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Complex Actions of Sex Steroids in Adipose Tissue, the Cardiovascular System, and Brain: Insights from Basic Science and Clinical Studies

Judith L. Turgeon, Molly C. Carr, Pauline M. Maki, Michael E. Mendelsohn, and Phyllis M. Wise

Division of Endocrinology, Clinical Nutrition, and Vascular Medicine, Department of Internal Medicine (J.L.T.), University of California, Davis, Davis, California 95616; Division of Endocrinology, Metabolism, and Molecular Medicine (M.C.C.), Northwestern University, Feinberg School of Medicine, Chicago, Illinois 60611; Department of Psychiatry and Psychology (P.M.M.), University of Illinois at Chicago, Chicago, Illinois 60612; Molecular Cardiology Research Institute, Department of Medicine, and Division of Cardiology (M.E.M.), New England Medical Center Hospitals and Tufts University School of Medicine, Boston, Massachusetts 02111; and Departments of Physiology and Biophysics, and Biology (P.M.W.), University of Washington, Seattle, Washington 98195

Recent publications describing the results of the Women's Health Initiative (WHI) and other studies reporting the impact of hormone therapy on aging women have spurred reexamination of the broad use of estrogens and progestins during the postmenopausal years. Here, we review the complex pharmacology of these hormones, the diverse and sometimes opposite effects that result from the use of different estrogenic and progestinic compounds, given via different delivery routes in different concentrations and treatment sequence, and to women of different ages and health status. We examine our new and growing appreciation of the role of estrogens in the immune system and the inflammatory response, and we pose the concept that estrogen's interface with this system may be at the core of some of the effects on multiple physiological systems, such as the adipose/metabolic system, the cardiovascular system, and the central nervous system. We compare and contrast clinical and basic science studies as we focus on the actions of estrogens in these systems because the untoward effects of hormone therapy reported in the WHI were not expected. The broad interpretation and publicity of the results of the WHI have resulted in a general condemnation of all hormone replacement in postmenopausal women. In fact, careful review of the extensive literature suggests that data resulting from the WHI and other recent studies should be interpreted within the narrow context of the study design. We argue that these results should encourage us to perform new studies that take advantage of a dialogue between basic scientists and clinician scientists to ensure appropriate design, incorporation of current knowledge, and proper interpretation of results. Only then will we have a better understanding of what hormonal compounds should be used in which populations of women and at what stages of menopausal/postmenopausal life. (*Endocrine Reviews* 27: 575–605, 2006)

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Abbreviations: AD, Alzheimer's disease; APC, activated protein C; AR, androgen receptor; BMI, body mass index; CEE, conjugated equine estrogens; CRP, C-reactive protein; CVD, cardiovascular disease; DRSP, drospirenone; E_1, estrone; E_2, 17β-estradiol; EE, 17α-ethinyl estradiol; eNOS, endothelial NOS; ER, estrogen receptor; ET, estrogen therapy; FVL, factor V Leiden; HDL, high-density lipoprotein; HERS, Heart and Estrogen/Progestin Replacment Study; HT, hormone therapy; iNOS, inducible NOS; LDL, low-density lipoprotein; LPL, lipoprotein lipase; LXR, liver X receptor; MCAO, middle cerebral artery occlusion; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MORE, Multiple Outcomes of Raloxifene Evaluation; MPA, medroxyprogesterone acetate; 3MSE, modified Mini-Mental State Examination; NF-κB, nuclear factor-κB; NMDA, *N*-methyl-D-aspartate; nNOS, neuronal NOS; NO, nitric oxide; NOS, NO synthase; OC, oral contraceptive; PPAR, peroxisome proliferator-activated receptor; PR, progesterone receptor; SAA, serum amyloid A; SERM, selective ER modulator; SRC, steroid receptor coactivator; SSHR, sex steroid hormone receptors; WHI, Women's Health Initiative; WHIMS, WHI Memory Study; WHISCA, WHI Study of Cognitive Aging.

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I. Introduction

WOMEN TYPICALLY UNDERGO the transition to postmenopause at approximately 51 yr of age. This dramatic physiological transition, which can be abrupt or can occur over several years, is associated with decreases in ovarian hormone secretion and secondary changes in multiple endocrine and metabolic parameters. Many women suffer from menopausal symptoms that have been attributed to the lack of ovarian steroids, including hot flushes, sleep disturbance, vaginal dryness, urinary symptoms, and emotional instability. In addition, postmenopausal women are thought to be at increased risk for several diseases compared with age-matched cycling women such as cardiovascular disease (CVD), cerebrovascular stroke, osteoporosis, hip fracture, dementia, and Alzheimer's disease (AD).

Over the past 20 yr, numerous observational, retrospective, interventional, and meta-analytic studies (reviewed in Refs. 1–3) as well as studies using animal models (reviewed in Refs. 4–9) have supported the hypothesis that ovarian steroids exert important protective actions in women and the absence of these hormones after the menopause makes postmenopausal women more vulnerable than younger premenopausal women to CVD, central nervous system imbalances, neurodegenerative diseases, osteoporosis, and immune dysfunction. However, the recent release of several studies (10–17) has forced us to reassess these conclusions and reevaluate the benefits and risks of hormone therapy (HT) in older women. The study of the Women's Health Initiative (WHI) (11) (see http://www.nih.gov/PHTindex.htm for a complete list of publications) has caught the attention of researchers, physicians, and the lay public. The WHI was comprised of two large, randomized, placebo-controlled clinical trials for HT in postmenopausal women and had three significant design characteristics: 1) only one type of hormone regimen was studied, *i.e.*, oral conjugated equine estrogens (CEE) at a single concentration without or with continuous oral medroxyprogesterone acetate (MPA); 2) the average age of the participants at entry was 63 yr, and, for a majority of the women, estrogen deficiency had been present for more than a decade; and 3) nearly 70% of the subjects were either overweight or obese. These studies were expected to settle the question of whether postmenopausal women would benefit from HT and would solve the dilemma of what women should do after the menopause once and for all. Instead, they have raised many questions and caused much reanalysis and reinterpretation of the results of previous studies.

Fallout from the WHI has been an indictment of all menopausal HT. The unfortunate consequence is that women and their physicians are left with, at best, unsettling choices. In attempting to reconcile the disparate conclusions derived from the WHI and observational studies/basic research, three points emerge that may account for the disparities: 1) different estrogens (or progestins) are not recognized in the same way in all cells and do not have equivalent functions; 2) hormone delivery regimens have a major impact on outcome measures; and 3) age is a critical interactive factor in hormone action, particularly in the timing of the initiation of HT relative to the onset of menopause. An additional and perhaps crucial element in accounting for the study disparities is our emergent understanding of estrogen's interface with inflammation and with the adipose/metabolic system. There are direct effects of estrogen on the inflammatory process and on adipose tissue distribution and function, and the question is how does this contribute to the phenotypic shifts in dyslipidemia, diabetes, and cardiovascular and neurodegenerative diseases in women through their life span. Recent discoveries in these areas allow us to consider novel concepts regarding the varied roles of estrogen and the design of future hormone therapies.

Here, we review the basic pharmacology of estrogens and progestins and provide an overview of the inflammatory response. On this background, we examine the points raised above with a focus in three major areas: adipose/metabolic, cardiovascular, and central nervous systems.

II. Pharmacology of Estrogens and Progestins

A. Menopausal hormone therapy (HT)

Publication of results from clinical trials, such as the WHI and the Heart and Estrogen/Progestin Replacement Study (HERS) (10), has prompted an extremely useful reexamination of the roles of ovarian hormones outside of the reproductive axis. An equally constructive result has been an increased awareness of lapses that can occur in the dialogue between clinical and basic scientists on estrogen and progestin actions.

In comparing clinical studies and animal models of HT, the focus most often is on subject characteristics and main outcome measurements and less frequently on the specifics of the intervention. Here, we address the principle that not all estrogens or progestins are equal and briefly review the consequences of differences in receptor activation, delivery modes, and timing of hormone treatment. The reader is also directed to reviews by Shoham and Kopernik (18, 19) that provide a wonderfully lucid and insightful perspective on the history, pharmacology, and bioactivity of HT formulations.

1. Are all estrogens or progestins equal in action? Clearly, the answer is no. "Estrogen" and "progestin" are generic terms covering an array of endogenous hormones and synthetic compounds. Although useful as shorthand, these nonspecific terms obscure the importance of the unique actions of individual estrogens or progestins. As reviewed in Refs. 3, 20, and 21, binding of ligand to a steroid receptor induces distinct structural alterations in the receptor. Instead of functioning as simple switches for receptor activation, ligands have a more complex role in that different ligands induce unique conformations in a receptor. This is crucial to the ultimate cellular response because recruitment of appropri-

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ate coregulators to the ligand-receptor complex is conformation-dependent. The coregulator complement, in turn, confers cell specificity. The final conformation of the ligand-receptor-coregulator complex affects how its activity is modified (by phosphorylation, *etc.*) and ultimately determines target-gene promoter specificity or, in the case of extranuclear receptors, signaling targets (21–26). This versatile system is made more so by at least two different forms of the estrogen receptor (ERα and ERβ) and the progesterone receptor (PR-A and PR-B). Within their ligand binding domains, the ER isoforms differ by more than 40% in amino acid sequence, thus providing further opportunity for different estrogens to induce distinct conformational changes in an ER-coregulator complex to elicit discrimination in function (25). The isoforms within a receptor type are differentially expressed and regulated across cell types and have both common and distinct target genes (27–34).

The significance of ligand-dependent receptor conformation cannot be overstated in comparing the actions of different types of estrogens or progestins. Examples of the influence that structural differences in ligand have on outcome measures can be seen with selective ER modulators (SERMs), such as tamoxifen or raloxifene, which function as either ER agonists or antagonists depending on the cell type and coregulator complement (29, 35). SERMs, acting through ER isoforms, have been shown to regulate different gene subsets compared with the classic ovarian estrogen, 17β-estradiol (E_2) (36). Receptor isoform-specific regulation of gene expression, which has been demonstrated for the PR isoforms as well as for ER isoforms, is being exploited for structure-based design for ligands with individualized tissue effects (34, 37–41). This work and other research, for example investigating specific coregulator recruitment (42, 43), use mechanism-based approaches that show great promise for producing therapeutic tools that are function-specific. These approaches also are being explored for extranuclear steroid receptors, for example in the design of ER ligands that are cytosolic signaling pathway-selective, with the goal of target-specific therapy (44). The area of extranuclear steroid receptors and rapid signaling, including the possibility of nonreceptor-mediated effects, is too complex and unique a topic to be succinctly summarized here, but we strongly recommend recent excellent and comprehensive reviews of the subject (45, 46).

Although SERMs and selective PR modulators represent well-known examples of compounds that induce unique receptor conformations and exhibit distinct properties in specific cell types (35), what about the estrogens and progestins commonly used in HT? By far the most frequently prescribed treatment in the United States for menopausal HT is oral CEE, with or without MPA (Table 1) (47). CEE, which is derived from the urine of pregnant horses, is comprised of at least 10 different estrogens, as well as some androgens and progestins (48, 49). In addition to sulfated forms of estrone (E_1) and E_2, the estrogen component of CEE includes several sulfated estrogens that are unique to the horse, the most prominent being equilin (49, 50). The critical question is what are the functional, tissue-specific effects of the CEE component estrogens on the function of ER isoforms in women? The few studies that directly addressed this question suggest a wide range of activities reflecting individual metabolic clearance rates as well as estrogen type-specific activity at the ER that can translate into cell type-specific responses (48, 50, 51). For example, a CEE component, $\Delta^{8,9}$-dehydroestrone sulfate, has a tissue selectivity and activity profile distinct from that for E_2 with full agonist effects on several central nervous system endpoints and little or no efficacy in certain hepatic or vascular endpoints (48, 52). In general, the CEE components demonstrate the principle that binding affinity for the ER does not necessarily predict biological activity (48). Thus, the clinical response to CEE is a composite of the pharmacokinetic profiles and individual actions of the steroid components as well as the interactions of the components at ER isoforms that could either mitigate or magnify a response.

TABLE 1. Prescriptions dispensed annually for HT in the U.S.

Delivery route	% of total prescriptions	
	1997	2003[a]
Oral estrogen[b]	70	65
Oral estrogen plus progestin	19	18
Transdermal HT	8	11
Vaginal HT	3	6

Data are derived from Ref. 47.

[a] Overall HT prescriptions have declined since publication of the WHI results; between July 2002 and July 2003, total HT use decreased by ~40% (47, 406).

[b] Of total oral estrogen prescriptions, CEE represents 73% in 1997 and 62% in 2003.

The common synthetic progestins used in HT can be divided into those structurally related to progesterone (*e.g.*, MPA and nomegestrol) or those structurally related to testosterone. Included in the latter category are norethindrone (norethisterone in Europe) and levonorgestrel, which have activity at the androgen receptor (AR) as well as at the PR, and therefore their composite and tissue-specific effects would be expected to be more complex than progesterone, which has minimal activity at the AR (49, 53–55). MPA, which is the most common synthetic progestin component in HT in the United States (47), also has been shown to have properties distinct from endogenous progesterone. For example, *in vivo* and *in vitro* studies in primate or rat models suggest differential actions of MPA and progesterone in endothelial cells, in vascular smooth muscle cells, and in neurons (56–61). The divergent activities of progesterone and MPA include differences in signaling at cytosolic as well as nuclear PRs (46). An additional complexity in comparing responses is the ability of MPA to activate glucocorticoid receptors, whereas progesterone does so minimally (61–65). MPA also has been found to act as a weak agonist for human AR *in vitro* (66).

Drospirenone (DRSP) is a progestin with antialdosterone and antiandrogenic activities that is in active use in HT and contraceptive formulations in many countries outside the United States, and especially Europe (67). In clinical trials, DRSP is effective against menopausal symptoms and is able to provide endometrial protection and maintain amenorrhea in a majority of women. In the doses used in HT formulations, DRSP also has a modest blood pressure-lowering effect, which may prove to be beneficial in some patients. The role of the mineralocorticoid receptor in cardiovascular

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physiology currently is receiving increasing attention, and the presence of functional mineralocorticoid receptors regulating gene expression in vascular tissues has been reported recently (68), raising the possibility that some of the protective effects of antialdosterone compounds like spironolactone or eplerenone in CVDs (69, 70) may be exerted in nonrenal tissues, including the blood vessel (71, 72).

2. HT delivery route. In postmenopausal literature, the term "HT" frequently is used as if it were a single entity. However, in addition to the profound effect that the specific estrogen or progestin formulation may have on outcome, a perhaps equally critical variable is how HT is delivered. In 2003, an estimated 80% of prescriptions dispensed in the United States for HT were for estrogen +/− progestin formulations delivered orally, and the majority of these contained CEE as the estrogen component (Table 1) (47). In Europe, micronized E_2 is one of the most common estrogens used orally, but transdermally administered E_2 is widely used as well (19, 73–75). For basic research in animal models, steroid hormones are usually given as E_2 and progesterone in sc or im depots and are rarely administered orally. This difference in drug delivery methods is a critically important factor when extrapolating knowledge gained from animal models to humans, for which oral HT is by far the most common delivery route.

Oral CEE and oral E_2 are similar in that a peak in plasma concentration is reached within the first 3 h, followed by a decline, and both oral formulations result in higher plasma E_1 levels than E_2, which is unlike the normal menstrual cycle ratio. The pharmacokinetics of oral CEE is complex due to the multiple estrogen components, with varying binding affinities for transport proteins and different metabolic clearance rates (49). For transdermal HT, the estrogen component is E_2. Transdermal application of E_2 avoids the exaggerated peaks and nadirs in plasma estrogen concentration that are a consequence of oral HT and results in a rate of conversion to E_1 that produces an E_2:E_1 ratio more like that found in a menstrual cycle.

It has long been recognized that estrogens taken orally have the potential to modulate liver function due to first-pass effects on hepatic ERs. Clinical studies reporting side-by-side or crossover comparisons provide specific and striking examples of effects induced by oral but not transdermal estrogen on liver production of proteins involved in inflammation, lipid profile, thrombosis, and metabolism (Table 2). A key to these differences is the high concentration of oral estrogen that is required to escape first-pass hepatic metabolism and achieve a therapeutic concentration in peripheral circulation. For E_2, oral administration requires doses that are 20- to 40-fold higher than doses used for transdermal therapy to achieve comparable systemic E_2 levels. For the examples shown in Table 2 comparing oral and transdermal E_2, the resulting circulating E_2 levels averaged 40–150 pg/ml for either treatment modality; the difference was in the dose delivered to the liver.

A succinct example of the route/concentration relationship comes from a study by Friend *et al.* (76) that addressed whether the hepatic effect of oral E_2 on IGF-I in postmenopausal women was indeed route-specific or could be accomplished with transdermal E_2 that achieved sufficiently elevated circulating E_2 levels, thus exposing hepatic cells to increased E_2 as well. The rationale is that endogenous E_2 is known to modulate the production of certain liver proteins during the late follicular/midcycle phase of the menstrual cycle and during pregnancy when systemic E_2 levels are elevated about 2-fold and more than 20-fold above the therapeutic level. The Friend study (76) found that transdermal delivery achieving circulating E_2 at 2- to 4-fold the usual therapeutic level resulted in a decrease in IGF-I indistinguishable from that with oral E_2. Other liver proteins also were affected to varying degrees by the elevated circulating E_2 protocol, underscoring the importance of HT concentration at different targets (76, 77).

Recent studies have generated insight into mechanisms responsible for some of the hepatic actions of estrogen. For example, the cytokine signaling pathway used by GH to

TABLE 2. Effect of postmenopausal estrogen on liver proteins

Liver protein target	Estrogen delivery route			Potential consequences	Examples (Ref.)
	Transdermal	Oral			
	E_2[a]	E_2[b]	CEE[c]		
CRP	No change	↑	↑	Risk for atherosclerosis, ischemic stroke	56, 190, 407–409
Lipoproteins				Antiatheroma formation	83, 407, 408, 410, 411
LDL	↓ or no change	↓	↓		
HDL	↑ or no change	↑	↑		
SAA	↓		↑	Elevated SAA: can promote atherosclerosis, vascular inflammation; may interfere with HDL function	195
APC resistance	No change	↑	↑	Increased risk for venous thrombosis	100, 101, 412, 413
GH-induced IGF-I	No change	↓	↓	Decrease in lean body mass	409, 414, 415
Serum binding proteins				Change in bioavailability of estrogens, androgens, corticosteroids, and thyroid hormones	410, 416, 417
SHBG	No change	↑	↑		
TBG	No change		↑		
CBG	No change	↑	↑		
Angiotensinogen	No change	↑	↑	Sodium retention, vasoconstriction	410, 418

↑, Increased; ↓, decreased; TBG, T_4 binding globulin; CBG, corticosteroid binding globulin.
[a] 0.05 or 0.10 mg/d.
[b] 1–2 mg/d.
[c] 0.625 mg/d.

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stimulate production of IGF-I by liver cells has a built-in brake system via a parallel induction of suppressor proteins from the SOCS (suppressor of cytokine signaling) family. *In vitro* studies now show that E_2, acting through nuclear ER, can suppress GH signaling by induction of SOCS-2, thus providing one possible target mechanism for elevated hepatic estrogen (78). It will be important to establish that a concentration-dependent estrogen suppression of GH signaling occurs *in vivo* in the liver and to determine whether this estrogen-stimulated mechanism is operative at other GH targets, *e.g.*, affecting GH-stimulated lipolysis in adipose tissue.

Determining the impact of oral progestins on hepatic proteins is complex due to the multiple formulations and delivery routes used therapeutically and also to the experimental challenge of sorting out effects attributable to the androgenicity of the compounds. It has been shown, however, that the most commonly prescribed progestin, oral MPA, blunts the increase in high-density lipoprotein (HDL) induced by oral CEE in a dose-dependent manner, yet oral micronized progesterone has no effect on this endpoint (79, 80). For the more androgenic progestins, such as norethindrone or levonorgestrel, both of which can be taken either orally or transdermally, the few side-by-side trials in postmenopausal women suggest that the differences in outcomes such as thromboembolic events or effects on lipid profile are more related to chemical structure than to delivery route (81–83).

3. HT timing. Two design aspects of the WHI and HERS that have been particularly controversial are the age of the participants and the duration of estrogen deficiency before HT was initiated. With a mean age of 63 yr (with nearly 70% between the ages of 60 and 79) and estrogen deficiency for more than a decade in the majority of the women, the WHI in effect becomes a study more of aging than of menopause (84, 85).

The physiological consequence of estrogen reintroduction depends on an interaction between the target system, the health of that system, and the duration of estrogen deprivation. For example in the cardiovascular system, premenopausal ovariectomy or premature ovarian failure has been associated with an increase in peripheral vascular resistance and blood pressure, impaired endothelial function, and increased risk of coronary heart disease that can be reversed by early replacement therapy with estrogen (86–88). That the timing of estrogen therapy (ET) initiation is critical was demonstrated in a monkey model of diet-induced atherosclerosis. After surgically induced menopause in these monkeys, if ET was initiated immediately there was a reduction in coronary artery atherosclerosis compared with placebo-treated ovariectomized monkeys, but delaying ET by an equivalent of about 6 human years completely eliminated the beneficial reduction in atherosclerosis (89). The effects of estrogen reintroduction after a disease process has begun will vary depending on the end organ target, its plasticity, and any consequences aging may have had in the interim. Evaluation of these system-dependent variables must be made before conclusions can be drawn regarding beneficial, neutral, or harmful effects of HT.

In summary, the initial aim of HT was to alleviate the obvious signs of estrogen withdrawal such as vasomotor flushes and vaginal dryness. Over the years, these relatively simple aims have grown into a long and complex list of targets outside of the reproductive system for which a "one formulation/one size fits all" approach is not tenable. CEE/MPA or other HT formulations were not meant to be a panacea for aging, nor were they designed to be optimal for the physiological/endocrinological status of all women over the age of 50. When some risk to health for HT users is suggested, as in the WHI, all estrogens and progestins should not be denounced because each of the formulations has a unique action profile and pharmacokinetic properties. Additionally, oral HT has a wide range of effects on liver proteins, and the consequences of these changes and their impact on other organ systems must be a central consideration in the interpretation of clinical studies as well as in comparing human studies with animal model studies, for which the steroids rarely are administered orally. Other influential factors include a woman's age and health status if HT is initiated after a lapse of several years. To paraphrase Stevenson and Whitehead (90), survival of the human species over 2 million years implies that ovarian hormones by themselves are not a health hazard; if harm is suspected, the judicious research inquiry should focus on the types and doses of hormone substitutes being used as well as their delivery routes and treatment protocols. And we would add that an additional focus should be to understand the underlying physiology for the many targets of E_2 and progesterone outside of the reproductive system and the adaptive roles they have in the aging population.

B. Estrogen/progestin therapy before menopause

The most common use of exogenous estrogens and progestins in premenopausal women is for contraception. The pharmacology of the wide-ranging choices for types of contraceptive drugs and delivery routes is outside the scope of this review, but a limited examination of common oral formulations and the recently available transdermal preparation is presented for comparison with the regimens and outcomes of postmenopausal HT.

1. Oral contraceptive (OC) hormones. Preparations containing 17α-ethinyl estradiol (EE) and a progestin (*e.g.*, norethindrone, levonorgestrel, or norgestimate) are the most frequently prescribed oral hormonal contraceptives. Since U.S. approval 46 yr ago, the primary changes in OCs have been a decrease in EE content and the development of new generations of progestins in an effort to reduce side effects while maintaining LH/FSH inhibition to prevent ovulation. Current low-dose oral EE is no greater than 35 μg/d.

How does EE compare to E_2? At the nuclear ER, the two ligands have similar binding affinity (91, 92) and ability to induce rat uterine target genes (93), although this similarity in efficacy has not been established for all systems or for nonnuclear ERs. In fact, there are surprisingly few studies evaluating specific actions of EE outside of the reproductive system. Earlier studies suggested that EE had enhanced hepatic effects and systemic potency compared with that fol-

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lowing oral E_2, but this is likely related to the increased bioavailability of EE due to its marked resistance to metabolism by hepatic 16α-hydroxylation as well as reduced conversion to E_1, thus resulting, in effect, in a higher concentration of EE presented to hepatic cells (see Refs. 49, 93, and 94 and references therein). Another crucial contribution to the bioavailability of EE is its minimal binding to SHBG, instead being transported by albumin (49). A 35-μg dose of oral EE in premenopausal women results in an average total serum concentration of about 50 pg/ml, a large fraction of which is free due to the low-affinity binding of EE to albumin (95). In comparison, at least 1000 μg of oral micronized E_2 would be required to achieve comparable average total serum levels. However, in contrast to EE, less than 5% of total E_2 in circulation is free due to SHBG binding (96).

