Nutrition, insulin and polycystic ovary syndrome

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The adverse effects of obesity on reproductive function in women are well recognized, but women with polycystic ovary syndrome (PCOS), the most common cause of anovulatory infertility, seem particularly vulnerable to the effects of excessive intake of calories. Polycystic ovary syndrome is associated with hyperinsulinaemia and insulin resistance, the causes of which remain unclear. These metabolic abnormalities are, in turn, related to a disorder of energy expenditure, characterized by reduced post-prandial thermogenesis. It is proposed that these closely interlinked phenomena that, particularly in overweight subjects, are associated with anovulation, may confer a biological advantage for women with PCOS at times of food deprivation, when such women may reproduce more successfully than those without PCOS. A possible causal link between hyperinsulinaemia and ovulation is explored by reference to the interaction of insulin and LH in granulosa cells.

It has long been recognized that nutritional status and body weight can have profound effects on reproductive function. The mechanism of the adverse influence of undernutrition on ovarian function is reasonably well understood. This is best illustrated, in women, by the phenomenon of weight loss-related amenorrhoea, in which there is a characteristic disturbance of the hypothalamic control of gonadotrophins by gonadotrophin-releasing hormone (GnRH) (Boyar *et al.*, 1974; Frisch and McArthur, 1974; Knuth *et al.*, 1977). At present, however, the humoral factors that mediate the effects of nutrition on the hypothalamus remain unclear. The effects of obesity on reproductive function are more complex; there is little evidence for an important disturbance of GnRH secretion (Kopelman, 1988) and it seems likely that peripheral effects – i.e. those on the ovary itself – are more relevant. The possible role of circulating insulin in this context will be examined at length in this review.

Obesity and reproductive function

An early reference to the influence of obesity on menstruation can be found in the writings of Hippocrates (edited by Chadwick and Mann, 1978). In an essay on the Scythians, which appears under the heading 'the influence of climate, water supply and situation on health', their reproductive function is described in the following terms: "The girls get amazingly flabby and podgy… People of such constitution cannot be prolific…fatness and flabbiness are to blame. The womb is unable to receive the semen and they menstruate infrequently and little". The 'control subjects' are the serving wenches: "As good proof of the sort of physical characteristics that are favourable to conception, consider the case of serving wenches. No sooner do they have intercourse with a man than they become pregnant, on account of their sturdy physique and their leanness of flesh".

In more recent times, there have been a number of reports describing menstrual disturbance in obese women (Hartz *et al.*, 1979; Harlass *et al.*, 1984; Kopelman, 1988). Oligomenorrhoea or amenorrhoea are common but, in general, there is no characteristic derangement of the normal pattern of gonadotrophin secretion in such subjects. Clinical and biochemical evidence of hyperandrogenism has been described in obese women with menstrual disturbances (Hartz *et al.*, 1979; Harlass *et al.*, 1984) but the relevance of these findings to the mechanism of anovulation is not obvious. Interpretation of these studies is made more difficult by the fact that patients with polycystic ovary syndrome (who, almost certainly, constitute a significant subgroup of these subjects) have not been specifically identified.

Obesity may affect not only ovulation but also, in fertile women, the outcome of pregnancy. Thus, in an analysis of over 13 000 pregnancies recorded on the Northwest Thames Obstetric Data Base, it was found that the relative risk of miscarriage (odds ratio, adjusted for maternal age) in only moderately overweight women (body mass index [BMI] 25–27.9 kg m^{-2}) was 1.27 (95% confidence interval (CI), 1.11–1.14) compared with a reference group with a normal BMI (19–25 kg m^{-2}) and was 1.36 (CI:1.17–1.59) in the group with a BMI greater than 28 kg m^{-2} (Hamilton Fairley *et al.*, 1992).

Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, accounting for 73% of cases of anovulatory infertility (Hull, 1987; Franks, 1989, 1995). The typical clinical features are hyperandrogenism – hirsutism, androgen-dependent alopecia, or acne – associated with anovulation and a characteristic ovarian morphology. However, it is well-recognized that there is considerable heterogeneity in the clinical presentation of women with polycystic ovaries, ranging from anovulatory women without hirsutism (but who are hyperandrogenaemic) to hirsute women with regular, ovulatory cycles (Adams *et al.*, 1986). It seems likely that, despite the variability in clinical presentation, these subjects represent opposite ends of the same spectrum of the disorder of PCOS. This is borne out by the trend towards high serum concentrations of testosterone and LH, common to both ovulatory and anovulatory women
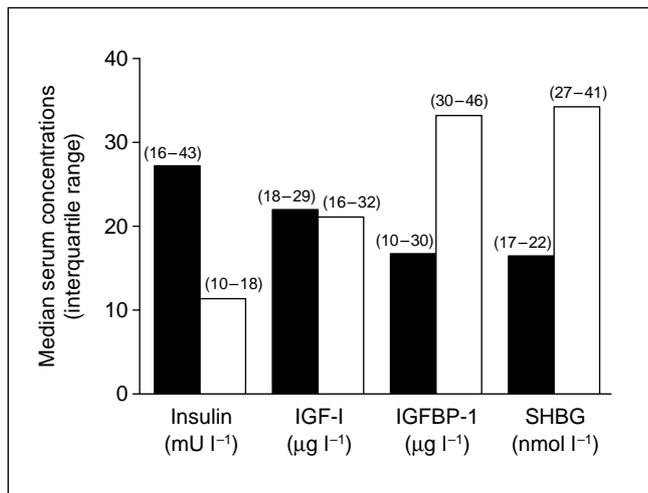
Fig. 1. Median and interquartile range of serum concentrations of insulin, insulin-like growth factor I (IGF-I), IGF-binding protein 1 (IGFBP-1) and sex hormone binding globulin (SHBG) in 10 anovulatory women with polycystic ovary syndrome (PCOS) (■) and 10 weight-matched controls with normal ovaries and regular cycles (□). Differences between values for women with PCOS and controls were significant for insulin ($P<0.01$), IGFBP-1 ($P<0.01$) and SHBG ($P<0.007$). (Data from Hamilton-Fairley *et al.*, 1995.)

with PCOS (Franks, 1989, 1995). The aetiology of PCOS remains unclear but there is evidence for primary hypersecretion of ovarian androgens (Gilling-Smith *et al.*, 1994), which may have a genetic basis (Carey *et al.*, 1993).

Insulin resistance in polycystic ovary syndrome

Although the reproductive consequences of PCOS have been recognized for at least 60 years, there has, more recently, been considerable interest in the metabolic implications of PCOS. Women with the 'classic' form of PCOS (i.e. those with anovulation as well as hyperandrogenism) are relatively hyperinsulinaemic compared with weight-matched controls (Burghen *et al.*, 1980; Chang *et al.*, 1983; Dunaif *et al.*, 1987; Conway *et al.*, 1990). Fasting serum insulin concentrations are typically higher than normal in women with PCOS but the differences in insulin concentrations between patients with PCOS and weight-matched controls are best illustrated by examination of glucose-stimulated insulin secretion (Burghen *et al.*, 1980; Chang *et al.*, 1983; Dunaif *et al.*, 1987; Conway *et al.*, 1990) or 24 h profiles of serum insulin (Hamilton-Fairley *et al.*, 1995) (Fig. 1).

It has now been demonstrated that hyperinsulinaemia in PCOS is a marker of peripheral insulin resistance. Insulin sensitivity – whether measured by the short insulin tolerance test or by the more traditional hyperinsulinaemic, euglycaemic clamp technique – is significantly reduced in both lean and obese subjects with PCOS, compared with controls of similar BMI (Dunaif *et al.*, 1989; Peiris *et al.*, 1989; Robinson *et al.*, 1992; Holte *et al.*, 1994). The results of insulin sensitivity measurements using the short insulin tolerance test are shown (Fig. 2a).

