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Exploration of biological causes of psychological problems in polycystic ovary syndrome (PCOS)

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PhD thesis (by prior publication)

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Because all the papers presented here are products of co-authorship, a few words are required to clarify my contribution: as first author of all papers, each project was conceived, initiated, ethics applied for, patient materials & protocols written, patients recruited/data collected (with collaboration in the papers in chapters 3 and 5), data analysed, first draft written, and papers submitted for publication, by the first author i.e. John Barry. The exception to this is the paper in chapter 3 which was submitted for publication by Dr Adam Kay. Other authors contributed mainly in terms of participant recruitment and contribution to some aspects of design, and to later drafts of the papers.

DECLARATION

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THESIS ABSTRACT

Background: Polycystic ovary syndrome (PCOS) affects up to 10% of women, and is characterised by elevated testosterone (T) levels. Women with PCOS have higher scores than healthy women on a range of measures of psychological problems.

Objective: To test the hypotheses that: 1/ The female fetus in a PCOS pregnancy experiences elevated T levels; 2/ T causes mood disturbance in women with PCOS. 3/ women with PCOS show more signs of mood disturbance typical of symptoms of reactive hypoglycaemia than healthy controls.

Design: Mainly between-groups cross-sectional studies. Also two meta-analyses.

Setting: The research took place mainly in two London gynaecology clinics, University College London Hospital (UCLH) and the Royal Free Hospital, Hampstead (RFH). Some of the research was conducted online, and at three other gynaecology and fertility clinics.

Participants: Participants were recruited from hospital clinics, support groups for women with PCOS, or the internet. Most participants were women aged 18-40.

Outcome Measures: Testosterone; psychometric measures of mood disturbance.

Results: 1/ Elevated T was found in the umbilical cord blood of the female fetus in PCOS pregnancies; 2/ Mood problems in PCOS were not directly caused by T. 3/ Women with PCOS showed higher levels of mood problems typical of hypoglycaemia than controls.

Conclusions: The findings suggest the female fetus in a PCOS pregnancy may be exposed to relatively high levels of T. Mood problems in adults with PCOS are possibly caused by the direct effects of low blood glucose and indirect effects of T (e.g. obesity) than direct effects of T. Further research using the gold-standard biochemical assessment methods is required for any replications of these findings.

KEY TO ABBREVIATIONS

ACU Assisted Conception Unit (Eastman Dental Hospital)

ANOVA Analysis of Variance

ANCOVA Analysis of Covariance

BMI body mass body index (metric weight divided by height squared).

CAH congenital adrenal hyperplasia

CGM continuous glucose monitor

CV coefficient of variation

DHEAS dehydroepiandrosterone sulphate

DHT dihydrotestosterone

E2 estradiol, measured in nmol/l

ECL electrochemiluminescence assay

EPQ Eysenck Personality Questionnaire,

FAI free androgen index calculated as (T*100)/SHBG.

HADS Hospital Anxiety and Depression Scale

HSC-7 Hypoglycemia Symptom Checklist – 7

ICC intra-class correlation coefficients

IR insulin resistance

GI glycaemic-index (as in 'low GI diet')

MACL Mood Adjective Check List

MS mass spectrometry

NHS National Health Service

nmol/l nanomoles per litre

OGTT Oral Glucose Tolerance Test

Pco polycystic ovaries (multiple ovarian cycsts)

PCOS polycystic ovary syndrome

PCOSQ PCOS Quality of Life Questionnaire

QoL quality of life

RH Reactive hypoglycaemia

RFH Royal Free Hospital, London

RH Reactive (or 'postprandial') hypoglycemia

RIA radioimmunoassay

RLCQ Recent Life Changes Questionnaire

SD Standard Deviation

SHBG sex hormone binding globulin, measured in nmol/l

SEC Socio-economic classification

STAXI State Trait Aggression Inventory

T testosterone (total i.e. bound + unbound to SHBG) measured in nmol/l

UCLH University College London Hospital.

UVT umbilical venous T (testosterone from umbilical vein)

UAT umbilical arterial T (testosterone from umbilical arteries)

CHAPTER 1

General introduction to the thesis

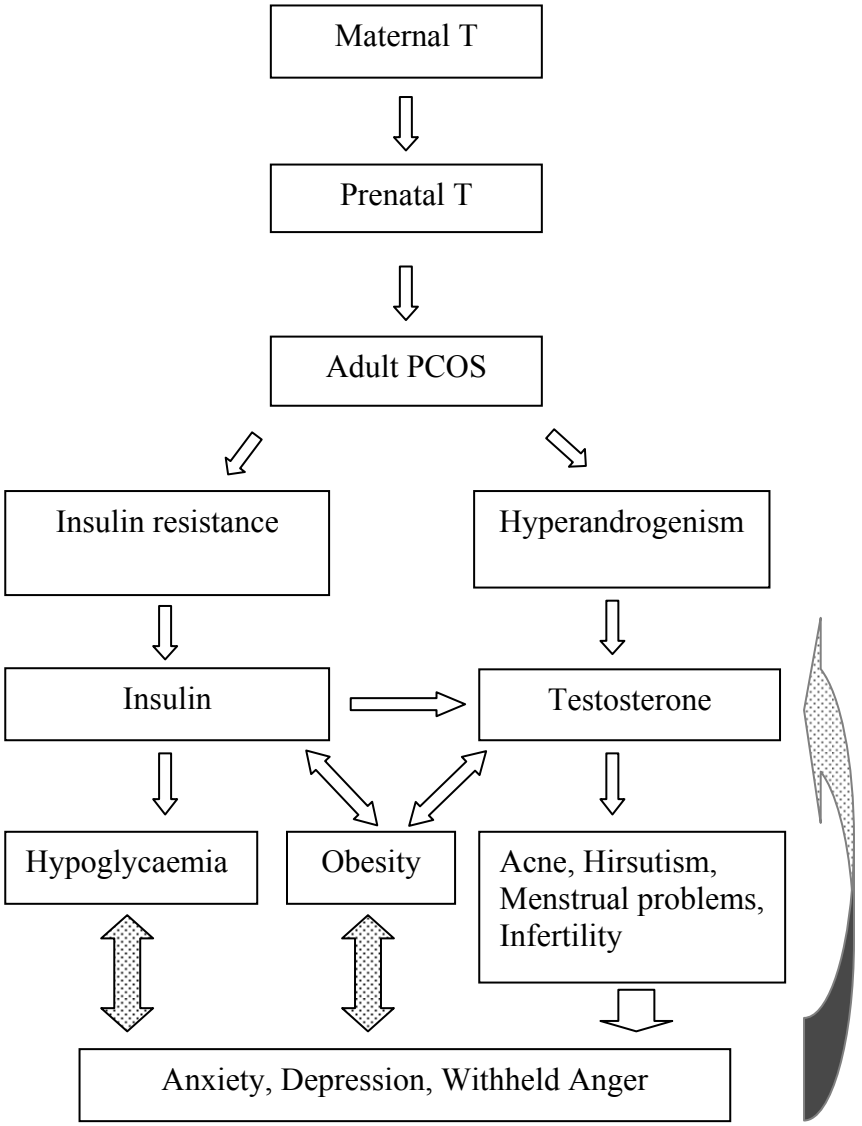
1.1 Overview of this thesis

Polycystic ovary syndrome (PCOS) is a medical condition that affects 5-10% of women (Franks, 1995). One of the characteristics of PCOS is elevated testosterone (T) levels. Research suggests that anxiety, depression, aggression, and various other psychological problems are greater in PCOS than in healthy women (Farrell and Antoni, 2010). However the cause of these problems has been a matter of debate.

Three hypotheses regarding the cause of psychological problems in PCOS are explored in the present thesis. The first hypothesis is that exposure to elevated levels of T prenatally has organizational effects on neuroanatomical structures, and this has consequences for psychological functioning in adults with PCOS (Ingudomnukul et al, 2007). The second hypothesis is that psychological problems are caused by elevated T circulating in the bloodstream of adults (Weiner et al, 2004). In general, the second hypothesis is that the effect of T is indirect; T causes distressing conditions such as obesity, infertility, and skin problems, and these symptoms of elevated T produce psychological problems. The third hypothesis is that low blood sugar, which is common in women with PCOS, is a direct cause of mood problems (Brand-Miller, Farid & Marsh, 2004). The three published papers in this thesis will explore key aspects of these three hypotheses.

The flowchart in Figure 1 shows the pathway to psychological problems in PCOS suggested by the findings of the author's PhD research programme.

Figure 1. A model of the main pathways to psychological problems in PCOS. The double headed shaded arrows indicate that comfort eating may feed back from mood to hypoglycaemia and obesity. The shaded arrow at bottom right of the figure indicates that anxiety may increase adrenal androgen production.



In this thesis, the basis for the first hypothesis is explored in Chapter 3 by testing the key issue of whether total T levels are higher in PCOS pregnancies than healthy pregnancies. Dumesic, Abbott & Padmanabhan (2007) review animal studies in several species where the pregnant mother is given large doses of T, resulting in developmental dysregulation of the hypothalamus & pituitary glands in the offspring. Prenatal exposure to T has also been found to masculinise brain structure in female sheep (Roselli et al, 2007). Therefore exposure to elevated T prenatally may be a risk for the development of PCOS in later life and could in theory lead to psychological problems. The aim of the study in Chapter 3 was to establish whether there was evidence that T was raised in the prenatal environment of daughters of women with PCOS compared to healthy pregnancies. The main outcome of interest was whether total T levels in the umbilical cord blood of newborns of mothers with PCOS was higher than healthy pregnancies.

To provide some background to the first study, a meta-analysis and systematic review of research on umbilical cord total T levels in healthy pregnancies is presented in Chapter 3. This review is original in that it establishes that there is usually a sex difference in umbilical cord T, and offers the first evidence supporting the suggestion that T in the umbilical vein is of maternal/placental origin, an important point in identifying the etiology of PCOS. The meta-analysis also evaluates the quality of methods of measuring umbilical cord T.

Chapter 5 tests the second hypothesis – suggested by the findings of Weiner et al (2004) - that circulating total T is related to anxiety, depression, and other mood problems in women with PCOS. Background material on this topic is presented in a meta-analysis and systematic review of research into self-reported anxiety and depression in PCOS (Chapter 2).

The finding in Chapter 5 that mood was not related to total T in PCOS prompted a rejection of the second hypothesis and the search for plausible new

hypothesis. The third published study (Chapter 6) represents the result of this reappraisal, and tests the hypothesis that low blood glucose levels might be related to mood problems in PCOS. In this pilot study the outcome measures were psychological symptoms of low blood glucose. The third hypothesis links into the first hypothesis in that animal studies have found that fetal exposure to T in early gestation programmes for insulin resistance and type 2 diabetes in later life (Dumesic, Abbott & Padmanabhan, 2007), and chapter 6 suggests that it is this type of metabolic dysregulation that causes mood problems seen in adults with PCOS.

The final chapter presents a discussion of the implications of the research presented in this thesis. An evaluation of theory, methodology, and clinical applications is also made. Conclusions and suggestions for future research are also presented.

The following sections will firstly review the psychological dimensions associated with PCOS and the symptoms of PCOS, secondly explore the role of testosterone in PCOS, and finally look at hypotheses regarding the etiology of PCOS.

1.2 The Impact of PCOS on Quality Of Life

Health-related quality of life (HRQoL, or HRQL, or simply QoL) is defined as "a multidimensional concept that encompasses physical, emotional and social aspects associated with a specific disease or its treatment" (Jones et al 2008, p.15). Many of the studies in the following sections focus on various psychological aspects of PCOS, and although not all are QoL, it might be considered that QoL is implicitly measured in studies of anxiety and depression because there is some evidence that the three constructs are related. For example, Lipton et al (2006) found that QoL and Hospital Anxiety and Depression Scale (HADS) scores were highly correlated in women with PCOS, indicating that the poorer the QoL the more anxiety and

depression was experienced. Similarly, Barnard et al (2007) found that all PCOSQ (Polycystic Ovary Syndrome Questionnaire) domains were significantly correlated with depression ratings.

Using the SF-36 (Short Form-36), a scale for measuring HRQoL for illnesses in general, Coffey et al (2006) found that women with PCOS scored similarly for physical problems to women with diabetes, epilepsy, back pain and asthma, but much lower (i.e. worse QoL) on the psychological dimension.

1.3 Anxiety and Depression in PCOS

In one of the earliest studies of the psychological aspects of PCOS Monzani et al. (1994) found that 23 PCOS patient had significantly higher levels of anxiety and depression on the Crown & Crisp Experiential Index than 20 age–matched healthy controls. Research since then has very often found evidence for greater depression in women with PCOS than controls or normative populations. Although anxiety is less usually a focus of research, women with PCOS are sometimes significantly more anxious too.

Some recent examples of studies in this area are Weiner et al (2004) who found depression was higher in women with PCOS than in healthy controls matched for BMI, age, ethnicity and educational level, and using as covariates body dissatisfaction, acne, hisutism, and stress. Using logistic regression Hollinrake et al (2007) found that 103 women with PCOS were significantly more at risk of clinical depression than 103 controls. In a recent study of 49 women with PCOS and 49 age-matched controls, Mansson et al (2008) found higher rates of depressive episodes, social phobia, and antidepressant and anti-anxiety medication use in the PCOS group. They also found suicide attempts were 7 times more likely in the PCOS group. Manlove et al (2008) found that 34 women with PCOS reported less happiness than 27 controls, but this finding is undermined by the fact that happiness was measured using only one item.

Although it is clear that PCOS is a risk for depression, attempts to identify the specific cause of depression in PCOS have not provided a clear answer, probably because many variables could contribute (weight, acne, hirsutism, T etc) to depression in PCOS.

1.4 Depression, QoL, and obesity in PCOS

In their review of the QoL literature on PCOS Jones et al (2008) found that weight problems had the greatest negative impact on QoL. This problem starts in adolescence when the symptoms of PCOS manifest and continue into adulthood: using the CHQ-CF87 (Child Health Questionnaire) Trent et al (2005) found that 97 adolescents with PCOS had worse HRQoL for weight issues than 186 healthy adolescent girls. McCook (2005) found that weight was the main PCO SQ domain affecting adult women (mean age 30.4 $\pm$ 5.5). McCook (2005) and Hahn et al (2005) found a significant correlation between QoL weight scores and BMI suggesting that a higher BMI causes worse quality of life regarding weight. Although Trent et al (2005) and McCook (2005) didn't measure depression specifically – the PCOSQ measures the emotional impact of weight problems rather than BMI - most studies that have measured depression have found obesity related to depression, both in healthy women (Stunkard et al, 2003) and in PCOS (Rasgon et al, 2003). However not all studies have found this relationship, for example, McCook (2002) and Hahn et al (2005).

It is common to use BMI as a covariate in studies of PCOS (see discussion below regarding the pitfalls in doing so) and when BMI is controlled group differences in depression scores are reduced (for example, Elsenbruch et al, 2003) suggesting that BMI is a contributor to depression. However women with PCOS often still have significantly higher depression than normative samples (Keegan et al, 2003) and age/weight-matched controls (Weiner et al, 2004). Hollinrake et al (2007) found that a subgroup of obese women with PCOS (n=73) were

significantly more at risk of clinical depression than obese controls. It could be that the other symptoms of PCOS add to the depression risk, but it could also be that the type of obesity seen in PCOS is more distressing because android (or central) obesity has a less feminine appearance than gynoid obesity (weight gain on the hips) and is more resistant to ordinary dieting (Marsh and Brand-Miller, 2005).

There is evidence that in general central obesity ('apple shaped' body rather than 'pear shaped') is considered unattractive in many cultures (Brown, 1991). This body shape is caused by elevated androgens and is typical of PCOS. This cross-cultural evidence suggests that although it maybe be secondary in QoL terms to other issues, BMI is an issue for women with PCOS regardless of cultural background.

Jones et al (2008) point out that BMI does not necessarily affect QoL domains other than weight (for example, McCook et al, 2005). Obesity contributes to higher androgen levels via the decrease in aromatase activity in adipose tissue (Wake et al, 2007) so we should expect that BMI has a global impact on features affected by T i.e. the other PCOSQ domains of acne, hirsutism, fertility, and (to some extent) emotions (see section on effects of T, below). However the fact that a direct correlation between BMI and other QoL variables does not always exist indicates the complexity of the interrelationships between the many variables involved in PCOS. Psychological factors may ameliorate a relationship between BMI and QoL; for example, good general coping skills might reduces the QoL impact of BMI. A measurement issue that might contribute to the lack of direct BMI/QoL correlation is the possibility that the waist:hip ratio (WHR) is a better measure of android or central obesity than BMI (Carranza-Lira et al, 2006) thus the WHR might be more sensitive to QoL issues than BMI.

Overall the evidence suggests that obesity contributes to depression in PCOS, but one or more other factors are also contributors. However the fact that there is at least some evidence that HRQoL is improved by weight loss in both

women with PCOS (Hahn et al, 2006) and in the general population (Kolotkin et al, 2001b) underlines the importance of QoL.

1.5 Depression, QoL and hirsutism in PCOS

Hirsutism is associated with elevated androgen levels in general, and varies with ethnicity (Young & Sinclair, 1998). Kitzinger & Willmott (2002) interviewed 30 women with PCOS, and it emerged that the condition is associated with feeling poorly adjusted to the feminine gender role. This is largely due to the affects of hyperandrogenism for example, hirsutism. Using the HADS, Lipton et al (2006) found that 74% of 88 women who had problems with facial hair showed clinical levels of anxiety and 30% had clinical levels of depression. However self-esteem (measured using the Rosenberg Self-Esteem Scales and WHOQoL-BREF) was not lower than normal in this group.

Elsenbruch et al (2003) found that 50 women with PCOS felt particularly unattractive if hirsutism was a problem, and this caused them to experience sex as less satisfactory than 50 female controls. This was true even though the rates of sexual intercourse was similar in both groups, and when BMI was used as a covariate.

Clayton et al (2005) found that laser treatment for facial hirsutism improved HRQoL and HADS anxiety and depression scores compared to a control group. In contrast Keegan et al (2003) did not find a significant correlation between distress and hirsutism in their study of 53 women with PCOS. Also there is not a consistent relationship between objective measures of hirsutism and QoL for hirsutism; while McCook et al found the expected correlation, Trent et al (2002) and Guyatt et al (2004) did not. The sometimes inconsistent relationship between objective versus subjective measures is a recurring theme in QoL research.

1.6 Depression, QoL and infertility in PCOS

Infertility is clinically defined as being unable to conceive within 12 months (Abma et al, 1997) and fertility problems are common in PCOS because elevated T and BMI disrupt the menstrual cycle. Although Trent (2003) found that adolescents with PCOS were 3.4 times more likely than healthy controls to have worries about future fertility, McCook (2002) and Himelein & Thatcher (2006a) found no significant difference between the depression ratings of fertile and infertile women with PCOS.

McCook (2005) found that women with PCOS who had miscarried had lower PCOSQ scores than women with PCOS who had not ever succeeded in becoming pregnant. van Wely et al (2004) randomized 168 women with PCOS into two fertility treatment conditions (laproscopic electrocautory Vs recombinant follicle stimulating hormone) and found that successful fertility treatment improved QoL Short Form 36 (SF-36) scores for emotional and mental health problems, though also increased role limitations (for example, feeling that they had accomplished less) due to normal physical restrictions of pregnancy.

1.7 Anxiety and stress in PCOS

In their review of PCOS and stress Eggers & Kirechenhast (2001) suggest that PCOS may be a cause of distress to the sufferer (due to symptoms) as well as be exacerbated by psychosocial stressors (for example, major life events). As mentioned previously, stress activates the adrenal glands via the HPA (Reiche et al., 2004) and because women produce roughly 25% of their T from their adrenal glands (Burger, 2002) the level of stress a woman experiences in her daily life may exacerbate her PCOS symptoms.

As with depression, obesity has been found to contribute to psychological distress in PCOS (Elsenbruch et al, 2006). Several studies have found that women with PCOS experience raised anxiety levels (Mansson et al, 2008; Elsenbruch et al, 2003; McCook, 2002; Greiner et al, 2005; and Keegan et al, 2003). Barnard et al

(2007) found that almost one third of women with PCOS reported experiencing anxiety compared to under 10% of healthy controls. This represents a significant difference, but the validity, reliability, and even the items used in Barnard et al's bespoke measure of anxiety are uncertain because this measure has not been published.

There is evidence that women with PCOS show a stronger HPA (hypothalamic-pituitary adrenal axis) response to a stressor than healthy women. Mociali et al (1990) found that although a baseline measure of trait anxiety was similar between groups, after undertaking mental arithmetic problems state anxiety and various hormones were disrupted in 13 women with PCOS compared to 13 controls. Using the Stroop task as a stressor, Gallinelli et al (2000) found that cortisol levels became higher in their PCOS group compared to controls.

Benson et al (2009) compared 32 women with PCOS to 32 BMI- and age-matched controls in their HPA responsivity to a stress-inducing public speaking task. They found that in the PCOS group, ACTH, cortisol, and heart rate were all significantly higher than controls. Use of metformin did not have an impact on these differences.

Greiner et al (2005) found that 18 women with PCOS scored significantly higher than 12 healthy controls for anxiety on Goldberg's General Health Questionnaire (GHQ-30), and although depression was higher in the PCOS group on the Hamilton Depression Rating Scale the difference did not reach significance. Also, although the circadian cortisol pattern was not different between groups, a significantly lower percentage of the PCOS group secreted low levels of urinary cortisol and a significantly higher percentage of the PCOS group were unable to suppress cortisol below 1 ug/mL in response to an overnight 1 mg dexamethasone suppressor test; both of these results are indicators of increased sympathetic tone in the PCOS group. The authors conclude that the biochemical alterations caused the

anxiety, but how strong this relationship might be was not explored - the authors did not statistically correlate the psychological and biochemical outcomes.

1.8 **Neuroticism**

A character trait is a consistent pattern of thinking, feeling, and behaving (Perwin et al, 2004). Neuroticism is a character trait that combines features of anxiety, depression, and anger. Eysenck (1967) suggests that neuroticism is caused by lability of the ANS (autonomic nervous system) and predisposes a person to cope poorly with stressful events. Recent evidence has found a strong correlation between anxiety and neuroticism (Hettema et al, 2004).

Although neuroticism has not been measured in a PCOS population before, it has been linked to three conditions that are associated with PCOS: hirsutism, cardiovascular disease (CVD), and fertility problems. Barth et al (1993) studied 69 hirsute patients and found high EPQ Neuroticism scores. None were identified as having PCOS, and the authors did not compare the results to a control group. However the mean score of 14.6 ± 4.0 is roughly 25% higher than the norm for women (Eysenck et al, 1985). It is also higher than the scores for obese women; in a study of 7889 English men and women aged 30 to 50 years old (Faith et al (2001).

Neuroticism is associated with health outcomes too. In a 21 year prospective survey of 5424 adults in the general population of men and women, Shipley et al (2007) found that after controlling for gender, age, social class, education, smoking, alcohol consumption, physical activity, and health, high neuroticism scores were significantly related to mortality from cardiovascular disease. Verhaak et al (2005) studied 187 women who had recently failed to conceive after one treatment cycle with either IVF or ICSI. The causes of infertility were: 33% idiopathic (i.e. of spontaneous or unknown cause), 22% female causes, 35 % male causes, and 10% a combination of male and female causes. The mean

age was 34.3 years, and the mean duration of fertility problems was 3.3 years. Measures included the EPQ, STAI (State Trait Anxiety Inventory) and BDI (Beck Depression Inventory). The infertile women scored slightly below the norm for neuroticism at baseline (before failing the first cycle) 4.7 (sd 3.0) Vs 5.72 (sd 2.91). However after the failed cycle there was a significant increase in anxiety and depression ($p<.001$ for both variables). The largest correlation between the change in the anxiety score from baseline to time 2 was for neuroticism ($p<.001$). The change in depression was also correlated with neuroticism ($p<.001$). Multiple regression showed that baseline neuroticism scores were the best predictors of anxiety and depression after the failed cycle.

The above studies demonstrate that neuroticism has known links with conditions that are associated with PCOS: hirsutism, CVD, and infertility.

1.9 Anxiety, stress, depression and fertility

Fertility problems are clearly distressing, but it is important to note that the causality may be reciprocal i.e. there is evidence that psychological stress can impair fertility (Boivin and Takefman, 1995). In his classic studies Selye (1950) found that exposure to stressors caused ovarian atrophy in rats. Psychological anxiety can activate the HPA axis, and because stress hormones interact with fertility hormones (for example, LH (luetinising hormone, and FSH (follicle stimulating hormone)) the HPG (hypothalamic-pituitry gonadal) axis is vulnerable to the affects of stress (Berga, 1996). Rivest and Rivier (1995) found that cortisol reduces levels of sex hormones by disrupting their synthesis. Anxiety and high cortisol levels, depression, avoidance and high expression of emotion also have been associated with lower pregnancy rates from fertility treatment (Demyttenaere et al, 1992; Smeenk et al, 2001, 2005). Smeenk et al (2001) found that anxiety and to a lesser extent depression were significantly negatively correlated with

pregnancy outcome in their study of 291 women who underwent fertility treatment with IVF/ICSI (in vitro fertilization/intracytoplasmic sperm injection). Klonoff-Cohen et al (2001) suggest that while the anxiety generated by fertility treatment procedures affects biological outcomes (for example, number of oocytes fertilized) baseline measures of acute and chronic stress are related to pregnancy outcomes such as birthweight.

Cwikel et al (2004) suggest that chronic stress should be treated prior to fertility treatment, and although there has been very little research in this area there is evidence that psychological interventions improve fertility rates. For example Sarrel and DeCheney (1985) found that a psychotherapeutic interview yielded a pregnancy rate of 60% for 10 infertile couples compared to 10% for 10 controls. Hosaka et al (2002) found that 5 weekly 90-minute sessions of relaxation, guided imagery, and stress management reduced emotional distress and improved the pregnancy rate compared to controls (38% Vs 14%). Natural Killer (NK) cell activity was also lower in the treatment group (48% Vs 34%). NK cell activity is not usually measured in fertility studies but high NK cell activity has been observed in idiopathic infertile women and unexplained recurrent miscarriage (Matsubayashi et al, 2001). Levitas et al (2006) used a single session of hypnosis with 89 patients during the embryo transfer procedure and found a significantly higher pregnancy rate than 96 no-hypnosis controls (58% Vs 30%, $p<.05$). The hypnosis group also had a higher implantation rate (28% Vs 14%, $p<.001$). Domar (2000) however found no significant effect of CBT + relaxation on pregnancy outcomes in a randomized controlled trial (RCT).

As is seen with the QoL measures, self-reported anxiety is not always reflected in objective measures (for example, Gold et al, 2003). There is also the methodological issue of establishing the amount of variance in anxiety ratings that could be a cause (trait/chronic anxiety) or result (state anxiety) of fertility issues;

this is not an easy task because the relationship between infertility and psychological problems is reciprocal.

1.10 Social avoidance

There is evidence that from an early age women with PCOS may have interpersonal difficulties. Manlove et al (2008) found that compared to controls, women with PCOS reported that as children they felt less cared for by both their mother and father, and resented or disliked their brother more. This same group reported less sociability in adolescence, and reported more time spent reading and babysitting, and less time playing sports or dating boys.

In a non-PCOS group Barth et al (1993) found that patients with hirsutism suffered from social and emotional stress, and it could be that the symptoms of PCOS are embarrassing and make socialising uncomfortable. In support of this suggestion, Mansson et al (2008) found higher rates of social phobia in women with PCOS compared to age-matched controls. Elesenbruch et al (2003) found that women with PCOS scored significantly higher than controls on the SCL-90 (Symptom Check List-90) Interpersonal Tender Mindedness scale. This difference remained even after Bonferroni correction and use of BMI as a covariate in ANCOVA, suggesting that BMI was not the cause of interpersonal sensitivity, though other PCOS symptoms may have been.

The evidence is mixed regarding the degree to which PCOS symptoms cause social unease. Keegan et al (2003) did not find women with PCOS scored higher than normative levels on the Social Avoidance and Distress Scale. Using the SF-36, Hahn et al (2006) found that although obesity worsened physical QoL, social and emotional QoL were not related to obesity. Coffey et al (2006) found that 22 women with PCOS scored worse than 96 healthy controls on all PCOSQ

and SF-36 subscales, apart from physical and social functioning. Clayton et al (2005) found that laser treatment for facial hirsutism improved HRQoL and HADS anxiety and depression scores compared to controls, but no significant improvements in the QoL social or environmental domain were seen, nor self-esteem.

Overall, while there is some evidence of a negative impact of PCOS on social QoL, the evidence is not overwhelming. BMI does not appear to be the root cause. The direction of any causal relationship between social avoidance and factors such as anxiety or depression is open to speculation; Thatcher and Himelein (2006b) suggest depression causes social avoidance, but it could equally be suggested that the symptoms of PCOS cause anxiety, and this causes social avoidance.

1.11 T and psychological disturbance

The evidence relating T to psychological and emotional disturbance is not as clearcut as might be expected. Some studies of the general population have observed a raised free androgen index (FAI, a measure of bioavailable T) in women suffering from premenstrual syndrome (PMS, for example Eriksson et al, 1992), whereas other studies have found that androgen levels were not significantly different in women with PMS compared to women without PMS (for example, Rubinow et al, 1989). Indeed some research has found that T is associated with positive mood in both men and women. For example Dabbs et al (1997) found that men and women who were naturally higher in T than their same-sex peers reported having a restless energy to socialize with friends compared to low-T sex-matched controls, but they did not feel more anger or aggression. Indeed assumptions that T causes rather than results from mental states may be erroneous. There is evidence from studies of humans (e.g. Booth et al, 1989) and non-human primates (e.g.

Bernstein et al 1983) that T increases as a result of winning in competitive encounters. Motivation before a competition is also associated with rising T (Salvador et al, 2003).

In women with PCOS, three studies failed to find a link between T levels and depression (Hahn et al, 2005; McCook, 2002; Rasgon et al, 2003). In a tightly controlled study, Weiner et al (2004) found that, contrary to expectations, the FAI was positively correlated with positive mood states in the PCOS group and negatively correlated for the control group; in other words free T seemed to have a calming affect on the PCOS group but not the controls. Thus for example State Trait Anxiety Inventory (STAI) ratings were negatively related to the FAI for the PCOS group ($r=-.39$, $n=27$, $p<.05$) but positively correlated to the FAI in the control group ($r= .55$, $n=27$, $p<.01$). Similar though less marked contrasts were seen for most of the State Trait Aggression Inventory (STAXI) and Aggression Questionnaire subscales. For example, for STAXI anger expression the correlation for the PCOS group was negative ($r=-.37$, $n=27$, $p<.05$) and for the controls was positive ($r=.37$, $n=27$, $p<.05$). However Weiner et al's conclusion that there is a cubic relationship between the FAI and mood states seems poorly founded given that this pattern of relationship was based on the combination of two different populations with significantly different FAI levels and with opposite patterns of correlations i.e. the pattern is bound to be nonlinear as the left side of the scatterplot is ascending (showing the positive relationship with FAI and disturbed mood for controls), and the right side descending (negative relationship with FAI and disturbed mood for PCOS).

Barnard et al (2007) found that two thirds of women with PCOS rated themselves on the Zung depression scale (Zung, 1965) as experiencing mild depression or worse, compared to less than one third of healthy controls. The rates were similar regardless of whether the women were taking anti-androgen

medication or not (for example, contraceptive pill) in both the PCOS and control groups, suggesting that either the anti-androgens did not alter androgen levels, or that androgen levels do not affect depression levels.

1.12 T and aggression in PCOS and healthy people

Monzani et al. (1994) found that compared to 20 age−matched healthy controls, 23 women with PCOS showed elevations (though non-significant) on Type A personality, a personality type associated with dominance, time urgency, hostility, competitiveness/leadership, and CHD. Although they did not measure T, the underlying assumption is that elevations in ratings related to dominance and aggression are caused by the elevated T often seen in PCOS.

A methodological issue regarding this and other studies of PCOS is whether the source of anger in these studies is T, or the effects of T (i.e. troubling symptoms like hirsutism) or the other effects of having PCOS. It is easy to understand that having the troubling symptoms of PCOS might cause negative feelings, and Sills et al. (2001) found that 67% of 657 women with PCOS associated their diagnosis with “frustration”. Elesenbruch et al (2003) found that the significantly higher aggression scores of their PCOS group was negated when BMI was used as a covariate and Bonferroni corrections were made, though it could be argued that the group difference was legitimate but negated by statistical overcorrection.

Assessing aggression in humans is a vast and complex area, and not central to the present thesis. Although males of a given species are generally perceived as being more aggressive than females, a review by Albert et al (1993) of the literature on aggression in humans concluded that there is little basis for the idea that testosterone causes aggression. Albert et al also cast doubt on the idea that

women are inherently less aggressive than men, and cite evidence from domestic violence studies of women's violence (against partners and children) that show rates of violence that are generally equal to those from men. This is supported by the meta-analysis by Archer (2000) finding that although in heterosexual partnerships women are more likely to be injured, women are also slightly more likely to use physical aggression and to do so more frequently.

In a double-blind cross-over study of surgically menopausal women (oophorectomized i.e. had their ovaries removed), Sherwin and Gelfand (1985) found that administration of T or T and E2 combined caused increased hostility. As mentioned above, Weiner et al (2004) found that aggression was negatively correlated with FAI for 27 women with PCOS, but positively correlated for age- and weight-matched controls. Archer et al (2005) found that the correlation between T and aggression was stronger in women than in men.

Bjorkquist & Niemela (1992) suggest that research on T and aggression has tended to look for overt forms of aggression (for example, physical rather than indirect or verbal aggression), and because men generally display the former type of aggression more than women, some researchers have erroneously concluded that men are more aggressive than women.

Condry and Condry (1976) found that people are more inclined to label a baby's crying in response to being startled as fear if they are led to believe it's a girl, but interpret the response as anger if they think it's a boy. Sometimes researchers in psychology appear to have viewed the world through a gendered schema when studying aggression.

1.13 PCOS, CAH and the masculinising effects of testosterone

PCOS is the commonest cause of androgen excess in women and is estimated to affect about 10% of women (Ledger & Clark, 2003). It affects similar rates of women across the world (Azziz, 2006). Despite its high prevalence, most aspects of this condition (e.g. etiology) are only partially known, and reliably effective treatment remains elusive. Polycystic ovaries (or *pco* – multiple small cysts on the ovaries) may be present in the full-blown syndrome (PCOS), but is not a necessary or sufficient condition according to the generally accepted definition, the *Rotterdam Criteria* (described in detail below).

Broadly speaking, the effects of elevated T are known as *hyperandrogenism.* Elevated T is one of the characteristics of PCOS, and there is growing evidence that PCOS is associated with various physical and psychological conditions, largely thought to be caused by elevated T. It should be noted, however, that although it is normal and healthy for women to have some T in their bodies, levels over the norm can be problematic.

Elevated T has been found to have various physical and psychological effects in a condition with some similarities to PCOS, classical congenital adrenal hyperplasia (CAH). CAH is a relatively rare condition (1 in 15 000 live births) caused by enzymatic deficiency in the glucocorticoid pathway and resulting in overproduction of adrenal androgens prenatally (Miller and Levine, 1987) and continuing until identified and treated, usually shortly after birth in females. The levels of androgens experienced by the fetus with CAH are difficult to estimate but probably much higher than seen in the fetus in a PCOS pregnancy. Forest et al (1981) found that T in the fetal environment of girls with CAH was in same range as that of normal male pregnancies at their highest point during the 'testosterone surge' of prenatal weeks 8-24 (Smail et al 1981). In CAH these levels are high enough to cause females to be born with ambiguous genitalia. PCOS has not been found to affect the external genitals of newborns, though can on rare occasions cause *clitoromegaly* (enlargement of the clitoris) in adulthood (Marshall, 2001).

The main findings from CAH research regarding psychology are that girls with CAH are more aggressive than their unaffected sisters (Pasterski et al, 2007), show increased male-typical play behaviour (e.g. Berenbaum and Hines, 1992), reduced heterosexual orientation (e.g. Hines et al, 2004), better targeting ability (Hines et al, 2003), and reduced interest in infants (Leveroni & Berenbaum, 1998; Mathews et al, 2008). The degree of influence on male-typical play behaviour has been found to relate to the severity of the CAH disorder (e.g. Nordenstrom et al, 2002).

Sample sizes in CAH research are small because the condition is rare. However the literature on CAH provides a reasonably sound analogue to the kinds of effects of T that might be seen in PCOS, and indeed there is some evidence for similar types of effects of T in PCOS as CAH. For example, Manlove et al (2008) found that women with PCOS reported less female-typical behaviour in childhood, though not at adolescence or adulthood. Agrawal et al (2004) found a significantly higher prevalence of PCOS and higher T levels in lesbians compared with heterosexual women, but this finding has not been replicated by Sutter et al (2008). Regarding aggression, Elsenbruch et al (2003) found that women with PCOS were significantly more aggressive than controls, but this difference became non-significant when BMI was statistically controlled for. Ingudomnukul et al (2007) found that PCOS was more common in women with autistic spectrum disorder than healthy controls. However it should be noted that the difference was because PCOS was unusually low in the control group sample (2.7%), and normal rather than high in the ASD group (11.3%). Regarding cognition, women with PCOS have been found to perform less well than controls on verbal fluency, a cognitive task that usually favours women over men (Schattmann & Sherwin, 2007b), although other cognitive outcomes that often show sex differences (e.g. three dimensional mental rotation) showed no difference between PCOS and control women.

The majority of studies of boys with CAH show no difference between subjects and controls. There are two published exceptions: one study found reduced rough & tumble play (Hines & Kaufman, 1994), and another found reduced self-reported male-typical behaviour in boys with CAH (Slijper, 1984), but these anomalies in the CAH literature may be caused by the boys' frequent hospitalisation during their first two years of life (Hines, 2004). Similarly, Effects of PCOS in males seem minor compared to consequences for females, and are unreplicated. For example, early baldness has been suggested (Dusková & Stárka, 2006) as have raised dehydroepiandrosterone sulfate (DHEAS), and a tendency to insulin resistance (IR) (Sam et al 2008), but as yet none of these suggestions have been substantiated. However amongst two relatively large samples of non-PCOS participants a significant relationship has been found between maternal T during pregnancy and gender-role behavior in the offspring. Hines et al (2002) found markers of high maternal androgen associated with masculinized gender-role behavior in female offspring, and Udry et al (1995) found that gender-typical behavior was related to hormones from maternal serum only during the second trimester,

1.14 Biochemical aspects of testosterone

The principle gonadal hormones are T and estradiol (E2). T is an anabolic steroid and the prototypic hormone of the androgen family of sex hormones. Androgens affect masculine sexual development and function. T is considered a male sex hormone because the testes predominantly produce T, and T levels are normally about 10 times higher in men than women. Similarly E2 is considered a female hormone as the ovaries predominantly produce estrogens, and E2 levels are usually much higher in women than men. T is synthesised from androstendione and androstenediol, both of which come from DHEA. T can be synthesised into E2 or the androgen dihydrotestosterone (DHT) which is roughly three times more potent

than T. It is of note that DHEAS is a product of the adrenal glands and a precursor of T; thus not only is T produced by the gonads (testes and ovaries) but is also a byproduct of the adrenal glands. It is estimated that in healthy women roughly 25% of T is of adrenal origin (Burger, 2002), though the percentage is probably higher in PCOS because the adrenals are hyperresponsive to the stress hormone adrenocorticotropic hormone (ACTH) in PCOS (McKenna and Cunningham, 1995; Moran et al 2004).

E2 is the principal estrogenic hormone. The other main estrogen is estrone (E1). Perhaps surprisingly, T is the biochemical precursor of E2. T is converted to E2 by the enzyme aromatase, and it is possible that a dysfunction in aromatase contributes to the higher T levels seen in PCOS (Xita et al, 2008).

Sex hormone binding globulin (SHBG) is the main substance that attaches to sex hormones, and in binding to T and E2 renders them biologically inert. Its binding affinity is roughly twice as strong for T than E2 (Rosner, 1991). Thus higher levels of circulating SHBG will reduce androgenic activity more than estrogenic. Knowledge of binding by SHBG has led to the use of measurements of *free androgens,* for example, the free androgen index (FAI, sometimes known as the free testosterone index, or FTI) which calculates the amount of T that is biologically available in the blood once the amount of bound T has been taken into account. The FAI is often considered a better measure of the androgenic potential in serum than total T. However the FAI is not always used in because of the cost and inconvenience of the extra step of measuring SHBG is not always considered to outweigh the benefit of knowing the FAI as opposed to the total T level.

