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Bioidentical Hormone Replacement Therapy (BHRT) Position Paper & References

As a primary care physician who specializes in optimal aging medicine, I think the most important role doctors can play is that of teacher—to provide complete, unbiased information so that patients can make their own informed decisions.

Following is a list of facts I provide my patients when they seek help for hormone-related symptoms:

- Many women and men have significant symptoms as they age.
- No two people are identical in terms of their hormone production or the symptoms they experience.
- Eating a healthy diet, exercising regularly, minimizing stress, and avoiding environmental toxins are the foundations for preventing and managing hormone-related symptoms.
- Hormone replacement—an option that contains benefits and risks—can enhance the above foundations for healthy aging.
- People have different medication needs and drug detoxifying capacities. Testing baseline hormone levels and following up with repeat testing is a reasonable way to help determine hormone dosages, and to assess whether a person is receiving too much hormone. However, lab work is not a substitute for clinical decisions based on signs and symptoms. Symptoms of excess or deficient hormones are more indicative of the body's exposure to hormones over time, rather than the moment in time when hormones are tested.
- Synthetic hormones (especially Provera) have been shown to have serious health consequences including increased risk of breast cancer, blood clots, heart disease, and stroke. Synthetic oral testosterone has been shown to increase the risk for liver inflammation and liver cancer in men.
- Bioidentical hormones are identical in structure to those made by the body. There is a large body of research involving the effectiveness of bioidentical estradiol, progesterone, and testosterone [please see references below]. Bioidentical hormones do carry risks, especially when administered in excessive dosages, outside of physiological levels; overall, however, they have a lower risk profile than their synthetic counterparts (this is especially true for bioidentical progesterone vs. progestins, and bioidentical testosterone vs. methyltestosterone). More research about long-term effects of bioidentical hormones needs to be done.
- Bioidentical hormones are found in pharmaceuticals (e.g., bioidentical estradiol patches such as Climara or Vivelle, bioidentical progesterone such as Prometrium and Crinone, and bioidentical testosterone such as Androderm, Androgel, or Testopel) as well as in individual preparations made by compounding pharmacists.

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- Compounded medications have been available since the 1930s. Organizations such as the Professional Compounding Centers of America (PCCA) provide continuing education seminars for pharmacists and physicians, as well as a source of FDA-approved ingredients subjected to quality assurance standards. There are significant differences in quality among compounding pharmacies. Choosing a pharmacy that is approved by the Professional Compounding Accreditation Board (PCAB) provides assurance that the pharmacy has demonstrated superior quality and safety in compounding practices.

- Treating hormone imbalances requires a comprehensive understanding of endocrinology and gynecology, as well as significant clinical experience.

Following are opinions I share with my patients:

- It makes sense to test baseline hormone production, and then, if low levels and/or hormone-related symptoms deem necessary, it's reasonable to prescribe dosages of bioidentical hormones that eliminate or minimize symptoms, or to bring a patient's hormone levels to within physiological range. There is no established protocol for such treatment and potential risks exist.

- It is a patient's right to use bioidentical hormones to maintain optimal health and possibly prevent chronic diseases of aging. If a person chooses this, it is the physician's responsibility to monitor the patient and provide new research that may impact the patient's health or hormone choices.

- Choosing an experienced physician who listens, provides you with information, and respects your treatment decisions is your right and responsibility. Expect your physician to provide you with available research, benefits, and risks of any treatment you choose. Do not be afraid to question any treatment or to make your own healthcare decisions.

Bioidentical Hormone References

Compiled from International Hormone Society, Thierry Hertoghe, MD (www.intlhormonesociety.org), Women in Balance (www.womeninbalance.org), Rebecca Glaser, MD (www.hormonebalance.org), David Zava, PhD (www.zrtlab.com), Neil Rouzier, MD (www.worldlinkmedical.com), and personal pubmed searches.

Topics:

Bioidentical Estrogens & Progesterone Combined Safety

Bioidentical Estrogens

- Brain, Nervous System, Mood
- Bone
- Breast Cancer
- Cardiovascular System or Lipids
- Genitourinary Tract
- Uterus
- Immune Function

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- Vasomotor Symptoms
- Mode of Delivery
- Estriol

Bioidentical Progesterone

- Breast Cancer
- General Health
- Safety
- Ovaries
- Mode of Delivery
- Cancer (other than Breast)
- PMS
- Bone
- Fertility and Pregnancy
- Cardiovascular Disease & Lipids
- Menopausal Symptoms
- Sleep
- Quality of Life
- Brain, Nervous System, Mood
- Uterus
- Progesterone vs Progestins

WOMEN — Androgens (DHEA & Testosterone)

- General
- Brain, Nervous System, Mood
- Breast
- Bone
- Cardiovascular System & Lipids
- Libido

MEN —Testosterone

- General
- Body Composition
- Bone
- Erectile Dysfunction
- Prostate
- Cardiovascular System, Metabolic Syndrome, Diabetes, & Obesity
- Brain, Nervous System, Mood
- DHEA & Immune Function

WOMEN — Pellets (Subcutaneous Estradiol/Testosterone Implants)
MEN — Pellets (Subcutaneous Testoterone Implants)

Bioidentical Estrogens & Progesterone Combined – Safety:

- Holtorf K. The bioidentical hormone debate: are bioidentical hormones (estradiol, estriol, and progesterone) safer and more efficacious than commonly used synthetic versions in hormone replacement therapy? *Postgraduate Medicine.* 2009;121(1):73-85.

This paper reviews the evidence comparing bioidentical estradiol, estriol, and progesterone with commonly used synthetic HRT in terms of efficacy, physiological action on breast tissue, and risk for breast cancer and cardiovascular disease. Results were that patients reported greater satisfaction with BHRT, especially progesterone over progestins. In addition, BHRT was associated with lower breast cancer and cardiovascular disease risk.

- L'Hermite M, Simoncini T, Fullller S, et al. Could transdermal estradiol + progesterone be a safer postmenopausal HRT? A review. *Maturitas.* 2008. 60:185-201.

  Hormone replacement therapy (HRT) in young postmenopausal women is a safe and effective tool to counteract climacteric symptoms and to prevent long-term degenerative diseases, such as osteoporotic fractures, cardiovascular disease, diabetes mellitus and possibly cognitive impairment. The different types of HRT offer to many extent comparable efficacies on symptoms control; however, the expert selection of specific compound and doses or routes of administration can provide significant clinical advantages. This paper reviews the role of the non-oral route of administration of sex steroids in the clinical management of postmenopausal women. Non-orally administered estrogens, minimizing the hepatic induction of clotting factors and others proteins associated with the first-pass effect, are associated with potential advantages on the cardiovascular system. In particular, the risk of developing deep vein thrombosis or pulmonary thromboembolism is negligible in comparison to that associated with oral estrogens. In addition, recent indications suggest potential advantages for blood pressure control with non-oral estrogens. To the same extent, a growing literature suggests that the progestins used in association with estrogens may not be equivalent. Recent evidence shows that natural (bioidentical) progesterone displays a favorable action on blood vessels and on the brain, while this might not be true for some synthetic progestins. Compelling indications also exist that differences might also be present for the risk of developing breast cancer, with recent trials indicating that the association of natural progesterone with estrogens confers less or even no risk of breast cancer as opposed to the use of other synthetic progestins. In conclusion, while all types of hormone replacement therapies are safe and effective and confer significant benefits in the long-term when initiated in young postmenopausal women, in specific clinical settings the choice of the transdermal route of administration of estrogens and the use of natural progesterone might offer significant benefits and added safety.

Bioidentical Estrogens & the Brain, Nervous System, & Mood:

- Asthana S, Baker LD, Craft S. et al. High-dose estradiol improves cognition for women with AD. *Neurology*. 2001;57(4):605-- 612.

  Twenty postmenopausal women with Alzheimer's disease were randomized to receive either 0.10 mg/day of 17 beta-estradiol patch or placebo for 8 weeks. Subjects were evaluated at baseline, at weeks 3, 5, and 8 during treatment, and again 8 weeks after treatment termination. During each visit, cognition was assessed with a battery of neuropsychological tests, and blood samples were collected to measure plasma estradiol as well as several other neuroendocrine markers of interest. Significant effects of estrogen treatment were observed on attention (Stroop Color Word Interference Test), verbal memory (Buschke Selective Reminding Test), and visual memory (Figure

Copy/Memory). In addition, women treated with estrogen demonstrated improved performance on a test of semantic memory (Boston Naming Test) compared with subjects who received a placebo. Conclusion was that administration of a 0.10 mg estradiol patch may enhance attention and memory for postmenopausal women with AD.

- Behl C, Skutella T, Lezoualch F, et al. Neuroprotection against oxidative stress by estrogens: structure-activity relationship. *Mol Pharmacol*. 1997;51: 535-541.

  Oxidative stress-induced neuronal cell death has been implicated in different neurological disorders and neurodegenerative diseases; one such ailment is Alzheimer's disease. Using the Alzheimer's disease-associated amyloid beta protein, glutamate, hydrogen peroxide, and buthionine sulfoximine, the authors investigated the neuroprotective potential of estrogen against oxidative stress-induced cell death. Results showed that 17-beta-estradiol, its nonestrogenic stereoisomer, 17-alpha-estradiol, and some estradiol derivatives can prevent intracellular peroxide accumulation and, ultimately, the degeneration of primary neurons, clonal hippocampal cells, and cells in organotypic hippocampal slices.

- Bruce-Keller AJ, Keeling JL, Keller JN, Huang FF, Camondola S, Mattson MP. Anti-inflammatory effects of estrogen on microglial activation. *Endocrinology* 2000 Oct;141(10):3646-56.

  This study identified new pathways for the estrogenic anti-inflammatory effects on brain function, potentially leading to identification of new methods for improving neurodegenerative disease, specifically involving the microglial cells.

- Cohen, L, Soares, C, Poitras, J, et al. Short-term use of estradiol for depression in perimenopausal and postmenopausal women: a preliminary report. *Am J Psychiatry*. 2003 Aug;160(8):1519-22.

  22 peri or postmenopausal women with median age of 50 years experiencing moderate severity depression (DSM-IV major depression, minor depression, or dysthymia) were enrolled in a 4-week open-label clinical trial of 100 micrograms of transdermal 17β estradiol. Results showed decreased score on Montgomery-Asberg Depression Rating Scale (20 to 11.50) and Beck Depression Inventory. Greene-Climacteric Scale scores showed measured improvement during the 4-week study. Changes in depression scales and climacteric scales were not significantly correlated. Perimenopausal (6) women showed greater improvement in depression scales than postmenopausal women (2). Authors suggested this study supports previous results showing that the effect of estrogen therapy on mood may be independent of antidepressant effects mediated by alleviation of vasomotor symptoms and that estrogen therapy may be of benefit to perimenopausal women experiencing moderately severe depression.

- Janicki SC, Schupf N. Hormonal influences on cognition and risk for Alzheimer's disease. *Curr Neurol Neurosci Rep*. 2010;10(5):359-66.

  Research increasingly suggests that changes in estrogen levels during aging may increase risk for Alzheimer disease, the most common type of dementia. This update

reviews the newest information about estrogen and cognitive aging, including information regarding the role of bioavailable estrogen in older women and men, use of selective estrogen receptor modulators (SERMs) to improve cognition, and studies of genetic risk factors to elucidate the effects of endogenous estrogen on aging and cognition. Future trials are needed to determine whether alternate timing, dosage, formulation, or method of administration of hormone replacement can reduce risk of dementia.

While individual study results are inconsistent, overall data from epidemiologic studies, observational studies and clinical trials of hormone replacement therapy, studies of endogenous hormones and evaluations of genetic variants involved in estrogen biosynthesis and receptor activity indicate that estrogen plays an important role in the pathogenesis of cognitive decline and risk for AD in both men and women.

- Jacobs DM, Tang MX, Stern Y, et al. Cognitive function in nondemented older women who took estrogen after menopause. *Neurology*.1998;50(2):368-73.

  This study evaluated the relationship between a history of estrogen use and cognitive test performance in 727 women participating in a large community-based study. Participants were followed longitudinally for an average of 2.5 years. Estrogen use history was obtained at baseline. Standardized tests of memory, language, and abstract reasoning were administered at baseline and at follow-up. Results indicated that women who had used estrogen replacement scored significantly higher on cognitive testing at baseline than nonusers, and their performance on verbal memory improved slightly over time. The effect of estrogen on cognition was independent of age, education, ethnicity, and APOE genotype. Results suggest that estrogen replacement therapy may help to maintain cognitive function in nondemented postmenopausal women.

- Joffe H, Hall JE, Gruber S, et al. Estrogen therapy selectively enhances prefrontal cognitive processes: a randomized, double-blind, placebo-controlled study with functional magnetic resonance imaging in perimenopausal and recently menopausal women. *Menopause*. 2006;13(3):411-22.

  Estrogen therapy (ET) seems to differentially effect cognitive processes in younger versus older postmenopausal women, suggesting a window of opportunity when ET is most beneficial. Cognitive improvement in younger postmenopausal women has been attributed to ET's influence on hot flushes and sleep, but empiric examination of the mediating role of menopause symptoms versus direct effects of ET on the brain is limited. In this double-blind trial, 52 women were randomly assigned to estradiol 0.05 mg/day or placebo transdermal patches for 12 weeks. Women completed tests of memory, learning, and executive functioning, and hot flush and sleep assessments at baseline and study end. A subset of women (five ET treated, six placebo treated) also underwent blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) studies. Nondepressed perimenopausal and postmenopausal women were studied. The majority had hot flushes and sleep impairment. Compared with placebo, ET selectively reduced errors of perseveration during verbal recall, a frontal system-mediated function, but did not influence other cognitive processes. Women with baseline hot flushes had greater cognitive benefit with ET. Cognitive benefit was not associated with sleep problems or its improvement. Measures of fMRI BOLD activation

during tests of verbal and spatial working memory showed significant increases in frontal system activity with ET. Conclusion was that ET selectively improves executive functioning as demonstrated by reduced perseverative errors and prefrontal cortex activation during verbal recall tasks. Cognitive improvement with ET is associated with hot flushes, but not with sleep, suggesting that ET has a direct central nervous system effect, rather than an indirect effect mediated through improvement of sleep.

- Maki PM. Minireview: effects of different HT formulations on cognition. *Endocrinol*. 2012 June 6. [Epub ahead of print]

  Evidence from preclinical studies, randomized clinical trials (RCT), and observational studies underscores the importance of distinguishing among the different forms of estrogen and progestogens when evaluating the cognitive effects of hormone therapy (HT) in women. Despite this evidence, there is a lack of direct comparisons of different HT regimens. To provide insights into the effects of different HT formulations on cognition, this minireview focuses on RCT of verbal memory because evidence indicates that HT affects this cognitive domain more than others and because declines in verbal memory predict later development of Alzheimer's disease. Some observational studies indicate that estradiol confers benefits to verbal memory, whereas conjugated equine estrogens (CEE) confer risks. RCT to date show no negative impact of CEE on verbal memory, including the Women's Health Initiative Study of Cognitive Aging. Similarly, the Women's Health Initiative Memory Study showed no negative impact of CEE on dementia. Transdermal estradiol in younger postmenopausal women improved verbal memory in one small RCT but had no effect in another RCT. RCT of oral estradiol in younger and older postmenopausal women had neutral effects on cognitive function. In contrast, RCT show a negative impact of CEE plus medroxyprogesterone acetate on verbal memory in younger and older postmenopausal women. Small RCT show neutral or beneficial effects of other progestins on memory. Overall, RCT indicate that type of progestogen is a more important determinant of the effects of HT on memory than type of estrogen.

- Montgomery J, Brincat M, Tapp A, et al. Effect of oestrogen and testosterone implants on psychological disorders in the climacteric. *The Lancet.* 1987:297-299.

  Double-blind, placebo-controlled trial assessing psychological symptoms involving 3 treatment groups of peri and postmenopausal women (N=70): estradiol and testosterone implants, estradiol implant only, or placebo. Depression and anxiety were significantly lower in the pellet treated groups.

- Paganini-Hill A, Henderson VW. Estrogen deficiency and risk of Alzheimer’s disease in women. *Am J Epidemiol*. 1994;140(3):256-61.

  The authors explored the possibility that estrogen loss associated with menopause may contribute to the development of Alzheimer's disease by using a case-control study nested within a prospective cohort study. The Leisure World Cohort includes 8,877 female residents of Leisure World Laguna Hills, a retirement community in southern California, who were first mailed a health survey in 1981. From the 2,529 female cohort members who died between 1981 and 1992, the authors identified 138 with Alzheimer's

disease or other dementia diagnoses likely to represent Alzheimer's disease (senile dementia, dementia, or senility) mentioned on the death certificate. Four controls were individually matched by birth date (+/- 1 year) and death date (+1 year) to each case. The risk of Alzheimer's disease and related dementia was less in estrogen users relative to nonusers. The risk decreased significantly with increasing estrogen dose and with increasing duration of estrogen use. Risk was also associated with variables related to endogenous estrogen levels; it increased with increasing age at menarche and (although not statistically significant) decreased with increasing weight. This study suggests that the increased incidence of Alzheimer's disease in older women may be due to estrogen deficiency and that estrogen replacement therapy may be useful for preventing or delaying the onset of this dementia.

- Rocca WA, Bower JA, Maraganore DM, et al. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. *Neurology.* 2007; 11;69(11):1074-83.

  This study established that both unilateral and bilateral oophorectomy preceding the onset of menopause are associated with an increased risk of cognitive impairment or dementia. The effect is age-dependent and suggests a critical age window for neuroprotection.

- Shaywitz S, Shaywitz B, Pugh K, et al. Effect of estrogen on brain activation patterns in postmenopausal women during working memory tasks. *JAMA*. 1999;281(13):1197-1202.

  Estrogen in a therapeutic dosage alters brain activation patterns in postmenopausal women in specific brain regions during the performance of the sorts of memory function that are called upon frequently during any given day. These results suggest that estrogen affects brain organization for memory in postmenopausal women.

- Singh M. Ovarian hormones elicit phosphorylation of Akt and extracellular-signal regulated kinase in explants of the cerebral cortex. *Endocrine* 2001 Apr;14(3):407-15.

- Soares CN, Almeida OP, Joffe H, Cohen LS. Efficacy of estradiol for the treatment of depressive disorders in perimenopausal women: a double-blind, randomized, placebo-controlled trial. *Arch Gen Psychiatry* 2001;58(6):529-34.

  Results of previous studies suggest that estrogen improves somatic and mild depressive symptoms experienced by perimenopausal women. This study investigated the efficacy of 17beta-estradiol for the treatment of clinically significant depressive disorders in endocrinologically confirmed perimenopausal women. Fifty perimenopausal women (aged 40-55 years, with irregular menstrual periods and serum concentrations of follicle-stimulating hormone >25 IU/L), meeting criteria for major depressive disorder, dysthymic disorder, or minor depressive disorder, according to DSM-IV, were randomized to receive transdermal patches of 17beta-estradiol (100 microgram) or placebo in a 12-week, double-blind, placebo-controlled study. Subjects responded similarly to estradiol treatment, regardless of DSM-IV diagnosis. Patients treated with estradiol sustained antidepressant benefit of treatment after the 4-week washout period, although somatic complaints increased in frequency and intensity. Treatment was well tolerated and

adverse events were rare in both groups. The authors concluded that transdermal estradiol replacement is an effective treatment of depression for perimenopausal women.

- Singer CA, Figueroa-Masot XA, Batchelor RH, Dorsa DM. The mitogen-activated protein kinase pathway mediates estrogen neuroprotection after glutamate toxicity in primary cortical neurons. *J Neuroscience* 1999;19: 2455-2463.

  This randomized, double-blind, placebo-controlled trial investigated the efficacy of 17beta-estradiol for the treatment of clinically significant depressive disorders in 50 perimenopausal women (FSH >25 IU/L, irregular menses), meeting criteria for major depressive disorder, dysthymic disorder, or minor depressive disorder. Women received transdermal patches of 17beta-estradiol (100 microgram) or placebo for 12-weeks. A 4-week washout period followed the 12-week treatment phase. Outcome measures were the Montgomery-Asberg Depression Rating Scale and Blatt-Kupperman Menopausal Index scores. RESULTS: Remission of depression was observed in 17 (68%) women treated with 17beta-estradiol compared with 5 (20%) in the placebo group (P =.001). Subjects responded similarly to estradiol treatment, regardless of DSM-IV diagnosis. CONCLUSION: Transdermal estradiol replacement is an effective treatment of depression for perimenopausal women.

- Wharton W, Baker LD, Gleason CE, et al. Short-term hormone therapy with transdermal estradiol improves cognition for postmenopausal women with Alzheimer's disease: results of a randomized controlled trial*. J Alzheimers Dis.* 2011;26(3):495-505.

  This double-blind, placebo-controlled, parallel-group study evaluated efficacy of hormone therapy in postmenopausal women with mild to moderate Alzheimer's disease (AD). Treatment arms included transdermal estradiol with or without medroxyprogesterone acetate (MPA, Provera) for 12 months. Results showed that treatment improved cognition in multiple cognitive domains. Plasma estradiol levels positively correlated with improvements in visual memory.

- Wharton W, Gleason C, Lorenze K, et al. Potential role of estrogen in the pathobiology and prevention of Alzheimer's disease*. Am J Transl Res*. 2009;1(2):131-147.

- Wise PM. Estradiol: a protective factor in the adult brain. *J Pediatr Endocrinol Metab* 2000;13 Suppl 6:1425-9.

  Clinical studies suggest that estradiol acts as a protective factor in the adult brain. Postmenopausal women suffer from an increased risk of brain injury associated with neurodegenerative diseases such as Alzheimer's disease, and estrogen replacement therapy appears to decrease the risk and severity of this neurodegenerative condition. Studies using animal models show that estradiol exerts similar effects in rodents and can enhance cell survival. The authors designed experiments to determine whether estradiol treatment can decrease brain injury induced by an experimental model of ischemia. The experiments used a permanent middle cerebral artery occlusion model and physiological levels of estradiol replacement therapy. The results demonstrated that estradiol exerts profound protective effects against ischemic brain injury induced by cerebral artery

occlusion and that this protective action correlates with changes in the level of gene expression of estradiol receptors and members of the Bcl-2 family. These data suggest that estrogen replacement therapy may provide important protection against age- and disease-related degenerative processes in the brain.

- Wise P. Estradiol exerts neuroprotective actions against ischemic brain injury: insights derived from animal models. *Endocrine*. 2003 Jun;21(1):11-5.

- Yaffe K, Lui LY, Grady D, et al. Cognitive decline in women in relation to non-protein-bound oestradiol concentrations*. Lancet.* 2000;356(9231):708-12.

  Previous studies found no association between serum concentrations of total oestradiol and cognitive function, but these measurements may not reflect concentrations of hormone available to the brain. This study tested the hypothesis that concentrations of non-protein-bound (free) and loosely bound (bioavailable) sex hormones are associated with cognitive function in older women. Cognitive performance was measured at baseline and 6 years later in 425 women (65 years or older). Concentrations of non-protein-bound and bioavailable oestradiol and total and non-protein-bound testosterone were measured by RIA in serum samples taken at baseline.Women with high serum concentrations of non-protein-bound and bioavailable oestradiol, but not testosterone, were less likely to develop cognitive impairment than women with low concentrations. This finding supports the hypothesis that higher concentrations of endogenous oestrogens prevent cognitive decline.

- Zandi PP, Carlson MC, Plassman BL, et al. Hormone replacement therapy and incidence of Alzheimer disease in older women: the cache county study. *JAMA* 2002;288:2123-2129.

  Previous studies have shown a sex-specific increased risk of Alzheimer disease (AD) in women older than 80 years. Basic neuroscience findings suggest that hormone replacement therapy (HRT) could reduce a woman's risk of AD. Epidemiologic findings on AD and HRT are mixed. The purpose of this study was to examine the relationship between use of HRT and risk of AD among elderly women. This was a prospective study of dementia incidence among 1357 men (mean age, 73.2 years) and 1889 women (mean age, 74.5 years) residing in a single county in Utah. Participants were first assessed in 1995-1997, with follow-up conducted in 1998-2000. History of women's current and former use of HRT, as well as of calcium and multivitamin supplements, was ascertained at the initial contact. Thirty-five men (2.6%) and 88 women (4.7%) developed AD between the initial interview and time of the follow-up (3 years). Incidence among women increased after age 80 years and exceeded the risk among men of similar age. Women who used HRT had a reduced risk of AD (26 cases among 1066 women) compared with non-HRT users (58 cases among 800 women). Risk varied with duration of HRT use, so that a woman's sex-specific increase in risk disappeared entirely with more than 10 years of No similar effect was seen with calcium or multivitamin use. Almost all of the HRT-related reduction in incidence reflected former use of HRT. There was no effect with current HRT use unless duration of treatment exceeded 10 years. Conclusion was that prior HRT use is associated with reduced risk of AD, but there is no apparent benefit with current HRT use unless such use has exceeded 10 years.

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Bioidentical Estrogens & Bone:

- Anderson CHM, Raju KS, Forling ML, Wheeler MJ. The effects of surgical menopause and parenteral hormone replacement therapy on bone density, menopausal symptoms, and hormone profiles. Department of Gynaecology, St. Thomas Hospital, London, UK.

  45 women undergoing complete hysterectomies were randomized to receive 50-mg estradiol implants, 50 mcg estradiol patches, or 50 mg estradiol and 100 mg testosterone implants. After one year, there was a significant decrease in bone density in the patch group; no decrease in bone density in the pellet implant groups.

- Arrenbrecht S, Boermans AJ. Effects of transdermal estradiol delivered by a matrix patch on bone density in hysterectomized, postmenopausal women: a 2-year placebo-controlled trial. *Osteoporosis Int* 2002;13(2):176-83.

- Collette J, Viethel P, Dethor M, et al. Comparison of changes in biochemical markers of bone turnover after 6 months of hormone replacement therapy with either transdermal 17 beta-estradiol or conjugated equine estrogen plus nomegestrol acetate. *Gynecol Obstet Fertil*. 2003 May;31(5):434-41.

- Ettinger B, Genant HK, Steiger P, Madvig P. Low-dosage micronized 17 beta-estradiol prevents bone loss in postmenopausal women. *American Journal of Obstetrics and Gynecology*. 1992 Feb;166(2):479-88.

  The authors evaluated the effects of 17 beta-estradiol in a random double blind, dose-ranging study of 41 postmenopausal women conducted in 2 phases. Phase one included phased E2 doses (0.5mg, 1.0mg, 2.0mg) plus calcium supplementation (to serum value of 1500 mg). Phase two included E2 doses plus random cessation of calcium supplementation. Progestins were added during phase two (total study time of 18 months). Results showed very little change in bone density results for placebo group (0.5 – 0.9%) whereas treatment group showed significant increases from baseline bone density. In phase two, the treatment groups showed an annual change in bone density of 2.0. There was a positive correlation between total calcium intake and the change in bone density results. The study showed a continuous dose-response effect on bone density results. Authors concluded that low dose (1.0mg) beta-estradiol and 1000mg of calcium prevented bone loss in postmenopausal women.

- Evans SF, Davie MW. Low and conventional dose transdermal oestradiol are equally effective at preventing bone loss in spine and femur at all post-menopausal ages. *Clin Endocrinol* (Oxf) 1996 Jan;44(1):79-84.

- Garnett T, Studd J, Watson N, et al. The effects of plasma estradiol levels on increases in vertebral and femoral bone density following therapy with estradiol and estradiol with testosterone implants. *Obstet Gynecol* 1992;79:968-72.

- Hayashi T, Ito I, Kano H, Endo H, Iguchi A. Estriol (E3) replacement improves endothelial function and bone mineral density in very elderly women. *J Gerontol A Biol Sci Med Sci* 2000 Apr;55(4):B183-90; discussion B191-3.

- Miller BE, De Souza MJ, Slade K, Luciano AA. Sublingual administration of micronized estradiol and progesterone, with and without micronized testosterone: effect on biochemical markers of bone metabolism and bone mineral density. *Menopause* 2000 (5):318-26.

  The purpose of this investigation was to evaluate the relative efficacy of the sublingual administration of micronized estradiol (E2), progesterone (P4), and testosterone (T) on bone mineral density and biochemical markers of bone metabolism. In this double-blind, prospective study, postmenopausal women were randomly assigned to one of four treatment groups: hysterectomized women were assigned to either 1) micronized E2 (0.5 mg) or 2) micronized E2 (0.5 mg) + micronized T (1.25 mg). Women with intact uteri were assigned to either 3) micronized E2 (0.5 mg) + micronized P4 (100 mg) or 4) micronized E2 (0.5 mg) + micronized P4 (100 mcg) + micronized T (1.25 mg). For the purpose of this study, the four treatment groups were combined into two groups for all comparisons. The E2 and E2+P4 groups were combined into the HRT alone group (n=30), and the E2+T and E2+P4+T groups were combined into the HRT + T group (n=27). Hormones were administered sublingually as a single tablet twice a day for 12 months. The subjects were of similar age, height, weight and had similar baseline follicle-stimulating hormone, E2, P4, total T, and bioavailable T levels. During therapy, serum levels increased for each hormone. Conclusion was that sublingual micronized HRT favorably decreases serum and urine markers of bone metabolism, prevents bone loss, and results in a slight increase in spine and hip bone mineral density. Although the addition of testosterone to HRT for 1 year did not result in added benefit to the spine bone mineral density, it did result in a significant increase in hip bone mineral density. Longer duration of therapy may have further improved these outcomes.

- Nozaki M, Hashimoto K, Inoue Y, Sano M, Nakano H. [Usefulness of estriol for the treatment of bone loss in postmenopausal women] *Nippon Sanka Fujinka Gakkai Zasshi*. 1996 Feb;48(2):83-8.

- Prestwood KM, Kenny AM, Unson C, Kulldorff M. The effect of low dose micronized 17b-estradiol on bone turnover, sex hormone levels, and side effects in older women: a randomized, double blind, placebo-controlled study. *Journal of Clinical Endocrinology and Metabolism* 2000 Dec;85(12):4462-9.

  This study determined that oral low-dose estrogen (0.25mg/day) had similar beneficial effects on bone health in elderly (mean age 75 years) postmenopausal women without the breast tenderness and bleeding associated with higher doses.

- Savvas M, Studd J, Fogelman I, et al. Skeletal effects of oral oestrogen compared with subcutaneous oestrogen and testosterone in postmenopausal women. *BMJ* 1988;297:331-333.

  This study compared different oral vs pellet implants for estrogen replacment. Women who received estradiol pellet implants also received testosterone pellet implants. Oral treatment group was 37 postmenopausal women compared with 41 women given oestrogen implants and 36 controls. Weight was not significantly different among the groups. Implant group was given subcutaneous implants of oestradiol 50 mg combined with testosterone 100 mg, on average six monthly for a median of 8.5 years. Results of

this study showed that estradiol implants were more effective at increasing bone density than oral.

Bioidentical Estrogens & Breast

Note: most of these studies used synthetic equine estrogens with or without synthetic progestins. Very few studies have examined breast cancer risk in women given bioidentical estrogen plus progesterone plus testosterone (in other words, mimicking youthful hormone levels). For more information, see "Hormones & Breast Cancer FAQ" handout.

- Batur P, Bixen CE, Moore HC, et al. Menopausal hormone therapy (HT) in patients with breast cancer. *Maturitas.* 2006;53(2):123-32.

  The authors performed a quantitative review of all studies reporting experience with menopausal HT for symptomatic use after a diagnosis of breast cancer. Rates of reoccurrence, cancer-related mortality, and overall mortality were calculated in this entire group. A subgroup analysis was performed in studies using a control population to assess the odds ratio of cancer reoccurrence and mortality in hormone users versus non-users. Fifteen studies encompassing 1416 breast cancer survivors using HT were identified. Seven studies included a control group comprised of 1998 patients. Among the 1416 HT users, reoccurrence was noted in 10.0% (95% CI: 8.4-11.6%). Cancer-related mortality occurred at a rate of 2.6% (95% CI: 1.8-3.7%), while overall mortality was 4.5% (95% CI: 3.4-5.8%). Compared to non-users, patients using HT had a decreased chance of reoccurrence and cancer-related mortality with combined odds ratio of 0.5 (95% CI: 0.2-0.7) and 0.3 (95% CI: 0.0-0.6), respectively. The authors concluded that menopausal HT use in breast cancer survivors was not associated with increased cancer reoccurrence, cancer-related mortality or total mortality. In addition, future trials should focus on better ways to identify breast cancer survivors who may safely benefit from HT versus those who have a substantial risk of reoccurrence with HT use.

  Note that many of these studies did not use bioidentical estradiol or progesterone.

- Beral V; Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet.* 2003;362:419-27.

  Current use of hormone-replacement therapy (HRT) increases the incidence of breast cancer. The Million Women Study was set up to investigate the effects of specific types of HRT on incident and fatal breast cancer. 1,084,110 UK women aged 50-64 years were recruited into the Million Women Study between 1996 and 2001, provided information about their use of HRT and other personal details, and were followed up for cancer incidence and death. Half the women had used HRT; 9364 incident invasive breast cancers and 637 breast cancer deaths were registered after an average of 2.6 and 4.1 years of follow-up, respectively. Current users of HRT at recruitment were more likely than never users to develop breast cancer (relative risk 1.66) and die from it (1.22). Past users of HRT were, however, not at an increased risk of incident or fatal disease (1.01 and 1.05 respectively). Incidence was significantly increased for current users of preparations containing oestrogen only (1.30), oestrogen-progestagen (2.00), and

tibolone (1.451), but the magnitude of the associated risk was substantially greater for oestrogen-progestagen than for other types of HRT). Results varied little between specific oestrogens and progestagens or their doses; or between continuous and sequential regimens. The relative risks were significantly increased separately for oral (1.32), transdermal (1.24), and implanted oestrogen-only formulations (1.65). In current users of each type of HRT the risk of breast cancer increased with increasing total duration of use. 10 years' use of HRT is estimated to result in five additional breast cancers per 1000 users of oestrogen-only preparations and 19 additional cancers per 1000 users of oestrogen-progestagen combinations. Conclusion was that current use of HRT is associated with an increased risk of incident and fatal breast cancer; the effect is substantially greater for oestrogen-progestagen combinations than for other types of HRT.

- Bergkvist L, Hans-Olov A, Persson I, et al. The risk of breast cancer after estrogen and estrogen-progestin replacement. *N Engl J Med.* 1989;321:293-7.

  This was a study of 23,244 women 35 years of age or older who had had estrogen prescriptions filled in the Uppsala region of Sweden. During the follow-up period (mean, 5.7 years) breast cancer developed in 253 women. Compared with other women in the same region, the women in the estrogen cohort had an overall relative risk of breast cancer of 1.1. The relative risk increased with the duration of estrogen treatment 1.7 after nine years. Estradiol (used in 56 percent of the treatment periods in the cohort) was associated with a 1.8-fold increase in risk after more than six years of treatment. No increase in risk was found after the use of conjugated estrogens (used in 22 percent of the treatment periods) or other types, mainly estriols (used in 22 percent of the treatment periods). Although the numbers of women were smaller, the risk of breast cancer was highest among the women who took estrogen and progestin in combination for extended periods. The relative risk was 4.4 in women who used only this combination for more than six years. Among women who had previously used estrogens alone, the relative risk after three years or more of use of the combination regimen was 2.3. The authors concluded that long-term perimenopausal treatment with estrogens (or at least estradiol compounds) seems to be associated with a slightly increased risk of breast cancer, which is not prevented and may even be increased by the addition of progestins.

- Chang KJ, et al. Influences of percutaneous administration of estradiol and progesterone on human breast epithelial cell cycle in vivo. *Fertil Steril* 1995; 63(4):785-91.

  The effect of transdermal estradiol (1.5 mg), transdermal progesterone (25 mg), and combined transdermal estradiol and progesterone (1.5 mg and 25 mg) on human breast epithelial cell cycles was evaluated in vivo. Results demonstrated that estradiol significantly increases cell proliferation, while progesterone significantly decreases cell replication below that observed with placebo. Transdermal progesterone was also shown to reduce estradiol-induced proliferation.

- Chen W, Manson J, Hankinson S, et al. Unopposed estrogen therapy and the risk of invasive breast cancer. *Arch Intern Med.* 2006;166(9):1027-1032.

  Although short-term unopposed estrogen use does not seem to increase breast cancer risk, the effect of longer-term estrogen use remains unclear. This study sought to assess

the relationship between longer-term use of unopposed estrogen and the risk of invasive breast cancer over an extended follow-up period. Within the Nurses' Health Study, a prospective cohort study, 11,508 postmenopausal women who had a hysterectomy and reported information on estrogen use at baseline (1980) were observed. The study population was expanded every 2 years to include women who subsequently became postmenopausal and had a hysterectomy, so that 28,835 women were included in the final follow-up period (2000-2002). A total of 934 invasive breast cancers were included in the analysis. Breast cancer risk increased with duration of unopposed estrogen use. The relative risks (RRs) for breast cancer with current use of unopposed estrogen for less than 5 years was 0.96; 5 to 9.9 years was 0.90; 10 to 14.9 years was 1.06' 15 to 19.9 years was 1.18; 20 years or longer was 1.42. Conclusion was that users of unopposed estrogen were at increased risk of breast cancer but only after longer-term use.

- Colditz GA, Stampfer MJ, Willett Wc, et al. Type of postmenopausal hormone use and risk of breast cancer: 12-year follow-up from the Nurses' HealthStudy. *Cancer Causes Control.* 1992; 3(5):4333-9.

  Note: this study used the term "progesterone" when referring to synthetic progestins.

  This prospective study examined the use of hormone replacement therapy in relation to breast cancer incidence in a cohort of women 30 to 55 years of age in 1976. During 12 years of follow-up (480,665 person-years) among postmenopausal women, 1,050 incident cases of breast cancer were documented. Overall, past users of estrogen were not at increased risk. After adjustment for established risk factors, type of menopause, age at menopause, and current age, the relative risk was 0.91. The risk of breast cancer was higher among current users (RR = 1.33); after adjusting for age, the authors observed no evidence of increasing risk with increasing duration of use among current users or among past users (P trend = 0.46). Women currently using unopposed estrogen (RR = 1.42), estrogen and progesterone (RR = 1.54), or progesterone alone (RR = 2.52, were all at increased risk of breast cancer compared with never users. These data suggest that long-term past use of estrogen replacement therapy is not related to risk, that current estrogen use increases risk of breast cancer to a modest degree, and that the addition of progesterone does not remove the increased risk observed with current use of unopposed estrogen.

- Davelaar EM, Gerretsen G, Relyveld J. [No increase in the incidence of breast carcinoma with subcutaneous administration of estradiol.] *Ned Tijdschr Geneeskd* 1991;135(14):613-5.

  Between 1972 and mid-1990 the frequency of breast cancer was studied in a group of 261 mostly premenopausal women of the gynaecological department of the Municipal Hospital in The Hague, the Netherlands. All patients had had a total hystero-adnexectomy and received estradiol implants. On the basis of a stratified life table giving the cumulative incidence of breast cancer in the Netherlands, an expected incidence of 2 per 1000 person-years was estimated for the observed group (mean observation period: 8.25 years). There were three cases of breast cancer in the observed group. This means

an incidence density of 1.4 per 1000 person-years. It is concluded that this form of oestrogen substitution does not increase the risk of breast cancer.

- Dew J, Wren B, Eden J. A cohort study of topical vaginal estrogen therapy in women previously treated for breast cancer. *Climacteric.* 2003;6:45-52.

