Estrogens and Body Weight Regulation in Men

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Abstract Our understanding of the metabolic roles of sex steroids in men has evolved substantially over recent decades. Whereas testosterone once was believed to contribute to metabolic risk in men, the importance of adequate androgen exposure for the maintenance of metabolic health has been demonstrated unequivocally. A growing body of evidence now also supports a critical role for estrogens in metabolic regulation in men. Recent data from clinical intervention studies indicate that estradiol may be a stronger determinant of adiposity than testosterone in men, and even short-term estradiol deprivation contributes to fat mass accrual. The following chapter will outline findings to date regarding the mechanisms, whereby estrogens contribute to the regulation of body weight and adiposity in men. It will present emergent clinical data as well as preclinical findings that reveal mechanistic insights into estrogen-mediated regulation of body composition. Findings in both males and females will be reviewed, to draw comparisons and to highlight knowledge gaps regarding estrogen action specifically in males. Finally, the clinical relevance of estrogen exposure in men will be discussed, particularly in the context of a rising global prevalence of obesity and expanding clinical use of sex steroid-based therapies in men.

Introduction

Obesity has become a global epidemic. Overweight and obesity have an estimated global prevalence of over 2 billion people, surpassing that of undernutrition for the first time in history (Popkin et al. 2012). Although women are at higher risk for obesity than men, men are more likely to experience obesity-related disorders including insulin resistance, type 2 diabetes mellitus (T2DM), nonalcoholic fatty liver disease, and cardiovascular disease (Kautzky-Willer and Handisurya 2009). Thus, identifying risk factors for the development of obesity and its associated

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complications specifically in men has been a growing area of interest. Over the past two decades, sex steroid deprivation in men has garnered increased attention as a key risk factor for metabolic disease. Thus, men with physiologic hypogonadism or those undergoing androgen deprivation treatment (ADT) for prostate cancer are at higher risk for the incident development of obesity and associated metabolic disorders including insulin resistance, nonalcoholic fatty liver disease, and T2DM (Ding et al. 2006; Hamilton et al. 2011; Keating et al. 2012). A contributory, causal role for testosterone deprivation in these metabolic disorders is supported by clinical data demonstrating that exogenous testosterone therapy reverses the increased adiposity evident in hypogonadal men and may improve insulin sensitivity in hypogonadal men with T2DM (Bhasin et al. 2003, 2010; Isidori et al. 2005; Jones et al. 2011).

Importantly, both clinical and preclinical data indicate that the beneficial metabolic effects of testosterone in men are not solely androgen mediated. Testosterone can undergo conversion to 17β-estradiol by the enzyme aromatase, and estradiol deficiency now appears a key facet of the metabolic risk conferred by either physiologic or iatrogenic hypogonadism. This recognition of the metabolic importance of estrogens in men was initially generated when rare syndromes of congenital estrogen deficiency in men were first reported about two decades ago (Jones et al. 2006). These clinical observations were followed by a succession of animal models demonstrating the critical, protective roles of estrogens in regulating body weight, adiposity, and glucose homeostasis in male as well as female mice. Notably, male mice with genetic deletion of estrogen receptor-α (ERα) or aromatase exhibit a more pronounced phenotype of obesity and metabolic dysregulation than do male mice with disruption of androgen receptor (AR) signaling (Fan et al. 2005; Heine et al. 2000; Jones et al. 2000). These preclinical findings more recently have been corroborated by clinical intervention studies in men that have suggested a stronger effect of estradiol than testosterone in suppressing fat mass accrual in men. Greater understanding of the mechanisms by which estrogens regulate body weight and body composition in men is essential. These mechanistic insights will be critical for (1) formulating optimal strategies for sex steroid replacement in hypogonadal men, (2) avoiding potential harm of sex steroid-based interventions in clinical practice, and (3) developing novel interventions for the prevention and treatment of obesity in men.

Estrogen Production and Metabolism

In men, ~15% of circulating estrogens derive directly from testicular production with the remainder generated from androgens through peripheral activity of the enzyme aromatase (Hemsell et al. 1974). The predominant form of circulating estrogen in men is 17β-estradiol, which is generated by aromatization of testosterone. Estrone is also found in circulation and formed from the aromatization of androstenedione. Estrogens can mediate effects through multiple pathways, with

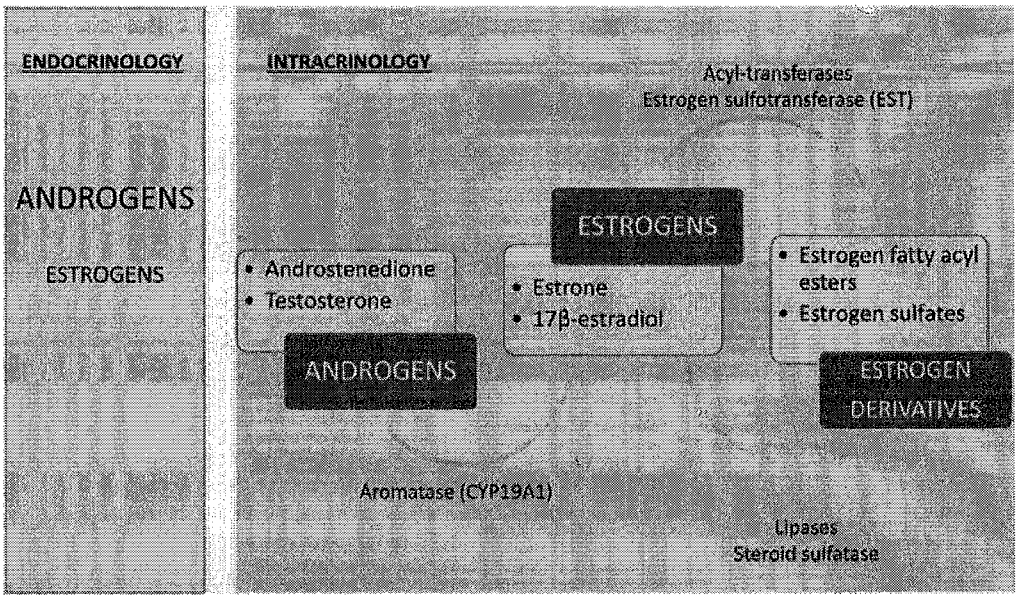

Fig. 1 Extensive regulation of estrogen production and metabolism within peripheral tissues is enabled by local expression of aromatase, which converts androgens to estrogens. Estrogens further can be converted to estrogen sulfates and estrogen fatty acyl esters through the activity of estrogen sulfotransferase and acyltransferases, respectively. Finally, these estrogen derivatives can be converted back to parent estrogens by steroid sulfatase and lipase activity

both genomic and non-genomic effects conferred through binding of its canonical receptors ERα and ERβ. Estrogens also can signal through a membrane-bound G-protein-coupled receptor (GPER). Further, ERα and ERβ can mediate both genomic and non-genomic ligand-independent effects (Foryst-Ludwig and Kintscher 2010). Of note, all three receptors have been implicated in the regulation of body weight and adiposity in both males and females (Cooke et al. 2001; Davis et al. 2014; Heine et al. 2000). Adipose tissue in particular is rich in estrogens and other sex steroids, with markedly higher concentrations of both estrogens and androgens than are found in serum (Deslypere et al. 1985).

