

Acupuncture in Polycystic Ovary Syndrome: Current Experimental and Clinical Evidence

E. Stener-Victorin,* E. Jedel† and L. Mannerås*

*Institute of Neuroscience and Physiology, Department of Physiology, Sahlgrenska Academy, Göteborg University, Göteborg, Sweden.

†Osher Center for Integrative Medicine, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden.

Journal of Neuroendocrinology

This review describes the aetiology and pathogenesis of polycystic ovary syndrome (PCOS) and evaluates the use of acupuncture to prevent and reduce symptoms related with PCOS. PCOS is the most common female endocrine disorder and it is strongly associated with hyperandrogenism, ovulatory dysfunction and obesity. PCOS increases the risk for metabolic disturbances such as hyperinsulinaemia and insulin resistance, which can lead to type 2 diabetes, hypertension and an increased likelihood of developing cardiovascular risk factors and impaired mental health later in life. Despite extensive research, little is known about the aetiology of PCOS. The syndrome is associated with peripheral and central factors that influence sympathetic nerve activity. Thus, the sympathetic nervous system may be an important factor in the development and maintenance of PCOS. Many women with PCOS require prolonged treatment. Current pharmacological approaches are effective but have adverse effects. Therefore, nonpharmacological treatment strategies need to be evaluated. Clearly, acupuncture can affect PCOS via modulation of endogenous regulatory systems, including the sympathetic nervous system, the endocrine and the neuroendocrine system. Experimental observations in rat models of steroid-induced polycystic ovaries and clinical data from studies in women with PCOS suggest that acupuncture exert long-lasting beneficial effects on metabolic and endocrine systems and ovulation.

Correspondence to:

Elisabet Stener-Victorin, Institute of Neuroscience and Physiology, Department of Physiology/Endocrinology, Sahlgrenska Academy, Göteborg University, Box 434, SE-405 30 Göteborg, Sweden (e-mail: elisabet.stener-victorin@neuro.gu.se).

Key words: acupuncture, insulin resistance, metabolic syndrome, obesity, opioids, ovulation, physical exercise, polycystic ovary syndrome, sympathetic nerve activity.

doi: 10.1111/j.1365-2826.2007.01634.x

Polycystic ovary syndrome (PCOS), the most common female endocrinopathy, is associated with hyperandrogenism, ovulatory dysfunction and obesity (1). PCOS increases the risk of hyperinsulinaemia, insulin resistance and type 2 diabetes. Type 2 diabetes results primarily from insulin resistance and is correlated with both hyperandrogenaemia and obesity (1). Women with PCOS often develop hypertension and have an increased likelihood of developing cardiovascular risk factors (2). They also appear to have impaired mental health (3, 4). Common features of PCOS can be highly distressing to patients and body image appears to be strongly associated with depression (5), and there are also indications that anorexia nervosa coexists with PCOS (6). Taken together, PCOS-related symptoms lead to higher levels of depression, psychological and psychosexual morbidity, and increased exposure to stressful stimuli.

Data on the dietary history of women with PCOS are limited but there are indications that women with PCOS consume: (i) more

saturated fat and less dietary fibre than age-matched control women (7) and (ii) larger quantities of specific foods with a high glycaemic index compared to matched controls (8).

This review aims to describe the aetiology and pathogenesis of PCOS and to evaluate the use of acupuncture to prevent and reduce symptoms related with PCOS.

Polycystic ovary syndrome aetiology

The aetiology of PCOS is incompletely understood, despite high prevalence of the syndrome, morbidity from its metabolic, reproductive and hyperandrogenic features, and the associated cardiovascular risk. The most consistent endocrine feature is hyperandrogenaemia from a predominantly ovarian source, which likely plays a key aetiological role (9, 10). Insulin sensitivity is decreased by 30–40% in women with PCOS, predominantly in overweight women, and the compensatory hyperinsulinaemia

increases ovarian androgen production and further exacerbates symptoms of PCOS (11). Adiposity is also important in the pathogenesis of PCOS (1), as shown by improved menstrual regularity after weight reduction (12–14). Visceral fat, which is metabolically more active than subcutaneous adipose tissue, tends to accumulate in women with PCOS (15) and the increased fat mass and central adiposity are related to hyperinsulinaemia from prepuberty to postmenarche (16). In line with these observations, PCOS is associated with two strong predictors of insulin resistance: enlarged adipocytes and reduced lipolytic activity (17). Thus, lipid mobilisation, a process tightly regulated by insulin and the sympathetic nervous system, appears to be disturbed in women with PCOS.

Furthermore, PCOS appears to have a genetic basis, but the genes involved are unknown. Likely candidates include genes that regulate ovarian steroidogenesis or influence body mass index and adiposity (18). The symptoms and severity of PCOS vary, suggesting subpopulations among women with the syndrome.

The disturbances in PCOS have been attributed to defects in different organ systems. These include androgen synthesis defects that enhance ovarian androgen production and alter cortisol metabolism resulting in enhanced adrenal androgen production, neuroendocrine defects with exaggerated luteinising hormone (LH) pulsatility and defects in insulin action and secretion leading to hyperinsulinaemia and insulin resistance (2) (Fig. 1).

Sympathetic nervous system in the pathogenesis of polycystic ovary syndrome

The primary aetiology of this complex disease remains a hen-and-egg mystery; thus, the sympathetic nervous system may be an important aetiological factor, as described below. PCOS is associated with peripheral and central factors that influence sympathetic nerve activity. The central β -endorphin system exerts tonic inhibitory control on the gonadotrophin-releasing hormone (GnRH) pulse generator, on pituitary LH release and modulates sympathetic tone. Absolute LH augmentation is dependent on baseline LH levels and may account for difference in LH responsiveness in women with PCOS. LH responses to GnRH and LH pulse amplitude do not correlate with LH pulse frequency, suggesting the involvement of other factors, such as GnRH together with androgens and/or body mass index, to account for the increased LH release in PCOS (19). Tonic augmented release of LH may be caused by diminished or exaggerated β -endorphin and dopaminergic control of GnRH release (20, 21).