T has a masculinising effect and can cause females to gain male-typical characteristics or lose female typical ones. The effects of T can be subdivided roughly into two types: 1/ *organizational effects* occur pre- or neonatally and are permanent, and 2/ *activational effects* occur after puberty and are reversible. Structural changes in the brain can result from either type of influence.

Three models describe the effects of sex hormones: the gradient model, the classic model, and the multidimensional model. The *gradient model* suggests that the effects of sex hormones are dose-dependant and the level of exposure to a hormone will affect the degree to which masculinisation or feminization occurs. The *classic model* suggests that T causes masculinisation and reduces feminization, and absence of T causes feminization and reduces masculinization, and there is a lot of evidence to support this model (Pfaff et al, 2002). For example, all mammalian fetuses will develop as phenotypic females unless the Y chromosome (called SRY) causes the gonads to differentiate as testes and produce T (Wilson et al, 1981). Prenatal exposure to T can alter brain structure, for example, creating a male-typical development of aromatase-expressing neurons in the sexually dimorphic nucleus of female sheep (Roselli et al, 2007). The *multidimensional model* (see below) allows for expansion beyond the gradient and classic models.

Although estrogens are necessary for female-typical maturation and function in adulthood, they appear to have little or no role in promoting female development of the fetus. However active feminization of behaviour by estrogens occurs at other times, for example, Dunlap et al (1978) found that in rats near puberty, ovarian hormones fix some aspects of female typical sexual behaviour.

There is also evidence that there are critical or sensitive periods for the influence of T on development in animals, though evidence in humans is limited (principally due to ethical constraints in research this area). For example, female mammals exposed to T during pre- or neonatal critical periods show increased sexual behaviour in adulthood toward females and reduced sexual responding to males; likewise depriving males of T during pre- or neonatal critical periods results in reduced male-typical and increased female-typical sexual behaviour in adulthood (Goy and McEwen, 1980; Beach 1975).

There is evidence that masculinization and feminization are separate dimensions, and the simple idea of T causing male-typical development and the

absence of T causing female- typical development is an oversimplification; there are examples in rodents that demonstrate that in many cases T needs to be converted to E2 before acting on the E2 receptors to create male-typical neural and behavioural development (McCarthy, 2008). However it is likely that the type of pathway will differ by species and by type of behaviour, and Hines (2009) suggests this sexual differentiation is best understood by a *multidimensional model* that resolves apparent contradictions between earlier models. "This multidimensional conceptualization allows not only for differences in critical periods for hormones to act on specific behaviors, but also for different mechanisms… to be involved in differentiation of each characteristic. A multidimensional model is also consistent with evidence that individuals can vary in sex-related behavior from one dimension to another, being strongly sex-typical in some respects, but less so, or even sex-atypical, in others" (Hines 2009, p.5).

It can be difficult to differentiate between the effect of nature and the postnatal environment, especially when studying human behaviour. Traditionally, the literature on sex difference uses the term 'gender' to identify sex differences with cultural causes (for example the wearing of trousers rather than skirts) and the term 'sex' for difference due to biology (for example, having internal ovaries rather than external testes). However in many cases it is very difficult to establish whether a sex difference is mainly due to nature, nurture, or is some roughly equal combination of both. For this reason some authors (for example, Maccoby 1988) use the terms sex and gender interchangeably and with these caveats.

1.15 The role of insulin in androgen production

Insulin causes androgen production in the ovaries (Yen, 1991), and there is a positive correlation between T and insulin levels in women with PCOS (Buffington et al, 1991). Indeed insulin and T are in a cyclical relationship: insulin promotes T,

T promotes visceral fat and insulin resistance, and this elevates insulin levels (Stanley et al, 2008).

Insulin resistance (IR) can be genetic or the result of a lifestyle characterised by a lack of exercise, high carbohydrate diet, and stress. IR is caused when the number of insulin receptors on cell walls is reduced, and in PCOS this reduction can be up to 75% (reduced from roughly 20 000 receptors to 5 000 receptors). This means that the ability of serum glucose (blood sugar) to enter the cell to be converted to energy is reduced, and must instead remain in the bloodstream until converted by the liver into fat. The accumulation of fat leads to obesity, which in itself can contribute to PCOS. In women, carrying weight on the stomach rather than hips is known as central (or android) obesity, and is related to elevated androgen levels. Although IR occurs in non-obese women (Dunaif et al, 1989) non-diabetic women with central obesity typically have elevated androgens, IR, and hyperinsulinaemia (elevated blood insulin levels) (Kissebah et al, 1982). It seems likely then that insulin plays an important role in PCOS, though whether it is more important than T is open to debate (Azziz et al 2008) and may depend on the PCOS phenotype in question (see below) or the level at which etiology is being considered.

Drug treatment of PCOS usually aims to reduce T levels, either indirectly by using the diabetic drug metformin or directly by using anti-androgens or contraceptives that have an anti-androgenic effect (for example, Dianette). Metformin is also used with the primary aim of reducing insulin resistance in PCOS, as insulin resistance is not uncommonly comorbid with PCOS (Dunaif et al., 1989). Hunter and Sterrett (2000) suggest that metformin reduces testosterone levels and restores normal menstrual cyclicity. Pasquali et al. (2000) found that metformin decreased testosterone levels in women with PCOS, and that both women with PCOS and healthy controls treated with metformin experienced a reduction in their body mass index (BMI, calculated using metric units as weight

divided by height squared). In a case study of a woman with untreated PCOS and major depression, Rasgon et al. (2002) found that treatment with metformin and the spironolactone (an anti-androgen also used for liver and heart complaints) resolved both the depression and PCOS. Despite IR often being a feature of PCOS, the ovaries remain sensitive to insulin; there is as yet no explanation for this phenomenon (Diamanti-Kandarkis et al, 2008).

1.16 Types of PCOS

PCOS is today seen as a heterogeneous syndrome where a range of symptoms may be present or absent, and no distinct categories are very evident. For example, in their influential paper Hunter and Sterrett state that PCOS "is perhaps best viewed as a spectrum of symptoms, pathologic findings and laboratory abnormalities" (Hunter and Sterrett 2000, paragraph 10).

Identifying types, or more specifically *phenotypes* (observable physiological characteristics created by the genes and environment) is a complex task because the most recent definition of PCOS, the Rotterdam criteria (Rotterdam ESHRE/ASRM (2003), explained in detail below) allows for a range of expression of the syndrome. Diamanti-Kandarakis et al (2006a) note that one of the problems of research in this area is that there is not simply one type of PCOS, and genes for one type (e.g. obese/ anovulatory) may not contribute to the development of another (e.g. lean/ ovulatory). In other words the fact that there are several overlapping phenotypes makes it difficult to identify the underlying genotype(s).

There are important distinctions to be made in the types of problem experienced by women with PCOS e.g. insulin resistant Vs non-insulin resistant (Acien et al, 1999) but there is no consensus as to whether these basic dichotomies constitute types. The most immediately obvious distinction that has some

agreement amongst clinicians is the distinction between the obese type that features insulin resistance (and possibly hyperandrogenism) and the lean type that is often hyperandrogenic. The lean type is far less common; in Spain the estimated rate of PCOS in lean women is 5.5% (Barclay & Murata, 2006).

Although these two broad types are reasonably well established, there is a lot of speculation about the degree to which there are other distinct categories of PCOS, for example, types based on three differential responses to human corticotropin-releasing hormone suggested by Kondoh et al (1999). Differences in type can also be seen cross-culturally. For example, women from the Pacific Islands with PCOS are less inclined to acne and hirsutism than European women (Williamson et al, 2001). There is also the question of and the degree to which there is comorbidity between PCOS and the metabolic syndrome ('syndrome x'), and how to categorise cases where overlapping occurs.

Diamanti-Kandarakis et al (2008) describe two types of PCOS which they suggest are manifest from birth. The possibility of diagnosis at birth represents a significant advance, not least because early diagnosis offers the important possibility of early treatment. The first phenotype is expressed in low birth weight, but the weight is regained within 12 months. Premature adrenarche occurs, and at adolescence hyperandrognesim and anovulation occur, but with normal ovarian morphology. They second type is overweight at birth, is obese in childhood, and experiences the complete 'triad' of problems of PCOS i.e. hyperandrogenism, anovulation and pco (see also section on PCOS in childhood, below).

More longitudinal research in this area is necessary, and a good example of the insights that can be gained by taking a longitudinal approach is a recent study by Franceschi et al (2009). They followed up 46 adolescent girls who had precocious puberty (with no identifiable pathological cause) and found that at age 18 ($\pm$3 years) 32% of them had PCOS, mainly characterised by pco and elevated T (either by clinical signs e.g. hirsutism, or assay). Obviously this cohort will be

tested again, but the evidence so far suggests that diagnosis of a specific type of PCOS (pco + hyperandrogenism) might be possible at a young age. The importance of this is that the earlier the type is identified, the earlier appropriate treatment can begin. In the case of this particular phenotype, it would seem likely that anti-androgens (e.g. spironolactone) would be more appropriate than insulin sensitizers (e.g. metformin). However without the knowledge of the type of PCOS that is likely to develop from precocious puberty, there would be no way of deciding whether treatment with either antiandrogens or insulin sensitizers would be more appropriate. From different combinations of these three criteria five different types can be made, and means that neither pco nor hyperandrogenism are necessary components for diagnosis. By contrast, the National Institutes for Health definition of PCOS (NIH, 1990) which was used prior to the Rotterdam revision, defined PCOS using criteria 1 and 2 above only. In other words, the Rotterdam criteria are more inclusive and suggest a greater prevalence of PCOS (up to 12% of women according to Azziz et al, 2004) than the NIH definition (up to 8%, Norman et al, 2007).

The inclusion of pco in the Rotterdam definition not only makes sense at face value, but also biochemically because the cysts (immature ovarian follicles) prevent the conversion of T to E2. In the PCOS ovary an abnormal number of ovarian follicles develop, but don't develop to maturity. Mature follicles contain granulosa cells that express aromatase, but in PCOS (and pco) this stage is of development is not reached (Gougeon, 1996). Note that insulin resistance does not figure in either definition despite the high rates of insulin resistance seen in PCOS and the fact that insulin leads to increased T levels in PCOS (Buffington et al, 1991).

Not everyone accepts the Rotterdam criteria e.g. Azziz (2006). Indeed in October 2008 a new Androgen Excess-PCOS Society Task Force which was set up

to define PCOS concluded that "PCOS should be defined by the presence of hyperandrogenism (clinical and/or biochemical), ovarian dysfunction (oligo-anovulation and/or polycystic ovaries), and the exclusion of related disorders" (Azziz et al 2008, paragraph 1). Predictably, not all members of the Task Force agreed; a minority thought it important that future research not rule out the possibility of non-hyperandrogenic PCOS.

One problem facing the creation of a typology of PCOS is that many cases need to be seen in a single study before different categories become obvious, but research in PCOS has been hampered by small sample sizes (Escobar-Morreale et al, 2005). Although perhaps 10% of women have PCOS, because a smaller proportion have each type (for example, only perhaps 0.5% of women have lean-type PCOS) the sample sizes required for an epidemiological study are quite large. All studies in the present thesis used the Rotterdam criteria to diagnose PCOS in all cases, and the limitations of this definition should be borne in mind.

1.17.1 Theoretical perspectives: the evolutionary perspective

Some authors have speculated that – paradoxically - there is adaptive value in being infertile, whether due to PCOS or other reasons. Eggers et al (2007) draw upon the kin selection hypothesis to explain how having PCOS offers a selective advantage. The kin selection hypothesis (Hamilton, 1963, 1964a,b) suggests that a person might help the offspring of a relative to survive, and because the helper shares genes with the relative's offspring, at least some part of the helper's genes survive into the next generation. Because raising human newborns is more demanding in time and energy than raising newborns in other species, it is especially useful to the survival of the human child to have a 'helper at the nest'. Amongst primates, this helper is most likely to be nulliparous females, regardless of age (Hrdy, 1999). In humans, these females may be women who have not been reproductively successful in their own right, but they may show interest in caring

for the child of a sister, cousin, or niece. Indeed this provides an explanation for the adaptive value of old age in women too; 'the grandmother hypothesis' suggests that postmenopausal women have an important adaptive role (Hawkes et al, 1998). With help from non-reproductive female relatives, fertile women are more free to reproduce more frequently thus increasing the representation of the family's genes – including the helper's - in the gene pool. Having links to more children also benefits the helpers in increasing their chances of having someone to take care of them when they are ill or old.

The metabolic abnormality often seen in PCOS that leads to weight gain can also confer an adaptive advantage, according to Shaw and Elton (2008). They suggest that the tendency to store fat confers a survival advantage on women during times when food is scarce. This advantage also applies to the fetus when such women are pregnant, consistent with the greater birth weight sometimes seen in newborns born to mothers with PCOS.

1.17.2 Theoretical perspectives: the genetic perspective

In the general population of healthy women, 40% of the variance in T levels is inherited (Harris et al, 1998), and more so in White people than Black (Hong et al, 2001). The rate of inheritance is likely to be at least this high for PCOS, and Legro et al (1998) have found that 50% of sisters of women with PCOS have elevated free or total T. In their study of PCOS twins Jahanfar et al (2004) found that pco is less heritable than androgen and insulin levels. In a PCOS twin study (1332 monozygotic and 1873 dizygotic twins) Vink et al (2006) found a concordance rate for PCOS symptoms of .71 for MZ compared to .38 for DZ twins and other sisters, indicating a contribution of heredity in PCOS. Dunaif and Thomas (2001) found that 50% of sisters of women with PCOS have pco and hyperandrogenemia. Kahsar-Miller et al (2001) found that 24-32% of first-degree relatives (mothers,

sisters, daughters) of women with PCOS also have PCOS. The prevalence rate of PCOS also varies by ethnicity; Williamson et al (2001) found that PCOS is more prevalent in Indian women and less prevalent in Chinese women than European women. However the specific genetic factors associated with PCOS are complex and as yet poorly understood.

Diamanti-Kandarakis et al (2006a) suggest five obstacles to understanding the contribution of genetics to PCOS. Firstly there is the problem of overlapping phenotypes (outlined above). Secondly there is the obvious problem of finding evidence of heredity in a condition that works against having children. Thirdly, evidence for the phenotype can only be seen in women of reproductive age. Fourthly it is only possible to see the phenotype in females, not in males. Finally, inherited conditions can usually be modelled in mice - a very useful process known as 'genetic mapping'. However PCOS is not known to occur spontaneously in any species other than humans, thus precluding the use of genetic mapping.

As yet no gene has been identified that significantly contributes to the development of any one specific type of PCOS. Several sites have been proposed for PCOS genes including CYP11A, the insulin gene, the follistatin gene, and the insulin receptor, but to date the evidence implicating these areas is not particularly strong. The strongest case for linkage can be made for the region near (but not directly involving) the insulin receptor gene at chromosome 19p13.3 (Diamanti-Kandarakis et al, 2006a). However the specific gene remains unidentified

What has been established is that genes related to PCOS have defects that impact androgen biosynthesis, steroid genesis, insulin resistance, expression of the metabolic syndrome, and cancer. Recent research has found that women with PCOS have polymorphisms on the AKT2 gene, a gene which affects glucose metabolism, mitogenic signaling, and mediates cell survival in the ovary (Goodarzi

et al, 2008). AKT2 also interacts with another gene associated with PCOS, the glycogen synthase kinase 3beta (GSK3B) which Geraldes et al (2008) found is implicated in post-insulin receptor signalling and inhibition of apoptosis.

1.17.3 Theoretical perspectives: the environmental perspective

The first documented description of polycystic ovaries was made by Vallisieri in 1721 (cited in Eggers et al, 2007). The observation was made in relation to infertility, and until very recently PCOS has been seen as solely a fertility problem (Hunter and Sterrett, 2000). Fertility problems have existed for as long as mankind has existed, and the social importance of infertility is evidenced by the prevalence of fertility rituals and fertility goddesses from mankind's earliest history (Gelis, 1989; Schenker, 2000). The perceived importance of fertility exists cross-culturally too (Husain, 1998). Given that infertility is by its nature a condition that acts against being inherited, its historical prevalence suggests that its cause is not primarily genetic, and may therefore have environmental causes.

PCOS is not simply an inherited condition; it appears to follow the diathesis-stress model i.e. it is a genetic syndrome most likely to become manifest under certain environmental conditions. One of the principal stressors is diet. In their review of the genetic and molecular basis of PCOS, Escobar-Morreale et al (2005) conclude that the phenotype is modified by ethnicity, diet, and lifestyle factors. The modern Western diet (plentiful, and high in calories, saturated fats, sugars and carbohydrates) is well established as a risk factor for PCOS. A lack of exercise compounds the problem caused by diet. Unsurprisingly then, interventions that improve diet and exercise tend to have good outcomes for women with PCOS although long-term outcomes need more research (Moran et al, 2006).

Other environmental factors that have been suggested to trigger PCOS are medication, viruses, and stress.

Valproic acid is a medication used to treat epilepsy, bipolar disorder and migranes, and there is some evidence that long-term use can cause PCOS, and that discontinuation will reduce the PCOS symptoms (Isojarvi, 1998). However this view has been contested on the grounds that the evidence is retrospective and relies on small samples (Genton et al, 2001).

There is evidence that the adenovirus-36 (Ad-36) causes obesity in rhesus and marmoset monkeys (Dhurandhar, 2002) and is related to obesity in humans (Dhurandhar, 1997). Although there is no evidence that Ad-36 is associated with the development of PCOS, its potential as a contributor and deserves research.

Eggers & Kirchenghast (2001) suggest that psychological stress may contribute to the development of PCOS. This is possible via a number of psychological and behavioural pathways e.g. if an adolescent girl engages in comfort eating due to stress this may cause obesity (Keski-Rahkonen et al, 2007) a known risk for PCOS. Stress is a sufficient cause for the development of functional hypothalamic secondary amenorrhea (FHSA), a condition characterised by elevated adrenal androgens (Gallinelli et al, 2000). Similarly, psychological distress may activate the adrenal glands releasing DHEAS, which will raise T levels, especially in PCOS patients whose adrenal glands are hyperresponsive to the stress hormone ACTH (Moran et al, 2004). However although stress is potentially a risk factor for PCOS, the contribution of ovarian androgens alone is unlikely to be sufficient to cause the range and severity of metabolic and endocrinological problems that constitute any PCOS phenotype.

1.18 The fetal environment

A special case of possible environmental causes of PCOS is the fetal environment. Barker (2004) proposed that some diseases of adulthood may have their origin in conditions in the fetal environment, and women with PCOS have been found to have much higher levels of T than controls during the first trimester (Hu et al,

2007). Although placental aromatase is traditionally thought to protect the fetus from raised maternal T (Abbott et al, 2002), the results of animal research suggest otherwise. For example, Resko et al (1984; 1987) gave doses of T (10 to 15 mg) to pregnant rhesus monkeys. The doses were high for a female fetus but normal for a male, and the resulting female offspring were twice as likely to develop PCOS than a control group of untreated females. Based on this and similar evidence, Dumesic & Abbott (2007) hypothesise that PCOS develops as a result of exposure to abnormally high levels of T prenatally.

Insulin resistance in PCOS offers another possible pathway for prenatal androgenisation, because aromatase activity is inhibited by insulin (Nestler, 1990). Aromatase normally converts T into E2, and interference with this process could create an androgen-rich fetal environment. However it remains unclear how much maternal T contributes to fetal levels in human pregnancies, or whether it is possible to control maternal T levels. Vanky et al (2006) found that taking metformin – an insulin sensitizer – reduced pregnancy complications (e.g. premature birth) in women with PCOS without reducing T levels. Similarly, Glueck et al (2004) did not find that metformin significantly reduced T during PCOS pregnancies, though they found that T levels were remained fairly stable over the three trimesters (56.5, 54.3, and 63.8 ng/dl) on metformin.

1.19 Evidence of PCOS in childhood

If PCOS has its origins in prenatal development, then it stands to reason that there could be signs of PCOS evident in childhood. Diamanti-Kandarakis et al (2008) review the literature on the early expression of PCOS. They suggest that signs can be seen at birth (high birth weight, or low birth weight with postnatal catch-up within a year) and the classic symptoms of PCOS begin to show in adolescence. However a criticism of Diamanti-Kandarakis et al's description of postnatal weight catch-up as an early sign of PCOS is that the evidence for this is almost entirely

based on animal studies e.g. sheep, experiencing supraphysiological doses of T (Manikkam et al, 2003) thus not necessarily applicable to humans with only moderately elevated T. However there is research evidence in humans that postnatal catch-up in early childhood is associated with many problems at three years of age, including obesity and insulin resistance (Inuguez et al, 2006).

Precocious puberty (early onset) is also seen as a sign of the potential development of PCOS (e.g. Franceschi et al, 2009). However diagnosing PCOS at puberty can lead to Type 1 errors. Diamanti-Kandarakis et al (2008) suggest that because some of the signs of a normal puberty are similar to those of PCOS (acne, irregular menstrual cycles) caution in diagnosis of PCOS at puberty is warranted. This is especially true as in puberty it is not unusual to see multiple cysts on at least one ovary in 10% of normally menstruating adolescents. Also, because of the increase in growth hormone in adolescence, insulin resistance is not uncommon. For these reasons the presence of four of five criteria (menstrual irregularity, acne or hirsutism, elevated T, insulin resistance, pco) are recommended to diagnose PCOS in adolescence (Sultan and Paris, 2006).

Franks (2008) suggests that the rise in childhood obesity is responsible for triggering symptoms of PCOS, and of exacerbating insulin-related problems at adolescence. Franks also suggests that elevated serum androgens should be considered the hallmark of PCOS in adolescents. However Diamanti-Kandarakis et al (2008) caution against biochemical measures of T in diagnosis because of the perceived low reliability of some types of T assays.

1.20 Concluding comments for the introduction

As can be seen, PCOS is a complex condition and completing our knowledge of even the most basic aspects of it – the causes and the most reliable treatments - still requires a great deal of research. There is a long way to go before we know whether PCOS is caused by prenatal exposure to T, the cause of any such exposure,

and whether such exposure has orgnanizational effects on development. It also remains to be seen whether psychological factors in PCOS are directly caused by T (due to prenatal organization, or activation by circulating T), or other factors such glucose fluctuations, or are simply caused by the stress of having PCOS symptoms. This thesis describes studies that attempt to address these issues.

REFERENCES

Abbott DH, Eisner JR, Goodfriend T, Medley RD, Peterson EJ, Colman RJ, et al. (2002). Leptin and total free fatty acids are elevated in the circulation of prenatally androgenized female rhesus monkeys. *Abstract P2–329. 84rd Annual Meeting of The Endocrine Society, San Francisco, CA*, June 19–22.

Agrawal R, Sharma S, Bekir J, Conway G, Bailey J, Balen AH, Prelevic G. (2004). Prevalence of polycystic ovaries and polycystic ovary syndrome in lesbian women compared with heterosexual women. *Fertil Steril, 82*, 1352-7.

Albert DJ, Walsh ML, Jonik RH. (1993). Aggression in humans: what is its biological foundation? Neurosci Biobehav Rev, 17, 405-25.

Archer, J., Graham-Kevan, N. & Davies, M. (2005) Testosterone and aggression: A reanalysis of Book, Starzyk & Quinsey. *Aggression and Violent Behavior. 10,* 241-261.

Auyeung B, Baron-Cohen S, Ashwin E, Knickmeyer R, Taylor K, Hackett G. (2009). Fetal testosterone and autistic traits. *Br J Psychol, 100*, 1-22.

Azziz R. (2006). Controversy in clinical endocrinology: diagnosis of polycystic ovarian syndrome: the Rotterdam criteria are premature. *J Clin Endocrinol Metab, 9,* 781-5.

Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE, Witchel SF; (Task Force on the Phenotype of the Polycystic Ovary Syndrome of The Androgen Excess and PCOS Society) (2008). The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril, 91*, 456-88

Acién P, Quereda F, Matallín P, Villarroya E, López-Fernández JA, Acién M, Mauri M, Alfayate R. (1999). Insulin, androgens, and obesity in women with and without polycystic ovary syndrome: a heterogeneous group of disorders. *Fertil Steril, 72,* 32-40.

Azziz R, Woods, KS, Reyna, R, Key, TJ, Knochenhauer, ES, Yilidz, BO (2004). The prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab, 89*, 2745-9

Barclay, L, and Murata, P. (2006). Obese premenopausal women are at high risk for PCOS. *Medscape Today,* October. Accessed 16th Oct 2008 http://www.medscape.com/viewarticle/546731

Barker DJ. (2004). The developmental origins of chronic adult disease. *Acta Paediatr Suppl, 93*, 26-33.

Barnard L, Ferriday D, Guenther N, Strauss B, Balen AH, Dye L. (2007). Quality of life and psychological well being in polycystic ovary syndrome. Hum Reprod, 22, 2279-86

Barth JH, Catalan J, Cherry CA, Day A. (1993). Psychological morbidity in women referred for treatment of hirsutism. J Psychosom Res. 37, 615-9.

Beach, F.A. (1975). Hormonal modification of sexually dimorphic behavior. *Psychoneuroendocrinology, 1*, 3-23

Berenbaum SA, Hines M. Early androgens are related to childhood sex-typed toy preferences. *Psychological Science, 3,* 203-206

Berga SL. (1996). Stress and ovarian function. *Am J Sports Med. 24*, :S36-7

Booth, A., G. Shelley , A. Mazur , G. Tharp and R. Kittok (1989). Testosterone, and Winning and Losing in Human Competition. *Hormones and Behavior* , *23*, 556-571.

Bjorkquist & P. Niemela (Eds.) (1992). *Of mice and women: Aspects of female aggression.* Academic Press.

Bjorkquist, K., Nygren, T., Bjorklund, A.C., and Bjorkquist, S. E. (1994). Testoserone intake and aggressiveness - Real effect or anticipation. *Aggressive Behavior, 20*, 517-522.

Brand-Miller, J. Farid, N.R. and Marsh, K. (2004). The low GI guide to managing PCOS. London: Hodder & Stoughton.

Brown, P.J. (1991). Culture and the evolution of obesity. *Hum. Nat. 2*, 31–57.

Buffington CK, Givens JR, Kitabchi AE. (1991). Opposing actions of dehydroepiandrosterone and testosterone on insulin sensitivity. In vivo and in vitro studies of hyperandrogenic females. *Diabetes, 40,* 693-700.

Burger, H.G. (2002). Androgen production in women. *Fertil Steril., 77*, S3-5

Carranza-Lira S, Velasco Díaz G, Olivares A, Chán Verdugo R, Herrera J. (2006). Correlation of Kupperman's index with estrogen and androgen levels, according to weight and body fat distribution in postmenopausal women from Mexico City. *Int J Fertil Womens Med, 51*, 83-8.

Cashdan, E. (2008).Waist-to-Hip Ratio across Cultures: Trade-Offs between Androgen- and Estrogen-Dependent Traits. *Current Anthropology*, 6, 20-28.

Coffey S, Bano G, Mason HD. (2006). Health-related quality of life in women with polycystic ovary syndrome: a comparison with the general population using the Polycystic Ovary Syndrome Questionnaire (PCOSQ) and the Short Form-36 (SF-36). *Gynecol Endocrinol, 22*, 80-6.

Clayton WJ, Lipton M, Elford J, Rustin M, Sherr L. (2005). A randomized controlled trial of laser treatment among hirsute women with polycystic ovary syndrome. Br J Dermatol, 152, 986-92.

Cohen-Bendahan, C.C., van de Beek, C., and Berenbaum, S.A. (2005). Prenatal sex hormone effects on child and adult sex-typed behavior: methods and findings, *Neurosci Biobehav Rev, 29,* 353–384.

Condry, J., Condry, S., (1976). Sex Differences: A study in the Eye of the Beholder. *Child Development, 47*, 812-819

Boivin J, Takefman JE. (1995). Stress level across stages of in vitro fertilization in subsequently pregnant and nonpregnant women. *Fertil Steril*, 64, 802-10

Dabbs J.M.; Strong R.; Milun R. (1997). Exploring the mind of testosterone: a beeper study. *Journal of Research in Personality, 31*, 577-587

Demyttenaere K, Nijs P, Evers-Kiebooms G, Koninckx PR. (1992). Coping and the ineffectiveness of coping influence the outcome of in vitro fertilization through stress responses. *Psychoneuroendocrinology, 17*, 655-65.

Diamanti-Kandarakis E, Kandarakis H, Legro RS (2006a). The role of genes and environment in the etiology of PCOS. *Endocrine, 30,* 19-26

Diamanti-Kandarakis E, Economou F. (2006b). Stress in women: metabolic syndrome and polycystic ovary syndrome. *Ann N Y Acad Sci, 1083*, 54-62.

Diamanti-Kandarakis E, Christakou C, Palioura E, Kandaraki E, Livadas S. (2008). Does polycystic ovary syndrome start in childhood? *Pediatr Endocrinol Rev, 5*, 904-11

Domar AD, Friedman R, Zuttermeister PC. (1999). Distress and conception in infertile women: a complementary approach. *J Am Med Womens Assoc, 54*, 196-8.

Domar AD, Clapp D, Slawsby EA, Dusek J, Kessel B, Freizinger M. (2000). Impact of group psychological interventions on pregnancy rates in infertile women.*Fertil Steril, 73*, 805-11.

Domar, A. D. (2002). Conquering Infertility. London: Penguin.

Dumesic DA, Abbott DH, Padmanabhan V. (2007). Polycystic ovary syndrome and its developmental origins. *Rev Endocr Metab Disord, 8*, 127-41

Dunaif A, Segal KR, Futterweit W, Dobrjansky A. (1989). Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome.*Diabetes, 38,* 1165-74.

Dunaif A, Thomas A. (2001). Current concepts in the polycystic ovary syndrome. *Annu Rev Med, 52*, 401-19.

Dhurandhar NV, Whigham LD, Abbott DH, Schultz-Darken NJ, Israel BA, Bradley SM, Kemnitz JW, Allison DB, Atkinson RL. (2002). Human adenovirus Ad-36

promotes weight gain in male rhesus and marmoset monkeys. *J Nutr, 132*, 3155-60.

Dhurandhar NV, Kulkarni PR, Ajinkya SM, Sherikar AA, Atkinson RL. (1997). Association of adenovirus infection with human obesity. *Obes Res, 5*, 464-9.

Dunlap JL, Gerall AA, and Carlton SF (1978). Evaluation of prenatal androgen and ovarian secretions on receptivity in female and male rats. *Journal of Comparative and Physiological Psychology, 92*, 280–288.

Dusková M, Stárka L. (2006). The existence of a male equivalent of the polycystic ovary syndrome--the present state of the issue. *Prague Med Rep;107*, 17-25

Eggers S, Kirchengast S. (2001). The polycystic ovary syndrome--a medical condition but also an important psychosocial problem. *Coll Antropol, 25,* 673-85.

Eggers S, Hashimoto DM, Kirchengast S. (2007). An evolutionary approach to explain the high frequency of the polycystic ovary syndrome (PCOS). *Anthropol Anz, 65*, 169-79.

Elsenbruch S, Hahn S, Kowalsky D, Offner AH, Schedlowski M, Mann K, Janssen OE. (2003). Quality of life, psychosocial well-being, and sexual satisfaction in women with polycystic ovary syndrome. *J Clin Endocrinol Metab, 88*, 5801-7

Elsenbruch S, Benson S, Hahn S, Tan S, Mann K, Pleger K, Kimmig R, Janssen OE. (2006). Determinants of emotional distress in women with polycystic ovary syndrome. *Hum Reprod, 21*, 1092-9

Eriksson E, Sundblad C, Lisjö P, Modigh K, Andersch B. (1992). Serum levels of androgens are higher in women with premenstrual irritability and dysphoria than in controls. *Psychoneuroendocrinology. 17,* 195-204.

Escobar-Morreale HF, Luque-Ramírez M, San Millán JL. (2005). The molecular-genetic basis of functional hyperandrogenism and the polycystic ovary syndrome. *Endocr Rev, 26*, 251-82.

Eysenck, H. J. (1967). *The biological basis of personality.* Springfield, IL: Charles C. Thomas.

Eysenck, S.B.G., Eysenck, H.J., and Barrett, P.T. (1985) A revised version of the Psychoticism scale. *Personality and Individual Differences, 6*, 21-29.

Faith MS, Flint J, Fairburn CG, Goodwin GM, Allison DB. (2001). Gender differences in the relationship between personality dimensions and relative body weight. *Obes Res, 9*, 647-50

Forest MG, Bétuel H, Couillin P, Boué A. (1981). Prenatal diagnosis of congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency by steroid analysis in the amniotic fluid of mid-pregnancy: comparison with HLA typing in 17 pregnancies at risk for CAH. *Prenat Diagn, 1,* 197-207.

Farrell K, and Antoni MH. Insulin resistance, obesity, inflammation, and depression in polycystic ovary syndrome: biobehavioral mechanisms and interventions. (2010). *Fertil Steril, 9,* 1565-1574.

Franceschi, R., Gaudino, R., Marcolongo, A., Chiara Gallo, M., Rossi, L., Antoniazzi, F., and Tatò, L. (2009). Prevalence of polycystic ovary syndrome in young women who had idiopathic central precocious puberty. *Fertility and Sterility,* In Press, Corrected Proof, Available online 9 January 2009.

Franks S. Polycystic ovary syndrome (1995). *N Engl J Med*, *333*, 853-861.

Franks S. (2008). Polycystic ovary syndrome in adolescents. *Int J Obes (Lond), 32,*1035-41.

Gallinelli A, Matteo ML, Volpe A, Facchinetti F. (2000). Autonomic and neuroendocrine responses to stress in patients with functional hypothalamic secondary amenorrhea. *Fertil Steril, 73*, 812-6

Gelis J. (1989). The Secret of Birth. *Hist Sci Med, 23*, 109-14.

Genton P, Bauer J, Duncan S, Taylor AE, Balen AH, Eberle A, Pedersen B, Salas-Puig X, Sauer MV. (2001). On the association between valproate and polycystic ovary syndrome. *Epilepsia, 42*, 295-304

Geraldes P, Yagi K, Ohshiro Y, He Z, Maeno Y, Yamamoto-Hiraoka J, Rask-Madsen C, Chung SW, Perrella MA, King GL. (2008). Selective Regulation of Heme Oxygenase-1 Expression and Function by Insulin through IRS1/Phosphoinositide 3-Kinase/Akt-2 Pathway. *J Biol Chem, 283*, 34327-36.

Gerall AA, Stone LS, Hitt JC. (1972). Neonatal androgen depresses female responsiveness to estrogen. *Physiol Behav, 8*, 817-20.

Glueck CJ, Goldenberg N, Wang P, Loftspring M and Sherman A (2004). Metformin during pregnancy reduces insulin, insulin resistance, insulin secretion, weight, testosterone and development of gestational diabetes: prospective longitudinal assessment of women with polycystic ovary syndrome from preconception throughout pregnancy. *Hum Reprod 19*, 510–521.

Gold SM, Schulz H, Mönch A, Schulz KH, Heesen C. (2003). Cognitive impairment in multiple sclerosis does not affect reliability and validity of self-report health measures. *Mult Scler, 9*, 404-10.

Goodarzi MO, Jones MR, Chen YD, Azziz R. (2008). First evidence of genetic association between AKT2 and polycystic ovary syndrome. *Diabetes Care, 31,* 2284-7.

Gougeon A. (1996). Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev, 17*, 121-55.

Goy RW, McEwen BS. Sexual Differentiation of the Brain. Cambridge, Massachusetts: MIT Press, 1980

Guyatt, G, Weaver, B., Cronin, L., Dooley, JA, and Azziz, R (2004). Health related quality of life in PCOS. *J Clin Epidemiology, 57,* 1279-87

Hahn S, Benson S, Elsenbruch S, Pleger K, Tan S, Mann K, Schedlowski M, van Halteren WB, Kimmig R, Janssen OE. (2006). Metformin treatment of polycystic ovary syndrome improves health-related quality-of-life, emotional distress and sexuality. *Hum Reprod, 21*, 1925-34

Hahn S, Janssen OE, Tan S, Pleger K, Mann K, Schedlowski M, Kimmig R, Benson S, Balamitsa E, Elsenbruch S. (2005). Clinical and psychological correlates of quality-of-life in polycystic ovary syndrome. *Eur J Endocrinol, 153*, 853-60.

Hamilton, W. G. 1963. The evolution of altruistic behavior. *American Naturalist, 97*, 354-356.

Hamilton, W. G. 1964a. The genetical evolution of social behavior. I. *Journal of Theoretical Biology, 7,* 1-16.

Hamilton, W. G. 1964b. The genetical evolution of social behaviour. II. *Journal of Theoretical Biology, 7*, 17-27.

Harris JA, Vernon PA, Boomsma DI. (1998). The heritability of testosterone: a study of Dutch adolescent twins and their parents. *Behav Genet, 28*, 165-71.

Hawkes K, O'Connell JF, Jones NG, Alvarez H, Charnov EL. (1998). Grandmothering, menopause, and the evolution of human life histories. *Proc Natl Acad Sci, 95,* 1336-9.

Himelein MJ, Thatcher SS. (2006a). Depression and body image among women with polycystic ovary syndrome. *J Health Psychol, 11,* 613-25.

Himelein MJ, Thatcher SS. (2006b). Polycystic ovary syndrome and mental health: A review. *Obstet Gynecol Surv, 61,* 723-32

Hines, M. (2002). Sexual differentiation of human brain and behaviour. In Pfaff, D., Arnold, A.P., Etgen, A.M., Fahrbach, S.E. & Rubin, R.T. (Eds.) (2002). Hormones, Brain and Behavior, Volume 4, pp. 425-462. Academic Press, New York.

Hines, M., Johnston, K.J., Golombok, S., Rust, J., Stevens, M., and Golding, J. (2002b). Prenatal Stress and Gender Role Behavior in Girls and Boys: A Longitudinal, Population Study. *Hormones and Behavior, 42,* 126–134.

Hines, M., Fane, B.A., Pasterski, V.L., Mathews, G.A., Conway, G.S., Brook, C. (2003). Spatial abilities following prenatal androgen abnormality: Targeting and mental rotations performance in individuals with congenital adrenal hyperplasia (CAH). *Psychoneuroendocrinology 28*, 1010-1026

Hines M, Brook C, Conway GS. (2004). Androgen and psychosexual development: Core gender identity, sexual orientation and recalled childhood gender role behavior in women and men with congenital adrenal hyperplasia (CAH). *Journal of Sex Research 41*, 1-7.

Hollinrake E, Abreu A, Maifeld M, Van Voorhis BJ, Dokras A. (2007). Increased risk of depressive disorders in women with polycystic ovary syndrome. *Fertil Steril, 87*,1369-76.

Hosaka T, Matsubayashi H, Sugiyama Y, Izumi S, Makino T. (2002). Effect of psychiatric group intervention on natural-killer cell activity and pregnancy rate. Gen Hosp Psychiatry, 24, 353-6.

Hu S, Leonard A, Seifalian A, Hardiman P. (2007). Vascular dysfunction during pregnancy in women with polycystic ovary syndrome. *Hum Reprod, 22*, 1532-9

Hong Y, Gagnon J, Rice T, Pérusse L, Leon AS, Skinner JS, Wilmore JH, Bouchard C, Rao DC (2001). Familial resemblance for free androgens and androgen glucuronides in sedentary black and white individuals: the HERITAGE Family Study. Health, Risk Factors, Exercise Training and Genetics. *J Endocrinol, 70,* 485-92.

Hrdy, S.B. (1999). Mother Nature. Berlin: Verlag.

