI-1 (p=0.034) and sICAM-1 (p=0.047) at week 0 and elevated PAI-1 (p=0.047), sVCAM-1 (p=0.009) and sICAM-1 (p=0.032) at week 8 compared to controls. No changes with PAI-1 or ADMA and similar decreases in sVCAM-1 (p=0.026) and sICAM-1 (p=0.04) occurred with weight loss for women with or without PCOS (Table 1). For all women combined, the change in sICAM-1 correlated with the change in sVCAM-1 (r=0.490 p=0.009) and waist circumference (r=0.463 p=0.017).

Discussion

We confirm elevations in vascular inflammatory markers in PCOS compared to controls (Moran et al., 2009, Diamanti-Kandarakis et al., 2006, Orio et al., 2004) and report for the first time decreased sVCAM-1 and sICAM-1 with weight loss in PCOS. These changes occurred in association with decreases in central adiposity. Furthermore, sVCAM-1 reductions have been previously reported with metformin in PCOS independent of changes in BMI (Diamanti-Kandarakis et al., 2006) which is consistent with metformin's anti-inflammatory effects and the role of inflammatory cytokines on increasing endothelial CAM expression.

We report for the first time no differential effect on weight loss on vascular inflammatory markers in women with or without PCOS. Conversely, we previously reported weight loss decreased C-reactive protein for women without but not with PCOS (Moran et al., 2007). Given that C-reactive protein is elevated in PCOS (Orio et al., 2004) and the role of C-reactive protein both as a marker for low-grade chronic inflammation, a potential active role in atherosclerosis and its association clinical cardiovascular events (Pai et al., 2004), the implications of these findings with regards to CVD risk in PCOS is unclear. This may reflect the heterogeneity of metabolic risk in PCOS or an improvement in vascular as opposed to systematic inflammation following weight loss. Weight loss may also not consistently improve CVD risk in PCOS, supported by previous findings of no changes in lipids with weight loss (Andersen et al., 1995, Moran et al., 2007). Furthermore, PAI-1 and ADMA did not decrease with weight loss in contrast to some (Andersen et al., 1995) but not all studies (Rissanen et al., 2001). This may be related to the intensity and duration of our dietary intervention as PAI-1 decreases previously occurred in association with >5-10% but not <5% weight losses over 3 months (Rissanen et al., 2001).

We note here the small sample size, lack of power for sVCAM-1 and ADMA and potential selection bias through selecting a subset of subjects from a larger study. However, there were no differences in baseline parameters for women included and excluded from this sub-study. While we assessed circulating rather than functional markers of vascular function, these surrogate markers have been previously associated with cardiovascular morbidity or mortality. We report for the first time improvements in vascular inflammatory markers in overweight women with PCOS with modest weight loss which supports lifestyle intervention as a key initial treatment for PCOS. However, longer-term intensive lifestyle interventions may be required to achieve consistent improvements in CVD risk. Further research is warranted in larger studies examining the

heterogeneity of metabolic improvements and the appropriate amount of weight loss for optimising metabolic health in overweight women with PCOS.

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231 **Table 1: Changes in anthropometric, reproductive and metabolic variables with weight loss in overweight women with and without polycystic**

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	PCOS (n=14)		Control (n=13)		Mean difference between PCOS and control (95% CI)	P Intervention	P Intervention by PCOS status
	Week 0	Week 8	Week 0	Week 8			
Age (years)	32.3±5.9	N/A	36.4±4.6	N/A	N/A	N/A	N/A
Weight (kg)	94.5±19.8	90.6±17.7	93.7±15.4	89.0±14.4	0.9 (-2.3, 4.0)	<0.001	0.486
BMI (kg/m^2)	35.3±5.7	33.9±5.5	34.9±4.8	33.2±4.6	0.5 (-0.7, 1.5)	<0.001	0.944
WC (cm)	114.0±14.8	106.9±15.8	110.1±11.8	103.0±11.6	0.2 (-4.1, 4.4)	<0.001	0.944
Fat mass (kg)	34.5±8.6	31.8±7.6	34.1±7.7	30.7±6.5	0.8 (-1.3, 2.9)	<0.001	0.438
Fat free mass (kg)	59.9±11.9	58.3±11.7	60.4±9.2	58.4±8.5	0.9 (-0.8, 2.6)	0.001	0.285
FAI†	22.0±18.9*	17.3±18.4*	8.9±4.5	6.8±3.3	-2.5 (-5.4, 0.3)	<0.001	0.954
Insulin (mU/l)	22.4±14.4*	18.3±14.1*	12.2±6.9	8.5±5.8	-0.3 (-7.1, 6.6)	0.028	0.939
HOMA	5.5±3.8*	4.4±3.5*	2.9±1.8	2.0±1.7	-0.3 (-2.0, 1.6)	0.035	0.782
ADMA (µmol/L)	0.79±0.15	0.78±0.21	0.80±0.15	0.76±0.18	0.03 (-0.1, 0.2)	0.372	0.636

PAI-1 (ng/mL) †	251.2±94.1*	228.8±74.0*	178.2±72.6	174.8±61.3	-19.1 (-49.5, 11.4)	0.337	0.392
sVCAM-1 (ng/mL)	1261.9±337.8	1187.2±257.8*	1038.6±274.1	948.6±168.6	15.2 (-128.2, 158.5)	0.026	0.829
sICAM-1 (ng/mL) †	192.6±74.3*	172.1±51.2*	147.3±61.1	137.2±37.5	-10.3 (-34.0, 13.3)	0.040	0.377

ADMA: Asymmetric dimethylarginine, FAI: Free androgen index, HOMA: Homeostasis model assessment of insulin resistance, PAI-1: Plasminogen activator inhibitor-1, sICAM-1: Soluble intercellular adhesion molecule-1, sVCAM-1: Soluble vascular cell adhesion molecule-1, WC: Waist circumference

* Significant difference between women with and without PCOS for FAI (p=0.002), insulin (p=0.030), HOMA (p=0.032), PAI-1 (p=0.034) and sICAM-1 (p=0.047) at week 0 or FAI (p=0.002), insulin (p=0.028), HOMA (p=0.035), PAI-1 (p=0.047), sVCAM-1 (p=0.009) and sICAM-1 (p=0.032) at week 8

† Data that were non-normally distributed were log transformed prior to analysis.

Data are expressed as mean±SD and mean difference and 95% confidence interval and analysed by one-way ANOVA for baseline comparisons and repeated-measures ANOVA for changes with time with P values presented both for the effect of the intervention on outcomes (within subject factor) and the interaction effect of intervention by PCOS status on outcomes (between subject factor).