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Chapter 42

The New Science of Anti-Aging Hormone Replacement Therapy: A Multidimensional Approach

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ABSTRACT

Since ancient times, humans have been concerned with developing and preserving youthful vigor, stopping the running age clock, and extending lifespan. Today there is a great progress in understanding the aging process in attempt to delay it. This presentation considers the main popular and easily obtainable hormones: estradiol, testosterone, DHEA, thyroid hormone, melatonin, growth hormone, and progesterone. Many of the benefits of using these hormones are equivocal thus far, but we are seeing an increasing number of studies which, at least, recommend these hormones as viable therapies to slow down the aging process, to stop the development age-related diseases, and to stay vital and fit in the second half of life.

Keywords: Estradiol; Testosterone; DHEA; Thyroid hormone; Melatonin; Progesterone

INTRODUCTION

At present, it appears that the only way of significantly extending human lifespan is with caloric restriction. However, there are a number of factors, which seemingly can improve lifespan.

In his presentation on behalf of The Endocrine Society and The Hormone Foundation, Dr Robert B Jaffe, the Fred Gellert Professor of Reproductive Medicine and Biology at the University of California, San Francisco, discussed the impact that recently released hormonal treatment studies, such as the Women's Health Initiative (WHI), have had on patients who take or are considering taking combined estrogen and progestin treatment.

"The new research that has come out over the past few months has caused distress and confusion for millions of women, and it has changed the way that doctors practice medicine when it comes to menopause," said Dr. Jaffe. "I believe that it is essential for the medical community to translate these new data so that patients can understand how this information will impact their healthcare and doctors will understand the best ways to treat postmenopausal patients in the future."

Jaffe also discussed the need to examine lifestyle issues, such as good nutrition, weight loss when appropriate, adequate exercise, no smoking and drinking in moderation as important for optimizing postmenopausal health, saying: "As we further examine lifestyle and postmenopausal health, I think that we will find that a healthy lifestyle may be as important as pharmaceuticals."

Following the results of the WHI and Heart and Estrogen/Progestin Replacement (HERS) Studies, both The Endocrine Society and The Hormone Foundation worked to communicate the findings to doctors and patients.

This article presents a new approach to an endocrinological treatment strategy, which should encompass the following key features:

- Individual
- Custom-attracted
- Secure and multidimensional

There has been a lot of progress since Charles Edouard Brown-Sequard transplanted young guinea pigs testes into old dogs and injected himself a mixture of crushed dog and pig testicles. The great advances in medicine in the last years can realize this idea for the near future. Women and men are increasingly interested in initiating a custom-attracted and individualized HRT. We can observe a change of paradigm in nearly all medical subject areas. The standard of an individualized therapy for our patients requires more and more comprehensive epidemiological investigations, meta analyses, and genetic analysis, which has been supported by the detection of the human genome.

HORMONE REPLACEMENT THERAPY

Sexual hormones are of essential importance for reproduction, and later in life for metabolism, the cardiovascular system, and the general well being of the women (see Table 1).. Menopause, which is characterized by a dramatic decrease in estrogen secretion, and is accompanied by diminishing progesterone and androgen levels, triggers a multiple loss of functions. Especially in regions of the brain (central vegetative neuronal system, psyche, libido, cognition, memory), the bone, the skin, connective tissue, peripheral vessels, and cardiovascular system. Ultimately, this causes alterations in those systems; these alterations can lead to an increase in oxidation and a weakened lipid metabolism, resulting in high cholesterol levels and an increased risk of Alzheimer's disease, myocardial infarction, and apoplexy. From this point of view, it seems sensible to consider a long-term HRT with progestins and estrogens.

Table 1. Women's Health Initiative Findings

Specific Risks - Specific Benefits	Overall Risks - Overall Benefits
↑ 29% cardiovascular diseases (MI) ↓ 37% colon cancer	↑ 22% total CVD events ↓ 24% total rate fractures
↑ 26% breast cancer ↓ 17% endometrial cancer	↑ 3% total cancer rate ↓ 2% total mortality
↑ 41% apoplexies ↓ 34% hip fractures (osteoporosis)	↑ 15% "Global Index"
↑ 113% embolic diseases (lung) ↓ 8% mortality (other reasons)	
↑ denotes Increased risk; ↓ denotes Decreased risk	

In the last few years we have been bombarded by a lot of data from studies (e.g. WHI, HERS), which concluded that the risks associated with HRT composed with progestins far outweigh any benefits (see figure). This was in contrast to the findings of other studies, which praised the benefits of HRT.

It seems to be that the results of WHI and HERS showed the typical problems of a non-individual, custom-tailored HRT, that leads to problems in a definite percentage of the female population. Many of whom have genetic or lifestyle dependent alterations in their steroid-hormone metabolism, which increases their risk of CVD and cancer, especially of the breast and endometrial mucous membranes

This presentation displays the new approach to an endocrinological treatment strategy, which as we said before, should be individual, custom attracted, and secure. The great advances in medicine in the last few years will make this new type of HRT a reality in the near future.

This is the hypothesis:

- The individual reaction to HRT is caused by the genetic conditions.
- Long time HRT is correlated with higher risk of breast cancer and CV.
- Breakdown mechanism and productive mechanism of steroidgenesis are correlated with higher tissue levels of estrogens.

Today we are able to answer three main questions:

1. Cardiovascular disease: Who will benefit the most from hormone replacement therapy?
2. Thrombosis: Which patients have a high risk of clotting and cardiovascular complications?
3. High plasma levels of estradiol, DHEA, and testosterone: How can we avoid supraphysiological hormone plasma levels, which have been recognized as a high risk for breast cancer, thrombosis, and eventually CVD? In men: how can we have impact on the risk of prostate cancer in testosterone therapy?

The very interesting overview by Clemons and Goss and their presented studies substantiates the assumption that the continued increased burdening of the organism by supraphysiological doses of 17-ß-estradiol increases the proliferation pressure of the mammary glands, increases the radiological density of the mammogram, and increases the lifetime risk of breast cancer. Furthermore, it appears that the risk of breast cancer is dependent upon the cumulative risk of lifetime estrogen exposure.