Not unexpectedly, because of the first-pass hepatic phenomenon and increased bioavailability, oral EE affects liver proteins similarly to oral E_2 (Table 2). For example, OCs are associated with increased incidence of thromboembolic disease (81, 82, 97). One of the most common risk factors for venous thromboembolism is hereditary resistance to hepatic protein C, which when activated essentially down-regulates thrombin formation. OCs have been shown to induce resistance to activated protein C (APC) in the absence of a hereditary mutation, so-called acquired APC resistance, and this extends to oral postmenopausal HT as well (Table 2) (98–101). Although oral estrogen by itself can lead to increased APC resistance, the progestin component, particularly third generation progestins such as norgestimate, can contribute to the thrombotic effects of oral estrogen (81, 82, 98, 99, 102). In addition to its role in antithrombin formation, APC has been shown to have antiinflammatory activity (103, 104), but whether this function is affected by OC- or oral HT-induced APC resistance has not been established.

Venous thrombosis is a complex process involving procoagulant, anticoagulant, and fibrinolytic elements, and oral estrogen has some beneficial effect on other components of the pathway despite its overall adverse effect on thrombosis. This becomes more apparent when first-pass liver effects are bypassed; studies in postmenopausal women suggest that transdermally administered E_2 has beneficial effects on several hemostatic markers for anticoagulant activity (105). It is significant that transdermal E_2 used for HT, unlike oral E_2, is not associated with risk for venous thrombosis and does not induce APC resistance (Table 2) (75, 100, 106). It remains to be determined whether the same applies to transdermal EE (see *Section II.B.2*) and to premenopausal women.

2. Transdermal contraceptive hormones. A recent addition to the contraceptive armamentarium is a combined estrogen/progestin formulation given as a transdermal patch. Instead of containing E_2 as in the patch used for HT, the currently available contraceptive patch contains EE. The formulation results in average serum levels of 50–70 pg/ml with the same increased bioavailability as oral EE but without the peaks and troughs and, consequently, an overall higher 24-h level of serum EE compared with that found with oral EE as described above (95, 107). The progestin in the contraceptive patch is norelgestromin, the primary active metabolite of a common component of OCs (norgestimate).

Given the importance of overall health status and the indeterminate effects of aging in the response of organ systems to the modulating actions of estrogen, the premenopausal population represents an excellent opportunity for a direct comparison of oral *vs.* transdermal administration of the same estrogen/progestin formulation in younger women. This would be particularly useful for the examination of cardiovascular and central nervous system endpoints affected by the confounding variable of oral estrogen's hepatic action. For example, a study of oral *vs.* transdermal E_2 rather than EE in premenopausal women to evaluate indicators of venous and arterial thromboses could be highly informative for comparison with existing data for oral *vs.* transdermal E_2 in postmenopausal women.

In summary, hormone treatment can be considered as a continuum from contraception through postmenopause. Although similarities are many, the differences may be informative. For example, unlike most regimens for HT, the standard protocol for contraceptive hormones includes a periodic interruption for a week without exogenous hormones, which may have consequences for ER isoform expression level and ratios. Additionally, some differences in formulation and dose are found between contraceptive hormones and HT, but the significance of these variances to targets outside the reproductive system has not been firmly established. Confounding such analysis are endogenous hormones. Unlike that in postmenopausal women, the ovarian contribution to circulating estrogen levels still can be significant in premenopausal women on contraceptive HT. However, the current trend toward an increase in body mass index (BMI) for both pre- and postmenopausal women may mute this difference in endogenous hormone levels because of conversion of adrenal androgens to estrogens by adipocyte aromatase. And perhaps the most critical difference between premenopausal and postmenopausal populations is that of overall health status and background into which HT is introduced, a consideration particularly relevant for subclinical disease progression when HT initiation is delayed.

III. The Inflammatory Process

A. Inflammation in brief

Inflammation research has experienced remarkable progress over the last decade and has generated increased appreciation for the complexity of the inflammatory process. A detailed presentation of the area is well beyond the scope of this review, but an outline of some of what is known of the ground rules and the players in inflammation is necessary for considering how sex steroid hormones might affect this process.

As a simple working definition, inflammation is a well-coordinated response of the immune, endocrine, and metabolic systems to tissue damage. Although an acute inflammatory response, for instance to a skin injury or infection, is the type that most readily comes to mind, at the other end of the spectrum is chronic inflammation, which is thought to be at the base of disorders such as arteriosclerosis, osteoarthritis, inflammatory bowel diseases, and several neurodegenerative diseases. The highly regulated acute inflammatory re-

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sponse that results in damage control and tissue restoration normally includes antiinflammatory mechanisms that contribute to the termination of the response. However, if the initiating injury or perturbation is recurrent or nonresolving or if a genetic alteration interferes with the sequence, the result is chronic inflammation.

The primary signaling molecules in the inflammatory response are cytokines produced on site (by, *e.g.*, macrophages, monocytes, lymphocytes, activated microglial cells) and peripherally (*e.g.*, visceral adipose tissue). The proinflammatory cytokines (*e.g.*, TNFα, IL-1, IL-6, and interferon-γ) along with growth factors (*e.g.*, macrophage colony-stimulating factor) and chemokines (*e.g.*, IL-8) initiate a coordinated sequence including, for example, recruitment of appropriate leukocytes and immune cells, up-regulation of pattern-recognition receptors for innate immunity including scavenger receptors and Toll-like receptors, positive feedback for continued production of proinflammatory cytokines, and increased synthesis of prostaglandins and leukotrienes. In contrast to this group of mediators, as part of the turn-off mechanism, antiinflammatory cytokines (*e.g.*, IL-4, IL-10) inhibit production or block actions of the proinflammatory cytokines.

The initiating signals that stimulate production of proinflammatory molecules can range from peroxidation products from excessive lipid oxidation to mechanical stress or a combination of cell-specific insults. Regardless of the particular perturbation, nuclear factor-κB (NF-κB)/Rel transcription factors play pivotal roles in transducing a signal that leads to production of cytokines. The NF-κB system also acts as mediator of inflammatory cytokine action, as do the MAPK signaling pathways and c-Jun N-terminal kinase (JNK1) (reviewed in Refs. 108–111). In addition to cytokines such as TNF-α, JNK1 also is activated by free fatty acids, and the activity of this kinase is abnormally elevated in mouse models of obesity (112).

Another key player in the regulation of inflammatory events is nitric oxide (NO), a diffusible uncharged gas with a half-life in seconds and having multiple molecular targets, for example cytokine production, inhibition of immune cell proliferation, antimicrobial activity, cyclooxygenase-2 induction, and vascular permeability and vasodilation (see Refs. 113–115 and references therein). Depending on its concentration and the cell- and event-specific activity, NO can have either pro- or antiinflammatory effects. NO production is regulated by isoforms of NO synthase (NOS): inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS). All three isoforms operate in the immune system and are expressed in multiple cell types. Regulation of iNOS is via transcription activated by cytokines and costimulatory molecules signaling through several pathways, including the NF-κB system. Activation of eNOS and nNOS predominantly is via a change in activity of existing protein triggered by a wide array of stimuli, including vasoactive substances, neurotransmitters, shear stress, and cytokines.

B. Estrogen and the inflammatory process

Experimental evidence for the role of estrogen in inflammation within the adipose/metabolic, cardiovascular, and neural systems will be reviewed in subsequent sections, but it is useful here to consider recent studies that establish the mechanistic potential for estrogen to affect the inflammatory process. ERα and, in some cases, ERβ are present in front line immune and cytokine-producing cells, such as macrophages and microglia, and E_2-activated ER has been shown *in vitro* to affect release of proinflammatory cytokines from these cells and to interfere with the action of cytokines (see Refs. 116–120 and references therein). These critical E_2 actions can be explained, at least in part, by the ability of ER to function as a transcriptional repressor by inhibiting the activity of NF-κB through a protein-protein interaction of agonist-bound ER with a subunit of activated NF-κB (121–123). The extent of the inhibitory action of E_2 on NF-κB function may be context- and target gene-selective, thus opening intriguing possibilities for mechanism-based design of SERMs (30, 123, 124).

E_2 also is implicated in the activation of eNOS and nNOS as well as the regulation of expression of all three NOS isoforms in the immune, cardiovascular, and central nervous systems (see Refs. 37, 115, and 125–127 and references therein). Both nuclear and extranuclear pathways are used by E_2 to affect the NOS isoforms. For example, the rapid release of NO in endothelial cells that results in vasodilation can be accomplished by E_2 activation of membrane ER and subsequent signaling through a phosphatidylinositol 3-kinase/Akt signaling pathway leading to eNOS activation (reviewed in Refs. 45 and 46). Progesterone has been shown to potentiate E_2 effects on the generation of NO in human endothelial cells *in vitro*, whereas MPA impairs E_2 signaling in this model (61).

In summary, the widespread presence and participation of ERs in multiple cell types of the immune system and the inflammatory response is remarkable and represents just one facet of the pleiotropic action of estrogens. Although much work remains to be done in defining the complex interaction of estrogens with the inflammatory process, the studies to date establish E_2 as a significant player, as will be explored in the following.

IV. Adipose/Metabolic System and Estrogens

A. Visceral adipose tissue

Visceral fat produces an array of hormones involved in energy homeostasis, the fibrinolytic system, the immune system, vascular homeostasis, and the inflammatory response. This multifunctional endocrine/paracrine organ has been the focus of many excellent reviews (for example, Refs. 128–131), and only a few highlights will be considered here.

Recent interest has been sparked, in part, by the observation that increased visceral adiposity is closely associated with insulin resistance, hypertension, dyslipidemia, and CVD. Adipose-derived hormones (adipokines) involved in these disorders have been examined in clinical, animal knockout, and *in vitro* studies, and prominent on any list have been the inflammatory cytokines and related proteins (Fig. 1) (131–133). For many of the adipokines shown in Fig. 1, there is a direct relationship between visceral adipose mass and the amount of product. An important exception is adi-

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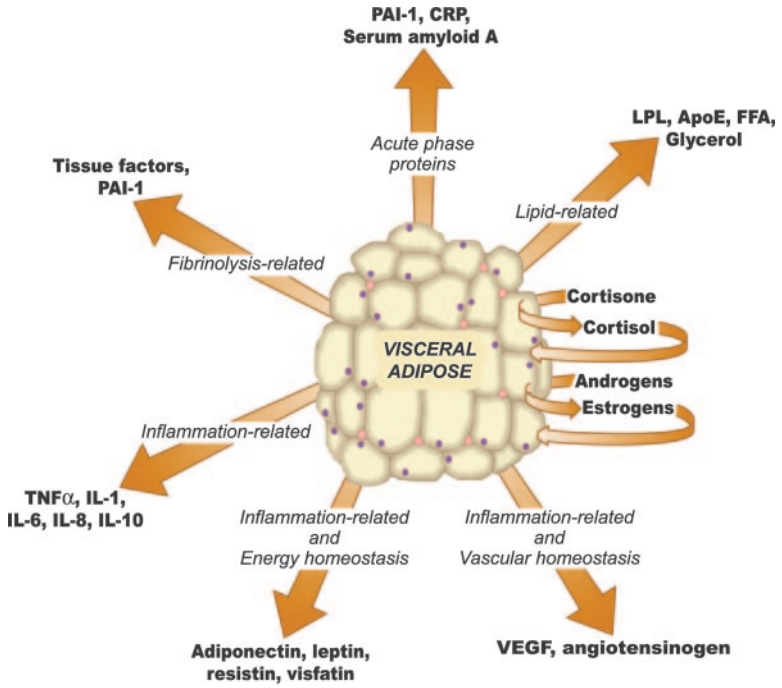

FIG. 1. Overview of some of the endocrine and paracrine products of visceral adipose tissue. The products are grouped into broad functional categories with examples shown for each category. Many of the secretory products (adipokines) have targets within visceral fat as well as in the periphery. The enzyme 11β-hydroxysteroid dehydrogenase-1 expressed in adipocytes is responsible for the conversion of circulating cortisone (inactive) to cortisol (active); adipose aromatase converts circulating androgens to estrogens. Receptors for cortisol and for E_2 are present in adipocytes. PAI-1, Plasminogen activator inhibitor-1; FFA, free fatty acids; VEGF, vascular endothelial growth factor.

ponectin, which has an inverse relationship to fat mass; in addition, an inverse association between plasma adiponectin levels and both insulin resistance and chronic inflammation has been reported (131, 134).

Visceral adipose tissue is thought to be metabolically and functionally different from sc fat tissue (see Ref. 135 and references therein). Epidemiological studies show that increased waist circumference, independent of total body fat, is associated with insulin resistance, dyslipidemia, hypertension, and low-grade inflammation (136). Waist circumference appears to be a better predictor of coronary events than BMI in men and women (137). The INTERHEART study, a large (~30,000) case-control study of cardiovascular risk factors carried out in 52 countries, recently showed that abdominal obesity accounted for a greater proportion of population-attributable risk than that associated with smoking in high- and middle-income countries (138). In addition, the study of metabolically obese, normal-weight individuals, with an adverse metabolic profile (insulin resistance, dyslipidemia) despite normal weight, has consistently revealed increased visceral adiposity (139). Brochu *et al.* (140) compared metabolically normal to metabolically abnormal (insulin resistant) obese postmenopausal women with similar age, BMI, and percent body fat. They found 50% more visceral adipose tissue in the metabolically abnormal obese women but no differences in sc or total percent body fat, compared with the metabolically normal obese women. Klein *et al.* (141) recently showed in humans that removal of sc fat by liposuction did not lead to improvements in metabolic parameters (insulin resistance, lipids).

Rodent models have revealed that the selective surgical resection of visceral fat pads led to marked improvements in insulin resistance, which was not seen with the removal of equivalent amounts of sc adipose tissue (142), supporting the concept that sc adipose tissue is a less important determinant of insulin action and lipid metabolism than visceral adipose tissue. Consistent with the functional distinctiveness of adipose depots is the recent identification of visfatin, a novel adipokine preferentially produced by human abdominal visceral adipose that facilitates adipogenesis but intriguingly also acts as an insulin-mimetic (143–145). Although much work remains in establishing its physiological roles, visfatin offers new prospects in obesity research.

The regulation of visceral adipose activity is complex, which is not surprising given its multifunctional and integrative characteristics. In addition to receptors for multiple cytokines and metabolic hormones, visceral adipocytes express most members of the nuclear hormone receptor family, notably peroxisome proliferator-activated receptor-γ (PPARγ), liver X receptor (LXR), thyroid hormone receptor, glucocorticoid receptor, AR, PR, and ER. An example of visceral adipose as a complex, functionally integrated unit comes from studies of glucocorticoid receptor and its ligand cortisol, which is produced locally from inactive cortisone by 11β-hydroxysteroid dehydrogenase-1 (11βHSD-1) (Fig. 1). This enzyme, which is highly expressed in human adipocytes, results in increased cortisol concentration within adipose without significantly affecting systemic cortisol levels (reviewed in Refs. 128, 146, and 147). In addition to its effect on adipocyte lipolysis and sensitivity to insulin, cortisol promotes differentiation of human preadipocytes into mature adipocytes (148). Although questions remain about the regulation and dysregulation of 11βHSD-1 and its role as cause or effect in visceral obesity (146, 149, 150), this cortisol-generating system within adipose is significant for its local integration of energy homeostasis under normal conditions and for its potential as a target for pharmaceutical manipulation.

B. Influence of estrogen on adipose tissue

1. Estrogen and body fat distribution. It is now clear that sex steroid hormones are major determinants of body fat distri-

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bution and that reproductive hormones in general may be considered adiposity signals. There is a gender dimorphism in body fat distribution, because women generally accumulate sc fat in the gluteal and femoral regions, whereas men develop an android pattern of body fat distribution with weight gain. Women generally have a higher percentage of total body adiposity, whereas BMI-matched men have about twice as much visceral adipose tissue as premenopausal women (151). The lower amount of visceral adipose tissue in women is thought to contribute to the lower prevalence of dyslipidemia, hypertension, diabetes, and CVD in premenopausal women compared with men and postmenopausal women.

The influence of hormones on body fat distribution appears to be related both to adipose tissue-specific expression of steroid receptors and to local tissue steroid hormone metabolism. ERα and ERβ are expressed in both sc and visceral fat tissues; however, ERβ appears to be preferentially expressed in sc adipose (152). Steroidogenic enzymes that metabolize sex steroid hormones, *e.g.*, 17α-hydroxylase and aromatase, are differentially expressed in sc and visceral adipose depots and regulate local steroid conversion (153). Adipose aromatase itself can be regulated, for example, by locally produced cytokines and prostaglandins (154). In human adipose stromal cells, cortisol produced locally from cortisone is a potent stimulator of the conversion of androgens to estrogens through an action on aromatase (155).

Lipoprotein lipase (LPL), a triglyceride hydrolase that directs the deposition of triglyceride into adipocytes cells, is thought to play an important role in regional body fat distribution. Tchernof *et al.* (156) recently showed regional differences in adipocyte metabolism with menopausal status, independent of age, body fat mass, and visceral adipose tissue accumulation. The authors found that omental adipocytes from postmenopausal women were larger and had higher LPL activity compared with premenopausal women, but they found no menopause-related differences in sc adipocytes, reflecting a shift toward visceral fat storage. A role for estrogen in LPL regulation is supported by studies in aromatase knockout mice, which have a marked increase in visceral adiposity associated with an increase in LPL expression and a metabolic syndrome phenotype. E_2 treatment resulted in a marked decrease in visceral fat mass and a profound inhibition of LPL expression in the aromatase knockout (reviewed in Ref. 157).

2. Estrogen deficiency. Menopausal estrogen deficiency is associated with significant changes in metabolic parameters that appear to be partially related to the shifts in body fat distribution with menopause (158). Premenopausal women have a less "atherogenic" lipid profile than men due to higher HDL, higher levels of large antiatherogenic HDL_2 subspecies, and lower triglyceride levels, all of which are closely associated with lower central fat accumulation. Menopause, either natural or surgical, is coupled with rapid adverse changes in lipid metabolism, because reduced HDL and HDL_2 and increased triglyceride and low-density lipoprotein (LDL) are seen within 3 months of amenorrhea (159, 160). The rapidity of the lipid changes implies that there may be both direct effects of sex steroid hormones and indirect effects of visceral fat accumulation on lipid metabolism.

Cross-sectional (156, 161) and longitudinal studies (162, 163) have shown that the menopausal transition is associated with an increase in visceral adiposity, and this effect of menopause is independent of the effect of age and total body adiposity or BMI (164–166). Establishing a mechanistic relationship in humans between the absence of E_2 and an increase in visceral adipose has been elusive, due in part to the complexities contributed by a paradoxical increase in serum E_2 associated with increases in adiposity in older menopausal women (see Ref. 167 and references therein). An additional factor in attempting to sort out the cause/effect role of estrogen and adiposity is the negative correlation of serum SHBG levels with BMI in peri- and postmenopausal women, resulting in a greater fraction of bioactive E_2 in circulation in obese individuals (168, 169). Another consideration is the source of postmenopausal E_2, which primarily is from extragonadal conversion of testosterone by aromatase. Consistent with the positive correlation of serum E_2 and BMI in postmenopausal women is the observation that adipose aromatase activity increases with age (170). So influential is the regulation by aromatase that the argument has been made that, in postmenopausal women, E_2 functions not as a circulating hormone but as a paracrine factor at its sites of production (*e.g.*, adipose, brain, breast, and bone) (157). Clearly, the availability of testosterone and its derivation from adrenal and ovarian androstenedione and dehydroepiandrosterone must be major considerations in attempting to decipher the complex relationship of E_2, body weight, and metabolism in menopausal women.

3. Exogenous estrogen. Contrary to popular belief, neither postmenopausal ET nor OCs have been shown to cause weight gain (171, 172), and OCs do not appear to have an effect on body fat distribution (173). However, oral ET has been shown to have a modest effect in reducing the postmenopausal weight gain (174). Mattiasson *et al.* (175) showed a selective reduction in visceral fat in early postmenopausal women treated with oral E_2 plus cyclic MPA that was not seen in the placebo-treated women. The HERS and WHI studies showed that combination HT (oral CEE + MPA) led to improvements in BMI and waist circumference in women with (372) and without (176) coronary heart disease.

Rodents rapidly become obese after ovariectomy, and E_2 administration is known to prevent the increase in adiposity (177). Recent data from D'Eon *et al.* (178) showed that E_2 treatment in ovariectomized mice promoted a reduction in adipose tissue mass and adipocyte size compared with pair-fed ovariectomized controls. The reductions in adiposity were seen in the intraabdominal fat but not the sc fat depots. The authors found increased levels of adipocyte lipolysis and down-regulation of associated genes such as LPL and LXR-α in intraabdominal fat from the E_2-treated mice. Conversely, in muscle they found that E_2 led to up-regulation of LPL and muscle-specific PPARγ, as well as several of its downstream targets, suggesting that E_2 promotes the use of lipid as a fuel by promoting fat oxidation in the muscle and enhancing adipocyte lipolysis (178).

If ET is associated with decreased visceral adipose, the

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critical question is whether there are beneficial metabolic consequences in humans. When insulin sensitivity is used as an endpoint, results are conflicting with reports of either increases, decreases, or no change depending on the age of the women, treatment route, and methodology for assessing insulin resistance (175, 176, 179). Similar inconsistencies are found with other endpoints and underscore the complexity of interactions between the paracrine and the endocrine functions of adipose, their integration with liver metabolic activity and inflammation, and the multiple estrogen targets within these tissues. Studies to disentangle this complex area are beginning to produce some answers, but much work remains in understanding the roles for estrogen in these relationships.

C. The influence of estrogen on markers of inflammation

Acute phase proteins serve as examples of the interface between adipose tissue and the inflammatory response. Markers of subclinical inflammation, such as C-reactive protein (CRP), IL-6, and serum amyloid A (SAA), have been linked to insulin resistance, diabetes mellitus 2, and CVD. *In vivo* and association studies have shown a strong link between obesity and increased inflammatory proteins, and obesity is associated with increased infiltration of adipose tissue by macrophages (180, 181).

CRP is a marker of the presence of subclinical inflammation that has been proposed to be an independent risk factor for cardiac events (182) and is a hallmark of the inflammatory processes that convert "fatty streak" plaques into complex atherosclerotic lesions. Although CRP is synthesized mainly in the liver, several extrahepatic sources of CRP have been reported, for example, adipocytes, macrophages, coronary artery smooth muscle cells, and neurons (131, 183). Circulating CRP levels are positively associated with total body fat and visceral adipose, and weight loss by hypocaloric diet or surgical intervention has been shown to reduce CRP levels (reviewed in Ref. 131). Oral estrogen significantly increases CRP levels through the first-pass hepatic effect, and some have hypothesized that increased CRP may have played a role in the higher rates of cardiovascular events and stroke observed in the HERS (10) and WHI (11) trials. Transdermal E_2, however, has no effect on systemic CRP levels (Table 2). It would be important to determine whether E_2 can affect local production of CRP within adipose or other target sites.

IL-6 is a proinflammatory cytokine produced at many sites, for example monocytes/macrophages, microglia, bone, and adipose, and has multiple functions in the inflammatory cascade, including increasing CRP and SAA production. Elevated IL-6 levels have been associated with increased BMI and risk of cardiovascular death (184). IL-6 has been shown to be preferentially secreted by visceral adipocytes, and removal of sc fat by liposuction did not reduce IL-6 levels (141). In addition to the positive association with BMI, circulating levels of IL-6 correlate positively with age (116, 186). Whether menopausal ET can reverse elevated serum IL-6 levels has been the subject of many studies with conflicting results, depending on treatment formulations and the age and BMI status of the women (116, 186–189). For example, data from the Postmenopausal Estrogen Progestin Intervention (PEPI) trial and other randomized clinical trials have shown no significant effect of oral ET (CEE), with or without progestin, on IL-6 levels (188), but several observational studies have shown variable effects (no change, reduction, or elevation) on IL-6 levels in postmenopausal women using oral ET (reviewed in Ref. 190). In contrast, *in vitro* studies establish that E_2 can decrease IL-6 production and interfere with its actions in several cell types secondary to a cell-type specific disruption of NF-κB signaling (116, 123, 191, 192). That E_2 modulates IL-6 activity primarily at the local tissue level is an important consideration and requires further study.

SAA is a marker of systemic inflammation that predicts future cardiovascular events and responds simultaneously with CRP to inflammatory stimuli. The SAA family is a group of differentially expressed apolipoproteins that are synthesized primarily in the liver and associate with HDL particles (187). The association of elevated SAA with HDL has been hypothesized to promote a proatherogenic phenotype by impairing reverse cholesterol transport (193). During inflammation, SAA-associated HDL particles become depleted of ApoA-1 (the apolipoprotein of the more antiatherogenic HDL subspecies), have a lower affinity for hepatocytes than HDL, and induce activity of phospholipases that lower HDL levels (187). Like CRP and IL-6, SAA is positively associated with BMI and waist circumference and has been found to be expressed in adipose tissue (194). Both IL-6 and SAA are up-regulated in adipocytes in the insulin-resistant state (see Ref. 129 and references therein). Abbas *et al.* (195) recently compared the effects of 8 wk of oral estrogens (CEE) or transdermal E_2 to placebo in postmenopausal women. They found that oral estrogen significantly increased total SAA and HDL-associated SAA levels (proatherogenic phenotype), whereas transdermal E_2 resulted in a significant reduction in SAA levels compared with placebo. Thus, presumed beneficial effects of oral estrogens resulting from an increase in HDL particles may be obviated by the parallel increase in SAA and interference with HDL function. The observation that transdermal E_2 is associated with a decrease in SAA reinforces the need to establish the action of E_2 on the production of acute phase proteins at nonhepatic sites.

