Original Article: The Effects of Esreradiol on Leptin and other Factors

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ABSTRACT

In this article, the Effects of esreradiol on leptin and other factors as investigated. Gonadal hormones potently control food intake and body weight. In female animals, deactivation effects of estradiol acutely and chronically influence body weight homeostasis. In rats and mice, estrogen exerts a tonic inhibitory effect on meal size and daily food intake throughout the ovarian cycle and a cyclic inhibitory effect during the peri-ovulatory phase. Removal of estrogen leads to changes in meal size and duration, hyperphagia, and obesity. Estrogen has similar effects in humans where it modulates periovulatory decreases in daily food intake. Additionally, reductions in estrogen are associated with changes in body weight and fat distribution in humans, which parallel the findings in animals. Estrogen has the ability to control energy balance, food intake, and body fat distribution and this may be mediated through its interaction with orexigenic and anorexigenic hormones. This review aims to explore these interactions and discuss the link between estrogen and obesity. Differences in adipose mass and distribution and glucose homeostasis in males and females have been attributed at least partly to sex steroids. Menopause is characterized by reduced estrogen production and a shift in adipose distribution from peripheral to central accumulation. Hyper androgenization in polycystic ovarian disease is associated with central (visceral) obesity, insulin resistance, and type 2 diabetes. Moreover, ERα deficiency resulted in a decrease in energy expenditure with no change in food intake. Estrogen regulates energy balance through the central nervous system, as evidenced by the development of hyperphagia and reduced energy expenditure in response to injection of ERα RNAi into the ventromedial nucleus of the hypothalamus. Therefore, to determine the local effect of estrogen deficiency on leptin and insulin we choose testosterone in female to levels found in males instead of ovariectomy and to determine the effect of androgen deficiency we choose esreradiol injection in male rats to levels found in females instead of orchiectomy.

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Introduction

A large number of people who suffer from lower limb trauma survive, and a significant number of them suffer from physical disabilities and mental problems during the treatment process and even after (1). The most common mental disorders after organ trauma include post-traumatic depression, anxiety, mental distress, and alcohol abuse (2) and usually, mental disorders caused by traumas decrease with the passage of time and appropriate support measures. Merkarti et al. (2003) in a study showed that the prevalence of mental disorders in people who suffered lower limb traumas decreased from 48% to 42% between 3 and 12 months after the accident (3). Sauper and his colleagues (2012) also showed in a study that after 5 years of the occurrence of traumas, the severity of physical and mental disorders in traumatized people has decreased, but the prevalence of these disorders is still significantly higher than other people in the society (4). Chani Sober (2015) in another study showed that in the time interval of 10 years after the trauma, the prevalence of mental disorders in injured people is similar to other people in the society, but the physical health of these people is lower than other people in the society (5).

Reviewing the texts of the articles

In a study conducted by Abbas Ali Ebrahimian and colleagues on the trend of changes in depression, anxiety and stress in men with lower limb traumas, they showed that movement restrictions can be effective on the prevalence of mental disorders, but usually during treatment (6). Lower limb traumas are more focused on the physical problems and disabilities of the patients, and less attention is paid to the treatment of mental disorders of such patients.

Therefore, a study was conducted with the aim of investigating the changes in depression, anxiety and stress in men suffering from lower limb trauma. In this prospective study, the injured who were hospitalized from June 2013 to August 2015 due to lower limb traumas in Amir al-Momenin (AS) and Kosar Semnan hospitals were selected and studied by simple random method. Data collection tools were two demographic and accident questionnaires and depression, anxiety and stress questionnaires (7). At first, the patients were asked to complete the first stage questionnaires based on their psychological characteristics in the month leading up to the trauma. Then in time intervals 1 and 3 months after the trauma, these questionnaires were completed by the patients.