High plasma β -endorphin levels may contribute to PCOS and seem to be related to hyperinsulinaemia (22) and stress (23). Increased sympathetic nerve activity is the most common risk factor for cardiovascular disease and mortality (24, 25), and women with PCOS are more prone to develop hypertension and other cardiovascular disturbances (26).

Obesity, particularly abdominal obesity, and insulin resistance are risk factors for hypertension and cardiovascular disease in women

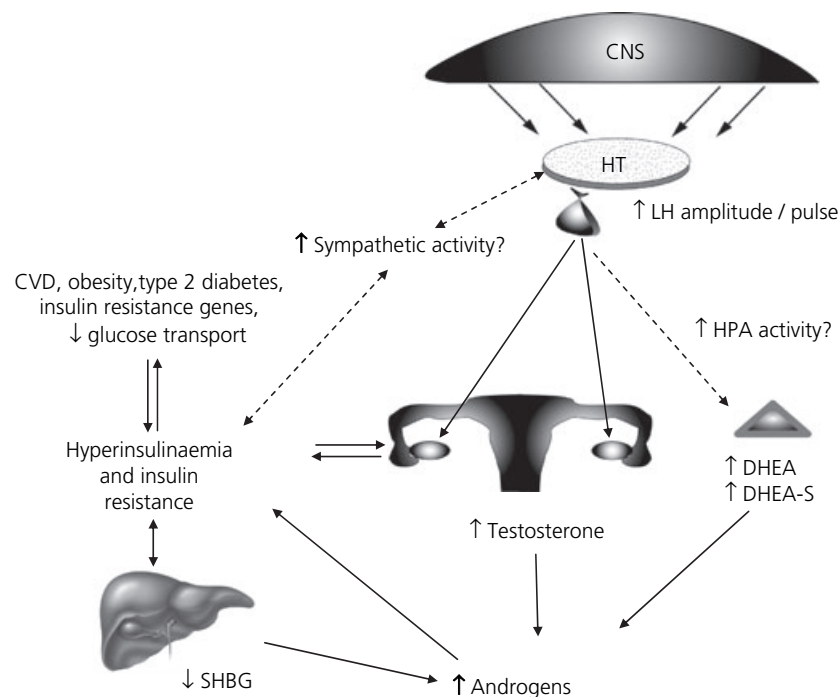


Fig. 1. Abnormalities in polycystic ovary syndrome (PCOS) have been attributed to primary defects in the hypothalamic-pituitary-adrenal (HPA) axis, the ovarian microenvironment, the adrenal gland, and the insulin/insulin growth factor-1 metabolic regulatory system. Furthermore, the potential contribution of the sympathetic nervous system to PCOS has been suggested by several observations because the syndrome is associated with both peripheral and central factors that influence sympathetic nerve activity. CNS, Central nervous system; CVD, cardiovascular disease; DHEA, dehydroepiandrosterone; HT, hypothalamus; P, pituitary; SHBG, sex hormone binding globulin.

with PCOS (27). In patients with congestive heart failure, visceral obesity appears to be the most important determinant of excessive sympathetic activation (28). Increased sympathetic nerve activity may also be linked to hyperandrogenism (29, 30), hyperinsulinaemia (31) and hypertension (32). Furthermore, growth hormone (GH) and insulin growth factor-1 (IGF-1) play central roles in regulating sympathetic nerve activity. A recent study found disturbances in the somatotrophic axis (GH/IGF-1) in PCOS (33). Sympathetic nervous system involvement is further supported by the greater density of catecholaminergic nerve fibres in polycystic ovaries (PCO) (34, 35). Increased ovarian sympathetic nerve activity might contribute to PCOS by stimulating androgen secretion (36). This would explain why ovarian wedge resection or laparoscopic laser cauterisation (37), which likely disrupt ovarian sympathetic innervation, increase ovulatory responses in women with PCOS. The importance of the sympathetic nervous system in PCOS pathophysiology needs to be investigated further. Table 1 summarises the relation between sympathetic nervous system and PCOS.

Experimental evidence of involvement of sympathetic nervous system in polycystic ovaries

Validated animal models are valuable for therapeutic screening, pre-clinical trials and studying the pathogenesis of complex reproductive disorders such as PCOS. Observations in prenatally androgenised rhesus monkeys and sheep (38, 39) suggest that androgen hypersecretion from the ovaries or adrenals, most likely before puberty, can cause clinical and biochemical features of human PCOS.

In rats, a single i.m. injection of oestradiol valerate (EV) induces an anovulatory state with endocrinological and morphological characteristics of human PCOS (40); ovarian sympathetic nerve activity is higher in this model than in normal rats (41–43). The sympathetic nerves appear to be involved in controlling ovarian secretory activity and are important regulators of ovarian function because noradrenaline and vasoactive intestinal peptide potently stimulate steroid secretion (44). EV-induced PCO in rats is associated with increased peripheral sympathetic outflow, as demonstrated by increases in noradrenaline release and ovarian noradrenaline

Table 1. Clinical Signs and Symptoms in Polycystic Ovary Syndrome (PCOS) Associated With Increased Activity in the Sympathetic Nervous System.

Signs and symptoms in PCOS
Hyperandrogenism
Hypersecretion of LH → anovulation
Increase in ovarian catecholaminergic nerve fibres
Visceral obesity
Insulin resistance
Hypertension
Disturbed GH/IGF-1 axis
Psychological stress

LH, Luteinising hormone; GH, growth hormone; IGF, insulin growth factor.

content and a reduced number of β_2 -adrenoceptors in the ovarian compartment receiving catecholaminergic innervation (41, 42). The increased ovarian sympathetic nerve activity may reflect augmented ovarian production of nerve growth factor (NGF), a target-derived neurotrophin (43, 45, 46). Further, α_1 -adrenoceptor subtypes are up-regulated in the ovaries of PCO rats (47), which further supports the involvement of the sympathetic nervous system in the regulation of ovarian function.