Importantly, circulating estrogen levels are not the sole determinant of tissue estrogen levels, highlighting the distinction between endocrinology (hormones released into circulation) and intracrinology, the cell-specific regulation of sex steroid metabolism (Fig. 1). Indeed, several clinical studies have demonstrated dissociations between circulating and intra-adipose estrogen levels, including studies with male subjects (Blankenstein et al. 1992; Bélanger et al. 2006; Deslypere et al. 1985; Wang et al. 2013). Aromatase is expressed broadly throughout central and peripheral tissues including brain and adipose tissue and skeletal muscle; therefore, through local aromatase activity in key metabolic tissues, estrogen production is regulated in tissue-specific fashion (Matsumine et al. 1986; Simpson 2004). Estrogen metabolism also is locally regulated, predominantly through the enzyme estrogen sulfotransferase (EST), which inactivates 17β-estradiol through sulfoconjugation.

Estrogens alternatively can undergo conversion to fatty acyl esters mediated by acyl-transferases. Estrogen fatty acyl esters are found in both serum and peripheral tissues, as are estrogen sulfates. Further, estrogen fatty acyl esters and estrogen sulfates can undergo conversion to their bioactive counterparts through lipase and steroid sulfatase activity, respectively. Adipose tissue is particularly enriched in estrogen fatty acyl esters and consequently has an extensive buffering system that enables local regulation of estrogen production and metabolism. Notably, in a study of obese men, 17β-estradiol fatty acyl ester concentrations did correlate in serum and fat (Wang et al. 2013), possibly indicating that serum estrogen levels influence stored estrogen content in adipose tissue, but conversion to bioactive forms is locally regulated. These findings highlight the limitations of circulating levels as an index of tissue-specific estrogen regulation and underscore the need to carefully delineate tissue-specific pathways of estrogen metabolism and signaling in order to fully define estrogen-mediated mechanisms of body weight regulation in men.

Genetic Mutations in Men and Mice

In the 1990s, an initial series of cases were published detailing men with rare genetic mutations in the genes encoding aromatase or ERα that led to partial or complete loss of estrogen signaling (Carani et al. 1997; Morishima et al. 1995; Simpson 1998; Smith et al. 1994). Although initially reported as predominantly a skeletal phenotype, congenital estrogen deficiency soon was identified as a syndrome of metabolic perturbations characterized by increased central adiposity, insulin resistance, and nonalcoholic fatty liver disease (Maffei et al. 2007). Further, in the case of aromatase deficiency, these metabolic disturbances could be reversed with exogenous estradiol but not testosterone treatment (Maffei et al. 2007).

The development of genetically modified mice used to model estrogen deficiency provided further, compelling evidence of the metabolic importance of estrogen signaling in males. These preclinical models also provided new insights into the mechanisms whereby estrogens contribute to energy balance and body weight regulation in male as well as female mice. Thus, a striking metabolic phenotype first was observed for both male and female mice with global ERα deficiency. ERα-deficient male mice exhibited increased adiposity that became more pronounced with age, such that older mice had more than double the white adipose tissue mass of wild-type controls without differences in brown fat mass (Cooke et al. 2001; Heine et al. 2000; Ohlsson et al. 2000). This phenotype was ascribed to reduced energy expenditure in ERα-deficient mice rather than differential food intake (Heine et al. 2000). Male mice with adipocyte-specific ERα deficiency similarly exhibited significant increases in white adipose tissue mass, suggesting that loss of ERα signaling specifically within adipose tissue leads to changes in energy metabolism that favor increased adiposity.

In contrast to models of ERα deficiency, male mice with global ERβ deficiency exhibited comparable body weight and insulin sensitivity to wild-type mice on a

chow diet (Ohlsson et al. 2000). When female ERβ-deficient mice were exposed to a high-fat diet, however, they exhibited greater adiposity but less insulin resistance than wild-type controls, a phenotype that was ascribed to enhanced PPARϒ activation (Foryst-Ludwig et al. 2008). To date, a parallel interaction between high-fat feeding and ERβ deficiency has not been established for male mice.

Similar to male mice with abrogated ERα signaling, male mice with aromatase deficiency were shown to exhibit increased adiposity (Jones et al. 2001). In these mice, increased fat mass was attributable to reductions in spontaneous physical activity and glucose oxidation and found in association with insulin resistance and hepatic steatosis (Jones et al. 2000; Takeda et al. 2003). Liver from aromatase-deficient male mice showed increased expression of genes involved in fatty acid synthesis, and steatosis was reversed with administration of either 17β-estradiol or an ERα but not ERβ agonist (Cooke et al. 2001). In a second model of male mice with aromatase deficiency, hyperglycemia was attributed specifically to hepatic insulin resistance and increased gluconeogenesis (Van Sinderen et al. 2014). This liver phenotype again was reversed with exogenous estradiol. In female aromatase-deficient mice, adipose tissue exhibited increased expression of lipoprotein lipase (Lpl), consistent with increased fatty acid uptake, though this finding has not yet been corroborated in male mice (Misso et al. 2003).

More recently, potential metabolic roles of the membrane estrogen receptor GPER (GPR30) have been supported through genetic models. Male mice with global GPER deficiency showed greater body weight and fat mass accrual than wild-type controls. Increased adiposity was found in association with greater adipocyte size and ascribed to reduced energy expenditure rather than differential food intake (Davis et al. 2014). Notably, obesity due to GPER deficiency evolved earlier in male than female mice (Davis et al. 2014).

Central Mechanisms of Estrogen-Mediated Body Weight Regulation

Estrogens could regulate body weight in men through numerous mechanisms in both central and peripheral tissues (Fig. 2). Extensive work has demonstrated that central estradiol signaling plays key roles in the regulation of appetite, energy expenditure, and body weight (Brown and Clegg 2010; Mauvais-Jarvis et al. 2013). Aromatase is broadly expressed in the brain, but expression is particularly enriched in the hypothalamus, the principal regulatory site of appetite and energy expenditure as well as reproductive behavior (Abdelgadir et al. 1994; Roselli et al. 2009). The hypothalamus also has abundant ER expression, particularly in the arcuate, paraventricular, and ventromedial nuclei, with ERα expression generally higher than ERβ (Brown and Clegg 2010; Merchenthaler et al. 2004; Simerly et al. 1990). The central nervous system further is a site of de novo estrogen generation, as

