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REVIEW

# Flaxseed and its lignan and oil components: can they play a role in reducing the risk of and improving the treatment of breast cancer?[1]

Julie K. Mason and Lilian U. Thompson



**Abstract:** Flaxseed (FS), rich in the phytoestrogen lignans and α-linolenic acid-rich oil, has been suggested to have an anticancer effect. Questions remain whether FS and its lignan and oil components are effective in reducing breast cancer risk and tumour growth, and can interact beneficially with breast cancer drugs. To find answers, in vitro, animal, observational, and clinical studies on FS and its lignan and oil components were reviewed. The majority of studies in various rodent models show that 2.5%–10% FS diet or the equivalent amount of lignan or oil reduces tumour growth. Ten percent FS and equivalent lignans do not interfere with but rather increase the effectiveness of tamoxifen (80 mg/day) while the 4% FS oil increases trastuzumab/Herceptin (2.5 mg/kg) effectiveness. Observational studies show that FS and lignan intake, urinary excretion, or serum levels are associated with reduced risk, particularly in postmenopausal women. Lignans reduce breast cancer and all-cause mortality by 33%–70% and 40%–53%, respectively, without reducing tamoxifen effectiveness. Clinical trials show that FS (25 g/day with 50 mg lignans; 32 days) reduces tumour growth in breast cancer patients and lignans (50 mg/day; 1 year) reduces risk in premenopausal women. Mechanisms include decreased cell proliferation and angiogenesis and increased apoptosis through modulation of estrogen metabolism and estrogen receptor and growth factor receptor signalling pathways. More clinical trials are needed but current overall evidence indicates that FS and its components are effective in the risk reduction and treatment of breast cancer and safe for consumption by breast cancer patients.

*Key words:* flaxseed, lignan, flaxseed oil, α-linolenic acid, breast cancer, chemoprevention, drug–diet interaction.

**Résumé :** La graine de lin (« FS ») posséderait des propriétés anticancéreuses : elle est riche en lignanes, des phytoestrogènes, et en huile renfermant de l'acide linolénique. On ne sait cependant pas si la FS, ses lignanes et les constituants de son huile peuvent efficacement diminuer le risque de cancer du sein et la croissance de la tumeur en plus de bien interagir avec les médicaments pour le cancer du sein. On effectue une analyse documentaire, des études in vitro, animales, observationnelles et cliniques portant sur la FS, ses lignanes et son huile. La majorité des études réalisées sur divers modèles de rongeurs révèlent qu'un régime comportant de 2,5 à 10 % de FS ou l'équivalent en lignanes ou en huile diminue la croissance de la tumeur. Un taux de 10 % de FS et l'équivalent en lignanes n'entravent pas seulement l'efficacité du tamoxifène (80 mg/jour), ils l'améliorent; un taux de 4 % d'huile de FS améliore aussi l'efficacité du trastuzumab/Herceptin (2,5 mg/kg). Les études observationnelles révèlent que l'apport, l'excrétion urinaire et les niveaux sériques de FS et de lignanes sont associés à une diminution du risque, particulièrement chez les femmes postménopausées. Les lignanes diminuent la mortalité due au cancer du sein et toutes causes confondues de 33 à 70 % et de 40 à 53 %, respectivement, et ce, sans diminuer l'efficacité du tamoxifène. Les essais cliniques révèlent que la FS (25 g/jour plus 50 mg de lignanes; 32 jours) diminue la croissance de la tumeur chez les femmes aux prises avec un cancer du sein et les lignanes (50 mg/jour; un an) diminuent le risque de cancer chez les femmes préménopausées. Parmi les multiples mécanismes proposés, il y a la diminution de la prolifération cellulaire et de l'angiogenèse, l'augmentation de l'apoptose médiée par la modulation du métabolisme des œstrogènes et des récepteurs d'œstrogènes et des voies de signalisation des récepteurs du facteur de croissance. Il faut effectuer d'autres essais cliniques; néanmoins, l'ensemble des données probantes actuelles indique que la FS et ses constituants sont efficaces pour diminuer le risque et contribuer au traitement du cancer du sein tout en étant sécuritaire sur le plan alimentaire pour les patientes aux prises avec le cancer du sein. [Traduit par la Rédaction]

*Mots-clés :* graine de lin, lignanes, huile de graine de lin, acide α-linolénique, cancer du sein, chimioprévention, interaction médicament-aliment.

## Introduction

Flaxseed (FS), an oilseed rich in the *n*-3 polyunsaturated fatty acid (PUFA) α-linolenic acid (ALA, 18:3*n*-3) and phytoestrogens called lignans, is widely available in the food supply and is often used by consumers for their potential health benefits and by breast cancer patients to improve treatment and to prevent recurrence (Boon et al. 2007; Boucher et al. 2012; Rausch et al. 2011). There is an interest in the healthcare, research, and patient communities to answer the questions of whether FS consumption is effective and safe for breast cancer risk reduction and for breast cancer patients, whether FS promotes or reduces tumour growth, and if and how it interacts with drugs used in the treatment of breast cancer (Patterson 2011). This paper aims to address these questions by reviewing the current evidence from in vitro, animal, observational, and clinical studies. A brief overview of

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breast cancer, which focuses on statistics, risk factors, and pathophysiology, including molecular subtypes and current treatment approaches, is initially provided. Information on FS composition and the results from studies on FS and its lignan and oil components from various designs, including animal, observational, and clinical studies are then described. These results are integrated with those from in vitro studies to give an overview of proposed mechanisms of action. The safety and current regulatory status of FS are also included. Finally, a general discussion of the limitations and conclusions of the studies are provided.

## Breast cancer

### Statistics and risk factors

In the western world, breast cancer is the most commonly diagnosed cancer site in women. With major improvements in screening, early detection, and treatment, breast cancer mortality rates have declined. Despite this promising survival trend, breast cancer remains the second-leading cause of a cancer-related death in women (American Cancer Society 2013; Canadian Cancer Society's Advisory Committee on Cancer Statistics 2013; World Cancer Research Fund/American Institute for Cancer Research 2007). The high personal, societal, and economic burdens of breast cancer render research into strategies for prevention and treatment a high priority.

Like all cancers, breast cancer is a multifactorial disease. Established nonmodifiable risk factors include early menarche, late menopause, and family history/genetics. Several modifiable factors, including dietary factors, alcohol consumption, hormone replacement therapy use, radiation exposure, physical activity, and lactation, have all been suggested to influence breast cancer risk. This review will focus on FS as a potential dietary factor used to prevent and treat cancer.

### Breast cancer pathophysiology, molecular subtypes, and treatment

Breast cancer is characterized by uncontrolled proliferation of breast epithelial cells, a loss of cellular differentiation, and the ability of cells to migrate/metastasize to other parts of the body. This complex process consists of 3 stages: initiation, promotion, and progression/metastasis (Fig. 1) and has been previously reviewed elsewhere (Pitot 1993; Russo et al. 2000; Welsh 2007). This paper will review the evidence from preclinical studies of FS and its components during all stages of carcinogenesis.

While the carcinogenic process is similar among subtypes, breast cancer is not one disease. Tumours vary in expression levels of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). ER and PR influence cell growth through transcriptional regulation of genes involved in cell cycle regulation and through activation of signalling pathways both directly and indirectly through cross-talk with growth factor signalling pathways. HER2, a member of the epidermal growth factor receptor family, signals through the proproliferative and anti-apoptotic PI3K-Akt and MAPK pathways. On the basis of ER, PR, and HER2 status, a number of molecular subtype classifications for breast cancer have been proposed (Carlson et al. 2011; Sorlie et al. 2003; Yang et al. 2007). The National Comprehensive Cancer Network (NCCN) uses the following classifications: Luminal A and B (ER+/PR+/HER2–), HER2-overexpressing (ER/PR+ or –/HER2+), basal (ER–/PR–/HER2–), and normal breast-like (have characteristics similar to normal breast tissue) (Carlson et al. 2011). Progression beyond the "one size fits all" approach to treatment has significantly improved care.

Treatment of breast cancer is complex and varies based on tumour stage and molecular subtype and approaches are outlined in the NCCN guidelines (Carlson et al. 2011; Theriault et al. 2013). In general, approaches considered include surgery and (or) radiation along with or followed by single agent or combination systemic therapies (i.e., chemotherapy, endocrine therapy, and biologic agents). Molecular subtype dictates the systemic therapies selected; ER+ tumours are treated with the ER antagonist tamoxifen (TAM); and HER2-overexpressing tumours are treated with the HER2-targeted agents trastuzumab (TRAS; Herceptin (Genentech, San Francisco, Calif., USA)) and pertuzumab. As breast cancer is further characterized, novel strategies can be developed. Like targeted pharmaceuticals, dietary agents may behave differently depending on the molecular subtype. How and if dietary agents interact with these systemic therapies is an area of research interest. Thus this paper will discuss the existing preclinical evidence for the effect of FS and its components in different subtypes of breast cancer and for the interactions between FS and its components with both TAM and TRAS.