However, programming with EV in adult rats does not cause the metabolic disturbances seen in PCOS (48) and few rat models have focused on the metabolic disturbances that are a major feature of human PCOS.

Female rats exposed to testosterone (49), or the aromatase inhibitor letrozole (50), develop cystic follicles and endocrine features similar to human PCOS but it is unclear whether they develop metabolic disturbances.

Because PCOS symptoms usually develop during early puberty, when androgen production commences, we assessed the contribution of androgens to PCOS by evaluating the effects of prepubertal administration of dihydrotestosterone (DHT), a non-aromatisable androgen, or letrozole in rats during adulthood (51). Adult DHT rats presented irregular cycles and polycystic ovaries characterised by cysts formed from atretic follicles with a diminished granulosa layer. They also displayed metabolic disturbances, including increased body weight, body fat, mesenteric adipocyte size and leptin levels, as well as insulin resistance. All letrozole rats became anovulatory with polycystic ovaries, including structural changes strikingly similar to those in human PCOS, although they were not insulin resistant or obese. Thus, the letrozole model was concluded to be suitable for studies of the ovarian features of PCOS whereas the DHT model was suitable for studies of the ovarian and metabolic features (51). Evidence is required to demonstrate whether these new models are related to increased sympathetic nerve activity. Hypothetically, sympathetic nerve activity is increased in the ovaries and in adipose tissue.

Acupuncture in polycystic ovary syndrome

Many women with PCOS require prolonged treatment. Pharmacological approaches are effective but have adverse effects. First-line therapy in PCOS is often oral contraceptives, which reduce hirsutism and acne but adversely affect glucose tolerance, coagulability and fertility (52). Therefore, treatment strategies such as acupuncture need to be evaluated in PCOS. Acupuncture, a treatment that dates back 3000–5000 years, is an integral part of traditional Chinese medicine and has become more established in Western medicine as a complement or alternative to conventional therapies.

What is the physiological basis for using acupuncture in PCOS? Intramuscular needle insertion cause a particular pattern of afferent activity in peripheral nerves. Depending on the intensity, stimulation of the acupuncture needles activate muscle afferents to the spinal cord and the central nervous system (53). In electro-acupuncture (EA), low-frequency (1–15 Hz) electrical stimulation excite ergoreceptors in the muscles (54), which are physiologically activated during muscle contractions. Low-frequency EA cause release of a

needles were placed in the abdomen and hind limb, which have the same somatic innervation as the ovaries and uterus (65, 66). The response was mediated via ovarian sympathetic nerves as a reflex response and controlled by supraspinal pathways (65, 66).

Whether the release of β -endorphin is increased or possibly decreased in women with PCOS is not fully understood. However, opioid antagonist treatment has been shown to improve PCOS related symptoms via regulation of insulin and/or LH secretion (67–69) which point to an exaggerated secretion of β -endorphin in the disease. Our hypothesis is that low-frequency EA restores opioid tone and improve related symptoms in PCOS (Fig. 2).

Clearly, acupuncture and specifically low-frequency EA affect PCOS symptoms via modulation of endogenous regulatory systems, including the sympathetic nervous system, the endocrine system and the neuroendocrine system (55, 70). The changes are most likely mediated via the endogenous opioid system (55, 70). Figure 2 shows a hypothetical model of the effects of acupuncture in PCOS.

Effects on metabolic pattern

Low-frequency EA with repetitive muscle contractions activate physiological processes similar to those resulting from physical exercise. Daily low-frequency EA treatment induces weight loss and increases insulin sensitivity, reducing blood glucose and lipid levels (71, 72). Repeated low-frequency EA reduces food intake and body weight, possibly by increasing leptin levels in both rats (71) and humans (72–75). Low-frequency EA also stimulates glucose transport in skeletal muscle independently of insulin and increase the insulin sensitivity of glucose transport in rats (76–78). In female rats with PCOS, induced by continuous prepubertal administration of DHT (51), we found that both repeated low-frequency EA treatment and 4–5 weeks of voluntary exercise reduced insulin resistance (unpublished data). Exercise also reduced body fat and body weight and increased lean body mass. Thus, repeated low-frequency EA may reduce insulin resistance, a central feature of PCOS.

Effects on ovulation

The effect of acupuncture on ovulation in PCOS has been evaluated only in case-control studies. In one study, 12 of 24 women with undefined ovulatory dysfunction treated with manual acupuncture (average, 30 treatments) had marked improvements in menstruation and biphasic basal body temperature for more than two cycles or became pregnant (79). Regulatory effects were also shown on LH, follicle-stimulating hormone (FSH) and 17β -oestradiol, indicating an influence on the HPG axis. In another study, 11 anovulatory women (nine with PCOS) received low-frequency EA (three sessions/day, 3 days/cycle) to induce ovulation (80). Ovulation was induced in six of 13 menstrual cycles. EA had no effect in controls. In the anovulatory women, high plasma β -endorphin levels and low hand skin temperature, indicating increased sympathetic nervous activity, were improved by EA, likely reflecting inhibition of the sympathetic nervous system (80). In another study, one single acupuncture

treatment along with human menopausal gonadotrophin treatment induced ovulation in infertile women as effectively as human chorionic gonadotrophin treatment (81). However, acupuncture had the advantage of reducing the occurrence of ovarian hyperstimulation syndrome. In infertile women with hormonal disturbances, auricular acupuncture demonstrated pregnancy rates equivalent to those induced by hormonal treatment (82) but with fewer side-effects and miscarriages.

