Sex steroids and insulin resistance

Callum LIVINGSTONE* and Mary COLLISON†
*Centre for Clinical Science and Measurement, School of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey GU2 5XH, U.K., and †Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, U.K.

ABSTRACT

There is extensive experimental evidence that sex steroids and insulin interact in their actions on tissues. At physiological levels, testosterone and oestradiol are thought to be involved in maintaining normal insulin sensitivity. However, outside this ‘physiological window’ these steroids may promote insulin resistance. Considerable research has been carried out on polycystic ovarian syndrome, a common disorder associated with excessive androgen production and insulin resistance. Hyperinsulinaemia in patients with this condition is believed to stimulate ovarian androgen production, and there is also evidence that androgens act directly on peripheral tissues to promote insulin resistance. There is the potential for a vicious circle to develop with increasing androgen production and insulin resistance. The molecular basis of this insulin resistance has been reported to involve reduced insulin receptor autophosphorylation, reduced expression and translocation of insulin-responsive glucose transporters and defects of the insulin signalling pathway distal to the insulin receptor. These defects await full characterization. Insulin-sensitizing agents can reverse many of the effects of insulin resistance and may have a future place in the treatment of polycystic ovarian syndrome and other conditions associated with steroid-induced insulin resistance. Recognition and treatment of sex steroid-associated insulin resistance at an early stage in patients may reduce their risk of developing Type II (non-insulin-dependent) diabetes mellitus, hypertension and dyslipidaemia, and so may improve fertility and reduce cardiovascular risk. Here we review the interplay between sex steroids and insulin resistance, and consider the implications this has for clinical conditions.

INTRODUCTION

There is a wealth of clinical and experimental data demonstrating that sex steroids and insulin interact in their actions on tissues. For example, high circulating concentrations of sex steroids appear to contribute to the development of insulin resistance, and women with low serum levels of female sex steroids or high serum testosterone are at greater risk of developing Type II (non-insulin-dependent) diabetes mellitus [1,2]. While it is clear that the serum levels of sex steroids are closely linked to insulin sensitivity, the nature of the link is uncertain, especially in humans. After outlining the molecular basis of insulin action, we will go on to consider how this can be disrupted by sex steroids and by disease states involving abnormal levels of sex steroids. The adrenal androgens dehydroepiandrosterone (DHEA) and its sulphated form dehydroepiandrosterone sulphate (DHEAS) are included in the discussion, as their actions are inextricably linked with those of the sex
steroids, and there is evidence that they influence insulin sensitivity [3]. The effects of sex steroids on insulin’s actions other than glucose uptake have received less attention, but we will consider these briefly. We finish off by describing the implications of this work for therapeutics and the horizons for research work in this area.

INSULIN ACTION AND RESISTANCE

Insulin has a wide range of acute metabolic and anabolic actions. Of the acute metabolic effects, the most important are stimulation of glucose uptake into peripheral insulin-sensitive tissues, suppression of hepatic glucose output, stimulation of glycogen synthesis and an antilipolytic effect on adipose tissue [4]. In all cases of insulin resistance, there is a subnormal biological response to a given level of insulin in its target tissues. The term ‘insulin resistance’ is generally assumed to include insulin’s action on lipid and protein metabolism and glucose uptake, but also encompasses its myriad of other actions, including those on vascular tissue and cellular growth [5]. Impairment of each of these actions contributes to the consequences of insulin resistance for the organism.

Among the various actions of insulin, glucose metabolism is the most straightforward to investigate by means of clinical studies. Glucose clamping can readily provide information on insulin sensitivity as regards insulin-stimulated glucose uptake [6]. This, along with the importance of glycaemic control in diabetes, has inevitably resulted in a disproportionate amount being known about the regulation of glucose metabolism by insulin and how it is adversely affected in conditions associated with insulin resistance.

Insulin resistance initially results in lower levels of uptake of glucose from the blood into the target tissues, predominantly skeletal muscle and to a lesser extent adipose tissue [7]. Therefore blood glucose levels become raised, a phenomenon known as hyperglycaemia. In a bid to overcome the hyperglycaemia, more insulin is secreted from pancreatic β-cells, resulting in characteristic hyperinsulinaemia. In the majority of pre- or early-phase diabetics, this elevated secretion of insulin is sufficient to overcome the tissue insensitivity, and patients can exist in a hyperinsulinaemic but euglycaemic state. Eventually, due to pancreatic β-cell dysfunction, the hyperinsulinaemic response may become inadequate to overcome the deficit, and these subjects become both hyperinsulinaemic and hyperglycaemic. At this point, patients will be classified as suffering from Type II diabetes mellitus.

In quantitative terms, skeletal muscle is the most important peripheral tissue in whole-body glucose homoeostasis [8]. Adverse influences on insulin sensitivity in skeletal muscle are therefore likely to have a considerable hyperglycaemic effect on the organism as a whole, and so skeletal muscle is a particularly important tissue in which to study pathological influences on glucose homoeostasis. Nevertheless, the role of adipose tissue must also be considered, as it plays a vital role in the integration of carbohydrate and lipid metabolism and hence energy balance.

Insulin signalling

Insulin’s actions are brought about by intracellular events following the binding of insulin to its cell surface transmembrane receptor (Figure 1) [9]. The insulin receptor is a tetramer, composed of two extracellular insulin-binding α-subunits and two transmembrane β-subunits. Binding of insulin to the α-subunits results in activation and autophosphorylation of intracellular portions of the β-subunits, which serves as a signal for recruitment of intracellular signalling proteins [10]. The insulin receptor substrate (IRS) proteins are the first to bind, and specific Src-homology 2 (SH2) domains in these proteins serve as docking sites for other signalling proteins [11]. Thereafter the signalling pathway increases rapidly in complexity as it diverges to bring about the different effects of insulin. The next step in the pathway leading to stimulation of glucose uptake is activation of the enzyme phosphatidylinositol 3-kinase (PI3-K), following its recruitment to the IRS–insulin-receptor complex [12]. Like IRS, PI3-K is in fact a heterogeneous group of proteins widely implicated in insulin’s actions. PI3-K catalyses the phosphorylation of phosphoinositides at the 3-position of the inositol ring, the major product being phosphatidylinositol 3,4,5-trisphosphate.