V. Cardiovascular System and HT

In postmenopausal women, the effect of HT on cardiovascular events has become an intensely controversial issue. Several major recent clinical trials, especially the WHI study, have caused basic and clinical scientists to reconsider the fundamental biology and physiological complexities that impact upon HT in postmenopausal women (3). The lack of an effect of HT on cardiovascular endpoints in WHI and several other recent prospective clinical trials has caused confusion and controversy because the outcomes of these trials seem in opposition to a large body of observational data that demonstrate a protective cardiovascular effect of hormone replacement in postmenopausal years. However, recent clinical trials like the WHI studied cardiovascular endpoints after *de novo* initiation of HT to a mostly older postmenopausal population without a prior history of hormone use, which contrasts sharply with most observational cohorts

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studied in which the initiation of HT occurred in younger women who usually were enrolled in these trials for treatment of active perimenopausal symptoms.

The strengths and weaknesses of the WHI trial have been reviewed recently (3). Consensus now has emerged that it is important to acknowledge the strengths and limitations of WHI, but also to move past the study by emphasizing the enormous need that exists to strengthen interactions between preclinical and clinical research scientists and to improve our understanding of menopause and of the biology of age and gender differences in the cardiovascular system (3, 196, 197). The biology of the perimenopause remains poorly understood at present, and enormous gaps remain in our knowledge of the gender differences of relevance to cardiovascular function that may occur during this transition (197). It remains unclear, for example, whether or not there is a critical window for hormonal treatment that will confer cardioprotection, whether the duration of any such therapeutic advantage conferred by HT is limited, and what postmenopausal time course underlies the loss of efficacy and/or emergence of adverse effects seen with administration of HT to older women. The cardiovascular implications of discontinuous *vs.* continuous administration of HT also are unclear, as are the hormonal formulations and selective receptor modulators that may optimize cardiovascular functions and protection (197, 198). To address these issues, it will be necessary to understand the mechanisms of sex steroid hormone effects on cardiovascular tissues in both normal physiology and disease.

A. Sex steroid hormone receptor action and cardiovascular physiology

Steroid hormones all activate members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. The biology of these receptors has been reviewed in *Section II.A* and will not be discussed in detail here. The general presence of functional sex steroid hormone receptors (SSHR) in the cardiovascular system is well established, their expression in both heart and blood vessels having been recognized for several decades (reviewed in Refs. 199–202). E_2, progesterone, testosterone, and dihydrotestosterone all bind specifically to atrial and ventricular myocardial fibers and to vascular endothelial and smooth muscle cells of human and nonhuman primates (203–209). In a classic study, specific saturable receptors for estrogens, androgens, and glucocorticoids were demonstrated in canine coronary arteries, and PRs, absent at baseline, were induced by treatment with physiological E_2 concentrations (205). PRs also are expressed early in life, in human term fetoplacental vessels (210). ERα and ERβ expression in vascular smooth muscle and endothelial cells is regulated by injury and by E_2 (see Refs. 199, 200, and 202 and references therein). Aromatase, the enzyme that produces estrogens from androgens, is expressed in primate coronary arteries (211) and human venous and arterial tissues, including vascular smooth muscle cells and, in some but not all studies, endothelial cells (212–216). Vascular aromatase mediates normal vasomotion in males, and normal young males given aromatase inhibitors develop endothelial dysfunction that is reversible upon cessation of the drug (202, 217–219). Surprisingly, the effects of aromatase inhibition on vascular function in females have not yet been studied, despite the increasing use of these compounds in the therapy of breast cancer. Furthermore, the precise distribution and regulation of expression of SSHR and aromatase in heart and vascular tissues is not known, nor is it clear yet whether vascular beds differ, whether there are gender differences in the levels of expression of these proteins, and whether their level of expression is modified by the presence of atherosclerosis.

The hypothalamic-pituitary-gonadal axis undergoes perinatal maturation. Mammalian hypothalamic-pituitary-gonadal axis function begins *in utero*, when testosterone induces male reproductive organ development, whereas ovarian endocrine activity begins after birth (220). However, the cardiovascular developmental effects of sex steroid hormones and the time course of expression of SSHR in the development of cardiovascular tissues have not been studied. An important hypothesis in maternal-fetal medicine, the Barker or fetal programming hypothesis arose from recognition of a statistical correlation between conditions prevailing at the time of birth and morbidity and mortality due to chronic diseases late in life, such as the strong and inverse relationship observed between coronary heart disease mortality and perinatal weight (221). However, little data examining this hypothesis at the level of cardiovascular endocrinology and physiology exist at present. One recent epidemiological report suggested that low birth weight is only associated with increased carotid intimal medial thickening in young adulthood in subjects who have experienced both severe intrauterine growth retardation and exaggerated postnatal growth (222).

Another interesting perinatal issue is the recently described presence of fetal cells in myocardial tissues of the mother (223). The potential biological effects of fetal microchimerism on adult physiology have been suggested, but the cardiovascular role of these cells in gender differences for vascular or myocardial disorders also has not been explored.

Sex steroid hormones are critical determinants of cardiovascular gender differences. The majority of research has focused on the effects of estrogens and ER on cardiovascular physiology and disease, whereas progesterone and testosterone, their receptors, and the enzyme Cyp19 or aromatase all have received far less attention (202). Although SSHR interactions with their respective hormones, transcriptional coregulatory proteins, and specific DNA response elements have been studied extensively in reproductive target organs, these interactions have not yet been explored sufficiently in cardiovascular physiology. One common mechanism regulating hormone action that is likely to be of major importance for cardiovascular physiology involves coactivator and corepressor proteins that interact directly with the SSHR. The regulation of these coregulator proteins, in turn, through posttranslational modifications allows cells to alter the genes regulated by SSHR and the timing of transcriptional events (3, 21, 43). Coregulator biology also is important to the development of novel cardiovascular selective SSHR modulators (43, 198). Examples of cardiovascular coregulator specificity include the relatively specific myocardial AR coactivator, FHL2 (224) and the vascular role of the coacti-

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vator protein steroid receptor coactivator 3 (SRC-3; also known as AIB1, pCIP, ACTR, and TRAM-1) in mediating estrogen inhibition of vascular injury (225).

Yuan *et al.* (225) recently studied the role of SRC-3 in estrogen-mediated vascular protection after vascular injury. These authors created mice harboring a knock-in of the LacZ reporter into the SRC-3 gene. Heterozygous mice were phenotypically normal and provided a sensitive marker to characterize SRC-3 expression within the cardiovascular system, where SRC-3 expression was noted in vascular smooth muscle cells and endothelial cells but not in myocardial cells. A carotid ligation injury model was used to examine the extent of neointima formation in mice homozygous for SRC-3 disruption (SRC-$3^{-/-}$ mice). Vascular injury was more exuberant in SRC-$3^{-/-}$ mice than in wild-type mice, and this difference was diminished after ovariectomy. E_2 treatment after ovariectomy in wild-type mice inhibited the neointimal response, as has been reported previously (226, 227), but the ability of E_2 to inhibit the neointimal response and cellular proliferation in SRC-$3^{-/-}$ mice was attenuated. Thus, the loss of SRC-3 function interfered with the protective effects of estrogen in this vascular injury model, suggesting the potential importance of this coactivator in vascular biology. SRC-3 has been shown recently to integrate genomic responses to multiple cellular signaling pathways (228), suggesting that it may be an especially critical coactivator deserving of further study in cardiovascular cells and tissues (Fig. 2).

Another mechanism by which sex steroid hormones influence cardiovascular physiology is through the regulation of other nuclear hormone receptors. The PPARs, which mediate metabolic pathways relevant to CVD, are examples of nuclear hormone receptors that appear to be regulated differently in males and females, but very little work has appeared thus far that sheds light on these gender differences. Estrogen deficiency in humans causes alterations of both carbohydrate and lipid metabolism, and men with defective estrogen synthesis or action tend toward insulin resistance and dyslipidemia (229). PPARα-deficient male mice have metabolic defects far more severe than their female littermates, and these defects are rescued by ET (230). Cardiac disruption of the gene for the PPARγ coactivator protein PGC-1α, which regulates mitochondrial biogenesis and fatty acid oxidation, causes early sudden cardiac death in male but not female mice (231).

Another type of nuclear hormone receptor that has not yet been adequately explored for regulation by sex steroid hormones and as a contributor to the gender differences in CVDs is the LXRs that regulate cholesterol homeostasis. Like the SSHR, LXRs are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors, and there is evidence that LXR expression can be affected by E_2 (232–234) (see below). The LXRs regulate genes whose protein products control cholesterol homeostasis, such as proteins in the reverse cholesterol transport pathway involved in cholesterol removal from peripheral tissues, as well as proteins involved in the breakdown and excretion of cholesterol from the body, which can directly influence the rate and progression of atherosclerosis (232). LXR-α and LXR-β, the two known LXRs, are highly expressed in liver, adipose tissue, and macrophages, and LXR-β is expressed as well in many other tissues. Lundholm *et al.* (234) have recently used a gene profiling approach to examine estrogen-regulated genes in mouse adipose tissue. In these studies, E_2 decreased mRNA expression of LXRα, as well as several known LXRα target genes involved in cholesterol homeostasis, including

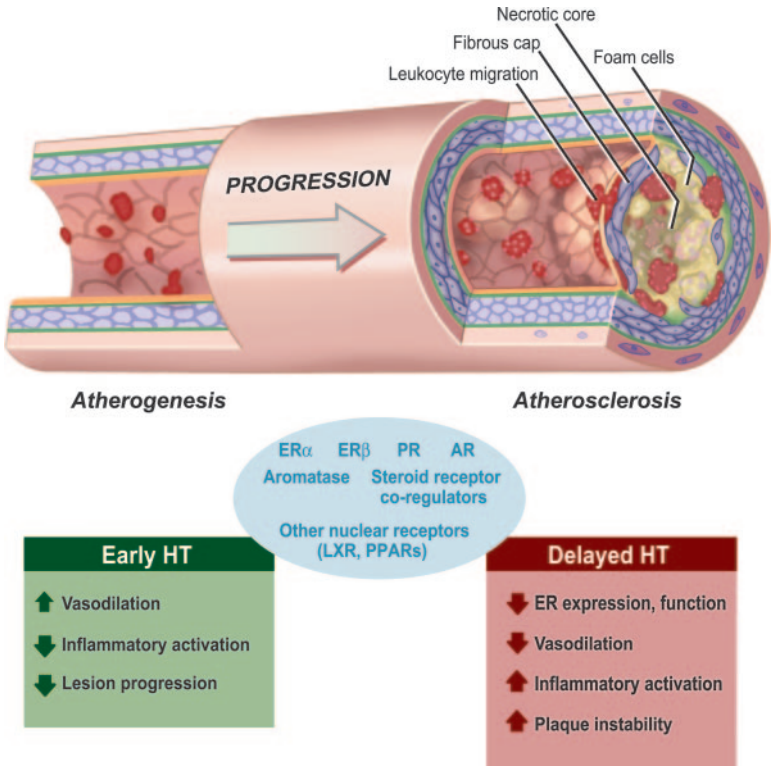

FIG. 2. Overview of the vascular and blood cell types that are influenced by estrogens. The effects of estrogens on the blood vessel wall depend upon the extent and complexity of atherosclerotic disease present at the time therapy is initiated. As atherosclerosis evolves, the early vascular protective mechanisms of estrogen ("early HT"; *e.g.*, increased NO and cyclooxygenase-2 and decreased TNF-α, cell adhesions molecules, LDL oxidation/binding, platelet activation, and vascular smooth muscle cell proliferation) recede and are replaced by estrogen responses in the setting of underlying advanced and unstable lesions that may be deleterious ("delayed HT"). The pleiotropic effects of HT on the vascular endothelium, vascular smooth muscle cells, and inflammatory cells thus differ, depending on the stage of atherosclerosis in the underlying blood vessel (the Timing Hypothesis, Ref. 202). The relative roles of steroid hormone receptors, other than ERs, and of the coregulatory molecules involved in nuclear receptor action in the vasculature remain less well understood.

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sterol regulatory element-binding protein 1c, apolipoprotein E, and the ATP-binding cassette A1. That E_2 can decrease expression of LXRα and several of its target genes in adipose tissue suggests that further study of LXR regulation by sex steroid hormones may be fruitful. This sort of crosstalk between SSHR and other nuclear hormone receptors important to cardiovascular physiology and disease deserves greater attention (Fig. 2). In summary, better understanding of the emerging regulatory mechanisms for SSHR is central to advancing our understanding of sex steroid hormones in cardiovascular physiology and disease (202).

B. Vascular effects of sex steroid hormones, atherosclerosis, and ischemic cardiovascular diseases

The importance of the timing of HT to the effects on vascular function has been reviewed recently (202). The largest body of scientific evidence in the field, the seminal primate studies of Clarkson and colleagues (89, 235), supports the conclusion that HT can be beneficial when initiated in the early stages of atherogenesis but that its effects are lost in the setting of more advanced atherosclerosis (202) (Fig. 2). Sex steroid hormone therapies will require better attention to timing, as well as dose and formulation of hormone, like other hormonal replacement therapies for endocrine organs that are losing or have lost their capacity to synthesize hormone (such as in thyroid disease). The effects of endogenous and exogenous hormones on cardiovascular SSHR have been discussed at length (201, 202). Here, we highlight several newer areas of hormone action in cardiovascular tissues that have not been discussed as thoroughly.

Two important systems regulated by SSHR that impact on cardiovascular physiology and disease are located principally outside of the cardiovascular tissues: those controlling lipoprotein metabolism and hemostasis-thrombosis. The role of SSHR in regulating lipoprotein synthesis and circulating lipid levels is well studied, especially for estrogens and progesterone, and has been reviewed extensively (201, 236–238). The liver expresses ERα but not ERβ and also expresses AR. Hormonal stimulation of hepatic receptors is largely responsible for the decreases in LDL and total cholesterol, the modest rise in HDL, and the increase in triglycerides that characterize oral HT.

Oral HT regimens and SERMs studied to date all lead to an increase in venous thromboembolic events, which may be relevant to the increased incidence of stroke seen in older women started *de novo* on HT in the WHI. Surprisingly, however, too little is known about the effects of sex steroid hormones on coagulation, fibrinolysis, and arterial thrombosis, and this area is ripe for increased research attention (196, 237, 239). Oral HT and OCs alter coagulation-fibrinolytic pathway protein expression, increasing levels of factor VIIc and VIIa and platelet fibrinogen binding, while decreasing circulating fibrinogen and the tissue plasminogen activator inhibitor, plasminogen activator inhibitor-1 (reviewed in Ref. 239). Simple correlations are not likely to be informative (for example, oral estrogen decreases fibrinogen levels, but increases thrombosis). Genetic variants of coagulation and fibrinolytic mediators also need greater exploration (196), because early evidence supports the hypothesis that specific variants in several of these proteins predisposes to arterial thrombosis (239). A leading candidate for increased venous thrombosis seen with ET is the common variant factor V Leiden (FVL), which may predispose to thrombosis by causing functional resistance to the fibrinolytic protein C, as a recent meta-analysis suggests (240). Oral but not transdermal estrogen has been shown to induce resistance to APC in the absence of FVL, but the risk of thrombotic events increases severalfold further in women with FVL, supporting the importance of further studies of FVL in peri- and postmenopausal women (75, 98, 106, 239, 241).

Genotypic-phenotypic studies are just beginning to move past identification of simple associations to the current preferred standards of: 1) confirmation of associations in two or more independent DNA datasets; and 2) *in vitro* and *in vivo* studies of the physiological consequences of different variants identified. There also are few studies of hormonal effects on thrombosis with *in vivo* models or at the menopausal transition. Studies of carotid injury in male ERα knockout mice and their wild-type littermates have been technically limited by frequent early thrombosis at the site of injury and the failure of the ERα knockout male mice to survive the procedure (M. E. Mendelsohn, unpublished results). Megakaryocytes contain ERβ and AR mRNA, but not ERα or PR (242). Platelet aggregation and secretion change with sexual maturity differently in females and males (243). Platelets of female mice are more reactive than those of males (244), but studies of platelet function in gonadectomized animals or in mice harboring disruptions of various SSHR are lacking.

C. Sex steroid hormones and the myocardium in health and disease

The effects of hormones on SSHR receptors in the heart also have been discussed at length (199, 201, 202). Several newer areas of hormone action in the heart have not been discussed as thoroughly and are now highlighted. E_2, progesterone, and testosterone all bind specifically to animal and human atrial and ventricular myocardial tissue (201–203, 206, 245). Although recent controversy has arisen as to whether murine hearts express ERβ (246), both ERα and ERβ have been identified in animal and human myocardial cells (247–252). Local production of estrogen occurs in murine cardiac myocytes (250). Myocardial cells and tissue of lower animals and human and nonhuman primates also express ARs, which respond to agonist stimulation with a hypertrophic response (245, 253–255). In diseased hearts, AR expression is increased, and testosterone can alter expression of cardiac-specific genes in diseased hearts, suggesting that heart-specific steroid metabolism influences cardiac hypertrophy (255).

The electrical (conduction) system of the heart also is influenced by sex steroid hormones. There are clear gender differences in cardiac electrophysiological function, the subject of several excellent recent reviews (256–258). Women normally have higher resting heart rates, due in part to intrinsic differences in the sinus node pacemaker (257). This difference is apparent at birth, when newborn boys display lower baseline heart rates than girls (259). Evidence also

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supports the fact that women have differences in autonomic nervous system activity and in myocardial repolarization (the electrocardiographic QT interval) that are hormonally influenced (257–259). The QT interval is consistently found to be longer in women than in men, and women are more susceptible to drug-induced ventricular tachycardia due to this QT prolongation. At puberty, QT shortening is noted in boys, suggesting that androgens may be responsible for this gender difference. Heart rate is lower during menses than during the follicular or luteal phases of the menstrual cycle, and spontaneous episodes of tachycardia appear to be correlated directly with progesterone levels and inversely with E_2 levels, although the mechanism is unknown. Several atrial and ventricular arrhythmias are more common in women, including especially supraventricular atrial tachycardias and ventricular tachycardias due to prolonged myocardial repolarization. Ion channel effects of sex steroid hormones likely contribute to gender differences in arrhythmias. Myocardial L-type calcium channel density is down-regulated via an estrogen-ER mechanism. E_2 also decreases potassium channel currents and prolongs repolarization in animal models, whereas androgen shortens repolarization in mice (260), supporting roles for both sex steroid hormones in the gender differences. More studies are needed of SSHR expression in cardiac atrial and ventricular tissues and the effects of different HT preparations on ion channel function and cardiac electrophysiology.

There are clear gender differences in myocardial hypertrophy and heart failure that have been noted mainly in animal and *in vitro* models (reviewed in Refs. 261 and 262). SSHR-mediated changes in the levels and regulation of myocardial calcium-contractility coupling proteins in the heart are likely involved in the effects of sex steroid hormones on myocardial hypertrophy and heart failure. Testosterone induces expression of both AR and the Na/Ca exchanger in isolated rat ventricular myocytes (263). Cardiac myocytes from male rats have an enhanced response to β-adrenergic stimulation with a greater influx of calcium than females (264). Disruption in mice of the gene for the FK506 immunosuppressant binding protein FKBP12.6, which interacts with cardiac ryanodine receptors important to regulation of intracellular calcium, results in hypertension and cardiac hypertrophy in male but not female mice (265). Treatment of female FKBP12.6-null mice with the mixed ER agonist-antagonist tamoxifen induces cardiac hypertrophy, suggesting that estrogen has vascular and/or cardiac effects that oppose loss of the FKBP12.6 gene (265). These and other notable studies underscore the potential for sex steroids to profoundly affect myocardial function, the gender difference in that action, and the need for continuing mechanistic studies that can form the basis for therapeutic exploitation.

VI. Central Nervous System and Sex Steroids

This component of the review will focus on our understanding of the role of estrogens on the central nervous system. The first portion will emphasize studies that were performed using *in vivo* and *in vitro* models. The second portion will focus on studies performed in humans, to illustrate instances in which these animal models provide insights that will help to improve the design of clinical and epidemiological studies in the future. Over the past 20 yr, we have come to understand that the presence of ovarian steroids is essential to the normal development and function of the brain, spinal cord, and peripheral nervous system (266–268). In addition, a significant, but not universal literature suggests that ovarian steroids are involved in the prevention of neurodegeneration brought about by disease or injury.

One of the great paradoxes today is that the vast majority of *in vivo* and *in vitro* studies performed using laboratory animals clearly demonstrates that E_2 is critical in the development, maintenance, and recovery of normal neural structures and function; E_2 is important in maintaining normal neuronal morphometry, synapse formation and function, and morphological and functional relationships among neurons, astrocytes, and microglia (267, 269, 270). Furthermore, these basic science studies show that E_2 is a potent neuroprotective factor that attenuates neuronal cell death and proliferation of new neurons and helps to maintain normal neural function in the face of injury or disease. The relationship between trophic actions of E_2 and its ability to protect against neurodegeneration is not clear. Moreover, the apparent dichotomy between the beneficial actions of E_2 on the brains of experimental animals and the WHI report that hormone treatment increases the risk of probable dementia in humans (271, 272) or risk of cerebrovascular stroke (273) is a great conundrum. A review of the basic science literature may offer insights into the complexities of hormone actions and provide a rationale for the design of future clinical trials. The majority of the studies in animal models have used sc E_2, and not CEE or SERMs. However, a growing body of experimental studies have begun to use these compounds (6, 274–278). Many of these recent studies continue to support the concept that estrogens are efficacious neuroprotective agents, although a few studies report contradictory results in terms of whether these compounds mimic the actions of E_2 (276–283). For example, Rossberg *et al.* (282) and Ciriza *et al.* (275) found that raloxifene protected the brain against kainic acid or stroke injury in rats; however, Lacreuse *et al.* (276) found that it does not improve aspects of spatial working memory in rhesus monkeys. Also, studies performed *in vitro* are inconsistent in terms of demonstrating that SERMs act as estrogens in protecting against cell death (277, 278). Interestingly, a recent study conducted in older women showed that raloxifene protected against the risk of mild cognitive impairment and developing AD but that it was dose related (see *Section VI.G*) (283).

A. Comparisons between basic science studies and the WHI

Why should animal studies and human studies draw such opposing conclusions? It is critical to consider several differences between the design of most of the animal studies, differences in physiology of the animal models and humans, and the design of the WHI.

First, most studies performed in animals induce a stroke-like environment and then examine the extent of injury after the experimental manipulation. In contrast, the WHI as-

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sessed the risk of a stroke in the future if women are exposed to HT.

Second, the vast majority of experiments performed in animals initiate hormone treatment before or immediately after they are ovariectomized. Ovariectomy of younger animals is used to mimic the postmenopausal state, because all ovarian hormones are removed. However, this model may have inherent problems as a model of the menopause, because the effects of aging are not considered, the abruptness of the removal of steroids is unlike the normal gradual sequence of events that accompany the menopausal transition, and the changes in gonadotropin levels that accompany ovariectomy are not identical to those that accompany the menopause. Other animal models of the menopause are being developed; however, they are not without their own challenges, and they have not been thoroughly characterized (284, 285).

Finally, extrapolations from animal models to humans always must be done with the full appreciation that there are inherent species differences. *In vitro* models, while simplifying the cellular environment, by necessity, do not mimic the complexity of neural interactions but do provide an excellent starting point for investigating that complexity.

B. Studies performed using animal models

During fetal development and the early postnatal period, E_2 is a potent trophic factor that influences brain development and differentiation by modulating neurogenesis (286), cell death (287), cell migration (288, 289), neuronal somatic and dendritic growth (289), synapse formation and elimination (290), and glial differentiation and neuronal morphology (291). Several investigators have found that addition of E_2 *in vitro* to organotypic cultures or dispersed cells derived from embryonic or neonatal rodent hippocampus, cerebral cortex, or hypothalamus stimulates neurite outgrowth, arborization of their branches, and synaptogenesis (for reviews, see Refs. 292–294). More recently, we recognized that these neurotrophic actions extend into adulthood. The elegant work of Woolley and McEwen and their colleagues (reviewed in Ref. 295) over the past 15 yr clearly shows that removal of endogenous ovarian hormones results in a reduced number and density of dendritic spines and axospinous synapses in the CA1 region of the hippocampus. This decrease can be either prevented or reversed by treatment with E_2 and requires activation of *N*-methyl-D-aspartate (NMDA) receptors. The electrophysiological consequences of the structural changes appear to be greater sensitivity to NMDA receptor-mediated input that is directly correlated with the density of dendritic spines. A recent study by Hao *et al.* (296) showed that the E_2 treatment has similar morphological and functional effects related to increased dendritic spine density and NMDA receptor-mediated activity in the prefrontal cortex of nonhuman primates (see *Section VI.F*).

