April 2002 Issue | Fritz Parl, MD, PhD

http://jeffreybland.com/knowledgebase/april-2002-issue-fritz-parl-md-phd/

DOWNLOAD AUDIO |

Welcome to Functional Medicine Update for April 2002. Excitement is building for the Ninth Annual Symposium on Functional Medicine, which will take place next month. We will meet at the five-star Diplomat Hotel & Spa at Fort Lauderdale. I hope you plan to attend.

The Symposium pre-course, Making Functional Medicine Work in the Office Practice, will begin on Saturday, May 25. This useful course will explain how to integrate functional medicine effectively into standard care so it becomes procedurally as well as clinically successful. That pre-course continues on May 26. As an alternative on that day, registrants can attend a basic nutritional neuroendocrinology course. The plenary sessions, which will take place May 27 through 29, focus on the gastrointestinal tract and functional neurology (the gut/brain connection) and functional approaches to depression. The third day's curriculum features psychoneuroendocrinology. This symposium will be information-packed, but it will also be a lot of fun. Please join us for our first East Coast symposium. For more information, call 1-800-843-9660.

We have talked at length in the past few years about the concept of Mendelian genetic determinism model of disease versus a new paradigm that is being driven by the Human Genome Project and which focuses on the plasticity of genomic and proteomic expression . This new model represents a wholly different view of how disease originates. We previously cited a paper from a 2000 issue of the *New England Journal of Medicine*, which described a study done at the Karolinska Institute in Stockholm, Sweden. Researchers examined twin registries of homozygous twins and the relative appearance of cancer. The researchers sought to determine if cancer was a familial or environmentally related condition.[1] If it were clearly familial and genetically determined, you would see concordance between identical twins as they grew up. If one twin had cancer, we would expect the other to have it as well.

The Multi-Gene Concept
According to the results of this study, no more than 25 percent of cancer incidence could be statistically traced to a hard-wired gene for cancer; the remaining 75 percent appeared to be related more to an interaction between the environment and the genetic blueprint. This study supports a multigene concept rather than that of single-point gene mutations giving rise to cancer.

The multiple messages present in our genes may not appear as a cancerous phenotype unless the genome is plunged into an environment detrimental for the particular individual. This plasticity model supports the value of functional medicine as a method of dealing with early warning markers for later-stage disease in that patient.

An article in the British Medical Journal describes a similar theme related to the relative importance of genetic effects in rheumatoid arthritis.[2] This Danish nationwide twin study looked at 37,338 pairs of twins who returned questionnaires about rheumatic diseases. Clinical examination and medical records verified self-reported rheumatoid arthritis. The response rate was quite high, 84.7 percent, and rheumatoid arthritis was verified in 13 monozygotic and 36 dizygotic twins. According to the authors, based on capture/recapture methods, the probability of ascertainment of a conclusion was about 78 percent, with confidence limits that could be statistically significant. The proband concordance rate was 0 in monozygotic twins and 8.8 in dizygotic twins. The researchers concluded that genes are of minor importance in the development of rheumatoid arthritis. It is the combination of genetic uniqueness with environment that gives rise to the outcome we call rheumatoid arthritis.

This paper is another in the series of observations that the chronic, age-related diseases we typically develop in midlife are not genetically locked in stone . We can modify the outcome in their phenotype by our habits, environment, and lifestyle.

A similar theme is mirrored in a recent paper that appeared in the Journal of the American Medical Association. It is titled "Effects of Diet and Simvastatin on Serum Lipids, Insulin, and Antioxidants in Hypercholesterolemic Men." [3] This paper shows once again the power of diet in modifying gene expression and reducing relative risk of age-related diseases in the phenotype.

This study consisted of 120 patients randomized to four groups. One group received simvastatin, a cholesterol-lowering statin drug with a habitual diet, and one group received simvastatin with an active diet. There were 30 men in each of four groups, so one can pick out the diet relationship versus the drug HMG CoA reductase relationship. There were two groups in the dietary intervention group, one placebo and one simvastatin, and two in a habitual non-diet intervention group, one simvastatin and one placebo-simvastatin. The objective was to determine, in these four groups, what relationship, if any, diet has to conditions of serum lipids, insulin, and antioxidants, and how it compares to the HMG CoA reductase statin-inhibiting drug.

Modifying Phenotype through Diet
The results are interesting. The therapeutic diet was lower in total fat and higher in polyunsaturated fat, higher in fiber, and richer in plant protein, grains, and fruits and vegetables. As one might expect, this diet resulted in lowering cholesterol, but not as much as the simvastatin when the drug was examined alone. Simvastatin decreased levels of total cholesterol by 20.8 percent and LDL cholesterol by 29.7 percent. LDL cholesterol reduction was about proportionately the same between the two groups, 11 percent LDL reduction in the diet-only group, and approximately 30 percent in the simvastatin group.

There were some interesting differences in coenzyme Q10. The simvastatin group had significant reduction in coQ10 compared to the diet group, which had elevated coQ10 at the end of the trial. A marked difference occurred with insulin and insulin sensitivity as well. The simvastatin group had decreased insulin sensitivity and increased insulin levels, and the diet-only group had decreased insulin level and increased insulin sensitivity.

Effects of Drug-Diet Combination
The effects were additive when therapeutic diet intervention was combined with the simvastatin. There were also antioxidant differences in susceptibility to LDL oxidation between the two groups. The diet and

therapeutic intervention group had higher levels of carotenoids, higher levels of tocopherol (vitamin E), lowered LDL oxidation, and lowered conjugated diene information, a measurement of damaged unsaturated lipids, than the simvastatin group.

The authors conclude the modified Mediterranean diet, which is rich in omega-3 fatty acids, fiber, carotenoids, and natural polyphenol antioxidants, potentiates the cholesterol-lowering effect of simvastatin. This diet counteracted the fasting insulin-elevating levels of the drug simvastatin. It did not decrease serum levels of β-carotene, ubiquinol-10, or vitamin E. We are beginning to see published studies that show the power of diet as an intervention tool to modify the phenotypic expression of characteristics that give rise to early-stage, age-related diseases and lowered life expectancy.

The same result occurred in another paper, titled "Reduction in the Incidence of Type 2 Diabetes with Lifestyle Intervention or Metformin," published in the New England Journal of Medicine.[4] The authors hypothesized that modifying the following factors--elevated plasma glucose concentrations in the fasting state and after an oral glucose load, overweight, and a sedentary lifestyle--with a lifestyle-intervention program or the administration of metformin would prevent or delay the development of diabetes.

This is another interesting paper. We now have enough information to enable us to compare the results of diet and lifestyle programs to those of drugs. We can review the risk/benefit or cost/benefit tradeoff of diet and lifestyle to modify gene susceptibilities and compare it to drugs that modify the outcome of effects. We are starting to see that diet and lifestyle are winning the battle. They are more cost-effective and with equal or greater clinical efficacy than single pharmaceutical agents. This is certainly one such study that suggests that result.

