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Chapter 5

Estrogen Receptors Are Pivotal Regulators of Glucose Uptake and Energy Expenditure

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Abstract

Glucose is the most important fuel of mammalian cells and its uptake is crucial for cellular metabolism, differentiation, survival and proliferation. The insulin signaling pathway regulates the uptake of glucose and whole-body metabolic homeostasis by transducing extracellular signals through the insulin receptor (IR) to downstream intracellular targets. Dysregulation of insulin secretion or alterations of IR signal transduction pathways is associated with self-generating, progressive insulin resistance in thorough interplay with an imbalance of sexual steroid production. Insulin resistance and the associated metabolic and hormonal alterations predispose patients to a variety of life threatening diseases; such as metabolic syndrome, type-2 diabetes, cardiovascular lesions and malignancies at different sites. Estrogens have beneficial effects on energy metabolism and glucose homeostasis by several pathways. In the central nervous system, hypothalamic nuclei are the pivotal regulators of food intake and energy expenditure by means of their estrogen receptors (ERs). In the pancreatic islet cells, ERs advantageously regulate the biosynthesis and secretion of insulin and maintain the equilibrium of glycogen synthesis and glycogenolysis in the liver by the balanced activation of glycogen synthase and glycolytic enzymes. In the peripheral tissues, ERs advantageously modulate the insulin stimulated glucose uptake through regulation of the phosphorylation of IR protein and increase the expression and translocation of intracellular glucose transporters (GLUTs). There are strict crosstalks and feedback mechanisms between estrogen synthesis and ER signaling; however, pathologic states may develop when the

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interactions of these players cannot restore each other's defect. Failures of ER signal transduction result in disturbances in cellular glucose uptake and the appearance of clinical biomarkers, forecasting manifestation of serious diseases, such as type 2 diabetes, cardiovascular diseases, cognitive disturbances and malignancies.

Introduction

A famous hypothesis of Reaven established a causal association between cellular insulin resistance and compensatory hyperinsulinemia, which were then regarded as basic disorders for several human diseases [1]. Earlier, cardiovascular lesions, hypertension, dyslipidemia, obesity and elevated fasting glucose level were attributed to be the complications of type 2 diabetes. Nevertheless, the sequence of these alterations was frequently inconsequent and contradictory. The new theory revealed that many divergent symptoms and findings all have a common soil; the defective glucose uptake of mammalian cells [2].

Actions of female sexual steroids are much wider than having crucial roles in female physiology and reproduction [3]. Physiological estrogen levels in healthy premenopausal women supply protection against insulin resistance, hypertension, cardiovascular diseases and malignancies as compared with men of the same age [4, 5]. However, after menopause a decreased ovarian estrogen synthesis will rapidly deteriorate the glucose uptake and increases the prevalence of life threatening diseases.

New findings on functions of tissular estrogen receptors indicate that estradiol plays important role in the maintenance of glucose homeostasis and energy expenditure [6]. It beneficially modulates the expression of genes that are involved in insulin secretion, cellular insulin sensitivity and glucose uptake at different sites [6, 7]. Nevertheless, there are conflicting data concerning estradiol action in women and its relation to glucose homeostasis and insulin sensitivity in pre- and postmenopausal cases.

Recent studies suppose that a functional imbalance between the signals of estrogen receptor (ER) isoforms and gene polymorphism of ERs may have important implications for the development of dysmetabolism, such as metabolic syndrome and type 2 diabetes [8]. Moreover, hyperestrogenism is erroneously supposed to disrupt the glucose uptake in the adipose tissue mass in obese women and is presumed to contribute to insulin resistance and the associated co-morbidities [9, 10].

Results of animal experiments support that estradiol administration improves the metabolic functions in insulin resistance and obesity [11, 12]. Elevated estrogen level is advantageous against metabolic disorders and decreases the risk for associated diseases. In experimental animals, pregnancy analogue estradiol administration counteracts to the growth of chemically induced or transplanted malignant tumors [13-15].

Moreover, high dose estrogen administration as ovulation provocation and good hormonal equilibrium in multiparous women prove to be metabolically advantageous and exhibit anticancer capacities [16]. In human metabolic diseases the beneficial effects of estradiol treatment justify that ERs conduct advantageous intracellular signals if the estrogen supply is sufficient [17]. Conversely, in estrogen deficient states or in case of defective ER signals an increased prevalence of metabolic syndrome, type 2 diabetes and their comorbidities is characteristic [7].

The purpose of this study is to examine the real significance of estradiol supply in cellular metabolic functions. Experimental studies on disabled ER knockout mice help to evaluate the unique and apparently controversial functions of each ER isoform. Nevertheless, understanding the crosstalk and interplay between them illuminates the fact that there is no good or bad ER isoform, but they construe a complex system so as to achieve an ideal internal milieu.

Role of Estradiol and Its Receptors in the Regulation of Cellular Glucose Uptake and Energy Homeostasis

The metabolic state of the body is controlled by a central regulation of the brain through signals arriving from the pancreas, liver, adipose tissue, skeletal muscle, and gut [8]. A wide variety of these signals includes hormones (insulin, leptin, adiponectin, etc.), cytokines (TNF- α , IL-6) and nutrients [glucose, free fatty acids, lipids].

Estrogens have pivotal effects on energy metabolism and glucose homeostasis [6]. Two receptor isoforms were identified; estrogen receptor alpha (ER α) and estrogen receptor beta (ER β), which belong to the steroid-thyroid hormone nuclear receptor supergene family [17, 18]. The classic genomic mechanism of estrogen binding activates ERs in the nucleus and they act as transcriptional modulators in the promoter region of target genes. ERs can also regulate gene expression without direct binding to DNA through interaction with transcription factor proteins in the nucleus [3]. Estrogen action has also non-genomic signaling cascades through cell membrane associated ERs [19]. Finally, genomic and non-genomic pathways of estrogen receptor signaling converge on the target genes.

ER α and ER β regulates in thorough interplay the metabolic processes, the cell growth and the cell proliferation of mammalian cells. They may oppose the activities of each-other eliciting sometimes quite opposite reactions in the presence of estradiol [20], which may be crucial for the dynamism of regulatory mechanisms.

Discovery of selective agonists for ER α and ER β yielded new possibilities to research the specificity of estrogen receptors [21]. PPT, an ER α selective agonist provoked an increase in cell number nearly 50%, while DPN, an ER β selective ligand caused a decrease of 30%. Selective activation of each receptor alone increased cell metabolism independently as either mitotic activity or cell growth require enhanced cellular metabolism. However, the simultaneous activation either by estradiol or by PPT-DPN combination produced equilibrium in metabolic activity. Development of the three knockout mouse lines: estrogen receptor α and β knockout (ER α KO, ER β KO) and aromatase enzyme knockout (ARKO) mice provided new opportunities for understanding the individual actions of estrogen receptor isoforms. ER α knockout mice exhibit insulin resistance, impaired glucose tolerance and obesity affecting both males and females [22]. Ovariectomy of insulin resistant ER α knockout mice (i.e. removal of the action of estradiol on ER β) improved glucose and insulin metabolism [23], suggesting that ER β might have a diabetogenic, opposing action against ER α . In the maintenance of normal glucose homeostasis ER α mainly enhances the capacity for glucose uptake, whereas ER β exerts contraregulatory activity [6].