We studied the effect of low-frequency EA treatments on endocrinological and neuroendocrinological parameters and anovulation in 24 anovulatory women with PCOS (62). EA increased ovulation in nine women (38%) from this group. The mean monthly rate of ovulation/woman increased from 0.15 before EA to 0.66 during and afterwards ($P = 0.004$). Three months after the last treatment, the LH/FSH ratio and testosterone concentrations were significantly decreased (62).

These studies demonstrate that low-frequency EA affects endocrine, neuroendocrine and metabolic disturbances in PCOS without any negative side-effects. Indeed, EA can be a suitable alternative or complement to pharmacological induction of ovulation. However, controlled randomised studies are required to verify these results. None of these studies has revealed the mechanisms responsible for the beneficial effects of EA.

Effects on the sympathetic nervous system

Transection of the superior ovarian nerve in EV-induced PCO rats reduces the steroid response, increases β_2 -adrenoceptor expression to more normal levels and restores oestrous cyclicity and ovulation (41). Furthermore, blockade of endogenous NGF action restore the EV-induced changes in ovarian morphology and expression of the sympathetic markers α_1 - and β_2 -adrenoceptors, p75 neurotrophin receptor p75^{NTR}, NGF-tyrosine kinase receptor and tyrosine hydroxylase. These data confirm that there is a close interaction between NGF and the sympathetic nervous system in the pathogenesis of steroid-induced PCO rats (83).

In line with these observations, repeated low-frequency EA reduces high ovarian concentrations of NGF (45, 46), CRF (61) and endothelin-1 (46) in EV-induced PCO. It also increases low hypothalamic β -endorphin concentrations and immune function (63) in the same rat PCO model. Low-frequency EA stimulates ovarian blood flow, indicating decreased ovarian sympathetic activity (84). To investigate the hypothesis that repeated low-frequency EA treatments and physical exercise modulate sympathetic nerve activity in rats with EV-induced PCO, we studied the expression of mRNA and protein of α_{1a} -, α_{1b} -, α_{1d} - and β_2 -adrenoceptors and the NGF receptor p75^{NTR} and immunohistochemical expression of TH. Four weeks of physical exercise almost normalised ovarian morphology (85), and both EA and exercise normalised the expression of NGF, NGF receptors and α_1 - and α_2 -adrenoceptors (86). The results indicate that low-frequency EA and physical exercise both modulate sympathetic nervous activity and demonstrate functional interactions between the nervous and endocrine systems, consistent with a therapeutic effect.

Effects on mental health

A recent review examined the efficacy and adverse effects of acupuncture in the treatment of depression (87). There is a lack of well designed randomised controlled trials to evaluate the role of acupuncture in the treatment of depression. However, in a recent study, 33 women with depression were treated with acupuncture for 8 weeks (88). The response rate and relapse rate were similar to those reported with other validated treatments, indicating that acupuncture might be helpful in the treatment of depression (88). This comprises an important field for further exploration with a focus on women with PCOS.

Factors that influence the outcome of acupuncture

Evidence exists that the physiological responses produced by acupuncture vary depending on site, intensity and duration of stimulation (70). Acupuncture intensity can vary enormously depending on the form of stimulation used (e.g. superficial skin needling, sham non-acupuncture point needling, or placebo needling where a blunt tip of a needle touches skin without penetrating) (89, 90). Other acupuncture confounders that may influence outcome are duration of stimulation, number of needles inserted and needle diameter, as well as psychological factors and the environment.

Is acupuncture real or is it a placebo effect?

The control situation in acupuncture studies has widely been discussed and the following biases challenge the concept of acupuncture study designs.

When a needle penetrates the skin, it should be considered as a form of sensory stimulation that activates afferent nerve fibres. Recently, light touch of the skin has been shown to stimulate mechanoreceptors coupled to slow conducting unmyelinated (C) afferents (91) that modulate activity in the central nervous system (91).

In many acupuncture studies, two different acupuncture techniques have been compared (e.g. superficial skin acupuncture needling and deep muscle acupuncture needling). We believe that it is the intensities of acupuncture that are compared; the acupuncture procedure itself is not controlled because the control treatment is not a physiologically inert treatment. It is likely that the control procedures in many acupuncture studies, which are meant to be inert, actually activate C tactile afferents and consequently elicit physiological responses (92). This could explain why superficial, sham acupuncture is as effective as deep muscle acupuncture (93); superficial, sham acupuncture and placebo needling acupuncture cannot be considered as placebo procedures because they are not inert.

Future perspectives

The effect of acupuncture depends on the stimulation and amount of needles, the frequency, duration and amount of acupuncture treatments, as well as environmental and psychological factors.

With this in mind, we think that standardisation and fixed study protocols, where all patients receive the same treatment, will increase the validity of acupuncture studies. Fixed study protocols may bias the outcome, but we believe that they are necessary.

More precise standards for reporting randomised controlled trials of acupuncture are needed to overcome difficulties in analysis and interpretation. The revised recommendations for improving the quality of reports of parallel-group randomised trials (CONSORT) statement addresses general difficulties (94). However, certain aspects are insufficiently covered. The Standards for reporting interventions in controlled trials of acupuncture (STRICTA) group have made recommendations to improve reporting of interventions in controlled trials of acupuncture. The STRICTA checklist should be used in conjunction with CONSORT to improve critical appraisal, analysis, and replication of trials (95).

Conclusion

Despite the lack of a large body of evidence, we should not ignore the fact that many women with PCOS use acupuncture. This alone is a compelling reason to investigate the method. In the hands of competent registered health-practitioners, acupuncture is safe (96). Clinical and experimental evidence shows that acupuncture can be a suitable alternative or complement to pharmacological induction of ovulation in women with PCOS and may also relieve other symptoms, without adverse side-effects. Clearly, acupuncture modulates endogenous regulatory systems, including the sympathetic nervous system, the endocrine system and the neuroendocrine system. However, randomised controlled trials are needed to evaluate the effect of acupuncture in women with PCOS.