  To estimate the risk of recurrence of breast cancer associated with the use of topical vaginal estrogen therapy in the management of vaginal atrophy in women previously treated for breast cancer. The study group comprised 1472 women with histologically confirmed breast cancer. In these women, poorly absorbed topical vaginal estrogen cream or tablets were used. Hormone usage was entered as a time-dependent covariate with disease-free interval as the outcome. Although the small numbers of this study preclude a definitive result, topical estrogen usage does not appear to be associated with an increased risk of recurrence of breast cancer.

- Foidart JM, Colin C, Denoo X, Desreux J, Beliard A, Fournier S, de Lignieres B. Estradiol and progesterone regulate the proliferation of human breast epithelial cells. *Fertil Steril* 1998 May;69(5):963-9.

  In this double-blind randomized study, 40 postmenopausal women received daily topical application of a gel containing either placebo, estradiol, progesterone, or estradiol + progesterone for two weeks prior to esthetic breast surgery or the excision of a benign breast lesion. The results showed that increased estrogen concentration increased the number of cycling epithelial cells, whereas exposure to progesterone for 14 days reduced the estrogen-induced proliferation of normal breast epithelial cells.

- Fournier A, Berrino F, Riboli E, et al. Breast cancer risk in relation to different types of hormone replacement therapy in the E3N-EPIC cohort. *Int J Cancer*. 2004;114:448-454.

  This study assessed the risk of breast cancer associated with HRT use in 54,548 postmenopausal women who had never taken any HRT 1 year before entering the E3N-EPIC cohort study (mean age at inclusion: 52.8 years); 948 primary invasive breast cancers were diagnosed during follow-up (mean duration: 5.8 years). In this cohort where the mean duration of HRT use was 2.8 years, an increased risk in HRT users compared to nonusers was found (relative risk (RR) 1.2. The RR was 1.1 for estrogens used alone and 1.3 when used in combination with oral progestogens. The risk was significantly greater with HRT containing synthetic progestins than with HRT containing micronized progesterone, the RRs being 1.4 and 0.9], respectively. When combined with synthetic progestins, both oral and transdermal/percutaneous estrogens use were associated with a significantly increased risk; for transdermal/percutaneous estrogens, this was the case even when exposure was less than 2 years. Our results suggest that, when combined with synthetic progestins, even short-term use of estrogens may increase breast cancer risk. Micronized progesterone may be preferred to synthetic progestins in short-term HRT.

- Gambrell RD. Hormone replacement therapy and breast cancer risk. Arch Fam Med. 1996;5(6):341-8.

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The role of estrogen therapy in the risk of breast cancer has been a concern for both physicians and patients. There is some evidence that women taking estrogen who develop breast cancer have a better prognosis. During 8 to 18 years of follow-up of 256 postmenopausal women with breast cancer from our hospital, median survival time was 84 months for those who never used estrogen, 80 months for past users, and 143 months for current users. More than 50 studies have shown that there is no increased risk of breast cancer even with long-term estrogen use, while some studies suggest an increased risk. Several studies indicate that when progestogens are added to estrogen therapy, there is a significant reduction in the risk of breast carcinoma. Indirect evidence is accumulating to show why added progestogen should decrease the risk of breast cancer. Preliminary studies further indicate that estrogen therapy, which has been contraindicated in breast cancer survivors in the past, may be safe, and added progestogens may decrease recurrences and deaths. Some medical oncologists and surgeons now advocate estrogen use in women with previous carcinoma of the breast.

- Holmberg L, Iversen OE, Rudenstam CM, et al. Increased risk of recurrence after hormone replacement therapy in breast cancer survivors. J Natl Cancer Inst. 2008;100(7): 475-82.

  This randomized HABITS study, compared HT for menopausal symptoms with best management without hormones among women with previously treated breast cancer, was stopped early due to suspicions of an increased risk of new breast cancer events following HT. Most patients who received HT were prescribed continuous combined or sequential estradiol hemihydrate and norethisterone.Of the 447 women randomly assigned, 442 could be followed for a median of 4 years. Thirty-nine of the 221 women in the HT arm and 17 of the 221 women in the control arm experienced a new breast cancer event (HR = 2.4, 95% CI = 1.3 to 4.2). Cumulative incidences at 5 years were 22.2% in the HT arm and 8.0% in the control arm. By the end of follow-up, six women in the HT arm had died of breast cancer and six were alive with distant metastases. In the control arm, five women had died of breast cancer and four had metastatic breast cancer. After extended follow-up, there was a clinically and statistically significant increased risk of a new breast cancer event in survivors who took HT.

  Note that most women in this study received synthetic estrogen and synthetic progestin.

- Holtorf K. The bioidentical hormone debate: are bioidentical hormones (estradiol, estriol, and progesterone) safer and more efficacious than commonly used synthetic versions in hormone replacement therapy? *Postgraduate Medicine.* 2009;121(1):73-85.

  This paper reviews the evidence comparing bioidentical estradiol, estriol, and progesterone with commonly used synthetic HRT in terms of efficacy, physiological action on breast tissue, and risk for breast cancer and cardiovascular disease. Results were that patients reported greater satisfaction with BHRT, especially progesterone over progestins. In addition, BHRT was associated with lower breast cancer and cardiovascular disease risk.

- Lemon HM. Pathophysiologic considerations in the treatment of menopausal patients with oestrogens; the role of oestriol in the prevention of mammary carcinoma. *Acta Endocrinol Suppl* (Copenh) 1980;233:17-27.

At menopause, several abnormalities in oestrogen metabolism have been reported, which may increase the likelihood of cancer development in the breast or uterus following oestrone or oestradiol-17 beta supplementation. Occult hypothyroidism reduces the rate of oestrogen inactivation by C2 hydroxylation, and 15-20% of women have low rates of C16 hydroxylation to oestriol. Reduced sex hormone binding globulin concentration occurs in association with obesity, thereby increasing the biologically active unbound fraction of oestradiol in plasma. Since oestriol undergoes minimal metabolism after absorption, does not bind to sex hormone binding globulin, and has an anti-oestradiol action by decreasing the duration of nuclear binding of oestradiol-receptor proteins, it is less likely to induce proliferative changes in target organs of cancer-prone women than oestrone or oestradiol. Intermittent non-conjugated oestriol treatment has demonstrated the most significant anti-mammary carcinogenic activity of 22 tested compounds as well as anti-uterotropic activity in intact female Sprague Dawley rats fed either of two dissimilar carcinogens (7, 12 dimethylbenz(a) anthracene, procarbazine) and followed for their natural life span. The protective effect was specific for mammary carcinomas only and has been decreased in rats with a 20% increase in growth curves. Clinical experience thus far with oral oestriol therapy of post-menopausal women has indicated little hazard of cancer development.

- Lyytinen H, Pukkala E, Ylikorkala O. Breast cancer risk in postmenopausal women using estrogen-only therapy. *Obstet Gynecol*. 2006;108(6):1354-1360.

  All Finnish women older than age 50 years using oral or transdermal estradiol (n=84,729), oral estriol (n=7,941), or vaginal estrogens (n=18,314) for at least 6 months during 1994-2001 were identified from the national medical reimbursement register. They were followed for breast cancer with the aid of the Finnish Cancer Registry to the end of 2002. Altogether, 2,171 women with breast cancer were identified. The standardized incidence ratio of breast cancer with systemic estradiol for less than 5 years was 0.93, and for estradiol use for 5 years or more, 1.44. Oral and transdermal estradiol was accompanied by a similar risk of breast cancer. The risk was most prominent with the dose greater than 1.9 mg/d orally; whereas the risk associated with transdermal route was not dose-dependent. Conclusion was that estradiol for 5 years or more, either orally or transdermally, means 2-3 extra cases of breast cancer per 1,000 women who are followed for 10 years. Oral estradiol use for less than 5 years, oral estriol, or vaginal estrogens were not associated with a risk of breast cancer.

- Natrajan P, Gambrell D. Estrogen replacement therapy in patients with early breast cancer. *Obstet Gynecol.* 2002;187:289-95.

  This study looked at 123 early breast cancer patients. Most patients received estradiol pellets, testosterone pellets, or both. Neither estradiol nor testosterone pellets increased the risk of recurrence or death in these patients.

Bioidentical Estrogens & Skin:

- Brincat MP, Baron M, Galea R. Estrogens and the skin. *Climacteric* 2005;8:110–123.

  This paper presented conclusions from a meta-analysis of the available research regarding estrogen and skin changes in menopausal women. Estradiol supplementation,

including estradiol pellet implants, have been shown to increase collagen content, dermal thickness and elasticity, as well as skin water content. In addition, studies on estrogen and wound healing suggest that estradiol supplementation may play a beneficial role in cutaneous injury repair.

- Punnonen R, Vaajalahti P, Teisala K. Local oestriol treatment improves the structure of elastic fibers in the skin of postmenopausal women. *Ann Chir Gynaecol Suppl* 1987;202:39-41.

- Schmidt JB, Binder M, Demschik G, Bieglmayer C, Reiner A. Treatment of skin aging with topical estrogens*. Int J Dermatol* 1996 Sep;35(9):669-74.

- Schmidt JB, Binder M, Macheiner W, et al. Treatment of skin aging symptoms in perimenopausal females with estrogen compounds. A pilot study. *Maturitas* 1994 Nov;20(1):25-30.

Bioidentical Estrogens & the Cardiovascular System or Lipid Effects

- Chan HY, Yao X, Tsang SY, Chan FL, Lau CW, Huang Y. Different role of endothelium/nitric oxide in 17beta-estradiol- and progesterone-induced relaxation in rat arteries. *Life Sci* 2001 Aug 24;69(14):1609-17.

- Crews JK, Khalil RA. Antagonistic effects of 17 beta-estradiol, progesterone, and testosterone on Ca2+ entry mechanisms of coronary vasoconstriction. *Arterioscler Thromb Vasc Biol* 1999 Apr;19(4):1034-40.

- Davis SR, Walker KZ, Strauss BJ. Effects of estradiol with and without testosterone on body composition and relationships with lipids in postmenopausal women. *Menopause* 2000 Nov-Dec;7(6):395-401.

  33 postmenopausal women were randomized to receive either estradiol 50 mg (E) alone or estradiol 50 mg plus testosterone 50 mg implants (E&T) every 3 months for 2 years in conjunction with cyclic oral progestins for women with an intact uterus. Both therapies were associated with sustained reductions in total cholesterol and low-density lipoprotein (LDL) cholesterol. In women who received E but not E&T, hip and abdominal circumferences and fat mass:fat-free mass ratio over the abdomen declined. E&T but not E resulted in increased FFM and a reduced FM:FFM ratio. For E but not E&T, the decrease in LDL cholesterol was significantly related to changes in total and compartmental body fat and to change in the FM:FFM ratio. Estrogen replacement has effects on body fat distribution in postmenopausal women that are associated with improved lipid parameters. Addition of parenteral testosterone does not negate the favorable effects of estrogen on LDL cholesterol levels but may attenuate the reduction in centralized body fat achieved with E implants.

- De Kleijn MJ, van der Schouw YT, Verbeek AL, et al. Endogenous estrogen exposure and cardiovascular mortality risk in postmenopausal women. *Am J Epidemiol* 2002 Feb 15;155(4):339-45.

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In this study, the authors investigated whether combined information on reproductive factors has additive value to the single reproductive factor age at menopause for assessing endogenous estrogen exposure and cardiovascular mortality risk in postmenopausal women. They conducted a population-based cohort study that included 9,450 postmenopausal women from Nijmegen, the Netherlands, who were aged 35--65 years at enrollment in 1975, with a median follow-up of 20.5 years. Women aged 52 years or more at menopause had an 18% reduction in cardiovascular mortality compared with those aged 44 years or less. Women with more than 18 years of exposure to endogenous estrogen had a statistically significant 20% reduction in cardiovascular mortality compared with those who had 13 years of exposure or less. This study shows that age at menopause is related to cardiovascular disease mortality and that a newly developed composite measure of endogenous estrogen exposure does not add to the predictive value of age at menopause for cardiovascular mortality.

- Haines, C, Chung, T, Chang, A, Masarei, J, Tomlinson, B, Wong, E. Effect of oral estradiol on Lp(a) and other lipoproteins in postmenopausal women. A randomized, double-blind, placebo-controlled, crossover study. *Arch Intern Med* 1996 Apr 22;156(8):866-72.

  In a randomized, double-blind, placebo-controlled, crossover study, 91 surgically postmenopausal women received either 6 months of 2 mg daily oral estradiol followed by 6 months of placebo or the opposite regimen. During treatment phase, Group One showed decreased Lp(a) lipoprotein concentration (10.78 to 6.44 mg/dL) and LDL-C with increase in HDL-C and TG while Group Two showed a less pronounced decrease (12.74 to 10.75). 53 women continued oral estrogen therapy for an additional 12 months. Lp(a) levels were essentially unchanged from previous measures at the end of the treatment phase after 12 months of additional therapy. Authors suggested that reduced Lp(a) lipoprotein levels with extended oral estrogen therapy support a cardioprotective effect of HRT in postmenopausal women.

- Hayashi T, Ito I, Kano H, Endo H, Iguchi A. Estriol (E3) replacement improves endothelial function and bone mineral density in very elderly women. *J Gerontol A Biol Sci Med Sci* 2000 Apr;55(4):B183-90; discussion B191-3.

- Itoi H, Minakami H, Iwasaki R, Sato I. Comparison of the long-term effects of oral estriol with the effects of conjugated estrogen on serum lipid profile in early menopausal women. *Maturitas* 2000 Oct 31;36(3):217-22.

  This was a randomized comparison study (N=67) with three arms: 2.0mg estriol (E3) + 2.5mg medroxyprogesterone, 0.625mg conjugated estrogen (CE) + 2.5mg medroxyprogesterone, and a vitamin D and calcium combination (control). The study looked at changes in serum lipid profiles in early menopausal women. After 48 months on the randomized protocol, the serum lipid profiles showed that those in the E3 group decreased total cholesterol and triglycerides compared to the control and CE group. The E3 group showed less significant changes in HDL cholesterol and LDL cholesterol when compared to the CE protocol. The results show the improvement of serum lipid profiles in response to estrogen. The authors suggested that in women where bleeding has been a problem with certain estrogen protocols, low-dose estriol may be an alternative treatment for those at risk of cardiovascular disease.

- Lindoff C, Peterson F, Lecander I, et al. Transdermal estrogen replacement therapy: beneficial effects on hemostatic risk factors for cardiovascular disease. *Maturitas* 1996 May;24(1-2):43-50.

- Perera M, Sattar N, Petrie JR, et al. The effects of transdermal estradiol in combination with oral norethisterone on lipoproteins, coagulation, and endothelial markers in postmenopausal women with Type 2 diabetes: a randomized, placebo-controlled study. *J Clin Endocrinol Metab.* 2001 Mar;86(3):1140-3.

  This study showed that transdermal estradiol and oral norethisterone reduce plasma triglyceride and total cholesterol levels, factor VII activity and vonWillebrand factor antigen levels in women with Type 2 diabetes without a concurrent change in adiposity or glycemic control. The authors suggest that this protocol might be of benefit for women at high risk of cardiovascular disease.

- Riedel M, Oeltermann A, Mugge A, Creutzig A, Rafflenbeul W, Lichtlen P. Vascular responses to 17 beta-oestradiol in postmenopausal women. *Eur J Clin Invest.* 1995;25(1):44-7.

  The vascular responses to 17 beta-oestradiol were examined in 23 postmenopausal women using a placebo-controlled double-blind crossover design. All women received 1 mg 17 beta-oestradiol or placebo sublingually on consecutive days in random order. Oestradiol induced a vasodilation of femoral, the vessel diameter was unchanged with placebo. The blood flow rate increased significantly after oestradiol application, but not after placebo. Mean blood pressure and heart rate remained constant with both drugs. Despite its vasodilatory effect, ISDN significantly reduced the arterial blood flow after pretreatment with oestradiol and placebo, probably through cardiac preload reduction. In conclusion, 17 beta-oestradiol alters the vascular tone of systemic arteries resulting in a vasodilation and increase of blood flow. The authors suggest that these direct vascular actions may contribute to the preventive properties of oestrogens on cardiovascular diseases in postmenopausal women.

- Sendag F, Karadadas N, Ozsener S, Bilgin O. Effects of sequential combined transdermal and oral hormone replacement therapies on serum lipid and lipoproteins in postmenopausal woman. *Arch Gynecl Obstet*. 2002;266(1):38-43.

  The aim of this study was to compare the effects of sequential combined transdermal and oral postmenopausal hormone replacement therapies on serum lipid-lipoprotein profiles risk markers for cardiovascular disease. 96 healthy nonhysterectomised postmenopausal women were randomized to receive either transdermal continuous 17beta-estradiol, 0.05 mg/d with transdermal sequential norethisterone acetate 0.25 mg/d, or oral continuous conjugated equine estrogens, 0.625 mg/d, with oral sequential medroxyprogesterone acetate, 10 mg/d. 84 women completed the trial, 42 in oral and 42 in the transdermal group. The serum levels of total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, apolipoproteins AI and apolipoproteins B at 6 months after starting treatment were compared with baseline values for both therapies. Both oral and transdermal therapies significantly reduced serum levels of total and LDL-cholesterol. The serum levels of triglycerides did not show any significant change with oral therapy, whereas this lipid fell significantly with transdermal therapy. We found significant

decrease in HDL-cholesterol with transdermal therapy while there was no significant change with oral therapy. Apolipoproteins AI, the major protein component of HDL2 subfraction, was increased by oral therapy and lowered by transdermal therapy. The conclusion was that that serum total cholesterol and LDL-cholesterol were lowered by both therapies, with no significant differences between treatments, whereas there were significant differences between treatments according to effects on serum triglycerides and apolipoproteins AI.

- Snabes, M, Payne, J, Kopelen, et al. Physiologic estradiol replacement therapy and cardiac structure and function in normal postmenopausal women: a randomized, double-blind, placebo-controlled crossover trial. *Obstet Gynecol*, 1997;89(3):332-39.

  In a randomized, double-blind, placebo-controlled, crossover clinical trial of 31 postmenopausal women, average age 59.7 years, using 2.0mg of oral estradiol (E2) daily, the authors investigated the effects of estradiol on cardiac function and structure. This study did not include the use of progestins with estrogen. 12 weeks of E2 therapy showed no change in left ventricular thickness or mass, left atrial size or aortic size. There was a small but significant increase in left ventricular end-diastolic volume but it was not associated with change in end-systolic volume or ejection fraction changes. Heart rate and systolic and diastolic pressures were unchanged after 3 months of treatment. Time-velocity integral of flow and peak flow velocities were unaffected by E2 treatment. Authors concluded that estrogen replacement therapy did not affect cardiac structure or size in normal postmenopausal women (after 12 weeks of treatment).

- Vehkavaara S, Silveira A, Hakala-Ala-Pietilä T, et al. Effects of oral and transdermal estrogen replacement therapy on markers of coagulation, fibrinolysis, inflammation and serum lipids and lipoproteins in postmenopausal woman. *Thromb Haemost*. 2001;85(4):619-25.

  This study compared the effects of oral estradiol (2 mg), transdermal estradiol (50 microg), and placebo on measures of coagulation, fibrinolysis, inflammation and serum lipids and lipoproteins in 27 postmenopausal women at baseline and after 2 and 12 weeks of treatment. Oral and transdermal estradiol induced similar increases in serum free estradiol concentrations. Oral therapy increased the plasma concentrations of factor VII antigen (FVIIag) and activated factor VII (FVIIa), and the plasma concentration of the prothrombin activation marker prothrombin fragment 1+2 (F1+2). Oral but not transdermal estradiol therapy significantly lowered plasma plasminogen activator inhibitor-1 (PAI-1) antigen and tissue-type plasminogen activator (tPA) antigen concentrations and PAI-1 activity, and increased D-dimer concentrations, suggesting increased fibrinolysis. The concentration of soluble E-selectin decreased and serum C-reactive protein (CRP) increased significantly in the oral but not in the transdermal or placebo groups. In the oral but not in the transdermal or placebo estradiol groups low-density-lipoprotein (LDL) cholesterol, apolipoprotein B and lipoprotein (a) concentrations decreased while high-density-lipoprotein (HDL) cholesterol, apolipoprotein AI and apolipoprotein AII concentrations increased significantly. LDL particle size remained unchanged. In summary, oral estradiol increased markers of fibrinolytic activity, decreased serum soluble E-selectin levels and induced potentially antiatherogenic changes in lipids and lipoproteins. In contrast to these beneficial effects, oral estradiol changed markers of coagulation towards hypercoagulability, and increased serum CRP

concentrations. Transdermal estradiol or placebo had no effects on any of these parameters. These data demonstrate that oral estradiol does not have uniformly beneficial effects on cardiovascular risk markers and that the oral route of estradiol administration rather than the circulating free estradiol concentration is critical for any changes to be observed.

- Zegura B, Guzic-Salobir B, Sebestien M, Keber I. The effect of various menopausal hormone therapies on markers of inflammation, coagulation, fibrinolysis, lipids, and lipoproteins in healthy postmenopausal women. *Menopause.* 2006;13(4):643050.

  Androgenic progestins such as norethisterone acetate (NETA) may influence the effect of estradiol (E(2)) therapy. This study compared the influence of oral E(2), with and without NETA, and transdermal E(2) on markers of coagulation, fibrinolysis, and inflammation and on lipids and lipoproteins in healthy postmenopausal women. 112 healthy postmenopausal women were randomized to receive treatment with either oral E(2), with or without NETA, transdermal E(2), or placebo. At baseline and after 28 weeks, levels of serum lipids and lipoproteins and markers of coagulation, fibrinolysis, and inflammation were determined. Results were that oral E(2), with or without NETA, produced no net activation of coagulation but improved fibrinolysis. Both modes of oral menopausal hormone therapy have a greater impact on markers of inflammation, coagulation, fibrinolysis, lipids, and lipoproteins than transdermal E(2). NETA attenuates some E(2) effects.

- Zegura B, Keber I, Sebestjen M, Borko E. Orally and transdermally replaced estradiol improves endothelial function equally in middle-aged women after surgical menopause. *Am J Obstet Gynecol*. 2003 May;188(5):1291-6.

  Improvement in endothelial function may be an important mechanism by which estrogen replacement therapy protects postmenopausal women against coronary artery disease. The aim of this study was to determine whether the vascular effects of estradiol depend on the route of administration. Six weeks after surgically induced menopause, 43 healthy women were assigned randomly to 28 weeks of treatment by either orally or transdermally replaced estradiol. Endothelium-dependent dilation increased after oral estradiol replacement from 6% +/- 3.9% to 13.2% +/- 4.4% (P <.0001) and after transdermal estradiol replacement from 7% +/- 4.9% to 14.9% +/- 5.6%(P <.0001). Endothelium-independent dilation did not change significantly in either group. The improvements in endothelium-dependent dilation after estrogen substitution were independent of the changes in blood lipids and lipoproteins. Conclusion was that both oral and transdermal long-term replacement of estradiol leads to improved endothelial function in healthy middle-aged women after surgically induced menopause.

- Zegura B, Keber I, Sebestjen M, Koenig W. Double blind, randomized study of estradiol replacement therapy on markers of inflammation, coagulation and fibrinolysis. *Atherosclerosis*. 2003 May;168(1):123-9.

  Estrogen replacement therapy (ERT) has been found to be associated with increased cardiovascular risk in the first year after initiation of ERT. This study compared the effects of oral and transdermal estradiol (E2) replacement therapy on markers of

inflammation, coagulation and fibrinolysis in a randomized double-blind trial. 43 healthy women were randomized 6 weeks after surgically induced menopause to receive treatment with either oral or transdermal E2 over a period of 28 weeks. At baseline and after 28 weeks, levels of serum lipids and lipoproteins, and markers of coagulation, fibrinolysis and inflammation were determined. Among fibrinolytic parameters, oral E2 shortened euglobulin clot lysis time and reduced tissue type plasminogen activator antigen and plasminogen activator inhibitor activity. Among coagulation parameters, both routes of E2 replacement decreased fibrinogen levels. Oral E2 resulted in an increase in C-reactive protein (CRP) from 2.15 to 3.41 mg/l, while transdermal E2 showed no effect. Levels of serum amyloid A (SAA), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) did not change significantly after oral and transdermal E2. Oral E2 significantly improved the lipid profile, while transdermal E2 had a less pronounced effect. Both oral and transdermal E2 significantly reduced fasting glucose. Oral E2 was associated with a pro-inflammatory response, but at the same time improved fibrinolytic capacity, showed no pro-coagulatory effects, and acted beneficially on lipids and lipoproteins. There was no influence of transdermal E2 on markers of coagulation activation, fibrinolysis and inflammation, but it decreased fibrinogen levels significantly.

- Zegura B, Keber I, Sebestjen M, Borko E. Orally and transdermally replaced estradiol improves endothelial function equally in middle-aged women after surgical menopause. *Am J of Obstet Gynecol.* 2003 May;188(5):1291-6.

  Forty-three surgically induced (6 weeks postop) menopausal women were randomly assigned in a double-blind study to 28 weeks of 2.0 mg oral or 50 mcg transdermal estradiol. Looking at blood flow through the brachial artery, flow-mediated dilation (ultrasound) in the oral group increased 6.0 to 13.2% and in the transdermal group increased 7.0 to 14.9% Results indicate that both oral and transdermal administration had equal effect on arterial endothelium independent of lipid profiles and increased vasodilation.

Bioidentical Estrogens & the Genitourinary Tract

- Iosif CS. Effects of protracted administration of estriol on the lower genitourinary tract in postmenopausal women. *Arch Gynecol Obstet* 1992;251(3):115-20.

  This study contained 80 postmenopausal women, 48 of whom (60%) agreed to undergo long-term treatment with estriol suppositories. All had symptoms of vaginal atrophy and urinary incontinence. Endometrial samples were taken after 8-10 years of therapy. Estriol had induced slight proliferative changes in the endometrium in 7 of 48 patients studied by endometrial sampling. 75% of the women reported significant subjective improvement of stress incontinence. The authors' conclusion was that the risk of estriol treatment is insignificant.

- Koloszar S, Kovacs L. [Treatment of climacteric urogenital disorders with an estriol-containing ointment] *Orv Hetil* 1995 Feb 12;136(7):343-5.

- Lose G, Englev E. Oestradiol-releasing vaginal ring versus oestriol vaginal pessaries in the treatment of bothersome lower urinary tract symptoms. *BJOG* 2000 Aug;107(8):1029-34.

- Manonai J, Theppisai U. Effect of oral estriol on urogenital symptoms, vaginal cytology, and plasma hormone level in postmenopausal women. *J Med Assoc Thai* 2001 Apr;84(4):539-44.

- Raul, R, Stamm, W. A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. *New Engl J Med.* 1993;329(11):753-56.

  This randomized, double blind, placebo-controlled trial looked at the incidence of urinary tract infections (UTI) in 93 postmenopausal women using 0.5 mg estriol vaginal cream once nightly for two weeks followed by twice weekly application or placebo. Results showed significantly lower UTI rates in treatment group (0.5 infections per patient-year vs. 5.9 for placebo group). The mean vaginal pH fell from 5.5+-0.7 to 3.6+-1.0 for treatment group and 5.8+-1/2 to 6.1+-2.0 in placebo group and there was an increase in vaginal colonization with lactobacilli in the treatment group. Authors recommend use of topical vaginal estriol in preventive treatment of women with frequent UTI as possible replacement for long-term use of nitrofurantoin, co-trioxazole, trimethoprim, cephalexin or fluoroquinolones.

- Tomaszewski J, Adamiak A, Skorupski P, Rzeski W, Rechberger T. [Effect of 17 beta-estradiol and phytoestrogen daidzein on the proliferation of pubocervical fascia and skin fibroblasts derived from women suffering from stress urinary incontinence] *Ginekol Pol*. 2003 Oct;74(10):1410-4.

- Yoshimura T, Okamura H. Short term oral estriol treatment restores normal premenopausal vaginal flora to elderly women. *Maturitas* 2001 Sep 28;39(3):253-7.

  This study looked at short term (14 days) oral estriol (2.0mg/day) treatment for atrophic vaginitis in 59 postmenopausal women aged 50-75 years. The results showed that in the majority of women in the study group the oral estriol restored normal vaginal flora by the end of the treatment period.

Bioidentical Estrogens & Mode of Delivery

- Canonico M, Oger E, Plu-Bureaun G, et al. Hormone therapy and venous thromboembolism among postmenopausal women – impact of the route of estrogen administration and progestogens: the ESTHER study. *Circulation* 2007; 115:840-845.

  This study showed that oral <u>not</u> transdermal (patch) estrogens increase the risk of blood clots. In addition, it suggested that synthetic progestins increase the risk of clots, whereas micronized, bioidentical progesterone does not.

- Callantine MR, Martin PL, Bolding OT, Warner PO, Greaney MO Jr. Micronized 17 beta-estradiol for oral estrogen therapy in menopausal women. *Obstet Gynecol* 1975 Jul;46(1):37-41.

- Chetkowski RJ, Meldrum DR, Steingold KA, et al. Biologic effects of transdermal estradiol. *N Engl J Med* 1986 Jun 19;314(25):1615-20.

  Twenty-three postmenopausal women were randomly assigned to use of transdermal estradiol in four increasing doses (25, 50, 75, 100 micrograms per 24 hours) followed by daily oral dose of conjugated equine estrogens in two doses (0.625 mg, 1.25 mg) or to use oral conjugated equine estrogens followed by transdermal estradiol. Results showed a dose-response relation between the amount of estradiol delivered and the serum measure of the hormone. Estrone concentrations also rose with transdermal application. At the 50 and 100-microgram transdermal dose levels, results were comparative to the 0.625 and 1.25 mg conjugated equine estrogen results. Nonhepatic markers (serum gonadotropin, vaginal cytologic studies, urinary calcium levels and urinary calcium/creatinine ratios all increased in dose-dependent fashion. Hepatic markers (hepatic protein level, lipid metabolism, clotting factors, renin substrate) were not affected by transdermal doses of estradiol. Transdermal estradiol provided benefit of increased serum hormone levels without hepatic protein effects of oral conjugated equine estrogens.

- Darj E, Axelsson O, Carlstrom K, Nilsson S, von Schoultz B. Liver metabolism during treatment with estradiol and natural progesterone. *Gynecol Endocrinol* 1993 Jun;7(2):111-4.

- Friel PN, Hinchcliffe C, Wright JV. Hormone replacement with estradiol: conventional oral doses result in excessive exposure to estrone. *Altern Med Rev*. 2005 Mar;10(1):36-41.

- Hargove JT, Maxson WS, Wentz AC, et al. Menopausal hormone replacement therapy with continuous daily oral micronized estradiol and progesterone. *Obstetr Gynecol* 1989; 73:606-612.

  The safety and efficacy of a daily combination of micronized estradiol (E2) (0.7-1.05 mg) and progesterone (200-300 mg) were evaluated in ten menopausal women with moderate to severe vasomotor symptoms and/or vaginal atrophy over a 12-month study interval. For comparison, five similar women were placed on conjugated estrogens, 0.625 mg daily, and medroxyprogesterone acetate, 10 mg daily, for the first 10 days of each calendar month for 12 months. Patients were evaluated at 0, 1, 3, 6, and 12 months. Estrogens rose significantly from baseline in both groups (P less than .01). Progesterone increased significantly above baseline in the E2 and progesterone group (P less than .01), but did not change in the conjugated estrogens and medroxyprogesterone acetate users. All women on E2 and progesterone had a decrease in total cholesterol and an increase in high-density lipoprotein cholesterol from baseline (P less than .01). Those on conjugated estrogens and medroxyprogesterone acetate had no significant change from baseline in total cholesterol; however, they did have an increase in high-density lipoprotein cholesterol values (P less than .01). In the E2 and progesterone group, the endometrial histology became completely quiescent and there was no uterine bleeding after 6 months of observation. Four of five women on conjugated estrogens and medroxyprogesterone acetate continued regular withdrawal bleeding throughout the study period, but no endometrial hyperplasia was encountered. This study demonstrates that the daily administration of a combination of micronized E2

and progesterone results in symptomatic improvement, minimal side effects, an improved lipid profile, and amenorrhea without endometrial proliferation or hyperplasia in menopausal women.

- Jarupanich T, Lamlertkittikul S, Chandeying V. Efficacy, safety and acceptability of a seven-day, transdermal estradiol patch for estrogen replacement therapy. *J Med Assoc Thai* . 2003 Sep;86(9):836-45.

- Kainz C, Gitsch G, Stani J, Breitenecker G, Binder M, Schmidt JB. When applied to facial skin, does estrogen ointment have systemic effects? *Arch Gynecol Obstet* 1993;253(2):71-4.

- Nahoul K, Dehennin L, Jondet M, Roger M. Profiles of plasma estrogens, progesterone and their metabolites after oral or vaginal administration of estradiol or progesterone. *Maturitas* 1993 May;16(3):185-202.

  Doses of 100 mg of micronized progesterone (P) and of 0.5 mg of micronized estradiol (E2) were administered vaginally and orally, respectively, in the early follicular phase of the menstrual cycle in six premenopausal women. In the second cycle, the same doses were administered in the same subjects, orally for P and vaginally for E2. Serial blood samples were collected. P and E2 levels were higher after vaginal than after oral administration, while those of E1 (estrone) were similar after either route. Metabolites of P were higher after oral administration. Concerning estrogen sulfates, E1S concentrations were similar whichever the route, while those of E2S were lower after oral than after vaginal administration. The authors concluded that "in view of the metabolic pathways which are operative and of the peripheral plasma levels which were found, the vaginal route appears to be more adequate than the oral one for hormone replacement therapy."

- Suvanto-Luukkonen E, Sundstrom H, Penttinen J, et al. Percutaneous estradiol gel with an intrauterine levonorgestrel releasing device or natural progesterone in hormone replacement therapy. *Maturitas* 1997 Apr;26(3):211-7.

- Wren BG, Day RO, McLachlan AJ, Williams KM. Pharmacokinetics of estradiol, progesterone, testosterone and dehydroepiandrosterone after transbuccal administration to postmenopausal women. *Climacteric*. 2003 Jun;6(2):104-11.

  This study looked at whether or not sublingual troche use of bioidentical estradiol, progesterone, testosterone, and DHEA raised serum levels. Each troche contained estradiol (0.5 mg), progesterone (200 mg), testosterone (2.0 mg) and dehydroepiandrosterone (10 mg). A half troche was administered to each of six women and the plasma concentration-time profiles determined over 24 hrs. Thereafter, a one-half troche was taken twice daily for 2 weeks and concentrations determined over a dosage interval (12 h). Each of the hormones was readily absorbed via the buccal mucous membrane. Peak plasma concentrations of estradiol and progesterone were comparable to those found normally in young menstruating women.

Bioidentical Estrogens & Vasomotor Symptoms (Hot Flashes & Night Sweats)

- Archer DF; EstroGel Study Group. Percutaneous 17beta-estradiol gel for the treatment of vasomotor symptoms in postmenopausal women. *Menopause* 2003 Nov-Dec;10(6):516-21.

Bioidentical Estrogens & Endometrium (Uterine Lining)

- Callantine MR, Martin PL, Bolding OT, Warner PO, Greaney MO Jr. Micronized 17 beta-estradiol for oral estrogen therapy in menopausal women. *Obstet Gynecol* 1975 Jul;46(1):37-41.

- Granberg S, Eurenius K, Lindgren R, Wilhelmsson L. The effects of oral estriol on the endometrium in postmenopausal women. *Maturitas* 2002 Jun 25;42(2):149-56.

This study conducted endometrial evaluation using both transvaginal ultrasound and histological biopsy by Pipelle in postmenopausal women taking a low-dose oral estriol (1 or 2 mg daily) for a mean duration of 4.3 years. Mean endothelial thickness in the study group after one year was 3.0mm and in the control group was 2.4mm. There was a noted increase in atrophic vaginal epithelium in the control group. There was a noted increased incidence of endometrial polyps in the study group (14.1%) compared to the control group (2.9%) although this was not determined to be clinically significant.

Bioidentical Estrogens & Immune Function

- Salem ML, Hossain MS, Nomoto K. Mediation of the immunomodulatory effect of beta-estradiol on inflammatory responses by inhibition of recruitment and activation of inflammatory cells and their gene expression of TNF-alpha and IFN-gamma. *Int Arch Allergy Immunol* 2000 Mar;121(3):235-45.

Bioidentical Estriol:

- Haspels AA, Luisi M, Kicovic PM. Endocrinological and clinical investigations in post-menopausal women following administration of vaginal cream containing oestriol. *Maturitas* 1981 Dec;3(3-4):321-7.

- Head KA. Estriol: safety and efficacy. *Altern Med Rev*. 1998. 3(2):101-13.

While conventional hormone replacement therapy provides certain benefits, it is not without significant risks. Estriol has been found to provide some of the protection without the risks associated with stronger estrogens. Depending upon the situation, estriol may exert either agonistic or antagonistic effects on estrogen. Estriol appears to be effective at controlling symptoms of menopause, including hot flashes, insomnia, vaginal dryness, and frequent urinary tract infections. Results of research on its bone density-maintaining effects have been contradictory, with the most promising results coming from Japanese studies. Estriol's effect on cardiac risk factors has also been somewhat equivocal; however, unlike conventional estrogen prescriptions, it does not seem to contribute to hypertension. Although estriol appears to be much safer than estrone or estradiol, its continuous use in high doses may have a stimulatory effect on both breast and endometrial tissue.

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- Melamed M, Castano E, Notides AC, Sasson S. Molecular and kinetic basis for the mixed agonist/antagonist activity of estriol. *Mol Endocrinol* 1997 Nov;11(12):1868-78.

- Schiff I, Tulchinsky D, Ryan KJ, Kadner S, Levitz M. Plasma estriol and its conjugates following oral and vaginal administration of estriol to postmenopausal women: correlations with gonadotropin levels. *Am J Obstet Gynecol* 1980 Dec 15;138(8):1137-41.

  This study administered 4 mg of estriol (E3) either orally or vaginally to six postmenopausal women. Blood samples were collected every hour for 6 hours and five different estriol fractions as well as gonadotropins were measured. Vaginal E3 administration resulted in a decline of 45% in luteinizing hormone (LH) levels and 17% in follicle-stimulating hormone (FSH) levels at 6 hours after treatment. In contrast, the administration of 4 mg of E3 orally did not produce a decline of LH and FSH, despite the fact that the serum levels of E3-3-sulfate, E3-3-sulfate-16-glucosiduronate, estriol-3-glucosiduronate, and estriol-16-glucosiduronate were all fourfold to 24-fold higher after oral administration than after vaginal estriol administration. However, since the levels of unconjugated E3 were higher after the vaginal than after the oral administration of estriol, the authors concluded that only unconjugated E3 suppresses gonadotropins. The study shows that E3 possesses biologic activity in women as evidenced by the prompt suppression of LH following vaginal E3 administration. The biologic activity of E3 is attributed mainly to the levels of unconjugated E3 and not to the levels of various conjugates. It is not known whether estrogen replacement therapy with E3 is safer or as effective as other estrogen preparations.

- Takahashi K, Manabe A, Okada M, et al. Efficacy and safety of oral estriol for managing postmenopausal symptoms. *Maturitas* 2000 Feb 15;34(2):169-77.