The mechanism of insulin resistance in PCOS has not been fully elucidated, but some interesting observations have been made. First, resistance to the action of insulin affects peripheral glucose uptake and insulin-mediated suppression of lipolysis, but does not appear to extend to the hepatic actions of insulin (Peiris *et al.*, 1989; Dunaif, 1993). This is borne out by the well recognized, inverse relationship of serum concentrations of insulin and sex hormone binding globulin (SHBG), which is presumed to be a reflection of the direct inhibitory action of insulin on SHBG synthesis by the liver (Plymate *et al.*, 1988; Conway *et al.*, 1990; Sharp *et al.*, 1991; Hamilton-Fairley *et al.*, 1995). The involvement of other target organs for insulin (for example the ovary) remains uncertain (see below). Second, the distribution of body fat seems to have an important bearing on insulin sensitivity. If examined using covariate analysis, there is a significant interaction between BMI and PCOS in determining the deposition of abdominal fat (Holte *et al.*, 1994). Thus, for a given increase in BMI, a woman with PCOS will deposit more adipose tissue in the central abdominal region than will a woman without PCOS. The differences in insulin sensitivity between PCOS and BMI-matched control groups are no longer significant if the distribution of body fat is taken into account (Robinson *et al.*, 1992; Holte *et al.*, 1994). Third – and this is not surprising, given the apparently tissue-selective nature of insulin resistance – there is little evidence for a primary abnormality in the insulin receptor in PCOS (Dunaif, 1993). While it is true that genetically determined defects in the insulin receptor can lead to the development of a PCOS-like syndrome, it must be conceded that women with these receptor disorders constitute only a very small proportion of those presenting with hyperandrogenism (Dunaif, 1993). Data from studies of adipocytes derived from women with PCOS and controls suggest that there is a post-receptor abnormality in the insulin signalling pathway (Ciaraldi *et al.*, 1992; Dunaif *et al.*, 1992; Rosenbaum *et al.*, 1993). In particular, there is evidence that up to 50% of patients with PCOS demonstrate constitutive abnormalities in receptor autophosphorylation, which is an integral part of the normal intracellular signalling function of the insulin receptor (Book *et al.*, 1995). At present, it is not clear how these findings relate to those regarding fat distribution. Do patients with the proposed post-receptor defect form a discrete subgroup of women with PCOS or is there a link between this phenomenon and deposition of body fat?

What are the implications of insulin resistance in terms of long-term health? It is estimated that between 10 and 20% of obese young women with PCOS have either impaired glucose tolerance (IGT) or frank, non-insulin-dependent diabetes (NIDDM) (Dunaif *et al.*, 1987; Conway *et al.*, 1990). Analysis of a population of middle-aged women in whom a diagnosis of PCOS had been made during their reproductive years revealed a 13% prevalence of NIDDM compared with only 2% in a carefully matched, reference population (Dahlgren *et al.*, 1992a). Detailed examination of the early-phase insulin response to a glucose challenge (i.e. the rise in serum insulin within 10 min of giving an intravenous glucose bolus of 300 mg kg^{-1} body weight) resulted in further insight into the mechanism of IGT and NIDDM in PCOS. Women with PCOS have abnormalities in the 'first phase' insulin response to an intravenous glucose load, which is highly suggestive of a disorder of pancreatic β-cell function (O'Meara *et al.*, 1993; Holte *et al.*, 1994; Ehrmann *et al.*, 1995). It remains to be seen whether this represents a primary abnormality of insulin secretion in PCOS and in what way this relates to peripheral insulin sensitivity and body fat distribution.

Initial analysis, however, favours the notion that the β-cell dysfunction is independent of insulin resistance (Holte *et al.*, 1994, 1995).