Hunter MH, Sterrett JJ. (2000). Polycystic ovary syndrome: it's not just infertility. *Am Fam Physician, 62*, 1079-88

Hyde, J.S. (1984). How large are gender differences in aggression? A developmental meta-analysis. *Developmental Psychology, 20*, 722-736

Ingudomnukul E, Baron-Cohen S, Wheelwright S, Knickmeyer R. (2007). Elevated rates of testosterone-related disorders in women with autism spectrum conditions. *Horm Behav, 51*, 597-604

Isojärvi JI, Rattya J, Myllyla VV, et al. (1998). Valproate, lamotrigine, and insulin-mediated risks in women with epilepsy. *Ann Neurol*, *43,* 446–51

Jahanfar S, Maleki H, Mosavi AR, Jahanfar M. (2004). Leptin and its association with polycystic ovary syndrome: a twin study. *Gynecol Endocrinol, 18*, 327-34

Jones GL, Hall JM, Balen AH, Ledger WL. (2008). Health-related quality of life measurement in women with polycystic ovary syndrome: a systematic review. *Hum Reprod Update, 14*, 15-25.

Kahsar-Miller MD, Nixon C, Boots LR, Go RC, Azziz R. (2001). Prevalence of polycystic ovary syndrome (PCOS) in first-degree relatives of patients with PCOS. *Fertil Steril, 75,* 53-8

Keegan A, Liao LM, Boyle M. 'Hirsutism': a psychological analysis. *J Health Psychol, 8,* 327-45

Keski-Rahkonen A, Bulik CM, Pietiläinen KH, Rose RJ, Kaprio J, Rissanen A. (2007). Eating styles, overweight and obesity in young adult twins. *Eur J Clin Nutr, 61,* 822-9

Kissebah AH, Vydelingum N, Murray R, Evans DJ, Hartz AJ, Kalkhoff RK, Adams PW. (1982). Relation of body fat distribution to metabolic complications of obesity. *Clin Endocrinol Metab, 54*, 254-60

Kitzinger, C. and Willmott, J. (2002). 'The thief of womanhood': women's experience of polycystic ovarian syndrome. *Soc Sci Med, 54*, 349-61

Klonoff-Cohen H, Chu E, Natarajan L, Sieber W. (2001). A prospective study of stress among women undergoing in vitro fertilization or gamete intrafallopian transfer. *Fertil Steril, 76*, 675-87.

Knickmeyer R, Baron-Cohen S, Fane BA, Wheelwright S, Mathews GA, Conway GS, Brook CG, Hines M. (2006). Androgens and autistic traits: A study of individuals with congenital adrenal hyperplasia. *Horm Behav, 50*, 148-53.

Kolotkin RL, Meter K, Williams GR. (2001b). Quality of life and obesity. *Obes Rev, 2,* 219-29

Kondoh Y, Uemura T, Ishikawa M, Yokoi N, Hirahara F. (1999). Classification of polycystic ovary syndrome into three types according to response to human corticotropin-releasing hormone. *Fertil Steril, 72*, 15-20

Ledger, W.L., & Clark, T. (2003). Long−term consequences of polycystic ovary syndrome. Royal College of Obstetrician and Gynaecologists Physicians Guidelines, No.33

Legro RS, Driscoll D, Strauss JF 3rd, Fox J, Dunaif A. (1998). Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. *Proc Natl Acad Sci U S A.,* 95, 14956-60

Leveroni CL, Berenbaum SA. (1998). Early androgen effects on interest in infants: Evidence from children with congenital adrenal hyperplasia. *Developmental Neuropsychology, 14*, 321-340

Levitas E, Parmet A, Lunenfeld E, Bentov Y, Burstein E, Friger M, Potashnik G. (2006). Impact of hypnosis during embryo transfer on the outcome of in vitro fertilization-embryo transfer: a case-control study. *Fertil Steril, 85*, 1404-8.

Lipton MG, Sherr L, Elford J, Rustin MH, Clayton WJ. Women living with facial hair: the psychological and behavioral burden. *J Psychosom Res, 61*, 161-8

Maccoby, E.E. (1988). Gender as a social category. *Dev. Pscyhol., 24,* 755-65

Manikkam M, Crespi EJ, Doop DD, Herkimer C, Lee JS, Yu S, Brown MB, Foster DL, Padmanabhan V. (2004). Fetal programming: prenatal testosterone excess leads to fetal growth retardation and postnatal catch-up growth in sheep. Endocrinology, 145, 790-8.

Manikkam M, Steckler TL, Welch KB, Inskeep EK, Padmanabhan V. (2006). Fetal programming: prenatal testosterone treatment leads to follicular persistence/luteal

defects; partial restoration of ovarian function by cyclic progesterone treatment. *Endocrinology, 147*, 1997-2007.

Manlove HA, Guillermo C, Gray PB. (2008). Do women with polycystic ovary syndrome (PCOS) report differences in sex-typed behavior as children and adolescents?: Results of a pilot study. *Ann Hum Biol, 35,* 584-95

Månsson M, Holte J, Landin-Wilhelmsen K, Dahlgren E, Johansson A, Landén M. (2008). Women with polycystic ovary syndrome are often depressed or anxious--a case control study. *Psychoneuroendocrinology, 33*, 1132-8.

Marsh, K., and Brand-Miller, J. (2005). The optimal diet for women with polycystic ovary syndrome? *British Journal of Nutrition, 94*, 154–165

Marshall K. (2001). Polycystic ovary syndrome: clinical considerations. *Altern Med Rev, 6,* 272-92.

Mathews GA, Fane BA, Conway GS, Brook CG, Hines M. (2009). Personality and congenital adrenal hyperplasia: possible effects of prenatal androgen exposure. *Horm Behav, 55,* 285-91.

McCarthy MM (2008) Estradiol and the developing brain. *Physiological Reviews, 88*, 91–134.

McCook, J. G. (2002). The influence of hyperandrogenism, obesity and infertility on the psychosocial health and wellbeing of women with polycystic ovary syndrome. Unpublished doctoral dissertation, University of Michigan.

McCook JG, Reame NE, Thatcher SS. (2005). Health-related quality of life issues in women with polycystic ovary syndrome. *J Obstet Gynecol Neonatal Nurs, 34*, 12-20.

McKenna TJ, Cunningham SK. (1995). Adrenal androgen production in polycystic ovary syndrome. *Eur J Endocrinol., 133,* 383-9.

Miller WL, Levine LS. (1987). Molecular and clinical advances in congenital adrenal hyperplasia. *J Pediatr, 111*, 1-17

Monzani et al. (1994). Psychological and psychopathological correlates in the polycystic ovary syndrome (PCOS). *Medicina−Psicosomatica, 39*, 225−236.

Moran C, Reyna R, Boots LS, Azziz R. (2004). Adrenocortical hyperresponsiveness to corticotropin in polycystic ovary syndrome patients with adrenal androgen excess. *Fertil Steril., 81,* 126-31.

Moran LJ, Brinkworth G, Noakes M, Norman RJ. (2006). Effects of lifestyle modification in polycystic ovarian syndrome. *Reprod Biomed Online, 12*, 569-78.

Nestler JE. (1990). Insulin-like growth factor II is a potent inhibitor of the aromatase activity of human placental cytotrophoblasts. *Endocrinology, 127*, 2064-70

Nordenstrom A, Servin A, Bohlin G, Larsson A, Wedell A. Sex-typed toy play behavior correlates with the degree of prenatal androgen exposure assessed by CYP21 genotype in girls with congenital adrenal hyperplasia. *Journal of Clinical Endocrinology and Metabolism, 87*, 5119-5124

Norman RJ, Dewailly D, Legro RS, Hickey TE. (2007). Polycystic ovary syndrome. *Lancet, 370*, 685-97.

Pasterski V, Hindmarsh P, Geffner M, Brook C, Brain C, Hines M. (2007). Increased aggression and activity level in 3- to 11-year-old girls with congenital adrenal hyperplasia (CAH). *Horm Behav, 52*, 368-74.

Pfaff, D., Arnold, A.P., Etgen, A.M., Fahrbach, S.E. & Rubin, R.T. (Eds.) (2002). Hormones, Brain and Behavior, Volume 4, pp. 425-462. Academic Press, New York.

Rasgon NL, Rao RC, Hwang S, Altshuler LL, Elman S, Zuckerbrow-Miller J, Korenman SG. (2003). Depression in women with polycystic ovary syndrome: clinical and biochemical correlates. *J Affect Disord, 74*, 299-304

Resko JA, Ellinwood WE. (1984). Sexual differentiation of t he brain of primates. In: Serio M, Motta M, Zanisi M, Martini L, editors. Sexual differentiation: basic and clinical aspects. New York: Raven Press;. p. 169–81.

Resko JA, Buhl AE, Phoenix CH. (1987). Treatment of pregnant rhesus macaques with testosterone propionate: observations on its fate in the fetus. *Biol Reprod, 37*, 1185–91.

Rivest S, Rivier C. (1995). The role of corticotropin-releasing factor and interleukin-1 in the regulation of neurons controlling reproductive functions. Endocr Rev, 16, 177-99.

Roselli CE, Stadelman H, Reeve R, Bishop CV, Stormshak F. (2007). The ovine sexually dimorphic nucleus of the medial preoptic area is organized prenatally by testosterone. *Endocrinology, 148*, 4450-7.

Rosner, W. (1991). Plasma steroid binding proteins. *Endocrinol Metab Clin North Am, 20,* 697-720.

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, 19*, 41-47

Rubinow DR, Schmidt PJ. (1989). Models for the development and expression of symptoms in premenstrual syndrome. Psychiatr Clin North Am, 12, 53-68

Salvador, A., Suay, F., González-Bono, E., and Serrano, M. A. (2003) Anticipatory cortisol, testosterone and psychological responses to judo competition in young men. *Psychoneuroendocrinology, 28*, 364-375

Sam S, Sung YA, Legro RS, Dunaif A. (2008). Evidence for pancreatic beta-cell dysfunction in brothers of women with polycystic ovary syndrome. *Metabolism, 57*, 84-9.

Sarrel PM, DeCherney AH. (1985). Psychotherapeutic intervention for treatment of couples with secondary infertility. Fertil Steril, 43, 897-900.

Schattmann L, Sherwin BB. (2007b) Testosterone levels and cognitive functioning in women with polycystic ovary syndrome and in healthy young women. *Horm Behav, 51,* 587-96

Schenker JG. (2000). Women's reproductive health: monotheistic religious perspectives. *Int J Gynaecol Obstet, 70*, 77-86.

Selye, H. (1950) *Stress*. *A Treatise Based on the Concepts of the General-Adaptation-Syndrome and the Diseases of Adaptation*. Acta Inc. Medical Publishers, Montreal.

Shaw LM, Elton S. (2008). Polycystic ovary syndrome: a transgenerational evolutionary adaptation. *BJOG*, 115, 144-8.

Sherwin BB, Gelfand MM. (1985). Sex steroids and affect in the surgical menopause: a double-blind, cross-over study. *Psychoneuroendocrinology, 10*, 325-35.

Sills et al. (2001). Diagnostic and treatment characteristics of polycystic ovary syndrome: descriptive measurements of patient perception and awareness from 657 confidential self−reports. *BMC Womens Health. 1*, 1-3.

Shipley BA, Weiss A, Der G, Taylor MD, Deary IJ. (2007). Neuroticism, extraversion, and mortality in the UK Health and Lifestyle Survey: a 21-year prospective cohort study. Psychosom Med, 69, 923-31

Sir-Petermann T, Hitchsfeld C, Maliqueo M, Codner E, Echiburú B, Gazitúa R, Recabarren S, Cassorla F. (2005). Birth weight in offspring of mothers with polycystic ovarian syndrome. *Hum Reprod, 20,* 2122-6

Smail PJ, Reyes FI, Winter JSD, Faiman C 1981 The fetal hormonal environment and its effect on the morphogenesis of the genital system. In: Kogan SJ, Hafez ESE, eds. Pediatric andrology. The Hague: Martinus Nijhoff; 9–19

Smeenk JM, Verhaak CM, Eugster A, van Minnen A, Zielhuis GA, Braat DD. (2001). The effect of anxiety and depression on the outcome of in-vitro fertilization. Hum Reprod, 16, 1420-3.

Smeenk JM, Verhaak CM, Vingerhoets AJ, Sweep CG, Merkus JM, Willemsen SJ, van Minnen A, Straatman H, Braat DD. (2005). Stress and outcome success in IVF: the role of self-reports and endocrine variables. Hum Reprod, 20, 991-6

Stanley T, Misra M. Polycystic ovary syndrome in obese adolescents. *Curr Opin Endocrinol Diabetes Obes, 15,* 30-6.

Stein, I. F., and Leventhal, M. L. (1935). *Amenorrhea associated with bilateral polycystic ovaries. American Journal of Obstetrics and Gynecology, 29*, 181-191.

Stunkard, AJ, Faith, AS, Allison, KC (2003). Depression and obesity. *Biol Psychiatry, 54*, 330-7

Sultan C, Paris F. (2008). Clinical expression of polycystic ovary syndrome in adolescent girls. *Fertil Steril, 86,* Suppl 1:S6

Trent M, Austin SB, Rich M, Gordon CM. (2005). Overweight status of adolescent girls with polycystic ovary syndrome: body mass index as mediator of quality of life. *Ambul Pediatr, 5*, 107-11.

van Wely M, Bayram N, Bossuyt PM, van der Veen F. (2004). Laparoscopic electrocautery of the ovaries versus recombinant FSH in clomiphene citrate-resistant polycystic ovary syndrome. Impact on women's health-related quality of life. *Hum Reprod,19,* 2244-50.

Vanky E, Salvesen KA, Heimstad R, Fougner KJ, Romundstad P, Carlsen SM. (2004). Metformin reduces pregnancy complications without affecting androgen levels in pregnant polycystic ovary syndrome women: results of a randomized study. *Hum Reprod, 19,* 1734-40.

Verhaak CM, Smeenk JM, van Minnen A, Kremer JA, Kraaimaat FW. (2005). A longitudinal, prospective study on emotional adjustment before, during and after consecutive fertility treatment cycles. *Hum Reprod, 20,* 2253-60

Vink JM, Sadrzadeh S, Lambalk CB, Boomsma DI. (2006). Heritability of polycystic ovary syndrome in a Dutch twin-family study. *J Clin Endocrinol Metab, 91*, 2100-4

Voet, D, and Voet, J.G. (1995). Biochemistry (2nd Edition). New York: John Wiley and Sons Inc.

Wake DJ, Strand M, Rask E, Westerbacka J, Livingstone DE, Soderberg S, Andrew R, Yki-Jarvinen H, Olsson T, Walker BR. (2007). Intra-adipose sex steroid

metabolism and body fat distribution in idiopathic human obesity. *Clin Endocrinol (Oxf), 66*, 440-6.

Weiner CL, Primeau M, Ehrmann DA. (2004). Androgens & mood dysfunction in women: comparison of women with PCOS to healthy controls. *Psychosom Med, 66*, 356-62.

Wilson JD, George FW, and Griffin JE (1981). The hormonal control of sexual development. *Science, 211*, 1278–1284.

Women's Health Resource (2008) http://www.wdxcyber.com/dxinf001.htm Accessed 17th Dec 2008

Xita, I. Georgiou, L. Lazaros, V. Psofaki, G. Kolios, and A. Tsatsoulis (2008). The synergistic effect of sex hormone-binding globulin and aromatase genes on polycystic ovary syndrome phenotype. *Eur. J. Endocrinol, 158,* 861 - 865.

Yen, S.S.C., 1991. Chronic anovulation caused by peripheral report. J. Clin. Psychiatry 61, 173–178. endocrine disorders. In: Yen, S.S.C., Jaffe, R.B. (Eds.), Reproductive Endocrinology: Physiology, Pathophysiology and Clin. Management, 3rd Edition. W.B. Saunders, Philadelphia, pp. 576–630.

Young R, Sinclair R. (1998). Hirsutes. I: Diagnosis. *Australas J Dermatol, 39,* 24-8.

Zung WW. (1965). A Self-rating depression scale. *Arch Gen Psychiatry, 12*, 63-70.

Chapter 2

Anxiety and depression in polycystic ovary syndrome: a systematic review and meta-analysis.

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Abstract

BACKGROUND:

Our aim was to assess differences in anxiety and depression between women with and without (controls) PCOS.

METHODS: Systematic review and meta-analysis of published literature comparing women with PCOS to control groups on anxiety and depression. Electronic databases were searched up to December 17th 2010. The inverse variance method based, as appropriate, on a random- or fixed-effects model in Review Manager, Version 5 was used to analyse the data.

RESULTS: Twelve comparative studies were included; all studies assessed depression (910 women with PCOS and 1347 controls) and six also assessed anxiety (208 women with PCOS and 169 controls). Analysis revealed higher depression ($Z = 17.92$, $p<.00001$; Hedges' $g = 0.82$; 95% CI 0.73 to 0.92) and anxiety ($Z = 5.03$, $p<.00001$; Hedges' $g = 0.54$; 95% CI 0.33 to 0.75) scores in the participants with, than without, PCOS. Studies controlling for BMI showed a smaller difference between women with PCOS and controls on anxiety and depression scores than studies not controlling for BMI.

CONCLUSIONS: Women with PCOS on average tend to experience mildly elevated anxiety and depression, significantly more than women without PCOS. Women with lower BMI tended to have slightly lower anxiety and depression scores, suggesting that having a lower BMI reduces anxiety and depression. Future studies might consider (i) controlling for BMI, (ii) stratifying by medication use in order to control for any anti-androgenic effects of medication, and (iii) excluding women with polycystic ovaries from control groups.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common hormone disorders affecting women, with a prevalence of 5-10% in women of reproductive age (Franks, 1995). It is the most common cause of androgen excess in women and the most common cause of ovulatory failure. Clinically, the androgen excess presents as hirsutism and acne, whereas anovulation presents as subfertility and menstrual irregularity. In addition, PCOS is associated with obesity. It is therefore perhaps not surprising that women with PCOS experience mood dysfunction and psychiatric problems to a greater degree than women without PCOS (Farrell and Antoni, 2010). Many studies, for example, Hollinrake et al. (2007) have found that anxiety and depression are higher in PCOS than healthy women. Most studies have focused on depression but others, such as Månsson et al. (2008), have found that anxiety in PCOS is also an important issue.

It has been suggested that mood problems in PCOS are caused by the distress associated with the symptoms often seen in PCOS (obesity, hirsutism etc; Eggers and Kirchengast, 2001). In general, studies find that obesity is related to depression, both in healthy women (Stunkard et al., 2003) and in PCOS (Rasgon et al., 2003). In their review of the quality of life (QoL) literature on PCOS Jones et al. (2008) found that weight problems had the greatest negative impact on QoL. However not all studies of obesity in PCOS have found this (Hahn et al., 2005) and some studies, such as Weiner et al. (2004), find that depression is higher in women with PCOS even after controlling for BMI.

Women vary in how much their mood is affected by the various PCOS symptoms. For example, Farrell and Antoni (2010) suggest that PCOS symptoms that affect appearance are more likely to cause distress in younger women than older women. Because PCOS involves so many potentially distressing symptoms, it is difficult to identify the main cause of distress or the relative contribution of causal factors. The physical symptoms of PCOS are largely the result of elevated testosterone; for example, hirsutism is caused by elevated androgen levels (Young and Sinclair, 1998). Hirsutism is also associated with distress in women with PCOS (Kitzinger and Willmott, 2002). Thus it is tempting to view distress in

PCOS as mainly an indirect effect of testosterone and the cure for distress, therefore, to lower testosterone levels.

Two drugs widely used to treat the symptoms of PCOS, metformin and the oral contraceptive co-cyprindiol (commonly marketed under the brand name 'Dianette'), differ in their action; metformin acts primarily to increase insulin sensitivity, whereas co-cyprindiol is composed of oestrogen and anti-androgen. Some authors use the umbrella term 'anti-androgens' to describe these and other medications of this kind used to treat PCOS (Barnard et al., 2007). In 52 women with PCOS, Harborne et al. (2003) found that co-cyprindiol was more effective than metformin in treating acne, whereas hirsutism responded better to metformin than co-cyprindiol.

Some authors suggest that the drugs commonly used to reduce symptoms of PCOS may also reduce the distress associated with these symptoms (Bruce-Jones et al., 1993; Farrell and Antoni, 2010). In support of this suggestion, Rasgon et al. (2003) found that women with PCOS taking oral contraceptives were significantly less depressed than those with PCOS not taking oral contraceptives. In contrast, Barnard et al. (2007) found that rates of depression were slightly higher in women with PCOS taking anti-androgens (71%) than women with PCOS not taking anti-androgens (67%). Depression rates were lower in controls and showed the opposite pattern; 30% taking anti-androgens and 37% not taking anti-androgens showed some sign of depression. Thus there is, at present, uncertainty regarding the benefits of medications on mood in PCOS.

The primary objective of the present paper is to assess the degree to which women with PCOS score differently to women without PCOS on measures of anxiety and depression. The secondary objective is to determine whether there is an impact of BMI and PCOS medication use (AUTHOR: you do not seem to present results – some discussion only - on use of medication. Please would you check and delete this mention of medication here?) on anxiety and depression scores. All between-groups designs were considered.

Materials and Methods

Literature search

All studies that measured anxiety and depression in women with PCOS listed in Pubmed and Medline published up to Dec 17th 2010, and EMBASE from 1980 to Dec 17th 2010, were identified. The Cochrane Reviews database was also searched up to Dec 17th 2010. The keyword search terms 'polycystic ovary syndrome' and 'anxiety' or 'polycystic ovary syndrome' and 'depression', were entered simultaneously. From a search of Pubmed from 1968 to 2010, 98 articles emerged related to depression and 32 related to anxiety. A Medline search from 1950 produced 95 depression and 31 anxiety papers but none in addition to those cited in Pubmed. An EMBASE search from 1980 produced 79 depression and 36 anxiety papers, two of which were not found in other searches and met the inclusion criteria (see below) for the present meta-analysis. The 'related article' function was used to widen the results. Additionally two Medical Subject Heading (MeSH) searches were performed using firstly the terms 'polycystic ovary syndrome' AND 'depression' and 'polycystic ovary syndrome' AND 'anxiety'. This retrieved 27 and 15 publications, respectively, all of which were previously found using the Pubmed keyword search. The Cochrane Reviews database did not produce any published reviews of PCOS and anxiety or depression. A hand search of relevant articles referenced in these publications was performed, which produced two publications not previously found, neither of which met the inclusion criteria. Also included was a paper by the present authors (Barry et al., 2011). Each article was assessed by the first author and articles that fitted the main criteria (measuring anxiety and/or depression in PCOS) were accessed. Methodological quality was assessed by the first author based on the criteria of the Newcastle-Ottowa Quality Assessment Scale (NOS) for case-control studies (Wells et al., 2000) adapted for cross-sectional studies. This adaptation meant changing the 'ascertainment of exposure' criterion to 'ascertainment of diagnosis'. The Cochrane Non-Randomized Studies Methods Working Group consider the NOS one of the best tools

available for assessment of non-randomized studies (Reeves et al., 2008). A protocol for the present review has not been registered.

Inclusion and exclusion criteria

Studies that compared women with PCOS to controls were eligible for inclusion provided that:

a. The studies reported a quantitative outcome on a standardized measure of depression or anxiety
b. Comparison groups did not have a severe illness (for example, cancer) which would undermine the equivalence of the groups.
c. The studies reported outcomes as mean and SD.
d. The studies reported other relevant data e.g. *n* values in each group

Papers with titles or abstracts that indicated that they were not relevant (for example, reviews, single case studies etc) were excluded. A table of excluded studies (Supplementary Table 1) is available online on the *Human Reproduction* website [appendix in thesis]**.**

Statistical analysis

Statistical analyses were performed using Review Manager, Version 5 (RevMan 5). Anxiety and depression scores were meta-analysed using the fixed effect or random effects model, as appropriate. The inverse variance method was used. Heterogeneity was assessed using I^2 and Chi Square statistics. I^2 values of 30% or above were considered likely to represent moderate heterogeneity, and Chi Square *p* values <.10 were considered to represent significant heterogeneity, thus studies showing I^2 values < 30% and Chi Square *p* values >.10 were analysed using fixed effects models. The effect size was measured as the standard mean difference, calculated using Hedges' *g*. Like Cohen's *d*, a Hedges' *g* of 0.2 can be considered a small difference, 0.5 a moderate difference, and 0.8 or more a large difference between groups. It should be noted that these values indicate statistical differences measured in terms of effect size; clinical differences must be assessed by reference to scores on

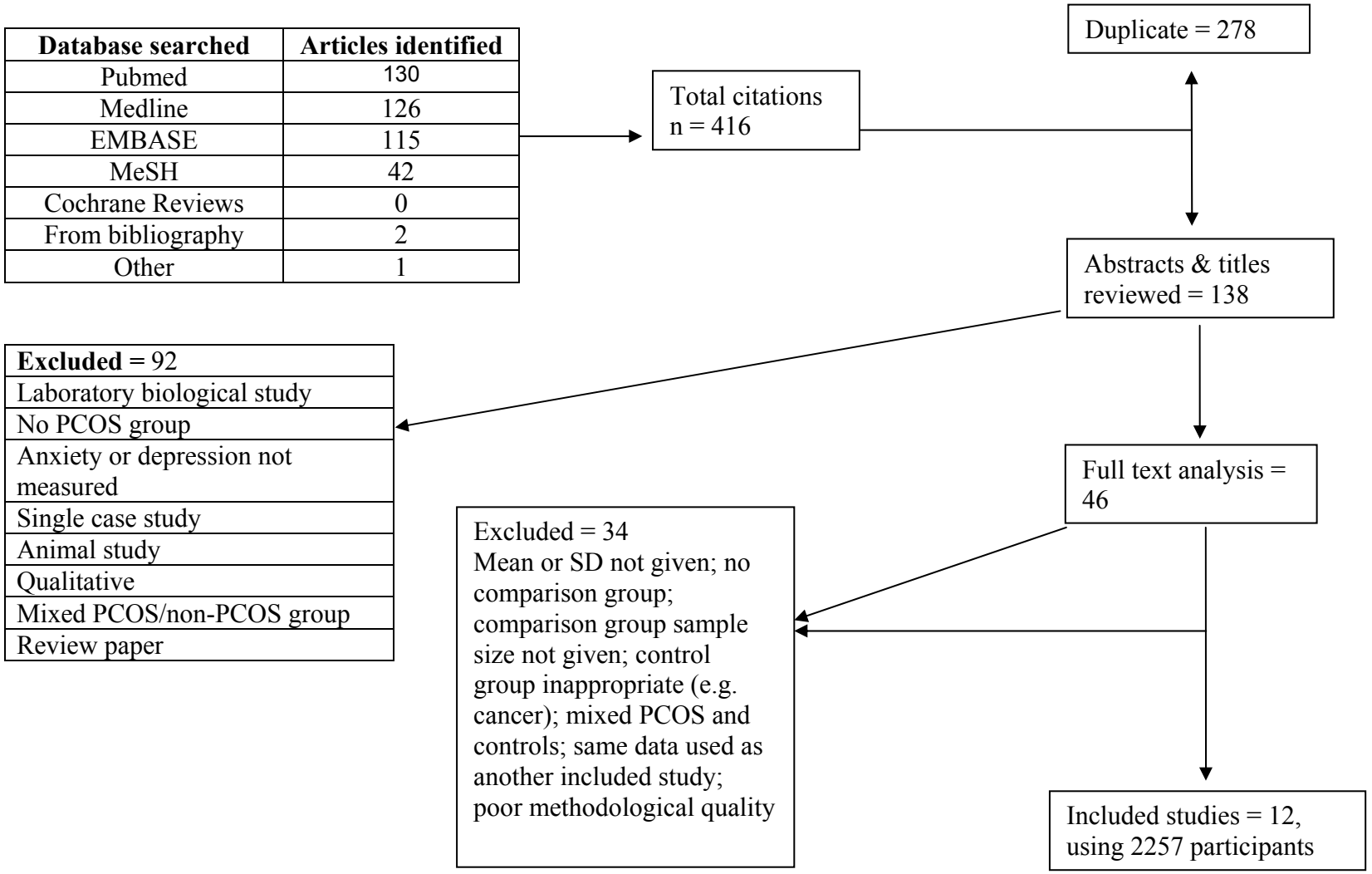
clinical measures of anxiety and depression (see Supplementary Table 2 and Supplementary Table 3 on the *Human Reproduction* website [in appendix in thesis]).

Results

Studies of anxiety and/or depression in PCOS (n=138) were retrieved, mostly from the electronic databases (Fig. 1). Of these, 126 studies were excluded, thus 12 studies including 2257 participants (910 women with PCOS and 1347 controls), qualified for review according to the inclusion criteria. For all of these studies, those that measured depression also measured anxiety. There were no separate studies that examined only anxiety.

Figure I Literature search and study selection for systematic review of studies of anxiety and depression in women with polycystic ovary syndrome (PCOS) compared to controls.

Database searched	Articles identified
Pubmed	130
Medline	126
EMBASE	115
MeSH	42
Cochrane Reviews	0
From bibliography	2
Other	1



Two of the included studies (Rocco et al., 1991; Weiner et al., 2004) used the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch & Lushene 1970) and reported scores for both state and trait anxiety and depression. The results were similar on state and trait measures in both studies. Because the most frequently used measure of depression in the included studies was a measure of state depression ,the Beck Depression Inventory (BDI; Beck, Ward, Mendelson, Mock & Erbaugh, 1961), for the sake of consistency only state anxiety and depression scores were included in the meta-analysis.

Himelein and Thatcher (2006) had two control groups, one infertility group and one community control. Because women with PCOS may experience problems with fertility, in the interests of maximising between-group comparability only, the infertility control group was included in the present analysis.

Table 1 shows the characteristics of the included studies and Table II shows the methodological quality of the included studies.

Table I Characteristics of the twelve included studies of anxiety and depression in polycystic ovary syndrome (PCOS) compared to controls.

Study	Age (years)		BMI(kg/m^2)		Control group features	Controls: ovarian cysts excluded?	Psychiatric cases excluded	Diagnosis PCOS	Medication status		Duration on medication	Variables controlled[a, b]	Outcome measure
	PCOS	Control	PCOS	Control					Metformin	OCP			
Rocco et al (1991)	20.2 (2.75)	21.7 (2.21)	22.7 (1.81)	21.01 (0.95)	Healthy medical students	Yes	No	3 symptoms	Not stated	Not stated	Not stated	Age, culture	MMPI, STAI
Weiner et al (2004)	28.19 (4.84)	30.07 (6.48)	37.7 (3.46)	36.89 (7.24)	Advert. Normal menstruation & Testosterone	No	Yes	3 symptoms; some self report	none	none	off minimum 2 months	Age, BMI, ethnicity, education	DACL, STAI
Himelein & Thatcher (2006)	32.1 (5.5) (all participants).		33.8 (8.5)	23.4 (3.6)	2 groups; infertile & healthy. 98% all Participants White	Unclear	No	Rotterdam-like[f]	78% PCOS report receiving "some type of treatment for PCOS"		Not stated	Fertility. Also age, demographics	BDI-SF
Hollinrake et al (2007)	29.8 (6.2)	30.7 (8.5)	34.9 (8.5)	24.5 (4.7)	Healthy	No	No	Rotterdam	Not stated	Same rate in both groups	Not stated	Age, demographics	BDI-II
Barnard et al (2007a) *No AA* [g]	31 (6.5)	31 (7.9)	31 (10.23)	25 (5.12)	Internet	No	No	Rotterdam (self-report)	none	none	Not stated	AA	Zung
Barnard et al (2007a) *AA* [g]	29 (5.6)	26 (5.11)	32 (9.4)	23 (4.3)	Internet	No	No	Rotterdam (self-report)	AA	AA	Not stated	AA	Zung
Soyupek et al (2008)	24.1 (6.1)	26.1 (5.7)	24.5 (6.5)	22.5 (2.6)	Healthy	No	No	Rotterdam	No PCOS on meds for PCOS. Not stated controls.		Not stated	Age [d]	BDI
Adali et al (2008)	23.54 (3.13)	24.45 (2.47)	28.42 (4.3)	24.11 (4.14)	No PCOS features	Yes	Yes	Rotterdam	All Ps clear for minimum 6 months			Age, demographics	BDI
Benson et al (2008)	28.9 (0.7)	29.9 (1.2)	29.6 (1.0)	23.6 (0.7)	Advert.	No	No	Rotterdam	Not stated	Not stated	Not stated	Age	BDI
Ozenli et al (2008)	27.58 (7.66)	26.54 (5.16)	25.43 (5.58)	24.76 (5.37)	Friends & family of staff	No	Yes	Rotterdam	None	Not stated	PCOS off for min. 3 months	BMI, age, demographics	BDI, STAI

Benson et al (2009)	30.1 (0.9)	31.5 (1.1)	29.8 (1.6)	28.7 (1.5)	Advert; healthy	No	Yes	Rotterdam	44% PCOS; controls?	0% controls; PCOS?	Not stated	BMI, age, demographics, psych. problems	BDI, STAI
Laggari et al (2009)	16.95 (2.0)	17.04 (2.16)	24.63 (6.42)	20.7 (2.97)	Schools, colleges	No	No	Rotterdam	Not stated	none	Never	Age	BDI, STAI
Barry et al (2011)	28.8 (4.81)	35.12 (4.37)	27.87 (7.36)	24.69 (7.08)	Subfertility	No	Severe psychiatric cases excluded	Rotterdam	76% PCOS & 11% controls on AA. Status of 33% of total sample unknown.		Not stated	Infertility[c]	HADS

[a] Variables controlled prior to statistical manipulation.

[b] Demographics here indicates that one or more of the following were controlled or were not significantly different in each group: race, education, income. Other demographics factors may be included in some studies e.g. religion, city or rural dwelling.

[c] Barry et al (2011) has also performed a subgroup analysis controlling for age and BMI but because medians were presented for depression, data from the total group analysis is presented here.

[d] Groups were matched for age. Although age was significantly different in both groups, the difference was not of clinical significance.

[e] "Infertile women with PCOS were not included in the study because infertility imputes depressive characters for these patients" (Ozenli et al 2008, p. 191).

[f] Rotterdam-like indicates that PCOS diagnosis was based on 2 of 3 PCOS symptoms, but not as defined by the Rotterdam criteria (2004).

[g] Barnard et al (2007a) has two rows, one for participants taking anti-androgen (AA) medication and one for those not taking AA.

AA = anti-androgen medication; OCPs, metformin, or other medication capable of reducing testosterone levels; OCP = oral contraceptive pill, or other anti-androgen; Metf. = metformin, or other insulin sensitiser;
MMPI = Minnesota Multiphasic Personality Inventory
STAI = State-Trait Anxiety Inventory
DACL = *Depression* Adjective Check Lists
BDI = Beck Depression Inventory; BDI-II = Beck Depression Inventory-II
BDI-SF = Beck Depression Inventory Short Form
Zung = Zung Self-Rating Depression Rating
HADS = Hospital Anxiety and Depression Scale

Table II Evaluation of the methodological quality of the studies using the Newcastle-Ottowa Quality Assessment Scale (NOS).

Study	Case definition adequate	Representativeness of cases	Selection of Controls	Definition of Controls	Comparability of both groups	Ascertainment of diagnosis	Same ascertainment method for both groups	Non-Response rate	**NOS Score**
Rocco et al (1991)	*	x	*	* [a]	*	*	x	x	5
Monzani et al (1994) [b]	*	x	x	x[a]	*	x	x	x	2
Weiner et al (2004)	x	*	*	*	**	x	x	x	5
Himelein & Thatcher (2006)	*	*	*	* [a]	*	*	x	*	7
Hollinrake et al (2007)	*	*	x	*	*	*	x	*	6
Barnard et al (2007a)	x	x	*	x	**	x	*	x	4
Soyupek et al (2008)	*	*	x	*	**	*	x	x	6
Adali et al (2008)	*	*	x	* [a]	**	*	*	x	7
Benson et al (2008)	*	x	*	*	*	*	*	x	6
Ozenli et al (2008)	*	x	*	*	**	*	x	x	6
Benson et al (2009)	*	x	*	*	**	*	x	x	6
Laggari et al (2009)	*	*	*	*	*	*	x	x	6
Barry et al (2011)	*	x	*	*	*	*	x	*	6

[a] Controls screened for presence of polycystic ovaries; [b] Excluded from meta-analysis because of low NOS score; * NOS quality assessment star

The funnel plots in Supplementary Figure I (see online supplement [appendix in thesis]) suggest that publication bias was probably not a significant problem for this meta-analysis. The plot on the right shows that all of the anxiety studies are (a) closely grouped, (b) reasonably symmetrical, and (c) the larger studies (near the top of the graph) tend towards the mean more than the smaller studies. The plot on the left is less symmetrical than the plot on the right, indicating that the studies reporting on only depression (rather than anxiety and depression) may show signs of possible publication bias. Only two of the 12 studies included fewer than 50 participants, making the possibility of small study effects unlikely.

Main outcomes

Table III shows the results of the meta-analyses. Figures II and III show forest plots and test statistics for the main outcomes, depression and anxiety, respectively, and subgroup analyses are shown in Supplementary Figures -II to VII (online supplement [appendix in thesis]). Most analyses used fixed effects models, except the three subgroups that used random effects models due to showing levels of heterogeneity outside the accepted limits for the use of fixed effects models (Supplementary Figures II, V and VI). The findings were fairly similar regardless of whether fixed or random effects models were applied.

Table III. Results of meta-analyses for all included studies and the subgroups for women with PCOS compared to controls.

Analysis	Number of studies	Hedges' *g* [95% CI][a]	Z *(p)*	Chi² *(p)*	I²
Depression	12	0.82 [0.73, 0.92]	Z = 17.92 (P < 0.00001)	14.76 (P = 0.19)	25%
Depression [b]	13	0.82 [0.61, 1.03]	Z = 7.65 (P < 0.00001)	40.14 (P = 0.0001)	70%
Anxiety	6	0.54 [0.33, 0.75]	Z = 5.03 (P < 0.00001)	5.45 (P = 0.36)	8%
Anxiety [b]	7	0.76 [0.35, 1.17]	Z = 3.64 (P = 0.0003)	23.03 (P = 0.0008)	74%
Depression on AA [c d]	5	0.84 [0.64, 1.04]	8.20 (P < 0.00001)	7.55 (P = 0.11)	47%
Depression no AA [c]	5	0.74 [0.61, 0.88]	10.76 (P < 0.00001)	2.36 (P = 0.67)	0%
Depression matched BMI	3	0.65 [0.37, 0.94]	4.48 (P < 0.00001)	0.54 (P = 0.76)	0%
Depression BMI unmatched [d]	9	0.77 [0.62, 0.93]	9.64 (P < 0.00001)	12.61 (P = 0.13)	37%
Anxiety matched BMI[d]	3	0.48 [0.08, 0.87]	2.38 (P = 0.02)	3.60 (P = 0.17)	44%
Anxiety BMI unmatched	3	0.63 [0.33, 0.93]	4.11 (P < 0.0001)	1.28 (P = 0.53)	0%

[a] 95% CI = 95% confidence intervals

[b] These results show the effect on heterogeneity of including data from Monzani et al (1994); I^2 is increased above 30% and the Chi^2 *p* value becomes significant, thus the random effects model has been used. This study also had a low score for methodological quality and was excluded from the meta-analysis based on the criteria described by Xiong et al. (2010)[c] AA = anti-androgen medication, i.e. medication commonly used to treat PCOS, mostly metformin or oral contraceptives.

[d] The random effects model was used, as I^2 was >30% and the Chi^2 *p* value was almost 0.10

Figure II Forest plot of all included studies of depression in women with PCOS compared to controls.