  The purpose of this study was to assess the therapeutic efficacy and safety of oral estriol for the treatment of climacteric symptoms in postmenopausal women. 68 postmenopausal women with climacteric symptoms received oral estriol, 2 mg/day, daily for 12 months. Climacteric symptoms, serum levels of gonadotropins, estradiol (E2) and lipids, biochemical markers of bone metabolism, blood pressure, and side effects at baseline and during treatment were measured. Oral estriol therapy significantly reduced total MI scores. The greatest relief was noted for hot flushes, night sweats, and insomnia. Estriol treatment significantly lowered serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations but did not affect any of the other parameters (lipids, bone, liver and blood pressure) during the study period. Slightly vaginal bleeding occurred in 14.3% of those who underwent natural menopausal women. Histologic evaluation of the endometrium and ultrasound assessment of the breasts following 12 months of estriol treatment found normal results in all women. Conclusion was that estriol is a safe and effective alternative for relieving climacteric symptoms in postmenopausal Japanese women.

- Takahashi K, Okada M, Ozaki T, Kurioka H, Manabe A, Kanasaki H, Miyazaki K. Safety and efficacy of oestriol for symptoms of natural or surgically induced menopause. *Hum Reprod* 2000 May;15(5):1028-36.

- Tzingounis VA, Aksu MF, Greenblatt RB. Estriol in the management of the menopause. *JAMA* 1978 Apr 21;239(16):1638-41.

- Yang TS, Tsan SH, Chang SP, Ng HT. Efficacy and safety of estriol replacement therapy for climacteric women. *Zhonghua Yi Xue Za Zhi* (Taipei) 1995 May;55(5):386-91.

Progesterone and Breast:

- Campagnoli C, Clavel-Chapelon F, Kaaks R, et al. Progestins and progesterone in hormone replacement therapy and the risk of breast cancer*. J Steroid Biochem Mol Biol*. 2005;96(2):95-108.

  This paper reviews data regarding lack of increase in breast cancer risk with bioidentical progesterone vs synthetic progestins with estrogen replacement. The paper also reviews the non-progesterone effects of synthetic progestins, which potentiate the proliferative action of estrogens (such as decreased insulin sensitivity, increased levels and activity of IGF-1, and decreased levels of SHBG).

- Chang KJ, et al. Influences of percutaneous administration of estradiol and progesterone on human breast epithelial cell cycle in vivo. *Fertil Steril* 1995; 63(4):785-91.

  The effect of transdermal estradiol (1.5 mg), transdermal progesterone (25 mg), and combined transdermal estradiol and progesterone (1.5 mg and 25 mg) on human breast epithelial cell cycles was evaluated in vivo. Results demonstrated that estradiol significantly increases cell proliferation, while progesterone significantly decreases cell replication below that observed with placebo. Transdermal progesterone was also shown to reduce estradiol-induced proliferation.

- Cowan LD, Gordis L, Tonascia JA, et al. Breast cancer incidence in women with a history of progesterone deficiency. *Am Journal of Epidemiol* 1981; 114(2):209-17.

  1083 white women who had been evaluated and treated for infertility from 1945-1965 were followed prospectively through April 1978 to determine breast cancer incidence. These women were categorized as to the cause of infertility into two groups, those with endogenous progesterone deficiency (PD) and those with nonhormonal causes (NH). Women in the PD group had 5.4 times the risk of premenopausal breast cancer compared to women in the NH group. This excess risk could not be explained by differences between the two groups in ages at menarche or menopause, history of oral contraceptive use, history of benign breast disease or age at first birth. Women in the PD group also experienced a 10-fold increase in deaths from all malignant neoplasms compared to the NH group. The incidence of postmenopausal breast cancer did not differ significantly between the two groups.

- de Lignieres B. Effects of progestogens on the postmenopausal breast. *Climacteric* 2002; 5(3):229-35.

  In this review, the author highlights the differences between progesterone and synthetic progestins in the breast and cautions that progestogens not be "all put in the same bag"

with respect to safety. A strong case is made for the protective effect of progesterone on the breast.

- Desreux J, Kebers F, Noel A, et al. Progesterone receptor activation- an alternative to SERMs in breast cancer. *Eur J Cancer* 2000 Sep;36 Suppl 4:S90-1.

  This review emphasizes progesterone's role in supporting healthy breast homeostasis and opposing the proliferative effects of estradiol in the breast, unlike synthetic progestins.

- Flesch-Janys D, et al. Risk of different histological types of postmenopausal breast cancer by type and regimen of menopausal hormone therapy. *Int J Cancer.* 2008;123:933-941.

  In a large population-based case-control study in Germany, including 3,464 breast cancer cases aged 50-74 at diagnosis and 6,657 population based and frequency matched controls, the effects of menopausal hormone therapy (HT) by type, regimen, timing and progestagenic constituent on postmenopausal breast cancer risk overall and according to histological type was evaluated. Risks for current users varied significantly by type and regimen of HT, OR (odds ratio) 1.05 for continuous combined estrogen-progestagen, 1.03 for cyclical EP and 1.01 for estrogen-only therapy. No statistically significant increase in risk was observed after 5 years of cessation of HT use for any histological type. Analyses of progestagenic content by regimen revealed a significantly higher risk for continuously administered norethisterone- or levonorgestrel-derived progestagens than for continuously administered progesterone. These data suggest that the risks associated with menopausal HT differ by type and regimen of HT and histological type of breast cancer and may vary by progestagenic component.

- Foidart JM, Colin C, Denoo X, Desreux J, Beliard A, Fournier S, de Lignieres B. Estradiol and progesterone regulate the proliferation of human breast epithelial cells. *Fertil Steril* 1998 May;69(5):963-9.

  In this double-blind randomized study, 40 postmenopausal women received daily topical application of a gel containing either placebo, estradiol, progesterone, or estradiol + progesterone for two weeks prior to esthetic breast surgery or the excision of a benign breast lesion. The results showed that increased estrogen concentration increased the number of cycling epithelial cells, whereas exposure to progesterone for 14 days reduced the estrogen-induced proliferation of normal breast epithelial cells.

- Formby B, Wiley TS. Bcl-2, surviving and variant CD44 v7-v10 are down regulated and p53 is unregulated in breast cancer cells by progesterone: inhibition of cell growth and induction of apoptosis. *Mol Cell Biochem* 1999 Dec;202(1-2):53-61.

  The purpose of this study was to determine the mechanism by which progesterone inhibits the proliferation of breast cancer cells. Utilizing breast cancer cell lines with and without progesterone receptors (T47-D and MDA-231, respectively) in vitro, the authors looked at apoptosis (programmed cell death) in response to progesterone exposure as a possible mechanism. The genetic markers for apoptosis - p53, bcl-2 and surviving, were utilized to determine whether or not the cells underwent apoptosis. The results

demonstrated that progesterone does produce a strong antiproliferative effect on breast cancer cell lines containing progesterone receptors, and induced apoptosis. The relatively high levels of progesterone utilized were similar to those seen during the third trimester of human pregnancy.

- Formby B, Wiley TS. Progesterone inhibits growth and induces apoptosis in breast cancer cells: inverse effects on Bcl-2 and p53. *Ann Clin Lab Sci* 1998 Nov-Dec;28(6):360-9.

  This study explored the mechanism by which progesterone inhibits breast cancer cell proliferation (growth). In progesterone receptor positive T47-D breast cancer cells, the mechanism of apoptosis appeared to be through the regulation of the genes p53 and bcl-2 by progesterone. These genes control the apoptotic process. It was demonstrated that at progesterone levels that approximate the third trimester of pregnancy, there was a strong antiproliferative effect in at least 2 breast cancer cell lines.

- Laidlaw IJ, Clarke RB. The proliferation of normal breast tissue implanted into athymic nude mice is stimulated by estrogen, but not by progesterone. *Endocrinology* Jan 1995;136(1):164-71.

  Normal human breast tissue was implanted subcutaneously into athymic nude mice. The mice were then treated with estradiol or progesterone such that serum levels approximated those seen in normal menstruating women. Immunocytochemical measures were made of proliferative activity and steroid receptor expression of the tissue implants. It was found that physiologic levels of estradiol significantly stimulated the proliferation of human breast epithelial cells and increased progesterone receptor expression 10-20-fold. Progesterone failed to affect proliferation alone or after estradiol priming.

- Lin VC, Ng EH, Aw SE, Tan MG, Ng EH, Chan VS, Ho GH. Progestins inhibit the growth of MDA-MB-231 cells transfected with progesterone receptor complementary DNA. *Clin Cancer Res* 1999 Feb;5(2):395-403.

  Progesterone is mainly thought to exert its effects via the estrogen-dependent progesterone receptor (PR), the effects of which may be overshadowed by the presence of estrogen. In order to study the independent effects of progesterone on breast cancer cell lines, PR expression vectors were transfected into a PR and ER negative cell line (MDA-MB-231). The growth of these cells was then studied in response to progesterone and several progestins. Progesterone was found to significantly inhibit DNA synthesis and cell growth in a dose-dependent fashion. The results of this study indicate that progesterone and progestins independent of estrogen have an antiproliferative effect on breast cancer cells via the progesterone receptor. This suggests a possible role in the treatment of PR negative breast cancer via re-activation of the PR receptor.

- Malet C, Spritzer P, Guillaumin D, Kuttenn F. Progesterone effect on cell growth, ultrastructural aspect and estradiol receptors of normal human breast epithelial (HBE) cells in culture. *J Ster Biochem Mol Biol* 2002; 73: 171-181.

  In a culture system, progesterone was found to have an inhibitory effect on breast cell

growth. When given following estradiol (E2), it limited the stimulatory effect of E2 on cell growth.

- Mauvais-Jarvis P, Kuttenn F, Gompel A. Antiestrogen action of progesterone in breast tissue. *Horm Res* 1987;28(2-4):212-8.

  In a review of the literature on the cellular effects of progesterone on both normal breast cells and breast cancer cell lines, the authors conclude that most data indicate progesterone and progestins have an antiestrogenic effect on the breast, as reflected in the decrease in estradiol receptor content, the decrease in cell proliferation, and an increase in a marker of cell differentiation, 17 beta-hydroxysteroid activity, which is mediated by the progesterone receptor.

- Mohr PE, Wang DY, Gregory WM, Richards MA, Fentiman IS. Serum progesterone and prognosis in operable breast cancer. *British Journal of Cancer* 1996;73:1532-1533.

  Higher blood levels of progesterone measured during surgical treatment of breast cancers were associated with significantly better survival, especially in women who were node-positive (P<0.01). There was no significant relationship between E2 levels and survival. This study demonstrated that a higher level of progesterone at time of excision is associated with improved prognosis in women with operable breast cancer.

- Plu-Bureau G, Le MG, Thalabard JC, Sitruk-Ware R, Mauvais-Jarvis P. Percutaneous progesterone use and risk of breast cancer: results from a French cohort study of premenopausal women with benign breast disease. *Cancer Detect Prev* 1999;23(4):290-6.

  This cohort study followed 1150 premenopausal French women diagnosed with benign breast disease. Topical progesterone cream, a common treatment for mastalgia in Europe, had been prescribed to 58% of the women. Follow-up accumulated 12,462 person-years. There was no association noted between progesterone cream use and breast cancer risk. Furthermore, women who had used both progesterone cream and an oral progestogen had a significant decrease in breast cancer risk (RR= 0.5) as compared to women who did not use progesterone cream. There was no significant difference in the risk of breast cancer in percutaneous progesterone users versus nonusers among oral progestogen users. These results suggest there are no deleterious effects caused by percutaneous progesterone use in women with benign breast disease.

Progesterone and General Health:

- Dalton K. Prenatal progesterone and educational attainments. *British Journal of Psychiatry* 1976; 126:438-42.

  This study compares educational attainments of 34 children whose mothers received prenatal progesterone with 37 normal and 12 toxemic controls. Results at ages 17-24 showed that progesterone children were more likely to continue schooling after 16 years, a higher number left school with 'O' and 'A' level grades and more obtained entrance to university. The best academic results were found for children whose mothers had

received over 5 grams of progesterone for a minimum of eight weeks, with treatment beginning before week sixteen.

- Fitzpatrick LA, Pace C, Wiita B. Comparison of regimens containing oral micronized progesterone or medroxyprogesterone acetate on quality of life in postmenopausal women: a cross-sectional survey. *J Womens Health Gend Based Med.* 2000;9(4):381-7.

  A cross-sectional survey was conducted to examine quality of life (QOL) related to physiological, somatic, and vasomotor effects of changing progestogen treatment from medroxyprogesterone acetate (MPA) to micronized progesterone in postmenopausal women. Eligible women (n = 176) were currently using hormone replacement therapy (HRT) containing micronized progesterone for 1-6 months and had previously received HRT containing MPA. When compared with the MPA-containing regimen, women using micronized progesterone-containing HRT experienced significant improvement in vasomotor symptoms, somatic complaints, and anxiety and depressive symptoms. Women reported improved perceptions of their patterns of vaginal bleeding and control of menopausal symptoms while on the micronized progesterone-containing regimen. Approximately 80% of women reported overall satisfaction with the micronized progesterone-containing regimen.

- Hargrove J, Maxson W, Wentz A, et al. Menopausal hormone replacement therapy with continuous daily oral micronized estradiol and progesterone. *Obstet Gynecol.* 1989;73:606.

  This study demonstrated that the daily administration of a combination of micronized estradiol and progesterone results in symptomatic improvement, minimal side effects, an improved lipid profile, and amenorrhea without endometrial proliferation or hyperplasia in menopausal women.

- Mahesh VB, Brann DW, and Hendry LB. Diverse modes of action of progesterone and its metabolites. *J Steroid Biochem Molec Biol* 1996;56(1-6):209-219.

  Progesterone and its metabolites have a variety of diverse effects in the brain, uterus, smooth muscle, sperm and the oocyte. The effects include changes in electrophysiological excitability, induction of anesthesia, regulation of gonadotropin secretion, regulation of estrogen receptors, modulation of uterine contractility and induction of acrosome reaction and oocyte maturation. The classical mechanism of steroid hormone action of intracellular receptor binding has been supplemented by the possibility of the steroid acting as a transcription factor after the binding of the receptor protein to DNA. Other mechanisms include influence of the steroids on membrane fluidity and acting through other cell signaling systems, membrane receptors and GABA(A) receptors. Of particular interest are multiple mechanisms for the same types of action. For example the effect of progesterone on gonadotropin release is largely exerted via the classical intracellular receptor as well as membrane receptors, whereas 3(alpha),5(alpha)-tetrahydroprogesterone-induced LH release occurs via the GABA(A) receptor system. The inhibition of uterine contractility by progesterone is regulated by progesterone receptors while the action of 3(alpha),5(alpha)-tetrahydroprogesterone on uterine contractility is regulated by GABA(A) receptors. The regulation of the differences

in the pattern of progesterone effects on estrogen receptor dynamics in the anterior pituitary and the uterus in the same animal are also of considerable interest.

- Nappi C, Affinito P. Double-blind controlled trial of progesterone vaginal cream treatment for cyclical mastodynia in women with benign breast disease. *J Endocrin Invest* 1994;15(11):801-6.

  80 regularly menstruating women with mastodynia were studied to evaluate the clinical effectiveness of vaginally administered micronized progesterone. Subjects were randomly assigned to one of two groups, with all participating in a control cycle prior to treatment. One group received 4 grams of vaginal cream containing 2.5% natural progesterone for six cycles from day 19 to day 25 of the cycle. The other group was similarly treated with placebo. Both subjective reporting on a daily basis and clinical examination revealed a significant reduction in breast pain, defined as 50% reduction, in 64.9% of subjects receiving progesterone and 22.2% of subjects receiving placebo. Effects of breast nodularity were not significant. No side effects were detected.

- Sitruk-Ware R, Bricaire C, De Lignieres B, Yaneva H, Mauvais-Jarvis P. Oral micronized progesterone. Bioavailability pharmacokinetics, pharmacological and therapeutic implications--a review. *Contraception* 1987 Oct; 36(4): 373-402.

  This paper reviews the effects and benefits of oral micronized progesterone. Progesterone exhibits anti-estrogenic effects, anti-androgenic effects, and anti-mineralcorticoid effects in addition to its progestational effects. No side effects have been reported for micronized progesterone with respect to lipid profile, coagulation, or blood pressure, leading the authors to recommend micronized progesterone as suitable for treatment of PMS, menopause, irregular cycles, and pregnancy maintenance.

- Sofuoglu M, Babb DA, Hatsukami DK. Progesterone treatment during the early follicular phase of the menstrual cycle: effects on smoking behavior in women. *Pharmacol Biochem Beh*av 2001 May-Jun;69(1-2):299-304.

  In this unique randomized controlled study, administration of progesterone (200 mg oral) demonstrated a decrease in craving for and subjective effects of cigarette smoking in female smokers. With progesterone treatment, there was a noted trend to decrease smoking.

Progesterone Safety

Although the two hormones are vastly different in their molecular structure and effects, progesterone and synthetic progestins are often lumped together with respect to their safety profiles. Further complicating the understanding of individual safety, early reports of concerns for progestins were not often separated from those of combination hormone therapies. Since the pivotal Women's Health Initiative (WHI) study results of 2002, there has been heightened interest by researchers for determining the safety of progesterone—alone, in combination, and in contrast to synthetic progestins. This research suggests a good safety profile for progesterone with respect to the cardiovascular system, breast, brain, and other target tissues. Numerous human studies evaluating progesterone reported the treatments were well tolerated, with few side effects.

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General Safety:

- Arafat ES, Hargrove JT, Maxson WS, Desiderio DM, Wentz AC, Andersen RN. Sedative and hypnotic effects of oral administration of micronized progesterone may be mediated through its metabolites. *Am J Obstet Gynecol* 1988 Nov; 159(5): 1203-9.

  This small pilot study evaluated progesterone and its metabolites following administration of oral micronized progesterone in eight postmenopausal women. Progesterone and its metabolites were measured in serum extracts by radioimmunoassay and gas chromatography-mass spectrometry. Evaluation of serial blood samples showed elevated levels of serum progesterone and its metabolites from baseline, reaching a peak between 2 and 6 hours after oral administration. The following compounds: progesterone, 5 beta-pregnan-3 alpha, 5 alpha-pregnan-3 alpha-ol-20-one, 5 beta-pregnan-3 alpha-ol-20-one, 20 beta-diol, and 5 beta-pregnan-3 alpha-ol-11,20-dione, were identified. These compounds have reported anesthetic qualities, which may contribute to the sedative and hypnotic effects seen with oral administration of progesterone. The authors reported that, in one subject, 400 mg of oral micronized progesterone induced a hypnotic state lasting approximately 2 hours.

- Burry KA, Patton PE, Hermsmeyer K. Percutaneous absorption of progesterone in postmenopausal women treated with transdermal estrogen. *Am J Obstet Gynecol* 1999 Jun;180(6 Pt 1):1504-11.

  This pilot study demonstrated a significant increase in serum progesterone levels in 6 women receiving topical progesterone cream (Progest®; 30-60 mg P4/day) and 17beta estradiol (0.05mg patch). The absorption of progesterone via a topical cream correlated well with estrogen absorption (p< 0.001). They concluded that progesterone cream appeared to be a safe and effective route of application.

- de Wit H, Schmitt L, Purdy R, Hauger R. Effects of acute progesterone administration in healthy postmenopausal women and normally-cycling women. *Psychoneuroendocrinology* 2001 Oct;26(7):697-710.

  This randomized controlled study investigated the effects of acute progesterone administration (25, 50, 100 mg, intramuscularly, 1 dose/wk) on mood. Contrary to the investigators' expectations, very few unwanted behavioral effects were noted, and only in the highest dose (100 mg) did women slightly increase their self-rating of "sluggishness."

- Darj E, Axelsson O, et al. Liver metabolism during treatment with estradiol and natural progesterone. *Gynecological Endocrinology.*1993; 7(2):111-4.

  Thirty postmenopausal women were treated daily for four months with 2 mg micronized 17 beta-estradiol and micronized progesterone orally in doses of 50, 100 and 200 mg daily. Serum concentrations of sex hormone-binding globulin (SHBG), corticosteroid binding globulin (CBG), ceruloplasmin, lipoprotein A and liver enzymes were measured. Serum SHBG and CBG increased during treatment with a weak association shown between progesterone and serum CBG. Levels of Lp(a) and liver enzymes did not

change, concluding that natural progesterone supplementation in postmenopausal women does not appear to cause any side effects to the liver.

- Fitzpatrick LA, Good A. Micronized progesterone: clinical indications and comparison with current treatments. *Fertil Steril* 1999 Sep;72(3):389-97.

  The literature reviewed in this tutorial indicates a potential use for oral micronized progesterone for the treatment of secondary amenorrhea, dysfunctional uterine bleeding, luteal phase disorders, premenopausal bleeding disorders, and as a component of hormone replacement therapy that may provide a better safety profile than commonly utilized synthetic progestins.

- Shantha S, Brooks-Gunn J, Locke RJ, Warren MP. Natural vaginal progesterone is associated with minimal psychological side effects: a preliminary study. *J Women Health Gend Based Med* 2001 Dec;10(10):991-7.

  This 3 month, multicenter randomized study evaluated the psychological side effects of a vaginally applied progesterone gel in reproductive aged women treated for hypothalamic amenorrhea or premature ovarian failure. No differences were noted in psychometric measures as evaluated by the Hopkins Symptom Checklist. Natural progesterone in a vaginal gel can be an effective treatment for women requiring hormone therapy.

- Sitruk-Ware R, Bricaire C, De Lignieres B, Yaneva H, Mauvais-Jarvis P. Oral micronized progesterone. Bioavailability pharmacokinetics, pharmacological and therapeutic implications--a review. *Contraception* 1987 Oct; 36(4): 373-402.

  This paper reviews the effects and benefits of oral micronized progesterone. Progesterone exhibits anti-estrogenic effects, anti-androgenic effects, and anti-mineralcorticoid effects in addition to its progestational effects. No side effects have been reported for micronized progesterone with respect to lipid profile, coagulation, or blood pressure, leading the authors to recommend micronized progesterone as suitable for treatment of PMS, menopause, irregular cycles, and pregnancy maintenance.

Progesterone and the Ovaries:

- Hu Z, Deng X. [The effect of progesterone on proliferation and apoptosis in ovarian cancer cell] *Zhonghua Fu Chan Ke Za Zhi* 2000 Jul;35(7):423-6. [Article in Chinese]

  In this in vitro study, researchers demonstrated that administered progesterone had a dose-dependent effect causing inhibition of growth of epithelial ovarian cancer cells, suggesting an anti-cancer effect.

- Yu S, Lee M, Shin S, Park J. Apoptosis induced by progesterone in human ovarian cancer cell line SNU-840. *J Cell Biochem* 2001;82(3):445-51.

  Although the mechanism is not fully understood, progesterone has been used as an anticancer therapy for the treatment of ovarian cancer. This study evaluates the effects of progesterone on ovarian cancer cells (SNU-840). Following incubation with 100 microM progesterone, viability of the cancer cells was evaluated by MTT assay, resulting

in 45% of the cells being viable after 48 h. Additionally, [(3)H] thymidine incorporation assay showed that progesterone completely inhibited the proliferation of the cells at the same duration and concentration. Cell death was by apoptosis as seen by fragmentation of the chromosomal DNA via colorimetric TUNEL assay. The level of the p53 mRNA reached its maximum at 12 h, then decreased following incubation with progesterone as determined by northern blotting assay. This is consistent with the fact that many apoptosis processes are mediated by up-regulation of the p53 expression. The authors conclude that progesterone inhibits the proliferation of and elicits apoptosis of the SNU-840 line of ovarian cancer cells and up-regulates p53 expression.

Progesterone Modes of Delivery:

- de Ziegler D, Fanchin R. Progesterone and progestins: applications in gynecology. *Steroids* 2000 Oct-Nov;65(10-11):671-9.

  This paper reviews the use of a transvaginal progesterone gel as a viable option to other routes of application of natural progesterone (intramuscular, oral micronized), and offered it as a viable option to synthetic progestins given the low incidence of side effects noted in existing studies.

Progesterone and Cancer (other than Breast)

- Horita K, Inase N, Miyake S, Formby B, Toyoda H, Yoshizawa Y. Progesterone induces apoptosis in malignant mesothelioma cells. Anticancer Res 2001 Nov-Dec;21(6A):3871-4.

  In this study, researchers demonstrated that progesterone administration suppressed cell proliferation and induced apoptosis (programmed cell death) in malignant mesothelioma cells (21 1H). This is consistent with an earlier in vitro study that found administered progesterone induced apoptosis in the breast cancer cell line, T47-D.

Progesterone and PMS:

- Dennerstein L, Spencer-Gardner C, Gotts G, et al. Progesterone and the premenstrual syndrome: a double blind crossover trial. *Br Med J* (Clin Res Ed) 1985 Jun 1; 290(6482): 1617-21.

  In this double-blind, placebo-controlled randomized crossover trial, oral micronized progesterone demonstrated effectiveness in alleviating premenstrual complaints. Twenty-three women completed a Beck depression inventory, Moos's menstrual distress questionnaire, Spielberger, state anxiety inventory, and daily symptom diary before and during each treatment. There was an overall benefit of treatment for all variables, except positive moods, restlessness, and interest in sex. For most parameters, maximum benefit was seen within the first month of treatment, demonstrating an effectiveness of progesterone as a viable treatment option for women with PMS.

- Magill PJ. Investigation of the efficacy of progesterone pessaries in the relief of symptoms of premenstrual syndrome. Progesterone Study Group. *Br J Gen Pract* 1995

Nov; 45(400): 589-93.

This multi-center, prospective, double-blind, randomized parallel study undertook to compare progesterone vaginal suppositories (400 mg twice daily) with placebo for the relief of premenstrual symptoms. Ninety-three participants completed the study. A clinically and statistically significant reduction of symptoms was consistently demonstrated in the women receiving the suppositories who had experienced symptoms in the moderately to severe categories. Adverse events were slightly higher in the active group (51 vs. 43%) and were limited to headache, irregular bleeding, and vaginal itching.

Progesterone and Bone Health:

- Lee JR. Osteoporosis reversal; the role of progesterone. *International Clinical Nutrition Review* 1990;10(3):384-91.

  Transdermal progesterone supplementation with and without conjugated estrogens was evaluated in a clinical setting using 100 women aged 38 to 83 years. The average time from onset of menopause was 16 years. 63 women were followed for three years with dual photon absorptiometry. Treatment also included dietary changes, nutritional supplements, and exercise. All individuals showed an increase in bone mineral density over the three years, with the greatest increase occurring in the first year. There was no difference noted between estrogen/progesterone and progesterone only groups. Subjective changes included increased libido, diminished hot flushes, reduced joint pain, and increased mobility and energy. No side effects were noted during treatment protocol.[Note: women in this study received weight-bearing exercise recommendations and calcium supplementation. There was no arm comparing these recommendations vs. those who also received progesterone supplementation. Therefore, it cannot be concluded that progesterone supplementation improves bone density over calcium plus exercise alone.]

- Liang M, Liao EY, Xu X, Luo XH, Xiao XH. Effects of progesterone and 18-methyl levonorgestrel on osteoblastic cells. *Endocr Res* . 2003 Nov;29(4):483-501.

  This study evalutated the effects of progesterone (P4) and levonorgestrel (LNG) on markers of bone growth, utilizing normal human osteoblasts as well as the osteosarcoma cell line, MG-63. Their study found that, compared with placebo, both P4 and LNG increased the proliferation and differentiation of human osteoblasts through osteocalcin gene transcription.

- Prior JC, Vigna Y, Alojado N. Progesterone and the prevention of osteoporosis. *Can J Obstet Gynecol and Women's Health Care* 1991;3(4):178-84.

  In this review article, the authors propose that cyclic progesterone both prevents bone loss and acts as a bone-builder. The studies discussed focus on abnormal menstrual cycles as an important risk factor for osteoporotic fractures. The author's conclusion is that the first step in preventing osteoporosis is treating ovulation disorders.

- Prior JC. Progesterone as a bone-trophic hormone. *Endocrine Reviews* 1990;11(2): 386-398.

- Prior JC, Vigna YM, Schecter MI, Burgess AE. Spinal bone loss and ovulatory disturbances. *N Engl J Med.* 1990; 323:1221-7.

  A review of the available data indicates that progesterone acts to promote bone metabolism. It appears to be independent of estrogen by either acting directly at progesterone receptors, or indirectly through competition at glucocorticoid receptors in the osteoblasts.

Progesterone and Fertility and Pregnancy:

- Ferre F, Uzan M, Janssens Y, Tanguy G, et al. Oral administration of micronized natural progesterone in late human pregnancy. Effects on progesterone and estrogen concentrations in the plasma, placenta, and myometrium*. Am J Obstet Gynecol* 1984 Jan 1; 148(1): 26-34.

  Levels of progesterone, 17 beta-estradiol, and estrone were measured in the plasma, in the placenta, and at different sites in myometrium following a single dose of micronized oral progesterone administered to 15 pregnant women immediately prior to elective cesarean section. In comparison to a control group, progesterone levels in the treated women increased in the plasma and myometrium 150 minutes after administration. Placenta progesterone levels did not demonstrate any change. No change was seen in 17 beta-estradiol levels in the plasma or the myometrium, however placental levels were increased. Estrone levels were decreased in the myometrium and in the placenta, and unchanged in the plasma.

- Hajek Z, Uhlir M. [Micronized progesterone in the treatment of imminent necrosis of a myoma during pregnancy. Ultrasound changes during treatment] *Ceska Gynekol* 1999 Jun;64(3):189-92. [Article in Czech]

  Progesterone has a role in increasing blood flow to the uterus during pregnancy. As such, these researchers studied the effect of progesterone treatment to resolve imminent necrosis of a myoma in two cases. Both resolved within several days following oral and vaginal doses of progesterone (300-600 mg/day). Both women went on to deliver healthy, full-term infants.

- Lydon JP, DeMayo FJ, Conneely OM, and O'Malley BW. Reproduction phenotypes of the progesterone receptor null mutant mouse. *J Steroid Biochem Molec Biol* 1996; 56(1-6):67-77.

  In an attempt to better understand the diversity of progesterone's effects, a novel mouse strain homozygous for the absence of progesterone receptors has been studied. Female PR null mice were found to have extensive reproductive abnormalities, and results provide evidence for progesterone's diverse role as the coordinator of events that ensure female fertility. Future studies of this animal model may help redefine progesterone's role as not just a sex steroid, but as a key player and regulator in a variety of physiological processes.

- Massai R, Miranda P, et al. Preregistration study on the safety and contraceptive efficacy of a progesterone-releasing vaginal ring in Chilean nursing women.

Contraception 1999 Jul;60(1):9-14.

In this long-term controlled study, the safety and efficacy of a progesterone-releasing vaginal contraceptive device was compared to that of the copper-T 380A IUD in nursing mothers. There was no difference in breastfeeding performance or infant growth between groups. The participants using the progesterone-releasing ring had a longer period of lactational amenorrhea than did the group using the copper T. Women were tracked for over 2000 women-months of exposure in both groups. The Chilean government found the progesterone-releasing ring to be a safe and effective contraceptive alternative.

- Pouly JL, Bassil S, Frydman R, et al. Luteal support after in-vitro fertilization: Crinone 8%, a sustained release vaginal progesterone gel, versus Utrogestan, an oral micronized progesterone. *Human Reprod* 1996;11:2085-89.

  Two progesterone presentations, a vaginal application of 90 mg progesterone per day (Crinone) or 300 mg progesterone administered orally (Utrogestan), were compared for luteal phase support of patients undergoing an in-vitro fertilization (IVF) procedure. 283 patients were randomly allocated to either treatment. The treatment started within 24 h after the embryo transfer procedure and continued until day 30 in cases of implantation. The pregnancy rates per transfer were not significantly different in the Crinone and Utrogestan groups at days 12 (Crinone 35.3%, Utrogestan 29.9%, P = 0.55), 30 (Crinone 28.5%, Utrogestan 25.0%, P = 0.61) and 90 (Crinone 25.9%, Utrogestan 22.9%, P = 0.69). No differences in the spontaneous abortion rates were seen thereafter. The delivery rates (number of deliveries per patient; Crinone 23.0%, Utrogestan 22.2%, P = 1.00), as well as the ratio of newborn babies per embryo transferred (Crinone 11.7%, Utrogestan 11.1%, P = 0.91), were not significantly different. Safety parameters were similar in both groups, except for drowsiness, which was more significantly frequent in the oral progesterone group than in the Crinone group at all time points. No serious adverse events were recorded in this study. The fact that Crinone matches the efficacy of the larger doses of progesterone used orally reflects an advantage of the transvaginal route of administration which avoids the metabolic inactivation of progesterone during its first liver pass.

Progesterone and Cardiovascular Disease and Lipids:

- Carmody BJ, Arora S, Wakefield MC, Weber M, Fox CJ, Sidawy AN. Progesterone inhibits human infragenicular arterial smooth muscle cell proliferation induced by high glucose and insulin concentrations. *J Vasc Surg* 2002 Oct;36(4):833-8.

  In vitro, progesterone was shown to have antiproliferative effects on vascular smooth muscle after proliferation was induced by models simulating hyperinsulinemia and hyperglycemia. Progesterone may, therefore, have a protective role against the atherosclerotic changes seen with diabetes (type II).

- Cheng W, Lau OD, Abumrad NA. Two antiatherogenic effects of progesterone on human macrophages; inhibition of cholesterol ester synthesis and block of its enhancement by glucocorticoids. *J Clin Endocrinol Metab* 1999 Jan;84(1):265-71.

This study evaluated the effects of estradiol and progesterone on cholesterol ester(CE) formation. Progesterone blocked CE formation, while estradiol had no effect. In comparison, cortisol and prednisolone (a widely prescribed glucocorticoid) both increased CE formation from 2-fold to 5-fold. This study demonstrated a role for progesterone in the decrease of cardiovascular risk factors that was not mediated by the progesterone receptor.

- Hermsmeyer RK, Mishra RG, Pavcnik D, et al. Prevention of coronary hyperreactivity in preatherogenic menopausal rhesus monkeys by transdermal progesterone. *Arterioscler Thromb Vasc Biol* . 2004 May;24(5):955-61.

  Previous studies by Hermsmeyer, et al demonstrated a reduction of coronary reactivity in response to subphysiological levels of progesterone in non-atherogenic monkeys. In this study, the authors sought to determine if transdermal progesterone cream conferred coronary vascular protection in surgically menopausal preatherosclerotic rhesus monkeys. Compared with monkeys receiving placebo cream (n= 5), treated monkeys (n= 7) experienced reduced Lp (a) levels, and an attenuation of coronary vasoconstriction, which was artificially stimulated by intracoronary serotonin plus U46619. Coronary hyperreactivity is a component of coronary artery disease and was demonstrated in this study to be prevented in preatherosclerotic primates by progesterone cream treatment.

- Lee WS, Harder JA, Yoshizumi M, Lee ME, Haber E. Progesterone inhibits arterial smooth muscle cell proliferation. *Nat Med* 1997 Sep;3(9):1005-8.

  Premenopausal women have a lower mortality from atherosclerotic cardiovascular disease than age-matched men. Progesterone receptors have been found in human and rat aortic smooth muscle cells in vivo and in vitro. This study examined the effect of progesterone on the proliferation of vascular smooth muscle cells. At physiologic levels, progesterone dose-dependently inhibited DNA synthesis and proliferation. RU486, a progesterone antagonist, blocked inhibition. This inhibition of arterial smooth muscle suggests a protective effect of progesterone against atherosclerosis.

- Molinari C, Battaglia A, Grossini E, Mary DA, Surico N, Vacca G. Effect of progesterone on peripheral blood flow in prepubertal female anesthetized pigs. *J Vasc Res* 2001 Nov-Dec;38(6):569-77.

  To determine the effects of progesterone on the peripheral circulation, prepubertal female pigs were anesthetized with sodium pentobarbitone and changes in the superior mesenteric, left renal and left external iliac flow caused by intravenous infusion of progesterone were assessed using electromagnetic flow meters. Increased blood flows in the mesenteric, renal, and iliac arteries were demonstrated in all 20 subjects. In 4 additional subjects, a dose dependent effect was noted. This effect was blocked by the injection of N(omega)-nitro-L-arginine methyl ester. Results demonstrated a vasodilatory effect of progesterone, with the mechanism being that of nitric oxide release.

- Otsuki M, Saito H, Xu X, Sumitani S, Kouhara H, Kishimoto T, Kasayama S. Progesterone, but not medroxyprogesterone, inhibits vascular cell adhesion molecule-1 expression in human vascular endothelial cells. *Arterioscler Thromb Vasc Biol* 2001

Feb;21(2):243-8.

This study utilizing human umbilical vein endothelial cells (HUVEC's) demonstrated that progesterone, but not medroxyprogesterone acetate (MPA) inhibited expression of vascular cell adhesion molecule-1 (VCAM-1), demonstrating a role for progesterone in the prevention of atherosclerosis. The differing effects of progesterone and MPA are clinically important, as MPA is widely used in hormone replacement therapy, when, as this research suggests, progesterone might be a more appropriate option.

- Rosano GM, Webb CM, Chierchia S, et al. Natural progesterone, but not medroxyprogesterone acetate, enhances the beneficial effect of estrogen on exercise-induced myocardial ischemia in postmenopausal women. *J Am Coll Cardiol* 2000 Dec;36(7):2154-9.

  This randomized crossover study compared the effects of estradiol (E2) (2mg/day), estradiol + progesterone (P4) vaginal gel (2 mg/day + 90 mg on alternate days), and estradiol + medroxyprogesterone acetate (MPA) (2 mg/day + 10 mg/day) on exercise-induced myocardial ischemia in eighteen postmenopausal women with coronary artery disease (CAD) or previous myocardial infarction (MI). Utilizing treadmill testing, patients were evaluated for exercise tolerance after each estradiol phase and at day 10 of each progestogen phase. The results demonstrated an increase in exercise tolerance with both E2 and E2 + progesterone, but not by E2 + MPA as compared to baseline. Furthermore, E2 + P4 demonstrated a significant increase in exercise tolerance when compared to MPA. The results suggest that progesterone may be the progestogen of choice for hormone replacement therapy for women at risk for cardiovascular disease.

- Rylance PB, Brincat M, Lafferty K, De Trafford JC, Brincat S, Parsons V, Studd JW. Natural progesterone and antihypertensive action. *Br Med J* (Clin Res Ed) 1985 Jan 5;290(6461):13-4.

  In a placebo controlled, double blind crossover study, increasing doses of natural progesterone was given orally to six men and four postmenopausal women with mild to moderate hypertension who were not receiving any other antihypertensive drugs. Compared to before treatment values and to placebo, progesterone caused a significant reduction in blood pressure, suggesting that progesterone has an antihypertensive action rather than a hypertensive one as has been previously thought. The authors suggest this protective effect of progesterone should be investigated further.

- Tsuda K, Kinoshita Y, Nishio I. Synergistic role of progesterone and nitric oxide in the regulation of membrane fluidity of erythrocytes in humans: an electron paramagnetic resonance investigation. *Am J Hypertens* 2002 Aug;15(8):702-8

  Progesterone increased red blood cell membrane fluidity in this in vitro study, in part by a nitric oxide-dependent mechanism. It has been demonstrated that progesterone may play various roles in the regulation of blood pressure and other cardiovascular activities. The findings of this study suggest a positive role for progesterone in the improvement of microcirculation in humans.