The metabolic abnormalities in PCOS may also constitute a risk factor for the later development of cardiovascular disease. A number of studies have reported that there is a characteristic dyslipidaemia in women with PCOS which is closely related to both hyperinsulinaemia and insulin sensitivity (Wild and Bartholomew, 1988; Graf *et al.*, 1990; Conway *et al.*, 1992; Franks and Robinson, 1994). Typical features are high serum triglycerides and reduced serum concentrations of high density lipoprotein (HDL)-cholesterol, a profile thought to be predictive of cardiovascular risk in women. Most, but not all, studies suggest that the dyslipidaemia is independent of obesity but, clearly, there is an important interaction between PCOS and body weight which affects lipid metabolism. An important question is whether the unfavourable lipid profile in women with PCOS translates into a real increase in the risk of cardiovascular disease in later life. Data from the study of middle-aged women with PCOS, referred to earlier, seem to suggest that there is an increased risk of coronary artery disease (Dahlgren *et al.*, 1992b) but few of the subjects in this study had reached an age at which there was likely to be overt (i.e. symptomatic) evidence of cardiovascular pathology and the increased risk (estimated at sevenfold) was computed from a risk factor 'profile' including lipid concentrations and body topography. There is clearly a need for long-term prospective studies to resolve this important issue that has major implications for the health of the female population. This is especially relevant when simple interventional measures such as calorie restriction have been shown to reverse many of the metabolic abnormalities (Pasquali *et al.*, 1989; Kiddy *et al.*, 1992; Holte *et al.*, 1995).

An abnormality of energy expenditure in polycystic ovary syndrome

Obesity and insulin-resistant states are associated with abnormalities in energy expenditure, notably in post-prandial thermogenesis (PPT) (Ravussin *et al.*, 1983, 1985). We therefore studied women with PCOS and compared PPT (defined as the increase over resting energy expenditure (REE) following a standard mixed meal) with that in a weight-matched control group (Robinson *et al.*, 1992). There was no difference between the groups in REE. Post-prandial thermogenesis was significantly reduced in the PCOS group (Fig. 2b) and there was a direct correlation of PPT with insulin sensitivity. The difference in PPT between obese PCOS subjects and controls was 42 kJ. The test meal represented about one fifth of the daily calorie intake. If this difference in energy balance between PCOS and controls were maintained in the long term (as seems likely), it can be calculated that, over a year, women with PCOS would have an excess of 73 500 kJ or 1.9 kg of fat. It should be mentioned that the data from this study are at variance with those reported by Segal and Dunaif (1990), who found no significant difference in PPT between PCOS and control groups. This is likely to reflect both different experimental protocols and differences in the populations of patients studied, particularly with respect to ethnic background and range of BMI. Despite these disparities, Segal and Dunaif (1990) observed, as we did, a negative correlation between percentage fat mass and PPT in PCOS and controls.
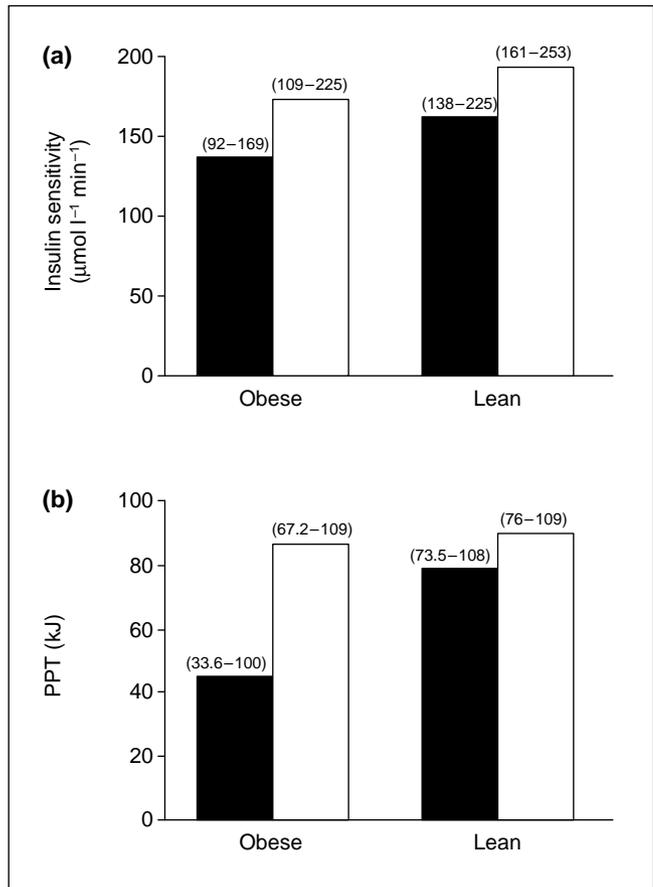