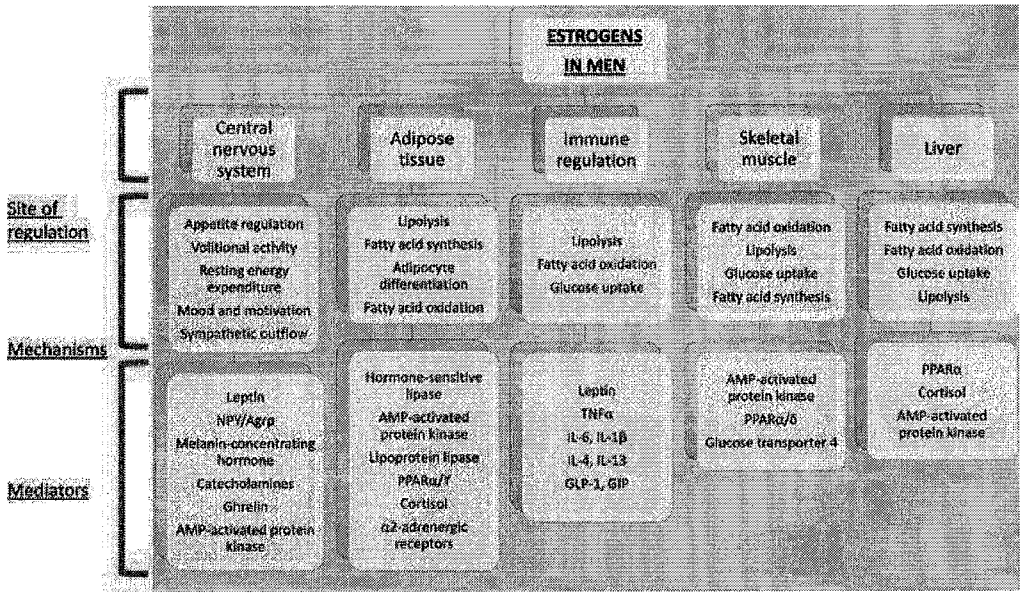

Fig. 2 Estrogens influence body weight and body composition in men through myriad pathways in key metabolic tissues

astrocytes and neurons have all the requisite enzymes to synthesize estradiol from cholesterol (Gillies and McArthur 2010).

Estradiol is well recognized as a critical regulator of both food intake and energy expenditure in females, with extensive research demonstrating its signaling effects particularly in the arcuate and ventromedial nuclei of the hypothalamus (Mauvais-Jarvis et al. 2013). Although most work to date has focused on the central mechanisms whereby estradiol regulates body weight in females, considerable evidence supports a parallel role for estradiol in males, as well. Aromatase expression in most brain regions is comparable in men and women (Stoffel-Wagner et al. 1999), and hypothalamic ER expression is comparable in male and female rodents, including within both the arcuate and paraventricular nuclei (Brown et al. 1992; Chakraborty et al. 2008).

Preclinical findings in males suggest an interaction between central estradiol and leptin signaling, as has been well established in females. The anorexigenic effects of leptin are more pronounced in females than in males, a phenomenon that has been ascribed to estradiol-mediated sensitization to leptin signaling. Supporting this idea, intact male rats did not exhibit changes in food intake after central leptin administration. However, a significant reduction in food intake after leptin administration was evident in orchiectomized male rats treated with subcutaneous estradiol (Clegg et al. 2006). Although increased leptin sensitivity was seen in males only with pharmacological estradiol treatment, these observations do not exclude a leptin-sensitizing effect of estradiol at physiological levels in males; thus, additional

work is needed to determine whether diminished leptin sensitivity is found in males with selective estradiol deprivation. Further supporting a dynamic interaction between estradiol and leptin in males, both males and females exhibited ERα upregulation in the arcuate nucleus of the hypothalamus in the setting of leptin deficiency (Chakraborty et al. 2008). Importantly, leptin is a critical regulator not only of energy intake but also energy expenditure. Thus, leptin also increases energy expenditure, in part through enhanced activity of the sympathetic nervous system (Morton and Schwartz 2011).

However, the anorexigenic effects of estradiol in males clearly are not mediated solely through leptin, as genetically leptin-deficient obese male mice exhibited diminished food intake and body weight loss after estradiol treatment (Gao et al. 2007). This phenomenon was ascribed to estradiol-mediated stimulation of POMC neurons in the arcuate nucleus through a leptin-independent, ERα-STAT3 pathway. The anorexigenic effects of supraphysiologic estradiol administration in males have been ascribed to other pathways, as well. Thus, in male rats, reductions in food intake after estradiol treatment were thought to be due in part to inhibition of the orexigenic neuropeptide melanin-concentrating hormone (MCH) (Mystkowski et al. 2000). Estradiol-mediated regulation of MCH may reflect an indirect interaction; although ERα did not co-localize with MCH neurons in male rats, both neuronal populations were found in abundance within the lateral hypothalamic area (Muschamp and Hull 2007). Nonetheless, estradiol-induced hypophagia has been observed in both MCH- and leptin-deficient male mice, and the hypophagic effect became more pronounced when mice were exposed to a high-fat diet (Tritos et al. 2004). Additional postulated effects of estradiol-mediated hypophagia in males include regulation of cannabinoid and ghrelin signaling. Thus, estradiol has been shown to downregulate cannabinoid receptor expression in the hypothalamus and attenuate cannabinoid-induced hyperphagia (Kellert et al. 2009; Riebe et al. 2010). Further, 17β-estradiol was found to inhibit the hyperphagic effect of central administration of the orexigenic hormone ghrelin in male rats (Clegg et al. 2007).

Notably, animal data do not uniformly demonstrate that estradiol-mediated effects on appetite regulation are similar in males and females with comparable degrees of estradiol exposure. A clear sexual dimorphism in hypothalamic estradiol signaling was illustrated by an experimental model that examined in parallel female sheep and castrated male sheep administered exogenous estradiol. Females and estradiol-supplemented males showed pronounced differences in the expression of hypothalamic genes implicated in appetite regulation in response to both light cycle and food restriction (Archer et al. 2004). A sexual dimorphism in the role of hypothalamic ERα signaling also has been demonstrated in mice, as ERα silencing in the ventromedial nucleus of the hypothalamus led to body weight gain in female but not male mice (Frank et al. 2014). Similarly, knockdown of ERα in POMC neurons in the arcuate nucleus of the hypothalamus promoted increased food intake and body weight gain exclusively in female mice (Xu et al. 2011).

In addition to the direct regulation of appetite through hypothalamic signaling, estradiol also could mediate changes in energy balance through indirect effects on

mood and motivation in men. Thus, these affective effects could produce changes in appetite and volitional activity that influence overall energy balance and, consequently, body weight. In male rats subject to orchiectomy, testosterone replacement conferred changes in behavior indicative of antianxiety and antidepressant effects, but these behavioral changes were abrogated with concurrent administration of an aromatase inhibitor (Carrier et al. 2015). Lower serum 17β-estradiol levels correlated with more depressive symptoms in a cohort of older men (Castanho et al. 2014), as well as in a population of men with obesity (Monteagudo et al. 2016).