Acknowledgements

There is no conflict of interest that would prejudice the impartiality of this scientific work.

Received: 27 June 2007,
revised 2 October 2007,
accepted 15 October 2007

References

- 1 Barber TM, McCarthy MI, Wass JA, Franks S. Obesity and polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2006; **65**: 137–145.
- 2 Tsilchorozidou T, Overton C, Conway GS. The pathophysiology of polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2004; **60**: 1–17.
- 3 Himelein MJ, Thatcher SS. Polycystic ovary syndrome and mental health: a review. *Obstet Gynecol Surv* 2006; **61**: 723–732.
- 4 Barnard L, Ferriday D, Guenther N, Strauss B, Balen AH, Dye L. Quality of life and psychological well being in polycystic ovary syndrome. *Hum Reprod* 2007; **22**: 2279–2286.
- 5 Himelein MJ, Thatcher SS. Depression and body image among women with polycystic ovary syndrome. *J Health Psychol* 2006; **11**: 613–625.
- 6 Pinhas-Hamiel O, Pilpel N, Carel C, Singer S. Clinical and laboratory characteristics of adolescents with both polycystic ovary disease and anorexia nervosa. *Fertil Steril* 2006; **85**: 1849–1851.

- 7 Carmina E, Legro RS, Stamets K, Lowell J, Lobo RA. Difference in body weight between American and Italian women with polycystic ovary syndrome: influence of the diet. *Hum Reprod* 2003; **18**: 2289–2293.
- 8 Douglas CC, Norris LE, Oster RA, Darnell BE, Azziz R, Gower BA. Difference in dietary intake between women with polycystic ovary syndrome and healthy controls. *Fertil Steril* 2006; **86**: 411–417.
- 9 Gilling-Smith C, Story H, Rogers V, Franks S. Evidence for a primary abnormality of thecal cell steroidogenesis in the polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 1997; **47**: 93–99.
- 10 Abbott DH, Dumesic DA, Franks S. Developmental origin of polycystic ovary syndrome – a hypothesis. *J Endocrinol* 2002; **174**: 1–5.
- 11 Dunaif A, Thomas A. Current concepts in the polycystic ovary syndrome. *Annu Rev Med* 2001; **52**: 401–419.
- 12 Tang T, Glanville J, Hayden CJ, White D, Barth JH, Balen AH. Combined lifestyle modification and metformin in obese patients with polycystic ovary syndrome. A randomized, placebo-controlled, double-blind multi-centre study. *Hum Reprod* 2006; **21**: 80–89.
- 13 Hoeger KM. Obesity and lifestyle management in polycystic ovary syndrome. *Clin Obstet Gynecol* 2007; **50**: 277–294.
- 14 Norman RJ, Davies MJ, Lord J, Moran LJ. The role of lifestyle modification in polycystic ovary syndrome. *Trends Endocrinol Metab* 2002; **13**: 251–257.
- 15 Gambineri A, Patton L, Vaccina A, Cacciari M, Morselli-Labate AM, Cavazza C, Pagotto U, Pasquali R. Treatment with flutamide, metformin, and their combination added to a hypocaloric diet in overweight-obese women with polycystic ovary syndrome: a randomized, 12-month, placebo-controlled study. *J Clin Endocrinol Metab* 2006; **91**: 3970–3980.
- 16 Ibanez L, Ong K, de Zegher F, Marcos MV, del Rio L, Dunger DB. Fat distribution in non-obese girls with and without precocious pubarche: central adiposity related to insulinaemia and androgenaemia from prepuberty to postmenarche. *Clin Endocrinol (Oxf)* 2003; **58**: 372–379.
- 17 Faulds G, Ryden M, Ek I, Wahrenberg H, Arner P. Mechanisms behind lipolytic catecholamine resistance of subcutaneous fat cells in the polycystic ovarian syndrome. *J Clin Endocrinol Metab* 2003; **88**: 2269–2273.
- 18 Urbanek M. The genetics of the polycystic ovary syndrome. *Nat Clin Pract* 2007; **3**: 103–111.
- 19 Patel K, Coffler M, Dahan M, Malcom P, Deutsch A, Chang R. Relationship of GnRH-stimulated LH release to episodic LH secretion and baseline endocrine-metabolic measures in women with polycystic ovary syndrome. *Clin Endocrinol* 2004; **60**: 67–74.
- 20 Cumming DC, Reid RL, Quigley ME, Rebar RW, Yen SS. Evidence for decreased endogenous dopamine and opioid inhibitory influences on LH secretion in polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 1984; **20**: 643–648.
- 21 Hayden BJ, Balen AH. The role of the central nervous system in the pathogenesis of polycystic ovary syndrome. *Minerva Ginecol* 2006; **58**: 41–54.
- 22 Carmina E, Dittkoff EC, Malizia G, Vijod AG, Janni A, Lobo RA. Increased circulating levels of immunoreactive beta-endorphin in polycystic ovary syndrome is not caused by increased pituitary secretion. *Am J Obstet Gynecol* 1992; **167**: 1819–1824.