Therefore, it seems necessary to maintain low estrogen levels. The effort to maintain estrogen concentrations in the sex-steroid dependent tissues at as low as level as is possible is a demand on the evidence based medicine. However, the demand for estrogen replacement is based upon the experience that estrogens have a positive influence on many age-related health problems, especially on the support of bone metabolism, and the improvement of the lipid profile, which can affect the risk of cardiovascular disease.

17-ß estradiol acts directly on the genome, and is bound by the estrogen receptor complex. Supraphysiological increased estradiol levels has an impact on cell metabolism, cell division, and transcription of DNA. Therefore, should the HRT practice of administering female sex steroids without knowledge of the production and breakdown of estrogens and other sex steroids hormones, especially the genetically dependent influence of the steroid metabolism, belong to the past?

Since we know that the difference between individuals is based upon genetic mutations and polymorphisms, the HRT managing physician should utilize the following when assessing a patient for HRT in order to obtain an overview of their estrogen history and ascertain their individual risk if prescribed HRT:

1. Personal history (of particular importance is weight gain in pregnancy, endometriosis, breast cysts, and ovarian cysts).
2. Clinical profile, for example hormone plasma tests (be on the look out for high estrone and/or high estradiol levels).
3. Image producing diagnostics; such as a bone density scan mammogram and ultrasound. (High bone density, high tissue density, cysts, and a high endometrium are all suggestive of increased breast cancer risk.)
4. Polymorphism diagnostics.

POLYMORPHISMS

The possibilities of the new molecular genetic diagnostics, especially the gene chip techniques allow us to weigh up the risk of HRT. These technologies mean that we can estimate the benefits of HRT for women in terms of cardiovascular disease risk reduction. As we know, weight reduction, exercise, and smoking cessation lower our cardiovascular disease risk. We also know that the possibility of cardiovascular disease increases dramatically after the menopause, with 10 years latency compared with men.

When assessing a patient for HRT is it important to be aware of several very important polymorphisms. These include:

- **17-Beta Hydroxysteroid Dehydrogenase Type 1 (17 ß-HSD1):** This is a key enzyme in the production of estradiol. 17-ß HSD1 works by converting the less active estrone into 17-ß estradiol. A mutation in the promotor region of HSD1 (-27A→C) leads to 45% decrease of enzymatic activity, which therefore affects estradiol levels.

  The change of T by C in the Promotor area of the CYP17 gene in position 34 produces a new "SP1-type (CCACC box) promotor site." Women, who carry cytosine in this position (A2/A2), have significantly higher estradiol, estron, progesterone, and DHEA plasma levels. Feigelson et al showed that A2/A2-women are half as likely as A1/A1 women to be HRT users (odds ratio = 0.52) because of side-effects such as breast tenderness and weight gain.
- **Cytochrome P450, 19 Gene, CYP19, Aromatase:** Knowledge of the activity of aromatase, which is coded by the CYP19 gene, is very important. High activity of this enzyme leads to a faster conversion rate from testosterone to estradiol and from androstenedione to estrone, which increases tissue and plasma estrogen levels. We are aware of several polymorphisms of this gene, which are all of great practical importance. The C1558 T Mutation doubles the risk of breast cancer, while another CYP19 mutation significantly decreases the risk of developing breast cancer over lifetime.

GOOD NEWS: CARDIOVASCULAR RISK

Diabetic women who use hormone replacement therapy (HRT) are more likely to have their blood glucose under control, and have lower cholesterol levels than women who never used hormone therapy, a study by University at Buffalo, the State University of New York, epidemiologists has found. Furthermore, the study results also showed that non-diabetic women who were using HRT had lower total cholesterol levels, as well as higher levels of beneficial cholesterol.

A new study, published in the current issue of Diabetes Care, adds yet another twist to the murky risks-benefits scenario surrounding HRT. The federal government suspended a nationwide clinical trial of HRT in July, citing, among other concerns, that the combination of estrogen and progesterone used in the trial did not protect against cardiovascular disease as expected. Yet, the University at Buffalo researchers found that HRT had a positive effect on two important risk factors for heart disease – blood levels of fats and glucose – in a population-based study of 2,786 diabetic and non-diabetic postmenopausal women between the ages of 40 and 74. Carlos Crespo, associate professor of social and preventive medicine at University at Buffalo School of Medicine and Biomedical Sciences, noted that the national HRT clinical trial did not include women with diabetes, and that scientists haven't researched the benefits or risks of hormone replacement in this group.

"Although there may be some risk in using certain types of HRT among certain women, there might be a segment of women who would be better off using HRT," Crespo said. "These findings indicate that diabetic women may be one such segment."

The study, based on data from the Third National Health and Nutrition Examination Survey (NHANES III), compared lipid profiles, glucose and insulin levels, and concentrations of selected blood components known to increase or decrease the risk of heart disease in diabetic and non-diabetic women. Participants were grouped into one of three HRT-use categories: current, previous, or never.

Results showed that diabetic women on HRT had significantly lower fasting levels of total cholesterol compared to diabetic women who were previous or never users: 225 mg/dl, 247 mg/dl, and 241 mg/dl, respectively. The difference in fasting glucose levels among diabetic women according to HRT status were equally significant: 112 mg/dl for current users, compared to 151 mg/dl and 154 mg/dl for previous and never users.

Among non-diabetic women, current HRT users had significantly higher levels of beneficial high-density lipoprotein (HDL) than previous or never users -- 64 mg/dl, 57 mg/dl, and 55 mg/dl, respectively.

HRT also appeared to have a beneficial effect on several additional markers of heart health and glycemic control in both diabetic and non-diabetic women:

- **Fibrinogen** – a protein associated with increased risk of coronary heart disease, stroke, and peripheral artery disease through its role in blood clotting and platelet aggregation was lower among HRT users in both groups of women compared to never users.
- **ApoA** – a protein component of HDL that allows it to remove excess cholesterol from the bloodstream, was higher among HRT users in both groups of women, compared to never users.
- **ApoB** – associated with vessel blockage, was lower among HRT users in both groups of women, compared to never users.
- **HbA1c (GHb), or glycosylated hemoglobin** – an indicator of poor glycemic control, was lower among diabetic women using HRT, compared to previous and never users.