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- Bagis T, Gokcel A, Zeyneloglu HB, Tarim E, Kilicdag EB, Haydardedeoglu B. The effects of short-term medroxyprogesterone acetate and micronized progesterone on glucose metabolism and lipid profiles in patients with polycystic ovary syndrome: a prospective randomized study. *J Clin Endocrinol Metab* 2002 Oct;87(10):4536-40.

  This randomized prospective study evaluated and compared the effects of ten days treatment with oral and vaginal progesterone (MP) and medroxyprogesterone acetate (MPA) on glucose metabolism, lipid profiles, and hormonal parameters in 28 patients with polycystic ovary syndrome (PCOS). Oral MPA and oral MP decreased LH (P = 0.028, P = 0.009, respectively) and total testosterone (P = 0.013, P = 0.037, respectively) levels. There was no change in hormonal parameters with vaginal MP. Basal insulin decreased (P = 0.021) and insulin sensitivity increased significantly in the oral MPA group. Low- density lipoprotein cholesterol (LDL) and lipoprotein (a) levels decreased only in the MPA group. This study concluded that MPA and oral MP may reduce insulin sensitivity in patients with PCOS. Vaginal MP had no effect on glucose metabolism and lipid profiles.

- Bolaji II, Grimes H, Mortimer G, et al. Low-dose progesterone therapy in oestrogenised postmenopausal women: effects on plasma lipids, lipoproteins, and liver function parameters. Eur *J Obstet Gynecol Reprod Biol* 1993 Jan;48(1):61-8.

  This 12 month prospective, open, non-comparative study measured the effects progesterone (oral micronized 100mg/day) paired with 0.625 mg conjugated equine estrogens (CEE) and found progesterone had no adverse effects on the lipid profile when combined with CEE. This lack of effect differs from other studies that noted adverse effects on lipid profiles when synthetic progestins were utilized with CEE.

- Hargrove JT, Maxson WS, Wentz AC, Burnett LS. Menopausal hormone replacement therapy with continuous daily oral micronized estradiol and progesterone. Obstetrics & Gynecology April 1989; 73(4): 606-12.

  Fifteen menopausal subjects were studied to determine the efficacy and safety of hormone replacement therapy with micronized estradiol (E2) and progesterone. Ten subjects were given estradiol (0.7-1.05 mg daily) and progesterone (200-300 mg daily) and evaluated over one year at month 0, 1, 3, 6, and 12. Five subjects were administered conjugated estrogens (0.625mg daily) and medroxyprogesterone acetate (10 mg daily) and evaluated at the same intervals. Results showed all 10 women on E2 and progesterone had a decrease in total cholesterol with an increase in HDLs and sustained amenorrhea with no endometrial hyperplasia or withdrawal bleeding after six months of observation. Four of five women in the conjugated estrogen group continued to have withdrawal bleeding without endometrial hyperplasia. HDLs also increased in this group but no significant change in total cholesterol was found.

- Mather KJ, Norman EG, Prior JC, Elliott TG. Preserved forearm endothelial responses with acute exposure to progesterone: A randomized cross-over trial of 17-beta estradiol, progesterone, and 17-beta estradiol with progesterone in healthy menopausal women. J *Clin Endocrinol Metab* 2000 Dec;85(12):4644-9.

  Regularly menstruating women enjoy relative protection from cardiovascular disease.

Until recently, this has been attributed to the function of estrogen, despite the fact that progesterone is also present. This study evaluated the differing acute effects of 17-beta estradiol with and without progesterone with progesterone alone on endothelial function in a randomized crossover trial. Endothelial function was evaluated via endothelium dependent and independent forearm blood flow (FBF) using venous occlusion plethysmography. Flow responses were measured during brachial artery infusions achieving physiological levels of E2, E2 + P4, or P4 respectively along with either acetylcholine (an endothelium-dependent vasodilator), or sodium nitroprusside (an endothelium-independent vasodilator) in 27 healthy menopausal women with no cardiovascular disease risk factors. Small, statistically non-significant increases in endothelium-dependent flow responses were seen with all treatments. No impairment in response was seen with P4 alone or in combination with E2. The authors concluded that progesterone does not have detrimental vascular effects in humans.

- Ottosson UB, Johansson BG, et al. Subfractions of high-density lipoprotein cholesterol during estrogen replacement therapy: A comparison between progestogens and natural progesterone. *Am J Obstet Gynecol.* 1993;151(6):746-50.

  Fifty-eight postmenopausal women were followed with respect to subfractions of high-density lipoprotein during 3 cycles of unopposed estrogen. The women received either levonorgestrel, medroxyprogesterone acetate, or natural progesterone during the last ten days of the treatment period. Both progestogens significantly lowered HDL cholesterol, whereas natural progesterone had no effect on HDL levels.

- Rosano GM, Webb CM, Chierchia S, Morgani GL, Gabraele M, Sarrel PM, de Ziegler D, Collins P. Natural progesterone, but not medroxyprogesterone acetate, enhances the beneficial effect of estrogen on exercise-induced myocardial ischemia in postmenopausal women. *J Am Coll Cardiol* 2000 Dec;36(7):2154-9

  Eighteen postmenopausal women were randomized to receive 17-beta estradiol with a synthetic progestin (medroxyprogesterone acetate) or a progesterone vaginal gel for 4 weeks, then crossed over to the alternate treatment. Researchers found through treadmill testing that estrogen plus progesterone significantly increased exercise time before myocardial ischemia, when compared to estradiol plus synthetic progestin. In addition, 2 patients on the synthetic progestin arm had to discontinue due to unstable angina. This research suggests that women at risk for cardiovascular disease need to consider progesterone as a safer alternative to synthetic progestins as a part of their hormone replacement therapy regime.

- Saarikoski S, Yliskoski M, Penttila I. Sequential use of norethisterone and natural progesterone in pre-menopausal bleeding disorders. *Maturitas* 1990 Jun;12(2):89-97.

  This randomized controlled study evaluated the effects of norethisterone (NET) and micronized progesterone (MP) on bleeding disorders in pre-menopausal women. 80 patients were randomized to the trial and all were found via endometrial morphology to need progestogen therapy. They were subsequently treated with NET or MP. In both treatment groups, hyperplastic changes disappeared during the first three cycles, with the duration of treatment being 6 months. NET decreased follicle-stimulating hormone, luteinizing hormone, estradiol and sex-hormone-binding globulin levels ($P < 0.001$)

whereas no changes were seen during MP treatment. High-density-lipoprotein cholesterol and triglyceride levels were also lowered by NET (P< 0.001-0.02) slightly decreased phospholipids. MP treatment had no effect on lipid profiles suggesting it may be a preferred progestogen for the treatment of bleeding disorders.

- Sitruk-Ware R. Progestins and cardiovascular risk markers. Steroids 2000 Oct-Nov;65(10-11):651-8.

  This article reviewed the effects of various synthetic progestins and progesterone on cardiovascular health. Many synthetic progestins, especially 19-nortestosterone and some 17-hyroxyprogesterones, have negative effects on cardiovascular risk factors, whereas natural progesterone does not. Further studies utilizing natural and other steroids should be considered.

Progesterone and Menopausal Symptoms:

- Burry KA, Patton PE, Hermsmeyer K. Percutaneous absorption of progesterone in postmenopausal women treated with transdermal estrogen. *Am J Obstet Gynecol* 1999;180: 1504-1511

- Fitzpatrick LA, Pace C, Wiita B. Comparison of regimens containing oral micronized progesterone or medroxyprogesterone acetate on quality of life in postmenopausal women: a cross-sectional survey. *J Women Health Gend Based Med* 2000 May;9(4):381-7.

  A cross-sectional survey was conducted to examine quality of life (QOL) related to physiological, somatic, and vasomotor effects of switching progestogen treatment from medroxyprogesterone acetate (MPA) to micronized progesterone in postmenopausal women already using hormone replacement therapy (HRT). One hundred seventy-six women who were currently using hormone replacement therapy (HRT) containing micronized progesterone for 1-6 months and had previously received HRT containing MPA were surveyed to assess QOL. Women using micronized progesterone-containing HRT experienced significant improvement in vasomotor symptoms, anxiety, somatic complaints, and depressive symptoms. Women reported improved control of menopausal symptoms and perceptions of their vaginal bleeding patterns while on the micronized progesterone-containing regimen. Approximately 80% of women reported satisfaction with the progesterone-containing therapy. A micronized progesterone-containing HRT therapy offers the potential for improved QOL with respect to menopausal symptoms.

- Leonetti HB, Longo S, Anasti JN. Transdermal progesterone cream for vasomotor symptoms and postmenopausal bone loss. *Obstet Gynecol* 1999 Aug;94(2):225-8.

  In this randomized controlled trial, 102 menopausal women were treated with topical progesterone (Pro-Gest® , 20 mg daily) or placebo and monitored for 1 year. Improvement in vasomotor symptoms was seen in 83% of the women in the treatment group who had experienced hot flashes, compared to 19% in the placebo group (p<

.001). There was no difference noted in bone mineral densities between groups after one year. All women studied received a daily multivitamin and 1200 mg calcium.

- Stephenson, Kenna; Price, Carol; Kurdowska, Anna; et al. Topical progesterone cream does not increase thrombotic and inflammatory factors in postmenopausal women. Session Type: "Publication Only" *Blood* 2004 Nov;104(11).

  In this prospective, double-blinded, crossover study, the authors examined the short-term effect of topical progesterone cream on menopausal symptom relief in 30 healthy postmenopausal women. Potential adverse effects of topical progesterone on hemostatic and inflammatory factors and cortisol levels were also examined. Subjects received either 20 mg of topical progesterone cream or placebo cream for 4 weeks. Following a subsequent 4-week washout period, subjects were crossed over to either placebo cream or active drug for an additional 4-week period. In each case, progesterone and cortisol levels were monitored by salivary sampling. Baseline values, 4-week follow-up values and end-of-study values were also obtained for the Greene Climacteric Scale, total factor VII:C, factor VIIa, factor V, fibrinogen, antithrombin, PAI-1, CRP, TNFá, and IL-6. Subjects receiving 20 mg of topical progesterone cream for 4 weeks improved menopausal symptoms, without adversely altering prothrombotic potential.

- Wetzel W. Micronized progesterone: a new option for women's health care. *Nurse Pract* 1999 May;24(5):62-6, 71, 75-6.

  This paper discusses the use of micronized progesterone as a safe, effective, and well-tolerated therapy and reviews indications for use. It also includes case studies and issues of patient compliance and the need for an individualized treatment plan for women receiving hormone therapy.

- Wren BG, Champion SM, Manga RZ, et al. Transdermal progesterone and its effect on vasomotor symptoms, blood lipid levels, bone metabolic markers, moods, and quality of life for postmenopausal women. *Menopause* 2003;10(1): 13-18.

Progesterone & Sleep

- Montplaisir J, Lorrain J, Denesle R, Petit D. Sleep in menopause: differential effects of two forms of hormone replacement therapy. *Menopause* 2001; 8(1):10-16.

  This randomized clinical trial compared the effects of conjugated equine estrogen (CEE) and medroxyprogesterone acetate to CEE and oral micronized progesterone. Twenty-one postmenopausal women were studied in a sleep lab, with results demonstrating an improvement in subjective measures of menopausal symptoms and sleep in both groups. The group receiving natural progesterone had significantly improved sleep efficiency, whereas the medroxyprogesterone acetate group did not, suggesting that the former might better improve sleep in postmenopausal women.

Progesterone & Quality of Life

- Ryan N, Rosner A. Quality of life (QOL) and costs associated with micronized progesterone and medroxyprogesterone acetate in hormone replacement therapy for non-hysterectomized, postmenopausal women. *Clin Ther* 2001 Jul;23(7):1099-115.

  This prospective, multicenter, randomized, parallel-group study enrolled 182 postmenopausal women 45 to 65 years of age and evaluated the quality of life and menopausal symptoms associated with the use of medroxyprogesterone acetate vs oral micronized progesterone when used as a part of a regular hormone replacement therapy. Menopausal symptoms improved in both groups from baseline to 9 months, as did QOL measures. In addition, patients using micronized progesterone had specific improvements in the areas of cognition and menstrual problems whereas the patients using MPA did not. Micronized progesterone was seen as an effective, cost-comparable alternative to MPA as well as being better tolerated.

- Sherwin BB. Progestogens used in menopause. Side effects, mood and quality of life. J *Reprod Med* 1999 Feb;44(2 Suppl):227-32.

  This review summarizes the effects of progesterone on mood and other brain functions. Progesterone receptors are present in many of the same areas of the brain as estrogen receptors, including the limbic system and hypothalamus. The limbic system plays a prominent role in regulating mood and emotion. As a comparison, progesterone decreases brain excitability, while estrogens increase it. This relates to why women with epilepsy have a higher frequency of seizures during the part of the cycle when estrogen levels are high, and a reduced frequency when progesterone levels are high. Estrogen and progesterone may also have differing effects on MAO, thereby affecting concentration of serotonin (a mood elevator) in the brain.

Progesterone and the Brain, Nervous System, and Mood:

- Baulieu E, Schumacher M. Progesterone as a neuroactive neurosteroid, with special reference to the effect of progesterone on myelination. *Steroids* 2000;65(10-11):605-12.

  Some steroids are synthesized within the central and peripheral nervous system, mostly by glial cells. These are known as neurosteroids. In the brain, certain neurosteroids have been shown to act directly on membrane receptors for neurotransmitters. For example, progesterone inhibits the neuronal nicotinic acetylcholine receptor, whereas its 3alpha,5alpha-reduced metabolite 3alpha, 5alpha-tetrahydroprogesterone (allopregnanolone) activates the type A gamma-aminobutyric acid receptor complex. Besides these effects, neurosteroids also regulate important glial functions, such as the synthesis of myelin proteins. Thus, in cultures of glial cells prepared from neonatal rat brain, progesterone increases the number of oligodendrocytes expressing the myelin basic protein (MBP) and the 2',3'-cyclic nucleotide-3'-phosphodiesterase (CNPase). An important role for neurosteroids in myelin repair has been demonstrated in the rodent sciatic nerve, where progesterone and its direct precursor pregnenolone are synthesized by Schwann cells. After cryolesion of the male mouse sciatic nerve, blocking the local synthesis or action of progesterone impairs remyelination of the regenerating axons, whereas administration of progesterone to the lesion site promotes the formation of new myelin sheaths.

- Cummings JA, Brizendine L. Comparison of physical and emotional side effects of progesterone or medroxyprogesterone in early postmenopausal women. *Menopause* 2002 Jul-Aug;9(4):253-63.

  Twenty-three early postmenopausal women were randomized to either medroxyprogesterone acetate (MPA) or oral micronized progesterone combined with conjugated equine estrogens (CEE) and followed for 91 days in a sequence of treatments. None of the hormone treatments had any noticeable effect on mood. Participants using MPA experienced more breast tenderness and bleeding than those using progesterone. This study debunks the belief that progesterone depresses mood in healthy individuals.

- De Nicola AF, Gonzalez SL, Labombarda F, et al. Progesterone treatment of spinal cord injury: Effects on receptors, neurotrophins, and myelination. *J Mol Neurosci.* 2006;28(1):3-15.

  In addition to its traditional role in reproduction, progesterone (PROG) has demonstrated neuroprotective and promyelinating effects in lesions of the peripheral and central nervous systems, including the spinal cord. The latter is a target of PROG, as nuclear receptors, as well as membrane receptors, are expressed by neurons and/or glial cells. When spinal cord injury (SCI) is produced at the thoracic level, several genes become sensitive to PROG in the region caudal to the lesion site. Although the cellular machinery implicated in PROG neuroprotection is only emerging, neurotrophins, their receptors, and signaling cascades might be part of the molecules involved in this process. In rats with SCI, a 3-d course of PROG treatment increased the mRNA of brain-derived neurotrophic factor (BDNF) and BDNF immunoreactivity in perikaryon and processes of motoneurons, whereas chromatolysis was strongly prevented. The increased expression of BDNF correlated with increased immunoreactivity for the BDNF receptor TrkB and for phosphorylated cAMP-responsive element binding in motoneurons. In the same SCI model, PROG restored myelination, according to measurements of myelin basic protein (MBP) and mRNA levels, and further increased the density of NG2+-positive oligodendrocyte progenitors. These cells might be involved in remyelination of the lesioned spinal cord. Interestingly, similarities in the regulation of molecular parameters and some cellular events attributed to PROG and BDNF (i.e., choline acetyltransferase, Na,K-ATPase, MBP, chromatolysis) suggest that BDNF and PROG might share intracellular pathways. Furthermore, PROG-induced BDNF might regulate, in a paracrine or autocrine fashion, the function of neurons and glial cells and prevent the generation of damage.

- Garay L, Deniselle MC, Lima A, et al. Effects of progesterone in the spinal cord of a mouse model of multiple sclerosis. *J Steroid Biochem Mol Biol*. 2007 Nov-Dec;107(3-5):228-37.

  The spinal cord is a target of progesterone (PROG), as demonstrated by the expression of intracellular and membrane PROG receptors and by its myelinating and neuroprotective effects in trauma and neurodegeneration. The authors studied PROG effects in mice with experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis characterized by demyelination and immune cell infiltration in the spinal cord. Female C57BL/6 mice were immunized with a myelin oligodendrocyte

glycoprotein peptide (MOG(40-54)). One week before EAE induction, mice received single pellets of PROG weighing either 20 or 100 mg or remained free of steroid treatment. On average, mice developed clinical signs of EAE 9-10 days following MOG administration. The spinal cord white matter of EAE mice showed inflammatory cell infiltration and circumscribed demyelinating areas, demonstrated by reductions of luxol fast blue (LFB) staining, myelin basic protein (MBP) and proteolipid protein (PLP) immunoreactivity (IR) and PLP mRNA expression. In motoneurons, EAE reduced the expression of the alpha 3 subunit of Na,K-ATPase mRNA. In contrast, EAE mice receiving PROG showed less inflammatory cell infiltration, recovery of myelin proteins and normal grain density of neuronal Na,K-ATPase mRNA. Clinically, progesterone produced a moderate delay of disease onset and reduced the clinical scores. Progesterone attenuated disease severity, and reduced the inflammatory response and the occurrence of demyelination in the spinal cord during the acute phase of EAE.

- Gibson CL, Murphy SP. Progesterone enhances functional recovery after middle cerebral artery occlusion in male mice. *J Cereb Blood Flow Metab*. 2004 Jul;24(7):805-13.

  Differences in outcomes following ischemia have been noted in the sexes, and is thought to be attributed to sex steroids. This study investigated the potential benefits of progesterone administration after focal cerebral ischemia of the middle cerebral artery of male mice. Male mice undergoing 60-minute middle cerebral artery occlusion (MCAO) received either progesterone or vehicle following occlusion. The mice receiving progesterone had significantly reduced lesion volume ($p< 0.05$) when compared with the vehicle treated mice (control). Progesterone treatment also improved survival rate, weight recovery, and motor ability when compared to the control group. In addition, mice treated with progesterone demonstrated motor ability comparable to mice that did not undergo MCAO. The authors suggest the need to further investigate the mechanisms of progesterone action on recovery from cerebral injury.

- González SL, Labombarda F, González Deniselle MC, et al. Progesterone up-regulates neuronal brain-derived neurotrophic factor expression in the injured spinal cord. *Neuroscience*. 2004;125(3):605-14.

  Progesterone (PROG) provides neuroprotection to the injured central and peripheral nervous system. These effects may be due to regulation of myelin synthesis in glial cells and also to direct actions on neuronal function. Recent studies point to neurotrophins as possible mediators of hormone action. In this study, the authors show that the expression of brain derived neurotrophic factor (BDNF) at both the mRNA and protein levels was increased by PROG treatment in ventral horn motoneurons from rats with spinal cord injury (SCI). Semiquantitative in situ hybridization revealed that SCI reduced BDNF mRNA levels by 50% in spinal motoneurons (control: 53.5+/-7.5 grains/mm(2) vs. SCI: 27.5+/-1.2, P<0.05), while PROG administration to injured rats (4 mg/kg/day during 3 days, s.c.) elicited a three-fold increase in grain density (SCI+PROG: 77.8+/-8.3 grains/mm(2), P<0.001 vs. SCI). In addition, PROG enhanced BDNF immunoreactivity in motoneurons of the lesioned spinal cord. Analysis of the frequency distribution of immunoreactive densities (chi(2): 812.73, P<0.0001) showed that 70% of SCI+PROG motoneurons scored as dark stained whereas only 6% of neurons in the SCI group belonged to this density score category (P<0.001). PROG also prevented the lesion-

induced chromatolytic degeneration of spinal cord motoneurons as determined by Nissl staining. In the normal intact spinal cord, PROG significantly increased BDNF inmunoreactivity in ventral horn neurons, without changes in mRNA levels. Our findings suggest that PROG enhancement of endogenous neuronal BDNF could provide a trophic environment within the lesioned spinal cord and might be part of the PROG activated-pathways to provide neuroprotection.

- Grossman KJ, Goss CW, Stein DG. Effects of progesterone on the inflammatory response to brain injury in the rat. *Brain Res*. 2004 May 15;1008(1):29-39.

  Progesterone has a known anti-inflammatory effect. In this study, male rats treated with progesterone (4 mg/kg) and/or vehicle, were examined with respect to cellular inflammatory response to frontal cortex injury on postsurgical days 1, 3, 5, 7 and 9. The treated mice suffered significantly less edema than untreated mice, as well as showed an increase in the accumulation of activated microglia, demonstrating a neuroprotective effect on the rat brain.

- Labombarda F, González SL, Lima A, et al. Effects of progesterone on oligodendrocyte progenitors, oligodendrocyte transcription factors, and myelin proteins following spinal cord injury. *Glia*. 2009;57(8):884-97.

  Progesterone is emerging as a myelinizing factor for central nervous system injury. Successful remyelination requires proliferation and differentiation of oligodendrocyte precursor cells (OPC) into myelinating oligodendrocytes, but this process is incomplete following injury. To study progesterone actions on remyelination, the authors administered progesterone (16 mg/kg/day) to rats with complete spinal cord injury. Results suggested early progesterone treatment enhanced the density of OPC and induced their differentiation into mature oligodendrocytes by increasing the expression of Olig2 and Nkx2.2. Twenty-one days after injury, progesterone favors remyelination by increasing Olig1 (involved in repair of demyelinated lesions), PLP expression, and enhancing oligodendrocytes maturation. Thus, progesterone effects on oligodendrogenesis and myelin proteins may constitute fundamental steps for repairing traumatic injury inflicted to the spinal cord.

- Leonelli E, Bianchi R, Cavaletti G, et al. Progesterone and its derivatives are neuroprotective agents in experimental diabetic neuropathy: a multimodal analysis. *Neuroscience*. 2007;144(4):1293-304.

  One important complication of diabetes is damage to the peripheral nervous system. However, in spite of the number of studies on human and experimental diabetic neuropathy, the current therapeutic arsenal is meagre. Consequently, the search for substances to protect the nervous system from the degenerative effects of diabetes has high priority in biomedical research. Neuroactive steroids might be interesting since they have been recently identified as promising neuroprotective agents in several models of neurodegeneration. We have assessed whether chronic treatment with progesterone (P), dihydroprogesterone (DHP) or tetrahydroprogesterone (THP) had neuroprotective effects against streptozotocin (STZ)-induced diabetic neuropathy at the neurophysiological, functional, biochemical and neuropathological levels. Using gas chromatography coupled to mass-spectrometry, the authors found that three months of

diabetes markedly lowered P plasma levels in male rats, and chronic treatment with P restored them, with protective effects on peripheral nerves. In the model of STZ-induced of diabetic neuropathy, chronic treatment for 1 month with P, or with its derivatives, DHP and THP, counteracted the impairment of nerve conduction velocity (NCV) and thermal threshold, restored skin innervation density, and improved Na(+),K(+)-ATPase activity and mRNA levels of myelin proteins, such as glycoprotein zero and peripheral myelin protein 22, suggesting that these neuroactive steroids, might be useful protective agents in diabetic neuropathy. Interestingly, different receptors seem to be involved in these effects. Thus, while the expression of myelin proteins and Na(+),K(+)-ATPase activity are only stimulated by P and DHP (i.e. two neuroactive steroids interacting with P receptor, PR), NCV, thermal nociceptive threshold and intraepidermal nerve fiber (IENF) density are also affected by THP, which interacts with GABA-A receptor. Because, a therapeutic approach with specific synthetic receptor ligands could avoid the typical side effects of steroids, future experiments will be devoted to evaluating the role of PR and GABA-A receptor in these protective effects.

- Leonelli E, Ballabio M, Consoli A, et al. Neuroactive steroids: A therapeutic approach to maintain peripheral nerve integrity during neurodegenerative events*. J Mol Neurosci.* 2006;28(1):65-76.

  It is now well known that peripheral nerves are a target for the action of neuroactive steroids. This review summarizes observations obtained so far, indicating that through the interaction with classical and nonclassical steroid receptors, neuroactive steroids (e.g., progesterone, testosterone and their derivatives, estrogens, etc.) are able to influence several parameters of the peripheral nervous system, particularly its glial compartment (i.e., Schwann cells). Interestingly, some of these neuroactive steroids might be considered as promising neuroprotective agents. They are able to counteract neurodegenerative events of rat peripheral nerves occurring after experimental physical trauma, during the aging process, or in hereditary demyelinating diseases. On this basis, the hypothesis that neuroactive steroids might represent a new therapeutic strategy for peripheral neuropathy is proposed.

- Morali G, Montes P, Hernandez-Morales L, et al. Neuroprotective effects of progesterone and allopregnanolone on long-term cognitive outcome after global cerebral ischemia. *Restor Neurol Neurosci.* 2011; 29(1):1-15.

- Movaghar B, Tiraihi T, Mesbah-Namin SA. Transdifferentiation of bone marrow stromal cells into Schwann cell phenotype using progesterone as inducer. *Brain Res*. 2008 May 7;1208:17-24.

  Bone marrow stromal cells (BMSCs) were reported to transdifferentiate into Schwann cells by a two-stage protocol, using beta-mercaptoethanol and retinoic acid (BME-RA) as preinducers (preinduction stage: PS) and platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF), forskolin (FSK) and heregulin (HRG) as inducers (induction stage: IS). In this study, six groups were used, group one was used as control (PS: BME-RA; IS: PDGF, bFGF, FSK and HRG). In group 2, the preinducer was similar to group 1, and in the induction stage, progesterone replaced HRG. In groups 3 and 4, the preinducer was progesterone; and at the induction stage, the inducer was similar to groups 1 and 2. Accordingly, in groups 5 and 6, the preinducer was FSK. The

immunohistochemical differentiation markers were S-100 and P0, and RT-PCR markers were OCT-4 and P0 at the preinduction stage, while at the induction stage P0 and NeuroD were used. The results of the study showed that S-100 was expressed in the groups after the induction stage, however, P0 was not expressed in any group. There was not any significant difference between the percentage of S100 positive cells in the 1st and 2nd groups. P0 was expressed at the mRNA level in the undifferentiated BMSCs and in the 3rd and 4th groups after the preinduction and the induction stages. The conclusion of this study is that progesterone can induce BMSCs into Schwann cell phenotype.

- Nilson J, Brinton Rd. Divergent impact of progesterone and medroxyprogesterone acetate (Provera) on nuclear mitogen-activated protein kinase signaling. *Proc Natl Acad Sci.* 2003;100(18):10506-11.

  Estrogen and progesterone are neuroprotective against excitotoxicity, whereas medroxyprogesterone acetate (MPA or Provera) is not. This paper demonstrates that estradiol and progesterone treatment of hippocampal neurons attenuates the excitotoxic glutamate-induced rice in intracellular calcium concentration. MPA completely antagonized the estradiol-induced attenuation of intracellular calcium concentration.

- Roglio I, Bianchi R, Gotti S, et al. Neuroprotective effects of dihydroprogesterone and progesterone in an experimental model of nerve crush injury. *Neuroscience*. 2008;155(3):673-85.

  A satisfactory management to ensure a full restoration of peripheral nerve after trauma is not yet available. In this study using rats, the authors demonstrate that the levels of neuroactive steroids, such as pregnenolone and progesterone (P) metabolites (i.e. dihydroprogesterone, DHP, and tetrahydroprogesterone, THP) present in injured sciatic nerve were significantly decreased. On this basis, they focused attention on DHP and its direct precursor, P, analyzing whether these two neuroactive steroids may have neuroprotective effects on biochemical, functional and morphological alterations occurring during crush-induced degeneration-regeneration. The authors demonstrated that DHP and/or P counteract biochemical alterations (i.e. myelin proteins and Na(+),K(+)-ATPase pump) and stimulate reelin gene expression. These two neuroactive steroids also counteract nociception impairment, and DHP treatment significantly decreases the upregulation of myelinated fibers' density occurring in crushed animals. Altogether, these observations suggest that DHP and P (i.e. two neuroactive steroids interacting with progesterone receptor) may be considered protective agents in case of nerve crush injury.

- Schumacher M, Guennoun R, Stein DG, De Nicola AF. Progesterone: therapeutic opportunities for neuroprotection and myelin repair. *Pharmacol Ther*. 2007;116(1):77-106.

  Progesterone and its metabolites promote the viability of neurons in the brain and spinal cord. Their neuroprotective effects have been documented in different lesion models, including traumatic brain injury (TBI), experimentally induced ischemia, spinal cord lesions and a genetic model of motoneuron disease. Progesterone plays an important

role in developmental myelination and in myelin repair, and the aging nervous system appears to remain sensitive to some of progesterone's beneficial effects. Thus, the hormone may promote neuroregeneration by several different actions by reducing inflammation, swelling and apoptosis, thereby increasing the survival of neurons, and by promoting the formation of new myelin sheaths. Recognition of the important pleiotropic effects of progesterone opens novel perspectives for the treatment of brain lesions and diseases of the nervous system. Over the last decade, there have been a growing number of studies showing that exogenous administration of progesterone or some of its metabolites can be successfully used to treat traumatic brain and spinal cord injury, as well as ischemic stroke.Progesterone can also be synthesized by neurons and by glial cells within the nervous system. This finding opens the way for a promising therapeutic strategy, the use of pharmacological agents, such as ligands of the translocator protein (18 kDa) (TSPO; the former peripheral benzodiazepine receptor or PBR), to locally increase the synthesis of steroids with neuroprotective and neuroregenerative properties. A concept is emerging that progesterone may exert different actions and use different signaling mechanisms in normal and injured neural tissue.

- Schumacher M, Guennoun R, Robert F, et al. Local synthesis and dual actions of progesterone in the nervous system: neuroprotection and myelination. *Growth Horm IGF Res.* 2004 Jun;14 Suppl A:S18-33.

  This paper reviews of the effects of progesterone as an autocrine/paracrine hormone in the brain. The brain, spinal cord and peripheral nerves all synthesize progesterone from the precursor, pregnenolone. Macroglial cells, including astrocytes, oligodendroglial cells and Schwann cells, also have the capacity to synthesize progesterone. This production is regulated by cellular interactions. Recent research has suggested the role progesterone plays in the brain is likely a significant one, supporting the viability of neurons and the formation of myelin sheaths. In mice and rat studies, progesterone also demonstrated a neuroprotective effect. These actions of progesterone suggest viable therapeutic possibilities for the prevention and treatment of neurodegenerative diseases, as well as for repair processes and for preserving cognitive functions with age.

- Stein DG. Progesterone exerts neuroprotective effects after brain injury. *Brain Res Rev*. 2008 Mar;57(2):386-97.

  This article reviews published preclinical and epidemiologic studies that examine progesterone's role in the central nervous system. Its effects on the reproductive and endocrine systems are well known, but a large and growing body of evidence, including a recently published pilot clinical trial, indicates that the hormone also exerts neuroprotective effects on the central nervous system. We now know that it is produced in the brain, for the brain, by neurons and glial cells in the central and peripheral nervous system of both male and female individuals. Laboratories around the world have reported that administering relatively large doses of progesterone during the first few hours to days after injury significantly limits central nervous system damage, reduces loss of neural tissue, and improves functional recovery. Although the research published to date has focused primarily on progesterone's effects on blunt traumatic brain injury, there is evidence that the hormone affords protection from several forms of acute central nervous system injury, including penetrating brain trauma, stroke, anoxic brain injury, and spinal cord injury. Progesterone appears to exert its protective effects by protecting

or rebuilding the blood-brain barrier, decreasing development of cerebral edema, down-regulating the inflammatory cascade, and limiting cellular necrosis and apoptosis. All are plausible mechanisms of neuroprotection.

- Stein DG, Wright DW, Kellermann AL. Does progesterone have neuroprotective properties? *Ann Emerg Med*. 2008 Feb;51(2):164-72.

  Progesterone, although still widely considered primarily a gender hormone, is an important agent affecting many central nervous system functions. This review assesses recent, primarily in vivo, evidence that progesterone can play an important role in promoting and enhancing repair after traumatic brain injury and stroke. Although many of its specific actions on neuroplasticity remain to be discovered, there is growing evidence that this hormone may be a safe and effective treatment for traumatic brain injury and other neural disorders in humans.

- Veiga S, Leonelli E, Beelke M, et al. Neuroactive steroids prevent peripheral myelin *alterations induced by diabetes. Neurosci Lett. 2006 Jul 10;402(1-2):150-3.*

  The effect of the neuroactive steroids progesterone, dihydroprogesterone and tetrahydroprogesterone on myelin abnormalities induced by diabetes was studied in the sciatic nerve of adult male rats treated with streptozotocin. Streptozotocin increased blood glucose levels and decreased body weight gain, parameters not affected by steroids. Streptozotocin increased the number of fibers with myelin infoldings in the axoplasm, 8 months after the treatment.. Chronic treatment for 1 month with progesterone and dihydroprogesterone resulted in a significant reduction in the number of fibers with myelin infoldings to control levels. Treatment with tetrahydroprogesterone did not significantly affect this myelin alteration. These results suggest that neuroactive steroids such as progesterone and dihydroprogesterone may represent therapeutic alternatives to counteract peripheral myelin alterations induced by diabetes.

Progesterone and the Uterus:

- Anasti JN, Leonetti HB, Wilson KJ. Topical progesterone cream has antiproliferative effect on estrogen-stimulated endometrium. *Obstet & Gynecol* 2001; 97(4 Suppl.):10S and *Fertil Steril* 2003;79(1):221-2.

  This randomized, controlled study involving 58 postmenopausal women demonstrated that topically applied progesterone cream (Pro-Gest®) had an antiproliferative effect in postmenopausal women who had been given oral estrogens x 14 days prior to progesterone treatment. Treatment with topical progesterone did not differ in effects from vaginally applied progesterone (Crinone®), and both progesterone applications demonstrated a significant effect over placebo. Patients preferred the topical application of progesterone cream.

- Casanas-Roux F, Nisolle M, Marbaix E, et al. Morphometric, immunohistological and three-dimensional evaluation of the endometrium of menopausal women treated by oestrogen and Crinone ® , a new slow-release vaginal progesterone. Human Reprod 1996;11:357-63.

Twenty estrogen-deprived women were given oral estrogen for 12 days followed by oral estrogen- vaginal progesterone gel for 12 days. Endometrial evaluation occurred before treatment, after the estrogen-only phase and after estrogen-progesterone gel treatment. Atrophy was present before treatment in all patients. Typical proliferative changes occurred after estrogen-only treatment, and secretory transformation occurred after estrogen-progesterone treatment, indicating that sustained-release progesterone gel can effectively counteract the proliferative effects of estrogen treatment in postmenopausal women.

- Cicinelli E, de Ziegler D, Galantino P, et al. Twice-weekly transdermal estradiol and vaginal progesterone as continuous combined hormone replacement therapy in postmenopausal women: a 1-year prospective study. *Am J Obstet Gynecol* 2002 Sep;187(3):556-60.

  In this study of 35 postmenopausal women, twice-weekly administration of a progesterone vaginal gel (45 mg P4/day) sufficiently protected the endometrium in women receiving transdermal estradiol (0.05 mg/d) as revealed by endometrial thickness and histology. The authors present vaginally applied progesterone as a viable option for hormone replacement therapy at menopause.

- Dai D, Wolf DM, Litman ES, White MJ, Leslie KK. Progesterone inhibits human endometrial cancer cell growth and invasiveness: down-regulation of cellular adhesion molecules through progesterone B receptors. *Cancer Res* 2002 Feb;62(3):881-6.

  This in vitro study demonstrated that progesterone acts through progesterone receptor B to inhibit endometrial cancer cell invasiveness via the down-regulation of adhesion molecules.

- Fanchin R, De Ziegler D, Bergeron C, et al. Transvaginal administration of progesterone. *Obstet Gynecol* 1997;90:396-401.

  Three different doses of transvaginal progesterone gel were administered to 40 estrogen-deprived women aged 25-41 years. Estradiol was administered orally for 28 days, with progesterone added vaginally on alternate days from days 15-27. Plasma gonadotropins, E1, E2 and progesterone were measured, and an endometrial biopsy was obtained to assess endometrial status, estrogen, and progesterone receptor determinations. Transvaginal progesterone induced normal secretory transformation despite low serum progesterone levels, suggesting a direct transit of progesterone into the uterus, or "first uterine pass effect."

- Hodges LC, Houston KD, Hunter DS, Fuchs-Young R, Zhang Z, Wineker RC, Walker CL. Transdominant suppression of estrogen receptor signaling by progesterone receptor ligands in uterine leiomyoma cells. *Mol Cell Endocrinol* 2002 Oct 31;196(1-2):11-20.

  Although estrogen is known to stimulate the growth of uterine fibroids, the effect of progesterone is unclear. The role of progesterone in the development of uterine fibroids (leiomyoma) is examined in this study in an in vivo/in vitro mouse model. Progestins and antiprogestins were utilized to investigate progesterone receptor (PR) signaling in a

leiomyoma cell line. Both progestins and antiprogestins inhibited estrogen-mediated growth. PR ligands were also shown to suppress estrogen receptor signaling and leiomyoma cell growth.

- de Lignieres B. Endometrial hyperplasia: risks, recognition and the search for a safe hormone replacement regimen. *J Reprod Med.* 1999;44:191-96.

  This article discusses the use of oral micronized progesterone with estrogen replacement rather than synthetic progestins. Conclusion is that 100 mg/day micronized progesterone for 25 days per month appears to be nearly ideal from the standpoint of endometrial safety, efficacy, and tolerability.

- Leonetti HB, Anasti JN, Landes J. Topical progesterone cream: an alternative progestin in hormone replacement therapy. *Obstet & Gynecol* 2003; 101(4 Suppl.):85.

  20 women completed a 1 year randomized, controlled, cross-over study comparing conjugated equine estrogen (Premarin® , 0.625 mg) paired with progesterone cream (Pro-Gest ® , 20 mg) vs. conjugated equine estrogen paired with medroxyprogesterone acetate (Prempro ®). Endometrial biopsies were performed at the end of each 6-month arm of the study. No hyperplasia was found in either group. Incidence of spotting was similar in both groups. Participants preferred the progesterone cream composition (76% vs 5%, p<0.001).

- Lindenfeld EA, Langer RD. Bleeding patterns of the hormone replacement therapies in the postmenopausal estrogen and progestin interventions trial. *Obstet Gynecol*. 2002;100(5 Pt1):853-63.

- Montz FJ, Bristow RE, Bovicelli A, Tomacruz R, Kurman RJ. Intrauterine progesterone treatment of early endometrial cancer. *Am J Obstet Gynecol* 2002 Apr;186(4):651-7.