Effectiveness of Lifestyle Intervention
The authors of this study randomly assigned 3,234 nondiabetic persons with elevated fasting and post-load plasma glucose concentrations to placebo, metformin, (850 mg twice daily) or a lifestyle modification program. The goal was at least a 7 percent weight loss and at least 150 minutes of physical activity per week. About 20 minutes of exercise daily, such as a regular walking program, was all that was required to meet these objectives.

The patients in the study were individuals who had elevated body mass index, meaning they were by definition modestly obese with a mean average BMI of 34. Sixty-eight percent of them were women, and 45 percent were members of minority groups. The average follow-up was about 2.8 years. The incidence of diabetes during the follow-up period was 11.0, 7.8, and 4.8 cases per 100 person-years in the placebo, metformin, and lifestyle groups respectively. You will notice an almost twofold reduction, relative to metaformin, in the appearance of diabetes in the lifestyle intervention group. Incidence of diabetes in the lifestyle intervention group declined by 58 percent, and in the metformin group by 31 percent. The authors concluded that lifestyle changes and treatment with metformin both reduced incidence of diabetes in persons at high risk, but lifestyle intervention was the more effective of the two.

Healthy lifestyle choices work by modifying genetic function. The improved function that results from alteration of the variables that wash over the genes results in a new phenotype of improved insulin sensitivity, improved glucose transport, and a reduction in the likelihood of type II diabetes. We are witnessing the emergence of a genetic/ molecular model of functional medicine that can be translated into an observable clinical effects. Individuals who have stated that diet is of little importance in overall

treatment in the practice of medicine will have to reevaluate their presumptions based on these types of studies

Macronutrients are not the only important dietary factors. An array of micro- and phytonutrients participate in the modulation of gene expression. Some have profound effects through their influence as nuclear regulatory agents and hormonal messengers. For instance, vitamin D and vitamin A are not simply vitamins. They are also prohormones that are converted by metabolic processes in the body. Vitamin D is converted to 1-25-dihydroxycholecalciferol, a hormonal modulator. It is a member of the nuclear orphan receptor agonist family.

Similarly, vitamin A, or retinol, is converted to retinoic acid. Retinoic acid exerts its biological effects when bound to one of several members of the retinoic acid receptor family or the so-called RAR or RXR nuclear transcription factor family. These substances are found in nearly every cell, and the RAR and RXR transcription factors increase or decrease gene expression by binding the specific DNA response elements. Therefore, the ability of retinoic acid to regulate gene products in spatial and temporal patterns accounts for the programmed cell growth, differentiation, and apoptosis function essential for normal embryonic development, cellular differentiation, and immunity.

The Power of Retinoic Acid

Retinoic acid is a powerful mediator of cell function. As with all of the powerful mediators, neither insufficiency nor excess is good for physiological function. We have a dose/response parabola of activity with a safe zone of concentration leading to optimal function. Therefore, too much or too little vitamin A as a precursor to retinoic acid could disrupt these tightly regulated cell communication and cell differentiation processes.[5]

That might explain the results described in a recent paper in the Journal of the American Medical Association. titled "Vitamin A Intake and Hip Fractures among Postmenopausal Women," an epidemiological study by Dr. Walter Willett and his colleagues at Harvard.[6] They examined the Nurses' Health Study data in 72,337 postmenopausal women aged 34 to 77 years. The researchers found the quintile of those who consumed the highest level of vitamin A (greater than 3000 µg of vitamin A equivalent per day), had a significantly elevated relative risk of hip fracture, about 1.5 times that of those with the lowest level. This would suggest excess vitamin A may have a disadvantageous effect upon trabeculae or bone function, and increased relative risk to bone fracture.

Vitamins D, Estrogen, and Bones

A number of aspects of this study do not seem completely to make sense. The statistical data from the study indicated that calcium, vitamin D, and vitamin K, which are normally associated with decreased bone demineralization and fracture risk, did not appear to confer any beneficial protection when examined statistically. It was also interesting to note that the higher risk of hip fracture was seen only in postmenopausal women not taking estrogens. This observation, as the editorial states, adds further intrigue. Estrogens are known to block several steps in osteoclast formation and function, including differentiation, activation, and programmed cell death. This research, therefore, supports the possibility that estrogen could oppose the type of effects expected of high levels of retinoic acid.

The takeaway suggestion is that excessive vitamin A intake may put the skeleton at risk, particularly in postmenopausal women, of increased fracture. We should not jump to the conclusion that if a little is

good a whole lot more ought to be better. Generally, amounts considered to be in the safe range are 800 µg per day level for men and 700 µg per day for women.

Higher doses may be effective in some cases, however. In those cases one should follow liver function and calcium status to make sure the patient does not move into a relative risk category. This is a general theme in nutrition. We cannot assume that molecules with high biological activity will have no adverse side effects when taken in excess, regardless of the source of the molecules. They may come from food, supplements, or pharmaceuticals. In the case of most nutritional products, the range of safety is quite broad, but with regard to fat-soluble prohormone vitamins like vitamins A and D, caution is advised

Many factors influence gene expression. Stress certainly is one of those factors. As Dr. Hans Selye explained, psychosocial stress is a major precipitating event for a number of metabolic sequelae associated with dysfunction at a number of organ sites. Psychosocial stressors are reflected, perceived, and translated into physiological function according to the uniqueness of the individual. Both one's genomic inheritance and experiential background can modify the expressed response at the physiological level, the neuroendocrine immune response to stress.

An interesting recent paper discusses stress following the events of September 11, 2001. This paper, titled "Post-Traumatic Stress Disorder," appeared in the November 2001 issue of the New England Journal of Medicine.[7] It discusses the increased number of stress-related symptoms appearing throughout the United States after September 11. The authors talk about the physiology of post-traumatic stress disorder. A diagram in this paper (Figure 1) depicts the HPA axis and what happens to it when it is triggered with very significant psychosocial stress.

Stress and the HPA Axis
The diagram shows HPA axis activation with increased corticotropic releasing factor, CRF, coming from the hypothalamus, driving the anterior pituitary to produce more corticotropin, which then drives the adrenal cortex. Marked elevation occurs in the production of cortisol, which keeps driving in a feedback process the cycle. The result can be hypersensitivity to the adrenal cortex so that even though ACTH levels may go down, there is still a hyper-cortisol output.

