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FOR CLINICIANS WHO PROVIDE CARE FOR WOMEN

Testosterone Treatment: Psychological and Physical Effects in Postmenopausal Women

Susan R. Davis, M.B.B.S., F.R.A.C.P., Ph.D.
Associate Professor, Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia; Director of Research, The Jean Hailes Foundation, Clayton, Victoria, Australia

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INTRODUCTION

There is no agreed upon definition of androgen deficiency in women, nor is there a clear-cut parameter, such as free testosterone level, with an accepted limit below which biochemical testosterone deficiency can be diagnosed. However a cluster of symptoms appear to characterize androgen deficiency in women: loss of sexual desire, diminished well-being, lowered mood, loss of energy (or feeling of vitality), decreased assertiveness (often associated with decreased confidence) and, over time, lowered bone mass and reduced muscle strength.[1] There is substantial evidence that judicious androgen replacement, usually in the form of testosterone, is effective in improving well-being and libido in postmenopausal women, in addition to increasing bone mineral density (BMD).

Currently, the availability of products for the treatment of androgen replacement in women is limited, although prescription of androgens to women is widespread and likely to increase; hence the necessity to review the rationale for therapy, suggest guidelines that will help physicians identify the women most likely to benefit, and consider the available options and the risks of therapy.

WHICH WOMEN EXPERIENCE ANDROGEN DEFICIENCY?

In contrast to the acute fall in circulating estrogen at the time of menopause, the decline in circulating testosterone and the adrenal pre-androgens (androstenedione [A] and dehydroepiandrosterone [DHEA]) commences in the decade leading up to the average age of natural menopause, and most closely parallels increasing age.[2] An absolute decline in free testosterone with age has been reported. Women may experience symptoms of androgen deficiency in their late reproductive years, as early as late 30s and beyond. Acutely, across the perimenopausal period, neither A, dihydrotestosterone (DHT), or the ratio of total testosterone to sex hormone binding globulin (SHBG) (the free androgen index, [FAI]) change.[3] The mean plasma concentrations of testosterone in women transiting the menopause are however significantly lower than younger ovulating women sampled in the early follicular phase. Androgen deficiency symptoms usually develop insidiously and most

FROM THE EDITOR

David F. Archer, M.D.

A frequently asked question concerning menopausal women is, "What is the role of testosterone?" Dr. Susan Davis, a well-known investigator in this area, presents the information that should allow you to make the appropriate decision for diagnosis and treatment of androgen deficiency in postmenopausal women.

Over the last 30 years, there has been increasing concern relative to the amount of potentially endocrine-disrupting chemicals in the environment. Drs. Claude Hughes and Warren Foster provide the reader with an extensive review of the role of these compounds across the lifespan of women, including menopause. Their conclusion should be encouraging to both physicians and consumers alike in that there is no evidence, as yet, of a significant impact on these endocrine-disrupting chemicals during the woman's menarchal and postmenopausal years.

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1209 Montgomery Hwy, Birmingham, AL35216
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women are not aware that their symptoms have a biological basis. That many do not report symptoms until the menopause does not mean that the symptoms have not been present earlier. Indeed, it is well known that women who report loss of libido following menopause have commonly had a similar problem in the premenopausal years.

Symptoms are more pronounced in women who have undergone a surgical menopause because of the abrupt cessation of testosterone production by the ovaries. Symptoms also occur in women who have experienced premature ovarian failure, premenopausal women with either spontaneous or iatrogenic androgen deficiency (following radiotherapy or chemotherapy), and naturally menopausal women. The oral contraceptive pill or oral estrogen replacement therapy increase SHBG and suppress LH, reducing the stimulus for the ovarian stromal production of testosterone; hence these therapies also reduce free testosterone.

Other circumstances in which androgen replacement may be appropriate in women include postmenopausal- and glucocorticosteroid-induced bone loss, and possibly premenopausal osteopenia. Novel potential indications for androgen therapy in postmenopausal women include alleviation of the symptoms of systemic lupus and rheumatoid arthritis.

EVALUATING ANDROGEN LEVELS IN WOMEN

In women, androgens may act directly via the androgen receptor or indirectly via the estrogen receptors after conversion to estrogen. Androgens are the precursor hormones for estrogen production not only in the ovaries but also in extra gonadal tissues including bone, adipose, and brain. Therefore, maintenance of physiological circulating androgen levels in women ensures adequate supply of substrate for estrogen biosynthesis in tissues such as bone in which high tissue estrogen concentrations may be required physiologically (for example, maintenance of bone mineralization and prevention of bone loss). This may explain the gender discrepancy between the development of osteoporosis and dementia, which occur much later in life in men than in women, since men maintain adequate circulating testosterone levels for the extra gonadal production of estrogen in bone and brain well into their latter years.

The adrenal glands produce the pre-androgens A, DHEA, and DHEA sulfate whereas the ovaries make A, DHEA, and testosterone. Approximately half of the circulating testosterone is produced by conversion of the pre-androgens to testosterone, with A being the main precursor. Testosterone is further metabolized to either the potent androgen DHT or to estradiol in various sites.

Plasma levels of A and testosterone vary during the menstrual cycle in regularly ovulating women, with the lowest levels during the early follicular phase (menstruation) and, as a consequence of ovarian production, there is an increase in the mean circulating levels of both of these hormones in the middle third of the menstrual cycle.[4] This is followed by a second rise in A production by the corpus luteum during the late luteal phase. Testosterone also exhibits a diurnal variation with higher levels in the morning. Under normal physiological conditions, only 1% to 2% of total circulating testosterone is free or unbound. The rest is bound by SHBG, which binds 66%, and albumin. Exogenous estradiol and thyroxine increase SHBG, whereas testosterone, glucocorticosteroids, growth hormone, and insulin suppress SHBG production.

Therefore in order not to obtain falsely low values when investigating women for testosterone depletion, blood should be drawn before midday, because of the diurnal variation, and after the early follicular phase in menstruating women. Biochemical measurements that should be performed for evaluation include total testosterone, SHBG, free androgen index, and any other clinically indicated investigations such as thyroid function and iron studies. Free testosterone is not particularly useful as it does not indicate total testosterone production and how much is unavailable because of high binding. Furthermore, most free testosterone assays have a large co-efficient of variation with limited accuracy at the lower end of the female range. In general, total testosterone is measured with assays that do not discriminate low testosterone from mid- to low-normal range.

If a woman on postmenopausal oral estrogen has a normal testosterone level but high SHBG (therefore low FAI), she should be initially changed to non-oral therapy and the profile repeated with clinical review after six to eight weeks. This may obviate the need for testosterone therapy.

CONSEQUENCES OF LOW TESTOSTERONE

Diminished libido

"Sexual interest" or libido in women is generally less goal oriented than in men, and more diffuse and distractible. It may be more related to a desire to be intimate, to sensuousness and passion. Therefore, low libido is not sexual dysfunction, in contrast to anorgasmia, but sexual disinclination, and it may be more appropriate to classify this symptom as a mood disorder. This then fits in well with the important changes in mood (see below) that appear to characterize testosterone insufficiency.