POLYMORPHISMS THAT AID CARDIOVASCULAR RISK ASSESSMENT

Despite this good news, we should consider the possible polymorphisms of women, who use HRT, before starting with this therapy, because by doing so we can estimate the risk of possible CV events in our patients, and thus avoid the danger.

- **Plasminogen Activator Inhibitor Type 1 (PAI-1):** Mutation of the promoter region of the PAI-1 gene (del/ins 4G/5G) leads to increased PAI-1 plasma levels. Increased activity of PAI-1 leads to reduced fibrinolysis, which in turn increases the risk of blood clotting.
- **Angiotensinogen (AGT):** AGT and its products AT I, II, III, IV play a very important part in the regulation of sodium homoeostasis and fluid retention and excretion. Polymorphism of the AGT gene (M 235T) is related to hypertension.
- **Endothelial Nitric Oxide Synthetase (NOS3):** NOS3 encourages vasorelaxation. A mutation of the promotor region of the NOS3 gene reduces nitric oxide levels and is related to coronary spasms and hypertension.

We can further estimate the risk of thrombosis in candidates for HRT by considering Prothrombin Factor II (F2) and Factor V Leiden (F5).

- **Prothrombin Factor II (F2):** Prothrombin factor II is a vitamin K-dependent blood clotting factor. Increased expression of F2 by mutation of the F2 gene (G20210) causes an increased risk of blood clotting. (Psaty et al. JAMA 2001;285:906-913)
- **Factor V Leiden (F5):** The G1691A mutation of the F5 gene is known to cause activated protein C resistance (APC resistance). Carriers of this mutation are at high risk of thrombosis.

BREAST CANCER

Polymorphisms that Aid Breast Cancer Risk Assessment

Being aware of the polymorphisms of the genes of steroid metabolizing enzymes gives us the possibility to estimate the risk of non-familiar dependent breast cancer. Personal history, mammogram (high density), ultrasound (ovarian cysts, high build up endometrium), and bone density (increased bone density leads to the suspicion of elevated lifelong estradiol levels) are all helpful in determining breast cancer risk. The following polymorphisms may also help to clarify a woman's risk:

- **CYP 1A1 and 1B1 Gene Polymorphism:** These genes code for the two major enzymes controlling the break down of estrogens and xenobiotics from toxic environment. Mutations in those genes lead to higher levels of estradiol, and increased pressure on the proliferation of female hormone dependent tissues. Furthermore, it cuts the estrogens to form 2 OH-estrogens and 16 OH-estrogens
- **CYP 1A1: Hydroxylation to 2-OHE (2 Polymorphisms: T3801C and A2455G):** CYP 1A1 has anti-carcinogenic effects against the estrogen agonist 16 alpha OHE1, however smokers have a higher risk for breast cancer if they display the A2455G Polymorphism. CYP 1A1 is inducible by dietary modification and supplementation with active components of cruciferous vegetables, such as indole-3-carbinol (I3Carbinol), or diindolmethane (DIM).
- **4-OH Estradiol:** Displays over-expression in breast cancer cells. Murray et al found that P450 CYP1B1 is a tumor-specific antigen. According to Liehr et al, 4-Hydroxylation of estrogens can be used as a marker of human mammary tumors.

- **4-Hydroxylation of Estradiol – Estrogen Metabolism and the Risk of Breast Cancer**: To learn more about this I recommend reading the following articles: Muti et al (*Epidemiology*. 2000;11:635-640), and Liehr et al (*Proc Natl Acad Sci U S A*. 1995;92:9220-9224.*)*
- **CYP 1B1:** Metabolizes polyaromatic hydrocarbons, aromates, and different xenobiotics from a toxic environment, however it also produces the carcinogen 16 - alpha OH-estrone. We have a very easy Elisa test from morning urine to measure the content of 2-OHE2 and OHE1 ratio to 16-alpha OHE1, which seems to be a model for the risk of breast cancer and other epithelial cancers. Women with breast or endometrial cancer have increased estrogen-16-alpha hydroxylase activity. 16-OHE1 has the unique capacity to bind covalently and irreversibly with the endoplasmic reticulum. Agents that increase 2-OHE1 inhibit carcinogenesis. Exposure of mammary epithelial cells to 16-alpha OHE1 results in genotoxic DNA damage and increased cell proliferation, similar to that induced by the carcinogen DMBA (7,12,-dimethylbenzanthracene.) Data from women with breast cancer and age-matched controls shows a strong inverse association of the 2/16alpha ratio with cancer. Cancer Gene Activation by CYP 1B1:
    - Dibenzoypyrene: Pottenger et al (*Arch Biochem Biophys*. 1991;286:488-497.)
    - Aflatoxin B1: Crespi et al (*Mutagenesis*. 1997;12:83-89.)
    - Dietary Heterocycloamines
    - Hydroxylation of Testosterone
- **Catechol-Ortho-Methyl-Transferase (COMT) and CYP 1A1, CYP 1B1**: COMT is responsible for the breakdown of catechol-estrogens by hydroxylation. These are converted from estradiol by P450 oxidases (Cytochrome P450 1A1 (CYP 1A1) and (P450 CYP 1B1). The intermediary produced catechol-estrogens are highly carcinogenic.

  Polymorphisms of the CYP 1A1 gene leads to increased levels of estrogens and catechol-estrogens, which are themselves a risk factor for the development of breast cancer. Mutation of the COMT gene leads to a slower breakdown rate of catechol-estrogen, thus a greater amount of cancer stimulating intermediary estrogen products is present in the breast cells.
- **Steroid-5 alpha Reductase Type II (SRD 5A2):** This is responsible for the breakdown of testosterone. A mutation of this gene (89val→leu) is known, which reduces the enzymatic activity of SRD 5A2, thus people carrying this mutation have increased levels of testosterone. Women who carry this mutation can develop androgen side effects, while men are at higher risk of prostate cancer.