High levels of cortisol have adverse impact on the metabolism of thyroid hormone, increasing reverse T3, the brake of thyroid hormone, and decreasing T3, which is the accelerator effect from thyroid hormone. An individual can develop what looks like a hypothyroid condition, but it is really a secondary underconversion hypothyroidism that relates to altered ratios of T4 to T3 to reverse T3 at the cellular level. It may not be seen at the plasma level. It is not like euthyroid sick syndrome, but rather a cellular alteration in the way that thyroid hormone interacts with the thyroid receptor site. The T3 receptor heterodimerizes with the retinoic acid molecule we discussed above, to stimulate specific gene expression patterns in sensitive cells.

Post-traumatic stress syndrome can be a precipitating event that locks into a feed-forward cycle of altered cortisol, altered thyroid hormone metabolism, and altered psychological and cellular function. It can have one other impact, which is altered blood lipid patterns. Often, the patient will start to show hyperlipidemias of different types, including, ironically, hypercholesterolemia, because cholesterol elevations can be a manifestation of the secondary effects from hypercortisolemia and this stress-induced dysfunction.

This information makes a paper published in the American Heart Journal even more interesting.[8] It is a discussion of policosanol, the long-chain waxy alcohols from sugar cane or beeswax, which have important potential clinical effects on the metabolism, synthesis, and removal of cholesterol.

Policosanol is a mixture of primarily aliphatic long-chain alcohols. Its main component is a compound called octacosanol. Research in the 1970s demonstrated that octacosanol improved exercise endurance and functional activity. From our 2002 perspective, we now know that improvement may originate at the mitochondria. The policosanol mixture lowered cholesterol in animal models, in healthy volunteers, and in patients with type 2 hypercholesterolemia when given at doses of 10 mg per day.

Delivery Forms and Applications of Policosanols
One historic difficulty with policosanols is that, due to their waxy characteristics, their solubility and absorption have generally been quite low. They have to be put together into formulations that allow them to be emulsified and absorbed effectively. Tableting policosanols often leads to reduced absorption. If you have an absorbable form, it appears as if a10 mg daily dose of policosanols can lower total cholesterol from 17 to 21 percent, and LDL cholesterol from 21 to 29 percent, while raising HDL 8 to 15 percent. This is an interesting effect. Its mechanism of action does not appear to relate directly to HMG CoA reductase. In fact, it appears to work at a step before mevalonate generation. It also tends to reduce LDL oxidation.

Policosanols may, therefore, have an antiinflammatory, antioxidative effect different from that of the statin drugs. This may also explain why it does not appear to lower coenzyme Q10, as the statins do. Policosanols may represent a new class of nutritional agents that modify genetic expression in relation to cholesterol synthesis and metabolism. They may be useful for individuals with modest elevations of cholesterol for whom statins may not be the best choice and for whom the stress-modulated alteration in lipids has been a major factor.

Health Risks Associated with Elevated Homocysteine Levels in Postmenopause
When postmenopausal women are under high stress conditions, their homocysteine levels may increase, a factor that may be associated with increased risk of postmenopausal heart disease. This fairly new series of discoveries results from such studies as the HERS Study on female-related cardiovascular risk. It appears that homocysteine elevation after menopause may be observed in those women who carry certain genetic polymorphisms. The polymorphisms do not become evident in the phenotype until menopause, at which time they may appear as alterations on folate metabolism. The MTHFR, 5,10-methylenetetrahydrofolate reductase polymorphism, for example, more obvious by the modest elevations of homocysteine that are now observed.

A recent paper in the New England Journal of Medicine indicates elevated homocysteine levels are risk factors for dementia and Alzheimer's disease.[9] You might not see some of these characteristics in a woman's phenotype until she has gone through the menopause, her estrogen metabolism has been modified, and suddenly the expression of these characteristics is more obvious. This might explain the higher post-menopausal incidence of Alzheimer's dementia, heart disease, and possibly even other hormone-related problems pertaining to methylation pathways.

Homocysteine’s Role in the Body
Homocysteine’s role in dementia may be related to the fact that it is converted to homocysteic acid,

which has neuronal excitotoxicity, increasing the potential for apoptotic cell death of the neuron. It also has an influence on vascular oxidative stress that damages the endothelia and can lead to thrombotic effects and produce CNS ischemia, neuronal hypoxia. Finally, amyloid fibrils themselves might be initiated through homocysteine. Elevated homocysteine levels, therefore, may operate by a number of mechanisms to influence dementia and Alzheimer's disease in genetically susceptible individuals.

Some individuals have folate metabolic uniqueness as a consequence of genetic polymorphisms such as the MTHFR polymorphism. One might ask what level of folate, vitamin B12, and other methylating nutrients might help these individuals overcome this genetic "small pipe." We are talking about a single transition mutation, in which the cytosine at nucleotide 677 (in cDNA) is changed to a thymine the so-called MTHFR 677C>T mutation. In the heterozygous form, this mutation may be present in 35 percent of the population. The homozygous form, called the MTHFR $677^{T/T}$ form, may occur in 10 to 15 percent of the population. In other words, 10 percent of the 280 million individuals in the United States, or 28 million people, may be at much higher risk for these conditions as a consequence of uniquenesses in folate metabolism.

MTHFR Genotype and Folate Supplementation
An interesting paper titled "5, 10- Methylenetetrahydrofolate Reductase Genotype Determines the Plasma Homocysteine-Lowering Effect of Supplementation with 5-Methyltetrahydrofolate or Folic Acid in Healthy Young Women" was recently published in theAmerican Journal of Clinical Nutrition.[10] The metabolic uniqueness in the MTHFR 677C>T polymorphism reveals itself at the stage where 5, 10-methylenetetrahydrofolate is converted into 5-methyltetrahydrofolate. That is the "small pipe," so to speak, or the "small wire."

One is asked to consider the effects on subsequent metabolic dysfunction of administering the downstream substance or metabolite, which is 5-methyltetrahydrofolate that bypasses that metabolic block. Would the result be improved function, lowered homocysteine, and reduced risk to metabolic dysfunctions that cut across the disease diagnostic codes and specialties of peri- or neonatal, medicine pediatrics, oncology, cardiology, rheumatology, diabetes management, neurology, or psychiatry? Patients of doctors in all of those fields have illnesses associated with potential folate interruptions. They come to their doctors with signs and symptoms of specific dysfunctions of unknown origin that may be connected to these MTHFR polymorphisms, particularly the $677^{T/T}$ homozygous form.

Homocysteine and MTHFR Genotype
The authors of the paper mentioned above evaluated the homocysteine-lowering potential of folic acid and racemic 5-methyltetrahydrofolate with regard to the MTHFR genotype. This was a randomized, placebo-controlled, double-blind trial with 160 women who received either 400 µg of folic acid, an equal molar amount of 5-methyltetrahydrofolate, or placebo during an eight-week treatment trial.