It is fatuous to state that there are numerous factors that influence sexual interest, frequency of sexual activity, and sexual pleasure. When depression coexists, many would elect to treat the depression on the basis that either this is the primary problem or that it should take priority. An alternative approach is that if the woman has low testosterone, then treatment with testosterone alone or concurrently with antidepressant therapy is appropriate. Similarly, many are reluctant to treat a woman with low libido and partnership discord. However, effective intervention may result not only in restoration of libido but also of well-being, and this will flow over into the partnership issues, which also need to be addressed separately.

Is loss of libido "natural" for women?

The cause(s) of sexual disinterest and avoidance may not be pathological but perhaps physiological in the late reproductive years and beyond. Lowered sexual desire and receptivity due to diminished testosterone after several years of fertility may have had an evolutionary survival value which is redundant now that women spend on average 65% of their adult life beyond their fortieth year. If an age-related change in circulating testosterone results in the symptoms listed, should this be classified as sexual dysfunction or physiological hormone insufficiency responsive to therapeutic intervention? A similar logic applies for estrogen insufficiency.

Libido in women does not universally decline with increasing age. Although most women who have a natural menopause do not report loss of sexual desire, erotic pleasure, or orgasm, an age-related reduction in sexual interest and lessening of coital frequency has been associated with the menopausal transition independent of age.

Androgens appear to be important in female sexuality, with declining androgen levels contributing to the decline in sexual interest experienced by a proportion of women. Compared with premenopausal women, women in their postmenopausal years report fewer sexual thoughts or fantasies, have less vaginal lubrication during coitus, and are less satisfied with their partners as lovers. A low testosterone level is most closely correlated with reduced coital frequency. In a study of sexagenarian women, the only hormone positively correlated with sexual desire was circulating free testosterone.

In an Australian study, 31% of women aged 45 to 55 years reported a decrease in sexual interest which was significantly associated with menopause rather than with age. The greatest predictor of postmenopausal sexual satisfaction is the quality of the sexual aspects of a woman's life in her premenopausal years, consistent with important changes preceding menopause. Having a satisfying sexual relationship prior to menopause does not preclude a woman from experiencing androgen-responsive sexual dysfunction subsequently.

Table 1: CLINICAL INDICATIONS FOR TESTOSTERONE REPLACEMENT IN POSTMENOPAUSAL WOMEN

- Symptomatic testosterone deficiency following natural menopause
- Symptomatic testosterone deficiency due to surgical menopause, chemotherapy, or irradiation
- Premature ovarian failure

Potential indications for use in women:

- Glucocorticosteroid-induced bone loss
- Postmenopausal bone loss
- Adjunctive therapy for rheumatoid arthritis or SLE

Central Effects of Sex Steroids

Estradiol and testosterone, and their receptors, are present in the human female brain with the highest concentrations in the hypothalamus, the preoptic area, and the substantia nigra.[5] This distribution corresponds with high aromatase activity found in these regions in animals. In human females, the concentration of testosterone is several-fold higher than estradiol in each of these sites.[5] It is most probable that androgen effects within the central nervous system are mediated both directly via androgen receptors and as a consequence of local conversion by aromatization of androgen precursors to estrogen. Mice with no functional estrogen receptors (ER α/ß double knock out mice) and mice unable to synthesize estrogens (aromatase knock out mice) exhibit loss of sexual activity,[6] lending further weight to this hypothesis.

Support for specific direct androgen actions within the brain comes from cross-sex hormone therapy studies in transsexuals in whom the administration of androgens to female-to-male transsexuals led to increases in sexual motivation and arousability, whereas the combination of anti-androgens and high dose estrogen given to male-to-female transsexuals had the opposite effect.[7] Similarly, a major side-effect of anti-androgens in the treatment of hirsutism is loss of libido.

Testosterone Therapy Improves Libido in Women

Estrogen replacement improves vasomotor symptoms, vaginal dryness, and general well-being, but has minimal effect on libido except in women with atrophic vaginitis causing dyspareunia who may experience improvement in sexual gratification with estrogen replacement.[8,9] Women treated with intramuscular estradiol and testosterone have improvements in sexual motivational behaviors (desire, fantasy, and arousal), increased rates of coitus and orgasm, and enhancement of sexual parameters co-vary with plasma testosterone, not estradiol.[10] In postmenopausal women described as being "dissatisfied" with their hormone therapy regimens, the addition of testosterone resulted in significant improvements in sexual desire, satisfaction, and frequency, where as those treated with estrogen alone had no improvement.[11] Subcutaneous testosterone implants[1] and most recently transdermal testosterone therapy[12] significantly improve sexual activity, satisfaction, pleasure, and orgasm over and above the effect achieved with estrogen alone with no adverse effects on blood lipids or virilization effects.

Testosterone and Mood

Clinical experience indicates that testosterone exerts a major positive influence on mood, but conclusive data are lacking. We have hypothesized that the fall in tes-

tosterone during the reproductive years may contribute significantly to the symptoms diagnosed as depression in many women.

In men, mood is significantly and inversely associated with bioavailable testosterone and DHT independent of age and weight,[13-15] and testosterone replacement restores mood and alleviates depression in hypogonadal men.[16]

Following surgical menopause, the addition of intramuscular testosterone therapy to estrogen replacement results in women feeling more composed, elated, and energetic than with estrogen alone. Other studies have reported positive effects of testosterone in peri- and naturally postmenopausal women.

Transdermal testosterone replacement in surgically menopausal women significantly improves the Psychological General Well-Being Index score over placebo, with the greatest change being in improved general well-being and less depressed mood.[12] DHEA, currently available over-the counter in the USA, given orally (50mg/day) or transdermally (by a 10% DHEAcream) is associated with a marked improvement in well-being over placebo.[17,18] Oral DHEA improves well-being and depression and anxiety scores in women with adrenal insufficiency.[19] However, not all DHEAtrials have been positive.[20] Larger prospective trials with this steroid are required before definitive guidelines can be made for its use clinically.

Table 2: EVALUATION OF ANDROGEN DEFICIENCY IN WOMEN

Clinical suspicion of androgen deficiency
- Gradual loss of sexual desire in otherwise satisfying sexual relationship
- Persistent fatigue with no clear cause
- Premature ovarian failure
- Bilateral oophorectomy

Exclusion of other causes of symptoms
- Full psychosocial history
- Assess adequacy of estrogen therapy in post-menopausal women
- Exclude other causes of fatigue (e.g., iron deficiency, hypothyroidism)

Tests to establish androgen deficiency
- Total testosterone
- Sex hormone binding globulin (SHBG)
- Free androgen index (total T/SHBG x100)
- DHEA-S

Consider androgen therapy for women with:
- Symptomatic testosterone deficiency after natural menopause
- Symptomatic testosterone deficiency following oophorectomy, chemotherapy, or radiotherapy
- Premature ovarian failure – primary or secondary

Androgens and Bone

Androgenic steroids have an important physiologic role in the development and maintenance of bone in women and men. The skeletal effects of androgens appear to be mediated in part via local metabolism of androgens to estrogen within bone. Thus men with mutations in either the estrogen receptor gene or the aromatase gene (therefore no estrogen production) suffer osteoporosis.[21] Androgens also act directly in bone. Androgen receptors have been demonstrated in human osteoblast-like cell lines, and androgens have been shown to directly stimulate bone cell proliferation and differentiation. Total and bioavailable testosterone and DHEA-S, not estradiol, are the greatest predictors of bone mineral density (BMD) and bone loss in premenopausal women.[18,22-24] Women who experience bone loss confined to the hip prior to menopause have significantly lower total and free testosterone concentrations than those who do not significantly lose bone.[23] Consistent with these findings, hyperandrogenic women have higher BMD, after correction for body mass index, than their normal female counterparts. In the premenopausal years, BMD is also strongly positively correlated with body weight. In obesity, SHBG is suppressed with a resultant increase in free testosterone, and this may partially explain the relationship between obesity, free testosterone, and increased BMD, with the greater endogenous levels of biologically active free testosterone in more corpulent women directly enhancing bone mass.