Interestingly, in male mice fed a diet high in phytoestrogens, decreased adiposity was found despite increased food intake (Cederroth et al. 2007). This phenotype was ascribed in part to increased voluntary activity and resting energy expenditure consequent to changes in hypothalamic neuropeptides. Hypothalamic gene expression was notable for increased mRNA expression of the orexigenic orexin A and MCH but reduced mRNA expression of agouti-related peptide (Agrp) (Cederroth et al. 2007). Whereas increases in orexin A and MCH expression were thought to underlie the increased food intake in these animals, increased basal metabolic rate, lipid oxidation, and volitional activity were ascribed to reduced Agrp expression. Of note, in immortalized hypothalamic neurons treated with 17β-estradiol, ERα-mediated signaling reduced whereas ERβ-mediated signaling augmented Agrp expression (Titolo et al. 2006). An ERα-dependent increase in voluntary activity manifest as wheel running also was seen in male mice subject to orchiectomy followed by estradiol treatment (Ogawa et al. 2003).

Thus, findings to date support a role for central estradiol signaling in the regulation of appetite and energy expenditure in males. However, most findings are predicated on models that expose males to supraphysiologic doses of exogenous estradiol. These models underscore the importance of not viewing males simply as estrogen-deficient females and the corresponding need for continued investigation of physiologic estrogen signaling specifically in males. Indeed, some experimental models argue against a potent role for estradiol in appetite regulation at physiologic levels in males. Thus, additional work is necessary to better discriminate between concentration-dependent effects and true sexual dimorphisms in centrally mediated effects of estradiol in body weight regulation.

Adipose-Specific Mechanisms of Estrogen-Mediated Body Weight Regulation

Among peripheral metabolic tissues, adipose tissue is particularly enriched in both aromatase and ER expression. Adipose tissue aromatase is found predominantly in preadipocytes and is part of the pro-adipogenic program coordinated by glucocorticoid signaling (Simpson 2004). Similar to the CNS, adipose tissue generally exhibits higher expression of ERα than ERβ, and expression levels are comparable in men and women (Pedersen et al. 2001). ERα has been identified in both mature

adipocytes and preadipocytes, whereas ERβ was found exclusively in mature adipocytes (Dieudonné et al. 2004). The regulation of ER in adipose tissue is partially dependent on estradiol and exhibits both cell type specificity and sexual dimorphisms. Whereas 17β-estradiol upregulated expression of both ERα and ERβ in adipocytes harvested from women, it selectively upregulated ERα expression in adipocytes from men (Dieudonné et al. 2004). Further, ERα expression in preadipocytes was not estradiol-responsive in cells from either sex.

Preclinical and clinical data collectively support an overall anti-obesogenic role for estradiol action in adipose tissue. Fat mass accrual occurs through adipocyte hypertrophy as well as adipocyte hyperplasia, the generation of new adipocytes through preadipocyte differentiation. Adipocyte hypertrophy results from progressive accumulation of lipid within the cell, either through lipogenesis or uptake of extracellular lipid. One described mechanism by which estradiol can suppress adiposity is inhibition of Lpl, which hydrolyzes triglycerides and thereby releases free fatty acids for cellular uptake. Estradiol has been shown to inhibit Lpl activity in women and suppress Lpl expression in cultured 3T3-L1 cells, an immortalized cell line that can be induced to differentiate into cells that phenotypically resemble adipocytes (Homma et al. 2000; Price et al. 1998). Increased Lpl-mediated fatty acid uptake also has been proposed as a primary mechanism underlying the increased adiposity seen in aromatase-deficient mice though this was proposed on findings limited to female mice (Misso et al. 2003). Illustrating the dose dependency of estrogen-mediated effects, high-dose estradiol treatment inhibited Lpl protein expression in adipocytes isolated from women, whereas the lowest treatment dose enhanced Lpl expression (Palin et al. 2003). Again, however, parallel dose-dependent responses in adipocytes harvested from men have not been determined.

Although orchiectomy has been used extensively in rodent studies to investigate the metabolic effects of gonadal steroids in males, few studies to date have carefully discriminated between androgen- and estrogen-mediated outcomes. In a recent study, male mice were subject to orchiectomy with testosterone replacement with or without an aromatase inhibitor. Orchiectomy led to increases in adiposity in conjunction with increased adipose tissue expression of Lpl and the lipogenic genes fatty acid synthase and sterol regulatory element-binding protein-1 (SREBP-1). These changes in both fat mass and gene expression were fully reversed by testosterone replacement (Holland et al. 2016). Adipose tissue expression of Lpl and fatty acid synthase appeared at least partially suppressed by estrogen exposure, whereas SREBP-1 expression exhibited more pronounced estrogen-mediated inhibition. Thus, treatment with testosterone and an aromatase inhibitor led to an intermediate phenotype, with less adiposity than seen in orchiectomized animals but persistent increases in fat mass and lipogenic gene expression relative to animals administered testosterone replacement alone (Holland et al. 2016). Diminished expression of lipogenic genes regulated by SREBP-1 also was seen in vitro in cultured murine adipocytes treated with 17β-estradiol (D'Eon et al. 2005). Further supporting an anti-obesogenic role for estrogen in males, male mice fed a diet enriched in phytoestrogens exhibited reduced adiposity, with enhanced AMP-activated protein

kinase (AMPK) signaling in adipose tissue (Cederroth et al. 2008). As AMPK promotes fatty acid β-oxidation and suppresses lipogenesis, its regulation by estrogens may be another mechanism whereby estrogen favors lipid utilization over lipid uptake, synthesis, and storage. Collectively, these preclinical findings indicate that adequate androgen and estrogen exposure are likely both required to restrain adiposity in males.

Estradiol signaling in males has been shown to contribute to the regulation of preadipocyte proliferation and differentiation, central facets of fat mass accrual. Again, sexual dimorphisms are evident in the effects of estradiol on adipogenesis. Thus, 17β-estradiol increased preadipocyte proliferation and enhanced differentiation in cells harvested from female but not male rats. In mature adipocytes from rats of both sexes, however, estradiol treatment upregulated expression of PPARϒ, the central transcriptional regulator of adipocyte differentiation (Dieudonne et al. 2000). In a clinical study, 17β-estradiol increased proliferation of preadipocytes harvested from both men and women, although the rate of proliferation was significantly faster in preadipocytes from women (Anderson et al. 2001). One mechanism underlying estradiol's role in adipogenesis may be regulation of glucocorticoid metabolism. Although glucocorticoids induce aromatase expression and estrogen production during preadipocyte differentiation, this may reflect a key role for estradiol in the restraint of adipogenesis through negative feedback regulation of glucocorticoid signaling. Thus, in male mice, exogenous 17β-estradiol administration reduced body weight gain in a model of diet-induced obesity. This lower adiposity occurred in association with reduced adipose tissue mRNA expression and activity of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), the enzyme that generates cortisol from cortisone (Dakin et al. 2015). These findings led the authors to propose that inhibition of cortisol generation and its associated pro-adipogenic effects is a primary mechanism through which estradiol suppresses fat accrual in males (Dakin et al. 2015). Supporting this conclusion, treatment with 17β-estradiol rapidly inhibited 11β-HSD activity in cultured 3T3-L1 adipocytes; interestingly, this effect did not appear dependent on ER signaling (Tagawa et al. 2009).