Events downstream of PI3-K leading to stimulation of glucose uptake are less clear, but probably involve activation of protein kinase B, a protein also involved in activation of glycogen synthesis in response to insulin [13]. Interaction of protein kinase B with phosphatidylinositol 3,4,5-trisphosphate is thought to render it susceptible to phosphorylation and activation by phosphoinositide-dependent protein kinases [14]. The atypical protein kinase C λ and ζ isoforms, which may themselves be activated by phosphoinositides, are probably also involved downstream of PI3-K in events leading to activation of glucose transport [15].

Glucose uptake

The facilitated diffusion of glucose into mammalian cells is mediated by a family of homologous glucose transporter (GLUT) proteins [16]. Under basal conditions, glucose entry into the cell is mediated by the ubiquitously expressed GLUT1 protein. Upon stimulation by insulin there is a rapid and massive increase in glucose entry into the cell. Although GLUT1 levels at the plasma membrane are increased in response to insulin, the effect is modest, and only accounts for a small percentage of the extra
Sex steroids and insulin resistance

EVIDENCE FOR INTERACTION BETWEEN SEX STEROIDS AND INSULIN ACTION

Human studies
Considerable evidence has emerged from human studies strongly suggesting that sex steroids are able to influence insulin sensitivity. Indeed, these hormones have been reported to either enhance or reduce insulin sensitivity, depending on the particular situation.

Physiological exposure to sex steroids
The elevated serum levels of sex steroids associated with normal puberty are associated with a decrease in insulin sensitivity, and this effect appears to be restricted to peripheral glucose metabolism [19,20]. Girls with premature adrenarche have been noted to have reduced insulin sensitivity and may be at increased risk of developing polycystic ovarian syndrome (PCOS) later in life [21]. Normal pregnancy, which is associated with high circulating levels of both oestrogens and progesterone, is also associated with reduced insulin sensitivity [22]. Similarly, a fall in insulin sensitivity has been reported in normal women during the luteal phase of the menstrual cycle.

Reduced glucose uptake in response to insulin is observed in insulin-resistant states, and various molecular mechanisms can potentially account for this. It has emerged that these mechanisms are species-, tissue- and/or disease-dependent. For example, the expression of GLUT4 can be significantly reduced, as observed in adipose tissue from certain animal models of insulin resistance and in some humans with Type II diabetes [18]. Alternatively, the specific insulin-mediated translocation of GLUT4 may be impaired, either as a result of a defect in signalling events leading to translocation or because defects in the actual machinery involved in translocation prevent normal movement of transporter proteins. Defects in the insulin signalling system can, of course, potentially affect all of insulin’s actions.

© 2002 The Biochemical Society and the Medical Research Society
when serum progesterone and oestrogen levels are both elevated.

Treatment with sex steroids

Numerous studies on the administration of sex steroids to humans have shown that these hormones are capable of inducing peripheral insulin resistance. Administration of testosterone to female transsexuals or of ethinyl oestradiol to male transsexuals caused a reduction in peripheral glucose uptake in the absence of any change in endogenous glucose production. This indicates that the steroids have a peripheral site of action [23], and since skeletal muscle is responsible for the majority of peripheral glucose disposal, it would appear that sex steroids have a direct action on skeletal muscle to reduce insulin sensitivity. In other studies, administration of testosterone or its derivatives to women resulted in impaired glucose tolerance and hyperinsulinaemia, indicative of insulin resistance [24–26]. Androgen treatment in patients with aplastic anaemia resulted in glucose intolerance and raised insulin levels [27]. Similarly, abuse of anabolic steroids has also been observed to result in reduced insulin sensitivity [28].

The use of oral contraceptives containing oestrogens and progestins is associated with the development of insulin resistance, especially in women with a history of gestational diabetes [29,30]. However, the doses of ethinyl oestradiol used in oral contraceptive preparations are low, and it is likely that, in many women, any adverse effect on insulin sensitivity caused by these oral contraceptives would be outweighed by the beneficial effect on lipid levels [23]. This does not apply to women at greater risk of diabetes, e.g. those with a past history of gestational diabetes or with established insulin resistance who are more susceptible to hypertriglyceridaemia.

Obesity in male patients is associated with higher insulin levels and lower serum testosterone levels, i.e. relative hypogonadism as compared with lean controls [31]. Testosterone replacement in such patients has been shown to improve insulin sensitivity, as measured by the glucose clamp technique, and glucose and lipid profiles also improved [6,32]. This suggests that, at physiological levels, testosterone has a role in maintaining normal insulin sensitivity in men, an effect that is lost at supraphysiological concentrations.

Oestrogen replacement therapy decreases hyperandrogenism and improves glucose homoeostasis in post-menopausal women with Type II diabetes [33]. As in the situation with androgens in males, the level of the hormone appears to be important in determining whether its action on insulin sensitivity is beneficial or adverse. At physiological levels oestrogen may have a role in maintaining normal insulin sensitivity. These findings, along with the data on replacement therapy and administration of supraphysiological doses of sex steroids, suggest that there is a ‘physiological window’ for the action of sex steroids on insulin sensitivity [34].

PCOS

PCOS is a common endocrine disorder associated with anovulation and subfertility in women [35,36]. It is also characterized by hyperandrogenaemia, i.e. elevated serum levels of testosterone and of the adrenal androgens DHEA and its sulphated derivative DHEAS. This hyperandrogenism manifests itself clinically as hirsutism and menstrual irregularity. PCOS is well recognized to be associated with insulin resistance and hyperinsulinaemia [35]. Consequently, women with the disorder are at increased risk of developing Type II diabetes mellitus, hypertension and dyslipidaemia leading to atherosclerosis [37,38].

PCOS and its accompanying insulin resistance are believed to have genetic components, and their propensity to run in families suggests a dominant mode of inheritance [39,40]. However, progress in defining the genetics of PCOS has been slow, firstly because the heterogeneous nature of the clinical syndrome has resulted in the lack of a consensus on the clinical phenotype and secondly because it is likely that multiple genetic defects are involved. However, linkage and association studies using affected sibling pairs have implicated a region close to the insulin gene on chromosome 19 [39]. There are many individual candidate genes, such as those involved in the regulation of insulin secretion and steroidogenesis, and further study will clarify the extent to which these genes are involved. It has been suggested that PCOS is the result of ‘thrifty’ genes that promote energy storage [41]. These genes may provide a survival advantage in times of starvation, but lead to obesity and PCOS when energy supplies are abundant.