Several laboratories have shown that E_2 not only acts during development and adulthood to establish and then maintain normal brain structure and function, but exerts protective actions when the neuron is vulnerable to cell death when faced with injury or disease. Thus, E_2 exerts neuroprotective actions in adult animals and protects against a wide variety of injuries, such as cerebral contusion (297), hypoxia (298), administration of excitatory amino acids (299), and drug-induced toxicity (300). Studies using animal models of stroke have been developed and used extensively as a model of neural injury. Results from these studies provide particularly strong evidence that E_2 is a neuroprotective factor that attenuates the degree of ischemic brain injury (301–306). The studies in animal models clearly establish that females uniformly suffer less stroke injury than males. For example, intact female rats sustain over 50% less injury than intact males and ovariectomized female rats after ischemia induced by transient middle cerebral artery occlusion (MCAO) (305, 307). Furthermore, gonadectomized animals given ET (301–303, 308, 309) have smaller infarcts than E_2-depleted controls. Estrogens appear to protect against β-amyloid-induced cell death (300, 310, 311) and to enhance memory (312) in animal models and *in vitro* culture models. Together, these studies are particularly perplexing because, on first glance, they lead to opposite conclusions compared with the results of the WHI: both CEE (Premarin) and the combination of CEE with MPA (Prempro) were found to exacerbate the risk of stroke and dementia.

In vitro studies complement and confirm the results of *in vivo* studies and clearly establish that estrogens exert protective effects against a variety of neurotoxic insults. Studies have induced injury through conditions that mimic AD toxicity (300, 310, 313), hypoxia and oxidative stress (314–317), excitotoxicity (317–319), and physical injury. Thus, studies have examined whether E_2 can salvage cells from death induced by inhibition of mitochondrial function, suppression of glucose metabolism, alteration of NO production, or administration of substances such as β-amyloid peptide, excitatory amino acids, free radicals, and gp120. Although the modes of injury are variable, they may share similar mechanisms of toxicity and face final common pathways in the induction of cell death. It remains to be determined whether E_2 protects against cell death through parallel or divergent pathways in the different modes of neuronal injury.

C. Mechanisms of estrogen action in the central nervous system

What are the mechanisms by which E_2 exerts its effects? What determines which mechanism is used? Are similar mechanisms used when estrogen-like compounds are used? Answers to these questions are only beginning to emerge.

It appears that when low physiological concentrations of E_2 exert trophic or protective actions, ERs are critical and they appear to act either as transcription factors that induce or repress the expression of genes that favor neuronal differentiation and survival (300, 320–322) or in conjunction with membrane-associated second messenger systems (292, 318, 323–328). In these cases, trophic effects and neuroprotective actions require hours to days of exposure, and the exposure must precede the injury by at least 12–24 h (301, 316, 329). The absence of the receptor (330) or the presence of receptor antagonists (316, 325) blocks the trophic and protective actions of the hormone. However, it is also clear that receptors

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are not the only element that is critical, but that they may interact with other growth factors (331) to activate nongenomic actions. Thus, using cultured hippocampal neurons, Murphy and colleagues (327, 332–334) showed that E_2 induced a coordinated multistep sequence of cellular events: activation of NMDA receptors, decreased γ-aminobutyric acid neurotransmission, increase in cAMP binding protein and phosphorylated cAMP-response element binding protein, and a decrease in brain-derived neurotrophic factor. All of these signals were required to mediate the influence of E_2 on synapse formation and turnover. Several other E_2-induced changes may contribute to changes in excitability and synaptogenesis (335, 336). However, their roles in this experimental paradigm have not been tested.

When higher, pharmacological concentrations are used, E_2 appears to act in a receptor-independent manner. When these concentrations are used, treatment before injury is not necessary; in fact, E_2 protects against stroke-like injury when it is administered as late as 3 h after MCAO (308). Pharmacological levels of E_2 can rapidly and reversibly decrease NMDA induced currents (319), suggesting that it may reduce excitatory cell death caused by neurodegenerative injury. Furthermore, estrogens can influence members of the NOS family to induce vasodilatory actions on cerebral blood vessels (337) and thus improve blood flow to compromised brain regions. Estrogens can also act as potent antioxidants and inhibit lipid peroxidation (304, 313, 338–340) through actions that have been shown to occur via the C3 hydroxyl group located on the phenolic A ring of the steroids (341). These studies demonstrate that this antioxidant mechanism requires supraphysiological levels of estrogens, findings that may be key in the development of therapeutic approaches aimed at achieving neuroprotection against injury induced by oxidative stress.

More recently, we have begun to appreciate the importance of inflammation in neurodegeneration (342–344) and the role of E_2 acting as an antiinflammatory factor (117–119, 125). The inflammatory response associated with stroke is complex, but significant experimental evidence shows that estrogens may directly or indirectly regulate three components of the inflammatory response: 1) microglial activation; 2) activation of the enzyme, iNOS; and 3) the activation of cytokines/chemokines (345). These components of inflammation may interact with each other and are not mutually exclusive. Microglia are the resident immune cells of the central nervous system (Fig. 3). They become activated in response to injury and initiate a pattern of events marked by proliferation, migration to the site of injury, and changes in both morphology and cell surface markers (346). Recent work establishes that microglia play a critical role in response to injury (347, 348). Both *in vivo* and *in vitro* studies have shown that E_2 suppresses microglial activation and that this response is regulated by ERs (117, 119, 125, 349). The region surrounding the ischemic core (penumbra) is vulnerable but responsive to protective factors. Experimental results suggest that microglial activation in the ischemic penumbra is a critical component of the inflammatory response associated with cerebral ischemic injury. Infiltrating monocytes and macrophages from the periphery also migrate to the penumbra. Microglial activation can be either deleterious or beneficial, depending on the microenvironment and time course of the stroke injury (343, 350, 351). Currently, we

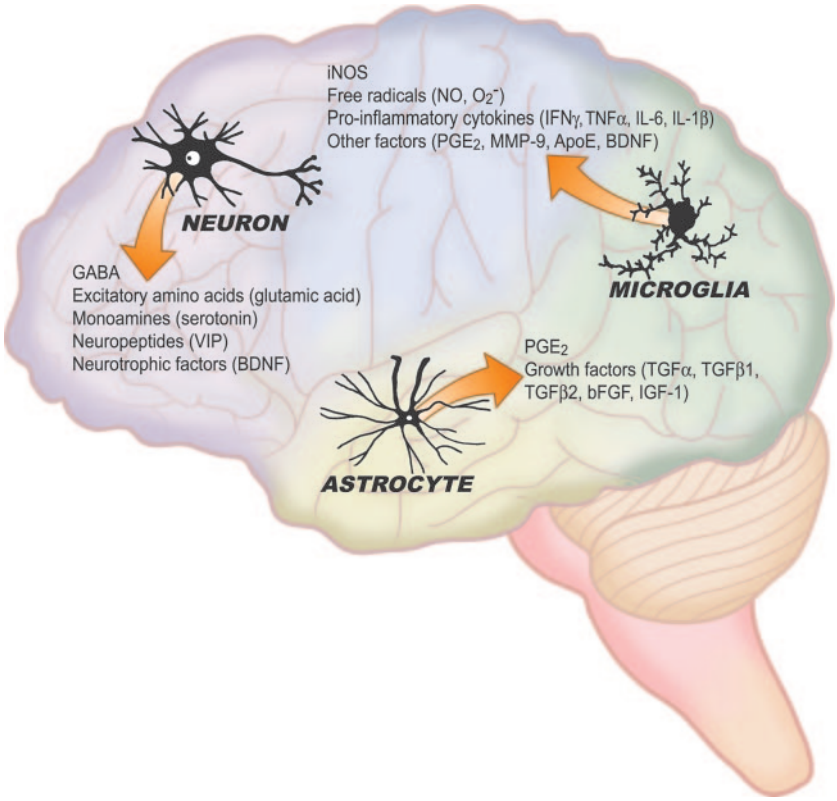

FIG. 3. Overview of cell types and examples of neurotransmitters and neuromodulators that are influenced by estrogens. The ability of estrogens to exert trophic and protective actions depends upon their ability to alter the birth and death of neurons, synaptogenesis, and neuritogenesis. Their effects on microglia, through modulation of the number of microglia and synthesis and secretion of pro- and antiinflammatory cytokines, result in altering the inflammatory state of the brain. Finally, estrogen effects on astrocytes influence astrocyte morphometry and function as well as neuron-neuron, neuron-astrocyte, and astrocyte-astrocyte interactions. VIP, Vasoactive intestinal peptide; BDNF, brain-derived neurotropic factor; PGE_2, prostaglandin E_2; bFGF, basic fibroblast growth factor; IFNγ, interferon-γ; MMP-9, matrix metalloproteinase-9.

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believe that E_2 may suppress deleterious microglial activation pathways, leading to decreased cell death in the ischemic penumbra.

E_2 regulates iNOS in various disease paradigms. Many studies show that physiological levels of E_2 suppress the iNOS-mediated immune response. At these concentrations, E_2 decreases lipopolysaccharide-mediated NO production in human monocytes, a murine microglial cell line, and rat primary microglial cultures (349). ERs have been implicated in the actions of E_2 on this component of the inflammatory response. The pan-ER antagonist ICI 182,780 blocks E_2 attenuation of NO production (349), with studies implicating both ERα (119) and ERβ (125). Finally, iNOS may possess an imperfect estrogen response element (352), because many estrogen responsive genes have been shown to possess similar estrogen response elements (353, 354). An E_2-induced increase in iNOS and subsequent increase in production of NO contributes to postischemic secondary cell injury (354, 355). iNOS knockout mice (356–358) and wild-type mice treated with inhibitors of iNOS demonstrate that NOS2 is a deleterious gene in models of both permanent and transient MCAO (358, 359).

Cytokines are secreted proteins that communicate the immune status of the organism between tissues and organs. They appear to play a critical role in the pathophysiology of human cerebral ischemia: there is a positive correlation between high levels of proinflammatory cytokine in serum and greater stroke severity. Conversely, increased levels of antiinflammatory cytokines correlate with diminished stroke severity and an improved outcome (345, 360–362). Cytokines in the brain perform pleiotropic functions in inflammation and are synthesized primarily by microglia and astrocytes, but also can be produced by neurons (363) (Fig. 3). In several different brain injury paradigms, sc E_2 generally suppresses proinflammatory cytokines and enhances the production of antiinflammatory cytokines (364, 365). Both TNFα (366–368) and IL-6 (360) display critical roles as part of the proinflammatory response, but also display pleiotropic roles as mediators of neuronal survival.

It is important to remember that even in animal models, estrogens do not always exert beneficial effects (297, 369–371). Several points should be emphasized. First, although a large body of literature suggests that estrogens are potent neuroprotective factors under numerous experimental and clinical circumstances *in vivo* and in many *in vitro* experimental conditions, in other experimental and disease/injury paradigms, estrogens either fail to protect or even exacerbate neural injury. Some authors have shown that E_2 fails to attenuate cell death in some animal models (297, 370, 371). It appears that when the degree of injury is too severe, as may be the case in the hippocampus after prolonged global ischemia (370) or when a disease state is already in progress (369), estrogens cannot overcome this degree of injury.

D. Evaluation of the Women's Health Initiative Memory Study (WHIMS): design and findings

The starting point for any discussion of the clinical effects of HT on cognitive function is WHIMS because it is the only randomized clinical trial to date to examine the effects of HT on dementia risk (272, 373). WHIMS was an ancillary study to the WHI. The WHI enrolled over 161,000 women across an observational study and included three partially overlapping randomized trials: dietary modification, calcium/vitamin D, and postmenopausal HT. There were over 27,000 women enrolled in the HT arm, and of these 29% were also enrolled in the dietary modification trial, 59% were enrolled in the calcium/vitamin D trial, and 18% were in all three trials (374). WHIMS volunteers were drawn from the HT study and met additional screening criteria relevant to the study of HT and primary prevention of dementia, including absence of dementia at screening and age 65 and older. Inclusion of only older women in WHIMS was due to the practical need for a sufficient number of incident dementia cases over the planned 8.5-yr study.

As in the main WHI trial, treatment assignment in WHIMS was carried out in two separate arms. Older hysterectomized women were randomized to receive CEE or placebo, and older naturally menopausal women were randomized to receive CEE/MPA or placebo. Dementia evaluations took place in four phases. In phase I, the modified Mini-Mental State Examination (3MSE) was given to screen for probable dementia and mild cognitive impairment (MCI)—the preclinical phase of dementia—and to assess global cognitive function. Patients who scored below designated 3MSE cut points underwent additional clinical evaluation in phase II, including a neuropsychological evaluation and interviews with the patient and an informant. Phase III involved a clinical dementia evaluation that yielded a determination of no dementia, MCI, or probable dementia. Phase IV included laboratory and imaging studies to aid in diagnosis of specific dementia subtypes (*e.g.*, AD, vascular dementia). Central adjudication by three experts was carried out to reach one consensus on diagnosis (375).

Results from the two HT arms combined indicated that the risk for all-cause dementia was significantly increased with HT compared with placebo (373). In the combined estrogen plus progestin arm, dementia risk was doubled with HT (40 of 2229 *vs.* 21 of 2303) (272). In the estrogen-alone arm, dementia risk was not significantly increased with active treatment (373). The risk for MCI did not increase significantly in the two arms combined or in either arm individually (272, 373). A direct statistical comparison between the CEE/MPA and CEE alone arms found no difference in the effect of active treatment on risk of dementia (373).

E. WHIMS validity: internal and external

The internal validity of WHIMS was high, with no evident biases or confounding factors. Perhaps the most important questions remaining after WHIMS pertain to external validity: Do the results generalize to all women and HT regimens that were not directly tested in WHIMS? At the forefront of this issue is the question of whether these results apply to women for whom HT remains an indication, that is, younger women with menopausal symptoms. WHIMS addressed a critically important question: Can older women initiate HT after age 65 to reduce risk of dementia. Whether this interpretation of the data can be generalized to younger women who initiate earlier in the menopausal transition for the treat-

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ment of menopausal symptoms is unknown. This question is not likely to be addressed by a randomized clinical trial because such a study would be prohibitively expensive because it would require early initiation (around age 50) and a long-term follow-up until women were at an age of increased risk for dementia (until age 65 and older). However, the generalizability of the WHIMS findings to young women could be addressed in follow-up analyses because WHIMS dementia evaluations continued even after treatment was discontinued. Therefore, it will be possible to examine whether the risk of dementia remains once treatment is stopped. If the risk is no longer present after discontinuation, then this would suggest that early initiators are not at long-term risk for developing dementia. In light of the very low incidence of AD among women in their early 50s, concerns about both immediate and long-term risk could be appreciably alleviated.

F. Differences with previous clinical and observational studies: age of initiation of treatment

Other insights into the external validity of WHI can be found in observational studies of HT and dementia, randomized clinical trials of HT and cognitive test performance, and basic science studies. The observational studies that gave rise to the hypothesis that HT might reduce the risk of AD involved women with typical patterns of HT use early in the menopause. These observational studies suggested that HT might decrease the risk for AD by 39–50% (376–378). This contrasts with the 75% increase observed in WHI (373). What factors might explain such a dramatic inconsistency in findings? One possibility is that WHIMS controlled for confounders that were ubiquitous in prior nonrandomized studies and that when those were controlled, the "true" effect became evident. Confounding differences in education levels between hormone users and nonusers have been noted as important biases, and low education (<8 yr) is a risk factor for dementia, particularly in women (379). To date, there have been three prospective studies of HT and incident dementia cases, and of these, two reported no significant differences between hormone users and nonusers in education (376, 378), and the third reported modest but significant differences (380). In each, however, HT users were younger than nonusers.

Recent data suggest that age of initiation of therapy may explain this magnitude of difference in dementia risk. For example, data from Cache County, Utah, revealed an increased risk for AD in older women who initiated HT after age 64 in contrast to a decreased risk for dementia in older women who initiated HT before age 64. This is similar to reports from observational studies (378). This pattern of findings led to the "critical period hypothesis," which states that HT confers optimal neuroprotection when initiated close in time to the menopausal transition (381). Additional evidence consistent with the critical period hypothesis was found in a recent case-control study of women participating in the MIRAGE (Multi-Institutional Research in Alzheimer Genetic Epidemiology) study, which found a reduced risk for AD only in the youngest age group (aged 50–63) (382). One interpretation of these studies is that early initiation protects against AD; those in the youngest age group necessarily initiated HT at a younger age, but those in the older age groups could have initiated HT at any age.

Other insights into the critical period hypothesis come from studies examining performance on neuropsychological tests. For example, women in the Melbourne Midlife Women's Health Study who initiated HT before the final menstrual period showed better verbal memory compared with women who initiated HT sometime after menopause (383). These findings parallel seminal findings from small randomized clinical trials that estrogen withdrawal, either through surgery or pharmacological suppression, is associated with decreased verbal memory, and add-back estrogen can reverse those deficits (384–386). Two recent randomized clinical trials reported enhanced verbal memory in women aged 50–65 randomized to receive HT (185, 387). Other trials showed neutral or detrimental effects of estrogen alone (388) or combined HT on verbal memory in women with an average age in the early 70s to 80s at randomization (389–391). There is some evidence from recent randomized clinical trials that HT enhances other cognitive measures, even when initiated later in life. For example, the largest clinical trial of HT and cognitive function, the Women's Health Initiative Study of Cognitive Aging (WHISCA), recently published findings from a sample of 1416 women (mean age, 74) participating in the CEE/MPA arm of WHIMS (391). Results revealed a significant detrimental effect of HT on verbal memory and a significant beneficial effect on figural memory as measured by the Benton Visual Retention Test. Overall, clinical trials provide some support for the critical period hypothesis as applied to estrogen effects on tests of verbal memory but suggest that the critical period hypothesis does not apply to all cognitive domains.

Secondary analyses from large-randomized trials in elderly women suggest that the effects of HT on global cognitive function may depend on baseline cognitive function. A beneficial effect of E_2 alone on the MMSE was observed in the Women's Estrogen for Stroke Trial (WEST) in a subset of women who had normal MMSE scores at baseline (388). WEST, a 3-yr trial of E_2 alone involving 664 women (mean age, 70) with a history of stroke, showed no detrimental effect of treatment on any cognitive function. Among women with a normal baseline MMSE score, those randomized to receive E_2 had a significantly lower rate of decline compared with the placebo group. The results are similar to findings from WHIMS showing that baseline 3MSE score significantly modified the effect of treatment on cognitive performance over time. In WHIMS, the detrimental effect of CEE on global cognitive function was evident among the 10% of women showing cognitive impairment at baseline. In contrast, for the 90% of women in the CEE alone arm who showed no cognitive impairment at baseline, CEE had no negative effect on global cognitive function (373). A similar effect was observed in the CEE/MPA arm, where women showing cognitive impairment at baseline showed significant declines in global cognitive function with treatment, whereas those with normal cognitive function at baseline showed no significant declines with treatment (272). These studies suggest that HT may be especially detrimental for women with impaired cognitive function and counter the earlier view that HT may

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be effective in the treatment of existing cognitive impairment. This set of findings is also consistent with the general view that estrogen appears better able to maintain healthy tissue than to repair damaged tissue (85).

This general view has been discussed with respect to HT and cardioprotection (see *Sections II.A.3* and *V.B*; also reviewed in Refs. 85 and 202). The CEE alone arm of the WHI showed a 50% reduction in adverse coronary events in women ages 50–59 who were assigned to active treatment (12). Longitudinal studies report an increased risk for AD among those with cardiovascular risk factors, including hypercholesterolemia and hypertension, and a decreased risk among statin users (392, 393). The parallel between potential early benefit of HT on the cardiovascular system and brain raise interesting questions about possible common mechanisms of benefit and risk. The Kronos Early Estrogen Prevention Study, a 5-yr randomized, placebo-controlled trial, is planned to examine the effects of transdermal and oral HT on cardiovascular outcomes in women in early menopause (menses absent for at least 6 and not more than 36 months; E_2 and FSH levels in postmenopausal range) (394). Cognitive outcomes will be evaluated in an ancillary study with verbal memory as the primary outcome. This will be the first head-to-head trial comparing different forms and delivery methods of estrogen on cognition in a sample of early menopausal women.

Two of the primary mechanisms by which HT is thought to exert neuroprotection appear to be age-dependent, namely, effects on the hippocampus and interactions with the cholinergic system. Early studies performed using rats showed that estrogens exert direct effects on the morphology and connectivity of the hippocampus, a brain area critical to memory functioning. The density of dendritic spines in the hippocampus fluctuates with circulating E_2 over the estrous cycle and increases with ET in female rats. The mechanisms by which E_2 induces increases in hippocampal spine density are age-dependent, with younger but not older female rats showing the increase (395). E_2 interacts with the cholinergic system to reverse memory deficits in younger rats (396), but not in older rats (397). These studies suggest two mechanisms by which ET might confer protection to the hippocampus and memory in younger, but not older animals.

Important recent studies in aged female rhesus monkeys addressed the question of the impact of E_2 on the neocortex. Two experimental design characteristics for these studies are noteworthy: first, the monkeys were comparable in age to 55- to 65-yr-old women when they were made surgically menopausal; and second, HT was low-dose E_2 administered im in a cyclical pattern over 2–3 yr. Compared with vehicle control, E_2 treatment substantially reversed the marked age-related impairment in the delayed response test of spatial working memory, a behavioral assessment that critically requires the prefrontal cortex (398). Investigation of possible neurobiological substrates showed that in E_2-treated monkeys there was a profound effect on spine morphology and an increase in spine density of pyramidal neurons in an area of the prefrontal cortex that mediates corticocortical integration responsible for cognitive processes (296). These results are highly significant in considering E_2-induced plasticity and potential for reversal of age-related decline in cognition. The characteristics of E_2 dose, treatment regimen, and time of initiation in this primate study have implications for clinical trial design.

G. Potential of SERMs as HT: efficacy on central nervous system parameters

There is accumulating evidence that some SERM compounds may protect against age-associated declines in cognition, even when initiated later in life. This evidence provides justification for continued investigation of SERMs as potential cognitive enhancers. For example, the impact of raloxifene on cognitive function was evaluated in a sample of almost 7500 postmenopausal women with osteoporosis (mean age, 66 yr; range, 31–80) enrolled in the Multiple Outcomes of Raloxifene Evaluation (MORE) (399). Women randomized to raloxifene showed a trend toward protection against significant declines in verbal memory and psychomotor speed over a 3-yr interval compared with women randomized to placebo. This effect reached significance in women age 70 and older, those who were at the highest risk for cognitive impairment. A subsequent report from MORE indicated that the risk of mild cognitive impairment was significantly lower among women randomized to receive 120 but not 60 mg/d of raloxifene compared with those randomized to receive placebo (283). This underscores the importance of further efforts to develop SERMs as treatments in the primary prevention of mild cognitive impairment and, consequently, primary prevention of dementia. The inclusion of cognitive outcomes in clinical trials of SERMs under development, including bazedoxifene, lasofoxifene, droloxifene, and idoxifene, would help to advance our understanding of potential cognitive benefits of these agents.