Estradiol further has been implicated in the regulation of adipocyte lipolysis, another mechanism by which it can serve as a critical determinant of the balance between lipid storage and mobilization. Both pro- and anti-lipolytic roles for estradiol have been reported. Thus, upregulation of α2-adrenergic receptors was found after estradiol treatment of subcutaneous adipocytes from women, suggesting inhibition of lipolysis (Pedersen et al. 2004). In apparent contrast, however, 17β-estradiol treatment amplified catecholamine-induced lipolysis in murine adipocytes (D'Eon et al. 2005). Consistent with both seemingly discordant findings, testosterone treatment was shown to simultaneously upregulate both β- and α2-adrenergic receptors in adipocytes harvested from castrated male hamsters (Giudicelli et al. 1993). Whether these respective effects were androgen or estrogen dependent was not established. In subcutaneous adipocytes isolated from women, estradiol also was shown to exert pro-lipolytic effects through dose-dependent stimulation of hormone-sensitive lipase (Palin et al. 2003). It remains to be established whether estradiol confers similar effects in adipocytes isolated from men.

Estrogen concentrations are regulated within adipose tissue not only through local production by aromatase but also estrogen metabolism mediated primarily by EST. The role of EST in body weight and fat mass regulation has been examined, and interestingly, EST exhibits sexually dimorphic expression and may exert divergent effects on adipogenesis in rodents and humans. EST expression was identified in white adipose tissue from male but not female rodents, and EST deficiency in male mice led to increases in epididymal fat mass with larger adipocyte size (Khor et al. 2008). These findings are consistent with in vitro data demonstrating that EST expression declined with the differentiation of both 3T3-L1 cells and primary mouse adipocytes, whereas EST overexpression inhibited 3T3-L1 cell differentiation (Wada et al. 2011). In humans, EST expression initially was detected in subcutaneous adipose tissue from obese men and women and subsequently in adipose tissue from nonobese women, as well (Ahima et al. 2011; Ihunnah et al. 2014). In striking contrast to preclinical findings, EST overexpression in human adipose tissue stem cells promoted adipocyte differentiation in association with increased expression of lipogenic genes, whereas differentiation was inhibited by EST knockdown. Further, these effects were shown to be ER dependent, indicating that they were conferred specifically through the enzymatic activity of EST that leads to inactivation of estradiol (Ihunnah et al. 2014). The authors also showed a positive correlation between adipose tissue EST expression and BMI. Critically, however, these findings were generated with adipose stem cells isolated exclusively from women and need corroboration in men, as well.

Other Peripheral Mechanisms of Estrogen-Mediated Body Weight Regulation

Although aromatase and ER expression are particularly enriched in adipose tissue, estrogen signaling in other peripheral metabolic tissues also may make a significant contribution to estrogen-mediated regulation of energy metabolism and body weight. In female mice, estradiol was found to downregulate expression of lipogenic genes in both skeletal muscle and liver. Further, estradiol increased activation of AMPK and PPARδ in skeletal muscle, putative mechanisms through which estradiol appeared to cause a shift away from lipid storage and toward fatty acid oxidation (D'Eon et al. 2005). ERα deficiency in skeletal muscle led to increases in gonadal fat mass and impaired glucose tolerance in female mice, findings ascribed to dysregulated mitochondrial turnover and function (Ribas et al. 2016). Both ERα and ERβ have been shown to regulate glucose transporter 4 (GLUT4) expression in skeletal muscle as well as adipose tissue in male mice with divergent effects of the two receptor subtypes; GLUT4 expression is upregulated by ERα but downregulated by ERβ (Barros et al. 2006, 2009). Although the ERα-mediated increase in GLUT4 expression may be seen as beneficial with regard to enhanced glucose

disposal, it nonetheless also contributes to increased energy uptake in adipocytes that could manifest as fat mass accrual in the setting of positive energy balance.

An emergent area of research involves the roles of sex steroids in regulating intestinal flora. The composition of the gut microbiome has received substantial attention as a potential contributor to obesity and associated metabolic dysregulation (Khan et al. 2016). A recent mouse model offers initial evidence that sex steroid exposure may influence the composition of the gut microbiome. Thus, in male mice fed a high-fat diet, castration led to increases in visceral adiposity in association with alterations in intestinal microflora (Harada et al. 2016). Further, castration-induced increases in visceral fat were blocked with antibiotic administration. Importantly, changes in the intestinal microflora were not seen on a regular chow diet, suggesting these changes may reflect not a direct, sex steroid-mediated effect but rather an interaction between sex steroids and diet and/or excess energy intake. Further, this study did not discriminate between androgen- and estrogen-mediated effects on intestinal flora. Clinical data regarding sex steroids and the gut microbiome are scant. In a cross-sectional study that included healthy men, urinary estrogen levels exhibited a strong, positive correlation with diversity in intestinal flora and fecal β-glucuronidase activity, leading the authors to posit that the gut microbiome may regulate circulating estrogen levels (Flores et al. 2012). The possibility exists, too, that this relationship may be inverse or bidirectional. Thus, estrogen signaling in these extra-adipose sites of energy metabolism could also contribute to estrogen-mediated changes in energy balance, body weight, and adiposity in males. Critically, however, estrogens mediate dose-, context-, and sex-dependent roles, and additional work is therefore necessary to establish and characterize these estrogen-mediated effects specifically in males.

The Immunomodulatory Effects of Estradiol

A growing area of research is focused on defining how the immunomodulatory effects of estradiol might contribute to estrogen-mediated regulation of energy balance and body composition. Estradiol is known to influence immune cell function, with extensively described effects on cellular differentiation, phenotype, and function in both adaptive and innate immunity (Cunningham and Gilkeson 2011; Kovats 2012, 2015). Resident immune cells are present within adipose tissue and undergo dynamic changes in both number and phenotype during states of both positive and negative energy balance with associated adipose tissue remodeling (Gerriets and MacIver 2014; Mathis 2013; Olefsky and Glass 2010; Suganami and Ogawa 2010; Weisberg et al. 2003). Animal models demonstrate that adipose tissue immune cells – through the secretion of paracrine effectors including growth and angiogenic factors, matrix metalloproteinases, and cytokines – are critical mediators of lipid and glucose metabolism, adipocyte differentiation, and tissue remodeling (Lacasa et al. 2007; Lu et al. 2010; Lumeng et al. 2008; Spencer et al. 2010; Suganami and Ogawa 2010; Xu et al. 2003; Ye and McGuinness 2013).