Obesity occurs in 50–80% of patients with PCOS, and generally occurs at a younger age in women with this condition (third to fourth decade) compared with women in the general population [42]. Although PCOS is not invariably associated with visceral obesity, both lean and obese women with PCOS have a greater incidence of insulin resistance than controls, and are at high risk of developing diabetes later in life [43]. It is also well recognized that there is an association between the android pattern of fat distribution, characterized by excessive abdominal adiposity and an increased waist/hip ratio, and increased mortality from coronary artery disease [44,45]. In another study, androgen excess in young women was demonstrated to be a predictor of coronary heart disease in later life [38].

While there is no doubt that hyperinsulinaemia and hyperandrogenism are strongly correlated in PCOS, there is debate about the nature of their interaction, i.e. the extent to which high insulin levels cause hyperandrogenism and high androgen levels themselves pro-
mote insulin resistance. The former hypothesis, whereby high insulin levels stimulate ovarian androgen production, gained popularity more quickly, particularly as insulin resistance appears to precede the increase in androgen levels [46,47]. It is likely that specific biochemical defects are present in some patients with PCOS that renders them susceptible to hyperandrogenism. For example, serine phosphorylation of the main regulatory enzyme of androgen biosynthesis, P450c17, is known to increase its 17,20 lyase activity, resulting in increased androgen output [48]. Enhanced serine phosphorylation may therefore underlie the hyperandrogenism of PCOS. In addition, phosphorylation of the insulin receptor on serine residues is known to induce insulin resistance [49]. It has therefore been suggested that a single biochemical defect causing excess serine phosphorylation may be responsible for both hyperandrogenism and insulin resistance in a proportion of women with PCOS [50]. Further evidence for insulin stimulation of androgen production has come from a study using diazoxide to suppress endogenous insulin secretion [51]. In this study, diazoxide treatment was found to reduce the serum testosterone level in obese women with PCOS. Despite this progress, questions remain unanswered with regard to the stimulation of androgen production by insulin. It is unclear, for example, why hyperinsulinaemia should result in hyperandrogenism in PCOS patients, but fail to stimulate androgen production in patients with Type I diabetes taking exogenous insulin [35].

The insulin-like growth factors (IGFs) and their binding proteins are involved in ovarian physiology, and there is evidence that the IGFs, like insulin, have a role in the pathophysiology of PCOS. In common with insulin, IGF-I stimulates ovarian growth and potentiates the action of gonadotropins on ovarian steroid synthesis. IGF-I has been shown to enhance DNA and androgen synthesis in human thecal monolayer cultures, and it synergizes with luteinizing hormone in androstenedione production [52,53]. It is likely that insulin and IGF-I act synergistically in vivo in the production of hyperandrogenism. Insulin reduces the serum level of sex hormone binding globulin (SHBG) and both serum and intrafollicular levels of IGF binding protein-1, actions that tend to increase the levels of bioactive IGF-I and androgens [54–56]. It has been proposed that it is the increasing activities of both insulin and IGF-I which occur during puberty that induce the development of PCOS in susceptible patients [57].

While hyperinsulinaemia appears to promote hyperandrogenism in patients with PCOS, evidence has also mounted for a direct effect of androgens on skeletal muscle, in line with the findings on exposure of healthy people to exogenous androgens [23]. Just as excessive exogenous sex steroids can promote insulin resistance in healthy people, one would predict that endogenous androgens present in excess could promote insulin resistance in patients with PCOS. Evidence that this is the case came from the observation that treatment of PCOS patients with the anti-androgen spironolactone caused androgen levels to fall and improved insulin sensitivity [58]. It is likely that both processes occur in vivo, with a vicious circle tending to develop, comprising increasing insulin and androgen levels (Figure 2) [23]. Such a vicious circle would account for the coexistence of hyperinsulinaemia and hyperandrogenism, and the tendency for temporal progression to PCOS. Treatment of hyperandrogenism may prevent the progression of PCOS as well as the progression of its associated insulin resistance, so reducing the risk of coronary heart disease and Type II diabetes and improving fertility. Hyperinsulinaemia can induce insulin resistance in its own right [59]. This makes it difficult to assess the extent to which hyperandrogenaemia contributes to insulin resistance in the clinical situation. Studies of isolated cells and tissues will clarify the situation, as these can be exposed to high insulin levels and high androgen levels separately or together. This is discussed in a later section.

### Congenital adrenal hyperplasia (CAH)

CAH is an inborn disorder of cortisol biosynthesis, most commonly due to 21-hydroxylase deficiency. Synthetic intermediates in the pathway of cortisol biosynthesis become diverted into androgen biosynthesis, and so the condition is associated with hyperandrogenism. In one study of six patients with CAH, all prepubertal and none treated with glucocorticoids at the time of the study, insulin sensitivity was found to be significantly lower than in control subjects [60]. This suggests that chronic androgen overproduction can induce insulin resistance to glucose uptake. There is one case report in the literature of a woman with CAH due to impaired 21-hydroxylase activity whose insulin resistance persisted after normalization of high androgen levels [61]. This was proposed as evidence that the hyperandrogenism was not contributing to insulin resistance in this case. However, it is likely that, once established, the insulin resistance would be maintained by mechanisms other than hyperandrogenism.

Although much rarer than PCOS, CAH is an attractive condition to study with respect to androgen-induced insulin resistance, as patients are less likely to have genetic factors predisposing them to insulin resistance and complicating the interpretation of peripheral androgen effects. Further studies of the insulin resistance associated with CAH will be needed in order to clarify its mechanism and the role of androgens in its genesis.