Androgen insufficiency may underlie bone loss in women with premature ovarian failure. Despite adequate standard estrogen-progestin therapy, most women with this condition have significantly reduced BMD to levels associated with increased hip fracture risk.

In postmenopausal women, low circulating free testosterone is predictive of subsequent height loss (a surrogate measure of vertebral compression fractures) and hip fracture,[25-30] and the progressive decline in DHEA with increasing age may contribute to senile osteoporosis. It is most likely that the effects of the adrenal pre-androgens are the result of conversion to estradiol, A, or testosterone. Suppression of the adrenal production of DHEA and DHEA-S with chronic glucocorticosteroid therapy may in part be the cause of the known complications of osteoporosis and osteopenia with this therapy. Hence, DHEA or testosterone administration may prevent glucocorticosteroid-induced bone loss.

Studies of both oral and parenteral estrogen and estrogen-plus-testosterone therapy in postmenopausal women have shown beneficial effects of testosterone replacement on BMD.[27,28,31,32] Combined estradiol and testosterone replacement with subcutaneous implant pellets increas-

Table 3: POTENTIAL RISKS OF TESTOSTERONE REPLACEMENT IN WOMEN

1. CARDIOVASCULAR DISEASE RISK
Parenteral testosterone therapy in women does not appear to be associated with the undesirable metabolic consequences seen in women with androgen excess or with an increase in cardiovascular risk as it does adversely affect lipoprotein lipids or endothelial function.[37,41]

2. ANDROGENS & BREAST CANCER
Androgen receptors are found in over 50% of breast tumors and are associated with longer survival in women with operable breast cancer and a favorable response to hormone treatment in advanced disease. There is also evidence that the mechanism by which high dose medroxyprogesterone acetate exerts a negative effect on breast cancer growth is mediated via the androgen receptor. Primate research indicates that testosterone may oppose any unfavorable effects of estrogen therapy on the breast.

3. TESTOSTERONE REPLACEMENT & CLINICALSIDE-EFFECTS
The potential masculinizing effects of androgen therapy include development of acne, hirsutism, deepening of the voice, and excessive libido. These cosmetic side-effects are rare if supra-physiological hormone levels are avoided. Fluid retention is uncommon and appears to be more idiosyncratic than dose related. Hirsutism, androgenic alopecia, and/or acne are relatively strong contradictions to androgen replacement. Enhancement of libido is currently the most common indication for testosterone therapy; however, circumstances in which this would be an undesirable effect is a relative contraindication to therapy. Absolute contraindications include pregnancy and lactation, as well as known or suspected androgen-dependent neoplasia.

es lumbar spine, hip, and total body bone mineral density in postmenopausal women[29,33] with the effects in each site being greater than with estradiol implants alone.[27,31] Estrogen plus testosterone not only suppresses biochemical markers of bone reabsorption (as seen with estrogen alone and other anti-reabsorptive drugs) but is also associated with increases in markers of bone formation.[30,34] Thus it appears that estradiol alone has an anti-reabsorptive effect on bone in postmenopausal women, whereas the addition of testosterone, either orally or parenterally, enhances bone formation.

Treatment of postmenopausal women with nandrolone decanoate has been shown to increase vertebral BMD and has been used successfully for many years to treat osteoporosis. This therapy still provides a conservative option for elderly women, as long as it is used judiciously, i.e., every two to three months.

Increasing BMD is only clinically important if it is associated with enhanced mechanical strength and a reduced fracture rate. As yet, no studies have addressed the impact of androgens on fracture incidence. However, the effects of androgens on the mechanical properties of bone have been studied in feral female cynomolgous monkeys.[31,35] In this primate model, increases in intrinsic bone strength and resistance to mechanical stress were associated with increased BMD following testosterone therapy. Treatment also resulted in increased bone torsional rigidity and bending stiffness.

In summary, current data indicate that androgen replacement, in the form of testosterone, and possibly DHEA, may be an effective alternative to the prevention of bone loss and the treatment of osteopenia and osteoporosis. The relative contributions of estrogen and androgen to bone density are not clear at this time. As prospective data confirming a reduction in fracture rate with such therapy are lacking, specific guidelines cannot be given for this sole indication.

Testosterone and Autoimmune Disease

Androgens appear to suppress both cell-mediated and humoral immune responses, and it has been proposed that higher testosterone levels in men may be protective against autoimmune disease.[32-34,36-38] The direct administration of testosterone replacement may result in symptomatic improvement in postmenopausal women with rheumatoid arthritis, and both pre- and postmenopausal women have been observed to have reductions in disease activity with DHEA therapy.[35,39] However these findings should be considered preliminary.

WHICH WOMEN ARE MOST LIKELY TO BENEFIT FROM TESTOSTERONE REPLACEMENT?

The indication for androgen replacement is most often low libido and diminished well-being, and hence based on clinical assessment, with evaluation of the outcome of treatment based upon the subjective self-assessment of response and reporting by the patient.

The clinical settings in which the administration of testosterone is most likely to enhance a postmenopausal woman's health and well-being and potential indications are listed in Table 1, and an approach to evaluating a potential candidate for therapy is described in Table 2.

A significant proportion of women will not raise the issue of diminished libido because they find the topic awkward. Following treatment with chemo- or radiotherapy, the symptoms of the iatrogenic menopause and androgen deficiency, including fatigue, loss of well-being, depression, and reduced libido, can be difficult to distinguish from the overall physical toll of cancer treatment, and frequently premature menopause and associated androgen insufficiency go undiagnosed and untreated. Therefore, all "at risk" women should be directly questioned about the symptoms of androgen deficiency and informed of the therapeutic possibilities available.

Testosterone replacement therapy is accepted for women who have undergone a surgical menopause. This therapy for women who have undergone natural menopause and especially women who have premature ovarian failure continues to be a neglected component of hormone replacement therapy.

Testosterone replacement should also be considered as part of the management of young women with premature ovarian failure, particularly Turner's Syndrome. Women who are sexually active when they develop premature ovarian failure are often very disturbed by their diminished libido. Alternatively, young women who have not become sexually active, who have either primary or secondary premature ovarian failure, should be fully informed about the option of androgen replacement or perhaps in some instances offered low dose androgen replacement as a component of their hormone replacement regimen.

Prescribing Testosterone

Testosterone therapy appears to be effective and safe, with the caveat that the doses given achieve circulating androgen levels close to the physiological range for young reproductive women. The current international therapeutic options in terms of androgen replacement therapy are listed in Table 3. The pharmacokinetcics of many of these preparations in women are still to be established.

