MINIREVIEW

Role of Estrogens in Adipocyte Development and Function

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Estrogen has historically been viewed as a major regulator of adipose tissue in adult females, but recent work has indicated that estrogen's role in adipose biology may be broader than initially appreciated and has also provided important insights into the mechanism of estrogen effects on adipose tissue. Estrogen has direct effects on adipocytes to inhibit lipogenesis and may also have direct effects on other cellular constituents of adipose tissue, as well as metabolic effects on other target organs that can regulate adipose tissue. Estrogen has central effects on food consumption and energy expenditure that contribute to its overall inhibitory effects on adipose deposition. Estrogen also plays an important role in regulating adipose deposition in males and recently has been shown to be an important factor in the determination of adipocyte number, indicating that it regulates key developmental events in adipogenesis. Although critical questions still remain in our understanding of the overall role of estrogen in adipose tissue, it is clear that estrogen plays a more important role in adipose tissue than originally realized and that it is a major regulator of adipose tissue in both sexes during development and adulthood. Exp Biol Med 229:1127–1135, 2004

Key words: hormones; phytoestrogen; adipose tissue; adipogenesis

Introduction

Obesity has become a major public health concern, and the continuing worldwide increases in obesity rates indicate that the crisis will worsen (1). Of additional concern is the rapid increase in childhood obesity that is accompanying the increase in overall obesity rate (2), as these individuals have a strong predisposition to remain obese throughout life and suffer from the deleterious metabolic sequelae of obesity at an earlier age. These public health problems have emphasized the necessity of understanding adipose biology as a prelude to understanding obesity. At its simplest level, obesity is an energy imbalance in which intake exceeds output, but we still do not understand the multiple factors involved in the etiology of obesity, nor do we understand how to prevent the development of obesity. The latter is critical, because obesity is treated with minimal success by diet and exercise approaches, so strategies to prevent its development may be the most medically promising.

Hormones are major regulators of adipose tissue and are critical for adipocyte development and function. An extensive array of hormones and growth factors modulate adipocyte development and activity, including growth hormone, thyroid hormone, glucocorticoids, catecholamines, glucagon, insulin, and insulin-like growth factor. Estrogen has long been recognized as a major factor in regulating adipose development and deposition in females. In recent years, it has become clear that estrogen's role in adipose biology may be broader and more complex than initially appreciated. Estrogen is now known to play an important role in regulating adipose deposition in males and recently has been shown to be an important factor in the determination of adipocyte number, indicating that it regulates key developmental events in adipogenesis. The classical estrogen receptor, estrogen receptor (ER) α, appears to be the major regulator of adipose tissue, but recent results have also indicated a possible role for the more recently discovered estrogen receptor, ERβ. This review will summarize estrogen effects on adipose tissue and will concentrate on the more recent developments in

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this area that have contributed to our still-evolving view of the role of estrogen in the regulation of this critical tissue.

Adipose Tissue Is Sexually Dimorphic and Is Regulated by Estrogen

Adipose tissue is sexually dimorphic in humans, with gender-specific differences in body fat distribution (3, 4). Women have more extensive subcutaneous fat (as well as substantial overall increases in adipose tissue) compared to men (5, 6) (Fig. 1), with average body fat percentages in young, nonobese men and women of about 15% and 25%, respectively (7). The increased subcutaneous fat in women develops pubertally, indicating that estrogen may preferentially promote subcutaneous adipose deposition. The increase in adipose mass in women results from increases in adipocyte number as well as adipocyte size. Adipocyte number in gluteal (subcutaneous) fat was increased by 34% in girls compared to boys at adolescence (8). Adipocyte size was also increased 45% compared to similar-aged boys, although adipocyte size shows substantial regional variation and is not necessarily greater in women in all adipose depots (9, 10). In contrast, the accretion of abdominal fat in premenopausal women appears to be inhibited by estrogen, whereas men tend to depot abdominal fat. However, in postmenopausal women, abdominal fat increases, which correlates with various increased health risks (11).

Increased overall adipose mass in women also partially reflects greater numbers of adipocytes compared to men (10). This indicates that estrogens could play a role in adipocyte development and the establishment of adult adipocyte number, as well as modulate adult adipocyte size in adult females. However, gender differences in other hormones (e.g., androgens) that also modulate adipose deposition clearly indicate that the sexual dimorphism in adipocyte number and adipose distribution may not solely reflect estrogen effects in men versus women. In addition, androgen-receptor expression also shows variations in different fat pads (12), which is of great importance because the ratio between ER and androgen receptors is also thought to be critical for the response of adipocytes to sex steroids.