Estradiol-mediated effects on immune function are highly context dependent and vary as a function of estradiol concentration and timing of exposure, the distinct microenvironment and concurrent signals, and target cell type (Straub 2007). Irrespective of the context-dependent magnitude and directionality of these estradiol-mediated effects, however, estradiol has been reproducibly shown to modulate cellular differentiation, survival, and chemokine and cytokine production in both lymphocytes and myeloid cells. Most of estradiol's immunomodulatory effects are thought to be mediated through ERα signaling, but ERβ and GPER also have been implicated in estradiol-mediated effects on immune cell function (Blasko et al. 2009; Monteiro et al. 2014; Straub 2007).

The regulation of cytokine secretion constitutes one general mechanism whereby estradiol can mediate indirect effects on energy metabolism, insulin sensitivity, and adipogenesis. Estradiol has been shown to regulate production of the predominantly macrophage-derived cytokines TNFα, IL-1β, and IL-6, with either stimulatory or inhibitory effects on secretion that depend largely on macrophage activation state. In general, at higher concentrations, these cytokines exert inhibitory effects on adipocyte differentiation and fatty acid synthesis while promoting lipolysis. Importantly, however, immune-derived mediators play complex roles in metabolic regulation and defy simple designation as good or bad for metabolic health (Wang and Ye 2015; Ye and McGuinness 2013). TNFα illustrates this principal, as its common designation as a "pro-inflammatory" or insulin resistance-promoting cytokine fails to capture the complexity and context dependence of its metabolic effects. Thus, the effects of TNFα exposure on GLUT4 expression and insulin signaling in adipocytes varied as a function of time and TNF dose (Stephens et al. 1997). Elevated circulating levels of TNFα were found in aromatase-deficient mice and thought to play a pathogenic role in the metabolic dysregulation conferred by aromatase deficiency. Strikingly, however, when TNF receptor type 1 (TNFR1) was knocked down in these mice, hepatic steatosis and insulin resistance not only failed to improve but rather were further exacerbated (Toda et al. 2010).

Estradiol-regulated cytokines other than TNF also appear to have significant metabolic roles. Evidence from both rodents and humans supports a role for IL-6 in promoting the secretion of glucagon-like peptide-1 (GLP-1), a gut-derived hormone that regulates satiety and glucose homeostasis (Kahles et al. 2014; Lebherz et al. 2016). The cytokines IL-4 and IL-13 have been implicated in the regulation of adipose tissue energy metabolism, as both were shown to increase expression of uncoupling protein 1 (UCP1) and thermogenic capacity in adipocytes (Qiu et al. 2014). Thus, through regulation of cytokine secretion alone, estradiol could mediate changes in adipocyte differentiation and energy metabolism that could substantially impact fat mass accrual. Moreover, in addition to direct effects on immune cell phenotype and function, estradiol also could modulate immune activity indirectly, through pathways involving leptin, glucocorticoids, or sympathetic nervous system function, all of which are regulated by 17β-estradiol and, in turn, influence immune cell function.

Findings in female mice further lend in vivo evidence that estradiol regulates adiposity and energy metabolism through immunomodulatory effects. Thus, ovari-

ectomy resulted in altered cytokine expression and immune cell populations in adipose tissue (Rogers et al. 2009). Lending more direct evidence to this proposed model, female mice were generated with selective ERα deficiency in either myeloid or all hematopoietic cells. In both models, ERα deficiency conferred significant increases in fat mass (Ribas et al. 2011). In mice with myeloid-specific ERα deficiency, increased fat mass was found in association with increased adipocyte size and greater tissue macrophage infiltration (Ribas et al. 2011). Thus, these findings indicate that the immunomodulatory effects of estradiol are at least partially responsible for the obesity and associated metabolic derangements seen in mice with global ERα deficiency. To date, however, parallel studies have not been performed in male mice.

Estrogens Beyond 17β-Estradiol: Estrone and 17α-Estradiol

Although 17β-estradiol is the predominant circulating estrogen in men and premenopausal women, it may not be the only relevant estrogen with regard to metabolic regulation in men. Among men enrolled in the Diabetes Prevention Program, positive associations were found between serum estrone concentrations and the incident development of T2DM (Mather et al. 2015). Another study similarly found that circulating estrone levels are associated with incident development of T2DM in men and, further, are a better predictor of diabetes development than serum estradiol levels (Jasuja et al. 2013b). Changes in serum estrone levels were shown to positively correlate with BMI, and increased prevalence of both diabetes and cardiovascular disease was found among men in the highest quintile of serum estrone levels (Jasuja et al. 2013a). Other studies have corroborated this positive association between body weight and serum estrone levels in obese men (Bélanger et al. 2006; Kley et al. 1980a, b).

This reproducible association between serum estrogen levels and adiposity in men has been ascribed to increased adipose tissue aromatase activity in the setting of fat mass accumulation (Brind et al. 1990). Rather than merely a marker of adipose tissue aromatase activity, however, estrone could mediate metabolic effects. Thus, both estrone and its fatty acyl ester oleoyl-estrone have been shown to influence body weight and adiposity in rodents as well as adipogenesis in in vitro models. Administration of oleoyl-estrone to female rats reduced fat mass through both decreases in food intake and enhanced lipolysis and fat oxidation (Sanchis et al. 1996, 1997). Similarly, oleoyl-estrone decreased food intake in male Zucker rats and produced even more marked loss of fat mass than seen in female rats (Grasa et al. 2001). The anti-adiposity effects of oleoyl-estrone were abrogated with corticosteroid treatment, suggesting inhibition of glucocorticoid signaling may be a key mechanism through which oleoyl-estrone reduces fat mass (Serrano et al. 2009). In contrast, however, elevated estrone exposure has been posited to contribute directly to increases in body weight and adiposity (Remesar et al. 1999). In acute food deprivation in rats, serum estrone levels rose whereas estrone fatty esters declined, find-

ings potentially consistent with their respective putative effects on energy conservation and utilization (Vilà et al. 1999).

Another estrogen that has been implicated in metabolic regulation is 17α-estradiol. A naturally occurring enantiomer of 17β-estradiol, 17α-estradiol is best described as a paracrine regulator in brain (Toran-Allerand et al. 2005) but more recently was found to play roles in body weight regulation and energy homeostasis. In male mice, systemic administration of 17α-estradiol led to reductions in adiposity greater than those seen with modest caloric restriction, with particular loss of visceral adipose tissue (Stout et al. 2017). Both central and peripheral mechanisms of action were supported. Thus, 17α-estradiol-treated mice exhibited lower food intake with associated changes in the expression of hypothalamic genes implicated in appetite regulation, and 3T3-L1 adipocytes showed changes in energy-sensing pathways after 17α-estradiol treatment in vitro. Male mice treated with 17α-estradiol further showed increased AMPK activation in adipose tissue but not liver or skeletal muscle (Stout et al. 2017). The regulation of 17α-estradiol production has not been elucidated, but its local production is indicated by persistent presence throughout the brain after gonadectomy and/or adrenalectomy in mice (Toran-Allerand et al. 2005). Thus, estrone and 17α-estradiol underscore the complexity of local estrogen metabolism. Careful, tissue-specific interrogation of an expanded scope of estrogens and their derivatives will therefore be essential to fully delineate the estrogen-mediated mechanisms of body weight regulation in men.