Methyltestosterone is available in combination with esterified estradiol (EE) in two doses, EE 0.625mg/methyltestos-

Table 4: ANDROGEN REPLACEMENT THERAPY FORMULATIONS USED FOR WOMEN

	Dose Range	Frequency	Route
Methyltestosterone* (in combination with esterified estrogen)	1.25-2.5 mg	daily	oral
Mixed testosterone esters	50-100 mg	4 - 6 Weekly	intramuscular
Testosterone implants	50 mg	3-6 Monthly	subcutaneous
Transdermal testosterone patch**	150-300 mcg	every 3.5 days	topical
Testosterone cream 1%**	5-10 mg	daily	topical
Testosterone undecanoate	40 mg	daily	oral
Nandrolone decanoate	50mg	8-12 weekly	intramuscular

Currently available in USA* Undergoing clinical trial**

terone 1.25mg or EE 1.25mg/methyltestosterone 2.5mg. Studies of up to two years duration have not resulted in adverse effects of these formulations on either hepatic enzymes or blood pressure.[36,40] Circulating levels of androgens may be supraphysiological with this preparation.

Testosterone implants are commonly administered in Australia and Europe. A dose of 50mg is extremely effective, allows slow release of the hormone over a period of three to six months, and does not tend to cause virilization. As there is variability in its absorption amongst different women, it is essential that its levels be monitored prior to administration of each subsequent implant. Occasionally in young oophorectomized women, 100mg is required, but levels and side-effects should be carefully monitored. Some women also develop a small patch of terminal hair above the implant site, but this rarely troubles the patient.

A transdermal testosterone matrix patch, designed specifically for use in women, is undergoing clinical trial. As with other hormone patches, some women may experience skin irritation or simply prefer a less conspicuous form of treatment.

A hydroalcoholic testosterone gel is available for hypogonadal men. However, a formulation of a dose appropriate for women is not yet available.

A 1% testosterone cream (10mg per day and then titrated down to 5mg per day) is available in Australia, and our experience to date with this formulation has been generally positive in terms of sexual desire, general well-being, and energy. We are currently conducting pharmacokinetic and clinical trials pertaining to its use to establish accurate therapeutic guidelines.

Mixed testosterone esters are also sometimes given as an intramuscular injection in a dose of 75mg to 100mg every four to six weeks. This dose regimen is purely empirical and to date no studies have involved this therapy in women.

Testosterone undecanoate is an oral androgen used in hypogonadal men. It has been little studied in women, although in some countries its prescription is widespread. Documentation of the safety and efficacy of this agent is required before its use can be recommended.

Nandrolone decanoate, a weakly aromatizable androgen, is approved in some countries for the treatment of postmenopausal osteoporosis. The dose, administered intramuscularly, should not exceed 50mg, and the frequency of treatment is best titrated against the patient's gross build. It is prudent that treatment is not given less than every six weeks and patients should be very carefully monitored for hirsutism and voice deepening.

The synthetic steroid tibolone and its four metabolites interact with androgenic receptors such that tibolone improves libido to a greater extent than continuous combined estrogen-progestin therapy. This therapy may be less effective in some women than the addition of testosterone to standard HRT.

Potential Adverse Effects

Menopause, both natural and surgically induced, is associated with the development of a more adverse lipoprotein profile, which is unrelated to endogenous testosterone levels.[1] Postmenopausal estrogen replacement therapy lowers total and low-density lipoprotein (LDL)-cholesterol, and these favorable effects are not diminished with either oral or parenteral testosterone replacement.[2-4] Parenteral testosterone replacement does not affect high-density lipoprotein (HDL)-cholesterol,[2] however, HDL-cholesterol and apolipoprotein A1 decrease significantly when oral methyltestosterone is administered with oral estrogen.[5,6] Whereas oral estrogen replacement is associated with increased triglyceride levels, concomitant administration of oral methyltestosterone has resulted in a reduction in triglycerides.[3]

Measurement of circulating lipids is a clinical surrogate for lipoprotein-lipid metabolism. More direct measurement of lipid metabolism is probably a better indicator of the effects of exogenous steroid therapy on cardiovascular risk. Combined oral esterified estrogen and methyltestosterone therapy reduces arterial LDL degradation and cholesterol ester content in cynomolgus monkeys which does not differ from the effects observed when estrogen is given alone.[7] Combined estrogen and methyltestosterone therapy is also associated with reduced plasma concentrations of apoplipoprotein B, reduced LDL particle size, and increased total body LDL catabolism.[7] Small LDL particles are more susceptible to oxidation and are hence considered to be more atherogenic. However, since estrogens appear to increase oxidative modification of LDL in the arterial wall,[7] the reduction in LDL particle size observed with both oral estrogen and combined therapy may not be deleterious but may merely reflect selective removal of large LDL particles from the circulation.

Women tend to be anxious regarding the risks associated with the use of androgens, particularly the masculinizing effects of hirsutism, acne, and voice deepening. Identifying patients with such conditions will minimize side-effects, and judicious dosing and careful patient monitoring should further reduce the risk of developing adverse events.

In general, postmenopausal testosterone replacement should only be prescribed with concurrent estrogen therapy, as there are no data regarding the use of testosterone alone. One would predict that such use would increase the risk of adverse metabolic and cosmetic side-effects. The only exception is the administration of nandrolone decanoate.

CONCLUSION

Controversy continues to surround the issue of testosterone therapy for women. This article briefly summarizes the data available, but clearly there are vast gaps that need to be filled. As more women enjoy longer and healthier lives, the demand to optimize quality of life will increase. Testosterone therapy is an important quality of life issue. No woman will die from testosterone deficiency, but if the link between testosterone depletion and depression and well-being is established, in addition to the effects on libido, testosterone therapy will be a therapy to be considered by all women. In the near future, specific formulations for women are likely to revolutionize this aspect of women's health, but in the interim, all women treated with testosterone should be carefully reviewed and blood testosterone levels in women treated with pure testosterone formulations and adverse effects should be regularly monitored.

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Available on Request from the Author

Associate Professor Susan R. Davis, M.B.B.S., F.R.A.C.P., Ph.D., Director of Research, The Jean Hailes Foundation, 173 Carinish Rd., Clayton, Victoria, 3168 Australia

Some Potential Health Effects of Endocrine-Disrupting Chemicals Across the Lifespan of Adult Women

Claude L. Hughes Jr., M.D., Ph.D.
Department of Obstetrics and Gynecology, Duke University Medical Center, Durham and Avalon Medical Group, Chapel Hill, NC

Warren G. Foster, Ph.D.
Department of Obstetrics and Gynecology, McMaster University Medical Center, Hamilton, Ontario, Canada

INTRODUCTION

Potential human reproductive effects of exposures to hormonally active environmental contaminants (commonly described as endocrine-disrupting chemicals, EDCs) such as environmental estrogens (EEs) and anti-androgens have become an area of increasing concern. These agents have been linked to a wide variety of adverse reproductive/developmental health outcomes in women including but not limited to the following: breast, ovarian, and endometrial cancers; endometriosis; infertility; prolonged time-to-pregnancy; increased rates of spontaneous abortion; decreased birth sex ratios (number of male births/number of births for both sexes); and precocious puberty. There are a number of reports in the literature of trends in reproductive outcomes that suggest that environmental contaminants may be causing adverse effects on human reproduction. For example, a decrease in the birth sex ratio (a decrease in the number of boys being born) has been reported in the United States[3,74] as well as Canada[3] and elsewhere. An increase in the incidence of breast cancer since the 1940s has also been documented.[59] Finally, there are also reports that body burdens of some contaminants are greater in women with endometriosis compared to a reference group.[56] These reports, among others, taken together raise concerns regarding potential adverse effects of environmental contaminants on the reproductive tract and subsequent function. However, the role of man-made chemicals and EDCs in particular in these adverse outcomes remains controversial.