### Molecular basis of sex steroid-induced insulin resistance in humans

Although evidence has accumulated from clinical studies that androgens and oestrogens can cause insulin resistance, there is relatively little information available on
Figure 2  Relationship between androgen levels and insulin resistance in PCOS

This figure summarizes factors leading to insulin resistance and factors leading to hyperandrogenism in patients with PCOS. In PCOS, high circulating insulin levels resulting from insulin resistance stimulate ovarian androgen production both by a direct action on the ovary and by stimulating the release of luteinizing hormone (LH). Hyperinsulinemia may also enhance the bioavailability of androgens. It can achieve this in two ways. First, it reduces the biosynthesis of SHBG, thus increasing the level of free androgens. Secondly, it decreases the biosynthesis of IGF binding protein-1 (IGFBP-1), elevating the levels of free IGF-I, which, like insulin, stimulates ovarian androgen production. Hyperandrogenaemia results in the menstrual abnormalities and hirsutism associated with PCOS. In turn, the hyperandrogenaemia may increase insulin resistance in adipose tissue and skeletal muscle, potentially resulting in a vicious circle of increasing androgen levels and worsening insulin resistance. Hyperinsulinaemia and insulin resistance may also lead to hypertension and dyslipidaemia, increasing cardiovascular risk in patients with PCOS. The cycle may potentially be interrupted by insulin-sensitizing agents which lower androgen output, resulting in improved fertility and reduced cardiovascular risk.

the molecular basis of this insulin resistance in humans. One study examined the effect of endogenous oestrogens (in pregnancy) and exogenous oestrogens (in the form of an oral contraceptive) on blood cells. The results suggested that oestrogens interfere with the binding of insulin to its transmembrane receptor [62].

The most detailed and interesting findings in this area have come from studies of patients with PCOS. Clearly the defects described may not represent direct actions of androgens, given that the tissues were removed from the body, where many other influences will be at work. Decreases in the number and affinity of insulin binding sites have been reported in monocytes and erythrocytes from patients with PCOS [63,64]. While these findings are interesting, they do not necessarily relate to the situation in insulin target tissues. In studies of adipocytes from PCOS patients, however, two groups have noted decreased insulin sensitivity in the absence of any changes in insulin binding [65,66]. This resulted in a decrease in insulin-stimulated glucose uptake. The underlying defect was later noted to be a reduction in GLUT4 expression [67]. There is one report of reduced autophosphorylation of the insulin receptor in the ovary of a patient with PCOS, and studies on fibroblasts from women with PCOS have demonstrated that autophosphorylation of the insulin receptor is reduced in about half of PCOS women [68–70]. Those patients in whom insulin receptor autophosphorylation was normal were still insulin resistant, and so it was considered likely that separate defects exist in the insulin signalling pathway of these patients, further downstream of the receptor. The nature of the signalling defect or defects remains elusive, as does that associated with peripheral insulin resistance in patients with Type II diabetes [8]. Exposure to high levels of insulin is known to cause mistargeting of GLUT4 in model systems [18]. This mechanism has also been
reported in adipocytes from patients with gestational diabetes mellitus, but whether it occurs in PCOS remains to be established. Fuller investigation of individual target tissues will be required in order to determine the molecular basis of sex steroid-induced insulin resistance in humans.

**Animal models**

More work investigating interactions between sex steroids and insulin action has been carried out in animal than in human tissues. It is clear that, in animal models, steroid hormones are important regulators of insulin-mediated events in skeletal muscle. In particular, insulin-stimulated glucose uptake and glycogen synthesis are influenced by sex steroids. The molecular mechanisms have been examined in greater detail than in human studies, although it should be emphasized that the molecular basis of steroid-induced insulin resistance may vary markedly between species, and data obtained from animal studies cannot be extrapolated to humans.

**Studies of ovariectomized and orchidectomized animals**

Ovariectomized female rats have been used as a model system for studying the effects of oestradiol and progesterone in the absence of endogenous production of these hormones. In studies on skeletal muscle in these rats, insulin sensitivity, as measured by the euglycaemic hyperinsulinaemic clamp technique, has been found to be diminished. Treatment with oestradiol restored glucose uptake, glycogen synthesis and insulin sensitivity, whereas treatment with progesterone alone resulted in insulin resistance [71]. These findings suggest that, at physiological concentrations, oestradiol is involved in the maintenance of normal insulin sensitivity in female rats. In a similar study, the absence of female sex hormones was found to result in decreased whole-body insulin-stimulated glucose disposal. This was reported to be due to an impairment of GLUT4 translocation and to be associated with reduced expression of glycogen synthase in skeletal muscle [72]. Similar findings were reported in a study of ovariectomized female mice [73]. The observed decrease in insulin-stimulated glucose uptake was reversed by oestradiol replacement, with a 2-fold enhancement of uptake; progesterone antagonized this action when both hormones were given together. Oestradiol replacement in ovariectomized rats has also been reported to enhance exercise tolerance and to have glycogen-sparing effects [74]. There was no effect on the level of GLUT4 at low oestradiol concentrations, but at supraphysiological doses GLUT4 mRNA levels were reduced in adipose tissue. In two other studies, low levels of oestradiol and progesterone in combination (but not alone) reduced GLUT4 mRNA levels in adipose tissue to half those observed in controls, but the same hormone treatment had no effect on GLUT4 mRNA levels in skeletal muscle [75,76].

Considered together, these findings suggest that, in female rats, oestradiol has a role in maintaining normal insulin sensitivity, an action that is not due to regulation of transporter expression. At higher levels, or in combination with progesterone, oestradiol appears to impair insulin sensitivity by reducing GLUT4 expression in adipose tissue and by a separate action in skeletal muscle, either on insulin signalling or on the process of GLUT4 translocation itself.

Activation of glycogen synthesis in response to insulin is known to be impaired in insulin-resistant states, and studies have investigated the influence of sex steroids on this important action of insulin [77]. There is considerable evidence from studies on female rats that androgen excess impairs glycogenesis and insulin-stimulated glucose uptake. Administration of testosterone to female rats for a period of 12 weeks resulted in severe insulin resistance, characterized by decreases in the uptake of 2-deoxyglucose and in glycogen synthesis of approx. 50% [78]. In a similar study using a shorter period of exposure (24 h), testosterone impaired 2-deoxyglucose uptake in the most insulin-sensitive muscles from ovariectomized female rats [79]. Increased androgenicity resulting from testosterone treatment led to a reduction in whole-body insulin-mediated glucose uptake and impairment of glycogen synthase expression in the same animal model [72].