- 23 Lobo RA, Granger LR, Paul WL, Goebelsmann U, Mishell DR Jr. Psychological stress and increases in urinary norepinephrine metabolites, platelet serotonin, and adrenal androgens in women with polycystic ovary syndrome. *Am J Obstet Gynecol* 1983; **145**: 496–503.
- 24 Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, Francis GS, Simon AB, Rector T. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. *N Engl J Med* 1984; **311**: 819–823.
- 25 Kaye DM, Lambert GW, Lefkowitz J, Morris M, Jennings G, Esler MD. Neurochemical evidence of cardiac sympathetic activation and increased central nervous system norepinephrine turnover in severe congestive heart failure. *J Am Coll Cardiol* 1994; **23**: 570–578.
- 26 Dahlgren E, Janson PO, Johansson S, Lapidus L, Lindstedt G, Tengborn L. Hemostatic and metabolic variables in women with polycystic ovary syndrome. *Fertil Steril* 1994; **61**: 455–460.
- 27 Franks S. Polycystic ovary syndrome. *N Engl J Med* 1995; **333**: 853–861.
- 28 Grassi G, Seravalle G, Quarti-Trevano F, Scopelliti F, Dell'Oro R, Bolla G, Mancia G. Excessive sympathetic activation in heart failure with obesity and metabolic syndrome: characteristics and mechanisms. *Hypertension* 2007; **49**: 535–541.
- 29 Ojeda S, Lara H. *Role of the Sympathetic Nervous System in the Regulation of Ovarian Function*. Berlin: Springer-Verlag, 1989.
- 30 Sir-Petermann T, Maliqueo M, Angel B, Lara HE, Perez-Bravo F, Recabarren SE. Maternal serum androgens in pregnant women with polycystic ovarian syndrome: possible implications in prenatal androgenization. *Hum Reprod* 2002; **17**: 2573–2579.
- 31 Fagius J. Sympathetic nerve activity in metabolic control—some basic concepts. *Acta Physiol Scand* 2003; **177**: 337–343.
- 32 Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities—the role of insulin resistance and the sympathoadrenal system. *N Engl J Med* 1996; **334**: 374–381.
- 33 Wu X, Sallinen K, Zhou S, Su Y, Pollanen P, Erkkola R. Androgen excess contributes to altered growth hormone/insulin-like growth factor-1 axis in nonobese women with polycystic ovary syndrome. *Fertil Steril* 2000; **73**: 730–734.
- 34 Semenova I. Adrenergic innervation of the ovaries in Stein-Leventhal syndrome. *Vestn Akad Med Nauk SSSR* (Abstract in English). 1969; **24**: 58–62.
- 35 Heider U, Pedal I, Spänzel-Borowski K. Increase in nerve fibers and loss of mast cells in polycystic and postmenopausal ovaries. *Fertil Steril* 2001; **75**: 1141–1147.
- 36 Greiner M, Paredes A, Araya V, Lara HE. Role of stress and sympathetic innervation in the development of polycystic ovary syndrome. *Endocrine* 2005; **28**: 319–324.
- 37 Balen A. Surgical treatment of polycystic ovary syndrome. *Best Pract Res* 2006; **20**: 271–280.
- 38 Abbott DH, Barnett DK, Bruns CM, Dumesic DA. Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? *Hum Reprod Update* 2005; **11**: 357–374.
- 39 Dumesic DA, Abbott DH, Padmanabhan V. Polycystic ovary syndrome and its developmental origins. *Rev Endocr Metab Disord*. 2007; **8**: 127–141.
- 40 Brawer JR, Munoz M, Farookhi R. Development of the polycystic ovarian condition (PCO) in the estradiol valerate-treated rat. *Biol Reprod* 1986; **35**: 647–655.
- 41 Barria A, Leyton V, Ojeda SR, Lara HE. Ovarian steroidal response to gonadotropins and beta-adrenergic stimulation is enhanced in polycystic ovary syndrome: role of sympathetic innervation. *Endocrinology* 1993; **133**: 2696–2703.
- 42 Lara HE, Ferruz JL, Luza S, Bustamante DA, Borges Y, Ojeda SR. Activation of ovarian sympathetic nerves in polycystic ovary syndrome. *Endocrinology* 1993; **133**: 2690–2695.
- 43 Lara HE, Dissen GA, Leyton V, Paredes A, Fuenzalida H, Fiedler JL, Ojeda SR. An increased intraovarian synthesis of nerve growth factor and its low affinity receptor is a principal component of steroid-induced polycystic ovary in the rat. *Endocrinology* 2000; **141**: 1059–1072.
- 44 Ahmed CE, Dees WL, Ojeda SR. The immature rat ovary is innervated by vasoactive intestinal peptide (VIP)-containing fibers and responds to VIP with steroid secretion. *Endocrinology* 1986; **118**: 1682–1689.
- 45 Stener-Victorin E, Lundeberg T, Waldenstrom U, Manni L, Aloe L, Gunnarsson S, Janson PO. Effects of electro-acupuncture on nerve growth factor and ovarian morphology in rats with experimentally induced polycystic ovaries. *Biol Reprod* 2000; **63**: 1497–1503.