Because SERMs can act as cell type-specific ER antagonists as well as agonists, another priority for research on women's cognitive health is to understand the possible detrimental cognitive effects of SERMs and other pharmacological tools such as aromatase inhibitors used in the treatment, for example, of breast cancer. Tamoxifen's antiestrogenic actions in breast tissue underlie its efficacy in preventing recurrence of breast cancer. Effects on cognition are less well understood, and clinical studies performed to date have provided conflicting data. In a study of breast cancer survivors, standard-term and long-term tamoxifen users were more than twice as likely to report having seen a physician for memory problems after diagnosis than nonusers (400). Moreover, women in that study who had taken tamoxifen for at least 5 yr performed worse on cognitive tests than those who had taken tamoxifen for shorter periods, suggesting that tamoxifen may have detrimental longer-term effects on the brain. Another study of breast cancer survivors found that women who received both chemotherapy and tamoxifen performed worse on certain cognitive tests (*i.e.*, visual memory, verbal learning, and visuospatial tests) compared with women who received chemotherapy alone (401). In contrast, tamoxifen use was associated with decreased risk of AD and enhanced decision-making skills in nursing home residents who had taken tamoxifen compared with those who had not (402). Thus, findings are inconsistent with respect to

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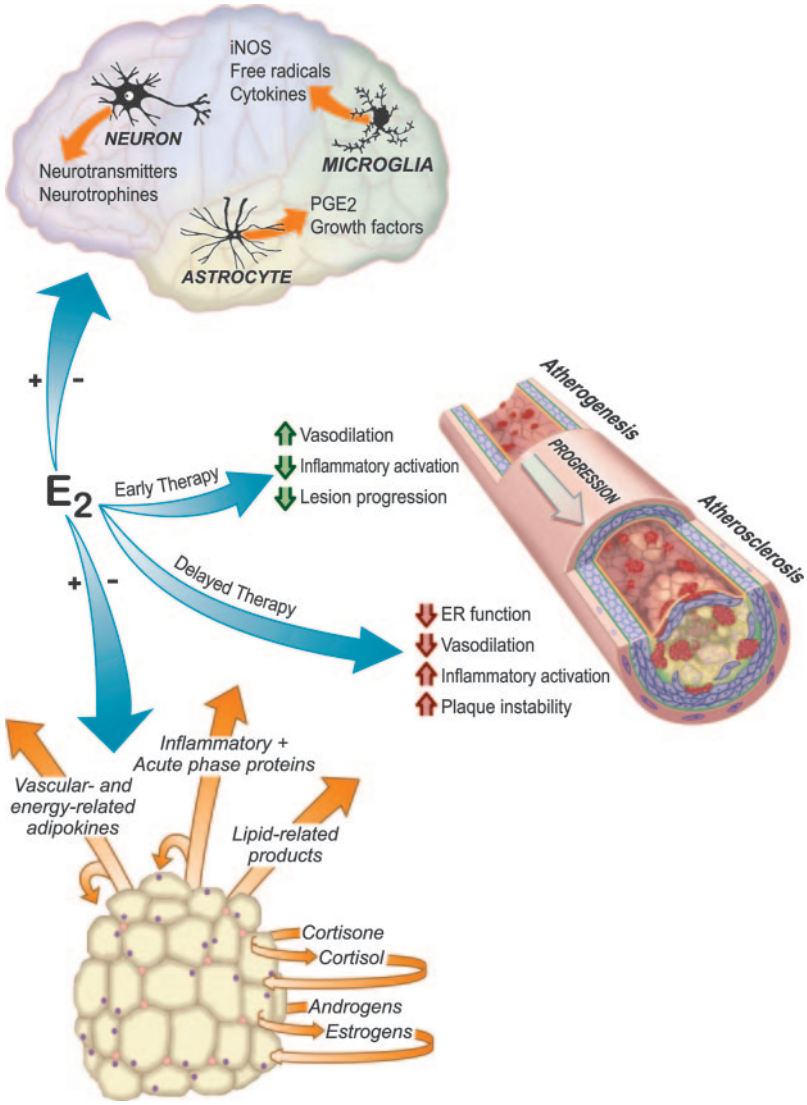

FIG. 4. Composite overview of some of the pleiotropic actions of E_2 on the adipose/metabolic, cardiovascular, and central nervous systems. Among its effects is a modulation of inflammatory processes within these three systems, thus placing E_2 in a potentially integrative role. Whether E_2 is stimulatory or inhibitory is a function of cell specificity, including ER type and expression level and coregulator complement, as well as E_2 concentration and timing.

the effects of tamoxifen on cognition. The ongoing trial, Cognition in the Study of Tamoxifen and Raloxifene, should provide insights into this important question.

Although aromatase inhibitors have become more important in standard adjuvant therapy for early hormone receptor-positive breast cancer, little is known about their cognitive effects. Preliminary insights into the effects of aromatase inhibitors and tamoxifen on cognitive function were drawn from pilot data from a small study of breast cancer patients receiving anastrozole, tamoxifen, or combined treatment compared with community-dwelling healthy controls. Although treatment assignment remained blinded at the time of this analysis, patients performed significantly worse on tests of verbal memory and psychomotor speed compared with controls (403). In light of the recent U.S. Food and Drug Administration approval for the use of another aromatase inhibitor, letrozole, in the postsurgery treatment of postmenopausal women with hormone receptor-positive early breast cancer, cognitive studies of aromatase inhibitors take on greater importance.

H. Effect of progestins on cognition

Arguably one of the highest priorities in clinical research on HT and cognition is the need for studies examining the combined effects of estrogens plus various progestins and administered in different regimens (*i.e.*, continuous *vs.* cyclic) on cognitive function. The pattern of results from WHIMS—a significant impact of CEE/MPA but not CEE alone on dementia risk—might seem to suggest that the progestin used in this study is responsible for the effect on dementia risk (272, 373). However, statistically significant differences in subject characteristics between the two studies at initial screening make cross-study comparisons difficult; compared with women in the CEE/MPA arm, those in the CEE alone arm were more likely to have used HT in the past, were more ethnically diverse, were more likely to have had a previous stroke or CHD, had lower educational attainment, and had lower 3MSE scores (373). Because of these cross-arm differences in factors that could influence dementia risk, WHIMS does

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not provide a clear answer with respect to how progestins may modulate the effects of estrogens on dementia.

To date, only two randomized, placebo-controlled studies have directly addressed the effects of estrogen plus progestin *vs.* estrogen alone on cognition test performance, and these studies are based on the same cohort of 49 early menopausal women (404, 405). The studies included a direct comparison of estrogen alone, estrogen plus progestin (estradiol valerate plus dinogest), and placebo. An advantage for estrogen alone over the other treatment conditions was observed in associative verbal memory, whereas an advantage for estrogen plus progestin was observed in numerical memory (404). This pattern is similar to the mixed pattern of effect observed in WHISCA in elderly women, *i.e.*, improved figural memory and decreased verbal memory with CEE/MPA (391). The scarcity of studies on combined estrogens and progestin in younger women is particularly troublesome given that new prescriptions for symptomatic naturally menopausal HT users will include a progestin with estrogen to protect against uterine cancer. Thus, there is a pressing need for clinical trials aimed at comparing the effect of different progestins and different progestin regimens on cognitive outcomes in early menopausal women.

VII. Summary/Conclusions

The goal of this review was to discuss the dichotomy between the results of many clinical and basic studies and the results of the WHI. We present a summary of current knowledge of the action of E_2 on three major physiological systems: the adipose/metabolic, the cardiovascular, and the central nervous systems. We highlight that 1) different estrogens (and progestins) can have disparate actions within each physiological system; 2) routes of delivery, concentrations, and sequence of HT administration determine differential actions at various targets; and 3) the age, genetic background, health status, and previous hormone treatment environment determine the actions of HT (Fig. 4). This perspective reveals the incredible challenge that faces physicians and researchers when a single treatment may be desired by the public.

Historically, the aim of HT during the postmenopausal years was to relieve the immediate symptoms of the menopause that plagued many women: vasomotor flushes and vaginal dryness. In addition, because it was clear that estrogens could slow the progress of osteoporosis, the leading cause of hip fractures/morbidity in older women, HT was considered a universal solution to multiple symptoms of ovarian failure associated with the menopause. Indeed, numerous clinical observational studies and a wealth of data based on animal models supported the hypothesis that ovarian steroids exert protective actions and their absence makes postmenopausal women more vulnerable to CVDs and neurodegenerative disorders, including dementia and cerebrovascular stroke.

In that milieu, the WHI results were surprising, as was their broad interpretation. However, when the data are evaluated within the narrow context of the study design, insights emerge that encourage us to reassess fundamental assumptions and to consider how future clinical trials could be better designed. Our knowledge of the complexities of hormone action, particularly related to different types of estrogen, would predict that mechanism-based hormone therapies might lead to different, more individualized treatments during the menopausal and postmenopausal years. Thus, therapy design would recognize that estrogen treatment outcomes can be radically altered by the subject's age and subclinical disease progression as well as her genetic or hormonal background, such as ER mutations or duration without E_2. For the future, clinical trials that balance empirical-based information and mechanism-based knowledge will allow better, more appropriate designs.

Finally, the results of the WHI and other recent prospective clinical trials emphasize the enormous need that exists to strengthen interactions between preclinical and clinical research scientists and to improve our understanding of menopause and of the biology of gender differences. In underscoring the complex actions of sex steroids and gaps in our knowledge regarding cardiovascular and neural function during the menopausal transition, postmenopause, and aging, the trial results provide a road map for future studies. We suggest that one of the most critical areas for focus is the interface of estrogens with the inflammatory response and the adipose/metabolic system and the consequences of this interface to cardiovascular and neural health in women through their lifespan. The need for basic studies that address these gaps and the role for HT is clear, as is the requirement for more clinical studies that build on this mechanism-based knowledge.

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Address all correspondence and requests for reprints to: Judith Turgeon, Ph.D., Department of Internal Medicine, Division of Endocrinology, Clinical Nutrition, and Vascular Medicine, University of California Davis, Davis, California 95616. E-mail: jlturgeon@ucdavis.edu

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References

1. **Sherwin BB** 2002 Estrogen and cognitive aging in women. Trends Pharmacol Sci 23:527–534
2. **Paganini-Hill A** 2001 Hormone replacement therapy and stroke: risk, protection or no effect? Maturitas 38:243–261
3. **Turgeon JL, McDonnell DP, Martin KA, Wise PM** 2004 Hormone therapy: physiological complexity belies therapeutic simplicity. Science 304:1269–1273
4. **Behl C** 2002 Oestrogen as a neuroprotective hormone. Nat Rev Neurosci 3:433–442
5. **Roof RL, Hall ED** 2000 Gender differences in acute CNS trauma and stroke: neuroprotective effects of estrogen and progesterone. J Neurotrauma 17:367–388
6. **Dhandapani KM, Brann DW** 2002 Protective effects of estrogen

and selective estrogen receptor modulators in the brain. Biol Reprod 67:1379–1385
7. **McCullough LD, Hurn PD** 2003 Estrogen and ischemic neuroprotection: an integrated view. Trends Endocrinol Metab 14:228–235
8. **Wise PM, Dubal DB, Wilson ME, Rau SW, Liu Y** 2001 Estrogens: trophic and protective factors in the adult brain. Front Neuroendocrinol 22:33–66
9. **Wise PM, Dubal DB, Rau SW, Brown CM, Suzuki S** 2005 Are estrogens protective or risk factors in brain injury and neurodegeneration: reevaluation after the Women's Health Initiative. Endocr Rev 26:308–312
10. **Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E** 1998 Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. JAMA 280:605–613
11. **Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J, Writing Group for the Women's Health Initiative Investigators** 2002 Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA 288:321–333
12. **Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, Bonds D, Brunner R, Brzyski R, Caan B, Chlebowski R, Curb D, Gass M, Hays J, Heiss G, Hendrix S, Howard BV, Hsia J, Hubbell A, Jackson R, Johnson KC, Judd H, Kotchen JM, Kuller L, LaCroix AZ, Lane D, Langer RD, Lasser N, Lewis CE, Manson J, Margolis K, Ockene J, O'Sullivan MJ, Phillips L, Prentice RL, Ritenbaugh C, Robbins J, Rossouw JE, Sarto G, Stefanick ML, Van Horn L, Wactawski-Wende J, Wallace R, Wassertheil-Smoller S** 2004 Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: The Women's Health Initiative randomized controlled trial. JAMA 291:1701–1712
13. **Viscoli CM, Brass LM, Kernan WN, Sarrel PM, Suissa S, Horwitz RI** 2001 A clinical trial of estrogen-replacement therapy after ischemic stroke. N Engl J Med 345:1243–1249
14. **Mulnard RA, Cotman CW, Kawas C, van Dyck CH, Sano M, Doody R, Koss E, Pfeiffer E, Jin S, Garnst A, Grundman M, Thomas R, Thal LJ** 2000 Estrogen replacement therapy for treatment of mild to moderate Alzheimer's disease. JAMA 283:1007–1015
15. **Henderson VW, Paganini-Hill A, Miller BL, Elble RJ, Reyes PF, Shoupe D, McCleary CA, Klein RA, Hake AM, Farlow MR** 2000 Estrogen for Alzheimer's disease in women. Randomized, double-blind, placebo-controlled trial. Neurology 54:295–301
16. **Marder K, Sano M** 2000 Estrogen to treat Alzheimer's disease: too little, too late? So what's a woman to do? Neurology 54:2035–2037
17. **Wang PN, Liao SQ, Liu RS, Liu CY, Chao HT, Lu SR, Yu HY, Wang SJ, Liu HC** 2000 Effects of estrogen on cognition, mood, and cerebral blood flow in AD: a controlled study. Neurology 54:2061–2066
18. **Kopernik G, Shoham Z** 2004 Tools for making correct decisions regarding hormone therapy. Part II. Organ response and clinical applications. Fertil Steril 81:1458–1477
19. **Shoham Z, Kopernik G** 2004 Tools for making correct decisions regarding hormone therapy. Part I. Background and drugs. Fertil Steril 81:1447–1457
20. **McDonnell DP** 2005 The molecular determinants of estrogen receptor pharmacology. Maturitas 48:S7–S12
21. **Smith CL, O'Malley BW** 2004 Coregulator function: a key to understanding tissue specificity of selective receptor modulators. Endocr Rev 25:45–71
22. **Beekman JM, Allan GF, Tsai SY, O'Malley BW** 1993 Transcriptional activation by the estrogen receptor requires a conformational change in the ligand binding domain. Mol Endocrinol 7:1266–1274
23. **Hall JM, McDonnell DP, Korach KS** 2002 Allosteric regulation of estrogen receptor structure, function, and coactivator recruitment by different estrogen response elements. Mol Endocrinol 16:469–486
24. **Kong EH, Pike AC, Hubbard RE** 2003 Structure and mechanism of the oestrogen receptor. Biochem Soc Trans 31:56–59
25. **Paige LA, Christensen DJ, Gron H, Norris JD, Gottlin EB, Padilla KM, Change C-Y, Ballas LM, Hamilton PT, McDonnell DP** 1999 Estrogen receptor (ER) modulators each induce distinct conformational changes in ER α and ER β. Proc Natl Acad Sci USA 96:3999–4004
26. **Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL** 1998 The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. Cell 95:927–937
27. **Giangrande PH, McDonnell DP** 1999 The A and B isoforms of the human progesterone receptor: two functionally different transcription factors encoded by a single gene. Rec Prog Hormone Res 54:291–313
28. **Gustafsson JA** 2003 What pharmacologists can learn from recent advances in estrogen signalling. Trends Pharmacol Sci 24:479–485
29. **Hall JM, Couse JF, Korach KS** 2001 The multifaceted mechanisms of estradiol and estrogen receptor signaling. J Biol Chem 276:36869–36872
30. **Harris HA, Albert LM, Leathurby Y, Malamus MS, Mewshaw RE, Miller CP, Kharode YP, Marzolf J, Komm BS, Winneker RC, Frail DE, Henderson RA, Shu Y, Keith Jr JC** 2003 Evaluation of an estrogen receptor-β agonist in animal models of human disease. Endocrinology 144:4241–4249
31. **Jacobsen BM, Richer JK, Sartorius CA, Horwitz KB** 2003 Expression profiling of human breast cancers and gene regulation by progesterone receptors. J Mammary Gland Biol Neoplasia 8:257–268
32. **Li X, O'Malley BW** 2003 Unfolding the action of progesterone receptors. J Biol Chem 278:39261–39264
33. **Smith LH, Coats SR, Qin H, Petrie MS, Covington JW, Su M, Eren M, Vaughan DE** 2004 Differential and opposing regulation of PAI-1 promoter activity by estrogen receptor α and estrogen receptor β in endothelial cells. Circ Res 95:269–275
34. **Stossi F, Barnett DH, Frasor J, Komm B, Lyttle CR, Katzenellenbogen BS** 2004 Transcriptional profiling of estrogen-regulated gene expression via estrogen receptor (ER) α or ERβ in human osteosarcoma cells: distinct and common target genes for these receptors. Endocrinology 145:3473–3486
35. **McDonnell DP, Connor CE, Wijayaratne A, Chang CY, Norris JD** 2002 Definition of the molecular and cellular mechanisms underlying the tissue-selective agonist/antagonist activities of selective estrogen receptor modulators. Rec Prog Hormone Res 57:295–316
36. **Kian Tee M, Rogatsky I, Tzagarakis-Foster C, Cvoro A, An J, Christy RJ, Yamamoto KR, Leitman DC** 2004 Estradiol and selective estrogen receptor modulators differentially regulate target genes with estrogen receptors α and β. Mol Biol Cell 15:1262–1272
37. **Sun J, Baudry J, Katzenellenbogen JA, Katzenellenbogen BS** 2003 Molecular basis for the subtype discrimination of the estrogen receptor-β-selective ligand, diarylpropionitrile. Mol Endocrinol 17:247–258
38. **Hillisch A, Peters O, Kosemund D, Muller G, Walter A, Schneider B, Reddersen G, Elger W, Fritzemeier KH** 2004 Dissecting physiological roles of estrogen receptor α and β with potent selective ligands from structure-based design. Mol Endocrinol 18:1599–1609
39. **Mulac-Jericevic B, Conneely OM** 2004 Reproductive tissue selective actions of progesterone receptors. Reproduction 128:139–146
40. **Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM, Horwitz KB** 2002 Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. J Biol Chem 277:5209–5218
41. **Zhi L, Tegley CM, Pio B, Edwards JP, Motamedi M, Jones TK, Marschke KB, Mais DE, Risek B, Schrader WT** 2003 5-Benzylidene-1,2-dihydrochromeno[3,4-f]quinolines as selective progesterone receptor modulators. J Med Chem 46:4104–4112
42. **Webb P, Nguyen P, Kushner PJ** 2003 Differential SERM effects on corepressor binding dictate ER_α activity in vivo. J Biol Chem 278:6912–6920
43. **Shang Y, Brown M** 2002 Molecular determinants for the tissue specificity of SERMs. Science 295:2465–2468
44. **Wessler S, Otto C, Wilck N, Stangl V, Fritzemeier KH** 2006 Identification of estrogen receptor ligands leading to activation of non-genomic signaling pathways while exhibiting only weak transcriptional activity. Mol Biol 98:25–35
45. **Simoncini T, Genazzani AR** 2003 Non-genomic actions of sex steroid hormones. Eur J Endocrinol 148:281–292

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46. **Edwards DP** 2005 Regulation of signal transduction pathways by estrogen and progesterone. Annu Rev Physiol 67:335–376
47. **Hersh AL, Stefanick ML, Stafford RS** 2004 National use of postmenopausal hormone therapy: annual trends and response to recent evidence. JAMA 291:47–53
48. **Dey M, Lyttle CR, Pickar JH** 2000 Recent insights into the varying activity of estrogens. Maturitas 34(Suppl 2:S25–S33
49. **Kuhl H** 2005 Pharmacology of estrogens and progestogens: influence of different routes of administration. Climacteric 8(Suppl 1): 3–63
50. **Bhavnani BR** 2003 Estrogens and menopause: pharmacology of conjugated equine estrogens and their potential role in the prevention of neurodegenerative diseases such as Alzheimer's. J Steroid Biochem Mol Biol 85:473–482
51. **Ansbacher R** 2001 The pharmacokinetics and efficacy of different estrogens are not equivalent. Am J Obstet Gynecol 184:255–263
52. **Baracat E, Haidar M, Lopez FJ, Pickar J, Dey M, Negro-Vilar A** 1999 Estrogen activity and novel tissue selectivity of Δ8,9-dehydroestrone sulfate in postmenopausal women. J Clin Endocrinol Metab 84:2020–2027
53. **Rosano GM, Vitale C, Silvestri A, Fini M** 2003 Metabolic and vascular effect of progestins in post-menopausal women. Implications for cardioprotection. Maturitas 46(Suppl 1):S17–S29
54. **Sitruk-Ware R** 2002 Progestogens in hormonal replacement therapy: new molecules, risks, and benefits. Menopause 9:6–15
55. **Hapgood JP, Koubovec D, Louw A, Africander D** 2004 Not all progestins are the same: implications for usage. Trends Pharmacol Sci 25:554–557
56. **Miller AP, Chen Y-F, Xing D, Feng W, Oparil S** 2003 Hormone replacement therapy and inflammation: interactions in cardiovascular disease. Hypertension 42:657–663
57. **Miyagawa K, Rosch J, Stanczyk F, Hermsmeyer K** 1997 Medroxyprogesterone interferes with ovarian steroid protection against coronary vasospasm. Nat Med 3:324–327
58. **Miyagawa K, Vidgoff J, Hermsmeyer K** 1997 Ca^{2+} release mechanism of primate drug-induced coronary vasospasm. Am J Physiol 272:H2645–H2654
59. **Nilsen J, Brinton RD** 2003 Divergent impact of progesterone and medroxyprogesterone acetate (Provera) on nuclear mitogen-activated protein kinase signaling. Proc Natl Acad Sci USA 100:10506–10511
60. **Otsuki M, Saito H, Xu X, Sumitani S, Kouhara H, Kishimoto T, Kasayama S** 2001 Progesterone, but not medroxyprogesterone, inhibits vascular cell adhesion molecule-1 expression in human vascular endothelial cells. Arterioscler Thromb Vasc Biol 21:243–248
61. **Simoncini T, Mannella P, Fornari L, Caruso A, Willis MY, Garibaldi S, Baldacci C, Genazzani AR** 2004 Differential signal transduction of progesterone and medroxyprogesterone acetate in human endothelial cells. Endocrinology 145:5745–5756
62. **Bamberger CM, Else T, Bamberger AM, Beil FU, Schulte HM** 1999 Dissociative glucocorticoid activity of medroxyprogesterone acetate in normal human lymphocytes. J Clin Endocrinol Metab 84: 4055–4061
63. **Herkert O, Kuhl H, Sandow J, Busse R, Schini-Kerth VB** 2001 Sex steroids used in hormonal treatment increase vascular procoagulant activity by inducing thrombin receptor (PAR-1) expression: role of the glucocorticoid receptor. Circulation 104:2826–2831
64. **Koubovec D, Berghe WV, Vermeulen L, Haegeman G, Hapgood JP** 2004 Medroxyprogesterone acetate downregulates cytokine gene expression in mouse fibroblast cells. Mol Cell Endocrinol 221:75–85
65. **Koubovec D, Ronacher K, Stubsrud E, Louw A, Hapgood JP** 2005 Synthetic progestins used in HRT have different glucocorticoid agonist properties. Mol Cell Endocrinol 242:23–32
66. **Kemppainen JA, Langley E, Wong CI, Bobseine K, Kelce WR, Wilson EM** 1999 Distinguishing androgen receptor agonists and antagonists: distinct mechanisms of activation by medroxyprogesterone acetate and dihydrotestosterone. Mol Endocrinol 13:440–454
67. **Krattenmacher R** 2000 Drospirenone: pharmacology and pharmacokinetics of a unique progestogen. Contraception 62:29–38
68. **Jaffe IZ, Mendelsohn ME** 2005 Angiotensin II and aldosterone regulate gene transcription via functional mineralocorticoid receptors in human coronary artery smooth muscle cells. Circ Res 96: 643–650
69. **Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, Palensky J, Wittes J** 1999 The effect of spironolactone on morbidity and mortality in patients with severe heart failure. N Engl J Med 341:709–717
70. **Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, Bittman R, Hurley S, Kleiman J, Gatlin M** 2003 Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. N Engl J Med 348:1309–1321
71. **Mendelsohn ME** 2005 In hypertension, the kidney is not always the heart of the matter. J Clin Invest 115:840–844
72. **Schriffin EL** 2006 Effects of aldosterone on the vasculature. Hypertension 47:312–318
73. **Jewelewicz R** 1997 New developments in topical estrogen therapy. Fertil Steril 67:1–12
74. **Rosano GM, Simon T, Mercuro G, Sans S, Schenck-Gustaffson K, Stevenson JC, Swahn E, Jaillon P** 2001 Hormone replacement therapy: where we stand in Europe. Eur Heart J 22:439–441
75. **Scarabin P-Y, Oger E, Plu-Bureau G** 2003 Differential association of oral and transdermal oestrogen-replacement therapy with venous thromboembolism risk. Lancet 362:428–432
76. **Friend KE, Hartman ML, Pezzoli SS, Clasey JL, Thorner MO** 1996 Both oral and transdermal estrogen increase growth hormone release in postmenopausal women–a clinical research center study. J Clin Endocrinol Metab 81:2250–2256
77. **Pirwany IR, Sattar N, Greer IA, Packard CJ, Fleming R** 2002 Supraphysiological concentrations of estradiol in menopausal women given repeated implant therapy do not adversely affect lipid profiles. Hum Reprod 17:825–829
78. **Leung KC, Johannsson G, Leong GM, Ho KK** 2004 Estrogen regulation of growth hormone action. Endocr Rev 25:693–721
79. **Writing Group for the PEPI Trial** 1995 Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. JAMA 273:199–208
80. **Wakatsuki A, Okatani Y, Ikenoue N, Fukaya T** 2002 Effect of medroxyprogesterone acetate on vascular inflammatory markers in postmenopausal women receiving estrogen. Circulation 105: 1436–1439
81. **Alhenc-Gelas M, Plu-Bureau G, Guillonneau S, Kirzin J-M, Aiach M, Ochat N, Scarabin P-Y** 2004 Impact of progestagens on activated protein C (APC) resistance among users of oral contraceptives. J Thromb Haemost 2:1594–1600
82. **Gomes MP, Deitcher SR** 2004 Risk of venous thromboembolic disease associated with hormonal contraceptives and hormone replacement therapy: a clinical review. Arch Intern Med 164:1965–1976
83. **Godsland IF** 2001 Effects of postmenopausal hormone replacement therapy on lipid, lipoprotein, and apolipoprotein (a) concentrations: analysis of studies published from 1974–2000. Fertil Steril 75:898–915
84. **Hays J, Ockene JK, Brunner RL, Kotchen JM, Manson JE, Patterson RE, Aragaki AK, Shumaker SA, Brzyski RG, LaCroix AZ, Granek IA, Valanis BG** 2003 Effects of estrogen plus progestin on health-related quality of life. N Engl J Med 348:1839–1854
85. **Naftolin F, Taylor HS, Karas R, Brinton E, Newman I, Clarkson TB, Mendelsohn M, Lobo RA, Judelson DR, Nachtigall LE, Heward CB, Hecht H, Jaff MR, Harman SM** 2004 The Women's Health Initiative could not have detected cardioprotective effects of starting hormone therapy during the menopausal transition. Fertil Steril 81:1498–1501
86. **Colditz GA, Willett WC, Stampfer MJ, Rosner B, Speizer FE, Hennekens CH** 1987 Menopause and the risk of coronary heart disease in women. N Engl J Med 316:1105–1110
87. **Kalantaridou SN, Naka KK, Papanikolaou E, Kazakos N, Kravariti M, Calis KA, Paraskevaidis EA, Sideris DA, Tsatsoulis A, Chrousos GP, Michalis LK** 2004 Impaired endothelial function in young women with premature ovarian failure: Normalization with hormone therapy. J Clin Endocrinol Metab 89:3907–3913
88. **Mercuro G, Zoncu S, Saiu F, Mascia M, Melis GB, Rosano GM** 2004 Menopause induced by oophorectomy reveals a role of ovar-