In order to investigate the action of testosterone on insulin sensitivity in male animals, studies have been carried out on orchidectomized male rats, which have no endogenous testosterone production. These animals when untreated or treated with high doses of testosterone show insulin resistance to both glucose uptake and incorporation of glucose into glycogen in skeletal muscle [80]. When treated with low doses of testosterone, however, all the metabolic abnormalities were corrected. Interestingly, no effects of testosterone were observed on liver glycogen synthesis, suggesting that this steroid action is confined to peripheral tissues. Muscle glycogen stores have also been reported to be diminished in skeletal muscle from orchidectomized animals [81]. Glycogen synthase activity was impaired and glycogen phosphorylase activity enhanced. Again, these abnormalities were reversed by testosterone replacement. This study [81] did not investigate the effect of pharmacological doses of testosterone, but treatment with oestradiol was found to have no effect. These results suggest that, at physiological concentrations, testosterone in male animals, like oestradiol in female animals, maintains muscle insulin sensitivity, but has deleterious effects on insulin sensitivity outwith this range. The molecular basis for the action of sex steroids on glycogen synthesis is not known.

**Other animal models**

In a study of streptozotocin-induced diabetes in female rats, oestradiols were found to inhibit the diabetogenic
effect of streptozotocin, whereas exposure to androgens resulted in an increased incidence of diabetes [82]. This is analogous to the situation with PCOS in women, where androgen excess predisposes to Type II diabetes. In studies of insulin-independent glucose uptake into the rat uterus, oestradiol was found to increase glucose uptake due to transcriptional activation of the glucose transporter GLUT1 [83]. No increase in translocation was observed for either GLUT1 or GLUT4. Oestradiol has also been reported to enhance insulin-independent glucose uptake at the blood–brain barrier in rats by increasing GLUT1 mRNA and protein in cerebral microvessels [84]. The effects of oestradiol on glucose uptake in rats therefore appear to be tissue- and transporter-specific.

Studies on animal cells

Cell lines provide excellent model systems for studying the effects of steroids on insulin sensitivity, as the hormone actions can be examined in isolation, free from interfering influences present in the whole animal. The in vitro approach also allows precise hormone concentrations to be achieved, something that is not possible in the whole animal or human subject.

Although the association between circulating oestrogen levels and impaired insulin action has long been appreciated, there has, until now, been a failure to demonstrate a direct effect of sex steroids on peripheral insulin-sensitive tissues. However, recent data obtained using a cell culture model of an insulin-sensitive peripheral tissue, the 3T3-L1 adipocyte, have demonstrated such an effect [85]. Culture of 3T3-L1 adipocytes with high levels of oestrogens (oestrone, oestradiol or oestriol) resulted in a reduced ability of insulin to stimulate glucose uptake, an effect that was maximal at 100 nmol/l oestrogen [85].

In the 3T3-L1 adipocyte model of steroid-induced insulin resistance, the impaired insulin-stimulated glucose uptake was accompanied by decreased translocation of GLUT4 to the plasma membrane, as assessed by the reduced presence of GLUT4 immunofluorescence in plasma membrane lawn sheets. Interestingly, however, the actual levels of the GLUT4 protein in the cell were not altered, reinforcing the idea that the defect lies at the level of translocation rather than protein expression [85]. Recently much work has focused on how alterations in the early insulin signalling events leading to GLUT4 translocation can also participate in the development of cellular insulin resistance. In a recent study also using 3T3-L1 adipocytes, Clark et al. [86] have shown that the IRS proteins appear to associate with a particular cytoskeletal fraction in the particulate fraction of cell membranes. It is believed that this complex may perform a unique function, allowing the IRS proteins to interact with the insulin receptor and providing a location for the interaction of IRS with downstream target molecules. IRS proteins are subsequently released from this scaffold after insulin stimulation occurs. Interestingly, release from this platform is also associated with the development of insulin resistance: the IRS proteins fail to interact with or are abnormally released from the scaffold, hence preventing normal interactions [86]. A similar cellular insulin resistance also appears to be present in the steroid-treated cells, whereby steroid treatment not only reduces the cellular levels of the three important insulin signalling molecules IRS-1, IRS-2 and PI3-K, but also causes the movement of IRS-1 and IRS-2 away from the particulate and into the cytosolic fraction.

Although the 3T3-L1 adipocyte is far removed from the adipocytes of human PCOS subjects, the results presented clearly have implications for the development of such disorders. Current lines of evidence do not favour a role for oestrogen action on peripheral tissues in initiating the onset of PCOS [87]. Nevertheless, it is thought that such an action is likely to have a role in propagating the disease once it has begun to develop. Indeed, some lines of evidence suggest that the persistent hyperinsulinaemia of PCOS is able to influence the elevated oestrogen secretion, and that the reverse is also true. With regard to the insulin resistance present in PCOS, Dunaif et al. [88] showed that this is a peripheral and not a hepatic insulin resistance. In other words, the insulin resistance is not related to any impairment in the ability of the liver to exert normal control over glucose homoeostasis, but more a decreased ability of peripheral tissues to respond normally to insulin. This therefore suggests impairment in some mechanism within the peripheral tissues that is responsible for mediating the steps between insulin binding and its biological effects. This fits well with the recent data, since oestrogen treatment also affects machinery within the 3T3-L1 adipocyte, resulting in the development of cellular insulin resistance. Rosenbaum et al. [67] have shown a similar decrease in sensitivity to insulin in PCOS patients, which is apparently due to an obesity-independent reduction in GLUT4 expression in adipocytes. It is quite likely, however, that the differences observed in the cellular system may relate to how the parameters are actually measured. Indeed, PCOS patients observed at the early stages of the disorder may show a lesser decrease in GLUT4 content; conversely, were it possible to treat 3T3-L1 adipocytes with steroid for much longer periods, reductions in GLUT4 expression might be observed.

The data presented also have implications for other disorders where insulin resistance is associated with an altered sex steroid profile. Pregnancy, long-term use of the combined oral contraceptive pill and the use of hormone replacement therapy can all result in a state of cellular insulin resistance. Although it is perhaps not ideal to group these conditions together, they all have the potential to result in the development of an insulin-resistant state by a similar mechanism, i.e. the presence of
Elevated circulating levels of oestrogens. Therefore it is quite possible that the development of insulin resistance in these conditions follows a pattern similar to that observed in the 3T3-L1 adipocyte cell system.

Further work is required to investigate more closely different aspects of the effects of the sex steroids on 3T3-L1 adipocytes. It would be of interest to study the IRS genes in detail, in order to determine the presence and location of specific oestrogen-responsive areas. It would also be appropriate to extend this work to human adipocytes, and to determine whether similar defects are observed in adipocytes obtained from PCOS subjects.