Many of the hormonally active environmental contaminants that have been the focus of human health concerns are persistent bioaccumulative chemicals with half-lives of many years. Therefore, the body burden of compounds with these physical properties tends to increase with age, because the duration of exposure via environmental media (food, water, workplace agents, etc.) is *de facto* ever-lengthening. So to some extent, the longer you live, the longer your interval of exposure and thus potential accumulation of some compounds, as well as the longer interval during which a particular target tissue could be affected. Although different physiological and pathological issues may come to the fore at different parts of the adult lifespan, the health of an aging woman is, to some degree, a sum total of what has gone before, what she presently faces, and what lies ahead in the future. Since we all will have borne some of these chemicals in our bodies across vast portions of our lifespans, we suppose that due consideration of the potential effects of these EDCs on the health of adult women should include any reproduction-related disorder for which there is both mechanistic plausibility and data supporting the contended association of exposure and outcome. However, the long latency for the development of diseases such as breast cancer and recognition that the developing fetus and neonate may be more sensitive to exposure to environmental contaminants than adults has lead to examination of early lifetime exposures and susceptible populations.

To test the weight of evidence supporting the hypothesis that EDCs are responsible for changes in the prevalence of, for example, subfertility, endometriosis, polycystic ovarian syndrome (PCOS), and breast cancer, three criteria were used to evaluate the literature; specifically, the strength of the data for: 1) a change in the prevalence of the health outcome of interest, 2) the epidemiological evidence linking exposure and health effects, and 3) available mechanistic data indicating receptor-mediated disruption of an endocrine signalling pathway. While it is concluded that the accumulated evidence supports the plausibility of the hypothesis, the data do not support a conclusion that, at the levels of exposure reported for the general population in contemporary studies, human reproductive health has been adversely affected. The literature, however, is sufficiently strong to support continued concern and to warrant further study of this problem. From this analysis a number of data gaps are identified and research priorities recommended.

FECUNDITY AND FERTILITY

The terms fecundity and fertility are often used interchangeably, although their meanings are distinct where "fecundity" refers to the potential or ability of a couple to conceive a child, i.e. how quickly they can conceive. The "fecundity" of a couple is therefore the sum of the fertility status of both partners[55] and is a reflection of the time necessary to achieve a pregnancy. Because of the complexity of human reproduction, it is often difficult to determine which partner has been affected and whether or not there has been an actual increase in age-specific infertility rates due to some exogenous factor such as an environmental exposure.

Perception that infertility rates have risen is paralleled by the growth in the number of fertility clinics, clinicians trained to assist couples with impaired fertility, and continued media attention for new reproductive technologies. Regardless, in the United States the pregnancy rate in 1996 was found to be 9% lower than in 1990;[85] however, factors such as decreased sexual activity, increased condom use, and the introduction of injectable and implantable contraceptive devices may have contributed to this decline. In Sweden, analysis of birth registries has shown that the number of infertile couples (failure to conceive after one year of unprotected intercourse) has decreased from 12.7% in 1983 to 8.3% in 1993.[2] This approach, however, is likely to overlook changes in susceptible subpopulations or at-risk occupational groups. Published studies on secular or geographical trends in human fecundity are sparse.

Another potentially useful approach is to review the total fecundity of a population of people with no predisposition for limitation of family size. For example, there has been a decreased age-specific fertility rate in the Hutterite population, a group in which reproductive practices are

unlikely to have changed over time.[62,72] These retrospective cohort studies revealed a decline in the total number of children born beginning with a cohort 1931 to 1935 and a continuing decline with subsequent birth cohorts. While genetic influences cannot be excluded, neither can extraneous factors such as man-made chemicals, although there is no clear link to any hypothesized exposure to endocrine disrupting chemicals.

"Time to pregnancy" (TTP) is another useful epidemiological tool to measure the fecundity of a population. TTP does not require categorization of subjects into fertile and infertile groups but determines the mean amount of time required for a population of couples that are actively trying to achieve a pregnancy to become pregnant. The model has been validated as a consistently effective tool for measuring the impact of exogenous agents that affect reproduction. For example, studies have shown that it can clearly demonstrate a difference in the TTP among smokers vs. nonsmokers.[64]

Differences in TTP have been found in a prospective study involving seven well-defined geographical areas in Europe.[45] The highest fecundity was observed in southern Italy and northern Sweden; the lowest fecundity was in east Germany. The differences in TTP remained significant after adjustment for regional differences in body mass, smoking, frequency of intercourse, and sexually transmitted disease. TTP has also been reported to be shorter among couples in Finland compared to those in the U.K.[42] It is interesting to note that sperm counts among men in Finland are reported to be among the highest in the world[82,87] and thus may account in part for the shorter TTP in this population.[42] By comparison, although a decline in semen quality has been reported in the U.K.,[40] an increase in fertility rather than a decline was revealed in a retrospective study of TTP for all births conceived after unprotected intercourse that began during 1961 to 1993 in the British population aged 16 to 59.[43] Thus, no clear picture emerges with respect to time trends and human fertility.

Occupational exposures are often cited as evidence of external impacts on fertility.[73,77,24] Published studies relate to the impact of both female and male occupations. In one study, in which 281 women with a diagnosis of infertility were compared to 216 postpartum women for chemical exposures, women with a history of working in the agricultural industry had an elevated risk of infertility.[26] In addition to occupationally exposed individuals, at-risk groups may also include those whose social or leisure activities lead to higher exposures than occur in the general public. The effect of consumption of sport fish containing PCBs and mercury on TTP has yielded equivocal results.[8,14] In these studies, lifetime exposure to PCBs and mercury is estimated by the numbers of fish meals consumed. The method has obvious weaknessses in that it assumes a constant pattern of consumption, that levels of contamination have remained constant in fish over this time frame, the same type of fish has been consumed throughout, and that individual susceptibility to adverse effects has been constant over the period under investigation. Regardless, an increased TTP was observed only with increased paternal consumption (adjusted odds ratios were 1.4, 1.8, and 2.8 for annual fish meals consumed: 1-114, 115-270, and 271-1127 meals/yr., respectively).[14] Note that only in the highest fish consumption group did the 95% CI for the OR exclude 1.0. These data suggest a weak association only for high fish consumption in men and conception delay.

Changes in fecundability were also investigated in the New York State Angler Cohort Study (NYACS), and lifetime exposure to PCBs was estimated from recent consumption of contaminated Great Lakes fish.[9] Maternal consumption of fish for three to six years was associated with reduced fecundability (OR 0.75, 95% CI 0.59 – 0.91); however, this effect was lost in those with fish consumption greater than seven years (OR 0.75, 95% CI 0.51 – 1.07). Maternal consumption of more than one fish meal a month was also associated with reduced fecundability (OR 0.73, 95% CI 0.54 – 0.98), whereas there was no association with paternal fish consumption or either maternal or paternal estimated lifetime exposure to PCBs. These data suggest that maternal but not paternal consumption of contaminated fish may reduce fecundability.