## Flaxseed and breast cancer

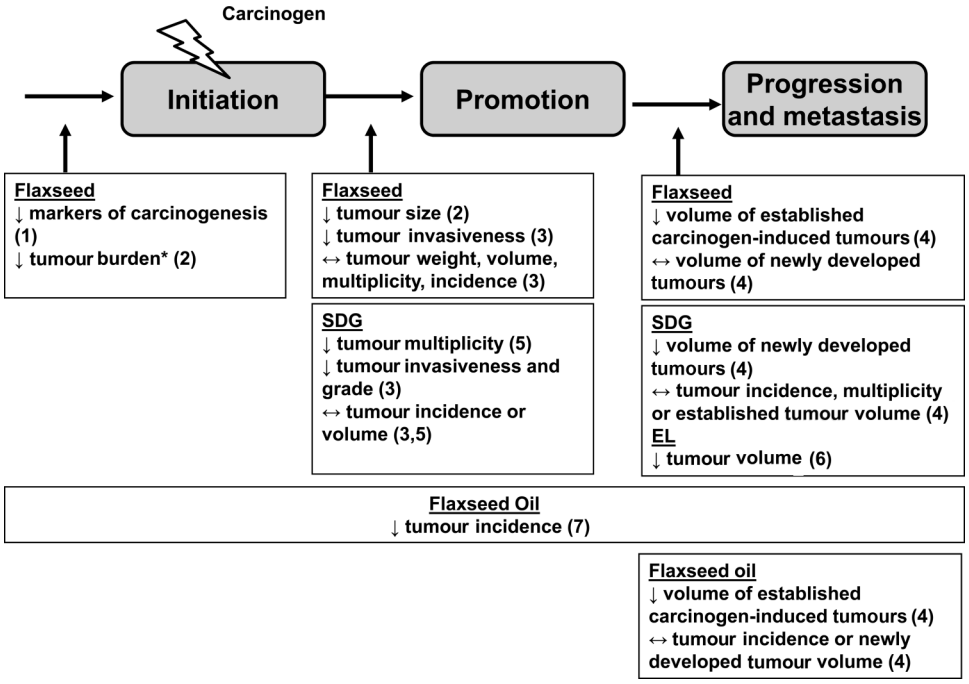
### Flaxseed composition

FS composition has previously been reviewed (Daun et al. 2003; Hall et al. 2006). Briefly, the composition of FS varies by growth location, year, environmental conditions, and cultivar, but typically its composition by dry weight is approximately 30% fiber, 20% protein, 40% fat, 4% ash, and 6% moisture (Daun et al. 2003), and 4% lignan, which is a type of phytoestrogen (Thompson et al. 2006).

Two components of FS that may play a role in breast cancer are the lignans and oil, which is rich in the *n*-3 PUFA ALA. FS is the richest dietary source of lignans. The predominant lignan in FS is secoisolariciresinol diglucoside (SDG; approximately 95%), although matairesinol, pinoresinol, and lariciresinol are also found in FS (Thompson et al. 2006). Upon consumption, SDG is metabolized by colonic bacteria to the enterolignans, enterodiol (ED) and enterolactone (EL), which are then absorbed in the colon, undergo enterohepatic circulation, and are excreted in the urine or are directly excreted in the feces. Urinary and serum levels of enterolignans (or their glucoronic and sulfate conjugates) are related to dietary plant food intake and have been used in epidemiological studies as indicators of lignan intake. Lignans are structurally similar to 17β-estradiol (E2) and are suggested to have estrogenic or anti-estrogenic effects in the body and thus have been studied in hormone-related diseases such as breast cancer (Adlercreutz 2007; Hall et al. 2006; Thompson 2003). Lignans have been shown to be incorporated into breast cancer tissues (Saarinen et al. 2008).

FS oil contributes approximately 40% of FS by weight. The approximate fatty acid profile of FS oil is 9% saturated fatty acids, 18% monounsaturated fatty acids, and 73% PUFA (Hall et al. 2006). The predominant PUFA is ALA (approximately 57%), followed by the *n*-6 PUFA linoleic acid (LA) (approximately 16%). ALA and LA, the parent *n*-3 and *n*-6 PUFA, respectively, can be converted by desaturase and elongase enzymes to their longer chain metabolites. The major long-chain *n*-3 PUFAs are eicosapentaenoic acid (EPA; 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3), which are found at high levels in fatty fish and have been suggested to have many health benefits including anticancer effects. The major long-chain *n*-6 PUFA is arachidonic acid (20:4*n*-6), which is found in animal-based foods such as red meat, eggs, dairy, and some type of fish (e.g., tilapia and mackerel). The extent of conversion of parent PUFA to their downstream metabolites is controversial and occurs to varying degrees in different tissues and depends on the level of *n*-3 PUFA in the diet. Kinetic studies in humans and rodents suggest that the conversion is low and we refer readers to the statement from the International Society for the Study of Fatty Acids and Lipids for details on this conversion (Brenna et al. 2009). However, we have previously demonstrated that consumption of a diet rich in FS results in higher ALA, EPA, and DHA levels in human breast tumour xenografts compared with a corn oil-rich basal diet, suggesting that tumour tissue may readily convert ALA to long-chain PUFA or preferentially take up *n*-3 PUFA (Mason et al. 2013). The health benefits and antitumour effects of FS may be

**Fig. 1.** The effects of adulthood exposure to flaxseed, secoisolariciresinol diglucoside and flaxseed oil on carcinogenesis in the carcinogen-induced rodent model. (1) Serraino and Thompson 1991; (2) Serraino and Thompson 1992; (3) Rickard et al. 1999; (4) Thompson et al. 1996*a*; (5) Thompson et al. 1996*b*; (6) Saarinen et al. 2002; (7) Cameron et al. 1989. EL, enterolactone; SDG, secoisolariciresinol diglucoside. *, Trend.




related to ALA specifically, the ALA metabolites EPA and DHA, or the overall fatty acid profile of the oil. Research is being done to further elucidate this point.

Other FS components may also contribute to its health effects. These include soluble and insoluble fiber and phenolic compounds. Of some concern is the presence of antinutritional compounds such as cyanogenic glycosides, phytic acid, and cadmium (Cd), which are present in raw FS. These antinutritional compounds will be further discussed in the Safety and regulatory status section.

## Flaxseed in the diet of breast cancer patients

Research suggests that breast cancer patients change their diet and lifestyle upon diagnosis (Boon et al. 2007; Boucher et al. 2012). Several studies have assessed FS intake in breast cancer patients and found that 12%–50% of breast cancer patients consume FS (Boon et al. 2007; Boucher et al. 2012; Greenlee et al. 2009; Rausch et al. 2011). One study showed that 70% of recently diagnosed breast cancer patients consume high lignan foods, 52% consume flax bread, and 33% consume 1 tablespoon of FS at least once per week (Boucher et al. 2012). Hence there is a large interest in finding the scientific evidence for the use of FS in the prevention and treatment of breast cancer (Patterson 2011).

## Flaxseed and the risk reduction and treatment of breast cancer

### *Animal studies*

Rodent models have allowed for the study of the role of FS at various stages of the life cycle and carcinogenesis pathway. These studies can be divided into 3 general categories: (*i*) early life exposures and risk of breast cancer in later life, (*ii*) adulthood exposures and risk of breast cancer, and (*iii*) exposures once breast cancer is established.

#### (i) *Early life exposures and risk of breast cancer in later life*

It has been hypothesized that early life exposures may affect the development of the mammary gland, which then alters breast cancer risk during adulthood (Hilakivi-Clarke et al. 2001). Mammary gland development has been reviewed elsewhere (Russo et al. 1982, 2000; Russo and Russo 2004). Two important structures that relate to breast cancer risk are the terminal end buds (TEB) and the alveolar buds (AB), which show differences in susceptibility to carcinogen-induced transformation: TEB are highly susceptible while AB are not susceptible. Thus, agents that can promote the transformation of TEB to AB may result in a mammary gland that is less susceptible to cancer development during adulthood (Hilakivi-Clarke et al. 2001; Russo et al. 1982) and research has been conducted to determine whether FS is such an agent.

The existing literature that relates early life exposure to FS with breast cancer risk is variable (Table 1). Several studies suggest that 10% FS exposure in utero (Tou and Thompson 1999), during suckling (Tan et al. 2004; Ward et al. 2000), from suckling until postnatal day 50 (Ward et al. 2000), and throughout life (Tou and Thompson 1999) may enhance mammary gland differentiation/morphogenesis, which is suggested to prevent carcinogenesis. Furthermore, exposure to 10% FS during suckling has been shown to result in significantly lower tumourigenesis after exposure to the mammary carcinogen dimethylbenz(α)anthracene (DMBA) (Chen et al. 2003), suggesting that the results observed on mammary gland morphology translate to protection against carcinogenesis. However, others have shown that exposure to 10% FS in utero or during suckling (Khan et al. 2007) or 15% defatted FS in utero (Yu et al. 2006) increases susceptibility to carcinogen-induced mammary tumourigenesis as indicated by increased tumour incidence and multiplicity and reduced tumour latency. The reason for this discrepancy is unclear but a potential explanation is the FS source: studies showing a cancer-promoting effect of FS used FS from the United States, whereas those showing a protective effect used the Linnott variety of FS from Canada. The different growth locations may lead to differences in the level of Cd in the seed and Cd is known to have estrogenic effects (Daun et al. 2003; Johnson et al. 2003; Khan et al. 2007; Kolodziejczk and Fedec 1993). Because of the discrepancies, caution should be taken when FS is consumed during pregnancy and (or) lactation.