Changes in plasma homocysteine concentration were found to depend both on the supplemented folate derivative and the MTHFR genotype. Those with the TT genotype had the greatest decrease in homocysteine after supplementation with folic acid and 5-methyltetrahydrofolate. Perhaps, because racemic 5-methyl-THF rather than the natural 6S-isomer was used, folic acid appeared to be more effective than the methylated vitamin.

Applied Orthomolecular Medicine

This field of research is attempting to identify individuals who are genetically most at risk, utilize the appropriate form of a nutrient that might bypass those metabolic uniquenesses, and produce a functional phenotype to reduce relative disease risk. This is consistent with Dr. Linus Pauling's concept of molecular medicine, later termed orthomolecular medicine, using orthomolecular substances unique to the individual's requirement.

It is also connected to Dr. Roger Williams's concept of genetotrophic disease, which he described in an article titled "The Concept of Genetotropic Disease," in a 1950 issue of theLancet. At that time Dr. Williams was proposing a whole new model of disease. He suggested the genotype of the individual was not being met with the appropriate nutrition to meet his or her need. The result was expressed in the phenotype of diseases across a wide range of clinical ICD9s that have not been identified as having a nutritional link. We are beginning to see the acceptance of this concept as a component of the newly emerging application of genomic-based medicine.

Let us look at the hormone replacement therapy approach to modify symptoms and conditions associated with menopause. A recent paper in the Journal of the American Medical Association is titled "Quality-of-Life and Depressive Symptoms in Postmenopausal Women after Receiving Hormone Therapy."[11] These were mixed conjugated equine estrogens and medroxyprogesterone acetate. It was the first study to evaluate outcome in terms of quality of life and neurocognitive function in women who had been taking these replacement therapies. The title of the editorial that follows this paper speaks volumes. It is "Postmenopausal Hormone Therapy and Quality of Life, No Cause for Celebration."[12]

The study found that unless a woman had very severe vasomotor flushing symptoms at perimenopause or menopause, the use of replacement therapy was actually not beneficial and might even have been deleterious. It might have produced more symptoms and more adverse outcomes for the woman. Therefore, according to this study, many women are being overtreated or inappropriately treated with mixed conjugated estrogens as a consequence of the general belief, once again, that one size fits all. Symptom suppression or symptom management in women with a lot of flushing might be a positive outcome. There are, however, a number of risks associated with this treatment. Recent studies suggest that some benefits ascribed to this therapy, such as protection against heart disease and bone fractures, do not apply to all women

In a risk/benefit tradeoff, the relative risk of breast cancer that results from hormonal replacement therapy may tip the equation toward risk over benefit. A recent paper in the British Medical Journal discusses recent developments in breast cancer and the understanding of its etiology,[13] not only that of BRCA1 and BRCA2 carriers. Individuals with this genetic mutation have a much higher familial risk of breast cancer. Even this may be modified by utilizing selective estrogen response modifiers.

A published study shows that tamoxifen, when given to women who had BRCA mutations, resulted in no significant increase in the appearance of breast cancer over women at large. It suggests you can even modulate genetic propensity by specific environmental risk factor reduction, in this case, selective estrogen response modifiers (SERMs).[14] Many SERMs, including the soy isoflavones, are natural and have a positive effect on modulating estrogen responsiveness.

In short, when we examine the risk of breast cancer in relation to the administration of equine estrogens,

the risk/benefit equation is not clearly on the side of benefit. For some women, based on their unique genetic susceptibilities, the relative risk may be much higher on the risk than the benefit side.

Breast Cancer and HRT Study

That theme is similarly discussed in a paper in the Journal of the American Medical Association, describing work at the Fred Hutchinson Cancer Research Center and the Department of Epidemiology and Center for Health Studies at the University of Washington.[15] This paper is titled "Hormone Replacement Therapy in Relation to Breast Cancer." It was a nested case control study of 705 postmenopausal women enrolled in Group Health Cooperative of Puget Sound, age 50 to 74 years, in whom primary invasive breast cancer was diagnosed between July, 1990, and December, 1995.

Researchers found the incidence of breast cancer of all histologic types combined was increased by 60 to 85 percent in recent, long-term users of HRT, with an increasing risk on years of duration of use. Therefore, one got cumulative increase in HRT in associated breast cancer. Long-term HRT use was associated with a 50 percent increase in non-lobular cancer. These data add to the growing body of evidence that recent long-term use of HRT is associated with an increased risk of breast cancer. The researchers concluded that such use may be related primarily to lobular tumors, reevaluating, therefore, some past presumptions about the safest and most effective way to manage women through perimenopause and into their menopausal years.

Modifying Cancer Gene Expression in High-Risk Women

That leads into identification of women who are uniquely at highest risk and those who carry multigene susceptibilities. It is not just BRCA as a single gene point mutation. A paper in the Lancet talks about identification of high-risk breast cancer patients by gene expression profiling.[16] We will discuss this topic in detail with our Researcher of the Month on side II of this issue of FMU. This paper looks at metastatic disease risk pertaining to specific gene profiles of susceptibility genes, the expression of which can be modified.

A paper that appeared in the New England Journal of Medicine, titled "Production and Actions of Estrogens," describes modification of that risk.[17] Many papers are now appearing on the theme of genetic susceptibilities, functional outcome as translated into phenotype, and ways of modifying a phenotype of potential, early-stage disease risk. The authors of this paper describe estrogen metabolism into the 2-, 4-, and 16-hydroxylated estrogen families. They discuss soy isoflavones genistein and daidzein, and the lignan metabolite enterolactone, and their effect on estrogen receptor activity, estrogenicity, and estrogen metabolism. They talk about the cascade of events relating to activation of specific genetic expression patterns due to estrogen metabolites, particularly the 2- and 4-hydroxylated estrogens, and how they interact with inflammatory mediators and the whole potential for cell replication.

Hormone Effects of Soy in Postmenopausal Women

A last paper in this family discusses the effect of soy on endogenous hormones in postmenopausal women. It connects the concept of genetic susceptibilities, metabolic types, multigene risk of breast cancer, hormonal effects that women may have from endogenous and exogenous hormones, and the ways that diet might be used to modify these risks and produce a phenotype of lowered breast cancer risk. We will come back to a discussion of that topic on side II and discuss this paper in greater detail.

It is time to move to our extraordinary Researcher of the Month interview on side II of this month's FMU

INTERVIEW TRANSCRIPT

Fritz Parl, MD, PhD

Professor of Pathology
The Vanderbilt-Ingram Cancer Center
Nashville, TN 37232

JB: Our Clinician/Researcher of the Month interview this month features an investigator whose work I have had the privilege of reading in the past year or so. Dr. Fritz Parl is a professor of pathology at the Vanderbilt University School of Medicine. He has been doing pioneering work on genetic polymorphisms related to estrogen metabolism, breast cancer risk, and the relationship to various estrogen metabolites. This is a topic we have been discussing on FMU. In December 2001 we interviewed Dr. Thomas Klug, who talked about 2- and 16-hydroxyestrogen metabolites and the ratio that, according to Leon Bradlow, may have value in predicting risk.