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ian estrogen on the maintenance of pressure homeostasis. Maturitas 47:131–138
89. **Mikkola TS, Clarkson TB** 2002 Estrogen replacement therapy, atherosclerosis, and vascular function. Cardiovasc Res 53:605–619
90. **Stevenson JC, Whitehead MI** 2002 Hormone replacement therapy. BMJ 325:113–114
91. **Anstead GM, Carlson KE, Katzenellenbogen JA** 1997 The estradiol pharmacophore: ligand structure-estrogen receptor binding affinity relationships and a model for the receptor binding site. Steroids 62:268–303
92. **Cheskis BJ, Karathanasis S, Lyttle CR** 1997 Estrogen receptor ligands modulate its interaction with DNA. J Biol Chem 272:11384–11391
93. **Hyder SM, Chiappetta C, Stancel GM** 1999 Synthetic estrogen 17α-ethinyl estradiol induces pattern of uterine gene expression similar to endogenous estrogen 17β-estradiol. J Pharmacol Exp Ther 290:740–747
94. **Goebelsmann U, Mashchak CA, Mishell Jr DR** 1985 Comparison of hepatic impact of oral and vaginal administration of ethinyl estradiol. Am J Obstet Gynecol 151:868–877
95. **Abrams LS, Skee D, Natarajan J, Wong FA** 2002 Pharmacokinetic overview of Ortho Evra/Evra. Fertil Steril 77:S3–S12
96. **Lobo RA, Cassidenti DL** 1992 Pharmacokinetics of oral 17 β-estradiol. J Reprod Med 37:77–84
97. **Rosendaal FR, Helmerhorst FM, Vandenbroucke JP** 2002 Female hormones and thrombosis. Arterioscler Thromb Vasc Biol 22:201–210
98. **Vandenbroucke JP, Rosing J, Bloemenkamp KW, Middeldorp S, Helmerhorst FM, Bouma BN, Rosendaal FR** 2001 Oral contraceptives and the risk of venous thrombosis. N Engl J Med 344:1527–1535
99. **Kemmeren JM, Algra A, Meijers JC, Tans G, Bouma BN, Curvers J, Rosing J, Grobbee DE** 2004 Effect of second- and third-generation oral contraceptives on the protein C system in the absence or presence of the factor V_{Leiden} mutation: a randomized trial. Blood 103:927–933
100. **Oger E, Alhenc-Gelas M, Lacut K, Blouch MT, Roudaut N, Kerlan V, Collet M, Abgrall J-F, Aiach M, Scarabin P-Y, Mottier D** 2003 Differential effects of oral and transdermal estrogen/progesterone regimens on sensitivity to activated protein C among postmenopausal women: a randomized trial. Arterioscler Thromb Vasc Biol 23:1671–1676
101. **Hoibraaten E, Mowinckel MC, de Ronde H, Bertina RM, Sandset PM** 2001 Hormone replacement therapy and acquired resistance to activated protein C: results of a randomized, double-blind, placebo-controlled trial. Br J Haematol 115:415–420
102. **Baillargeon JP, McClish DK, Essah PA, Nestler JE** 2005 Association between the current use of low-dose oral contraceptives and cardiovascular arterial disease: a meta-analysis. J Clin Endocrinol Metab 90:3863–3870
103. **Esmon CT** 2004 Crosstalk between inflammation and thrombosis. Maturitas 47:305–314
104. **Viles-Gonzalez JF, Fuster V, Badimon JJ** 2005 Thrombin/inflammation paradigms: a closer look at arterial and venous thrombosis. Am Heart J 149:S19–S31
105. **Martinez C, Basurto L, Zarate A, Saucedo R, Gaminio E, Collazo J** 2005 Transdermal estradiol does not impair hemostatic biomarkers in postmenopausal women. Maturitas 50:39–43
106. **Post MS, Christella M, Thomassen LG, van der Mooren MJ, van Baal WM, Rosing J, Kenemans P, Stehouwer CD** 2003 Effect of oral and transdermal estrogen replacement therapy on hemostatic variables associated with venous thrombosis: a randomized, placebo-controlled study in postmenopausal women. Arterioscler Thromb Vasc Biol 23:1116–1121
107. **van den Heuvel MW, van Bragt AJ, Alnabawy AK, Kaptein MC** 2005 Comparison of ethinylestradiol pharmacokinetics in three hormonal contraceptive formulations: the vaginal ring, the transdermal patch and an oral contraceptive. Contraception 72:168–174
108. **Hayden M, Ghosh S** 2004 Signaling to NF-κB. Genes Dev 18:2195–2223
109. **Kumar A, Takada Y, Boriek AM, Aggarwal BB** 2004 Nuclear factor-κB: its role in health and disease. J Mol Med 82:434–448
110. **Varfolomeev EE, Ashkenazi A** 2004 Tumor necrosis factor: an apoptosis juNKie? Cell 116:491–497
111. **Kaminska B** 2005 MAPK signalling pathways as molecular targets for anti-inflammatory therapy–from molecular mechanisms to therapeutic benefits. Biochim Biophys Acta 1754:253–262
112. **Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS** 2002 A central role for JNK in obesity and insulin resistance. Nature 420:333–336
113. **Bogdan C** 2001 Nitric oxide and the immune response. Nat Immun 2:907–916
114. **Guzik TJ, Korbut R, Adamek-Guzik T** 2003 Nitric oxide and superoxide in inflammation and immune regulation. J Physiol Pharmacol 54:469–487
115. **Karpuzoglu E, Fenaux JB, Phillips RA, Lengi AJ, Elvinger F, Ansar Ahmed S** 2006 Estrogen up-regulates inducible nitric oxide synthase, nitric oxide, and cyclooxygenase-2 in splenocytes activated with T cell stimulants: role of interferon-γ. Endocrinology 147:662–671
116. **Pfeilschifter J, Koditz R, Pfohl M, Schatz H** 2002 Changes in proinflammatory cytokine activity after menopause. Endocr Rev 23:90–119
117. **Vegeto E, Belcredito S, Ghisletti S, Meda C, Etteri S, Maggi A** 2006 The endogenous estrogen status regulates microglia reactivity in animal models of neuroinflammation. Endocrinology 147:2263–2272
118. **Dimayuga FO, Reed JL, Carnero GA, Wang C, Dimayuga ER, Dimayuga VM, Perger A, Wilson ME, Keller JN, Bruce-Keller AJ** 2005 Estrogen and brain inflammation: effects on microglial expression of MHC, costimulatory molecules and cytokines. J Neuroimmunol 161:123–136
119. **Vegeto E, Belcredito S, Etteri S, Ghisletti S, Brusadelli A, Meda C, Krust A, Dupont S, Ciana P, Chambon P, Maggi A** 2003 Estrogen receptor-α mediates the brain antiinflammatory activity of estradiol. Proc Natl Acad Sci USA 100:9614–9619
120. **Xing D, Miller A, Novak L, Rocha R, Chen YF, Oparil S** 2004 Estradiol and progestins differentially modulate leukocyte infiltration after vascular injury. Circulation 109:234–241
121. **Stein B, Yang MX** 1995 Repression of the interleukin-6 promoter by estrogen receptor is mediated by NF-κB and C/EBPβ. Mol Cell Biol 15:4971–4979
122. **Ghisletti S, Meda C, Maggi A, Vegeto E** 2005 17β-estradiol inhibits inflammatory gene expression by controlling NF-κB intracellular localization. Mol Cell Biol 25:2957–2968
123. **Kalaitzidis D, Gilmore TD** 2005 Transcription factor cross-talk: the estrogen receptor and NF-κB. Trends Endocrinol Metab 16:46–52
124. **Chadwick CC, Chippari S, Matelan E, Borges-Marcucci L, Eckert AM, Keith Jr JC, Albert LM, Leathurby Y, Harris HA, Bhat RA, Ashwell M, Trybulski E, Winneker RC, Adelman SJ, Steffan RJ, Harnish DC** 2005 Identification of pathway-selective estrogen receptor ligands that inhibit NF-κB transcriptional activity. Proc Natl Acad Sci USA 102:2543–2548
125. **Baker AE, Brautigam VM, Watters JJ** 2004 Estrogen modulates microglial inflammatory mediator production via interactions with estrogen receptor β. Endocrinology 145:5021–5032
126. **Grohe C, Kann S, Fink L, Djoufack PC, Paehr M, van Eickels M, Vetter H, Meyer R, Fink KB** 2004 17 β-estradiol regulates nNOS and eNOS activity in the hippocampus. Neuroreport 15:89–93
127. **Schulz E, Anter E, Zou MH, Keaney Jr JF** 2005 Estradiol-mediated endothelial nitric oxide synthase association with heat shock protein 90 requires adenosine monophosphate-dependent protein kinase. Circulation 111:3473–3480
128. **Kershaw EE, Flier JS** 2004 Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 89:2548–2556
129. **Flier JS** 2004 Obesity wars: molecular progress confronts an expanding epidemic. Cell 116:337–350
130. **Lyon CJ, Law RE, Hsueh WA** 2003 Minireview: adiposity, inflammation, and atherogenesis. Endocrinology 144:2195–2200
131. **Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S** 2005 Adipokines: molecular links between obesity and atherosclerosis. Am J Physiol Heart Circ Physiol 288:H2031–H2041
132. **Rajala MW, Scherer PE** 2003 Minireview: the adipocyte–at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology 144:3765–3773

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133. **Hansson GK** 2005 Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 352:1685–1695
134. **Goldstein BJ, Scalia R** 2004 Adiponectin: a novel adipokine linking adipocytes and vascular function. J Clin Endocrinol Metab 89:2563–2568
135. **Cnop M, Landchild MJ, Vidal J, Havel PJ, Knowles NG, Carr DR, Wang F, Hull RL, Boyko EJ, Retzlaff BM, Walden CE, Knopp RH, Kahn SE** 2002 The concurrent accumulation of intra-abdominal and subcutaneous fat explains the association between insulin resistance and plasma leptin concentrations: distinct metabolic effects of two fat compartments. Diabetes 51:1005–1015
136. **Wajchenberg BL** 2000 Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev 21:697–738
137. **Rexrode KM, Carey VJ, Hennekens CH, Walters EE, Colditz GA, Stampfer MJ, Willett WC, Manson JE** 1998 Abdominal adiposity and coronary heart disease in women. JAMA 280:1843–1848
138. **Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L** 2004 Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 364:937–952
139. **Ruderman N, Chisholm D, Pi-Sunyer X, Schneider S** 1998 The metabolically obese, normal-weight individual revisited. Diabetes 47:699–713
140. **Brochu M, Tchernof A, Dionne IJ, Sites CK, Eltabbakh GH, Sims EAH, Poehlman ET** 2001 What are the physical characteristics associated with a normal metabolic profile despite a high level of obesity in postmenopausal women? J Clin Endocrinol Metab 86: 1020–1025
141. **Klein S, Fontana L, Young VL, Coggan AR, Kilo C, Patterson BW, Mohammed BS** 2004 Absence of an effect of liposuction on insulin action and risk factors for coronary heart disease. N Engl J Med 350:2549–2557
142. **Gabriely I, Ma XH, Yang XM, Atzmon G, Rajala MW, Berg AH, Scherer P, Rossetti L, Barzilai N** 2002 Removal of visceral fat prevents insulin resistance and glucose intolerance of aging: an adipokine-mediated process? Diabetes 51:2951–2958
143. **Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R, Matsuzawa Y, Shimomura I** 2005 Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science 307:426–430
144. **Hug C, Lodish HF** 2005 Medicine. Visfatin: a new adipokine. Science 307:366–367
145. **Sethi JK, Vidal-Puig A** 2005 Visfatin: the missing link between intra-abdominal obesity and diabetes? Trends Mol Med 11:344–347
146. **Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Lavery GG, Cooper MS, Hewison M, Stewart PM** 2004 11β-Hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. Endocr Rev 25:831–866
147. **Seckl JR, Walker BR** 2004 11β-Hydroxysteroid dehydrogenase type 1 as a modulator of glucocorticoid action: from metabolism to memory. Trends Endocrinol Metab 15:418–424
148. **Hauner H, Schmid P, Pfeiffer EF** 1987 Glucocorticoids and insulin promote the differentiation of human adipocyte precursor cells into fat cells. J Clin Endocrinol Metab 64:832–835
149. **Hewitt KN, Walker EA, Stewart PM** 2005 Minireview: hexose-6-phosphate dehydrogenase and redox control of 11β-hydroxysteroid dehydrogenase type 1 activity. Endocrinology 146:2539–2543
150. **Morton NM, Paterson JM, Masuzaki H, Holmes MC, Staels B, Fievet C, Walker BR, Flier JS, Mullins JJ, Seckl JR** 2004 Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11 β-hydroxysteroid dehydrogenase type 1-deficient mice. Diabetes 53:931–938
151. **Enzi G, Gasparo M, Biondetti PR, Fiore D, Semisa M, Zurlo F** 1986 Subcutaneous and visceral fat distribution according to sex, age, and overweight, evaluated by computed tomography. Am J Clin Nutr 44:739–746
152. **Mayes JS, Watson GH** 2004 Direct effects of sex steroid hormones on adipose tissues and obesity. Obes Rev 5:197–216
153. **Belanger C, Luu-The V, Dupont P, Tchernof A** 2002 Adipose tissue intracrinology: potential importance of local androgen/estrogen metabolism in the regulation of adiposity. Horm Metab Res 34:737–745
154. **Simpson ER, Zhao Y, Agarwal VR, Michael MD, Bulun SE, Hinshelwood MM, Graham-Lorence S, Sun T, Fisher CR, Qin K, Mendelson CR** 1997 Aromatase expression in health and disease. Rec Prog Hormone Res 52:185–213
155. **Yang K, Khalil MW, Strutt BJ, Killinger DW** 1997 11 β-Hydroxysteroid dehydrogenase 1 activity and gene expression in human adipose stromal cells: effect on aromatase activity. J Steroid Biochem Mol Biol 60:247–253
156. **Tchernof A, Desmeules A, Richard C, Laberge P, Daris M, Mailloux J, Rheaume C, Dupont P** 2004 Ovarian hormone status and abdominal visceral adipose tissue metabolism. J Clin Endocrinol Metab 89:3425–3430
157. **Simpson ER, Misso M, Hewitt KN, Hill RA, Boon WC, Jones ME, Kovacic A, Zhou J, Clyne CD** 2005 Estrogen—the good, the bad, and the unexpected. Endocr Rev 26:322–330
158. **Carr MC** 2003 The emergence of the metabolic syndrome with menopause. J Clin Endocrinol Metab 88:2404–2411
159. **Jensen J, Nilas L, Christiansen C** 1990 Influence of menopause on serum lipids and lipoproteins. Maturitas 12:321–331
160. **Bruschi F, Meschia M, Soma M, Perotti D, Paoletti R, Crosignani PG** 1996 Lipoprotein(a) and other lipids after oophorectomy and estrogen replacement therapy. Obstet Gynecol 88:950–954
161. **Kanaley JA, Sames C, Swisher L, Swick AG, Ploutz-Snyder LL, Steppan CM, Sagendorf KS, Feiglin D, Jaynes EB, Meyer RA, Weinstock RS** 2001 Abdominal fat distribution in pre- and postmenopausal women: the impact of physical activity, age, and menopausal status. Metabolism 50:976–982
162. **Guthrie JR, Dennerstein L, Dudley EC** 1999 Weight gain and the menopause: a 5-year prospective study. Climacteric 2:205–211
163. **Carr MC, Brunzell JD,** Increased hepatic lipase activity and intraabdominal fat across the transition from pre- to postmenopause. Program of the 85th Annual Meeting of The Endocrine Society, Philadelphia, PA, 2003, p 374 (Abstract P2–280)
164. **Matthews KA, Meilahn E, Kuller LH, Kelsey SF, Caggiula AW, Wing RR** 1989 Menopause and risk factors for coronary heart disease. N Engl J Med 321:641–646
165. **Blumel JE, Castelo-Branco C, Rocangliolo ME, Bifa L, Tacla X, Mamani L** 2001 Changes in body mass index around menopause: a population study of Chilean woman. Menopause 8:239–244
166. **Bjorkelund C, Lissner L, Andersson S, Lapidus L, Bengtsson C** 1996 Reproductive history in relation to relative weight and fat distribution. Int J Obes Relat Metab Disord 20:213–219
167. **Key TJ, Allen NE, Verkasalo PK, Banks E** 2001 Energy balance and cancer: the role of sex hormones. Proc Nutr Soc 60:81–89
168. **Santoro N, Lasley B, McConnell D, Allsworth J, Crawford S, Gold EB, Finkelstein JS, Greendale GA, Kelsey J, Korenman S, Luborsky JL, Matthews K, Midgley R, Powell L, Sabatine J, Schocken M, Sowers MF, Weiss G** 2004 Body size and ethnicity are associated with menstrual cycle alterations in women in the early menopausal transition: The Study of Women's Health across the Nation (SWAN) Daily Hormone Study. J Clin Endocrinol Metab 89:2622–2631
169. **Verkasalo PK, Thomas HV, Appleby PN, Davey GK, Key TJ** 2001 Circulating levels of sex hormones and their relation to risk factors for breast cancer: a cross-sectional study in 1092 pre- and postmenopausal women (United Kingdom). Cancer Causes Control 12:47–59
170. **Misso ML, Jang C, Adams J, Tran J, Murata Y, Bell R, Boon WC, Simpson ER, Davis SR** 2005 Adipose aromatase gene expression is greater in older women and is unaffected by postmenopausal estrogen therapy. Menopause 12:210–215
171. **Norman RJ, Flight IH, Rees MC** 2000 Oestrogen and progestogen hormone replacement therapy for peri-menopausal and postmenopausal women: weight and body fat distribution. Cochrane Database Syst Rev (2):CD001018
172. **Gallo MF, Grimes DA, Schulz KF, Helmerhorst FM** 2004 Combination estrogen-progestin contraceptives and body weight: systematic review of randomized controlled trials. Obstet Gynecol 103:359–373
173. **Reubinoff BE, Grubstein A, Meirow D, Berry E, Schenker JG,**

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Brzezinski A 1995 Effects of low-dose estrogen oral contraceptives on weight, body composition, and fat distribution in young women. Fertil Steril 63:516–521
174. **Espeland MA, Stefanick ML, Kritz-Silverstein D, Fineberg SE, Waclawiw MA, James MK, Greendale GA** 1997 Effect of postmenopausal hormone therapy on body weight and waist and hip girths. Postmenopausal Estrogen-Progestin Interventions Study Investigators. J Clin Endocrinol Metab 82:1549–1556
175. **Mattiasson I, Rendell M, Tornquist C, Jeppsson S, Hulthen UL** 2002 Effects of estrogen replacement therapy on abdominal fat compartments as related to glucose and lipid metabolism in early postmenopausal women. Horm Metab Res 34:583–588
176. **Margolis KL, Bonds DE, Rodabough RJ, Tinker L, Phillips LS, Allen C, Bassford T, Burke G, Torrens J, Howard BV** 2004 Effect of oestrogen plus progestin on the incidence of diabetes in postmenopausal women: results from the Women's Health Initiative Hormone Trial. Diabetologia 47:1175–1187
177. **Cooke PS, Naaz A** 2004 Role of estrogens in adipocyte development and function. Exp Biol Med 229:1127–1135
178. **D'Eon TM, Souza SC, Aronovitz M, Obin MS, Fried SK, Greenberg AS** 2005 Estrogen regulation of adiposity and fuel partitioning: Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. J Biol Chem 280:35983–35991
179. **Sites CK, L'hommedieu GD, Toth MJ, Brochu M, Cooper BC, Fairhurst PA** 2005 The effect of hormone replacement therapy on body composition, body fat distribution, and insulin sensitivity in menopausal women: a randomized, double-blind, placebo-controlled trial. J Clin Endocrinol Metab 90:2701–2707
180. **Wisse BE** 2004 The inflammatory syndrome: the role of adipose tissue cytokines in metabolic disorders linked to obesity. J Am Soc Nephrol 15:2792–2800
181. **Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Jr AW** 2003 Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796–1808
182. **Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH** 1998 Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation 98:731–733
183. **Venugopal SK, Devaraj S, Jialal I** 2005 Effect of C-reactive protein on vascular cells: evidence for a proinflammatory, proatherogenic role. Curr Opin Nephrol Hypertens 14:33–37
184. **Pradhan AD, Manson JE, Rossouw JE, Siscovick DS, Mouton CP, Rifai N, Wallace RB, Jackson RD, Pettinger MB, Ridker PM** 2002 Inflammatory biomarkers, hormone replacement therapy, and incident coronary heart disease: prospective analysis from the Women's Health Initiative observational study. JAMA 288:980–987
185. **Shaywitz SE, Naftolin F, Zelterman D, Marchione KE, Holahan JM, Palter SF, Shaywitz BA** 2003 Better oral reading and short-term memory in midlife, postmenopausal women taking estrogen. Menopause 10:420–426
186. **Straub RH, Hense HW, Andus T, Scholmerich J, Riegger GA, Schunkert H** 2000 Hormone replacement therapy and interrelation between serum interleukin-6 and body mass index in postmenopausal women: a population-based study. J Clin Endocrinol Metab 85:1340–1344
187. **Uhlar CM, Whitehead AS** 1999 Serum amyloid A, the major vertebrate acute-phase reactant. Eur J Biochem 265:501–523
188. **Hu P, Greendale GA, Palla SL, Reboussin BA, Herrington DM, Barrett-Connor E, Reuben DB** 2006 The effects of hormone therapy on the markers of inflammation and endothelial function and plasma matrix metalloproteinase-9 level in postmenopausal women. The Postmenopausal Estrogen Progestin Intervention (PEPI) trial. Atherosclerosis 185:347–352
189. **Langer RD, Pradhan AD, Lewis CE, Manson JE, Rossouw JE, Hendrix SL, LaCroix AZ, Ridker PM** 2005 Baseline associations between postmenopausal hormone therapy and inflammatory, haemostatic, and lipid biomarkers of coronary heart disease. The Women's Health Initiative Observational Study. Thromb Haemost 93:1108–1116
190. **Davison S, Davis SR** 2003 New markers for cardiovascular disease risk in women: impact of endogenous estrogen status and exogenous postmenopausal hormone therapy. J Clin Endocrinol Metab 88:2470–2478
191. **Cheleuitte D, Mizuno S, Glowacki J** 1998 *In vitro* secretion of cytokines by human bone marrow: effects of age and estrogen status. J Clin Endocrinol Metab 83:2043–2051
192. **Rogers A, Eastell R** 2001 The effect of 17β-estradiol on production of cytokines in cultures of peripheral blood. Bone 29:30–34
193. **Chait A, Han CY, Oram JF, Heinecke JW** 2005 Thematic review series: the immune system and atherogenesis. Lipoprotein-associated inflammatory proteins: markers or mediators of cardiovascular disease? J Lipid Res 46:389–403
194. **Connelly PW, Hanley AJ, Harris SB, Hegele RA, Zinman B** 2003 Relation of waist circumference and glycemic status to C-reactive protein in the Sandy Lake Oji-Cree. Int J Obes Relat Metab Disord 27:347–354
195. **Abbas A, Fadel PJ, Wang Z, Arbique D, Jialal I, Vongpatanasin W** 2004 Contrasting effects of oral versus transdermal estrogen on serum amyloid A (SAA) and high-density lipoprotein-SAA in postmenopausal women. Arterioscler Thromb Vasc Biol 24:e164–e167
196. **Miller VM, Shuster LT, Hayes SN** 2003 Controversy of hormone treatment and cardiovascular function: need for strengthened collaborations between preclinical and clinical scientists. Curr Opin Investig Drugs 4:1220–1232
197. 2005 Biology of perimenopause: impact on health and aging workshop summary report. Bethesda, MD: National Institute on Aging/National Institutes of Health. http://www.nia.nih.gov/ResearchInformation/ConferencesAndMeetings/Biology+of+the+Perimenopause+-+Impact+on+Health+and+Aging+Workshop.htm
198. **McDonnell DP** 1999 The molecular pharmacology of SERMs. Trends Endocrinol Metab 10:301–311
199. **Mendelsohn ME, Karas RH** 1994 Estrogen and the blood vessel wall. Curr Opin Cardiol 9:619–626
200. **Farhat MY, Lavigne MC, Ramwell PW** 1996 The vascular protective effects of estrogen. FASEB J 10:615–624
201. **Mendelsohn ME, Karas RH** 1999 Mechanisms of disease: the protective effects of estrogen on the cardiovascular system. N Engl J Med 340:1801–1811
202. **Mendelsohn ME, Karas RH** 2005 Molecular and cellular basis of cardiovascular gender differences. Science 308:1583–1587
203. **Stumpf WE, Sar M, Aumuller G** 1977 The heart: a target organ for estradiol. Science 196:319–321
204. **McGill Jr HC, Anselmo VC, Buchanan JM, Sheridan PJ** 1980 The heart is a target organ for androgen. Science 207:775–777
205. **Horwitz KB, Horwitz LD** 1982 Canine vascular tissues are targets for androgens, estrogens, progestins, and glucocorticoids. J Clin Invest 69:750–758
206. **Ingegno MD, Money SR, Thelmo W, Greene GL, Davidian M, Jaffe BM, Pertschuk LP** 1988 Progesterone receptors in the human heart and great vessels. Lab Invest 59:353–356
207. **Karas RH, Patterson BL, Mendelsohn ME** 1994 Human vascular smooth muscle cells contain functional estrogen receptor. Circulation 89:1943–1950
208. **Venkov CD, Rankin AB, Vaughan DE** 1996 Identification of authentic estrogen receptor in cultured endothelial cells. A potential mechanism for steroid hormone regulation of endothelial function. Circulation 94:727–733
209. **Caulin-Glaser T, Watson CA, Pardi R, Bender JR** 1996 Effects of 17β-estradiol on cytokine-induced endothelial cell adhesion molecule expression. J Clin Invest 98:36–42
210. **Cudeville C, Mondon F, Robert B, Rebourcet R, Mignot TM, Benassayag C, Ferre F** 2000 Evidence for progesterone receptors in the human fetoplacental vascular tree. Biol Reprod 62:759–765
211. **Diano S, Horvath TL, Mor G, Register T, Adams M, Harada N, Naftolin F** 1999 Aromatase and estrogen receptor immunoreactivity in the coronary arteries of monkeys and human subjects. Menopause 6:21–28
212. **Bayard F, Clamens S, Meggetto F, Blaes N, Delsol G, Faye JC** 1995 Estrogen synthesis, estrogen metabolism, and functional estrogen receptors in rat arterial smooth muscle cells in culture. Endocrinology 136:1523–1529
213. **Sasano H, Murakami H, Shizawa S, Satomi S, Nagura H, Harada N** 1999 Aromatase and sex steroid receptors in human vena cava. Endocr J 46:233–242
214. **Mukherjee TK, Dinh H, Chaudhuri G, Nathan L** 2002 Testosterone attenuates expression of vascular cell adhesion molecule-1 by

