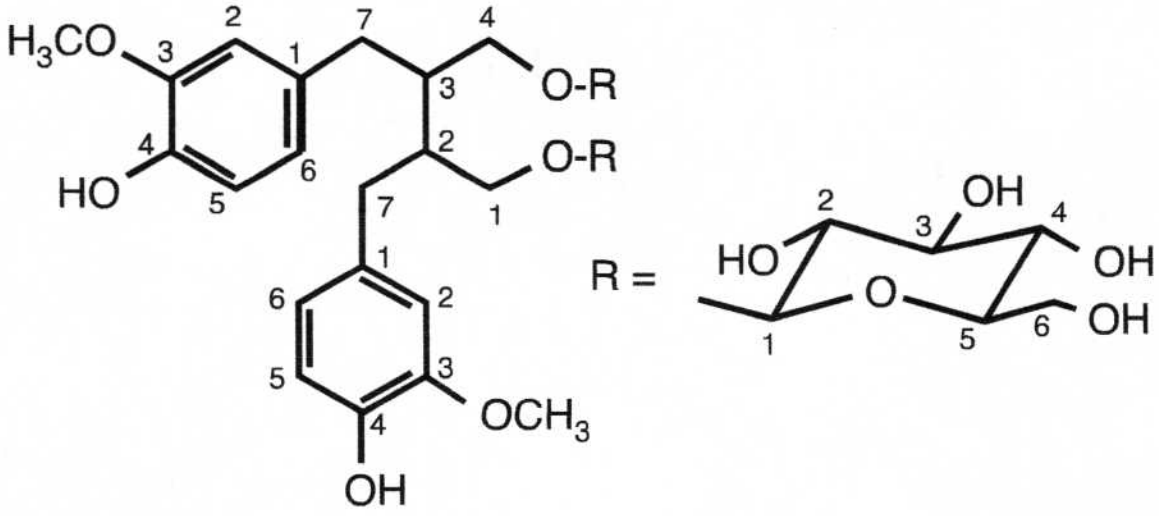
Brevail®

SDG precision standardized Flaxseed Extract



Scientific Research Monograph

Anthony L. Almada, B.Sc., M.Sc.

AUTHOR

Anthony L. Almada, B.Sc., M.Sc.

Mr. Almada has worked within the dietary supplement industry since 1975. He has a B.Sc. in physiology and nutritional biochemistry minor from California State University, Long Beach and an M.Sc. from Berkeley (with a research thesis in antioxidant-exercise biochemistry). He is the co-founder and past-president of Experimental and Applied Sciences (EAS), and is the founder and CSO of IMAGINutrition, Inc., (www.imaginutrition.com) a nutritional technology think tank/incubator. He has been a co-investigator on over 60 university animal and clinical trials, including breast cancer inhibition with natural products. He also is the author of a chapter on male breast cancer in book scheduled for publication in late 2003.

About this Publication

The information presented in this monograph is intended for professional education and is obtained from published research, articles, and books. This monograph is not intended to replace the care of a licensed health professional in the diagnosis and treatment of illness.

Key Points

Studies in animals with chemical-induced breast (mammary) cancer have found lignans (from whole flaxseed) and a pure lignan (e.g. secoisolariciresinol) to:

- Significantly slow the growth rate of tumors
- Significantly reduce tumor size
- Significantly reduce the number of tumors
- Increase the lifespan of animals with these tumors

The effects of flaxseed and its lignans on breast tumors appear within one month after their addition into the diet.

The primary active component found in flaxseed, which protects against breast cancer, appears to be the lignan secoisolariciresinol diglucoside, also known as "SDG".

Whole flaxseed, which contains other lignans and other biologically active components, may exert its breast cancer preventive actions through more than one of these components.

In randomized, double-blind, placebo controlled clinical trials flaxseed supplementation to premenopausal women can:

- Reduce recurring breast pain and tenderness associated with the menstrual period
- Decrease hormone concentrations (estrogens) during the menstrual cycle

In randomized, double-blind, clinical trials flaxseed supplementation to women with breast cancer has been shown to:

- Reduce markers of breast cancer cell proliferation

Supplementation with flaxseed and its lignans has not been associated with any noteworthy or significant adverse side effects. All adverse effects that have been reported in clinical trials have been mild, transient and clinically insignificant.

1. Introduction

Breast cancer is the leading form of cancer among American women over the lifetime, and trails behind only lung cancer in cancer-related deaths. The commonly communicated breast cancer risk estimate is one in eight: one in eight women will develop breast cancer over her lifetime. Through 1999 the number of new breast cancer cases diagnosed annually remained relatively unchanged[1], however, beginning in 2000, the American Cancer Society's annual projections indicate an alarmingly sharp increase[2] *(see chart below)*.

Year	Breast Cancer Estimated Diagnosis	Percentage Increase / Decrease From Prior Year
2003	211,300	+3.8%
2002	203,500	+5.9%
2001	192,200	+5.1%
2000	182,800	+4.5%
1999	175,000	-2.1%
1998	178,700	-0.8%
1997	180,200	

Currently available medical therapies focus upon agents that reduce the breast and uterine cell growth-promoting effects of estrogens while maintaining the cardiovascular and bone protective actions. Such agents are called Selective Estrogen Receptor Modulators, or SERMs. These are also used in the prevention of breast cancer among women who are at high risk, either prior to developing any breast cancer or those who have already experienced breast cancer. Tamoxifen is the most widely used SERM and anti-cancer agent, shown to prevent the occurrence of invasive breast cancers under the period of observation within clinical trials, in addition to treating primary breast cancer. However, as with any therapeutic agent adverse events are associated with their use. Endometrial cancer risk is elevated two to

three fold by tamoxifen use among postmenopausal women.[3,4] Moreover, vasomotor symptoms ("hot flashes") and thromboembolic events (stroke, pulmonary embolism, or deep vein thrombosis, similar to risks associated with oral contraceptive use) also accompany its use.[5]

The increasing research focus on genetic factors that increase a woman's risk for developing breast cancer has yet to uncover a genetic link that affects the majority of women in the U.S. population. Current research points to a genetically-determined increased risk among only 10-15% of women that develop breast cancer. Positioned another way, the remaining 85-90% of women (in the absence of finding other genetic risk-increasing factors) who develop breast cancer, arising from other "external" factors (diet, physical activity, estrogen-containing medications) do have an opportunity to adopt a preemptive strategy to reduce their risk.

For these women there exists a need for a safe, effective, and well tolerated breast cancer preventive agent.

2. Description

Brevail® is a chemically characterized proprietary extract of North American flaxseed (Linum usitatissimum), with a defined and consistent amount of the naturally-occurring lignan secoisolariciresinol diglucoside (SDG). Flaxseed is the richest source of lignans in the diet. Lesser amounts are found in garlic, carrots, broccoli, asparagus, as well as dried apricots and prunes.[6,7]

The amount of SDG and its proportion to the other naturally-occurring constituents present in Brevail® are tightly controlled and highly uniform from batch to batch, and are stable under suitable storage conditions. Each dosage of Brevail results in the endogenous production of the mammalian lignan enterolactone, equivalent to consuming approximately 25 grams of ground flaxseed.

3. Breast Cancer Risk Factors

Primary risk factors include age—the risk for developing breast cancer by age 40 is 0.5%, climbing to 10% by age 80—as well as lifetime exposure to "unopposed" estrogens (early menarche, late menopause, late first pregnancy, or prolonged use of estrogen-based oral contraceptives), the number of breast biopsies experienced, and atypical hyperplasia determined in a breast biopsy.[8,9] Additionally, women who have already sustained one breast cancer are at increased risk for a second primary tumor. One model has been developed to assess breast cancer risk—the Gail model.[10] The Gail model was used to identify women at high risk for breast cancer without any detectable disease who were given tamoxifen, which showed a reduction in breast cancer incidence. This model takes into account the following:

- number of first-degree relatives with breast cancer (0, 1, or 2)
- age at menarche (<12, 12 to 13, or 14 years)
- age at first live birth (<20 to 24, 25 to 29 or nulliparous, or 30 years)
- number of breast biopsies (0, 1, or 2) race (white, black, Hispanic, Asian/Pacific Islander, American Indian/Alaskan native, unknown)
- presence of atypical hyperplasia on breast biopsy

This model fails to incorporate age at breast cancer diagnosis, family history of ovarian cancer, family history of breast or ovarian cancer in other than first-degree relatives, or ethnic background more likely to be associated with mutations in breast cancer susceptibility (BCS) genes.

BCS genes are DNA housekeeping genes, which "code" for large proteins presumably involved in the repair of DNA and the integrity of genes. Mutations of the BCS genes BRCA1 and BRCA2 (and a few other rare BCS genes like CHEK2)[11] account for only 15–20% of breast cancer that clusters within families and less than 5% of breast cancer overall.[12] They can manifest as either a loss of control on cell division, cell death, or the lifespan of cells ("gatekeeper" role), fostering the growth of cancer cells, or a loss of gene stability ("caretaker" role), which can increase the number of disturbances in gatekeeper genes.[13]

Hormonal risk factors are likely to modulate genetic predisposition to breast cancer, although the extent of this contribution remains enigmatic. Nevertheless, because of the positive association between estrogen exposure[14] and breast cancer, and the common practice of using hormone-based therapies (via selective estrogen antagonism) to treat breast cancer, estrogens likely have a contributory or permissive role. Indeed, one of the most compelling lines of evidence implicating estrogens in breast cancer is the potent therapeutic effect of bilateral oophorectomy (removal of the ovaries, the primary source of estrogens) in breast cancer, first demonstrated in 1896,[15] and the far lower incidence of breast cancer among males (1 out of 100 men over a lifetime).[16] Recent studies indicate external (exogenous) estrogens, i.e. oral contraceptives, may have little contributory effects upon breast cancer.[17]

Taken together, the body of data supports the hypothesis that estrogen and its metabolites are related to both the initiation and the promotion of breast cancer but that these associations are complex. One useful metaphor that can be employed to unite the estrogen-related risks is an "estrogen window". Any life events or lifestyle choices that prolong the exposure of the breasts to estrogens (or "open the aperture of the window") can be viewed as risk-increasing. Conversely, any events or choices that abbreviate the breasts' exposure to estrogens can be viewed as risk-decreasing. Lifestyle strategies that attempt to close the window earlier and/or narrow the opening may provide greater insulation against the risk of cancer.

4. Estrogen Metabolism

The ovaries produce the majority of estrogens circulating within premenopausal women, while that circulating in postmenopausal women arises from peripheral tissues (fat, liver, and muscle) "aromatizing" androgen hormones (derived from both the adrenal glands and ovaries) into estrogens.[18]

The "breakdown" or catabolism of estrogens within the female body is far more complex than simple excretion through the kidneys or intestinal tract. Estrogens are broken down by hydroxylation reactions, wherein they can enter a number of different paths. Estradiol, the most potent estrogen produced in women, can enter 2-, 4-, or 16- hydroxylation pathways, giving rise to 2-hydroxy-, 4-hydroxy-, and 16-hydroxy-compounds, respectively. The 2- and 4-hydroxy metabolites (called catechol estrogens) can be inactivated by an enzyme called COMT (catechol O-methyl transferase), which uses S-adenosylmethionine (SAMe) to perform a methylation step (addition of a chemical methyl group). If this methylation step is incomplete, these catechol estrogens are oxidized to other chemically reactive compounds. The 4-hydroxyestrone and 16-hydroxyestradiol forms retain estrogenic properties and are considered to be carcinogenic . The 16-hydroxy product does not appear to be acted upon by any deactivating enzymes. The 2-hydroxylation pathway can be viewed as anti-carcinogenic while the 16-hydroxylation pathway can be viewed as pro-carcinogenic. This estrogen metabolism "balancing act" (See Figure1) points to women whose estrogen metabolism is preferential to the 16-hydroxylation pathway possibly being at higher risk for breast cancer.[19]

Estrogen Metabolism Pathways

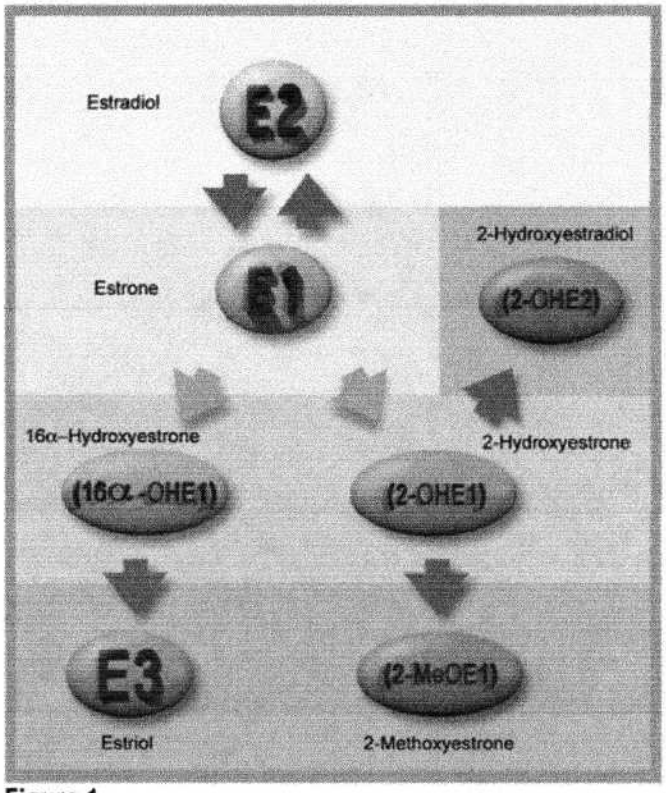


Figure 1

5. SDG: Digestion, Absorption, and Metabolism

The lignan secoisolariciresinol (SECO) is a phenolic compound, marked by the presence of ring structures bearing attached "hydroxyl" groups. Chemically it resembles endogenous steroid hormones. In flaxseed, SECO appears to exist as a complex attached to two glucose (sugar) molecules—SDG. After SDG enters the intestines, it has its two glucose molecules removed to form the "aglycone" SECO. SECO is converted by the intestinal microflora to enterolactone (ENL) (See Figure 2).[20] The microflora can also transform ENL into enterodiol (END). Due to ENL and END being produced by mammals and not by plants (although they are derived from plant precursors), they are also called mammalian lignans.

Metabolism of Brevail

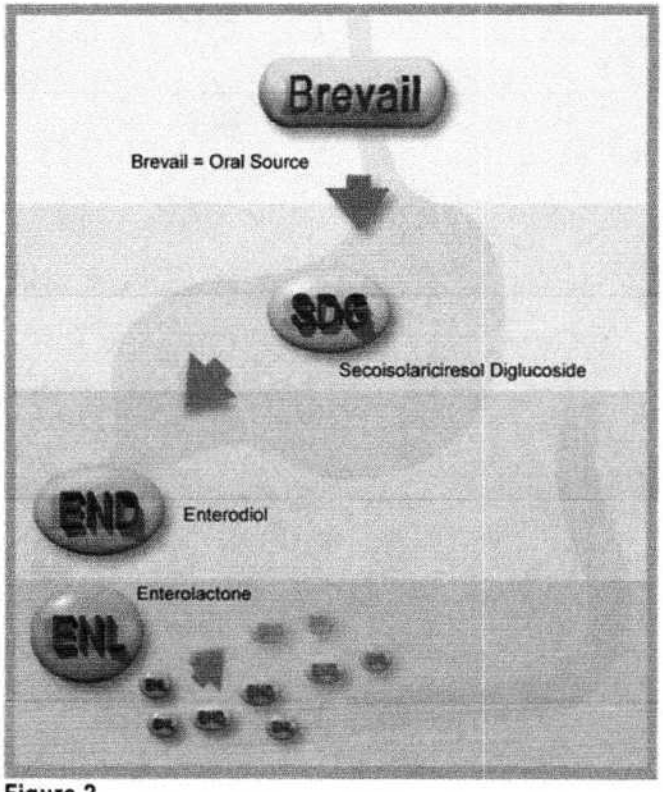


Figure 2

One study had healthy postmenopausal nuns consume a placebo, or 5 or 10 grams of ground flaxseed (incorporated into a muffin) daily for 7 weeks, with a 7 week "washout" period (NO supplements taken) between each treatment.[21] The supplement provided 10 and 20 mg of SECO for the 5 and 10 gram doses, respectively. This resulted in dramatic (4-16 times) and dose-dependent increases in the urinary concentrations of ENL and END. The primary mammalian lignan precursor SECO does not appear to enter the circulation after oral ingestion. However, the contents of the intestinal microflora may play an appreciable role in determining the extent of mammalian lignan production and absorption.

After mammalian lignans are absorbed they undergo reactions within the liver that render them more water soluble, and thus well suited for excretion in the urine. The metabolic reactions described here include sulfation and glucuronidation conjugation processes. Mammalian lignans thus appear in the urine as sulfate and glucuronide forms, in addition to the "free", unconjugated forms.[6] A much smaller fraction appears in the urine as oxidative metabolites.[22]

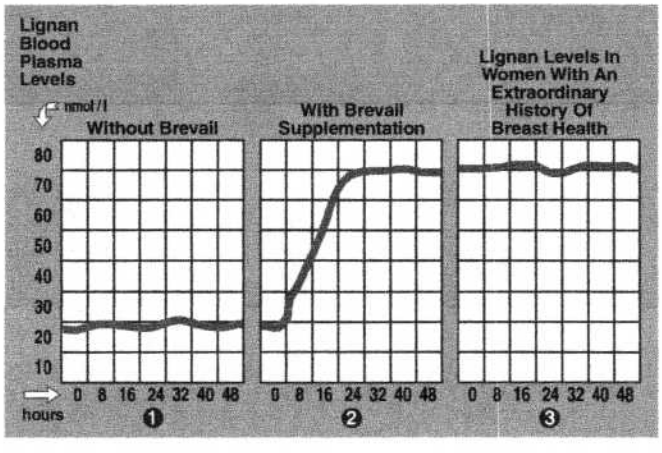
5.1 Brevail: Human Oral-dosing Pharmacokinetic Studies

Brevail was subject to stringent, university based, human oral-dosing pharmacokinetics and steady state studies in adult women.[23] A total of two studies were completed. The first as a means to establish plausible dose ranges and the second, chronicled below, to establish the exact dosage of Brevail necessary to raise and sustain lignan concentrations in the body to levels demonstrated in women who exhibit an extraordinary history of breast health.

Secoisolariciresinol (SDG) is the natural lignan found in high levels in flaxseed. When consumed in the diet, SDG converted by intestinal bacteria to the important phytoestrogen, enterodiol and enterolactone. These lignans when present in high levels in the blood and urine have been found to be associated with reduced risk of many chronic diseases including breast cancer and cardiovascular disease.

In order to evaluate the bioavailability of Brevail, a commercial SDG extract of flaxseed, 15 healthy postmenopausel women were randomized to doses of 25 mg, 50 mg, 100 mg and 200 mg (5 women/dose) of an extract of SDG which was taken as a single oral dose. Blood and urine samples were collected

at timed intervals over the next 5 days and the concentrations of SDG, enterodiol and enterolactone were determined by mass spectrometry. SDG was efficiently converted to enterodiol and then to enterolactone, and blood concentrations increased rapidly after Brevail was taken. Peak levels of SDG occurred 8 h after ingestion, and the maximum plasma concentration of enterodiol and enterolactone occurred after 12 h and 24 h, respectively, reflecting the time course for the intestinal metabolism of SDG *(see chart below)*. The lignans were eliminated from the circulation with a half-life of 10 h. When Brevail was taken on a daily basis for 7 consecutive days the plasma concentrations of enterolactone and enterodiol were maintained at a steady level consistent with those previously reported for women who are at low risk for breast cancer.



This pharmacokinetic study confirmed that the SDG in Brevail is bioavailable and at 200 mg/day, providing 50 mg of SDG, maintains plasma enterolactone levels in the range of 63+ 12 nmol/L comparable to those of women at low risk for breast cancer. Studies from Finland have shown that serum concentrations of enterolactone ranged 3-54 nmol/L and were inversely correlated with risk for breast cancer.[24] Those women with enterolactone levels of above 34 nmol/L showed a marked reduction (0.38) in the odds adjusted risk for breast cancer. A single 200 mg capsule of Brevail, providing 50 mg/day of SDG maintained blood concentrations in this range.

6. Efficacy In Vitro

Flaxseed and its primary lignan SDG exert a variety of complementary effects upon estrogen metabolism, receptors, and signaling through their intestinal transformation products ENL and END. Most studies have explored their effects on estrogen metabolism and the competitive interaction between naturally circulating estrogens and these mammalian lignans. Because mammalian lignans exert both estrogen-like and anti-estrogenic effects they are also referred to as phytoestrogens (the isoflavones found in soy foods are also classified as phytoestrogens). Collectively, these biological activities may contribute to their breast cancer prevention potential.

6.1 Mammalian Lignans: Breast Cancer Cell Growth

Human-derived breast cancer cells (MCF-7) were kept in culture and incubated with ENL or ENL plus estradiol.[25] Estradiol and low concentrations of ENL individually stimulated the proliferation of the cells, but when combined no stimulation was seen. This study suggested that ENL prevents or reduces the binding/metabolism of estradiol within these cells. However, other studies point to the concentrations of ENL found to inhibit breast cancer cell growth in vitro have generally been greater than those found in human blood serum.[26] This suggests that for ENL to exert a breast cancer protective effect, significant increases in ENL concentrations need to be achieved such as demonstrated in the Brevail pharmacokinetics studies.

6.2 Mammalian Lignans: Sex Steroid Binding Proteins

Within the blood, estrogens travel on a protein carrier called sex hormone binding globulin (SHBG) or sex steroid binding protein (SBP). SHBG, produced in the liver, tightly binds estrogens and acts as a delivery vehicle to tissues. Incubation of ENL with human liver cancer cells in culture stimulated the synthesis of SHBG, also acting synergistically in this respect with estradiol.[27] Incubation of ENL with human SBP was shown to efficiently displace estradiol from binding sites on the protein.[28] These results suggest that ENL can reduce the estrogen payload of SBP, thereby relieving the stimulatory effect of estrogens on tissue growth.

6.3 Mammalian Lignans: Sex Steroid Metabolizing Enzymes

Aromatase (also called estrogen synthetase) is a pivotal enzyme regulating the conversion of androgens into estrogens. This is the primary pathway through which estrogens are produced both in postmenopausal women and in men. The source of estrogens arises not only from aromatase activity in peripheral tissues (adipose, muscle, and liver) and the ovaries, but also within the healthy and cancerous breast.[29] Aromatase is inhibited in vitro when incubated with either ENL or END, with ENL being notably more potent than END.[30, 31] This may influence the amount of estrogens present within breast tissue (see Figure 3).

Estrogen Production Outside of the Ovaries

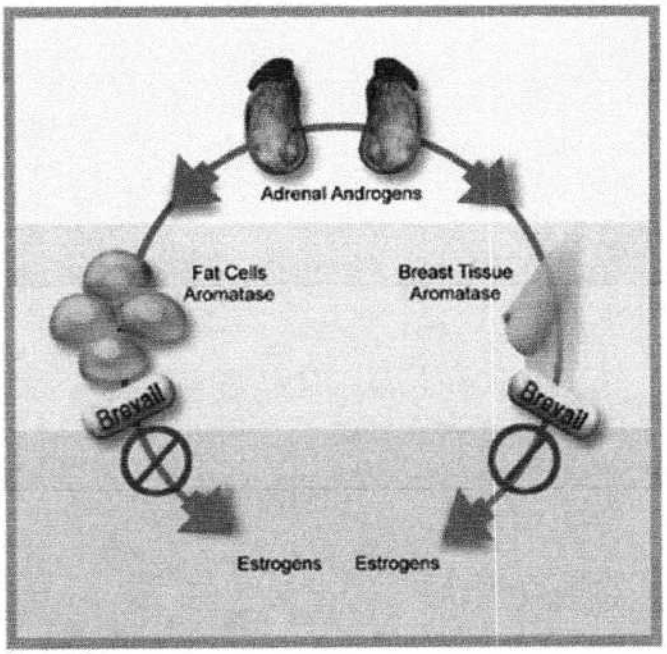


Figure 3

Antiestrogenic effects of phytoestrogens have also been observed. At concentrations 100-1000 times that of estradiol (the probable levels in human plasma after regular phytoestrogen consumption), it has been proposed that phytoestrogens may be able to compete effectively with endogenous mammalian estrogens, bind the ER, and prevent estrogen-stimulating growth in mammals.[32]

7. Efficacy In Vivo: Preclinical Studies

The highest phytoestrogen consumption and concentrations in biological fluids are found in subjects living in countries where cancer incidence is low, while the lowest concentrations were found in breast cancer patients or in women at high risk for breast cancer.[33] These findings led researchers to assess the effects of flax, its most abundant lignan SDG, and its mammalian counterpart ENL on the chemopreventive effects in animals, where other modifying variables can be rigorously controlled. These studies show noteworthy reductions in both the size and number of tumors.

7.1 Flaxseed Supplementation

Animals exposed to certain chemical carcinogens routinely develop breast cancer. Adding a flaxseed meal supplement to animals given a carcinogen and a high fat diet produced sharp reductions in the size and number of breast tumors.[34, 35] Because flaxseed contains both fiber and the omega-3 fatty acid alpha-linolenic acid (ALA) the cancer-protective components of flaxseed were not discernible from these studies (see "SDG Supplementation" below).

An important event in tumor growth, progression, and spreading (metastasis) is the manufacture of new blood vessels (capillaries) from pre-existing blood vessels.[36] This process, called angiogenesis, is facilitated in part by the synthesis of a protein growth factor called vascular endothelial growth factor (VEGF). VEGF can "float" in the spaces between cells, attaching to the interior lining of blood vessel cells, and then stimulate proliferation and migration of new blood vessel cells.[37] Animals implanted with a human-derived breast cancer cell line, displaying potent metastatic activity (and estrogen-independent growth) and supplemented with flaxseed meal, showed significant reductions in tumor growth rate and metastasis, accompanied by a sharp reduction in VEGF concentrations within large tumors.[38] This same research group has shown that supplementation with flaxseed meal inhibits breast cancer growth and metastasis by blunting the concentrations of insulin growth factor-I (IGF-I) and the receptor for epidermal growth factor, two

additional tumor growth promoters.[39] The breast cancer drug tamoxifen shares this IGF-I-lowering ability, in both animals and humans.[40, 41]

High plasma levels of IGF-I have been associated with increased breast cancer risk in many studies. The similar lowering effect of flaxseed and its equivalent dose of SDG on plasma IGF-I concentrations in both studies and the inverse relationship between urinary lignans and plasma IGF-I suggest that lignans were largely responsible.[42]

7.2 SDG Supplementation

In an attempt to dissect out the most chemopreventive component in flaxseed, two studies were undertaken. One study with animals given a breast cancer-causing chemical carcinogen and a high fat diet for 13 weeks then supplemented their diets with either flaxseed oil (rich in ALA and containing only trace amounts of SDG), flaxseed meal, or pure SDG (extracted from flaxseed).[35] All three diet supplements reduced tumor size but only the animals supplemented with pure SDG showed a reduction in both tumor size and the average number of new tumors formed.[35] This study also found a strong, inverse relationship between the amount of ENL and END excreted in the urine and tumor size, suggesting that lignans do mitigate, in part, tumor progression. In a second study, pure SDG extracted from flaxseed and fed to carcinogen-treated animals resulted in a 46% lower number of tumors compared to those not receiving SDG, with no apparent adverse effects on any of the other organs.[43]

Flaxseed contains very high amounts of secoisolariciresinol diglucoside (SDG), which is converted to enterodiol and enterolactone in the gut. This substance seems to inhibit the initiation and growth phases of mammary tumor development in rats, affecting both tumor size and proliferative ability.[44]

8. Efficacy In Vivo

A compelling case that prompted many researchers to directly examine the influence of flaxseed and its lignans on breast health resides in studies where breast cancer incidence in postmenopausal women is expressed in relation to dietary and/or urinary lignan concentrations.[33, 45] A recent study conducted among women in Eastern Finland (where dietary lignan intake is typically higher than in the U.S.), found that in 194 pre- and postmenopausal women, higher blood ENL concentrations were linked to a striking reduction in breast cancer risk.[24]

8.1 Flaxseed Lignans: Estrogen Metabolism

Because SDG can alter the metabolism and action of estrogens, the following question was asked: Could flaxseed supplementation alter the metabolism of estrogens in healthy postmenopausal women? Twenty-eight women (nuns, with no history of pregnancy) completed a series of 7-week supplementation periods where they **1:** ate their usual "control" diet, **2:** took a 5 gram/day supplement of flaxseed meal, or **3:** took a 10 gram/day supplement of flaxseed meal.[46] A "washout" period of at least 7 weeks followed each 7-week dietary treatment period. During the periods when the women consumed the flaxseed supplements the concentration of 2-hydroxy estrogen (2OH-E; the "safe" estrogen) in the urine increased, with the 10 g/day dose producing 2OH-E increases significantly greater than both the 5 g/day dose and the control diet. Additionally, the ratio of 2/16-OH estrone, indicative of breast cancer risk with lower values, increased with flaxseed, again the 10 g/day dose eliciting an increase significantly greater than the 5 g/day dose and the control diet.

In a follow-up study, the same research group evaluated the effects of flaxseed and wheat bran fiber supplementation on estrogen metabolism in 16 healthy premenopausal women over a period of 8 menstrual cycles.[47] The women were asked to consume their diet and specially prepared baked goods (cookies and muffins) containing either **1:** 10 g ground flaxseed, **2:** 28 g wheat bran fiber, **3:** 10 g ground flaxseed + 28 g wheat bran fiber, or **4:** no added lignan or fiber source for two consecutive cycles. No washout period was instituted between each treatment. Because of the added calories in the baked goods, the women were asked to substitute these for similar items in the diet. Only the two treatments where flaxseed baked goods were consumed produced significant alterations in urinary markers of estrogen metabolism. Both resulted

in significant increases in 2OH-E and 2/16-OH estrone ratio. Because the flaxseed used in this study was low in the omega-3 fatty acid alpha-linolenic acid and low in fiber (relative to the wheat bran), and because wheat bran has trivial amounts of lignans (as SDG), these findings reinforce the assertion that SDG is the primary estrogen metabolism modifier present in flaxseed. Because flaxseed and its mammalian lignan products enterolactone and, to a lesser extent, enterodiol have been shown to influence the early risk markers for and incidence of mammary and colonic carcinogenesis in animal models, decrease cell proliferation in vitro, and influence factors that affect the hormone concentrations in humans, increases in the metabolism and excretion of these compounds may offer increased protection against hormone-dependent cancers.[21]

Another type of phytoestrogen of clinical significance is isoflavones, abundant in soy foods. To compare the effects of flaxseed and soy upon estrogen metabolism,[49] postmenopausal women received a placebo muffin (control) or muffin with 25 g ground flaxseed or soy daily for 16 weeks.[48] At the end of supplementation, only the group receiving the flaxseed muffin showed significant increases in 2OH-E and the 2/16 OH estrone ratio.

8.2 Flaxseed Lignans: Cyclical Mastalgia

A not uncommon experience for women around the menstrual period is breast tenderness and pain. The recurring form of this condition is known as cyclical mastalgia. Recent studies suggest that cyclical mastalgia accompanied by breast swelling may also carry an elevated risk of breast cancer.[49] Because of the possible estrogen action-modifying effects of flaxseed and its predominant lignan, SDG, 116 women who had experienced severe cyclical mastalgia for a preceding 6 month interval were supplemented with flaxseed (25 g/muffin) or placebo muffins over 3 menstrual cycles.[50] After three cycles the reduction in breast pain, swelling, and lumpiness was significantly lower in the women eating the flaxseed muffin. These women also showed an increase in urinary mammalian lignans (ENL and END) and a change in blood estrogens. This led the authors to assert that the positive effects of flaxseed may be due to anti-estrogenic actions of the lignans in cyclical mastalgia.

It is concluded that flaxseed is effective in relieving symptoms of cyclical mastalgia without significant side effects and might be considered as an alternative treatment for cyclical mastalgia. Its putative mechanism of action may be via the anti-estrogenic effects of the lignans.[50]

8.3 Flaxseed Lignans: Breast Tumor Cell Biology

The effects of flaxseed lignans on breast tumor cell biology were investigated in 39 women with newly diagnosed cancerous breast tumors.[51] Six premenopausal and 17 postmenopausal women were asked to consume a muffin (one/day) containing 25 g of ground flaxseed meal, while 4 premenopausal and 12 postmenopausal consumed a placebo muffin. Prior to beginning the muffin supplementation, women had diagnostic biopsies performed. After this, supplementation began until the women had breast cancer surgery. At both times analyses were performed to assess the rate of tumor cell proliferation. The average period of supplementation was 39 days in the placebo group and 38 in the flaxseed group. In the postmenopausal women receiving the flaxseed muffin cellular factors indicative of tumor growth fell by 21-33%, accompanied by significant increases in urinary lignans (ENL and END), while neither were altered in the placebo group. The former findings are similar to those seen in women with breast cancer receiving tamoxifen under similar conditions.[52] The small number of premenopausal women receiving the flaxseed muffin (six) likely made it difficult to show a difference in relation to the equally small group of premenopausal women receiving placebo muffins (four). This was the first study to demonstrate that flaxseed supplementation could favorably alter the behavior of breast cancers in women, likely attributable to the lignan component.

No significant adverse effects of flaxseed were reported. This study showed, for the first time, the potential of dietary modification with flaxseed and its components such as the lignans, in reducing tumor growth in patients with breast cancer comparable to the effects seen with preoperative tamoxifen.[51]

9. Safety and Tolerability

The safety and tolerability of SDG, as present in ground flaxseed meal, has been assessed in numerous clinical trials, involving over 250 healthy female volunteers and women with benign breast conditions and breast cancer, for periods ranging from a few weeks to a 12 months. In all of these studies no clinically significant adverse effects have been observed or recorded and the vast majority of the subjects found the supplements well tolerated. No significant changes in any biochemical safety parameters have been noted. Additionally, the doses of ground flaxseed used (5-50 g/day) are equivalent to 10-350 mg of secoisolariciresinol, the absorbable lignan "SECO" component in SDG. Because of the sustained circulation of mammalian lignans in women up to 24 hours after ingestion of flaxseed, the added ease of once daily dosing with flaxseed lignans is afforded.[53]

Numerous animal studies with varying doses of pure SDG derived from flaxseed have found no adverse effects upon any organs.[35, 54] Studies with the mammalian lignans ENL and END, derived from dietary SDG, have revealed no mutagenic effects on mammalian cells in culture.[55] Brevail has undergone stringent toxicity testing with absolutely no toxicity detected even at dosages significantly higher than recommended.

10. Cautions

Because of the lack of studies done with flaxseed and its principal lignan SDG in pregnant or lactating women, or those attempting to conceive, and the possible anti-estrogenic effects of SDG, it appears prudent for such women to avoid consumption of large amounts of flaxseed or SDG.

11. Indications

An abundance of animal and human research suggests that the flaxseed lignan SDG exerts effects similar to the anti-estrogen drug tamoxifen with an apparently much greater degree of tolerability. Indeed, SDG is chemically similar to tamoxifen. Lignans are a promising class of compounds for use

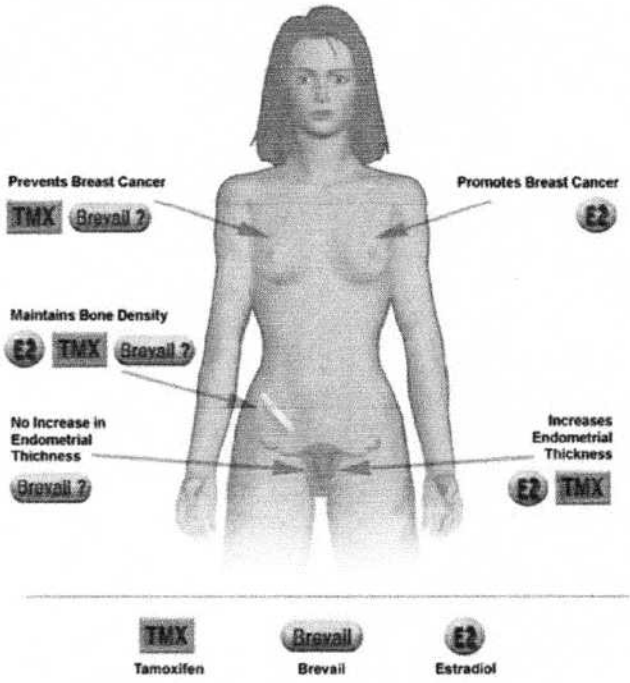


Figure 4

in breast cancer prevention[56] and may be considered as natural SERMs (See Figure 4).

The essential aspect of an ideal SERM is that its estrogenic effects are selectively limited to bone and blood vessel health while exerting anti-estrogenic actions within breast and uterine tissues. Higher dietary lignan intakes have been associated with increased flexibility of the aorta (the largest artery in the heart), which may reduce the risk of atherosclerosis.[57] Additional clinical studies on flaxseed and SDG are ongoing, destined to further illuminate their ability to act as a natural SERM and function as a judicious lifestyle choice for the woman seeking to minimize her risk of breast cancer.

It would seem very reasonable to propose that these weakly oestrogenic compounds [mammalian lignans] act as the natural tamoxifen in Asian and Mediterranean people.[58]

REFERENCES:

1. Edwards BK, Howe HL, Ries LAG, et al. Annual report to the nation on the status of cancer, 1973-1999, featuring implications of age and aging on U.S. cancer burden. Cancer 2002; 94:2766-2792.

2. American Cancer Society, Cancer Statistics 2002, 2001, 2000, 1999, 1998, 1997. ACS's annual estimates of expected numbers of new cancer cases and deaths.

3. Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. Lancet 1998; 351:1451-67.

4. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998; 90:1371-88.

5. Bernstein L, Deapen D, Cerhan JR, et al. Tamoxifen therapy for breast cancer and endometrial cancer risk. J Natl Cancer Inst 1999; 91:1654-62.

6. Adlercreutz H, Van Der Wildt J, Kinzel J, et al. Lignan and isoflavanoid conjugates in human urine. Journal of Steroid Biochemistry and Molecular Biology 1995; 52:97-103.

7. Horn-Ross PL, Barnes S, Lee M, et al. Assessing phytoestrogen exposure in epidemiologic studies: development of a database (United States). Cancer Causes Control 2000; 11:289-98.

8. Key TJ, Verkasalo PK, Banks E. Epidemiology of breast cancer. Lancet Oncol 2001; 2:133-40.

9. McPherson K, Steel CM, Dixon JM. ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. Bmj 2000; 321:624-8.

10. Gail MH, Costantino JP. Validating and improving models for projecting the absolute risk of breast cancer. J Natl Cancer Inst 2001; 93:334-5.

11. Meijers-Heijboer H, van den Ouweland A, Klijn J, et al. Low penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nat Genet 2002; 31:55-9.

12. Nathanson KL, Wooster R, Weber BL, Nathanson KN. Breast cancer genetics: what we know and what we need. Nat Med 2001; 7:552-6.

13. Kinzler KW, Vogelstein B. Cancer-susceptibility genes. Gatekeepers and caretakers. Nature 1997; 386:761, 763.

14. Clemons M, Goss P. Estrogen and the risk of breast cancer. N Engl J Med 2001; 344:276-85.

15. Beatson GT. On the treatment of inoperable cases of the carcinoma of the mamma: suggestions for a new method of treatment with illustrative cases. Lancet 1896; ii:104-7.

16. Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics, 1999. CA Cancer J Clin 1999; 49:8-31, 1.

17. Marchbanks PA, McDonald JA, Wilson HG, et al. Oral contraceptives and the risk of breast cancer. N Engl J Med 2002; 346:2025-32.

18. Santen RJ, Leszczynski D, Tilson-Mallet N, et al. Enzymatic control of estrogen production in human breast cancer: relative significance of aromatase versus sulfatase pathways. Ann N Y Acad Sci 1986; 464:126-37.

19. Bradlow HL, Telang NT, Sepkovic DW, Osborne MP. 2-h hydroxyestrone: the 'good' estrogen. J Endocrinol 1996; 150 Suppl:S259-65.

20. Axelson M, Sjovall J, Gustafsson BE, Setchell KD. Origin of lignans in mammals and identification of a precursor from plants. Nature 1982; 298:659-60.

21. Hutchins AM, Martini MC, Olson BA, Thomas W, Slavin JL. Flaxseed influences urinary lignan excretion in a dose dependent manner in postmenopausal women. Cancer Epidemiology Biomarkers & Prevention 2000; 9:1113-1118.

22. Jacobs E, Kulling SE, Metzler M. Novel metabolites of the mammalian lignans enterolactone and enterodiol in human urine. J Steroid Biochem Mol Biol 1999; 68:211-8.

23. Setchell, K.D., et al. Pharmacokinetics and bioavailability of secoisolariciresinol-diglycoside (SDG). Clinical Mass Spectrometry, Department of Pediatrics, Children's Hospital Medical Center, 2003.

24. Pietin p, Stumpf K, Mannisto S, Kataja, V, Uusitupa M, Adlercreutz H. Serum enterolactone and risk of breast cancer: A case-control study in Eastern Finland. Cancer Epidemiology Biomarkers & Prevention 2001; 10:339-344

25. Mousavi Y, Adlercreutz H. Enterolactone and estradiol inhibit each other's proliferative effect on MCF-7 breast cancer cells in culture. J Steroid Biochem Mol Biol 1992; 41:615-9.