Neuropeptide Y

Neuropeptide Y (NPY) is an important central regulator of energy homeostasis in a many hypothalamic neurons (8). NPY is a potent orexigen, which increases feeding behavior in fed and fasted animals (9). Estrogen acts via the estrogen receptors (ERs) in the hypothalamus to reduce feeding (10), and may mediate its anorectic effects by decreasing NPY expression or release (11). In addition, Sar et al. reported colocalization of estradiol and NPY immunoreactivity in ARC neurons, suggesting that estradiol may modulate NPY neurosecretion in the hypothalamus (12). However, it remains unclear whether estrogen directly affects NPY neurons and in which area of the brain this effect occurs (13). Ovariectomized (OVX) rats experience a rapid weight gain, which can be reversed by estradiol replacement (14). administered either peripherally or centrally (15). Estradiol deficiency results in increased NPY concentrations in the paraventricular nucleus (PVN) of the hypothalamus and elevated NPY mRNA expression in the arcuate nucleus (ARC) of the hypothalamus (16). Bonavera et al. reported that estradiol treatment decreased hypothalamic NPY levels in the PVN of OVX rats (17). NPY neurons in the hypothalamus not only affect feeding, but they also influence reproduction. Therefore, estradiol can modulate both of these neuroendocrine systems by regulating NPY gene expression. NPY neurons are activated by signals indicating reduced energy availability, such as decreased levels of circulating glucose, leptin or insulin, which increase NPY release in the PVN to stimulate feeding (18). This may be due to estradiol stimulating NPY, NPY Y1 receptor (20) expression and NPY release (19). In an *ex vivo* hypothalamic neuronal cell line, N-38, estradiol affected the expression of NPY in a biphasic

manner, which corresponded to changes in the ERa: ERa ratio. When the ERa: ERa ratio was high, NPY transcription was repressed; conversely, when the ratio was low, NPY transcription was stimulated. These results provided evidence that the ratio of ERs expression in the hypothalamus may differentially regulate NPY *in vivo* (21).

Ghrelin

Ghrelin is produced in the stomach and acts on growth hormone secretagogue receptors (GHSRs) to increase food intake. While mainly synthesized by the stomach, ghrelin is also found the hypothalamus, pituitary gland, hippocampus, brain cortex, adrenal gland, intestine, and pancreas (22). Exogenous ghrelin is less potent in intact female rats than in male rats or OVX females (23). Central intra-third ventricular (i3vt) or peripheral ghrelin administration reliably increased feeding in intact male rats and OVX females (24); however, the threshold for a significant increase in feeding using either administration route was significantly greater in intact females than in males or OVX females. When OVX rats were treated with estradiol, moderate intra-peritoneal (i.p.) or i3vt doses of ghrelin no longer stimulated eating. Together, these data demonstrate that estradiol reduces the orexigenic potency of ghrelin. Lastly, estradiol reduced the eating-stimulatory effect of i3vt ghrelin in male rats, indicating that the estrogenic effect exists in both sexes, which is a point of potential therapeutic relevance (25). Careful attention to sex differences and gonadal hormone status should be included in the development of any ghrelin-based clinical control for eating behaviors. The eating-stimulatory effect of ghrelin varies across different phases of the ovarian cycle in intact rats (26). Administration of i3vt ghrelin had no reliable overall effect when cycle day was not taken into account. However, when the cycle day was considered, i3vt ghrelin increased eating during diestrus 1 and diestrus 2, but not during proestrus or estrus. In addition, in estradiol-treated OVX rats, ghrelin increased food intake on the days that modeled diestrus in intact rats, but not on the days that modeled proestrus or estrus. This indicates that there are cyclic variation in eating in rats and mice, and spontaneous food intake is maximal during diestrus and minimal during estrus. Therefore, the analogous peri-ovulatory decreases in eating in women may be due to changes in estrogenic tone that affect ghrelin's eating-stimulatory action. To assess the importance of ghrelin signaling in OVX-induced hyperphagia and obesity, *Ghsr*−/− mice lacking GHSR were OVX [95]. The OVX mice, which were similar to wild-type mice in body weight and food intake pre-surgery, showed no increase in food intake or body weight gain after surgery. This indicates that estradiol tonically inhibits endogenous ghrelin signaling in mice and that release from this inhibition is necessary for OVX mice to increase food intake and body weight. This mechanism may account for other sex differences in eating and weight regulation previously reported in *Ghsr*−/− mice. For example, female *Ghsr*−/− mice accumulated less body weight and adiposity when given a high-fat diet (27). Also, the magnitude of the differences in adiposity observed between *Ghsr*−/− and wild-type mice were greater in females than in males (28). Ghrelin signaling appears to be a necessary component of the estrogenic control of eating and weight regulation (29). The site of the GHSR-mediated effects on eating remains unclear (30), so estradiol may influence ghrelin and its receptors either centrally or peripherally (31). In male brains, GHSR have been implicated in ghrelin's eating-stimulatory effect in the ARC, PVN, ventral tegmental area, and dorsal vagal complex (32). Since administration of exogenous ghrelin can reach all of these ER-containing sites, each area could mediate the observed sex differences (33). In contrast, Currie et al. failed to observe any sex difference following direct ghrelin microinjections to the ARC or PVN (34). However, since ovarian cycling was not monitored, it is possible that the female rats were acyclic, which may have caused an artificial increase in ghrelin-induced eating. It is also possible that estrogenic actions on nucleus tractus solitaries (NTS) neurons contribute to the observed effects (35).