It is a great privilege to have Dr. Parl as our guest today. Dr. Parl has written a fine book, titled Estrogens, Estrogen Receptor and Breast Cancer (IOS Press; Amsterdam, Netherlands: 2000). It is available in medical bookstores or through Amazon.com.

Polymorphisms in Breast Cancer Risk
Dr. Parl, welcome to FMU. Please tell us what caused you to begin looking at these polymorphisms you have been identifying as potentially having a relationship to breast cancer risk.

FP: Thank you, Dr. Bland, for giving me the opportunity to present my research. I will give you a brief overview of the endogenous and exogenous estrogen risk factors on breast cancer, because that’s what got me into the research.

The early studies go back to the 1960s and 1970s. Let’s talk about two sisters, one who had surgically induced menopause by removal of the ovaries at age 45, and the other who experienced natural menopause at age 55, so there is a 10-year difference of estrogen exposure. It turns out that the natural-menopause sister has about a twofold higher risk than her sibling. If the interval is even greater than 10 years, like 20 years, then the risk goes up threefold. In addition, in recent years, several prospective studies have shown that in postmenopausal women, the circulating estrogen level is significantly higher, by about 10 to 15 percent, and in those women who do develop breast cancer, so these are prospective studies.

Exogenous Risk Factors
As far as exogenous risk factors, of course there are the meta-analyses by the Collaborative Group on Hormonal Factors and Breast Cancer, which have shown that both oral contraceptives and hormone replacement therapy (HRT) are associated with increased breast cancer.18 These studies encompass more than 50 international studies involving more than 50,000 women and 100,000 controls.

Taking all this together, one is left with the evidence that estrogens do somehow cause breast cancer. The

two main questions are, first, how do estrogens cause breast cancer, and second, since all women are exposed to estrogens, why do some women get breast cancer and others do not? That's the main starting point.

Estrogen Binding and Interactions

If one looks at what estrogens do, what proteins they interact with, of course, there's the estrogen receptor. Having worked in the field for 15 years or so, however, I have found no real evidence that the estrogen receptor is causally involved (and I emphasize causally) in the development of breast cancer.

The only thing I think is important is that it is involved by binding the estrogen and driving the cell cycle, inducing cyclin D1 production, and in the G-1 phase, driving the cell cycle, so it leads to proliferation.

Estrogen-Metabolizing Enzymes

But if one looks at other proteins involved, a number of them have really only come into the limelight in recent years. These are primarily enzymes that metabolize estrogens. As it turns out, it is not the estrogens such as 17-b-estradiol and estrone that may be the culprits, but rather their metabolic products. These, in particular, are the catechol estrogens.19 The catechol estrogens are further metabolized to estrogen quinones, and these estrogen quinones are very labile, aggressive compounds that attack anything in sight, including DNA. This leads to DNA adduct formation, setting up mutations, and thereby establishing a link, at least experimentally, at this point, to cancer development.

So, how is estrogen metabolized? The key enzyme expressed in breast tissue is an enzyme called cytochrome P4501b1.20 The body has many cytochromes. This one, in particular, is expressed in breast tissue. It metabolizes estrone by oxidizing it, leading from estradiol to the catechol estrogens.

Catecholestrogens

Catecholestrogens are compounds; that's how they got their name. They have two OH hydroxyl groups next to each other, just like the catecholamines, so they are similar chemically. Of course, they have the steroid ring as part of the molecule.

These enzymes have polymorphisms, and to come back to your original question, we would have a mechanism whereby estrogens can cause cancer and some women have polymorphisms. Others have wild-type enzymes, and a difference would thereby arise over time, mainly to different catechol estrogen levels in the breast, and thereby set up a different milieu for mutations.

Risk Differences between 2-Hydroxylated and 4-Hydroxylated Estrogens

JB: In this emerging model you've described, I want to differentiate between the 2-hydroxylation and 4-hydroxylation patterns. They both go on to give potential quinones, but the apurinic effects of the 4-hydroxylated sterols or estrogens appear to be more potentially injurious or mutagenic/carcinogenic than the 2-hydroxylated. The 2-hydroxylated themselves are not completely benign, however, I would assume. Is there a relative ratio risk difference in terms of jeopardy to the breast tissue with the 4- versus the 2-family?

FP: That has not been established, except in vitro. I might point out that the 4-hydroxyestradiol, or estrones, were overlooked in past years for technical reasons, because the HPOC determination of catecholestrogens did not separate the 2-hydroxy from the 4-hydroxy compounds. With better techniques,

including gas chromatography and mass spectometry, you can clearly separate the 4- and the 2- hydroxy. The older literature for that reason is almost irrelevant because it didn't distinguish between these two compounds.

As you point out, the 4-hydroxy catechol estrogens and the quinones that are formed further on are more damaging, and there are several reasons for that. One is a half-life. There may also be some chemical reasons, as far as structural differences. So we can form a ratio. We did that, in fact, in one of our publications. Generally, the 4-hydroxyestradiol over the 2-hydroxyestradiol is between 2 and almost 4, and the difference depends on which polymorphism you are studying. Once again, it comes back to polymorphisms. One should keep in mind the fact that estrogens are in the tissue, in particular breast tissue in this case, throughout reproductive life. So even if a subtle difference exists over time and over the year, it may be quite significant.

Bradlow's Work

JB: We have heard about the Bradlow work on the 2-hydroxylated and 16-hydroxylated estrogen ratio and how that, from prospective work that was done in Britain and elsewhere, suggests that is in itself the major determinant of estrogen metabolite risk to breast cancer. If I understand what you're saying, this is built around information prior to being able to differentiate the 4- from the 2-, so the story will probably be changing. We'll have to add the 4-hydroxylated compounds into this mix to develop the appropriate assessment of risk.

FP: That is indeed correct.

Genotype and Breast Cancer Risk

JB: Many clinicians have heard about genotypes that may give rise to increasing risk of breast cancer. We have heard most about BRCA1 and BRCA2. Would you discuss that part of the story as it relates to polymorphisms and relative risk?

FP: In the overall picture, breast cancers can be divided into so-called sporadic breast cancer and familial or inherited forms. The latter represent around 5 percent of all breast cancers, and BRCA1, 2, and possibly 3 account for a portion of those familial forms. So we are dealing with individual proteins, and they are mutated. Because of what in genetic terms are called penetrants in genetic terms, they lead over time to breast cancer.

In the case of the more common sporadic cancers, no single gene has been identified. So the genes and the protein products, the enzymes I mentioned, act together, namely the cytochrome P450, and there are a set of associated genes called COMT which stands for catechol-O-methyltransference and GST, glutathione-S-transferase. They act together in this catechol estrogen pathway. No one of these genes by itself does the damage alone. If that were the case, then it would appear clinically as a familial breast cancer.