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conversion to estradiol by aromatase in endothelial cells: implications in atherosclerosis. Proc Natl Acad Sci USA 99:4055–4060
215. **Simpson ER, Davis SR** 2001 Minireview: aromatase and the regulation of estrogen biosynthesis—some new perspectives. Endocrinology 142:4589–4594
216. **Harada N, Sasano H, Murakami H, Ohkuma T, Nagura H, Takagi Y** 1999 Localized expression of aromatase in human vascular tissues. Circ Res 84:1285–1291
217. **Mendelsohn ME, Rosano GM** 2003 Hormonal regulation of normal vascular tone in males. Circ Res 93:1142–1145
218. **Sudhir K, Komesaroff PA** 1999 Clinical review 110: cardiovascular actions of estrogens in men. J Clin Endocrinol Metab 84:3411–3415
219. **Lew R, Komesaroff P, Williams M, Dawood T, Sudhir K** 2003 Endogenous estrogens influence endothelial function in young men. Circ Res 93:1127–1133
220. **Huhtaniemi I** 1995 Molecular aspects of the ontogeny of the pituitary-gonadal axis. Reprod Fertil Dev 7:1025–1035
221. **Barker DJP** 1995 Fetal origins of coronary heart disease. BMJ 311: 171–174
222. **Oren A, Vos LE, Uiterwaal CS, Gorissen WH, Grobee DE, Bots ML** 2006 Birth weight and carotid intimal-media thickness: new perspectives from the atherosclerosis risk in young adults (ARYA) study. Ann Epidemiol 14:8–16
223. **Khosrotehrani K, Bianchi DW** 2005 Multi-lineage potential of fetal cells in maternal tissue: a legacy in reverse. J Cell Sci 118:1559–1563
224. **Muller JM, Isele U, Metzger E, Rempel A, Moser M, Pscherer A, Breyer T, Holubarsch C, Buettner R, Schule R** 2000 FHL2, a novel tissue-specific coactivator of the androgen receptor. EMBO J 19: 359–369
225. **Yuan Y, Liao L, Tulis DA, Xu J** 2002 Steroid receptor coactivator-3 is required for inhibition of neointima formation by estrogen. Circulation 105:2653–2659
226. **Sullivan Jr TR, Karas RH, Aronovitz M, Faller GT, Ziar JP, Smith JJ, O'Donnell Jr TF, Mendelsohn ME** 1995 Estrogen inhibits the response-to-injury in a mouse carotid artery model. J Clin Invest 96:2482–2488
227. **Pare G, Krust A, Karas RH, Dupont S, Aronovitz M, Chambon P, Mendelsohn ME** 2002 Estrogen receptor-α mediates the protective effects of estrogen against vascular injury. Circ Res 90:1087–1092
228. **Wu RC, Qin J, Yi P, Wong J, Tsai SY, Tsai MJ, O'Malley BW** 2004 Selective phosphorylations of the SRC-3/AIB1 coactivator integrate genomic responses to multiple cellular signaling pathways. Mol Cell 15:937–949
229. **Grumbach MM, Auchus RJ** 1999 Estrogen: consequences and implications of human mutations in synthesis and action. J Clin Endocrinol Metab 84:4677–4694
230. **Djouadi F, Weinheimer CJ, Saffitz JE, Pitchford C, Bastin J, Gonzalez FJ, Kelly DP** 1998 A gender-related defect in lipid metabolism and glucose homeostasis in peroxisome proliferator-activated receptor α-deficient mice. J Clin Invest 102:1083–1091
231. **Czubryt MP, McAnally J, Fishman GI, Olson EN** 2003 Regulation of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) and mitochondrial function by MEF2 and HDAC5. Proc Natl Acad Sci USA 100:1711–1716
232. **Repa JJ, Mangelsdorf DJ** 2002 The liver X receptor gene team: potential new players in atherosclerosis. Nat Med 8:1243–1248
233. **Barish GD, Evans RM** 2004 PPARs and LXRs: atherosclerosis goes nuclear. Trends Endocrinol Metab 15:158–165
234. **Lundholm L, Moverare S, Steffensen KR, Nilsson M, Otsuki M, Ohlsson C, Gustafsson JA, Dahlman-Wright K** 2004 Gene expression profiling identifies liver X receptor α as an estrogen-regulated gene in mouse adipose tissue. J Mol Endocrinol 32:879–892
235. **Clarkson TB** 2002 The new conundrum: do estrogens have any cardiovascular benefits? Int J Fertil Womens Med 47:61–68
236. **LaRosa JC** 1996 The pharmacology and effectiveness of specific lipid-active drugs: estrogen/sex steroids. Coron Artery Dis 7:683–688
237. **Seed M, Knopp RH** 2004 Estrogens, lipoproteins, and cardiovascular risk factors: an update following the randomized placebo-controlled trials of hormone-replacement therapy. Curr Opin Lipidol 15:459–467
238. **Gorodeski GI** 2002 Update on cardiovascular disease in postmenopausal women. Best Pract Res Clin Obstet Gynaecol 16:329–355
239. **Braunstein JB, Kershner DW, Bray P, Gerstenblith G, Schulman SP, Post WS, Blumenthal RS** 2002 Interaction of hemostatic genetics with hormone therapy: new insights to explain arterial thrombosis in postmenopausal women. Chest 121:906–920
240. **Kim RJ, Becker RC** 2003 Association between factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T mutations and events of the arterial circulatory system: a meta-analysis of published studies. Am Heart J 146:948–957
241. **Herrington DM** 2003 Role of estrogen receptor-α in pharmacogenetics of estrogen action. Curr Opin Lipidol 14:145–150
242. **Khetawat G, Faraday N, Nealen ML, Vijayan KV, Bolton E, Noga SJ, Bray PF** 2000 Human megakaryocytes and platelets contain the estrogen receptor β and androgen receptor (AR): testosterone regulates AR expression. Blood 95:2289–2296
243. **Jayachandran M, Okano H, Chatrath R, Owen WG, McConnell JP, Miller VM** 2004 Sex-specific changes in platelet aggregation and secretion with sexual maturity in pigs. J Appl Physiol 97:1445–1452
244. **Leng XH, Hong SY, Larrucea S, Zhang W, Li TT, Lopez JA, Bray PF** 2004 Platelets of female mice are intrinsically more sensitive to agonists than are platelets of males. Arterioscler Thromb Vasc Biol 24:376–381
245. **McGill Jr HC** 1989 Sex steroid hormone receptors in the cardiovascular system. Postgrad Med, April, Spec No: 64–68
246. **Forster C, Kietz S, Hultenby K, Warner M, Gustafsson JA** 2004 Characterization of the ER$\beta-/-$ mouse heart. Proc Natl Acad Sci USA 101:14234–14239
247. **Saunders PT, Maguire SM, Gaughan J, Millar MR** 1997 Expression of oestrogen receptor β (ER β) in multiple rat tissues visualised by immunohistochemistry. J Endocrinol 154:R13–R16
248. **Savolainen H, Frosen J, Petrov L, Aavik E, Hayry P** 2001 Expression of estrogen receptor sub-types α and β in acute and chronic cardiac allograft vasculopathy. J Heart Lung Transplant 20:1252–1264
249. **Nuedling S, Karas RH, Mendelsohn ME, Katzenellenbogen JA, Katzenellenbogen BS, Meyer R, Vetter H, Grohe C** 2001 Activation of estrogen receptor β is a prerequisite for estrogen-dependent upregulation of nitric oxide synthases in neonatal rat cardiac myocytes. FEBS Lett 502:103–108
250. **Grohé C, Kahlert S, Löbbert K, Vetter H** 1998 Expression of oestrogen receptor α and β in rat heart: role of local oestrogen synthesis. J Endocrinol 156:R1–R7
251. **Jankowski M, Rachelska G, Donghao W, McCann SM, Gutkowska J** 2001 Estrogen receptors activate atrial natriuretic peptide in the rat heart. Proc Natl Acad Sci USA 98:11765–11770
252. **Pedram A, Razandi M, Aitkenhead M, Levin ER** 2005 Estrogen inhibits cardiomyocyte hypertrophy in vitro: antagonism of calcineurin-related hypertrophy through induction of MCIP1. J Biol Chem 280:26339–26348
253. **Shain SA, Lin AL, McGill Jr HC** 1988 Steroid receptors in the cardiovascular system: potential physiologic significance. In: Sheridan PJ, Blum K, Trachtenberg MC, eds. Steroid receptors and disease: cancer, autoimmune, bone and circulatory disorders. New York: Marcel Dekker, Inc.; 549–567
254. **Marsh JD, Lehmann MH, Ritchie RH, Gwathmey JK, Green GE, Schiebinger RJ** 1998 Androgen receptors mediate hypertrophy in cardiac myocytes. Circulation 98:256–261
255. **Thum T, Borlak J** 2002 Testosterone, cytochrome P450, and cardiac hypertrophy. FASEB J 16:1537–1549
256. **Villareal RP, Woodruff AL, Massumi A** 2001 Gender and cardiac arrhythmias. Tex Heart Inst J 28:265–275
257. **Larsen JA, Kadish AH** 1998 Effects of gender on cardiac arrhythmias. J Cardiovasc Electrophysiol 9:655–664
258. **Pham TV, Rosen MR** 2002 Sex, hormones, and repolarization. Cardiovasc Res 53:740–751
259. **Nagy E, Orvos H, Bardos G, Molnar P** 2000 Gender-related heart rate differences in human neonates. Pediatr Res 47:778–780
260. **Brouillette J, Rivard K, Lizotte E, Fiset C** 2005 Sex and strain differences in adult mouse cardiac repolarization: importance of androgens. Cardiovasc Res 65:148–157

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261. **Barnard DD** 2005 Heart failure in women. Curr Cardiol Rep 7:159–165
262. **Lund LH, Mancini D** 2004 Heart failure in women. Med Clin North Am 88:1321–1345
263. **Golden KL, Marsh JD, Jiang Y** 2004 Testosterone regulates mRNA levels of calcium regulatory proteins in cardiac myocytes. Horm Metab Res 36:197–202
264. **Vizgirda VM, Wahler GM, Sondgeroth KL, Ziolo MT, Schwertz DW** 2002 Mechanisms of sex differences in rat cardiac myocyte response to β-adrenergic stimulation. Am J Physiol Heart Circ Physiol 282:H256–H263
265. **Xin HB, Senbonmatsu T, Cheng DS, Wang YX, Copello JA, Ji GJ, Collier ML, Deng KY, Jeyakumar LH, Magnuson MA, Inagami T, Kotlikoff MI, Fleischer S** 2002 Oestrogen protects FKBP12.6 null mice from cardiac hypertrophy. Nature 416:334–338
266. **McEwen BS, Alves SE** 1999 Estrogen actions in the central nervous system. Endocr Rev 20:279–307
267. **McCarthy MM, Konkle AT** 2005 When is a sex difference not a sex difference? Front Neuroendocrinol 26:85–102
268. **Toran-Allerand CD** 2005 Estrogen and the brain: beyond ER-α, ER-β, and 17β-estradiol. Ann NY Acad Sci 1052:136–144
269. **Garcia-Segura LM, McCarthy MM** 2004 Minireview: role of glia in neuroendocrine function. Endocrinology 145:1082–1086
270. **Garcia-Ovejero D, Azcoitia I, DonCarlos LL, Melcangi RC, Garcia-Segura LM** 2005 Glia-neuron crosstalk in the neuroprotective mechanisms of sex steroid hormones. Brain Res Brain Res Rev 48:273–286
271. **Rapp SR, Espeland MA, Shumaker SA, Henderson VW, Brunner RL, Manson JE, Gass MLS, Stefanick ML, Lane DS, Hays J, Johnson KC, Coker LH, Dailey M, Bowen D** 2003 Effect of estrogen plus progestin on global cognitive function in postmenopausal women. JAMA 289:2663–2672
272. **Shumaker SA, Legault C, Rapp SR, Thal LJ, Wallace RB, Ockene JK, Hendrix SL, Jones BN, Annlouise RA, Jackson RD, Kotchen JM, Wassertheil-Smoller S, Wactawski-Wende J** 2003 Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women. JAMA 289:2651–2662
273. **Wassertheil-Smoller S, Hendrix SL, Limacher M, Heiss G, Kooperberg C, Baird A, Kotchen T, Curb JD, Black H, Rossouw JE, Aragaki A, Safford M, Stein E, Laowattana S, Mysiw WJ** 2003 Effect of estrogen plus progestin on stroke in postmenopausal women. JAMA 289:2673–2684
274. **Ogita H, Node K, Asanuma H, Sanada S, Liao Y, Takashima S, Asakura M, Mori H, Shinozaki Y, Hori M, Kitakazi M** 2002 Amelioration of ischemia- and reperfusion-induced myocardial injury by the selective estrogen receptor modulator, raloxifene, in the canine heart. J Am Coll Cardiol 40:998–1005
275. **Ciriza I, Carrero P, Azcoitia I, Lundeen SG, Garcia-Segura LM** 2004 Selective estrogen receptor modulators protect hippocampal neurons from kainic acid excitotoxicity: differences with the effect of estradiol. J Neurobiol 61:209–221
276. **Lacreuse A, Wilson ME, Herndon JG** 2002 Estradiol, but not raloxifene, improves aspects of spatial working memory in aged ovariectomized rhesus monkeys. Neurobiol Aging 23:589–600
277. **Nilsen J, Mor G, Naftolin F** 1998 Raloxifene induces neurite outgrowth in estrogen receptor positive PC12 cells. Menopause 2:211–216
278. **O'Neill K, Chen S, Brinton RD** 2004 Impact of the selective estrogen receptor modulator, raloxifene, on neuronal survival and outgrowth following toxic insults associated with aging and Alzheimer's disease. Exp Neurol 185:63–80
279. **Pinilla L, Gonzalez LC, Tena-Sempere M, Aguilar E** 2001 Evidence for an estrogen-like action of raloxifene upon the hypothalamic-pituitary unit: raloxifene inhibits luteinizing hormone secretion and stimulates prolactin secretion in ovariectomized female rats. Neurosci Lett 311:149–152
280. **Bethea CL, Mirkes SJ, Su A, Michelson D** 2002 Effects of oral estrogen, raloxifene and arzoxifene on gene expression in serotonin neurons of macaques. Psychoneuroendocrinology 27:431–445
281. **Pinilla L, Barreiro ML, Gonzalez LC, Tena-Sempere M, Aguilar E** 2002 Comparative effects of testosterone proprionate, oestradiol benzoate, ICI 182,780, tamoxifen and raloxifene on hypothalamic differentiation in the female rat. J Endocrinol 172:441–448
282. **Rossberg MI, Murphy SJ, Traystman RJ, Hurn PD** 2000 LY353381.HCI, a selective estrogen receptor modulator, and experimental stroke. Stroke 31:3041–3046
283. **Yaffe K, Krueger K, Cummings SR, Blackwell T, Henderson VW, Sarkar S, Ensrud K, Grady D** 2005 Effect of raloxifene on prevention of dementia and cognitive impairment in older women: the multiple outcomes of raloxifene evaluation (MORE) randomized trial. Am J Psychiatry 162:683–690
284. **Mayer LP, Pearsall NA, Christian PJ, Cevine PJ, Payne CM, McCluskey MK, Marion SL, Sipes IG, Hoyer PB** 2002 Long-term effects of ovarian follicular depletion in rats by 4-vinylcyclohexene diepoxide. Reprod Toxicol 16:775–781
285. **Thompson KE, Bourguet SM, Christian PJ, Benedict JC, Sipes IG, Flaws JA, Hoyer PB** 2005 Differences between rats and mice in the involvement of the aryl hydrocarbon receptor in 4-vinylcyclohexene diepoxide-induced ovarian follicle loss. Toxicol Appl Pharmacol 203:114–123
286. **Jacobson CD, Gorski RA** 1981 Neurogenesis of the sexually dimorphic nucleus of the preoptic area of the rat. J Comp Neurol 196:519–529
287. **Arai Y, Murakami S, Nishizuka M** 1994 Androgen enhances neuronal degeneration in the developing preoptic area: apoptosis in the anteroventral periventricular nucleus (AVPvN-POA). Horm Behav 28:313–319
288. **Breedlove SM** 1985 Hormonal control of the anatomical specificity of motoneuron to muscle innervation in rats. Science 227:1357–1359
289. **Sengelaub DR, Arnold AP** 1986 Development and loss of early projections in a sexually dimorphic rat spinal nucleus. J Neurosci 6:1613–1620
290. **Jordan CL, Letinsky MS, Arnold AP** 1989 The role of gonadal hormones in neuromuscular synapse elimination in rats. I. Androgen delays the loss of multiple innervation in the levator ani muscle. J Neurosci 9:229–238
291. **Mong JA, Glaser E, McCarthy MM** 1999 Gonadal steroids promote glial differentiation and alter neuronal morphology in the developing hypothalamus in a regionally specific manner. J Neurosci 19:1464–1472
292. **Toran-Allerand CD, Singh M, Setalo G** 1999 Novel mechanisms of estrogen action in the brain: new players in an old story. Front Neuroendocrinol 20:97–121
293. **Beyer C** 1999 Estrogen and the developing mammalian brain. Anat Embryol 199:379–390
294. **Woolley CS** 1999 Effects of estrogen in the CNS. Curr Opin Neurobiol 9:345–354
295. **Cooke BM, Woolley CS** 2004 Gonadal hormone modulation of dendrites in the mammalian CNS. J Neurobiol 64:34–46
296. **Hao J, Rapp PR, Leffler AE, Leffler SR, Janssen WG, Lou W, Mckay H, Roberts JA, Wearne SL, Hof PR, Morrison JH** 2006 Estrogen alters spine number and morphology in prefrontal cortex of aged female rhesus monkeys. J Neurosci 26:2571–2578
297. **Emerson CS, Headrick JP, Vink R** 1993 Estrogen improves biochemical and neurologic outcome following traumatic brain injury in male rats, but not in females. Brain Res 608:95–100
298. **Saiyed M, Riker WK** 1993 Cholinergic and anticholinergic drug effects on survival during hypoxia: significant gender differences. J Pharmacol Exp Ther 264:1146–1153
299. **Nishikawa T, Kirsch JR, Koehler RC, Miyabe M, Traystman RJ** 1994 Competitive N-methyl-D-aspartate receptor blockade reduces brain injury following transient focal ischemia in cats. Stroke 25: 2258–2264
300. **Pike CJ** 1999 Estrogen modulates neuronal Bcl-x_L expression and β-amyloid-induced apoptosis: relevance to Alzheimer's disease. J Neurochem 72:1552–1563
301. **Dubal DB, Kashon ML, Pettigrew LC, Ren JM, Finklestein SP, Rau SW, Wise PM** 1998 Estradiol protects against ischemic injury. J Cereb Blood Flow Metab 18:1253–1258
302. **Rusa R, Alkayed NJ, Crain BJ, Traystman RJ, Kimes AS, London ED, Klaus JA, Hurn PD** 1999 17β-Estradiol reduces stroke injury in estrogen-deficient female animals. Stroke 30:1665–1670
303. **Simpkins JW, Rajakumar G, Zhang Y-Q, Simpkins CE, Greenwald D, Yu CJ, Bodor N, Day AL** 1997 Estrogens may reduce mortality and ischemic damage caused by middle cerebral artery occlusion in the female rat. J Neurosurg 87:724–730