Alternative Therapies and Prevention of High Estradiol Levels and Breast Cancer

1. Progesterone (sulphatase inhibitor), because estradiol is sulphated in breast tissue.
2. Livial (Tibolone), is a synthetic steroid, which is fully absorbed after intake and is metabolized to the metabolites: delta 4-isomer, 3-alpha hydroxy-tibolone, and 3-beta hydroxy-tibolone. Tibolone activates estradiol in the brain, vagina, and bone. However, it inhibits estradiol in breast tissue (like progesterone). In a DMBA mouse model, Tibolone had a tamoxifen-like effect on tumor growth.

Breast Cancer and Smoking

Palmer et al's (Am J Epidemiol. 1991;134:1-13.) review of two case-controlled studies of women with breast cancer, revealed that women who had never smoked had a relative breast cancer risk of 1.0.Whereas, the odds ratio for women who smoked 25 or more cigarettes per day as compared

with never smokers was 1.2. In both studies, breast cancer risk was more strongly related to smoking at a young age. Among women who started smoking aged 16 and under the relative risk of breast cancer was 1.7 in the Canadian study, and 1.8 in the US study.

The Diet Connection

Important – No Alcohol: (Rosner and Colditz, J Natl Cancer Inst 1996;88:359-364.):

- directly toxic effects, weakened detoxification of xenobiotics, which take the same breakdown pathway as estrogens.
- Activated carcinogens by alcohol->acetaldehyde by stimulating CYP P450 enzymes.
- Increased oxidation.
- Inhibited DNA repair.
- Loss of B-vitamins, vitamin A and retinoids (anti-cancer vitamins).
- Energy→increased fat mass.
- Destroying immune system.
- Increased toxicity of tobacco.
- Increased estrogens.
- Stimulation of sulphatase.

An anti-cancer diet should be rich in fiber, cruciferous vegetables (indole-3-carbinol), and isoflavones (lentils, soy, red clover).

Estrogen Metabolism and the Diet-Cancer Connection Lord et al (*Altern Med Rev*. 2002;7:112-129; Lu et al (*Cancer Res*. 2000;60:1299-1305.)

- Indole-3-carbinol (I3C) reduces the frequency of carcinogen-induced mammary tumors in rats by 75%.
- Dietary indoles inhibit aromatic hydrocarbon-induced neoplasia in mice stomach.
- I3C strongly influences estradiol metabolism in humans by increasing 2-Hydroxylation.
- I3C has specific anti-growth effects on human breast cells but little effect on cells not responsive to estrogen.
- Dietary indoles and isothiocyanates generated from cruciferous vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines.
- I3C increases 2-hydroxylation in human cancer cell cultures.
- Human subjects taking I3C at 400 mg/d for 3 months show sustained elevation of 2-OH/16OH1-alpha ratio with no detectable side effects.
- Using the 2OHE/16alpha OHE1 ratio Elisa assay as the surrogate endpoint biomarker, an I3C minimum effective dose schedule of 300mg/d is proposed for long-term breast cancer chemoprevention.
- Metabolism of cigarette smoke carcinogen is increased by dietary supplementation with I3C in mice.

Omega-3 Fatty Acids

The Omega-3 class of polyunsaturated fats has emerged as a promising therapy for adjunctive cancer prevention. Possible mechanism:

- Favoring series 3- eicosanoid synthesis.
- Modulation of estrogen metabolism and estrogen receptor binding.

- Increased 2-hydroxylation of estradiol.
- DHA (Docosahexaenoic acid) causes decreased binding to estradiol.

A high intake of Omega-6 and arachidonic acid inhibits the CYP 1A1 detoxification of estrogens by 2-hydroxylation, and increases 16alpha OHE1. Omega-3 fatty acids suppress the growth of estrogen sensitive tumors. DHA specifically inhibits the growth of HPV-induced cervical cell growth.

Flaxseed Cancer Prevention

The lignans and phytoestrogens present in flaxseed are metabolized in the intestine by bacteria into enterolactone and enterodiol, which are have a structure similar to estradiol. Thus, flaxseed may:

- Inhibit breast cancer and colon cancer growth.
- Stimulate sex hormone-binding globulin (SHBG).
- Inhibit aromatase activity.
- Have direct chemo-protective effects, by helping remove endogenous estrogens via increased retention within the gut for elimination in the feces (entero-hepatic circulation decreased).

Flaxseed induces 2-hydroxylation of estrone. Dietary intake of 10 grams of ground flaxseed per day for seven weeks produced the most dramatic effects in estrogen metabolism. No change in 16 alpha OHE1 was observed.

Soy and Isoflavones Anti-Cancer Effects

Soy and isoflavones have been linked to breast cancer risk in epidemiological studies. Isoflavones are found in: beans, legumes, lentils, red clover, and soy. Soy isoflavones are referred to as natural selective estrogen receptor modulators (SERMs), molecules that interact with estrogen receptors, inhibit steroidogenic enzymes, and interfere with binding to SHBG.

Soy isoflavones reduce circulating levels of 17-ß estradiol in women, and activate both 2-hydroxylation and 16-alpha hydroxylation, but have a stronger effect upon 2-hydroxylation.
These effects have not been evidenced in postmenopausal women.

A definitive statement, that soy reduces cancer risk in women cannot be made at this time, but there is considerable evidence of protective effects on estrogen metabolism.

Beer (Milligan et al *J Clin Endocrinol Metab*. 2000;85:4912-4915; Rong et al (*Eur J Cell Biol*. 2001;80:580-585.)