The extensive gender-related differences seen in humans between subcutaneous and visceral fat in terms of estrogen responsiveness are not seen in rodents. Likewise, the pronounced sexual dimorphism in adipose tissue seen in humans does not occur in rodents, although rodents show marked sexual dimorphism in terms of adipose response to caloric restriction, exercise, and treatment with PPARγ agonists (13–15).

Further complicating an overall understanding of the role of estrogen in adipose tissue, adipose ER varies by depot and with different physiological states. The variations in ER expression in the adipose depots correlate with clear differences in the physiological responsiveness of adipocytes from various fat pads to estrogen (16). ER expression in adipose tissue shows changes with age, and ER expression is altered in response to various physiological states such as obesity (16).

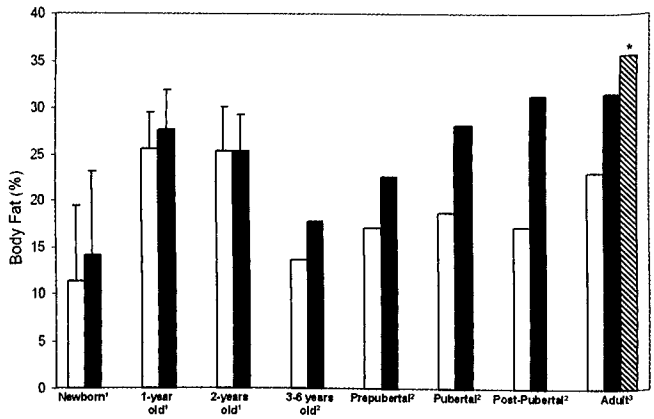

Figure 1. Gender differences in body fat in humans during development. The figure illustrates the changes in body fat percentage from infancy to adulthood in both men and women. Gender and estrogen status (puberty and pre-/postmenopause) play an important role in adipose deposition of humans. Data adapted from references (3, 4, 11) White and black bars indicate men and women, respectively. Bar with diagonal lines indicates postmenopausal women.

Understanding the role of ER signaling in adipose tissue also obviously necessitates taking into account the large developmental changes in estrogen concentrations in humans and laboratory animals during their lifetimes. For example, in women there are minimal levels of E2 in infancy and childhood, a sharp rise during puberty, continued high concentrations during the remainder of the reproductive lifetime, and then a decline to low levels during menopause and throughout subsequent life. Likewise, there normally is a marked difference in estrogen concentration between men and women. Therefore, when analyzing the overall role of estrogen signaling in adipose tissue, these variations in receptor levels and response or ligand availability must always be taken into account.

ER Expression in Adipose Tissue of Humans and Rodents

Human and rodent adipocytes express both ERα and ERβ (17–19), indicating that estrogen signaling may occur through either of these ERs in adipose tissue. In addition, there is also evidence that 17β-estradiol (E2) can act through the less well characterized membrane ER in adipocytes to induce rapid effects that do not involve the classical nuclear ERs (20).

Previous work has focused on the ability of estrogen to induce functional and morphological changes in adipocytes, though other literature clearly indicates that many, if not all, major cell types in adipose tissue are potentially direct estrogen targets. For example, a number of studies using human and animal tissue have indicated that ER is expressed in preadipocytes (18, 21), so E2 can potentially regulate preadipocyte development as well as act directly on adipocytes. In addition to the preadipocyte/adipocyte lineage, ERα or β are also expressed in other cell types

found in adipose tissue, such as vascular endothelium, vascular smooth muscle, and macrophages (22–24). Therefore, effects on many cell types must be considered in an analysis of overall estrogen effects on adipose tissue activity and lipid stores.

Estrogens are also capable of producing effects on adipose tissue by acting indirectly through other tissues that regulate appetite, energy expenditure or metabolism. ERs are widely distributed in the hypothalamus, the primary site in the brain that regulates energy balance, and effects of estrogens on both energy intake and expenditure are well known, as described later in this review. In addition, ERs are also present in organs such as the liver, where estrogen effects can produce metabolic changes that ultimately affect adipose deposition and overall adipose mass.

Although the literature has concentrated on estrogen effects in adipose tissue of females, adipocytes from males (of humans and other species) express ER at levels similar to those seen in females (21). Despite the typically greater circulating E2 concentrations in females, males from a variety of species also have measurable circulating concentrations of E2. Adipose tissue in males is therefore a direct target of estrogenic stimulation, as demonstrated by the following section highlighting extensive adipose changes in males lacking ERα.