Fig. 2. (a) Insulin sensitivity (median, range), as measured by the short insulin tolerance test, in obese and lean women with polycystic ovary syndrome (PCOS) (■) compared with weight-matched controls (□). Insulin sensitivity was calculated from the slope of the glucose curve between three and 15 min after a low dose (0.05 units kg^{-1}) bolus of insulin (Bonora *et al.*, 1989; Robinson *et al.*, 1992). (b) Post-prandial thermogenesis (PPT) (sum of increments above basal metabolic rate; median, range) after a standard mixed meal (10 kcal kg^{-1} lean body weight) in women with polycystic ovary syndrome (PCOS) (■) and matched controls (□). Differences between values from women with PCOS and controls were significant ($P<0.05$) in lean and obese subjects for both insulin sensitivity and PPT. (Data from Robinson *et al.*, 1992.)

Polycystic ovaries are common in women of reproductive age, with a prevalence of about 20% (Polson *et al.*, 1988) and PCOS appears to have a genetic basis (Simpson, 1992; Carey *et al.*, 1993). Is there, therefore, an evolutionary advantage in the decreased PPT which would favour enhanced survival at a time of food deprivation? In modern, Western society, the 'PCO gene(s)' may have an adverse effect on reproductive function if calorie intake is excessive but, as outlined in the next section, calorie restriction in obese women with PCOS leads to improved menstrual pattern and fertility. In other words, such women may continue to reproduce at times of relative calorie deprivation.

It will be evident from the above observations that there is a close interaction between calorie intake, body fat distribution, insulin resistance and PPT. Although it is known that reduction of abdominal fat and reciprocal changes in insulin sensitivity
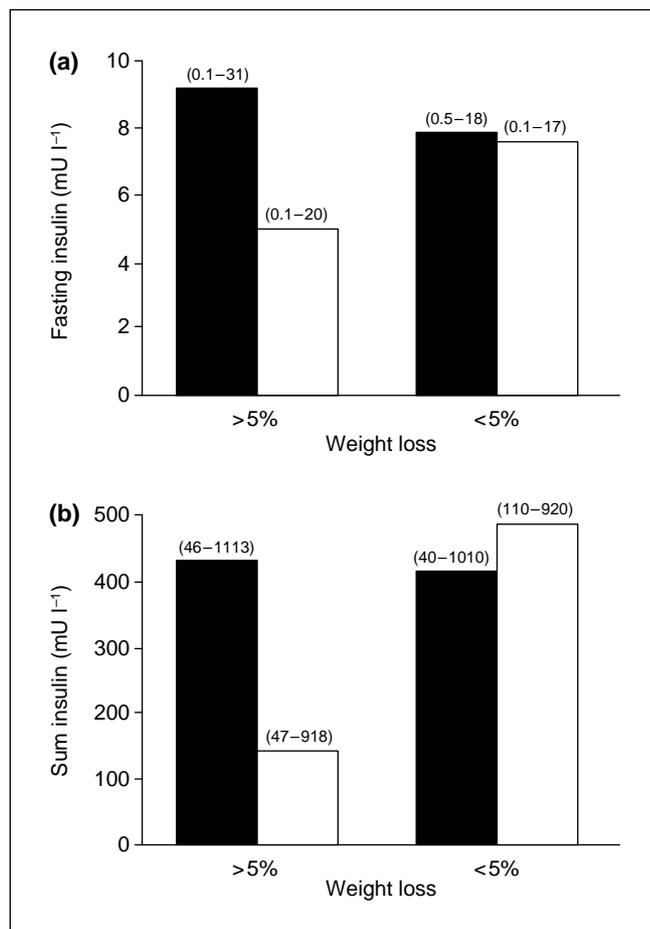
Fig. 3. (a) Fasting insulin concentrations and (b) sum of insulin concentrations after a 75 g oral glucose tolerance test before (■) and after (□) weight loss in women with polycystic ovary syndrome (PCOS), grouped according to the degree of weight loss. Values are median and ranges. Differences between before and after diet were significant ($P<0.02$, $P<0.03$, respectively) in the group ($n=13$) who lost >5% of their initial body weight but not in patients ($n=11$) who did not. (Data from Kiddy *et al.*, 1992.)