Study or Subgroup	PCOS Mean	PCOS SD	PCOS Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Fixed, 95% CI	Year
Rocco et al D	73	18.33	21	47.1	7.59	10	1.1%	1.60 [0.73, 2.46]	1991
Weiner et al D State	11.3	6.49	27	6.81	4.87	27	2.6%	0.77 [0.22, 1.33]	2004
Himelein & Thatcher D	7.5	7	40	4.56	5.03	40	4.1%	0.48 [0.03, 0.92]	2006
Barnard et al D	56	12.28	419	45.5	11	930	56.1%	0.92 [0.80, 1.04]	2007
Hollinrake et al D	11.9	11.1	103	4.5	5.9	103	10.0%	0.83 [0.54, 1.11]	2007
Soyupek et al D	9.78	8.05	41	6.42	5.03	35	3.9%	0.49 [0.03, 0.94]	2008
Benson et al D	10.1	7.55	57	5.9	7.41	28	3.8%	0.55 [0.09, 1.01]	2008
Adali et al D	11.69	9.49	42	5.8	4.58	42	4.1%	0.78 [0.34, 1.23]	2008
Ozenli et al D	14.71	7.67	35	10.5	5.26	35	3.5%	0.63 [0.15, 1.11]	2008
Benson et al 09 D	9.7	7.92	32	4.9	5.09	32	3.2%	0.71 [0.21, 1.22]	2009
Laggari et al D	12.82	7.86	22	10.32	7.19	22	2.3%	0.33 [-0.27, 0.92]	2009
Barry et al D	5.42	1.98	71	3.73	2.04	43	5.2%	0.84 [0.44, 1.23]	2011
Total (95% CI)			**910**			**1347**	**100.0%**	**0.82 [0.73, 0.92]**	

Heterogeneity: Chi² = 14.76, df = 11 (P = 0.19); I² = 25%

Test for overall effect: Z = 17.92 (P < 0.00001)

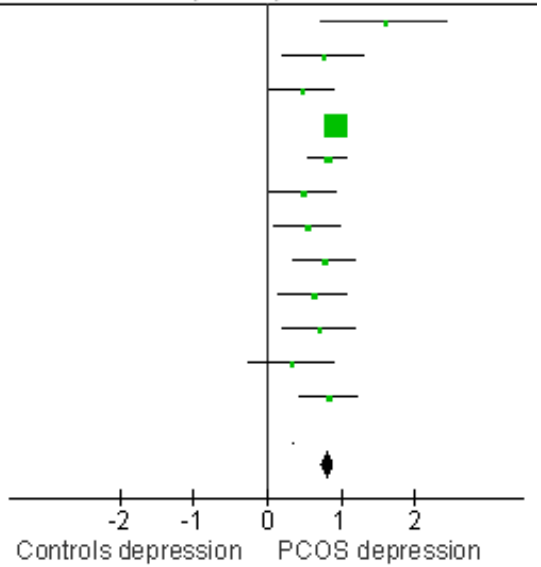
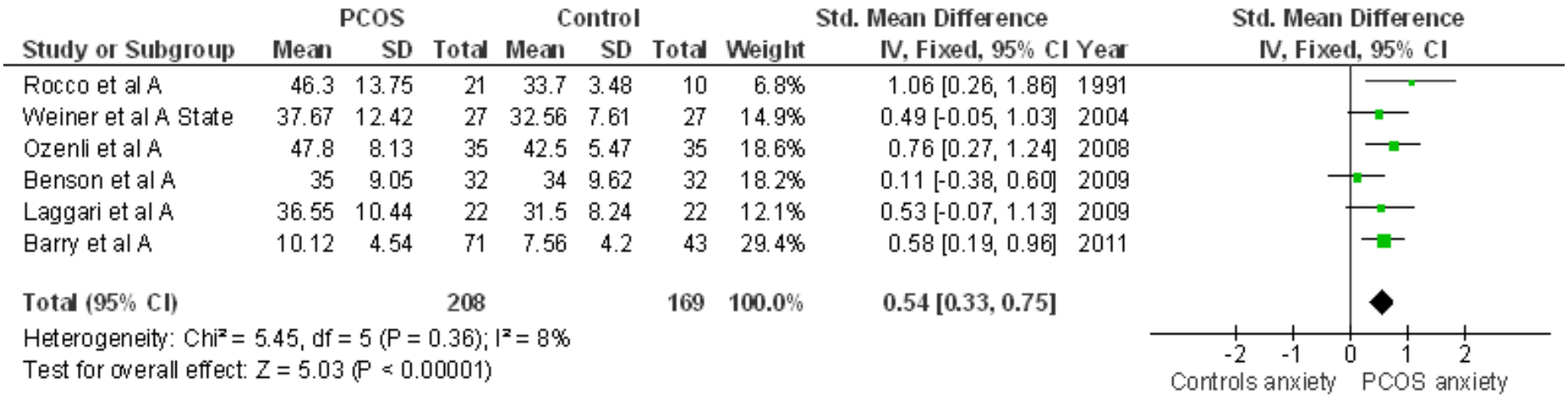


Figure III Forest plot of all included studies of anxiety in women with PCOS compared to controls.



The most commonly used outcome measure for assessing depression was the BDI, which was used in eight of the 12 studies (see Supplementary Table II). Six of these studies used comparable versions of the BDI, with mild depression being indicated when scores reach 11. For these six studies the mean BDI score for women with PCOS was 11.47, and for controls it was 7.31. This difference is statistically significant ($Z = 5.94$, $p<.00001$; Hedges' $g = 0.60$; 95% CI 0.40 to 0.80) with 0% heterogeneity ($I^2 = 0\%$). In clinical terms this represents the difference between, on average, mild depression in the PCOS groups and no depression in the control groups. For anxiety, the most commonly used outcome measure was the STAI-S (state anxiety measure), and was used in 5 of the 6 studies. In these five studies the women with PCOS indicated statistically significantly higher anxiety scores ($Z = 3.47$, $p<.0005$; Hedges' $g = 0.46$; 95% CI 0.20 to 0.73) with little heterogeneity ($I^2 = 14\%$). The control groups scored a mean of 34.6 on the STAI-S, which is slightly below the norm of 36.2 for women of this age group (Spielberger, 1983), and the mean score for women with PCOS was 40.7, which is mildly elevated.

Table III shows a slightly larger difference in depression scores between women with PCOS and controls for women with PCOS were taking anti-androgen medication (Hedges' $g = 0.84$; 95% CI 0.64 to 1.04) than for women with PCOS were not taking anti-androgen medication (Hedges' $g = 0.74$; 95% CI 0.61 to 0.88).

Discussion

Analysis of the 12 studies and subgroups revealed differences in the scores for anxiety and depression, which were significantly higher in the PCOS groups compared to controls. These findings support those of the previous research reviewed by Farrell and Antoni (2010). The confidence intervals for the included studies were generally narrow and showed effect sizes which may be clinically relevant. Of the 18 results (12 depression and 6 anxiety), four confidence intervals encompassed a zero value, which suggests that overall (14 of the 18 studies) the results were representative of those likely to be seen in the general population of women with PCOS and healthy women.

This meta-analysis helps to quantify the impact of BMI on anxiety and depression, and suggests that BMI has a small effect on anxiety and depression in PCOS. The three studies that controlled for BMI (Weiner et al., 2004; Özenli et al., 2008; Benson et al., 2009) found on average a smaller difference in depression between women with PCOS and controls (Hedges' $g = 0.65$) than the eight studies that did not control for BMI (Hedges' $g = 0.77$), a small difference in effect size ($g = 0.12$). This finding lends modest support to previous research suggesting an impact of obesity on depression (Rasgon et al, 2003) and perhaps also QoL (Jones et al., 2008). For anxiety, the three studies that controlled for BMI also found on average a smaller difference between women with PCOS and controls (Hedges' $g = 0.48$) than the three studies that did not control for BMI (Hedges' $g = 0.63$). The difference in anxiety when controlling for BMI represents a small effect size ($g = 0.15$), similar to the impact of BMI on depression.

The differences between women with PCOS and controls for anxiety and depression were statistically significant, but what is the clinical significance of these findings? The most commonly used outcome measure for assessing depression, the BDI, found that women with PCOS scored statistically significantly higher than women without PCOS. In clinical terms this represents the difference between, on average, mild depression in the PCOS group and no depression in the control group. This appears to suggest that the difference between the groups is, on average, only of limited clinical significance. However the SDs in Supplementary Table II indicate that some proportion of the women in these studies had depression scores of greater clinical interest, especially in the PCOS groups. For example, Benson et al (2008) found a mean PCOS BDI scores of 10.1 with an SD of 7.55; given that these scores passed tests of normality of distribution, and given the properties of the normal distribution, we can infer that roughly 15% of women with PCOS in this study had depression scores of 18 or more, which places them in the range of moderate to severe depression. However it should be noted that although the cut offs for BDI scores offer

guidance on depression severity, interpretation of scores should be based on clinical judgement (Beck, Steer & Garbin, 1988).

For anxiety, the most commonly used outcome measure was the STAI-S (state anxiety measure), used in 5 of the 6 studies. The control groups scored slightly below the norm, and the PCOS groups' score wasmildly elevated. Although this is perhaps only a modest clinical difference in anxiety scores, any increase in anxiety might be clinically significant in PCOS because of the potential impact of anxiety on testosterone levels; given that stress activates the adrenal glands via the hypothalamic-pituitary adrenal axis (HPA) (Reiche et al., 2004) and that women produce roughly 25% of the testosterone from their adrenal glands (Burger, 2002) the level of stress that a woman experiences in her daily life may potentially increase her testosterone levels, thus exacerbating the symptoms of PCOS. Furthermore, there is evidence that women with PCOS show a stronger HPA response to a stressor than healthy women (Benson et al., 2009; Gallinelli et al., 2000) which suggests that the elevations in anxiety ratings seen in the studies reviewed in the present meta-analysis are of special relevance. The potential impact of anxiety and stress on testosterone levels in PCOS is clearly of importance and could be explored in future research.

It should be noted that the control group participants from the included studies were drawn from a variety of sources, for example, the Internet, friends and family of the researchers, and medical students (Table 1). Although the NOS criteria specify that community controls are preferable to hospital controls, the wide variety of control groups in the present review implies a heterogenous selection process, which introduces uncertainty in interpretation of the findings because of the unknown influence of possible selection bias. Variation in the selection of participants is an issue often seen when reviewing comparative observational studies and the possible effects of this should be borne in mind when considering the findings of the present review. The NOS quality ratings ranged from four to seven, out of a maximum of nine. Eleven of the 12 studies scored five or more, which indicates reasonably good methodological quality overall. The NOS criteria specify that community controls are preferable to hospital controls but it could be argued that in studies

where women with PCOS were recruited from hospitals and clinics, a hospital or clinic control group may be the most appropriate comparison provided that the controls had medical conditions of comparable impact to PCOS. Nonetheless, in the interests of adherence to the NOS criteria, studies that used hospital control groups (Adali et al., 2008; Hollinrake et al., 2007; Barry et al., 2011) lost an NOS quality assessment star. Where a study presented more than one control group, the one best matched to the PCOS group was used in the presented analysis. Thus Himelein and Thatcher's (2006) infertility control group was used, which had the effect of reducing the size of the difference in depression between women with PCOS and controls. The Barry et al.(2011) matched group was not used because the median rather than mean was given for the depression outcome.

Only three studies (Adali et al., 2008; Himelein and Thatcher, 2006; Rocco et al., 1991) tested for polycystic ovaries in control groups. Although the findings from the present study suggest that screening controls for polycystic ovaries is not essential (see below), this raises the question of whether a woman with polycystic ovaries can be classified as a control in a study of PCOS. Controls can be defined by excluding menstrual problems and hyperandrogenism (Rotterdam criteria i and ii; Rotterdam ESHRE/ASRM, 2004) without reference to polycystic ovaries, but this leaves open the possibility that women in the control groups had one of the diagnostic conditions (polycystic ovaries) for membership of the PCOS group. Polycystic ovary morphology (as seen on ultrasound) has been found in around 23% of regularly menstruating women (Koivunen et al., 1999; Polson et al., 1988), and although this may represent one end of the spectrum of symptom severity in genetically predisposed individuals, this single symptom in asymptomatic women is not generally considered to be of clinical importance. Theoretically therefore the failure to screen controls for polycystic ovaries could reduce group differences on outcomes related to PCOS, but in the present meta-analysis the three studies that excluded polycystic ovaries in their control groups had a slightly *lower* mean Hedge's *g* for depression than the other nine studies (g = 0.74 versus 0.83, respectively): the smaller difference in depression score in these studies is not explained by other factors (study quality, BDI scores etc), thus it could be concluded that

screening for polycystic ovaries in control groups does not seem to be a confounding variable in studies of depression in PCOS. Only one of the three studies measured anxiety, so an assessment of the effect of screening for polycystic ovaries on anxiety scores cannot be made.

The finding that women with PCOS on medication for PCOS were slightly more depressed compared to controls than women with PCOS not on medication appears to suggest there is a small benefit in terms of depression of not taking PCOS medication, contradicting the finding by Rasgon et al., (2003) and supporting the finding of Barnard et al., (2007). However, because different outcome measures were used in the various studies (none of the on-medication PCOS groups used exactly the same scale), it is difficult to compare scores for the women with PCOS on medication to the women with PCOS not taking medication. Also the apparent benefit – relative to controls - of women with PCOS not taking anti-androgen medication is possibly caused in part by high depression scores in two of the control groups relative to their respective PCOS groups (Laggari et al., 2009; Özenli et al., 2008). Furthermore, medication use was not clearly defined in the studies (see below). For these reasons the small apparent affect of medication use (Hedge's $g = 0.1$) should be interpreted with caution. Regarding the effect of medication for PCOS on anxiety scores, there were too few studies of anxiety that differentiated groups by medication status to assess any effect.

A weakness of the present meta-analysis is that medication use was not clearly defined in the included studies. Whether oral contraceptives or other anti-androgenic medications were used by the control group is unclear in seven of the twelve control groups, perhaps because these researchers did not hypothesise that healthy women would be affected by anti-androgen medication use. In the five studies classified as 'on medication', the numbers of women with PCOS taking medication ranged from 100% to 44% (see Table 1). The study that most clearly stratified by medication use (100% on medication in Barnard et al., 2007) relied on self-report, and as always self-report introduces uncertainty as to the exact numbers. The type of medication was not stratified in any of the studies making it

impossible to assess, for example, whether insulin sensitisers affect women differently than anti-androgens, as found by Harborne et al., (2003). Four of the 12 studies stated the length of time that participants with PCOS had not been taking medication (minimum two months), and none stated the duration that the controls were on medication. Because the most common PCOS medications will have had their major effects within six months (Harborne et al., 2003), it would be useful to know at least whether they had been taking medication for a longer or shorter period than six months. Future studies would be improved by stratifying findings by medication type, duration of medication, and dose.

Some studies controlled for age. All studies were of adult women of childbearing age, and apart from the Laggari et al. (2009) sample who were in their mid to late teens, all studies were of women aged on average in their mid twenties to early thirties. The Hedges's *g* for the Laggari et al. (2009) sample was smaller than the mean Hedges's *g* for the other studies for depression (*g* =0.33 versus 0.84) but was the same for anxiety (*g* = 0.54 versus 0.54) suggesting that younger women with PCOS may be similarly anxious but less depressed than women with PCOS in their 20s and 30s. This finding undermines to some degree the suggestion that PCOS causes more distress to younger women because of the impact of PCOS on physical appearance (Farrell and Antoni 2010) and fertility (Trent et al., 2003). However more studies with young women with PCOS are needed to help clarify this issue.

Five of the 12 studies screened for psychiatric illness, and these studies showed slightly smaller differences between depression scores in women with PCOS and controls (Hedge's *g* = 0.76) compared to those with no screening (Hedge's g = 0.84). This suggests that screening for psychiatric illness in studies of anxiety and depression in PCOS does not have a major impact on results. However other factors that might affect mood in women with PCOS should also be accounted for in future research e.g. the presence of hypoglycaemia (Kasim-Karakas et al., 2007) or proinflammatory cytokines (González et al., 2010).

Because of the possibility that state anxiety might be raised simply by being in a hospital context, future studies might also consider stating the context in which the questionnaires were completed. Although none of the included studies published data on this issue, the present authors know that 59% of the Barry et al (2011) sample opted to fill in the questionnaire online, the remainder doing so at the clinic. However, context appeared to have little effect on Hospital Anxiety and Depression (HADS; Zigmond & Snaith, 1983) anxiety scores as there was no significant difference between those completing the survey at the clinic for the PCOS group, controls or both groups combined.

Conclusion

PCOS is generally seen as a reproductive endocrine disorder, with symptoms of modest importance compared to other conditions with more obvious effects on wellbeing but the results of this systematic review highlight the psychological distress experienced by the 5-10% of women affected by this condition. General practitioners, gynaecologists, endocrinologists and clinical psychologists should be more aware that levels of anxiety and depression are higher in women with PCOS than controls. The fact that differences in anxiety and depression scores between women with PCOS and controls are slightly lower when both groups have a similar BMI would suggest that mood in PCOS might be improved to some degree through weight control, and a recent study found that the low GI diet has some advantages for women with PCOS (Marsh et al., 2010). Future studies in this field should also be directed at assessing the potential to alleviate psychological distress using antiandrogens and insulin sensitisers, currently used to treat physical symptoms of PCOS, as well as conventional antidepressant and anxiolytic therapy. A 'talking treatment', such as cognitive behavioural therapy or counselling, should be considered too.

Author's roles

JAB conducted the literature search, assessed study characteristics, study quality assessment, conducted the data analysis, interpretation of statistics, and drafted the paper. AK critically

reviewed the paper, commenting on the clinical psychological interpretation of statistical findings. PJH commented on clinical/biological aspects of the findings, and revised the paper. All authors approved the final version.

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Conflict of interest

The authors report no conflicts of interest.

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References

Adali E, Yildizhan R, Kurdoglu M, Kolusari A, Edirne T, Sahin HG, Yildizhan B, and Kamaci M. The relationship between clinico-biochemical characteristics and psychiatric distress in young women with polycystic ovary syndrome. *J Int Med Res* 2008;**36**: 1188-1196.

Barry J, Hardiman PJ, Saxby BK, A Kuczmierczyk A. Testosterone and Mood Dysfunction in Women With Polycystic Ovarian Syndrome Compared to Subfertile Controls. *J Psychosom Obst Gyn* 2011 [Epub ahead of print]

Barnard L, Ferriday D, Guenther N, Strauss B, Balen AH, and Dye L. Quality of life and psychological well being in polycystic ovary syndrome. *Hum Reprod* 2007;**22**: 2279-2286.

Beck AT, Ward CH, Mendelson M, Mock J, and Erbaugh, J. An inventory for measuring depression. *Arch Gen Psychiatry* 1961;**4**: 561-571

Beck AT, Steer RA, and Garbin MG. Psychometric properties of the Beck Depression Inventory: twenty-five years of evaluation. *Clin Psychol Rev* 1988;**8**:77-100.

Benson S, Arck PC, Tan S, Hahn S, Mann K, Rifaie N, Janssen OE, Schedlowski M, and Elsenbruch S. Disturbed stress responses in women with polycystic ovary syndrome. *Psychoneuroendocrinology* 2009;**34**: 727-735.

Bruce-Jones W, Zolese G, and White P. Polycystic ovary syndrome and psychiatric morbidity. *J Psychosom Obstet Gynaecol* 1993;**14**: 111-116.

Burger HG. Androgen production in women. *Fertil Steril* 2002;**77 Suppl 4**: S3-5.

Eggers S, and Kirchengast S. The polycystic ovary syndrome--a medical condition but also an important psychosocial problem. *Coll Antropol* 2001;**25**: 673-685.

Farrell K, and Antoni MH. Insulin resistance, obesity, inflammation, and depression in polycystic ovary syndrome: biobehavioral mechanisms and interventions. *Fertil Steril* 2010;**94**: 1565-1574.

Franks S. Polycystic ovary syndrome. *N Engl J Med* 1995;**333**: 853-861.

Gallinelli A, Matteo ML, Volpe A, and Facchinetti F. Autonomic and neuroendocrine responses to stress in patients with functional hypothalamic secondary amenorrhea. *Fertil Steril* 2000;**73**: 812-816.

González F, Rote NS, Minium J, Weaver AL, and Kirwan JP. Elevated circulating levels of macrophage migration inhibitory factor in polycystic ovary syndrome. *Cytokine* 2010;**51**: 240-244.

Hahn S, Janssen OE, Tan S, Pleger K, Mann K, Schedlowski M, Kimmig R, Benson S, Balamitsa E, and Elsenbruch S. Clinical and psychological correlates of

quality-of-life in polycystic ovary syndrome. *Eur J Endocrinol* 2005;**153**: 853-860.

Harborne L, Fleming R, Lyall H, Sattar N, and Norman J. Metformin or antiandrogen in the treatment of hirsutism in polycystic ovary syndrome. *J Clin Endocrinol Metab* 2003;**88**: 4116-4123.

Himelein MJ, and Thatcher SS. Depression and body image among women with polycystic ovary syndrome. *J Health Psychol* 2006;**11**: 613-625.

Hollinrake E, Abreu A, Maifeld M, Van Voorhis BJ, and Dokras A. Increased risk of depressive disorders in women with polycystic ovary syndrome. *Fertil Steril* 2007;**87**: 1369-1376.

Jones GL, Hall JM, Balen AH, and Ledger WL. Health-related quality of life measurement in women with polycystic ovary syndrome: a systematic review. *Hum Reprod Update* 2008;**14**: 15-25.

Kasim-Karakas SE, Cunningham WM, and Tsodikov A. Relation of nutrients and hormones in polycystic ovary syndrome. *Am J Clin Nutr* 2007;**85**: 688-694.

Kitzinger C, and Willmott J. 'The thief of womanhood': women's experience of polycystic ovarian syndrome. *Soc Sci Med* 2002;**54**: 349-361.

Koivunen R, Laatikainen T, Tomás C, Huhtaniemi I, Tapanainen J, and Martikainen H. The prevalence of polycystic ovaries in healthy women. *Acta Obstet Gynecol Scand* 1999;**78**: 137-141.

Laggari V, Diareme S, Christogiorgos S, Deligeoroglou E, Christopoulos P, Tsiantis J, and Creatsas G. Anxiety and depression in adolescents with polycystic ovary syndrome and Mayer-Rokitansky-Küster-Hauser syndrome. *J Psychosom Obstet Gynaecol* 2009;**30**: 83-88.

Månsson M, Holte J, Landin-Wilhelmsen K, Dahlgren E, Johansson A, and Landén M. Women with polycystic ovary syndrome are often depressed or anxious--a case control study. *Psychoneuroendocrinology* 2008;**33**: 1132-1138.

Marsh KA, Steinbeck KS, Atkinson FS, Petocz P, and Brand-Miller JC. Effect of a low glycemic index compared with a conventional healthy diet on polycystic ovary syndrome. *Am J Clin Nutr* 2010;**92**: 83-92.

Monzani F, Pucci F, Caraccio N, Bagnolesi A, Molli D, Fenu A, and Prunetti C. Psychological and psychopathological correlates in the polycystic ovary syndrome (PCOS). *Med Psicosom* 1994;**39**: 225-236.

Özenli Y, Haydardedeoglu B, Micozkadıoğlu I, Şimşek E, Kılıçdağ E, and Bağış T. Anxiety, Depression and Ways of Coping Skills in Women with Polycystic Ovary Syndrome: A Controlled Study. *J Turkish German Gynecol Assoc* 2008;**9**: 190-193.

Polson DW, Adams J, Wadsworth J, and Franks S. Polycystic ovaries--a common finding in normal women. *Lancet* 1988;**1**: 870-872.

Rasgon NL, Rao RC, Hwang S, Altshuler LL, Elman S, Zuckerbrow-Miller J, and Korenman SG. Depression in women with polycystic ovary syndrome: clinical and biochemical correlates. *J Affect Disord* 2003;**74**: 299-304.

Reeves BC, Deeks JJ, Higgins JPT and Wells GA. Chapter 13: Including non-randomized studies. In: Higgins JPT, Green S (editors), *Cochrane Handbook for Systematic Reviews of Interventions.* Version 5.0.1 [updated September 2008]. The Cochrane Collaboration, 2008. Available from www.cochrane-handbook.org. Accessed 4th May 2011

Reiche EMV, Nunes SOV, and Morimoto HK. Stress, depression, the immune system, and cancer. *Lancet Oncol* 2004;**5**: 617-625.

Rocco A, Falaschi P, Perrone G, Pancheri P, Rosa M, and Zichella L. Psychoneuroendocrine aspects of polycystic ovary syndrome. *Journal of Psychosomatic Obstetrics & Gynecology* 1991;**12**: 169-179.

Rotterdam ESHRE/ASRM, 2004. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* **19**: 41-47

Spielberger CD, Gorsuch RL, and Lushene RE. Manual for the State-Trait Anxiety Inventory (STAI). Palo Alto, CA: Consulting Psychologists Press; 1970.

Spielberger C. Manual for the State-Trait Anxiety Inventory STAI (Form Y). Palo Alto, CA, Consulting Psychologists Press, 1983

Stunkard AJ, Faith MS, and Allison KC. Depression and obesity. *Biol Psychiatry* 2003;**54**: 330-337.

Trent ME, Rich M, Austin SB, and Gordon CM. Fertility concerns and sexual behavior in adolescent girls with polycystic ovary syndrome: implications for quality of life. *J Pediatr Adolesc Gynecol* 2003;**16**: 33-37.

Weiner CL, Primeau M, and Ehrmann DA. Androgens and mood dysfunction in women: comparison of women with polycystic ovarian syndrome to healthy controls. *Psychosom Med* 2004;**66**: 356-362.

Wells G, Shea B, O'Connell D. The Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomized studies in meta-analysis. Proceedings of the Third Symposium on Systematic Reviews. Beyond the Basics: Improving Quality and Impact. Oxford 2000, 3–5 July.

Xiong C, Miller JP, and Morris JC. Measuring Study-Specific Heterogeneity in Meta-Analysis: Application to an Antecedent Biomarker Study of Alzheimer's Disease. *Stat Biopharm Res* 2010;**2**: 300-309.

Young R, and Sinclair R. Hirsutes. I: Diagnosis. *Australas J Dermatol* 1998;**39**: 24-28.

Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. *Acta Psychiatr Scand* 1983;**67**:361-370.

APPENDIX

Supplementary Table I Excluded studies (N=126). 'Y' indicates that the full paper was reviewed, 'N' indicates that only the title &/or abstract were reviewed.

	Study and reason for exclusion	**Full article reviewed?**
1	Herbert et al (2010) no data on depression or anxiety	Y
2	Krysztof & Blazej (2010) no means/SDs	N
3	Loughlane (2010) non-research article	Y
4	Peraita-Adrados et al (2010) focus is sleep; one patient with PCOS	N
5	Rassi et al (2010) no means/SDs	Y
6	Teede et al (2010) review	N
7	Farrell K, Antoni MH (2010) review	N
8	Benson S, et al (2010) no comparison group	Y
9	Deeks et al (2010) no comparison group	Y
10	Peacock et al (2010) Review menstrual problems	N
11	Harrison et al (2010) non-research: hirsute. problems	Y
12	Rathnayake, Sinclair et al (2010) non-research	N
13	Cinar et al (2010) no means/SDs.	N
14	Cheang et al (2010) no means/SDs.	N
15	Danciulescu Miulescu et al (2010) no means/SDs	N
16	Sayyah Melli M., Kazemi-shishvan M. (2010) no comparison	N

	group	
17	Moreira et al (2010) psychosocial approach to PCOS	N
18	Jedel et al (2010) median & range	Y
19	Wheatley & Ahmed (2010) single case	N
20	Onno et al (2010) conference abstract (duplicates Benson et al, 2009)	N
21	Bhattacharya & Jha (2009) no means /SDs	Y
22	Vrbikova & Hainer (2009) not anxiety or depression	N
23	Thomson & Buckley (2010) no control group	Y
24	Mallappa & Hanji (2010) Review (medical info for women with PCOS)	N
25	Flanagan & Jensen (2009) Hidradenitis suppurativa patients	N
26	Benson et al (2009) normative groups & *N* of norm groups not stated.	Y
27	Moran et al (2009) no means /SDs	Y
28	Eyvazzadeh et al (2009) review	N
29	Bishop et al (2009) review	N
30	Manlove (2008) not anxiety or depression	Y
31	Marangell (2008) review of bipolar	N
32	Mansson et al (2008) no means /SDs	Y
33	Aylwin & Al-Zaman (2010) Review of surgery for BMI	N
34	Kapoor (2009) comment	Y
35	Battaglia et al (2009) no means /SDs	Y
36	Rofey et al (2009) no cont or norm	Y
37	Kerchner (2009) no comparison group	Y

38	Morgan (2008) mixed PCOS & hirsute group; no comparison group	Y
39	Castillo-Quan (2008) comment	N
40	Tan et al (2008) no comparison group	Y
41	Aylwin & Al-Zaman (2008) review of obesity	N
42	Janssen et al (2008) review	N
43	Szalat & Raz (2007) review diabetes	N
44	Barnard et al (2007b) women from 2007a sample (right handed only)	Y
45	Magnotti & Futterweit (2007) review BMI & PCOS	N
46	Blume-Peytavi et al (2007) review hirsutism	N
47	Barron (2007) review menstrual (biological)	N
48	Setji & Brown (2007) review PCOS care	N
49	Galletly et al (2007) low carb., no control group	Y
50	Peritogiannis et al (2007) single case	N
51	Pecori Giraldi et al (2007) Cushing's	N
52	Anger et al (2007) no control group	Y
53	Elsenbruch (2006) no means /SDs; norm comparison.	Y
54	Vgontzas et al (2006) sleep & BMI review	N
55	Himelein & Thatcher (2006) review	N
56	Lipton et al (2006) "suspected" PCOS & no controls	Y
57	Legato et al (2006) review diabetes	N
58	Lipton et al (2006) hirsutism survey (not specifically PCOS)	N
59	Biton (2006) review BMI	N
60	Daniels (2006) review BMI children	N

61	Sobjanek et al (2006) acne	N
62	Rasgon & Kenna (2005) Alzheimers etc	N
63	Dawber (2005) review hirsutism	N
64	McCook et al (2005) QoL only (uses data from McCook 02)	Y
65	Clayton et al (2005) RCT (PCOS in all groups)	Y
66	Penovich et al (2004) epilepsy review	N
67	Swann (2004) bipolar	N
68	Brown (2004) review depression & IR in PCOS	N
69	van Wely et al (2004) RCT (PCOS in all groups)	Y
70	Ozanne et al (2004) review of Barker hypothesis	N
71	Coffey & Mason (2004) review of HRQoL in PCOS	N
72	Boro & Haut (2003) epilepsy review	N
73	Hernandez-Escalante (2003) binge eating	N
74	Keegan et al (2003) norm group of cancer patients.	Y
75	Elsenbruch (2003) no means /SDs & compared to norms	Y
76	Rasgon (2003) no control group and no means/SDs given	Y
77	Joffe (2003) review	N
78	McIntyre (2003) bipolar	N
79	Childres et al (2003) not about anxiety & depression specifically	Y
80	Ledger (2003) review	N
81	McCook (2002) unpublished thesis	N
82	Rasgon (2002) single case study	N
83	Resch & Szendei (2002) review eating disorders	N
84	O'Brien & Dixon (2002) obesity review	N
85	Rohr (2002) review T & depression	N

86	Merke et al (2002) review CAH	N
87	Martin-Du Pan (2002) review (effects of aging)	N
88	Ernst (2002) bipolar	N
89	Kitzinger & Willmott (2002) no means/SDs	Y
90	Henmi et al (2001) rats	N
91	Sills (2001) no means/SDs	N
92	Eggers & Kirchengast (2001) review/theoretical paper	Y
93	Simon-Vermot & Keller (2000) review obesity	N
94	Graña-Barcia et al (1998) GnRH depression	N
95	Björntorp (1998) review obesity	N
96	Bragagni et al (1995) not PCOS	N
97	Monzani et al. (1994) Poor methodological quality NOS rating	Y
98	Cavagnini & Invitti (1994) Cushing's Syndrome	N
99	Matsunaga & Sarai M (1993) all psych patients, no PCOS	N
100	Matsunaga Taniguchi et al (1993) bipolar	N
101	Sonino et al (1993) PCOS group mixed with non-PCOS	N
102	Bruce-Jones (1993) no means/SDs	Y
103	Levin et al (1992) case study (psychiatric patient)	N
104	Matsunaga et al (1992) psychiatric, non-PCOS	N
105	Mason et al (1990) cell function	N
106	Macleod et al (1990) biological study, no controls	N
107	Johnson & Pearce (1990) miscarriage (no psychological measures)	N
108	Fleming et al (1990) biological study, no controls	N
109	Ghaziuddin (1989) case study, bipolar	N

110	Schlaff (1989) biological / non-psychological study	N
111	Orenstein et al (1989) Unstandardized outcome measure & PCOS diagnosis unclear	N
112	Fleming (1985) biological study, no controls	N
113	Mori (1985) biological study, no controls	N
114	Cumming et al (1984) biological study, no controls	N
115	Orenstein & Raskind (1983) two cases, no controls	N
116	Suginami et al (1982) biological study, no controls	N
117	Duignan (1976) review PCOS biological studies	N
118	Netter (1976) ovarian dystrophy, non-psychological	N
119	Beck et al (1976) biological study, no controls	N
120	Mendelsohn et al (1970) biological study	N
121	Lopez et al (1969) biological study	N
122	Wiese et al (1969) biological study	N
123	Rhodes (1968) biological study	N
124	Bardin et al (1968) biological study	N
125	Serment & Piana (1968) biological study	N
126	Hart et al (1968) biological study	N

SDs, Standard Deviations.

Norm, Normative

RCT, randomized controlled trial

HRQoL, health-related quality of life

CAH, Congenital adrenal hyperplasia

Supplementary Table II Mean and standard deviation (SD) depression scores in the PCOS and control groups of the included studies and Monzani et al (1994).[a]

		PCOS			Control		
Study	Outcome	Mean	SD	N	Mean	SD	N
Rocco et al (1991)	MMPI	73.0	18.33	21	47.1	7.59	10
Monzani et al (1994)	CCEI	8.25	2.04	23	2.69	1.14	20
Weiner et al (2004)	DACL	11.3	6.49	27	6.81	4.87	27
Himelein & Thatcher (2006)	BDI-SF	7.5	7.0	40	4.56	5.03	40
Hollinrake et al (2007)	BDI-II	11.9	11.1	103	4.5	5.9	103
Barnard et al (2007)	Zung	56.0	12.28	419	45.5	11.0	930
Adali et al (2008)	BDI	11.69	9.49	42	5.8	4.58	42
Soyupek et al (2008)	BDI	9.78	8.05	41	6.42	5.03	35
Benson et al (2008)	BDI	10.1	7.55	57	5.9	7.41	28
Ozenli et al (2008)	BDI	14.71	7.67	35	10.5	5.26	35
Laggari et al (2009)	BDI	12.82	7.86	22	10.32	7.19	22
Benson et al (2009)	BDI	9.7	7.92	32	4.9	5.09	32
Barry et al (2011)	HADS	5.42	1.98	71	3.73	2.04	43

MMPI = Minnesota Multiphasic Personality Inventory

CCEI = Crown-Crisp Experiential IndexDACL = Depression Adjective Check ListsBDI-SF = Beck Depression Inventory Short Form (scores of 5-7 = mild to moderate depression)

BDI-II = Beck Depression Inventory-II (scores of 14-19 = mild to moderate depression)BDI = Beck Depression Inventory (scores of 11-18 = mild to moderate depression)

Zung = Zung Self-Rating Depression Rating

HADS = Hospital Anxiety and Depression Scale[a] The Monzani et al (1994) data was excluded due to its affect on heterogeneity and the study's relatively low score on methodological quality.

Supplementary Table III Mean and standard deviation (SD) anxiety scores in the PCOS and control groups of the included studies and Monzani et al (1994).[a]

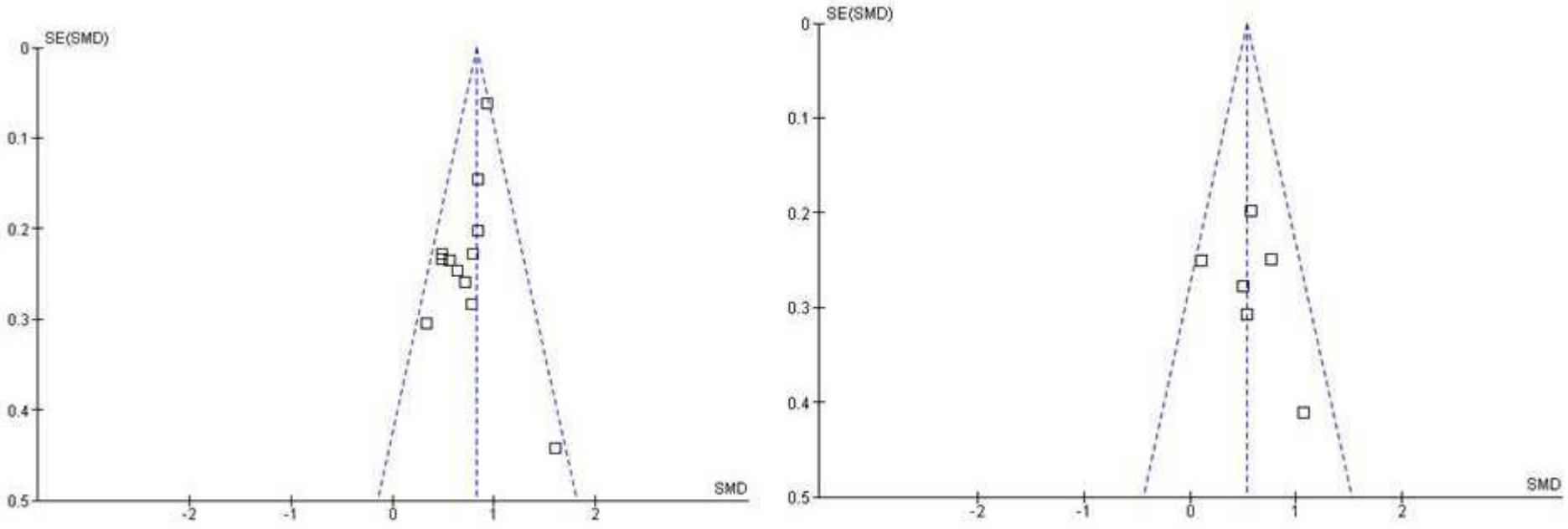
		PCOS			Control		
Study	Outcome	Mean	SD	N	Mean	SD	N
Rocco et al (1991)	STAI	46.3	13.75	21	33.7	3.48	10
Monzani et al (1994)	CCEI	9.95	3.11	23	4.08	1.64	20
Weiner et al (2004)	STAI	37.67	12.42	27	32.56	7.61	27
Ozenli et al (2008)	STAI	47.8	8.13	35	42.5	5.47	35
Laggari et al (2009)	STAI	36.55	10.44	22	31.5	8.24	22
Benson et al (2009)	STAI	35.0	9.05	32	34.0	9.62	32
Barry et al (2011)	HADS	10.12	4.54	71	7.56	4.2	43

STAI = State-Trait Anxiety Inventory
CCEI = Crown-Crisp Experiential Index

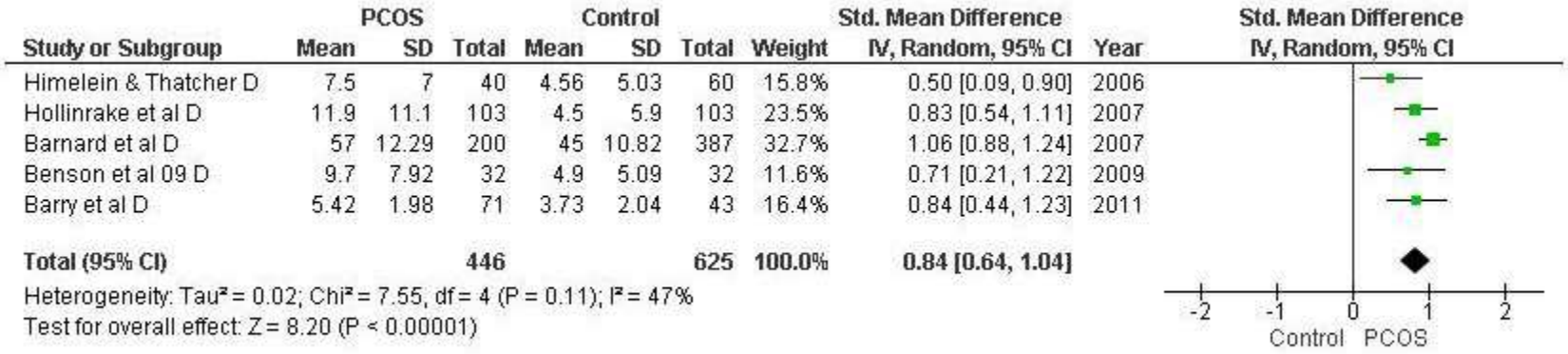
HADS = Hospital Anxiety and Depression Scale

[a] The Monzani et al (1994) data was excluded due to its affect on heterogeneity and the study's relatively low score on methodological quality.