In summary, estrogen can have direct effects on adipocytes and other cell types in adipose tissue of both sexes, as well as indirect effects on other tissues (e.g., brain, liver) that regulate adipose tissue. Understanding the totality of estrogen effects on adipose tissue involves elucidation of the roles of estrogen in the various target tissues that can effect adipose deposition.

Estrogen Regulates Adipose Tissue Deposition in Adult Females

It has been known for many years that estrogen is an important regulator of female adipose deposition in humans, rodents, and other species (25). Ovariectomy of experimental animals, or menopause in women resulting either from natural aging or surgical removal of the ovaries, results in increases in adipose tissue (25) (Fig. 2). The deleterious effects of estrogen deficiency in increasing adipose mass in women are compounded by the fact that loss of estrogen signaling produces a preferential increase in visceral fat.

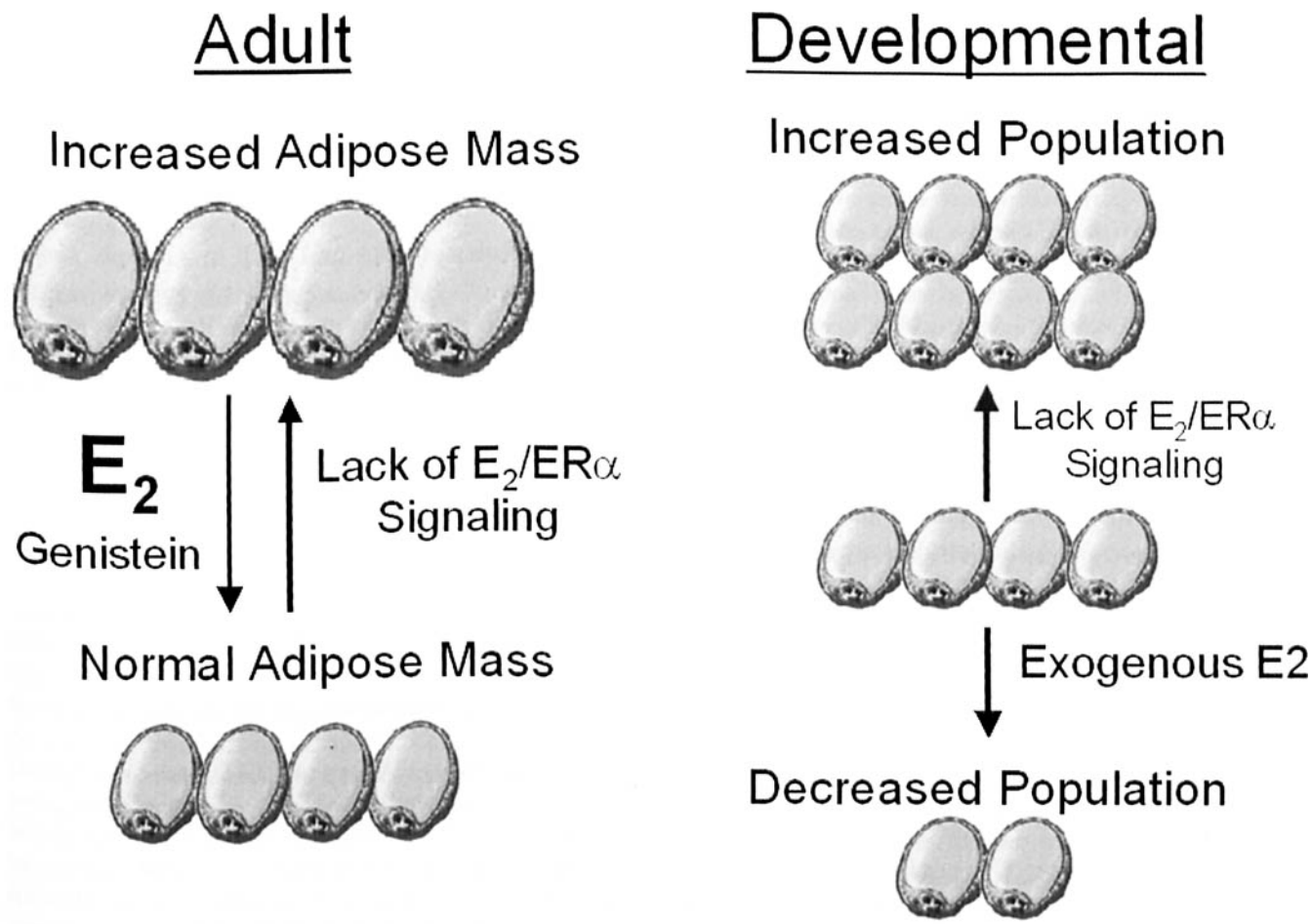

Figure 2. Influence of estrogen on adipogenesis and lipogenesis. Lack of estrogens or presence of exogenous estrogens like 17β-estradiol (E_2) or genistein can regulate the amount of adipose tissue during development and in adults by affecting adipocyte size or number. ER, estrogen receptor.