may follow calorie restriction (Holte *et al.*, 1995), these variables are so interwoven that it is not possible to determine which is the critical underlying (causal) abnormality.

Effects of calorie restriction in obese women with polycystic ovary syndrome

Dietary treatment may (at least, partially) reverse the biochemical abnormalities and improve reproductive function in obese women with PCOS (Pasquali *et al.*, 1989; Kiddy *et al.*, 1992; Holte *et al.*, 1995). In our own study, 24 obese women with PCOS (mean weight 91.5 [SD 14.7] kg) were treated for 6 months with a 1 000 kcal, low fat diet (Kiddy *et al.*, 1992). Nineteen of the 24 had menstrual abnormalities and 19 were hirsute. Thirteen of the 24 (11 of whom had menstrual disturbances) succeeded in losing more than 5% of their initial weight. Menstrual patterns were improved in nine of the 11, and five of seven previously infertile women conceived after spontaneous ovulation. By contrast, in

the group of 11 women who did not lose a significant amount of weight (i.e. less than 5%), only one of the eight women with irregular menses or amenorrhoea showed any change in menstrual pattern. The clinical improvement in the group who lost weight was mirrored by a significant and substantial fall in fasting and glucose-stimulated insulin concentrations (Fig. 3), raising the question of a causal link between hyperinsulinaemia (or insulin sensitivity) and ovarian dysfunction.

The association of these changes in reproductive function with those in insulin concentrations during calorie restriction prompted us to explore further the possible relationship between hyperinsulinaemia and menstrual pattern in women with PCOS.

Menstrual function and insulin

The 'classic' PCOS includes anovulation as an obligatory ingredient of the definition, but not all women with polycystic ovaries have menstrual disturbances (Adams *et al.*, 1987; Franks, 1995). It has been estimated that between 60% and 87% of women with hirsutism and regular menses (sometimes referred to as 'idiopathic hirsutism') have polycystic ovaries on ultrasound examination (Adams *et al.*, 1986; Franks, 1989; O'Driscoll *et al.*, 1994). Biochemically, these women appear to form part of the same spectrum as women with the classic syndrome, in that they not only have high serum concentrations of androgens, but also have significantly increased serum concentrations of LH. However, when serum insulin concentrations and insulin sensitivity in women with regular cycles and PCO are examined, it is clear that there are substantial differences between this group of PCO subjects and those with menstrual disturbance (Robinson *et al.*, 1993). In essence, hyperandrogenaemic women with regular cycles have fasting and glucose-stimulated insulin concentrations that are indistinguishable from those in control subjects, and which are therefore significantly lower than those in weight-matched, equally hyperandrogenaemic, anovulatory women with PCO. Furthermore, insulin sensitivity is also normal in the women with PCO who have regular menses (Fig. 4).

What is the nature of this intriguing relationship between menstrual pattern and hyperinsulinaemia in hyperandrogenaemic women? It is unlikely that either hyperandrogenaemia or menstrual disturbance causes hyperinsulinaemia. Insulin sensitivity varies during the normal menstrual cycle but this involves a small *decrease* during the luteal phase (Valdes and Elkind-Hirsch, 1991), so that it might be expected that acyclic women are, if anything, less insulin resistant than are those with intact cycles. Suppression of ovarian activity and serum androgen concentrations by the administration of a long-acting analogue of GnRH has no effect on insulin concentration or sensitivity (Geffner *et al.*, 1986; Dunaif *et al.*, 1990). Is it possible, therefore, that hyperinsulinaemia or insulin resistance in a woman with PCO causes, or at least contributes to, the mechanism of, anovulation?