Melanin-Concentrating Hormone (MCH)

Since its discovery in hypothalamic neurons, MCH has been recognized as an important

regulator of energy homeostasis (36). Central administration of MCH promotes feeding, while genetic ablation of the *Mch* gene produces a lean phenotype. In addition, *Ch* is upregulated by fasting. MCH neurons in the lateral hypothalamic area (LHA)receive inputs from NPY/AgRP and POMC neurons in the ARC (37). Therefore, MCHneurons are in a position to integrate the feeding response because they have projections from the ARC and to brain structures like the nucleus accumbent. Messina et al. investigated the effect of centrally injected MCH on feeding in estradiol and vehicle-treated OVX rats and male rats. MCH increased the meal size in all three groups. Additionally, intake increased more in rats in diestrus than rats in estrus, although MCH increased food intake in both groups. Overall, estradiol decreased the orexigenic effect of MCH, leading the authors to hypothesize that the decrease in food intake during estrus was mediated by a decrease in MCH signaling (38). In a second study, Santollo et al. examined whether the behavioral effects of MCH were sexually dimorphic. They observed a greater increase in food intake, meal size, and water intake following MCH treatment in male rats than in estradiol-treated OVX rats (39). Additionally, they observed that higher MCH doses were necessary to increase food intake in estradiol-treated OVX rats, suggesting that estradiol reduced MCH sensitivity in female rats. Given that circulating levels of estradiol are lower in males than in females, this sex difference may contribute to the increased sensitivity to MCH in male rats. There are several ways that estradiol could decrease MCH signaling. By acting on nuclear ERs in the lateral hypothalamus (LH) and zona incerta (ZI), estradiol can alter gene transcription. ERs can modulate gene expression locally by decreasing MCH synthesis. In support of this hypothesis, physiological doses of estradiol decreased pre-pro MCH mRNA expression in the ZI of OVX rats and the LH of obese male rats. In addition, chronic estradiol treatment in male rats blocked increases in LH MCH mRNA expression induced by fasting (40). In contrast, pharmacological doses of estradiol in male mice increased MCH mRNA within hypothalamic tissue punches. These discrepancies emphasize the need for additional research in intact, cycling rats to determine the role of endogenous estradiol in regulating MCH mRNA expression. It is also possible that estradiol, acting at nuclear ERs in brain regions that express MCH^{-1} receptors, can decrease MCH signaling by decreasing the number or binding affinity of MCH-1 receptors. A recent study demonstrated that LH neurons containing MCH-1 receptors and ERs are not expressed, but are in close proximity to one another. Several studies evaluated whether MCH affects meal size or number [29,30,134]. The size of the first meal increased following MCH treatment in male and estradiol- or vehicle-treated OVX rats. In male and vehicle-treated OVX rats, MCH increased the average meal size throughout the period of increased food intake. There was no change in meal size in the estradiol-treated OVX rats, and there were no differences in meal number in any group, indicating that MCH increases food intake by increasing meal size in both males and females.