  This study evaluated the use of a progestin-releasing IUD as a feasible treatment for early stage endometrial cancer (IA, grade 1). Twelve subjects were followed for 36 months. Results suggested IUD progestin appeared to resolve some cases of early endometrial cancer.

- Moyer DL, de Lignieres B, Driguez P, Pez JP. Prevention of endometrial hyperplasia by progesterone during long-term estradiol replacement: influence of bleeding pattern and secretory changes. *Fertil Steril* 1993 May;59(5):992-7.

  It is often presumed that progesterone levels must be high enough to induce endometrial bleeding by withdrawal in order to convey protection during estrogen replacement therapy. In this expanded observational study, the authors sought to determine the influence of withdrawal bleedings, secretory transformation, and reduction of mitosis on the prevention of endometrial hyperplasia during long-term estrogen-replacement therapy. Hysteroscopy and endometrial biopsies were utilized to establish maturation patterns, glandular epithelial mitosis rates, and macroscopic endometrial appearance. The results showed an increase in withdrawal bleeding with higher levels of progesterone, with those levels producing distinct secretory responses. However, incidence of endometrial hyperplasia after 5 yrs of E2/P therapy was independent of

secretory changes and withdrawal bleeding, and was more related to the control of mitosis, which was seen even with low doses of progesterone. The authors conclude that a relatively low dose of P may be offered to women seeking hormone replacement therapy with similar levels of endometrial safety.

- Stanczyk FZ, Paulson RJ, Roy S. Percutaneous administration of progesterone: blood levels and endometrial protection. *Menopause* 2005;12(2) 232-237.

  This article discusses the controversy about the use of topical progesterone cream and the assumption that serum progesterone levels achieved with progesterone creams are too low to have a secretory effect on the endometrium. Antiproliferative effects on the endometrium have been demonstrated with progesterone creams when circulating levels of progesterone are low. The article claims that effects of topical progesterone creams on the endometrium should not be based on serum progesterone levels, but on histological examination of the endometrium. Despite the low serum progesterone levels achieved with the creams, salivary progesterone levels are very high, indicating that progesterone levels in serum do not necessarily reflect those in tissues. The mechanism by which the serum progesterone levels remain low is not known. However, one explanation is that after absorption through the skin, the lipophilic ingredients of creams, including progesterone, may prefer saturating the fatty layer below the dermis. Because there appears to be rapid uptake and release of steroids by red blood cells passing through capillaries, these cells may play an important role in transporting progesterone to salivary glands and other tissues. In contrast to progesterone creams, progesterone gels are water-soluble and appear to enter the microcirculation rapidly, thus giving rise to elevated serum progesterone levels with progesterone doses comparable to those used in creams.

- Sager G, Orbo A, Jaeger R, Engstrom C. Non-genomic effects of progestins-inhibition of cell growth and increased intracellular levels of cyclic nucleotides. *J Steroid Biochem Mol Biol* 2003 Jan; 84(1):1-8.

  The anti-proliferative effects of three different progestins were compared using three human uterine cervix cell lines. In one cell line (C-41) devoid of progesterone receptors (PR) all progestogens studied inhibited growth in the following potency - progesterone (56%) > medroxyprogesterone (38%) > megestrol acetate (25%). Sensitivity demonstrated the same order, with progesterone being the most sensitive to inhibiting growth. This suggests there is a non-genomic action of progestogens that is anti-proliferative. The progestins studied also had anti-proliferative effects on the cell lines exhibiting PR.

- Whitehead MI, Fraser D, Schenkel L, et al. Transdermal administration of oestrogen/progestogen hormone replacement therapy. *Lancet* 1990;335:310-2.

  Sixteen estrogen-deficient women were evaluated on a course of transdermal estradiol and transdermal progestogen for five cycles. Regular withdrawal bleeding was noted in all but one patient. Fourteen endometrial biopsies were performed after the fifth cycle, with no evidence of endometrial hyperplasia.

Difference in Effects between Bioidentical Progesterone and Progestins:

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- Bagis T, Gokcel A, Zeyneloglu HB, Tarim E, Kilicdag EB, Haydardedeoglu B. The effects of short-term medroxyprogesterone acetate and micronized progesterone on glucose metabolism and lipid profiles in patients with polycystic ovary syndrome: a prospective randomized study. *J Clin Endocrinol Metab* 2002 Oct;87(10):4536-40.

  This randomized prospective study evaluated and compared the effects of ten days treatment with oral and vaginal progesterone (MP) and medroxyprogesterone acetate (MPA) on glucose metabolism, lipid profiles, and hormonal parameters in 28 patients with polycystic ovary syndrome (PCOS). Oral MPA and oral MP decreased LH (P = 0.028, P = 0.009, respectively) and total testosterone (P = 0.013, P = 0.037, respectively) levels. There was no change in hormonal parameters with vaginal MP. Basal insulin decreased (P = 0.021) and insulin sensitivity increased significantly in the oral MPA group. Low-density lipoprotein cholesterol (LDL) and lipoprotein (a) levels decreased only in the MPA group. This study concluded that MPA and oral MP may reduce insulin sensitivity in patients with PCOS. Vaginal MP had no effect on glucose metabolism and lipid profiles.

- Dalton K. The effects of progesterone and progestogens on the foetus. *Neuropharmacology* 1981; 20:1267-9.

  This article looks at the differing effects of progesterone and synthetic progestogens on the fetus. Of note in this article is evidence that progesterone supplementation may reduce episodes of pre-eclampsia. Synthetic progestogen supplementation during pregnancy may produce a variety of side effects. Several references are made to articles documenting cases of masculinization of external genitalia in female babies. There are two known cases of true hermaphroditism and several cases of behavioral problems developing in adolescent girls whose mothers took oral synthetic progestogens during pregnancy. More problematic may be administration of oral estrogen-progestogen preparations. Side effects may include spina bifida, esophageal anomalies, heart defects, and limb reduction deformities.

- de Lignieres B. Effects of progestogens on the postmenopausal breast. *Climacteric* 2002; 5(3):229-35.

  In this review, the author highlights the differences between progesterone and synthetic progestins in the breast and cautions that progestogens not be "all put in the same bag" with respect to safety. A strong case is made for the protective effect of progesterone on the breast.

- de Lignieres B. Oral micronized progesterone. *Clin Ther* 1999; 21(1):41-60.

  This review article examines the rationale for selecting oral micronized progesterone over synthetic progestins. It reviews research regarding efficacy and safety and concludes that oral micronized progesterone has fewer side effects than synthetic progestins and is a convenient way to deliver natural progesterone.

- Fitzpatrick LA, Pace C, Wiita B. Comparison of regimens containing oral micronized progesterone or medroxyprogesterone acetate on quality of life in postmenopausal women: a cross-sectional survey. *J Womens Health Gend Based Med* 2000

May;9(4):381-7.

A cross-sectional survey was conducted to examine quality of life (QOL) related to physiological, somatic, and vasomotor effects of switching progestogen treatment from medroxyprogesterone acetate (MPA) to micronized progesterone in postmenopausal women already using hormone replacement therapy (HRT). One hundred seventy-six women who were currently using hormone replacement therapy (HRT) containing micronized progesterone for 1-6 months and had previously received HRT containing MPA were surveyed to assess QOL. Women using micronized progesterone-containing HRT experienced significant improvement in vasomotor symptoms, anxiety, somatic complaints, and depressive symptoms. Women reported improved control of menopausal symptoms and perceptions of their vaginal bleeding patterns while on the micronized progesterone-containing regimen. Approximately 80% of women reported satisfaction with the progesterone-containing therapy. A micronized progesterone-containing HRT therapy offers the potential for improved QOL with respect to menopausal symptoms.

- Fournier A, et al. Unequal risks for breast cancer associated with different hormone replacement therapies: results from the E3N. *Breast Cancer Res Treat*. 2008;107:103-111.

- Greendale GA, Reboussin BA, Slone S, Wasilauskas C, Pike MC, Ursin G. Postmenopausal hormone therapy and change in mammographic density. *J Natl Cancer Inst* 2003 Jan 1;95(1):30-7.

  Breast cancer risk independently increases with mammographic density. Use of hormone replacement therapy (HRT) postmenopausally is associated with an increase in mammographic density, but the extent of the density increase is unknown. This study evaluated mammograms from 571 of the 875 women enrolled in the PEPI trial at baseline and after 12 months HRT. The women had been randomized to receive placebo, conjugated equine estrogens (CEE) + medroxyprogesterone acetate (MPA) in a continuous or cyclic fashion, or CEE + micronized progesterone (MP). Mammograms were analyzed digitally and a linear regression analysis was utilized to quantify breast density change in all four treatment arms. The adjusted absolute mean changes in mammographic percent density over 12 months were 4.76% (95% confidence interval [CI] = 3.29% to 6.23%), 4.58% (95% CI = 3.19% to 5.97%), and 3.08% (95% CI = 1.65% to 4.51%) for women in the CEE+MPA-cyclic, CEE+MPA-continuous, and CEE-MP groups, respectively. Each of those absolute mean changes was statistically significantly different from the adjusted absolute mean change in mammographic percent density for women in the placebo group, which was -0.07% (95% CI = -1.50% to 1.38%). Greater mammographic density was associated with the use of estrogen/progestin combination therapy, although the micronized progesterone containing arm appeared to induce less of an increase that that with MPA.

- Lobo RA. Progestogen metabolism. *J Reprod Med* 1999 Feb;44(2 Suppl):148-52.

  This review clearly elucidates what is known about the differences in metabolism of various progestins as compared with endogenous or natural progesterone. Not only are there different pathways for metabolism, but the route of administration also has a

significant effect. The physiologic and pathologic state of the patient further influences the metabolism, and there are measurable variations between patients. The authors also review the differences expressed by various tissues in metabolizing progestogens as well as the different biologic potencies of the various progestogens. Most importantly, the authors state the lack of knowledge about the synthetic progestins as compared to natural progesterone, which has a much better understood effect in the body.

- Martorano JT, Ahlgrimm M, Meyers D. Differentiating between natural progesterone and synthetic progestogens: clinical implications for PMS management. *Comprehensive Therapy* 1993; 19(3):96-8.

  Clinical observations demonstrate that patients suffering from PMS respond to treatment with natural progesterone, whereas synthetic progestins may exacerbate the condition. The authors review the differences between natural progesterone and synthetic progestins.

- Miyagawa K, Rosch J, Stanczyk F, and Hermsmeyer K. Medroxyprogesterone interferes with ovarian steroid protection against coronary vasospasm. *Nature Medicine* 1997;3(3): 324-327.

  Ovariectomized rhesus monkeys were treated with physiological levels of 17-beta estradiol in combination with either medroxyprogesterone or progesterone (oral micronized) for four weeks. Following pathophysiological stimulation without injury to induce coronary vasospasm, it was shown that progesterone plus estradiol was protective against vasospasm, whereas estradiol plus medroxyprogesterone allowed vasospasm, concluding that medroxyprogesterone increases risk of coronary vasospasm, while progesterone does not.

- Montplaisir J, Lorrain J, Denesle R, Petit D. Sleep in menopause: differential effects of two forms of hormone replacement therapy. *Menopause* 2001; 8(1):10-16.

  This randomized clinical trial compared the effects of conjugated equine estrogen (CEE) and medroxyprogesterone acetate to CEE and oral micronized progesterone. Twenty-one postmenopausal women were studied in a sleep lab, with results demonstrating an improvement in subjective measures of menopausal symptoms and sleep in both groups. The group receiving natural progesterone had significantly improved sleep efficiency, whereas the medroxyprogesterone acetate group did not, suggesting that the former might better improve sleep in postmenopausal women.

- Ojasoo T. Multivariate preclinical evaluation of progestins. *Menopause* 1995; 2(2): 97-107.

  Specificity profiles of numerous progestins were evaluated by multivariate analysis. Twenty steroid hormones, including natural progesterone, were tested for anti-estrogenic activity and for binding to the androgen, progesterone, and glucocorticoid receptors.

- Otsuki M, Saito H, Xu X, Sumitani S, Kouhara H, Kishimoto T, Kasayama S. Progesterone, but not medroxyprogesterone, inhibits vascular cell adhesion molecule-1 expression in human vascular endothelial cells. *Arterioscler Thromb Vasc Biol* 2001

Feb;21(2):243-8.

This study utilizing human umbilical vein endothelial cells (HUVEC's) demonstrated that progesterone, but not medroxyprogesterone acetate (MPA) inhibited expression of vascular cell adhesion molecule-1 (VCAM-1), demonstrating a role for progesterone in the prevention of atherosclerosis. The differing effects of progesterone and MPA are clinically important, as MPA is widely used in hormone replacement therapy, when, as this research suggests, progesterone might be a more appropriate option.

- Ottosson UB, Johansson BG, et al. Subfractions of high-density lipoprotein cholesterol during estrogen replacement therapy: A comparison between progestogens and natural progesterone. *Am J Obstetrics and Gynecol* 1993 Mar;151(6): 746-50.

  Fifty-eight postmenopausal women were followed with respect to subfractions of high-density lipoprotein during 3 cycles of unopposed estrogen. The women received either levonorgestrel, medroxyprogesterone acetate, or natural progesterone during the last ten days of the treatment period. Both progestogens significantly lowered HDL cholesterol, whereas natural progesterone had no effect on HDL levels.

- Osano GM, Webb CM, Chierchia S, Morgani GL, Gabraele M, Sarrel PM, de Ziegler D, Collins P. Natural progesterone, but not medroxyprogesterone acetate, enhances the beneficial effect of estrogen on exercise-induced myocardial ischemia in postmenopausal women. *J Am Coll Cardiol* 2000 Dec;36(7):2154-9

  Eighteen postmenopausal women were randomized to receive 17-beta estradiol with a synthetic progestin (medroxyprogesterone acetate) or a progesterone vaginal gel for 4 weeks, then crossed over to the alternate treatment. Researchers found through treadmill testing that estrogen plus progesterone significantly increased exercise time before myocardial ischemia, when compared to estradiol plus synthetic progestin. In addition, 2 patients on the synthetic progestin arm had to discontinue due to unstable angina. This research suggests that women at risk for cardiovascular disease need to consider progesterone as a safer alternative to synthetic progestins as a part of their hormone replacement therapy regime.

- Ryan N, Rosner A. Quality of life (QOL) and costs associated with micronized progesterone and medroxyprogesterone acetate in hormone replacement therapy for non-hysterectomized, postmenopausal women. *Clin Ther* 2001 Jul;23(7):1099-115.

  This prospective, multicenter, randomized, parallel-group study enrolled 182 postmenopausal women 45 to 65 years of age and evaluated the quality of life and menopausal symptoms associated with the use of medroxyprogesterone acetate vs oral micronized progesterone when used as a part of a regular hormone replacement therapy. Menopausal symptoms improved in both groups from baseline to 9 months, as did QOL measures. In addition, patients using micronized progesterone had specific improvements in the areas of cognition and menstrual problems whereas the patients using MPA did not. Micronized progesterone was seen as an effective, cost-comparable alternative to MPA as well as being better tolerated.

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- Saarikoski S, Yliskoski M, Penttila I. Sequential use of norethisterone and natural progesterone in pre-menopausal bleeding disorders. *Maturitas* 1990 Jun;12(2):89-97.

  This randomized controlled study evaluated the effects of norethisterone (NET) and micronized progesterone (MP) on bleeding disorders in pre-menopausal women. 80 patients were randomized to the trial and all were found via endometrial morphology to need progestogen therapy. They were subsequently treated with NET or MP. In both treatment groups, hyperplastic changes disappeared during the first three cycles, with the duration of treatment being 6 months. NET decreased follicle-stimulating hormone, luteinizing hormone, estradiol and sex-hormone-binding globulin levels (P < 0.001) whereas no changes were seen during MP treatment. High-density-lipoprotein cholesterol and triglyceride levels were also lowered by NET (P< 0.001-0.02) slightly decreased phospholipids. MP treatment had no effect on lipid profiles suggesting it may be a preferred progestogen for the treatment of bleeding disorders.

- Sager G, Orbo A, Jaeger R, Engstrom C. Non-genomic effects of progestins-inhibition of cell growth and increased intracellular levels of cyclic nucleotides. *J Steroid Biochem Mol Biol* 2003 Jan;84(1):1-8.

  The anti-proliferative effects of three different progestins were compared using 3 human uterine cervix cell lines. In one cell line (C-41) devoid of progesterone receptors (PR) all progestogens studied inhibited growth in the following potency - progesterone (56%) > medroxyprogesterone (38%) > megestrol acetate (25%). Sensitivity demonstrated the same order, with progesterone being the most sensitive to inhibiting growth. This suggests there is a non-genomic action of progestogens that is anti-proliferative. The progestins studied also had anti-proliferative effects on the cell lines exhibiting PR.

- Sitruk-Ware R. Progestins and cardiovascular risk markers. *Steroids* 2000 Oct-Nov;65(10-11):651-8.

  This article reviews the effects of various synthetic progestins and progesterone on cardiovascular health. Many synthetic progestins, especially 19-nortestosterone and some 17-hyroxyprogesterones, have negative effects on cardiovascular risk factors, whereas natural progesterone does not. Further studies utilizing natural and other steroids should be considered. Sitruk-Ware R. Progestogens in hormonal replacement therapy: new molecules, risks, and benefits. Menopause 2002;9(1):6-15. The classifications of various progestogens (natural and synthetic) are reviewed in terms of their risks and benefits. This review clearly elucidates the differences in the mode of action of various synthetic progestins as well as progesterone.

Androgens (DHEA and Testosterone) in Women—General

- Arlt W, Justl H, Callies F, et al. Oral dehydroepiandrosterone for adrenal androgen replacement: pharmacokinetics and peripheral conversion to androgens and estrogens in young healthy females after dexamethasone suppression. *J Clin Endocrinol Metab* 1998*;* 83: 1928–1934.

- Arlt W, Callies F, van Vlijmen JC, et al. Dehydroepiandrosterone replacement in women with adrenal insufficiency. *N Engl J Med* 1999 Sep 30;341(14):1013-20.

In this double-blind study, 24 women with adrenal insufficiency received 50 mg of DHEA. Results showed that DHEA raised the initially low serum levels of DHEA, DHEA-S, and testosterone into the normal ranges; serum concentrations of sex hormone-binding globulin, total cholesterol, and HDL cholesterol decreased significantly. DHEA significantly improved overall well-being as well as scores for depression and anxiety. As compared with placebo, DHEA significantly increased the frequency of sexual thoughts, interest, and satisfaction.

- Braunstein G. Androgen insufficiency in women. *Growth Horm IGF Res*. 2006;16 Suppl A:S109-17.

  Female androgen insufficiency syndrome includes low libido, persistant, unexplained fatigue, and decreased sense of well-being. Oral, but not parenteral or transdermal, testosterone may decrease HDL.

- Braunstein G, Cameron D. Postmenopausal androgen therapy. *The Female Patient*. 2004.29:40-45.

- Casson PR, Andersen RN, Herrod HG, et al. Oral dehydroepiandrosterone in physiologic doses modulates immune function in postmenopausal women. *Am J Obstet Gyneco l*1993 Dec;169(6):1536-9.

  This prospective, randomized, double-blind, crossover study of 11 subjects evaluated the immune impact of oral DHEA in postmenopausal women. The control group showed marked increase in natural killer cell activity and suppressed increased IL-6 production seen in the placebo group (IL-6 in vitro has been shown to be an important bone resorber). Authors concluded DHEA may have immune modulatory functions in older postmenopausal women and may additionally have an antioncogenic effect.

- Cardozo L, Gibb D, Tuck S, et al. The effects of subcutaneous hormone implants during the climacteric. *Maturitas* 1984;5:177-184.

  This study included 120 women with a total of 469 hormonal implants of 50-mg estradiol and 100-mg testosterone implants over four years. Patients with a uterus were given an oral progestogen. Hot flushes were improved in 100%; depression in 99%; and loss of libido in 92%.

- Chu M, Lobo R. Formulations and use of androgens in women. *Mayo Clinic Proc* April 2004(79)Suppl.

- Davis SR. The therapeutic use of androgens in women. *J Steroid Biochem Mol Biol*. 1999 Apr-Jun;69(1-6):177-84.

- Davis S, Burger H. Androgens and the postmenopausal woman. *J Clin Endocrin Metab* 1996;81(8):2759-2763.

  This paper is an excellent review of androgens in postmenopausal women. It discusses the role of androgens in women, and the decline of ovarian and adrenal androgens and

pre-androgens that can precede menopause by a decade. It also discusses the potential significant impact this decline can have on women's health. The authors conclude that side effects for androgen replacement (including testosterone subcutaneous implants) in symptomatic women are rare if patients are properly monitored.

- Gambrell RD, Natrajan PK. Moderate dosage estrogen-androgen therapy improves continuation rates in postmenopausal women: impact of the WHI reports. *Climacteric* 2006;9:224-233.

  According to the authors, the purpose of this paper was not to provide descriptive data for practice recommendations but to point the way to more liberal thinking than the conservatism of today. The patients in this historical practice were audited to determine the reasons for continuing hormone replacement. During the 3 years from 1996 to 1999, 814 women were followed prospectively. The records of the patients were reviewed in January 2005 to determine the impact of the Women's Health Initiative (WHI). Of the 814 patients, there were 573 surgically menopausal women with a mean age of 61.8+/-3.25 years and 241 naturally menopausal women with a mean age of 58.6+/-3.08 years. During the 3 years of observation, 692 women continued HRT while 122 discontinued their therapy. Of those continuing therapy, 606 were treated with the implantation of various combinations of estradiol and testosterone pellets, while 86 used injectables, patches or oral hormones. Continuation rates for pellet patients were 96.7% for 10 years, 88.8% for 20 years, and 21.9% for 40 or more years. Continuation rates for the other hormone users were 53.5% for 10 years and 20.9% for 20 years. Continuation rates in the 692 remaining patients declined to 66.7% during the next 5 years. The authors concluded that moderate dosages of estrogens, with androgens added when indicated, improve ontinuation rates.

- Glaser R, York AE, Dimitrakakis C. Beneficial effects of testosterone therapy in women measured by the validated Menopause Rating Scale (MRS). Maturitas. 2011;68(4):355-61.

  This study was designed to measure the beneficial effects of continuous testosterone therapy, delivered by subcutaneous implant, in the relief of somatic, psychological and urogenital symptoms in both pre-and post-menopausal patients, utilizing the validated Health Related Quality of Life (HRQOL), Menopause Rating Scale (MRS). 300 pre-and post-menopausal women took the MRS questionnaire at baseline and 3 months afert their first subcutaneous testosterone pellet implant. Both pre-menopausal and post-menopausal females reported similar hormone deficiency symptoms. Both groups demonstrated similar improvement in total score, as well as psychological, somatic and urogenital subscale scores with testosterone therapy. Better effect was noted in women with more severe complaints. Higher doses of testosterone correlated with greater improvement in symptoms.

- Glaser R, Kalantaridou S, Dimitrakakis C. Testosterone implants in women: pharmacological dosing for physiologic effect. *Maturitas*. 2013;74:179-84.

  The objectives of this study were to determine therapeutic serum testosterone (T) levels/

ranges and inter-individual variance in women treated with subcutaneous T implants. Study design: In study group 1, T levels were measured at two separate time intervals in pre- and postmenopausal women treated with subcutaneous T for symptoms of androgen deficiency: (i) four weeks after pellet insertion, and (ii) when symptoms of androgen deficiency returned. In a separate pharmacokinetic study (study group 2), 12 previously untreated postmenopausal women each received a 100 mg T implant. Serum T levels were measured at baseline, 4 weeks and 16 weeks following T pellet implantation. In study 'group' 3, serial T levels were measured throughout a 26 h period in a treated patient. Results: In study group 1, serum T levels measured at 'week 4' (299.36 ± 107.34 ng/dl, n = 154), and when symptoms returned (171.43 ± 73.01 ng/dl, n = 261), were several-fold higher compared to levels of endogenous T. There was significant inter-individual variance in T levels at 'week 4' (CV 35.9%) and when symptoms returned (CV 42.6%). Even with identical dosing (study group 2), there was significant inter-individual variance in T levels at 'week 4' (CV 41.9%) and 'week 16' (CV 41.6%). In addition, there was significant intra-individual circadian variation (CV 25%). Conclusions: Pharmacologic dosing of subcutaneous T, as evidenced by serum levels on therapy, is needed to produce a physiologic effect in female patients. Safety, tolerability and clinical response should guide therapy rather than a single T measurement, which is extremely variable and inherently unreliable.

- Jakiel G, Baran A. [Androgen deficiency in women] Article in Polish. Endokrynol Pol. 2005;56(6):1016-20.

  Androgens are defined as the steroids having a binding affinity of the androgen receptor. In the reproduction age a daily production of testosterone is equally divided between the ovaries and adrenal and local tissue conversion of androstenedione and DHEA. After menopause the 80% of testosterone is produced in ovaries, but majority of precursors for peripheral conversion is adrenal origin. Androgen receptors are present throughout in the body; over 200 cellular actions of androgens have been described. Androgenic action is determined by quantitative level of the androgen present in the circulation, its degree of binding to proteins, the degree of interconversion to other androgens and estrogens, and the biological potency and androgen receptor binding affinity of the androgen. The most common clinical symptoms of androgen deficiency are the reduction of sex motivation, sex fantasy, sex enjoyment, sex arousal, vaginal vasocongestion, but also reduction of pubic hair, bone mass, muscle mass, worsening of quality of life (mood, affect, energy), more frequent vasomotors symptoms, insomnia, depression, headache. All these signs and symptoms can be multifactorial. Most common conditions associated with hypoandrogenism in women are hypothalamic-pituitary abnormalities, lack or insufficiency of ovaries, adrenal insufficiency, glucocorticoid therapy, exogenous estrogen administration. Besides the clinical picture the free testosterone measuring is important for diagnosis. The method of choice of this measure is equilibrium dialysis assay. Despite of clinical importance of androgen insufficiency in women, none of methods of androgen substitution is approved by FDA.

- Kapetanakis E, Dmowski W, Auletta F, et al. Endocrine and clinical effects of estradiol and testosterone pellets used in long-term replacement therapy. *Int J Gynaecol Obstet* 1982;20:387-99.

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Ten menopausal women with estrogen deficiency symptoms received subcutaneous implants consisting of 25-75 mg estradiol (E2) with or without 75 mg testosterone (T). All had elevated plasma FSH, and LH, and low E2 prior to treatment. Plasma levels of FSH, LH, E2, T and estrone (E1) were measured three times a week for the first week and once a week for up to 76 weeks after implantation. Mean plasma E2 levels rose abruptly and reached a maximum of 190 +/- 35 pg/ml within 2 weeks. They fluctuated around 150 pg/ml for 46 weeks, then gradually declined, but remained above pretreatment values for more than 68 weeks. Plasma E1 increased to a lesser extent resulting in E2:E1 ratio between 1 and 5. Elevated FSH and LH titers became suppressed within 4--6 weeks. Plasma T rose abruptly to a peak mean level of 2.5 +/- 1.6 ng/ml within 2 weeks of implantation. A precipitous and steady decline with return to preimplantation titers between 17th and 18th week were then observed. The E2:E1 ratio during the first 18 weeks after implantation was significantly higher in women who received E2 implant alone than in those who received E2 + T implant. Clinically, all patients had symptomatic improvement within 24-48 hours. Regular withdrawal bleeding followed administration of oral progestogen for up to 76 weeks after implantation in six patients with intact uterus.

- Lasco et al. Metabolic effects of dehydroepiandrosterone replacement therapy in postmenopausal women. *Eur J Endocrinol* 2001;145:457-461.

- Lobo RA. Androgens in postmenopausal women: production, possible role, and replacement options. *Obstet Gynecol Surv.* 2001 Jun;56(6):361-76.

- Martin-Du Pan R. [Androgen deficiency in women: indication and risks of testosterone or DHEA treatment]. Article in French. Rev Med Suisse. 2007;28;3(108):792-6.

  Androgen deficiency syndrome is defined by impaired well being and libido in women with adequate estrogenization and low levels of total serum testosterone (T). The causes of low T levels are discussed. Seven placebo controlled studies have shown that percutaneous administration of T is able to increase sexual activity and libido in ovariectomized women (5 studies), after hypophysectomy and in natural menopause. DHEA (50 mg/d) is beneficial only in women with Addison disease or after hypophysectomy but not in natural adrenopause. Other beneficial and side effects of androgenotherapy are discussed in particular the breast cancer risk.

- Miller KK. Androgen deficiency in women. *J Clin Endocrinol Metab* 2001 Jun;86(6):2395-401.

  Physiological and pathological processes as well as iatrogenic interventions may result in androgen deficiency compared with levels in young healthy women. Whether relative androgen deficiency results in a clinical syndrome similar to that reported in men, including osteopenia, increased fat mass, decreased libido, and diminished quality of life, has not been definitively established. However, preliminary data in postmenopausal women suggest that physiological androgen replacement therapy, which involves substantially lower doses than those used in men, may result in increased bone mineral density, increased libido, and improved quality of life. The safety of androgen preparations that result in supraphysiological levels has not been established in women and would be expected to result in hirsutism, acne, and virilization with chronic use.

Androgen preparations that avoid liver metabolism and result in physiological serum androgen levels in women with androgen deficiency are not currently available, but are in development. Therefore, although widespread screening and hormone replacement for androgen deficiency cannot be recommended yet, increasing interest in this topic makes consideration of the available data important.

- Rivera-Woll LM, Papalia M, Davis SR, et al. Androgen insufficiency in women: diagnostic and therapeutic implications. *Human Reproduction Update* 2004;10(5):421–432.

- Female androgen insufficiency: the Princeton consensus statement on definition, classification, and assessment. *Fertility & Sterility* 2002;77(4): 660-5.

  Conclusion: A new definition of androgen insufficiency in women has been proposed along with consensus-based guidelines for clinical assessment and diagnosis. A simplified management algorithm for women with low androgen in the presence of clinical symptoms and normal estrogen status has also been proposed.

- Schneider HP. Androgens and antiandrogens. *Ann N Y Acad Sci*. 2003 Nov;997:292-306.

- Simon JA. Safety of estrogen/androgen regimens. *J Reprod Med* 2001 Mar;46(3 Suppl):281-90.

- Tagawa N, Tamanaka J, Fujinami A, et al. Serum dehydroepiandrosterone, dehydroepiandrosterone sulfate, and pregnenolone sulfate concentrations in patients with hyperthyroidism and hypothyroidism. *Clinical Chemistry* 2000;46(4):523-28.

  In a comparative study of 46 individuals with untreated thyroid disorders to 43 healthy controls, results showed a significant increase in serum DHEA-S but no change in DHEA for those with hyperthyroidism. In hypothyroidism, both DHEA and DHEA-S were significantly decreased. The serum PREG-S was increased in hyperthyroidism and decreased in hypothyroidism. Serum albumin was decreased in hyperthyroidism and serum SHBG was increased in hyperthyroidism.

- Zhu YS et al. Natural potent androgens: lessons from human genetic models. *Baillieres Clin Endocrinol Metab.* 1998 Apr;12(1):83-113.

- Yes SS, Morales AJ, Khorram O. Replacement of DHEA in aging men and women. Potential remedial effects. *Ann NY Acad Sci*. 1995;774:128-42.

- Zumoff B, Strain G, Miller L, et al. Twenty-four-hour mean plasma testosterone concentration declines with age in normal premenopausal women. *J Clin Endocrinol Metab* 1995; 80:1429-1430.

  This study looked at testosterone levels of 33, healthy, non-obese women ages 21 to 51 yrs. Women in their 40s had 50% less testosterone levels that women in their early 20s.

Androgens & the Brain, Nervous System, Mood

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- Barrett-Connor E, von Műhlen D, Laughlin GA, Kripke A. Endogenous levels of dehydroepiandrosterone sulfate, but not other sex hormones, are associated with depressed mood in older women: the Rancho Bernardo Study*. J Am Geriatr Soc*. 1999;47(6):685-91.

  The purpose of this study was to determine whether endogenous steroid hormone levels are associated with depressed mood in community-dwelling older women. A total of 699 non-estrogen using, community-dwelling, postmenopausal women (aged 50 to 90 years) from the Rancho Bernardo cohort who were screened for depressed mood and had plasma obtained for steroid hormone assays in 1984-1987. Plasma levels of total and bioavailable (non-SHBG-bound) estradiol and testosterone, estrone, androstenedione, cortisol, dehydroepiandrosterone, and (DHEA) and its sulfate (DHEAS) were measured by radioimmunoassay. Mood and depression were assessed using the Beck Depression Inventory. Only DHEAS levels were significantly and inversely associated with depressed mood, and the association was independent of age, physical activity, and weight change. Age, sedentary lifestyle, and weight loss were positively associated with depressed mood. Alcohol intake, cigarette smoking, marital status, type of menopause, and season of testing were unassociated with depressed mood. A subset of 31 women with categorically defined depression had lower DHEAS levels compared with 93 age-matched nondepressed women.

- Cardounel A, Regelson W, Kalimi M. Dehydroepiandrosterone protects hippocampal neurons against neurotoxin-induced cell death: mechanism of action. *Proc Soc Exp Biol Med* 1999, 222:145-149.

- Montgomery J, Brincat M, Tapp A, et al. Effect of oestrogen and testosterone implants on psychological disorders in the climacteric. *The Lancet* 1987:297-299.

  Double-blind, placebo-controlled trial assessing psychological symptoms involving 3 treatment groups of peri and postmenopausal women (N=70): estradiol and testosterone implants, estradiol implant only, or placebo. Depression and anxiety were significantly lower in the pellet treated groups.

- Scott LV, Salahuddin F, Cooney J, Svec F, Dinan TG. Differences in adrenal steroid profile in chronic fatigue syndrome, in depression and in health. *J Affect Disord* 1999 Jul;54(1-2):129-37.

- Sherwin B, Gelfand M. Transactions of the fortieth annual meeting of the society of obstetricians gynaecologists of Canada: Differential symptom response to parenteral estrogen and/or androgen administration in the surgical menopause. *Am J Obstet & Gynecol.* 1985 151:153-60.

  In this study, 41 women who underwent complete hysterectomies for benign conditions were randomized to receive estrogen alone, estrogen plus testosterone, or placebo. Women who received combined estrogen-androgen or androgen alone had higher energy levels, sense, or well-being, fewer psychological symptoms, and increased appetite.

- Wiebke, A, Callies, F, Van Vlijmen, et al. Dehydroepiandrosterone replacement in women with adrenal insufficiency. *New Engl J Med.* 1999;341(14):1013-19.

  This double-blind crossover study reviewed alternately the effects of 50mg of oral dehydroepiandrosterone (DHEA) daily with placebo in 24 women with adrenal insufficiency. Participants were evaluated using established well-being (depression and anxiety scores) and sexuality (thoughts, interest, satisfaction) scales and serum profiles. Results showed that serum DHEA, DHEA-S and active androgen increased to normal or low-normal levels during treatment. SHBG levels were significantly lower following treatment. IGF-I concentrations increased after treatment (only in women with primary adrenal insufficiency), but IGF-binding protein 3 levels did not change. Serum total and HDL lipoprotein cholesterol levels decreased significantly during treatment. LDL and triglyceride concentrations did not change significantly. Psychological testing scores for well-being and sexuality both improved significantly during treatment. These effects were noticed after treatment for four months, but not after treatment for one month. Authors recommended that treatment with DHEA should be part of hormone replacement therapy for women with adrenal insufficiency.

- Wolkowitz OM, Reus VI, Keebler A, et al. Double-blind treatment of major depression with dehydroepiandrosterone. *Am J Psychiatry* 1999, 156:646-649.

  Twenty-two patients with major depression, either medication-free or on stabilized antidepressant regimens, received either DHEA (maximum dose = 90 mg/day) or placebo for 6 weeks in a double-blind manner and were rated at baseline and at the end of the 6 weeks with the Hamilton Depression Rating Scale. Patients previously stabilized with antidepressants had the study medication added to that regimen; others received DHEA or placebo alone. DHEA was associated with a significantly greater decrease in Hamilton depression scale ratings than was placebo. Five of the 11 patients treated with DHEA, compared with none of the 11 given placebo, showed a 50% decrease or greater in depressive symptoms. These results suggest that DHEA treatment may have significant antidepressant effects in some patients with major depression.

Androgens & Breast

- Barrett-Connor E, Friedlander NJ, Khaw KT. Dehydroepiandrosterone sulfate and breast cancer risk. Cancer Res. 1990;50(20):6571-4.

  It has been suggested that dehydroepiandrosterone (DHEA) and its sulfate ester, dehydroepiandrosterone sulfate (DHEAS), have a protective effect against breast cancer. In this investigation, DHEAS levels were measured in plasma obtained and frozen in 1972-1974 from 534 women aged 50-79 yr. This group, which had been followed for 15 yr, included 21 incident cases of breast cancer, 20 cases with earlier diagnosis, and ten cases with unknown date of onset who were identified from death certificates only. Two sets of analyses were done: one using all women and one which excluded women using estrogen. No significant differences in age-adjusted DHEAS levels were found between any case type and noncases. Age-adjusted rates of breast cancer by DHEAS tertile also showed no significant trends or differences among tertiles for any case type. A multivariate model in which the DHEAS level was adjusted for age, body mass index, estrogen use, and cigarette smoking status also showed no significant

association between DHEAS and risk of breast cancer. These results do not support a protective role for plasma DHEAS in breast cancer risk in postmenopausal women.

- Dimitrakakis C, Jones R, Liu A, et al. Breast cancer incidence in postmenopausal women using testosterone in addition to usual hormone therapy. *Menopause* 2004; 11(5):531-5.

  This study looked at 508 patients who received 50 to 150 mg testosterone implants (dosage titrated to relieve symptoms and improve bone density and to minimize adverse effects – mean dosage 100-mg) in additional to usual hormone replacement in Australia. Average age at start of study was 56.4 years, and mean duration of follow-up was 5.8 years. Breast cancer incidence in testosterone users was close to that reported for hormone therapy never-users, suggesting that the addition of testosterone to conventional hormone therapy for postmenopausal women does not increase the risk of breast cancer. Because users of HRT are expected to have an increased risk, testosterone supplementation may reduce hormone therapy-associated breast cancer risk.

- Loeser A. Mammary carcinoma response to implantation of male hormone and progesterone. *The Lancet* 1941:698-700.

- Martin-Du Pan R. [Androgen deficiency in women: indication and risks of testosterone or DHEA treatment]. Article in French. Rev Med Suisse. 2007;28;3(108):792-6.

  Androgen deficiency syndrome is defined by impaired well being and libido in women with adequate estrogenization and low levels of total serum testosterone (T). The causes of low T levels are discussed. Seven placebo controlled studies have shown that percutaneous administration of T is able to increase sexual activity and libido in ovariectomized women (5 studies), after hypophysectomy and in natural menopause. DHEA (50 mg/d) is beneficial only in women with Addison disease or after hypophysectomy but not in natural adrenopause. Other beneficial and side effects of androgenotherapy are discussed in particular the breast cancer risk.

- Shufelt CL, Braunstein GD. Testosterone and the breast. *Menopause Int*. 28;14(3):117-22.

  Although women have been treated with testosterone (T) for female sexual dysfunction since the 1950s, the role of T in normal female physiology is not yet fully defined. One of the major safety concerns of androgen therapy is whether androgens have a stimulatory effect on the breast that could lead to breast carcinomas. The proposed mechanisms for such stimulation include local estrogen production from the aromatase enzyme complex present in the breast tissue or by the direct stimulation of the androgen receptor. Predominant data from in vitro studies have shown that androgens actually have apoptotic and antiproliferative effects and not stimulatory effects. Animal models have shown similar results to in vitro studies, finding that androgens inhibit breast cancer growth. Prospective and retrospective epidemiological analyses have shown mixed outcomes, with no clear consensus regarding androgen use and breast cancer risk. Hyperandrogenism in patients with polycystic ovarian syndrome with elevated levels of endogenous T is not associated with an increased risk of breast cancer and may, in fact,

be protective. Another human model with excess of T is female-to-male transgenderism, in which genotypic women are treated with large doses of exogenous T with no increased risk. High-dose androgen therapy also has been effective in treating patients with advanced breast cancer. Thus, the preponderance of data suggests that T use in females is not associated with an increased risk of breast carcinoma.

- Secreto G, Zumoff B. Abnormal production of androgens in women with breast cancer. *Anticancer Res* 1994 Sep-Oct;14(5B):2113-7.

- Somboonporn W, Davis S. Testosterone effects on the breast: implications for testosterone therapy for women. *Endocr Rev.* 2004 Jun;25(3):374-88.

  Androgens have important physiological effects in women. Postmenopausal androgen replacement, most commonly as testosterone therapy, is becoming increasingly widespread. This is despite the lack of clear guidelines regarding the diagnosis of androgen insufficiency, optimal therapeutic doses, and long-term safety data. With respect to the breast specifically, there is the potential for exogenous testosterone to exert either androgenic or indirect estrogenic actions, with the latter potentially increasing breast cancer risk. In experimental studies, androgens exhibit growth-inhibitory and apoptotic effects in some, but not all, breast cancer cell lines. Differing effects between cell lines appear to be due primarily to variations in concentrations of specific coregulatory proteins at the receptor level. In rodent breast cancer models, androgen action is antiproliferative and proapoptotic, and is mediated via the androgen receptor, despite the potential for testosterone and dehydroepiandrosterone to be aromatized to estrogen. The results from studies in rhesus monkeys suggest that testosterone may serve as a natural endogenous protector of the breast and limit mitogenic and cancer-promoting effects of estrogen on mammary epithelium. Epidemiological studies have significant methodological limitations and provide inconclusive results. The strongest data for exogenous testosterone therapy comes from primate studies. Based on such simulations, inclusion of testosterone in postmenopausal estrogen-progestin regimens has the potential to ameliorate the stimulating effects of combined estrogen-progestin on the breast. Research addressing this is warranted; however, the number of women that would be required for an adequately powered randomized controlled trial renders such a study unlikely.

- Somboonporn W, Davis S. Postmenopausal testosterone therapy and breast cancer risk. *Maturitas* 2004;49:267-275.

  This paper evaluated experimental and epidemiological studies pertaining to the role of testosterone in breast cancer. Main outcome measured were mammary epithelial proliferation, apoptosis and breast cancer. Results: In experimental studies, testosterone action is anti-proliferative and pro-apoptotic, and mediated via the AR, despite the potential for testosterone to be aromatized to estrogen. Animal studies suggest that testosterone may serve as a natural, endogenous protector of the breast and limit mitogenic and cancer promoting effects of estrogen on mammary epithelium. In premenopausal women, elevated testosterone is not associated with greater breast cancer risk. The risk of breast cancer is also not increased in women with polycystic ovary syndrome who have chronic estrogen exposure and androgen excess. However,

in postmenopausal women, who are oestrogen deplete and have increased adipose aromatase activity, higher testosterone has been associated with greater breast cancer risk. Conclusion: Available data indicate the inclusion of testosterone in estrogen–progestin regimens has the potential to ameliorate the stimulating effects of hormones on the breast. However, testosterone therapy alone cannot be recommended for estrogen deplete women because of the potential risk of enhanced aromatisation to estrogen in this setting.

- Stoll BA. Dietary supplements of dehydroepiandrosterone in relation to breast cancer risk. *Eur J Clin Nutr* 1999 Oct;53(10):771-5.

  This review examines reports of clinical, epidemiological experimental studies for evidence that DHEA may be a factor in promoting the growth of mammary cancer. Biological mechanisms which might be involved are identified. DHEA is reported to inhibit the growth of human mammary cancer cells in vitro and also the growth of chemically-induced mammary cancer in rats. However, growth inhibition occurs only in the presence of high oestrogen concentrations, and growth stimulation occurs in both models in the presence of a low-level oestrogen milieu. Epidemiological studies report a positive correlation between higher serum concentrations of DHEA and increased breast cancer risk in the case of postmenopausal but not premenopausal women.

- Traish A, Fetten K, Miner M, et al. Testosterone and risk of breast cancer: appraisal of existing evidence. *Horm Mol Biol Clin Invest* 2010;2(1):177-190.

  This paper examines the data from preclinical, clinical, and epidemiological studies to evaluate if testosterone (T) poses increased risk of breast cancer in women. Appraisal of the existing literature produced several lines of evidence arguing against increased breast cancer risk with T. These include (1) Data from breast tumor cell lines treated with androgens did not corroborate the notion that T increases breast cancer risk. On the contrary, androgens appear to be protective as they inhibit tumor cell growth. (2) Many of the epidemiological studies claiming an association between T and breast cancer did not adjust for estrogen levels. Studies adjusted for estrogen levels reported no association between T and breast cancer. (3) Data from clinical studies with exogenous androgen treatment of women with endocrine and sexual disorders did not show any increase in incidence of breast cancer. (3) Women afflicted with polycystic ovary disease, who exhibit high levels of androgens do not show increased risk of breast cancer compared with the general population. (5) Female to male trans-sexuals, who receive supraphysiological doses of T for long time periods prior to surgical procedures, do not report increased risk of breast cancer. (6) Finally, women with hormone responsive primary breast cancer are treated with aromatase inhibitors, which block conversion of androgens to estrogens, thus elevating androgen levels. These women do not experience increased tumor growth. The conclusion of the paper was that the evidence available strongly suggests that T does not increase breast cancer risk in women.

- Zeleniuch-Jacquotte A, Bruning PF, Bonfrer JM, et al. Relation of serum levels of testosterone and dehydroepiandrosterone sulfate to risk of breast cancer in postmenopausal women. *Am J Epidemiol* 1997 Jun 1;145(11):1030-8.

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Postmenopausal serum levels of testosterone and dehydroepiandrosterone sulfate (DHEAS) and subsequent risk of breast cancer were studied in a case-control study nested within the New York University Women's Health Study cohort. A specific objective of the analysis was to examine whether androgens had an effect on breast cancer risk independent of their effect on the biologic availability of estrogen. A total of 130 cases of breast cancer were diagnosed prior to 1991 in a cohort of 7,054 postmenopausal women who had donated blood and completed questionnaires at a breast cancer screening clinic. Testosterone was positively associated with breast cancer risk (odds ratio (OR) for the highest quartile. However, after including % estradiol bound to sex hormone-binding globulin (SHBG) and total estradiol in the statistical model, the odds ratios associated with higher levels of testosterone were considerably reduced, and there was no longer a significant trend. Conversely, breast cancer risk remained positively associated with total estradiol levels and negatively associated with % estradiol bound to SHBG after adjustment for serum testosterone levels. These results are consistent with the hypothesis that testosterone has an indirect effect on breast cancer risk, via its influence on the amount of bioavailable estrogen. No evidence was found of an association between DHEAS and risk of breast cancer in postmenopausal women.

Androgens & Bone:

- Anderson CHM, Raju KS, Forling ML, Wheeler MJ. The effects of surgical menopause and parenteral hormone replacement therapy on bone density, menopausal symptoms, and hormone profiles. Department of Gynaecology, St. Thomas Hospital, London, UK.

  45 women undergoing complete hysterectomies were randomized to receive 50 mg estradiol implants, 50 mcg estradiol patches, or 50 mg estradiol and 100 mg testosterone implants. After one year, there was a significant decrease in bone density in the patch group; no decrease in bone density in the pellet implant groups.

- Barlow DH, Abdalla HI, Roberts DG, et al. Long-term hormone implant therapy – hormonal and clinical effects. *Obstet Gynecol* 1986; 67:321.

  75 women were given estradiol or estradiol plus testosterone implants. Bone density was maintained in both groups and both groups had effective menopausal symptom improvement.

- Davis S, McCloud P, Strauss B, et al. Testosterone enhances estradiol's effects on postmenopausal bone density and sexuality. *Maturitas* 1995;227-236.

  This prospective, 2 year, single-blind, randomized trial evaluated bone mineral density (BMD) in 34 postmenopausal women who received either 50-mg estradiol pellets, or 50-mg estradiol and 50-mg testosterone pellets. Combined treatment was more effective at improving BMD, as well as improving libido.

- Garnett T, Studd J, Watson N, et al. The effects of plasma estradiol levels on increases in vertebral and femoral bone density following therapy with estradiol and estradiol with testosterone implants. *Obstet Gynecol* 1992;79:968-72.

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Percutaneous estradiol (E2) implants effectively preserve bone density in postmenopausal women. However, these implants are often given with testosterone, which may itself have an anabolic effect on bone. To determine whether testosterone confers any additional bone-sparing effect, the authors studied 50 postmenopausal women randomly allocated to receive E2 (75 mg) alone or with testosterone (100 mg) every 6 months for 1 year. Twenty-five untreated women were recruited to act as a reference group. Bone density was measured at the lumbar spine and proximal femur by dual x-ray densitometry. By 1 year, bone density at the lumbar spine had fallen by 1.8% in the reference group. In the women treated with E2 alone, it increased significantly by 7.8% and in those receiving E2 with testosterone, it increased by 6.3%. At the femoral neck, bone density decreased by 3% in the controls and increased by approximately 4% in both treated groups. The increase in bone density at these sites was unrelated to the woman's chronological age, menopausal age, or initial bone density. However, it correlated significantly with the serum E2 levels attained after 1 year of therapy. In no treated patients did bone density decrease significantly. These data suggest that testosterone confers no additional bone-sparing effect in postmenopausal women.

- Garnett T, Studd J, Watson N, et al. A cross-sectional study of the effects of long-term percutaneous hormone replacement therapy on bone density. *Obstet Gynecol* 1991;78:1002-1007.

  The effect of hormone implants on the bone density of postmenopausal women was studied in 110 patients who received hormone replacement in the form of estradiol (50-75 mg) and testosterone (100 mg) pellets at 6-month intervals for 2-24 years (mean 5.2). They were compared with 254 untreated). Subcutaneous estradiol and testosterone pellet implants prevented postmenopausal osteoporosis and maintained normal bone density for as long as treatment was continued.

- Labrie F, Diamond P, Cusan L, et al. Effect of 12-month dehydroepiandrosterone replacement therapy on bone, vagina, and endometrium in postmenopausal women. *J Clin Endocrinol Metab*. 1997;82(10):3498-35050.

  Women treated for 12 months with 10% DHEA cream did not cause endometrial thickening, but did improve vaginal epithelium and bone density in the hip (along with increased plasma osteocalcin, and decreased alkaline phosphatase and urinary hydroxyproline/creatinine ratio.)

- Miller BE, De Souza MJ, Slade K, Luciano AA. Sublingual administration of micronized estradiol and progesterone, with and without micronized testosterone: effect on biochemical markers of bone metabolism and bone mineral density. *Menopause* 2000 (5):318-26.

  The purpose of this investigation was to evaluate the relative efficacy of the sublingual administration of micronized estradiol (E2), progesterone (P4), and testosterone (T) on bone mineral density and biochemical markers of bone metabolism. In this double-blind, prospective study, postmenopausal women were randomly assigned to one of four treatment groups: hysterectomized women were assigned to either 1) micronized E2 (0.5 mg) or 2) micronized E2 (0.5 mg) + micronized T (1.25 mg). Women with intact uteri were assigned to either 3) micronized E2 (0.5 mg) + micronized P4 (100 mg) or 4)

micronized E2 (0.5 mg) + micronized P4 (100 mcg) + micronized T (1.25 mg). For the purpose of this study, the four treatment groups were combined into two groups for all comparisons. The E2 and E2+P4 groups were combined into the HRT alone group (n=30), and the E2+T and E2+P4+T groups were combined into the HRT + T group (n=27). Hormones were administered sublingually as a single tablet twice a day for 12 months. The subjects were of similar age, height, weight and had similar baseline follicle-stimulating hormone, E2, P4, total T, and bioavailable T levels. During therapy, serum levels increased for each hormone. Conclusion was that sublingual micronized HRT favorably decreases serum and urine markers of bone metabolism, prevents bone loss, and results in a slight increase in spine and hip bone mineral density. Although the addition of testosterone to HRT for 1 year did not result in added benefit to the spine bone mineral density, it did result in a significant increase in hip bone mineral density. Longer duration of therapy may have further improved these outcomes.

- Notelovitz M. Androgen effects on bone and muscle. *Fertility & Sterility* 2002;77(Suppl 4):S34-41.

  Bone health and strength are dependent on the coupling of bone resorption and bone formation. This process is governed by the interaction of osteoclasts and osteoblasts plus the modulating influence of the bone mechanicosensory cells-the osteocytes. Both sex steroids-estrogen (E) and testosterone (T) have receptors on all bone cells, with androgen dominance on osteoblasts and osteocytes. Specific receptors for the weaker androgens, such as DHEA have also been identified. The activity of the sex steroids, influenced by various enzymes found in bone, is reflective of the hormone ligand before its binding to the bone cells. As a result, T acts both directly and via its aromatization to estradiol. The activity of the androgens also varies with the bone surface; periosteal cells, for example, do not have 5alpha-reductase activity, indicating that T is the active metabolite at this clinically important site. Androgens influence bone cell function via local and systemic growth factors and cytokines. By enhancing osteoblast differentiation, androgens regulate bone matrix production, organization, and mineralization. Androgens also regulate osteoclast recruitment and activity. Endogenous androgens increase bone mineral density (BMD) in both adolescent and adult premenopausal women. Women with excess endogenous androgen-for example, those with hirsutism and polycystic ovary syndrome (PCOS)-have increased BMD compared with normal young women. E and androgen therapy increases BMD to a greater degree than does E therapy alone. This is true for both oral combinations of esterified E and methyltestosterone and for subcutaneous T implants. Androgenic progestins have an additive effect on BMD when combined with E therapy and have the further advantage of being protective to the endometrium in E-treated women. Androgens increase muscle mass and strength. The resulting improvement in physical activity leads to the activation of bone-forming sites and the stimulation of the bone formation-modulating cells, the osteocytes. Mechanical loading, when combined with hormone therapy, results in greater osteogenic response than does either alone.

- Savvas M, Studd J, Fogelman I, et al. Skeletal effects of oral oestrogen compared with subcutaneous oestrogen and testosterone in postmenopausal women. *BMJ* 1988;297:331-333.

This study compared different oral vs pellet implants for estrogen replacment;women who received estradiol pellet implants also received testosterone pellet implants. Oral treatment group was 37 postmenopausal women compared with 41 women given oestrogen implants and 36 controls. Weight was not significantly different among the groups. Implant group was given subcutaneous implants of oestradiol 50 mg combined with testosterone 100 mg, on average six monthly for a median of 8.5 years. Results of this study showed that estradiol implants were more effective at increasing bone density than oral.

Androgens &the Cardiovascular System & Lipids

- Bernini GP, Sgro M, Moretti A, et al. Endogenous androgens and carotid intimal-medial thickness in women. *J Clin Endocrinol Metab*. 1999.84(6):2008-12.

  This study concluded that, in women, serum DHEA-S, androstenedione, total and free testosterone levels decline with age, and normal hormonal levels were not associated with major cardiovascular risk factors. Higher DHEA-S and testosterone levels were associated with lower carotid wall thickness.

- Burger HG, Hailes J, Menelaus M, et al. The management of persistent menopausal symptoms with oestradiol-testosterone implants: clinical, lipid, and hormonal results. *Maturitas* 1984;6:351-58.

  Seventeen patients were treated with combined subcutaneous implants of oestradiol (40 mg) and testosterone (100 mg), because oral oestrogens had not provided adequate symptomatic relief, particularly of decreased libido. There were significant improvements in libido, enjoyment of sex and tiredness, and in lack of concentration, but there was no significant change in flushes, sweats and depression. Based on an analogue scale, libido increased from a mean basal score of 13.5 to a maximum of 86.1 at 3 mth. Symptomatic improvement was maintained for 4-6 mth. There were no significant changes in total serum cholesterol and triglycerides nor in cholesterol subfractions. The authors concluded that the hormonal implants provided substantial symptomatic relief, particularly of loss of libido, while causing rises to mid-follicular concentrations of oestradiol and maximal testosterone levels about three times normal, without significant effects on plasma lipids.

- Davis S, Goldstat R, Papalia M, et al. Effects of aromatase inhibition on sexual function and well-being in postmenopausal women treated with testosterone: a randomized, placebo-controlled trial. *Menopause* 2006;13(1):37-45.

  The extent to which aromatization of testosterone (T) to estradiol is required for the observed effects of testosterone therapy on sexual function and well-being are not known. Therefore, the authors investigated the effects of aromatase enzyme inhibition on sexual function, well-being, and mood in estrogen- and T-replete postmenopausal women in a double-blind, randomized, placebo-controlled study. Postmenopausal women using transdermal estrogen therapy for at least 8 weeks and reporting low sexual satisfaction with a total T value of less than 1.2 nmol/L were treated with 400 muL of a 0.5% T gel (total dose 2 mg) and were randomly assigned to receive treatment with

either 2.5 mg/day of letrozole or placebo. Total T and calculated free T increased from baseline in both groups, with no difference between groups. At 16 weeks, estradiol, sex hormone-binding globulin, fasting lipids, lipoprotein(a), and C-reactive protein did not differ from baseline or between groups. No adverse treatment effects were reported.Increases in total and free T in the physiologic range in postmenopausal women were associated with improved sexual satisfaction, well-being, and mood. In this study, aromatase inhibition did not influence any of these outcomes.

- Golden S, Maguire A, Ding J, et al. Endogenous postmenopausal hormones and carotid atherosclerosis: a case-control study of atherosclerosis risk in communities cohort. *Am J Epidemiol.* 2002;155(5):437-445.

  Endogenous postmenopausal hormone levels were compared in women with and without significant carotid atherosclerosis. After adjusting for cardiovascular risk factors, no association was found between the odds of atherosclerosis and increasing estrogen, DHEA-S, or androstenedione. Particpants with the highest total testosterone had lowest levels of atherosclerosis.

- Rako S. Testosterone deficiency: a key factor in the increased cardiovascular risk to women following hysterectomy or with natural aging? *J Womens Health*. 1998 Sep;7(7):825-9.

  The ovaries are a critical source not only of estrogen but also of testosterone. On removal of the uterus, even in instances where ovaries have been spared, their function can be compromised. Women who have had a simple hysterectomy (ovaries remaining intact), even if treated postsurgically with supplementary estrogen, have three times the risk of cardiovascular disease compared with women who have not had a hysterectomy. In men, testosterone has been demonstrated to have beneficial fibrinolytic effects and beneficial effects on blood vessel endothelium, in blood sugar and insulin metabolism, and in maintaining coronary artery circulation. Studies on the potential cardiovascular protective effects of physiologic levels of testosterone in women are critically needed. Restoring a physiologic level of testosterone to women after hysterectomy not only can improve quality of life in terms of sexual libido, sexual pleasure, and sense of well-being but also can build bones--and may be a key to protecting cardiovascular health. Women developing testosterone deficiency as a consequence of natural aging/menopause may similarly benefit from physiologic testosterone supplementation.

- Sands R, Studd J, Seed M, et al. The effects of exogenous testosterone on lipid metabolism and insulin resistance in postmenopausal women. Maturitas 1997;27(suppl 1):50.

Androgens & Libido & Sexual Health

- Bolour S, Braunstein G. Testosterone therapy in women: a review. *Int J Impot Res.* 205;17(5):399-408.

  Female sexual dysfunction is a complex problem with multiple overlapping etiologies. Androgens play an important role in healthy female sexual function, especially in stimulating sexual interest and in maintaining desire. There are a multitude of reasons

why women can have low androgen levels with the most common reasons being age, oophorectomy and the use of oral estrogens. Symptoms of androgen insufficiency include absent or greatly diminished sexual motivation and/or desire, that is, libido, persistent unexplainable fatigue or lack of energy, and a lack of sense of well being. Although there is no androgen preparation that has been specifically approved by the FDA for the treatment of Women's Sexual Interest/Desire Disorder or for the treatment of androgen insufficiency in women, androgen therapy has been used off-label to treat low libido and sexual dysfunction in women for over 40 y. Most clinical trials in postmenopausal women with loss of libido have demonstrated that the addition of testosterone to estrogen significantly improved multiple facets of sexual functioning including libido and sexual desire, arousal, frequency and satisfaction. In controlled clinical trials of up to 2 y duration of testosterone therapy, women receiving androgen therapy tolerated androgen administration well and demonstrated no serious side effects. The results of these trials suggest that testosterone therapy in the low-dose regimens is efficacious for the treatment of Women's Sexual Interest and Desire Disorder in postmenopausal women who are adequately estrogenized. Based on the evidence of current studies, it is reasonable to consider testosterone therapy for a symptomatic androgen-deficient woman with Women's Sexual Interest and Desire Disorder.

- Davis SR, et al. Efficacy and safety of a testosterone patch for the treatment of hypoactive sexual desire disorder in surgically menopausal women: a randomized, placebo-controlled trial. *Menopause* 2006; May-Jun;13(3):387-96.

  This study was a 24-week, randomized, double-blind, placebo controlled trial investigating the safety and effectiveness of a testosterone patch in surgically menopausal women receiving concurrent transdermal estrogen. Women were randomly allocated to placebo (n = 40) or testosterone 300 microg/day (n = 37) treatment. Results indicated that the testosterone-treated group had greater sexual desire score compared with. The domain scores for arousal, orgasm, decreased sexual concerns, responsiveness, and self-image as well as decreased distress were also significantly greater with testosterone therapy than placebo. The frequency of satisfactory sexual events increased but was not statistically different between treatment groups. Adverse events occurred with similar frequency in both groups, and no serious risks of therapy were observed.

- Davis S, Goldstat R, Papalia M, et al. Effects of aromatase inhibition on sexual function and well-being in postmenopausal women treated with testosterone: a randomized, placebo-controlled trial. *Menopause* 2006;13(1):37-45.

  The extent to which aromatization of testosterone (T) to estradiol is required for the observed effects of testosterone therapy on sexual function and well-being are not known. Therefore, the authors investigated the effects of aromatase enzyme inhibition on sexual function, well-being, and mood in estrogen- and T-replete postmenopausal women in a double-blind, randomized, placebo-controlled study. Postmenopausal women using transdermal estrogen therapy for at least 8 weeks and reporting low sexual satisfaction with a total T value of less than 1.2 nmol/L were treated with 400 muL of a 0.5% T gel (total dose 2 mg) and were randomly assigned to receive treatment with

either 2.5 mg/day of letrozole or placebo. Total T and calculated free T increased from baseline in both groups, with no difference between groups. At 16 weeks, estradiol, sex hormone-binding globulin, fasting lipids, lipoprotein(a), and C-reactive protein did not differ from baseline or between groups. No adverse treatment effects were reported.Increases in total and free T in the physiologic range in postmenopausal women were associated with improved sexual satisfaction, well-being, and mood. In this study, aromatase inhibition did not influence any of these outcomes.

- Hubayter Z, Simon JA. Testosterone therapy for sexual dysfunction in postmenopausal women. Climacteric 20008;11(3):181-91.

  BACKGROUND: After menopause, both surgical and natural, increases occur in the number of women experiencing sexual dysfunction. Although a direct link between sexual dysfunction and endogenous testosterone levels has not been clearly established, testosterone therapy is known to improve the signs and symptoms related to hypoactive sexual desire. However, testosterone supplementation is not approved in the United States for these clinical indications, primarily because of a lack of data evaluating the possible side-effects of these drugs. METHOD: A MEDLINE search was performed, with a priority for well-designed studies (randomized, controlled trials, meta-analysis), for published data related to the efficacy and safety of testosterone therapy in postmenopausal women. RESULTS: Randomized trials have demonstrated an improvement in sexual function with testosterone in postmenopausal women with hypoactive sexual desire disorder, particularly after oophorectomies. Side-effects have been well tolerated and reversible upon discontinuation. CONCLUSION: Exogenous testosterone treatment provides a rational therapeutic alternative to consider in women whose hypoactive sexual desire disorder negatively affects their quality of life and who have no biologic or psychosocial causes not related to decreased androgen levels for their sexual disorder. Women receiving testosterone should be monitored for clinical improvement and for adverse reactions. Transdermal patches and topical gels avoid the hepatic first-pass metabolism and are the preferred formulations. Testosterone therapy is usually administered concomitantly with estrogen therapy due to a lack of adequate safety and efficacy data on testosterone alone.

- Kingsberg SA, Simon JA, Goldstein I. The current outlook for testosterone in the management of hypoactive sexual desire disorder in postmenopausal women. J Sex Med 2008; Sep 2;5 Suppl 4:177-8.

  This paper reviews the current state of knowledge about the physiologic effects of testosterone in postmenopausal women, the effects of transdermal testosterone delivery in surgically menopausal women with hypoactive sexual desire disorder (HSDD), and ongoing studies of a transdermal testosterone gel. MAIN OUTCOME MEASURES: Results from the Women's International Study of Health and Sexuality; and studies utilizing the Brief Index of Sexual Functioning for Women, the Psychological General Well-Being Index, and validated instruments that assess female sexual function: the Sexual Activity Log, the Profile of Female Sexual Function, and the Personal Distress Scale. RESULTS: Surgically menopausal women receiving testosterone experience significant increases in total satisfying sexual activity vs. women receiving placebo, significant improvement in all domains of sexual function, and decreases in personal distress, with a favorable safety profile. CONCLUSIONS: Testosterone deficiency may

be considered among the underlying causes of HSDD. Currently, testosterone is available to women in the United States only via off-label prescribing or by unregulated compounding of testosterone preparations.

- Kingsberg S. Testosterone treatment for hypoactive sexual desire disorder in postmenopausal women. *J Sex Med* 2007 Mar;4 Suppl 3:227-34.

  This paper is a review of transdermal (patch) testosterone studies in women. A key feature of these studies was the use of validated study instruments to measure sexual function: Sexual Activity Log (SAL), Profile of Female Sexual Function (PFSF) and Personal Distress Scale. METHODS: The data from the Phase III studies, known as the Investigation of Natural Testosterone in Menopausal women Also Taking Estrogen in Surgically Menopausal women (INTIMATE SM) 1 and 2 were reviewed and the salient information is presented. RESULTS: Both INTIMATE 1 and 2 showed a significant increase in total satisfying sexual activity, via the SAL in those women receiving testosterone, compared with those women in the placebo group. The PFSF instrument demonstrated significant improvements in INTIMATE 1 and 2 in all domains of sexual function in testosterone-treated women compared with the placebo patients. In both studies, personal distress decreased in those patients receiving testosterone, compared with the placebo group. The most commonly reported adverse events were application site reactions. Eight-five percent of patients said they would probably or definitely continue treatment. Conclusions: the transdermal testosterone patch is an effective treatment for hypoactive sexual desire disorder in surgically postmenopausal women receiving concomitant estrogen therapy. The treatment has a favorable safety profile.

- Munarriz R, Talakoub L, Flaherty E, et al. Androgen replacement therapy with dehydroepiandrosterone for androgen insufficiency and female sexual dysfunction: androgen and questionnaire results. *J Sex Marital Ther* 2002; 28 (Suppl 1):165-173.

- Shifren JL, Braunstein GD, Simon JA, et al. Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. *N Engl J Med*. 2000;343:682-8.

  In women who'd undergone oophorectomy and hysterectomy, transdermal testosterone improved sexual function and psychological well-being.

Testosterone in Men: General

- Haring R, Volzke H, Steveling A, et al. Low serum testosterone levels are associated with increased risk of mortality in a population-based cohort of men aged 20-79. *Eur Heart J.* 2010;31(12):1494-1501.

- Maggio M, Lauretani F, Ceda G, et al. Relationship between low levesl of anabolic hormones and 6-year mortality in older men. The Aging in the Chianti Area (InCHIANTI) study. *Arch Intern Med*. 2007;167(20):2249-2254.

  Age-associated decline in anabolic hormone levels is a strong independent predictor of mortality in older men. Having mulitple hormoneal deficiencies (testosterone, IGF-1,

DHEA-S) rather than a single anabolic hormone is a reobust biomarker of health status in older persons.

- Lunenfeld B. Men's Health and Aging: The 5th World Congress on the Aging Male. *The Aging Male* 2006;9(1)1-70.

- Shores M, Smith N, Forsberg C, et al. Testosterone treatment and mortality in men with low testosterone levels. *J Clin Endocrinol Metab.* 2012, 97(6):0000 – 0000 jcem.endojournals.org

- Tan R, Culberson J. An integrative review on current evidence of testosterone replacement therapy for the andropause. *Maturitas* 2003;45: 15-/27.

- Tibblin G, Adlerberth A, Lindstedt G, et al. The pituitary-gonadal axis and health in elderly men: a study of men born in 1913. *Diabetes.* 1996 Nov;45(11):1605-9.

- Vermeulen A. Androgen replacement therapy in the aging male—a critical evaluation. *J Clin Endocrinol Metab* 2001;86(6)2380-90.

- Winters S. Current status of testosterone replacement therapy in men. *Arch Fam Med*. 1999;8:257-263.

Testosterone & Body Composition

- Steidle C, Schwartz S, Jacoby K, et al. AA2500 testosterone gel normalizes androgen levels in aging males with improvements in body composition and sexual function. *J Clin Endocrinol Metab.* 2003 ;88:2673–81.

Testosterone replacement in hypogonadal men improves body composition, mood, and sexual functioning. In this 90-d study, the authors compared the pharmacokinetics and treatment effectiveness of a topical testosterone gel (AA2500) at two concentrations, 50 mg/d and 100 mg/d, to a testosterone patch and placebo gel in 406 hypogonadal men. Pharmacokinetic profiles were obtained, body composition was measured, and mood and sexual function were monitored. AA2500 treatments resulted in dose-dependent improvements in all pharmacokinetic parameters, compared with testosterone patch and placebo. At d 90, the 100 mg/d AA2500 treatment improved lean body mass by 1.7 kg and percentage of body fat by 1.2% to a significantly greater degree than either control treatment. Significant improvements in spontaneous erections, sexual desire, and sexual motivation were also evidenced with the 100 mg/d AA2500 dose in comparison with placebo.

Testosterone & Bone in Men:

- Amory J, Watts N, Easley K, et al. Exogenous testosterone or testosterone with finasteride increases bone mineral density in older men with low serum testosterone. *J Clin Endocrinol Metab* 2004;89: 503–510.

Older men, particularly those with low serum testosterone (T) levels, might benefit from T therapy to improve bone mineral density (BMD) and reduce fracture risk. Concerns

exist, however, about the impact of T therapy on the prostate in older men. The authors hypothesized that the combination of T and finasteride (F), a 5 alpha-reductase inhibitor, might increase BMD in older men without adverse effects on the prostate. 70 men aged 65 yr or older, with a serum T less than 12.1 nmol/liter on two occasions, were randomly assigned to receive one of three regimens for 36 months: T enanthate, 200 mg im every 2 wk with placebo pills daily (T-only); T enanthate, 200 mg every 2 wk with 5 mg F daily (T+F); or placebo injections and pills (placebo). Low BMD was not an inclusion criterion. 50 men completed the 36-month protocol. T therapy for 36 months increased BMD at the lumbar spine and in the hip.Over 36 months, PSA increased significantly from baseline in the T-only group. Prostate volume increased in all groups during the 36-month treatment period, but this increase was significantly less in the T+F group compared with both the T-only and placebo groups. Results demonstrated that T therapy in older men with low serum T increases vertebral and hip BMD over 36 months, both when administered alone and when combined with F. This finding suggests that dihydrotestosterone is not essential for the beneficial effects of T on BMD in men. In addition, the concomitant administration of F with T appears to attenuate the impact of T therapy on prostate size and PSA and might reduce the chance of benign prostatic hypertrophy or other prostate-related complications in older men on T therapy. These findings have important implications for the prevention and treatment of osteoporosis in older men with low T levels.

- Arslanian S, Suprasongsin C. Testosterone treatment in adolescents with delayed puberty: changes in body composition, protein, fat, and glucose metabolism. *J Clin Endrocrinol Metab* 1997;82: 3213–3220.

- Vanderschueren D, Vandenput L, Boonen S, et al. Androgens and bone. *Endocrine Reviews* 2004; 25: 389–425.

Testosterone & Erectile Dysfunction

- Glina S. Testosterone and erectile dysfunction. *J Men's Health Gend* 2004;1(4):407-412.

- Shabsigh R, Kaufman JM, Steidle C, Padma-Nathan H. Randomized study of testosterone gel as adjunctive therapy to sildenafil in hypogonadal men with erectile dysfunction who do not respond to sildenafil alone. *J Urology*. 172(2):658-663.

The purpose of this randomized, placebo controlled, double-blind, multi-center study was to compare the efficacy of testosterone gel (T-gel) versus placebo as adjunctive therapy to sildenafil in hypogonadal men with erectile dysfunction who do not respond to sildenafil alone. 75 men were randomized to receive a daily dose of 1% T-gel or 5 gm placebo gel as adjunctive therapy to 100 mg sildenafil during a 12-week period. Testosterone treated subjects had greater improvement in erectile function compared to those who received placebo.

Testosterone & the Prostate

- Barrett-Connor E, Garland C, McPhillips JB, et al. *Cancer Res* 1990;50(1):169-73.

Endogenous androgens have been suggested as determinants of risk of prostatic cancer. To examine this possibility, baseline sex hormone levels were measured in 1008 men ages 40-79 years who had been followed for 14 years. There were 31 incident cases of prostatic cancer and 26 identified from death certificates with unknown dates of diagnosis. In this study, total testosterone, estrone, estradiol, and sex hormone-binding globulin were not related to prostate cancer, but plasma androstenedione showed a positive dose-response gradient.

- Calof OM, Singh AB, Lee ML, et al. Adverse events associated with testosterone replacement in middle-aged and older men: a meta-analysis of randomized, placebo-controlled trials. *J Gerontol A Biol Sci Med Sci* 2005;60:1451–7.

- Carter HB, Pearson JD, Metter EJ, et al. Longitudinal evaluation of serum androgen levels in men with and without prostate cancer. *Prostate*. 1995;27(1):25-1.

  Three age-matched groups of men who were part of the Baltimore Longitudinal Study of Aging (men with no prostate cancer; men with benign prostatic hyperplasia; men with prostate cancer) were tested for LH, total testosterone, free testosterone, and SHBG levels. There was no difference in serum testosterone levels among men with prostate cancer compared to men without prostate cancer.

- Dorgan JF, Albanes D, Virtamo J, et al. Relationships of serum androgens and estrogens to prostate cancer risk: results from a prospective study in Finland. *Cancer Epidemiol Biomarkers Prev.* 1998;7(12):1069-74.

  This was a prospective nested case-control study to evaluate the relationships of serum androgens and estrogens to prostate cancer using serum collected at baseline for the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. The 29,133 male smokers who participated in the trial were 50-69 years old at baseline. During 5-8 years of follow-up, 246 men were diagnosed with prostate cancer, and 116 of these were randomly selected for inclusion in the current study. For each case, two controls matched on age, date of blood collection, intervention group, and study center were selected. Hormones were measured in serum by RIA using standard procedures. None of the individual androgens or estrogens was significantly related to prostate cancer. These findings were unaltered by simultaneous evaluation of serum androgen and estrogen concentrations in multivariate models. These results do not support a strong relationship of serum androgens and estrogens with prostate cancer in smokers.

- Garcia-Cruz E, Piqueras M, Huguet J, et al. Low testosterone levels are related to poor prognosis factors in men with prostate cancer prior to treatment. *BJU Int.* 2012 doi: 10.1111/j.1464-410X.2012.11232.x. [Epub ahead of print]

  Prostate growth is ruled by testosterone. Nevertheless, the paradigm that high testosterone levels induce prostate cancer development or lead to a poor prognosis in prostate cancer is not supported by evidence. A growing number of studies suggest that, on the contrary, low testosterone levels are related to poor prognosis features in prostate cancer such as higher prostate-specific antigen or higher Gleason score. Our experience shows that testosterone levels are related to risk of progression of prostate cancer - those men with lower testosterone levels are at higher risk of progression of their

prostate cancer after treatment delivery. This study prospectively analysed 137 males with PCa with 5+5 core prostate. Free testosterone and bioavailable testosterone were calculated. Age, prostate-specific antigen (PSA), free to total PSA, PSA density, number of previous biopsies, digital rectal examination staging, Gleason score, percentage of tumour in the biopsy sample, bilaterality of the tumour and risk of progression group were prospectively recorded. Higher testosterone levels were related to lower digital rectal examination staging and lower PSA level. Higher testosterone was not related to lower Gleason score (P= 0.08. Testosterone was inversely related to PCa bilaterality and percentage of tumour in the biopsy. High testosterone levels were found in patients allocated to the low risk of progression group and inversely. Patients with PCa and lower testosterone levels have poor prognosis factors and higher tumour burden before treatment onset. These findings reinforce the idea that low testosterone levels pretreatment are related to a poor prognosis in PCa.

- Gustafsson et al. Dihydrotestosterone and testosterone levels in men screened for prostate cancer: a study of a randomized population. *Br J Urol* 1996;77(3):433-40.

- Harper et al. Carcinoma of the prostate: relationship of pretreatment hormone levels to survival. *Eur J Cancer Clin Oncol* 1984;20(4):477-82.

- Heikkilä et al. Serum testosterone and sex hormone-binding globulin concentrations and the risk of prostate carcinoma A longitudinal study. *Cancer.* 86(2):312-315.

- Isurugi et al. Responses of serum levels of testicular steroid hormones to hCG stimulation in patients with prostatic cancer and benign prostatic hypertrophy. *Prostate* 1981 Suppl;1:19-26.

- deJong et al. Peripheral hormone levels in controls and patients with prostatic cancer or benign prostatic hyperplasia: results from the Dutch-Japanese case-control study. *Cancer Res.* 1991; 51(13):3445-50.

- Marks LS, Mazer NA, Mostaghel E, et al. Effect of testosterone replacement therapy on prostate tissue in men with late-onset hypogonadism. *JAMA* 2006;296:2351-2361.

  Prostate safety is a primary concern when aging men receive testosterone replacement therapy (TRT), but little information is available regarding the effects of TRT on prostate tissue in men. This randomized, double-blind, placebo-controlled trial was designed to determine the effects of TRT on prostate tissue of aging men with low serum testosterone levels. 44 men with screening serum testosterone levels lower than 300 ng/dL were included. The primary outcome measure was the 6-month change in prostate tissue androgen levels (testosterone and dihydrotestosterone). Secondary outcome measures included 6-month changes in prostate-related clinical features, histology, biomarkers, and epithelial cell gene expression. Results were that TRT normalizes serum androgen levels but appears to have little effect on prostate tissue androgen levels and cellular functions.

- Miekle et al. Familial factors affecting prostate cancer risk and plasma sex-steroid levels. *Prostate* 1985;6(2):121-8.

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- Morgentaler A. Testosterone therapy in men with prostate cancer: scientific and ethical considerations. *J Urol* 2009;181:972-9.

  Purpose: Pertinent literature regarding the potential use of testosterone therapy in men with prostate cancer is reviewed and synthesized. This paper was a literature search of publications on testosterone administration in men with a known history of prostate cancer and investigation of the effects of androgen concentrations on prostate parameters, especially prostate specific antigen. Results: The prohibition against the use of testosterone therapy in men with a history of prostate cancer is based on a model that assumes the androgen sensitivity of prostate cancer extends throughout the range of testosterone concentrations. Although it is clear that prostate cancer is exquisitely sensitive to changes in serum testosterone at low concentrations, there is considerable evidence that prostate cancer growth becomes androgen indifferent at higher concentrations. The most likely mechanism for this loss of androgen sensitivity at higher testosterone concentrations is the finite capacity of the androgen receptor to bind androgen. This saturation model explains why serum testosterone appears unrelated to prostate cancer risk in the general population and why testosterone administration in men with metastatic prostate cancer causes rapid progression in castrated but not hormonally intact men. Worrisome features of prostate cancer such as high Gleason score, extracapsular disease and biochemical recurrence after surgery have been reported in association with low but not high testosterone. In 6 uncontrolled studies results of testosterone therapy have been reported after radical prostatectomy, external beam radiation therapy or brachytherapy. In a total of 111 men 2 (1.8%) biochemical recurrences were observed. Anecdotal evidence suggests that testosterone therapy does not necessarily cause increased prostate specific antigen even in men with untreated prostate cancer. Conclusions: Although no controlled studies have been performed to date to document the safety of testosterone therapy in men with prostate cancer, the limited available evidence suggests that such treatment may not pose an undue risk of prostate cancer recurrence or progression.

- Morgentaler A, Bruning CO, DeWolf WC, et al. Occult prostate cancer in men with low serum testosterone levels. *JAMA* 1996 276(23)1904-6.

  The purpose of this study was to determine the prevalence of occult prostate cancer in men with low serum total testosterone or free testosterone levels. Seventy-seven men with low total testosterone or free testosterone levels, with normal results of digital rectal examination and prostate-specific antigen (PSA) levels of 4.0 ng/mL or less. The mean age was 58 years. Results of prostate needle biopsies, transrectal ultrasound, prostate volume, PSA level, PSA density, total and free testosterone levels. Prostate cancer was identified in 14% (11/77) of the entire group and in 10 men (29%) aged 60 years or older. The median age for men with cancer was 64 years. Histologic examination showed Gleason scores of 6 or 7 for all cancers. No significant differences were noted between the cancer and benign groups with regard to PSA level, PSA density, prostate volume, total testosterone level, or free testosterone level. A high prevalence of biopsy-detectable prostate cancer was identified in men with low total or free testosterone levels despite normal PSA levels and results of digital rectal examination. These data suggest that (1) digital rectal examination and PSA levels are insensitive indicators of prostate cancer in men with low total or free testosterone levels, and (2) PSA levels may be altered by naturally occurring reductions in serum androgen levels.

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- Morales A. Androgen replacement therapy and prostate safety. *European Urology* 2002;41:113-120.

  Progress in the understanding of the action of exogenous testosterone has diminished many of the concerns that existed regarding its safety. The major interest is now focused on the effects of androgen supplementation on the prostate gland. Many such concerns have been addressed but others remain to be fully elucidated. It is well established that hypogonadal men receiving adequate androgen therapy develop a prostate with a volume similar to what would be expected from their eugonadal counterparts. Androgen therapy results in modest elevations in the PSA and minor changes in flow parameters. Prostate cancer, on the other hand, remains the most prominent of the safety concerns. Although there is no evidence that normal levels of testosterone promote the development of cancer of the prostate, it is clear that the administration of testosterone enhances a pre-existing prostatic malignancy. Androgen supplementation studies have been, in most cases, of short duration and lacked a control cohort. The current evidence does not support the view that appropriate treatment of hypogonadal elderly men with androgens has a causal relationship with prostate cancer. Larger experience, however, is needed. The same criteria applies to the use of other hormones such as dehydrotestosterone, dehydroepiandrosterone follicle stimulating and growth hormone. A set of recommendations regarding androgen replacement therapy and prostate safety is proposed.

- Nomura et al. Serum androgens and prostate cancer. *Cancer Epidemiol Biomarkers Prev.* 1996 Aug;5(8):621-5.

- Roddam A. Endogenous Sex Hormones and Prostate Cancer: A Collaborative Analysis of 18 Prospective Studies*. J Natl Cancer Inst* 2008;100: 170- 183.

  Sex hormones in serum have been hypothesized to influence the risk of prostate cancer. This paper was a meta analysis of the existing worldwide epidemiologic data to examine these associations in a uniform manner and to provide more precise estimates of risks. Data on serum concentrations of sex hormones from 18 prospective studies that included 3886 men with incident prostate cancer and 6438 control subjects were pooled by the Endogenous Hormones and Prostate Cancer Collaborative Group. Relative risks (RRs) of prostate cancer by fifths of serum hormone concentration were estimated by use of conditional logistic regression with stratification by study, age at recruitment, and year of recruitment. All statistical tests were two-sided. No associations were found between the risk of prostate cancer and serum concentrations of testosterone, calculated free testosterone, dihydrotestosterone, dehydroepiandrosterone sulfate, androstenedione, androstanediol glucuronide, estradiol, or calculated free estradiol. The serum concentration of sex hormone – binding globulin was modestly inversely associated with prostate cancer risk (RR in the highest vs lowest fifth = 0.86, 95% confidence interval = 0.75 to 0.98; P trend = .01). There was no statistical evidence of heterogeneity among studies, and adjustment for potential confounders made little difference to the risk estimates. Conclusion was that in this collaborative analysis of the worldwide data on endogenous hormones and prostate cancer risk, serum concentrations of sex hormones were not associated with the risk of prostate cancer.

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- Shin B, Hwang EC, Im CM, et al. Is a decreased serum testosterone level a risk factor for prostate cancer? A cohort study of Korean men. *Korean J Urol*. 2010 ;51(12):819-23.

  The purpose of this study was to investigate 568 patients who had transrectal ultrasonography (TRUS)-guided prostate biopsy to define the role of the serum testosterone level in predicting prostate cancer risk and its association with a high Gleason score. Patients with lower levels of serum testosterone had a higher risk of prostate cancer than did patients with high serum testosterone. Even though a lower serum testosterone level was a predictor of prostate cancer risk, it was not associated with an increased risk of high-grade prostate cancer.

- Sofikerim M, Eskicorapci S, Oruc O, Ozen H. Hormonal predictors of prostate cancer. *Urol Int* 2007;79(1):13-8.

  Androgens are necessary for the development and functioning of the prostate gland. The association of serum testosterone and pituitary hormone levels with prostate cancer development is not completely understood. This study evaluated the role of serum testosterone, free testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in predicting prostate cancer risk in 211 patients who had transrectal ultrasonography-guided prostate biopsy with the suspicion of prostate cancer. Conclusion was that patients diagnosed with prostate cancer have low levels of serum testosterone and high levels of serum FSH compared with the patients with BPH. No support was found for the theory that high levels of testosterone increase prostate cancer risk.

- Steiner MS, Raghow S. Antiestrogens and selective estrogen receptor modulators reduce prostate cancer risk. *World J Urol* 2003 May; 21(1): 31-6.

- Vatten et al. Androgens in serum and the risk of prostate cancer: a nested case-control study from the Janus serum bank in Norway *Cancer Epidemiol Biomarkers Prev.* 1997 Nov;6(11)967-9.

Testosterone & the Cardiovascular System, Metabolic Syndrome, Dlabetes, & Obesity

- Barrett-Connor, EL. Testosterone and risk factors for cardiovascular disease in men. *Diabetes & Metab*. 1995. 156-161.

  It has been assumed for years that male testosterone levels play a central role in worsening lipoprotein patterns and causing greater susceptibility to ischemic heart disease. Yet most clinical trials of quasi-physiologic doses of intramuscular testosterone in older men show no effect on high-density lipoprotein (HDL)-cholesterol, while cross-sectional epidemiologic studies almost uniformly find that endogenous testosterone levels are positively associated with HDL-cholesterol levels. Further work is required to determine whether and why physiologic testosterone levels in the high normal range appear to be conducive to optimal cardiovascular health for adult men.

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- Barud W, Piotrowska-Swirszcz A, Ostrowski S, et al. Association of obesity and insulin resistance with serum testosterone, sex hormone binding globulin and estradiol in older males. *Pol Merkur Lekarski.* 2005 Nov:19(113):634-7.

- Corona G, Rastrelli G, Monami M, et al. Hypogonadism as a risk factor for cardiovascular mortality in men: a meta-analytic study. *Eur J Endocrinol*. 2011;165(5):687-701

  The purpose of this study was to verify whether hypogonadism represents a risk factor for cardiovascular (CV) morbidity and mortality and to verify whether testosterone replacement therapy (TRT) improves CV parameters in subjects with known CV diseases (CVDs). Of the 1178 retrieved articles, 70 were included in the study. Among cross-sectional studies, patients with CVD have significantly lower testosterone and higher 17-β estradiol (E(2)) levels. Conversely, no difference was observed for DHEAS. The association between low testosterone and high E(2) levels with CVD was confirmed in a logistic regression model, after adjusting for age and body mass index (hazard ratio (HR)=0.763 (0.744-0.783) and HR=1.015 (1.014-1.017), respectively, for each increment of total testosterone and E(2) levels; both P<0.0001). Longitudinal studies showed that baseline testosterone level was significantly lower among patients with incident overall- and CV-related mortality, in comparison with controls. Conversely, we did not observe any difference in the baseline testosterone and E(2) levels between case and controls for incident CVD. Finally, TRT was positively associated with a significant increase in treadmill test duration and time to 1mm ST segment depression. CONCLUSIONS: Lower testosterone and higher E(2) levels correlate with increased risk of CVD and CV mortality. TRT in hypogonadism moderates metabolic components associated with CV risk. Whether low testosterone is just an association with CV risk, or an actual cause-effect relationship, awaits further studies.

- Dobs AS, Bachorik PS, Arver S, et al. Interrelationships among lipoprotein levels, sex hormones, anthropometric parameters, and age in hypogonadal me treated for 1 year with a permeation-enhanced testosterone transdermal system. *J Clin Endocrinol Metab*. 2001;86(3):1026-33.

  Serum lipoproteins and cardiovascular risk are affected by endogenous and exogenous sex hormones. As part of a multicenter evaluation of a permeation-enhanced testosterone transdermal system (TTD), the interrelationships among serum lipoproteins, hormone levels, anthropometric parameters, and age were investigated in 29 hypogonadal men. Subjects (aged 21-65 yr) were first studied during prior treatment with intramuscular testosterone esters (IM-T), then during an 8-week period of androgen withdrawal resulting in a hypogonadal state (HG), and finally during a 1-yr treatment period with the TTD. Compared with treatment with IM-T, the HG period produced increases in high density lipoprotein and total cholesterol and a decrease in the cholesterol/HDL ratio. Compared with the HG period, TTD treatment produced decreases in and increases in the cholesterol/HDL ratio and triglycerides.Serum HDL levels showed a strong negative correlation with body mass index and other obesity parameters in all three study periods During treatment with TTD, serum testosterone levels also correlated negatively with body mass index. As a consequence of these relationships, a positive trend was observed between HDL and testosterone levels during TTD. Changes in lipoprotein levels during TTD treatment indicated a more

favorable profile with increasing age of the patients. In hypogonadal men the effects of transdermal testosterone replacement on serum lipoproteins appear consistent with the physiological effects of testosterone in eugonadal men.

- Ding EL, Song Y, Malik VS, Liu S. Sex differences of exogenous sex hormones and risk of type II diabetes. *JAMA*. 2006 295(11):1288-99.

- English K, Steeds R, Jones T, et al. Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina. *Circulation.* 2000;102:1906.

  46 men with stable angina treated with testosterone. Results showed testosterone reduced exercise-induced myocardial ischemia.

- Fogari R, Zoppi A, Preti P, et al. Sexual activity and plasma testosterone levels in hypertensive males. *Am J Hypertension* 2002; 15:217–221.

  The aim of this study was to compare sexual activity and plasma testosterone levels of hypertensive men with those of healthy normotensive controls. 110 newly diagnosed, never treated hypertensive (blood pressure [BP] > or = 140/95 mm Hg) men and 110 healthy normotensive (diastolic BP <90 mm Hg) me, aged 40 to 49 years, married, without any previous sexual dysfunction, nondiabetic, nonobese (body mass index <28 kg/m2), nonsmoking, and not taking any drug. All subjects were evaluated in the morning after an overnight fast. Findings suggested a relationship between essential hypertension and impaired testosterone levels in men.

- Fukui M, Kitagawa Y, Nakamura N, et al. Association between serum testosterone concentration and carotid atherosclerosis in men with type 2 diabetes. Diabetes Care. 2003;26:1869-1873.

  This study looked at the relationship between serum testosterone concentrations and carotid atherosclerosis as well as major cardiovascular risk factors in men with type 2 diabetes. Results showed lower carotid artery atherosclerosis with higher testosterone levels.

- Hak AE, Witteman JC, deJong F, et al. Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. *J Clin Endocrinol Metab*. 2002;87(8):3632-3639.

  In both men and women, circulating androgen levels decline with advancing age. Until now, results of several small studies on the relationship between endogenous androgen levels and atherosclerosis have been inconsistent. In the population-based Rotterdam Study, we investigated the association of levels of dehydroepiandrosterone sulfate (DHEAS) and total and bioavailable testosterone with aortic atherosclerosis among 1,032 nonsmoking men and women aged 55 yr and over. Aortic atherosclerosis was assessed by radiographic detection of calcified deposits in the abdominal aorta, which have been shown to reflect intimal atherosclerosis. Relative to men with levels of total and bioavailable testosterone in the lowest tertile, men with levels of these hormones in the highest tertile had age-adjusted relative risks of 0.4 [95% confidence interval (CI),

0.2-0.9] and 0.2 (CI, 0.1-0.7), respectively, for the presence of severe aortic atherosclerosis. The corresponding relative risks for women were 3.7 (CI, 1.2-11.6) and 2.3 (CI, 0.7-7.8). Additional adjustment for cardiovascular disease risk factors did not materially affect the results in men, whereas in women the associations diluted. Men with levels of total and bioavailable testosterone in subsequent tertiles were also protected against progression of aortic atherosclerosis measured after 6.5 yr (SD +/- 0.5 yr) of follow-up (P for trend = 0.02). No clear association between levels of DHEAS and presence of severe aortic atherosclerosis was found, either in men or in women. In men, a protective effect of higher levels of DHEAS against progression of aortic atherosclerosis was suggested, but the corresponding test for trend did not reach statistical significance. In conclusion, we found an independent inverse association between levels of testosterone and aortic atherosclerosis in men. In women, positive associations between levels of testosterone and aortic atherosclerosis were largely due to adverse cardiovascular disease risk factors.

- Jones TH, Saad F. The effects of testosterone on risk factors for, and the mediators of, the atherosclerotic process. *Atherosclerosis.* 2009;207(2):318-27.

  It is becoming increasingly evident that the low serum levels of testosterone experienced by aging men are associated with increased all-cause mortality from CHD and other vascular disorders. Achieving a normal physiological testosterone concentration through the administration of testosterone therapy has been shown to provide beneficial effects on the pathophysiological markers and clinical symptoms of CHD. Many of the factors involved in the atherosclerotic process are interlinked with other, increasingly prevalent pathological conditions such as obesity, the metabolic syndrome (MetS), type 2 diabetes and erectile dysfunction, suggesting that testosterone therapy has potentially wide-ranging health benefits. As the number and scope of testosterone substitution and androgen deprivation studies increases and evidence accumulates, it is timely to assess available data and this review summarises the current understanding of the effects of testosterone on cardiovascular risk factors with particular emphasis on the relevance of testosterone treatment.

- Jones TH, Arver S, Behre H, et al. Testosteorne replacment in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 Study). *Diabetes Care*. 2001;34(4):828-837.

  Efficacy, safety, and tolerability of a 2% testosterone gel was evaluated over 12 months in 220 hypotonadal men with type 2 diabetes or metabolic syndrome in a multicenter, prospective, randomized, double-blind, placebo-controlled sutyd. TRT reduced HOMA-IR by 15.2% at 6 mos and 16.4% at 12 mos. In type 2 diabetics, glycemic control was better in TRT patients (HbA1c decreased 0.446%). Improvements in total and LDL cholesterole, Lp(a), body composition, libido, and sexual function occured in treated group. No signficiant adverse events were seen.

- Hyde Z, Norman P, Flicker L, et al. Low free testosterone predicts mortality from cardiovascular disease but not other causes: the health in men study. *J Clin Endocrinol Metab.* 2012;97(1):179-189.

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- Jones RD, Nettleship JE, Kapoor D, et al. Testosterone and atherosclerosis in aging men: purported association and clinical implications. Am J Cardiovasc Drugs. 2005;5(3):141-54.

  Two of the strongest independent risk factors for coronary heart disease (CHD) are increasing age and male sex. Despite a wide variance in CHD mortality between countries, men are consistently twice as likely to die from CHD than their female counterparts. This sex difference has been attributed to a protective effect of female sex hormones, and a deleterious effect of male sex hormones, upon the cardiovascular system. However, little evidence suggests that testosterone exerts cardiovascular harm. In fact, serum levels of testosterone decline with age, and low testosterone is positively associated with other cardiovascular risk factors. Furthermore, testosterone exhibits a number of potential cardioprotective actions. For example, testosterone treatment is reported to reduce serum levels of the pro-inflammatory cytokines interleukin (IL)-1beta and tumor necrosis factor (TNF)-alpha, and to increase levels of the anti-inflammatory cytokine IL-10; to reduce vascular cell adhesion molecule (VCAM)-1 expression in aortic endothelial cells; to promote vascular smooth muscle and endothelial cell proliferation; to induce vasodilatation and to improve vascular reactivity, to reduce serum levels of the pro-thrombotic factors plasminogen activator inhibitor (PAI)-1 and fibrinogen; to reduce low-density lipoprotein-cholesterol (LDL-C); to improve insulin sensitivity; and to reduce body mass index and visceral fat mass. These actions of testosterone may confer cardiovascular benefit since testosterone therapy reduces atheroma formation in cholesterol-fed animal models, and reduces myocardial ischemia in men with CHD. Consequently, an alternative hypothesis is that an age-related decline in testosterone contributes to the atherosclerotic process. This is supported by recent findings, which suggest that as many as one in four men with CHD have serum levels of testosterone within the clinically hypogonadal range. Consequently, restoration of serum levels of testosterone via testosterone replacement therapy could offer cardiovascular, as well as other, clinical advantages to these individuals.

- Kapoor D, Goodwin E, Channer KS, Jones TH. Testosterone replacment therapy improves insulin resistance, gycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. *Eur J Endocrinol.* 2006;154(6):899-906.

  Double-blind, placebo-controlled crossover study in 24 hypogonadal men with type 2 diabetes (10 treated with insulin) over the age of 30. Men were given 200 mg testosterone IM every 2 weeks for 3 months or placeob. Testosterone therapy improved fasting insulin senstivity, hemoglobin A1C, fasting glucose, and total cholesteerol. Testosterone also decreased visceral adiposity and waist to hip ratio. No change in blood pressure was observed.

- Kapoor et al. Androgens, insulin resistance and vascular disease in men. *Clin Endocrinol (Oxf)* 2005 Sep;63(3):239-50.

- Kay-Tee K, Fokerd E, Bingham S, et al. Endogenous testosterone and mortality due to all causes, cardiovascular disease, and cancer in men. *Circulation*. 2007;116:2694-2701.

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*Endogenous testosterone concentrations and mortality due to all causes, cardiovascular disease, and cancer was examined in a nested case-control study based on 11,606 men aged 40 to 79 years surveyed in 1993 to 1997 and followed up to 2003. Among those without prevalent cancer or cardiovascular disease, 825 men who subsequently died were compared with a control group of 1489 men still alive, matched for age and date of baseline visit. Endogenous testosterone concentrations at baseline were inversely related to mortality due to all causes (825 deaths), cardiovascular disease (369 deaths), and cancer (304 deaths). Odds ratios (95% confidence intervals) for mortality for increasing quartiles of endogenous testosterone compared with the lowest quartile were 0.75 (0.55 to 1.00), 0.62 (0.45 to 0.84), and 0.59 (0.42 to 0.85), respectively (*P*<0.001 for trend after adjustment for age, date of visit, body mass index, systolic blood pressure, blood cholesterol, cigarette smoking, diabetes mellitus, alcohol intake, physical activity, social class, education, dehydroepiandrosterone sulfate, androstanediol glucuronide, and sex hormone binding globulin). An increase of 6 nmol/L serum testosterone (≈1 SD) was associated with a 0.81 (95% confidence interval 0.71 to 0.92,* P*<0.01) multivariable-adjusted odds ratio for mortality. Inverse relationships were also observed for deaths due to cardiovascular causes and cancer and after the exclusion of deaths that occurred in the first 2 years.* **Conclusions—** *In men, endogenous testosterone concentrations are inversely related to mortality due to cardiovascular disease and all causes. Low testosterone may be a predictive marker for those at high risk of cardiovascular disease.*

- Kelly DM, Jones TH. Testosterone: a metabolic hormone in health and disease. J Endocrinol. 2013; 217(3):R25-45.

  Testosterone is a hormone that plays a key role in carbohydrate, fat and protein metabolism. It has been known for some time that testosterone has a major influence on body fat composition and muscle mass in the male. Testosterone deficiency is associated with an increased fat mass (in particular central adiposity), reduced insulin sensitivity, impaired glucose tolerance, elevated triglycerides and cholesterol and low HDL-cholesterol. All these factors are found in the metabolic syndrome (MetS) and type 2 diabetes, contributing to cardiovascular risk. Clinical trials demonstrate that testosterone replacement therapy improves the insulin resistance found in these conditions as well as glycaemic control and also reduces body fat mass, in particular truncal adiposity, cholesterol and triglycerides. The mechanisms by which testosterone acts on pathways to control metabolism are not fully clear. There is, however, an increasing body of evidence from animal, cell and clinical studies that testosterone at the molecular level controls the expression of important regulatory proteins involved in glycolysis, glycogen synthesis and lipid and cholesterol metabolism. The effects of testosterone differ in the major tissues involved in insulin action, which include liver, muscle and fat, suggesting a complex regulatory influence on metabolism. The cumulative effects of testosterone on these biochemical pathways would account for the overall benefit on insulin sensitivity observed in clinical trials. This review discusses the current knowledge of the metabolic actions of testosterone and how testosterone deficiency contributes to the clinical disease states of obesity, MetS and type 2 diabetes and the role of testosterone replacement.

- Khaw KT, Barrett-Connor E. Blood pressure and endogenous testosterone in men: an inverse relationship*. J Hypertension* 1998; 6:329–332.

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- Kiel DP, Baron CA, Plymate SR 1989 Sex hormones and lipoproteins in men. *Am J Med* 87: 35-39.

- Kyriazis J, Tzanakis I, Stylianou K, et al. Low serum testosterone, arterial stiffness, and mortality in male haemodialysis patients*. Nephrol Dial Transplant*. 2011;26(9):2971-2977.

- Laughlin G, Barrett-Connor E, Bergstrom J. Low serum testosterone and mortality in older men. *J Clin Endocrinol Metab*. 2008;93(1):68-75.

- Maggio M, Basaria S. Welcoming low testosterone as a cardiovascular risk factor. *Int J Impotence Res. 2009;21:261-4.*

  Male hypogonadism now has a new spectrum of complications. They are mainly cardiometabolic in nature. Low serum testosterone levels are a reisk factor for diabetes, metabolic syndrome, inflammation, and dyslipidemia. These metabolic and inflammatory complications are not without consequences. Recent studies have shown low serum testosteorne levels to be an independent risk factor of cardiovasular and all-cause mortality.

- Muller M, van der Schouw Y, Thijssen J, et al. Endogenous sex hormones and cardiovascular disease in men. *J of Clin Endocrinol & Metab* 2003;88(11):5076-5086.

  Unlike women, men do not experience an abrupt reduction in endogenous sex hormone production. It has, however, become clear that an age-associated decrease in the levels of (bioactive) sex hormones does occur. Whether endogenous sex hormones have an impact on cardiovascular disease has for many years remained largely unknown, but during the last decade more attention has been drawn to the importance of testosterone, estrogens, and adrenal androgens in etiology, prevention, and treatment of male cardiovascular disease. The purpose of this article is to summarize the evidence currently available on the association between endogenous sex hormones and cardiovascular disease in males. Published studies dealing with the relationship between circulating levels of sex hormones and cardiovascular disease in males were reviewed. The studies reviewed in this article suggest that circulating endogenous sex hormones and estrogens have a neutral or beneficial effect on cardiovascular disease in men.

- Muraleedharan V, Jones TH. Review: testosterone and the metabolic syndrome. *Ther Advances in Endocrinol Metab*. 2010;1(5):207-223.

- Muraleedharan V, Marsh H, Kapoor D, et al. Testosterone deficiency is associated with increased risk of mortality and testosterone replacement improves survival in men with type 2 diabetes. *Eur J Endocrinol*. 2013;169(6):725-33.

  581 men with type 2 diabetes who had testosterone levels performed between 2002 and 2005 were followed for mean period of 5.81 years. Mortaltiy increased ithn the low testosteorne group compared with the normal testosterone group. The authors concluded that low Low testosterone levels predict an increase in all-cause mortality during long-term follow-up. Testosterone replacement may improve survival in hypogonadal men with type 2 diabetes.

- Nettleship JE, Jones RD, Channer KS, Jones TH. Testosterone and coronary artery disease. *Front Horm Res*. 2009;37:91-107.

  The strongest independent risk factors for coronary artery disease (CAD) are increasing age and male gender. Whilst a wide variation in CAD mortality exists between countries, a male to female ratio of approximately 2:1 is consistently observed. These observations have led to the assumption that testosterone may exert a detrimental influence on the cardiovascular system. Despite this, coronary atherosclerosis increases with age, whilst a marked fall in serum bioavailable testosterone levels is observed. Similarly, low testosterone levels are also associated with other cardiovascular risk factors and increased expression of mediators of the atherosclerotic process. This in itself suggests that testosterone does not promote atheroma formation. Moreover, epidemiological studies show an inverse relationship between testosterone levels and surrogate markers of atherosclerosis, which suggests that it may be a testosterone deficient state, rather than male sex which is associated with CAD. In cholesterol-fed animal models, atherosclerosis is accelerated by castration and reduced after testosterone replacement therapy. Testosterone has also been shown to improve myocardial ischemia in men with angina pectoris. Consequently, increasing evidence suggests that the process of atherosclerosis is beneficially modulated by testosterone.

- Phillips GB, Pinkernell BH, Jing TY. The association of hypotestosteronemia with coronary artery disease in men*. Anterioscler Thromb.* 1994;14(5):701-6.

  Estradiol, testosterone, and risk factors for MI were measured in 55 men undergoing angiography (no previous MI). Neither estradiol, nor other risk factors except fro HDL correlated with the degree of coronary artery disease in the final rroup. Testosterone correlated negatively with risk factors fibrinogen, plasminogen activator inhibitor-1, and insulin and positively with HDL. Conclusion was that low testosterone may be risk factor for coronary atherosclerosis.

- Phillips GB, Jing TY, Resnick LM, et al. Sex hormones and hemostatic risk factors for coronary heart disease in men with hypertension. *J Hypertension* 1993; 11:699–702.

- Potenza M, Shimshi M. Male hypogonadism: the unrecognized cardiovascular risk factor*. J Clin Lipid*. 2008;2:71-78.

  Normal levels of male sex hormones are essential to men's health. Many studies demonstrate that hypogonadal men are at higher risk for developing a host of metabolic derangements, including dyslipidemia, type 2 diabetes mellitus, obesity, and hypertension. We examined the most recent studies supporting this notion of hypogonadism as a cardiac risk factor by reviewing all relevant PubMed data. Most studies showed an increase in metabolic disorders and cardiac events in hypogonadal men compared to their eugonadal counterparts. Mechanisms explaining this increased risk include adverse cytokine profiles produced by excess adipose tissue, abnormal lipid metabolism by understimulated hormone-sensitive lipase, and abnormal cellular respiration leading to insulin resistance. In contrast, some studies have not demonstrated such an increased cardiac risk. Conflicting data between studies is expected, given the complexity of testosterone and its metabolic effects. Additionally, the interaction of testosterone with the androgen receptor differs based on an individual

genome. Hypogonadism will affect individual men differently because of this genomic variance. The literature points toward true hypogonadism as a major cardiac risk factor. Men at risk of being hypogonadal should be screened and brought back to eugonadism with hormone replacement.

- Selvin E, Feinleib M, Zhang L, et al. Androgens and diabetes in men: results from the Third National Health and Nutrition Examination Survey (NHANES III). *Diabetes Care*. 2007;30(2):234-8.

  More than 1400 men tested for testosterone levels; low free and bioavailable testosterone concentration in the normal range were associated with diabetes, independent of adiposity. Conclusion was that low androgen levels may be a risk factor for diabetes.

- Simon D, Charles MA, Lahlou N, et al. Androgen therapy improves insulin sensitivity and decreases leptin level in health adult men with low plasma total testosterone: a 3-month randomized placebo-controlled trial. *Diabetes Care*. 2001;24(12):2149-51.

- Simon D, Charles M, Nahoul K, et al. Association between plasma total testosterone and cardiovascular risk factors in healthy adult men: The Telecom Study*. J of Clin Endocrin & Metab.* 1997 82(2): 682-5.

- Stellato RK, FEldman HA, Harndy O, et al. Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men: prospective results from the Massachusetts male aging study. *Diabetes Care* 2000;23: 490-94.

- Svartberg J, Braekkan SK, Laughlin GA, Hansen JB. Endogenous sex hormone levels in men are not associated with risk of venous thromboembolism: the Tromso study. *Eur J Endocrinol.* 2009;160(5):833-8.

  Low testosterone levels in men have been associated with cardiovascular risk factors and atherosclerosis and lately also an increased risk of both cardiovascular disease (CVD) and all-cause mortality. As arterial CVDs and venous thromboembolism (VTE) have been shown to share common risk factors, the purpose of the present study was to determine the impact of endogenous sex hormone levels on the incidence of VTE in a cohort of men. Sex hormone measurements were available in 1350 men, aged 50-84, participating in the Tromsø study in 1994-1995. First, lifetime VTE-events during the follow-up were registered up to September 1 2007. There were 63 incident VTE-events during a mean of 10.4 years of follow-up. Age was significantly associated with increased risk of VTE; men 70 years or older had a 2.5-fold higher risk of VTE, compared with those between 50 and 60 years of age. In this population-based study of middle-aged and older men, endogenous sex hormone levels were not associated with 10-year risk of VTE.

- Toma M, McAlister F, Coglianese E, et al. Testosterone supplementation in heart failure: a meta-analysis. *Circ Heart Fail*. 2012;5(3):315-21.

  This meta-analysis looked at randomized controlled trials (RCTs) reporting the effects of testosterone on exercise capacity in patients with HF. Reviewers determined the

methodological quality of studies and collected descriptive, quality, and outcome data. Four trials (n=198; men, 84%; mean age, 67 years) were identified that reported the 6-minute walk test (2 RCTs), incremental shuttle walk test (2 RCTs), or peak oxygen consumption (2 RCTs) to assess exercise capacity after up to 52 weeks of treatment. Testosterone therapy was associated with a significant improvement in exercise capacity compared with placebo. The mean increase in the 6-minute walk test, incremental shuttle walk test, and peak oxygen consumption between the testosterone and placebo groups was 54.0 m (95% CI, 43.0-65.0 m), 46.7 m (95% CI, 12.6-80.9 m), and 2.70 mL/kg per min (95% CI, 2.68-2.72 mL/kg per min), respectively. Testosterone therapy was associated with a significant increase in exercise capacity as measured by units of pooled SDs (net effect, 0.52 SD; 95% CI, 0.10-0.94 SD). No significant adverse cardiovascular events were noted. CONCLUSIONS: Given the unmet clinical needs, testosterone appears to be a promising therapy to improve functional capacity in patients with HF. Adequately powered RCTs are required to assess the benefits of testosterone in this high-risk population with regard to quality of life, clinical events, and safety.

- Traish A, Saad F, Guay A. The dark side of testosterone deficiency: II. type 2 diabetes and insulin resistance. *J Androl.* 2009;30(1): 23-32.

  A considerable body of evidence exists suggesting a link among reduced testosterone plasma levels, type 2 diabetes (T2D), and insulin resistance (IR). Hypogonadal men are at higher risk for T2D. Here we evaluate the relationships between testosterone, metabolic syndrome (MetS), T2D, and IR and discuss the relationships among androgen deficiency and these factors, especially as it ultimately relates to the development of cardiovascular disease and erectile dysfunction (ED). Thus, a comprehensive literature search was carried out using PubMed, and relevant articles pertinent to androgen deficiency, T2D, IR, MetS, and ED were reviewed and discussed. Low testosterone precedes elevated fasting insulin, glucose, and hemoglobin A1c (HbA1C) values and may even predict the onset of diabetes. Treatment of prostate cancer patients with surgical or medical castration exacerbates IR and glycemic control, strengthening the link between testosterone deficiency and onset of T2D and IR. Androgen therapy of hypogonadal men improves insulin sensitivity, fasting glucose, and HbA1c levels. We suggest that androgen deficiency is associated with IR, T2D, MetS, and with increased deposition of visceral fat, which serves as an endocrine organ, producing inflammatory cytokines and thus promoting endothelial dysfunction and vascular disease.

- Vlachopoulos C, Ioakeimidis N, Terentes-Printzios D, et al. Plasma total testosterone and incident cardiovascular events in hypertensive patients. *Am J Hypertens*. 2013;26(3):373-381.

- Vikan T, Schirmer H, Njølstad I, Svartberg J. Endogenous sex hormones and the prospective association with cardiovascular disease and mortality in men: the Tromso Study*. Eur J Endocrinol.* 2009.161(3):435-42.

  The purpose of this prospective study was to determine the impact of endogenous testosterone levels in 1568 men and later risk for myocardial infarction (MI) and all-cause, cardiovascular disease (CVD), and ischemic heart disease (IHD) mortality.Men with free testosterone levels in the lowest quartile had a 24% increased risk of all-cause mortality.

- Wehr E, Pilz S, Boehm B, et al. Low free testosterone is associated with heart failure mortality in older men referred for coronary angiography. *Eur J Heart Fail*. 2011;13(5):482-88.

- Webb C, McNeill J, Hayward C. Effects of Testosterone on Coronary Vasomotor Regulation in Men with Coronary Heart Disease. *Circulation* 1999;100:1690-1696.

- Zgliczynski S, Ossowski M, Slowinska-Srzednicka J, et al. Effect of testosterone replacement therapy on lipids and lipoproteins in hypotgonadal and elderly men. *Atherosclerosis*. 1996;121(1):35-43.

  Long term testosterone replacement decreased total cholesterol and LDL, without altering HDL or its subfractions. In addition, no effects on the prostate were observed.

Testosterone & the Brain, Nervous System, Mood

- Bialek M, ZarembaP, Borowicz K, et al.Neuroprotective role of testosterone in the nervous system. *Pol J Pharmacol*. 2004;56:509-518.

  Testosterone plays an important role in the central nervous system (CNS) development. One of the less known testosterone actions is neuroprotection. There are some evidences supporting the hypothesis that testosterone may act protectively in neurodegenerative disorders, e.g. Alzheimer's disease (AD), mild cognitive impairment (MCI) or depression. Androgens alter also the morphology, survival and axonal regeneration of motor neurons. These hormones accelerate the regeneration of hamster facial nerve and anterior tibialis sciatic nerve in rabbits following crush axotomy. Androgens exert trophic action in laryngeal motor nucleus of Xenopus laevis. Testosterone is linked to an increase in neuron somal size, neuritic growth, plasticity and synaptogenesis in both motoneurons of the spinal nucleus of the bulbocavernosus and several populations of pelvic autonomic neurons. The hormone reduced the extent of spinal cord damage in vitro. There are also evidences against the neuroprotective action of testosterone. Testosterone does not protect against methamphetamine-induced neurotoxicity of the dopaminergic system in mice and does not provide significant neuroprotection against glutamate-induced neurotoxicity. Androgens do not prevent striatal dopamine depletion induced by 1-methyl-4-phenyl- -1,2,3,6-tetrahydropyridine (MPTP) in mice. Although the role of testosterone in the CNS is still poorly understood, accumulating evidence suggests that testosterone may create a future treatment for MCI and related cognitive diseases, including dementia and may influence motor neuron regeneration in adulthood. Androgen replacement therapy in selected male populations may hold therapeutic promise for the prevention and/or treatment of age-related disorders associated with neuronal injury.

- Carnahan R, Perry P. Depression in aging men. *Drugs Aging.* 2004;21(6):361-376

  Age-related decline in testosterone levels is associated with a number of mild, nonspecific symptoms, including depressive symptoms. The relationship between depressive symptoms and testosterone levels is confounded by numerous factors, including medical illness, obesity, smoking, alcohol use, diet and stress, and is thus complex. Studies have not consistently supported an integral role of reduced

testosterone levels in major depressive disorder, although levels may often be reduced in men with treatment-refractory depression and older men with dysthymia. Low testosterone levels may also increase the risk of incident depression in older males, although this may depend upon androgen receptor genetic polymorphisms. Testosterone replacement has demonstrated short-term tolerability and efficacy in augmenting antidepressants to alleviate treatment-refractory depression in adult males. Case studies support the potential need for maintenance therapy to maintain response. In a placebo-controlled trial, testosterone monotherapy was not effective in treating major depressive disorder in men with hypogonadism. However, in an open-label, noncomparative study, testosterone monotherapy appeared effective in treating late-onset but not early-onset major depressive disorder in older males. Testosterone therapy is not without potential for adverse effects, the most worrisome of which is the worsening of pre-existing prostate carcinoma. Oral, short- and long-acting parenteral, and transdermal patch and gel formulations are available. Testosterone has demonstrated usefulness in the treatment of a number of depressed populations, but further studies are needed to fully elucidate its role in the treatment of depressive syndromes in the aging male.

- Carruthers M. The paradox dividing testosterone deficiency symptoms and androgen assays: a closer look at the cellular and molecular mechanisms of androgen action. *J Sex Med*. 2008;5:998–1012.

  Central to the diagnosis and treatment of testosterone deficiency syndrome in the adult male is the remarkable paradox that there is a very poor correlation between the characteristic symptoms and levels of serum androgens. Aim. Because androgen deficiency can be associated with severe symptomatology, as well as diverse conditions such as coronary heart disease, diabetes, and metabolic syndrome, the aim was to present an evidence-based working hypothesis to resolve this confusing clinical paradox. Methods. A review of the possible mechanisms in testosterone deficiency syndrome was carried out, and a hypothesis to explain this paradox and associated problems in the diagnosis and clinical management of androgen deficiency was established on the basis of a review of the literature. Main Outcome Measures. The mechanisms by which androgen deficiency could arise were studied at five different levels: 1. Impaired androgen synthesis or regulation. 2. Increased androgen binding. 3. Reduced tissue responsiveness. 4. Decreased androgen receptor activity. 5. Impaired transcription and ranslation. Results. As with insulin in maturity onset diabetes mellitus, there can be both insufficient production and variable degrees of resistance to the action of androgens operating at several levels in the body simultaneously, with these factors becoming progressively worse with aging, adverse lifestyle, other disease processes, and a wide range of medications. Conclusions. Using this model, androgen deficiency can be redefined as an absolute or relative deficiency of androgens or their metabolites according to the needs of that individual at that time in his life. There are important ways in which the considerations raised by this hypothesis affect the etiology, terminology, diagnosis, and treatment of androgen-deficient states.