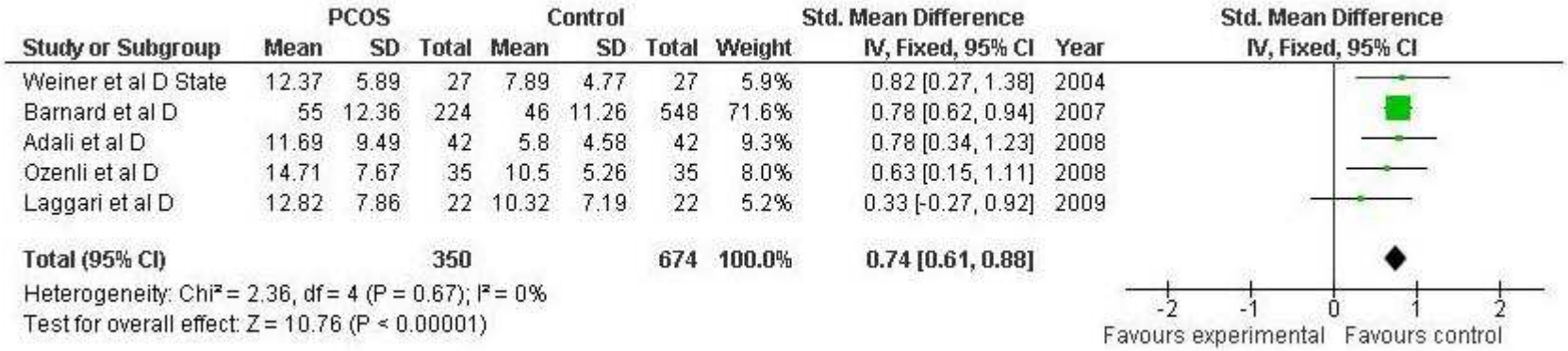
Supplementary Figure I Funnel plots for assessment of publication bias. Effect size is on the X axis and study size is on the Y axis.



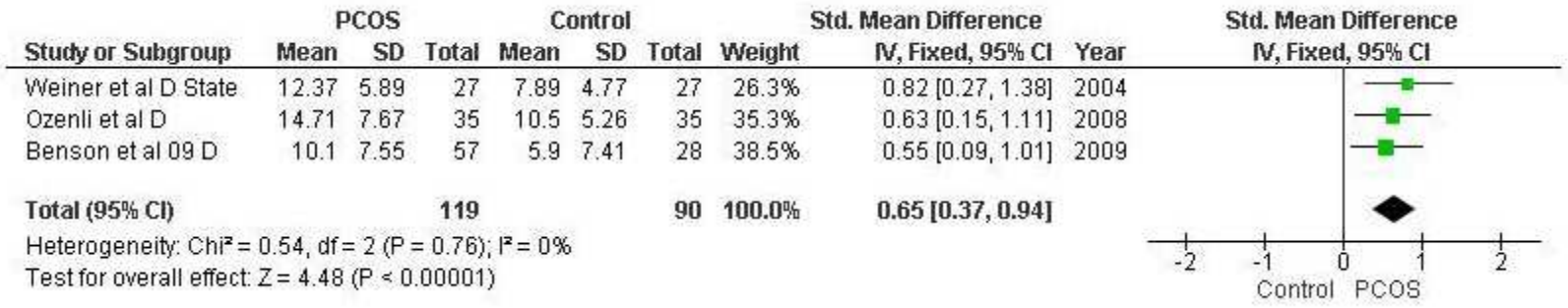
Supplementary Figure II. Forest plot of the difference in depression levels in studies of women with PCOS taking medication for PCOS, and controls.



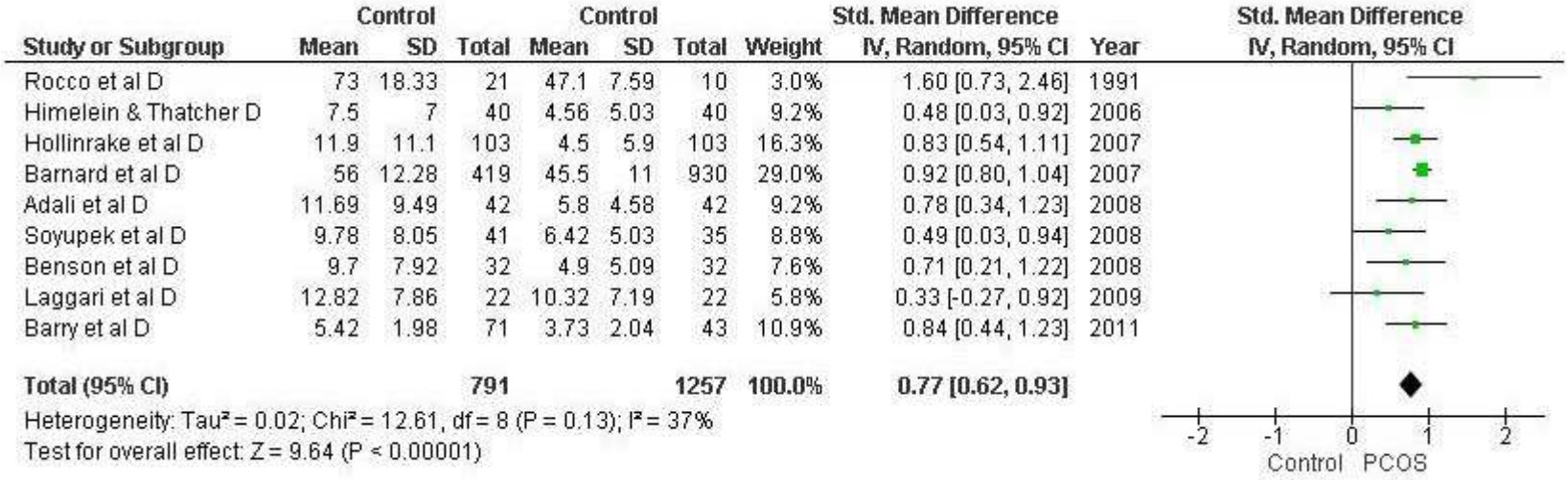
Supplementary Figure III Forest plot of the difference in depression levels in studies of women with PCOS not taking medication for PCOS, and controls.



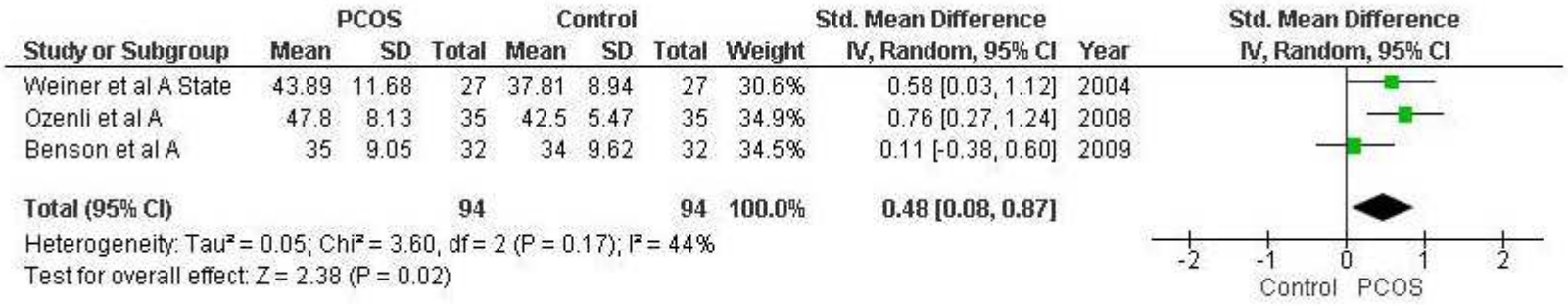
Supplementary Figure IV Forest plot of studies of depression in women with polycystic ovary syndrome (PCOS) compared to controls, where BMI was matched



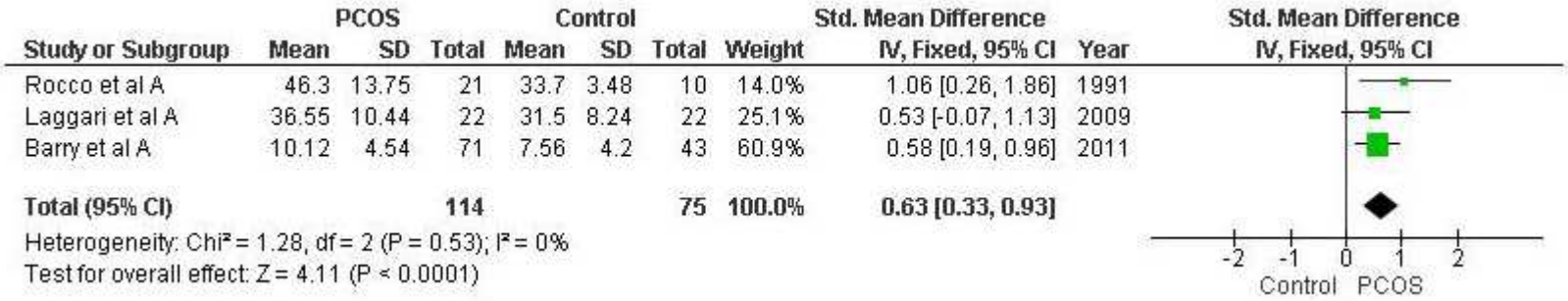
Supplementary Figure V Forest plot of studies of depression in women with polycystic ovary syndrome (PCOS) compared to controls, where BMI was not matched



Supplementary Figure VI Forest plot of studies of anxiety in women with polycystic ovary syndrome (PCOS) compared to controls, where BMI was matched



Supplementary Figure VII Forest plot of studies of anxiety in women with polycystic ovary syndrome (PCOS) compared to controls, where BMI was not matched



CHAPTER 3

Meta-analysis of sex difference in testosterone levels in umbilical cord blood.

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Summary

This meta-analysis reviewed published literature comparing human male and female umbilical cord total testosterone (T) levels. Eighteen studies using 1229 samples from 602 male and 627 female newborns were analyzed using the *RevMan 5* statistical package. Analysis using the inverse variance method based on a random-effects model revealed significantly higher cord T in boys than girls at a moderate effect size (Hedges' $g = 0.57$). There was significant heterogeneity between the 18 studies, though the five studies using direct assays showed no heterogeneity. For studies using extraction and chromatography, those that combined T from arterial and venous cord blood found a larger sex difference than those using only cord venous samples (Hedges' $g = 0.94$ *versus* 0.32); this suggests umbilical cord venous T is of maternal/placental origin and arterial T is of fetal origin. The wide range of T values between studies suggests high cross-reactivity in the assay methods reviewed.

Introduction

Barker (2004) proposed that some diseases of adulthood may have their origin in conditions in the fetal environment, and that low birth weight is associated with insulin resistance, type 2 diabetes, hypertension, and coronary heart diseases in adults. This hypothesis is supported by research showing that elevated maternal testosterone (T) is related to low birth weight in sheep (Manikkam et al., 2006) and humans (Carlsen et al., 2006). Elevated maternal T in rhesus monkeys in late pregnancy causes hyperinsulinemia, and in early pregnancy additionally causes type II diabetes (Abbott et al., 2009). Elevated maternal T in either early or late gestation doubles the risk of the female offspring of rhesus monkeys suffering from anovulation and polycystic ovaries in adulthood (Resko et al., 1987). Animal research suggests that females may be more sensitive to the effects of T on brain development than males (Roselli et al., 2007) so it is especially important to assess potential risks to the female fetus of exposure to T. However at present there is no definitive consensus as to whether T is normally lower at birth in the umbilical cord of healthy human females pregnancies than in male, so it has been difficult to make clinical judgements regarding prenatal risk of T exposure based on levels of umbilical cord T. It is hoped that the identification of normative sex differences in T levels in the present meta-analysis will contribute to our scientific and clinical understanding of this issue.

Polycystic ovary syndrome (PCOS) affects 5-10% of women (Franks, 1995) and is hypothesised to develop as a result of exposure to elevated T prenatally (Dumesic et al., 2006). Some recent research suggests that umbilical vein T is elevated in female newborns of mothers with PCOS (Barry et al., 2010). Placental aromatase is traditionally thought to protect the fetus from raised maternal T, but

animal research contradicts this hypothesis (Resko et al., 1987; Manikkam et al., 2006; Abbott et al., 2009).

To date, studies have produced mixed evidence regarding a sex difference in umbilical vein T. Some studies have found that compared to female newborns, male newborns have more umbilical vein T (Herruzo et al., 1993), less (Pardo et al., 1993), or virtually identical levels (van de Beek et al., 2004). Establishing a norm for umbilical cord T is difficult because the commonly used 'direct' assay methods (described in discussion section, below) lack the sensitivity to accurately detect T at levels below 10 nmol/l (Rosner et al., 2007); although umbilical vein T tends to be higher than circulating T in women of reproductive age (Baik et al., 2004) it is usually below the 10 nmol/l detection threshold suggested for direct assays (see Table II).

Fetal T can be measured from various sites. Although amniotic fluid is the best candidate to investigate the effects of early fetal androgen exposure (van de Beek et al., 2004), it is not comparable to cord T at the end of gestation because amniotic T is sampled around weeks 11 to 21 when a sex difference in T is likely to largest because of the testosterone surge in male fetuses (Smail et al., 1981). Most studies of fetal T sample from the umbilical vein at birth; fewer studies sample mixed (arteries + vein) cord blood, and very few sample directly from umbilical arteries. Almost all studies measure total T rather than free (unbound) T.

Some papers on this topic (Mathur et al., 1980; Bolton et al., 1989; Pardo et al., 1993; Troisi et al., 2003; Anderson et al., 2010) speculate or infer that umbilical arterial T is of fetal origin and umbilical vein T is of maternal/placental origin. This hypothesis has not been proved, but if male fetuses produce more T than females, and if the umbilical arteries carry hormones of fetal origin, then the umbilical arteries

of males should contain more T than the arteries of females (i.e. a sex difference in arterial T). By extension, because mixed cord T contains arterial T, mixed cord T should show a larger sex difference than the sex difference for venous T.

The objective of the present paper was to discover whether there is a sex difference in umbilical vein T across comparable studies..

Materials and Methods

Literature search

All studies that measured testosterone in umbilical cord listed in Pubmed and Medline published up to March 1st 2010, and EMBASE from 1980 to March 31st 2010, were identified. The Cochrane Reviews database was also searched. The keyword search terms, 'umbilical' 'cord' and 'testosterone', were entered simultaneously. This produced 115 articles from Pubmed from 1965 to 2010, and 73 from Medline. Medline produced no new papers in addition to those cited in Pubmed. EMBASE produced four additional studies, but these did not meet the inclusion criteria for the present meta-analysis. The "related article" function was used to widen the results. Additionally three Mesh searches were performed using firstly the terms "testosterone" AND "umbilical cord", "testosterone" AND "umbilical veins", and "testosterone" AND "umbilical arteries". This retrieved 52, 26, and 16 publications respectively, all of which were previously found using the Pubmed keyword search. The Cochrane Reviews database produced six publications, but these were not relevant. A hand search of relevant articles referenced in these publications were obtained, which produced nine publications not previously found. Each article was assessed by JAB and MT, and articles that fitted the main criteria (assaying T in umbilical cord vein in healthy pregnancies) were accessed.

Methodological quality was assessed based on the criteria of the Scottish Intercollegiate Guidelines Network (2010).

Inclusion and exclusion criteria

Human studies that compared male and female umbilical cord total T at birth were eligible for inclusion provided that:

a. The pregnancies were healthy
b. The deliveries were spontaneous, or else planned caesarean sections
c. The assay methods were IA, CLIA/ECL, a method using extraction, or mass spectrometry.

Papers with titles or abstracts that indicated that they were not relevant (for various reasons e.g. reviews, single case studies etc) were excluded. A literature search flow chart (S1), an additional Forest plot (S2), table of excluded studies (S3), conversion table for T to nmol from other units (S4), and tables of methodological quality (S5a, b, and c) are available as supplemental digital content on the *JOG* website [appendix in thesis].

Table 1 shows the characteristics of the included studies.

Table 1. Characteristics of the included studies.

Study	Assay	Original T units	Mother	Delivery	Newborn	T source
Simmer et al (1972)	C-X-IA	"ng %" (ng/100ml)	Not stated	Vaginal, term	Normal, primiparous	Vein
Forest et al (1973)	C-X-IA	ng/100ml	Not stated	Normal, vaginal	Normal	Mixed
Forest et al (1974)	C-X-IA	ng/100ml	Healthy	Normal, vaginal	Normal	Mixed
Abramovich et al (1974)	IA	ng/100ml	Not stated	Mix of C-S and vaginal	Not stated	"mainly venous"
Dawood & Saxena (1977)	X-IA	pg/ml	Not stated, (some had amnio.)	Spontaneous vaginal	Not stated	vein
Maccoby et al (1979)	C-X-IA	ng/ml	Not stated	Term, no C-S	Normal	"Predominantly venous"
Penny et al (1979)	C-X-IA	ng/100 ml	Healthy	Vaginal	Not stated	Mixed
Miyamoto (1981)	IA	ng/ml	Not stated	normal pregnancy & delivery	Term	Vein
Furuhashi et al (1982)	C-X-IA	ng/ml	Not stated	Normal, vaginal	Normal	Vein
Shinkawa et al (1983)	IA	ng/dl	Not stated	Not stated	Term	vein
Bolton et al (1989)	C-X-IA	nmol/l	Not stated	Not stated	Term	vein
Herruzo et al (1993)	IA	ng/ml	Not stated	Uneventful	Term	vein
Simmons et al (1994)	X-IA	nmol/l	Healthy	Normal	Not stated	vein
Maffeis et al (1999)	CLIA	nmol/l	Uncomplicated	Uncomplicated	Term	vein
Troisi et al (2003)	C-X-IA	ng/dl	healthy	Normal: SVD or C-S	Not stated	mixed
Van de Beek et al (2004)	C-X-IA	nmol/l	Healthy	SVD	Term	Vein
Gol et al (2004)	IA	ng/ml	Healthy	Uncomplicated, C-S only	Term	Not stated (mixed?)
Anderson et al (2010)	LCMS	ng/dl	Healthy	74% SVD; 26% C-S; uncomplicated	>35weeks gestation	mixed

IA = Direct immunoassay
CLIA = Direct CLIA
X-IA = IA, after extraction
C-X-IA = IA, after extraction and chromatography (TLC and/or column)
LCMS = Liquid Chromatography-Mass Spectrometry.
C-S = Caesarian section

Of the included studies, six used direct methods (five IA and one CLIA/ECL), eleven used extraction methods (9 with thin layer chromatography and two with di-ethyl ether extraction alone), and one used LCMS. There were no mixed cord X-IA studies, and only one direct assay mixed cord study. Two studies (Dawood & Saxena, 1977; Bolton et al., 1989) measured T in umbilical arteries and vein separately. The venous samples for these two studies were included in the venous subgroup, and the means of their arterial and venous samples combined were included in the mixed cord subgroup. Only the venous samples for these two studies were included in the 'all groups' analysis. Two studies used 'predominantly' (Abramovich, 1974) or 'mainly' (Maccoby et al., 1979) venous blood, so were classified as venous. One study did not state whether they differentiated between arteries and vein (Gol et al., 2004) and was classified as 'mixed'. Thirteen of the studies were classified as being of moderate quality, four were classified as poor (Forest et al., 1973; Abramovich, 1974; Dawood & Saxena, 1977; Shinkawa et al., 1983) and one classified as good (Troisi et al., 2003).

Statistical analysis

Statistical analyses were performed using Review Manager, Version 5 (RevMan 5). Heterogeneity tests suggested that a random effects model was appropriate to assess the sex difference in umbilical vein T levels in seven of the eight subgroups and the studies as a whole. The I^2 value in the direct assay subgroup was zero thus suggesting no problem with heterogeneity, but in the interests of not risking an underestimation of the heterogeneity between studies the more conservative random effects model was used rather than fixed effects. The inverse variance method was used. The effect size was measured as the standard mean

difference, calculated using Hedges' *g*. By convention, like Cohen's *d* the thresholds for small, moderate and large Hedges' *g* effect sizes are 0.2, 0.5, and 0.8 respectively. All T values are presented in nmol/l, and were converted from other units for most studies.

Results

Seventy studies of umbilical cord T were retrieved from the electronic databases. Eighteen studies using 1229 samples (602 male and 627 female) qualified for review according to the inclusion criteria. 834 samples were venous (410 male and 424 female), 395 were mixed (192 male and 203 female), and 41 were arterial (Bolton et al., 1989), (Dawood & Saxena, 1977) (21 male and 20 female). Fifty-two trials were excluded.

Table II shows cord T levels (nmol/l) for the included studies. It can be seen that most studies (16 of 18) found higher cord T in boys than girls, and that overall this difference was of a moderate effect size (Hedges' $g = .57$).

Table 2. Testosterone levels (nmol/l) for all of the included studies, in chronological order. '*g*' indicates Hedges' *g*, the standard mean difference between male and female umbilical cord T. Levels for 41arterial samples (Dawood & Saxena, 1977; Bolton et al., 1989) are not shown.

Study	Male			Female			Difference
	Mean	SD	n	Mean	SD	n	Hedges' *g* [95% CI]
Simmer et al (1972)	0.69	0.45	16	0.80	0.52	24	-0.22 [-0.85, 0.42]
Forest et al (1973)	1.17	0 .33	35	0.92	0.26	46	0.85 [0.39, 1.31]
Forest et al (1974)	1.24	0.36	51	0.93	0.28	53	0.96 [0.55, 1.36]
Abramovich et al (1974)	2.92	0.59	20	2.63	1.25	20	0.29 [-0.33, 0.91]
Dawood & Saxena (1977)	0.79	0.47	11	0.31	0.15	18	1.51 [0.65, 2.36]
Maccoby et al (1979)	0.97	0.24	58	0.74	0.15	58	1.14 [0.75, 1.54]
Penny et al (1979)	1.35	0.30	21	0.90	0.25	22	1.60 [0.91, 2.30]
Miyamoto (1981)	16.69	3.78	55	14.44	3.23	57	0.64 [0.26, 1.02]
Furuhashi et al (1982)	682.93	43.65	37	659.99	162.2	35	0.19 [-0.27, 0.66]
Shinkawa et al (1983)	7.67	4.23	45	6.80	2.01	44	0.26 [-0.16, 0.68]
Bolton et al (1989)	0.34	0.10	12	0.28	0.37	12	0.21 [-0.59, 1.02]
Herruzo et al (1993)	22.17	11.45	27	14.23	5.62	25	0.86 [0.29, 1.43]
Simmons et al (1994)	2.10	0.3	62	1.80	0.60	63	0.63 [0.27, 0.99]
Maffeis et al (1999)	10.40	5.54	48	8.50	4.24	50	0.38 [-0.02, 0.78]
Troisi et al (2003)	1.01	0.83	49	0.76	0.59	37	0.34 [-0.09, 0.77]
Van de Beek et al (2004)	4.01	2.50	19	3.72	3.30	18	0.10 [-0.55, 0.74]
Gol et al (2004)	875.13	121.2	29	809.2	100.2	31	0.59 [0.07, 1.11]
Anderson et al (2010)	0.49	0.35	7	0.66	1.01	14	-0.19 [-1.10, 0.72]
Subtotal (95% CI)			**602**			**627**	**0.57 [0.37, 0.77]**

Table III shows the results of meta-analyses. Although there was a lot of heterogeneity in the findings (evidenced by the large I^2 values) the various groups based on assay types and sources of serum all indicate significantly higher T in male umbilical cord than female.

Table 3. Results of meta-analysis for all studies and the subgroups

Group	Number of studies	Hedges' *g* [95% CI]	Z *(p)*	Chi² *(p)*	I²
All	18	0.57 [0.37, 0.77]	5.66 (P < 0.00001)	45.96 (P = 0.0002)	63%
All venous	12	0.50 [0.26, 0.73]	4.17 (P < 0.0001)	28.48 (P = 0.003)	61%
All mixed [a]	8	0.81 [0.48, 1.15]	4.77 (P < 0.00001)	17.38 (P = 0.02)	60%
All extraction	11	0.66 [0.36, 0.95]	4.31 (P < 0.0001)	36.79 (P < 0.0001)	73%
Venous extraction	7	0.51 [0.09, 0.92]	2.40 (P = 0.02)	24.06 (P = 0.0005)	75%
All C-X-IA	9	0.59 [0.24, 0.94]	3.31 (P = 0.0009)	33.03 (P < 0.0001)	76%
Venous C-X-IA	5	0.32 [-0.23, 0.86]	1.15 (P = 0.25)	18.76 (P = 0.0009)	79%
Mixed C-X-IA [b]	5	0.94 [0.53, 1.34]	4.51 (P < 0.00001)	11.31 (P = 0.02)	65%
Venous direct [c]	5	0.48 [0.27, 0.68]	4.62 (P < 0.00001)	3.98 (P = 0.41)	0%

95% CI = 95% confidence intervals
C-X-IA = IA after extraction and chromatography (TLC and/or column)
[a] Includes the mean of umbilical arterial T and umbilical vein T combined, for two studies , (Dawood & Saxena, 1977; Bolton et al., 1989).
[b] Includes the mean of umbilical arterial T and umbilical vein T combined for one study (Bolton et al., 1989)
[c] Using the fixed variance method improved results slightly (Z = 4.79, P < 0.00001; *g* = 0.52 [0.31, 0.73]; Chi² = 3.78, (P = 0.44); I² = 0%).

Figure 1 shows the Forest plot of sex difference in umbilical vein T for all included studies. The findings of the studies tend towards the right hand side of the vertical zero point, indicating the tendency of the studies to find higher T in the umbilical cord of boys.

Figure 1. Forest plot of sex difference in umbilical vein T for all included studies.

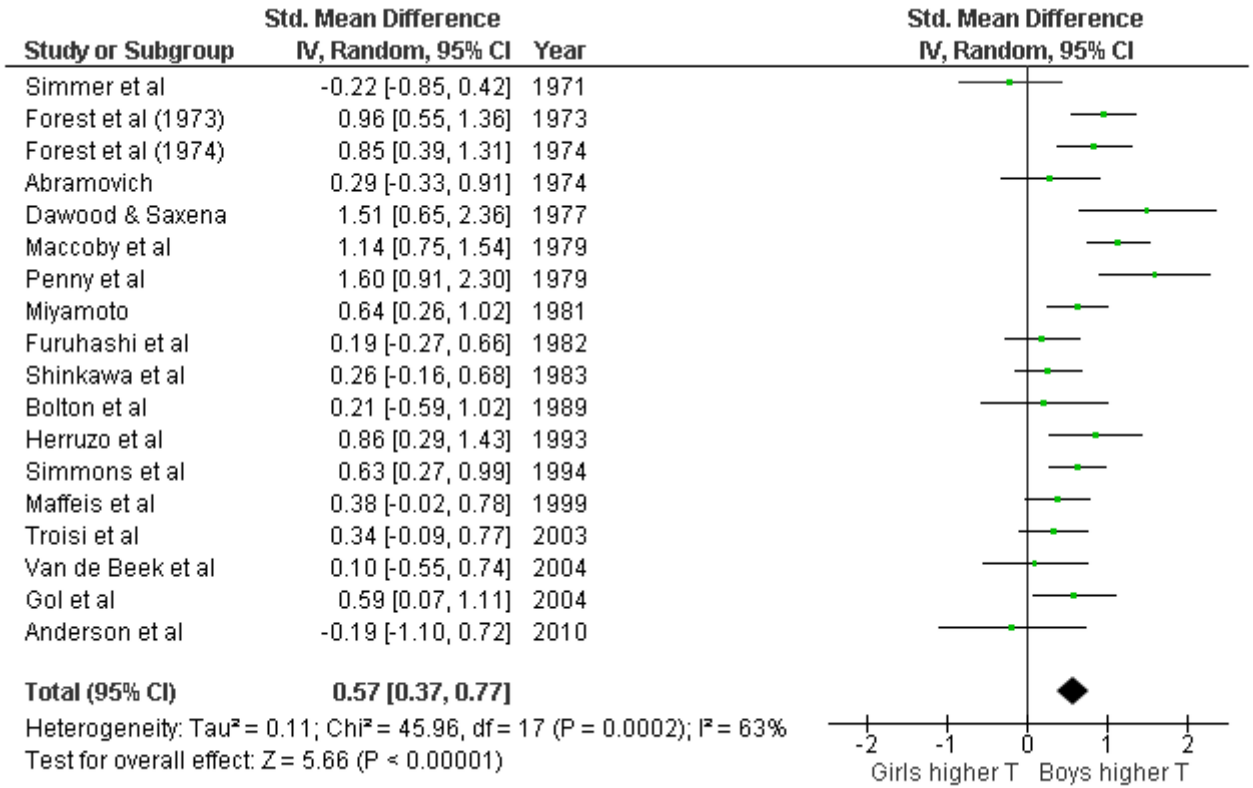
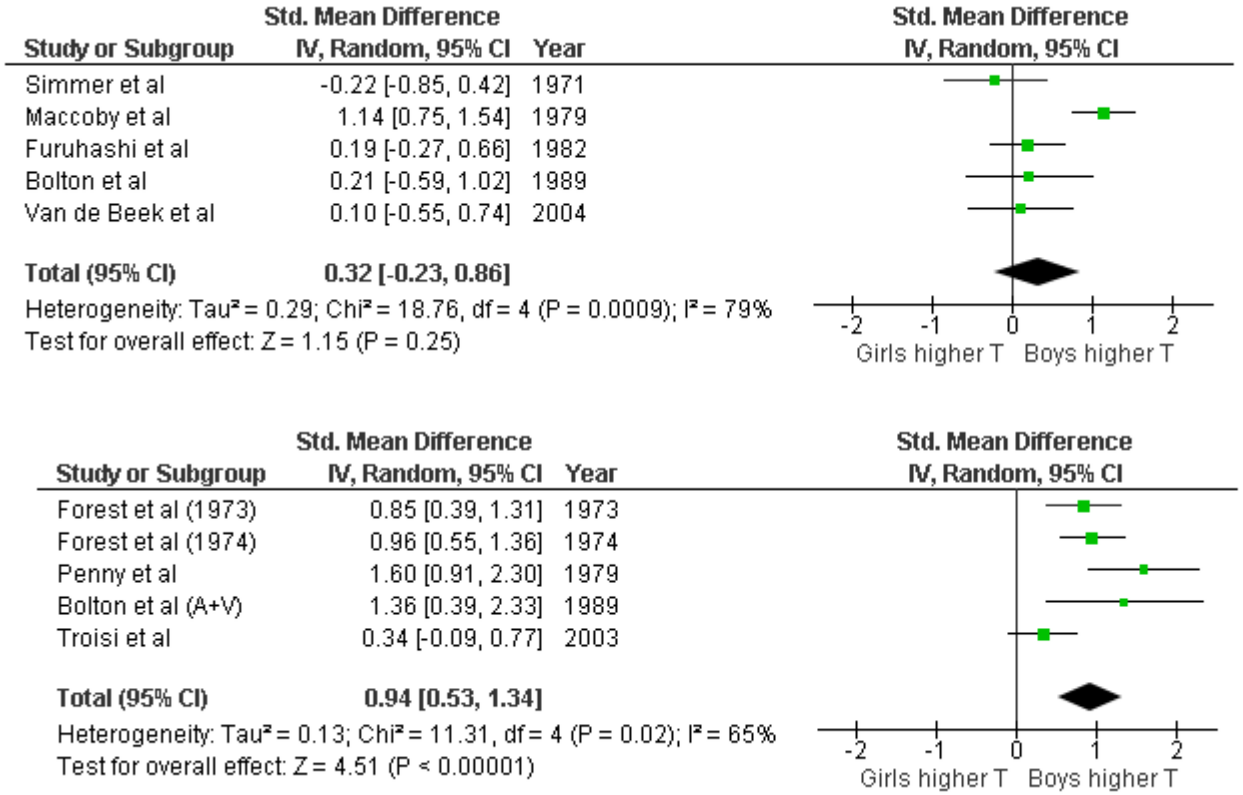


Figure 2 compares the magnitude of sex differences in venous compared to mixed cord blood in studies using chromatography with extraction. The findings in the lower Forest plot (mixed cord blood) are further to the right of the zero point than those in the upper Forest plot (venous cord blood); this indicates that T values for boys in the lower plot showed a greater sex difference than T values for boys in the higher plot.

Figure 2. Comparison of the magnitude of the sex differences in venous compared to mixed cord blood in studies using chromatography with extraction.



Discussion

Analysis of all eighteen studies combined, and all subgroups, revealed significantly higher umbilical cord T in boys than girls. There was significant heterogeneity between the 18 studies and in all subgroups except for the direct assay venous subgroup. The confidence intervals for the subgroups were generally narrow and showed effect sizes of clinical interest. With the exception of the venous chromatographic extraction subgroup the confidence intervals did not encompass a zero value, which suggests that overall the sex differences were representative of those likely to be seen in the general population of newborns. Four of the 18 studies were rated as poor, largely due to these papers lacking information regarding methodological quality rather than explicitly being of poor quality. The four 'poor' ratings were on the borderline for scores for moderate quality and excluding them from the overall analysis made almost no difference to the results, thus their inclusion is appropriate.

The most widely available assay methods, in descending order of accuracy, use mass spectrometry, thin layer chromatography (TLC), extraction with ethyl ether, and direct measurement (Rosner et al., 2007). A therefore unexpected finding of this meta-analysis is that, judging by heterogeneity measures, the direct method may be a more reliable indicator than extraction methods of the sex difference in cord T. However it is likely that the direct method assay detected more substances than T alone, and the interference of other steroids and substances may have simply masked the heterogeneity evidenced in the extraction group. The cross-reacting substances are most likely to have been androgens such as 11-keto-testosterone, 11-beta-OH-testosterone, and dihydrotestosterone (Roche Diagnostics, 2000); for this reason the direct assay might be viewed as an omnibus measure of androgens rather

than simply a measure of T. This interpretation remains to be confirmed by a superior assay method, though it is of interest that Legro et al (2010) recently found good correlations between T levels measured using a direct assay and using liquid chromatography mass spectrometry in women with PCOS. Only one study to date (Anderson et al., 2010) has measured T levels in umbilical cord samples using liquid chromatography mass spectrometry. Anderson et al found that 14 healthy girls had nonsignificantly higher mixed cord T than seven healthy boys (0.66±1.01 vs 0.49±0.35 nmol/l). Sixty-six percent of these samples (10 of the 14 female and four of the seven male) had T levels lower than the detection limit of 0.24 nmol/l. These samples were assigned a value of 0.24 nmol/l. The fact that the actual T level is unknown for 66% of these samples indicates that more sensitive mass spectrometry assays are needed.

Two of the 18 studies (11% of the studies) found that girls had higher cord T than boys, and across studies the large amount of variation in the observed T levels gives cause for concern. Rosner et al. (2007) found that some of their direct assays of T in healthy women were roughly 10 times higher than others; the present authors found that two studies (Gol et al., 2004), (Furuhashi, 1982) reported T values over 100 times higher than some other studies using comparable assays and sample sites. These studies were of moderate methodological quality, and the disparity is not explained by other features of the studies. Although the Hedges *g* values of the two studies are in keeping with the other sixteen studies, their relatively high observed T values are suggestive of the unreliability of these assay types. With the exception of one study (Furuhashi, 1982), the extraction methods showed generally lower mean T values with a smaller range than the direct assay studies. Because the studies included in this meta-analysis are similar in most relevant characteristics, it might be

concluded that although the direct assays give reliable findings in terms of the size of the sex difference, no method appears to yield reliable findings in terms of the absolute T levels, especially the direct immunoassay due to its poor specificity, cross reactivity with other steroids and matrix effects[1] (Rosner et al., 2007).

A finding of potential clinical importance is that, using comparable assays, a larger sex difference was seen in mixed cord samples (g = .94, a large effect size) than venous samples (g = .32, a small effect size). This in turn would suggest that the higher level in males is due at least part to fetal production. This has implications for understanding the etiology of conditions such as PCOS in which prenatal T exposure is theorised to be a causal factor. Future studies might compare T levels in the umbilical arteries and veins in newborns of women with PCOS, and compare these to healthy pregnancies; relatively high T in the umbilical arteries compared to the vein in PCOS, and higher umbilical arterial T in PCOS compared to healthy pregnancies, would suggest fetal T production in this condition. A recent study of PCOS and metformin - a medication that lowers T – had the potential to address this issue, but interpretation of the findings is difficult because the results were not presented for girls whose mothers had PCOS and were not taking metformin (Carlsen & Vanky, 2010)

The conclusion of this meta-analysis is that although there appears to be a reliable sex difference in umbilical vein T of a moderate effect size, the direct and extraction assay methods lack ecological validity; differences in steroid milieu outside that usually used for in vitro diagnostic purposes produce wide differences in results where such assays are employed to measure specific steroids in real-world samples. Current direct assays are known to be poorly discriminative of testosterone

[1] If there is interference that is not known to be caused by other factors (such as cross-reactivity), this interference is termed a 'matrix effect' until such time as its cause is identified.

at low values (Rosner et al., 2007). This being the case, our knowledge of gross T levels in cord blood remains limited, and norms for umbilical cord T at birth remain to be established through further research using more specific methods, such as tandem mass spectrometry. Nevertheless the findings of the present meta-analysis suggest that serum in the male umbilical cord at birth might contain a higher level of androgen than seen in the female cord at birth, and this might provide clinicians a rough index (i.e. relative to values seen in the opposite sex) as to whether a female newborn has experienced elevated androgen prenatally, or whether a male newborn has experienced reduced androgen prenatally. Future research might also compare sex hormones in both umbilical veins and arteries (not mixed) from male and female progeny as way of identifying the source (maternal/placental, or fetal) of hormones.

Acknowledgements

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References

Abbott DH, Tarantal AF, Dumesic DA. 2009. Fetal, infant, adolescent and adult phenotypes of polycystic ovary syndrome in prenatally androgenized female rhesus monkeys. American Journal of Primatology 71: 776-84.

Abramovich DR. Human sexual differentiation--in utero influences. 1974. Journal of Gbstetrics and Gynaecology of the British Commonwealth 81: 448-53.

Anderson H, Fogel N, Grebe SK, Singh RJ, Taylor RL, Dunaif A. 2010. Infants of women with polycystic ovary syndrome have lower cord blood androstendione and estradiol levels. Journal of Clinical Endocrinology & Metabolism 95: 2180-2186

Baik I, Liu Q, Sturgeon S, Stanek EJ 3rd, Okulicz W, Hsieh CC. 2006. Reproducibility of assays for steroid hormones, prolactin and insulin-like growth factor-1 in umbilical cord blood. Paediatric and Perinatal Epidemiology 20: 79-86.

Barker DJ. The developmental origins of chronic adult disease. 2004. Acta Pædiatrica Supplement 93:26-33.

Barry JA, Kay AR, Navaratnarajah R, Iqbal S, David AL, Bamfo JEAK, Hines M, Hardiman PJ. 2010. Umbilical vein testosterone in female infants born to mothers with Polycystic Ovary Syndrome is elevated to male levels. Journal of Obstetrics & Gynaecology 30: 444-6.

Bolton NJ, Tapanainen J, Koivisto M, Vihko R. 1989. Circulating sex hormone-binding globulin and testosterone in newborns and infants. Clinical Endocrinology 31: 201-7.

Carlsen SM, Jacobsen G, Romundstad P. 2006. Maternal testosterone levels during pregnancy are associated with offspring size at birth. European Journal of Endocrinology 155: 365-70.

Carlsen SM, Vanky E. 2010. Metformin influence on hormone levels at birth, in PCOS mothers and their newborns. Human Reproduction 25: 786-90.

Dawood MY, Saxena BB. 1977. Testosterone and dihydrotestosterone in maternal and cord blood and in amniotic fluid. American Journal of Obstetrics and Gynecology 129: 37-42.

Dumesic DA, Abbott DH, Padmanabhan V. 2006. Polycystic ovary syndrome and its developmental origins. Reviews in Endocrine & Metabolic Disorders 8: 127-41.

Forest MG Cathiard AM, Bertrand JA. 1973. Evidence of testicular activity in early infancy. Journal of Clinical Endocrinology & Metabolism 37: 148-51.

Forest MG, Sizonenko PC, Cathiard AM, Bertrand J. 1974. Hypophyso-gonadal function in humans during the first year of life. 1. Evidence for testicular activity in early infancy. Journal of Clinical Investigation 53: 819-28.