This is of significance because the size of the visceral fat stores is strongly correlated with insulin resistance and glucose intolerance and with increases in the serious health problems such as cardiovascular disease and type II diabetes that arise from obesity.

The effects of loss of estrogen on adipose tissue in female rodents closely parallels those described in women. There is an extensive literature documenting increases in adipose tissue in rats and mice following ovariectomy (25), and female ERα knockout (αERKO) mice have large increases in adipose mass (26). The adipose changes in humans, rodents, or other animals caused by a lack of estrogen can be reversed with estrogen replacement (27), and decreases in adipose tissue are one significant benefit of hormone replacement therapy given to postmenopausal women (28).

Direct Effects of Estrogen on Adipocytes

Estrogen can directly inhibit adipose deposition by decreasing lipogenesis. This action happens principally through decreasing activity of lipoprotein lipase (LPL), an enzyme that regulates lipid uptake by adipocytes. Ovariectomy increases LPL and lipid deposition within the adipocyte and administering physiological doses of E2 reverses this deposition (29). Recently, work on a 3T3 adipocyte cell line transfected with estrogen receptor showed that the LPL gene has a negatively controlled estrogen response element (30).

E2 can indirectly affect lipolysis by inducing the lipolytic enzyme hormone-sensitive lipase (31) or by increasing the lipolytic effects of epinephrine (32). Fatty acid-β oxidation might also be increased, which might contribute to the decrease in adipose tissue deposition induced by E2 (33). Contrary to its overall antilipogenic and lipolytic effect, estrogen site-specifically attenuates the effects of α2A-adrenergic receptors in the subcutaneous fat cells of humans and decreases lipolysis; this effect could partially account for the increased deposition of subcutaneous adipose tissue in women compared to men (34).

Estrogen Effects on Energy Intake and Expenditure

Estrogen has a negative effect on feeding through actions on the hypothalamus (25). When ovariectomized and sham-ovariectomized rats were pair-fed, the ovariectomized animals gained more weight compared to the shams, even in the absence of hyperphagia (35). This indicates that, though estrogen has an effect on food consumption, the central effects of estrogen related to decreasing adipose tissue deposition might not entirely be through decreases in energy intake. This other facet of estrogen's central actions may involve effects on voluntary activity as well as energy expenditure independent of voluntary exercise, both of which are increased by estrogen (25). αERKO mice show a decrease in energy expenditure, indicating that estrogen's actions on energy metabolism are through ERα (26).

In addition to its direct effects on the hypothalamus described above, estrogen may also regulate the production or response to adipose hormones such as leptin and, through this mechanism, affect processes such as food consumption and energy metabolism. Some data have indicated that the increase in adipose stores following estrogen withdrawal leads to increased circulating leptin, indicating that the leptin increase following loss of estrogen is secondary to increased adipose deposition, rather than directly driven by the lack of estrogen (36). However, other results (37, 38) have indicated that estrogen can regulate leptin and there is presently no consensus on the role of estrogen in regulating leptin.

Leptin is produced primarily by adipocytes and it acts through its receptors (Ob-R) in the hypothalamus to ultimately produce changes in energy intake and expenditure that are involved in the homeostatic control of adipose mass. The critical long form of the leptin receptor (Ob-Rb) in the hypothalamus is modulated by estrogen status, with lack of estrogen for approximately 5 months causing a decrease in Ob-Rb, which may partially explain continued obesity following loss of estrogen despite large increases in circulating leptin induced by ovariectomy (36).

Estrogens have effects on other organs, such as the liver, that are involved in various aspects of metabolism and that are altered in obesity. For example, estrogen effects on cholesterol uptake, biosynthesis, and catabolism have been documented in the aromatase knockout (ArKO) female, which lacks the enzymatic machinery to make estrogens, although these effects are sexually dimorphic and not seen in ArKO males (39).