Estrogen interacts with anorexigenic neuropeptides

Estrogen has been reported to have an inhibitory effect on body weight gain in animal models (40). ERa null mice are obese, insulin resistant, and have decreased energy expenditure. This model indicates that ERa is critical for the estrogenic control of feeding behaviors and body weight (41). Estrogen decreases food intake through its direct effects and through its interactions with other compounds that reduce food intake. In this section we will review the literature on estrogen's interactions with anorexigenic hormones.

Insulin

After Kennedy (1953) hypothesized that fat stores produced a hormone that functioned as a negative feedback control for adiposity (42), one early suggestion was that the hormone was insulin. Plasma insulin levels directly correlate with body weight and adiposity (43). Obese animals and humans have higher basal insulin levels and secrete more insulin in response to a meal than lean individuals. Insulin increases during meals and other periods of positive

energy balance and decreases during fasting and other periods of negative energy balance (44).
Insulin receptors are distributed in discrete brain areas, including the hypothalamus. Hypothalamic insulin receptors are thought to mediate food intake and body weight regulation via mechanisms similar to leptin (45-47). Hormones provide important regulatory signals to the brain. Manipulation of gonadal steroid levels can influence leptin and insulin sensitivities and body fat distribution (48-50). This implies that the relative amount of androgen and estradiol is a key determinant of the brain's sensitivity to the catabolic actions of insulin. When there is proportionally less estrogen, this favors insulin sensitivity. Thus, estradiol directly determines body fat distribution through its actions in the brain.
Hyperinsulinemia is considered indicative of insulin resistance. It plays an important role in the pathophysiology of diabetes. It has shown a future predictor of many health-related adverse outcomes including coronary artery disease, stroke, dyslipidemia, polycystic ovarian syndrome and premature adrenarche. Therefore, it is of great interest to quantify insulin sensitivity in humans to investigate the pathophysiology and epidemiology of major public health problems and to follow the clinical course of patients on various therapeutic regimens (51-53). There are many ways to measure insulin resistance, for example; Homeostasis Model Assessment for Insulin resistance(HOMA-IR) and Quantitative Insulin-Sensitivity Check Index (QUICKI), hyperinsulinemia euglycemic clamp tests and insulin suppression tests (54-56). The potential problems with using the fasting G/I ratio as a physiologically appropriate index of insulin sensitivity become apparent when fasting glucose levels are abnormal (57-59). When a normal subject is compared with a non-diabetic insulin resistant subject, simple indices of insulin sensitivity based on fasting values such as leptin/insulin, QUICKI, and G/I are all decreased in the insulin-resistant subject when compared with the normal subject. Likewise, HOMA increases as expected. A diabetic subject who has the same fasting insulin level as the non-diabetic insulin resistant subject is obviously even more insulin resistant because the same level of insulinemia does not appropriately compensate for fasting hyperglycemia. Importantly, in this diabetic subject, the value for QUICKI decreased even further and HOMA increased further, exactly as one might predict (60 62). The exact relationship between leptin and insulin is not clear and is sometimes controversial. The secretion of both hormones is influenced by overall amount of fat stores as well as by short-term changes in energy balance. Also, insulin receptors are located in the same key hypothalamic areas as leptin receptors. Leptin, product of obesity gene produced primarily in adipose tissue. It plays an important role in regulating food intake, reproduction, and immune function (63-65) Leptin has myriad effects on insulin signaling pathways.
Some of these effects include modulation of tyrosine phosphorylation of the insulin receptor substrate-1, association of receptor substrate-1 with downstream signaling molecules, and potentiation of insulin's ability to suppress phosphoenolpyruvate carboxy kinase, the rate-limiting enzyme in gluconeogenesis (66).
Strong evidence indicates that leptin along with insulin exerts an inhibitory effect on food intake, and an activation effect on the regulation of thermogenesis within the central nervous system (67-69). Collectively the aforementioned effects of leptin indicate that leptin may be a naturally occurring insulin sensitizer. As such, persons with insulin resistance could elaborate more leptin as a compensatory response, whereas insulin-sensitive subjects would have no need for leptin augmentation (70-72). The widely reported hyperleptinemia in obese subjects lends facile support to this thesis because obesity is associated with insulin resistance. Previous studies documented marked variability in plasma leptin levels among persons of comparable adiposity. Because leptin exerts potent insulin-sensitizing effects in rodents and humans, we hypothesized that the variations in fasting leptin levels in persons of similar body mass index (BMI) reflect differences in insulin sensitivity (73-75).