MELATONIN

Melatonin is synthesized from 5-Hydroxytryptophan (5-OH-Trp), after being converted to serotonin by the support of phenylalanine. This is done mostly in the pineal gland. ß-blocking agents cause a decline in melatonin secretion. At least two melatonin receptor subtypes have been recognized. Melatonin is a calcium or calmodulin antagonist, which indicates that it plays an important role in cellular homeostasis. Melatonin modulates the TRH-TSH-thyroid axis, and is one of the most potent free radical scavengers. It can accumulate in the nucleus of cells, where it can bind nuclear proteins and DNA, and can help to protect against protein over-expression or

excessive cell replication. It protects the Cytochrome I and IV complex in the mitochondria, together with Gingko, which effects complex III.

Melatonin supports age-related T3-decrease. Thus, there is a lot of interest in melatonin as a treatment for sub-clinical hypothyroidism. Melatonin has important effects on the menstrual cycle. We see an significant decrease of melatonin at the last step from peri to postmenopausal life phase. This correlates with FSH increase. On the other hand, melatonin normalizes the short hypergonadotropinemia in perimenopause. Women with amenorrhoea display significantly amplificated nocturnal melatonin secretion. There is a negative correlation between melatonin and estradiol. Women with secondary amenorrhoea display significantly higher levels of melatonin. On the other hand, we find an increase of melatonin secretion by adminstering estradiol in post menopause. Estrogens amplificate melatonin receptor density. Walter Pierpaoli believes that aging is undeniably initiated in the pineal gland. Exogenous melatonin has been shown to increase the lifespan of mice by 25%. Melatonin interacts with the immune system and inhibits platelet aggregation. It also decreases glucose tolerance and Insulin sensitivity. Nocturnal labile hypertension is an indication to administer melatonin. We find impaired melatonin secretion in coronary heart disease and prostate cancer. Finally, melatonin inactivates norepinephrine.

DHEA

The typical postmenopausal androgen is the adrenal DHEA, which is mostly converted to testosterone and sometimes further converted to estradiol by aromatase, especially in obese women. Peri and postmenopausal women, with the typical clinical profile of lack of sexual desire, decreased orgasm frequency, lack of well being, and blunted motivation virtually always have decreased free testosterone plasma levels. This may be caused by:

- Lack of ovarian production (LH levels increased, low T-testosterone).
- Lack of ovarian production (LH and FSH low, low T-testosterone induced by increased psychic stress).
- Normal ovarian production (increased SHBG levels, induced by oral contraceptives or oral HRT).
- Decreased DHEA production by the adrenal gland, with low ovarian testosterone production.

Study results have showed that pharmacological doses of 4x 400 mg DHEA /day in women, produce an increase in lean body mass, accompanied by a decrease in fat mass. Meanwhile, a daily oral dose of 50 mg DHEA, yielding DHEA-S levels as observed in young women, caused an increase in testosterone and androstenedione levels to high-normal range. IGF-1 levels increased and IGF-BP decreased, however BP3 remained unchanged. Most patients reported a remarkable increase in perceived physical and psychological well being. With the higher dose of 100 mg /day, DHEA-S levels increased to three to four times above the upper-normal limit.
Other research has shown that percutaneous administration of a DHEA cream to postmenopausal women for 6-months results in a significant increase in bone mineral density of the hip. This suggests that DHEA may act as a bone re-modeling agent. Some authors have found an inverse correlation between DHEA-S levels and the severity of osteoporosis.

There is an enormous increase of ß-endorphin levels after the first month of DHEA -therapy. This supports the theory that it has an estrogen-like effect on the brain. Treatment with DHEA induces a restoration of ß- endorphin reaction similar to that seen with clonidine and naloxone.

DHEA affects directly the CNS. DHEA and DHEA-S are considered to be neurosteroids because they are , in part, produced in the CNS. In fact, the concentration of DHEA-S in the CNS is five to ten times greater than plasma levels. Cytochrome P450, the enzyme involved in the

cleavage of cholesterol, pregnenolone, and progesterone to DHEA, is localized in the mitochondria of glia cells. This enzyme is coded by the same P450 gene that is expressed in the adrenal and gonads. DHEA exerts its effects upon the CNS by binding to the GABA receptor, thus blocking chloride transport in a dose dependent manner and increasing neuronal excitability. DHEA decreases continuously during the aging process. DHEA and DHEA-S are synthesized from cholesterol, via pregnenolone and 17 OH-hydroxyprogesterone, and are secreted almost exclusively by the adrenals – only about 10% of plasma DHEA is derived from the ovaries.

Quantitatively, DHEA-S is the major steroid hormone secreted by the adrenals, its plasma concentration reaches very high values in the fetus and then again in young adult (roughly 10-20 times the higher than cortisol levels). The half-life of DHEA-S is approximately +10 hours, and its metabolic clearance rate is about 15 l/day. This yields a blood production rate of 25 to 30 mg/day in young women. The concentration of DHEA is 500-1000 times lower than that of DHEA-S.

DHEA and DHEA-S are mutually interconvertible, under the influence of a sulfate, which is very widely distributed in the tissue, and a sulfotransferase, which has a more limited distribution, but is present in the steroid producing glands, in the liver, and – to a lesser extent – in muscle and in the brain.

DHEA and DHEA-S are controlled by adrenocorticotropic hormone (ACTH). This explains, why DHEA levels show both pulsatile and nyctohemeral variations in parallel with cortisol and ACTH pulses. Circadian variations disappear almost completely in old age.

The role of DHEA in aging stems from the observation that DHEA levels decline dramatically with age in humans. Studies have provided evidence to suggest that DHEA improves the quality of sleep, boosts energy levels, and increases the ability to handle stress. As it binds to GABA and NMDA receptors, DHEA has a positive impact upon depression, memory, and cognition, by opening calcium channels, and having antagonistic and agonistic effects upon pregnenolone and progesterone, respectively.

As part of the CYP 17 enzyme system, DHEA has a great impact on the conversion of androstenedione and testosterone to estradiol. In the same way, it blocks the manufacture of corticosterone and cortisol. DHEA has been shown to have positive effects on the immune system by affecting the PPAR receptor of lymphocytes, which decreases the production of free radicals by monocytes.