Relative Roles of ERα and ERβ in Adipose Tissue

The discovery of a second form of ER, ERβ (40), and the demonstration that adipose tissue expressed both ERα and ERβ (17) necessitated work to ascertain the relative roles of ERα and ERβ in adipose tissue. αERKO mice show over a 100% increase in adipose tissue compared with wild-type (WT) mice (26) (Fig. 3). These increases were similar to those reported concomitantly in ArKO mice (41) and soon afterward in FSH receptor knockout mice (42), both of which do not synthesize E2. These results indicated that loss of E2/ERα signaling in αERKO mice, and the lack of endogenous E2 in the ArKO and FSH receptor knockout mice, which should lead to lack of signaling through both ERα and ERβ, led to similar increases in adipose tissue. These results strongly indicate that ERα is the main regulator of estrogen effects on adipose tissue.

αERKO mice still express ERβ and have tenfold increases in circulating E2 (43), which could cause increased E2/ERβ signaling. To evaluate the potential role of E2/ERβ signaling in adipose tissue, αERKO mice were ovariectomized to determine whether loss of E2/ERβ signaling in animals already lacking E2/ERα signaling induced any demonstrable change in adipose tissue or other parameters. Ovariectomized αERKO mice showed a

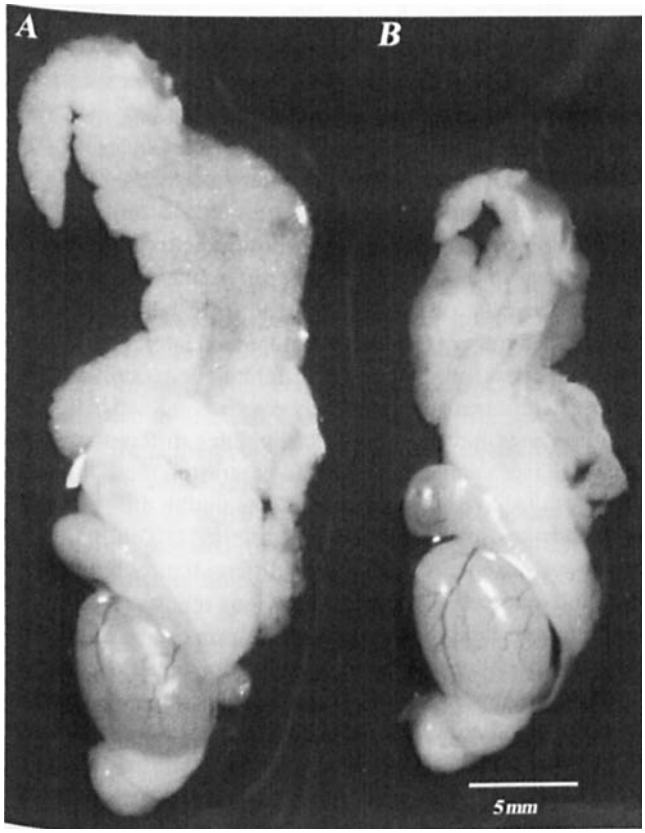

Figure 3. Epididymal fat pads of 120-day-old estrogen receptor α knockout (αERKO) (A) and wild-type (B) mice. αERKO mice show marked increases in adipose tissue compared to wild-type mice. This increase is a result of both hyperplasia and hypertrophy, which indicates that 17β-estradiol/estrogen receptor α signaling plays a role in regulating the size and number of adipocytes.

decrease in adipose tissue and body weight compared to sham-operated αERKO mice, indicating that ERβ might have an inhibitory effect on adipose deposition that is opposite that mediated through ERα (19). Therefore, although ERα is the predominant modulator of estrogenic effects in adipose tissue, ERβ may also play a role in the effects of estrogen on adipocytes.

Effects of Xenoestrogens on Adipose Tissue

Xenoestrogens are natural or man-made estrogens that mimic E2 effects by binding to ERα or β at varying levels and initiating transcription. Humans consuming soy products and supplements are exposed to high quantities of phytoestrogens, which are plant-derived estrogens. Genistein and diadzein are the principal phytoestrogen components in soy. Humans and animals are also exposed to varying degrees of equol, which is a metabolite of diadzein. Dietary genistein fed to mice results in serum-genistein concentrations comparable to those in humans consuming phytoestrogens and has an antilipogenic effect on adipose deposition (Fig. 2); this effect is brought about by the reduction of LPL expression, which is similar to the effect of E2 (44). These antilipogenic effects of genistein could also be obtained when approximately 6 mg/day of mixed isoflavones were fed to mice on a high-cholesterol diet (45). In this experiment, isoflavones plus exercise (treadmill running) totally abrogated the increase in body fat induced by ovariectomy. These results are consistent with previous *in vitro* studies that demonstrated that genistein decreased insulin-induced lipogenesis as measured by glucose incorporation into adipocytes, both in primary adipocyte cultures (46) as well as in 3T3 cell lines (47). Genistein also enhanced epinephrine-induced lipolysis in rat adipocytes (48).

