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Workshop on measuring estrogen exposure and metabolism: Summary of the presentations

1. Introduction

A workshop designed to address key clinical and research issues related to measuring estrogen exposure and metabolism was held in Bethesda, Maryland on March 20–21, 2014. This meeting brought together 25 experts as speakers, who are listed below, and an additional 75 attendees. Principal sponsors of the meeting included The Endocrine Society, American Association of Clinical Chemistry, and the Partnership for Accurate Testing of Hormones (PATH), with meeting coordination by Penn State Medical Center Department of Continuing Education. This manuscript summarizes the key aspects of each talk. Full manuscripts from most of these talks will be published in the journal *Steroids*.

2. Estrogen in health and disease: the need for accurate measurement of estradiol

2.1. Endogenous estrogens and risk of female cancers

Dr. Susan Hankinson reviewed data from the Nurses' Health Study and from multiple other prospective studies (also see data of Dr. Tim Key below) indicating that the relative risk of breast cancer in postmenopausal women is approximately 2.0–2.5 when comparing those with the highest 20% of circulating estrogen levels versus those with the lowest 20% of levels. Most studies report that these associations are stronger for estrogen receptor-positive breast cancer, but results for estrogen receptor-negative disease are limited and variable. Pooled studies suggest that estradiol levels are also associated with breast cancer risk in premenopausal women but the effect is modest reflecting a hazard ratio of 1.2–1.4 for a similar contrast in levels. Estradiol levels are also strongly associated with the risk of developing endometrial cancer. Only one small prospective study has evaluated the association of circulating estrogens and risk of ovarian cancer; hence no definitive conclusions can be drawn. A major challenge in prior epidemiologic studies is the lack of standardization of hormone assays such that the association of cancer risk with any absolute hormone level (versus simply high versus low levels) is still unknown.

2.2. Physiologic roles of androgens and estrogens in healthy adult men

Dr. Joel Finkelstein presented data derived from a novel suppression, add-back strategy that elucidated the specific and combined effects of estrogens and androgens in men (Finkelstein et al., N Engl J Med 369:1011–1022, 2013). Androgens acting as androgens are associated with increases in lean body mass, thigh muscle area, leg press strength, and levels of prostate-specific antigen, as well as with reductions in HDL cholesterol and leptin. Estrogens acting as estrogens are associated with decreases in percent body fat, subcutaneous fat, and intra-abdominal fat, as well as with increases in spine bone mineral density and insulin sensitivity. Both androgens and estrogens contribute to the regulation of bone resorption, sexual desire, and erectile dysfunction. Thus when men become severely hypogonadal with loss of lean mass and strength, an increase in HDL-cholesterol and a decrease in prostate-specific antigen are related to androgen deficiency *per se*; the increase in body fat, the decrease in insulin sensitivity, and most (if not all) of the bone loss is due to estrogen deficiency *per se*; and sexual dysfunction is related to both androgen and estrogen deficiency.

2.3. Estrogen and bone health in men and women

Dr. Jane Cauley reported that the greatest rate of bone loss in women was observed during the trans-menopausal period (i.e., 1 year before to 2 years after the start of menopause), a time characterized by falling estrogen levels. Later in menopause, a flattening of the slope of bone loss occurs, a phenomenon apparently not related to changes in estrogen levels. Estradiol levels predict fracture incidence in older women and older men. Total estradiol levels less than 5 pg/ml were associated with a 2.5-fold increase in hip and vertebral fractures, an association that was independent of age and body weight. This association did not vary by race. Most of the studies relied on radioimmunoassays with extraction and column chromatography methods for the measurement of estrogens. Menopausal hormone therapy reduced hip, spine, and other fractures in the Women's Health Initiative Hormone Trials with little difference observed between estrogen alone and estrogen plus progestin trials. The level of circulating estradiol before randomization did not predict the extent of fracture reduction observed with hormone therapy.

2.4. Considerations when testing estrogen effects on cognition in the rodent: a trip down memory lane

Dr. Heather Bimonte-Nelson presented data examining the effects of exogenous estrogens on memory and brain function in female rodents. She indicated that a systematic stepwise approach has identified positive cognitive effects of estrogen on specific parameters of learning and memory retention in rodents. Dr. Bimonte-Nelson noted that the cognitive efficacy of estrogens

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depends on many factors, including dose, mode of administration, and timing of treatment. Estradiol, progesterone, and androstenedione appear to exert specific, and at times interactive, effects on cognition. These studies provide preclinical models to better understand the effects of estrogen on cognition and the brain in women.

3. Estrogen measurement in clinical research and patient care

3.1. Why circulating estradiol is so low after menopause and how to measure it

Dr. Fernand Labrie focused on the mechanisms of *intracrinology*, defined as the conversion of steroid substrate precursors to active hormones in cells and in tissue. The level of expression of the steroid-forming and steroid-inactivating enzymes is specific to each tissue type, thus permitting control of local physiologic functions without affecting other tissues or inducing systemic effects. In postmenopausal women dehydroepiandrosterone (DHEA) is an important precursor that is converted in various tissues to estrogens and androgens. Serum levels of DHEA in postmenopausal women exhibit large individual variations ranging from barely detectable to normal premenopausal levels with no specific feedback mechanisms to modulate these levels. There is a steady decline in DHEA levels starting around age 30 and continuing until the 90s. A theoretical consequence of this is that women with low DHEA levels might benefit from external supplementation resulting in specific tissue production of estrogens and androgens.