GROWTH HORMONE

Acquired growth hormone (GH) deficiency results from the destruction of normal pituitary and/or hypothalamic tissue, usually from a tumor or secondary to surgical and/or radiation therapy. Diagnostic criteria and clinical sequelae of GH deficiency, although well established in children, are currently areas of active investigation in the adult. It is now apparent that acquired GH deficiency is associated with significant changes in body composition, bone density, lipid metabolism, cardiovascular function, and physical performance. In addition, new information is now available on the use of low doses of recombinant human growth hormone (rhGH) to reverse the sequelae of GH deficiency in adults. Patients with probability of GHD:

- Pituitary and peri-pituitary tumor.
- Intracranial radiation or tumor resection.
- Deficiency of other pituitary hormones.
- GHD from childhood.
- Elderly

Growth Hormone Deficiency of the Elderly

For each decade of life GH production drops by 14%. We also see a reduction of the altitude of the GH-amplitudes, and of the nightly spontaneous pulsatile GH-secretion. Such decreases in GH production can lead to:

- Anomalous body composition.
- Increased fat around the trunk.
- Dehydration
- Decreased muscle mass.
- Decreased bone density.
- Changes in heart muscle structure.
- Changes in cardiac function.
- Increase in cardiac risk factors, such as hyperlipidemia, reduced fibrinolysis, and increased atherosclerosis.
- Reduced strength and vital capacity.
- Reduced "well being."

Aging correlates significantly with changes of the biomarker insulin-like growth factor-1 (IGF-1) and its binding protein IGF-3 BP. GH deficiency is characterized by weight gain, increased fat mass, and decreased lean body mass. In one recent study, total body fat was shown to be increased by 7% in the GH-deficient population, while lean body mass was decreased to a similar degree. The increased fat mass is found in a truncal distribution, thereby increasing the waist:hip ratio. In addition, triglyceride levels are increased, and HDL levels decreased. The increased lipid levels may explain, in part, the observation of increased vascular wall thickness, as measured by carotid ultrasonography, in this population. These factors contribute to the increased incidence of cardiovascular mortality seen in patients with GH deficiency.

Muscle mass and muscle strength are diminished in GH-deficient patients. In the heart, these changes are manifested by a reduced left ventricular mass, decreased fractional shortening of cardiac myocytes, and decreased cardiac output. Such abnormalities may contribute to the striking decline in exercise capacity in this population. Exercise capacity, as assessed by cycle ergometry was decreased by 20-25%, compared to non-GH deficient controls.

Bone density is also known to be reduced in the GH-deficient patient. In a recent study, cortical bone density and spinal (trabecular) bone density were 2.8 and 1.5 standard deviations below the mean for age and sex matched controls.

HGH and the Brain

The level of IGF-1 in the cortex declines significantly with age. A lack of GH and IGF-1 in the brain leads to a decreases in learning ability, memory, and spatial memory, and delayed responses.

There is significant loss of dendrites in the aging brain, which may result from a decline of IGF-1. IGFs have an important role in neuronal repair, they stabilize tubulin-mRNA, stimulate neuronal and glial DNA, as well RNA synthesis and neurite formation. They also enhance oligodendrite proliferation, increase survival of neurons and glia, and increase synaptogenesis.

Finally, patients with GH deficiency appear to have impaired psychological well being and potentially significant neuropsychiatric manifestations, such as lack of concentration and memory impairment. Self rating questionnaires consistently demonstrate reduced vitality, fatigue, social isolation, and depression. However, it is unknown whether this impairment in psychological well being is associated specifically with GH deficiency or is due to another factor associated with hypopituitarism. Both, GH and IGF-1 stimulate endothelial cell proliferation, and angiogenesis in humans. In addition, adults with GH deficiency, have a increased rate of divorces, and a higher risk of unemployment Thus, GH deficiency is associated with:

- Atrophy of CNS.
- Accelerated atherosclerosis.
- Development of CNS tumors.
- Cardiovascular diseases.
- Decrease of muscle mass and strength.
- Osteoporosis
- Dysfunction of the immune system.
- Significant changes in the endocrine system.
- Depression
- Anxiety
- Loss of self confidence.
- Lack of motivation.
- Fatigue
- Social isolation.
- Marital problems.

As GH can pass through the blood-brain barrier it has direct effects upon the brain, these include:

- Inducing RNA-synthesis in neurons.
- Changing of homovanilic acid and free-T4 in CS fluid.
- Stimulating secretion of endorphins.
- Supporting myelinization.
- Essential functions in the embryonic brain.

Body Composition

GH therapy results in profound changes in body composition – fat mass is reduced while lean body mass increases. Growth hormone, at the relatively low dose of 0.003 mg/kg was shown to normalize lean body mass over 6 months in 24 adults with GH deficiency. The improvement in lean body mass is associated with increased protein synthesis, muscle mass, and muscle function. Total body fat mass also decreases after 6 months of GH administration. The decline in fat mass is most significant in visceral and trunk locations as compared to the arms, neck and legs, suggesting that GH replacement therapy will reverse the truncal redistribution of fat mass associated with GH deficiency, and thus impact on cardiovascular risk.

Protein synthesis declines with age, thus the elderly tend to have a decreased lean body mass and an increased body fat mass. Thus, patients with GH-decline display decreased strength, however treatment with GH improves muscle function and aerobic capacity. GH also changes fat mass distribution from central and visceral to peripheral parts of the body. The mean increase of muscle mass that occurs with GH-replacement therapy is 3-5 kg. While the mean loss of fat mass is 2-3 kg.

Lipid Metabolism

GH replacement in adults may have a beneficial effect on lipids. In a recent study, it was reported that short courses of GH reduced LDL cholesterol (and this reduction correlated with increased mRNA expression of the LDL receptor in the liver). The potential benefit of this interaction has yet to be investigated in longer term clinical trials, but it must be noted that dramatic changes in serum lipid levels are not consistently seen with GH administration.