In vitro studies have shown that genistein may also induce effects on adipogenesis, with inhibitory effects at low concentrations (49, 50) and stimulatory effects at high concentrations (50). These results indicate that genistein may have the capacity to alter adipocyte number either up or down at various concentrations, although the high levels of genistein required to induce stimulatory effects on adipogenesis make the physiological relevance of this finding questionable.

There have been varying reports on the effect of selective-estrogen receptor modulators such as tamoxifen and raloxifene on adipose tissue. The potential effects of these compounds on adipose tissue are important because of their widespread use for breast cancer prevention and treatment. Tamoxifen has been reported to reduce adipose tissue deposition and LPL activity in adipose tissue of rats in the absence of E2 (51). However, contrary to this report, women on tamoxifen treatment for breast cancer show an increase in body fat (52). A recent report showed that raloxifene inhibited the ovariectomy-induced adipose and serum leptin increase in rats similar to E2 (36). Likewise, EM-652, which is a pure antiestrogen in human breast and uterine cancer cells, appears to function as an estrogen agonist in adipose tissue, where it has antilipogenic effects, and it also produces beneficial effects on insulin resistance (53). The effect of xenoestrogens on adipose tissue depends on the levels of endogenous estrogens, binding efficiency to ERα and ERβ, and level of exposure (54). It appears that a number of selective-estrogen receptor modulators that have antiestrogenic effects in breast tissue may have beneficial estrogenic effects on adipose tissue. Clearly, more work is needed to understand the effects of these natural and man-made estrogen receptor agonists and antagonists on adipose tissue, and the ubiquity of these compounds in our environment and the extent of their use in human medicine emphasizes the importance of this area.

Estrogens Regulates Adipose Development and Mass in the Male

The presence of both ER in male adipose tissue and measurable circulating E2 in males raises the possibility that the E2/ERα signaling pathway could have effects on male adipose tissue. However, for many years it was unclear whether E2 played a significant role in adipose development or function in the male. The development of αERKO and ArKO mice provided a powerful tool to directly address the

effects of loss of E2/ERα signaling both on male adipose tissue as well as on various metabolic parameters such as insulin resistance that are affected by a loss of E2/ERα signaling in women and females of other species.

Both male αERKO and ArKO mice have modest increases in adult body weight compared to WT controls. Individual fat pad weights showed striking and age-related increases in αERKO and ArKO males compared to WT males (26, 41). Fat pads in αERKO males weigh 140%–185% more than those in WT mice at days 270–360 of age (26) (Fig. 3), and similar increases were seen in ArKO males (41). The increase in adipose tissue was a result of both adipocyte hypertrophy and hyperplasia in the αERKO (26) (Fig. 3) and ArKO (33, 41) males. As described above, in females E2 has effects on cholesterol uptake, biosynthesis, and catabolism, but these effects are not seen in males (39). However, ArKO males have hepatic steatosis and alterations in triglyceride and fatty acid homeostasis (41), which can be reversed by E2 replacement (39), indicating that E2 has important effects on nonadipose tissues that modulate the obesity phenotype and the metabolic changes seen in these animals.

The effects of estrogen on adipose deposition seen in male rodents appear to be similar to those seen in human males lacking aromatase and the ability to produce endogenous estrogens (55). However, the rarity of the inactivating mutation of the gene encoding aromatase in human males means that only a handful of such cases are known, and thus conclusions have to be drawn cautiously. Men lacking aromatase tend to have increased body fat, insulin resistance, and a proclivity toward type II diabetes, and these conditions are improved by estrogen treatment—findings that are totally consistent with the known role of estrogens in adipose deposition in laboratory rodents.

Effects of Estrogen on Adipose Development

Although the effects of E2 on adipose tissue in adults has historically been the focus of the most research, there has been an increasing interest in the role of estrogens in adipocyte development in recent years. Important insights into the effects of E2 on adipocyte development have come from *in vitro* studies of preadipocyte cell lines or primary cultures of the stromal-vascular cell fraction of adipose tissue, which contains preadipocytes and a variety of other cell types. More recently, phenotypic changes in knockout animals lacking E2 have been used to extend the *in vitro* studies and directly test the effects of loss of E2 signaling *in vivo* on adipose development.