Breast Cancer as a Multigene Disorder

JB: In the last couple of years, we have been developing the theme that the major age-related degenerative diseases that ultimately become the most common causes of death are not single-point gene mutational illnesses. We have suggested that they are, instead, multigene disorders related to both genomic expression and later proteomic outcome into the phenotype as a consequence of these genetic

patterns being thrust into environments that are harmful for that individual. That model seems to apply to breast cancer risk in the majority of cases of breast cancer, as you've just described it. Is that correct?

FP: I don't think breast cancer is an exception from other multifunctional, multigene diseases. What that means, of course, is that it will be very difficult to pinpoint individual genes now that we're past BRCA 1, 2, and 3. The complex task of figuring out which of these genes and in what form they act together represents a challenge for the next few years.

Estrogens, Estrogen Receptor and Breast Cancer

JB: In your book, Estrogens, Estrogen Receptor and Breast Cancer, you talk about the reviews of 17-b-hydroxysterol dehydrogenase and estrogen sulpholtransferases, the cytochrome P450 1A1 and 1B1 and the relationship also to COMT and the estrogen receptors.21 Would you give us an overview of the significance of this pattern?

FP: Estradiol and estrone are metabolized by cytochrome P450s, such as 1B1 and 1A1. At this point we think the 1B1 is more highly expressed, so it may be more important, just for quantitative reasons. The 2- and the 4-hydroxyestradiol (and I leave out estrone at this point because it is really parallel metabolism), the catechol estrogens, can go in two directions. They can go to the quinones, and that reaction is metabolized by the P450 enzymes, so they actually do two reactions in sequence. The side reaction is carried out by the catechol-O-methyltransferase, COMT.

It turns out that COMT has a polymorphism that is rather common. Approximately half the population has both alleles, the wild type and the variant allele, so it follows Mendelian genetics. About one third are homozygous variant; 25 percent are one-third homozygous wild type; and 50 percent are heterozygous. What that means is there is this variant that changes valine to a methionine, slows down the enzyme, and thereby decreases the metabolism from the 2 or 4-hydroxyestradiol to the methylated form. The methylated form appears to be an endproduct, so it is a side reaction that prevents the quinone formation. Thus a threefold difference between woman A and woman B may, over time, be quite significant.

The Methylation Pathway

JB: Is the methylation pathway the principal pathway for biotransformation of those 2- and 4-hydroxylated compounds?

FP: Yes. It is the first of two reactions of these enzymes. They are also called type two reactions. First, type one reactions are metabolized by the cytochrome P450s. The second reactions are carried out by glutathione-S-transferases, and there is a family of several enzymes called M1P1T1. Once again, each of these has polymorphisms. In fact, the GSTM1 has a very common polymorphism that affects not just one amino acid. It is a so-called null deletion, which means the gene itself is lacking. The GSTT1 also has a null deletion, and GSTP1 has a point mutation or polymorphism.

So the three enzymes together are expressed at different levels, or have different levels of activity in different women. Once again, the attachment of the glutathione is a side reaction, in this case to the quinone itself, which prevents the formation of DNA adducts. Once again, a threefold difference between woman A and woman B over time may be quite significant.

Polymorphic Risk to Quinones

JB: To review what you just said, the 2- and 4-hydroxylated compounds have one potential fate through COMT, which is methylation. If there is redox recycling of the hydroxylation compounds going to the quinones, such as the 4 quinones, then that has to be trapped by glutathione as a second step. Both of those steps have significant polymorphisms, with the glutathione conjugation step having no polymorphisms. This means there are people who can't do that well at all who would be significantly at risk to those quinones, or so it sounds.

FP: That is indeed correct. We are looking, so far, at only four enzymes, CYP1, A1, B1, COMT, and the GSTs of which there are at least three. We are looking at several enzymes, so we have a well-defined pathway. We know at this point which reaction occurs. We can biochemically determine the difference in activity. The task is to assess the clinical relevance of that information. So we have compared them in a clinical correlation study. When we initially got into this, we extracted DNA from women we had analyzed for estrogen receptor for clinical purposes.

We compared 200 women with sporadic breast cancer and 200 age-matched controls. We did a multigene analysis, using, in that case, a computer model developed by Jason Moore, one of my colleagues here at Vanderbilt. We are presently testing this multigene computer model to determine how relevant indeed these interactions are.

Pattern Recognition in Diagnosis

JB: I've had a chance to read your recent paper in the American Journal of Human Genetics, titled "Multifactor-Dimensionality Reduction Reveals High-Order Interactions among Estrogen-Metabolism Genes and Sporadic Breast Cancer."22 I think this article represents a brilliant new paradigm in medicine as it relates to diagnosis, assessment, or prognosis. It means looking at patterns, which is very different from the way pathology has been determined historically, which has been generally to look at a single point that indicates pathology. Philosophically, this is certainly a new way to look at potential risk of illness.

FP: Yes. I might point out there is an interaction between genes like BRCA1 and these genes that I mentioned, and so it should be. If you review the literature on BRCA1, you look at family trees in any given family, breast cancer may appear between the ages of 35 and 55 in women who have the same BRCA1 mutation. It has been very puzzling why that is so if they have the identical mutation and yet, one develops breast cancer at age 35 and the other at 55. It is likely that the estrogen metabolism kicks in somewhere along the line, and it would be interesting to see the genotypes of these siblings, how different are they with respect to these multigene polymorphisms having the same BRCA mutation.

16-Hydroxylated Estrogen

JB: That's very interesting. We will look forward to some of that information coming out of your work. I'd like to go back and pick up the 16-hydroxylated estrogen story. How does the 2- and 4- story contrast to the 16-, which we have been told is really mitogenic and has a very strong cell proliferative stimulatory effect? How would you weigh these in terms of the trajectory of this research?

FP: When we analyzed the activity of CYP1b1, that is when we exposed the enzyme to estradiol and to estrone, we found primarily 4-hydroxyestradiol and then 2-hydroxyestradiol, and as a minor fraction, 16-a-hydroxyestradiol. To date, no enzyme really has been identified that carries out this reaction, so quantitatively it seems that if B1 is indeed the main estrogen metabolizing enzyme, it would be a small

fraction in the breast compared to 4 hydroxy and 2 hydroxyestradiol.

Risk-Associated Analytes of Estrogen

JB: I don't want to jump to conclusions from what you've said. My takeaway from that information, however, is that if you looked at a ratio of 2- to 16-hydroxylated estrogen in either plasma or urine, 16-hydroxy is going to be kind of a consonance. It is almost like doing something in the urine versus creatine; it's going to be just not very changed. What really will change are the 2-hydroxylated levels, so your ratio would reflect that. It's like a denominator in the 16-hydroxy that is a constant, so the real action is probably going to be the 2- and the 4-hydroxylated derivatives in terms of analytes of estrogen that might have these relative risks.

FP: Yes, I think that is so. As you mentioned, these levels were determined in urine or serum, and that does not necessarily reflect what is going on in the breast. I make that point because pharmacologists for years have looked at liver enzymes and estrogen metabolism. In the liver, an entirely different set of cytochrome P450s is expressed.

Over the past few years we have come to appreciate the fact that the differences in tissue expression are quite significant and important. The CYP1b1 is the primary P450 enzyme in the breast, but it plays only a minor role in the liver. For that reason, I think the local level in breast (obviously that's where the action occurs over years), may not be mirrored at all in urine or circulating blood levels.

Hormone Replacement Therapy

JB: That leads me to a last question of perimenopausal or postmenopausal hormone replacement therapy (HRT). Is there any assumption at this point that exogenous hormones have an influence on breast hormone metabolism, or is it just endogenous differentiation that we are concerned about?

FP: This is an ongoing discussion. Some of your readers may have recently read the JAMA article in February 13 issue of this year by a group at Fred Hutchinson Cancer Research Center (see Reference 15). Again, they found a significant difference between women taking HRT compared to those that do not. These authors refer back to the Collaborative Group on Hormonal Factors and Breast Cancer study published in the Lancet in 1997, which is the meta-analysis where the authors pooled 54 international studies and showed that indeed there is a significant risk associated with HRT.

How significant is it? The risk increases by a factor of 1.023, that is by 2.3 percent, for each year of use. That is highly significant because these meta-analyses looked at data from over 50,000 women with breast cancer and twice as many controls, and the P value was 0.0002. You might believe that is not really that striking, but if you are exposed for five years, then if you multiply out, your risk goes up to 1.35, or 35 percent. These meta-analyses give a better picture of what's going on than some individual studies, which at times are outright contradictory.

Polymorphisms and HRT

JB: It would seem from your work that there may be differential risks for specific women who carry certain polymorphisms whose metabolic ability to manage estrogen would be different that that of other women. So it is a whole new concept: one size does not fit all.

FP: That is correct. These meta-analyses take all women, and if one would apply genetic studies, then one

could separate or identify women who are genetically at higher risk because their estrogen metabolism is, if you will, more damaging over time. One could thereby identify a high-risk group that should not be exposed to replacement therapy. But on the other hand, there is a group that genetically are at lower risk and thereby could safely respond to HRT. So it would be a benefit for the post-menopausal group where genotyping could be very important.

Medicine of the 21st Century

JB: Thank you, Dr. Parl. I compliment you on your work and that of your colleagues. It takes us in a direction that is consistent with the new concepts of personalized 21st century medicine, and genomic medicine. We like to think it will help improve function in individuals so they can live to the full limits of their biological potential. Once again, thank you for being with us today

I now return to discuss in greater detail the study of the effects of soy on endogenous hormones in postmenopausal women. Dr. Parl talked about the 2- and 4-hydroxylated estrogens. We know soy has an influence on several levels of this pathway, both estrogen responsiveness at the receptor site and the relative effects on hormone hydroxylation and metabolism. That information makes the conclusions in this particular paper even more significant in regard to information we might be giving women about modifying their relative risk.

This paper, which appeared in the American Journal of Clinical Nutrition, is titled "Effect of Soy Protein on Endogenous Hormones in Postmenopausal Women."[23] The authors, who are from multiple sites, are experts in the area of hormone metabolism and soy isoflavones. Stephen Barnes from Alabama, for example, is a well-known investigator. John Erdman, Jr., from the University of Illinois, has been in this field for a number of years. Dr. Victoria Persky has done extraordinary work in the pharmacology of flavonoids over the last several years. Susan Potter is a principal investigator in this area. And finally, Dr. H. Leon Bradlow is the originator of the 2-, 16-hydroxylated estrogen story. This is an esteemed group of investigators.

Clinical Trial of Soy Protein and Endogenous Hormones in Postmenopause

The study was designed to evaluate the influence of soy on endogenous hormones in postmenopausal women. The study included 73 hypercholesterolemic, free-living, postmenopausal women who participated in a six-month, double-blind trial in which they received 40 grams of protein as part of a National Cholesterol Education Program Step I diet. It was provided as casein from nonfat dry milk or isolated soy protein containing 56 mg isoflavones (mimicking what traditional Japanese and Chinese women might consume in their daily diet) or 90 mg isoflavones. This higher level might be more consistent with therapeutic intake of soy as soymilk, soy flour, and soy protein.

Endogenous hormone concentrations were measured at baseline and at three and six months. The number of parameters measured in this study indicates it is quite a remarkable endocrinological survey. The researchers measured not only sex hormone- binding globulin and estrogen as estradiol and estrone, but also the 2- and 16-hydroxylated estrogen metabolites, dehydroepiandrosterone sulfate, follicle-stimulating hormone, leutenizing hormone, cortisol, levels of isoflavones in the blood, and thyroid hormones including TSH, T4, T3, as well as insulin, glucagon and the insulin/glucagon ratio. This remarkable study looked at endocrine effects of soy intake against a placebo.

Effects of Soy on Thyroid Hormones

The outcome is quite fascinating. First we look at thyroid. A common belief is that thyroid hormones are somehow suppressed by consumption of soy on a regular basis in women taking thyroid. This effect was not evident at either the 56 mg or 90 mg isoflavone level per day. Thyroid hormone effects were modest to insignificant, and those that were modest showed increase in levels of thyroid hormone activity rather than decrease.

That information contradicts some people's belief that soy causes goiterous response and suppresses thyroid function. This presumption was not supported when the researchers looked at TSH levels at baseline versus TSH levels at three and six months. The TSH levels were indistinguishable statistically, and the thyroid index, thyroxine, and T3 all remained in the normal range. Although there was some modest increase in T3, it did not achieve statistical significance during the course of the intervention with soy.

We can conclude that at normal levels of intake in the diet of a postmenopausal woman, soy protein containing isoflavones at the level of 56 or 90 mg per day does not cause significant alteration in thyroid hormone. If anything, it causes a slight increase in T4 and T3.

Sex Steroid Hormones

Second, with regard to sex steroid hormone values and insulin sensitivity, there was some influence on a case-by-case basis, but no significant differences across the group for any of the endogenous estrogens, cortisol, dehydroepiandrosterone sulfate, insulin, glucagon, or FSH, after controlling for baseline hormone values. It does not appear, on a gross level, that soy protein containing 56 or 90 mg of isoflavones per day influences endocrine balance in women. Its effect is more likely to be at the genomic and cell physiological level and independent of gross endocrinological effects.

Soy isoflavones are best defined as adaptogens, which means they have agonist/antagonist activities, rather than as therapeutic phytoestrogens. I believe it is misleading to label these isoflavones as phytoestrogens. If a woman has high estrogens, they participate more as antagonists by bringing estrogenic activity down. If a woman has low estrogen, they serve more as agonists by increasing estrogenic activity at the estrogen receptor or cell transduction sites. Therefore, labeling them phytoestrogen is misleading.

Agonist/Antagonist Role of Soy Isoflavones

How can soy isoflavones participate as agonists/antagonists? They do so because of their weak interactions with receptor sites and cell membrane transport activities, as contrasted to estrogens that bind much more tightly and activate a much more profound gene expression response. One gets mild or more orchestrated, gentle effect with the isoflavones derived from soy.

We often hear soy isoflavones described as estrogens. Why would one give an estrogen to a woman who has an estrogen-positive receptor site in her breast? The answer is that these isoflavones do not behave like estrogens. They have post-estrogen receptor activities, different binding effect or ERα and ERβ. They influence the orchestration of gene expression through the nuclear receptor family transport processes differently, and they participate more as agonists/antagonists or adaptogens than as pharmacological estrogen replacements.

Clinical Relevance

Some things in this paper could be of clinical importance for practitioners involved in making decisions and counseling patients about estrogen and soy as part of their program. The authors state that in some women the possibility of the effect of soy on thyroid hormones may be related to properties that suggest changes in thyroid hormone were greatest in women with the lowest measures of estrogenicity at baseline. A postmenopausal woman whose adrenal glands could not take over, for example, might have very low baseline estrogen levels. In that case, supplementation with isoflavones might actually raise her thyroid function.

The association of changes in isoflavone concentration with changes in bone mineral density and HDL concentrations at six months in this study in women with the lowest baseline estrogen levels also supports the estrogenic effects of soy. The results suggest increased HDL and increasing bone mineral density, which are beneficial, not deleterious, effects of soy. There is evidence that estrogens may increase the sensitivity of the pituitary or thyroid gland to normal feedback mechanisms. It is possible that the agonist estrogenic effect of isoflavones in a woman with very low estrogens may have positive benefits.

Study Summary
In summary, the authors say the study does not show significant effects of soy protein or isoflavones on serum or urinary estrogens, sex hormone-binding globulin, follicle-stimulating hormone, cortisol, DHEA, insulin, or glucagon. The study did show small effects on thyroid hormones that are unlikely to be clinically important, and in very low estrogen women they showed effects that may be beneficial.

I hope that gives you some takeaway evidence that ties back to estrogens, estrogen receptors, and breast cancer. There are environmental modulators. We know about indole-3 carbinol's effect on increasing the 2-hydroxation at the extent of 4-hydroxylation. This is an exciting chapter in nutrition-focused genomic medicine.

We hope to see you at our symposium in May.

Bibliography

1. Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer. N Engl J Med. 2000;343(2):78-85.

2. Svendsen AJ, Holm NV, Kyvik K, Petersen PH, Junker P. Relative importance of genetic effects in rheumatoid arthritis: historical cohort study of Danish nationwide twin population. BMJ. 2001;323:1-5.

3. Jula A, Marniemi J, Huupponen R, Virtanen A, Rastas M, Ronnemaa T. Effects of diet and simvastatin on serum lipids, insulin, and antioxidants in hypercholesterolemic men. JAMA.2002;287(5):598-605.

4. Diabetes prevention program research group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or Metformin. New Engl J Med. 2002;346(6):393-403.

5. Denke MA. Dietary retinol--a double-edged sword. JAMA. 2002;287(1):102-104.

6. Feskanich D, Singh V, Willett WC, Colditz GA. Vitamin A intake and hip fractures among postmenopausal women. JAMA. 2002;287(1):47-54.

7. Yehuda R. Post-traumatic stress disorder. N Engl J Med. 200-2;346(2):108-114.

8. Gouni-Berthold I, Berthold H. Policosanol: clinical pharmacology and therapeutic significance of a new lipid-lowering agent. Am Heart J. 2002;143:356-365.

9. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. New Engl J Med. 2002;346(7):476-483.

10. Fohr IP, Prinz-Langenohl R, Bronstrup A, et al. 5, 10- Methylenetetrahydrofolate reductase genotype determines the plasma homocysteine-lowering effect of supplementation with 5-methyltetrahydrofolate or folic acid in healthy young women. Am J Clin Nutr.2002;75:275-282.

11. Hlatky MA, Boothroyd D, Vittinghoff E, Sharp P, Whooley MA. Quality-of-life and depressive symptoms in postmenopausal women after receiving hormone therapy. JAMA.2002;287(5):591-597.

12. Rexrode KM, Manson JE. Postmenopausal hormone therapy and quality of life. No cause for celebration. JAMA. 2002;287(5):641-642.

13. Morrow M, Gradishar W. Recent developments. Breast cancer. BMJ. 2002;321:410-414.

14. Narod SA, Brunet JS, Ghadirian P, et al. Tamoxifen and risk of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers: a case-control study. Lancet. 2000;356(9245):1876-1881.

15. Chen CL, Weiss NS, Newcomb P, Barlow W, White E. Hormone replacement therapy in relation to breast cancer. JAMA. 22002;287(6):734-741.

16. Ahr A, Karn T, Solbach C, et al. Identification of high risk breast-cancer patients by gene expression profiling. Lancet. 2002;359:131-133.

17. Gruber CJ, Tschugguel W, Schneeberger C, Huber JC. Production and actions of estrogens. N Engl J Med. 2002;346(5):340-351.

18. Collaborative Group on Hormonal factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52 705 women with breast cancer and 108 411 women without breast cancer. Lancet.1997;350:1047-1059.

19. Dawling S, Roodi N, Mernaugh RL, Wang X, Parl FF. Catechol-O-methyltransferase (COMT)-mediated metabolism of catechol estrogens: comparison of wild-type and variant COMT isoforms. Cancer Res. 2001;61:6716-6722.

20. Hanna IH, Dawling S, Roodi N, Guengerich FP, Parl FF. Cytochrome P450 1B1 (CYP1B1) pharmacogenetics: association of polymorphisms with functional differences in estrogen hydroxylation activity. Cancer Res. 2001;60:3440-3444.

21. Parl FF. Estrogens, Estrogen Receptor and Breast Cancer. Amsterdam, Netherlands; IOS Press:2000.

22. Ritchie MD, Hahn LW, Parl FF, et al. Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. Am J Hum Genet. 2001;69:138-147.

23. Persky VW, Turyk ME, Wang L, et al. Effect of soy protein on endogenous hormones in postmenopausal women. Am J Clin Nutr. 2002;75:145-153p>