Several epidemiological studies have been undertaken to study the fecundity and fertility of farmers exposed to pesticides. A retrospective study of 43 couples in the Netherlands whose respective male partner was a fruit grower included 91 pregnancies from 1978 to 1990.[18] Exposure to pesticides was determined by self-reported data. An adverse effect of pesticide exposure was found, mainly in highly exposed men who tried to conceive during the spraying season. The incidence of couples consulting a physician because of a fertility problem was also much greater in the high exposure group; however, there is no clear link to an endocrine disruption mechanism. In a retrospective study of 2,012 farm couples, no strong or consistent pattern of association of exposure to various classes of pesticides with time to pregnancy could be observed.[17] Similarly a large study on exposure to pesticides and a control group of agricultural workers in Denmark and France did not demonstrate any effect of pesticide exposure on time to pregnancy).[83]

Actual chemical exposure data in relation to fertility are limited. One example of what might be possible using new reproductive technology techniques is the isolation of persistent organochlorine chemicals from ovarian follicular fluid of women undergoing in vitro fertilization.[41] In this study, serum, adipose, and ovarian follicular fluid levels of persistent organochlorine contaminants were determined in women participating in fertility clinics from three Canadian cities. Geographical differences in body burdens for some of the contaminants were demonstrated and the association between exposure and adverse outcomes was explored; however the sample size of this study was too small to permit detection of effects if they were indeed present.

Animal studies designed to assess the effect of environmental contaminants on fertility and fecundity can be divided into government-approved test guidelines or apical studies and mechanistic studies conducted to characterize potential risk to human reproductive health. One of these, Reproductive Assessment through Continuous Breeding (RACB), has been used extensively by the U.S. EPA. The data for 72 chemicals contained in the RACB database was evaluated[10] to identify which of the definitive measures (sperm counts and necropsy results) were the most predictive of apical concerns (fertility). From this analysis, it was found that longer estrous cycles in mice were correlated with reduced numbers of pups, a relationship which was stronger in F_1 than in F_0 and not seen in the controls. Fertility was reduced if >15% of the sperm had abnormalities or sperm motility was <37%. While these studies point to target organs affected by the test compound, they do not

provide evidence that endocrine disruption is involved as the primary mechanism mediating the adverse effect. The potential for environmental contaminants to adversely affect fecundity and fertility involving an endocrine mechanism has been shown in a number of animal studies. Specifically, methoxychlor is an insecticide that displays both estrogenic and antiandrogenic activity in vitro and in vivo.[53] In female rodents, methoxychlor induces effects more typical of an estrogen, with induction of lordosis. Litter size was reduced in a study in which female rats were dosed by gavage (0, 5, 50, or 150 mg methoxychlor/kg/day) for the week before and the week after birth, whereupon the pups were directly dosed with methoxychlor from postnatal day (pnd) 7.[11] In another example, a multigeneration study[89] using Di-n-butyl phthalate (DBP), an androgen receptor antagonist, showed very marked effects on fertility of rats in the F_1 generation in comparison to their parents in the F_0 generation with fewer and smaller litters and a 50% decrease in sperm count.

In summary, the foregoing studies demonstrate an association between delayed conception and exposure to environmental contaminants through occupational settings or consumption of contaminated fish. However, the relationship between changes in the time to pregnancy and endocrine disruption is highly speculative, due in part to the inherent lack of specificity of this population-based model of fecundity as well as the complex array of issues that may alter normal human reproduction and result in a longer TTP. Moreover, although animal studies support the plausibility of the hypothesis that chemical contaminants can adversely affect reproduction, the dose levels required are in excess of those typically measured in contemporary exposure assessment studies of human tissues.

ENDOMETRIOSIS

Endometriosis is an estrogen-dependent disease characterized by the presence of endometrial glands and stroma outside the uterine cavity. It is a common gynecologic disorder affecting approximately 14% of women of all reproductive ages;[86] however, data concerning changes in the prevalence of this disease are lacking in the literature.

The etiology of endometriosis remains elusive, although retrograde menstruation or bleeding into the peritoneal cavity during menstruation is widely accepted as a major contributing factor in the pathogenesis of this disease. It is, however, a common phenomenon even in women without endometriosis.[29] Factors other than retrograde menstruation are thought to contribute to the development and progression of endometriosis.

Chemical contaminants have recently been inculpated in the pathobiology of endometriosis as a result of a series of clinical-observational and animal studies. An association between endometriosis and exposure to PCBs[27] and dioxins has been made. Apositive association between endometriosis and dioxin exposure was reported in a single case-control study in which 44 women with endometriosis were compared with 35 age-matched women with tubal infertility.[56] Note that while an OR of 7.6 was obtained, the 95% CI included unity (0.87-169.7). In another study, no association between organochlorine concentrations and endometriosis could be found in a case control study of 86 women with endometriosis and 70 controls matched for the indication of laparoscopy.[50] These studies are all relatively small, and thus may not have the statistical power to detect differences if they were indeed present. Hence, the human data at present neither confirm nor refute the hypothesis that environmental contaminants play a role in the pathobiology of endometriosis.

The notion that environmental contaminants are involved in the pathophysiology of endometriosis gained momentum with the demonstration that the incidence and severity of spontaneous endometriosis was increased in rhesus monkeys following treatment with 5 or 25 ppt TCDD.[69] Recently, potential involvement of TCDD in the pathophysiology of endometriosis was confirmed in Cynomolgus monkeys with surgically induced endometriosis.[94] However, the mode of action of TCDD on the non-human primate endometrium has yet to be determined. Recently, Rier et al[70] reported the results of residue analysis of the serum for TCDD and PCB congeners from the monkeys involved in the original endometriosis study.[69] Elevated serum levels of 3, 3', 4, 4' tetrachlorobipehnyl (TCB), 3, 3', 4, 4', 5–pentachlorobiphenyl (PnCB), and an increased total serum TEQ were associated with a higher prevalence of endometriosis, whereas the severity of endometriosis was correlated with the serum concentration of 3, 3', 4, 4'-TCB. In contrast, rhesus monkeys treated with PCBs failed to show any relationship between the incidence and/or severity of endometriosis and PCB dose.[4]

Surgical induction of endometriosis in rodents has suggested that the disease can be enhanced by the administration of TCDD.[16,44] The administration of very large doses of TCDD induced endometriosis in both the rat and the mouse.[16] In contrast, in ovariectomized and estrogen-replaced mice, TCDD treatment inhibited the growth of uterine implants.[93] These data suggest that ovarian factors in addition to estradiol contribute to the survival and growth of the implants. In another study, ovariectomized mice with autotransplanted uterine strips were used to demonstrate that administration of 4-chlorodiphenyl ether (an estrogenic compound) could facilitate implant growth.[92] Although the mechanism of action was not investigated in these studies, taken together these data suggest that environmental contaminants may play a role in the pathobiology of endometriosis, and one possible mechanism to be explored is that of endocrine disruption.