**Table 1.** Studies investigating the role of FS exposures in early life and risk of breast cancer in later life.

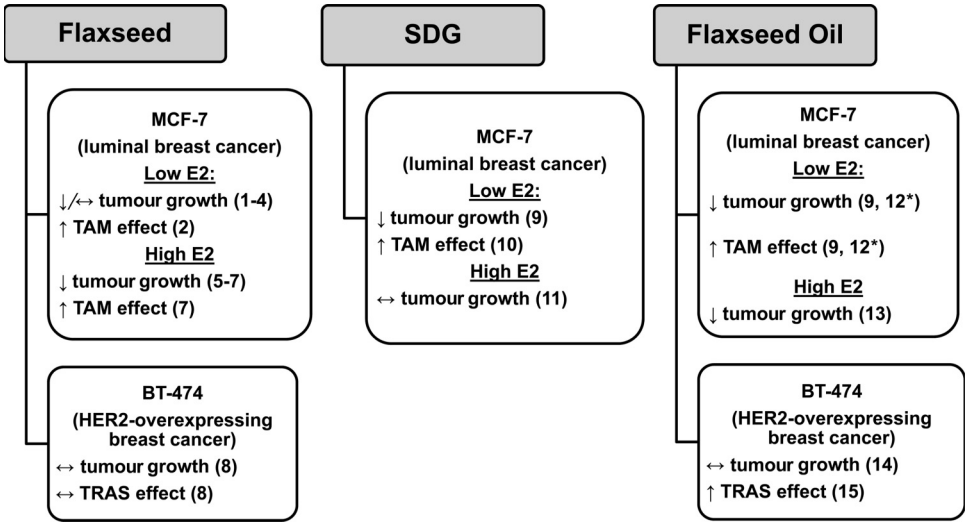
| Reference | Developmental stage | Diets | Major results |
|---|---|---|---|
| Khan et al. 2007 | (*i*) In utero and lactation; (*ii*) lactation alone | BD, 5% or 10% FS (American, Finnish FS) | • ↑ Tumourigenesis after DMBA administration (↓ latency, ↑ multiplicity) with 10% FS (in utero and lactation and lactation alone)<br>• ↔ No. of TEB or cell proliferation and apoptosis in mammary epithelial structures with 5% and 10% FS (in utero and lactation and lactation alone) |
| Yu et al. 2006 | In utero | BD, 15% defatted FS (American FS) | • ↑ Tumourigenesis after DMBA administration (↑ incidence, ↑ multiplicity) with 15% defatted FS<br>• ↔ No. of TEB or cell proliferation in mammary epithelial structures with 15% defatted FS<br>• ↑ Apoptosis in mammary epithelial cells with 15% defatted FS |
| Chen et al. 2003 | Lactation | BD, 10% FS, SDG (at levels present in 10% FS) (Canadian, Linnott variety FS) | • ↓ Tumourigenesis after DMBA administration with both FS and SDG (↓ tumour incidence, ↓ no. of tumours/rat, ↓ tumour load, ↓ tumour size) |
| Tan et al. 2004 | Lactation | BD, 10% FS, SDG (at levels present in 10% FS) (Canadian, Linnott Variety FS) | • No. of TEB: PND21 ↑ in FS group, ↔ in SDG group; PND49-51 ↓ in FS and SDG groups<br>• No. of TD: PND21 ↑ in FS group, ↔ in SDG group<br>• No. of LOB: PND49-51 ↔ in FS and SDG groups<br>• Ratio of LOB/TEB: PND49-51 ↑ in FS and SDG groups<br>• Mammary epithelial cell proliferation (PCNA and %G1/S): PND21 ↑ in FS and SDG groups; PND49-51 ↔ in FS and SDG groups |
| Ward et al. 2000 | (*i*) Lactation only; (*ii*) lactation to PND 50 | BD, 10% FS, SDG (at levels present in 10% FS) (Canadian, Linnott Variety FS) | • TEB density: ↓ in FS and SDG groups (lactation only and lactation to PND 50)<br>• AB density: ↑ in FS (lactation to PND 50) and SDG (lactation only and lactation to PND 50); ↔ in FS (lactation only)<br>• LOB density: ↑ in SDG (lactation to PND 50); ↔ in FS (lactation only and lactation to PND 50) and SDG (lactation only) |
| Tou and Thompson 1999 | (*i*) In utero and (*ii*) lactation after weaning (PND 21–50); (*iii*) lifetime (gestation to PND 50) | BD, 5% FS, 10% FS, FS oil, SDG (FS oil and SDG at levels present in 5% FS) (Canadian, Linnott variety FS) | • TEB density: ↓ with 5% and 10% FS fed during gestation and lactation or throughout the lifetime and with SDG fed during gestation; ↔ with 5% and 10% FS started after weaning and with FS oil fed during gestation<br>• AB density: ↑ with 10% FS fed during gestation and lactation or throughout the lifetime; ↓ with SDG fed during gestation; ↔ with 5% FS at any timing, 10% FS started after weaning and with FS oil fed during gestation |

**Note:** AB, alveolar bud; BD, basal diet (control); DMBA, 9,10 dimethyl-1,2-benzanthracene; FS, flaxseed; LOB, lobules; PCNA, proliferating cell nuclear antigen; PND, postnatal day; SDG, secoisolariciresinol diglycoside; TD, terminal ducts; TEB, terminal end buds.

*(ii) Adulthood exposures and risk of breast cancer*

Many studies have investigated the effect of whole ground FS fed later in life in rodent models of breast carcinogenesis. Figure 1 outlines the general study designs that use the carcinogen-treated rodent model to look at FS effect in the different stages of carcinogenesis. To assess the role of FS in the initiation stage, 5% and 10% FS diets were fed to rats for 4 weeks before DMBA administration and markers of carcinogenesis were measured 24 h after. Mitotic index, cell proliferation, and nuclear aberrations in the TEB were all significantly lower in the animals fed FS compared with those fed the control diet (Serraino and Thompson 1991). Next, the same model was used to determine the effect of a 5% FS diet fed at (*i*) initiation only, (*ii*) promotion only, and (*iii*) both initiation and promotion on tumour size and burden at 19 weeks after carcinogen administration and the results were complex. FS fed during initiation stage only tended to reduce tumour burden (number of tumours/tumour-bearing rat) but did not affect final tumour size compared with control while FS fed during the promotion stage only reduced tumour size compared with control but did not affect tumour burden. Interestingly, the tumours from rats fed at both initiation and promotion did not result in a difference in tumour burden or size compared with control but did result in a significantly lower tumour burden compared with the group fed FS at promotion stage only (Serraino and Thompson 1992). Next, the effect of 2.5% and 5% FS diets fed starting 13 weeks after DMBA administration during the tumour development and progression stage of carcinogenesis was determined. Tumours that were already established at the start of the dietary treatment significantly regressed in the FS groups compared with control and there was significantly lower total tumour volume (established at start of treatment + newly developed) with FS treatment. Interestingly, there were no effects of the FS diets on the incidence or volume of newly developed tumours (Thompson et al. 1996*a*). Finally, Rickard et al. (1999) observed significantly lower tumour invasiveness and grade but no effect on tumour weight, volume, multiplicity, and incidence when Sprague–Dawley rats were fed 2.5% and 5% FS diets for 22 weeks starting 2 days after administration of the mammary carcinogen *N*-nitrosomethyl-urea (NMU) (Rickard et al. 1999).

**Fig. 2.** The effects of adulthood exposure to flaxseed, secoisolariciresinol diglucoside and flaxseed oil on the growth of established human breast tumours in the athymic mouse model. (1) Chen et al. 2009; (2) Chen et al. 2007*a*; (3) Power et al. 2008; (4) Saarinen et al. 2006; (5) Bergman et al. 2007; (6) Chen et al. 2007*b*; (7) Chen et al. 2004; (8) Mason et al. 2013; (9) Saggar et al. 2010*b*; (10) Saggar et al. 2010*a*; (11) Truan et al. 2012; (12) Chen et al. 2011; (13) Truan et al. 2010; (14) Mason et al. 2010*b*; (15) Mason et al. 2010*a*. E2, estrogen; HER2, human epidermal growth factor receptor 2; SDG, secoisolariciresinol diglucoside; TAM, tamoxifen; TRAS, trastuzumab. *, Results related to FS cotyledon.




Transgenic mice present another useful model for investigating treatment effects on cancer prevention. The MMTV/c-neu transgenic mouse model, which spontaneously develops HER2+ tumours, has been used to investigate the role of FS in the prevention of HER2-overexpressing breast cancer (Birkved et al. 2011). Increasing levels of FS were incorporated into the diets and fed for 23 weeks and tumour incidence, burden, and number of large tumours were lower in mice fed the highest FS diet (0.054%) compared with control. None of the FS diets affected tumour multiplicity or number of tumour-bearing mice compared with control. The levels of FS used in this study were almost 100-fold lower than the previously outlined studies. It is possible that a greater FS effect would have been achieved with higher levels of FS in the diet. Together the results from studies using carcinogen-treated and transgenic rodent models suggest that FS reduces carcinogenesis and may be particularly effective at reducing the growth of established tumours.

*(iii) Exposures once breast cancer is established*

The next series of experiments evaluated the effect of FS on the growth of established human breast tumours using the ovariectomized athymic mouse model. This is a useful model for preclinical cancer studies because immunodeficient animals can accept xenografts without rejection and thus act as a vehicle for observing effects of dietary and drug treatments on the growth of human breast tumours representative of the different molecular subtypes of breast cancer. It also has an advantage over the carcinogen-treated rat model as human tumours are being tested rather than rat tumours. Furthermore, ovariectomy eliminates the production of endogenous E2 and allows for experimental manipulation of E2 levels to achieve levels that fall within the range of pre- and postmenopausal women. The effect of FS in this model is outlined in Fig. 2.

A series of experiments has shown that 10% dietary FS reduces the growth of established MCF-7 tumours, representative of luminal breast cancer (ER+/PR+/low HER2), when fed for 5–8 weeks with an E2 implant so circulating levels of E2 are high (Bergman Jungestrom et al. 2007; Chen et al. 2004; Chen et al. 2007*b*). Established MCF-7 tumours normally regress when circulating levels of E2 drop unless an estrogenic compound is introduced. After E2 pellet removal, dietary FS resulted in a greater regression compared with control in the short term (7 weeks) (Chen et al. 2009) although no differences in regression were observed between control and FS groups in longer term studies where tumour area was followed for 16 weeks (Chen et al. 2007*a*) and 25 weeks (Power et al. 2008; Saarinen et al. 2006). This indicates that FS has no estrogenic tumour-promoting effect under low E2 conditions typical of postmenopausal situations. One study has shown that feeding 10% dietary FS for 5 weeks does not affect the growth of BT-474 tumours, which represent the HER2-overexpressing subtype of breast cancer (ER+/HER2+) (Mason et al. 2013). Together these data suggest that FS has no estrogenic effect and can reduce the growth of human breast cancer; however, this is dependent on molecular subtype.