Franks S. Polycystic ovary syndrome. 1995. New England Journal of Medicine 333: 853-861.

Furuhashi N, Fukaya T, Kono H, Tachibana Y, Shinkawa O, Takahashi T. 1982. Correlation of birth weights with umbilical cord serum LH-hCG, FSH, beta-hCG, Estradiol, Cortisol and Testosterone levels. Gynecologic and Obstetric Investigation 13: 241-8.

Gol M, Altunyurt S, Cimrin D, Guclu S, Bagci M, Demir N. 2004. Different maternal serum hCG levels in pregnant women with female and male fetuses: does fetal hypophyseal--adrenal--gonadal axis play a role? Journal of Perinatal Medicine 32: 342-5.

Herruzo AJ, Mozas J, Alarcón JL, López JM, Molina R, Molto L, Martos J. 1993. Sex differences in serum hormone levels in umbilical vein blood. International Journal of Gynecology & Obstetrics 41: 37-41.

Legro RS, Schlaff WD, Diamond MP, Coutifaris C, Casson PR, Brzyski RG, Christman GM, Trussell JC, Krawetz SA, Snyder PJ, Ohl D, Carson SA, Steinkampf MP, Carr BR, McGovern PG, Cataldo NA, Gosman GG, Nestler JE, Myers ER, Santoro N, Eisenberg E, Zhang M, Zhang H; Reproductive Medicine Network. 2010. Total testosterone assays in women with polycystic ovary syndrome: precision and correlation with hirsutism. Journal of Clinical Endocrinology & Metabolism 95: 5305-13.

Maccoby EE, Doering CH, Jacklin CN, Kraemer H. 1979. Concentrations of sex hormones in umbilical-cord blood: their relation to sex and birth order of infants. Child Development 50: 632-42.

Maffeis C, Moghetti P, Vettor R, Lombardi AM, Vecchini S, Tatò L. 1999. Leptin concentration in newborns' cord blood: relationship to gender and growth-regulating hormones. International Journal of Obesity and Related Metabolic Disorders 23: 943-7.

Manikkam M, Steckler TL, Welch KB, Inskeep EK, Padmanabhan V. 2006. Fetal programming: prenatal testosterone treatment leads to follicular persistence/luteal

defects; partial restoration of ovarian function by cyclic progesterone treatment. Endocrinology 147: 1997-2007.

Mathur RS, Landgrebe S, Moody LO, Powell S, Williamson HO. 1980. Plasma steroid concentrations in maternal and umbilical circulation after spontaneous onset of labor. Journal of Clinical Endocrinology & Metabolism 51: 1235-8.

Miyamoto U. 1981. A sex difference of the concentrations of gonadotropins, its subunits and sex steroids in cord veins. Acta Obstetrica et Gynaecologica Japonica 33: 711-3.

Pardo IM, Geloneze B, Tambascia MA, Pereira JL, Barros Filho AA. 2004. Leptin as a marker of sexual dimorphism in newborn infants. Jornal de Pediatria 80: 305-8.

Penny R, Parlow AF, Frasier SD. 1979. Testosterone and estradiol concentrations in paired maternal and cord sera and their correlation with the concentration of chorionic gonadotropin. Pediatrics 64: 604-8.

Resko JA, Buhl AE, Phoenix CH. 1987. Treatment of pregnant rhesus macaques with testosterone propionate: observations on its fate in the fetus. Biology of Reproduction 37: 1185–91.

Roche Diagnostics. 2000. Elecsys Testosterone Product Information. Available at http://www.roche-diagnostics.ch/resource.php?id=Resourcefile-37084379e9754cf58 Retrieved 5th May 2010.

Roselli CE, Stadelman H, Reeve R, Bishop CV, Stormshak F. 2007. The ovine sexually dimorphic nucleus of the medial preoptic area is organized prenatally by testosterone. Endocrinology 148: 4450-7.

Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H. 2007. Position statement: Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. Journal of Clinical Endocrinology & Metabolism 92: 405-13.

Scottish Intercollegiate Guidelines Network (SIGN) guidelines. 2010. Available from: http://www.sign.ac.uk/guidelines/fulltext/50/checklist3.html Retrieved 4th June 2010.

Smail PJ, Reyes FI, Winter JSD Faiman C. 1981. The fetal hormone environment and. its effect on the morphogenesis of the genital. System. In Kogan SJ, Hafez ESE (Eds.), Pediatric Andrology. The Hague: Martinus Nijhoff; pp. 9–19.

Shinkawa O, Furuhashi N, Fukaya T, Kono H, Tachibana Y, Takahashi T, Suzuki M. 1983. A study on testosterone secretion in neonates. Nippon Sanka Fujinka Gakkai Zasshi 35: 266-8.

Simmer HH, Frankland MV, Greipel M. 1972. Neutral C_{19}-steroids and steroid sulphates in human pregnancy. VI. Quantification of plasma testosterone in cord venous blood. Steroids 19: 215-28

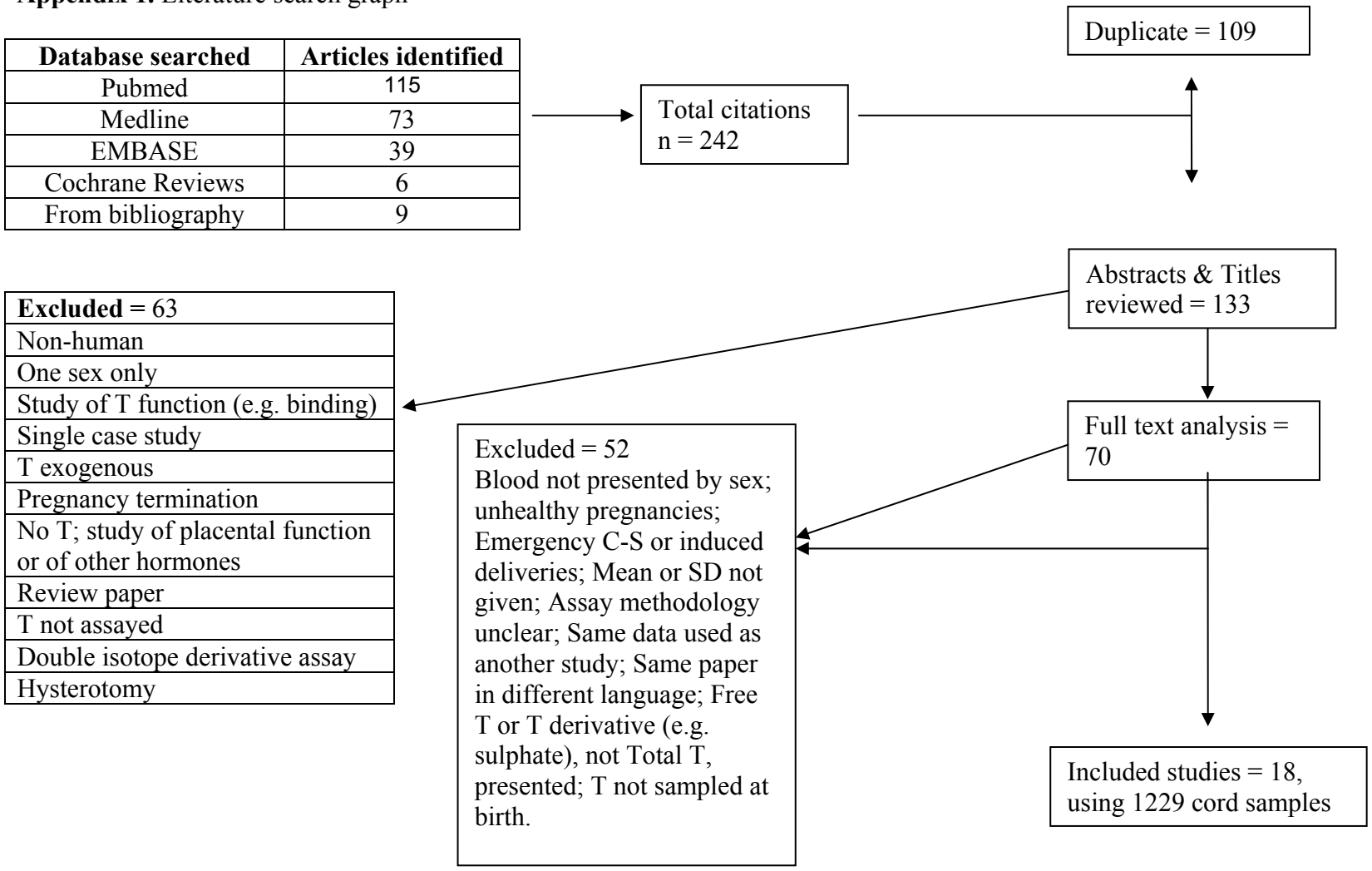
Simmons D, France JT, Keelan JA, Song L, Knox BS. 1994. Sex differences in umbilical cord serum levels of inhibin, testosterone, oestradiol, dehydroepiandrosterone sulphate, and sex hormone-binding globulin in human term neonates. Biology of the Neonate 65: 287-94.

Troisi R, Potischman N, Roberts JM, Harger G, Markovic N, Cole B, Lykins D, Siiteri P, Hoover RN. 2003. Correlation of serum hormone concentrations in maternal and umbilical cord samples. Cancer Epidemiology, Biomarkers & Prevention 12: 452-6.

van de Beek C, Thijssen JH, Cohen-Kettenis PT, van Goozen SH, Buitelaar JK. 2004. Relationships between sex hormones assessed in amniotic fluid, and maternal and umbilical cord serum: what is the best source of information to investigate the effects of fetal hormonal exposure? Hormones & Behavior 46: 663-9.

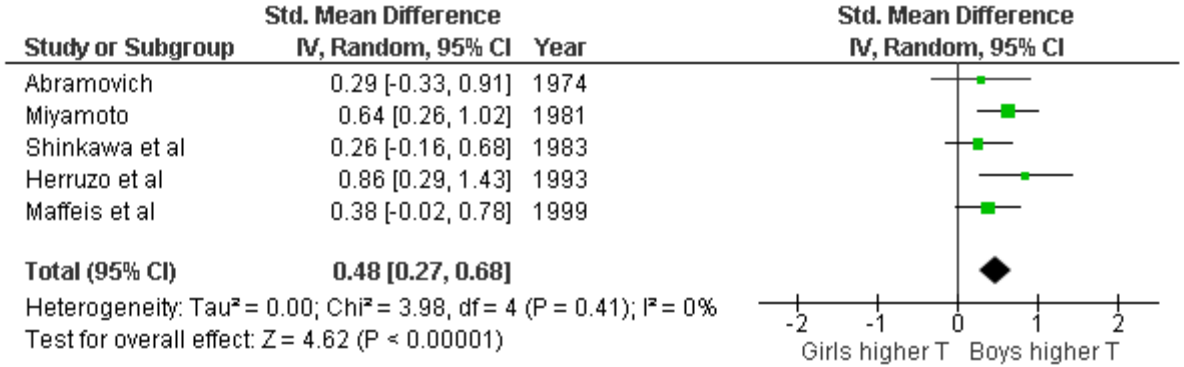
Appendix 1. Literature search graph

Database searched	Articles identified
Pubmed	115
Medline	73
EMBASE	39
Cochrane Reviews	6
From bibliography	9



Excluded = 63
Non-human
One sex only
Study of T function (e.g. binding)
Single case study
T exogenous
Pregnancy termination
No T; study of placental function or of other hormones
Review paper
T not assayed
Double isotope derivative assay
Hysterotomy

Appendix 2. Forest plot of sex differences in umbilical cord venous T for direct assay methods only (n=5).



Appendix 3. Exclusion criteria and excluded studies (N=115) presented in order of frequency and chronologically.

Studies of T (or aromatase etc) function (also usually not presented by sex) (N=24)	Cairrão et al (2008)
	Jin et al (2007)
	Perusquía et al (2007)
	Jin et al (2005)
	Zapata et al (2005)
	Yildiz et al (2005)
	Ijiri et al (2003)
	Zhang et al (2002)
	Nie et al (2001)
	Du et al (2001)
	Cid et al (1994)
	Loganath et al (1992)
	Gunasegaram (1991) (EMBASE)
	Higano et al (1989)
	Milewich et al (1987)
	Lewis et al (1986)
	Sybulski et al (1975)
	Ahluwalia et al (1974) (cocaine users)
	Swartz et al (1974)
	Stárka et al (1974)
	Simmer et al (1972)
	Rosenfield (1971)
	Heyns & De Moor (1971)
	Kobayashi et al (1969)
Animal studies (n=17)	Huang et al (2010)

	Hayashi et al (1997)
	Okamoto et al (1996)
	Heyns et al (1993)
	Mitchell et al (1986)
	Vreeburg et al (1986)
	Ford et al (1980)
	Ellinwood et al (1980)
	Resko (1977)
	Tseng et al (1975)
	Mongkonpunya et al (1975)
	Challis et al (1974)
	Resko (1974)
	Resko (1973)
	Milgrom et al (1973)
	Goy & Phoenix (1972)
	Dang and Meusy-Dessolle (1970) (EMBASE)
One sex only, or T not presented separately for each sex (N=17)	Hickey et al (2010)
	Whitehouse et al (2010)
	Whitehouse et al (2010)
	Hickey et al (2009)
	Rohrmann et al (2009)
	Troisi et al (2008)
	Nagata et al (2007) (also arteries only)
	Nagata et al (2006) (also arteries only)
	Baik et al (2006)
	Baik et al (2005)
	Schubring et al (1998)

	Sakai et al (1991) (same sex twins)
	Milewich et al (1990)
	Bradshaw et al (1986)
	Tapanainen et al (1984) (EMBASE)
	Tapanainen (1983)
	Mathur et al (1980)
Total T not measured or not	Toth et al (2009)
reported (N=12)	Clifton et al (2007)
	Tan & Tan (2001) (unclear if total or free T)
	Fausett et al (1999)
	Maffei et al (1998)
	Gemer et al (1997) (maternal T only)
	Adeyemo & Jeyakumar (1993) (Free T only)
	Ikegawa (1986) (Free T only)
	Tojo (1981)
	Plotti et al (1975)
	Nunez et al (1974)
	Ermini et al (1974) (T sulphate)
Same data from another	Faupel-Badger et al (2009) [Troisi et al's 2006a data]
study (N=12)	Savage (2009) (Cohrane cited twice)
	Troisi et al (2006) (b) [Troisi et al's 2006a data]
	Troisi et al (2006) (c) [Troisi et al's 2006a data]
	Zupan et al (2004) (Cohrane cited twice)
	Jacklin et al (1988) (same Maccoby et al (1979))
	Marcus et al (1985) (same Maccoby et al (1979))
	Jacklin et al (1984) (same Maccoby et al (1979))
	Jacklin et al (1983) (same Maccoby et al (1979))

	Abramovich & Rowe (1973) (almost identical to Abramovich et al (1974). (hand search)
	Gandy (1971) (same Gandy (1968)) (hand search)
	Mizuno et al (1968) (Japanese version of (1969) paper)
double isotope derivative assay (N=5)	Saez & Bertrand (1969) (hand search)
	Mizuno et al (1969)
	Rivarola et al (1968)
	Gandy (1968) (hand search)
	Bertrand & Saez (1968) (hand search)
Data from unhealthy women and/or children &/or pregnancy complications or healthy women and/or children not presented separately from other cases (N=6)	Carlsen & Vanky (2010)
	Jin et al (2009)
	Pardo et al (2004) (EMBASE)
	Su et al (1996)
	Simmons (1995)
	Forest et al (1980)
Single case study (N=4)	Bertalan et al (2007)
	da Silva et al (2007)
	Holt et al (2005)
	Hensleigh et al (1975)
Aborteses (N=3)	Stern et al (1975)
	Reyes et al (1974) (hand search)
	Reyes et al (1973) (hand search)
Amniotic (N=3)	Ahluwalia et al (1992) (also arteries)
	Nagamani et al (1979) (hand search)
	Caputo et al (1974)
Mean &/or SD not given	Adkins et al (2007)

(indicating a non-normal	Yuguang et al (2007) (geometric mean, no SD; hand search)
distribution of values)	Tan et al (1998)
(N=5)	Bammann et al (1980) (hand search)
	August et al (1969)
Sampled mid gestation	Abramovich (1974)
(N=2)	Ling et al (1974)
Other (N=7)	Savage (2009) (ENT, Cochrane review)
	Owens (2008) (vocal cords, Cochrane review)
	Zupan et al (2004) (cord hygiene, Cochrane review)
	Hofmeyr (1997) (cord complications, Cochrane review)
	Ghione et al (1993) (Review; not about cord T)
	Wei et al (1990) (assay methodology)
	Mitchell (1970) (Review)

Appendix 4. Table for conversion of testosterone to nmol/l from other units.

Units	Conversion factor
pg/mL	multiply by 0.00347
ng/dL	multiply by 0.0347
ng/100ml	multiply by 0.0347
ng %	multiply by 0.0347
ng/100ml	multiply by 0.0347
mμg/100 ml	multiply by 0.0347
μg/dl	multiply by .347
ng/mL	multiply by 3.47
μg/L	multiply by 3.47
pmol/L	multiply by .001

Appendix 5a. Methodological qualities of prospective studies included (Adapted from the Scottish Intercollegiate Guidelines Network). 1= yes; 0=no. Maximum score = 20. Scores of: 0-6 = poor methodology; 7-13 = fair; 14-20 =good.

Quality variables	**Simmer et al (1972)**	**Forest et al (1973)**	**Forest et al (1974)**	**Abramovich et al (1974)**	**Dawood & Saxena (1977)**	**Maccoby et al (1979)**
Inclusion Criteria	0	0	0	0	1	1
Exclusion Criteria	0	0	1	0	0	1
Demographics comparable	0	0	0	0	0	0
Can the number of participating centers be determined	0	0	1	0	0	1
Has the source of cord T been identified (e.g. vein)	1	1	1	1	1	1
Are the mother's baseline characteristics comparable in the two groups	0	0	1	0	0	0
Are the children's baseline characteristics comparable in the two groups	1	1	1	0	0	1
Can the number of hospital staff taking cord samples be determined	0	0	0	0	0	0
Can the reader determine how expert the sampler was	0	0	0	0	0	0
Can the reader determine how expert the lab technician (assayer) was	1	0	0	1	0	0
Is the cord sampling technique adequately described	1	0	1	1	1	1
Is the assay technique adequately described	1	1	1	1	1	1
Is there any way that they have tried to standardize the cord sampling technique	1	0	1	0	1	1
Is there any way that they have tried to standardize the assay technique	1	1	1	1	1	1
Is the delivery type identified	1	1	1	0	0	1
Is the delivery type comparable in the two groups	1	1	1	0	0	1
Do authors address whether there is any missing data	0	0	0	0	0	0
Was the study period stated	0	0	0	0	0	1
Is it clear whether all the patients asked to enter the study took part	0	0	0	0	0	1
Analysis by intention to treat	0	0	0	0	0	0
Score	**9**	**6**	**11**	**5**	**6**	**13**

Appendix 5b. Methodological qualities of prospective studies included (Adapted from the Scottish Intercollegiate Guidelines Network).
1= yes; 0=no. Maximum score = 20. Scores of: 0-6 = poor methodology; 7-13 = fair; 14-20 =good.

Quality variables	**Penny et al (1979)**	**Miyamoto (1981)**	**Furuhashi et al (1982)**	**Shinkawa et al (1983)**	**Bolton et al (1989)**	**Herruzo et al (1993)**
Inclusion Criteria	0	0	0	0	0	1
Exclusion Criteria	1	1	0	0	0	0
Demographics comparable	0	1	0	0	0	0
Can the number of participating centers be determined	0	0	0	0	0	1
Has the source of cord T been identified (e.g. vein)	1	1	1	1	1	1
Are the mother's baseline characteristics comparable in the two groups	1	0	0	0	0	0
Are the children's baseline characteristics comparable in the two groups	0	1	1	1	1	1
Can the number of hospital staff taking cord samples be determined	0	0	0	0	0	0
Can the reader determine how expert the sampler was	0	0	0	0	0	0
Can the reader determine how expert the lab technician (assayer) was	0	1	0	1	0	1
Is the cord sampling technique adequately described	1	0	1	0	1	1
Is the assay technique adequately described	1	1	1	1	1	1
Is there any way that they have tried to standardize the cord sampling technique	0	0	1	0	1	0
Is there any way that they have tried to standardize the assay technique	1	1	1	1	1	1
Is the delivery type identified	1	1	1	1	1	1
Is the delivery type comparable in the two groups	1	1	1	0	0	1
Do authors address whether there is any missing data	0	0	0	0	0	0
Was the study period stated	0	0	0	0	0	1
Is it clear whether all the patients asked to enter the study took part	0	0	0	0	0	0
Analysis by intention to treat	0	0	0	0	0	0
Score	**8**	**9**	**8**	**6**	**7**	**11**

Appendix 5c. Methodological qualities of prospective studies included (Adapted from the Scottish Intercollegiate Guidelines Network).
1= yes; 0=no. Maximum score = 20. Scores of: 0-6 = poor methodology; 7-13 = fair; 14-20 =good.

Quality variables	**Simmons et al (1994)**	**Maffeis et al (1999)**	**Troisi et al (2003)**	**Van de Beek et al (2004)**	**Gol et al (2004)**	**Anderson et al (2010)**
Inclusion Criteria	1	0	1	1	1	1
Exclusion Criteria	1	0	1	1	0	1
Demographics comparable	1	0	0	0	1	1
Can the number of participating centers be determined	0	0	1	1	0	0
Has the source of cord T been identified (e.g. vein)	1	1	1	1	0	1
Are the mother's baseline characteristics comparable in the two groups	1	1	1	1	1	1
Are the children's baseline characteristics comparable in the two groups	0	1	0	1	1	0
Can the number of hospital staff taking cord samples be determined	0	0	0	0	0	0
Can the reader determine how expert the sampler was	0	0	0	0	0	0
Can the reader determine how expert the lab technician (assayer) was	0	1	0	0	1	0
Is the cord sampling technique adequately described	1	1	1	0	0	1
Is the assay technique adequately described	1	1	1	1	1	1
Is there any way that they have tried to standardize the cord sampling technique	1	1	1	0	0	1
Is there any way that they have tried to standardize the assay technique	1	1	1	1	1	1
Is the delivery type identified	1	1	1	1	1	1
Is the delivery type comparable in the two groups	1	1	1	1	1	1
Do authors address whether there is any missing data	1	0	1	1	0	1
Was the study period stated	0	0	1	1	0	0
Is it clear whether all the patients asked to enter the study took part	0	0	1	0	0	0
Analysis by intention to treat	0	0	0	0	0	0
Score	**12**	**10**	**14**	**12**	**9**	**12**

CHAPTER 4

Umbilical vein testosterone in female infants born to mothers with Polycystic Ovary Syndrome is elevated to male levels

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Summary

The aetiology of Polycystic Ovary Syndrome (PCOS) is poorly understood, but an intrauterine hyperandrogenic environment has been implicated. This study was designed to assess whether the female offspring of mothers with PCOS are exposed to raised levels of testosterone (T) in utero. In this case-control study, three groups of pregnant women were recruited from Labour Ward: PCOS women with a female baby (n=10, PCOS girls); control women with a female baby (n=20, Control girls) and control women with a male baby (n=10, Control boys). Maternal and Umbilical Vein (UV) blood was assayed for T levels. UV T in PCOS girls was significantly raised, compared to control girls ($p<0.012$). The difference in UV T between PCOS girls and control boys was not significant ($p<0.254$). This is the first demonstration of a hyperandrogenic in utero environment in PCOS pregnancies; UV T in female infants is raised to male levels.

Keywords: Polycystic ovary syndrome, Testosterone, Fetal programming, Umbilical vein

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy of women of reproductive age, with a prevalence of 5-10% (Franks, 1995). The features of the syndrome are those associated with anovulation (amenorrhoea, irregular cycles) and with hyperandrogenism (hirsuitism, acne). Frequently presenting in adolescence, PCOS is associated with the development in later life of insulin resistance (Dunaif *et al.*, 1989), type 2 diabetes (Dahlgren *et al.,* 1992) and hypertension (Talbott *et al.,* 1995).

The aetiology of PCOS remains poorly understood. A genetic basis is supported by family studies showing first degree relatives of women with PCOS to have increased rates of hyperandrogenism (Goodarzi *et al.,* 2007) and metabolic disease (Billargeon *et al.*, 2007; Franks *et al.*, 2008). However, no genes have been conclusively implicated in the inheritance of PCOS (Nam and Strauss*,* 2007).

Familial clustering of the PCOS phenotype could also result from fetal programming in response to an abnormal intrauterine environment. Animal studies have shown that prenatal exposure to excess androgen results in the biochemical and morphological features of PCOS (Abbott *et al.*, 2005). A similar process in the human could result in vertical transmission of PCOS since hyperandrogenaemia persists during pregnancy in women with this syndrome (Sir-Petermann *et al.*, 2002).

Excess prenatal androgen exposure may also have important consequences for health in adult life. There is evidence of a sex difference in prenatal androgen levels; T levels in umbilical cord blood are higher in healthy male infants than in healthy female infants (Maccoby *et al*., 1979). Studies in experimental animals show that administration of androgens during pregnancy leads to insulin resistance and hypertension in the offspring (King *et al.*, 2007). There is evidence of a similar effect in the human; daughters of women

with PCOS have metabolic derangement (Sir-Petermann *et al.*, 2009).

Although there are a few studies of maternal androgen levels in pregnant women with PCOS, we are not aware of any study which has compared levels in the fetal circulation in such pregnancies with healthy controls. This study was designed to study the levels of total testosterone (T) in the umbilical vein (UV) of babies born to mothers with PCOS and in healthy controls (both male and female babies).

Methods

Design

In this case-control study, pregnant women were recruited from the Labour Wards of University College Hospital and the Royal Free Hospital, in London. Women were recruited either in early labour, or prior to an elective caesarean section and gave written informed consent.[1]

Three groups of women were recruited: women with PCOS, defined by Rotterdam criteria (ESHRE/ASRM 2003) who delivered a female baby (n=10, PCOS girls); control women without PCOS, who delivered a female baby (n=20, Control girls) and control women without PCOS, who delivered a male baby (n=10, Control boys).

Exclusion criteria were: multiple pregnancy, age under 18 years, hypertensive disorders, gestation under 38 weeks at delivery, fetal abnormality and endocrine disorders other than PCOS. The study was approved by the Royal Free Hospital and Medical School Research Ethics Committee and the Joint UCL/UCLH Committees on the Ethics of Human Research.

[1] All women who met the inclusion / exclusion criteria were approached. Their PCOS status – according to the Rotterdam criteria - was ascertained from their medical records subsequent to recruitment.

On admission to Labour Ward, anthropometric data, medical history, and obstetric history were obtained from the case-notes. A venous blood sample was obtained from the mother upon admission. Samples were centrifuged and serum stored at -80^{o}C .

Following delivery of the placenta, the umbilical cord was clamped and a 2 ml sample of umbilical cord venous blood was obtained. Samples were again centrifuged and stored at -80^{o}C, as above.

Gestational age, infant weight, duration of labour and mode of delivery were recorded.

Assays

T concentrations were measured using the Roche Elecsys system 1010/2010 Modular E170, using a direct electrochemiluminescence methodology.[2]

Statistics

The results are expressed as the mean +/- SD. *p* values of <0.05 were considered significant. Comparisons were made using Student's t-tests for parametric distributions, and Mann-Whitney tests for non-parametric distributions. Relationships between variables were assessed using Pearson's correlations for parametric distributions, and Spearman's

[2] For this assay: the intra-assay coefficients of variation were all less than 4.6%; reference intervals for women between 18 and 40 are 0.22–2.9 nmol/L; the dynamic range is 0.069 to 52.00 nmol/L. For this assay the cross-reacting substances are most likely to have been androgens such as 11-keto-testosterone, 11-beta-OH-testosterone, and dihydrotestosterone (Roche Diagnostics, 2000) thus this assay might be viewed as an omnibus measure of androgens rather than simply a measure of T. Because research such as that described in this thesis is interested in androgenic effects rather than T *per se*, whether what is measured is T or another androgen is not of primary importance.

correlations for nonparametric distributions. One-way between-groups ANOVA was used when comparing multiple groups. Statistical analyses were performed using SPSS version 15 (SPSS Inc., Chicago IL).[3]

Results

Patient characteristics (Table I)

None of the three groups differed significantly from any other in ethnicity, maternal age or BMI, or in gestational age at delivery or birthweight.

[3] Maccoby et al. (1979) found a sex difference for cord T with a Cohen's *d* effect size of 1.14; an a priori sample size estimate based on this suggests 11 per group would be required to show a group difference with power at 0.80 and alpha at .05. In the present study, with groups sizes of n=10 and n=20, power is exactly at 0.80 with alpha at .05, and the effect size for the main outcome of interest (PCOS girls compared to control girls) was 1.03.

Table I – Patient characteristics

		n	mean	SD	ANOVA
Age	PCOS girls	10	30.5	4.28	
	Control girls	20	30.5	6.30	*ns*
	Control boys	10	33.0	5.87	
BMI	PCOS girls	10	23.8	4.67	
(kg/m^2)	Control girls	20	24.0	3.00	*ns*
	Control boys	10	24.0	5.31	
Gestational age at	PCOS girls	10	277.0	8.10	
delivery (days)	Control girls	20	274.2	5.27	*ns*
	Control boys	10	276.5	8.25	
Birthweight (g)	PCOS girls	10	3318	492	
	Control girls	20	3172	543	*ns*
	Control boys	10	3406	678	

ns – not significant

There were 5 vaginal deliveries and 5 Caesarean sections (of which 4 were elective) in the PCOS girls group, 6 vaginal deliveries and 4 Caesarean sections (of which 3 were elective) in the control boys group and 9 vaginal deliveries and 11 Caesarean sections (of which 3 were elective) in the control girls group. Neither mode of delivery, nor whether the patient laboured, related significantly to levels of either maternal or UV T ($t=-0.666$, $df=37$, $p<0.510$, 2-tailed, and $t= 0.977$, $df=39$, $p<0.334$, 2-tailed).

Testosterone (Table II)

Although T was somewhat elevated in women with PCOS, there was no significant difference in maternal T between the three groups ($p<0.737$).

ANOVA showed a significant difference between the groups for UV T ($p<0.037$). UV T was significantly higher in the PCOS girls than the control girls ($p<0.012$, post-hoc analysis). The difference between PCOS girls and control boys was not significant ($p<0.254$, post-hoc analysis). The mean UV T in control boys was higher than in control girls but this did not reach statistical significance ($p<0.091$)

Table II – Maternal and UV testosterone in PCOS girls, control boys and control girls.

		n	**mean**	**SD**	**ANOVA**
Maternal T	PCOS girls	9	4.76	2.75	
(nmol/L)	Control girls	19	4.02	2.15	*ns*
	Control boys	10	4.00	2.96	
UV T (nmol/L)	PCOS girls	10	7.20	2.03	
	Control girls	20	5.34	1.54	$p<0.037$
	Control boys	10	6.26	2.09	

ns – not significant

UV testosterone and maternal testosterone were significantly positively correlated in PCOS girls ($r=0.653$, $n=9$, $p<0.028$).

Discussion

This study provides the first evidence of a hyperandrogenic fetal environment in pregnancies where the mother has PCOS. UV testosterone was not only significantly higher in female infants of PCOS mothers than of healthy mothers, but was observed to be as high as male levels. In our study, unlike that of Maccoby *et al*. (1979), the difference between control boys and control girls did not show statistical significance, possibly due to the sample size.

Post-hoc power calculations comparing UV T in PCOS girls and control populations, showed adequate power (0.84).

The finding of raised UV T in these female babies is compatible with the hypothesis of fetal programming in PCOS as a result of prenatal androgen excess. The sequelae of prenatal

androgen excess are not limited to development of the PCOS phenotype, hypertension and diabetes; there also is evidence of altered behavioural development. Exposure to raised concentrations of T prenatally has been linked to changes in childhood toy, playmate and activity preferences, to social behaviours related to autistic spectrum conditions and to sexual orientation and gender identity (Hines, 2004; Knickmeyer and Baron-Cohen, 2006).

Having shown that female fetuses of mothers with PCOS have elevated T concentrations, we are planning a follow up study which will address a number of limitations.

The current study used the electrochemilumesence T assay. Although the gold standard method is now isotope dilution mass spectrometry, the electrochemilumesence T assay is used routinely in clinical practice, as recognised by the 2007 Endocrine Society position statement (Rosner *et al.*, 2007). Nevertheless we are planning to use a mass spectrometry assay in our follow up study to eliminate errors from potential cross-reactivity of androgens.

We will modifiy sample sizes as appropriate to the mass spectrometry technique to give adequate power. An a priori sample size, based on previous work using this method (Maccoby et al., 1979) suggests 13 per group would be required to show a group difference (power 0.80). In addition, we will obtain paired arterial and venous umbilical cord blood samples in order to compare T levels before and after placental passage in order to investigate the source of the hyperandrogenaemia identified in the current study.

For future study, given the well-established influences of prenatal T on human behavioural development, our results suggest that studies of behaviour in daughters of women with PCOS might prove productive. Finally, in the light of the evidence (Rowan *et al*., 2008) showing the efficacy and apparent safety of metformin treatment in pregnancy it would be of interest to explore the effects of this treatment on T levels during pregnancy in women with PCOS and their fetuses. To date, the only study (Vanky *et al.*, 2004) of metformin treatment

throughout pregnancy did not assess fetal androgen exposure. Interestingly, in women receiving metformin, maternal levels of T at 36 weeks gestation were slightly below those in the first trimester, as compared to the increase of 0.9nmol/L in the untreated PCOS women.

In summary we have shown that female offspring of mothers with PCOS are subject to elevated androgen levels compared to babies whose mothers do not have this syndrome. There is evidence from human and animal studies that this exposure may have important consequences for future health and behaviour. These findings prompt additional research to investigate the mechanism responsible for the hyperandrogenaemia and its consequences for health and behaviour, as well as development of an intervention to normalise the prenatal environment in PCOS pregnancy.

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References

Abbott DH, Barnett DK, Bruns CM and Dumesic DA. 2005. Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? Hum Reprod Update 11: 357-374.

Billargeon JP and Carpentier AC. 2007. Brothers of women with polycystic ovary syndrome are characterized by impaired glucose tolerance, reduced insulin sensitivity and related metabolic defects. Diabetologia 50: 2424-2432.

Cattrall FR and Healy DL. 2004. Long-term metabolic, cardiovascular and neoplastic risks with polycystic ovary syndrome. Best Prac Res Clin Obs Gyn 18: 803-812.

Dahlgren E, Johansson S, Lindstedt G, Knutsson F, Odén A, Janson PO, Mattson LA, Crona N and Lundberg PA. 1992. Women with polycystic ovary syndrome wedge resected in 1956 to 1965: a long-term follow-up focusing on natural history and circulating hormones. Fertil Steril 57: 505-513.

Diamanti-Kandarakis E and Panidis D. 2007. Unravelling the phenotypic map of polycystic ovary syndrome (PCOS): a prospective study of 634 women with PCOS. Clin Endocrinol 67: 735-742.

Dunaif A, Segal KR, Futterweit W and Dobrjansky A. 1989. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes 38: 1165-1174.

ESHRE/ARSM. 2004. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Sterility 81: 19-25.

Franks S. 1995. Polycystic ovary syndrome. N Eng J Med 333: 853-861.

Franks S, Webber LJ, Goh M, Valentine A, White DM, Conway GS, Wiltshire S and McCarthy MI. 2008. Ovarian morphology is a marker of heritable biochemical traits in sisters with polycystic ovaries. J Clin Endocrinol Metab 93: 3396-3402.

Goodarzi MO, Guo X, Yildiz BO, Stanczyk FZ and Azziz R. 2007. Correlation of adrenocorticotropin steroid levels between women with polycystic ovary syndrome and their sisters. Am J Obstet Gynecol 196: E1-E5.

Hines M. 2004. Brain Gender. Oxford University Press, New York.

King AJ, Olivier NB, Mohankumar PS, Lee JS, Padmanabhan V and Fink GD. 2007. Hypertension caused by prenatal testosterone excess in female sheep. Am J Physiol Endocrinol Metab 292: E1837-E1841.

Knickmeyer RC and Baron-Cohen S. 2006. Fetal testosterone and sex differences in typical social development and in autism. J Child Neurol 21: 825-845.

Maccoby EE, Doering CH, Jacklin CN, Kraemer H. 1979. Concentrations of sex hormones in umbilical-cord blood: their relation to sex and birth order of infants. Child Dev 50: 632-642.

Nam MM and Strauss JF. 2007. Genetics of polycystic ovarian syndrome. Clin Obstet Gynecol 50: 188-204.

Rowan JA, Hague WM, Gao W, Battin MR, Moore MP and MiG Trial Investigators. 2008.

Metformin versus insulin for the treatment of gestational diabetes. N Engl J Med 358: 2003-2015.

Rosner W, Auchus RJ, Azziz R, Sluss PM and Raff H. 2007. Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. J Clin Endocrinol Metab. 92: 405-413.

Sir-Petermann T, Maliqueo M, Angel B, Lara HE, Pérez-Bravo F and Recabarren SE. 2002. Maternal serum androgens in pregnant women with polycystic ovarian syndrome: possible implications in prenatal androgenization. Hum Reprod 17: 2573-2579.

Sir-Petermann T, Codner E, Pérez V, Echiburú B, Maliqueo M, Ladrón de Guevara A, Preisler J, Crisosto N, Sánchez F, Cassorla F et al. 2009. Metabolic and reproductive features before and during puberty in daughters of women with polycystic ovary syndrome. J Clin Endocrinol Metab 94: 1923-1930.

Talbott E, Guzick D, Clerici A, Berga S, Detre K, Weimer K and Kuller L. 1995. Coronary heart disease risk factors in women with polycystic ovary syndrome. Arterioscler Thromb Vasc Biol 15: 821-826.

Vanky E, Salvesan KA, Heimstad R, Fougner KJ, Romundstad P, Carlsen SM. 2004. Metformin reduces pregnancy complications with affecting androgen levels in pregnant polycystic ovary women: results of a randomized study. Hum Reprod 19: 1734-1740.

Chapter 5

Testosterone and Mood Dysfunction in Women With Polycystic Ovarian Syndrome Compared to Subfertile Controls

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Abstract

Women with Polycystic Ovarian Syndrome (PCOS) have been found to suffer from fertility problems and mood dysfunction. To control for any effect of fertility problems, the present study compared mood dysfunction in women with PCOS to non-PCOS women with fertility problems. Seventy six women with PCOS and 49 subfertile controls reported their anxiety, depression, and aggression levels, and the relationship between mood and testosterone (T) was assessed. Controlling for age and BMI using MANCOVA, women with PCOS were significantly more neurotic (had difficulty coping with stress) than controls, had more anger symptoms, were significantly more likely to withhold feelings of anger, and had more quality of life problems related to the symptoms of their condition (acne, hirsutism, menstrual problems, and emotions). In a subgroup of 30 women matched on age, BMI and ethnicity, it was found that women with PCOS were significantly more anxious and depressed than controls. T was not generally correlated with mood states. Conclusion: This is the first study to identify problems with neuroticism and withholding anger in women with PCOS. These mood problems appear to be mainly attributable to PCOS symptoms, though other factors, such as hypoglycaemia, cannot be ruled out.

Introduction

Polycystic ovary syndrome (PCOS) affects 5-10% of women [1] and is characterised by elevated testosterone (T), menstrual irregularity, fertility problems, androgen excess, acne and hirsutism. Many studies have found that women with PCOS have greater mood dysfunction and psychiatric problems than women who do not have PCOS [2,3]. Levels of anxiety and depression are higher in PCOS than healthy women [4,5,6]. Some research suggests that aggression [7] and Type A personality [8] is more evident in women with PCOS than the norm, but this evidence is not particularly strong.

Although it has not been measured in a PCOS population before, neuroticism - a character trait that combines features of anxiety, depression, and anger - has been found in women with two conditions related to PCOS: hirsutism [9] and fertility problems [10]. Eysenck suggests that neuroticism is caused by lability of the autonomic nervous system (ANS) which predisposes a person to cope poorly with stressful events [11]. It is therefore of interest that women with PCOS show a stronger hypothalamic-pituitary adrenal axis (HPA) response to a stressor than healthy women [12,13].