Estrogen regulates adiposity

The accumulation of fat in a central distribution (intraabdominal) has emerged as a risk factor

for the metabolic syndrome which includes a higher risk of diabetes, hypertriglyceridemia, hypertension, and cardiovascular disease (76-78). Estrogen promotes the accumulation of subcutaneous fat, and the loss of estrogen with menopause is associated with an increase in central fat (79-81). The sexual dimorphism in adipose tissue distribution may partially explain the greater risk for the metabolic syndrome in men compared with premenopausal women.

Estrogen regulates adipose tissue distribution

Visceral fat varies inversely with estrogen levels (44). When estrogen levels become sufficiently low visceral fat accumulation occurs in females, possibly due to direct effects of estrogen, especially since progesterone and androgen receptors (PR and AR) as well as estrogen receptor (ER) are expressed in adipose tissues. Subcutaneous adipose tissue has higher concentrations of ER and PR; however, visceral adipose tissue has higher concentrations of AR. Additionally, subcutaneous adipose tissue has few androgen receptors, and estrogen down-regulates AR expression in subcutaneous fat (82-84). Adipose tissue-specific AR knockout mice have increased intra-adipose estradiol levels, which leads to increased subcutaneous obesity and hyperleptinemia (85-87). Ovariectomized (OVX) rats gain fat, specifically visceral fat with no change of subcutaneous fat. Peripheral or central administration of estradiol to OVX rats restores central leptin sensitivity and changes their body fat distribution to mirror that of intact females; altering the sex hormone milieu in males with estradiol administration increases sensitivity to central leptin and increases subcutaneous fat deposition. An important implication from these findings is that estrogen regulates body fat distribution, interacts with the integrated adiposity message conveyed to the brain by leptin, and enhances leptin's action in sympathetic activation to the visceral fat.