Rodent studies have also been useful for studying potential FS-drug interactions. One area of particular interest has been the interaction between FS and TAM. Several studies have shown that combining TAM treatment (5 mg, 60-day release pellet; 80 mg/day) with dietary 10% FS significantly enhances the antitumour effect of TAM in MCF-7 xenografts under both low and high circulating levels of E2 (Chen et al. 2004, 2007*a*, 2007*b*). These data suggest that FS does not interfere with TAM effect, it rather enhances TAM effect. Further, 1 study evaluated the potential interaction between FS (10%) and TRAS (2.5 mg/kg) (Mason et al. 2013). While no beneficial interaction was observed, dietary FS did not interfere with the action of TRAS in reducing the growth of HER2-overexpressing BT-474 tumours (Mason et al. 2013). Further studies are needed in breast cancer patients; however, the preclinical evidence supports the notion that dietary FS does not negatively affect breast cancer drugs.

***Observational studies***

The Ontario Women's Diet and Health Study, a case-control study, was the first to evaluate the association between FS intake and breast cancer risk (Lowcock et al. 2013). As shown in Table 2, both monthly and daily/weekly consumption of FS (1/4 cup serving, approximately 32.5 g) and flax bread (1 slice, approximately 2.5–5.0 g FS) consumption were both associated with significant reductions (18%–24%) in breast cancer risk in all women. Stratification by menopausal status showed that FS reduced breast cancer risk in postmenopausal women only while flax bread reduced risk in both pre- and postmenopausal women. The relationship between breast cancer risk and FS or flax bread intake is not associated with hormone receptor (ER, PR) expression. These results are supportive of findings from animal models of cancer

**Table 2.** Observational and clinical studies examining the relationship between FS, lignan, and ALA and breast cancer risk.

| Reference | Study design | Population | Exposure/treatment | Results |
|---|---|---|---|---|
| **Flaxseed** | | | | |
| Lowcock et al. 2013 | Case-control | 2999 breast cancer cases, 3370 controls | FS intake measured by FFQ (frequency of consuming a 1/4 cup serving of FS or 1 slice of flax bread) | • FS ↓ risk of breast cancer when consumed daily/weekly (OR = 0.82, CI = 0.69–0.97) and monthly or less (OR = 0.76, CI = 0.67–0.87) in all women<br>• Flax bread ↓ risk of breast cancer when consumed daily/weekly (OR = 0.77, CI = 0.67–0.89) and monthly or less (OR = 0.76, CI = 0.67–0.86) in all women |
| Thompson et al. 2005 | Randomized control trial | 32 postmenopausal breast cancer patients | 25 g FS/d (Canadian; Linnott variety) ($n$ = 19; mean treatment duration = 32 d) vs. placebo ($n$ = 13; mean treatment duration = 39 d) | • FS ↓ cell proliferation (Ki-67) by 34% and HER2 by 71% and ↑ apoptosis by 31%<br>• No changes in placebo group |
| **Lignans** | | | | |
| Fabian et al. 2010 | Clinical trial (1 arm) | 45 premenopausal women at high risk of breast cancer | 50 mg SDG/d (Brevail (Barleans Organic Oils Co., Ferndale Wash., USA)) for 12 mo | • SDG ↓ median cell proliferation (Ki-67) by 50%, tumour cell number, proportion of subjects with atypical cytomorphology. No effect on MBD. |
| Buck et al. 2010 | Meta-analysis | 11 prospective cohort/ nested case-control studies; 10 case-control studies | • Total lignan exposure;<br>• Plant lignan intake;<br>• Enterolignan exposure;<br>• Blood or urine concentrations of EL | • Total lignan exposure – overall: no significant association (RE = 0.92 (0.81–1.02)); premenopausal: no significant association (RE = 0.87 (0.66–1.08)); postmenopausal: ↓ breast cancer risk (RE = 0.86 (0.78–0.94))<br>• Dietary plant lignans – overall: no significant association (RE = 0.94 (0.82–1.05)); premenopausal: no significant association (RE = 1.01 (0.87–1.15)); postmenopausal: ↓ breast cancer risk (RE = 0.86 (0.77–0.94))<br>• Calculated dietary enterolignans – overall: ↓ breast cancer risk (RE = 0.84 (0.71–0.91))<br>• Blood and urinary EL – overall: no significant association (RE = 0.90 (0.69–1.10)); premenopausal: no significant association (RE = 0.70 (0.32–1.07)); postmenopausal: no significant association (RE = 0.85 (0.63–1.07)) |
| Velentzis et al. 2009 | Meta-analysis | 6 prospective cohort studies; 6 nested case-control studies; 10 case-control studies | • Plant lignan intake;<br>• Enterolignan exposure;<br>• Blood concentrations of EL | • Dietary plant lignans – overall: no significant association (OR = 0.93 (0.83–1.03)); premenopausal: no significant association (OR = 0.97 (0.82–1.15)); postmenopausal: ↓ breast cancer risk (OR = 0.85 (0.78–0.93))<br>• Calculated dietary enterolignans – overall: ↓ breast cancer risk (OR = 0.73 (0.57–0.92)); premenopausal: no significant association (OR = 0.67 (0.44–1.02)); postmenopausal: no significant association (RE = 0.85 (0.72–1.01))<br>• Blood EL – overall: no significant association (OR = 0.82 (0.59–1.14)); premenopausal: no significant association (OR = 0.85 (0.45–1.59)); postmenopausal: no significant association (RE = 0.86 (0.66–1.14)) |
| Zaineddin et al. 2012 | Meta-analysis | 8 prospective cohort/nested case-control studies; 5 case-control studies | • Blood or urine EL concentrations | • Blood and urinary EL – overall: ↓ breast cancer risk (RE = 0.72 (0.55–0.88)); postmenopausal: ↓ breast cancer risk (RE = 0.66 (0.55–0.77)) |

**Table 2** (*concluded*).

| Reference | Study design | Population | Exposure/treatment | Results |
|---|---|---|---|---|
| **FS oil/ALA** | | | | |
| Zheng et al. 2013 | Meta-analysis | 6 prospective cohort studies; 1 case-cohort study; 5 case-control studies | • Dietary intake of ALA<br>• Tissue or blood biomarker of ALA | • No association between ALA and breast cancer risk (overall RR = 0.97 (0.90–1.04)) |
| Saadatian-Elahi et al. 2004 | Meta-analysis | 3 cohort studies, 7 case-control studies | • Fatty acid composition of adipose tissue and serum | • Case control studies: ↓ breast cancer risk with increasing level of ALA (RR = 0.64 (0.46–0.89))<br>• Cohort studies: no association between breast cancer risk and level of ALA; ↑ risk in postmenopausal women (RR = 1.14 (1.03–1.26)) |
| Nkondjock et al. 2003 | Case-control | 414 breast cancer patient, 429 controls | • Dietary intake of ALA (FFQ)<br>• Median intake in population = 1.75 g/d | • No association between breast cancer risk and ALA intake (OR = 1.27 (0.85–1.89)) |
| De Stefani et al. 1998 | Case-control | 365 breast cancer patients, 397 controls | • Dietary intake of ALA (questionnaire and FFQ)<br>• Q4 (≥1.3 g/d) vs. Q1 (<0.8 g/d) | • ↑ Breast cancer risk with higher dietary ALA intake (OR = 3.79 (1.53–9.40)) |
| Kuriki et al. 2007 | Case-control | 103 breast cancer patients, 309 controls | • Erythrocyte ALA concentrations<br>• T3 (>0.39%) vs. T1 (<0.26%) | • No association between breast cancer risk and erythrocyte ALA (OR = 0.69 (0.37, 1.28)) |
| Shannon et al. 2007 | Case-control | 322 breast cancer patients, 1030 controls | • Erythrocyte fatty acid concentrations<br>• Q4 (>0.32%) vs. Q1 (≤0.18%) | • No association between breast cancer risk and erythrocyte ALA concentration (OR = 0.99 (0.54, 1.82)) |
| Bougnoux et al. 1994 | Cohort | 121 breast cancer patients | • Fatty acid composition of adipose tissue<br>• <0.38% vs. ≥0.38% | • ↓ Breast cancer metastases with higher ALA content of breast tissue (RR = 0.2 (0.1–0.6)) |

**Note:** ALA, α-linolenic acid; CI, confidence interval; EL, enterolactone; FFQ, food frequency questionnaire; FS, flaxseed; HER2, human epidermal growth factor receptor 2; MBD, mammographic breast density; OR, odds ratio; Q1, Q4, quartile 1 and 4, respectively; RE, risk estimate; RR, relative risk; SDG, secoisolariciresinol diglucoside; T1, T4, tertile 1 and 4, respectively.

prevention, which suggest that FS has anticancer effects particularly in postmenopausal women.

### *Clinical trials*

Only 1 randomized double-blind placebo-controlled trial has investigated the role of FS on markers of tumour biological markers in postmenopausal women (Table 2) (Thompson et al. 2005). Patients diagnosed with breast cancer for the first time were divided between 2 arms: 13 women consumed a placebo muffin daily for 39 days and 19 women consumed a muffin containing 25 g of ground flax (containing approximately 50 mg of SDG and 10 g of FS oil) for 32 days. At the beginning and end of the treatment, tumour biopsy tissue was taken and analyzed for tumour growth biomarkers. Cell proliferation, as measured by Ki-67 labelling index, and HER2 protein expression were significantly lower by 34% and 71%, respectively, and apoptosis was significantly higher by 31% at the end of the treatment in the FS group. There were no changes in the placebo group for any of these biomarkers. These results indicate that tumour growth is slowed by dietary FS in agreement with the results from animal models.