- 46 Stener-Victorin E, Lundeberg T, Cajander S, Aloe L, Manni L, Waldenstrom U, Janson PO. Steroid-induced polycystic ovaries in rats: effect of electro-acupuncture on concentrations of endothelin-1 and nerve growth factor (NGF), and expression of NGF mRNA in the ovaries, the adrenal glands, and the central nervous system. *Reprod Biol Endocrinol* 2003; **1**: 33.
- 47 Manni L, Holmang A, Lundeberg T, Aloe L, Stener-Victorin E. Ovarian expression of alpha (1)- and beta (2)-adrenoceptors and p75 neurotrophin receptors in rats with steroid-induced polycystic ovaries. *Auton Neurosci* 2005; **118**: 79–87.
- 48 Stener-Victorin E, Ploj K, Larsson BM, Holmang A. Rats with steroid-induced polycystic ovaries develop hypertension and increased sympathetic nervous system activity. *Reprod Biol Endocrinol* 2005; **3**: 44.
- 49 Beloosesky R, Gold R, Almog B, Sasson R, Dantes A, Land-Bracha A, Hirsh L, Itskovitz-Eldor J, Lessing JB, Homburg R, Amsterdam A. Induction of polycystic ovary by testosterone in immature female rats: modulation of apoptosis and attenuation of glucose/insulin ratio. *Int J Mol Med* 2004; **14**: 207–215.
- 50 Kafali H, Iriadam M, Ozardali I, Demir N. Letrozole-induced polycystic ovaries in the rat: a new model for cystic ovarian disease. *Arch Med Res* 2004; **35**: 103–108.
- 51 Mannerås L, Cajander S, Holmäng A, Seleskovic Z, Lystig T, Lönn M, Stener-Victorin E. A new rat model exhibiting both ovarian and metabolic characteristics of polycystic ovary syndrome. *Endocrinology* 2007; **148**: 3781–3791.
- 52 Lanham MS, Lebovic DI, Domino SE. Contemporary medical therapy for polycystic ovary syndrome. *Int J Gynaecol Obstet* 2006; **95**: 236–241.
- 53 Kagitani F, Uchida S, Hotta H, Aikawa Y. Manual acupuncture needle stimulation of the rat hindlimb activates groups I, II, III and IV single afferent nerve fibers in the dorsal spinal roots. *Jpn J Physiol* 2005; **55**: 149–155.
- 54 Kaufman MP, Waldrop TG, Rybycki KJ, Ordway GA, Mitchell JH. Effects of static and rhythmic twitch contractions on the discharge of group III and IV muscle afferents. *Cardiovasc Res* 1984; **18**: 663–668.
- 55 Andersson S, Lundeberg T. Acupuncture—from empiricism to science: functional background to acupuncture effects in pain and disease. *Med Hypotheses* 1995; **45**: 271–281.
- 56 Basbaum AI, Fields HL. Endogenous pain control systems: Brain-stem spinal pathways and endorphin circuitry. *Annu Rev Neurosci* 1984; **7**: 309–338.
- 57 Crine P, Gianoulakis C, Seidah NG. Biosynthesis of beta-endorphin from beta-lipotropin and a larger molecular weight precursor in rat pars intermedia. *Proc Natl Acad Sci U S A* 1978; **75**: 4719–4723.
- 58 Rivier C, Rivest S. Effects of stress on the activity of hypothalamic-pituitary-gonadal axis: peripheral and central mechanisms. *Biol Reprod* 1991; **45**: 523–532.
- 59 Harbach H, Moll B, Boedeker RH, Vigelius-Rauch U, Otto H, Muehling J, Hempelmann G, Markart P. Minimal immunoreactive plasma beta-endorphin and decrease of cortisol at standard analgesia or different acupuncture techniques. *Eur J Anaesthesiol* 2007; **24**: 370–376.
- 60 Chen BY. Acupuncture normalizes dysfunction of hypothalamic-pituitary-ovarian axis. *Acupunct Electrother Res* 1997; **22**: 97–108.
- 61 Stener-Victorin E, Lundeberg T, Waldenstrom U, Bileviciute-Ljungar I, Janson PO. Effects of electro-acupuncture on corticotropin-releasing factor in rats with experimentally-induced polycystic ovaries. *Neuropeptides* 2001; **35**: 227–231.
- 62 Stener-Victorin E, Waldenstrom U, Tagnfors U, Lundeberg T, Lindstedt G, Janson PO. Effects of electro-acupuncture on anovulation in women with polycystic ovary syndrome. *Acta Obstet Gynecol Scand* 2000; **79**: 180–188.
- 63 Stener-Victorin E, Lindholm C. Immunity and beta-endorphin concentrations in hypothalamus and plasma in rats with steroid-induced polycystic ovaries: effect of low-frequency electroacupuncture. *Biol Reprod* 2004; **70**: 329–333.
- 64 Sato A, Sato Y, Schmidt RF. *The Impact of Somatosensory Input on Autonomic Functions*. Heidelberg: Springer-Verlag, 1997.
- 65 Stener-Victorin E, Kobayashi R, Kurosawa M. Ovarian blood flow responses to electro-acupuncture stimulation at different frequencies and intensities in anaesthetized rats. *Auton Neurosci* 2003; **108**: 50–56.
- 66 Stener-Victorin E, Fujisawa S, Kurosawa M. Ovarian blood flow responses to electroacupuncture stimulation depend on estrous cycle and on site and frequency of stimulation in anesthetized rats. *J Appl Physiol* 2006; **101**: 84–91.
- 67 Fulghesu AM, Ciampelli M, Guido M, Murgia F, Caruso A, Mancuso S, Lanzone A. Role of opioid tone in the pathophysiology of hyperinsulinemia and insulin resistance in polycystic ovarian disease. *Metabolism* 1998; **47**: 158–162.
- 68 Lanzone A, Fulghesu AM, Cucinelli F, Ciampelli M, Caruso A, Mancuso S. Evidence of a distinct derangement of opioid tone in hyperinsulinemic patients with polycystic ovarian syndrome: relationship with insulin and luteinizing hormone secretion. *J Clin Endocrinol Metab* 1995; **80**: 3501–3506.
- 69 Ciampelli M, Fulghesu AM, Guido M, Murgia F, Muzj G, Belosi C, Fortini A, Cento R, Lanzone A. Opioid blockade effect on insulin beta-cells secretory patterns in polycystic ovary syndrome. Oral glucose load versus intravenous glucagon bolus. *Horm res* 1998; **49**: 263–268.
- 70 Stener-Victorin E, Wikland M, Waldenstrom U, Lundeberg T. Alternative treatments in reproductive medicine: much ado about nothing: Acupuncture—a method of treatment in reproductive medicine: lack of evidence of an effect does not equal evidence of the lack of an effect. *Hum Reprod* 2002; **17**: 1942–1946.
- 71 Kim SK, Lee G, Shin M, Han JB, Moon HJ, Park JH, Kim KJ, Ha J, Park DS, Min BI. The association of serum leptin with the reduction of food intake and body weight during electroacupuncture in rats. *Pharmacol Biochem Behav* 2006; **83**: 145–149.
- 72 Lee M, Kim J, Lim H-J, Shin B-S. Effects of abdominal electroacupuncture on parameters related to obesity in obese women: a pilot study. *Complement Ther Clin Pract* 2006; **12**: 97–100.
- 73 Cabioglu MT, Ergene N. Electroacupuncture therapy for weight loss reduces serum total cholesterol, triglycerides, and LDL cholesterol levels in obese women. *Am J Chin Med* 2005; **33**: 525–533.
- 74 Cabioglu MT, Ergene N. Changes in levels of serum insulin, C-Peptide and glucose after electroacupuncture and diet therapy in obese women. *Am J Chin Med* 2006; **34**: 367–376.
- 75 Cabioglu MT, Ergene N. Changes in serum leptin and beta endorphin levels with weight loss by electroacupuncture and diet restriction in obesity treatment. *Am J Chin Med* 2006; **34**: 1–11.
- 76 Holmang A, Mimura K, Lonnroth P. Involuntary leg movements affect interstitial nutrient gradients and blood flow in rat skeletal muscle. *J Appl Physiol* 2002; **92**: 982–988.
- 77 Chang SL, Tsai CC, Lin JG, Hsieh CL, Lin RT, Cheng JT. Involvement of serotonin in the hypoglycemic response to 2 Hz electroacupuncture of zusanli acupoint (ST36) in rats. *Neurosci Lett* 2005; **379**: 69–73.
- 78 Chang S-L, Lin K-J, Lin R-T, Hung P-H, Lin J-G, Cheng J-T. Enhanced insulin sensitivity using electroacupuncture on bilateral Zusanli acupoints (ST 36) in rats. *Life Sci* 2006; **79**: 967–971.
- 79 Xiaoming MO, Ding LI, Yunxing PU, Guifang XI, Xiuzhen LE, Zhimin FU. Clinical studies on the mechanism for acupuncture stimulation of ovulation. *J Tradit Chin Med* 1993; **13**: 115–119.
- 80 Chen BY, Yu J. Relationship between blood radioimmunoreactive beta-endorphin and hand skin temperature during the electro-acupuncture induction of ovulation. *Acupunct Electrother Res* 1991; **16**: 1–5.
- 81 Cai X. Substitution of acupuncture for HCG in ovulation induction. *J Tradit Chin Med* 1997; **17**: 119–121.