Estrogen regulates adiposity through estrogen receptors

Estrogen regulates body adiposity and fat distribution through its receptors, ER alpha (ERα) and beta (ERβ). However, only ERα has been reported to have a major influence on energy homeostasis. ERα is necessary for estradiol's genomic actions on body weight regulation while ERβ functions more as a modulator of estrogen actions. Rapid, nongenomic actions of estradiol also have been described and some of them appear to involve ERα. Heine et al., reported that male and female mice with total body deletion of ERα, ERα-knock-out (αERKO) mice, have increased adiposity in both male and female mice, suggesting an important role for this estrogen receptor in the regulation of body weight and adiposity. Recently, site-specific knockdown of ERα expression in the VMH, a brain region critical for body weight regulation, demonstrates the role of VMH ERα activity in the regulation of bodyweight homeostasis. Knockdown of VMH ERα results in obesity due to an anabolic process, with changes in energy expenditure primarily mediating the weight gain. These data are consistent to previous finding in the αERKO mice where it has been demonstrated that the obesity is primarily due to changes in energy expenditure rather than changes in food intake and those mice have increased visceral adiposity (unpublished data). These data suggest that estrogen signaling with critical hypothalamic nuclei is responsible for the regulation of body weight via modulating energy expenditure. Since ERa is expressed in hypothalamic areas that regulate energy homeostasis, the absence of ERa expression is consistent with changes in body weight. Furthermore, Era polymorphisms identified in humans have been associated with increased levels of visceral fat. A ventral medial nucleus (VMN) specific ERa knockdown in both female mice and rats resulted in phenotypes characteristic of a metabolic syndrome. Microinjections of low doses of estradiol directly into the brain were shown to inhibit food intake. Taken together, these observations suggest that the binding of estradiol to ERa in the hypothalamus, or elsewhere in the brain, may represent a mechanism by which estradiol regulates food intake, body weight, and possibly body fat distribution.

Estrogen regulates adiposity by decreasing inflammation

Obesity is a state of chronic inflammation, and inflammatory signaling pathways in obesity are linked to insulin resistance. Sex differences where females are protected have been reported in diet-induced obesity, insulin resistance and inflammatory response to a high-fat (HF) diet. This may be explained in part by the anti-inflammatory properties of estrogen. Recent studies have shown that estradiol may play a role in reducing the inflammatory response in adipose, cardiovascular, and neural systems, in addition to being neuroprotective both *in vivo* and *in vitro* (54).

ERa (and in some cases ERa) is expressed in immune and cytokine-producing cells including macrophages and microglia, and *in vitro* studies have shown estradiol-activated ERa decreases the number of pro-inflammatory cytokines (55). The anti-inflammatory properties of estradiol can be partially explained by the ability of ERs to act as transcriptional repressors by inhibiting the activity of nuclear factor kappa B (NFeB) through protein-protein interactions between agonist-bound ERs and activated NFeB subunits. Estradiol's inhibitory effect on NFeB function is not fully understood and may be target selective. Symptoms of a metabolic syndrome increase when animals are maintained on a high-fat (HF)diet or when females have low ovarian hormone levels. Free fatty acids (FFAs), particularly saturated fatty acids, increase inflammation by activating toll-like receptor 4 (TLR4).

Muscle and liver expression of tumor necrosis factor-alpha (TNFa), interleukin-6 (IL-6), and NFeB also increase with HF diets (59). Estradiol has been shown to be neuroprotective and to increase the expression of growth factors and proteins involved in apoptosis. Estradiol signaling pathways are active in monocytes and macrophages, and ERs are expressed by these cells (61). Therefore, the protective effects of estradiol in neurodegenerative diseases can be mediated by inhibiting the inflammatory response, and consequently, hormone withdrawal can increase inflammation.

Both in a model of brain inflammation and in primary cultures of microglial cells, estradiol inhibited the synthesis of inflammatory mediators induced by lipopolysaccharide (LPS) (63). Moreover, hormone loss in OVX mice resulted in increased microglial activation (64), while estradiol replacement decreased microglia activation (65). These data provide strong evidence that chronic inflammation in the brain can be regulated by estradiol.

Estrogen interacts with orexigenic neuropeptides

Estrogen has been proposed to act directly and indirectly to decrease orexigenic peptides and decrease food intake. In this section, we will review the literature and describing the interactions of estrogen and neuropeptides that increase food intake.