## Lignans and the risk reduction and treatment of breast cancer

### *Animal studies*

#### (i) *Early life exposures and risk of breast cancer in later life*

Studies suggest that SDG may affect the developing mammary gland (Table 1). SDG exposure at levels present in a 5%–10% FS diet in utero (Tou and Thompson 1999), during suckling (Chen et al. 2003; Tan et al. 2004; Ward et al. 2000) and from suckling to postnatal day 50 (Ward et al. 2000) have been shown to result in mammary glands that are less susceptible to carcinogenesis (i.e., fewer TEB and more AB) at time of carcinogen induction. Tou and Thompson (1999) found that both TEB and AB density were significantly lower in pups born to dams fed an SDG-containing diet during pregnancy and lactation suggesting that the SDG effect may be more related to TEB atrophy than differentiation to AB. Importantly, Chen et al. (2003) showed that exposure to SDG during suckling reduced tumourigenesis after DMBA administration providing greater support for the protective effects of SDG during mammary gland development. Overall, compared with FS which was discussed above, SDG has a more consistent effect on mammary gland development that is suggestive of protection from carcinogenesis.

#### (ii) *Adulthood exposures and risk of breast cancer*

Studies using the carcinogen-treated rodent model suggest that SDG has antitumour effects (Fig. 1). Thompson et al. (1996*b*) demonstrated that a gavage of 1.5 mg SDG/day (equivalent intake level from 5% FS) starting 1 week after DMBA administration resulted in significantly lower tumour multiplicity and total number of tumours per group compared with control but did not affect tumour volume after 20 weeks. To evaluate the effect of SDG during the early promotion stage, Sprague–Dawley rats were fed diets that contained 0.25% and 0.5% SDG (levels present in 2.5% and 5% FS) for 22 weeks starting 2 days after NMU administration. Both diets resulted in significantly lower tumour invasiveness and grade while 0.25% SDG promoted and 0.5% SDG reduced tumour multiplicity. Neither diet had effects on tumour weight, volume, or incidence (Rickard et al. 1999). Daily gavage of 1.5 mg of SDG (equivalent intake levels from 5% FS) starting 13 weeks after DMBA administration during the tumour development and progression stage of carcinogenesis significantly reduced the volume of new tumours that grew after the start of the treatment. However, SDG had no effect on tumour incidence or multiplicity or on the growth of tumours that were already established at the start of the dietary treatment (Thompson et al. 1996*a*). The SDG metabolite EL has also been tested for its effects in reducing the promotion stage

of carcinogenesis: treatment with 10 mg/kg of body weight of EL (similar to intake levels in a 10% FS diet) daily for 7 weeks commencing 9 weeks after DMBA administration resulted in significantly lower tumour volume compared with control with more pronounced reductions in the newly formed tumour growth (Saarinen et al. 2002). The tested lower dose of EL (1 mg/kg of body weight; similar to levels in a 1% FS diet) did not result in any significant differences in tumourigenesis, highlighting the importance of dose. Together these studies suggest that SDG can attenuate the development of tumours but has less effect in reducing established tumours.

The athymic mouse model has been useful in exploring the effect of SDG on the growth of established human breast tumours (Fig. 2). A comprehensive series of experiments have shown that compared with control, 0.1% SDG diets (level present in 10% FS diet) fed to mice for 7–8 weeks caused a 54% greater regression of ER+ MCF-7 tumours, representative of luminal breast cancer, with low circulating levels of E2 (Chen et al. 2009; Saggar et al. 2010*b*) but had no effect on tumour growth with high circulating levels of E2 (Truan et al. 2012). Interestingly, compared with control, FS hull, which is rich in SDG, did not significantly reduce the growth of MCF-7 tumours in athymic mice at low circulating levels of E2. SDG alone reduced tumour growth compared with control; however, there was no significant difference between the SDG and FS hull groups (Chen et al. 2009). As the level of SDG (0.076%) in the FS hull diet in this study was lower than in the SDG alone diet (0.1%), this suggests that the level of SDG in the diet is important to achieve maximal tumour reducing effect. To determine whether it is the mammalian lignan metabolites that are responsible for the tumour-reducing effect of SDG, ED, or EL (15 mg/kg of body weight; similar to intake levels in a 15% FS diet) were directly injected for 5 weeks in the presence of high circulating levels of E2 (Bergman Jungestrom et al. 2007). Both ED and EL showed significant reductions in the growth of MCF-7 tumours compared with control, suggesting that both ED and EL are responsible for the antitumour effect of SDG. Together these data strongly support the role of SDG in reducing the growth of ER + breast cancer.

Regarding drug interactions, under low circulating levels of E2, when TAM treatment (80 mg/day) was combined with 0.1% SDG diet in the athymic mouse model with ER+ MCF-7 xenografts, tumour area and cell proliferation were significantly reduced and apoptosis was significantly increased compared with TAM treatment alone, indicating that SDG does not interfere with TAM but rather increases its effectiveness (Saggar et al. 2010*a*).

### *Observational studies*

Several observational studies relating lignan exposure to breast cancer risk have been conducted. Lignan exposures were estimated from the intake of dietary plant lignan or the equivalent amount of enterolignans produced from those plant lignans recorded in dietary questionnaires, and measurements of enterolignan concentration in the urine or blood. Three meta-analyses of observational studies on the relationship between lignan or enterolignan exposure and breast cancer risk are summarized in Table 2 (Buck et al. 2010; Velentzis et al. 2009; Zaineddin et al. 2012). Velentzis and colleagues (2009) published the earliest meta-analysis of 22 observational studies, which showed no overall association between dietary plant lignan intake and breast cancer risk. However, when pre- and postmenopausal women were analyzed separately, a significant inverse association between dietary plant lignan intake and breast cancer risk was seen for postmenopausal women. A significant inverse association between enterolignan exposure and overall breast cancer risk was also seen. Buck and colleagues (2010) applied different inclusion criteria in their meta-analyses of 21 observational studies and only observed significant association of reduced breast cancer risk with total lignan exposure (i.e., all lignan exposure measurements combined) and intake of dietary plant lignan in subgroup analysis of postmenopausal women. The reduced risk of breast cancer with higher total lignan exposure did not differ based on ER status. Zaineddin and colleagues (2012) published an updated meta-analysis of 13 observational studies relating urinary and blood EL concentration with breast cancer risk. A significant 28% reduction in breast cancer risk was found when comparing the highest to lowest quantiles of EL and this reduction was most pronounced in postmenopausal women (44%). Interestingly, although the relationship was observed in ER+PR+ and ER–PR– tumours, the relationship was stronger for ER–PR–. Overall, the results from observational studies suggest that lignan exposure is associated with reduced risk of breast cancer and this relationship is particularly strong for postmenopausal women.

Another question that has been addressed in observational studies is whether lignans prolong the survival of breast cancer patients (Table 3). Five studies published between 2010 and 2011 that followed breast cancer patients from 6.1 to 10 years have all shown significant reductions in mortality (40%–53% reduction in all-cause mortality, 33%–70% reduction in breast cancer mortality) with increased lignan exposure measured by diet record or serum lignan level in postmenopausal women (Buck et al. 2011*a*, 2011*b*; Guglielmini et al. 2012; McCann et al. 2010; Olsen et al. 2011). Importantly, the protective association between lignan intake and survival was observed in populations with comparatively low (e.g., McCann et al. 2010; mean intakes of approximately 250 μg/day) and high (e.g., Olsen et al. 2011; mean intakes of approximately 730 μg/day) lignan intakes. Of particular interest is that in the study by Guglielmini and colleagues (2012) no interaction or interference was observed between lignans and TAM effect. Together, the consistent and strong associations suggest that dietary lignans are safe and beneficial for breast cancer patients.

There is a high level of heterogeneity in the observational studies. Sources of heterogeneity include exposure measurement (reported intakes, urine and blood biomarkers), menopausal status and tumour ER status. Urinary and blood enterolignan levels have been measured by different methods (chromatographic vs. time-resolved fluoroimmunoassay) with different sensitivities across the studies (Saarinen et al. 2010*b*). The level of exposure in the different populations studied varies widely and the range of intake/biomarker within a population is often very narrow. Moreover, an important limitation noted in the observational studies to date is the potential for confounding. Although lignans are the richest in FS, they are also found in many plant foods, including nuts, grains, fruits and vegetables (Thompson et al. 2006); therefore, it cannot be concluded that the significant association between lignans and breast cancer risk/survival is specifically due to FS. Rather, high lignan intake may be closely related to high plant food intake and the overall dietary pattern of high lignan consumers may be related to reduced risk and increased survival. The lignans may be acting synergistically with other healthful plant food components.

### *Clinical trials*

Although the beneficial association of lignans to breast cancer in observational studies cannot conclusively be attributed to FS, the specific beneficial effect of SDG, the main FS lignan, on biomarkers of breast cancer risk has been demonstrated in a single-armed clinical trial (Fabian et al. 2010). Forty-five premenopausal women with high breast cancer risk consumed 50 mg of SDG per day for 12 months. The SDG level (50 mg/day) was similar to the randomized controlled trial by Thompson et al. (2005) on the effect of FS described above. Results showed that median cell proliferation (Ki-67 expression) was significantly reduced by 50% by the end of the study. Furthermore, favourable changes in histological measures, including cell number and cytomorphology, were also seen. All these indicate that SDG may reduce the risk of breast cancer. No changes in mammographic breast density were seen, suggesting that in the short term it may not be the