Estrogen and Obesity in Men: Too Much or Too Little?

Estradiol deficiency clearly predisposes males to increased adiposity and metabolic dysregulation. In apparent contrast, however, obesity in men has been associated with hyperestrogenemia, and, further, excessive estradiol exposure has been postulated to play an exacerbating role in the progression of obesity and attendant metabolic dysregulation. Though not uniformly, obesity in men is often characterized by a profile of low circulating androgens but elevated levels of circulating estrone and 17β-estradiol (Schneider et al. 1979; MacDonald et al. 2010). The reasons for this variable co-occurrence of obesity and hyperestrogenemia in men are not well defined but may include repeat number of a TTTA polymorphism in the aromatase gene *CYP19A1* (Hammoud et al. 2010). Although overtly elevated serum estrogen levels are not found in all men with obesity, it has been proposed that obesity also may represent a state of relative rather than absolute estrogen excess. Thus, increased peripheral aromatization of testosterone in obese men may lead to enhanced central estradiol signaling that suppresses gonadotropin production and contributes to a sustained state of hypogonadotropic hypogonadism (Mah and Wittert 2010). This serum profile of sex steroids is normalized with weight loss, as serum testosterone and gonadotropin levels rise, whereas serum estradiol levels fall subsequent to weight loss effected either through bariatric surgery or behavioral change (Armamento-Villareal et al. 2016; Corona et al. 2013; Mihalca and Fica 2014;

Pellitero et al. 2012). Thus, whether absolute or relative, estrogen excess is believed to have a bidirectional relationship with obesity in men and to contribute to progressive adiposity and metabolic dysregulation (Corona et al. 2011; Mah and Wittert 2010).

Indeed, some preclinical evidence supports pro-adipogenic effects of estradiol. In male but not female preadipocytes, 17β-estradiol induced aromatase activity, leading the authors to postulate that excess estradiol within adipose tissue may be self-propagating and contribute to progressive increases in leptin and cortisol signaling with attendant pro-adipogenic effects (Dieudonné et al. 2006). Further, enhanced conversion of testosterone to estradiol could cause a relative androgen depletion and thereby limit the anti-adipogenic effects conferred by androgens within adipose tissue (Dieudonne et al. 2000). One possible way to reconcile the apparent paradox of intra-adipose estradiol as both pro- and anti-adipogenic is to view obesity as a dynamic rather than static state, one characterized by positive energy balance and continual delivery of excess glucose and lipids to peripheral tissue. Adipose tissue remains the primary sink for this excess energy intake, but fat mass expansion in obesity becomes increasingly limited by adequacy of blood and oxygen supply as well as physical constraints. Viewed in this context, increased estradiol generation within adipose tissue could be seen as an adaptation with variable effects on adipogenesis and lipid storage that serve to both restrain adipogenesis and maintain some capacity to store the excess glucose and lipid that otherwise would contribute to ectopic fat accrual.

Importantly, even as this increased estradiol production may be adaptive, it may nevertheless confer both harmful and beneficial effects with regard to metabolic regulation. In this model, estradiol is a regulatory mediator that can exert either pro- or anti-adipogenic effects or both concurrently. With regard to adipogenesis, for example, 17β-estradiol was shown to inhibit 11β-HSD1 activity in adipocytes from male rats, thus suppressing the formation of pro-adipogenic cortisol (Tagawa et al. 2009). However, it also increases leptin expression and secretion, which, in turn, enhances 11β-HSD1 expression in preadipocytes and, moreover, promotes further estradiol generation through upregulation of aromatase (Dieudonné et al. 2006). The concentration-dependent effects of estradiol on Lpl activity further demonstrate the potential for estradiol to play divergent roles in lipid uptake by adipocytes, again indicating estradiol could either inhibit or promote lipid accumulation in adipocytes. Consequently, the net impact on adipogenesis and fat mass will be contingent on local estradiol concentrations and concurrent signals in the tissue environment as well as the total delivery of energy substrate to adipose tissue (Fig. 3). These context-dependent effects of estradiol are also illustrated by estradiol-mediated regulation of TNFα. At higher concentrations, TNFα can inhibit adipocyte differentiation, promote lipolysis, and inhibit insulin-stimulated glucose uptake, thus acting as a potent anti-adipogenic signal (Ruan et al. 2002). Therefore, estradiol-mediated suppression of TNFα production would serve to promote adipogenesis, lipid storage, and glucose uptake in adipocytes. However, these anti-adipogenic effects of TNFα are concentration dependent, as are the inhibitory effects of estradiol on TNFα generation. Thus, pro-adipogenic effects of estradiol through TNFα suppres-

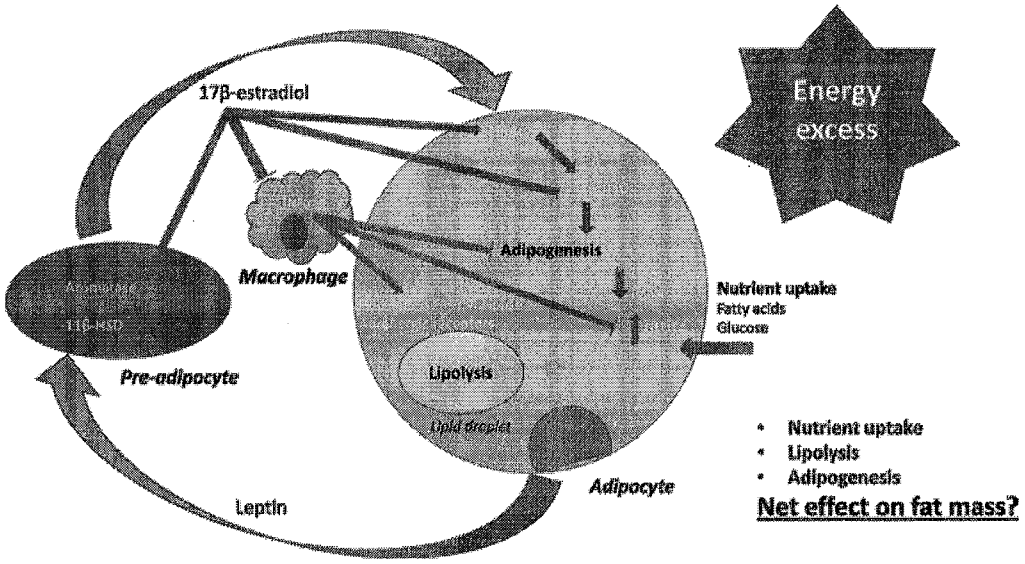

Fig. 3 Estradiol mediates myriad effects within adipose tissue and contributes to the regulation of adipocyte differentiation, nutrient uptake, and lipolysis. When viewed in isolation, these effects could be seen as either pro- or anti-obesogenic. The cumulative effect of estradiol on fat mass accrual is contingent upon context and, critically, overall energy balance

sion only would be evident in obesity or other states characterized by elevated production of both estradiol and TNFα within adipose tissue. Conversely, in a state of neutral or negative energy balance, TNFα production is generally lower, which may be one factor that allows the anti-adipogenic effects of estradiol to become manifest.