Effects of testosterone on insulin, leptin and other factors

Administration of aromatase inhibitors to men has recently been proposed as a potential therapeutic optioning elderly men with low serum testosterone (T) levels (66), and in obesity related male hypogonadism (66). However, questions remain about the long-term efficacy and safety of these compounds (67), and presently little is known on the short-term effects of aromatase inhibition on glucose and lipid metabolism. From epidemiologic data, we know that the ageing related decline in T levels in men is often associated within increase in (abdominal) fat mass, loss of muscle, and bone mass, impaired glucose tolerance, elevated lipid levels, and more prevalent atherosclerosis (68). Since all of these features are similar to those observed in patients with androgen deficiency, it has been hypothesized that the decrease in T levels contributes to at least some aspects of this detrimental metabolic profile. One hypothesis explaining this progressive hypogonadal–obesity–metabolic disease cycle is that increased aromatase activity caused by increasing fat mass accumulation leads to the depression of T production, further enhancing the deposition of abdominal fat (70). From this point of view, administration of aromatase inhibitors to men with (obesity-related) hypogonadotropic hypogonadism may offer an alternative therapeutic option to restore the disrupted balance betweenness steroid levels. T'Sjoen have previously reported that oral

administration for 28 days of an aromatase inhibitor markedly increased serum LH and T levels, while lowering estradiol (E2) levels, in both young and elderly men (71). In these two age groups, they examined and compared the effects of short-term aromatase inhibition on their metabolic profile by measuring serum levels of fasting insulin, glucose, lipids, leptin, insulin-like growth factor 1 (IGF1), and adiponectin. The rationale was that changes observed after short-term treatment are more likely to reflect direct effects of the hormonal changes induced by aromatase inhibition, whereas changes in metabolic profile after longer term treatment are more likely to reflect, at least for a substantial part, indirect effects resulting from changes in body composition. In young men, aromatase inhibition resulted in presumably lower fasting glucose levels, and markedly lower insulin and IGF1 levels. These findings corroborate with those of Wickman et al. who reported decreased insulin levels, correlating with changes inIGF1 levels, in boys treated long-term with T plus letrozole (72). By contrast, 2 years administration of letrozole to younger peripubertal boys did not improve insulin sensitivity (HOMA) (73). In line with our results in elderly men, Dougherty et al. found no changes in insulin sensitivity (HOMA) in elderly men with mild hypogonadism after 12 weeks of aromatase inhibition (anastrozole 1 mg daily or 2 mg weekly) (74).

Although Wickman et al. (75) and Hero et al. (76) reported decreased HDL-cholesterol levels in their patients, no changes in LDL-cholesterol levels were observed and no changes in lipid levels were found by Dougherty et al. (77). In another study, 6-weekadministration of test lactone (78) to adult men decreased HDL and APOA1 concentrations (78), which seems in line with our observations in elderly men. Together with our data, these observations might suggest that short-term effects of aromatase inhibition on glucose and lipid metabolism could be due to effect son GH and IGF1 metabolism, as endogenous estrogens are known to stimulate secretion of these hormones (80) and effects of IGF1 on glucose metabolism are well-established (118). This could also explain the discrepant effects of aromatase inhibition in young versus elderly men, since IGF1 metabolism is known to decrease with ageing (82). However, 10-week administration of anastrozole (1 mg/day) to eight healthy men did not affect insulin, glucose, or lipid levels, despite decreasingIGF1 concentrations (83), nor were lipid levels affected in another study (84).

In this regard, possible differences between studies might be explained by the higher aromatase-inhibiting potency of letrozole as compared with anastrozole. However, age-specific effects cannot be excluded, since despite similar changes in both of our age groups after active intervention, younger men presented higher absolute levels of FT and FE2 as compared with elderly men, both before and after treatment and in line with the well-known agerelatedchanges in sex steroid levels. In addition, there is evidence for a decreased number of androgen receptors, and thus androgen sensitivity, in various tissues in elderly men.

Conclusion

In summary, short-term aromatase inhibition with letrozole 2.5 mg daily affected glucose metabolism in young, but not elderly men, and lowered leptin levels in both age groups. Since the short duration of our intervention, these effects appear independent from changes in body composition. However, further research is needed regarding whether these effects are indeed direct due to sex steroid action, modulated by age, replicable in different populations and by changes within the normal physiological range or after even shorter-term intervention.

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