**Table 3.** Observational studies relating lignan measures with mortality in breast cancer patients.

| Reference | Location | Population | Exposure | Outcome | Median follow up | Results[a] |
|---|---|---|---|---|---|---|
| McCann et al. 2010 | USA | 1122 women with breast cancer | • Dietary lignan intake<br>• Q4 (>257 μg/d) vs. Q1 (<128 μg/d) | • Breast cancer mortality<br>• All-cause mortality | 7 y | • Premenopausal women: no associations<br>• Postmenopausal women: breast cancer HR = 0.29 (0.11–0.76); all-cause HR = 0.49 (0.26–0.91) |
| Buck et al. 2011*b* | Germany | 2653 postmenopausal women with breast cancer | • Dietary lignan intake<br>• ED: Q5 (857.5 μg/d) vs. Q1 (186.9 μg/d)<br>• EL: Q5 (502.0 μg/d) vs. Q1 (146.0 μg/d) | • Breast cancer mortality<br>• All-cause mortality | 6.4 y | • ED: breast cancer HR = 0.81 (0.51–1.29); all-cause HR = 0.63 (0.42–0.95)<br>• EL: breast cancer HR = 0.69–1.10); all-cause HR = 0.60 (0.40–0.89) |
| Buck et al. 2011*a* | Germany | 1140 postmenopausal women with breast cancer | • Serum EL<br>• Q5 (>64.1 nmol/L) vs. Q1 (<3.4 nmol/L) | • All-cause mortality | 6.1 y | • HR = 0.58 (0.34–0.99) |
| Olsen et al. 2011 | Denmark | 424 postmenopausal women with breast cancer | • Plasma EL<br>• ≤20.5 nmol/L vs. >20.5 nmol/L | • Breast cancer mortality<br>• All-cause mortality | 10 y | • Breast cancer HR = 0.56 (0.36–0.87); all-cause HR = 0.47 (0.32–0.68) |
| Guglielmi et al. 2012 | Italy | 300 women with breast cancer | • Serum EL<br>• <10 nmol/L vs. ≥10 nmol/L | • Breast cancer mortality<br>• Breast cancer-unrelated mortality | 23 y | • Breast cancer HR: 5-y HR = 0.42 (0.22–0.81); 10-y 0.67 (0.39–1.14)<br>• Breast cancer-unrelated: 5-y HR = 0.49 (0.27–0.91); 10-y HR = 0.65 (0.40–1.03)<br>• Above vs. below median |

**Note:** ED, enterodiol; EL, enterolactone; HR, hazard ratio; T1, T3, tertile 1 and 3, respectively; Q1, quartile or quintile 1; Q4, quartile 1; Q5, quintile 5.
[a]Results format: HR (95% confidence interval)



best indicator of cancer protective effect of lignans. The same research group is currently conducting a clinical trial including a placebo arm to confirm the results of this study (Trial NCT01276704).

## FS oil and risk reduction and treatment of breast cancer

### *Animal studies*

*(i) Early life exposures and risk of breast cancer in later life*

Few studies have investigated the role of FS oil specifically in mammary gland development and subsequent risk of breast cancer (Table 1). Exposure to FS oil (at levels present in 5% FS) during gestation does not appear to modify markers of breast cancer risk as indicated by TEB and AB density (Tou and Thompson 1999). One recent study showed that exposure to a FS oil diet from preconception to 6 weeks of age results in differential expression of genes involved in energy metabolism, immune response, and inflammation in the mammary gland compared with a corn oil control diet (Luijten et al. 2013). No measures of breast cancer risk (i.e., TEB number or response to carcinogen) were included and therefore it is difficult to associate the gene changes to breast cancer risk; however, these results support the idea that early life exposure to ALA-rich FS oil has lasting effects on the mammary gland at the gene level.

*(ii) Adulthood exposures and risk of breast cancer*

Studies suggest that FS oil-rich diets may inhibit breast carcinogenesis (Fig. 1). In a study comparing the effect of diets rich in various oils (all at 3% level) on mammary tumour growth in DMBA-treated C3H/Heston mice, *n*-3 PUFA-rich FS and fish oils reduced tumour incidence compared with *n*-6 PUFA-rich corn and safflower oils (Cameron et al. 1989). Thompson and colleagues (1996*a*) showed that a 2% FS oil diet (level found in 5% FS diet) fed to Sprague–Dawley rats 13 weeks after DMBA was administered reduced the growth of established tumours but had no effect on new tumour formation.

The MMTV-c-neu transgenic mouse model has also been used to investigate the role of FS oil in the prevention of HER2-overexpressing breast cancer. Mice were gavaged with 0.2 mL of oil that contained increased proportion of FS oil mixed into corn oil (0.05, 0.1, and 0.2 mL of FS oil) for 30 weeks. Interestingly, low-dose FS oil resulted in a nonsignificant increase in tumour incidence and number of tumours per mouse while there was a trend toward reduced tumour incidence with higher dose of FS oil. The high-dose FS oil-treated mice had lower overall weight of tumours per mouse compared with control (Rao et al. 2000). These results suggest that the *n*-6:*n*-3 ratio plays an important role in mediating the effect of flaxseed oil on HER2-overexpressing mammary tumourigenesis.

Studies using mouse models with implanted tumours have been valuable in determining the effect of FS oil in reducing established tumours. One study compared the effect of diets rich in various oils (from corn, FS, and fish) at 10% level on the growth of implanted tumours derived from mouse mammary tumours (410 and 410.1) (Fritsche and Johnston 1990). No differences were observed among diets on the growth of 410 tumours; however, FS oil had the greatest effect at reducing the growth and metastasis of 410.1 tumours. Xenograft studies have also determined the effect of ALA-rich FS oil diets on the growth of human breast tumours (Fig. 2). Four percent FS oil diets (level in 10% FS diet) fed for 7–8 weeks reduced the growth of ER+ MCF-7 tumours under both low (Saggar et al. 2010*b*) and high (Truan et al. 2010) E2 conditions. These results are further supported by results from Chen and colleagues (2011), where diets rich in FS cotyledon (level in 10% FS diet), which is the primary location of FS oil in the seed, were shown to reduce the growth of MCF-7 tumours at low circulating levels of E2. Finally, studies suggest that both 4% and 8% FS oil

(levels in 10% and 20% FS diets) do not affect the growth of HER2-overexpressing BT-474 tumours in athymic mice at high circulating levels of E2 (Mason et al. 2010*b*). Together these data suggest that FS oil is effective in reducing the growth of human breast tumours in the athymic mouse model; however, the results are dependent on the molecular subtype.

Regarding drug interactions, under low circulating levels of E2, when TAM treatment (80 mg/day) was combined with 4% FS oil diet (level in 10% FS diet), tumour area and cell proliferation were significantly reduced by 44% and 35%, respectively, and apoptosis was significantly increased by 76% compared with TAM treatment alone (Saggar et al. 2010*a*). Similarly, the combination of TAM with FS oil-rich cotyledon at level equivalent to amount present in 10% FS diet resulted in greater reduction in tumour cell proliferation compared with TAM treatment alone (Chen et al. 2011). Furthermore, under high circulating level of E2, when TRAS treatment (2.5 mg/kg) was combined with 8% FS oil diet (level in 20% FS), tumour area was 87% lower than TRAS treatment alone and cell proliferation was significantly reduced and apoptosis was significantly increased (Mason et al. 2010*a*). Together these studies support the role of FS oil in enhancing TAM and TRAS effect and the safety of consuming FS oil alongside cancer drug therapies. However, further research is needed in a clinical setting before recommendations can be made regarding the use of FS oil as a complementary treatment.

### *Observational studies*

There is a large body of literature on the association between breast cancer and total *n*-3 PUFA and long-chain *n*-3 PUFA (EPA and DHA), which can be read in several reviews (Gerber 2012; Sala-Vila and Calder 2011) and meta-analysis (Zheng et al. 2013). This paper will only review observational studies where ALA was used as an exposure.

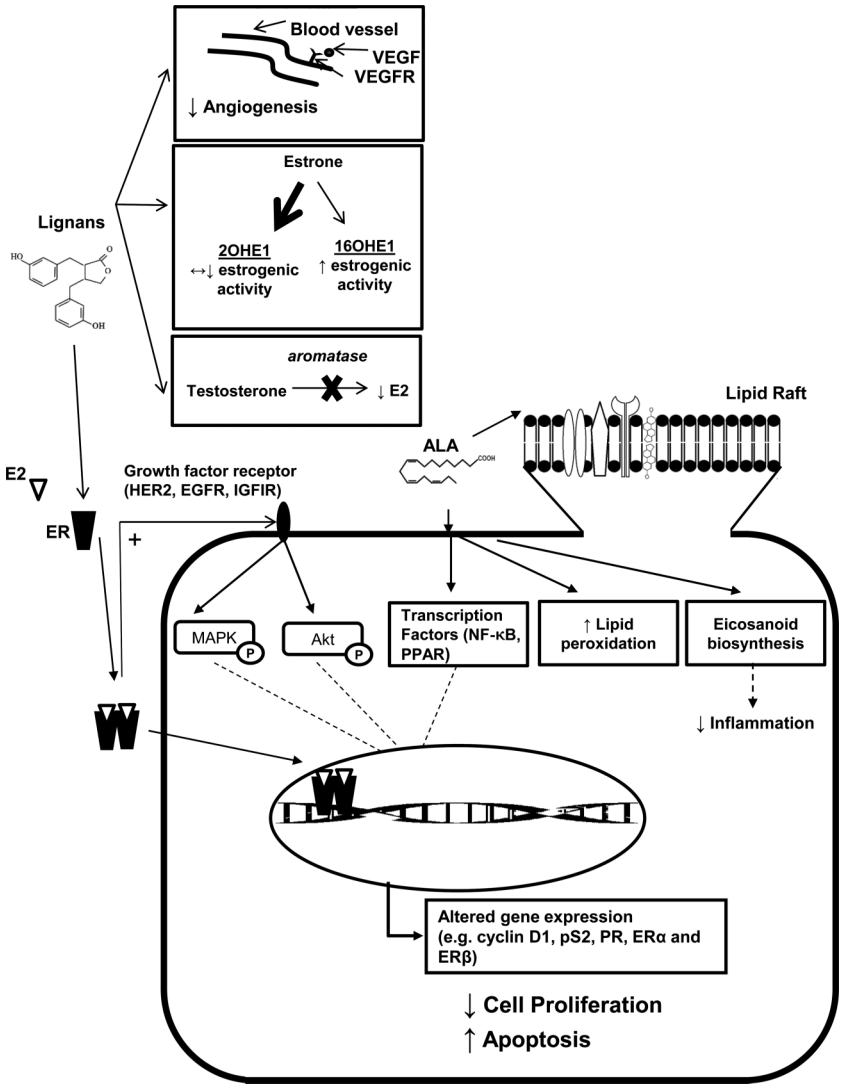
Table 2 shows the results of 2 meta-analyses (Saadatian-Elahi et al. 2004; Zheng et al. 2013) and additional observational studies that were not included in the meta-analyses. Zheng and colleagues (2013) showed that ALA exposure estimated from dietary intake and tissue or blood biomarkers had no significant association with breast cancer risk while an earlier meta-analysis by Saadatian-Elahi and colleagues (2004) showed that breast adipose tissue and blood levels of ALA were associated with a significant reduction in breast cancer risk in case-control studies but not in cohort studies. In fact, there was a significant increase in risk in postmenopausal women in cohort studies. There are a number of limitations that have been identified in the observational studies to date, including (*i*) measurement error of ALA exposure; (*ii*) differences in effect depending on food sources of ALA — for example, ALA intake from fruits, vegetables, and vegetable oils has been associated with reduced breast cancer risk while ALA from nuts and processed foods has been associated with increased risk (Thiebaut et al. 2009); and (*iii*) differences in effect depending on menopausal status. Overall, the research to date has generally shown a lack of effect or small risk-reduction associated with ALA with only 1 study showing an increased risk. It should be noted that this latter study was conducted in Uruguay where intake of red meat is very high and may be accounting for a high proportion of ALA intake rather than plant sources (Bougnoux and Chajes 2003). To our knowledge, no clinical trials have been completed investigating the effect of FS oil and ALA on breast cancer risk or treatment.