POLYCYSTIC OVARIAN SYNDROME (PCOS)

PCOS, a poorly understood condition, has been described as a self-perpetuating state of chronic anovulation. This syndrome is characterized by excess circulating androgen levels that lead to elevated levels of estrogens and LH, together with subnormal levels of FSH that are inadequate to support follicle maturation. Consequently, ovulation does not take place and the ovaries contain large numbers of small growing follicles. Because of the broad range of severity of the condition, there is a parallel variation in clinical findings, from the picture of the classical Stein-Leventhal syndrome to much milder forms of anovulation.[79,7] The syndrome is usually associated with the onset of puberty. The menses may at first be ovulatory, but shortly thereafter oligomenorrhea or amenorrhea ensues. Mild to moderate hirsutism may develop and obesity is common. The concomitant use of oral contraceptives may delay or reduce the appearance of symptoms. The actual incidence is not established because of the great variation in presentation and the very real possibility of under-diagnosis. However, Polson et al[67] have indicated through ultrasound evaluation that polycystic ovaries may be a fea-

ture in 22% of young women. Regardless, we are unaware of any evidence in the published literature to suggest that the prevalence of PCOS is increasing. We are also unaware of any reports of increased levels of environmental contaminants in the serum of patients with PCOS compared to an age-matched reference population.

Interest in this syndrome in relation to environmental considerations stems from the animal literature and the observation that some EDCs alter steroidogenesis in various target tissues. There is evidence that testosterone exposure has significant effects on the developing female brain, including induction of acyclicity and anovulation. Polycystic ovaries can also be induced in a variety of animal models through estrogen administration.[13] Recently, polycystic ovaries, chronically elevated serum LH, and anovulation were documented in aERKO mice.[15] These mice fail to express ERa in the hypothalamus-pituitary-gonadal axis. Consequently, these data suggest a role for ERa in follicle development, and we speculate that compounds in the environment that are selective estrogen receptor modulators may therefore play a role in the pathobiology of this disease.

BREAST CANCER

The incidence of breast cancer has increased steadily from the 1940s in many industrialized countries,[59] although recently there is evidence of a plateau in the rate of breast cancer occurring since the mid 1990s.[90] An etiological role for estrogens in breast cancer is supported by a number of lines of evidence. For example, an increased risk for breast cancer has been associated with an increased lifetime exposure to endogenous estrogen through earlier age at menarche, late onset of menopause, or not having children.[36] In addition, conditions or activities that increase the availability of estrogens have also been identified as risk factors for the development of breast cancer. For example, postmenopausal obesity, which favors the conversion of androgens to estrogens in peripheral fat tissue,[75] and alcohol consumption, which increases the amount of available estradiol,[68] have been linked with an increased risk of breast cancer. In contrast, breast cancer incidence is reported to be lower among women who have an oophorectomy at a young age.[22] Although breast cancer is clearly a hormonally related disease, only 30% to 50% of all breast cancer cases can be attributed to increased life-long exposure to endogenous estrogens.[66] Therefore, factors other than increased lifetime exposure to estrogens must play a role in the pathogenesis of breast cancer.

Environmental and dietary factors have arisen as potential factors following recognition of the large geographic differences in breast cancer incidence rates. The lowest incidence rates of breast cancer occur in Asian countries, while the highest in the world have been reported for the United States and northern Europe.[46] Moreover, breast cancer rates in Japan have been reported to be half as high as those in the United States.[12] It is also interesting to note that breast cancer rates in women who migrate from one geographical region and culture of the world to another experience a shift over a period of two or three generations to breast cancer rates similar to that of the host country's.[78] Thus, the inability for increased exposure to estrogens to account for the majority of breast cancer cases, large geographical differences in the incidence of breast cancer, and the higher rates of breast cancer amongst developed countries like the United States suggest that environmental factors are likely associated with the development of breast cancer.

The majority of data relating environmental contaminants to human breast cancer are limited to persistent organochlorine compounds, such as DDT and its metabolites, as well as the PCBs, which have been identified throughout the world in human tissue, blood, and milk.[1] Since the mid-1980s, a number of case-control studies have examined the potential association between exposure to organochlorines and breast cancer. These studies have been comprehensively reviewed elsewhere.[1,33,63]

Early case-control studies[88,61,23] of the involvement of DDT and its metabolites in breast cancer are of limited value due to sample size limitations and study design flaws. Subsequently, a nested case-control study was conducted to determine whether exposure to *p,p*'-DDE or PCBs is associated with breast cancer.[91] This study used serum collected from women participating in the New York University Women's Health Study, from which 58 women were diagnosed with breast cancer. Serum concentrations for *p,p*'-DDE or PCBs from 58 women with breast cancer were compared to those in 171 matched control subjects. This study revealed a four-fold increase in the relative risk for breast cancer for an elevation of serum *p,p*'-DDE from 2.0 ng/ml to 19.1 ng/ml. Asimilar increase in the relative risk for breast cancer and serum PCB concentration could not be demonstrated in this study.

In another study,[19] the concentrations of organochlorines including *p,p*'-DDE, PCBs, hexachlorobenzene, Mirex, b-hexachlorocyclohexane, oxychlordane, and transnanochlor were compared in the serum and adipose tissue of women with benign breast disease (controls, n=17) versus women with a diagnosis of mammary infiltrating adenocarcinoma (cases, n=20). The cases were further characterized on the basis of their estrogen receptor status. In ER-positive cases, the adipose tissue concentrations of *p,p*'-DDE were significantly higher than in the controls. The concentrations of organochlorines in ER-negative cases were lower than the levels documented in the control subjects. Therefore, these data suggest that *p,p*'-DDE may play a role in hormone responsive but not in non-hormone responsive breast cancer. Subsequently, four prospective case-control studies,[20,34,37,48] a retrospective study,[52] and two retrospective case-control studies performed with postmenopausal women[60,84] have failed to demonstrate that DDT, and more specifically *p,p*'-DDE, increase the risk for breast cancer.

Determining the association between PCB exposure and breast cancer is fraught with numerous difficulties. First, PCBs are a mixture of congeners, containing both estrogenic and anti-estrogenic compounds, some of which also act as immune suppressants. Consequently, exposure assessment poses unique challenges for the interpretation of study results and comparison across studies. For example, one approach is to examine the concentration of a few individual congeners,[20] whereas another sums the concentrations of the most important congeners according to their estrogenic potential,[80] or the percent of chlorinated carbons.[60] Another approach has been to sum selected congeners without regard to suspected hormone activity or to calculate the total area under the curve and report the total amount of PCBs.[34,37,48,60,91] Regardless, the relationship between PCB exposure and breast cancer in many studies has involved occupational exposure, and a positive association has not been found.[63] Accidental and/or occupational exposures to the industrial compounds TCDD and polybrominated biphenyl (PBB) com-

pounds have also been investigated with respect to increased risk of breast cancer. Equivocal results have been described in two studies of women exposed to PBBs in Michigan.[30,76] Several occupational studies on TCDD exposure have also shown equivocal results.[47,54]

The relationship between pesticide exposure and breast cancer has also been investigated. Arecent study[34] including 268 cases from a cohort of 7,712 Danish women found a two-fold increase in the risk of breast cancer with the highest plasma concentration of the pesticide dieldrin. However, there were no associations with the other measured 45 compounds, and the biological significance needs to be clarified by further studies. The triazine herbicides have been implicated as causative factors in breast cancer following the report that Atrazine significantly increased mammary tumor development in rats.[81] However, the carcinogenic effects of atrazine and related chemicals are rat strain, sex, and tissue-specific. Moreover, the mechanism of this effect appears to be related to ageing in this rat strain, and thus we suggest that these findings are not relevant to humans.