Hyperinsulinaemia and the mechanism of anovulation in polycystic ovary syndrome

Insulin has a gonadotrophic effect on ovarian steroidogenesis and has been shown to stimulate oestradiol production by cultured human granulosa cells (Garzo and Dorrington, 1984;
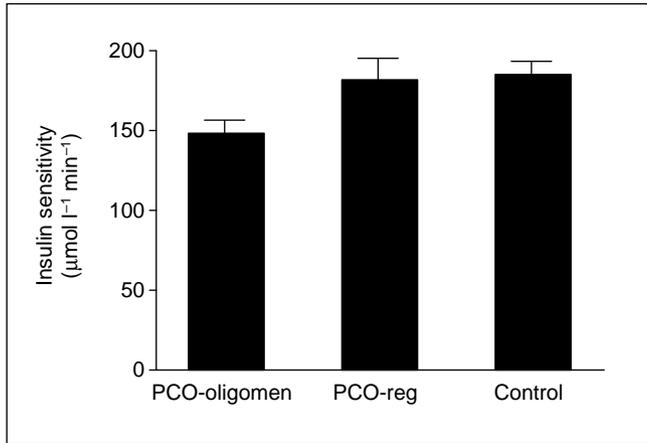
Fig. 4. Insulin sensitivity (mean + SEM), measured by the short insulin tolerance test, in women with polycystic ovary syndrome (PCOS) with oligomenorrhoea (PCO-oligomen) or regular cycles (PCO-reg) (early–mid-follicular phase) compared with normal subjects in the early–mid-follicular phase of the cycle. Groups were matched for body mass index; the PCO groups were equally hyperandrogenaemic. Insulin sensitivity was significantly lower ($P<0.01$) in the PCO-oligomen group than in controls but was similar to controls in the PCO-reg group. (Data from Robinson *et al.*, 1993.)

Fig. 5. Interaction of insulin and LH on oestradiol production by human granulosa cells in culture. Cells were preincubated with no hormones (■), insulin alone (□) or insulin with FSH (▨) before exposure to LH. (Data from Willis *et al.*, 1995.)

Willis *et al.*, in press). Recent data indicate that insulin enhances the effect of FSH on both oestradiol and progesterone (Willis *et al.*, in press). Importantly, it has been demonstrated that insulin is effective in augmenting FSH-stimulated steroidogenesis in granulosa cells derived from insulin-resistant women with PCOS, suggesting that the peripheral insulin resistance of PCOS does not extend to the ovary (Willis *et al.*, in press). The implication of these findings is that granulosa cells from insulin-resistant women with PCOS are, effectively, exposed to higher than normal circulating concentrations of insulin and that this may be expected to enhance steroidogenesis. This would be consistent with the observation that granulosa cells from anovulatory PCOS subjects have a greater capacity to produce oestradiol *in vitro* than do cells from either normal ovaries or ovulatory PCOS (Mason *et al.*, 1994). How can this proposed stimulatory effect of insulin be reconciled with the observation that PCOS is associated with disordered follicle maturation? We propose that the explanation for this apparent paradox is that while insulin enhances steroidogenesis it may, by virtue of its interaction with LH, bring about inappropriate advancement of granulosa cell differentiation and, hence, arrest of follicle growth.

Luteinizing hormone has two distinct actions on granulosa cells of the preovulatory follicle: amplification of steroidogenesis and (at relatively high doses) inhibition of further mitosis, and then terminal differentiation of granulosa cells (Yong *et al.*, 1992). These actions are, under physiological conditions, expressed at the onset of the LH surge, when serum LH concentrations exceed a notional 'ceiling' beyond which further mitosis of granulosa cells can no longer occur (Hillier, 1994). Studies in our laboratory have shown that insulin augments not only FSH action on granulosa cells but also that of LH (Willis *et al.*, in press). Furthermore, the interaction of insulin with LH on steroidogenesis seems to be synergistic (Fig. 5). Our
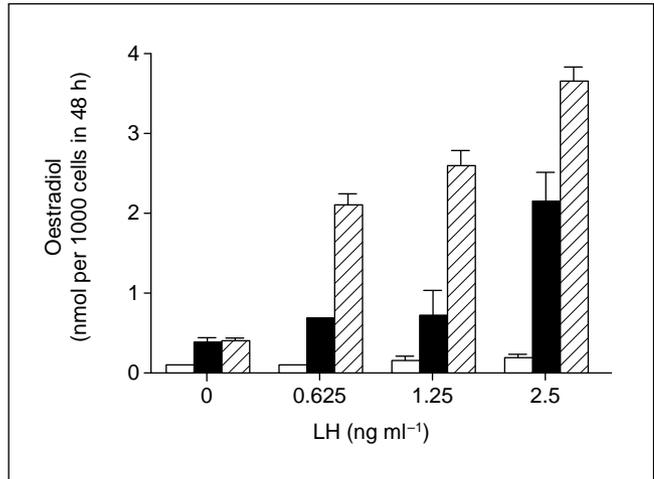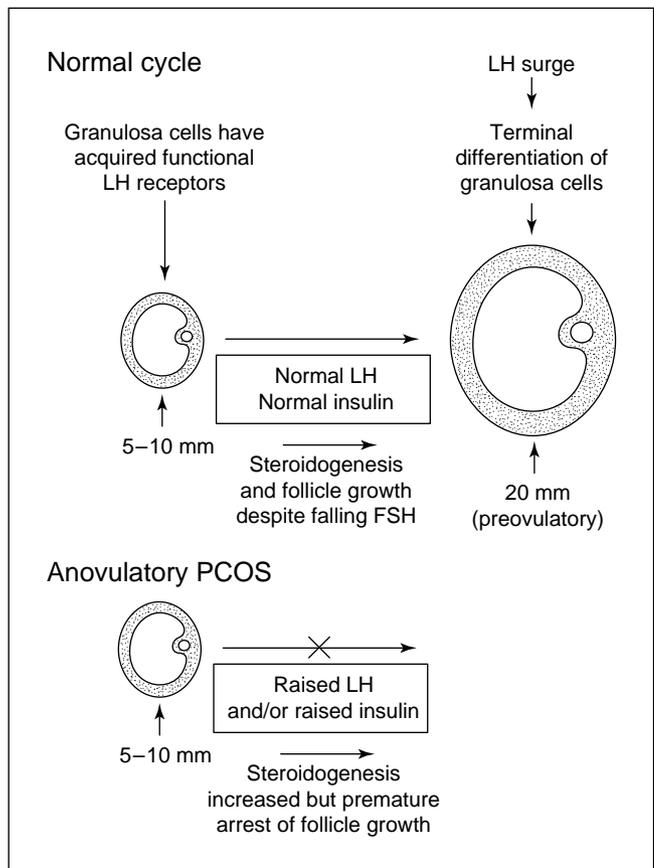

Fig. 6. Proposed mechanism for the role of hyperinsulinaemia in the aetiology of anovulation in polycystic ovary syndrome (PCOS). Insulin augments LH action on granulosa cells of the developing follicle leading to an increase in steroidogenesis but premature arrest of follicle growth.

hypothesis is that, in the presence of hyperinsulinaemia, the action of LH on granulosa cells in women with PCOS (who, typically, already have raised serum concentrations of LH) is greatly amplified. Thus the exposure of granulosa cells to LH in follicles that have acquired functional granulosa cell receptors for LH (i.e. 5–10 mm in diameter) may be effectively equivalent to that which, in the normal menstrual cycle, is attained only in mature follicles of about 20 mm in diameter. The predicted result would be enhanced oestradiol production (as indeed can be observed in studies *in vitro*, Mason *et al.*, 1994) but inhibition of further growth and arrest of follicles at 5–10 mm (Fig. 6). It remains to be determined whether these speculations can be supported by direct experimental evidence of the effects of insulin–LH interaction on growth and differentiation of human granulosa cells.

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