## Potential mechanisms of effect

A number of potential mechanisms of effect have been proposed for the anticancer effects of FS and its components and some are illustrated in Fig. 3. In vitro and animal studies have provided a great deal of insight into potential mechanisms of FS, SDG, and its metabolites ED and EL as well as ALA. Tumour analyses from the xenograft studies outlined above have suggested that the anticancer effects of FS and its components are related to reduced cell proliferation and increased apoptosis (Chen et al. 2004, 2007*a*, 2007*b*, 2009, 2011; Saggar et al. 2010*a*, 2010*b*; Truan et al. 2010), and reduced angiogenesis (Bergman Jungestrom et al. 2007; Dabrosin et al. 2002). While it is likely that the effect of FS is related to both the lignan and oil components, studies comparing the effect of SDG and FS oil suggest that the component most responsible for the observed tumour growth reduction may depend on the E2 environment and presence or absence of drugs. For example, SDG is more effective than FS oil in reducing the growth of ER+ MCF-7 tumours at low levels of E2 (Saggar et al. 2010*b*) while FS oil is more effective than SDG in reducing MCF-7 tumour growth at high levels of E2 (Thompson et al. 2010) and when fed in combination with TAM treatment (Saggar et al. 2010*a*). These findings can be interpreted a number of ways. First, SDG is likely acting through E2-related mechanisms since it is most effective in conditions where there is less E2 to compete for the ER. Second, FS oil may be acting through modulation of growth factor receptor signalling pathways such as through HER2. Prolonged treatment with TAM has been shown to result in upregulation of HER2 (Dowsett 2001). That FS oil was more effective when fed in combination with TAM treatment suggests that it may be reducing HER2 expression or signalling. These potential mechanisms, the anti-estrogenic effects of SDG in breast cancer and the growth-factor signalling reducing effects of FS oil, and others are further discussed below.

It is thought that SDG may affect breast cancer growth through its modulation of hormone levels, metabolism, and activity. FS lignans have been shown in vitro to inhibit the activity of several enzymes involved in hormone regulation and metabolism, including aromatase, 5α reductase, and 17β-hydroxysteroid dehydrogenase, suggesting that they may lower the levels of serum hormones and their metabolites (Adlercreutz et al. 1993; Brooks and Thompson 2005; Evans et al. 1995; Wang et al. 1994). Several clinical trials have determined the effect of FS consumption (5–25 g/day) on serum hormone levels. The majority of studies found no changes (Brooks et al. 2004; Frische et al. 2003; Sturgeon et al. 2008) with only 1 study showing a reduction in serum E2 and estrone sulfate but no changes in other hormone measures with dietary FS fed for 7 weeks (Hutchins et al. 2001). Clinical studies have also investigated the effect of FS lignans on circulating levels of E2 metabolites, 2-hydroxyestrone (2OHE1) which has little biological activity with some anti-estrogenic activity (Bradlow et al. 1996; Schneider et al. 1982) and 16α-hydroxyestrone (16αOHE1), which is known to have estrogenic agonistic and increased cell proliferation activities (Bradlow et al. 1996; Gupta et al. 1998). Higher 2OHE1:16αOHE1 is suggested to be protective against the development of breast cancer (Osborne et al. 1993; Schneider et al. 1982). Daily FS consumption (5–25 g/day) by both premenopausal (Haggans et al. 2000) and postmenopausal women (Brooks et al. 2004; Haggans et al. 1999; McCann et al. 2007) for 10 days to 3 months resulted in elevated 2OHE1:16αOHE1 in 3 studies while 1 study, where FS consumption (7.5 g/day for 6 weeks followed by 15 g/day for 6 weeks) by postmenopausal women, resulted in a reduction in 2OHE1:16αOHE1 (Sturgeon et al. 2010). In vitro work suggests that EL can bind to ERα and ERβ although with low affinity and preference for ERα (Penttinen et al. 2007). As observed in animal studies, this has led to a number of downstream effects, such as the modulation of E2-sensitive genes, including cyclin D1, pS2, PR, ERα and ERβ (Chen et al. 2009; Saggar et al. 2010*b*). Growth factor signalling was also involved in the effect of lignans as indicated by reduced expression of growth factor receptors, such as epidermal growth factor receptor (EGFR), insulin-like growth factor I receptor (IGF-IR), and HER2 (Chen et al. 2007*a*, 2009; Saggar et al. 2010*a*, 2010*b*; Truan et al. 2012). Processes involved in angiogenesis (Aberg et al. 2011; Bergman Jungestrom et al. 2007; Dabrosin et al. 2002; Lindahl et al. 2011; Saarinen et al. 2010*a*) and inflammation (Abrahamsson et al. 2012; Lindahl et al.

**Fig. 3.** Pathways and processes that may be influenced by flaxseed lignans and ALA-rich oil. Lignans have been shown to influence estrogen synthesis and metabolism (2OHE1:16αOHE1 ratio) and activity through binding to the ER. Altered ER activation alters cell proliferation and apoptosis through direct modulation of E2-sensitive genes and through cross-talk with the growth factor receptor signalling pathways. Lignans have also been shown to modulate angiogenesis. ALA is suggested to exert effects through regulation of transcription factors, alterations in the membrane phospholipid fatty acid profile and the downstream effects on eicosanoid biosynthesis and growth factor receptor expression and activity and lipid peroxidation. 2OHE1, 2-hydroxyestrone; 16OHE1, 16α-hydroxyestrone; ALA, α-linolenic acid; E2, estrogen; EGFR, epidermal growth factor receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IGFIR, insulin-like growth factor I receptor; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa B; PPAR: peroxisome proliferator-activated receptor; P, phosphate; PR, progesterone receptor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.




2011) were also reduced. Together, this suggests that FS lignans may influence breast cancer risk and progression through many mechanisms, including modulation of E2 metabolism and ER and growth factor cell signalling, which has pleiotropic downstream effects.

A number of potential mechanisms have been suggested for the anticancer effects of FS oil and ALA. ALA can be metabolized to a limited extent to the long-chain *n*-3 PUFA EPA and DHA. Therefore, the antitumour effects of FS oil may be related directly to ALA or indirectly to its metabolites EPA and DHA (Anderson and Ma 2009). A number of reviews have focused on the potential mechanisms of *n*-3 PUFA in breast cancer and we refer the reader to them for in-depth review (Calder 2012; Larsson et al. 2004; Wiggins et al. 2013). Proposed mechanisms of effect relate to (1) regulation of transcription factors; (2) alterations in the membrane phospholipid fatty acid profile and the downstream effects on growth factor receptor expression and activity and on eicosanoid biosynthesis; and (3) lipid peroxidation.

Altered activity of transcription factors including peroxisome proliferator-activated receptors (PPARs) and nuclear factor-kappa B (NF-κB) has been shown following treatment with fatty acids including ALA (Jump 2004). PPARs regulate the expression of genes implicated in cancer processes, such as cellular differentiation, proliferation, and inflammation, and as such have been studied as targets for ALA (Berger and Moller 2002; Larsson et al. 2004). Expression of NF-κB, a regulator of cell proliferation, apoptosis, inflammation, and angiogenesis, has been shown to be reduced with ALA treatment with a concomitant reduction in tumour necrosis factor alpha and cyclooxygenase 2 expressions (Hassan et al. 2010). Few studies have evaluated the role of ALA and FS oil on

regulation of transcription factors in breast cancer and research is warranted in this area.

Alterations in the phospholipid fatty acid profile influence cellular properties and functions, including growth factor signalling pathways and eicosanoid biosynthesis (Wang et al. 1994). Dietary FS and FS oil have been shown to increase the level of ALA, EPA, and DHA, and reduce *n*-6 PUFA content of serum and human breast tumours in the athymic mouse model (Mason et al. 2013; Truan et al. 2010). Changes in the membrane phospholipid fatty acid profile, specifically within the lipid raft microdomain, have been shown to alter the expression, localization, and activity of membrane bound receptors such as EGFR (Schley et al. 2007). Others have shown using in vitro and in vivo models of breast cancer that ALA and ALA-rich FS and FS oil have decreased the total expression of membrane-bound growth factor receptors, including HER2, IGF-IR, and EGFR (Chen et al. 2002; Menendez et al. 2006; Saggar et al. 2010*a*, 2010*b*; Truan et al. 2010). These changes are suggested to affect downstream signalling pathways as indicated by changes in total or phosphorylated Akt and MAPK (Saggar et al. 2010*b*; Truan et al. 2010). Additionally, membrane fatty acid profile changes are suggested to alter the synthesis of eicosanoids, which are synthesized from PUFA cleaved from membrane phospholipids. *n*-3-derived eicosanoids have anti-inflammatory or less potent inflammatory actions compared with *n*-6-derived eicosanoids (Wang and Dubois 2010). Few studies have directly linked dietary FS and FS oil to eicosanoid production; however, an early study demonstrated that *n*-6-derived prostaglandin-E2 was suppressed in rats fed a diet rich in FS oil (Marshall and Johnston 1982). This provides support for the idea that the enrichment of tumours with *n*-3 PUFA seen with FS suppresses *n*-6-derived and increases *n*-3-derived eicosanoid synthesis.