Estradiol excess therefore may not truly represent an aberrant response that promotes further fat mass accrual in the setting of obesity; rather, a more physiologically relevant model may be one of a propagated, amplified process consequent to a continued state of positive energy balance. Thus, the process may be wholly regulated and reversible, as supported by the normalization of serum estradiol levels as well as marked improvements in metabolic regulation in obese subjects after induction of negative energy balance leading to body weight reductions (Petersen et al. 2005; Viljanen et al. 2009; Wing and Group 2010). This model suggests that increased estradiol production within adipose tissue is an adaptive response to continual delivery of nutrient excess rather than a primary pathogenic driver of obesity in men. The increased estradiol production may be promoting continued – though not unrestrained – adipogenesis as well as nutrient uptake in order to handle excess lipid and glucose, thus maintaining adipose tissue as the primary reservoir for energy storage while addressing the constraints on adipose tissue expansion. An adaptive rather than pathogenic role of increased intra-adipose estradiol is further supported by clinical studies of aromatase inhibitors in obese men. Although aromatase inhibition reduces circulating estradiol levels and restores normal circulating testosterone levels, clinical intervention trials to date have failed to show any associated metabolic benefit (Burnett-Bowie et al. 2009; Loves et al. 2008, 2013).

These negative findings underscore the importance of understanding estradiol as a paracrine and intracrine mediator rather than solely assessing circulating levels (Simpson 2003).

Clinical Intervention Studies

Only over the past few years have clinical intervention studies begun to confirm preclinical evidence that estradiol contributes to body weight regulation and metabolic health in men. One small study examined the effects of testosterone replacement in obese men with low-normal baseline serum testosterone concentrations. Whereas treatment with testosterone gel led to significant reductions in adiposity, these changes were not seen when testosterone was co-administered with an aromatase inhibitor (Juang et al. 2014). In a larger study of healthy men, two subject cohorts were administered the GnRH analogue goserelin acetate to suppress endogenous sex steroid production. Simultaneously, subjects in the first cohort received either placebo gel or variable doses of add-back testosterone gel, and the second cohort of subjects received either placebo gel or testosterone gel with an aromatase inhibitor. Strikingly, whereas androgen exposure appeared to mediate changes in lean mass, estradiol rather than testosterone was found to be the primary determinant of changes in fat mass (Finkelstein et al. 2013). Subsequently, another clinical study similarly enrolled healthy, eugonadal men and rendered them medically castrate through use of the GnRH antagonist acyline. Subjects in this study variably received placebo gel, low-dose or full replacement dose testosterone gel, or full replacement dose testosterone gel with an aromatase inhibitor. In all three treatment groups rendered sex steroid deficient, significant increases in body fat mass were evident within only 4 weeks of drug treatment (Chao et al. 2016). Again, estradiol rather than testosterone deprivation exhibited a stronger correlation with the observed increases in adiposity.

Implications for Clinical Practice

Better understanding of the importance of estrogens for maintaining metabolic health in men is essential for optimal treatment of male hypogonadism and, further, for understanding the metabolic implications of sex steroid manipulation in clinical practice. Exogenous testosterone therapy in hypogonadal men restores circulating androgen levels but not fertility, prompting interest in identifying alternative therapeutic strategies. The ER antagonist clomiphene and its derivatives also have been proposed as therapeutic interventions for secondary hypogonadism in men (Wiehle et al. 2013). Clomiphene blocks estradiol-mediated gonadotropin suppression and

thereby helps restore normal testosterone production while preserving fertility (Kaminetsky et al. 2013; Kim et al. 2016; Wiehle et al. 2014). The use of aromatase inhibitors also has been proposed for men with hypogonadism associated with hyperestrogenemia, including subsets of men with late-onset and obesity-associated hypogonadotropic hypogonadism (de Boer et al. 2005; Tan et al. 2014; Zumoff et al. 2003). However, as appreciation grows of the critical metabolic roles of estrogens in men, caution may be warranted in the pursuit of such anti-estrogen-based approaches to treatment of male hypogonadism. Similarly, use of selective androgen receptor modulators for treatment of hypogonadism has been proposed (Bhattacharya et al. 2016; Thirumalai et al. 2017), but this strategy may not fully restore estradiol signaling and, therefore, fail to optimize metabolic health in hypogonadal men. Rather, selective estrogen receptor modulators may prove equally critical for improving body composition and metabolic regulation in male hypogonadism. Novel, targeted pharmacological strategies that promote ERα signaling may prove important not only for treatment of male hypogonadism, as a dual ERα/GLP-1 agonist has been developed as a potential therapeutic for metabolic disorders (Finan et al. 2012).

Another population for whom the metabolic effects of estradiol could prove highly relevant are men with prostate cancer. In the USA, prostate cancer affects 2 million men, and up to 50% of these men will undergo androgen deprivation therapy (ADT) at some point in their treatment course (Meng et al. 2002). The most common form of ADT involves GnRH analogues that confer central hypogonadism, and over the past decade, clinical evidence has compellingly demonstrated the men undergoing ADT are at substantially higher risk of increased adiposity, insulin resistance, T2DM, and cardiovascular disease than age-matched controls with or without prostate cancer (Cannata et al. 2012; Hamilton et al. 2011; Keating et al. 2006, 2012; Shahani et al. 2008). ADT-induced hypogonadism is a state of both androgen and estrogen deficiency, and the latter is now believed to contribute substantially to the metabolic dysregulation evident in men receiving GnRH analogues. Interestingly, estradiol therapy was among the ADT formulations originally used for treatment of prostate cancer (Cannata et al. 2012), and interest in estrogen-based ADT recently has been renewed as it could effectively suppress androgen production while reducing the metabolic sequelae of GnRH analogues (Phillips et al. 2014).

Finally, as efforts continue to develop an effective form of hormonal contraception for men (Ayoub et al. 2016; Zitzmann et al. 2017), these findings collectively underscore the need to carefully assess changes in estradiol exposure consequent to different contraceptive regimens. Thus, mounting evidence suggests that estradiol replacement is a pivotal facet of the treatment of male hypogonadism and, by corollary, states of estradiol deprivation – whether consequent to physiologic hypogonadism, androgen deprivation therapy, or hormonal forms of contraception – must be avoided as possible to optimize metabolic health in men.

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