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304. **Hall ED, Pazara KE, Linseman KL** 1991 Sex differences in post-ischemic neuronal necrosis in gerbils. J Cereb Blood Flow Metab 11:292–298
305. **Zhang Y-Q, Shi J, Rajakumar G, Day AL, Simpkins JW** 1998 Effects of gender and estradiol treatment on focal brain ischemia. Brain Res 784:321–324
306. **Wang Q, Santizo R, Baughman VL, Pelligrino DA, Iadecola C** 1999 Estrogen provides neuroprotection in transient forebrain ischemia through perfusion-independent mechanisms in rats. Stroke 30:630–637
307. **Alkayed NJ, Harukuni I, Kimes AS, London ED, Traystman RJ, Hurn PD** 1998 Gender-linked brain injury in experimental stroke. Stroke 29:159–166
308. **Yang S-H, Shi J, Day AL, Simpkins JW** 2000 Estradiol exerts neuroprotective effects when administered after ischemic insult. Stroke 31:745–750
309. **Back T, Ginsberg MD, Dietrich WD, Watson BD** 1996 Induction of spreading depression in the ischemic hemisphere following experimental middle cerebral artery occlusion: effect on infarct morphology. J Cereb Blood Flow Metab 16:202–213
310. **Green PS, Gridley KE, Simpkins JW** 1996 Estradiol protects against β-amyloid (25–35)-induced toxicity in SK-N-SH human neuroblastoma cells. Neurosci Lett 218:165–168
311. **Xu H, Gouras GK, Greenfield JP, Vincent B, Naslund J, Mazzarelli L, Fried G, Javonovic JN, Seeger M, Relkin NR, Liao F, Checler F, Buxbaum JD, Chait BT, Thinakaran G, Sisodia SS, Wang R, Greengard P, Gandy S** 1998 Estrogen reduces neuronal generation of Alzheimer β-amyloid peptides. Nat Med 4:447–451
312. **Fader AJ, Hendricson AW, Dohanich GP** 1998 Estrogen improves performance of reinforced t-maze alternation and prevents the amnestic effects of scopolamine administered systemically or intrahippocampally. Neurobiol Learn Mem 69:225–240
313. **Gridley KE, Green PS, Simpkins JW** 1997 Low concentrations of estradiol reduce β-amyloid (25–35)-induced toxicity, lipid peroxidation and glucose utilization in human SK-N-SH neuroblastoma cells. Brain Res 778:158–165
314. **Vedder H, Anthes N, Stumm G, Wurz C, Behl C, Krieg JC** 1999 Estrogen hormones reduce lipid peroxidation in cells and tissues of the central nervous system. J Neurochem 72:2531–2538
315. **Zaulyanov LL, Green PS, Simpkins JW** 1999 Glutamate receptor requirement for neuronal cell death from anoxia-reoxygenation: an in vitro model for assessment of the neuroprotective effects of estrogens. Cell Mol Neurobiol 19:705–718
316. **Wilson ME, Dubal DB, Wise PM** 2000 Estradiol protects against injury-induced cell death in cortical explant cultures: a role for estrogen receptors. Brain Res 873:235–242
317. **Regan RF, Guo Y** 1997 Estrogens attenuate neuronal injury due to hemoglobin, chemical hypoxia, and excitatory amino acids in murine cortical cultures. Brain Res 764:133–140
318. **Singer CA, Figueroa-Masot CD, Batchelor RH, Dorsa DM** 1999 The mitogen-activated protein kinase pathway mediates estrogen neuroprotection after glutamate toxicity in primary cortical neurons. J Neurosci 19:2455–2463
319. **Weaver Jr CE, Park-Chung M, Gibbs TT, Farb DH** 1997 17-β-Estradiol protects against NMDA-induced excitotoxicity by direct inhibition of NMDA receptors. Brain Res 761:338–341
320. **Dubal DB, Shughrue PJ, Wilson ME, Merchenthaler I, Wise PM** 1999 Estradiol modulates Bcl-2 in cerebral ischemia: a potential role for estrogen receptors. J Neurosci 19:6385–6393
321. **Rau SW, Dubal DB, Böttner M, Wise PM** 2003 Estradiol differentially regulates c-Fos after focal cerebral ischemia. J Neurosci 23:10487–10494
322. **Singer CA, Rogers KL, Dorsa DM** 1998 Modulation of Bcl-2 expression: a potential component of estrogen protection in NT2 neurons. Neuroreport 9:2565–2568
323. **Singh M, Setalo G, Guan X, Frail DE, Toran-Allerand CD** 2000 Estrogen-induced activation of the mitogen-activated protein kinase cascade in the cerebral cortex of estrogen receptor-α knock-out mice. J Neurosci 20:1694–1700
324. **Fitzpatrick JL, Mize AL, Wade CB, Harris JA, Shapiro RA, Dorsa DM** 2002 Estrogen-mediated neuroprotection against β-amyloid toxicity requires expression of estrogen receptor α or β and activation of the MAPK pathway. J Neurochem 82:674–682
325. **Wilson ME, Liu Y, Wise PM** 2002 Estradiol enhances Akt activation in cortical explant cultures following neuronal injury. Mol Brain Res 102:48–54
326. **Singh M, Setalo Jr G, Guan X, Warren M, Toran-Allerand CD** 1999 Estrogen-induced activation of mitogen-activated protein kinase in cerebral cortical explants: convergence of estrogen and neurotrophin signaling pathways. J Neurosci 19:1179–1188
327. **Murphy DD, Segal M** 1997 Morphological plasticity of dendritic spines in central neurons is mediated by activation of cAMP response element binding protein. Proc Natl Acad Sci USA 94:1482–1487
328. **Zhou Y, Watters JJ, Dorsa DM** 1996 Estrogen rapidly induces the phosphorylation of the cAMP response element binding protein in rat brain. Endocrinology 137:2163–2166
329. **Woolley CS, McEwen BS** 1992 Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. J Neurosci 12:2549–2554
330. **Dubal DB, Zhu B, Yu B, Rau SW, Shughrue PJ, Merchenthaler I, Kindy MS, Wise PM** 2001 Estrogen receptor-α, not -β, is a critical link in estradiol-mediated protection against brain injury. Proc Natl Acad Sci USA 98:1952–1957
331. **Miranda RC, Sohrabji F, Toran-Allerand CD** 1993 Neuronal colocalization of mRNAs for neurotrophins and their receptors in the developing central nervous system suggests a potential for autocrine interactions. Proc Natl Acad Sci USA 90:6439–6443
332. **Murphy DD, Cole NB, Segal M** 1998 Brain-derived neurotrophic factor mediates estradiol-induced dendritic spine formation in hippocampal neurons. Proc Natl Acad Sci USA 95:11412–11417
333. **Murphy DD, Cole NB, Greenberger V, Segal M** 1998 Estradiol increases dendritic spine density by reducing GABA neurotransmission in hippocampal neurons. J Neurosci 18:2550–2559
334. **Murphy DD, Segal M** 1996 Regulation of dendritic spine density in cultured rat hippocampal neurons by steroid hormones. J Neurosci 16:4059–4068
335. **Duenas M, Torres-Aleman I, Naftolin F, Garcia-Segura LM** 1996 Interaction of insulin-like growth factor-I and estradiol signaling pathways on hypothalamic neuronal differentiation. Neuroscience 74:531–539
336. **Naftolin F, Leranth C, Horvath TL, Garcia-Segura LM** 1996 Potential neuronal mechanisms of estrogen actions in synaptogenesis and synaptic plasticity. Cell Mol Neurobiol 16:213–223
337. **Pelligrino DA, Galea E** 2002 Estrogen and cerebrovascular physiology and pathophysiology. Japan J Pharmacol 86:137–158
338. **Subbiah MT, Kessel B, Agrawal M, Rajan R, Abplanalp W, Rymaszewski A** 1993 Antioxidant potential of specific estrogens on lipid peroxidation. J Clin Endocrinol Metab 77:1095–1097
339. **Green PS, Gridley KE, Simpkins JW** 1998 Nuclear estrogen receptor-independent neuroprotection by estratrienes: a novel interaction with glutathione. Neuroscience 84:7–10
340. **Culmsee C, Vedder H, Ravati A, Junker V, Otto D, Ahlemeyer B, Krieg J-C, Krieglstein J** 1999 Neuroprotection by estrogens in a mouse model of focal cerebral ischemia and in cultured neurons: evidence for a receptor-independent antioxidative mechanism. J Cereb Blood Flow Metab 19:1263–1269
341. **Behl C, Skutella T, Lezoualch F, Post A, Widmann M, Newton CJ, Holsboer F** 1997 Neuroprotection against oxidative stress by estrogens: structure-activity relationship. Mol Pharmacol 51:535–541
342. **Fassbender K, Masters C, Beyreuther K** 2000 Alzheimer's disease: an inflammatory disease? Neurobiol Aging 21:433–436
343. **Stoll G, Jander S, Schroeter M** 2002 Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. Adv Exp Med Biol 513:87–113
344. **Allen SM, Rothwell NJ** 1996 Cytokines and acute neurodegeneration. Nat Rev Neurosci 2:734–744
345. **Mergenthaler P, Dirnagl U, Meisel A** 2004 Pathophysiology of stroke: lessons from animal models. Metab Brain Dis 19:151–167
346. **Streit WJ** 2002 Microglia as neuroprotective, immunocompetent cells of the CNS. Glia 40:133–139
347. **Maggi A, Ciana P, Brusadelli A, Belcredito S, Bonincontro C, Vegeto E** 2001 Are there biological bases for a beneficial effect of estrogens in neural diseases? Horm Behav 40:203–209
348. **Maggi A, Ciana P, Belcredito S, Vegeto E** 2004 Estrogens in the

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nervous system: mechanisms and nonreproductive functions. Annu Rev Physiol 66:291–313
349. **Vegeto E, Bonincontro C, Pollio G, Sala A, Viappiani S, Nardi F, Brusadelli A, Viviani B, Ciana P, Maggi A** 2001 Estrogen prevents the lipopolysaccharide-induced inflammatory response in microglia. J Neurosci 21:1809–1818
350. **Stoll G, Jander S, Schroeter M** 1998 Inflammation and glial responses in ischemic brain lesions. Prog Neurobiol 56:149–171
351. **Danton GH, Dietrich WD** 2003 Inflammatory mechanisms after ischemia and stroke. J Neuropathol Exp Neurol 62:127–136
352. **Cuzzocrea S, Mazzon E, Sautebin L, Serraino I, Dugo L, Calabro G, Caputi AP, Maggi A** 2001 The protective role of endogenous estrogens in carrageenan-induced lung injury in the rat. Mol Med 7:478–487
353. **Gruber CJ, Gruber DM, Gruber IM, Wieser F, Huber JC** 2004 Anatomy of the estrogen response element. Trends Endocrinol Metab 15:73–78
354. **McDonnell DP** 2003 Mining the complexities of the estrogen signaling pathways for novel therapeutics. Endocrinology 144:4237–4240
355. **del Zoppo G, Ginis I, Hallenbeck JM, Iadecola C, Wang X, Feurstein GZ** 2000 Inflammation and stroke: putative role for cytokines, adhesion molecules and iNOS in brain response to ischemia. Brain Pathol 10:95–112
356. **Iadecola C, Zhang F, Casey R, Nagayama M, Ross ME** 1997 Delayed reduction of ischemic brain injury and neurological deficits in mice lacking the inducible nitric oxide synthase gene. J Neurosci 17:9157–9164
357. **Nagayama M, Abert T, Nagayama T, Ross ME, Iadecola C** 1999 Age-dependent increase in ischemic brain injury in wild-type mice and in mice lacking the inducible nitric oxide synthase gene. J Cereb Blood Flow Metab 19:661–666
358. **Iadecola C, Zhang F, Xu S, Casey R, Ross ME** 1995 Inducible nitric oxide synthase gene expression in brain following cerebral ischemia. J Cereb Blood Flow Metab 15:378–384
359. **Sugimoto K, Iadecola C** 2002 Effects of aminoguanidine on cerebral ischemia in mice: comparison between mice with and without inducible nitric oxide synthase gene. Neurosci Lett 331:25–28
360. **Acalovschi D, Wiest T, Hartmann M, Farahmi M, Mansmann U, Auffarth GU, Grau AM, Green FR, Grond-Ginsbach C, Schwaninger M** 2003 Multiple levels of regulation of the interleukin-6 system in stroke. Stroke 34:1864–1870
361. **Fassbender K, Rossol S, Kammer T, Daffertshofer M, Wirth S, Dollman M, Hennerici M** 1994 Proinflammatory cytokines in serum of patients with acute cerebral ischemia: kinetics of secretion and relation to the extent of brain damage and outcome of disease. J Neurol Sci 122:135–139
362. **Perini F, Morra M, Alecci M, Galloni E, Marchi M, Toso V** 2001 Temporal profile of serum anti-inflammatory and pro-inflammatory interleukins in acute ischemic stroke patients. Neurol Sci 22:289–296
363. **Hanisch U-K** 2002 Microglia as a source and target of cytokines. Glia 40:140–155
364. **Matejuk A, Adlard K, Zamora A, Silverman M, Vandenbark AA, Offner H** 2001 17 β-Estradiol inhibits cytokine, chemokine, and chemokine receptor mRNA expression in the central nervous system of female mice with experimental autoimmune encephalomyelitis. J Neurosci Res 65:529–542
365. **Salem ML** 2004 Estrogen, a double-edged sword: modulation of the TH1- and TH2-mediated inflammations by differential regulation of TH1/TH2 cytokine production. Curr Drug Targets Inflamm Allergy 3:97–104
366. **Barone FC, Arvin B, White RF, Miller A, Webb CL, Willette RN, Lysko PG, Feuerstein GZ** 1997 Tumor necrosis factor-α: a mediator of focal ischemic brain injury. Stroke 28:1233–1244
367. **Hallenbeck JM** 2002 The many faces of tumor necrosis factor in stroke. Nat Med 8:1363–1368
368. **Shohami E, Ginis I, Hallenbeck JM** 1999 Dual role of tumor necrosis factor α in brain injury. Cytokine Growth Factor Rev 10:119–130
369. **Santizo RA, Xu HL, Ye S, Baughman VL, Pelligrino DA** 2001 Loss of benefit from estrogen replacement therapy in diabetic ovariectomized female rats subjected to transient forebrain ischemia. Brain Res 956:86–95
370. **Harukuni I, Hurn PD, Crain BJ** 2001 Deleterious effect of β-estradiol in a rat model of transient forebrain ischemia. Brain Res 900:137–142
371. **Kondo Y, Suzuki K, Sakuma Y** 1997 Estrogen alleviates cognitive dysfunction following transient brain ischemia in ovariectomized gerbils. Neurosci Lett 238:45–48
372. **Kanaya AM, Herrington D, Vittinghoff E, Lin F, Grady D, Bittner V, Cauley JA, Barrett-Connor E** 2003 Glycemic effects of postmenopausal hormone therapy: the Heart and Estrogen/progestin Replacement Study. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 138:1–9
373. **Shumaker SA, Legault C, Kuller L, Rapp SR, Thal L, Fillit H, Stefanick ML, Hendrix SL, Lewis CE, Masaki K, Coker LH** 2004 Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's Health Initiative Memory Study. JAMA 291:2947–2958
374. **Hays J, Hunt JR, Hubbell FA, Anderson GL, Limacher M, Allen C, Rossouw AE** 2003 The Women's Health Initiative recruitment methods and results. Ann Epidemiol 13:18–77
375. **Shumaker SA, Reboussin BA, Espeland MA, Rapp SR, McBee WL, Dailey M, Bowen D, Terrell T, Jones BN** 1998 The Women's Health Iniative Memory Study (WHIMS): a trial of the effect of estrogen therapy in preventing and slowing the progression of dementia. Control Clin Trials 19:604–621
376. **Kawas CH, Corrada MM, Brookmeyer R, Morrison A, Resnick SM, Zonderman AB, Arenberg D** 2003 Visual memory predicts Alzheimer's disease more than a decade before diagnosis. Neurology 60:1089–1093
377. **Tang Y, Hao J, Mckay H, Allen PB, Rapp PR, Hof PR** 2004 Estrogen replacement increases spinopilin-immunoreactive spine number in the prefrontal cortex of female rhesus monkeys. Cereb Cortex 14:215–223
378. **Zandi PP, Carlson MC, Plassman BL, Welsh-Bohmer KA, Mayer LS, Steffens DC, Breitner JCS** 2002 Hormone replacement therapy and incidence of Alzheimer disease in older women: The Cache County Study. JAMA 288:2123–2129
379. **Launer LJ, Andersen K, Dewey ME, Letenneur L, Ott A, Amaducci LA, Brayne C, Copeland JR, Dartigues JF, Kragh-Sorensen P, Lobo A, Martinez-Lage JM, Stijnen T, Hofman A** 1999 Rates and risk factors for dementia and Alzheimer's disease: results from EURODEM pooled analyses. EURODEM Incidence Research Group and Work Groups. European Studies of Dementia. Neurology 52:78–84
380. **Tang MX, Jacobs D, Stern Y, Marder K, Schofield P, Gurland B, Andrews H, Mayeux R** 1996 Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. Lancet 348:429–432
381. **Resnick SM, Henderson VW** 2002 Hormone therapy and risk of Alzheimer's disease: a critical time. JAMA 288:2170–2172
382. **Henderson VW, Benke KS, Green RC, Cupples LA, Farrer LA** 2005 Postmenopausal hormone therapy and Alzheimer's disease risk: interaction with age. J Neurol Neurosurg Psychiatry 76:103–105
383. **Henderson VW, Guthrie JR, Dudley EC, Burger HG, Dennerstein L** 2003 Estrogen exposures and memory at midlife. Neurology 60:1369–1371
384. **Sherwin BB** 1988 Estrogen and/or androgen replacement therapy and cognitive functioning in surgically menopausal women. Psychoneuroendocrinology 13:345–357
385. **Sherwin BB, Tulandi T** 1996 "Add-back" estrogen reverses cognitive deficits induced by a gonadotropin-releasing hormone agonist in women with leiomyomata uteri. J Clin Endocrinol Metab 81:2545–2549
386. **Phillips SM, Sherwin BB** 1992 Effects of estrogen on memory function in surgically menopausal women. Psychoneuroendocrinology 17:485–495
387. **Woo J, Lau E, Ho SC, Cheng F, Chan C, Chan ASY, Haines CJ, Chan TYK, Li M, Sham A** 2003 Comparison on pueraria lobata with hormone replacement therapy in treating the adverse health consequence of menopause. Menopause 10:352–361
388. **Viscoli CM, Brass LM, Kernan WN, Sarrel PM, Suissa S, Horwitz RI** 2005 Estrogen therapy and risk of cognitive decline: results from

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the Women's Estrogen for Stroke Trial (WEST). Am J Obstet Gynecol 192:387–393
389. **Binder EF, Schechtman KB, Birge SJ, Williams DB, Kohrt WM** 2001 Effects of hormone replacement therapy on cognitive performance in elderly women. Maturitas 38:137–146
390. **Grady D, Yaffe K, Kristof M, Lin F, Richards C, Barrett-Conner E** 2002 Effect of postmenopausal hormone therapy on cognitive function: the heart and estrogen/progestin replacement study. Am J Med 113:543–548
391. **Resnick SM, Maki PM, Rapp SR, Espeland MA, Brunner R, Coker LH, Granek IA, Hogan P, Ockene JK, Shumaker SA** 2006 Effects of combination estrogen plus progestin hormone treatment on cognition and affect. J Clin Endocrinol Metab 91:1802–1810
392. **Scott H, Laake K** 2001 Statins for the prevention of Alzheimer's disease. Cochrane Database Syst Rev (4):CD003160
393. **Zamrini E, McGwin G, Roseman JF** 2004 Association between statin use and Alzheimer's disease. Neuroepidemiology 23:94–98
394. **Harman SM, Brinton EA, Cedars M, Lobo R, Manson JE, Merriam GR, Miller VM, Naftolin F, Santoro N** 2005 KEEPS: The Kronos Early Estrogen Prevention Study. Climacteric 8:3–12
395. **Adams MM, Shah RA, Janssen WG, Morrison JH** 2001 Different modes of hippocampal plasticity in young and aged female rats. Proc Natl Acad Sci USA 98:8071–8076
396. **Markowska AL, Savonenko AV** 2002 Effectiveness of estrogen replacement in restoration of cognitive function after long-term estrogen withdrawal in aging rats. J Neurosci 22:10985–10995
397. **Savonenko AV, Markowska AL** 2003 The cognitive effects of ovariectomy and estrogen replacement are modulated by aging. Neuroscience 119:821–830
398. **Rapp PR, Morrison JH, Roberts JA** 2003 Cyclic estrogen replacement improves cognitive function in aged ovariectomized rhesus monkeys. J Neurosci 23:5708–5714
399. **Yaffe K, Krueger K, Sarkar S, Grady D, Barrett-Conner E, Cox DA, Nickelsen T** 2001 Cognitive function in postmenopausal women treated with raloxifene. N Engl J Med 344:1207–1213
400. **Paganini-Hill A, Clark LJ** 2000 Preliminary assessment of cognitive function in breast cancer patients treated with tamoxifen. Breast Cancer Res Treat 64:165–176
401. **Castellon SA, Ganz PA, Bower JE, Petersen L, Abraham L, Greendale GA** 2004 Neurocognitive performance in breast cancer survivors exposed to adjuvant chemotherapy and tamoxifen. J Clin Exp Neuropsychol 26:955–969
402. **Breuer B, Anderson R** 2000 The relationship of tamoxifen with dementia, depression, and dependence in activities of daily living in elderly nursing home residents. Women Health 31:71–85
403. **Jenkins V, Shilling V, Fallowfield L, Howell A, Hutton S** 2004 Does hormone therapy for the treatment of breast cancer have a detrimental effect on memory and cognition? A pilot study. Psychooncology 13:61–66
404. **Linzmayer L, Semlitsch HV, Saletu B, Bock G, Saletu-Zyhlarz G, Zoghlami A, Gruber D, Metka M, Huber J, Oettel M, Graser T, Grunberger J** 2001 Double-blind, placebo-controlled psychometric studies on the effects of a combined estrogen-progestin regimen versus estrogen alone on performance, mood and personality of menopausal syndrome patients. Arzneimittelforschung 51: 238–245
405. **Saletu-Zyhlarz G, Anderer P, Gruber G, Mandl M, Gruber D, Metka M, Huber J, Oettel M, Graser T, Abu-Bakr MH, Gratzhofer E, Saletu B** 2003 Insomnia related to postmenopausal syndrome and hormone replacement therapy: sleep laboratory studies on baseline differences between patients and controls and double-blind, placebo-controlled investigations on the effects of a novel estrogen-progestogen combination (Climodien, Lafamme) versus estrogen alone. J Sleep Res 12:239–254
406. **Haas JS, Kaplan CP, Gerstenberger EP, Kerlikowske K** 2004 Changes in the use of postmenopausal hormone therapy after the publication of clinical trial results. Ann Int Med 140:184–188
407. **Strandberg TE, Ylikorkala O, Tikkanen MJ** 2003 Differing effects of oral and transdermal hormone replacement therapy on cardiovascular risk factors in healthy postmenopausal women. Am J Cardiol 92:212–214
408. **Vehkavaara S, Silveira A, Hakala-Ala-Pietila T, Virkamaki A, Hovatta O, Hamsten A, Taskinen MR, Yki-Jarvinen H** 2001 Effects of oral and transdermal estrogen replacement therapy on markers of coagulation, fibrinolysis, inflammation and serum lipids and lipoproteins in postmenopausal women. Thromb Haemost 85:619–625
409. **Vongpatanasin W, Tuncel M, Wang Z, Arbique D, Mehrad B, Jialal I** 2003 Differential effects of oral versus transdermal estrogen replacement therapy on C-reactive protein in postmenopausal women. J Am Coll Cardiol 41:1358–1363
410. **Chetkowski RJ, Meldrum DR, Steingold KA, Randle D, Lu JK, Eggena P, Hershman JM, Alkjaersig NK, Fletcher AP, Judd HL** 1986 Biological effects of transdermal estradiol. N Engl J Med 314:1615–1620
411. **Wakatsuki A, Okatani Y, Ikenoue N, Fukaya T** 2002 Different effects of oral conjugated equine estrogen and transdermal estrogen replacement therapy on size and oxidative susceptibility of low-density lipoprotein particles in postmenopausal women. Circulation 106:1771–1776
412. **Demirol A, Baykal C, Kirazli S, Ayhan A** 2001 Effects of hormone replacement on hemostasis in spontaneous menopause. Menopause 8:135–140
413. **Duschek EJ, Neele SJ, Thomassen MC, Rosing J, Netelenbos C** 2004 Effect of raloxifene on activated protein C (APC) resistance in postmenopausal women and on APC resistance and homocysteine levels in elderly men: two randomized placebo-controlled studies. Blood Coagul Fibrinolysis 15:649–655
414. **Kelly JJ, Rajkovic IA, O'Sullivan AJ, Sernia C, Ho KK** 1993 Effects of different oral oestrogen formulations on insulin-like growth factor-I, growth hormone and growth hormone binding protein in post-menopausal women. Clin Endocrinol (Oxf) 39:561–567
415. **O'Sullivan AJ, Crampton LJ, Freund J, Ho KK** 1998 The route of estrogen replacement therapy confers divergent effects on substrate oxidation and body composition in postmenopausal women. J Clin Invest 102:1035–1040
416. **Mashchak CA, Lobo RA, Dozono-Takano R, Eggena P, Nakamura RM, Brenner PF, Mishell Jr DR** 1982 Comparison of pharmacodynamic properties of various estrogen formulations. Am J Obstet Gynecol 144:511–518
417. **Vehkavaara S, Hakala-Ala-Pietilä T, Virkamäki A, Bergholm R, Ehnholm C, Hovatta O, Taskinen M-R, Yki-Järvinen H** 2000 Differential effects of oral and transdermal estrogen replacement therapy on endothelial function in postmenopausal women. Circulation 102:2687–2693
418. **Harvey PJ, Morris BL, Miller JA, Floras JS** 2005 Estradiol induces discordant angiotensin and blood pressure responses to orthostasis in healthy postmenopausal women. Hypertension 45:399–405

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