Despite the importance of skeletal muscle in whole-body glucose homoeostasis as outlined above, less work has been carried out on this tissue, mainly because of the difficulty in obtaining it in sufficient quantities for analysis. The availability of cell lines derived from skeletal muscle may help overcome this problem by providing more material for study. Such cell lines have been studied extensively with respect to other aspects of insulin action, and have proved to be useful model systems. Skeletal myoblasts (LD, C127 and Sol8 myoblasts), for example, have been reported to express functional oestrogen receptors, and so would appear to be suitable model systems for study [89]. This should be a fruitful area for future research.

EVIDENCE FOR AN INTERACTION BETWEEN DHEA AND INSULIN ACTION

The adrenal androgens DHEA and DHEAS are present in abundance in humans and primates. Their conversion into sex steroids occurs peripherally, and the effects of DHEA on a tissue depend on the metabolizing enzymes present locally. Levels of DHEA vary considerably between individuals, but are in general about 30% lower in young women than men, although the magnitude of this difference falls with age due to decreased production of DHEA. We will consider the evidence for an interaction between DHEA and insulin in human studies and studies on animal models.

Human studies

Decreased levels of endogenous DHEA in humans have been reported to be associated with diabetes, impaired glucose tolerance and insulin resistance, although much of the data have arisen from cross-sectional studies [90]. In a study to investigate the influence of advancing age on insulin action, it was shown that the negative relationship between age and insulin action, measured by whole-body glucose disposal, is related to the DHEAS concentration in plasma [91]. The decrease in the synthesis of DHEAS by the adrenals that occurs with age results in a fall in the peripheral formation of active sex steroids derived from this hormone, which may lead to the development of insulin resistance, obesity, cardiovascular disease and loss of muscle mass [92].

One study in women with PCOS concluded that insulin sensitivity is positively correlated with the serum DHEA level and the DHEA/testosterone ratio, and another study on hyperandrogenic women suggested that the DHEAS/testosterone ratio is a regulator of insulin sensitivity and glucose tolerance [30,93]. This ratio may therefore represent a surrogate marker for insulin sensitivity, but this hypothesis is difficult to pursue with clinical studies, both because of interfering factors that are present and because the investigator is not in a position to alter the ratio at will.

As with testosterone, there appear to be sex differences in the relationship between DHEA levels and insulin sensitivity, with the situation reversed in men. In non-diabetic men, elevated serum testosterone and DHEAS levels were shown to be associated with lower insulin concentrations, i.e. improved insulin sensitivity [3]. Sex differences have also been noted in the hormonal abnormalities associated with abdominal obesity. In women this condition is associated with raised serum testosterone and reduced SHBG levels as in PCOS, implying an elevated free testosterone concentration. In obese men the findings were of reduced serum testosterone, DHEA and SHBG [94].

Not all studies support an association between DHEA and insulin sensitivity. In one study on healthy men, no association was found between serum testosterone or DHEAS levels and insulin sensitivity [95]. DHEA was not shown to influence insulin sensitivity as measured by the euglycaemic clamp technique or to have a positive effect on insulin sensitivity in obese adolescents [96,97]. In a study on the effect of DHEA infusion on insulin sensitivity in women with PCOS and obese controls, DHEA had no demonstrable effect in vivo, but did have in vitro effects in the women with PCOS consistent with increased insulin action, namely enhanced insulin binding to lymphocytes and enhanced lymphocyte pyruvate dehydrogenase activity [98].

Trials are currently under way to evaluate a possible role for exogenous DHEA for replacement in men, postmenopausal women and patients with diabetes. At present there is little evidence in favour of the therapeutic use of DHEA in human insulin resistance, and until results of prospective studies are available its use in patients is not advised [99].

Animal models

While there is relatively little evidence for a beneficial effect of DHEA treatment in the prevention of metabolic disorders in humans, there is an abundance of evidence for its having a protective effect in animal models. However, the extremely low levels of DHEA present in normal rodents suggest that the effects of exogenous DHEA in rodents should be interpreted as being
pharmacological rather than physiological. DHEA treatment has been reported to reduce body fat content and maintain insulin responsiveness in male rats as effectively as exercise. The molecular basis of this action in skeletal muscle appeared to be on the insulin signalling cascade rather than on glucose transporter expression [100].

DHEA treatment of rodents has been demonstrated to protect against the development of visceral obesity and its associated insulin resistance in skeletal muscle [101]. DHEA decreased weight gain in young animals and decreased body fat in adult rats without a coincident reduction in energy intake. In addition, these animals had a higher basal metabolic rate than controls. DHEA has also been shown to increase glucose clearance rate in older rats, an indication of enhanced insulin sensitivity in peripheral tissues, especially muscle [102]. This is further evidence for DHEA having a direct effect on skeletal muscle in rodents.

A possible cause of increased insulin resistance in skeletal muscle associated with visceral obesity is the production of tumour necrosis factor-α (TNF-α) by adipose tissue. In a study measuring glucose disposal rates in obese Zucker rats, DHEA treatment appeared to have an ameliorating effect on insulin sensitivity, by reducing both body weight and serum TNF-α levels [103]. The decrease in visceral fat may protect against the development of muscle insulin resistance by reducing the amount of TNF-α released into the systemic circulation.

The effects of DHEA on rodent skeletal muscle cell lines have not yet been investigated, but some work has been carried out on rat adipocytes. DHEA caused an increase in basal and insulin-stimulated deoxyglucose uptake without having any discernable action on the insulin receptor [104]. It also increased deoxyglucose uptake in rat adipocytes by a mechanism believed to involve protein kinase C activation, and thus may mimic or enhance insulin action via activation of PI3-K [105].

**SEX STEROIDS AND OTHER ACTIONS OF INSULIN**

Relatively little study has been made of the effects of sex steroids on sensitivity to actions of insulin other than glucose uptake and glycogen synthesis. Below we summarize the work carried out in this area that has the most important clinical implications. As in the study of the effects of sex steroids on insulin-stimulated glucose uptake, one must be careful to distinguish between effects of replacement doses and pharmacological doses of sex steroids.