The results of some studies have shown that concerns about fertility problems [14] and miscarriage [15] can be a source of distress in those with PCOS. Other studies have not found this link [16,17,18]. To date only one study [19] has compared distress in women with PCOS to an infertility control group; it was found that women with PCOS were significantly more depressed than the infertility control group, who in turn were more depressed than a community control group of women without PCOS or fertility problems.

Because elevated T appears to be related to the physical symptoms of PCOS, and PCOS patients report greater mood disturbance, T could be implicated in causing mood disturbance. Nonlinear relationships between testosterone and depression have been found in adolescent girls [23] and women with PCOS [24]. However in women with PCOS, four studies failed to find a correlation between T levels and depression [5,16,20,21]. This is perhaps because correlation is typically assessed with tests based on the principles of the general linear model [22] which can only detect linear relationships, and studies that are only looking for linear relationships may overlook significant nonlinear relationships.

In summary, the results of previous studies suggest that women with PCOS have elevated T, suffer from fertility problems and experience mood dysfunction. It is however difficult to confirm a relationship between T and mood dysfunction because of the confounding effect of subfertility. The present study was designed to explore the association between T and mood dysfunction whilst controlling for the effect of fertility problems. This was done by comparing mood dysfunction in women with PCOS to non-PCOS women with fertility problems, recruited from the same clinics. To control for affects of age and BMI, multivariate analysis of covariance (MANCOVA) was used. To additionally control for any affects of ethnicity, as subset of the sample were matched. We also examined the relationship between T levels and mood dysfunction in these two populations using both linear and nonlinear statistical tests. Based on the findings of previous research, it was predicted that compared to the control group, women with PCOS will score higher on measures of distress (anxiety, depression, neuroticism) and aggression, and that T will be correlated with distress and aggression.

Methods

This study was conducted with the approval of the institutional Research Ethics Committees of participating clinics. Written, informed consent was given by all participants. Participants were identified only by a code to protect confidentiality,

Participants

All women were examined by an endocrinologist or gynaecologist.

PCOS group

Eighty-one women with PCOS were recruited from London gynaecology clinics (the Royal Free Hospital, University College London Hospital, St Mary's Hospital, Guy's Hospital, and the Centre for Reproductive and Genetic Health at the Eastman Dental Hospital).[1] Inclusion criteria: 1/ diagnosis of PCOS by the Rotterdam criteria [25] (broadly speaking, women exhibiting any two of the following three symptoms: (a) severe menstrual disruption,[2] (b) elevated androgens (e.g. T levels above 2.9 nmol/l) or clinical signs of such (e.g. acne, hirsutism) (c) multiple ovarian cysts); 2/ aged 18 – 45 years. Exclusion criteria: 1/ any condition other than PCOS affecting hormones (with the exception of hypothyroidism being controlled effectively by medication), for example, menopause, congenital adrenal hyperplasia, androgen-secreting tumours, Cushing's syndrome; 2/ being treated with any drugs that affect hormones (other than

[1] The sample size was based on the requirement to power between-groups statistical analyses sufficiently to detect at least a medium effect size difference (a Cohen's *d* of 0.5) with 80% power and alpha set to .05. With equal numbers in each group, the sample size required would have been 102. However having smaller numbers in the control group meant having larger numbers in the PCOS, and the eventual N for the present study (71 versus 43) gives 82.3% power to detect a Cohen's *d* of 0.5 with alpha at .05.

[2] This was identified by attending physicians as per the Rotterdam criteria i.e. oligo- and/or anovulation.

thyroid hormone, or drugs used to treat PCOS for example, Metformin or Dianette); 3/ history of psychotic illness; 4/ because questionnaires were used, participants who were not fluent in English were also excluded.

Control group

Fifty-four women with fertility issues not related to PCOS were recruited from the same London gynaecology clinics as the PCOS participants. Women who would qualify for a diagnosis of PCOS according to the Rotterdam criteria were excluded from the control group. All other inclusion and exclusion criteria for the PCOS group were also applied to the selection of the control group.

Questionnaires

Hospital Anxiety and Depression Scale (HADS) [26] (14 items). This scale measures state anxiety & depression in medical outpatients. Item responses are on a 4-point scale. An example of an item from the Anxiety scale is "I feel tense or 'wound up': Most of the time; A lot of the time; From time to time/ occasionally; Not at all". Scores of 8-10 indicate a mild problem; 11-14 moderate; 15-21 indicates a clinical problem.

Eysenck Personality Questionnaire (EPQ-R Short) [27] (Eysenck et al, 1985) (48 items). This instrument measures three personality traits: Extroversion, Neuroticism, Psychoticism and has a social desirability scale. Neuroticism is a measure of emotionality, and is measured with questions such as "Are your feelings easily hurt?"

Aggression Questionnaire [28] (19 items). Measures four aspects of trait aggression: Physical Aggression, Verbal Aggression, Anger, and Hostility. Items are

measured on a 5-point scale from ‘extremely uncharacteristic of me’ to ‘extremely characteristic of me’. An example of a Verbal Aggression item is “When people annoy me, I may tell them what I think of them”.

The State−Trait Anger Expression Inventory (STAXI) [29] (44 items). This measures various aspects of current (state) and long term (trait) personal expression of anger. The five subscales are State Anger, Trait Anger, Anger In (anger suppression), Anger Out (anger expression), and Anger Control. The three latter subscales are combined to denote extreme problems in dealing with anger, which is calculated as Anger In plus Anger Out, minus Anger Control (plus a constant).

Framingham Anger Measure [30] (12 items). Four aspects of anger are measured: Anger Symptoms (for example, “get tense or worried”), Anger In (for example, “keep it to myself”), Anger Out (for example, “take it out on others”) and Anger Discuss (for example, “talk to a friend or relative”). Answers are on a 3-point scale from ‘very likely’ to ‘not too likely’.

The Framingham Type A Behaviour Pattern Measure [30] (10 items). This scale measures traits such as hostility and competitiveness. Five traits are measured on a 4-point scale from ‘very well’ to ‘not at all’, and five are measured as either ‘yes’ or ‘no’.

Control measures

PCOS Quality of Life (QoL) Questionnaire (PCOSQ) [31] (26 items). The impact of emotions, hirsutism, weight, infertility, menstrual problems, and acne on the patient’s

QoL are assessed. Answers are on a Likert scale of 1 (‘severe problem’) to 7 (‘no problem’), thus the lower the rating, the worse the QoL.

Socio-economic classification (SEC) was defined by the Office for National Statistics’ [32] three-class hierarchy of Managerial & Professional, Intermediate Occupations and Routine & Manual jobs.

Information on the specific diagnosis (PCOS or control) and medication were confirmed from hospital records. Smoking / drinking level per week, prescription or over−the−counter medicines taken, height and weight were given by the participant in the questionnaire.

Hormone assays

Most of the serum T concentrations (83% of matched pairs and > 90% of the total sample) were measured using the Roche Elecsys system 1010/2010 Modular E170, a direct electrochemiluminescence (ECLIA) methodology (Roche Diagnostics, Indianapolis). The intra-assay coefficients of variation were all less than 4.6%. Reference intervals for women between 18 and 40 for this assay are 0.22–2.9 nmol/L. The dynamic range for this assay is 0.069 to 52.00 nmol/L. This assay was used in four different laboratories in the present study, and the United Kingdom National External Quality Assessment Service (UK NEQAS) suggests that the method yields comparable results from different laboratories, with coefficients of variation of around 10% [33]. Around 10% of T samples were assayed using a competitive CLIA on the Immulite

2500 analyser (Diagnostic Products Corporation, Los Angeles, CA). The T levels found in samples using the two assay methods did not differ significantly from each other.

Procedure

Patients who met the inclusion criteria were identified by the consultant or other attending doctors at the participating clinics.

Blood Collection

Blood was taken with a 21-gauge needle by the clinic's phlebotomist. Serum samples were transferred to 10ml gel activator clotting tubes. Samples were centrifuged and then frozen until being assayed.

Statistical analyses

The statistical package SPSS for Windows (Version 16) was used for all statistical analyses. In the full dataset, where distributions were non-normal a square root or log10 transformation was applied. Subsequently all of the distributions in the full dataset passed Levene's tests of homogeneity of variance, apart from QoL acne, hirsutism, infertility, and emotion which resisted transformation because only one group's distribution was skewed in each case. Most of the matched distributions passed the Kolmogorov-Smirnov test of normality. For normal distributions, matched t-tests were used to compare variables, and Pearson's correlations were used to assess relationships. Where a variable was found to be non-normal in either group (PCOS or control),

Wilcoxon tests were used to compare the groups, and Spearman's correlations were used for tests of association. The SPSS Curve Estimation function was used to test for nonlinear (quadratic and cubic) relationships between T and psychometric variables.

Demographic variables were assessed by independent t-tests and Fisher's exact tests. In all cases significance thresholds were 2-tailed and $p<.05$ was required for statistical significance.

Results

QoL for problems related to infertility were similar in each group (Table 1). The two largest women in each sample (with BMIs of 42.3 and 50.2) were matched on being 'morbidly obese' (BMI > 40) rather than being of similar BMI. One woman with diabetes in each group were matched to each other.

The matched data set consisted of Caucasian women only because no non-Caucasian women in the control group could be matched on age & BMI to non-Caucasians in the PCOS group.

Ten of the 81 (12%) of women with PCOS and 11 of the 54 (20%) control group were excluded because they did not fill in sections of the questionnaire, or gave insufficient data on some subscales for replacement to be possible. The number of women who omitted sections did not significantly differ by group (Chi Sq = 1.588, df=1, p<.208). Forty five (33%) of the participants did not have T measured.[3] For other variables in the full dataset an average of 2.54% of data was missing. Little's *Missing Completely at Random* (MCAR) test indicated that the missing data did not show a

[3] 20% (16) of the 81 women with PCOS and 29% (16) of 54 controls did not have a T measurement.

significant pattern (Chi-Square = 376.577, df = 367, p< .354). In the matched groups, an average of 3.9% of the data were missing and Little's MCAR test indicated that the missing data did not show a significant pattern (Chi-Square = 7.738, df = 230, p< 1.000). Missing data were replaced with the mean for each participant on the measure (subscale or single-domain scale) for which an item was missing, unless the participant had omitted 33% or more of data from the measure, in which case their data for the measure was deleted from analyses.

Table I shows descriptive statistics and statistical comparisons for the background variables of the PCOS and subfertile control group for the full sample, and Table III shows findings for the matched sample. The groups scored similarly for the QoL impact of fertility problems, both indicating “some problems” to “moderate problems” with fertility. Tables II and IV show the outcomes of comparisons for the total sample and matched sample.

Table I

Descriptive statistics (means or medians, and SDs or range) and between groups tests (t-tests, Mann-Whitney, or Chi Square) values for the PCOS and subfertile control group.

Variable		PCOS (*N*=76)[d]	Controls (*N*=49)[d]	*Test statistic*
Total testosterone (T) nmol/l		2.10 (0.20 – 5.80)	1.10 (0.40 – 4.20)	472*** [e]
Age		28.8 (4.81)	35.12 (4.37)	-7.38*** [f]
BMI		27.87 (7.36)	24.69 (7.08)	3.33*** [f]
QoL Weight		3.41 (2.16)	5.02 (1.88)	-4.31*** [f]
QoL Menstrual		3.5 (1 – 7)	5.3 (2 – 7)	969.5*** [e]
QoL Emotions		4.1 (1 – 7)	5 (2 – 7)	1408* [e]
QoL Infertility		3.65 (2.06)	3.98 (1.71)	-.932
QoL Acne		4 (1 – 7)	7 (1 – 7)	1076.5*** [e]
QoL Hirsutism		3.4 (1 – 7)	7 (2 – 7)	557.5*** [e]
SEC	Professional	25 (42%)	18 (40%)	2.87[c]
	Intermediate occupation	10 (17%)	14 (30%)	
	Routine/Manual	24 (41%)	14 (30%)	
Ethnic Group[a]	White	41 (64%)	33 (72%)	4.52[b]
	Black	7 (11%)	1 (2%)	
	Indian	5 (8%)	2 (4%)	
	Mixed Race	7 (11%)	5 (11%)	
	Other	4 (6%)	5 (11%)	

Note. * $p < .05$. ** $p < .01$. Significance values are 2-tailed.

T = Total Testosterone; BMI = Body Mass Index; QoL = Quality of Life; SEC = Socioeconomic Classification

[a] Ethnicity was not identified for 12 of the PCOS and 3 of the control participants.

[b] Fisher's Exact test statistic.

[c] Chi Square

[d] For total testosterone the sample size was 65 PCOS and 38 controls.

[e] Mann-Whitney U test

[f] Independent groups t-test

Table II

Comparisons between the PCOS and subfertile control group on the mood measures (anxiety, depression, anger) controlling for age and BMI using MANCOVA.

Variable	PCOS (*N*=76)[a]	Controls (*N*=49)[a]	*F*
Anxiety	9.99 (4.56)	7.57 (4.12)	2.25
Depression[b]	4.88 (1.98)	2.76 (2.04)	1.54
Neuroticism	7.89 (3.37)	5.61 (3.41)	3.95*
AQ Physical[b]	16.55 (6.98)	14.78 (6.37)	0.01
AQ Verbal	12.89 (4.57)	11.87 (4.85)	1.64
AQ Anger	17.05 (5.92)	15.59 (6.89)	0.01
AQ Hostile[c]	18.92 (7.77)	14.98 (6.07)	1.72
AQ Total[c]	16.48 (3.60)	14.36 (4.29)	0.87
STAXI State Anger	12.50 (5.46)	11.80 (4.55)	0.55
STAXI Trait Anger[b]	18.98 (6.01)	18.63 (6.76)	0.56
STAXI Trait Temper[b]	6.40 (1.55)	6.40 (1.51)	1.60
STAXI Reactivity	9.51 (2.96)	8.65 (2.81)	0.01
STAXI Anger In	17.55 (4.87)	15.93 (4.98)	2.00
STAXI Anger Out	15.04 (4.10)	14.98 (4.40)	2.10
STAXI Anger Control	22.10 (5.67)	22.44 (5.34)	1.87
STAXI Extreme	26.49 (10.45)	24.48 (10.30)	0.43
Fram. Anger Symptoms	2.82 (1.36)	2.02 (1.36)	2.69*
Fram. Anger In	1.41 (0.96)	0.84 (0.92)	4.71*
Fram. Anger Out[c]	0.65 (0.57)	0.40 (0.48)	0.29
Fram. Anger Discuss	1.19 (0.60)	1.28 (0.56)	1.29
Fram. Type A	0.54 (0.23)	0.51 (0.23)	0.24

Note. * $p < .05$. Significance values are 2-tailed.

AQ = Aggression Questionnaire; STAXI = State-Trait Anger Expression Inventory; Fram. = Framingham Anger Measure.

[a] For Framingham measures, n=71 PCOS and 43 controls.

[b] log transformed (log 10).

[c] square root transformed.

Table III

Descriptive statistics (means and SDs, or medians and ranges) and inferential statistics for the PCOS and subfertile control group.

Variable		PCOS (*N*=15) [a, b]	Subfertile controls (*N*=15) [b]	*Test statistic*
T		1.96 (0.76)	1.68 (1.03)	0.84 [c]
Age		32.27 (3.22)	34.33 (2.92)	- 1.84 [c]
BMI		23.10 (19.80 - 42.47)	23.53 (18.67 - 50.15)	107.00 [d]
QoL Weight		4.4 (1 – 7)	5.6 (1.2 – 7)	- 0.88 [d]
QoL Menstrual		4.18 (1.38)	4.87 (1.30)	1.58 [c]
QoL Emotions		4.67 (1.72)	5.54 (0.98)	1.81 [c]
QoL Infertility		3.85 (2.08)	3.47 (1.55)	0.511 [c]
QoL Acne		6 (1 – 7)	7 (5 - 7)	-2.75** [d]
QoL Hirsutism		5 (1 – 7)	7 (1 – 7)	-1.30 [d]
SEC	Professional	9	4	
	Intermediate occupation	3	4	3.93 [e]
	Routine/Manual	2	6	

Note. * $p < .01$. Significance values are 2-tailed.

T = Total Testosterone; BMI = Body Mass Index; QoL = Quality of Life; SEC = Socioeconomic Classification

[a] N=14 in the PCOS group for socially desirable responding

[b] N=14 in both groups for SEC

[c] matched t-test

[d] Wilcoxon

[e] Fisher's Exact Test

Table IV

Comparisons between the matched PCOS and subfertile control group on the mood measures (anxiety, depression, anger) using paired t-tests or Wilcoxon tests. Participants are matched on age, BMI, and ethnicity.

Variable	PCOS (*N*=15) [a, b]	Controls (*N*=15) [a, b]	*Test statistic*
Anxiety	9.61 (4.33)	5.67 (3.22)	2.91* [c]
Depression	4.00 (1-12)	2.00 (0-7)	-2.29*
Neuroticism[a]	3.06 (0.82)	2.44 (0.65)	2.91* [c]
AQ Physical[a]	12.5 (9 - 43)	13.00 (9 – 25.4)	0.00 [d]
AQ Verbal[a]	12.29 (3.20)	12.14 (3.98)	0.09 [c]
AQ Anger[a]	17.14 (6.21)	14.21 (5.10)	1.33 [c]
AQ Hostile[b]	19.0 (8.14)	14.77 (7.46)	1.37 [c]
AQ Total[b]	15.98 (5.93)	13.35 (4.36)	1.24 [c]
STAXI State Anger	10 (10 - 40)	10 (10 - 12)	-0.74 [d]
STAXI Trait Anger[b]	17 (13 - 40)	15 (10 - 23)	-1.30 [d]
STAXI Trait Temper[b]	6.00 (4 - 16)	6.00 (4 - 9)	-1.07 [d]
STAXI Reactivity[a]	8.93 (2.92)	7.64 (2.53)	1.26 [c]
STAXI Anger In[b]	16.00 (8 - 28)	16.00 (8 - 16)	-2.35* [d]
STAXI Anger Out[a]	15.25 (3.89)	15.36 (3.75)	-0.06 [d]
STAXI Anger Control[a]	21.79 (5.96)	22.14 (5.96)	-0.17 [c]
STAXI Extreme[b]	25.12 (12.63)	19.92 (7.57)	1.11 [c]
Fram. Anger Symptoms[b]	2.64 (1.51)	1.65 (1.09)	2.01 [c]
Fram. Anger In[b]	1.25 (0 - 3)	0.00 (0 - 2)	-2.26* [d]
Fram. Anger Out[b]	1.00 (0 - 1)	0.5 (0 – 1.5)	-1.22 [d]
Fram. Anger Discuss[b]	1.15 (0.72)	1.39 (0.51)	-0.92 [c]
Fram. Type A[b]	0.37 (0.13 - 0.87)	0.37 (0.13 - 0.87)	-0.04 [d]

Note. * $p < .05$. Significance values are 2-tailed.

AQ = Aggression Questionnaire; STAXI = State-Trait Anger Expression Inventory; Fram. = Framingham.

[a] n=14 per group

[b] n=13 per group

[c] matched t-test

[d] Wilcoxon and median (range)

QoL for acne, hirsutism, weight, menstrual problems and emotions related to the patients' medical condition were significantly different, so were used as covariates in MANCOVA in addition to age & BMI. After controlling for these seven variables using MANCOVA, all group differences were reduced to non-significance.

Medication

Of the 46 women with PCOS whose medication status was identified, 35 (76%) were taking PCOS medication (insulin sensitizer or an anti-androgen) as were four of the 36 controls (11%). In the matched groups, of the 13 women with PCOS whose medication use was identified, six were taking medication that can potentially lower T levels (five taking metformin and one taking an anti-androgen). In the matched groups, one of the control group women was taking insulin and metformin to control diabetes. In comparing the women with PCOS on medication and those not on medication, no significant effect of medication on T was seen.

Correlations

In the main dataset there were two significant correlations between T and psychometric variables in the PCOS group (QoL hirsutism r_s=-.32, n=65, p<.009; QoL menstrual problems r_s=-.26, n=59, p<.047). There were no significant correlations with T in the control group. ANOVA for nonlinear model fit was nonsignificant in most cases, but some significant trends were found. In the PCOS group the following trends reached significance: QoL hirsutism (linear p<.006; quadratic p<.005; cubic p<.014), QoL infertility (cubic p<.031); QoL menstrual (linear p<.03; quadratic p<.041), QoL acne

(linear p<.047; cubic p<.042), Framingham Anger Symptoms (quadratic p<.032; cubic p<.045), Framingham anger-in (quadratic p<.018; cubic p<.037). In the control group the following were significant: AQ hostility (linear p<.047; quadratic p<.018; cubic p<.028), STAXI temper trait (cubic p<.047); Framingham anger-out (cubic p<.011).

The hypothesis regarding greater distress in the PCOS group had some support, so this hypothesis is accepted. More aggression was not seen in the PCOS group, so this hypothesis is rejected. There was little evidence of a relationship between T and anxiety, depression, or aggression, so this hypothesis is also rejected.

Discussion

We found that PCOS patients had higher neuroticism and anger-in scores than subfertile controls. Both groups had a similar QoL impact of fertility problems, thus the group differences are unlikely to be caused by the impact of fertility problems.

In both the full sample and matched subsample, the PCOS group scored higher than the subfertile controls on neuroticism and withholding anger. Neither of these variables have been reported as significantly different in previous PCOS research, though the neuroticism finding echoes findings of greater anxiety [4], depression [34], and anger [7] in previous research. Weiner et al's [24] finding of a nonlinear relationship between T and mood was only weakly supported.

When group differences in PCOS symptoms were controlled using MANCOVA, the group differences on mood problems became non-significant, suggesting that most of the mood problems seen in PCOS are caused by the distressing symptoms of the syndrome. However in the matched groups comparison, none of the PCOS symptoms

apart from acne were significantly different between the groups, yet neuroticism and anger-in remained higher in the PCOS group. In other words, these differences in neuroticism and anger-in cannot be explained by fertility problems, age, BMI, most PCOS symptoms, or ethnicity, so another factor (or factors) must be responsible. One possible explanation is the psychological effects of hypoglycaemia (low blood sugar), a condition with a high rate of prevalence in women with PCOS [35]. Experimentally induced hypoglycaemia in healthy people can increase anger, anxiety, and low mood [36,37], in other words create a pattern of mood disturbance similar to that measured by the neuroticism scale. Hypoglycaemia causes autonomic arousal [36,37] and HPA activation [38], and because there is evidence of greater HPA activation in women with PCOS than controls [12] [13] it is possible that the mood disturbance seen in the present study and the HPA abnormalities seen in other PCOS studies are caused by hypoglycaemia. Blood sugar levels were not measured in the present study, but future studies might investigate the possible role of hypoglycaemia in altering mood in women with PCOS.

The PCOS group scored higher than controls on both measures of anger-in (STAXI and Framingham) suggesting that they suppress anger more than the control group. Campagne [39] suggests that dealing with stress caused by fertility issues is a highly complex task, thus basing treatment solely on expressing emotions might not help women with PCOS. Furthermore it is possible that the cause of the anger is simply cognitive labelling of HPA activation, as classically described by Schachter & Singer [40], and further research is needed to explore the causes of unexpressed anger in women with PCOS, and to develop appropriate ways of helping.

Although the PCOS group on average scored significantly higher on depression in the matched groups comparison, the levels were in the normal range for both groups. Previous studies (for example, [7]) found more psychopathology in their PCOS samples than the present study. In the present study women with a history of psychosis were excluded, and their exclusion could have lowered a difference in psychopathology between the PCOS and subfertile control group. However this will have primarily excluded symptoms of psychosis from the present study, and as these were not the focus of the present study their exclusion is not of direct relevance.

There were few linear correlations between T and mood variables for the PCOS group and none for the controls. Unlike Weiner et al [24], nonlinear relationships did not appear to be noticeably more prominent than linear. The general lack of association between T and psychometric measures suggests that T had little activational effect on mood in the present sample.

Log10 transformation of distributions was necessary for some of the variables in order to meet the assumption of normality of distribution required for MANCOVA. However it is notable that the log transformed variables tended to show a non-significant difference, even when – in the case of depression – the means and SDs indicated a large effect size. For this reason the results from the matched groups, which did not require transformation, are probably more important than those of the MANCOVA. Some authors suggest that matching is a more efficient method than ANCOVA for controlling for the effects of variables because of the fewer assumptions required for matching and better statistical power (e.g. [41]). ANCOVA remains

appealing to researchers however, because matching can be a difficult process and the more variables matched for the more difficult it is to find suitable matches.

At 34% the response rate was not high. However the response rate was similar in each group, and not dissimilar to response rates in some other questionnaire studies of PCOS (e.g. [42]). Many patients declined participation citing time pressure as the reason, and 16% of those who participated failed to complete some sections of the questionnaire. It could be that the patients who declined participation were different in some relevant psychological respect to the patients who participated, but this cannot be established because the decliners, inevitably, left no tangible information about their psychological state. The issue of response rate should not be given undue weight because low response rates don't necessarily compromise data validity [43].

The findings of the present study suggest that women with PCOS experience more neuroticism and anxiety than women with comparable levels of fertility problems, and tend to withhold feelings of anger. Assessment of mood state could be considered as part of routine care in PCOS. Care for these women might involve a combination of medication (e.g. metformin or an anti-androgen) to treat physical symptoms related to PCOS, and the development of a psychological treatment or support programme to help with deal with emotional issues. If future studies find that hypoglycaemia is a causal factor in mood disturbance in PCOS, a nutritional intervention – the low GI diet [44] – might be an appropriate part of a treatment plan because it has the potential to improve biochemical and physiological parameters, thus reducing PCOS symptoms and improving mood functioning.

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References

[1] Franks S. Polycystic ovary syndrome. N Engl J Med 1995;333:853-861.
[2] Himelein MJ, Thatcher SS. Polycystic ovary syndrome and mental health: a review. Obstet Gynecol Surv 2006;61:723-732.
[3] Farrell K, Antoni MH. Insulin resistance, obesity, inflammation, and depression in polycystic ovary syndrome: biobehavioral mechanisms and interventions. Fertil Steril 2010;94:1565-1574.
[4] Månsson M, Holte J, Landin-Wilhelmsen K, Dahlgren E, Johansson A, Landén M. Women with polycystic ovary syndrome are often depressed or anxious--a case control study. Psychoneuroendocrinology 2008;33:1132-1138.
[5] Hollinrake E, Abreu A, Maifeld M, Van Voorhis BJ, Dokras A. Increased risk of depressive disorders in women with polycystic ovary syndrome. Fertil Steril 2007;87:1369-1376.
[6] Bhattacharya SM, Jha A. Prevalence and risk of depressive disorders in women with polycystic ovary syndrome (PCOS). Fertil Steril 2010;94:357-359.
[7] Elsenbruch S, Hahn S, Kowalsky D, Offner AH, Schedlowski M, Mann K, Janssen OE. Quality of life, psychosocial well-being, and sexual satisfaction in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2003;88:5801-5807.
[8] Monzani F, Pucci F, Caraccio N, Bagnolesi A, Molli D, Fenu A, Prunetti CA. Psychological and psychopathological correlates in the polycystic ovary syndrome (PCOS). Med Psicosom 1994;39:225-236.
[9] Barth JH, Catalan J, Cherry CA, Day A. Psychological morbidity in women referred for treatment of hirsutism. J Psychosom Res 1993;37:615-619.
[10] Verhaak CM, Smeenk JMJ, Evers AWM, van Minnen A, Kremer JAM, Kraaimaat FW. Predicting emotional response to unsuccessful fertility treatment: a prospective study. J Behav Med 2005;28:181-190.
[11] Eysenck HJ. The biological basis of personality. New Brunswick, NJ: Transaction; 1967.
[12] Gallinelli A, Matteo ML, Volpe A, Facchinetti F. Autonomic and neuroendocrine responses to stress in patients with functional hypothalamic secondary amenorrhea. Fertil Steril 2000;73:812-816.
[13] Benson S, Arck PC, Tan S, Hahn S, Mann K, Rifaie N, Janssen OE, Schedlowski M, Elsenbruch S. Disturbed stress responses in women with polycystic ovary syndrome. Psychoneuroendocrinology 2009;34:727-735.
[14] Benson S, Hahn S, Tan S, Mann K, Janssen OE, Schedlowski M, Elsenbruch S. Prevalence and implications of anxiety in polycystic ovary syndrome: results of an internet-based survey in germany. Hum Reprod 2009;24:1446-1451.
[15] McCook JG, Reame NE, Thatcher SS. Health-related quality of life issues in women with polycystic ovary syndrome. J Obstet Gynecol Neonatal Nurs 2005;34:12-20.
[16] McCook JG. The influence of hyperandrogenism, obesity and infertility on the psychosocial health and wellbeing of women with polycystic ovary syndrome. Unpublished Doctoral Dissertation, University of Michigan 2002.
[17] Tan S, Hahn S, Benson S, Janssen OE, Dietz T, Kimmig R, Hesse-Hussain J, Mann K, Schedlowski M, Arck PC, Elsenbruch S. Psychological implications of

infertility in women with polycystic ovary syndrome. Hum Reprod 2008;23:2064-2071.
[18] Kerchner A, Lester W, Stuart SP, Dokras A. Risk of depression and other mental health disorders in women with polycystic ovary syndrome: a longitudinal study. Fertil Steril 2009;91:207-212.
[19] Himelein MJ, Thatcher SS. Depression and body image among women with polycystic ovary syndrome. J Health Psychol 2006;11:613-625.
[20] Hahn S, Janssen OE, Tan S, Pleger K, Mann K, Schedlowski M, Kimmig R, Benson S, Balamitsa E, Elsenbruch S. Clinical and psychological correlates of quality-of-life in polycystic ovary syndrome. Eur J Endocrinol 2005;153:853-860.
[21] Rasgon NL, Rao RC, Hwang S, Altshuler LL, Elman S, Zuckerbrow-Miller J, Korenman SG. Depression in women with polycystic ovary syndrome: clinical and biochemical correlates. J Affect Disord 2003;74:299-304.
[22] Cohen, J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale, NJ: Erlbaum; 1988.
[23] Angold A, Costello EJ, Erkanli A, Worthman CM. Pubertal changes in hormone levels and depression in girls. Psychol Med 1999;29:1043-1053.
[24] Weiner CL, Primeau M, Ehrmann DA. Androgens and mood dysfunction in women: comparison of women with polycystic ovarian syndrome to healthy controls. Psychosom Med 2004;66:356-362.
[25] 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;19:41-47.
[26] Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. Acta Psychiatr Scand 1983;67:361-370.
[27] Eysenck SBG, Eysenck HJ, Barrett PT. A revised version of the psychoticism scale. Personality and Individual Differences 1985;6:21-29.
[28] Buss AH, Perry M. The Aggression Questionnaire. J Pers Soc Psychol 1992;63:452-459.
[29] Spielberger CD. Manual for the state−trait anger expression inventory (staxi). Odessa, FL: Psychological Assessment Resources; 1988.
[30] Haynes SG. The relationship of psychosocial factors to coronary heart disease in the framingham study: i. methods and risk factors. American Journal of Epidemiology 1978;107:362-383.
[31] Cronin L, Guyatt G, Griffith L, Wong E, Azziz R, Futterweit W, Cook D, Dunaif A. Development of a health-related quality-of-life questionnaire (PCOSQ) for women with polycystic ovary syndrome (PCOS). J Clin Endocrinol Metab 1998;83:1976-1987.
[32] Office for National Statistics ELASA. NS-SEC classes and collapses 2004. Available online at http://www.ons.gov.uk/about-statistics/classifications/current/ns-sec/cats-and-classes/ns-sec-classes-and-collapses/index.html Accessed 29th October 2010
[33] Lamph S, Wheeler M, Halloran S. DPC Immulite and Immulite 2000. MHRA evaluation report, MHRA 04027. 2004. Available online at http://www.pasa.nhs.uk/evaluation/publications/per/clinical_biochemistry.asp Accessed 15th Feb 2008.

[34] Bhattacharya SM, Jha A. Prevalence and risk of depressive disorders in women with polycystic ovary syndrome (PCOS). Fertil Steril 2010;94:357-359.
[35] Altuntas Y, Bilir M, Ucak S, Gundogdu S. Reactive hypoglycemia in lean young women with PCOS and correlations with insulin sensitivity and with beta cell function. Eur. J. Obstet Gynecol Reprod Biol 2005;119:198-205.
[36] McCrimmon RJ, Ewing FM, Frier BM, Deary IJ. Anger state during acute insulin-induced hypoglycaemia. Physiol Behav 1999;67:35-39.
[37] McCrimmon RJ, Frier BM, Deary IJ. Appraisal of mood and personality during hypoglycaemia in human subjects. Physiol Behav 1999;67:27-33.
[38] Donald RA, Espiner EA. The plasma cortisol and corticotropin response to hypoglycemia following adrenal steroid and ACTH administration. J Clin Endocrinol Metab 1975;41:1-6.
[39] Campagne DM. Should fertilization treatment start with reducing stress? Hum Reprod 2006;21:1651-1658.
[40] Schachter S, Singer JE. Cognitive, social, and physiological determinants of emotional state. Psychol Rev 1962;69:379-399.
[41] Keele L, McConnaughy C, White I. Matching in randomized experiments 2008. Unpublished manuscript available online at http://polmeth.wustl.edu/media/Paper/ANCOVA%20and%20Matching.pdf Accessed 29th October 2010.
[42] Sharma A, Walker D, Atiomo W. National survey on management of weight reduction in PCOS women in the united kingdom. Eur J Obstet Gynecol Reprod Biol 2010;152:181-185.
[43] PWGSC. Best practices in public opinion research. Public Works and Government Services Canada; 2007. Available online at http://www.pwgsc.gc.ca/por/text/pebptel-intro-e.html Accessed 3rd March 2008
[44] Marsh KA, Steinbeck KS, Atkinson FS, Petocz P, Brand-Miller JC. Effect of a low glycemic index compared with a conventional healthy diet on polycystic ovary syndrome. Am J Clin Nutr 2010;92:83-92.

Chapter 6

The impact of eating behavior on psychological symptoms typical of reactive hypoglycemia: a pilot study comparing women with polycystic ovary syndrome to controls.

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ABSTRACT. The idea that diet can affect mood and behavior in women with polycystic ovary syndrome (PCOS) by altering blood glucose levels has become popular in recent years. This paper describes an online survey (N=462) of 24 women with PCOS, 299 healthy control women, 47 women who possibly had undiagnosed PCOS, and 92 men. The groups were compared for symptoms of mood and behavioral symptoms typical of reactive (postprandial) hypoglycemia. The outcome measures were two questionnaires that measure states associated with hypoglycemia: the Hypoglycemia Symptom Checklist -7 (HSC-7), which measures behavioral symptoms and the Mood Adjective Checklist (MACL), which measures emotional states. Controlling for age and body mass index (BMI) using between-groups analysis of covariance (ANCOVA), the women with PCOS scored significantly higher than the other three groups ($p < 0.001$) on the outcome measures. These differences remained statistically significant in a subset of twelve women with PCOS compared to twelve healthy control women closely matched for age, BMI, and eating behavior. The findings are suggestive of hypoglycemia-related mood and behavioral problems in PCOS. Future research should test whether blood glucose levels correlate with these symptoms in PCOS, and whether a low glycemic index ('low-GI') diet improves the symptoms.

Keywords: polycystic ovary syndrome, eating behavior, mood, hypoglycemia, low-GI diet.

Introduction

Polycystic ovary syndrome (PCOS) affects up to 10% of women (Ledger & Clark, 2003). As well as the classical symptoms of menstrual irregularity and testosterone excess, women with PCOS might report light headedness, trembling or faintness that they may attribute to low blood glucose levels (Marsh, Steinbeck, Atkinson, Petocz, & Brand-Miller, 2010). Women with PCOS are hyperinsulinemic compared to healthy women of a similar body mass index (BMI) (Diamanti-Kandarakis & Papavassiliou, 2006) and it is plausible that some of them experience symptoms caused by a sudden fall in blood glucose. In support of the hypoglycemia hypothesis, some PCOS patients say that their supposed hypoglycemia symptoms are associated with a craving of sweet food or drinks. Anecdotally, many treating physicians are skeptical that such symptoms are related to hypoglycaemia.

Reactive (or 'postprandial') hypoglycemia (RH) occurs when blood glucose levels drop two to five hours after a meal, especially a meal high in carbohydrates. Reactive hypoglycemia is under-researched, but there is evidence to suggest that this condition is more common in women with PCOS than in other women. For example, Sorensen & Johansen (2010) found a 12.4% rate of reactive hypoglycemia in women with no reported history of plasma glucose dysregulation, and by contrast a study of 64 lean women with PCOS found a 50% rate of reactive hypoglycemia (Altuntas, Bilir, Ucak, & Gundogdu, 2005). Kasim-Karakas, Cunningham, & Tsodikov (2007) assessed 28 obese women with PCOS using a 5-hour oral glucose tolerance test (OGTT) and found a reactive hypoglycemia rate of 66%.

Reactive hypoglycemia (typically mid-afternoon) may contribute to weight gain in PCOS (Magnotti & Futterweit, 2007); this could occur because insulin induces the production of androgens such as testosterone, that in turn increase visceral fat (Stanley & Misra, 2008). Reactive hypoglycemia has been described as one of the "subtle symptoms" of PCOS (Brand-Miller, Farid & Marsh 2004, p.9) and a low glycemic index (GI) diet may be a way of controlling this problem. Marsh, Steinbeck, Atkinson, Petocz & Brand-Miller

(2010) found that the low-GI diet significantly improved insulin resistance, menstruation (probably due to improved insulin sensitivity) and emotional quality of life in moderately overweight women with PCOS.Findings from two other recent studies (Galletly et al 2007; Herriot, Whitcroft, & Jeanes, 2008) appear to support the efficacy of the low-GI diet for women with PCOS in relation to mood and other symptoms related hypoglycemia.

Individuals with suspected postprandial hypoglycemia tend to have higher levels of psychological distress, including anxiety and depression, than other people (Berlin, Grimaldi, Landault, Cesselin, & Puech, 1994). The mood state typical of hypoglycemia has been described as 'tense-tiredness' (Thayer, 1989). Classic well-controlled studies of the effects of experimental induction of hypoglycemia in healthy participants found evidence of this 'tense-tired' mood state (Gold, MacLeod, Frier, & Deary, 1995; McCrimmon, Frier, & Deary, 1999). More recent research also suggests an effect of hypoglycemia on psychological functioning, for example, Bie-Olsen, Pedersen-Bjergaard, Kjaer, Lonsdale, Law, and Thorsteinsson (2010) induced hypoglycemia in twenty healthy men and found a statistically significant difference between scores before the induction and during induced hypoglycemia on the Edinburgh Hypoglycaemia Symptom Score Questionnaire (Hepburn, Deary, MacLeod, and Frier, 1994), a scale which includes psychological symptoms typical of hypoglycaemia, such as anxiety and tiredness. Although there is subjective and objective evidence of reactive hypoglycemia in women with PCOS, and despite considerable evidence of anxiety and depression in PCOS (for example, Himelein & Thatcher, 2006) the possible link between reactive hypoglycemia and mood problems in PCOS has received surprisingly little scientific attention.

The present study was designed to test the hypothesis that women with PCOS have more symptoms of mood and behavioral disturbance typical of reactive hypoglycemia than women who do not have PCOS.

Method

Design

An online questionnaire survey was used to identify PCOS cases and controls. Comparisons were made between four groups: women with PCOS, healthy control women, women who possibly had undiagnosed PCOS, and men. From this sample, matches were found between women with PCOS and non-PCOS controls on age, self-reported BMI, and eating behavior. The main grouping variables were PCOS status and eating behavior. The outcome variables were questionnaires measuring symptoms typical of reactive hypoglycemia.

Questionnaires

Two measures of signs of reactive hypoglycemia were used:

The UWIST Mood Adjective Check List (MACL) (Matthews, Jones, & Chamberlain, 1990). This scale assesses happiness, tension, and energy levels using three 8-item subscales, and changes in scoring on this measure has been found to reflect changes in arterialized venous blood glucose levels in studies of experimentally induced hypoglycaemia (Gold et al., 1995; McCrimmon et al., 1999). Similar mood changes are hypothesised to be seen in postprandial hypoglycemia because it is hypothesised that low blood glucose causes the mood changes regardless of whether hypoglycaemia is naturally occurring or experimentally induced. To increase sensitivity to degrees of mood, the present study expanded Matthews et al's checklist from a 'yes/no' format to a 4-point Likert scale prefixed with “I generally feel…” and response options of 'never, rarely, sometimes, often' e.g. ‘I feel cheerful’. Higher scores indicate positive hedonic tone (happy mood), more tension, and more energy. On each subscale the maximum score is 32, and the minimum is eight.