3.2. Estradiol measurement in translational studies of breast cancer

Dr. Per Lønning described labor-intensive but highly accurate radiometric methods used for measuring estrogen production in women. These involve administration of tritiated androstenedione and C-14 estrone in women to determine the rate of steady state conversion of androgens to estrogens via aromatization by measuring isotope ratios in plasma or urinary estrogens, a parameter termed the "rho value". As an example of studies with this technique, the most potent aromatase inhibitors – letrozole, anastrozole, and exemestane – block aromatization by 99.1%, 97.9% and 97.9%, respectively. Assessing aromatization by immunoassay requires a comparison of basal and suppressed values. Dr. Lønning reported that radioimmunoassay methodology, when using very stringent techniques, can validly measure low levels of estrogen and demonstrate substantial levels of suppression with aromatase inhibitors closely approaching the degree of aromatase inhibition assessed by using the isotope techniques. His radioimmunoassay for estrogen employs a multistep extraction process, column chromatography purification, and radioimmunoassay for determining estrogens both in plasma and in tissue. His data demonstrated that plasma estradiol radioimmunoassays, when they include multiple pre-purification steps, can achieve detection limits of 0.67 pM.

3.3. Measurement of estrogens in patients on aromatase inhibitors

Dr. James Ingle reported the results of estradiol levels measured by gas chromatography/tandem mass spectrometry in 649 patients receiving the aromatase inhibitor, anastrozole, as treatment for breast cancer. He found that estrone and estradiol concentrations were below the lower limit of detection (i.e., 0.625 pg/ml) in 70% and 79% of patients, respectively. Surprisingly, those subjects with reliably detectable levels of estradiol and estrone exhibited a broad range of values from 1.56 to 183 pg/ml for estrone and 0.627 to 234 pg/ml for estradiol, respectively. By measuring blood levels of anastrozole, he established that these patients were compliant in taking their medication. These findings suggested resistance to the blocking effects of the aromatase inhibitor with the clinically relevant conclusion that a standard 1 mg daily dose of anastrozole is sub-optimal for a substantial proportion of women with breast cancer. Accordingly, plasma levels of estradiol require measurement in patients on aromatase inhibitors, a caveat not routinely practiced by oncologists treating women with breast cancer.

3.4. Estrogen levels in adolescents

Dr. Alan Rogol described the metabolic actions of low dose estrogens in adolescents with hypogonadism (Turner syndrome) as a possible model for postmenopausal women. Estradiol administration, whether transdermal or oral, was metered to attain those levels that would be the approximate daily average (approximately 80–100 pg/mL) over a full ovarian cycle in a reproductive age woman. Although estradiol levels were indistinguishable during either mode of administration, the estradiol metabolites, estrone and estrone sulfate were approximately 10-fold higher during oral therapy. Bio-estrogen concentrations (see method by Karen Klein below) were approximately 1.5-fold higher with oral versus transdermal administration. Despite these markedly different levels there were no significant differences in luteinizing hormone levels, bone density *z*-scores, lipid profiles, body composition or energy expenditure. Sex hormone-binding globulin levels were approximately 1.5-fold greater but insulin-like growth factor-I levels were 20% lower with oral versus transdermal administration after one year of therapy. It should be noted that many postmenopausal women may be treated with estradiol for decades and that these one-year results in prepubertal adolescent girls with Turner syndrome may not describe the long term outcomes of estradiol therapy in postmenopausal women.

4. Accurate measurement of estradiol, especially at low concentrations

4.1. Estrogen measurement: radioimmunoassay to mass spectrometry

Dr. Ravinder Singh presented a critical review of the various methods used for plasma estradiol measurements. Methods for estradiol have ranged from bioassays to manual immunoassays and instrument-based automated immunoassays. Bioassays are clinically desirable, but not very practical for clinical laboratories. Estradiol circulates bound to sex hormone-binding globulin to a large extent and needs to be extracted completely for accurate and precise measurements. Automated immunoassays are very convenient and efficient but have huge variability and accuracy issues. In 2013, the College of American Pathology (CAP) quality assurance program detected a wide range of variability in plasma estradiol levels (184–609 pg/ml) between 14 different assay methods measuring a single standard premenopausal sample. Dr. Singh provided data comparing seven immunoassays and a tandem mass spectrometry assay for the measurement of low levels of estradiol in postmenopausal samples, revealing a lack of agreement between different labs and different types of assays. Immunoassays uniformly measure higher levels than mass spectrometry, in part due to the detection of cross-reacting substances. While mass spectrometry is considered a reference method, it too has some limitations, including susceptibility to interference from non-estrogen compounds embedded within estradiol peaks in the mass spectra. High resolution mass spectrometry methods may overcome interferences observed with routine mass spectrometry methods. Mass spectrometry assays are also considered tedious to perform and involve expensive equipment too costly for routine clinical laboratories. Critical attention, however, needs to be directed toward the accurate measurement of low levels of estrogen. Ultimately, the goal for the laboratory measurement of estrogens