- 82 Gerhard I, Postneek F. Auricular acupuncture in the treatment of female infertility. *Gynecol Endocrinol* 1992; **6**: 171–181.
- 83 Manni L, Holmang A, Cajander S, Lundeberg T, Aloe L, Stener-Victorin E. Effect of anti-NGF on the ovarian expression of α 1- and β 2-adrenoceptors, TrkA, p75NTR, and tyrosine hydroxylase in rats with steroid-induced polycystic ovaries. *Am J Physiol Regul Integr Comp Physiol* 2005; **290**: R826–R835.
- 84 Stener-Victorin E, Kobayashi R, Watanabe O, Lundeberg T, Kurosawa M. Effect of electro-acupuncture stimulation of different frequencies and intensities on ovarian blood flow in anaesthetised rats with steroid-induced polycystic ovaries. *Reprod Biol Endocrinol* 2004; **2**: 16.
- 85 Manni L, Cajander S, Lundeberg T, Naylor AS, Aloe L, Holmang A, Jonsdottir IH, Stener-Victorin E. Effect of exercise on ovarian morphology and expression of nerve growth factor and α 1- and β 2-adrenergic receptors in rats with steroid-induced polycystic ovaries. *J Neuroendocrinol* 2005; **17**: 846–858.
- 86 Manni L, Lundeberg T, Holmang A, Aloe L, Stener-Victorin E. Effect of electro-acupuncture on ovarian expression of α 1- and β 2-adrenoceptors, and p75 neurotrophin receptors in rats with steroid-induced polycystic ovaries. *Reprod Biol Endocrinol* 2005; **3**: 21.
- 87 Smith C, Hay P. Acupuncture for depression (Review). *The Cochrane Library* 2005; 1–21.
- 88 Gallagher SM, Allen JJ, Hitt SK, Schnyer RN, Manber R. Six-month depression relapse rates among women treated with acupuncture. *Complement Ther Med* 2001; **9**: 216–218.
- 89 Streitberger K, Kleinhenz J. Introducing a placebo needle into acupuncture research. *Lancet* 1998; **352**: 364–365.
- 90 White P, Lewith G, Hopwood V, Prescott P. The placebo needle, is it a valid and convincing placebo for use in acupuncture trials? A randomised, single-blind, cross-over pilot trial. *Pain* 2003; **106**: 401–409.
- 91 Olausson H, Lamarre Y, Backlund H, Morin C, Wallin BG, Starck G, Ekholm S, Strigo I, Worsley K, Vallbo AB, Bushnell MC. Unmyelinated tactile afferents signal touch and project to insular cortex. *Nat Neurosci* 2002; **5**: 900–904.
- 92 Lund I, Lundeberg T. Are minimal, superficial or sham acupuncture procedures acceptable as inert placebo controls? *Acupunct Med* 2006; **24**: 13–15.
- 93 Linde K, Streng A, Jurgens S, Hoppe A, Brinkhaus B, Witt C, Wagenpfeil S, Pfaffenrath V, Hammes MG, Weidenhammer W, Willich SN, Melchart D. Acupuncture for patients with migraine: a randomized controlled trial. *JAMA* 2005; **293**: 2118–2125.
- 94 Moher D, Schulz KF, Altman D. The CONSORT Statement: revised recommendations for improving the quality of reports of parallel-group randomized trials 2001. *Explore (NY)* 2005; **1**: 40–45.
- 95 MacPherson H, White A, Cummings M, Jobst K, Rose K, Niemtow R. Standards for reporting interventions in controlled trials of acupuncture: the STRICTA recommendations. *Complement Ther Med* 2001; **9**: 246–249.
- 96 Vincent C. The safety of acupuncture. *BMJ* 2001; **323**: 467–468.