

Direct effects of sex steroid hormones on adipose tissues and obesity

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Received 3 May 2004; revised 22 July 2004; accepted 3 August 2004

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Summary

Sex steroid hormones are involved in the metabolism, accumulation and distribution of adipose tissues. It is now known that oestrogen receptor, progesterone receptor and androgen receptor exist in adipose tissues, so their actions could be direct. Sex steroid hormones carry out their function in adipose tissues by both genomic and nongenomic mechanisms. In the genomic mechanism, the sex steroid hormone binds to its receptor and the steroid-receptor complex regulates the transcription of given genes. Leptin and lipoprotein lipase are two key proteins in adipose tissues that are regulated by transcriptional control with sex steroid hormones. In the nongenomic mechanism, the sex steroid hormone binds to its receptor in the plasma membrane, and second messengers are formed. This involves both the cAMP cascade and the phosphoinositide cascade. Activation of the cAMP cascade by sex steroid hormones would activate hormone-sensitive lipase leading to lipolysis in adipose tissues. In the phosphoinositide cascade, diacylglycerol and inositol 1,4,5-trisphosphate are formed as second messengers ultimately causing the activation of protein kinase C. Their activation appears to be involved in the control of preadipocyte proliferation and differentiation. In the presence of sex steroid hormones, a normal distribution of body fat exists, but with a decrease in sex steroid hormones, as occurs with ageing or gonadectomy, there is a tendency to increase central obesity, a major risk for cardiovascular disease, type 2 diabetes and certain cancers. Because sex steroid hormones regulate the amount and distribution of adipose tissues, they or adipose tissue-specific selective receptor modulators might be used to ameliorate obesity. In fact, hormone replacement therapy in postmenopausal women and testosterone replacement therapy in older men appear to reduce the degree of central obesity. However, these therapies have numerous side effects limiting their use, and selective receptor modulators of sex steroid hormones are needed that are more specific for adipose tissues with fewer side effects.

Keywords: Adipose, androgen, estrogen, obesity.

obesity reviews (2004) **5**, 197–216

Introduction

Obesity causes major health problems (1). It is a risk factor for non-insulin-dependent diabetes, cardiovascular disease, osteoarthritis, some types of cancer, and certain reproductive and metabolic disorders. This risk generally relates

more to the central (abdominal, omental, visceral) distribution of fat than to the amount of fat. The distribution of fat is different between males and females (2). Men have a more central accumulation of fat, whereas women have a more gluteal/femoral accumulation. Men also have a higher incidence of cardiovascular disease than women,

and menopause in women increases the incidence of cardiovascular disease and the central distribution of adipose tissue (3). This epidemiological and clinical evidence strongly suggests a major role for sex steroid hormones in the regulation of adipose tissue distribution. In developed countries, the prevalence of obesity is increasing, and recently it was reported that as much as 61% of the US population is overweight or obese (4).

The mechanism for the regulation of the amount and distribution of adipose tissues by sex steroid hormones is not clear. One possible mechanism would be the regulation of key proteins in adipose tissues at the genomic level by transcriptional means. This would require a steroid receptor to be present in adipose tissues, and convincing evidence now exists that oestrogen receptor (OR), progesterone receptor (PR) and androgen receptor (AR) are present in adipose tissues (5–9). Another possible mechanism would be the regulation of secondary messengers at the cell membrane by nongenomic effects. This would require a sex steroid receptor to be present in the plasma membrane, and there is now evidence of such receptors (10). A combination of genomic and nongenomic mechanisms of regulation by sex steroid hormones probably exists in most tissues (11). This review will discuss the mechanisms of action by sex steroid hormones on adipose tissues, their role in adipose accretion and metabolism, and how they might be used to ameliorate obesity.

Sex steroid receptors in adipose tissues

Rats

One of the first indications that OR exists in adipose tissues was reported by Wade & Gray in 1978 (5). They were able to show oestrogen-specific, high affinity [3H]-oestradiol binding in cytosolic extracts of ovariectomized rat adipose tissues. Binding was highest in parametrial fat and lowest in inguinal fat. It could be destroyed by treatment with pronase. This data indicated that OR existed in rat adipose tissues with similar properties to OR in reproductive tissue, but at a much lower concentration. In a subsequent paper, Gray & Wade (12) were able to show oestrogen binding sites in male rat adipose tissues. Gray *et al.* (13) also demonstrated binding of injected [3H]-oestradiol in the nuclei from adipose tissues. Using ovariectomized and adrenalectomized rats for conditions of low endogenous sex steroid production, Rebuffe-Scrive (14) demonstrated cytoplasmic oestradiol binding in adipose tissues from these animals that decreased dramatically after oestrogen administration. Pedersen *et al.* (15) showed nuclear binding of [3H]-oestradiol in isolated rat adipocytes. The binding was specific for oestradiol and showed regional variation. Using immunogold-electron microscopic localization, Echeverria *et al.* (16) were able to show significant labelling of OR in adipose

cells from various locations of both male and female rats. A higher density of label was found in the nucleus, but some label was observed in the cytoplasm of the adipose cells.

Progesterone receptor has not been extensively studied in rat adipose tissues. Gray & Wade (12) were not able to detect progestin binding sites in male adipose tissues. However, they (17) observed progestin binding sites in adipose tissues from ovariectomized–adrenalectomized rats that were primed with oestrogen. Androgen receptor also has not been extensively studied in rat adipose tissues. De Pergola *et al.* (18) used intact cultured cells from male rat epididymal fat pads to demonstrate androgen (R-1881) binding. These cells contained high affinity, specific binding sites for androgens. Exposure of the cells to R-1881 and testosterone, but not to dihydrotestosterone, markedly enhanced the androgen binding. Sjogren *et al.* (19) observed high affinity, specific androgen (R-1881) binding in nuclei from adipocytes. The binding showed regional differences with higher binding in epididymal and mesenteric adipose tissues than in retroperitoneal. All these results support the hypothesis that oestrogens, progestins and androgens may have a direct effect on adipose tissues.

Humans

In initial experiments, it was not possible to detect OR or PR in subcutaneous or omental fat from normal women (20,21). In more recent experiments with increased sensitivity of molecular techniques, low levels of OR, PR and AR have been found in human adipose tissues. Price & O'Brien (22) demonstrated by polymerase chain reaction (PCR) the presence of OR messenger RNA (mRNA) in human subcutaneous abdominal tissue. A higher amount of OR mRNA was found in adipocytes than in adipose stromal cells. Mizutani *et al.* (23) identified OR protein and OR mRNA in subcutaneous abdominal tissue. The OR protein had a molecular weight of 66 000, which is similar to the OR receptor in reproductive tissues. Pedersen *et al.* (24) demonstrated by ligand binding and reverse transcriptase (RT)-PCR the presence of OR in human adipose tissue and mature adipocytes, but not in preadipocytes. The receptor had a molecular weight of 65 kDa, which is similar to OR from other tissues. Cytosols from visceral adipose tissue and subcutaneous abdominal tissue of women showed about the same amount of OR binding, whereas cytosol of subcutaneous abdominal tissue from men showed a higher amount of binding than those from visceral adipose tissue. Recently, OR subtypes have been detected in human adipose tissues. Crandall *et al.* (25) identified OR-beta by RT-PCR in breast and subcutaneous abdominal tissues. OR-beta mRNA was expressed in the adipose tissue samples and in adipocytes and stroma/vasculature, but was not expressed in preadipocytes. Pedersen *et al.* (26) showed that both OR-alpha and OR-beta were present in cytosols of

adipose tissues from both men and women. Expression of OR- α was about the same in subcutaneous gluteal, subcutaneous abdominal and intra-abdominal adipose tissue, and was similar between men and women. The amount of OR- β -1 was lower in intra-abdominal adipose tissue than in the subcutaneous adipose tissues, with a fivefold lower concentration in women and a threefold lower concentration in men. Expression of OR- β -4 and OR- β -5 was higher in subcutaneous gluteal than in subcutaneous abdominal. Anwar *et al.* (27) have looked at the expression of OR- α and OR- β in stromal cells and adipocytes in human adipose tissues. By immunohistochemistry and RT-PCR analyses, OR- α and OR- β were detected in both stromal cells and adipocytes from subcutaneous and omental adipose tissues. Oestradiol treatment produced an up-regulation of OR- α and OR- β in stromal cells from both adipose tissues. However, in adipocytes from subcutaneous adipose tissue, oestradiol treatment produced a decrease in the expression of OR- α , while OR- β expression was increased. In omental adipocytes, the decrease in the expression of OR- α with oestradiol treatment was not observed. Joyner *et al.* (28) showed by RT-PCR that human preadipocytes express OR- α mRNA, but not OR- β mRNA, and possessed OR- α protein by Western blot. There were no regional differences between subcutaneous or visceral samples and no gender differences between males and females.

Besides OR, both PR and AR have been detected in human adipose tissues. In early experiments, PR was not detected in adipose tissue, mature adipocytes or preadipocytes (8). In contrast, O'Brien *et al.* (29) were able to identify PR by Northern blot analysis in subcutaneous abdominal adipose tissue of premenopausal women. The PR mRNA was higher in the stromal/vascular fraction than in the adipocyte fraction. Both the PR-A and PR-B isozymes were present in the human subcutaneous adipose tissue. Pedersen *et al.* (8) showed the presence of AR in adipose tissue, mature adipocytes and preadipocytes of subcutaneous abdominal fat from both men and women. The presence of AR in mature adipocytes and preadipocytes of both men and women has also been reported by Dieudonne *et al.* (30). Androgen binding sites were higher in intra-abdominal preadipocytes than in subcutaneous preadipocytes. There was a decrease of AR expression during adipogenesis and an up-regulation of AR by androgens *in vitro*. Joyner *et al.* (31) confirmed the presence of AR in subcultured human preadipocytes, and more AR was detected in visceral preadipocytes than in subcutaneous preadipocytes.

Sheep

Sheep is an excellent animal model to study the relationship of sex steroids to obesity. Obesity in sheep is easily induced

and maintained, and the animals develop many of the same deleterious effects, such as hypertension, insulin resistance and hyperinsulinemia, as seen in humans (32,33). Domestic sheep easily become obese simply by overeating and lack of exercise, as is the case for most human obesity. These animals are also large enough to collect serial tissue samples for experimentation. We (6) have studied OR in subcutaneous and internal fat depots from ovariectomized-adrenalectomized ewes. Scatchard, sucrose gradient and Western blot analyses all confirmed the presence of OR in the cytosolic fraction of gluteal, omental and perirenal adipose tissues. The content of OR was highest in gluteal fat and lowest in omental fat. These results support the presence of specific OR in adipose tissues of sheep with characteristics similar to those of OR from uterus. When oestrogen was given to ovariectomized-adrenalectomized ewes, it increased the PR in these adipose tissues (7). By using the same techniques as with OR, PR from oestrogen-treated sheep was induced in uterus >>> gluteal > adipose perirenal adipose > omental adipose. This up-regulation of PR by oestrogen is strong evidence that the OR in adipose tissues is functional.

In recent experiments, we (9) have concentrated on AR in sheep omental adipose tissue. Androgen receptor was determined in subcellular fractions by Western blot analyses. As anticipated, the receptor was found in the cytosolic fraction (presumably leaked from the nucleus), but a high concentration was also present in the microsomal fraction. A lesser amount was found in the plasma membrane fraction, and only a small amount was left in the nuclear-cell debris fraction. Three immunostaining bands with approximate molecular weights of 250, 140 and 110 kDa were detected in the cytosolic fraction. The 110-kDa band was predominant in the membrane fractions. A 104-kDa band was sometimes observed but appears to be a degradation product. Treatment with glycosidases resulted in the loss of the 250- and 140-kDa bands. To substantiate that the 250- and 140-kDa isoforms were glycoproteins, the cytosolic fraction was chromatographed on Con A Sepharose. The 110 kDa band was eluted in the 0.4-M KCL salt wash, while the 250- and 140-kDa bands were eluted with alpha-methylmannoside. Treatment of the glycoprotein (alpha-methylmannoside) peak with glycosidases converted the 250- and 140-kDa bands to the 110-kDa band. This data confirm the presence and distribution of AR in subcellular fractions of adipose tissue from sheep and suggest that it exists in various glycosylated isoforms.

Mice

The major advantage of working with mice is the development of techniques that allow for the manipulation of their genome. These techniques have been utilized to generate transgenic mice that lack functional OR (34). Oestrogen

receptor knockout mice have abnormalities in their adipose tissues (35). Heine *et al.* (36) showed significant increases in white adipose tissue, but not in brown adipose tissue, with advancing age in both male and female OR-alpha knockout mice. The OR-alpha knockout caused both adipocyte hyperplasia and hypertrophy. Energy intake was similar in wild-type and OR-alpha knockout male mice, indicating that obesity was not the result of hyperphagia. However, energy expenditure was reduced in the knockout mice, which may be related to the obesity in these animals. Both male and female OR-alpha knockout mice also caused insulin resistance and glucose intolerance. Ohlsson *et al.* (37) demonstrated an increase in total body fat after sexual maturity in OR-alpha knockout and double (OR-alpha/OR-beta) knockout mice, but not in OR-beta knockout male mice. An increase in serum cholesterol and a change in the lipoprotein profile were also observed in the mature OR-alpha knockout male mice. By studying oestrogen replacement in ovariectomized OR-alpha and/or OR-beta knockout mice, Lindberg *et al.* (38) were able to show a reduction of fat in these animals with oestrogen replacement that was mediated mainly by OR-alpha. From these studies, it is clear that OR-alpha is important in female and male white adipose deposition and metabolism, and may have important clinical implications. However, OR-beta may also have a role in adipose tissue, as Naaz *et al.* (39) reported that the loss of oestrogen/OR-beta signalling resulted in phenotypical and biochemical changes in adipose tissues opposite from those of oestrogen/OR-alpha signalling.

Androgen receptor knockout (ARKO) mice have recently been described by Yeh *et al.* (40). Total body weight of the ARKO male mice was less than the wild type and was similar to the body weight of normal females. Histology of subcutaneous adipose indicated that the size and number of adipocytes was different between wild-type and ARKO mice starting at 5 weeks and becoming more obvious at 8 weeks. In contrast, there was no difference in adipocytes from infrarenal adipose tissue, showing that androgen action is adipose tissue specific. Sato *et al.* (41) have also developed AR-deficient mice. Their ARKO male mice had growth curves similar to wild-type female littermates until the 10th week of age. Thereafter, the deficient male mice showed an increase in growth and the development of obesity. Wet weights of subcutaneous, infrarenal and intraperitoneal white adipose tissues were increased in the 30-week-old male ARKO mice, but not in gonadal white adipose tissue, again showing that androgen action is site specific.

A summary of sex steroid hormone receptors in adipose tissues is given in Table 1. It is clear that all three sex steroid hormone receptors (OR, PR, AR) are present in adipose tissues, suggesting that the action of sex steroid hormones could be direct. The OR and PR are higher in concentration

Table 1 Expression of sex steroid hormone receptors in adipose tissues

Receptor	Gender	Visceral fat	Subcutaneous fat
OR-alpha	Female	+	++
OR-beta	Female	+	+++
OR-alpha	Male	+	+
OR-beta	Male	+	+++
PR-A	Female	-	+
PR-B	Female	+	++
PR-A	Male	-	-
PR-B	Male	-	-
AR	Female	++	+
AR	Male	++	+

OR, oestrogen receptor; PR-A, progesterone receptor; PR-B, xxxxxx; AR, androgen receptor.

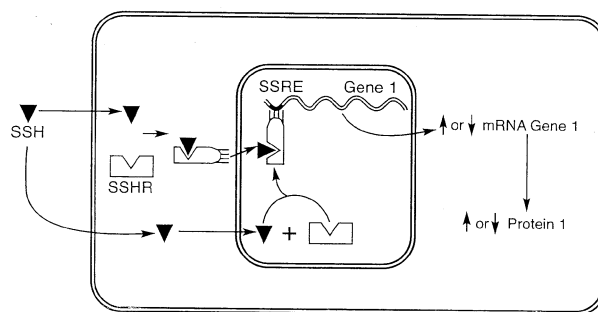


Figure 1 Mechanism of action of sex steroid hormones as transcriptional regulators. SSH, sex steroid hormone; SSHR, sex steroid hormone receptor; SSRE, sex steroid response element.

in subcutaneous fat than in visceral fat in both males and females, whereas the opposite is true for AR, with visceral fat having a higher concentration than subcutaneous fat in both males and females. These differences in concentrations of OR, PR and AR in visceral adipose tissue and subcutaneous adipose tissue may offer a possible explanation why men have a more central accumulation of fat, whereas women have a more gluteal/femoral accumulation.

Transcriptional regulation of proteins in adipose tissues by sex steroid hormones

In the classical mechanism of action of sex steroid hormones (Fig. 1), the steroid enters the cell and binds with high affinity to a specific receptor. The steroid-receptor complex then undergoes a conformation change and binds to a specific response element on the DNA. Once bound, it regulates the transcription of a given gene. Transcription may be up-regulated or down-regulated depending upon the gene, the steroid and the coregulatory proteins. There may be two possibilities for this process. In one route, the steroid combines with its receptor in the

cytoplasm and the steroid–receptor complex is then transported to the nucleus, possibly involving the cytoskeletal network or the motor protein complexes, such as the non-muscle myosin and actin motor, the kinesin and microtubule motor, or the dynein and microtubule motor. Heinlein & Chang (42) have reviewed the possibility that androgens enter the cell, binds to AR along with coregulators, and the complex is translocated along actin filaments to the nucleus. Because adipocytes have little cytoplasm due to the presence of large lipid deposits, these cells display a different type of polymerized actin, commonly known as cortical actin (43). Cortical actin lines the inner surface of the plasma membrane and could serve as a receptor attachment point within these cells. In a second route, the steroid binds to the receptor in the nucleus. It is generally accepted that OR is constitutively nuclear and free oestrogen passes through the cytoplasm to bind with the nuclear OR (44). Because of the lipophilic nature of steroids, it is harder to conceive of free steroid trafficking through the cytoplasm of the adipocyte. The adipocyte is packed with lipid droplets and the free steroid should readily partition into this lipid compartment. Further research is needed to define the intracellular trafficking of sex steroid hormones and their receptors in this unique cell type. Many genes in adipose tissues are no doubt transcriptionally regulated by sex steroid hormones. Two key proteins that are involved in lipid deposition are lipoprotein lipase and leptin.

Lipoprotein lipase

Lipoprotein lipase (LPL) is the key enzyme for the hydrolysis of circulating triglycerides into free fatty acids and glycerol (45). Although LPL is found in many tissues, its highest activity is located in adipose tissues, heart and skeletal muscle. It plays a major role in adipocyte lipid storage and muscle fuel supply and hence the regulation of lean muscle mass and obesity. It is synthesized and secreted by the adipocyte and then transported to the capillary endothelium, where it hydrolyses the circulating triglycerides into free fatty acids, which in turn are stored in the adipose tissues. Heparin releases LPL from the capillary endothelium into the bloodstream, so LPL is often assayed in postheparin plasma.

The hormonal control of LPL in adipose tissues is very complex, involving several hormones, and regulation occurs at the transcriptional and post-transcriptional level (46). In general, cortisol and insulin appear to promote lipid accumulation by increasing the activity of LPL, while growth hormone and oestrogen appear to exert opposite effects (2). Although it is well documented that sex steroid hormones regulate the activity of LPL, it is not clear whether the regulation is direct or indirect, and if direct, whether the regulation is genomic or nongenomic.

Rats

In female rats, ovariectomy leads to obesity, and oestrogen replacement inhibits the obesity triggered by ovariectomy. The mechanism of this oestrogen action may be the regulation of LPL. Several studies have shown that ovariectomy of female rats results in increased adipose tissue LPL, while oestrogen replacement decreased the LPL activity (47–55). Progesterone alone appeared to increase LPL activity (49,51). However, progesterone in combination with oestrogen attenuated the oestrogen effects in these animals (53). Androgens may also play a role in the regulation of LPL. Postheparin LPL activity was much greater in female rats than in males (56). However, the gender difference in LPL activity appears to be breed specific as it was observed in Wistar rats, but not in Sprague-Dawley rats (57). Postheparin LPL activity was decreased by testosterone administration in both male and female rats (58). Oestradiol administration to male rats significantly reduced adipocyte LPL activity (59). Adipose tissue LPL activity was increased in orchidectomized rats (60). In contrast, Peinado-Onsurbe *et al.* (61) reported that orchidectomy had no significant effect on adipose tissue LPL activity or mRNA levels. Androgen replacement showed no significant effect on adipose tissue LPL activity or mRNA levels, but oestrogen treatment of the orchidectomized male rats substantially decreased the LPL activity and mRNA levels. Therefore, some of the effects of androgens on LPL activity may be caused by the aromatization of testosterone to oestradiol, as adipose tissues have aromatase activity. Vikman-Adolfsson *et al.* (62) suggested that growth hormone, but not gonadal steroids, influence LPL activity in hypophysectomized rats. Hypophysectomy decreased LPL activity, and neither oestrogen nor testosterone had any effect on LPL, but growth hormone normalized the LPL activity. From these results, they conclude that gonadal steroids regulate LPL activity indirectly through their influence on growth hormone.

Humans

In human subjects, postheparin LPL activity has been a convenient way to study LPL regulation by sex steroid hormones. In obese women, fasting postheparin plasma LPL activity showed an inverse correlation with plasma oestradiol levels and a positive correlation with plasma-free testosterone (63). The administration of oestrogen–progestin containing contraceptives (64–68) or oestrogen alone (69) to women is known to increase serum triglyceride concentrations and to depress postheparin lipolytic activity. A transdermal oestradiol patch placed in the gluteal region of women decreased LPL in adipose tissue from beneath the patch as compared to tissue beneath the placebo patch (70). However, there was no difference in LPL mRNA levels between these two groups, suggesting that a possible post-transcriptional modification of LPL led to the differ-

ence. In addition, the effect of oestrogen on LPL activity may be concentration dependent, as Palin *et al.* (71) found that a high concentration (10^{-7} mol L⁻¹) of oestrogen reduced LPL expression in isolated adipocytes from subcutaneous abdominal fat of women, while lower concentrations increased LPL expression.

In other studies, postmenopausal women who underwent treatment with both oestrogen and progestins had higher LPL activity in subcutaneous femoral adipocytes (72), and the local application of progesterone resulted in increased LPL activity in human femoral adipose tissue (73), suggesting that progestins might also be involved in the regulation of LPL. These results, when taken with data on OR presence and up-regulation of PR by oestrogen in gluteal/femoral adipose tissue (6,7), perhaps offer an explanation for this adipose depot accretion in women.