Human and animal preadipocytes express both ERα and β (18, 19, 21), as described above. E2 was originally reported to stimulate proliferation of human preadipocytes in the 1970s (56) and similar results have been obtained in a variety of species and experimental systems since that time. For example, Lea-Currie *et al.* (57) demonstrated that 1 μM E2 stimulated proliferation in the mouse NIH 3T3-L1 preadipocyte cell line. Similarly, Dieudonne *et al* (58) reported that E2 stimulated proliferation of subcutaneous rat preadipocytes from females, but not males. The rodent data indicating a stimulatory effect of E2 on preadipocyte proliferation is corroborated by human studies indicating that E2 stimulated proliferation of preadipocytes derived from men and women, though some sex-specific responses to E2 stimulation were noted (59).

Preadipocytes can remain undifferentiated, or they can differentiate into postmitotic fully differentiated adipocytes; this process is obviously critical in establishing final adipocyte number. Present literature indicates that E2 can have an effect on adipocyte differentiation, although the reports in this area are not entirely consistent. Studies with rat preadipocytes indicate that E2 stimulates differentiation of these cells into adipocytes (58). In contrast to these findings, other results have indicated that E2 can inhibit differentiation of adipocytes in the 3T3-L1 cell line (57). The inhibitory effects of estrogen on adipocyte differentiation reported in this study are consistent with numerous reports (49, 60, 61) that estrogen inhibits adipogenesis in primary bone marrow stromal cell cultures or bone marrow stroma cell lines.

Despite reports that E2 stimulates preadipocyte proliferation and conflicting reports on the effects of E2 on adipocyte differentiation, recent studies using both the αERKO and ArKO gene knockout mouse models have clearly indicated that E2 normally has an inhibitory effect on overall adipocyte number. Heine *et al.* (26) reported that both male and female αERKO mice have large increases in fat pad weights, as described above. There was a modest hypertrophy of the adipocytes in these animals, but the most striking change was an adipocyte hyperplasia. For example, adipocyte number was up to 170% greater in the fat pads of αERKO compared to control mice, and adipocyte hyperplasia was seen in all fat pads examined. Similar increases were seen in ArKO mice (33, 41). Thus, lack of estrogen signaling in these knockout animals led to large increased adipocyte numbers, indicating that estrogen normally plays an inhibitory role during adipogenesis to limit ultimate adipocyte number (Fig. 3).

Neonatal Estrogen Treatment Decreases Adult Adipose Mass and Adipocyte Number

Data from αERKO and ArKO mice indicated that lack of signaling through ERα could result in large increases in adipocyte number and obesity. The converse question, whether exposure to exogenous estrogens during development can cause decreases in adipocyte number, is also an important one that has clinical implications and that is presently being addressed in a number of laboratories. Preliminary studies have shown that neonatal treatment of rats and mice with synthetic estrogens such as diethylstilbestrol or E2 can result in large decreases in adipose mass (up to 90%) in these animals when they reach adulthood (62; Koosuru and Cooke, unpublished data). In addition, these decreases appear to result primarily from decreases in adipocyte number (Naaz, Goyal, and Cooke,

unpublished data). Thus, early estrogen exposure produces decreases in adipocyte number, effects that are the opposite of those seen in αERKO and ArKO mice, further indicating the critical role of ERα during the neonatal period in the establishment of adult adipocyte number.

How Does Estrogen Regulate Adipocyte Number?

Altering normal E2/ER signaling during development induces large changes in adipocyte number, but the mechanism by which estrogens act to alter adipocyte development and ultimate adult population is unknown. During development, certain mesenchymal cells become committed to the adipogenic lineage. The preadipocytes formed from mesenchymal cells can proliferate, withdraw from the cell cycle but remain as preadipocytes, or differentiate as adipocytes; little is known about how this process is regulated. In contrast, preadipocyte differentiation into the mature adipocytes has been well delineated (63). However, there is little understanding of how overall adipocyte number is established. Determining the mechanism of action for estrogen or other treatments that affect adipocyte number is complex because these agents could act at more than one stage of adipogenic development to alter adipocyte number and may have opposite effects during various stages of adipogenesis, as indicated by the data that estrogen stimulates preadipocyte proliferation but may inhibit adipocyte differentiation.