Cardiovascular Function

Improvements in exercise capacity and cardiac function have been demonstrated among GH-deficient patients receiving GH replacement in several recent studies. Such patients show increased oxygen uptake and power output during cycle ergometry associated with increased

skeletal muscle mass and improved cardiac function. Echocardiography has shown that left ventricular mass index, fractional shortening, and fiber shortening velocity all improve after 6 months of low-dose GH therapy. Cardiovascular symptoms of GH-deficiency include:

- Hardened vessels (Sclerosis of vessel wall).
- Hyperlipidemia (LDL,HDL).
- Reduced NO-production.
- Increased fibrinogen and PAI-1.
- Increased frequency of action potentials of sympathetic neurons (hypertension).

Bone Density

The potential role of GH in the maintenance of the skeleton has recently been investigated. GH-deficient patients display lower bone mineral density, lower mineral content, and therefore increased risk of osteoporosis. GH is known to stimulate osteoblast proliferation and thymidine incorporation *in vitro*. Furthermore, GH stimulates systemic and local production of IGF-I, another known bone mitogen. In a recent study, GH replacement was shown to significantly increase bone Gla-protein, a sensitive indicator of osteoblast function. Less consistent changes in bone density have been demonstrated with GH administration. However, in a recent study using the sensitive techniques of quantitative tomography and single photon absorptiometry, significant increases of 5% and 4% were demonstrated in spinal and cortical bone density over 12 months of therapy in GH-deficient adults. Therefore, it seems that GH administration may act to reverse the osteopenia present in the GH-deficient patient. Long-term HGH replacement (2-5 years) will lead to improvements in bone mineral content and bone mineral density.

Side Effects Associated with Low-Dose GH Replacement

The dose of rhGH is an important consideration in the therapy of acquired GH-deficiency. Large, pharmacological doses of GH are often associated with the clinical sequelae of GH excess, including fluid retention and hypertension. However, increasingly smaller, physiological, doses of rhGH are currently being used for replacement in GH-deficient patients without such sequelae. At a dose of 0.03 mg/kg/week, Bengtsson et al (*J Clin Endocrinol Metab*. 1993;76:309-317) demonstrated only minor side effects including fluid retention and mild arthralgias in the majority of patients, but did report carpal tunnel syndrome in one patient. In all cases, further reduction of the GH dosage resulted in amelioration of side effects. In another recent study in which a smaller dose of GH was used, 0.01 mg/kg was administered three times per week without any reported side effects. It remains unknown, however, whether chronic administration of GH at doses that keep IGF-I levels within the normal range will also improve key metabolic variables

Before starting replacement:

- Correlate oral testosterone or estrogen therapy: if no effects choose multi-step therapy.
- Arginine, ornithine, alpha-ketoglutarate.
- High dose (10 g) evening time, if this has no effect carry out an arginine stimulation test:
    - Positive increase – A.
    - HGH 0.2 mg 4 weeks (after 4 weeks control).
- Sex
- BMI
- IGF-BP3
- Start: low-dose (0.1-0.2 mg/day).
- 12 hours after injection: blood sample.

- After 4 weeks therapy: control of IGF-1.
- Increase dose at 4-weekly intervals.
- Increase dose every 4 weeks under strict control of IGF-1 (0.1 mg).
- Maintain maximum dose of 0.6 mg.
- Control:
- Myalgia
- Carpal tunnel syndrome.
- Arthralgia
- Edema
- Other side effects.
- IGF-1 in 50th percentile.

Supraphysiological levels of IGF-1 are correlated with:

- Increased incidence of prostate cancer.
- Increased incidence of breast cancer.

Short-term over-dosages of HGH and supraphysiological levels of IGF-1 are also linked with increased frequency of short-duration side effects, such as myalgia, arthralgia, edema etc.

Concluding Remarks – HGH

HGH regulates length, growth, muscle mass, fat mass, lipids, glucose, and has effects on general well-being. In humans, GH levels drop by 14% each decade, and there is also a decrease in spontaneous nightly secretion peaks with age. However, even with the low GH levels present in elderly people, the pituitary will respond to stimulation with growth hormone releasing hormone (GHRH), ghrelin, arginine, and insulin.

Aging also correlates with decreasing levels of the HGH biomarkers IGF-1 and IGF-BP3. The symptoms of GH deficiency include: muscle mass loss, increased visceral and central obesity, brain atrophy, osteoporosis, negative impacts on the immune system, and significantly changes in the endocrine system. People with low hGH levels also display higher rate of divorce and unemployment.

Diagnosis of GH-deficiency must be done by excluding pituitary or hypothalamic tumor, trauma, or acquired deficiency. If we find isolated lack of hGH. Responsible anti-aging conduct two tests:

- Insulin test
- GHRH- test with arginine or pyridostigmine.

GH replacement therapy can increase heart muscle mass and improve function (increased ejection fraction), and improve spatial memory and learning. GH also improves muscle mass, lipid profile, bone mass, immune system and well being, and reduces fat mass.

To avoid side effects (arthralgia, fluid retention, impaired glucose sensitivity, hypertension, congestive heart failure, carpal tunnel syndrome) one should use low doses and recommend that the patient eats correctly and takes regular exercise.

THYROID HORMONE

National guidelines for thyroid hormone replacement give the following recommendations:

- Patients with stable status: TSH testing is more sensitive than T4.
- Unstable status: T4 measurements offer a more reliable indication of thyroid status than TSH (during the first months of treatment of hypo or hyperthyroidism).

Moreover, there is evidence that T3 should be measured together with T4 and TSH. This is based on the experience, that T3 levels tend to decrease with age.

Therapy: T4 treatment of 1.6 µg per kilogram of body weight per day. Older persons require T3 and lower doses of T4 (1.0 µg per kilogram of body weight per day). Post-menopausal women beginning thyroid replacement require higher doses to keep TSH levels at the therapeutic target. (especially, if autoimmune disease needs TSH suppression). TSH is slow to equilibrate to a new thyroid status. 6-8 weeks is needed for re-testing after changing T4 or T4/T3 dosages, or changing brand of medication.

