Leptin, Estrogens and Cancer

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Abstract: Obesity is a state of leptin resistance in which the membrane leptin receptor and the JAK-STAT pathway are blocked. This leads to increased intracellular concentrations of lipid metabolites, increased non-oxidative metabolism by adipocytes, and stimulation of the cell estrogen cycle. These factors are potentially oncogenic via the shared mitogen-activated protein kinase (MAPK), mitogen/extracellular signal-regulated kinase (MEK) and extracellular signal-regulated kinase (ERK) cellular pathways.

Keywords: Cancer, leptin, estrogen receptors.

LEPTIN, ESTROGENS AND CANCER

Obesity is related with the incidence of and prognosis for cancer. Certain types of cancer have also been shown to be related with estrogens, and the association between these cancers and obesity, particularly for endometrial and breast carcinomas, is well known.

Obviously, because leptin increases with adiposity, this hormone is related with cancer and with estrogens. The association, however, may be merely circumstantial, or leptin itself may play a role in the development of cancer. The truth is that the interactions between leptin and cancer appear to be contradictory. On one hand leptin plays a beneficial role since its effect on fat metabolism leads to a decrease in potentially oncogenic chemical agents derived from excess fat deposits in non-adipose cells. Moreover, its central action also plays a protective role against cancer to the extent that it prevents obesity. On the other hand, however, leptin can stimulate cell signaling pathways that activate progression of the cell cycle, and can thus increase the potential risk of carcinogenesis. Through this action leptin can potentiate the effect of estrogens on the development of cancer.

1. LEPTIN

The discovery of leptin [1] led to the identification of the role of adipose tissue in nutrition and immunity. This hormone is the product of the *ob* gene, located in chromosome 6 in mice and chromosome 7 in humans. The gene encodes a protein with a high degree of homology across species. The gene product, a 167-amino acid protein, circulates in the blood stream as a nonglycosylated 16-kDa molecule for which two main actions have been identified so far: its action on the central nervous system (CNS) decreases appetite, and its action on peripheral tissues has antisteatotic effects since it stimulates the oxidation of fatty acids and thus prevents the toxicity that excess fat deposits can cause in cells unsuited to lipid deposition [2]. When the *ob/ob* mutation occurs (rarely, in humans) it leads to obesity, overeating, hypothermia, hyperinsulinemia, hyperglycemia, and metabolic and endocrine alterations [1,3].

This adipokine with endocrine and paracrine activities is produced mainly in adipose tissue, but has also been found in the gastric mucosa, skeletal muscle, breast epithelium, placenta, bone marrow and pituitary, and in osteoblasts [3-5]. It is somewhat similar in molecular structure to certain interleukins (IL-6, IL-11 and IL-12), leukemia-inhibiting factor, ciliar neurotrophic factor, oncostatin-M, cardiotrofin-1, and granulocyte-stimulating factor [6,7]. In the blood stream it occurs in two forms: as a free bioactive molecule and bound to its soluble receptor (sOb-R). The free/bound ratio of leptin varies during an individual's lifetime depending on the amount of body fat: the more adipose tissue, the greater the production of free leptin. The free form can cross the blood-brain barrier to reach the hypothalamus via the cerebrospinal fluid (CSF).

The central effects of leptin include not only appetite reduction but also stimulation of thermogenesis via sympathetic pathways. Leptin also interacts with the hypothalamus-pituitary-adrenal axis, influencing sexual maturation, reproduction and development [4,8]. This adipokine is also a sign of adaptation to food deprivation, since it triggers increases in the concentration of serum triglycerides, which block leptin transport across the blood-brain barrier and lead to a decrease in leptin concentrations in the CSF [9]. This in turn increases appetite to ensure survival. Diminished leptin activity is related with an increase in adrenal glucocorticoid activity along with a decrease in gonadal and thyroid hormones and the depressed immune system activity seen in the complex mechanisms of food deprivation [10,11].

1.1. Leptin Receptors

For its effects on different tissues to take place, leptin must interact with its membrane receptor Ob-R, encoded by the *ob* gene [12]. The Ob-R, like receptors for IL-6, leukemia inhibitory factor (LIF), granulocyte colony stimulating factor

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(G-CSF), gonadotrophin-releasing hormone (GRH), prolactin and erythropoietin [13], belongs to the class I cytokine family of receptors, which bear 4-residue extracellular cysteine motifs and Trp-Ser-X-Trp-Ser (WSXWS), and contain different fibronectin type III domains [14,15].

The extracellular region of the Ob-R consists of four fibronectin type III domains and two cytokine receptor domains [16] which form homodimers. When the region is activated by its ligands, conformational changes take place. The proximal-transmembrane region comprises 29 intracellular amino acid residues that include the box 1 motif. Of the seven known isoforms of the receptor, at least six are generated by splicing of the RNA transcript of the *db* gene. All isoforms bear the same extracellular binding domain [17], but only five isoforms have transmembrane and cytoplasmic regions; these isoforms differ in the length of the intracellular domain as a result of differences in the number of additional cytoplasmic regions [18]. Of all known isoforms the one with the greatest functional capacity is the longest form (Ob-Rb), which contains 301 intracellular amino acids and the strongest signaling capacity.

The short isoforms (Ob-Ra, Ob-Rc and Ob-Rd) have limited signaling capacity, but although their transmembrane portion contains a single box 1 motif, they are nonetheless able to recruit the Janus kinase (Jak) and trigger the signaling cascade [19]. The isoform lacking the intracellular and transmembrane domain is the soluble receptor (sOb-R) [20-22], which binds to plasma leptin with high affinity [23, 24] to regulate the level of free leptin in plasma [25]. The Ob-Ra isoform acts as a membrane transporter of leptin from the plasma to the CSF across the blood-brain barrier, where it reaches highest concentrations in the choroid plexus of the hypothalamus [12,26,27].

Upon reaching the CSF, leptin binds to Ob-Rb on the membrane of hypothalamic neurons, specifically in the arcuate nucleus [20]. The intracellular portion of Ob-Rb contains a number of sequences of elements that are required to initiate post-receptor events. The Ob-Rb receptor has no intrinsic tyrosin kinase domain, and therefore requires the cytoplasmic kinase Jak2 for phosphorylation [28]. Its intracellular amino acids 6 to 17 constitute a proline-rich box 1 motif [20,29], and amino acids 49-60 and 202-213 comprise two box 2 motifs [28,30]. Box 1 and box 2 appear to be the regions that recruit Jak [19,31], although box 1 and amino acids 31 to 36 which flank this region are also essential for the activation of Jak [30,32]. Amino acids 37 to 48 enhance the signal, but can be substituted by other amino acids [30]. In these regions, two amino acids were identified as crucial for signal transmission: Leu896 and Phe897. These amino acids are thus conserved in different vertebrate species [32]. Although an intact box 2 motif is not required for Jak activation [30,32], this motif is necessary to induce the signal transduction and activation of transcription (STAT) factor [19].

Because it is able to form homodimers and transmit signals when the box 2 motif is mutated, Ob-R can be classified as a member of the GH receptor subclass within the class I cytokine family of receptors [33]. For signal transmission to trigger post-receptor events, tyrosine residues are required at positions 985 and 1138, the sites of molecular interaction [34]. The Ob-Rb receptor is expressed mainly in the hypothalamus [20], although it is also present in cells of the lung, kidney, stomach, muscle, liver, Islets of Langerhans, endometrium and placenta, and in adipocytes, endothelial cells, keratinocytes, mononuclear cells, T lympho-cytes, osteoblasts and germinal cells of the testicle [35-43]. Levels of this receptor vary with age and hormonal cycles [44,45]. The lack of functional leptin receptors leads to results similar to those noted above for the *ob/ob* mutation: obesity, overeating, hypothalamic hypogonadism, hyperglycemia, elevated circulating levels of corticosteroids, and hypothermia [3].

1.2. Intracellular Pathways of Action of Leptin: the Jak-STAT Pathway

Once leptin interacts with Ob-Rb receptors in the arcuate nucleus of the hypothalamus, signal transmission activates the Jak-STAT pathway. This pathway is regulated by the chaperone proteins HSP90, hTid1 and GRP58 [46]. The Jak proteins are cytoplasmic tyrosine kinases that bind to a specific domain of the proximal portion of the leptin receptor on the cell membrane [14]. When Jak2 [30] is activated, transphosphorylation of the other Jak kinases and other tyrosin residues of the receptor occurs (Tyr985 and Tyr1138) [34,47]. The Tyr1138 residue acts as the docking site for STAT proteins, especially for STAT3 [48]. The STAT factor then dissociates from the receptor to form homodimers or heterodimers which act in turn as a transcription factor in the cell nucleus, upon binding to promotor-specific response elements of the target genes such as sis-inducible element (SIE), acute-phase-response element (APRE) and other gamma-interferon-activated sequence (GAS) elements [14,48,49].

The STAT3 factor, present in the neurons of the paraventricular and arcuate nuclei, and in the lateral hypothalamic area [50,51], inhibits peptide Y and agouti-related proteins. This reduces appetite and increases energy expenditure. Experimental studies in *db/db* and *ob/ob* mice have shown that the STAT signal is absent [52,53], thus the mechanism of control of body weight is impaired and the result is obesity or morbid obesity. Activity of the STAT3 factor is also seen in orexin-secreting and glandin-secreting neurons; both factors are known to influence food intake [54]. Another source of STAT3 activity has been found in conjunction with the Ob-R receptor in vagal afferent neurons of the nodose ganglion, the solitary tract nucleus, and the dorsal motor nucleus of the vagus nerve [36].

In addition, the Jak-STAT pathway, mediated by the Ob-Rb receptor, is also involved in the peripheral action of leptin, which can therefore be considered a systemic action [35,36,37,41]. As a result of the action of leptin on the hypothalamus, the α-adrenergic system is activated and peripheral lipolysis is induced [55]. Leptin also stimulates the activity of uncoupling thermogenic proteins (UCP-1,2) which transform the energy in fat into heat [56,57]. Leptin also participates in the onset and progression of puberty, the control of the hypothalamus-pituitary-gonadal axis, hemato-poiesis and stimulation of the immune system [4,8], and its serum concentration decreases at higher altitudes [58].

With age the STAT signal and hence the anorexic behavior produced by leptin are diminished [45], a phenomenon that has been related with the alterations in lipid metabolism seen with aging. Moreover, the expression of molecules related with a reduction in body fat, i.e., acyl-CoA-oxidase, carnitine-palmitoyl-transferase-1 and peroxisome-proliferator receptor-α (PPAR-α), also decline with age.

In addition to the mechanisms described above, the cytokine signal regulator SOCS-3 increases in subcutaneous adipose tissue with age [59]. A member of a family of proteins with a small domain that contains SH2, SOCS-3 inhibits the Jak-STAT pathway by binding to tyrosin-phosphorylated residues in this pathway [60,61] and thus blocking Jak-induced phosphorylation and autophosphorylation of the receptor [62]. Thus SOCS-3 has been implicated in the leptin resistance seen in obesity [63].

Leptin resistance was postulated when it was found that obesity occurred together with elevated serum concentrations of leptin, a potent anorexic. Moreover, elevated leptin concentrations were not accompanied by increased concentrations of leptin in the CSF, a finding that identified the blood-brain barrier as the main site of resistance. It was recently shown that serum triglycerides can play a fundamental role in this phenomenon [9]. Only triglycerides with fatty acids in position sn-1, i.e., those of animal origin, are able to inhibit leptin transport across the blood-brain barrier. Triglycerides of plant origin or triglyceride-derived free fatty acids do not lead to resistance to leptin transport. [9]. It is not inconceivable that inhibition results from triglyceride binding to plasma leptin, although an action of triglycerides on leptin-cell receptor binding seems more likely. In epidemiological terms a role for triglycerides in the origin of leptin resistance and thus obesity is plausible as the epidemic of obesity currently in progress has coincided with the massive adoption by western societies of diets rich in saturated fats. (Suffice it to say that in the last 30 years the average energy intake in these societies has increased by more than 500 kilocalories per day).

Two other molecules are also able to inhibit this pathway: protein-tyrosine phosphatase 1B (PTP1B) [64], which recognizes a Jak2 motif and triggers its dephosphorylation [65], and activated STAT3-specific inhibitory protein (PIAS3), which blocks the binding of STAT3 to target cell DNA [66].

1.3. Intracellular Pathways of Action of Leptin: the MAPK (Mitogen-Activated Protein Kinase) Pathway

The mitogen-activated protein kinase (MAPK) pathway is activated by leptin [20,34], with maximal activation via the long receptor Ob-Rb. The Tyr985 residue in the long receptor is fundamental for leptin to induce full activation of the extracellular signal-regulated kinase (ERK) [34]. Phosphorylation of ERK is followed by recruitment of Jak1-2 to form a docking site for the SH2 domain of protein tyrosine phosphatase (SHP-2). After the SH2 domain binds to the Tyr985 tyrosine residue it is phosphorylated at its C terminal. This, in conjunction with the adaptor molecule termed "growth factor receptor binding protein 2" (Grb-2), activates the cascade of subsequent signals [34].

Another mechanism exists for the induction of the ERK pathway in a manner independent from the Tyr985 residue, via SHP-2, involving the short leptin receptor [34]. The Jak2 factor activates the ERK pathway via the Ob-R receptor independently from phosphorylation of the receptor [20]. To achieve this, Jak2 binds to the SH2 domain in the Grb-2 protein and SHP-2 [34,67], thus activating signals that involve—depending on the system—the ras and fas molecules. This is followed by activation of mitogen/extracellular signal-regulated kinase (MEK1) [68], which may require the aid of integrins [69].

Once MEK1 is activated, it phosphorylates ERK1/2, a transcription factor with a zinc finger involved in cell growth and differentiation, and the *c-fos* and *erg-1* genes are expressed, [3,70]. Through the MAPK pathway, leptin stimulates cell growth and placental trophism. For example, in human pancreatic β cell line MIN6, activation of the MAPK pathway by leptin induces cell proliferation, and inhibition of this pathway blocks leptin-induced DNA synthesis and cell viability, a process consistent with the Islet of Langerhans hypertrophy seen in obesity [71]. Another example of stimulation of cell proliferation is the influence of leptin on angiogenesis. Histochemical studies have found that increased Ob-R activity is important in the pathogenesis of intimal neovascularization in atherosclerosis lesions [72,73].

The SHP-2 protein appears not to affect gene induction in the STAT3 pathway [70]. The SOCS-3 factor is able to induce gene expression, and acts as a competitor with SHP-2 since both bind to Tyr985. Thus, SHP-2 indirectly activates the STAT3 pathway. Activation of the MAPK pathway has been described in the hypothalamus, liver and adipose tissue [34,70,74,75]. In monocytes, leptin induces, via the MAPK pathway, the expression and secretion of an interleukin-1 receptor antagonist, which activates the NFkB binding site in the promotor via an as yet unidentified factor [76]. In osteoblast precursor cells, leptin induces apoptosis via the MAPK pathway: ERK1 and ERK2 activate cytosol phospholipase A2 (cPLA2), which leads to the release of cytochrome C and activation of caspase-3 and caspase-9, which in turn trigger cell death.

1.4. Leptin in Lipid Oxidation

In peripheral tissues, leptin acts upon pathways of lipid metabolism to stimulate oxidation. It also prevents steatosis by stimulating mitochondrial oxidation of fatty acids in adipocytes and non-adipocyte cells, an action that protects the cells against the fat overload that can lead to cell lipoapoptosis and lipotoxicity in non-adipose tissues [77]. Leptin has been shown to activate the α2 subunit of 5'-AMP and to activate AMP-activated protein kinase (AMPK), which blocks the effect of acetyl-coenzyme A-carboxylase (ACC) in the muscle and stimulates lipid oxidation [78]. The AMPK protein kinase phosphorylates ACC-β, inhibiting the action of the carboxylase and increasing fat oxidation by disinhibiting carnitine-palmitoyl-transferasa (CPT) [79].

In non-adipocyte cells, leptin administration leads to activation of peroxisome proliferator-activated receptor-alpha

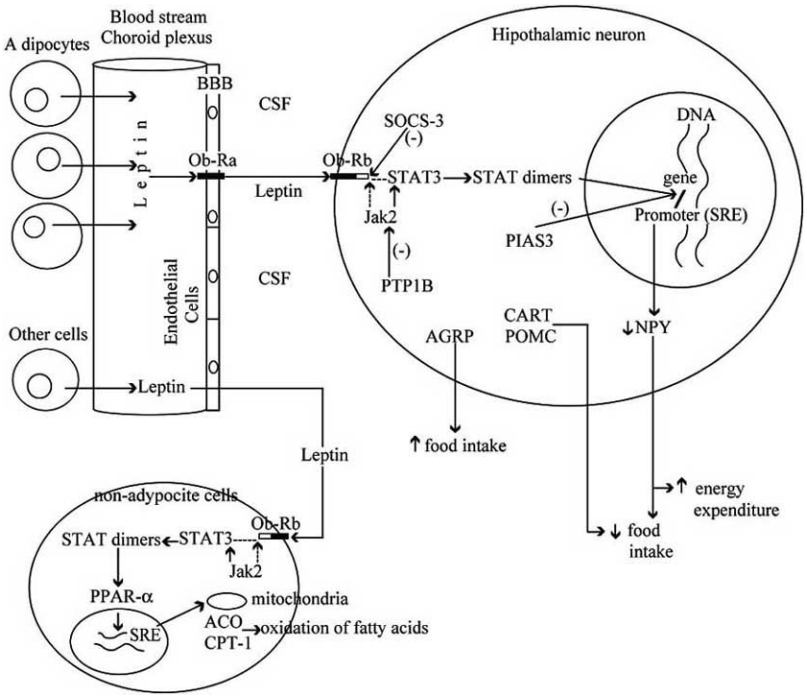

Fig. (1). Scheme of the Jak-STAT pathway.

(PPAR-alpha) by STAT3. Upon binding to its response element, STAT3 induces the expression of acyl-CoA-oxidase and CPT-1, which leads to fat oxidation [77]. In cells that lack functional leptin receptors, levels of PPAR-gamma and lipogenic enzymes such as ACC and fatty acid synthase are increased [77].

1.5. Obesity, Hyperleptinemia and Cancer

A causal association can be said to exist between obesity and cancer. The relationship between weight increase and death from cancer was identified more than 25 years ago [80]. Since then a number of studies have shown consistently that adiposity is associated with the risk of endometrial, kidney, and colon cancer, and with postmenopausal breast cancer [81,82]. Esophageal adenoma has also been related with obesity [83-85]. Studies that reported an association between hepatocellular carcinoma and obesity found an increased risk in both women and men [86,87]. An epidemiological follow-up study of a cohort of almost one million persons over 20 years [88] found incontrovertible evidence of the relationship between obesity and increased mortality from several kinds of cancer. It is estimated that 90 000 deaths from cancer could be avoided yearly in the USA if men and women maintained their normal body weight. Clearly, overweight and obesity are associated with an increased risk of death from all cancers globally, and with the risk of death from cancers in specific organs and tissues.

It was noted above that fat homeostasis is dependent on leptin and that resistance to leptin leads to lipotoxicity and cellular lipoapoptosis, changes that increase the risk of neoplasia. When leptin function is impaired, excess fat is stored in non-adipose tissues where it undergoes oxidation. These cells are thus exposed to large amounts of lipid metabolites such as ceramides, nitric oxide and peroxides which can lead to oncogenesis or lipoapotosis. Aside from the consequences of impaired leptin activity, other consequences can be attributed directly to its ability to stimulate cell proliferation. Specific examples are the high levels of leptin receptor mRNA in prostate epithelial cells with high-grade prostate intraepithelial neoplasia (PIN) [89] and the direct influence of leptin on prostate cancer cell progression [90]. The leptin-induced stimulation of angiogenesis by this hormone [91-93] can also influence prostate cancer indirectly.

The underlying cause of obesity and hyperlipidemia is usually the intake of excessive amounts of saturated fat, and this makes it important to consider the role of delta-9-desaturase (δ-9-d). The fat content of the diet has important effects on δ-9-d activity, and high levels of saturated fatty acids increase the activity of this enzyme two- or threefold, whereas polyunsaturated fats reduce its activity [94,95]. In other words, the same dietary patterns that favor obesity, leptin resistance and excess levels of ovarian estrogen (see below) also increase δ-9-d activity. In mammalian tissues most oleic acid (a monounsaturated fatty acid) is derived from stearic acid (a saturated fatty acid) [96,97]. The key to this conversion is controlled by δ-9-d, which also regulates the transformation of other common saturated fatty acids such as myristic and palmitic fatty acid to their monounsaturated forms. A number of studies have suggested a strong link between δ-9-d activity and tumor growth. The formation of mouse breast cancer cells [98], hepatoma cells [99] and human leukemia and lymphoma cells [100] requires high levels of monounsaturated fats produced from the

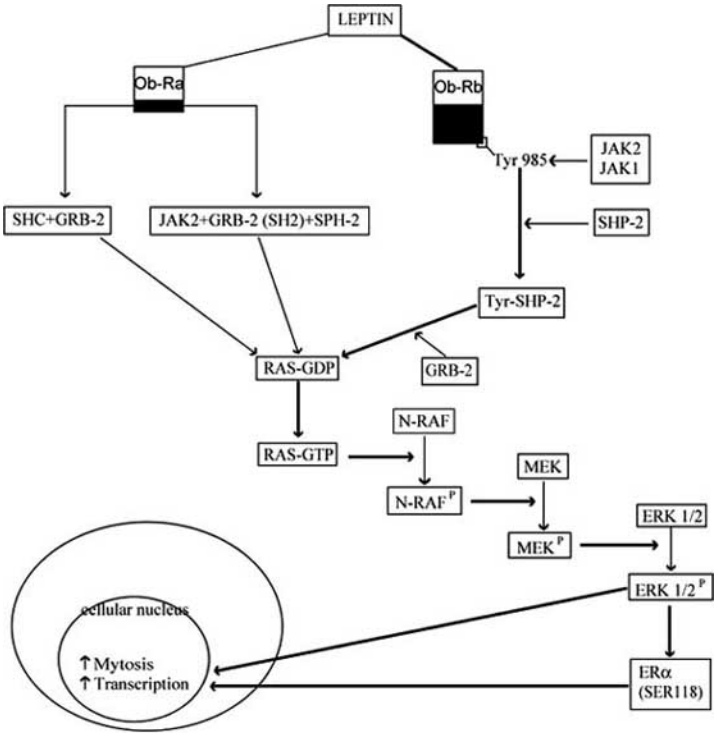

Fig. (2). Scheme of the MAPK pathway.

conversion of saturated fats, a process mediated by elevated δ-9-d activity. In these examples, adequate levels of monounsaturated fats are ensured by overexpression of the genes that encode δ-9-d. In rats, the inhibition of δ-9-d activity and consequently inhibition of the conversion of saturated fatty acids to monounsaturated fats blocks the growth of breast tumor transplants [101]. Furthermore, breast cancer has been slowed "in vitro" by adding a δ-9-d inhibitor to the growth medium [102]. The administration of insulin [95,103], estrogens [104,105] or testosterone [106] also activate δ-9-d, and there is conclusive evidence that high levels of these hormones significantly increase the risk of breast cancer [107].

2. ESTROGENS AND CANCER

The relationship between sexual steroid hormones and certain types of cancer has been well known for some time, particularly for breast and endometrial cancers. As explained above, obesity is characterized by increased leptin concentrations, leptin resistance, and altered lipid metabolism. Along with metabolic processes which predispose individuals to cancer, obesity also involves increased estrogen levels, which can reinforce the relationship between obesity and dysplasia-inducing events derived from the mechanism of action of steroid hormones. The increase in estrogens of peripheral origin in obesity is the result of intracellular enzymes responsible for the capacity of sex hormones for autocrine synthesis. The major enzymes involved in this relationship are described below.

1) Aromatase is the most important of these enzymes in functional terms, and is also the most abundant. The term actually refers to a complex of enzymes that include cytochrome P450-CYP19 AROM [108], which contains a heme group and catalyzes steroid oxidation reactions, and the flavoprotein NADPH cytochrome P450 reductase. Aromatase is expressed in ovarian tissue and the placenta, in many other tissues such as the hypothalamus, liver, muscle, subcutaneous adipose tissue, and in the fibroblasts and epithelial cells of normal and tumoral breast tissue, although its distribution varies [109,110]. After menopause, adipose tissue becomes the main producer of circulating estrogens through the action of this enzyme, and plasma estradiol levels correlate clearly with body mass index. Aromatase catalyzes three distinct hydroxylation reactions which convert testosterone into estradiol. Two reactions lead to the formation of 19-hydroxy and 19-aldehyde structures, and consume 2 NADPH + H with the release of two water molecules. The third reaction, which remains controversial, catalyzes the loss of methyl 19 with the release of formic acid, and aromatizes the A ring, a process that consumes NADH + H [111]. Several cytokines stimulate aromatase, e.g., IL-6 [112], TNFα and IGF-I [113]. Aromatase is also able to use androstenedione as a substrate to produce estrone. This substrate, produced by the suprarenal cortex, is converted to estrone by extra-ovarian aromatase during postmenopausal life. Estrone is then converted to estradiol (E2) by 17β-hydroxysteroid dehydrogenase.

2) Sulfatase converts a portion of estrone to estrone sulfate, a hydrophilic molecule that circulates more freely and acts as a reservoir for the formation of active estrogens. Estrogen sulfate is the most abundant estrogen during postmenopausal life. The expression of E1-STS (estrone sulfatase) mRNA is significantly elevated in breast tumors, and increased levels of this enzyme correlate with a poor prognosis [114].

3) Estradiol-17β-hydroxysteroid dehydrogenase converts estrone to estradiol to ensure maximal potency of estrogen. In breast tumors the activity of this enzyme is increased and as a result estrone is converted to estradiol in situ [115]. Like aromatase, this enzyme is stimulated by IL-6 [116] and TNF-α [117], cytokines produced by adipose tissue.

These relationships endow extraovarian tissues with the entire enzymatic machinery needed to ensure the local estrogen supply. Obesity gives rise to the circumstances necessary for the coordinated stimulation of this machinery by different cytokines. Thus obesity is a state in which the presence of increased amounts of potentially oncogenic metabolites coincides with an increase in steroid hormones able to favor tumor growth via their specific surface and intracellular receptors [118-120]. Estrogens, as inducers of cell proliferation [121-124], facilitate the evasion of apoptosis if an error in DNA duplication occurs. This would explain the high concentrations of estrogens found in breast cancer and other types of cancer [125]. The finding that most estrogen-dependent cancers occur after menopause, when circulating levels of ovarian estrogens are considerably diminished [126,127], suggests that extraovarian estrogens play a primordial role in the development of cancer. Oncogenic exposure is dose- and time-dependent, a finding consistent with the fact that early menarche, delayed menopause and hormone replacement therapy (phenomena that increase the dose and duration of exposure to estrogens) are associated with an increased risk for hormone-dependent cancers [128-130]. Obesity involves not only an increase in the dose and duration of exposure to estrogen-induced cell stimulation, but also stimulation of cell growth by leptin. Moreover, the dose and duration of exposure to oncogenic factors derived from the alterations in lipid metabolism described above are also increased.

In addition, the action of sexual xenohormones—external physicochemical agents such as pesticides, herbicides, plastics, solvents and detergents—can modify the chemical composition of nutrients found in dietary patterns that favor obesity (diets rich in saturated fats, such as the so-called western dietary pattern). As a result these nutrients may be transformed into sex-hormone-like substance and acquire a degree of mitogenic capacity that may potentiate the mitogenic capacity of the individuals own sex hormones [131].

2.1. Estrogen Receptors

Estrogens function in target cells through estrogen receptors (ER) of two types, called α and β. Both appear to be widely albeit irregularly distributed in different tissues. An abundance of ER ensures the cell's optimum response to estrogens. It has been known since the 1970s that estrogen-dependent breast carcinoma cells are richer in ER than the neighboring nontumoral cell, which makes the former more sensitive to proliferative stimuli.

The basis of estrogen-induced processes of carcinogenesis lies in the ability of these hormones—among the most potent mitogens known in some cell lines—to induce epithelial cells to enter the cell division cycle. However, as noted above, this physiological action is insufficient in itself to produce neoplastic alterations. In fact, breast and endometrial cells withstand this stimulus for years without undergoing transformation, indicating that the proliferative stimulus, enhanced in cells with a larger complement of ER, requires some other source of disruption of the cell cycle for neoplasia to result.

Estrogen receptors are highly active proteins that are soluble in the cytosol. The α and β forms are the products of different genes, and their tissue distribution and properties therefore differ although they share many characteristics, including several functional regions of their molecule. Estrogen receptor α is a single-chain 595-amino acid, 66-kDa protein whereas ERβ comprises 530 amino acids and has a molecular mass of 62 kDa. The α receptor is better characterized and appears to be bound in its native state to several proteins such as HSP90 and P59, which act as chaperones and keep the receptor in an inactive state. Briefly, the most interesting functional regions of the ER are as follows [132]:

1. In the Amino-Terminal Region:

- Presence of zinc fingers forming crosslinks with DNA, in the promotor region of estrogen-dependent genes where palindromic estrogen response elements are located
- Transcription activation or transactivation region (TAF1) binding to different nuclear transcription factors to complete the gene transcription machinery. One of the functional characteristics of TAF1 is its ability to act with these factors in the absence of estrogen bound to its receptor.

2. In the Carboxy-Terminal Region:

- The estrogen-binding domain
- A dimerization zone
- Inactivation protein-binding zone (HSP90, etc.)
- A hinge region containing basic amino acids that confer affinity of the nucleus to the receptor and also participate in dimerization

When estrogen reaches the binding domain it triggers interactions with at least three ER amino acids. The resulting conformational changes lead to loss of affinity for proteins, giving rise to the ERα monomer. This change also displays previously inaccessible regions, making them available for protein-protein interactions. The first result is receptor dimerization (to form homodimers or heterodimers) and receptor activation. The dimer has much stronger affinity for the nucleus than the inactive ER, and is usually found in the cell nucleus, where it recognizes and binds to estrogen

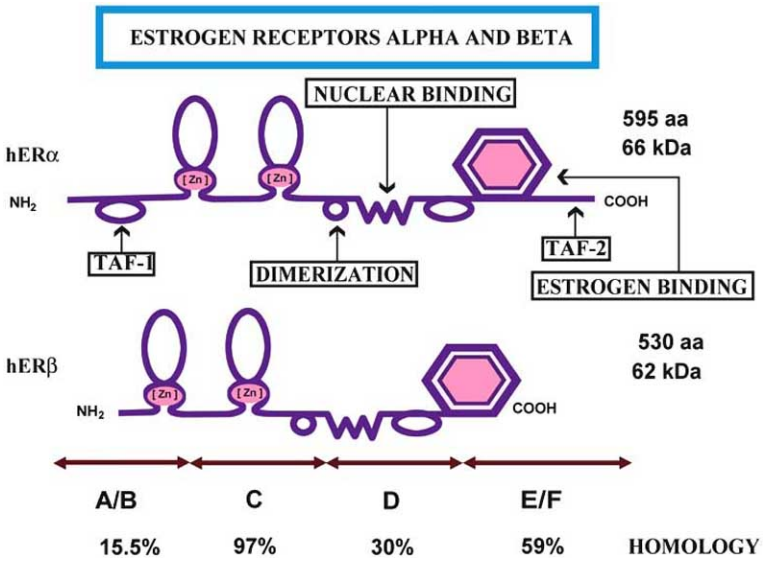

Fig. (3). Estrogen receptors .

response element (ERE) regions of the DNA that encode genes whose expression is under the control of estrogen. These genes contain one or more ERE in their promotor regions.

The TAF1 and TAF2 regions are able to interact with other proteins that co-activate or co-repress transcription. Activity of these regions is triggered when the ERE regions are recognized, and as a result RNA-polymerase transcription activity is accelerated and multiple copies of the gene mRNA are produced.

Although the magnitude of the transcriptional response is determined to a large extent by co-regulatory proteins [133], it also depends on whether ERα or ERβ is involved. Although their DNA binding domains are similar, ERα and ERβ differ in their capacities to activate different genes [134]. In addition, their affinities for ERE differ in many genes that respond to estrogen, such as *c-fos*, *c-jun*, *pS2* and *cathepsin D* [134]. An alternative mechanism for the interaction of ER with DNA has been described as the interaction of ER with activating protein-1 (AP-1) sites where the receptor binds through the Fos/Jun complex [135,136].

2.2. Effects of Activation of Estrogen Receptors

The first detectable consequence of ER activation is transcription of *c-myc*, a gene with mitogenic, apoptotic and transformation activities [137]. The *c-myc*-mediated action of estrogen consists of activation of cdk2 activity, which allows cells to enter the S phase of the cell cycle. Activation of cdk2 can result from cyclinA-cdk2 or cyclinE-cdk2 complexes [138]. Apparently, estrogen-induced carcinogens share the ability to induce centrosome amplification. Although the precise sequence of events remains unclear [139], it is evident that there is an early disruption of centrosome homeostasis in conditions involving dysplasia, such as intraductal carcinoma and breast carcinomas in rats. This process may involve the estrogen-mediated over-expression of *c-myc*, with disruption of the cell cycle cascade. According to the mechanism proposed by Li and colleagues [139] the sequence of events is as follows: The formation of E2-REα complexes induces *c-myc*/ myc protein overexpression, which in turn leads to overexpression of cyclin E-cdk2, of Aurora A (a centrosome-kinase), centro-some amplification, chromosomal instability, and eventually aneuploidy and cancer.

Activation of ERα also leads to stimulation of the *cyclin D1* gene [140]. As a result the ER binds to a cAMP-dependent response element (CRE). This would account for the overexpression of *cyclin D1* (both mRNA and the gene product) in tumor tissues, a finding that indicates that in cancer, cyclin is one of the targets for the action of estrogen. The cyclin D1-cd4/6 complex is also responsible for cyclinE-cdk2 activation.

Another family of genes that are regulated by estrogen is the *bcl2* family [141], whose members, like *c-myc*, have been shown to be important mediators in the action of steroids. Another factor involved in the action of steroids is the EGF family of ligands, which mediate cell proliferation and survival [142]. Other sites of estrogen activation are found in mitochondrial DNA [143]. A final site of ER activation is located on the plasma membrane, where activation triggers mitogenic activity through MAPKinase transduction signals, allowing crosstalk between ER and other cell signaling pathways such as the leptin pathway (see above) or modulation of IGF-I receptor (IGF-IR) activity [144].

A number of studies have documented the ability of different growth factors such as EGF and insulin-like growth factor to stimulate ER activity and alter the agonist/ antagonist balance of selective ER modulators (SERMS) [145,146]. Stimulation of ER transcriptional activity by IGF-I or EGF is associated with phosphorylation of the multiple serine residues in the TAF1 region of both ERα

and ERβ [147,148]. This includes the phosphorylation of Ser-118 in ERα, a MAPKinase site with mitogenic activity [147,149], and phosphorylation of ligand-dependent Cdk7 [150].

The cAMP-enhanced phosphorylation of ER can take place in different residues [151] which play an important role in the transactivation capacity of ER [147,151]. The tumorigenic action of IGF-I through its IGF-IR receptor is based on its potent antiapoptotic and mitogenic activity. The IGF-IR and ER signaling systems share some intracellular pathways. Specifically, ERα activation leads to over-regulation of the expression of insulin receptor substrate 1 (IRS-1), IGF-IR and IGF-1, which leads in turn to enhanced response to IGF-I. In a reciprocal manner, stimulation of IGF-IR increases ERα phosphorylation and activity [144].

The ERβ receptor has been found in many types of tumors including breast cancer [152], where its presence is associated with a favorable prognosis [152]. In breast cancer the ERβ receptor tends to be expressed in cases that are also ERα-positive. Breast carcinomas show a higher REα/REβ ratio in comparison to normal breast tissue or benign tumors [153,154]. Moreover, increased levels of ERβ correlate inversely with Ki-67, a cell proliferation marker; this association suggests that ERβ plays a protective role against the mitogenic effects of estrogens. In this connection Zhao and colleagues [155] have suggested that in breast tissue, loss of ERβ expression is a marker of carcinogenesis, which may be triggered by a methylation process.

2.3. Interaction Between the α-Leptin Estrogen Receptor and Breast Cancer

The relationship between the leptin estrogen receptors and breast cancer merits particular attention as it has been well characterized at the molecular level. The long and short isoforms of the leptin receptor are expressed on epithelial cells of normal breast tissue and in breast carcinoma cell lines [156]. This suggests a role for leptin in the development of the mammary gland and in tumors derived from this tissue. Breast carcinoma is characterized by increased levels of estrogens and leptin [157]. A number of studies in different tissues have shown that leptin stimulates the production of estrogens directly by stimulating aromatase in addition to the IGF-I pathway [158-160]. In the CMF-7 breast cancer cell line, leptin, via MAPK and STAT signaling pathways, stimulates the expression of aromatase via the aromatase promotor response element (AP1), a finding that demonstrates leptin's important role in raising estradiol levels in situ and promoting cell proliferation [161]. Estrogens are also known to modulate the expression of the leptin gene in adipose tissue [162,163].

Noting that unbound ERα is an important effector of the MAPK signaling pathway and that leptin is able to activate the *ras*-dependent MAPK [75], Catalano and colleagues [164] have shown that leptin is able to induce functional transactivation of ERα in the CMF-7 cell line, enhancing TAF1 activation independently of receptor binding to its ligand. These findings are evidence that two components are involved in the enhancement of leptin-induced estrogen signals: 1) increased aromatase activity, and 2) direct activation of ERα in the absence of its ligand.

In addition, leptin potentiates the effects of estradiol-induced ERα activation, indicating that two different functional domains that act as effectors for different signals can cooperate through a synergistic pathway. This emphasizes the role of leptin as a promotor of estrogen-dependent carcinomas in obesity.

Other cellular pathways that respond the stimulation by leptin with cell growth in cell line CMF-7 are transcription 3 activators and the extracellular signaling pathways regulated by kinases1/2 and Akt/GSK3/pRb [165]. Further-more, leptin may also play a role in promoting aggressive non-estrogen-dependent breast carcinomas via activation of transcription factor NFκB [166].

2.4. Transcriptional Regulation of Adipose Tissue Proteins by Estrogens

The presence in adipose cells of ERα and ERβ has been widely documented [167-169] and it is unquestionable that the transcriptional activity of many adipose tissue genes is regulated by sexual hormones. The two key proteins involved in fat deposits are lipoprotein-lipase and leptin. The mechanism that controls leptin's role in this activity is summarized below.

Concentrations of leptin are higher in women than in men [170]. In particular, during postmenopuasal life the levels of bioavailable estrogen correlate directly and significantly with leptin concentrations, but this does not appear to be the case before menopause or in women on hormone replacement therapy [171]. The most convincing evidence of the influence of sex hormones on the levels of leptin comes from studies of hormone treatment of transsexuals [172]. Elbers and colleagues [172] showed that the administration of estrogens and antiandrogens increased serum leptin levels whereas the administration of testosterone diminished leptin levels.

In summary, if steroid hormones and estrogens in particular directly regulate leptin production, ERE should be found in the promotor regions of the leptin gene. This was confirmed by analyses of the cDNA sequence of the gene [162]. Another study that provided support for the presence of ERE was the experiment reported by O'Neil and colleagues [173] in breast cancer cell line MCF-7 (ER-positive) and JEG-3 choriocarcinoma cells (ER-negative), which normally produce leptin. After transfection of these two cell lines with leptin-luciferase, estradiol increased leptin-luciferase activity in choriocarcinoma cells co-transfected with ERα but not in the transfected MCF-7 cells. When JEG-3 cells were co-transfected with ERβ, estradiol failed to stimulate leptin-luciferase activity. The authors concluded that the leptin gene contained an estrogen response element and that whether the promotor was activated or inhibited may depend on the presence of co-activators or repressors in leptin-producing cells. The discrepant effects of estrogens in this study may have been due to the differential expression of ERα or ERβ in the target cells.

It has also been noted that a nongenomic pathway of regulation may be involved in estrogen's influence on lipid metabolism. In this pathway the hormone binds to a plasma membrane receptor, and a second messenger effects it action. Although estrogen receptors have been characterized on the plasma membrane of several types of tissue [174,175], the mechanism of action of steroid hormones via membrane receptors is still poorly understood and probably involves several systems [176-179]. Through this pathway estrogens are able to interact with various components of the membrane signaling system, including the cAMP and phosphoinositide cascades [180-182]. Given the present state of knowledge, it seems likely that membrane receptors involved in the regulation by estrogen of hormone secretion in adipose tissue are controlled by a combination of genome-based ER-DNA and nongenomic mechanisms [183,184].

In conclusion, obesity involves leptin resistance and hyperleptinemia, failure of the anorexigenic and antesteatotic action of leptin, and the intracellular accumulation of potentially oncogenic metabolites. Hyperleptinemia stimulates the production of extraovarian estrogen, and excess estrogen in turn increases the expression of the leptin gene in adipose tissue. In cells that bear estrogen receptors, the increase in estrogen levels induces cell division and leads to centrosome amplification, chromosomal instability and aneuploidy. Therefore leptin and estrogens potentiate each other's capacity to stimulate cell proliferation, making obesity an environment that favors tumor growth.

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REFERENCES

[1] Zhang, Y.; Proenca, R.; Maffei, M.; Barone, M.; Leopold, L.; Friedman, J.M. *Nature*, **1994**, *372,* 425.
[2] Unger, R.H. *Regul. Pept*., **2000**, *92*, 87.
[3] Ahima, R.S.; Fliers, J. *Annu. Rev. Physiol*., **2000**, *62,* 413.
[4] Wauters, M.; Considine, R.;Van Gaal, L.F. *Eur. J. Endocrinol*., **2000**, *143*, 293.
[5] Reseland, J.E.; Syversen, U.; Bakke, I.; Qvigstad, G.; Eide, L.G.; Hjertner, O.; Gordeladze J.O.; Drevon, C.A. *J. Bone Miner. Res*., **2001**, *16*,142.
[6] Madej, T.; Boguski, M.S.; Bryant, S.H. *FEBS Lett.,* **1995**, *373*, 13.
[7] Prolo, P.; Wong, M.; Licino, J. *Int. J. Biochem. Cell Biol*., **1998**, *30*, 1285.
[8] Ducy, P.; Schinke, T.; Karsenty, G. *Science,* **2000**, *289*,1501.
[9] Banks, W.A.; Coon, A.B.; Robinson, S.M.; Moinuddin, A.; Shultz, J.M.; Nakaoke, R.; Morley, J.E. *Diabetes*, **2004**, *53*,1253.
[10] Ahima, R.S.; Prabacaran, D.; Mantzoros, C.; Qu, D.; Lowell, B.B.; Maratos-Flier, E.; Fliers J.S. *Nature*, **1996**, *382*, 250.
[11] Lord, G.M.; Matarese, G.; Howard, J.K.; Baker, R.J.; Bloom, S.R.; Lechler, R.I. *Nature*, **1998**, *394*, 897.
[12] Tartaglia, L.A.; Dembski, M.; Weng, X.; Deng, N.; Culpepper, J.; Devos, R.; Richards, G.J.; Campfield, L.A.; Clark, F.T.; Deeds, J.; Muir, C.; Sanker, S.; Moriarty, A.; Moore, K.J.; Smutko, J.S.; Mays, G.G.; Wool, E.A.; Monroe, C.A.; Tepper, R.I. *Cell*, **1995**, *83*, 1263.
[13] Bazan, J.F. *Proc. Natl. Acad. Sci. USA,* **1990**, *87*, 6934.
[14] Heim, M.H. *Eur. J. Invest*., **1996**, *26*, 1.
[15] Kishimoto, T.; Akira, S.; Narazaki, M.; Taga, T. *Blood*, **1995**, *86*, 1243.
[16] Heshka, J.T.; Jones, P.J. *Life Sci*., **2001**, *69*, 987.
[17] Gainsford, T.; Willson, T.A.; Metcalf, D.; Handman, E.; McFarlane, C.; Ng, A.; Nicola, NA; Alexander, W.S.; Hilton, D.J. *Proc. Natl. Acad. Sci. USA,* **1996**, *93*, 14564.
[18] Chua, S.C. Jr.; Chung, W.K.; Wu-Peng, X.S.; Zhang, Y.; Liu, S.M.; Tartaglia, L. *Science*, **1996**, *271*, 994.
[19] Murakami, M.; Narazaki, M.; Hibi, M.; Yawata, H.; Yasukawa, K.; Hamaguchi, M.; Taga, T; Kishimoto, T. *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 11349.
[20] Bjorbaek, C.; Uetani, S.; da Silva, B.; Fliers, J.S. *J. Biol. Chem*., **1997**, *272*, 32686.
[21] Lee, G.H.; Proenca, R.; Montez, J.M.; Carrol, K.M.; Darvishzadeh, J.G.; Lee, J.I.; Friedman, J.M. *Nature*, **1996**, *379*, 632.
[22] Wang, M.Y.; Zhou, Y.T.; Newgard, C.B.; Unger, R.H. *FEBS Lett.,* **1996**, *392,* 87.
[23] Lammert, A.; Kiess W.; Bottner, A.; Glasow, A.; Kratzsch, J. *Biochem. Biophys. Res. Commun*., **2001**, *283*, 982.
[24] Li, C.; Ioffe, E.; Fidahusein, N.; Connolly, E.; Friedman, J.M. *J. Biol. Chem*., **1998**, *273*, 10078.
[25] Chan, J.L.; Bluher, S.; Yiannakouris, N.; Suchard, M.A.; Kratzsch, J.; Mantzoros, C.S. *Diabetes*, **2002**, *51*, 2105.
[26] Uotani, S.; Bjorbaek, C.; Tornoe, J.; Fliers, J.S. *Diabetes*, **1999**, *48*, 279.
[27] Hileman, S.M.; Tornoe, J.; Flier, J.S.; Bjorbaek, C. *Endocrinology*, **2000**, *141*, 1995.
[28] Ghilardi, N.; Skoda, R.C. *Mol. Endocrinol*., **1997**, *11*, 393.
[29] White, D.W.; Kuropatwiski, K.K.; Devos, R.; Bumann, H.; Tartaglia, L.A. *J. Biol. Chem.*, **1997**, *272*, 4065.
[30] Kloek, C.; Haq, A.K.; Dunn, S.L.; Lavery, H.J.; Banks, A.S.; Myers, M.G. *J. Biol. Chem*., **2002**, *277*, 41547.
[31] Jiang, N.; He, T.C.; Myajima, A.; Wojchowski, D.M. *J. Biol. Chem*., **1996**, *271*, 16472.
[32] Bahrenberg, G.; Behrmann, I.; Barthel, A.; Hekerman, P.; Heinrich, P.C.; Joost, H.G.; Becker, W. *Mol. Endocrinol*., **2002**, *16*, 859.
[33] Heldin, CH. *Cell*, **1995**, *80*, 213.
[34] Banks, A.S.; Davis, S.M.; Bates, S.H.; Myers, M.G. Jr. *J. Biol. Chem*., **2000**, *275*, 14563.
[35] Akerman, F.; Lei, Z.M.; Rao, C.V. *Gynecol. Endocrinol*., **2002**, *16*, 299.
[36] Buyse, M.; Ovesjo, M.L.; Goiot, H.; Guilmeau, S.; Peranzi, G.; Moizo, L.; Walker, F.; Lewin, M.J.; Meister, B.; Bado, A. *Eur. J. Neurosci.* , **2001**, 14, 64.
[37] Ebenbichler, D.F.; Kaser, S.; Laimer, M.; Wolf, H.J.; Patsch, J.R.; Illey, N.P. *Placenta*, **2002**, *23*, 516.
[38] Frank, S.; Stallmeyer, B.; Kampfer, H.; Kolb, N.; Pfeilschifter, J. *J. Clin. Invest*., **2000**, *106*, 501.
[39] Goiot, H.; Attoub, S.; Kermorgant, S.; Laigneau, J.P.; Lardeux, B.; Lehy, T.; Lewin, M.J.; Bado, A. *Gastroenterology*, **2001**, *121*, 1417.
[40] Kim, Y.B.; Uotani, S.; Pierroz, D.D.; Fliers, J.S.; Kahn, B.B. *Endocrinology*, **2000**, *141*, 2328.
[41] Lee, Y.J.; Park, J.H.; Ju, S.K.; You, K.H.; Ko, J.S.; Kim, H.M. *FEBS Lett*., **2002**, *528*, 43.
[42] Morton, N.M.; Emilsson, V.; de Groot, P.; Pallet, A.l.; Cawthorne, M.A. *J. Mol. Endocrinol*., **1999**, *22*, 173.
[43] El-Hefnawy, T.; Ioffe, S.; Dym, M. *Endocrinology*, **2000**, *141*, 2624.
[44] Koshiba, H.; Kitawaki, J.; Ishihara, H.; Kado, N.; Kusuki, I.; Tsukamoto, K.; Honjo, H. *Mol. Hum. Reprod*., **2001**, *7*, 567.
[45] Scarpace, P.; Matheny, M.; Tumer, N. *Neuroscience*, **2001**, *104*, 1111.
[46] Sehgal, P.B. *Acta Biochim. Pol*., **2003**, *50*, 583.
[47] Eyckerman, S.; Waelput, W.; Verhee, A.; Broekaert, D.; Vandekerckhove, J.; Tavernier, *J. Eur. Cytokine New*., **1999**, *10*, 549.
[48] Baumann, H.; Morella, K.K.; White, D.W.; Dembski, M.; Bailon, P.S.; Kim, H.; Kim, H.; Lai, C.F.; Tartaglia, L.A. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 8374.
[49] Bendinelli, P.; Maroni, P.; Pecori Giraldi, F.; Piccoletti, R. *Mol. Cell Endocrinol*., **2000**, *168*, 11.
[50] Hakansson, M.L.; Meister, B. *Neuroendocrinology*, **1998**, *68*, 420.
[51] Hubschle, T.; Thom, E.; Watson, A.; Roth, J.; Klaus, S.; Meyerhof, W. *Neurosci*., **2001**, *21*, 2413.
[52] Ghilardi, N.; Ziegler, S.; Wiestner, A.; Stoffel, A.; Heim, M.H.; Skoda, R.C. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 6231.
[53] Hakansson-Ovesjo, M.L.; Collin, M.; Meister, B. *Endocrinology*, **2000**, *141*, 3946.
[54] Hakansson, M.; de Lecea, L.; Sutcliffe, J.G.; Yanagisawa, M.; Meister, B. *J. Neuroendocrinol*., **1999**, *11*, 653.

[55] Hosoi, T.; Kawagishi, T.; Okuma, Y.; Tanaka, J.; Nomura, Y. *Endocrinology*, **2002**, *143*, 3498.

[56] Zhou, Y.T.; Shimabukuro, M.; Koyama, K.; Lee, Y.; Wang, M.Y.; Trieu, F.; Newgard, C.B.; Unger, R.H. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 6386.

[57] Fleury, C.; Neverova, M.; Collins, S.; Raimbault, S.; Champigny, O.; Levi-Meyrueis, C.; Bouillaud, F.; Seldin, M.F.; Surwit, R.S.; Ricquier, D.; Warden, C.H. *Nat. Genet*., **1997**, *15*, 269.

[58] Cabrera de León, A.; González, D.A.; Méndez, L.I. ; Aguirre-Jaime, A.; Rodriguez Perez, M.C.; Coello, S.D.; Trujillo, I.C. *Obes. Res*., **2004**, *12*, 1492.

[59] Wang, Z.W.; Pan, W.T.; Lee, Y.; Kakuma, T.; Zhou, Y.T.; Unger, R.H. *FASEB J*., **2001**, *15*, 108.

[60] Endo, T.A.; Masuhara, M.; Yokouchi, M.; Suzuki, R.; Sakamoto, H.; Mitsui, K.; Matsumoto, A.; Tanimura, S.; Ohtsubo, M.; Misawa, H.; Miyazaki, T.; Leonor, N.; Taniguchi, T.; Fujita, T.; Kanakura, Y.; Komiya, S.; Yoshimura, A. *Nature*, **1997**, *387*, 921.

[61] Starr, R.; Willson, T.A.; Viney, E.M.; Murray, L.J.; Jenkins, B.J.; Gonda, T.J.; Alexander, W.S.; Metcalf, D.; Nicola, N.A.; Hilton, D.J. *Nature*, **1997**, *387*, 917.

[62] Hansen, J.A.; Lindberg, K.; Hilton, D.J.; Nielsen, J.H.; Billestrup, N. *Mol. Endocrinol*., **1999**, *13*, 1832.

[63] Bjorbaek, C.; El-Haschimi, K.; Frantz, J.D.; Flier, J.S. *J. Biol. Chem*., **1999**, *274*, 30059.

[64] Cheng, A.; Uetani, N.; Simoncic, P.D.; Chaubey, V.P.; Lee-Loy, A.; McGlade, C.J.; Kennedy, B.P.; Tremblay, M.L. *Dev. Cell*, **2002**, *2*, 497.

[65] Zabolotny, J.M.; Bence-Hanulec, K.K.; Stricker-Krongrad, A.; Haj, F.; Wang, Y.; Minocoshi, Y.; Kim, Y.B.; Elmquist, J.K.; Tartaglia, L.A.; Kahn, B.B.; Neel, B.G. *Dev. Cell*, **2002**, *2*, 489.

[66] Chung, C.D.; Liao, J.; Liu, B.; Rao, X.; Jay, P.; Berta, P.; Shuai, K. *Science*, **1997**, *278*, 1803.

[67] Stofega, M.R.; Herrington, J.; Billestrup, N.; Carter-Su, C. *Mol. Endocrinol*., **2000**, *14*, 1338.

[68] Blenis, J. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 5889.

[69] Fujioka, Y.; Matozaki, T.; Noguchi, T.; Iwamatsu, A.; Yamao, T.; Takahashi, N.; Tsuda, M.; Takada, T.; Kasuga, M. *Mol. Cell Biol*., **1996**, *16*, 6887.

[70] Bjorbaek, C.; Buchholz, R.M.; Davis, S.M.; Bates, S.H. ; Pierroz, D.D.; Gu, H.; Neel, B.G.; Myers, M.G. Jr.; Flier, J.S. *J. Biol. Chem*., **2001**, *276*, 4747.

[71] Tanabe, K.; Okuya, S.; Tanizawa, Y.; Matsunani, A.; Oka, Y. *Biochem. Biophys. Res. Commun*., **1997**, *241*, 765.

[72] Kang, S.M.; Kwon, H.M.; Hong, B.K.; Kim, D.; Kim, I.J.; Choi, E.Y.; Jang, Y,; Kim, H.S.; Kim, M.S.; Kwon, H.C. *Yonsei. Med. J*., **2000**, *41*, 68.

[73] Sierra-Honingmann, M. R.; Nath, A. K.; Murakami, C.; Garcia-Cardena, G.; Papapetropoulos, A.; Sessa, W.C.; Madge, L.A.; Schechner, J.S.; Schwabb, M.B.; Polverini, P.J.; Flores-Riveros, J.R. *Science*, **1998**, *281*, 1683.

[74] Figenschau, Y.; Knutsen, G.; Shahazeydi, S.; Johansen, O.; Sveinbjornsson, B. *Biochem. Biophys. Res. Commun*., **2001**, *287*, 190.

[75] Machinal-Quelin, F.; Dieudonne, M.N.; Leneveu, M.C.; Pecquery, R.; Giudicelli, Y. *Am. J. Physiol. Cell. Physiol*., **2002**, *282*, C853.

[76] Dreyer, M.G.; Juge-Aubry, C.E.; Gabay, C.; Lang, U.; Rohner-Jeanrenaud, F.; Dayer, J.M.; Meier, C.A. *Biochem. J*., **2003**, *370*, 591.

[77] Unger, R.H.; Yhou, Y-T.; Orci, L. *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 2327.

[78] Minokoshi, Y.; Kim, Y.-B.; Peroni, O.D.; Fryer, L.G.; Muller, C.; Carling, D.; Kahn B.B. *Nature*, **2002**, *415*, 339.

[79] Kahn, B.B.; Flier, J.S. *J. Clin. Invest*., **2000**, *106*, 473.

[80] Lew, E.A.; Garfinkel, L. J. *Chronic Dis*., **1979**, *32*, 563.

[81] World Cáncer Research Fund. Washington, DC: *American Institute for Cáncer Research*, **1997**, 371.

[82] Lukanova, A.; Bjor, O.; Kaaks, R.; Lenner, P.; Lindahl, B.; Hallmans, G.; Stattin, P. *Int. J. Cancer*, **2006,** *118*,458.

[83] IARC handbooks of cancer prevention. Weight control and physical activity. Lyon, France: International Agency for Research on Cáncer, **2002**, Vol *6*.

[84] Chow, W-H.; Blot, W.J.; Vaughan, T.L.; Risch, H.A.; Gammon, M.D.; Stanford, J.L.; Dubrow, R.; Schoenberg, J.B.; Mayne, S.T.; Farrow, D.C.; Ahsan, H.; West, A.B.; Rotterdam, H.; Niwa, S.; Fraumeni, J.F. Jr. *J. Natl. Cáncer Inst*., **1998**, *90*, 150.

[85] Vaughan, T.L.; Davis, S.; Cristal, A.; Thomas, D.B. *Cancer Epidemiol. Biomarkers Prev*. **1995**, *4*, 85.

[86] Wolk, A.; Gridley, G.; Svensson, M.; Nyren, O; McLaughlin, J.K.; Fraumeni, J.F.; Adam, H.O. *Cancer Causes Control*, **2001**, *12*, 13.

[87] Moller, H.; Mellemgaard, A.; Lindvig, K.; Olsen, J. *Eur. J. Cancer*, **1994**, *30*, 344.

[88] Calle, E.E.; Rodríguez, C.; Walter-Thurmond, K.; Thun, M.J. *N. Engl. J. Med.*, **2003**, *348*, 1625.

[89] Stattin, P.; Söderberg, S.; Hallmans, G.; Bylund, A.; Kaaks, R.; Stenman, U.-H.; Bergh, A.; Olsson, T. *J. Clin. Endocrinol. Metab*., **2001**, *86*, 1341.

[90] Nam, S.Y.; Lee, E.J.; Kim, K.R.; Cha, B.S.; Song, Y.D.; Lim, S.K.; Lee, HC; Huh, K.B. *Int. J. Obes. Relat. Metab. Disord*., **1997**, *21*, 355.

[91] Andersson, S.O.; Wolk, A.; Bergstrom, R.; Adami, H.O.; Engholm, G.; Englund, A.; Nyren, O. *J. Natl. Cancer. Inst*., **1997**, *89*, 385.

[92] Gronberg, H.; Damber, L.; Damber, J.E. *J. Urol*., **1996**, *155*, 969.

[93] Scacchi, M.; Pincelli, A.I.; Cavagnini, F. *Int. J. Obes. Relat. Metab. Disord*., **1999**, *23*, 260.

[94] Gellhorn, A.; Benjamín, W. *Biochem. Biophys. Acta*, **1996**, *116*, 460.

[95] Waters, K.M.; Ntambi, J.M. *Lipids*, **1996**, *31*, 33.

[96] Brenner, R.R. In *The role of fats in human nutrition,* Vergroesen, A.J.; Crawford, M., Eds.; Academic Press: London, (UK) **1989**; pp. 46-79.

[97] Zoeller, R.A.; Wood, R. *Biochim. Biophys. Acta*, **1985**, *845*, 380.

[98] Lu, J.; Pei, H.; Kaek, M.; Thompson, H.J. *Mol. Carcinog*., **1997**, *20*, 204.

[99] de Alaniz, M.J.; Marra, C.A. *Mol. Cell. Biochem*., **1994**, *137*, 85.

[100] Marzo, I.; Martínez-Lorenzo, M.J.; Anel, A.; Desportes, P.; Alava, M.A.; Naval, J.; Pineiro, A. *Biochim Biophys Acta*, **1995**, *1257*, 140.

[101] Khoo, D.E.; Fermor, B.; Millar, J.; Wood, C.B.; Apostolov, K.; Barker, W.; Williamson, R.C.; Habib, N.A. *Br. J. Cancer*, **1991**, *63*, 97.

[102] Habib, N.A.; Wood, C.B.; Apostolov, K.; Barker, W.; Hershman, M.J.; Aslam, M.; Heinemann, D.; Fermor, B.; Williamson, R.C.; Jenkins, W.E. *Br. J. Cancer*, **1987**, *56*, 455.

[103] Ntambi, J.M.; Sessler, A.M.; Takova, T. *Biochem. Biophys. Res. Commun*., **1996**, *220*,990.

[104] Thorling, E.B.; Hansen, H.S. *Biochem. Biophys. Acta*, **1995**, *1258*, 195.

[105] Hermier, D.; Catheline, D.; Legrand, P. *Comp. Biochem. Physiol. A. Physiol*., **1996**, *115*, 259.

[106] Brenner, R.R. *Biochem. Soc. Trans*., **1990**, *18*, 773.

[107] Berrino, F.; Muti, P.; Micheli, A.; Bolelli, G.; Krogh, V.; Sciajno, R.; Pisani, P.; Panico, S.; Secreto, G. *J. Natl. Cancer Inst*., **1996**, *88*, 291.

[108] Simpson, E.R.; Ackerman, G.E.; Smith, M.E.; Mendelson, C.R. *Proc. Natl. Sci. USA*, **1981**, *78*, 5690.

[109] Sasano, H.; Harada, N. *Endocr. Rev*., **1998**, *19*, 593.

[110] Esteban, J.M.; Warsi, Z.; Haniu, M.; Hall, P.; Shirvely, J.E.; Chen, S. *Am. J. Pathol*., **1992**, *140*, 337.

[111] Simpson, E.R.; Davis, S.R. *Endocrinology*, **2001**, *142*, 4589.

[112] Singh, A.; Purohit, A.; Wang, D.Y.; Duncan, L.J.; Ghilchik, M.W.; Reed, M.U. *J. Endocrinol*. **1995**, *147*, R9.

[113] Zhao, Y.; Nichols, J.E.; Bulnn, S.E.; Mendelson, C.R.; Simpson, E.R. *J. Biol. Chem*., **1995**, *270*, 16449.

[114] Utsumi, T.; Yoshimura, N.; Takeuchi, S.; Ando, J.; Maruta, M.; Maeda, K.; Harada, N. *Cancer. Res*., **1999**, *59*, 377.

[115] McNeill, J.M.; Reed, M.J.; Beranek, P.A.; Bonney, R.C.; Ghilchik, M.W.; Robinson, D.J.; James, V.H. *Int. J. Cancer*, **1986**, *38*, 193.

[116] Adams, E.F.; Rafferty, B.; White, M.C. *Int. J. Cancer*, **1991**, *49*, 118.

[117] Duncan, L.J.; Coldham, N.G.; Reed, M.J. *J. Steroid Biochem. Mol. Biol*., **1994**, *49*, 63.

[118] Osborne, C.K.; Schiff, R.; Fuqua, S.A.; Shou, J. *Clin. Cancer Res*., **2001**, *7*, 4338s.

[119] Lee, A.V.; Cui, X.; Oesterreich, S. *Clin. Cancer Res*., **2001**, *7*, 4429s.

[120] Palmieri, C.; Cheng, G.J.; Saji, S.; Zelada-Hedman, M.; Warri, A.; Weihua, Z.; Van Noorden, S.; Wahlstrom, T.; Coombes, R.C.; Warner, M.; Gustafsson, J.A. *Endocr. Relat. Cancer*, **2002**, *9*, 1.

[121] Henderson, E.; Feigelson, H.S. *Carcinogenesis*, **2000**, *21*, 427.

[122] Persson, I. *J. Steroid Biochem. Mol. Biol.*, **2000**, *74*, 357.

[123] Grover, P.I.; Martin, F.L. *Carcinogenesis*, **2002**; *23*, 1095.
[124] Thompson, P.A.; Ambrosone, C. *J. Natl. Cancer Inst. Monogr*., **2000**, *27,*125.
[125] Purohit, A.; Newman, S.P.; Reed, M.J. *Breast Cancer Res*., **2002**, *4*, 65.
[126] Jefcoate, C.R.; Liehr, J.G. ;Santen, R.J.; Sutter, T.R.; Yager, J.D.; Yue, W.; Santner, S.J.; Termal, R.; Demers, L.; Pauley, R.; Naftolin, F.; Mor, G.; Berstein, L. *J. Natl. Cancer Inst. Monogr*., **2000**, *27*, 95.
[127] Simpson, E. R.; Clyne, C.; Speed, C.; Rubin, G.; Bulun, S. *Ann. Y. Acad. Sci*., **2001**, *949*, 58.
[128] Kotsopoulos, J.; Lubinski, J.; Lynch, H.T.; Neuhausen, S.L.; Ghadirian, P.; Isaacs, C.; Weber, B.; Kim-Sing, C.; Foulkes, W.D.; Gershoni-Baruch, R.; Ainsworth, P.; Friedman, E.; Daly, M.; Garber, J.E.; Karlan, B.; Olopade, O.I.; Tung, N.; Saal, H.M.; Eisen, A.; Osborne, M.; Olsson, H.; Gilchrist, D.; Sun, P.; Narod, S.A. *Cancer Causes Control*, **2005**, *16*, 667.
[129] Rosner, B.A.; Colditz, G.A.; Webb, P.M.; Hankison, S.E. *Epidemiology*, **2005**, *16*, 508.
[130] Krieger, N.; Lowy, I.; Aronowitz, R.; Bigby, J.; Dickersin, K.; Garner, E.; Gaudilliere, J.P.; Hinestrosa, C.; Hubbard, R.; Johnson, P.A.; Missmer, S.A.; Norsigian, J.; Pearson, C.; Rosenberg, C.E.; Rosenberg, L; Rosenkrantz, B.G.; Seaman, B.; Sonnenschein, C.; Soto, A.M.; Thornton, J.; Weisz, G. *J. Epidemiol. Community Health*, **2005**, *59*, 740.
[131] Sugimura, T. *Carcinogenesis*, **2000**, *21*, 387.
[132] Ikeda, K.; Inoue, S. *Arch. Histol. Cytol*., **2004**, *67*, 435.
[133] Shibata, H.; Spencer, T.E.; Onate, S.A.; Jenster, G.; Tsai, S.Y.; Tsai, M-J.; O´Malley, B.W. *Rcent. Prog. Horm. Res*., **1997**, *52*, 141.
[134] Hyder, S.M.; Chiappetta, C.; Stancel, G.M. *Biochem. Pharmacol*., **1999**, *57*, 597.
[135] Paech, K.; Webb, P.; Kuiper, G.G.J.M.; Nilsson, S.; Gustafsson, J-A.; Kushner, P.J.; Scanian, T.S. *Science*, **1997**, *277*, 1508.
[136] Webb, P.; Nguyen, P.; Valentine, C.; Lopez, G.N.; Kwok, G.R.; Mclenerney, E.; Katzenellenbogen, B.S.; Enmark, E.; Gustafsson, J-A.; Nilsson, S.; Kushner, P.J. *Mol. Endocrinol*., **1999**, *13*, 1672.
[137] Oster, S.K.; Ho, C.S.; Soucie, E.L.; Penn, L.Z. *Adv. Cancer Res*. **2002**, *84*, 81.
[138] Keyomarsi, K.; Tucker, S.; Buchholz, T.A.; Callister, M.; Ding, Y.; Hortobagyi, G.N.; Bedrossian, I.; Knickerbocker, C.; Toyofuku, W.; Lowe, M.; Herliczek, T.W.; Bacus, S.S. *N. Engl. J. Med*., **2002**, *347*, 1566.
[139] Li, J.J.; Weroha, S.J.; Lingle, W.L.; Papa, D.; Salisbury, J.L.; Li, S.A. *Proc. Natl. Acad. Sci. USA,* **2004**, *101*, 18123.
[140] Resnitzky, D.; Goosen, M.; Bujard, H.; Reed, S.I. *Mol. Cell Biol*., **1994**, *14*, 1669.
[141] Nass, S.J.; Dickson, R.B. *Breast Cancer Res Treat*., **1997**, *44*, 1.
[142] Rosfjord, E.C.; Dickson, R.B. *J Mammary Gland Biol Neoplasia*, **1999**, *4*, 229.
[143] Chen, J.Q.; Yager, J. *Ann. N.Y. Acad. Sci*., **2004**, *1028*, 258.
[144] Surmacz, E.; Bartucci, M. *J. Exp. Clin. Cancer Res*., **2004**, *23*, 385.
[145] Aronica, S.M.; Katzenellenbogen, B.S. *Mol. Endocrinol*., **1993**, *7*, 743.
[146] Ignar-Trowbridge, D.M.; Nelson, K.G.; Bidwell, M.C.; Curtis, S.W.; Washburn, T.F.; McLachlan, J.A.; Korach, K.S. *Proc. Natl. Sci. USA*, **1992**, *89*, 4658.
[147] Kato, S.; Endoh, H.; Masuhiro, Y.; Kitamoto, T.; Uchiyama, S.; Sasaki, H.; Masucshige, S.; Gotoh, Y.; Nishida, E.; Kawashima, H.; Metzger, D.; Chambon, P. *Science*, **1995**, *270*, 1491.
[148] Ali, S.; Metzger, D.; Bornert, J-M.; Chambon, P. *EMBO J.,* **1993**, *12*, 1153.
[149] Trowbridge, J.M.; Rogatsky, I.; Garabedian, J. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 10132.
[150] Chen, D.; Riedl, T.; Washbrook, E.; Pace, P.E.; Coombes, R.C.; Egly, J.M.; Ali, S. *Mol. Cell*, **2000**, *6*, 127.
[151] Le Goff, P.; Montano, M.M.; Schodin, D.J.; Katzenellenbogen, B.S. *J. Biol. Chem*., **1994**, *269,* 4458.
[152] Omoto, Y.; Kobayasi, S.; Inoue, S.; Ogawa, S.; Toyama, T.; Yamashita, H.; Muramatsu, M.; Gustafsson, J.A.; Iwase, H. *Eur. J. Cancer*, **2002**, *38*, 380.
[153] Roger, P.; Sahala, M.E.; Makela, S.; Gustafsson, J.A.; Baldet, P.; Rochefort, H. *Cancer Res.*, **2001**, *61*, 2537.
[154] Bardin, A.; Boulle, N.; Lazennec, F.; Vignon, F.; Pujol, P. *Endocr. Relat. Cancer.*, **2004**, *11*, 537.
[155] Zhao, C.; Lam, W.F.E.; Sunters, A.; Enmark, E.; Tamburo De Bella, M.; Coombes, R.C.; Gustafsson, J.A.; Dahlman-Wright, K. *Oncogene*, **2003**, *22*, 7600.
[156] Dieudonne, M.N.; Machinal-Quelin, F.; Serazin-Leroy, V.; Leneveu, M.C.; Pecquery, R.; Giudicelli, Y. *Biochem. Biophys. Res. Commun*., **2002**, *26*, 622.
[157] Tessitore, L.; Vizio, B.; Jenkins, O.; De Stefano, I.; Ritossa, C.; Argiles, J.M.; Benedetto, C.; Mussa, A. *Int. J. Mol. Med*., **2000**, *5*, 421.
[158] Kitawaki, J.; Kusuki, I.; Koshiba, H.; Tsukamoto, K.; Honjio, H. *Mol. Hum. Reprod*., **1999**, *8*, 708.
[159] Mannucci, E.; Ognibene, A.; Becorpi, A.; Cremasco, F.; Pellegrini, S.; Ottanelli, S.; Rizzello, S.M.; Massi, G.; Messeri, G.; Rotella, C.M. *Eur. J. Endocrinol*. **1998**, *139*, 198.
[160] Magoffin, D.A.; Weitsman, S.R.; Aagarwal, S.K.; Jakimiuk, A.J. *Ginekol. Pol.,* **1999**, *70*, 1.
[161] Catalano, S.; Marsico, S.; Giordano, C.; Mauro, L.; Rizza, P.; Panno, M.L.; Ando, S. *J. Biol. Chem*., **2003**, *278*, 28668.
[162] Shimizu, H.; Shimomura, Y.; Nakanishi, Y.; Futawatari, T.; Ohtani, K.; Sato, N.; Mori, M. *J. Endocrinol*., **1997**, *154*, 285.
[163] Casabiell, X.; Piñeiro, V.; Peino, R.; Lage, M.; Camiña, J.; Gallego, R.; Garcia Vallejo, L.; Dieguez, C.; Casanueva, F.F. *J. Clin. Endocrinol. Metab*., **1998***, 83*, 2149.
[164] Catalano, S.; Mauro, L.; Marisco, S.; Giordano, C.; Rizza, P.; Rago, V.; Montanaro, D.; Maggiolini, M.; Panno, M.L.; Ando, S. *J. Biol. Chem*., **2004**, *279*, 19908.
[165] Garofalo, C.; Sisci, D.; Surmacz, E. *Clin. Cancer Res*., **2004**, *10*, 6466.
[166] Rose, D.P.; Gilhooly, E.M.; Nixon, D.W. *Int. J. Oncol*., **2002**, *21*, 1285.
[167] Watson, G.H.; Manes, J.L.; Mayes, J.S.; McCann, J.P. *J. Endocrinol*., **1993**, *139*, 107.
[168] Pedersen, S.B.; Fuglsig, S.; Sjogren, P.; Richelsen, B. *Eur. J. Clin. Invest*., **1996**, *26*, 1051.
[169] Kristensen, K.; Pedersen, S.B.; Richelsen, B. *Biochem. Biophys. Res. Commun*., **1999**, *259*, 624.
[170] Armellini, F.; Zamboni, M.; Bosello, O. *Int. J. Obes. Relat. Metab. Disord*., **2000**, 24, S18.
[171] Thomas, T.; Burguera, B.; Melton, L.J.; Atkinson, E.J.; O´Fallon, W.M.; Riggs, B.L.; Khosla, S. *Metabolism*, **2000**, 49, 1278.
[172] Elbers, J.M.; Asscheman, H.; Seidell, J.C.; Frolich, M.; Meinders, A.E.; Gooren, L.J. *J. Clin. Endocrinol. Metab*., **1997**, *82*, 3267.
[173] O´Neil, J.S.; Burow, M.E.; Green, A.E.; McLachlan, J.A.; Henson, M.C. *Mol. Cell. Endocrinol.*, **2001**, *176*, 67.
[174] Pietras, R.J.; Nemere, I.; Szego, C.M. *Endocrine*, **2001**,*14*, 417.
[175] Sreeja, S.; Thampan, R. *Indian J. Exp. Biol*., **2003***, 41*, 740.
[176] Giudicelli, M.; Dieudonne, D.; Lacasa, D.; Pasquier, Y.N.; Pecquery, R. *Prostaglandins Leukot. Essent. Fatty Acids*, **1993**, *48*, 91.
[177] Watson, C.S.; Gametchu, B. *Proc. Soc. Exp. Biol. Med.*, **1999**, *220*, 9.
[178] Nadal, A.; Diaz, M.; Valverde, M.A. *News Physiol. Sci*., **2001**, *16*, 251.
[179] Razandi, M.; Pedram, A.; Park, S.T.; Levin, E.R. *J. Biol. Chem*., **2003**, *278*, 2701.
[180] Levin, E.R. *Steroids*, **2002**, *67*, 471.
[181] Segars, J.H.; Driggers, P.H. *Trends. Endocrinol. Metab*., **2002**, *13*, 343.
[182] Driggers, P.H.; Segars, J.H. *Trends. Endocrinol. Metab*., **2002**, *13*, 422.
[183] Luconi, M.; Forti, G.; Baldi, E. *J. Steroid Biochem. Mol. Biol*., **2002**, *80,* 369.
[184] Pedram, A.; Razandi, M.; Aitkenhead, M.; Hughes, C.C.; Levin, E.R. *J. Biol. Chem*., **2002**, *277*, 50768.

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