ALA is unsaturated and therefore is susceptible to oxidation. Oxidation of ALA produces free radicals and reactive oxygen species (Larsson et al. 2004). It has been suggested that the anticancer effect of ALA and *n*-3 PUFA is related to lipid peroxidation (Cognault et al. 2000; Gonzalez et al. 1991). The tumour-reducing effect of FS oil is lower in the presence of antioxidants and greater in the presence of pro-oxidants (Cognault et al. 2000), which further supports this idea.

Evidently, there are a number of potential mechanisms that may contribute to the anticancer effects of FS and its components. Further research is needed to more completely understand these mechanisms to support the use of FS as an anticancer agent.

## Safety and regulatory status

In addition to the many beneficial components of FS, it also contains some antinutritional factors (mentioned in the Flaxseed and breast cancer section), which may affect the safe use of FS for breast cancer prevention and treatment. However, very high levels of FS would need to be consumed in order for these factors to be of concern. For example, FS contains cyanogenic glycosides, which can produce toxic hydrogen cyanide. However, FS has been estimated to contain cyanide equivalents between 190 to 1000 mg HCN/kg and it has been suggested that adults can detoxify between 30 and 100 mg of cyanide per day (Daun et al. 2003). Furthermore, heat treatment can destroy cyanogens; thus, the amount present in baked or toasted products may be negligible. FS also contains approximately 0.8%–1.5% (*w*/*w*) phytic acid, which is known to affect the bioavailability of minerals such as zinc. The levels in FS are comparable to those found in peanuts and soybean (Daun et al. 2003) and consumption of a 40% FS diet did not affect zinc status in rats (Ratnayake et al. 1992). The presence of Cd is also a concern and the majority of published data on FS Cd content have levels (0.02–1.70 mg/kg FS) higher than the limit set by the Codex Alimentarius Commission (0.1 mg/kg dry weight of cereals) (Kymalainen and Sjoberg 2006). National studies show that estimated intakes of Cd are 40%–60% of the provisional tolerable weekly intake, which is 7 μg/kg of body weight (Joint FAO/WHO Expert Committee on Food Additives 2003). Kymäläinen and Sjöberg (2006) analyzed the Cd content of 85 Finnish FS samples (mean = 0.61 mg/kg of FS) and, using estimated daily intakes measured in various European countries and towns, estimated that greater than 30 g of FS could be safely consumed with the exception of those with low body weight (<50 kg). Canadian FS samples have Cd in the range of 0.2–0.6 mg/kg (Daun et al. 2003), which are values lower than those found in the Finnish study. Adverse reactions to FS are rare and the reported gastrointestinal discomfort is likely related to the high dietary fiber when a large amount of FS is consumed (Demark-Wahnefried et al. 2001; Thompson et al. 2005). The FS interventions in breast cancer studies have used 25 g/day, which is evidently a safe level in terms of antinutritional factors.

Flax 2015 and the Flax Council of Canada made a request to the United States Food and Drug Administration (FDA) to consider whole and milled FS for Generally Recognized as Safe status and the FDA provided a no objections letter (Cheeseman 2009). Furthermore, Health Canada's Food Directorate has recently recommended the approval of a health claim for the cholesterol-lowering effect associated with the consumption of 30 g of FS/day (Flax Council of Canada, Personal Communications). These further attest to the safe intake of reasonable levels of FS. Currently in North America, there is no specific regulation in place regarding the level of FS that can be added to foods.

## General discussion and conclusions

Animal models have provided a great deal of insight into the role of FS, SDG, and FS oil exposure during early life and adulthood. There is heterogeneity in the design of animal studies, including the cancer stage, model (carcinogen-induced, xenograft with various cell lines), dose used (2.5%–20% FS or equivalent levels of FS oil or SDG), cultivar of FS, study duration, and outcomes that have been evaluated. Despite this heterogeneity, the majority of studies suggest that FS and its components at the above levels may prevent and reduce the growth of breast tumours. In fact, of the 18 studies describing the effect of FS on various stages of breast tumourigenesis, 13 showed beneficial effects on some measure of tumour growth (Birkved et al. 2011; Bergman Jungestrom et al. 2007; Chen et al. 2003; Chen et al. 2004, 2007*b*, 2009; Rickard et al. 1999; Serraino and Thompson 1991, 1992; Tan et al. 2004;Thompson et al. 1996*a*; Tou and Thompson 1999; Ward et al. 2000), 3 showed no effect (Chen et al. 2007*a*; Mason et al. 2013; Power et al. 2008), and only 2 showed negative effects (Khan et al. 2007; Yu et al. 2006). Similarly 11 of the 12 studies looking at the role of SDG showed beneficial effects (Bergman Jungestrom et al. 2007; Chen et al. 2003, 2009; Rickard et al. 1999; Saarinen et al. 2002; Saggar et al. 2010*b*; Tan et al. 2004; Thompson et al. 1996*a*, 1996*b*; Tou and Thompson 1999; Ward et al. 2000) while 1 showed no effect (Truan et al. 2012). Seven studies showed beneficial effects of FS oil (Cameron et al. 1989; Chen et al. 2011; Fritsche and Johnston 1990; Rao et al. 2000; Saggar et al. 2010*b*; Thompson et al. 1996*a*; Truan et al. 2010) and 3 showed no effect (Mason et al. 2010*a*, 2010*b*; Tou and Thompson 1999). Of note, the beneficial effect of FS, SDG, and FS oil appear to be more specific to ER+ tumours with low HER2 expression (MCF-7) as the 2 studies conducted in HER2-overexpressing breast cancer (BT-474) have all shown no effect of FS or FS oil alone. Studies have consistently shown that FS, SDG, and FS oil all enhance the effectiveness of TAM in ER+ tumours (Chen et al. 2004, 2007*a*, 2007*b*, 2011; Saggar et al. 2010*a*). In contrast, only FS oil and not FS has been shown to enhance the effectiveness of TRAS in the treatment of HER2-overexpressing breast cancer (Mason et al. 2010*a*, 2013). Ten percent FS, or equivalent levels of FS oil (4%) and SDG (0.1%), was the dose shown to be most efficacious and was used most consistently in animal models. These intakes in the animal diet have been

estimated to be equivalent to intake of 25–50 g of FS, 10–20 g of FS oil, and 25–50 mg of SDG by humans depending on how much other food they eat.

The few clinical studies on FS and SDG conducted to date agreed with the results seen in animal models; however, more studies are needed. Daily consumption of 25 g of FS (containing 50 mg SDG) for 32 days by postmenopausal breast cancer patients and 50 mg of SDG for 12 months by premenopausal women at high risk of breast cancer have both been shown to have beneficial effect on biomarkers of tumour cell growth (Fabian et al. 2010; Thompson et al. 2005). Both of these studies were relatively small and the study by Fabian and colleagues did not include a placebo comparison group. Despite these limitations, they provide promising support for the potential of the many observations seen in animal studies to be translated to humans. To our knowledge, no clinical studies have evaluated the role of FS oil in women at high risk of breast cancer or in breast cancer patients; this merits future work.

Observational studies are also generally supportive of the role of FS and lignans in breast cancer. One study has shown a significant association between FS and flax bread intake and reduced risk of breast cancer risk (Lowcock et al. 2013). However, the levels of intake associated with reduced risk of breast cancer in this study (e.g., monthly intake of a minimum of 1/4 cup of FS, approximately 32.5 g) were much lower than those used in animal (estimated human equivalent at 25–50 g/day) and clinical studies (25 g/day). Despite the limitations of observational studies of the association between lignans and breast cancer risk discussed in the Lignans and the risk reduction and treatment of breast cancer – Observational studies section, the overall findings are suggestive of a protective effect of FS and lignan intake, particularly for postmenopausal women. The consistency in the observational studies associating lignan intake to longer survival in breast cancer patients is very promising. In contrast, the observational studies on the association between ALA intake and breast cancer risk are much more variable and inconclusive as 1 meta-analyses showed no associations (Zheng et al. 2013) while another showed an inverse association in case-control studies and a positive association in cohort studies of postmenopausal women (Saadatian-Elahi et al. 2004).

In conclusion, current overall evidence from animal, observational, and clinical studies suggest that FS with its lignan and oil components is safe for consumption by healthy individuals to reduce the risk of breast cancer and by breast cancer patients to potentially reduce tumour growth, prevent recurrence, and improve outcomes. A daily dose of 25 g of FS and 50 mg of SDG appears effective in clinical trials. However, more and larger clinical studies need to be conducted to further substantiate these results and particularly the observed beneficial interaction of FS, lignans, or its oil with breast cancer drugs, such as tamoxifen and trastuzumab, and the effect of FS oil, where to our knowledge none has been conducted on breast cancer patients. Although many mechanisms of action, including ability to reduce cell proliferation and angiogenesis and increase apoptosis in part through reduced ER and HER2 cell signalling pathways have been suggested from animal, in vitro, and limited clinical studies, other molecular mechanisms of action, including those related to regulation of transcription factors, lipid peroxidation, and inflammation, should further be investigated in future clinical trials.

## References


Aberg, U.W., Saarinen, N., Abrahamsson, A., Nurmi, T., Engblom, S., and Dabrosin, C. 2011. