Botanical influences on estrogen metabolism - a literature review in the field of complementary cancer care

Chanchal Cabrera MNIMH, AHG

Incidence of breast cancer

Breast cancer is the most common form of cancer among women in Europe, North and South America and Australasia; approximately 1 in 10 women in Western countries will develop breast cancer during their lifetime. It is estimated that the disease will affect five million women worldwide over the next decade, and the incidence of breast cancer is increasing on average by about 1% per year in industrialized countries and at a greater rate in developing countries.

Breast cancer is the second leading cause of death among American women and by the year 2000 it was estimated that 1 billion women worldwide would have breast cancer. 180,000 new cases of breast cancer are expected to be diagnosed in the USA in 2001. Figures compiled by the World Health Organization over the past 40 years show a steady increase in both the incidence and the death rate from breast cancer and only in the past 5 years has this been seen to come down.

Table 1
Incidence of breast cancer morbidity and mortality in UK, USA and Australia 1955 – 1997

<table>
<thead>
<tr>
<th>Year</th>
<th>Australia - population in millions</th>
<th>Number of deaths</th>
<th>Deaths per million</th>
<th>UK – population in millions</th>
<th>Number of deaths</th>
<th>Deaths per million</th>
<th>USA – population in millions</th>
<th>Number of deaths</th>
<th>Deaths per million</th>
</tr>
</thead>
<tbody>
<tr>
<td>1955</td>
<td>9</td>
<td>1107</td>
<td>120.3</td>
<td>50</td>
<td>9595</td>
<td>188.3</td>
<td>164</td>
<td>21,945</td>
<td>133.6</td>
</tr>
<tr>
<td>1960</td>
<td>10</td>
<td>1151</td>
<td>112.0</td>
<td>52</td>
<td>10,267</td>
<td>196.0</td>
<td>179</td>
<td>23,970</td>
<td>133.7</td>
</tr>
<tr>
<td>1965</td>
<td>11</td>
<td>1285</td>
<td>113.3</td>
<td>54</td>
<td>10,957</td>
<td>201.3</td>
<td>193</td>
<td>27,048</td>
<td>139.6</td>
</tr>
<tr>
<td>1970</td>
<td>12</td>
<td>1497</td>
<td>119.4</td>
<td>55</td>
<td>12,121</td>
<td>217.5</td>
<td>204</td>
<td>29,917</td>
<td>146.5</td>
</tr>
<tr>
<td>1975</td>
<td>13</td>
<td>1656</td>
<td>120.2</td>
<td>56</td>
<td>13,081</td>
<td>233.8</td>
<td>215</td>
<td>32,435</td>
<td>150.5</td>
</tr>
<tr>
<td>1980</td>
<td>14</td>
<td>1803</td>
<td>123.4</td>
<td>56</td>
<td>13,671</td>
<td>242.8</td>
<td>227</td>
<td>35,641</td>
<td>156.9</td>
</tr>
<tr>
<td>1985</td>
<td>15</td>
<td>2195</td>
<td>139.3</td>
<td>56</td>
<td>15,073</td>
<td>266.7</td>
<td>239</td>
<td>40,093</td>
<td>167.9</td>
</tr>
<tr>
<td>1990</td>
<td>17</td>
<td>2421</td>
<td>141.9</td>
<td>57</td>
<td>15,179</td>
<td>266.4</td>
<td>256</td>
<td>43,391</td>
<td>169.7</td>
</tr>
<tr>
<td>1995</td>
<td>18</td>
<td>2598</td>
<td>143.8</td>
<td>58</td>
<td>14,114</td>
<td>240.8</td>
<td>263</td>
<td>43,844</td>
<td>166.9</td>
</tr>
<tr>
<td>1997</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>59</td>
<td>13,399</td>
<td>227.1</td>
<td>268</td>
<td>41,943</td>
<td>156.7</td>
</tr>
</tbody>
</table>

Source: World Health Organization

Although not confirmed it is thought that the recent decline in incidence and mortality may be due to several factors including but not limited to earlier detection, increased knowledge by the patients of contributory and protective dietary factors and the use of modern endocrine-modulating drugs. However, these statistics must be viewed critically in light of the fact that each country may apply different criteria to the diagnosis of cancer and that these criteria may change over time.
**Etiology of breast cancer**

Although the specific etiology of breast cancer remains unknown, a number of factors are recognized which increase a woman's risk of developing the disease. Genetic predisposition, or family history of breast cancer, is known to be responsible for 5% of all cases. The breast cancer gene BRCA1 confers a 59% risk of developing breast cancer by the age of 50 as compared to only 2% risk in the non-gene carrying population. Up to 75% of women with breast cancer have no significant family history of the disease. However, the variation in incidence throughout populations, and changes relating to population migration and adoption of altered lifestyles, all point to the critical importance of non-genetic determinants. Such factors include early menarche, late menopause, late age at birth of first child or nulliparity, a history of benign breast disease, breast density, environmental exposures especially to xeno-estrogens, and diet. Dietary modification with the introduction of soy products, curcumin, cruciferous vegetables and low fat could be beneficial in reducing the risk of developing cancer and possibly the effects of DDT.