26. Setchell KD, Lawson AM, McLaughlin LM, Patel S, Kirk DN, Axelson M. Measurement of enterolactone and enterodiol, the first mammalian lignans, using stable isotope dilution and gas chromatography mass spectrometry. Biomed Mass Spectrom 1983; 10:227-35.

27. Adlercreutz H, Mousavi Y, Clark J, et al. Dietary phytoestrogens and cancer: in vitro and in vivo studies. J Steroid Biochem Mol Biol 1992; 41:331-7.

28. Martin ME, Haourigui M, Pelissero C, Benassayag C, Nunez EA. Interactions between phytoestrogens and human sex steroid binding protein. Life Sci 1996; 58:429-36.

29. Reed MJ, Purohit A. Aromatase regulation and breast cancer. Clin Endocrinol (Oxf) 2001; 54:563-71.

30. Adlercreutz H, Bannwart C, Wahala K, et al. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. J Steroid Biochem Mol Biol 1993; 44:147-53.

31. Wang C, Makela T, Hase T, Adlercreutz H, Kurzer MS. Lignans and flavonoids inhibit aromatase enzyme in human preadipocytes. J Steroid Biochem Mol Biol 1994; 50:205-12.

32. Kurzer MS, Xu X. Dietary phytoestrogens. Annu Rev Nutr 1997; 17:353-81.

33. Adlercreutz H, Mazur W. Phyto-oestrogens and Western diseases. Ann Med 1997; 29:95-120.

34. Serraino M, Thompson LU. The effect of flaxseed supplementation on the initiation and promotional stages of mammary tumorigenesis. Nutr Cancer 1992; 17:153-9.

35. 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; 17:1373-6.

36. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. Faseb J 1999; 13:9-22.

37. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev 1997; 18:4-25.

38. Dabrosin C, Chen J, Wang L, Thompson LU. Flaxseed inhibits metastasis and decreases extracellular vascular endothelial growth factor in human breast cancer xenografts. Cancer Lett 2002 (in press).

39. Chen J, Stavro M, Thompson LU. Dietary flaxseed inhibits human breast cancer growth and metastasis and downregulates expression of insulin-like growth factor and epithelial cell growth factor receptor. Nutr Cancer 2002 (in press).

40. Huynh HT, Tetenes E, Wallace L, Pollak M. In vivo inhibition of insulin-like growth factor I gene expression by tamoxifen. Cancer Res 1993; 53:1727-30.

41. Pollak MN, Huynh HT, Lefebvre SP. Tamoxifen reduces serum insulin-like growth factor I (IGF-I). Breast Cancer Res Treat 1992; 22:91-100.

42. 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; 161:47-55.

43. Thompson LU, Seidl MM, Rickard SE, Orcheson LJ, Fong HH. Antitumorigenic effect of a mammalian lignan precursor from flaxseed. Nutr Cancer 1996; 26:159-65.

44. Adlercreutz H. Phyto-oestrogens and cancer. Lancet Oncol 2002; 3:364-73.

45. Adlercreutz H, Fotsis T, Heikkinen R, et al. Excretion of the lignans enterolactone and enterodiol and of equol in omnivorous and vegetarian postmenopausal women and in women with breast cancer. Lancet 1982; 2:1295-9.

46. Haggans CJ, Hutchins AM, Olson BA, Thomas W, Martini MC, Slavin JL. Effect of flaxseed consumption on urinary estrogen metabolites in postmenopausal women. Nutr Cancer 1999; 33:188-95.

47. Haggans CJ, Travelli EJ, Thomas W, Martini MC, Slavin JL. The effect of flaxseed and wheat bran consumption on urinary estrogen metabolites in premenopausal women. Cancer Epidemiol Biomarkers Prev 2000; 9:719-25.

48. Brooks J, Ward WE, Hilditch J, et al. Flaxseed, but not soy, saignificantly altered urinary estrogen metabolite excretion in healthy post-menopausal women. FASEB Journal 2002; 16:A1005.

49. Deschamps M, Band PR, Coldman AJ, Hislop TG, Longley DJ. Clinical determinants of mammographic dysplasia patterns. Cancer Detect Prev 1996; 20:610-9.

50. Goss PE, Li T, Theriault M, Pinto S, Thompson L. Effects of dietary flaxseed in women with cyclical mastalgia. Breast Cancer Research and Treatment 2000; 64:49.

51. Thompson LU, Li T, Chen J, Goss PE. Biological effects of dietary flaxseed in patients with breast cancer. Breast Cancer Research and Treatment 2000; 64:50.

52. Clarke RB, Laidlaw IJ, Jones LJ, Howell A, Anderson E. Effect of tamoxifen on Ki67 labelling index in human breast tumours and its relationship to oestrogen and progesterone receptor status. Br J Cancer 1993; 67:606-11.

53. Nesbitt PD, Lam Y, Thompson LU. Human metabolism of mammalian lignan precursors in raw and processed flaxseed. American Journal of Clinical Nutrition 1999; 69:549-555.

54. Orcheson LJ, Rickard SE, Seidl MM, Thompson LU. Flaxseed and its mammalian lignan precursor cause a lengthening or cessation of estrous cycling in rats. Cancer Lett 1998; 125:69-76.

55. Kulling SE, Jacobs E, Pfeiffer E, Metzler M. Studies on the genotoxicity of the mammalian lignans enterolactone and enterodiol and their metabolic precursors at various endpoints in vitro. Mutat Res 1998; 416:115-24.

56. Giridharan P, Somasundaram ST, Perumal K, et al. Novel substituted methylenedioxy lignan suppresses proliferation of cancer cells by inhibiting telomerase and activation of c-myc and caspases leading to apoptosis. Br J Cancer 2002; 87:98-105.

57. van der Schouw YT, Pijpe A, Lebrun CEI, et al. Higher usual dietary intake of phytoestrogens is associated with lower aortic stiffness in postmenopausal women. Arterioscler Thromb Vasc Biol 2002 (in press).

58. Morton MS, Wilcox G, Wahlqvist ML, Griffiths K. Determination of lignans and isoflavonoids in human female plasma following dietary supplementation. Journal of Endocrinology 1994; 142:251-259.