The process by which preadipocytes differentiate into adipocytes also involves cell proliferation, at least in the *in vitro* systems in which this is normally studied. This process represents another potential target in which a hormone such as E2 could act to alter ultimate adipocyte numbers. Knockout studies using animals lacking either the estrogen receptor ligand or the receptor itself indicate that the overall effect of abolishing ER signaling is to produce an adipocyte hyperplasia and obesity, but clearly additional work is required to elucidate the specific estrogen effects on the various developmental processes involved in adipogenesis.

Much of our understanding of adipogenesis comes from 3T3-L1 preadipocytes, which are initially fibroblastic but undergo adipogenic differentiation when confluent cultures are exposed to differentiation media. During differentiation, these cells undergo a period of proliferation, termed mitotic clonal expansion, characterized by increased cyclin expression. D- and E-type cyclins bind to their cyclin-dependent kinase (cdk) partners and play a critical role in cell-cycle progression from G1 to S. Functional activity of cyclin/cdk complexes is regulated by cyclin-dependent kinase inhibitors (CDKIs), which bind and inactivate cyclin/cdk complexes and therefore inhibit progression from G1 to S phase. CDKIs consist of two families, the Cip/Kip family (p27, p21, and p57) and the Ink4 family (64). Morrison and Farmer (65) reported that p27 and p21 decreased at the start of mitotic clonal expansion in 3T3-L1 preadipocytes, and termination of mitotic clonal expansion was accompanied by increases in both p27 and p21; these results indicate that p27 and p21 play an important role in adipogenesis of 3T3-L1 cells, but their role in adipogenesis *in vivo* has not been clear (66).

Table 1. Estrogen Effects on Adipose Tissue

Direct Effects
Lipogenesis
↓ Lipoprotein lipase mRNA and protein expression (29)
Lipolysis
↑ Hormone sensitive lipase activity (31)
↑ Epinephrine-induced lipolysis (32)
Site-specifically attenuates subcutaneous tissue lipolysis (34)
Adipogenesis
↑ Adipocyte precursor proliferation (57)
↓ Expression of adipocyte differentiation factors (49, 60, 61)
Central Effects
CNS/hypothalamic effects
↓ Feed consumption (25)
↓ Leptin secretion (36)
↑ Activity and energy expenditure (25, 26)
Lipid profile and hepatic effects
Absence of estrogen causes hepatic steatosis and altered lipid homeostasis

Note. Estrogen affects on adipose tissue may be direct by affecting lipogenesis, lipolysis, or adipogenesis of the adipocyte, or secondary to its affects on the central nervous system or liver.

To test the hypothesis that p27 or p21 regulate adipocyte number, we have compared adipose development and metabolic parameters in p27 and p21 knockouts (p27KO and p21KO, respectively), p27/p21 double knockout and WT mice. At 120 days of age, inguinal and parametrial fat pads showed 80%, 90%, and 400% increases in wet weight in p27KO, p21KO, and double knockout mice, respectively, compared to WT (67). Adipocyte numbers in parametrial fat pads of p27KO, p21KO, and double knockout mice were 2-, 2-, and 6-fold those in WT, respectively. Despite these striking increases in adipocyte number, average adipocyte size was not significantly different in any of the groups, and the observed increases in adipose pad weights reflect the adipocyte hyperplasia that occurs with the loss of one or more of these CDKIs.

The important role for p27 and p21, separately and together, in regulating adipocyte number and adipose mass indicates that these CDKIs are potential targets for E2 or other hormonal signals that may result in alterations in final adipocyte number. In addition, other recent reports have demonstrated the critical role of proteins such as sirtuin 1 in regulating adipogenesis (68), and work to establish whether estrogen effects on adipocyte number are mediated through p27 and p21 or other target proteins that play critical roles in adipogenesis is ongoing.

Summary and Conclusions

In summary, recent research has provided significant insights into the mechanisms of estrogen action on adipose tissue and has also indicated that estrogen effects on adipose tissue are broader than realized previously. Estrogen can have direct effects on adipocytes and cellular constituents of adipose tissue, as well as central effects on food consumption and energy expenditure that contribute to the overall effects on adipose deposition (Table 1). Though previous work has focused on females, estrogen is also critical in regulating adipose deposition in males. Estrogen plays an important role in regulating adipocyte number in the developing animal, but the mechanism of this effect is unknown. Understanding estrogen effects on adipose tissue is complicated by regional, sex-related, and species-specific differences in adipose ER expression and responsiveness, as well as by variations caused by age and physiological state. Although critical questions still remain in our understanding of the overall role of estrogen in adipose tissue, it is clear that estrogen plays a more important role than originally realized and is a major regulator of adipose tissue in both sexes during development and adulthood.

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