Half-year testing of people receiving thyroid therapy is recommended. The optimal TSH testing is not influenced by the time of day that the T4/T3 dose is ingested. Ideally, thyroid medication should be taken before a meal, and at least four hours apart from medications that influence absorption of thyroid hormones, such as cholestyramine, ferrous sulfate, sucralfate, antacids containing aluminum hydroxide, anticonvulsants, and Rifampin.

Abnormal serum FT4 and FT3 test result often is due to an abnormality in the concentration or affinity of TBG. Abnormal FT4 levels could result from pre-analytical, or analytical assay artifacts, the presence of medications that influence and displace T4 from TBG (phenytoin, Carbamazepine, Furosemide), and during critical non-thyroidal illness. When TSH is high ask the lab to re-measure the specimen diluted, and send another sample to a different lab. However, always use your clinical proof.

PROGESTERONE

Progesterone binds to GABA receptor. It can be used as tranquilizer, to regulate PMS, ease migraine and headaches, and it also has anti-depressive effects. Progesterone inhibits sulphatase, which displaces estrone and estradiol from its binding sulphate part, and thus increases the risk of breast-cell proliferation. It also decreases the density of the IGF-1 receptor.

Progesterone is mainly used topically or vaginally. The typical dose for the treatment of headaches before menstruation and convulsions is 400 mg / day. Because of its fluid displacing effect, progesterone can be used for treatment of edema, and its vasoconstrictor effects mean that it has a great impact on the venous system, and it can be used to treat phlebopathy and hemorrhoids. Progesterone inhibits matrix-metallo-proteinases. This effect is used to slow down pelvic descents, and the development of wrinkles.

TESTOSTERONE

Male sexual function declines with age. The effects of androgens in men and women are both very similar and very different (interest in sexuality, sebum production, hair growth and loss, Epo-production, improvement of lipid profile in men, but not in women).

Testosterone deficiency in men is manifested typically by symptoms of hypogonadism, including decreases in erectile function and libido. Testosterone also has an important role in the regulation of normal growth, bone metabolism, and body composition.

Specifically, testosterone deficiency is an important risk factor for osteoporosis and fractures in men. In men older than 65 years of age, the incidence of hip fracture is 4-5/1000, and approximately 30% of all hip fractures occur in men. Men with testosterone deficiency have significant decreases in bone density, particularly in the trabecular bone compartment. Testosterone deficiency has been reported in over half of elderly men with a history of hip fracture.

Men with testosterone deficiency also have alterations in body composition, including an increase in body fat. Using quantitative CT scans to assess fat distribution, we have shown that testosterone deficiency is associated with an alteration in site-specific adipose deposition with

increased deposits in all areas, particularly in the subcutaneous and muscle areas. Because truncal fat correlates with glucose intolerance and cardiovascular risk, hypogonadism may have important implications with regard to overall health and mortality. In one study, the alteration in skeletal muscle composition was associated with a decrease in muscle strength. Therefore, testosterone deficiency is associated with an increased risk for osteoporosis, altered body composition (including increases in truncal fat), and, possibly, decreases in muscle performance.

Administration of adequate testosterone replacement therapy leads to improvements in libido and erectile function. Following testosterone replacement, men note an increase in energy and mood, which may reflect either direct behavioral effects of androgens, and/or, an elevation of hematocrit due to rising testosterone levels. Testosterone therapy also leads to important beneficial effects on the skeleton and lean tissue mass. Testosterone replacement increases bone density in hypogonadal men with the most dramatic effects seen in the trabecular bone compartment. These effects may be seen as early as 6-months following initiation of testosterone therapy.

In one recent study of the long-term benefits of testosterone therapy, the greatest benefits in trabecular bone were seen in the first few years of therapy. With regard to body composition, testosterone replacement therapy results in a dramatic reduction in adipose content, with the greatest effects seen in the subcutaneous and skeletal muscle areas. Androgen therapy leads to a significant increase in lean skeletal muscle mass and strength. Therefore, there are beneficial effects of testosterone replacement on body composition and bone mineral density in adult hypogonadal men that may serve as indications for therapy, in addition to libido and sexual function.

Because testosterone levels decline with age, and aging is accompanied by body changes including loss of muscle and increases in fat, there is a great interest in the potential benefits of testosterone administration in elderly men. In a recent study, Snyder et al (*J Clin Endocrinol Metab*. 1999;84:2647-2653.) administered testosterone via a scotral patch in a randomized, placebo-controlled trial to 108 elderly men for 3 years. Testosterone administration resulted in beneficial effects on lean and fat mass. Therefore, there may be a role for androgens in improving body composition and function in elderly men.

Testosterone in women is mainly produced in the ovarian stroma cells, including a midcycle peak, when the woman is ovulating. This peak responds to the LH-peak of the pituitary and increases the libido of women for reproduction. Pro-hormones in the cascade of testosterone are pregnenolone, 17-OH -progesterone, progesterone and dehydroepiandrosterone (DHEA), which are then converted to androstenedione or androstenediol.

Androgen levels vary over the female life span with both changing age and reproductive organ changes. Although women in their prime produce roughly two-thirds of amount of testosterone that men do, they are rarely given supplementary androgens after loosing their androgen production capacity. Typically, androgen therapy is only given to postmenopausal women in special situations if it is deemed absolutely necessary, and then it is only given for as short as time as possible. There is the question as to if oral or parenteral administration is suitable for androgen replacement. Furthermore, there is concern about possible side effects on the lipoprotein profile of women, especially that of decreasing HDL-cholesterol levels and the impact that may have on cardiovascular disease risk. Women may also be concerned about two other difficult to control side effects of androgen replacement – hirsutism and acne.

Women taking an oral estradiol substitution and who have elevated SHBG levels should firstly be transferred to transdermal estrogen therapy, and then reviewed after 6-8 weeks to prove the need for androgen therapy. It should be clear, that androgen replacement in women is nearly forgotten by the dominance of estrogen replacement, and that there is a great need and demand to change the idea of a mono-therapy with E2 alone. Like all anti-aging interventions, androgen replacement should be done with protecting health, maintaining the benefits of life, and to prevent age-related disease in mind.

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