Androgens may also play a key role in the regulation of LPL and the distribution of fat in both men and women (74–76). Lipoprotein lipase activity in femoral adipose tissue from men has been shown to be inversely correlated with plasma levels of testosterone, bioavailable testosterone, dihydrotestosterone and oestradiol, while LPL activity in abdominal adipose tissue was inversely correlated with bioavailable testosterone only (77). These results suggest differential regulation of specific adipose depots and possible regulation of central fat accumulation. When moderately obese middle-aged men were given testosterone, LPL activity was down-regulated in subcutaneous abdominal adipose tissue, but the activity was not regulated in subcutaneous femoral adipose tissue, and dihydrotestosterone had no effect on LPL activity (78,79). These changes in LPL activity by androgens could be caused by aromatization of testosterone to oestradiol. Zmuda *et al.* (80) studied this possibility in men who were given the aromatase inhibitor testolactone. Postheparin plasma LPL activity was unaffected by testosterone or testolactone alone, but with combined testosterone and testolactone treatment, there was an increase in postheparin plasma LPL activity. This suggests that aromatization of testosterone to oestradiol may counteract some of the effects of androgens. In order to study a more localized effect, Anderson *et al.* (81) investigated the effect of androgens on LPL activity in cultured subcutaneous abdominal adipocytes from women. Dihydrotestosterone stimulated LPL protein expression in these cells, and flutamide, an antiandrogen, blocked the dihydrotestosterone (DHT) stimulation of LPL protein expression. Postheparin LPL has also been studied in transsexual subjects undergoing cross-sex hormone therapy (82). In 20 male-to-female transsexuals, treatment with oestrogen and antiandrogen for one year decreased the postheparin LPL activity. In 17 female-to-male transsexuals, postheparin LPL activity was unaffected by treatment with testosterone for one year.

In conclusion, oestrogen generally decreases the production of LPL in adipose tissues in both rats and humans. The

effects of androgens on LPL in adipose tissues are less clear because of the aromatization of androgen to oestrogen. However, when an aromatase inhibitor was given to men along with testosterone, LPL activity was increased in postheparin plasma, suggesting that androgens may increase LPL production in adipose tissues. If sex steroid hormones are going to directly regulate LPL, a sex steroid hormone response element should be present in the LPL genome. Homma *et al.* (83) prepared a pLPL(1980)-CAT construct, along with an OR expression vector, and introduced this into differentiated fat (3T3-L1) cells. Oestrogen markedly decreased the LPL mRNA in the genetically manipulated cells. A search of the LPL promoter for an oestrogen response element did not find a classical ORE but demonstrated an AP-1-like TGAATTC sequence that was responsible for the suppression of the LPL gene transcription by oestrogen.

Leptin

Leptin is a recently discovered protein hormone that is the product of the obese gene. It plays a key role in the regulation of food intake, energy expenditure and body weight homeostasis (84). It is produced mainly in adipose tissues. Therefore, the amount of leptin is related to the amount and distribution of body fat.

Humans

Leptin blood levels are much higher in women than in men (85). Whether this is related to sex steroid hormones remains unclear. Because women have more body fat and different distribution of fat than men, it has been difficult to relate sex steroid hormones to blood leptin concentrations. In support of oestrogen regulating leptin production, Hardie *et al.* (86) reported a correlation between serum leptin levels and serum oestrogen levels. As oestrogen levels decrease with menopause, a decrease in leptin levels might be expected with menopause. Some investigators (87–89) have reported a decrease in serum leptin with menopause, while others (90–94) reported no change in leptin levels. Similar discrepancies exist in results with hormone replacement therapy (HRT) in postmenopausal women and leptin levels, with some authors (89,95) reporting an increase in leptin with HRT and others (90,94,96–100) reporting no change in the level with HRT. Thomas *et al.* (101) found that bioavailable oestrogen levels in postmenopausal women not on HRT were significantly and directly related to serum leptin levels, but in premenopausal women or in women on HRT, variations in oestrogen level were not associated with the higher leptin levels, suggesting a possible threshold effect. In a recent report, Tommaselli *et al.* (102) showed that leptin levels on days 5 and 15 after bilateral ovariectomy did not differ from baseline values even though oestrogen levels were greatly reduced. With

HRT in these women, the leptin levels were unmodified after 6 months of treatment. However, the untreated control group did show an increase in leptin levels, which may have been caused by changes in fat patterns. It is possible that the effects of menopause and HRT on leptin levels may be a result of increases in body weight, body mass index, and central fat distribution during menopause and reversal of these by HRT (103). The most convincing evidence that the sex steroid hormones affect blood leptin levels come from studies on cross-sex administration of sex steroid hormones to transsexuals. Elbers *et al.* (104) were able to show that oestrogen and antiandrogen administration to male-to-female transsexuals greatly increased the serum leptin levels and testosterone administration to female-to-male transsexuals decreased the serum leptin levels. They concluded that the sex steroid milieu plays an important role in the regulation of blood leptin levels.

Studying human omental adipose tissue *in vitro*, Casabiell *et al.* (105) demonstrated that leptin secretion was higher in tissue from women than in tissue from men. Dexamethasone and oestradiol stimulated leptin secretion in fat from women, but not in fat from men. In contrast, Kristensen *et al.* (106) could show no direct effects of oestrogens or androgens on adipose tissue leptin production in female human subcutaneous abdominal tissue explants *in vitro*.

As androgens are known to affect fat accretion and metabolism (74), an effect on leptin production might be expected. In males, several studies (107–109) have reported an inverse relationship between serum testosterone and leptin concentrations. Administration of testosterone to men appears to decrease the levels of leptin. Jockenhovel *et al.* (110) found that serum leptin levels were three times higher in hypogonadal men than in normal men and that testosterone administration normalized the leptin levels in the hypogonadal men. Testosterone treatment resulted in an increase in serum testosterone, dihydrotestosterone and oestradiol. Using multiple regression analysis, the androgen (testosterone plus dihydrotestosterone)/oestrogen ratio was the only significant determinant of serum leptin levels. These results could have been caused by aromatization of androgens to oestrogen, but Luukkaa *et al.* (111) reported that inhibition of oestrogen biosynthesis with an aromatase inhibitor did not affect serum leptin levels in young men. Nowicki *et al.* (112) were able to show that androgen blockage in patients with advanced prostate cancer greatly increased leptin levels. Lambert *et al.* (113) showed in elderly men ingesting megestrol acetate for weight gain that testosterone replacement decreased the level of leptin. In females, this inverse relationship between testosterone and leptin levels may not exist, as Blum *et al.* (107) did not observe this inverse relationship in girls undergoing puberty and adolescents. In human omental adipose tissue *in vitro*, Pineiro *et al.* (114) were able to show that dihy-

drotestosterone, stanozolol, androstenedione and dehydroepiandrosterone sulphate inhibited leptin secretion in fat samples from women without affecting the secretion in samples from men. Testosterone did not affect secretion in either gender. Recently, Machinal-Quelin *et al.* (115) have studied the direct *in vitro* effects of androgens and oestrogens on leptin secretion in human adipose tissue. In subcutaneous adipose tissue fragments from men, only dihydrotestosterone at high concentration (100 nM) reduced leptin secretion, but not dihydrotestosterone at 10 nM, testosterone, dehydroepiandrosterone (DHEA), or oestradiol. In subcutaneous adipose tissue fragments from women, oestradiol increased leptin secretion, but not dihydrotestosterone or testosterone when combined with an aromatase inhibitor. They concluded that the difference in plasma leptin levels between men and women was mainly the result of stimulation of leptin expression by oestrogen in adipose tissue of women.