- Fargo K, Foecking E, Jones K, et al. Neuroprotective actions of androgens on motoneurons. *Frontiers in Neuroendocrinology* 2009; 30:130-141.

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Androgens have a variety of protective and therapeutic effects in both the central and peripheral nervous systems. This article reviews these effects as they related specifically to spinal and cranial motoneurons. Early in development, androgens are critical for the formation of important neuromuscular sex differences, decreasing the magnitude of normally occurring cell death in select motoneuron populations. Throughout the lifespan, androgens also protect against motoneuron death caused by axonal injury. Surviving motoneurons also display regressive changes to their neurites as a result of both direct axonal injury and loss of neighboring motoneurons. Androgen treatment enhances the ability of motoneurons to recover from these regressive changes and regenerate both axons and dendrites, restoring normal neuromuscular function. Androgens exert these protective effects by acting through a variety of molecular pathways. Recent work has begun to examine how androgen treatment can interact with other treatment strategies in promoting recovery from motoneuron injury.

- Moffat SD, Zonderman AB, Metter EJ, et al. Free testosterone and risk for Alzheimer's disease in older men. *Neurology* 2004;62:188-193

- Hogervorst E, et al. Low free testosterone is an independent risk factor for Alzheimer's disease. *Exp Gerontol* 2004;39(11-12):1633-9.

- Margolese HC. The male menopause and mood: testosterone decline and depression in the aging male—is there a link? *J Geriatr Psychiatry Neurol* 2000;13(2):93-101 .

- Muller M, Aleman A, Grobbee DE, et al. Endogenous sex hormone levels and cognitive function in aging men: is there an optimal level? *Neurology*. 2005;8:64(5):866-71.

  Testosterone and estradiol levels were measured and correlated with cognitive function. Higher testosterone levels (but not estradiol levels) were associated with better cognitive performance in the oldest age category.

- Moffat SD, Zonderman AB, Metter EJ, et al. Free testosterone and risk for Alzheimer disease in older men. *Neurology*. 2004;62:188-93.

- Okun MS; McDonald WM; DeLong MR. Refractory nonmotor symptoms in male patients with Parkinson disease due to testosterone deficiency: a common unrecognized comorbidity. *Arch Neurol* 2002; 59(5):807-11.

- Okun MS. Beneficial effects of testosterone replacement for the nonmotor symptoms of Parkinson disease. *Arch Neurol* 2002;59(11)1750-3.

- Ready RE. Testosterone deficiency and apathy in Parkinson's disease: a pilot study. *J Neurol Neurosurg Psychiatry* 2004;;75(9):1323-6.

- Rubinow DR, Schmidt, PJ. Androgens, brain, and behavior. *Am J Psychiatry* 1996; 153:974-984.

- Sicotte N, Giesser B, Tandon V, et al. Testosterone treatment in Multiple Sclerosis: A Pilot Study. *Arch Neurol.* 2007;64:683-688.

- Tan RS. A pilot study on the effects of testosterone in hypogonadal aging male patients with Alzheimer's disease. *Aging Male*. 2003; 6(1):13-7.

- Wang C, Alexander G, Berman N. Testosterone replacement therapy improves mood in hypogonadal men: a clinical research center study. *J Clin Endocrinol Metab.* 1996;81:3578- 3583.

- Wang C, Swedloff RS, Iranmanesh A, et al. Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. Testosterone Gel Study Group. *J Clin Endocrinol Metab* 2000;85(8):2839-53.

- Zonderman AB. Predicting Alzheimer's disease in the Baltimore longitudinal study of aging. *J Geriatr Psychiatry Neurol.* 2005;18(4):192-5.

This study initiated studies of dementia in the 1980s. This work suggested that hormone replacement and use of nonsteroidal anti-inflammatory drugs reduced the risk of Alzheimer's disease and that risk for Alzheimer's disease could be predicted from cognitive performance as many as 20 years prior to its onset. More recently, we showed that premorbid levels of free testosterone were lower in men who developed Alzheimer's disease and premorbid depressive symptomatology was a risk for Alzheimer's disease in men but not women as many as 6 years before the onset of dementia.

Androgens & Immune Function

- Khorram O, Vu L, Yen SS. Activation of immune function by dehydroepiandrosterone (DHEA) in age-advanced men. *J Gerontol A Biol Sci Med Sci* 1997 Jan;52(1):M1-M7.

Substantial data from animal studies have demonstrated a stimulatory effect of dehydroepiandrosterone (DHEA) on immune function. However, little is known about the effects of DHEA on the human immune system. Since aging is associated with a decline in immune function and in DHEA production, the authors proposed that oral administration of DHEA to elderly men would result in activation of their immune system. Nine healthy age-advanced men (mean age of 63 years) with low DHEA-sulfate levels participated in this study. They were treated nightly with an oral placebo for 2 weeks followed by DHEA (50 mg) for 20 weeks. When compared with placebo, DHEA administration resulted in a 20% increase in serum IGF-I, a decreasing trend in IGFBP-I, and a 32% increase in the ratio of IGF-I/IGFBP-I. Conclusion was that administration of oral DHEA at a daily dose of 50 mg to age-advanced men with low serum DHEAS levels significantly activated immune function. The mechanism(s) to account for the immunoenhancing properties of DHEA are unclear. Consideration is given to the potential role of an increase in bioavailable IGF-I, which by virtue of its mitogenic effects on immune cell function, may mediate the DHEA effects. While extended studies are required, these findings suggest potential therapeutic benefits of DHEA in immunodeficient states.

Subcutaneous Hormone Pellets in General

- Greenblatt R, Suran R. Indications for hormonal pellets in the therapy of endocrine and gynecic disorders. *Am J of Obstet and Gynec* 1949;57:294-301.

Research & Articles Regarding Estradiol & Testosterone Implants in Women

- Anderson CHM, Raju KS, Forling ML, Wheeler MJ. The effects of surgical menopause and parenteral hormone replacement therapy on bone density, menopausal symptoms, and hormone profiles. Department of Gynaecology, St. Thomas Hospital, London, UK, 1997.

  45 women undergoing complete hysterectomies were randomized to receive 50-mg estradiol implants, 50-mcg estradiol patches, or 50-mg estradiol and 100-mg testosterone implants. After one year, there was a significant decrease in bone density in the patch group; no decrease in bone density in the pellet implant groups.

- Barlow DH, Abdalla HI, Roberts DG, et al. Long-term hormone implant therapy – hormonal and clinical effects. *Obstet Gynecol* 1986; 67:321.

  75 women were given 50-mg estradiol (N=36) or 50-mg estradiol plus 100-mg testosterone (N=39) implants every 6 months for 3 years. Both groups had effective menopausal symptom improvement. Estradiol levels in both groups at 3 years were higher than baseline due to accumulation of implanted estradiol. In addition, testosterone levels were higher with each implantation due to accumulation of testosterone. There was no significant weight gain in either treatment group. Liver function and blood pressure did not change in either group. Bone density significantly increased in the ET arm, whereas the E-only arm maintained bone density.

- Brincat M, Versi E, Moniz CF, et al. Skin collagen changes in postmenopausal women receiving different regimens of estrogen therapy. *Obstet Gynecol* 1987; 70:123.

- Brincat M, Kabalan S, Studd JW, et al. A study of the decrease of skin collagen content, skin thickness, and bone mass in the menopausal woman. *Obstet Gynecol* 1987; 70:840.

- Brincat M, Muscat Baron Y, Galea R. Estrogens and the skin. *Climacteric* 2005; 8:110-123.

- Brincat M, Studd JW, O'Dowd T, et al. Subcutaneous hormone implants for the control of climacteric symptoms. *The Lancet* 1984;16-18.

  55 menopausal women were treated with either 50-mg estradiol and 100-mg testosterone pellets or placebo. All symptoms (hot flushes, heart palpitations, headaches, irritability, lack of concentration, insomnia, depression, dyspareunia, loss of libido, urethral syndrome, and lethargy) improved in the treatment arm; no symptoms improved in the placebo arm. The only symptom that did not improve in either arm was "aches and pains".

- Buckler HM, Kalsi PK, Cantrill JA, Anderson DC. An audit of oestradiol implants and implant frequency in women undergoing subcutaneous implant therapy. *Maturitas* 1985;22:263.

- Burger HG, Hailes J, Menelaus M, et al. The management of persistent menopausal symptoms with oestradiol-testosterone implants: clinical, lipid, and hormonal results. *Maturitas* 1984;6:351-58.

  17 patients were treated with combined subcutaneous implants of oestradiol (40 mg) and testosterone (100 mg), because oral oestrogens had not provided adequate symptomatic relief, particularly of decreased libido. There were significant improvements in libido, enjoyment of sex and tiredness, and in lack of concentration, but there was no significant change in flushes, sweats and depression. Based on an analogue scale, libido increased from a mean basal score of 13.5 to a maximum of 86.1 at 3 mth. Symptomatic improvement was maintained for 4-6 mth. There were no significant changes in total serum cholesterol and triglycerides nor in cholesterol subfractions. The authors concluded that the hormonal implants provided substantial symptomatic relief, particularly of loss of libido, while causing rises to mid-follicular concentrations of oestradiol and maximal testosterone levels about three times normal, without significant effects on plasma lipids.

- Cardozo L, Gibb D, Tuck S, et al. The effects of subcutaneous hormone implants during the climacteric. *Maturitas* 1984;5:177-184.

  This study included 120 women with a total of 469 hormonal implants of 50-mg estradiol and 100-mg testosterone implants over four years. Patients with a uterus were given an oral progestogen. Hot flushes were improved in 100%; depression in 99%; and loss of libido in 92%.

- Chu M, Lobo R. Formulations and use of androgens in women. *Mayo Clin Proc* 2004;79 (Supplement).

- Cravioto M, Larrea F, Delgado N, et al. Pharmacokinetics and pharmacodynamics of 25-mg estradiol implants in postmenopausal Mexican women. *Menopause;*8(5):353-360.

- Cronje WH, Vashisht A, Studd JW. Hysterectomy and bilateral oopherectomy for severe premenstrual syndrome. *Human Reproduction* 2004;19(9):2152-2155.

- Davelaar EM, Gerretsen G, Relyveld J. [No increase in the incidence of breast carcinoma with subcutaneous administration of estradiol.] *Ned Tijdschr Geneeskd* 1991;135(14):613-5.

  Between 1972 and mid-1990 the frequency of breast cancer was studied in a group of 261 mostly premenopausal women of the gynaecological department of the Municipal Hospital in The Hague, the Netherlands. All patients had had a total hystero-adnexectomy and received estradiol implants. On the basis of a stratified life table giving

the cumulative incidence of breast cancer in the Netherlands, an expected incidence of 2 per 1000 person-years was estimated for the observed group (mean observation period: 8.25 years). There were three cases of breast cancer in the observed group. This means an incidence density of 1.4 per 1000 person-years. It is concluded that this form of oestrogen substitution does not increase the risk of breast cancer.

- Davis S, Walker K, Strauss B. Effects of estradiol with and without testosterone on body composition and relationships with lipids in postmenopausal women. *Menopause* 2000;7(6):395-401.

  33 postmenopausal women were randomized to receive either 50-mg estradiol implants or 50-mg estradiol and 50-mg testosterone every 3 months for 2 years (women with an intact uterus were given cyclic oral progestins). Women were not re-inserted with estradiol or testosterone implants if levels were high at the time of reinsertion. 32 women completed the study (17 in E group; 15 in E&T group). Neither group experienced weight gain, although the E & T group had higher fat free mass at 2 years. Both groups had lower total and LDL cholesterol levels.

- Davis S. Androgen treatment in women. *MJA* 1999;170:545-9.

- Davis S, Burger H. Androgens and the postmenopausal woman. *J Clin Endocrin Metab* 1996;81(8):2759-2763.

  This paper is an excellent review of androgens in postmenopausal women. It discusses the role of androgens in women, and the decline of ovarian and adrenal androgens and pre-androgens that can precede menopause by a decade. It also discusses the potential significant impact this decline can have on women's health. The authors conclude that side effects for androgen replacement (including testosterone subcutaneous implants) in symptomatic women are rare if patients are properly monitored.

- Davis S, McCloud P, Strauss B, et al. Testosterone enhances estradiol's effects on postmenopausal bone density and sexuality. *Maturitas* 1995;227-236.

  This prospective, 2 year, single-blind, randomized trial evaluated bone mineral density (BMD) in 34 postmenopausal women who received either 50-mg estradiol implants, or 50-mg estradiol and 50-mg testosterone implants every 3 months for 2 years. E plus T was more effective at improving BMD and libido than E alone.

- Dimitrikakis C, Jones R, Liu A, Bondy L. Breast cancer incidence in postmenopausal women using testosterone in addition to usual hormone therapy. *Menopause* 2004;11(5):531-5.

  This study looked at 508 patients who received 50 to 150 mg testosterone implants (dosage titrated to relieve symptoms and improve bone density and to minimize adverse effects – mean dosage 100-mg) in additional to usual hormone replacement in Australia. Average age at start of study was 56.4 years, and mean duration of follow-up was 5.8 years. Breast cancer incidence in testosterone users was close to that reported for hormone therapy never-users, suggesting that the addition of testosterone to

conventional hormone therapy for postmenopausal women does not increase the risk of breast cancer. Because users of HRT are expected to have an increased risk, testosterone supplementation may reduce hormone therapy-associated breast cancer risk.

- Dimitrikakis C, Zhou J, Wang J, et al. A physiologic role for testosterone in limiting estrogenic stimulation of the breast. *Menopause*;10(4)292-8.

- Dow M, Hart D, Forrest C. Hormonal treatments of sexual unresponsiveness in postmenopausal women: a comparison study. *Br J of Ob & Gyn* 1983;90:361-6.

- Gambrell RD, Natrajan PK. Moderate dosage estrogen-androgen therapy improves continuation rates in postmenopausal women: impact of the WHI reports. *Climacteric* 2006;9:224-233.

  This paper looked at the continuation rates for hormone replacement in 814 menopausal women. During the 3 years of observation, 85% of women continued HRT More than 87% of these women used estradiol and testosterone implants; the remaining women used injectables, patches, or oral hormones. Continuation rates for pellet implant users were 96.7% for 10 years and 88.8% for 20 year, suggesting a high degree of satisfaction with pellet implants. Pellet dosage ranged from 25 to 75-mg estradiol & 75 to 150-mg testosterone. The majority of women received pellets every 4 ½ to 6 months.

- Garnett T, Studd J, Watson N, et al. The effects of plasma estradiol levels on increases in vertebral and femoral bone density following therapy with estradiol and estradiol with testosterone implants. *Obstet Gynecol* 1992;79:968-72.

- Garnett T, Studd J, Watson N, et al. A cross-sectional study of the effects of long-term percutaneous hormone replacement therapy on bone density. *Obstet Gynecol* 1991;78:1002-1007.

- Glaser R, Kalantaridou S, Dimitrakakis C. Testosterone implants in women: pharmacological dosing for physiologic effect. *Maturitas*. 2013;74:179-84.

  The objectives of this study were to determine therapeutic serum testosterone (T) levels/ ranges and inter-individual variance in women treated with subcutaneous T implants. Study design: In study group 1, T levels were measured at two separate time intervals in pre- and postmenopausal women treated with subcutaneous T for symptoms of androgen deficiency: (i) four weeks after pellet insertion, and (ii) when symptoms of androgen deficiency returned. In a separate pharmacokinetic study (study group 2), 12 previously untreated postmenopausal women each received a 100 mg T implant. Serum T levels were measured at baseline, 4 weeks and 16 weeks following T pellet implantation. In study 'group' 3, serial T levels were measured throughout a 26 h period in a treated patient. Results: In study group 1, serum T levels measured at 'week 4' (299.36 ± 107.34 ng/dl, n = 154), and when symptoms returned (171.43 ± 73.01 ng/dl, n = 261), were several-fold higher compared to levels of endogenous T. There was significant inter-individual variance in T levels at 'week 4' (CV 35.9%) and when symptoms returned (CV 42.6%). Even with identical dosing (study group 2), there was

significant inter-individual variance in T levels at 'week 4' (CV 41.9%) and 'week 16' (CV 41.6%). In addition, there was significant intra-individual circadian variation (CV 25%). Conclusions: Pharmacologic dosing of subcutaneous T, as evidenced by serum levels on therapy, is needed to produce a physiologic effect in female patients. Safety, tolerability and clinical response should guide therapy rather than a single T measurement, which is extremely variable and inherently unreliable.

- Goel N. Hormone replacement therapy part I: prescribing HRT – recent trends. file:///D|/hormone/data/Pellets Hormone Implants/Goel Dose India E 50 25 older T 100.htm (1 of 11)3/11/2006 .

- Holland EF, Leather AT, Studd JW. The effect of 25-mg percutaneous estradiol implants on the bone mass of postmenopausal women. *Obstet Gynecol* 1994;83:43-6.

- Hunter D, Akande E, Carr P, Stallworthy J. The clinical and endocrinological effect of oestradiol implants at the time of hysterectomy and bilateral salpingo-oophorectomy. *Obstet Gynecol* 1973;80:827-833.

- Kapetanakis E, Dmowski W, Auletta F, et al. Endocrine and clinical effects of estradiol and testosterone pellets used in long-term replacement therapy. *Int J Gynaecol Obstet* 1982;20:387-99.

- Khastgir G, Studd JW, Fox SW, et al. A longitudinal study of the effect of subcutaneous estrogen replacement on bone in young women with Turner's syndrome. *J Bone Mineral Res* 2003;(5):925-32.

- Khastgir G, Studd J, Holland N. Anabolic effect of estrogen replacement on bone in postmenopausal women with osteoporosis: histomorphometric evidence in a longitudinal study. *J Clin Endocrinol Metab* 2001;86:289-295.

- Khastgir G, Studd J. Patient's outlook, experience, and satisfaction with hysterectomy, bilateral oophorectomy, and subsequent continuation of hormone replacement therapy. *Am J Obstet Gynecol* 2000;183(6):1427-33.

- Kuhl H. Pharmacology of estrogens and progestogens: influence of different routes of administration. Climacteric 2005;8(Suppl 1):3-63.

  This is a comprehensive review of the pharmacokinetics and pharmacodynamics of natural and synthetic estrogens and progestogens used in contraception and HRT. The paper describes the mechanisms of action, the relation between structure and hormonal activity, differences in hormonal pattern and potency, peculiarities in the properties of certain steroids, tissue-specific effects, and the metabolism of the available estrogens and progestogens. The influence of the route of administration on pharmacokinetics, hormonal activity and metabolism is presented, and the effects of oral and transdermal treatment with estrogens on tissues, clinical and serum parameters are compared. The effects of oral, transdermal (patch and gel), intranasal, sublingual, buccal, vaginal, subcutaneous (pellets) and intramuscular administration of estrogens, as well as of oral,

vaginal, transdermal, intranasal, buccal, intramuscular and intrauterine application of progestogens are discussed.

- Lobo R. Androgens in postmenopausal women: production, possible role, and replacement options. *Obstet & Gynecol* 2001;56:361-376.

- Lobo R, March C, Goebelsmann U, et al. Subdermal estradiol pellets following hysterectomy and oophorectomy. Obstet & Gynecol 1980;138:714-9.

  This study looked included 22 women (ages 29-50 years) who received 25-mg estradiol pellets after complete hysterectomy. Serum estradiol levels remained steady in the follicular range, HDL cholesterol levels increased, and women remained symptom-free for 5-6 months after insertion. The estradiol to estrone ration remained >1 (as it is in ovulatory, menstruating women), unlike with oral ERT. The authors conclude that "estradiol pellets are an effective form of parenteral ERT and offer both practical and theoretical advantages over forms of ERT."

- Loeser A. Mammary carcinoma response to implantation of male hormone and progesterone. *The Lancet* 1941:698-700.

- Magos A, Zilkha K, Studd K. Treatment of menstrual migraines by oestradiol implants. J of *Neurology, Neurosurgery, and Psychiatry* 1983;46:1044-46.

  24 women with menstrual migraines were given estradiol pellets for up to 5 years. 23 of the women improved, and 20 (83%) became completely or almost completely headache-free. The results support the theory that estrogen withdrawal in the late luteal phase can precipitate migraines, and that preventing hormonal fluctuations with estradiol implants can prevent them.

- Mishell D. *A* clinical study of estrogenic therapy with pellet implantation. *Obstet Gynecol* 1941;41:1009-1017.

- Montgomery J, Brincat M, Tapp A, et al. Effect of oestrogen and testosterone implants on psychological disorders in the climacteric. *The Lancet* 1987:297-299.

  Double-blind, placebo-controlled trial assessing psychological symptoms involving 3 treatment groups of peri and postmenopausal women (N=70): 50-mg estradiol and 100-mg testosterone implants, 50-mg estradiol implant only, or placebo. Depression and anxiety were significantly lower in the implant treated groups.

- Nagamani M, Lin T, McDonough P, et al. Clinical and endocrine studies in menopausal women after estradiol implantation. *Obstet Gynecol* 1977;50:541-547.

- Naessen T. Maintained bone density at advanced ages after long term treatment with low dose oestradiol implants. *Br J Obstet Gynecol* 1993;100: 454-459.

  35 women receiving 20-mg estradiol pellets were compared with age-matched controls. Bone densities in the forearm, spine, and hip were 20-25% higher in women with estradiol pellets.

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- Natrajan P, Gambrell D. Estrogen replacement therapy in patients with early breast cancer. *Obstet Gynecol* 2002;187:289-95.

  This study looked at 123 early breast cancer patients. Most patients received estradiol pellets, testosterone pellets, or both. Neither estradiol nor testosterone pellets increased the risk of recurrence or death in these patients.

- Natrajan P, Soumakis K, Gambrell D. Estrogen replacement therapy in women with previous breast cancer. *Obstet Gynecol* 1999;181:288-295.

  This review discusses how testosterone supplementation (pellet or methyl) plus ERT improves bone density to a greater extent than ERT alone.

- Nezhat C, Karpas A, Greenblatt R, et al. Estradiol implants for conception control. *Obstet Gynecol* 1980;138:1151-1156.

- Notelovitz M, Johnston M, Smith S, et al. Metabolic and hormonal effects of 25-mg and 50-mg 17 beta-estradiol implants in surgically menopausal women. *Obstet Gynecol* 1987;70:749.

  This study included 12 surgically menopausal women. Results showed that estradiol implants improved bone density without any adverse cardiovascular side effects.

- Notelovitz M. Androgen effects on bone and muscle. *Fertility & Sterility* 2002;77(Suppl 4):S34-41.

- Oettinger M, Barak S, et al. Subcutaneous implantation of pure crystalline estradiol pellets for conception control. *Gynecol Obstet Invest* 2005;59:119-125.

- Owen E, Siddle N, McGarrigle H, et al. 25-mg oestradiol implants – the dosage of first choice for subcutaneous oestrogen replacement therapy? *Br J Obstet Gynaecol* 1992;99:671-75.

- Panay N, Versi E, Savvas M. A comparison of 25 and 50-mg oestradiol implants in the control of climacteric symptoms following hysterectomy and bilateral salpingo-oophorectomy. *Br J Obstet Gynaecol* 2000;107:1012-1016.

  This double-blind, randomized trial of 44 women showed that 25-mg and 50-mg estradiol pellets were equally effective at controlling menopausal symptoms. There was no difference in duration of effectiveness between the two dosages.

- Panay N, Zamblera D, Sands R, et al. Low dose 25 mg oestradiol implants and 1 mg norethisterone as continuous combined hormone therapy: a prospective study. *BJOG* 2002;109:958-960.

- Pereda C, Hannon R, Naylor K, et al. The impact of subcutaneous oestradiol implants on biochemical markers of bone turnover and bone mineral density in postmenopausal women. *BJOG* 2002;109:812-820.

- Pirwany I, Sattar N, Greer I, et al. Supraphysiological concentrations of estradiol in menopausal women given repeated implant therapy do not adversely affect lipid profiles. *Human Reproduction* 2002;17:825-829.

- Purdie DW, Ballard PA, Wahab M, Cooper A. Bone mineral density (BMD) at lumbar spine and femoral neck in hysterectomized women treated with chronic oestradiol implantation.

- Rufford J, Hextall A, Cardozo L, et al. A double-blind placebo-controlled trial on the effects of 25 mg estradiol implants on the urge syndrome in postmenopausal women. *International Urogynecology Journal and Pelvic Floor Dysfunction* 2003;14(2):78-83.

- Sands R, Studd J, Seed M, et al. The effects of exogenous testosterone on lipid metabolism and insulin resistance in postmenopausal women.

- Savvas M, Studd J, Fogelman I, et al. Skeletal effects of oral oestrogen compared with subcutaneous oestrogen and testosterone in postmenopausal women. *BMJ* 1988;297:331-333.

  Results of this study showed that estradiol implants were more effective at increasing bone density than oral ERT.

- Savvas M, Studd J, Norman S, et al. Increase in bone mass after one year of percutaneous oestradiol and testosterone implants in post-menopausal women who have previously received long-term oral estrogens. *Br J of Ob & Gyn*1992;99:757-760.

- Seeds M, Sands R, McLaren M, et al. The effect of hormone replacement therapy and route of administration on selected cardiovascular risk factors in postmenopausal women. *Family Practice* 2000;17(6):497-507.

- Servy EJ, Bryner JR, Scholer J. Effects of subcutaneous estradiol implants after oopherectomy. *Advances is Contraceptive Delivery Systems* 1991;2:1-19.

- Somboonporn W, Davis S. Postmenopausal testosterone therapy and breast cancer risk. *Maturitas* 2004;49:267-275.

  This paper evaluated experimental and epidemiological studies pertaining to the role of testosterone in breast cancer. Main outcome measured were mammary epithelial proliferation, apoptosis and breast cancer. Results: In experimental studies, testosterone action is anti-proliferative and pro-apoptotic, and mediated via the AR, despite the potential for testosterone to be aromatized to estrogen. Animal studies suggest that testosterone may serve as a natural, endogenous protector of the breast and limit mitogenic and cancer promoting effects of estrogen on mammary epithelium. In premenopausal women, elevated testosterone is not associated with greater breast cancer risk. The risk of breast cancer is also not increased in women with polycystic ovary syndrome who have chronic estrogen exposure and androgen excess. However, in postmenopausal women, who are oestrogen deplete and have increased adipose aromatase activity, higher testosterone has been associated with greater breast cancer

risk. Conclusion: Available data indicate the inclusion of testosterone in estrogen–progestin regimens has the potential to ameliorate the stimulating effects of hormones on the breast. However, testosterone therapy alone cannot be recommended for estrogen deplete women because of the potential risk of enhanced aromatization to estrogen in this setting.

- Staland B. Treatment of menopausal oestrogen deficiency symptoms in hysterectomised women by means of 17-B-oestradiol pellet implants. *Acta ObGyn Scand* 1978;57:281-85.

  94 women were treated with subcutaneous estradiol implants (20-mg) for menopausal symptoms (589 implantations total). Women reported very good resolution of symptoms with only 2 patients reporting unsatisfactory results regarding sweating and hot flushes. Many patients had previously used other forms of ERT and nearly all preferred pellet implantation.

- Stanczyk F. Editorial: parenteral versus oral treatment of postmenopausal women with estrogen. *Menopause* 2007 14(6)968-70.

- Stanczyk F, Shoupe D, Nunez V, et al. A randomized comparison of non-oral estradiol delivery in postmenopausal women. *Am J ObGyn* 1988;159:1540-6.

- Studd JW. The dose response of per-cutaneous oestradiol implants on the skeletons of postmenopausal women. *Br J ObGyn* 1994;101:787-791.

- Suhonen SP, Sipinen S, Lahteenmaki P, et al. Postmenopausal oestrogen replacement therapy with subcutaneous estradiol implants. *Maturitas* 1993;16:123-131.

- Suhonen SP, Lahteenmaki P, Rauramo I. Sustained-release estradiol implants in HRT: one year results on hormone levels and menopausal symptoms. Steroid Research Laboratory, Institute of Biomedicine, University of Helsinki 1997.

- Thom M, Collins WP, Studd JW. Hormonal profiles in postmenopausal women after therapy in subcutaneous implants. British *J of Obstetrics and Gynaecology* 1988;88:426-433.

- Vedi S, Purdie W, Ballard P, et al. Bone remodeling and structure in postmenopausal women treated with long-term, high-dose estrogen therapy. *Osteoporosis Int* 1999;10:52-58.

- Worboys S, Kotsopoulos D, Teede H, et al. Evidence that parenteral testosterone therapy may improve endothelium-dependent and independent vasodilation in postmenopausal women already receiving estrogen. *J Clin Endocrinol Metab* 2001;86:158-61.

  In this study, 33 women received 50-mg testosterone pellets in addition to HRT; 15 women received HRT only. Six weeks after implantation, the treated group had improved

endothelium-dependent (flow-mediated) and endothelium-independent (glyceryl trinitrate-mediated) brachial artery vasodilation.

Research and articles regarding testosterone pellet implants in men

- Brady BM, Waltoni M, Hollowi N, et al. Depot testosterone with etonogestrel implants result in induction of azoospermia in all men for long-term contraception. *Human Reproduction* 2004 19(11):2658–2667.

- Cantrill JA, Dewis P, Large DM, et al. Which testosterone replacement therapy? *Clin Endocrinol (Oxf)* 1984; 21:97-107.

- Conway A, Boylin L, Howe C, et al. Randomized clinical trial of testosterone replacement therapy in hypogonadal men. *Int J Andrology* 1988;11:247-264.

  15 men were given 3 treatment periods, each separated by a washout period. The treatments included IM injections of testosterone esters every 2 weeks, oral testosterone undecanoate, and subcutaneous testosterone pellets. Pellet implants produced the most prolonged, elevated total and free testosterone levels for up to 4 months. The authors concluded that pellet implants gave the closest approximation to steady-state, physiological delivery of the methods tested.

- Dunning T, Ward G. Testosterone replacement therapy – perceptions of participants and partners. *Issues and Innovations in Nursing Practice* 2004:467-74.

  This study evaluated sense of well-being and sexual function in 10 men receiving testosterone pellet implants, 5 with partners and 5 without partners. Decreased testosterone levels had a statistically significantly different effect on libido at time zero between men with and without partners and on ability to sustain an erection, but the ability to achieve an erection persisted over the 6 months in both male groups.

- Gooren L. New long-acting androgens. *World J Urol* 2003;21:306-10.

  This article acknowledges the major goal of testosterone replacement therapy is to replace testosterone levels at as close to physiological concentrations as is possible. General agreements about such an androgen replacement therapy are (1) a delivery of the physiological amount of testosterone (3-10 mg/d); (2) consistent levels of testosterone, 5a-dihydrotestosterone (DHT) and 17b-estradiol (E2) within normal physiological ranges; (3) a good safety profile without adverse effects on the prostate, serum lipids, liver or respiratory function; and (4) convenience in usage, patient-friendly, with a relative independence of medical services. The article discusses that pellets replicate the daily dosage of testosterone production in eugonadal men, with consistent levels for 4-6 months after implantation.

- Gooren L, Bunk M. Androgen replacement therapy: present and future. *Drugs* 2004;64(17):1861-1891.

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- Hair W, Wu F, Lincoln G. An investigation of the effectiveness of testosterone implants in combination with the prolactin inhibitor quinagolide in the suppression of spermatogenesis in men. *Human Reproduction* 2003;18(4):749-755.

- Handelsman DJ, Mackey MA, Howe C, et al. An analysis of testosterone implants for androgen replacement therapy. *Clin Endocrinol (Oxf)* 1997; 47: 311-6

  Review of 13 years of experience (220 men, 973 implant procedures) using testosterone pellets in order to identify pattern of usage, including continuation rates, and adverse events, including extrusion. Bleeding, infection, and fibrosis were rare; extrusion was related to work or procedure problems. Overall continuation rate with pellets increased with duration of use, 88% after the first implantation, 95% after the third.

- Handelsman DJ, Conway AJ, Howe CJ, et al. Establishing the minimum effective dose and additive effects of depot progestin in suppression of human spermatogenesis by a testosterone depot. *J Clin Endocrinol Metab* 1996;81:4113-4121.

- Handelsman DJ, Conway AJ, Boylan LM. Suppression of human spermatogenesis by testosterone implants. *J Clin Endocrinol Metab* 1992;175:1326-1332.

- Handelsman DJ, Conway AJ, Boylan LM. Pharmacokinetics and pharmacodynamics of testosterone pellets in man. *J Clin Endocrinol Metab* 1990;71:216-222.

  Pharmacokinetics and pharmacodynamics of subcutaneous testosterone pellets were compared in this prospective, cross-over clinical trial. Plasma, free and total testosterone, SHBG, LH, and FSH were measured before and at monthly intervals for at least 6 months after 111 implantations in 43 men. Total and free testosterone levels were shown to peak at month one, and were maintained for 4-6 months depending on dosage. The authors conclude that testosterone pellets provide "very satisfactory depot androgen replacement exhibiting many desirable features for androgen replacement."

- Jockenhovel F, Blum W, Vogel E, et al. Testosterone substitution normalizes elevated serum leptin levels in hypogonadal men. *J Clin Endocrinol Metab* 1997;82:2510-2513.

- Jockenhovel F, Vogel E, Kreutzer M, et al. Pharmacokinetics and pharmacodynamics of subcutaneous testosterone implants in hypogonadal men. *Clin Endocrinol (Oxf)* 1996;45:61-71.

  50 men received testosterone implants for a total of 112 implantations. The only side effect noted was extrusion of pellets in 3 men. When given the choice, all patients except one preferred testosterone pellets to previous testosterone replacement method The authors conclude that testosterone pellets are the androgen formulation with the longest biological action and strongest pharacodynamic efficacy in terms of gonadotrophin suppression. The pharmacokinetic features are advantageous compared to other testosterone preparations and patient acceptance is high.

- Kelleher S, Howe C, Conway A, Handelsman. Testosterone release rate and duration of action of testosterone pellet implants. *Clin Endocrinol* 2004;60:420-428.

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Testosterone pellets are a highly effective subdermal depot administered at regular intervals with the timing individualized depending upon return of the patient's characteristic androgen deficiency symptoms. Yet the in vivo testosterone release rate and effective duration of action of these pellets has been little studied systematically. This study analyzed prospectively collected data from three randomized controlled clinical trials. Patients included androgen-deficient men (n = 136) undergoing long-term androgen replacement therapy with a standard dose (800 mg) of testosterone pellets implanted subdermally at intervals from 5 to 7 months. The loss of dry weight of intact pellets was strongly correlated with time in situ providing an estimate of daily testosterone release rate per 200 mg for the first 3 months. After 756 implantations of the standard dose, men return for re-implantation at 5.8 calendar months following no or only a single pellet extrusion. Testosterone pellet implants release testosterone at a steady rate of 1.3 mg/200 mg implant/day. The duration of action is about 6 months in an uncomplicated cycle.

- Kelleher S, Conway A, Handelsman D. A randomized controlled clinical trial of antibiotic impregnation of testosterone pellet implants to reduce extrusion rate. *European J Endocrinol* 2002;146:513-518.

- Kelleher S, Conway A, Handelsman D. Influence of implantation site and track geometry on the extrusion rate and pharmacology of testosterone implants. *Clin Endocrinol* 2001;55,:531-536.

- Kinniburgh D, Zhu H, Cheng L, et al. Oral desogestrel with testosterone pellets induces consistent suppression of spermatogenesis to azoospermia in both Caucasian and Chinese men. *Human Reproduction* 2002;17:1490-1501.

- Leichtnam M, Rolland H, Wuthrich P, et al. Testosterone hormone replacement therapy: state-of- the art and emerging technologies. *Pharmaceutical Research* 2006;23:1117-1132.

- Nieschlag E, Behre H, Bouchard P, et al. Testosterone replacement therapy: current trends and future directions. *Human Reproduction Update* 20-4;10:409-419.

- Oettel M. The endocrine pharmacology of testosterone therapy in men. *Naturwissenschaften.* 2004;91:66–76.

- Sader M, McCredie R, Griffiths K, et al. Oestradiol improves arterial endothelial function in healthy men receiving testosterone. *Clin Endocrinol* 2001;54:175-81.

- Schubert M, Bullmann C, Minnemann T, et al. Osteoporosis in male hypogonadism: responses to androgen stimulation differ among men with primary and secondary hypogonadism. *Hormone Research* 2003;60:21-28.

- Vest S, Howard J. Clinical experiments with androgens. *JAMA* 1939;113(21):1869-1872.

  This article was published in 1939 discussing the use of testosterone pellet implants and case studies of two hypogonadal males.

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- Wang C, Swerdloff R. Male contraception. *Best Practice and Research Clinical Obstetrics and Gynaecology* 2002;16(2):193-202.

- Zacharin M, Pua J, Kanumakala S. Bone mineral density outcomes following long-term treatments with subcutaneous testosterone pellet implants in male hypogonadism. Clin Endocrin 2003;58:691-95.

  37 men with primary or secondary hypogonadism received long-term (mean 6.6 yrs) subcutaneous testosterone pellet implants. Bone density for treated men was the same as age-matched men not needing treatment.The authors conclude that subcutaneous testosterone pellet implants are safe and acceptable to the patient, and result in adequate bone mass and maintenance of normal bone mineral density. They also surmise that sustained physiological levels of testosterone via pellets may contribute to increased androgen effect at the receptor level.

- Zacharin MR, Warne GL. Treatment of hypogonadal adolescent boys with long acting subcutaneous testosterone pellets. *Arch Dis Child* 1997; 76: 495-9.

  Long acting subcutaneous testosterone pellets are of proved efficacy for the treatment of hypogonadal men, but have not been reported as a treatment modality in adolescent boys. Pharmacodynamic studies of subcutaneous testosterone release have shown prolonged normalisation of testosterone levels for at least four months. Administration of a long acting, safe, effective, and convenient form of treatment is desirable when life-long treatment is indicated. In this study, 18 boys (aged 13.9-17.5 years at the start of treatment)-seven with primary hypogonadism, nine with secondary hypogonadism, and two boys being treated with testosterone for tall stature--were given testosterone pellets every six months for 18 months. Height, weight, pubertal status, and psychosocial parameters were assessed and follicle stimulating hormone, luteinising hormone, testosterone, prolactin, and lipids were measured at 0, 1, 3, 6, 12, and 18 months. Bone age was measured at 0 and 12 months. In all boys growth velocity continued appropriately for bone age. Puberty continued to progress in all boys and in two boys the amount of virilisation exceeded that seen with previous treatment with intramuscular testosterone. After testosterone administration, follicle stimulating hormone and luteinising hormone suppressed incompletely in the boys with primary hypogonadism. Serum testosterone ranged from 4.3 to 26.7 nmol/l at three months to less than 10 nmol/l at six months after implantation. Prolactin and lipid levels were normal throughout the study. By report, there was an improvement in mood and emotional wellbeing. No pellet extrusions occurred in a total of 156 pellet insertions. All boys preferred this mode of testosterone administration to intramuscular injections. Long acting subcutaneous testosterone pellets are safe, efficacious, well tolerated, and convenient, and result in normal physical growth and improved psychological outlook in adolescent hypogonadal boys.