However, the triazine herbicides have recently been shown to up-regulate the expression and activity of aromatase, the enzyme responsible for the conversion of androgens to estrogens, in a human adrenocortical carcinoma cell line.[71] These findings suggest that in addition to direct estrogenic effects of EDCs, there is the potential for these compounds to increase target tissue exposure to estrogens through increased conversion of circulating androgens to estrogens. Consequently, we speculate that up-regulation of aromatase activity offers a novel mechanism of endocrine disruption and thus contributes to the pathogenesis of estrogen dependent tumors. Inconsistency in the findings of the various studies described above leads us to conclude that the data do not now support the view that organochlorine compounds increase the risk of breast cancer. However, the biological mechanisms of breast cancer and estrogen receptor status are issues that should be considered in future investigations designed to determine whether exposure to organochlorines are associated with an increase in breast cancer risk.

Any analysis of the association between environmental factors and breast cancer would be incomplete without consideration of dietary phytoestrogens. Phytoestrogens are hormone-mimicking compounds naturally present in over 300 species of plants. Different phytoestrogens have been shown to have both estrogenic and anti-estrogenic properties and thus may also contribute to an increased exposure to estrogens. Isoflavones found in soy products have been generally thought to confer reduced breast cancer risk. Indirect evidence from both human and laboratory studies support the notion that consumption of certain plant-based foods, particularly soy foods, may reduce the risk of developing breast cancer. For example, a lower risk of breast cancer has been found for Japanese women who traditionally consume more soy products than most women in western countries and have higher concentrations of phytoestrogens in their blood and urine.[32]

In an Australian study,[39] an inverse relationship was found with excretion of phytoestrogen metabolites and decreased breast cancer risk. However, equivocal results were found when the association between soy consumption and a breast cancer risk was evaluated in pre- and/or post-menopausal women[51,95,58]

We propose that despite the hypothesized beneficial effects of phytoestrogens in human cancer, phytoestrogens may not have beneficial effects for all women depending on age and individual susceptibility. The basis of this proposal comes from recent studies which reported that soy supplementation in women increased the proliferation rate of breast cells.[65,57] Genistein administration in rats during gestation results in a dose-dependent increase in mammary tumor susceptibility in the adult animal.[31] However, in seven out of nine animal studies, a lower number of mammary tumors were observed in rats whose diet was supplemented by soy.[6] Genistein exposure in newborn rats before sexual maturity results in protection against mammary tumor development, later induced by administration of the carcinogen DMBA in fully-grown animals.[49,25] Consequently, the safety of phytoestrogens in women at risk for the development of breast cancer is uncertain at this stage and thus further investigation is required.

The two-year carcinogenicity bioassay in laboratory rodents remains the cornerstone for identifying those chemicals most likely to cause cancer in humans.[35,38] A review by Dunnick et al[21] found that out of 450 chemicals tested, 34 caused mammary gland tumors. However, none of the chemicals that caused mammary gland tumors were uniquely carcinogenic to that organ system. These carcinogenicity assays do not include exposure during pregnancy and lactation that can influence expression of mammary gland carcinogenesis.[28] The effect of developmental exposure to EDCs and subsequent hormone responsivity of mammary tissue is unknown.

RESEARCH NEEDS

If there is an association between exposure to environmental contaminants and health outcomes in women, such as subfertility, endometriosis, PCOS, and breast cancer, then it is essential that accurate determination of exposure be conducted, particularly exposure at the level of individual target tissues. Moreover, consideration of the etiology of the disease under study together with the known or assumed mechanism of action of test compounds must be considered in future investigations. This is particularly relevant in estrogen dependent diseases, given the presence of estrogen receptor subtypes as well as the potential for environmental contaminants to act as selective estrogen receptor modulators and antagonists.

Genetic susceptibility of individuals and racial factors are also considerations that must be included in future epidemiological studies. Development of suitable animal models for the study of endometriosis, PCOS, and breast cancer continues to be a priority research need. Rodents are considered by many to be poor models for endometriosis since they do not spontaneously develop endometriosis. While the primate studies have provided insight into the role of environmental chemicals in this disease, they are too expensive for routine studies and require too much time for the spontaneous induction of endometriosis. The physiology of implanted uterine fragments is unlikely to accurately reflect the physiology of human endometrial cells that spontaneously implant and result in endometriosis. The applicability to human health of animal models of breast cancer that require induction of tumors by treatment with DMBAor MNU is questionable. It would seem that the stage is set for the use of transgenic animal models such as those containing the *neu* transgene or for innovative use of ER knockouts.

SUMMARYAND CONCLUSIONS

In summary, with the exception of occupational and accidental exposures, the available evidence does not support a conclusion that at levels of exposure in the general population, man-made chemicals have

measurably decreased human fecundability or induced reproductive hormone-related diseases in adult women. Reports in the literature do, however, suggest that changes in reproductive physiology, development, and target tissue response due to exposure to EDCs are indeed plausible even though the mechanism(s) underlying and driving these changes remain to be determined. On one hand, demonstration that any of the adverse outcomes discussed in this report are mediated by exposure to man-made chemicals acting via an endocrine pathway has yet to be established with certainty, while on the other hand many such plausible possibilities cannot even tentatively be excluded.

The potential for environmental contaminants to interact with the endocrine system and induce adverse effects in the human population is an issue that will remain important for some time to come. It will be necessary for investigators who routinely measure human exposures to recruit people from the general population into their studies and, wherever possible, to forge collaborations with epidemiologists so that exposure and effects may ultimately be linked. Furthermore, it is essential that in addition to hazard identification, the mechanisms of action be determined utilizing the most appropriate animal models. In some cases, this will require the development of new animal models of human diseases such as endometriosis and improved animal models of human diseases such as breast cancer. It is expected that identification of the relevant events in the receptor-mediated pathways influenced by exposure to EDCs will contribute to evidence-based risk assessment decisions, and will yield useful information for the elucidation of the basic physiology underlying normal reproductive function and development.

REFERENCES

Available on Request from the Author:

Claude Hughes, M.D., Ph.D., Avalon Medical Group, 1001 S. Hamilton Rd., Chapel Hill, NC 27514

Erratum: The following author photographs were omitted from the Spring 2001 issue and are printed below.

Ovarian Preservation for Women with Malignant Diseases: New Technologies May Be Around the Corner

John A. Schnorr, M.D.
Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, Virginia

Risks of Deep Vein Thrombosis in Hormone Users

Robert A. Wild, M.D., M.P.H.
Professor and Chief, Section of Reproductive Endocrinology, Adj. Professor of Medicine/Epidemiology, Oklahoma University Health Sciences Center, Oklahoma City, Oklahoma

HRT Adherence Issues

Margery Gass, M.D.
Professor, Clinical Obstetrics and Gynecology, Director, University Hospital Menopause and Osteoporosis Center, University of Cincinnati College of Medicine, Cincinnati, Ohio

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