The Hypoglycemia Symptom Checklist – 7 items (HSC-7). This short scale was designed for the present study to be a quick test for behavioral symptoms of hypoglycemia. The HSC-7 consists of four items related to symptoms of neuroglycopenia and three items related to autonomic symptoms of hypoglycemia. The neuroglycopenia symptoms are:

clumsiness, confusion, sudden weakness, and difficulty in speaking. The autonomic symptoms are: unexplained palpitations, sweating, and shivering. The symptoms are rated for frequency of occurrence on a Likert scale from 1 (never) to 4 (often).

The symptoms listed in the HSC-7 have been variously identified in several sources (e.g. Ross, 1975; Deary, Hepburn, MacLeod, & Frier, 1993), which contributes to the face validity of the HSC-7. The HSC-7 also demonstrates good internal reliability; for the sample in the present study the Cronbach's alpha is 0.776. A principal components analysis of the HSC-7 using Varimax rotation with Kaiser normalization indicated sound underlying components (Kaiser-Meyer-Olkin index = 0.830), with a good average factor loading (0.655). As mentioned above, the MACL has been validated against biological measures of hypoglycemia, and in the present study the HSC-7 subscales show moderate concurrent validity with the MACL (mean Pearson's $r = 0.44$).

Eating behavior was assessed by asking participants (a) whether they followed a specific kind of diet (e.g. low-GI, calorie controlled, the Atkins diet etc), and (b) to say a few words about their eating behavior (e.g. whether they eat healthily, binge eat etc). Responses to these two questions underwent content analysis, and consequently participants were classified into one of four categories: healthy eating, unhealthy eating (e.g. "I eat a lot of junk food"), binge &/or comfort eating, or 'other'. Note that the resulting categories are a broad assessment of eating habits, not diagnoses of eating disorders. Those who reported binging or comfort eating were combined into one group because both eating behaviors were often reported by the same participant, possibly supporting the suggestion by Grucza, Przybeck, & Cloninger (2007) that binging and comfort eating share a common underlying mood dysregulation. Eating was categorised as 'other' if a participant's response did not fall into any of the three aforementioned categories e.g. "I eat whatever is in the fridge". This measure relies on conventional content analysis, a well established approach to categorization based on qualitative methods (Graneheim & Lundman, 2004). As a qualitative

measure it is not amenable to psychometric validation using quantitative methods such as Cronbach's alpha or principal components analysis.

Demographics, lifestyle (e.g. alcohol consumption), presence of medical conditions and medication use were also assessed.

Participants

Women with PCOS were recruited from the *Verity* PCOS support group website. Controls were recruited from *Psychological Research on the Net,* a website dedicated to online research in a range of topics in psychology. Responses were anonymous. Because the control group website is accessed by the general public, up to 10% of the respondents from this source may have had PCOS. However all potential cases of PCOS – including undiagnosed cases - were identifiable because the survey included questions based on the diagnostic criteria for PCOS.

Inclusion to the PCOS group was based upon self-report of (a) having PCOS, and (b) having two of the three necessary conditions for PCOS, as defined by the Rotterdam criteria (1/ multiple ovarian cysts; 2/ elevated testosterone, or hirsutism or acne; 3/ irregular periods).[1] Twenty-four women fulfilled these criteria. A further 47 women reported either (a) having two of the three diagnostic criteria but did not identify as having PCOS or (b) identified as having PCOS but only reported having one of the three diagnostic criteria; these cases were categorised as 'Possible PCOS'. The female control group consisted of 299 women who reported one or no symptoms associated with PCOS, and did not identify as having PCOS.

In total, 536 people accessed the questionnaire between March and June 2009. Of

[1] Although not practical for an internet survey, it is undoubtedly preferable to have a confirmable diagnosis by a gynaecologist or endocrinologist. However it can be argued that there must be some validity in self report of symptoms, whether online or in a clinical interview. Nonetheless in the present study, if a participant did not understand a question regarding symptoms they had less opportunity to clarify the issue than in an clinical interview. Thus the answers regarding PCOS and PCOS symptoms (see Appendix 1) may have less validity than answers confirmable by a clinician or from medical records.

these, 32 did not go on to fill in the form, leaving an uptake rate of 94%. A further 42 were excluded because they did not give key information e.g. their sex. This left 462 people (24 women with PCOS, 299 controls, and 47 'Possible PCOS', and 92 men), a completion rate of 86%. One woman with PCOS was excluded from matching as she did not report her weight, but she was included in any total-sample (N=462) analyses that did not require this information.

Ethics

This study was approved by the Department of Psychology Ethics Committee, City University, London. To indicate their consent, participants read an information sheet and consent form and ticked a checkbox.

Data analysis

All variables passed the Kolomogorov-Smirnov test of normality and all ANCOVA models passed Levene's tests of homogeneity of variance, thus comparisons of continuous data were performed using parametric tests. For categorical outcomes, Fisher's exact test was used because expected frequencies were less than five in all cases. Less than 5% of data was missing, and missing data was replaced with the median for each participant's score on the relevant scale or subscale.

Results

The results are presented in two sections. The first section describes comparisons across the four groups, and the second section compares 12 women with PCOS to 12 healthy control women matched for age, self-reported BMI, and eating behavior.

Comparisons across the four groups

The mean ($\pm$ *SD*) ages of the participants were PCOS (31.3 $\pm$ 7.7), Possible PCOS (26.6 $\pm$

9.0), healthy control women (24.5 ± 9.1), healthy control men (29.1 ± 8.0). The mean BMIs were PCOS (29.5 ± 6.3), Possible PCOS (19.2 ± 3.3), healthy control women (21.1 ± 3.6), healthy control men (25.7 ± 5.2).

Twenty-two of the women with PCOS and 283 of the healthy control women gave information regarding their eating behavior. The different categories of eating behavior were represented at significantly different rates in the PCOS group compared to healthy control women (Fisher's Exact = 10.294, $p < 0.025$, 2-sided). Of the 22 women with PCOS who indicated their eating behavior, 21% reported healthy eating compared to 28% of the control women; nobody in the PCOS group reported unhealthy eating compared to 17% of the control women, and 58% of the PCOS group reported binge &/or comfort eating compared to 32% of control women. Five of the 22 women with PCOS (23%) and two of the 283 healthy control women (0.7%) were on a low-GI diet. Three of the five women with PCOS on a low-GI diet also binged &/or comfort ate.[2]

Of the 462 participants, 52.8% reported that they were not taking any medication, 13.2% were taking contraception, 5.0% were taking psychiatric medication, 2.2% were taking metformin, 10% were taking other medications (mostly vitamins or allergy medications), 1.5% were taking more than one type of medication, 10.2% said they were taking medication but did not indicate what type, and 5.2% did not state whether they were taking medication or not. Regarding medical conditions, of the 462 participants 13.2.0% reported a psychological or behavioural issue (mostly depression or anxiety), 4.3% reported hypoglycemia, 2.8% reported hypothyroid, 2.8% reported endometriosis, and 1.9% reported insulin resistance. The PCOS group's scores showed more evidence of mood and behavioral effects of hypoglycemia than the other three groups (Table 1).

[2] The five women with PCOS who were on a low-GI diet also binged &/or comfort ate. This would probably negate any benefits of this diet, thus neutralizing any confounding effect of more women in the PCOS group than controls being on a low-GI diet. Potential confounding effects of higher BMI in the PCOS group were controlled for through the use of ANCOVA and matching.

Table 1

Mean (± Standard Deviation) scores on the three Mood Adjective Checklist subscales (MACL) and Hypoglycemia Symptom Checklist – 7 (HSC-7) in the four groups constituting the total sample (*N*=462).

Outcome measure	PCOS (*n*=24)	Possible PCOS (*n*=47)	Control Women (*n*=299)	Men (*n*=92)
Energy [a]	16.8 ± 3.0	19.2 ± 3.3	21.1 ± 3.6	21.4 ± 3.7
Tension [a]	20.9 ± 5.2	19.2 ± 3.3	17.3 ± 3.8	17.2 ± 3.7
Hedonic Tone [a]	18.9 ± 5.0	22.1 ± 4.7	24.3 ± 4.3	24.2 ± 4.1
HSC-7 [b]	2.6 ± 0.6	2.5 ± 0.5	2.1 ± 0.6	2.0 ± 0.7

[a] Mood Adjective Checklist (MACL) subscale

[b] Hypoglycemia Symptom Checklist – 7

Using ANCOVA to control for any effect of age and BMI, the four groups were compared on the psychometric measures. Significant group differences were found for the Mood Adjective Checklist subscales: energy (F (3, 458) = 14.965, $p < 0.001$), tension (F (3, 458) = 9.385, $p < 0.001$), hedonic tone (F (3, 458) = 14.127, $p < 0.001$). Significant group differences were also found for the Hypoglycemia Symptom Checklist -7 (F (3, 458) = 10.067, $p < 0.001$). The PCOS group had more symptoms typical of reactive hypoglycemia than the other groups for each outcome measure. Least Significant Difference (LSD) comparisons of the main effects found that the PCOS and Possible PCOS groups both had significantly more symptoms typical of reactive hypoglycemia than the healthy female controls on all measures (minimum $p < 0.003$) and than the healthy men on all measures (minimum $p < 0.009$). Compared to the Possible PCOS group, the PCOS group had significantly lower energy ($p < 0.007$), more negative hedonic tone ($p < 0.003$), nonsignificantly more tension ($p < 0.059$) and nonsignificantly more symptoms on the HSC-

7 ($p < 0.352$). Healthy control women didn't score significantly differently to the healthy control men on any of the measures (maximum $p < 0.143$).

Matching 12 healthy control women to 12 women with PCOS

Twelve healthy control women were matched to 12 women with PCOS on age, BMI and eating behavior. Four of the matched pairs reported healthy eating behavior, six pairs binged &/or comfort ate, and the eating behavior of two pairs was classified as 'other'. Mean ($\pm SD$) age and BMI were closely matched (PCOS age 31.5 $\pm$ 8.9; matched controls 30.1 $\pm$ 9.2 years old; PCOS BMI 28.8 $\pm$ 6.1; matched controls 29.4 $\pm$ 5.9). One woman with PCOS and one healthy control woman who reported experiencing hypoglycemia were paired; all other medical conditions were excluded. Irritable bowel syndrome (IBS) was not excluded because IBS may, at least in part, have a psychological aetiology (Choung, Locke, Zinsmeister, Schleck, & Talley, 2009). Fewer than half of the matched participants consumed alcohol, and those who did were light drinkers (PCOS = 5.0 $\pm$ 2.7 units per week, and controls = 3.2 $\pm$ 4.1) compared the average of 9.0 units per week for women in England & Wales (Office of National Statistics, 2008).

The PCOS group reported significantly more symptoms typical of reactive hypoglycemia than matched controls on all measures (Table 2).[3]

[3] The PCOS group had less energy, more tension, less happiness (hedonic tone) and more psychological and behavioural symptoms typical of reactive hypoglycemia.

Table 2

Comparison of the 12 PCOS and 12 healthy matched control women on symptoms typical of hypoglycemia.

Outcome measure	Group	*M*	*SD*	*t*	*p**
Energy [a]	PCOS	16.8	3.4	-3.883	0.002
	Control	21.4	2.5		
Tension [a]	PCOS	19.7	5.8	2.078	0.031
	Control	16.3	2.6		
Hedonic Tone [a]	PCOS	19.2	5.1	-5.472	0.001
	Control	26.2	2.6		
HSC-7 [b]	PCOS	2.6	0.6	2.214	0.025
	Control	2.0	0.7		

* Probability value is one-tailed

[a] Mood Adjective Checklist (MACL) subscale

[b] Hypoglycemia Symptom Checklist – 7

Discussion

Women with PCOS reported significantly more mood and behavioral symptoms associated with reactive hypoglycemia than healthy women. This is the first time that such differences have been demonstrated. The differences remained statistically significant after controlling for age, BMI and eating behavior. Before and after matching, the PCOS group reported significantly less energy, more tension, less happiness, with more behavioural symptoms that may be associated with hypoglycemia. Women with PCOS also demonstrated the pattern of 'tense-tiredness' combined with lower mood that has been observed in studies of experimentally induced hypoglycaemia (Gold et al., 1995; McCrimmon et al., 1999).

Based on recent evidence regarding the high rates of hypoglycemia in PCOS and the benefits of a low-GI diet, it might be speculated that the type of food eaten may have an impact on the symptoms typical of hypoglycemia reported by the women with PCOS in this study. A limitation of the present study is that the number of women in the present sample following a low-GI diet was not sufficient to assess its affect on the outcome measures. Also the five women with PCOS who were on a low-GI diet also binged &/or comfort ate, which might obscure any observable benefit of eating low-GI foods. A future study should assess blood glucose levels and psychological outcomes in women with PCOS on a low-GI diet compared to women with PCOS on other diets, controlling for eating behavior.

One of the strengths of the present study is the novelty of the research focus, the use of the internet to maximise the numbers recruited, and the use of a bespoke assessment measure of eating habits. However these strengths also have corresponding limitations. For example, although the internet can increase the statistical power of analyses because of greater numbers, self-report is relied on more heavily than studies recruiting in hospital clinics where objective assessments can be made. Thus although some studies have found that self-report of height and weight can be reasonably accurate (Goodman et al, 2000; Dahl et al., 2010), research ideally should assess these variables objectively where possible.

Another limitation is that although the categorisation of eating habits using qualitative methods may have the advantage of increasing sensitivity to unique properties of the sample in question, future researchers might consider using a validated questionnaire to assess eating habits because this will make their findings more easily comparable to studies using similar measures. Similarly, the HSC-7 has not yet been validated against a physiological measure of hypoglycaemia. In the present study the HSC-7 has proved to have good psychometric properties and sensitivity to symptoms seen in hypoglycaemia, but a future study might seek to validate the HSC-7 against an objective measure of blood glucose.

The findings of this study suggest that effectively controlling diet and weight in PCOS is not only an important health issue, but also has implications for improving the troubling symptoms that may be caused by reactive hypoglycemia. When women with PCOS report hypoglycemia symptoms to their clinicians, these symptoms warrant investigation in the form of an extended oral glucose tolerance test. Readings should be taken every 30 minutes over 4 hours; reactive hypoglycemia is diagnosed if there is a sharp peak in capillary glucose after an hour followed by a sharp trough, or a trough that goes below 54 mg/dl (Marks, 1987). This test may on occasions be inconclusive because outside the laboratory hypoglycemia symptoms appear at higher blood glucose levels (Brun, Fedou, & Mercier, 2000). However where the diagnosis of reactive hypoglycemia is confirmed, a low-GI diet is a logical management option.

In conclusion, this is the first study to demonstrate that women with PCOS have significantly more mood and behavioural symptoms that may be associated with reactive hypoglycemia than healthy women, and this finding is potentially of clinical importance to women with PCOS and the health professionals who help them.

References

Altuntas, Y., Bilir, M., Ucak, S., Gundogdu, S. (2005). Reactive hypoglycemia in lean young women with PCOS and correlations with insulin sensitivity and with beta cell function. *European Journal* of Obstetrics & Gynecology and Reproductive *Biology, 119*, 198-205.

Berlin, I., Grimaldi, A., Landault, C., Cesselin, F., Puech, A.J. (1994). Suspected postprandial hypoglycemia is associated with beta-adrenergic hypersensitivity and emotional distress. *Journal of Clinical Endocrinology & Metabolism, 79*,1428-33.

Bie-Olsen, L.G., Pedersen-Bjergaard, U., Kjaer, T.W., Lonsdale, M.N., Law, I., Thorsteinsson, B. (2010). Differences in cortical and pituitary activity in response to hypoglycaemia and cognitive testing in healthy men with different basal activity of the renin-angiotensin system. *Journal of the Renin-Angiotensin-Aldosterone System,* 11, 173-9.

Brand-Miller, J. Farid, N.R. and Marsh, K. (2004). The low GI guide to managing PCOS. London: Hodder & Stoughton.

Brun, J.F., Fedou, C., Mercier, J. (2000). Postprandial reactive hypoglycemia. *Diabetes & Metabolism*, *26*, 337-51.

Choung, R.S., Locke, G.R. 3rd, Zinsmeister, A.R., Schleck, C.D., Talley, N.J. (2009). Psychosocial distress and somatic symptoms in community subjects with irritable bowel syndrome: a psychological component is the rule. *American Journal of Gastroenterology, 104,*1772-9.

Dahl, A.K., Hassing, L.B., Fransson, E.L., Pederson, N.L. (2010). Agreement between self-reported and measured height, weight, and body mass index in old age – a longitudinal study with 20 years of follow-up. *Age and Aging, 39,* 445-51.

Deary, I.J., Hepburn, D.A., MacLeod, K.M., Frier, B.M. (1993). Partitioning the symptoms of hypoglycaemia using multi-sample confirmatory factor analysis. *Diabetologia, 36,* 771-7.

Diamanti-Kandarakis, E., and Papavassiliou, A.G. (2006). Molecular mechanisms of insulin resistance in polycystic ovary syndrome. *Trends in Molecular Medicine, 12,* 324-32.

Galletly, C., Moran, L., Noakes, M., Clifton, P., Tomlinson, L., Norman, R. (2007). Psychological benefits of a high-protein, low-carbohydrate diet in obese women with polycystic ovary syndrome - a pilot study. *Appetite, 49,* 590-3.

Gold, A.E., MacLeod, K.M., Frier, B.M., Deary, I.J. (1995). Changes in Mood During Acute Hypoglycemia in Healthy Participants. *Journal of Personality and Social Psychology, 68,* 498-504.

Goodman, E., Hinden, B.R., Khandelwal, S. (2000). Accuracy of teen and parental reports of obesity and body mass index. *Pediatrics, 106,* 52-58.

Graneheim, U.H., Lundman, B. (2004). Qualitative content analysis in nursing research: concepts, procedures and measures to achieve trustworthiness. *Nursing Education Today, 24*, 105-12

Grucza, R.A., Przybeck, T.R., Cloninger, C.R. (2007). Prevalence and correlates of binge eating disorder in a community sample. *Comprehensive Psychiatry, 48*,124-31

Hepburn, D.A., Deary, I.J., MacLeod, K.M., Frier, B.M. (1994). Structural equation modelling of symptoms, awareness and fear of hypoglycemia, and personality in patients with insulin-treated diabetes. *Diabetes Care. 17*, 1273-80.

Herriot , A.M., Whitcroft, S., Jeanes, Y. (2008). A retrospective audit of patients with polycystic ovary syndrome: the effects of a reduced glycaemic load diet. *Journal of*

Human Nutrition and Dietetics, 21, 337-45.

Himelein, M.J., Thatcher, S.S. (2006). Polycystic ovary syndrome and mental health: A review. *Obstetrical & Gynecological Survey, 61*, 723-32.

Kasim-Karakas, S.E., Cunningham, W.M., Tsodikov A. (2007). Relation of nutrients and hormones in polycystic ovary syndrome. *American Journal of Clinical Nutrition, 85,* 688-94.

Ledger, W.L., Clark, T. (2003). Long−term consequences of polycystic ovary syndrome. *Royal College of Obstetrician and Gynaecologists Physicians Guidelines. No.33.*

Magnotti, M., Futterweit, W. (2007). Obesity and the Polycystic Ovary Syndrome. *Medical Clinics of North America, 91,*1151-68, ix-x.

Marks, V. (1987). Glycaemic stability in healthy subjects: fluctuations in blood glucose concentration during the day. In: Andreani, D., Marks, V., and Lefebvre, P.J. (Eds.), Hypoglycaemia (pp. 19–24). New York: Serona Symposia Publications from Raven Press.

Marsh, K.A., Steinbeck, K.S., Atkinson, F.S., Petocz, P., Brand-Miller, J.C. (2010). Effect of a low glycemic index compared with a conventional healthy diet on polycystic ovary syndrome. *American Journal of Clinical Nutrition, 92*, 83-92

Matthews, G., Jones, D.M., Chamberlain, G. A. (1990). Refining the measurement of mood: The UWIST Mood Adjective Checklist. *British Journal of Clinical Psychology, 81,* 17-42.

McCrimmon, R.J., Frier, B.M., Deary, I.J. (1999). Appraisal of mood and personality during hypoglycaemia in human subjects. *Physiology & Behavior*, *67,* 27-33.

Office of National Statistics (2008). Men drink twice as much alcohol as women.

http://www.statistics.gov.uk/pdfdir/ghs0108.pdf (Accessed 2nd May 2010).

Ross, H.M. (1975). Fighting depression. New York: Larchmont Books.

Sørensen, M., Johansen, O.E. (2010). Idiopathic reactive hypoglycaemia - Prevalence and effect of fibre on glucose excursions. *Scandinavian Journal of Clinical and Laboratory Investigation, 70,* 385-91.

Stanley, T., Misra, M. (2008). Polycystic ovary syndrome in obese adolescents. *Current Opinion in Endocrinology*, *Diabetes and Obesity, 15,*30-6.

Thayer, R.E. (1989). The Biopsychology of Mood and Arousal. London: Oxford University Press.

Appendix 1. The questionnaire used in the survey. Note that the original questionnaire was formatted for online use, and this formatting has been lost in the Word document version below. For example, the asterisks below were originally radio buttons in the survey and the response scale options boxes are missing.

Thank you for your interest in this survey. Please take a moment to read this information:
Some people say that certain foods and eating habits effect how they feel. The aim of this survey is to see whether certain foods or lifestyles are associated with specific mood states that might be associated with the metabolism of food, especially in women PCOS (polycystic ovary syndrome) but also other women.

The survey is entirely voluntary, anonymous, and confidential. It has been given ethical approval by the Dept of Psychology at City University (London) and approved by the research officer for the PCOS support group, Verity. All data will be kept safe and secure in accordance with the Data Protection Act 1998. There are no immediate benefits to you of filling the survey, apart from knowing that you are adding to knowledge on the relationship between food and wellbeing in PCOS.

If you have any questions about the survey, would like feedback on your responses, or wish to withdraw your answers from the survey please email John Barry at j.a.barry@city.ac.uk Note that if you would like feedback, please indicate this in the text box at the very end of this questionnaire by stating 'I will email you for feedback on my answers to the Food and Wellbeing Questionnaire. I submitted my form at [state exact time] on [state day and date]'.
* I understand the above information and am happy to take part in this survey

This questionnaire is mainly for women, but some men might like to participate too.
Are you male or female?
* Male
* Female

How old are you?

Do you follow any special diet? Please tick any that apply to you:
* Low GI (Glycaemic Index)
* Vegetarian
* Vegan
* Calorie controlled
* Weightwatchers
* Detox diet
* Atkins or low carb diet
* Mediterranean diet

Please say a few words about your eating habits (e.g. eat healthily, binge eat, comfort eat, eat unhealthily etc) if not covered by the previous question:

I feel anxious
Never
Rarely
Sometimes
Often

I feel contented
Never
Rarely
Sometimes
Often

I feel jittery
Never
Rarely
Sometimes
Often

I feel active
Never
Rarely
Sometimes
Often

I feel energetic
Never
Rarely
Sometimes
Often

I feel cheerful
Never
Rarely
Sometimes
Often

I feel satisfied
Never
Rarely
Sometimes
Often

I feel tense
Never
Rarely
Sometimes
Often

I feel alert
Never
Rarely
Sometimes

Often

I feel happy
Never
Rarely
Sometimes
Often

I feel nervous
Never
Rarely
Sometimes
Often

I feel vigorous
Never
Rarely
Sometimes
Often

I feel dissatisfied
Never
Rarely
Sometimes
Often

I feel calm
Never
Rarely
Sometimes
Often

I feel unenthusiastic
Never
Rarely
Sometimes
Often

I feel depressed
Never
Rarely
Sometimes
Often

I feel restful
Never
Rarely
Sometimes
Often

I feel sluggish
Never
Rarely
Sometimes
Often

I feel sad

Never
Rarely
Sometimes
Often

I feel relaxed
Never
Rarely
Sometimes
Often

I feel tired
Never
Rarely
Sometimes
Often

I feel sorry
Never
Rarely
Sometimes
Often

I feel composed
Never
Rarely
Sometimes
Often

I feel passive
Never
Rarely
Sometimes
Often

I am clumsy
Never
Rarely
Sometimes
Often

My heart pounds for no obvious reason
Never
Rarely
Sometimes
Often

I feel sweaty
Never
Rarely
Sometimes
Often

I feel confused
Never
Rarely
Sometimes
Often

I feel suddenly weak
Never
Rarely
Sometimes
Often

I have difficulty in speaking
Never
Rarely
Sometimes
Often

I shiver
Never
Rarely
Sometimes
Often

Please tick if any of the following apply to you. (Leave blank if you are not sure):
* Diabetes
* Polycystic ovary syndrome
* Acne or problems with body/facial hair
* Irregular periods
* Overweight
* Insulin resistance
* Cysts on ovaries (diagnosed by ultrasound scan)
* Psychiatric problem (e.g. clinical depression)
* Eating disorder (e.g. bulimia or anorexia)
* Low blood sugar (hypoglycaemia)
* Endometriosis
* Hypothyroid (underactive thyroid gland)

Please say if you have any other medical condition

How much do you weigh?

How tall are you?

Do you exercise regularly?
* Yes
* No

On average, how much alcohol do you consume per week? Please answer in units if possible (e.g. one small glass of wine = 1 unit. There is an online calculator here http://www.bupa.co.uk/health_information/asp/healthy_living/lifestyle/alcohol/alctest.asp)

Are you taking any medication at present?

Thank you so much for taking part in this survey!

Is there anything else you would like to add? Please feel free to write anything you think might be useful.

CHAPTER 7

General discussion: implications of findings from published papers & programme

Theoretical implications

PCOS is caused by maternal T during pregnancy

Any psychological theory proposing activational effects of prenatal T needs first to establish that the fetus in a PCOS pregnancy is exposed to elevated T. One of the most important implications arising from the material presented in Chapters 3 and 4 is that not only does it appear likely that the female fetus in a PCOS pregnancy is exposed to elevated T (or other androgen), but the source of the T is probably maternal. This latter point is suggested by the evidence in two ways. Firstly, the PCOS cord T study found that T was higher in the umbilical cord vein of female infants of mothers with PCOS than the vein of female infants of mothers who didn't have PCOS. The fact that the T levels were measured in the umbilical vein suggests that the T was of maternal/placental origin; this hypothesis is supported by the cord T meta-analysis which found evidence that T in the umbilical arteries is of fetal origin. Secondly, the PCOS cord T study also found that the T levels in maternal circulation were significantly correlated with T levels in the umbilical cord of their female newborns. This correlation did not exist in the male group or female control group. Although these findings are not conclusive proof that PCOS is caused by maternal T, both pieces of evidence taken together are supportive of the hypotheses that psychological problems in adults with PCOS might be traced to elevated prenatal T, and that maternal T is causal in PCOS itself.

Future studies might replicate Barry et al (2010) using a larger sample, assaying for T and other relevant biochemicals using mass spectrometry. The recommendation by Rosner et al (2007) of using mass spectrometry when measuring T in women, or T in the umbilical cord, raises questions regarding the validity of all previous studies not using mass

spectrometry with these populations. It might be reasonable to suggest that previous research may have been measuring androgens in general rather than T specifically. If this is true however, only studies that sought to make a distinction between the specific effects of T as opposed to the specific effects of other androgens may be regarding as being of questionable validity. In any case future studies in this field should measure levels of the major androgens, as these may have as great an impact on the fetus as T, or more so. This is especially true of dihydrotestosterone (DHT) which is bioconverted from T by the enzyme 5-alpha-reductase. Two recent studies underline the importance of DHT. Carlsen & Vanky (2010) studied PCOS pregnancies and found a correlation between maternal and cord levels of DHT, but not T. Similarly Anderson et al (2010) found that DHT was nonisignificantly higher and E2 significantly lower in the cord blood of daughters of mothers with PCOS, suggesting that maternal T has been converted to DHT rather than being transferred directly to the fetus as T. In both studies DHT played a more obvious role than T, underlining the importance of measuring more than just T in studies of PCOS.

T does not directly cause mood dysfunction

There were few significant correlations – linear or nonlinear - between T and mood variables for the PCOS group, and none for the controls. This suggests that T had little direct effect on mood, at least in the women in the sample in Chapter 5. The finding of Weiner et al (2004) of a complex relationship between T and mood states is probably an artifact of combining two populations with significantly different T levels when there are also opposite patterns of correlations between T and mood in each population; the resulting pattern will inevitably give the appearance of being nonlinear. However, based on findings from animal research, it is possible that prenatal T may programme for the development of insulin resistance and type 2 diabetes in later life, and these metabolic issues could cause the hypoglycaemia which

may be responsible for the mood problems seen in Chapter 6. Longitudinal studies in humans are required to test this hypothesis.

Future studies of this kind should use mass spectrometry if measuring T or other androgens. Also, to test whether mood is related to blood glucose levels, future studies could monitor blood glucose levels.

Methodological implications

The most important methodological issues raised by the present research programme relate to the measurement of T in populations where low levels are expected. As stated previously, assays should be performed using mass spectrometry. A further issue is to measure cord T from the arteries and vein separately, in order to be able to identify the relative contribution from maternal and fetal sources. Also the measurement of other hormones – especially DHT – is advised. A study that is making these modifications is being conducted by Hardiman, Barry et al. and is almost complete.

Testing for blood sugar problems in PCOS presents methodological challenges. The traditional test of hypoglycaemia - an oral glucose tolerance test – might also be passed over for more modern methods. A continuous blood glucose monitor (CGM) is a superior method and has the advantage of being an unobtrusive way of measuring glucose levels over several days, so that the effect of various meals (low GI, high GI) can be assessed. Recruitment for a study using CGM and low versus high GI meals is scheduled to begin in July 2011 (by Barry, Hardiman et al.).

Clinical implications

Protect the fetus from maternal T during pregnancy

Evidence from the cord T meta-analysis suggests that T in the umbilical vein is of maternal/placental origin, and the PCOS cord T study suggests that for females, fetal T is correlated with maternal T. If it is established by further research that maternal T causes PCOS, then the modification of maternal T during pregnancy might be considered, especially as it would be impractical to try to modify fetal T levels in utero directly. To date the only method of reducing T in pregnant women with PCOS has been the use of metformin. Metformin is an insulin sensitiser rather than an anti-androgen, and is generally prescribed to reduce diabetes in pregnancy rather than specifically to reduce T levels. Although this medication will indirectly reduce T levels, any such reduction may take several months, as seen in non-pregnant women (Harborne et al., 2003). The slowness of the effect of metformin on T is possibly the reason that the evidence to date does not clearly show an anti-androgenic effect of metformin in PCOS pregnancies.

Recognise mood problems in PCOS and treat using a low-GI diet or anti-androgen

The evidence pointing to mood problems in PCOS is clear, as demonstrated by the meta-analysis of anxiety and depression in PCOS in the present thesis. Clinicians and therapists should be aware of this and be sensitive to the fact that women with PCOS may display more emotionality than other patients. The origin of these mood problems should be treated as potentially multifactorial, and a treatment approach of any appropriate combination of medication, counselling and diet should be considered. The meta-analysis examining anxiety and depression in PCOS illustrates that improvements in body weight should have at least some slight impact on mood. In support of these suggestions, the first test of the low-GI diet in women with PCOS, the diet has been found to improve quality of life (QoL) for emotions, hirsutism and menstruation, and improved insulin resistance in moderately overweight women with PCOS (Marsh, Steinbeck, Atkinson, Petocz & Brand-Miller, 2010).

Evaluation

Despite the strengths of the studies presented in this thesis, there are inevitably limitations.

The research programme would have been more complete had it used a longitudinal design. This is because a longitudinal design has the advantages of (a) eliminating the possibility of individual differences across measurement occasions, and (b) allows for the use of more statistically powerful within-groups tests. However this was not feasible given the time limitations of a PhD programme and it was decided that a series of cross-sectional studies was the best that could be achieved. In terms of completeness of the present thesis as a representation of the overall research programme, it would have been useful to include all of the research undertaken, especially the study of behavioural characteristics of children of mothers with PCOS. However the paper based on this study was not accepted for publication and its inclusion here could not be justified, given the word limitations.

A question sometimes raised in regards meta-analyses is whether the conclusions of the meta-analysis are the result of publication bias. More than just a moot question of a 'file drawer effect', meta-analyses are sometimes scrutinised regarding whether an overall large effect size is simply an artefact of studies with small sample sizes and null or small effect sizes being less likely to be published. Neither of the meta-analyses in this thesis showed signs of any serious problems of this kind, as can be seen by the fact that the studies with larger sample sizes didn't tend to have smaller between-groups effect sizes. This can be seen by a simple inspection of Table 2 (page 121) in the cord testosterone meta-analysis, which shows no relationship between sample size and effect size, and the funnel plots in Supplementary Figure I (page 105, with commentary on page 81) for the anxiety and depression studies.

One possible criticism is that the studies in this thesis were natural experiments rather than true experiments i.e. none of the participants were randomised into the groups, but were assigned to groups by virtue of their PCOS status. On the other hand, in human

studies of PCOS a true experimental design is not possible, principally because it is unethical to, for example, expose a fetus to elevated T levels and observe subsequent developmental phenomena. Thus the studies presented in the present research programme might be considered the best that can be done given sensible ethical restrictions. However, as a result of the quasi-experimental nature of the research, it is difficult to justify the inference of causal relationships between grouping and outcome variables, because factors other than group membership have not been reduced to chance levels by the process of random allocation. On the other hand, relevant variables were controlled for by other methods, principally matching and ANCOVA, and therefore causal inferences might be considered acceptable, given the inevitable experimental limitations when working with human participants.

Some of the key findings of relevance to theories of the etiology of PCOS can be questioned on the grounds of being circumstantial or correlational. Thus although the evidence for the transmission of maternal T via the umbilical vein is suggested by the evidence presented in the cord T meta-analysis, the evidence is indirect. Furthermore, although the correlation between maternal and umbilical cord T in the PCOS cord T study is suggestive of the maternal transfer of T to the fetus, the evidence is correlational, thus open to other interpretations e.g. that T is transferred from the fetus to maternal circulation, or that a third variable (e.g. genetic factors) causes both maternal and fetal T levels. However because the evidence from the five published papers represents some of the first attempts to identify the biological etiology of PCOS in humans, and as such criticism should not be so strong as to negate these early attempts at understanding this complex and multifaceted condition.

The sample size in the PCOS cord T study was small. However it should be noted that the only other published study of this kind (Anderson et al, 2010) had fewer observed female cord T values than Barry et al (2010). It might also be fair to say that small sample

sizes are virtually inevitable given the difficulties of recruiting a specific patient group whose delivery times are unknown, and possibly in the early morning hours. Recruiting under these circumstances properly requires a team who are on-site 24 hours per day, seven days per week.

The hypothesis that low blood glucose causes mood problems in PCOS is not proven in the absence of actual measurement of blood glucose. However the study in Chapter 6 is high in originality, and the fact that the findings strongly supported the hypothesis after controlling for other variables suggests that the glucose hypothesis is well worth further exploration. Another strength is the development of a brief scale for measuring behavioural symptoms of hypoglycaemia, the Hypoglycaemia Symptom Checklist – 7 (HSC-7). This was validated against an existing validated measure of mood symptoms of hypoglycaemia (the Mood Adjective Checklist, MACL) and the HSC-7 will be further validated against observed blood sugar levels in the forthcoming study by Barry, Hardiman et al.

It could be said that the scope of this thesis was too wide, attempting to take in too many aspects of such a complex problem for a single PhD programme. On the other hand the breadth of the programme might be considered a positive, especially given the originality of many aspects of the research. For example, before 2004 when the present PhD programme began there were less than a dozen published papers addressing psychological aspects of PCOS. The PCOS cord T paper was almost a first on this subject, second only in publication by three months to Anderson et al's (2010) paper. The PCOS cord T paper has been cited three times, two of these by leading international researchers of PCOS etiology (Veiga-Lopez et al, 2011; Goodarzi et al, 2011). The PCOS and mood paper is the first to conceptualise PCOS mood problems as 'emotionality', and the first to identify the tendency to withholding anger in women with PCOS. The exploration of psychological factors typical of hypoglycaemia in PCOS is a first.

Concluding comments

About 18 000 girls per year are born to mothers with PCOS in the UK (see Appendix 1). The prevalence of PCOS means that the costs in terms of health, quality of life, and resources to the health services are not inconsiderable. It is the author's hope that this PhD programme contributes to the improvement of quality of life for women with PCOS until such time as a cure for this condition is made available.

References

Anderson H, Fogel N, Grebe SK, Singh RJ, Taylor RL, Dunaif A. 2010. Infants of women with polycystic ovary syndrome have lower cord blood androstendione and estradiol levels. Journal of Clinical Endocrinology & Metabolism 95: 2180-2186

Barry JA, Kay AR, Navaratnarajah R, Iqbal S, David AL, Bamfo JEAK, Hines M, Hardiman PJ. 2010. Umbilical vein testosterone in female infants born to mothers with Polycystic Ovary Syndrome is elevated to male levels. Journal of Obstetrics & Gynaecology 30: 444-6.

Vanky E, Salvesen KA, Heimstad R, Fougner KJ, Romundstad P, Carlsen SM. (2004). Metformin reduces pregnancy complications without affecting androgen levels in pregnant polycystic ovary syndrome women: results of a randomized study. Hum Reprod. Aug;19(8):1734-40. Epub 2004 Jun 3

Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. 2011. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. Nat Rev Endocrinol. 2011 Apr;7(4):219-31.

Harborne L, Fleming R, Lyall H, Sattar N, and Norman J. Metformin or antiandrogen in the treatment of hirsutism in polycystic ovary syndrome. *J Clin Endocrinol Metab* 2003;**88**: 4116-4123.

Marsh, K.A., Steinbeck, K.S., Atkinson, F.S., Petocz, P., Brand-Miller, J.C. (2010). Effect of a low glycemic index compared with a conventional healthy diet on polycystic ovary syndrome. *American Journal of Clinical Nutrition, 92*, 83-92

Office for National Statistics (2009). Key Population and Vital Statistics 2007, Series VS No 34, PPI No 30 http://www.statistics.gov.uk/downloads/theme_population/KPVS34-2007/KPVS2007.pdf Accessed 11th April 2010

Pregnancy Info.Net (2005). Miscarriage. http://www.epigee.org/pregnancy/miscarriage.html Accessed 11th April 2010

Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H. 2007. Position statement: Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. Journal of Clinical Endocrinology & Metabolism 92: 405-13.

Vanky E, Salvesen KA, Heimstad R, Fougner KJ, Romundstad P, Carlsen SM. (2004). Metformin reduces pregnancy complications without affecting androgen levels in pregnant polycystic ovary syndrome women: results of a randomized study. Hum Reprod. Aug;19(8):1734-40. Epub 2004 Jun 3

Veiga-Lopez A, Steckler TL, Abbott DH, Welch KB, MohanKumar PS, Phillips DJ, Refsal K, Padmanabhan V. Developmental programming: impact of excess prenatal testosterone on intrauterine fetal endocrine milieu and growth in sheep. Biol Reprod. 2011 Jan;84(1):87-96

Weiner CL, Primeau M, and Ehrmann DA. Androgens and mood dysfunction in women: comparison of women with polycystic ovarian syndrome to healthy controls. *Psychosom Med* 2004;**66**: 356-362.

Appendix 1

Estimate of the number of girls born per year in the UK to mothers with PCOS

The Office of National Statistics (2009) indicates that the total percentage of maternities in the UK by women aged 15-44 in 2007 was 6.1%. The number of women in the UK aged 16-44 is roughly 12.12 million, thus 720 000 births per year. If 5% of these births are from mothers with PCOS (based on 10% of women having PCOS, and the miscarriage rate being close to half of these pregnancies, according to the Pregnancy Info.Net, 2005) this represents 36000 PCOS births per year. If half the newborns are girls, this amounts to 18 000 girls born to mothers with PCOS per year in UK.