Rats

As in humans, female rats have higher circulating levels of leptin than do males (116–118). Ovariectomy of female rats has been shown by some investigators to lower circulating leptin levels, and oestrogen replacement in these animals has been found to return the levels back to normal (88,119). Other investigators, however, found no effect on circulating leptin levels after ovariectomy and oestrogen replacement (116–118,120–122). These discrepancies could be caused by the time of the blood samples, as Chu *et al.* (123) showed that leptin levels declined during the first 7 weeks after ovariectomy. Subsequently, its concentration increased, and by the end of 13 weeks the value was higher than the level before ovariectomy. The fluctuation of serum leptin levels with ovariectomy was eliminated by oestrogen replacement. These discrepancies could also be caused by weight gain in the ovariectomized animals, as leptin levels are related to the amount of fat, which was not always controlled in these experiments.

Ovariectomy has also been reported by some authors to decrease leptin gene expression in white adipose tissues of rats, and this decrease can be reversed by oestrogen replacement (88,119,120,122). In contrast, other authors have reported no effect of oestrogen replacement on leptin gene expression in white adipose tissues (116,121). These discrepancies could be caused by differences in leptin secretion and regulation in various adipose sites, as Shimizu *et al.* (88) showed that although leptin gene expression decreased in subcutaneous and retroperitoneal white adipose tissues of ovariectomized rats as compared to controls, leptin gene expression actually increased in mesenteric white adipose tissues of the ovariectomized rats. Machinal *et al.* (122) also observed that ovariectomy resulted in a 25% decrease in leptin gene expression in perirenal adipocytes and a small but insignificant decrease in subcutaneous adipo-

cytes. Therefore, the regional distribution of adipose tissues may greatly influence leptin levels and its regulation by sex steroid hormones.

Androgens could also be involved in the regulation of leptin production in rats. Wu-Peng *et al.* (116) found that administration of testosterone to ovariectomized female rats decreased the expression of leptin mRNA but did not change plasma leptin levels. However, Pinilla *et al.* (117) found that testosterone given to ovariectomized female rats decreased the serum leptin levels as compared to ovariectomized only. In male rats of similar body weights, orchidectomy caused a rise in plasma leptin levels, which was abolished with testosterone administration (117,118). In contrast, Shimizu *et al.* (124) showed that serum leptin levels were decreased by orchidectomy in fatty rats, and testosterone supplement reversed this decrease. Nazian (125) showed that serum leptin rises in male rats from immaturity to young adults as the serum testosterone also rises. Castration of immature rats with or without testosterone replacement for 1 week did not result in a change of serum leptin levels. Machinal *et al.* (122) observed that castration caused a twofold increase in leptin gene expression in perirenal adipocytes, but a 50% decrease in expression in subcutaneous adipocytes. Watanobe & Habu (126) have recently suggested that a difference in neonatal gonadal steroid milieu might permanently affect leptin secretion. In neonatally castrated male rats, a twofold increase in leptin levels was observed on postnatal day 57 as compared to intact males. This increase in leptin levels was prevented by testosterone when given neonatally, but not on day 29. Neonatal testosterone had no effect on leptin titres in female rats in later life.

Leptin production has been studied *in vitro* in order to determine a more direct action of sex steroids on adipose tissues. Machinal *et al.* (122) carried out *in vitro* studies on isolated adipocytes. In cells from normal female rats, obese (*ob*) gene expression was reduced by 20% after a 24-h exposure to dihydrotestosterone in both perirenal and subcutaneous adipocytes. This decrease was prevented by the antiandrogen, cyproterone acetate and actinomycin D. However, leptin secretion from cells of both adipose tissues was unchanged after exposure to dihydrotestosterone. In adipocytes from ovariectomized female rats, a 24-h exposure to 17-beta oestradiol caused an increase in *ob* gene expression and an increase in leptin secretion in cells from subcutaneous, perirenal and parametrial adipose tissues. These increases could be prevented by antiestrogen and actinomycin D. Kristensen *et al.* (127) also studied the steroid regulation of leptin secretion and expression in rat adipose tissue fragments *in vitro*. Basal leptin production was equal in epididymal fat fragments from males and parametrial fat fragments from females. Oestradiol increased leptin secretion in both male and female adipose fragments, but the effect was greater in female adipose

tissue. Progesterone, testosterone, dihydrotestosterone and dehydroepiandrosterone-sulphate had no effect on leptin secretion. Parametrial fat from ovariectomized rats had lower leptin secretion compared to sham-operated controls, and oestradiol treatment of ovariectomized rats maintained a normal leptin secretion in fat fragments. Nazian (125) showed that cultured epididymal fat pads from adult rats with higher testosterone levels secreted more leptin than those from immature rats. Castration of immature rats with or without testosterone replacement for 1 week did not change the ability of the epididymal fat to secrete leptin. However, exposure of the epididymal fat *in vitro* to testosterone resulted in an enhanced secretion of leptin into the media.

Mice

Sex steroid hormone regulation of leptin production has also been studied in normal and mutant mice. Nedvidkova *et al.* (128) showed a decrease of serum leptin levels in oestrogen-treated male mice. However, in female mice Pelleymounter *et al.* (129) reported that ovariectomy or oestradiol administration did not alter leptin levels. Kronfeld-Schor *et al.* (130) demonstrated that subcutaneous implants of oestradiol or corticosterone into lactating mice stimulated leptin secretion rates of omental adipose tissue *in vitro*, and corticosterone, but not oestradiol, stimulated leptin secretion rates when added to isolated omental adipose tissue. From this study, the authors suggest that oestradiol could be acting indirectly on the omental adipose tissue possibly through an increase in plasma corticosterone levels. In OR-alpha knockout mature male mice, Ohlsson *et al.* (37) demonstrated an enhanced serum leptin level as compared to controls that was not seen in OR-beta knockout mice. Jones *et al.* (131) showed an elevation of leptin levels in aromatase knockout male and female mice. However, both the OR-alpha knockout mice and aromatase knockout mice had an increase in fat mass, which could account for the increased leptin levels.

In summary, oestrogen would appear to increase leptin production, while androgen would appear to decrease its production. However, many discrepancies exist in the data. If sex steroid hormones are going to directly regulate the production of leptin, a sex steroid hormone response element should be present in the leptin gene. After reviewing the cDNA sequence of the leptin gene, Shimizu *et al.* (88) concluded that this gene had a consensus sequence of the oestrogen response element in its promoter region. O'Neil *et al.* (132) transfected a leptin-luciferase reporter construct into OR-positive MCF-7 breast cancer cells and in OR-negative JEG-3 choriocarcinoma cells, which normally produce leptin. Oestradiol stimulated leptin-luciferase activity and antiestrogens inhibited leptin-luciferase activity in the JEG-3 choriocarcinoma cells that were cotransfected with OR-alpha, but did not stimulate leptin-luciferase activity in

the MCF-7 breast cancer cells. In JEG-3 cells cotransfected with OR-beta, leptin-luciferase was not stimulated by oestradiol. These results suggest that the leptin gene has an oestrogen response element; that leptin promoter activation may depend upon coactivators present in leptin-producing cells; and that different effects of oestrogen may be due to the type of OR expressed in target tissue.

Nongenomic regulation of lipid metabolism by sex steroid hormones

Although transcriptional regulation by steroid hormones has been the most studied mechanism of steroid action, recent studies have shown that a nongenomic mechanism also exists for steroid hormone action (133). Nongenomic actions of steroids are generally rapid responses occurring in less than 10 min, while transcriptional regulation requires several hours to days (134–136). In nongenomic action, the hormone binds to a receptor in the plasma membrane and a second messenger carries out its action (Fig. 2). Sex steroid membrane receptors have been characterized in many different tissues (10), but little is known about the receptors in adipose tissues. Anwar *et al.* (27) were able to show immunostaining for both OR-alpha and OR-beta in the cellular membranes of subcutaneous abdominal and omental human adipose tissues. We (9) demonstrated by Western blot analyses the presence of a small amount of AR in the plasma membrane fraction of sheep omental adipose tissue and Dos Santos *et al.* (137) found immunoreactive OR-alpha in the membrane fraction from cultured rat adipocytes, but not in the membrane fraction from preadipocytes. These plasma membrane steroid receptors are associated with caveolae endocytic vesicles. Caveolin-1 was shown to co-immunoprecipitate with OR-alpha and to bind directly to OR-alpha (138,139). Caveolin-1 has also been shown to interact with AR (140). Caveolin-1 has also been reported to bind to endoplasmic

reticulum membranes (141). Under these conditions, one would expect to find sex steroid receptors in the microsomal fraction of the cell. In early work, Watson & Muldoon (142) demonstrated the presence of OR in the microsomal fraction from rat uterus. Steroid receptors have since been shown to occur in the microsomal fraction from different target tissues (143). We (9) have recently reported a high concentration of AR in the microsomal fraction from sheep omental adipose tissue, which would support a physiologic role at this site.

The mechanism of action of sex steroid hormones by membrane sex steroid receptors is still not completely understood but probably involves multiple systems (144–147). Sex steroid hormones appear to regulate various components of the membrane signalling systems, including both the cAMP cascade and the phosphoinositide cascade (148–150).

CAMP cascade

Rats

Early studies showed an increase in cAMP and lipolysis within 5 min when isolated rat adipocytes were treated with oestradiol (151) and adenylate cyclase activity in fat cell mass increased by oestradiol treatment of ovariectomized rats (152). This response to oestrogen appears to be depot specific as ovariectomy caused a decrease in cAMP production in rat parametrial adipocytes, but not in femoral subcutaneous adipocytes (153). These studies with rats would suggest that oestrogen increased cAMP in adipose tissues, resulting in stimulation of hormone-sensitive lipase and increased lipolysis. Androgens also appear to regulate the cAMP cascade in rat adipose tissues. In castrated male rats, the production of cAMP in cultured preadipocytes was lower in deep intra-abdominal (epididymal) preadipocytes, but not subcutaneous preadipocytes, and testosterone replacement partially restored the decreased cAMP production (154). In adipose precursor cells from male rats, testosterone, but not dihydrotestosterone, stimulated catecholamine-induced lipolysis (155). Both testosterone and dihydrotestosterone stimulated forskolin-induced lipolysis, suggesting an effect on adenylate cyclase. Aromatase inhibitors did not have any effect on these changes, suggesting they were not mediated by conversion of androgen to oestrogen. In male rat adipocytes, castration decreased the catecholamine-induced, as well as the forskolin-induced, lipolysis and testosterone treatment normalized lipolysis (156). In female rats, ovariectomy decreased lipolysis in adipocytes that was stimulated by beta-adrenergic reagents, and treatment of these animals with testosterone normalized lipolysis (157). Isoproterenol-induced cAMP accumulation was decreased in rat white adipocytes by castration and restored by testosterone treatment (158). The lipolytic effect of N⁶-monobutyryl-

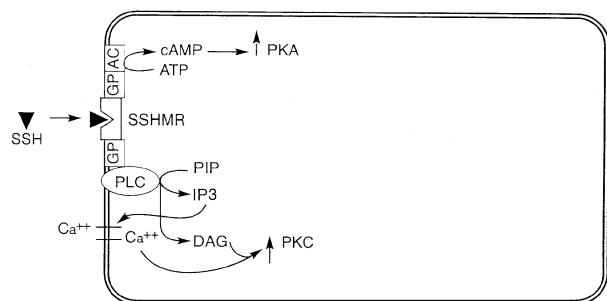


Figure 2 Scheme for nongenomic action of sex steroid hormones on adipose tissues. SSH, sex steroid hormone; SSHMR, sex steroid hormone membrane receptor; GP, G-protein; AC, adenylate cyclase; PKA, protein kinase-A; PLC, phospholipase-C; PIP, phosphatidyl inositol 4,5-bisphosphate; IP3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PKC, protein kinase C.

cAMP was also reduced after castration and was restored by testosterone, providing additional support for androgen regulation of this pathway.

Hamsters

Hamsters appear to have an opposite lipolytic response from rats to oestrogen on adipose tissues as oestrogen decreased lipolysis in adipocytes through a reduction in adenylate cyclase activity (159).

Humans

In humans, oestrogens also appear to decrease lipolysis in adipose tissues. Lindberg *et al.* (160) found that women who were given oral ethinyl oestradiol had a decrease in noradrenaline-stimulated lipolysis in subcutaneous abdominal adipocytes. To study the effect of oestrogens on lipolysis in women, Pedersen *et al.* (161) used adipocytes that were isolated from adipose tissue fragments, which were incubated *in vitro* with or without oestradiol. A decrease in adrenaline-stimulated lipolysis was observed in adipocytes from subcutaneous oestradiol-treated adipose tissue fragments. Oestradiol also increased the number of antilipolytic α 2A-adrenergic receptors in subcutaneous adipocytes. These results suggested that oestrogen lowered lipolysis by increasing the number of antilipolytic α 2A-adrenergic receptors. In contrast, no effect of oestrogen was observed in adipocytes from intra-abdominal fat. This difference in the oestradiol effect on α 2A-adrenergic receptors between subcutaneous fat and intra-abdominal fat may be one factor to explain how oestrogen maintains the gynoid fat pattern in women. This increase in α 2A-adrenergic receptors with oestrogen in subcutaneous fat from women may also explain the difference in response to oestrogen between humans and rats as adipose tissue from rats do not have α 2A-adrenergic receptors (160). Further studies by Elbers *et al.* (162) on transsexuals undergoing cross-sex hormone therapy support an effect of sex steroid hormones on lipolysis. In male-to-female transsexuals, treatment with oestrogen and antiandrogen for one year resulted in a reduction of basal lipolytic activity in adipocytes from both gluteal and abdominal subcutaneous adipose tissues. In female-to-male transsexuals, treatment with testosterone for one year resulted in an increase of basal lipolysis in adipocytes from abdominal subcutaneous adipose tissue, but not in adipocytes from gluteal subcutaneous adipose tissue. The cross-sex hormone therapy in male-to-female transsexuals resulted in an increase in the area of subcutaneous fat on magnetic resonance imaging (MRI), while testosterone treatment in female-to-male transsexuals resulted in a decrease in the area of subcutaneous fat. This supports the concept that oestrogens may play a role in a gynoid distribution of fat in women, while androgen may play a role in an androidal distribution of fat in men.

Phosphoinositide cascade

Rats

The phosphoinositide cascade also appears to be regulated by sex steroid hormones. Protein kinase C content of rat subcutaneous and parametrial fat cells were reduced by ovariectomy and restored to normal by oestradiol and progesterone treatment (163). Lacasa *et al.* (164) demonstrated that ovariectomy increased proliferation, mitogen-activated protein (MAP) kinase activity and c-fos protein in preadipocytes from rat perirenal fat depots. Treatment of the ovariectomized animals with oestradiol and progesterone reversed the increased proliferation and c-fos protein induction, but not the MAP kinase activation. The ovarian status had no effect on these parameters in subcutaneous preadipocytes, indicating that this regulation is depot specific. Recently, Dos Santos *et al.* (137) found that oestrogen activated p42/p44 MAP kinase by phosphorylation in rat white adipocytes through a rapid nongenomic pathway. This pathway was postulated to involve membrane OR-alpha, G-protein, protein kinase C, raf and MAP kinase kinase (MEK). The phosphoinositide cascade appears to be involved in adipose proliferation and differentiation. This cascade may also be regulated by androgens. Protein kinase C was decreased by castration in rat intra-abdominal (epididymal) adipocytes and was restored to normal by testosterone, but androgen status did not alter protein kinase C in subcutaneous adipocytes (165). This decrease in protein kinase C by castration was accompanied by a decrease in adipose tissue weight in epididymal fat pads, and testosterone partly restored this weight reduction. The regulation of protein kinase C appears to involve the MAP kinase/c-fos signalling pathway as castration resulted in an increase in MAP kinase activity and upstream activators raf-1 and MEK, along with a reduction in c-fos protein (166). Testosterone treatment corrected the effect of castration on raf-1 and c-fos, but not on MEK and MAP kinase. Dieudonne *et al.* (167) have reported opposite effects of androgens and oestrogens on adipogenesis in rat preadipocytes, with androgens eliciting an antiadipogenic effect and oestrogens having a proadipogenic effect. They showed that these opposite effects of androgens and oestrogens may be related to changes in insulin-like growth factor 1 (IGF-1) receptor and peroxisome proliferator-activated receptor γ 2. In summary, the androgenic status would appear to affect the proliferation and differentiation of specific adipose tissue.

As our knowledge of the action of sex steroid hormones expands, it seems probable that a combination of genomic (receptor DNA interactions) and nongenomic (membrane receptors) mechanisms are involved in sex steroid regulation of adipose accretion and metabolism (11,168). A summary of sex steroid regulation of key proteins and pathways in adipose tissues is shown in Table 2. It is based

Table 2 Hormonal regulation by sex steroids of proteins and pathways in adipose tissues

Protein or pathway	Action estrogen			Action androgen		
	bl	sc	vis	bl	sc	vis
Lipoprotein lipase*	↓	↓↑ [‡]	↓	↑	↑	↓
Leptin [†]	↑	↑	↑	↓	↓	↓
cAMP cascade	–	↓ [§]	↑ [¶]	–	↑ [§]	↑ [¶]
Phosphoinositide cascade	–	↑ [¶]	↑ [¶]	–	ud	↑ [¶]

bl, blood levels; sc, subcutaneous adipose tissue; vis, visceral adipose tissue; ud, undertermined.

*Based on studies in rats and humans including blood levels, *in vivo* and *in vitro* adipose tissues, and cross-sex hormone therapy in transsexuals.

[†]Based on studies in rats, humans, and mice including blood levels, *in vivo* and *in vitro* adipose tissues, and cross-sex hormone therapy in transsexuals.

[‡]Based on studies with oestrogen and progesterone.

[§]Studies in humans.

[¶]Studies in rats.

on studies in different species using many different techniques. This table represents a consensus of opinion of the authors that best fits the data, realizing that different results may exist for different species, sexes, adipose sites and body conditioning (i.e. normal vs. clinically obese). In the genomic regulation of the proteins, oestrogen and androgen generally have opposite effects. When androgens are shown to have actions similar to oestrogen, aromatization of testosterone to oestradiol may be involved and could explain the results. Oestrogens generally have a negative action on lipoprotein lipase. However, when progesterone was added with oestrogen, a positive action on lipoprotein lipase was seen in subcutaneous femoral adipose tissue. Androgens generally have a positive action on lipoprotein lipase in subcutaneous adipose tissue, but a negative action in visceral adipose tissue. Oestrogens appear to have a positive action on leptin production, while androgens have a negative action. As is the case many times, it is probably the oestrogen/androgen ratio that is important in the net regulating effect. As might be expected, the nongenomic regulation by sex steroid hormones of the cAMP cascade and the phosphoinositide cascade is very complex. Confounding data in this area makes the task attempted in Table 2 difficult, as there are species variations as well as tissue variables. The data for the cAMP cascade reflect a shift in the sensitivity of these tissues responsiveness to lipolytic hormones such as adrenaline. The phosphoinositide cascade data only reflect the relative activity of this multicomponent cascade as influenced by sex steroid hormones. The unique effects that appear to occur in the different types of adipose tissues as reflected by the subcutaneous and visceral grouping are dependent on a number of variables. For example in the gluteal/femoral adipose

depot in women, oestrogen and progesterone appear to work together to increase LPL, decrease lipolysis via changes in the cAMP cascade and push adipogenesis via the phosphoinositide cascade, which may explain the higher regional subcutaneous fat generally present in the normal female. It would appear that the data in general support opposite roles for androgens and oestrogens, with the latter being proadipogenic. It is clear that sex steroid hormones have a direct effect on key proteins and pathways in adipose tissues, but further experiments will be required to determine how this affects adipose accretion and obesity.

Role of sex steroid hormones in prevention and treatment of obesity

Because obesity and its associated health-related problems have become so prevalent in developed countries, many programs and strategies have been developed to prevent and/or treat this affliction but without much success (169–175). The three current major strategies to prevent and/or treat obesity are nutritional, pharmacological and surgical (Fig. 3). Another possibility could be hormonal intervention, involving the sex steroid hormones. Although sex steroid hormones have a major role in lipid metabolism and deposition in adipose tissues, they or their derivatives have not been extensively used to prevent or treat obesity. One area where sex steroid hormones appear to have a positive effect on obesity is in HRT in older women (3). Menopause in women causes a large decrease in oestrogens, progestins and androgens with a concomitant increase in total and central obesity (176–179). This increase in central obesity leads to an increased risk in cardiovascular, diabetes and other disorders in postmenopausal women (1). A relationship of central obesity and its complications to the decrease in sex steroid hormones is unclear. If it is related, HRT in postmenopausal women should reduce the occurrence of these problems (180). Several studies were able to show that HRT did decrease central obesity in postmenopausal women and hence prevent at least some of the problems of menopause (177,181–188). In a recent study of oestrogen treatment in postmenopausal women, Mattiasson *et al.* (189) showed by computed tomography that oestradiol treatment for 1 year decreased intra-abdominal and intrapelvic fat compartments, but no effect was shown in subcutaneous fat. In contrast, other studies have not been able to confirm the decrease in central obesity with HRT (190–192). Reubinoff *et al.* (193) observed that HRT did not prevent the weight gain in early postmenopausal women, but HRT did minimize the shift from gynoid to android fat distribution in these women. A recent report showed that HRT in postmenopausal women results in an increased risk for coronary heart disease, stroke, thromboembolic events and

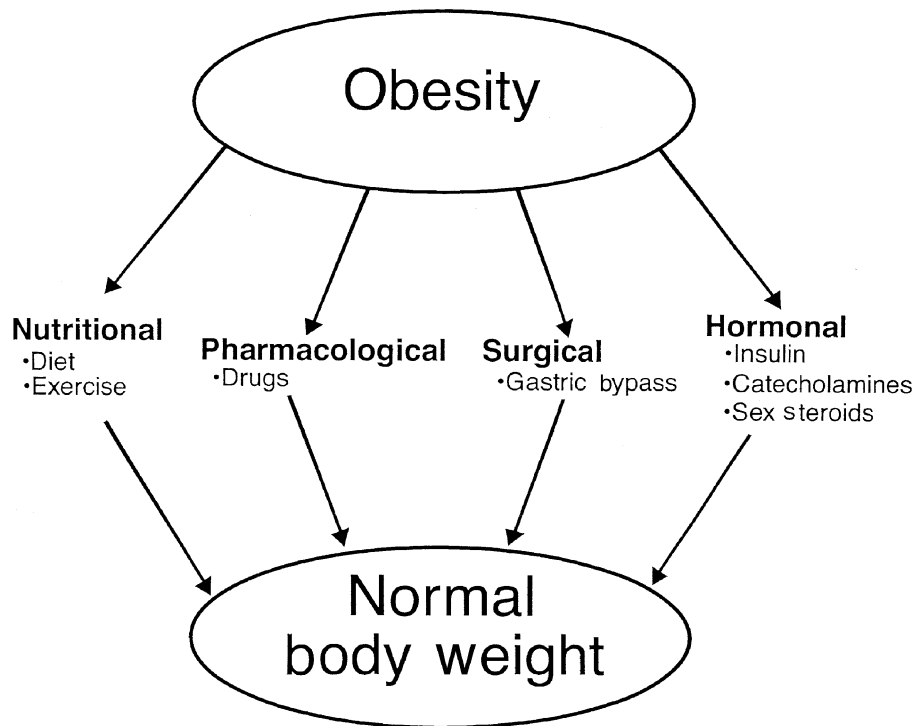


Figure 3 Strategies for the treatment of obesity.

breast cancer (194), suggesting caution when prescribing HRT. For the amelioration of obesity by sex steroid hormones, it would be helpful to develop compounds that would be specific for adipose tissues and that would not have the adverse side effects of oestrogen and/or androgens. In a recent review by Smith & O'Malley (195) on selective receptor modulators (SRMs), considerable time was devoted to the need for specific SRMs or STEARs (selective tissue oestrogenic activity regulators) for safe intervention in a number of endocrine-regulated conditions. Selective receptor modulators for obesity regulation may well be an option for the future. Several oestrogenic compounds, such as estren (196) and CP-336,156 (197), have recently been shown to prevent bone loss in animal models without serious side effects, and CP-336,156 was shown to prevent the increase in body fat that is seen in ovariectomized rats.

Androgens may also play a key role in central obesity in women (198). The hyperandrogenism that is seen in patients with polycystic ovary syndrome is associated with obesity of the abdominal phenotype. Furthermore, weight loss programs in patients with polycystic ovary syndrome have been shown to have greater efficiency when antiandrogens are utilized in the programs (199). This effect of androgen is also seen in patients receiving HRT. When oestradiol is given alone, women frequently realize a reduction in central body fat. However, when testosterone was added to the HRT, the androgen antagonized or reduced the oestrogen effect (200).

Male menopause is not as abrupt as menopause in women, and there is some question if male menopause even exists (201). However, in ageing men a progressive decrease in androgen production is common (202,203). This low testosterone has been associated with an increase in central obesity (202,204–207). This again raises the question of using sex steroid hormones to treat or prevent the central obesity seen in older men (208). Testosterone treatment in men has been shown to decrease central obesity (209–213) and total fat content (203). This prevention of central obesity by testosterone may be site specific, as Marin *et al.* (214) have shown that testosterone inhibits triglyceride assimilation in intra-abdominal fat depots, but not in subcutaneous fat depots. Although testosterone has been the focus of most replacement studies in men, other sex hormones, such as oestradiol (215) and DHEA (216), may also be of importance. Testosterone replacement in men may increase the risk of prostate cancer and other problems. However, Marin (210) did not detect changes in prostate volume, prostate specific antigen, genitor-urinary history or urinary flow measurements after men were on testosterone treatment for 9 months. Whether the risk of testosterone treatment will outweigh the benefits will require more studies (217), but the development of SRMs for these systems may also play an important role in the future.

Regulation of some key proteins in adipose tissues by sex steroid hormones may also be a mechanism for the treatment and/or prevention of obesity. McCarty (218) has suggested that the regulation of LPL may be a way to prevent

or treat central obesity. The approach would be to specifically down-regulate LPL in visceral adipose tissue. Although catecholamines, insulin and IGF-I may play important roles, sex steroid hormones appear to also play a role. Oestrogen is known to decrease LPL activity at least in gluteal adipose tissue, which may be important in body fat distribution (68), and oestrogen may cause a decrease in IGF-I production (219), which would favour a decrease in LPL activity and central obesity. Testosterone replacement therapy is known to decrease LPL activity in visceral adipocytes, but not in subcutaneous adipocytes (78). Therefore, androgens or their derivatives could be used to possibly prevent or treat central obesity.

Leptin is another adipose protein that could be a target for regulation by sex steroid hormones to control central obesity. In mice, a mutation in the leptin (*ob*) gene results in obesity, and treatment with exogenous leptin causes a weight reduction in the *ob* mutant mice (220–222), as well as in diet-induced obese mice (220). In rats, the intracerebroventricular injections of leptin for 4 d caused a decrease in food intake and a loss in body weight, and a reduction in white fat pad masses of 67% in retroperitoneal, 40% in inguinal and 20% in epididymal, respectively (223). Intraperitoneal injections of leptin in pregnant rats on days 8, 10, and 12 of gestation resulted in a reduction in adipose tissue weights of both males and females at adult age (224). The extension of leptin therapy in experimental animals to humans has been only partially successful (225–227). In patients with a genetic defect of leptin production, leptin therapy greatly reduces the obesity in these individuals (228). However, these individuals only represent a small percentage of the obese population and in the common diet-induced obesity, leptin therapy remains questionable. Heymsfield *et al.* (229) reported on a clinical trial with recombinant leptin therapy in 54 lean and 73 obese subjects. After 24 weeks of treatment, there was a dose-dependent small weight loss in the obese subjects with no apparent side effects. Hukshorn *et al.* (230) investigated the use of long-acting pegylated recombinant human leptin on weight loss in obese subjects. Weekly injection of pegylated leptin did not result in a significant weight loss after 8 weeks of treatment. Because leptin levels are very high in obese individuals, the high leptin levels in obese individuals might suggest that exogenous leptin therapy would be ineffective in decreasing fat stores. So possibly, regulation of endogenous leptin by sex steroid hormones would be more effective than exogenous leptin.

Conclusions

The amount and distribution of body fat is different between men and women. Men have a leaner body composition and a more central distribution of fat, whereas women have a higher amount of body fat and a more

gluteal/femoral pattern of fat distribution. These differences would suggest that the sex steroid hormones could have an effect on adipose tissues, and it is now evident that sex steroid hormones have an action on the metabolism, distribution and accretion of adipose tissues. Direct effects seem plausible, as sex steroid hormone receptors have now been found in adipose tissues. The amount of the receptor varies with the fat depot, with subcutaneous adipose tissue having a higher concentration of OR and PR and intra-abdominal adipose having a higher concentration of AR consistent with gender deposition. Sex steroid hormones carry out their action on adipose tissues by both genomic and nongenomic mechanisms. Lipoprotein lipase and leptin are two key adipose proteins that appear to be regulated by sex steroid hormones in a classical genomic mechanism, and the presence of membrane receptors for steroids would support the modulation of second messenger pathways via the cAMP and phosphoinositide cascades. Normal amount of oestrogens and androgens favours homeostasis in adipose tissues. However, decreases in the amount of oestrogen and/or androgen, as occur with ageing or gonadectomy, usually result in central obesity.

The effects of sex steroid hormones on adipose accretion and metabolism are obviously complex as one looks at the diversity of changes seen under a changing hormonal milieu. Besides the direct actions discussed in this review, one must factor in the effects these hormones play in supporting a lean body mass and a general, vital, and good overall quality of life, each of which influence metabolic rates and caloric turnover.

Hormone replacement therapy in older women and testosterone therapy in ageing men appear to have positive effects on central obesity. However, these therapies have numerous side effects. Future development of SRMs that are specific for adipose tissues and pathways related to lipid metabolism may find utility in the prevention and/or treatment of obesity when unwanted side effects or actions can be minimized.

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