The National Cancer Institute has estimated that only 18% of American adults consume the recommended amount (5 – 7 servings) of fruit and vegetables daily, and much of what is consumed comprises French fries, potato chips, tomato sauce and other processed foodstuffs of dubious nutritional value. There is also evidence that hormones play a major role in the etiology of breast cancer, with the risk of developing malignancies related to the cumulative exposure of the breast to estrogen and progesterone, which stimulate the growth of tumor cells. Estrogen receptors are expressed in approximately 35-55% of all breast tumors but up to 80-90% of tumors from women older than 55 years.

A bewildering number of factors play into the formation, development and progression of breast cancer and many of them offer opportunity for intervention. Of these processes, the estrogen signaling specifically is the focus of the present research proposal. Other mechanisms are mentioned for completeness. (see chart 1)

1) Hormonal (estrogen) signaling may be modulated by regulating the formation, function and excretion of estrogens.

2) Angio-genesis and consequent metastasis may be inhibited in many ways:
   * Signal transduction pathways may be modulated by inhibiting a variety of growth factors. Epidermal Growth Factor (EGF), Fibroblast Growth Factors (FGF), Transforming Growth Factor (TGF), Platelet-Derived Growth Factor (PDGF), Insulin-Like growth factors(ILGF), Protein Tyrosine Kinase (PTK), Protein Kinase C (PKC), tumor suppressor genes, Vascular Endothelial Growth Factor (VEGF) and reticular activating system (RAS).
   * Angio-genesis may also be inhibited by regulating the process of inflammation that the tumor uses to promote angiogenesis. This is achieved through several mechanisms including cyclo-oxygenase 2 (COX 2), 5, 12 & 15 lipoxygenase (LOX), platelet aggregation and Hydroxyl-3-methylgutaryl coenzyme A reductase (HMG CoA reductase) and by chelating copper from the system. Many compounds exert anti-inflammatory and chemo-protective effects, including Curcumin from Turmeric.
3) Urokinase type plasminogen activator (uPA) and matrix metalloproteinease (MMP) are two families of enzyme modulators that tumor cells use to alter the extra-cellular fluid matrix (ECF) such that they can spread more easily. Their expression is regulated by oncogenes, tumor promoters and growth factors. In health their expression is prevented by specific inhibitors in the matrix. Stabilizing the ECF, which is a form of connective tissue, can reduce cancer spread. The use of glycosaminoglycans (GAGS) as a nutritional supplementation has shown promise in treating cancer for this reason.

4) Cell adhesion molecules (CAM) are cell receptors that control intra-cellular and inter-cellular communication. CAMs regulate organ architecture, cell migration, cell differentiation, apoptosis (programmed cell death), mitosis, platelet aggregation and the activity of the immune system. There are 4 main classes of CAMs: cadherins, integrins, cell surface lectins and Immuno-globulin Super Family Cell Adhesion Molecules (ISCAMs).

**Allopathic interventions – an overview**

At the time of diagnosis, approximately 50% of patients will be diagnosed with early breast cancer. This proportion is increasing as a consequence of the introduction of early detection programs. Surgery remains the primary treatment for early breast cancer, and the frequency of radical mastectomy has been replaced by breast conserving surgery. After surgery, other therapeutic modalities such as radiation, chemotherapy or endocrine therapy may be given in the adjuvant setting. Surgical cure rates vary for patients with early breast cancer; the US figure is approximately 40%, and there are no definitive means to predict those who will be cured and those who will have recurrent disease.

Breast cancer research has developed at a rapid pace over the last decades. Age, race, tumor size, histological tumor type, axillary nodal status, standardized pathological grade, and hormone-receptor status are accepted as established prognostic and/or predictive factors for selection of systemic adjuvant treatment of breast cancer. The role of other promising new factors, such as p53 mutations, HER-2 status, plasminogen activator system, histological evidence of vascular invasion, and quantitative parameters of angiogenesis are currently or will soon be determined in prospective studies. Treatment options to date have raised almost as many questions as they have provided answers and research today is focusing more and more on natural substances and synthetic analogues as mechanisms for cell function alteration.

**Estrogen receptor site modulation and Aromatase inhibition**

Since 1896, when Sir George Beatson demonstrated that ovariectomy induced regression of mammary tumors in women, the aim of endocrine breast cancer therapy has been to selectively deprive the body of estrogen. Ovariectomy accomplished this by removing the gland that is the predominant source of estrogens in premenopausal women. Since the avoidance of such surgery is preferable, emphasis is devoted to the pharmacological inhibitors of estrogen production. Substantial evidence supports the concept that estrogens cause breast cancer in animals and in women but the precise mechanism is unknown.
How estrogen might cause breast cancer
- Estrogens stimulate proliferation of breast cells and thus statistically increase the chances for genetic mutations.
- Estrogen metabolism generates oxygen-free radicals and quinones which produce both stable and unstable DNA adducts and result in genetic mutations which accumulate and could ultimately cause cancer.
- *In situ* synthesis of estrogen due to the over-expression of intra-cellular aromatase. 40

Like other steroid hormones, the two circulating estrogens (estrone and estradiol) are produced from cholesterol. The process of transformation from cholesterol through pregnenolone, progesterone and androstenedione to testosterone largely occurs in the liver and adrenal glands. Testosterone then travels to distant tissues, notably adipose tissue and the skin, where it is converted by the action of aromatase into estrogens. There are many possible points of pharmacological interference in this process. In particular, aromatase inhibition has shown great potential, as has interference in the binding of testosterone or estrogen to target tissues through competitive inhibition mechanisms. One study of several naturally occurring and synthetic flavones found inhibition of the aromatization of androstenedione and testosterone to estrogens by several compounds including chrysin, apigenin, Quercitin and flavones. Chrysin was a potent competitive inhibitor and induced spectral changes in the aromatase cytochrome P-450 indicative of substrate displacement. The authors conclude that flavones may thus compete with steroids in their interaction with certain mono-oxygenases and thereby alter steroid hormone metabolism. 41

Inhibiting the enzymes that are involved at earlier steps in the branching pathway of steroidogenesis could have an undesirable impact on the production of other physiologically important hormones such as aldosterone and cortisol. Aromatase is a cytochrome P450 enzyme, with both an iron (heme)-containing and a steroid-binding site. Since aromatase catalyzes the last step in estrogen production, it makes an ideal target for the development of selective and potent inhibitors.

Two mechanisms for inhibiting aromatase
1) Occupying the steroid-binding site of the enzyme with a compound such as formestane (Lentaron)
2) Binding the iron with nitrogen-containing compounds such as aminogluthethimide (Orimeten), the oldest aromatase inhibitor.

Aminogluthethimide was one of the earliest pharmaceutical agents to inhibit adrenal steroid biosynthesis and block the conversion of cholesterol to pregnenolone, therefore reducing levels of adrenal androgens, which are a source of estrogens in both pre-menopausal and postmenopausal women. Aminogluthethimide has produced anti-tumor response rates of 35% in unselected patients, most of whom have undergone prior therapy with either chemotherapy or hormonal manipulation. Although aminogluthethimide has long been used to treat advanced breast cancer, its aromatase inhibition is not selective. Consequently, aminogluthethimide also binds to and thereby inhibits several other cytochrome P450 enzymes in the steriodogenesis pathway. Aminogluthethimide does have the known side effect of impaired mineralo-corticoid and gluco-corticoid activity. 42 An ideal aromatase inhibitor would fit the catalytic site of aromatase optimally and would thus interact only with aromatase. The affinity of letrozole (Femara) for the heme group of aromatase makes it a selective and potent inhibitor. In fact, studies show that
Femara has little effect on the other adrenal steroids, and is the most selective aromatase inhibitor available today.\textsuperscript{43} Flavone and isoflavone phytoestrogens are plant chemicals and are known to be competitive inhibitors of cytochrome P450 aromatase with respect to the androgen substrate.\textsuperscript{44} In one study the flavonoids catechin, daidzein, equol, genistein, beta-naphthoflavone (BNF), quercetin and rutin exerted no effect on aromatase activity. Eleven flavonoid compounds were compared with aminoglutethimide (AG) for their abilities to inhibit aromatase enzyme activity. Three naturally-occurring flavonoids, chrysin, flavone, and genistein 4'-methyl ether (Biochanin A) showed \textit{I}_{50} values of 4.6, 68, and 113 \textit{microM}, respectively, while AG showed an \textit{I}_{50} value of 7.4 \textit{microM}. Kinetic analyses showed that both AG and the flavonoids acted as competitive inhibitors of aromatase. Chrysin, the most potent of the naturally-occurring flavonoids, was similar in potency and effectiveness to AG.\textsuperscript{45, 46}

**Metabolism of estrogens**

Estrogen is a steroid hormone, manufactured in the body from cholesterol by way of pregnenolone, progesterone, androstenedione and testosterone. The final step in the pathway is conducted in the ovaries, adrenal glands and skin. Exogenous estrogens may contribute to the total load although the extent to which this is significant is unproven.\textsuperscript{47, 48} Xeno-hormones may work bi-functionally, through genetic or hormonal paths, depending on the periods and extent of exposure. Xeno-hormones can modify DNA structure or function. Two distinct mechanisms can influence the potential for aberrant cell growth: compounds can directly bind with endogenous hormone or growth factor receptors affecting cell proliferation or compounds can modify breast cell proliferation altering the formation of hormone metabolites that influence epithelial-stromal interaction and growth regulation. Beneficial xeno-hormones, such as indole-3-carbinol, genistein, and other bioflavonoids, may reduce aberrant breast cell proliferation, and influence the rate of DNA repair or apoptosis and thereby influence the genetic or hormonal micro-environments.\textsuperscript{49, 50}

Estrogen is comprised of three main types: Estrone, Estradiol and Estriol, with Estrone conferring the least cell-proliferant activity and Estriol conferring the most. Estrogen manufactured and active in peripheral tissues and organs eventually ends up in the liver where it is metabolized for excretion. Hydroxylation reactions in phase I detoxification yields either 16alpha hydroxyestrone (16alpha-OH), 4 hydroestrone (4-OH) or 2 hydroxyestrone (2-OH). (see charts 2 & 4).

16alpha-OH goes on to be reduced and form Estriol which is the most cell proliferant of the 3 endogenous estrogens although each of the estrogens are capable of binding to target tissues. All three hydroxyestrones can be excreted via glucuronidation, methylation or sulfation and are eliminated in bile and urine. However, studies suggest that elevated 2:OH : 16alpha -OH ratio is protective in breast cancer. In the presence of certain critical co-factors, (L-methionine, B12, B6, S-adenosyl methionine, S-adenosyl homocysteine, choline, folate, magnesium) 4-OH is cycled into 2-OH in the liver. In the absence of these co-factors, and in the absence of adequate levels of anti-oxidants, then 4-OH may oxidize into toxic quinines. Unavailability or deficiency of glutathione-S-transferases, the family of iso-enzymes that act as intra-cellular anti-oxidants, permits the onwards formation quinoones into toxic mercapturates.\textsuperscript{51} In this study we shall
research the extent to which 2-OH formation can be promoted using specific botanical extracts and nutritional supplies. The therapeutic intent is to promote estrogen clearance.

(See charts 2 – 5)

**Botanical & nutritional intervention in breast cancer development and progression**

Much recent research has focused on the influence of a variety of naturally occurring substances or their synthetic analogues. Phyto-estrogens, many of them flavonoids in nature, as well as other flavonoids, lignans, catechins, progestins and essential fatty acids have been investigated and many have shown significant promise in reducing the occurrence and spread of breast cancer. An extensive literature review revealed thousands of supportive citations for the idea of supplementing the diet with specific botanicals and nutritional supplements and a few inconclusive or dismissive.

In many cases the results of research have demonstrated variable results according to dose or potency of test material. At low concentrations, genistein and coumestrol significantly enhanced E2-induced and tyrosine kinase-mediated DNA synthesis; at high concentrations, inhibition was observed. Differing effects are observed with other compounds. 52

**Opportunities to inhibit estrogen formation**

- Inhibition of the sulphotransferases that sulphate both estrogenic steroids and a variety of environmental chemicals, including dietary pro-carcinogens. Circulating steroid sulphates are thought to be the major source of estradiol in post-menopausal breast tumors and sulphation is a key step in the activation of some dietary pro-carcinogens. 53

- The enzyme 17beta-hydroxysteroid dehydrogenase type 5 (17beta-HSD 5) converts estradiol (the more active form) to estrone (the less active form). Inhibition of reductive and oxidative activities of 17beta-HSD 5 is seen in the presence of many dietary compounds, especially zearalenone, coumestrol, quercetin and biochanin A. Inhibitor potency increases with an increasing number of hydroxylations in the flavonoids molecule. 54

- Inhibition of aromatase through use of lignans and flavonoids. 55 56 Wine has been shown to contain phytochemicals that are capable of suppressing aromatase. Red wine was shown to be much more effective than white wine in the suppression of aromatase activity. 57 In another study, among several flavonoids tested, only 7-methoxyflavanone and 7,8-dihydroxyflavone at high concentrations (50 microM) possessed anti-estrogenic and anti-proliferative activities. These results suggest that two hydroxyls (in positions 7 and 8) or 7-methoxy substitutes are essential for the anti-estrogenic activity of flavonoids. However, the authors emphasize that flavonoids at high concentrations appear to exert their anti-proliferative activity through other estrogen receptor-independent mechanisms as well. 58
Opportunities to inhibit estrogen function

- Human estrogen receptors (ER) exist as two subtypes, ER alpha and ER beta, which differ in the C-terminal ligand-binding domain and in the N-terminal transactivation domain. Competition with estrogens at the receptor site of the target tissue can modulate estrogen activity at the functional level. Several lignans and the isoflavonoids daidzein and equol were found to compete with estradiol for binding to the rat uterine type II estrogen binding site. Some phytoestrogens such as coumestrol, genistein, apigenin, naringenin, and kaempferol compete stronger with E2 for binding to ER beta than to ER alpha and the effect of this on clinical outcomes has yet to be investigated. The compound Indole-3-carbinol (I3C), extracted from cabbage, and its more metabolically available and active form, diindolyl-methane (DIM) have received much attention recently as potent mechanisms for influencing estrogen function at the receptor site. DIM is a major in vivo product of I3C, and can inhibit the proliferation of both estrogen-dependent and -independent breast tumor cells. It is an antagonist of estrogen receptor function and a weak agonist of aryl hydrocarbon (Ah) receptor function.

- Green tea extract inhibited protein kinase C activation by teleocidin, a tumor promoter, as did (-)-epigallocatechin gallate (EGCG), the main physiologically active polyphenol in green tea extract. In addition, EGCG and green tea extract showed inhibitory effects on the growth of lung and mammary cancer cell lines with similar potencies. An experiment using the estrogen-dependent MCF-7 cell line showed the mechanisms of action of these compounds to be inhibiting the interaction of estrogen with its receptors. The authors suggest that EGCG and compounds in green tea extracts may block the interaction of tumor promoters, hormones and growth factors with their receptors.

- Inhibition of estrogen activity through other mechanisms: several flavonoids, including kaempferide, apigenin, and flavone, are distinct, in that their anti-estrogenic activity does not appear to correlate with binding to ER, and therefore their suppression of estrogen-mediated gene trans-activation and proliferation may occur independent of direct antagonism of the receptor. It is suggested that receptor binding-independent anti-estrogenic chemicals may function through alternate signaling pathways as indirect ER modulators in a receptor- and cell type-specific manner.

- Research with coumestrol, genistein, biochanin A, apigenin, luteolin, kaempferol, and enterolactone showed conflicting results. Induction of DNA synthesis in estrogen-dependent cell lines but not in estrogen-independent cell lines is consistent with an estrogenic effect of these compounds. Inhibition of estrogen-dependent and -independent breast cancer cells at high concentrations suggests additional mechanisms independent of the estrogen receptor. One interesting piece of research found that genistein and curcumin together exerted a significantly greater effect than the individual substances given in isolation.

- Stimulation of SHBG synthesis. Maintenance or elevation of plasma Sex Hormone Binding Globulin (SHBG) which carries testosterone and estrogen and makes it
unavailable for initiating biological mechanisms. Isoflavones and lignans support SHBG formation and function.\textsuperscript{69, 70, 71}

**Opportunities to increase estrogen excretion and elimination**

- Estrogen is metabolized along three pathways to form the 2-hydroxylated, the 4 hydroxylated and the 16alpha-hydroxylated metabolites. Based on proposed differences in biological activities, the ratio of 2-hydroxyestrogen:16alpha-hydroxyestrone (2:16alpha-OHE1), has been used as a biomarker for breast cancer risk. Women with an elevated 2:16alpha-OHE1 ratio are hypothesized to be at a decreased risk of breast cancer. Flaxseed, the most significant source of plant lignans, and wheat bran, an excellent source of dietary fiber, have both been shown to have chemo-protective benefits. In one study urinary excretion of 2-hydroxyestrogen and 16alpha-hydroxyestrone, as well as their ratio, 2:16alpha-OHE1, were measured by enzyme immunoassay. Flaxseed supplementation significantly increased the urinary 2:16alpha-OHE1 ratio ($P = 0.034$), but wheat bran had no effect.\textsuperscript{72} In another study flaxseed supplementation significantly increased urinary 2-OHEstrone excretion ($p < 0.0005$) and the urinary 2/16 alpha-OHE1 ratio ($p < 0.05$) in a linear, dose-response fashion. There were no significant differences in urinary 16 alpha-OHE1 excretion. These results suggest that flaxseed may have chemo-protective effects in postmenopausal women.\textsuperscript{73} Soluble fiber such as is found in fruits, vegetables and certain grains including oats, undergoes metabolism in the small and large intestine and has an appreciable effect on modifying carcinogens in the colon. Insoluble fiber such as is found in wheat and rice bran, does not alter carcinogenic metabolites but does give bulk to the stool, thus diluting potential toxins and speeding the transit time and hence reducing toxin exposure overall.\textsuperscript{74}

- Rat studies have demonstrated that administration of green tea stimulated liver microsomal glucuronidation of estrone and estradiol by as much as 37%. Enzyme kinetic analysis indicates that the inhibition of estrone glucuronidation by 10 microM (-)-epigallocatechin gallate was competitive while inhibition by 50 microM (-)-epigallocatechin gallate was noncompetitive. Similarly, several flavonoids (naringenin, hesperetin, kaempferol, quercetin, rutin, flavone, alpha-naphthoflavone and beta-naphthoflavone) also inhibited rat liver microsomal glucuronidation of estrone and estradiol to varying degrees. Naringenin and hesperetin displayed the strongest inhibitory effects. These two hydroxylated flavonoids had a competitive mechanism of enzyme inhibition for estrone glucuronidation at a 10 microM inhibitor concentration and a predominantly noncompetitive mechanism of inhibition at a 50 microM inhibitor concentration.\textsuperscript{75, 76} Research into chrysin-induced UDP-glucuronosyltransferase (UGT) activity and expression in human intestinal cell lines, demonstrated that flavonoids may be important for the glucuronidation and detoxification of cells.\textsuperscript{77}

- Potential exists to promote the preferential excretion of 2-OH through the use of several compounds, notably Indole-3-carbinol (IC3) or its more potent and bio-available metabolite, Di-indolyl-methane (DIM). Similar effects can be obtained with flax, kudzu
and soya. Supplementation with L-methionine, S-adenosyl-methionine (SAM), vitamin B12, helps the inter-conversion of 2-OH and 4-OH in the liver. 4-OH may convert into quinones which are potentially carcinogenic and need lots of free radical quenching. Anti-oxidants can inhibit the formation of these compound.

- Maintain or elevate plasma Sex Hormone Binding Globulin which has been correlated to increased urinary excretion of 16 alpha-hydroxyestrone and estriol. High intakes of caffeinated coffee, green tea, and total caffeine were commonly correlated with increasing sex hormone-binding globulin after controlling for potential confounders. Although the effect of caffeine cannot be distinguished from effects of coffee and green tea, consumption of caffeine-containing beverages appeared to favorably alter hormone levels associated with the risk of developing breast cancer.

- Unquenched quinones become mercapturates. Formation and excretion of these products is mediated by glutathione-s-transferase (GST) which can be supported by supplementation with glutathione, selenium and N-acetyl-cysteine (NAC). Reduced glutathione and N-acetylcysteine can inhibit both apoptosis and necrosis of several cell types, suggesting a critical role for reactive oxygen species (ROS) in cell death. However, research into the effects of NAC is equivocal and more investigation is called for. Recent research has indicated that certain GST genotypes may have greater susceptibility to malignant changes than others and in future this may be used as a clinical test to evaluate the need or potential benefit from supplementation with GST or its pre-cursors.

- An enzyme in the bowel called glucuronidase can de-conjugate estrogen waste metabolites and allow them to be re-absorbed in an oxidized and highly reactive form.

- The body of evidence suggests that supplementation of the diet with potent botanical and nutritional extracts provides positive health benefits.

### Other mechanisms of reducing mammary tumorigenesis

As described in the section on the etiology of breast cancer, many factors play into the formation, development and progression of breast cancer and many of them offer opportunity for intervention. The research study we are proposing will look exclusively at estrogen metabolism, but it is interesting to note how many other mechanisms are advantageously influenced by flavonoids and lignans.

- Reduction of Insulin-Like Growth Factor I (IGF1) levels which are associated with abnormal cell turnover. Reduced plasma levels of IGF1 are inversely related to urinary lignan excretion after supplementation with flaxseed. Both the oil and the seed have been investigated. Flaxseed, a rich source of lignan precursor secoisolariciresinol-diglycoside (S.D.) and alpha-linolenic acid (ALA), has been shown to be protective at the early promotion stage of carcinogenesis. Reduction in tumor size is due in part to the lignans. The effect of flaxseed oil may also be related to its high ALA content. The S.D.
In flaxseed appears to be beneficial throughout the promotional phase of carcinogenesis whereas the oil component is more effective at the stage when tumors have already been established. 87

- Involvement of non-hormonal mechanisms such as may be triggered by lignans. Research with rats into the lignan hydroxymatairesinol (HMR) from Norway Spruce, the most abundant single component of spruce lignans, has demonstrated that it is metabolized to enterolactone (ENL) as the major metabolite in rats after oral administration. HMR decreased the number of growing tumors and increased the proportion of regressing and stabilized tumors. HMR (50 mg/kg body wt) did not exert estrogenic or anti-estrogenic activity. Neither ENL nor enterodiol showed estrogenic or anti-estrogenic activity via a classical alpha- or beta-type estrogen receptor-mediated pathway. HMR was an effective antioxidant in vitro. 88

- Many flavonoids are strongly anti-oxidant and this may reduce tumorigenesis by inhibiting DNA damage and promoting DNA repair. Regulation of cell protein content and inhibition of protein, DNA and RNA synthesis has been demonstrated by quercitin. 89 Novel 1H-cyclopenta[b]benzofuran lignans extracted from Aglaia elliptica are potent cytostatic inhibitors of protein biosynthesis and are capable of delaying tumor growth in an in vivo model. 90

- Increased intracellular reduced glutathione (GSH) content and hence quenching of free radicals and inhibition of oxidative damage has been demonstrated by quercitin and myricetin. 91

- Investigation into a number of naturally occurring chemo-preventive agents such as curcumin, quercetin, auraptene, 1'-acetoxychavicol acetate (ACA) and indole-3-carbinol showed generation of apoptosis as well as inhibition of cell proliferation. 92

- Progestins may also exert direct anti-estrogenic action by increasing the oxidative activity of 17 beta-hydroxy-steroid-dehydrogenase, thereby facilitating the conversion of estradiol (the more active form) to estrone (the less active form). Progestins may exert additional anti-estrogenic effects by suppressing estrogen receptor levels. They also cause estrogen deprivation indirectly through suppression of pituitary ACTH secretion, resulting in reduced production of adrenal androgen precursors. Aromatase inhibition in pre-menopausal women interrupts estrogen biosynthesis; the reflex rise in FSH then stimulates production of new aromatase enzyme, and the LH increment results in enhanced ovarian steroidogenesis, counteracting the inhibitory action of aromatase-blocking drugs on the ovary. 93

- Dihydrobenzofuran lignans (2-phenyl-dihydrobenzofuran derivatives) constitute a new group of anti-mitotic and potential anti-tumor agents that inhibit tubulin polymerization. A dimerization product of caffeic acid methyl ester, showed promising activity. It inhibited mitosis at micromolar concentrations in cell culture through a relatively weak interaction at the colchicine binding site of tubulin. 94
- Reduction of the highly proliferative terminal end bud (TEB) structures in the developing mammary gland by differentiation to alveolar buds (ABs) and lobules has been suggested to be protective against mammary cancer and may be achieved through the ingestion of flaxseed and exposure to protective lignans. Research showed that flax seed also caused endocrine changes, as suggested by early puberty onset and lengthened cycles due to prolonged estrus. This increased exposure to endogenous estrogens and stimulated mammary gland differentiation, as indicated by fewer TEBs and more ABs.  

- Two citrus flavonoids, hesperetin and naringenin, found in oranges and grapefruit, respectively, and four non-citrus flavonoids, baicalein, galangin, genistein, and quercetin, showed inhibitory effects on proliferation and growth of a human breast carcinoma cell line. The most potent single flavonoids was baicalein and the addition of quercitin to any of the other flavonoids increased their potency. Although tumor incidence and tumor burden (grams of tumor/rat) were somewhat variable in the different groups, rats given orange juice had a smaller tumor burden than controls, and they grew better than any of the other groups. 

- In vitro, anti-proliferative effects of different progesterone antagonists or anti-progestins (PAs) are observed, mainly in estrogen-stimulated growth of PR-positive tumor cell lines. In various experimental animal tumor models, different PAs showed a greater anti-tumor activity than tamoxifen. Combination treatment of different PAs, or progesterone receptor modulators (PRMs) with different anti-estrogens or with an aromatase inhibitor showed greater antitumor efficacy than treatment with each single type of drug alone. In some studies, these effects were accompanied by additive effects on several cell biologic parameters. 

- Inhibition of cyclin-dependent kinases has been demonstrated experimentally using Flavopiridol, a novel semisynthetic flavone analogue of rohitukine, a leading anticancer compound from an Indian tree. 

- Both genistein and equol interfere with signal transduction pathways but in one study genistein was 15-fold more growth-inhibitory than equol. At 100 mumol/l they both decreased c-fos levels, by 75 and 67%, respectively. Enterolactone and enterodiol had only a weak inhibitory effect. suggest that inhibition by genistein of epidermal-growth-factor (EGF)-induced c-fos mRNA transcription is probably related to its interruption of EGF receptor-linked protein tyrosine kinase, whereas genistein-induced growth arrest is not. 

- Aberrant hyperproliferation (AH) is a late occurring post-initiational event that precedes mammary tumorigenesis in vivo. Treatment of initiated cells with naturally occurring tumor inhibitors eicosapentaenoic acid (EPA), indole-3-carbinol (I3C), (epigallocatechin gallate (EGCG), squalene (SQE), and perillyl alcohol (PA) (analog of limonene) at non-toxic doses, resulted in a 70-99% inhibition of AH, depending on the initiator and the chemopreventive test compound. Up-regulation of AH in initiated mammary epithelial cells in vitro prior to tumorigenesis in vivo, and persistent inhibition of AH by diverse
naturally occurring tumor inhibitors, provides evidence for AH as a cellular surrogate endpoint for induction and modulation of mammary neoplastic transformation.


5 McVie G, Cancer Research Campaign symposium, April 2001


15 Kagawa Y, Impact of westernisation on nutrition in Japan – changes in physique, *Preventative Medicine, 1978, 7: 205*


38 Murphy MJ Jr, *Oncologist* 1998, 3(2):129-130


41 Kellis JT Jr, Vickery LE., Inhibition of human estrogen synthetase (aromatase) by flavones, Science 1984 Sep 7;225(4666):1032-4


43 Murphy MJ Jr,ibid


47 Nakagawa Y, Suzuki T, Tayama S., Metabolism and toxicity of benzophenone in isolated rat hepatocytes and estrogenic activity of its metabolites in MCF-7 cells, Toxicology 2000 Dec 7;156(1):27-36

48 McDougal A, Safe S, Induction of 16alpha-/2-hydroxyestrone metabolite ratios in MCF-7 cells by pesticides, carcinogens, and antiestrogens does not predict mammary carcinogens, Environ Health Perspect 1998 Apr;106(4):203-6


52 Wang C, Kurzer MS. Effects of phytoestrogens on DNA synthesis in MCF-7 cells in the presence of estradiol or growth factors, Nutr Cancer 1998;31(2):90-100


59 Adlercreutz H, Mousavi Y, Clark J, Hockerstedt K, Hamalainen E, Wahala K, Makela T, Hase T, ibid


70 Schottner M, Gansser D, Spiteller G, Lignans from the roots of Urtica dioica (Nettles) and their metabolites bind to human sex hormone binding globulin, *Planta Medica*, 1997; 63: p529-532

71 Adlercreutz H, Mousavi Y, Clark J, Hockerstedt K, Hamalainen E, Wahala K, Makela T, Hase T, *ibid*


72 Adlercreutz H, Mousavi Y, Clark J, Hockerstedt K, Hamalainen E, Wahala K, Makela T, Hase T, ibid


78 Gorbach SL, Goldin BR, Diet and the excretion and enterohepatic cycling or estrogens, Preventative Medicine, 1987, 16: 525-531

79 Goldin BR, Adlercreutz H, Gorbach

80 Goldin BR, Adlercreutz H, Gorbach

81 Estrogen patterns and plasma levels in vegetarian and omnivorous women, New England Journal of Medicine, 1982, 307: 1542 - 1547

82 Rickard SE, Yuan YV, Thompson LU., Plasma insulin-like growth factor I levels in rats are reduced by dietary supplementation of flaxseed or its lignan secoisolariciresinol diglycoside, Cancer Lett 2000 Dec 8;161(1):47-55

83 Thompson LU, Rickard SE, Orcheson LJ, Seidl MM., Flaxseed and its lignan and oil components reduce mammary tumor growth at a late stage of carcinogenesis, Carcinogenesis 1996 Jun;17(6):1373-1376


87 Rodgers EH, Grant MH, ibid


95  Tou JC, Thompson LU., Exposure to flaxseed or its lignan component during different developmental stages influences rat mammary gland structures, Carcinogenesis 1999 Sep;20(9):1831-1835