**Vascular endothelium**

The vascular endothelium is recognized to be a complex endocrine organ, producing a variety of different hormones and also possessing receptors for many hormones [106]. It regulates vascular tone and so determines the balance between vasoconstriction and vasodilatation. Interest in the association of endothelial function with insulin resistance has arisen following recognition of the role of endothelial dysfunction in vascular disease [107]. The physiological action of insulin on the vascular endothelium is to act as a vasodilator by stimulation of endothelial nitric oxide synthesis, and endothelial function can be assessed by measurement of endothelium-dependent vasodilatation [108]. In states of insulin resistance, the vasorelaxant effects of insulin are known to be impaired, predisposing to vascular disease [109].

Postmenopausal women are at increased risk of macrovascular disease, and hormone replacement therapy is known to reduce this risk [110]. One study investigated whether oestradiol replacement at physiological levels might enhance the vascular actions of insulin [111]. Although oestradiol did increase peripheral blood flow, it was concluded to have a distinct haemodynamic effect from that of insulin. Other biological mechanisms may therefore explain oestrogen-induced cardioprotection, e.g. improved metabolic profiles, and anti-atherogenic and antioxidant properties [110].

As discussed above, women with PCOS are believed to be at increased risk of macrovascular disease due to the presence of insulin resistance and multiple cardiovascular risk factors. Studies have therefore addressed the question of whether endothelium-dependent vasodilatation is impaired in PCOS patients and whether sex steroids may be involved. A recent study on women with PCOS showed an association with impaired endothelium-dependent vasodilatation, indicative of endothelial dysfunction, but also that there was resistance to the vasodilatory action of insulin [112]. The endothelial dysfunction was associated with insulin resistance and hyperandrogenaemia. However, there are other data which show no evidence of endothelial dysfunction in women with PCOS as compared with age-matched controls and no correlation with insulin resistance or hyperandrogenism [113]. In a study examining the thickness of the carotid artery wall and the prevalence of atherosclerotic plaques in women with PCOS, it was found that PCOS was associated with premature atherosclerosis and increased wall thickness [114]. This association could not be explained fully by the risk factors present, and it was suggested by the authors that PCOS, through its abnormal hormonal profile, may have an independent effect predisposing to increased wall thickness. Interestingly, long-term treatment with metformin, a recognized treatment for the insulin resistance associated with PCOS, has recently been reported to correct vascular insulin resistance and improve endothelium-dependent vasodilatation in patients with...
androgens in the regulation of triacylglycerol metabolism activities [122]. This suggests a physiological role for testosterone substitution in hypogonadal male subjects showed a marked increase in LPL and hepatic lipase action is opposite to that of oestradiol [121]. A study of free testosterone levels, suggesting that testosterone tissue LPL activity was positively correlated with plasma energy utilization rather than storage [116]. In the diabetic state, where insulin is deficient, the reduction in adipose tissue LPL activity predisposes to hypertriglyceridaemia because of reduced catabolism of circulating lipoproteins [117]. Insulin has the opposite effect on LPL activity in skeletal muscle, promoting energy utilization rather than storage [116].

It has become clear that sex steroids, by their effects on LPL activity, can modulate the action of insulin and influence body fat distribution. Various studies have suggested roles for sex steroids in the regulation of LPL activity. Treatment of rats with oestrogen at pharmacological doses caused a reduction in LPL activity in adipose tissue, while cardiac and diaphragmatic LPL activities were increased [118]. Thus exogenous oestrogens present at high levels appear to shift the flux of triacylglycerol from storage in adipose tissue to usage in skeletal muscle, an anti-insulin action. This action appears to be specific for oestrogen, as orchidectomy of male animals failed to reproduce the effect. These findings have been borne out by later studies. A suppressive effect of oestradiol upon adipose tissue LPL activity has also been observed in lean (but not obese) Zucker rats and exercised male rats [119,120]. In human subjects, fasting adipose tissue LPL activity was found to correlate inversely with plasma oestradiol levels, in line with the findings in rats and consistent with oestradiol being a negative regulator of human adipose tissue LPL activity [121]. Oestradiol appears to increase the availability of lipids to muscle from adipose tissue stores. Its action on lipid metabolism therefore opposes that of insulin, whose effects on adipose tissue are anabolic, promoting energy storage.

There is evidence of a role for androgens in the regulation of human LPL activity. In women, adipose tissue LPL activity was positively correlated with plasma free testosterone levels, suggesting that testosterone action is opposite to that of oestradiol [121]. A study of testosterone substitution in hypogonadal male subjects showed a marked increase in LPL and hepatic lipase activities [122]. This suggests a physiological role for androgens in the regulation of triacylglycerol metabolism by maintenance of LPL activity and, like insulin, promotion of anabolism. The effect of pharmacological doses of testosterone was not investigated.

The situation in visceral adipose tissue and abdominal adipocytes is distinct from that in adipocytes from other subcutaneous sites. Visceral adipocytes have relatively high rates of lipolysis compared with subcutaneous adipocytes and show a relatively low responsiveness to insulin, both to its anti-lipolytic effect and to its stimulatory effect on glucose uptake [123]. Indeed the non-esterified fatty acids that are released as a result of visceral adipose tissue lipolysis are implicated in the development of hepatic insulin resistance. Testosterone replacement is known to decrease visceral adipose tissue LPL activity in men, as does postmenopausal oestrogen therapy in women [124]. These observations may explain the improvement in insulin sensitivity and the decrease in visceral fat that occur upon sex steroid replacement in middle-aged patients. In summary, testosterone present at physiological levels or as replacement therapy exerts inhibitory effects on lipid accumulation in visceral adipose tissue and abdominal fat stores. Similarly, oestrogen has a protective effect against visceral fat accumulation in postmenopausal women.

**EFFECTS OF ANDROGEN-LOWERING TREATMENTS ON INSULIN SENSITIVITY**

**Metformin**

The biguanide metformin is known to be efficacious in the treatment of PCOS [125]. It improves insulin sensitivity, allowing androgen levels to fall, i.e. total testosterone, free testosterone, androstenedione and DHEAS [126,127]. This can reverse the menstrual abnormalities and result in increased fertility and pregnancy, as well as combatting features of the metabolic syndrome [128]. However, it is unclear how much of the action of metformin is due to a direct improvement in peripheral insulin sensitivity and how much occurs indirectly through an improvement in hyperinsulinaemia and a consequent improvement in hyperandrogenaemia. Further work will clarify the mechanism of action of metformin and its effects on dyslipidaemia, hypertension and cardiovascular risk.

**Thiazolidinediones**

The thiazolidinedione group of drugs were initially developed as lipid-lowering agents, but were later found to have beneficial effects on glucose metabolism [129]. They decrease fasting hyperglycaemia and hyperinsulinaemia and increase insulin-stimulated glucose disposal. This effect is brought about at the molecular level via an increase in the expression of GLUT1 and GLUT4 and actions on the insulin signalling cascade [130]. They also enhance glycogen synthase activity [131]. Thiazo-
Lidinediones are believed to impair the development of atherosclerotic lesions, not only by their metabolic actions but also by exerting direct effects on the vascular wall [129]. The thiazolidinediones already have a place in the treatment of insulin resistance associated with Type II diabetes. Patients with PCOS are at risk of Type II diabetes, and 80% of subjects with the latter disease die of cardiovascular disease. Clearly any treatment ameliorating metabolic abnormalities and reducing cardiovascular risk in PCOS patients would represent a significant advance.

One member of this group of drugs, troglitazone, has been found to be effective in the treatment of hyperandrogenism. A study on its use in the treatment of PCOS patients showed that it enhanced insulin action, as measured using the frequently sampled glucose tolerance test, and decreased circulating levels of DHEAS, androstenedione and oestrone [132]. Luteinizing hormone levels fell and SHBG levels increased, changes that would tend to reduce the bioactive proportion of androgens. Another study using troglitazone demonstrated its ability to reverse metabolic abnormalities in obese women with PCOS [133]. Both total and free testosterone levels were noted to decline after treatment, and this occurred independently of any change in gonadotropin levels. It is likely that a significant part of the mechanism of troglitazone’s effect on androgen levels is via its enhancement of insulin sensitivity, allowing high insulin levels to fall and so reversing the augmentation of steroidogenesis that occurs in insulin resistance. However, troglitazone is also known to directly inhibit the steroidogenic enzymes P450c17 and 3β-hydroxysteroid dehydrogenase [134].

Troglitazone itself has unfortunately had to be withdrawn from use as a result of adverse actions on the liver, but other drugs in this family have become available which share the same metabolic actions. Thiazolidinediones certainly have a potential role in the treatment of a range of metabolic abnormalities associated with PCOS, and further clinical trials will clarify this role. In the meantime weight reduction, another treatment that improves insulin sensitivity and results in lower androgen levels, remains the first line of treatment for PCOS [36].

**Gonadotropin-releasing hormone**

Lowering of androgen levels in patients with PCOS by means of gonadotropin-releasing hormone analogues has been shown to improve insulin sensitivity in patients with mild insulin resistance, but not in patients with severe insulin resistance [24,135]. This normalization of insulin sensitivity upon androgen lowering provides strong evidence for a direct action of androgens on glucose disposal in skeletal muscle. However, another study showed no effect of androgen lowering on insulin sensitivity [136]. The reason for the disagreement between studies may be that PCOS is a heterogeneous condition, with different levels of insulin sensitivity between patients and different mechanisms of insulin resistance. Thus the role of androgens in determining insulin sensitivity may be more important in some patients than others. The results of the above studies suggest that androgen-lowering treatment may only be effective at an early stage in the pathogenesis of the disorder, when insulin resistance is mild. Hyperglycaemia is well recognized to induce insulin resistance in vivo, and once established it will tend to promote insulin resistance independently of any other effects, such as those of androgens or TNF-α. As a result, androgen lowering at this late stage may no longer have a beneficial effect. This emphasizes the importance of recognizing and treating insulin resistance at an early stage in the process.

**FUTURE DIRECTIONS**

Although many clinical studies have suggested a link between sex steroids and insulin action, relatively few studies to date have investigated the molecular basis of these interactions, particularly in human cells. Future work on isolated human tissues and cell lines will undoubtedly clarify the role of oestrogens and androgens in the maintenance of normal insulin sensitivity, as well as their pathological role at supraphysiological levels. As such, these studies will usefully complement ongoing clinical investigations. A fuller understanding of the molecular basis of the effects of sex steroids on insulin sensitivity will suggest how these effects might be open to therapeutic intervention.

As insulin resistance underlies the metabolic syndrome and has a central position in the development of vascular disease, any conditions giving rise to it will remain a major focus for research. The recognition that PCOS is not just a disorder of fertility but has a variety of metabolic sequelae has led to an exponential increase in the amount of research carried out on this disorder in recent years. However, important questions remain to be answered about PCOS. What are the genetic factors predisposing to PCOS? How can the process leading to vascular disease be influenced in these individuals? Prospective studies in patients with PCOS will need to be carried out to investigate the effects of interventions on cardiovascular outcome in these patients. The long-term aims of research would be to identify at-risk individuals at an early stage by means of genetic testing, rather than on the basis of risk factor screening, and to be in a position to interrupt the progression towards cardiovascular disease. The ability to do this would clearly have huge epidemiological implications, given the prevalence of PCOS.
Since insulin-sensitizing agents are known to reverse insulin resistance and to modulate multiple cardiovascular risk factors, it is likely that a place will be established for them in the future management of PCOS as well as other conditions associated with the metabolic syndrome. These agents may also have potential uses in other situations where elevated levels of sex steroids are present for prolonged periods, e.g. CAH and the therapeutic use of sex steroids. At the present time less is known about other conditions associated with the metabolic syndrome. For them in the future management of PCOS as well as insulin resistance and to modulate multiple cardiovascu- 
14 Alessi, D. R., Andjelkovic, M., Caudwell, B. et al. (1996) Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J. 15, 6541–6551

REFERENCES


105 Tooke, J. (1999) The association between insulin resistance and endothelialopathy. Diabetes Obes. Metab. 1, (Suppl. 2), S17–S22


133 EHrmann, D. A., Schneider, D. J., Sobel, B. E. et al. (1997) Troglitazone improves defects in insulin action, insulin secretion, ovarian steroidogenesis and fibrinolysis in women with polycystic ovary syndrome. J. Clin. Endocrinol. Metab. 82, 2108–2116
134 Arlt, W., Autsch, R. J. and Miller, W. L. (2001) Thiazolidinediones but not metformin directly inhibit the steroidogenic enzymes P450c17 and 3 beta-hydroxysteroid dehydrogenase. J. Biol. Chem. 276, 16767–16771