Role of Estrogen Signaling in the Central Regulation of Metabolism and Energy Homeostasis

The central nervous system may induce metabolic and behavioral changes by anorexigenic and orexigenic stimuli so as to maintain the serum glucose level and energy homeostasis [8]. The hypothalamic nuclei are pivotal regulators of food intake and energy expenditure by means of their estrogen receptors [24].

The main hypothalamic areas involved in feeding behavior are the arcuate nucleus (AN), the lateral hypothalamus (LH), the paraventricular nucleus (PVN), the ventromedial nucleus (VMN) and the dorsomedial nucleus (DMN) [25] (Figure 1). Both ERs have been identified in the AN, LH and DMN [25, 26]. The PVN shows the highest expression of ER β isoform [27], whereas VMN is mainly ER α regulated having important function in food intake inhibition [28]. In animal experiments, direct estrogen actions in the hypothalamus lead to anorexia. In ovariectomized mice, increase in food intake, body weight and abdominal fat mass are characteristic, which are reversible by estradiol administration [29].

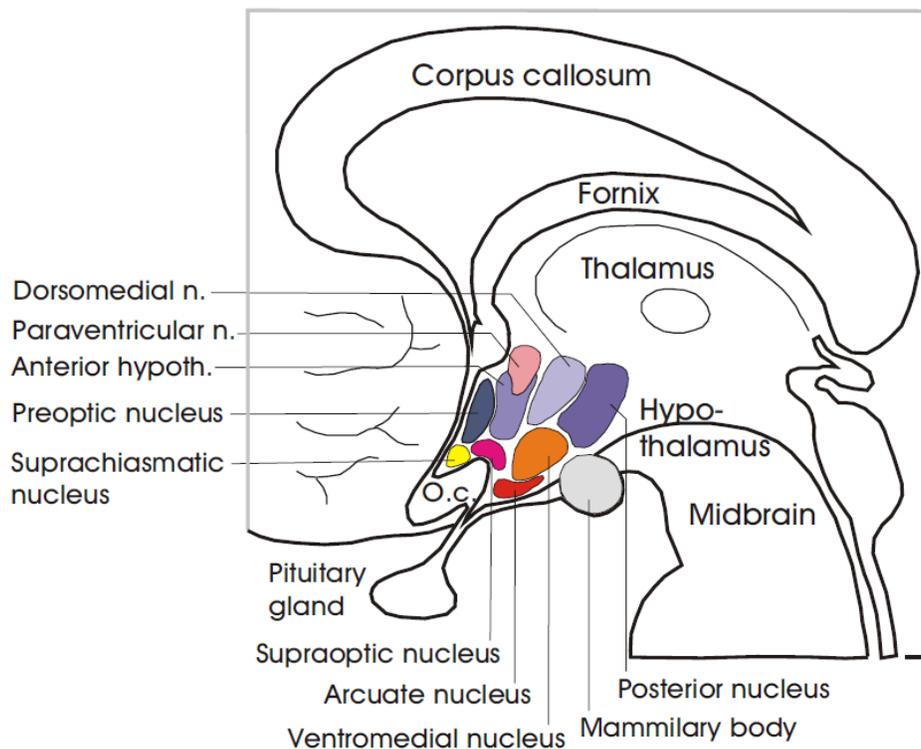


Figure 1. Hypothalamic nuclei. Linkage between the central nervous system and the pituitary gland. O. c.: Optic chiasm. (Reprinted with the permission of Omics Group.)

Leptin is an adipocytokine secreted by adipose tissue in direct proportion to body fat mass. It has crucial role in the central regulation of metabolism and transfers catabolic signals to the hypothalamic nuclei to inhibit food intake and increase energy expenditure [30, 31]. Estrogen modulates leptin synthesis and secretion via ER-dependent transcriptional mechanisms [32] and increases the leptin sensitivity of the brain [33]. Ovariectomy reduces leptin sensitivity and this effect can be reversed by estradiol substitution [34].

In rats and mice the ER α -selective agonist, PPT, rapidly results in a decrease in food intake [35], whereas silencing of ER α leads to hyperphagia, obesity, decreased glucose tolerance and reduced energy expenditure [36]. In ovariectomized rodents treatment with an ER α -specific ligand (PPT) resulted in an inhibition of eating and reduced body weight.

The exact role of ER β in the central regulation of feeding is less known [8]. In ovariectomized rats, estradiol and ER β anti-sense oligodeoxynucleotides (ODN) administered into the third ventricle in the brain reduced the inhibitory effect of estrogen on food intake [37]. This result suggests that in some hypothalamic areas ER β mediates the central anorexic effect of estradiol.

The hypothalamus has a wide control over insulin, glucocorticoid and gonadal hormone functions, which have important role in defining insulin sensitivity and consequently in peripheral carbohydrate and lipid metabolism [38]. The ratio of male to female sexual hormone levels affects peripheral fat distribution attributed to a modulation of insulin sensitivity. Estrogen deficiency or defective estrogen signaling results in insulin resistance and an increase in the adipose tissue mass, preferentially in visceral location [39].

Estrogen Receptor Actions on Pancreatic Islets and Insulin Production

Estradiol and its receptors are key players in the physiology and insulin production capacity of the β cells of pancreatic islets [40]. Estradiol administration is associated with pancreatic islet hypertrophy and increased insulin release from the β cells in rats. Islet cells isolated from ovariectomized mice respond to glucose with a smaller insulin release than islet cells from intact mice. Estradiol replacement in ovariectomized mice normalizes the insulin response to glucose ingestion [6]. ER α activation promotes β -cell mass proliferation and insulin biosynthesis in diabetic and obese cases, whereas ER β activation improves glucose stimulated insulin secretion [41].

After menopause, estrogen loss decreases the insulin secretion, which is transitorily compensated by its reduced elimination [42]. During postmenopausal hormone replacement therapy or contraceptive use estradiol improves insulin secretion, sensitivity and elimination.

Estrogen signaling seems to be essential for structural and functional β cell adaptation, especially during high metabolic demand and insulin resistant periods [8]. Recognition of these correlations leads to novel therapies for β -cell related diseases as estrogen administration preserves functional β -cell mass in patients with diabetes mellitus.

Estrogen Receptors and Energy Homeostasis in the Liver

Liver function disturbances are in close correlation with insulin resistance, hyperglycemia and dyslipidemia. Estrogen plays a pivotal role in the regulation of hepatic glucose homeostasis. ER α is the predominant receptor isoform in human hepatocytes and ER α signal is essential for the glucose tolerance of the liver [43, 44].

Insulin modulates hepatic glucose uptake by the activation of glycogen synthase and glycogen phosphorylase leading to glucose storage as glycogen, whereas it may stimulate glycolysis through the activation of several hepatic enzymes [45].

PPT, a selective ER α agonist, improved glucose tolerance and insulin sensitivity in genetically obese mice suggesting that estradiol has antidiabetogenic impact via ER α [46]. In

the liver of ER α knock out (ER α KO) mice, hepatic insulin resistance, increased glucose production and lipid synthesis as well as decreased lipid transport were observed [44]. By contrast ER β might be diabetogenic as ER β knock out (ER β KO) mice with increased body weight exhibited improved hepatic and muscular insulin sensitivity due to reduced accumulation of triglycerides [46].

Estradiol have pivotal interactions with growth hormone (GH)-regulated endocrine (e.g., IGF-I), metabolic (e.g., lipid and glucose metabolism) and sex-differentiated (e.g., endo- and xenobiotic metabolism) functions in liver [47]. Estrogens modulate GH action at the level of pituitary GH secretion and have pivotal role in the regulation of GH activity, receptor expression and signaling as well [48].

Estrogen induced suppression of cytokine signaling negatively regulates GHR-Janus kinase (JAK)-2-signal transducer and activator of transcription (STAT)-5 signaling pathway [48, 49]. Estrogen induced disruption of GHR-JAK2-STAT5 signaling may be associated with hepatic metabolic changes that include fatty liver, fibrosis, and hepatocellular carcinoma [50]. Complex interplay between GH and estrogen seemed to be disquieting because of the physiological roles of these hormones in mammals, and the widespread use of estrogen-related compounds [47].

Conversely, 17 β -estradiol administration could suppress tumor growth in mice with hepatocellular carcinoma [51]. Estradiol suppressed the alternative macrophage activation and tumor progression by keeping ER β away from interacting with ATPase-coupling factor 6, a part of ATPase, thus inhibiting the JAK1-STAT6 signaling pathway. This study introduced a novel mechanism for the estrogen suppression of male-predominant hepatocellular carcinoma.

ER α confers estradiol mediated protection of the liver from inflammatory injuries [52]. Ovariectomized mice were treated by IL-18 to induce hepatic inflammation and estradiol hampered the expression of cytokines in ER β KO but not in ER α KO mice. Estrogen may suppress the production of hepatocyte growth factors and interleukin-6, which modulates the inflammatory environment of hepatocellular carcinoma in rats and inhibits metastatic spread [53].

Estradiol replacement in postmenopausal women increased HDL and decreased LDL, total cholesterol, lipoprotein, fasting insulin and glucose levels, exhibiting antidiabetogenic and antiatherogenic impacts [54]. Conversely, estrogen deficiency, such as antiestrogen (tamoxifen) therapy or ovariectomy resulted in atherogenic lipid profile and hepatic steatosis increasing the risk of metabolic syndrome and cardiovascular diseases [55].

Estradiol Actions on the Energy Homeostasis of Skeletal Muscles

Skeletal muscle mass is responsible for 75% of the insulin-mediated glucose uptake in the body and consequently, physical activity is in close correlation with insulin sensitivity [8].

Insulin receptors (IRs) help the active glucose transport through the double lipid layer of the cell membrane (Figure 2). IRs have outer α -subunits with binding sites for insulin and two transmembrane β -subunits. Insulin interaction with the external α -subunits induces autophosphorylation of the β subunits at multiple tyrosines resulting in an activation of signal transduction [56]. The phosphorylation cascade provokes translocation of glucose transporter (GLUT4) containing cytoplasmic vesicles to the cell membrane. GLUT4 anchored and

incorporated into the cell membrane enables the facilitated diffusion of glucose from the extracellular space into the cell [57]. Alterations in this mechanism, such as insulin deficiency, disturbance in insulin signal transduction, GLUT4 expression and/or translocation result in insulin resistance.

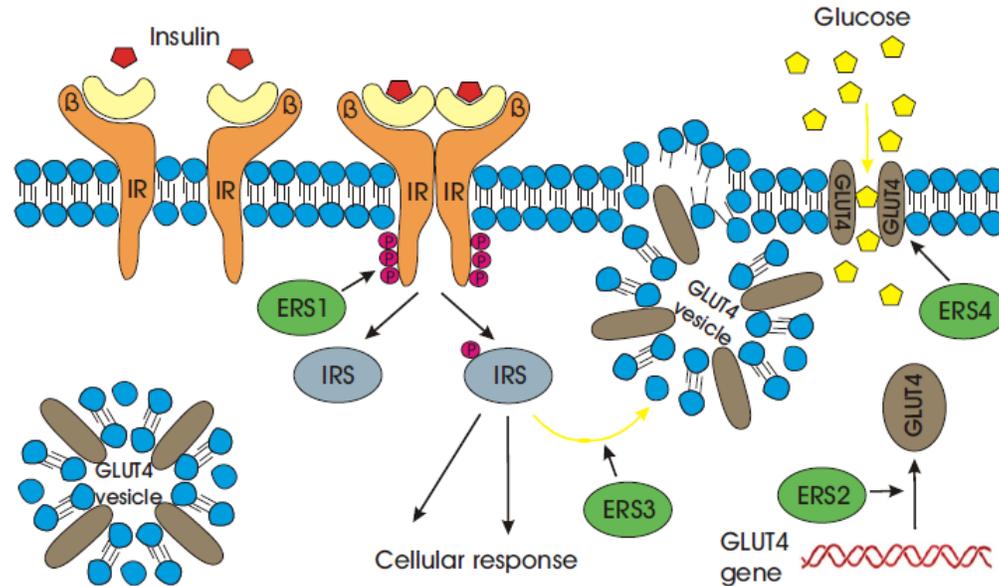


Figure 2. Insulin receptor (IR) activation and glucose uptake. Insulin binding to α subunits induces the phosphorylation (P) of transmembrane β subunits generating insulin receptor signal transduction (IRS). Glucose transporter (GLUT4) vesicle translocation to the cell membrane allows intramembraneous incorporation of GLUT4 and it enables the facilitated diffusion of glucose into the cell. Estrogen receptor signal (ERS) regulates the phosphorylation of IR protein (ERS1), participates in GLUT4 expression (ERS2) and translocation (ERS3), as well as in the intramembraneous incorporation of GLUT4 (ERS4). (Reprinted with the permission of Omics Group.)

Estradiol stimulates the phosphorylation of Akt, AMPK and the Akt substrate in soleus muscle [58]. Estradiol administration to insulin resistant rats or mice increases the insulin receptor substrate content and the concentration of the phosphorylated form of Akt in muscles, restoring the action of insulin [59].

ERs advantageously modulate insulin stimulated glucose uptake through the regulation of the tyrosine phosphorylation of insulin receptor protein [60]. ERs promote GLUT4 expression and intracellular translocation as well. Moreover, estradiol improves glucose homeostasis through the facilitation of GLUT4 incorporation into the cell membrane (Figure 2). By this way, estradiol treatment increases the GLUT4 content of the cell membrane [61]. In ovariectomized rats, the decreased amount of GLUT1 protein in the blood-brain barrier was increased after estradiol substitution [62].

Aromatase knockout (ARKO) mice with inactivation of the enzyme for estrogen synthesis, exhibit reduced glucose oxidation, increased adiposity and hyperinsulinemia both in males and females [63, 64]. Glucose intolerance and insulin resistance can be reversed by estradiol administration even in male ARKO mice [65].

Skeletal muscle expresses both ERs, and in mice ER β is the predominant isoform [8]. Treatment with the ER α selective agonist, PPT increased GLUT4 translocation to the cell

membrane of L6 myoblasts, and when ER α was silenced the translocation decreased [66]. ER α knockout (ER α KO) mice are glucose intolerant and insulin resistant [22] as the absence of ER α involves a reduced glucose uptake in muscles [44]. ER β is a repressor of GLUT4 expression and translocation. In ER β KO mice both glucose tolerance and insulin release remains normal or better than in wild type mice [44, 46]. These data exhibit that a steadily balanced activation of both receptors may ensure the ideal glucose tolerance and energy expenditure.

During estrogen loss in the postmenopausal period, muscle strength exhibits a striking decline that can be reversed by hormone replacement therapy (HRT), suggesting that estrogens are important modulators of muscle physiology [67, 68].

Some authors have established that hyperestrogenism is also related to insulin resistance similarly, to estrogen deficiency [69]. In women with irregular menstrual cycles and gestational diabetes, hyperestrogenism was presumed to be a contributor to insulin resistance [9, 10]. Nevertheless, today these pathologic insulin resistant states are attributed to hyperandrogenism and deficient estrogen synthesis rather than to estrogen excess [7, 16].

Taken together, estrogens increase insulin sensitivity, whereas an estrogen deficient milieu endangers the balanced glucose uptake and energy expenditure of skeletal muscles leading to insulin resistance [7].

Role of Estrogen Receptors in the Energy Homeostasis of Adipose Tissue

Fatty tissue participates in a variety of metabolic, endocrine and immunologic processes and interacts with CNS and peripheral organs by means of adipokine secretion. The size of the fatty tissue compartment clearly reflects the balance between whole-body energy intake and expenditure [70]. Increased adipose tissue in visceral location has been linked to a self-generating process of insulin resistance [16].

In rats, ovariectomy increased body weight, intra-abdominal fat mass, fasting glucose, insulin levels and insulin resistance. Estradiol substitution restored normoglycemia, increased the expression of adiponectin and decreased resistin expression resulting in improvement of insulin resistance [71].

In healthy premenopausal women, estradiol counteracts to the accumulation of visceral fat, and decreases the lipogenic activity of lipoprotein lipase in adipose tissue [34]. By contrast, irregular or long menstrual cycles in young women are associated with insulin resistance and predict occurrence of type 2 diabetes attributed to the defective estrogen synthesis [72, 73]. Postmenopausal decreasing estradiol production, deepening insulin resistance and decreased lipid utilization result in visceral fat mass accumulation [74].

The presence of ER α and ER β isoforms was confirmed in human adipocytes from both subcutaneous and intra-abdominal fat, with clear predominance of ER α [75]. Although the separated functions of the two ERs in adipose tissue may be studied on disabled ER α KO and ER β KO mice, these results may hardly be extrapolated to human practice. A clinical case was published with defective ER α who presented with glucose intolerance, hyperinsulinemia and obesity [76]. Hyperestrogenism in this patient seemed to be a contra-regulatory effect. Failure of his ER α signal led to premature coronary artery disease and decreased HDL cholesterol level [77].

In male mice deletion of ER α induces insulin resistance and progressive increase in adipose tissue with advancing age. In female ER α KO mice insulin resistance, increased adiposity, higher leptin and cholesterol levels and smaller LDL particles are characteristic [22].

Estradiol substitution in ovariectomized mice kept on high-fat diet preserved glucose tolerance and insulin sensitivity [12]. Studies on ER α and ER β knockout mice suggest that ER α is the main regulator of GLUT4 expression in adipose tissue [8] and the two ER isoforms seem to have opposite functions on fat metabolism [23]. In human adipocytes GLUT4 abundance is highly correlated with insulin responsiveness. In polycystic ovarian syndrome (PCOS) cases with ovarian overproduction of testosterone and defective estrogen synthesis, insulin stimulated glucose uptake was reduced due to decreased amounts of GLUT4 on adipocyte membrane [78].

Lifelong Changes in the Sex Hormone Levels of Women and Their Correlation with Insulin Resistance, Type-2 Diabetes and Obesity

In premenopausal women, the equilibrium of sexual steroid synthesis defines somatic health and reproductive capacity, whereas symptom-free adaptation to the estrogen deficient environment is a prerequisite of postmenopausal health [16, 79]. Changes in the sexual hormone equilibrium during women's lives strongly influence insulin sensitivity and the associated risk for type 2 diabetes and obesity (Table 1).

Table 1. Lifelong changes in the sex hormone levels of women and their correlations with insulin resistance, type-2 diabetes and obesity

Hormonal changes in the life periods of women	Estrogen level	Androgen level	Insulin resistance	Risk for type-2 diabetes and obesity
Adolescence				
menstrual disorder	↓	↑	↑	↑
anovulatory cycles	↓	↑	↑	↑
Premenopausal women				
anovulatory infertility	↓	↑	↑	↑
nulliparity	↓	↑	↑	↑
PCOS	↓	↑	↑	↑
hyperprolactinemia	↓	↑	↑	↑
antiestrogen treatment	↓	↑	↑	↑
contraceptive use	↑	↓	↓	↓
Postmenopausal women				
HRT use	↑	↓	↓	↓
non HRT use	↓	↑	↑	↑
antiestrogen treatment	↓↓	↑↑	↑↑	↑↑
hysterectomy	↓↓	↑↑	↑↑	↑↑

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Changes in Sexual Hormone Levels and Insulin Sensitivity in Puberty

In puberty, the extreme somatic growth and explosion-like sexual development mean a great challenge for the metabolic and hormonal systems. In this period there is a higher risk for development of insulin resistance, particularly in overweight cases [16]. Recent results support that poor natural light exposure in puberty also mediates insulin resistance and hormonal alterations by excessive melatonin secretion [80].

In adolescent girls, the developing insulin resistance leads to abnormal ovarian sexual steroidogenesis as well, resulting in excessive androgen and defective estrogen production associated with menstrual irregularity and anovulatory cycles [81-83]. Hyperandrogenism and insulin resistance in adolescents are preserved into adulthood and result in defective fertility patterns and dysmetabolism at least until 30 years of age [84-86]. This prolonged hormonal and metabolic imbalance might be a defining, dangerous factor for adult metabolic syndrome, type 2 diabetes and their comorbidities.

Correlations between Changes in Estrogen Levels and Insulin Sensitivity in Premenopausal Women

In premenopausal young women, defective estrogen synthesis is frequently associated with anovulation and infertility. Clinical signs of ovarian insufficiency are the long and/or irregular menstrual cycles [87, 88]. These reproductive dysfunctions are related to hyperinsulinism and excessive androgen production.

Among premenopausal hormonal disorders with insulin resistance and fertility failure, the polycystic ovarian syndrome (PCOS) is the most prevalent, presumably caused by a large number of different genetic abnormalities [89]. PCOS may usually be manifested by menstrual disorders, anovulatory infertility, hirsutism and obesity or overweight. Nevertheless, polycystic ovaries are common findings in symptom-free cases with normal menstrual cycles as well; only the laboratory findings of hyperinsulinemia and hyperandrogenism reveal the early phase of metabolic and hormonal disturbances [90, 91].

PCOS is not only an infertility disease but also represents a high systemic health risk for the affected women. Increased levels of insulin and insulin like growth factors (IGFs) and androgen excess are directly related to the high risk of cardiovascular lesions in women with PCOS [92]. Moreover, the excessive ovarian androgen production and defective estrogen synthesis are strong risks for cardiovascular diseases as the gender dependent equilibrium of male to female sexual hormone level is the prerequisite of vascular health [4]. Atherosclerotic complications were directly related to hyperandrogenism in PCOS cases [92].

Increased prevalence of type 2 diabetes, hypertension and cardiovascular complications were observed in a follow up study of a Dutch population of women with PCOS [72]. Close associations between PCOS and premature coronary and aortic atherosclerosis were revealed in middle-aged women [93, 94]. A retrospective Swedish study found a 7.4-fold risk of myocardial infarction among women suffering of PCOS [95].

In young infertile, nulliparous women with or without PCOS, an elevated risk for endometrial cancer was observed [96]. The high prevalence of endometrial cancer is frequently associated with synchronous primary cancers of the ovary or breast [96, 97].

This female organ triad has the highest estrogen demand showing peculiar cancer risk even in a slightly estrogen deficient environment [7, 16, 98].

Earlier, some authors presumed that elevated estrogen levels unopposed by progestin continuously stimulate estrogen receptors in women with PCOS. This postulation explained the high risk of endometrial and breast cancers observed in these cases based on the concept of the carcinogenic capacity of estrogen [99]. Recently, insulin resistance and hyperinsulinemia in PCOS patients are regarded as concomitants of high ovarian and adrenal androgen synthesis at the expense of defective estrogen production [16, 100].

Hyperprolactinemia is also associated with insulin resistance, obesity, cycle disorders, reproductive dysfunction and hyperandrogenism in women. Glucose intolerance and obesity are characteristic in hyperprolactinemia, suggesting that the associated hormonal disturbances might also be modulators of insulin sensitivity and body weight [101, 102]. In a population based cohort study the overall cancer risk was elevated in patients with hyperprolactinemia [103].

Oral contraceptive (OC) use replaces the natural menstrual cycle with relatively steady levels and fluctuations of artificial sex hormones. In PCOS cases, hormone treatment by oral contraceptives reduces the volume of cystic ovaries, decreases testosterone secretion and improves the carbohydrate and lipid metabolism as well [104]. Similarly, administration of the insulin sensitizing metformin in PCOS cases primarily lowers the high insulin level and at the same time improves menstrual abnormalities, ovulatory dysfunction and hirsutism [100].

Epidemiologic studies have confirmed that combined oral contraceptives provide substantial protection against endometrial and ovarian cancers in endangered anovulatory women [105]. A recent patent disclosed a method for treating hyperandrogenic states, including PCOS by an estrogen derivative compound, which is more advantageous against the dangerous dysmetabolism of PCOS cases than oral contraceptive administration [106].

Tamoxifen is a nonsteroidal anti-estrogenic drug used for adjuvant therapy of breast cancer and recently as a chemopreventive agent for breast cancer and for other cancers as well [107]. Nevertheless, worldwide administration of antiestrogen compounds yielded thorough disappointment [108].

Antiestrogens are cytostatic agents blocking the most important regulatory mechanisms associated with estrogen signaling. They have ambiguous impacts on mammary tumor development, however, the estrogen deprivation induces several life-threatening side-effects and exhibits strong carcinogenic capacity, particularly in the highly estrogen dependent endometrium [108]. Results of case-control studies demonstrated an increased prevalence of fatty liver, intraabdominal fat accumulation and type 2 diabetes in breast cancer cases receiving tamoxifen [107, 109, 110]. Artificial blocking of estrogen signaling pathways seems to confer serious insulin resistance leading to metabolic syndrome and type 2 diabetes. These disorders are well-known promoters of cancer initiation and progression affecting preferentially the endometrium [111, 112].

Correlations between Estrogen Loss and the Risk of Increasing Insulin Resistance in Postmenopausal Women

Menopause at 50-52 years of age means a sudden loss of ovarian estrogen synthesis and confers further decline in the circulating hormone level.

Postmenopausal women never using HRT are obviously insulin resistant and exhibit increasing inclination to the associated comorbidities. With ageing, every year after menopause is associated with continuous estrogen loss and parallel advancing insulin resistance [113]. For women aged 55-65 years, weight gain and obesity are their major health risks [114]. In postmenopausal women, deepening dysmetabolism, obesity and disturbance of male to female sexual steroid levels are associated with increased prevalence of metabolic syndrome, type 2 diabetes, cardiovascular disease and malignancies.

In HRT user postmenopausal cases, the protective effect of estrogen substitution may counteract to the developing insulin resistance and their metabolic and hormonal equilibrium becomes reminiscent to that of young women with preserved circulatory estrogen levels [16]. Estradiol administration increases insulin sensitivity [6], yields favorable changes in plasma lipid levels [115] and its anti-obesity effect decreases fat-accumulation, particularly in visceral location [116, 117]. All these impacts justify that HRT use is beneficially protective against insulin resistance and its comorbidities in postmenopausal women.

Hysterectomy and bilateral oophorectomy mean an abrupt, shocking hormone deprivation as compared with natural menopause. These patients are highly endangered as they have no possibility for gradual adaptation to estrogen loss by a compensatory hormone synthesis of the peripheral tissues [79, 98]. Bilateral oophorectomy is used as a risk reduction strategy in BRCA1/2 mutation carriers, although data on long-term side effects are not yet available. In the US population, oophorectomy, particularly at a young age, has been associated with highly increased overall and cardiovascular disease mortality [118].

In 2011 the WHI Randomized Controlled Trial substantiated that estrogen treatment in women with prior hysterectomy resulted in a significantly lower breast cancer risk than in untreated controls [119]. Hysterectomy seems to be a near uniformly high breast cancer risk for women, thus HRT studies on these homogenously selected cases proved to be methodologically strong and yielded unexpectedly correct results [7].

In postmenopausal women, antiestrogen administration is a worldwide practice as either therapeutic or cancer preventive agent. Both ER-blocker and aromatase inhibitor types of antiestrogens further aggravate the estrogen deficiency and insulin resistance of aged female patients [108].

Correlations among Endogenous Serum Estrogen Levels, Estrogen Receptor Reactivity and Cellular Estrogen Surveillance

There is a prevailing concept suggesting a linear cause-effect relationship between serum levels of sexual steroids and the clinical manifestations of certain symptoms and diseases [120, 121]. This belief seems to be a simplification if we realize the fact that the serum levels of steroid hormones may act indirectly via the inserted receptor signal transduction mechanism, evoking transcriptional activity in the nuclear genes [122]. These gene activations maintain cellular health, while defective interplay between available sexual steroid ligands and ligand activated receptor signaling results in disease development.

Proper estrogen receptor (ER) signaling is essential for the health of mammalian cells as estrogens are the chief regulators of cellular metabolism, growth, differentiation and

proliferation [3, 122]. Difficulties in either the synthesis of estrogens or ER signal transduction mechanism may increase the risk of defective estrogen surveillance [123, 124]. There are strict crosstalks and mutual feedback mechanisms between estrogen synthesis and ER signaling, but the insufficiency of these defense mechanisms leads to serious risk for diverse diseases. Critically low serum estrogen levels may induce alarming reactions so as to increase the estrogen independent transcriptional activity of ERs [125, 126]. Conversely, reactively increased endogenous estrogen supply may break through the deficient estrogen receptor signaling to save cellular estrogen surveillance and to prevent the development of diseases [124].

Failures of ER signal transduction result in disturbances in cellular glucose uptake and the appearance of clinical biomarkers, forecasting manifestation of serious diseases, such as type 2 diabetes, cardiovascular diseases, cognitive disturbances and malignancies [123, 124]. In conclusion, so as to evaluate the complicated correlations between serum levels of sexual steroids and breast cancer development, one should be aware of all stimulatory and inhibitory players having roles in the complex mechanisms of cellular estrogen surveillance.

Low Estrogen Exposure and Hyperandrogenism

Insufficient estrogen synthesis and decreased serum hormone concentrations may have many pathologic consequences. The most serious form of estrogen deficiency is associated with missing or defective aromatase P450 enzyme activity, which may be attributed to mutation of CYP19A1 gene [127, 128]. Aromatase deficiency is a rare autosomal recessive inheritance syndrome. Its worldwide incidence is unknown, and there are few case reports in the literature [129]. Mutation of the CYP19A1 gene inhibits the conversion of androgens to estrogen by aromatase enzyme and it results in elevated levels of androgens and abdominal obesity [130].

Mutation in the single human gene encoding aromatase P450 (CYP19) in a sister and brother was published [127]. The 28-year-old girl with XX chromosome presented the cardinal features of aromatase deficiency syndrome. By the age of puberty, she developed progressive signs of virilization with no signs of estrogen action; hypergonadotropic hypogonadism, polycystic ovaries, and tall stature. The basal concentrations of plasma testosterone, androstenedione, and 17-hydroxyprogesterone were highly elevated, whereas plasma estradiol was extremely low. Hormone replacement therapy led to breast development, menstrual cycles and resolution of ovarian cysts.

The XY male sibling with aromatase coding gene (CYP19) mutation was examined at 24 years of age. He was 204 cm tall with eunuchoid skeletal proportions [127]. He was fully mature sexually and presented with macroorchidism. The plasma concentrations of androgens were elevated; while estradiol and estrone levels were less than 7 pg/mL. Plasma FSH and LH concentrations were more than 3 times the mean value. Hyperinsulinemia, increased serum total and low density lipoprotein cholesterol, high triglyceride and decreased high density lipoprotein cholesterol levels were detected suggesting insulin resistance.

Aromatase activity and healthy estrogen synthesis may be endangered by various endogenous and exogenous factors, such as ageing [131], insulin resistance [7, 16, 132], diabetes [133], low physical activity [131], and deficient natural light exposure with melatonin excess [80, 134].

Insulin resistance and the compensatory excessive insulin synthesis inhibit aromatase activity and the accumulation of precursor androgens results in hyperandrogenism [100]. At the same time, decreased estrogen concentration hampers all phases of glucose metabolism from insulin synthesis to intracellular glucose transporter (GLUT) expression, provoking a further deepening of cellular insulin resistance [123].

In women with type 2 diabetes, the ability of the ovaries to convert androgen to estrogen is reduced, possibly attributed to a reduction of the ovarian aromatase activity [133]. As estrogens protect against atherogenesis, it is speculated that the decreased ability of the ovaries to produce estradiol in women with diabetes might be highly involved in the development of macroangiopathy, which often complicates this disease [4].

Fatty tissue distribution in obese patients strongly defines the dysmetabolism and sexual hormone imbalance. Male-like central obesity in women is in close correlation with insulin resistance, hyperinsulinism and excessive androgen synthesis resulting in high morbidity [114]. In centrally obese women, insulin resistance associated sex hormone imbalance with defective estrogen synthesis is accompanied by increased risks of breast, endometrium and ovarian cancers [16].

In women with polycystic ovarian syndrome (PCOS), anovulatory infertility, obesity, and hirsutism are characteristic clinical symptoms associated with the laboratory findings of hyperinsulinism and androgen excess [92]. In PCOS cases, dysmetabolism and ovarian failure are improved by either insulin sensitizing Metformin or oral contraceptive administration [135, 136]. Moreover, in highly endangered nulliparous women with anovulatory infertility, pregnancy mimicking excessive hormone treatment [137] and childbirth associated with ovulation provocation and in vitro fertilization proved to be preventive against breast cancer [138].

Hyperestrogenism in Health and Disease

The physiologic example of excessive estrogen production is pregnancy, in which state estrone, estriol and estradiol levels are extremely high, even a 200- to 300-fold serum estradiol concentration can be observed as compared with the follicular phase of menstrual cycle [139]. This physiologically increased hormone level supervises the safety, explosion-like proliferation of fetal structures as chief regulator of abundant growth hormones, growth factors, and other important biologic mediators. Unique features of estrogens are their beneficial impacts on all the cellular mechanisms of privileged healthy cells [122], while estrogens are capable of recognizing mitotic failures and malignant cells and neutralize them by apoptosis or promotion of their differentiation [140].

Pathologic, reactive hyperestrogenism may occur as a feedback mechanism against defective ER signaling [141, 142]. In these cases, excessive estrogen production is not causal factor for diseases but serves as a breakthrough of blocked ER signaling mechanisms [124].

In young women, mild point mutations of ER regulator genes may be compensated by slightly elevated estrogen synthesis, and the patient remains clinically healthy until the maintenance of extra estrogen level. With ageing, dramatically decreasing serum estrogen levels may lead to the manifestation of earlier hidden point mutations of ER genes resulting in signaling defects, especially in elderly women. Two single-nucleotide polymorphisms in ER β

gene were associated with increased breast cancer risk in hormonally challenged postmenopausal, but not in premenopausal women [143].

In a recent study, relatively elevated estrogen level was proposed to be a high risk for Alzheimer disease in elderly women, besides insulin resistance and diabetes. The impact of endogenous estrogen level on the risk of dementia was examined using data from a French population-based prospective study (the Three-City Study) including 5,644 postmenopausal women aged 65 years or older [144]. Results of this study showed a near U-shaped relationship between total estradiol level and risk of dementia. Total-estradiol values in both lower and upper quartiles were associated with increased dementia risk (HR = 2.2 and HR = 2.4, respectively). The risk associated with the upper estradiol concentration quartile was dramatically increased in women with diabetes (HR=14.2) when compared with nondiabetic women (HR=3.4).

In elderly women, total baseline serum estradiol concentrations are typically low; the quartiles exhibit only relative values. The upper quartile suggests a defective ER signaling and reactively increased but not sufficient estrogen synthesis. In these elderly cases the majority of ER signaling defects was disguised by compensatory good estrogen synthesis at younger ages. With ageing, the relatively higher but insufficient estradiol levels are not enough for the breakthrough of ER signal transduction defects. Lowest quartile of estradiol level means severely deficient estrogen synthesis attributed to the inhibition of aromatase enzyme activity, which is strongly associated with insulin resistance, hyperinsulinism and accumulation of androgen precursors [7, 16]. In elderly women with estrogen levels in the lower quartile, estrogen effect may be insufficient in any case of ER signal transduction capacity. Inappropriate cellular estrogen surveillance results in an enhanced risk for glucose intolerance and its comorbidities [123].

Considering the complex mechanisms affecting correlations between serum estradiol levels and cellular estrogen surveillance, one can understand that in elderly women, strongly decreased estradiol levels in either the lower or upper quartile may be associated with defective estrogen signaling.

Hyperestrogenism may be an embarrassing finding in certain young women with clinical symptoms of estrogen deficiency. In these women, a feedback mechanism aims to break through the inherited or acquired defective ER signaling by increased estrogen synthesis; however, if this defensive effort is insufficient, the result will be an estrogen deficiency disease. Among BRCA mutation carrier patients, low reactivity to fertility treatments [145] and earlier age at natural menopause, less than 40 years [146, 147] support the defect of estrogen surveillance. Clinical examinations on the reproductive factors of BRCA mutation carrier women suggest that their defective estrogen signaling may be in strong correlation with the preferential cancer risk of breast and ovaries. [124]. Defective ligand activated transcriptional activity of estrogen receptors in BRCA1/2 carrier women was associated with a 2-fold increase in the risk of type-2 diabetes, which was highly aggravated by a coexistent central obesity [148].

Correlations between elevated endogenous estradiol levels and BRCA gene mutations were investigated in women with breast cancer. Although, BRCA gene mutation carriers exhibited clinical symptoms of defective estrogen signaling, the serum estrogen levels of these patients were consequently elevated. Median luteal phase titers for estradiol were 33% higher in BRCA1/2 mutation carrier women than in BRCA mutation negative cases [149], suggesting a compensatory but insufficient increase in estrogen synthesis.

Genetic Defects of Estrogen Receptor Signaling

Much research has focused on identifying alterations within the coding sequence of estrogen receptors in clinical samples. A large number of naturally occurring splice variants of both ER α and ER β have been identified in both normal epithelium and diseased tissues. By contrast, only a few point mutations have been identified to be pathogenic in human patient samples from a variety of diseases, including breast cancer, endometrial cancer and psychiatric diseases [150].

Inherited estrogen receptor mutations may cause complete or partial blocking of estrogen signaling. The first mutation in ERs was reported 20 years ago in a 28-year-old man with knock-knees and signs of insulin resistance, obesity and premature cardiovascular lesions [76]. Laboratory examinations showed that his testosterone levels were normal and, although his estrogen hormone levels were compensatory high, he had essentially no response to estrogen.

Recently a case of an 18-year-old girl was published, who experienced no breast development or menstruation, the classic symptoms of too low estrogen level, the usual cause of delayed puberty [141]. Subsequent studies revealed sky-high levels of estrogens in her blood. In laboratory studies, 240 times the normal estrogen level was required to get a response out of the patient's ERs. Without estrogen reactivity, insulin levels were also typically increased, putting her at risk for diabetes. The patient exhibited an unusual response to oral glucose test, indicative of possible future glucose intolerance problems.

Rapid development of new methods for genetic investigations provided possibilities for the analysis of ER α gene (ESR1) polymorphism, which presumably may give the rational explanation of puzzling links among estrogen, metabolic disorders and the associated morbidities.

In PCOS cases, PvuII and XbaI polymorphisms of ESR1, as well as AluI and RsaI polymorphisms of ESR2 were genotyped, and no difference was found in the distribution of these gene variants between patients and healthy controls [151]. Nevertheless, in PCOS women, carriers of TC and TT genotypes of PvuII polymorphism had lower fasting glucose to insulin ratio compared with carriers of CC genotype. These results suggest some associations of ER α polymorphisms with insulin resistance in PCOS.

Apparently controversial results were published in relation to ESR1 polymorphism and the prevalence of myocardial infarct. In the Framingham Heart study, male carriers of the common ESR1 IVS1-397T>C C/C genotype, were found to have a substantial increase in the risk of myocardial infarction [152]. Conversely, in the Rotterdam study, postmenopausal female carriers of the ESR1 haplotype 1 (IVS1-397T>C*T allele and IVS1-351A>G*A allele), were shown to have an increased risk of myocardial infarct and ischemic heart disease, whereas in men an absence of this association was reported [153].

In postmenopausal women with HRT use, homozygous for the C allele at the IVS1-397T>C site, the severity of atherosclerosis was milder and less progressive [154]. By contrast, negative effects of the same allele have also been described; it was associated with risk of aortic valve sclerosis and coronary atherosclerosis at angiography in women [155]. Nevertheless, age distribution, body weight, HRT use and other factors of the examined population may thoroughly influence the cardiovascular risk besides the mutations of ER genotype [123], suggesting that presence or absence of compensatory estrogen synthesis may also be important players in health and disease.

Correlations between hormone replacement therapy (HRT) and mortality of women were examined to determine whether the risk of HRT associated mortality varies depending on the genetic variability of ERs [156]. These studies suggested that some women are genetically more vulnerable to the effects of HRT in terms of their estrogen receptor genotype. In these highly endangered women, as alternative possibility, the mutation associated severe lack of ER responsiveness emerges requiring increased compensatory hormone doses so as to achieve proper estrogen surveillance.

Correlations between genetic polymorphisms in the ESR1 gene and breast cancer risk are highly inconclusive. PvuII polymorphism was associated with a moderately increased risk, whereas, XbaI polymorphism was related to non-significantly elevated risk confined to older postmenopausal women in Shanghai [157]. Conversely, in a Korean study PvuII genotype distribution did not show any differences between breast cancer cases and controls, while the adjusted odds ratio for the XbaI allele containing genotypes was advantageously decreased (OR=0.4) [158].

In a further study, correlations among estrogen receptor alpha gene (ESR1) polymorphisms, estrogen levels and risk of breast cancer were studied in postmenopausal women [159]. The selection of these gene polymorphisms was based on previously published associations with osteoporosis and spontaneous abortions, suggesting defective estrogen surveillance. PvuII polymorphism in combination with higher estradiol levels was associated with increased breast cancer risk in postmenopausal women, which was interpreted as harmful collaboration between defective ERs and increased estrogen levels. Nevertheless, relatively increased estrogen level in elderly cases may be regarded as insufficient counteraction against the functional alterations of ERs instead of breast cancer initiator.

In severe defects of ligand activated ER signaling, both increased endogenous estrogen synthesis [142] and enhanced ligand independent ER activation mechanisms [160] may provide compensatory improvement for cellular estrogen surveillance. Failures of these compensatory actions result in defective estrogen surveillance, disturbances in glucose uptake and clinical manifestations of the associated morbidities.

Results of gene polymorphism studies suggest that mainly postmenopausal state and ageing associated estrogen deficiency may amplify the mutation associated defects of estrogen signaling, while in young cases sufficient or reactively high estrogen synthesis disguises the malfunction of ESR1 gene [79, 123].

Controversial Correlations between Endogenous Sex Steroid Levels and Breast Cancer Risk

Correlations between circulating endogenous sexual steroid levels and breast cancer risk seem to be highly controversial. Sexual steroid level measurements among breast cancer cases were performed on randomly selected patients disregarding their risk factors either for insufficient estrogen synthesis or defective ER signaling pathways. Analyzing the alteration pattern in the sexual steroid levels of breast cancer cases provides possibility to estimate the frequency of the different pathologic mechanisms, which may result in mammary malignancies.

In the majority of *young premenopausal women* with breast cancer, insulin resistant syndromes are the preponderant hormonal alterations with androgen excess. This prevailing

pathomechanism is reflected in the majority of studies suggesting a presumed causal correlation between androgen excess and breast cancer risk [161-165]. In these women, both insulin sensitizing Metformin and exogenous estrogen treatment may advantageously suppress the overwhelming excess of androgens.

In the second group of young breast cancer cases, hyperandrogenism may be coupled with reactive estrogen overproduction in a later stage of insulin resistance when growth factor abundance inhibits both the expression and transcriptional activity of ERs. Studies, supporting the carcinogenic capacity of increased levels of both estrogens and androgens, may preponderantly include these types of young breast cancer cases [121, 166]. In this group, a high dose of estrogen treatment is necessary to break through the ER resistance.

In the third, smaller group of premenopausal breast cancer cases, reactive but insufficient hyperestrogenism is associated with inherited, severe ER signaling defects. These types of patients are predominant in studies supporting the pivotal role of elevated levels of estrogens and their metabolites in malignant transformation without excessive androgen synthesis [167]. In these women, pregnancy mimicking high dose of estrogen may be a countermeasure against ER blockage.

In the vast majority of *postmenopausal breast cancer cases*, relatively increased circulating concentrations of both androgens and estrogens seem to be characteristic [120, 168-178]. Among these older breast cancer cases, tumor development was initiated by insulin resistance and the associated androgen excess. In such patients, slightly elevated estrogen concentration within the low postmenopausal hormone range may be regarded as an ineffective remnant of defensive estrogen synthesis against refractory ER signaling at younger age. These cases require increased exogenous doses of estrogen, since usual hormone treatment may be ineffective.

In the second group of postmenopausal breast cancer cases, relatively high levels of circulating androgens define the hormonal imbalance without increase in estrogens [179, 180]. ER signaling of these older women is not blocked either by progression of insulin resistant state or by ESR gene mutation. In this group of postmenopausal patients, even the usual dose of exogenous estrogen treatment may be effective.

In the third, smaller group of postmenopausal breast cancer cases, a reactively increased level of unconjugated estradiol without androgen excess may be strongly associated with refractory ERs [159]. Patients included into this group, exhibit estrogen resistance and need extra-high exogenous estrogen dose in order to get a response out of their defective ERs. Paradoxically, the higher the increase in compensatory endogenous estrogen level, the higher the promising dose of estrogen therapy. In the future, the quantitative evaluation of ER refractoriness in breast cancer patients will gain crucial importance in the therapeutic schedule.

Conclusion

Correlations between estrogen signaling and human morbidity seems to be very unclear and difficult topic. Nowadays, the opinions of scientists have been partially changed regarding “estrogen induced diseases” however; the overwhelming literary data are still contradictory.

Individual and population specific ER associated gene polymorphisms were presumed to illuminate the highly controversial clinical and experimental results on the role of estrogens in health and disease. Nevertheless, the puzzling biology behind estrogen action was not reassuringly clarified by studies on either disabled ERKO animals or patients with genotyping of their ERs.

Diverse disorders associated with insulin resistance are usually well treatable by estradiol substitution both in pre- and postmenopausal women as well as in animal experiments. ERs seem to have balanced interplay in the maintenance of ideal glucose uptake and energy expenditure with continuous adaptation to the momentarily changing intra and extracellular stimuli. This equilibrium may be shattered in case of a defective estrogen supply or by the derangement of ER signaling pathways.

Recently, new therapeutic options are planned based on the development of tissue specific selective estrogen receptor modulators (SERMs) by the pharmaceutical industry. Nevertheless, considering the beneficial results of estradiol substitution in diverse diseases, we should understand the marvelous network of ER signal transduction pathways instead of trying to manipulate it.

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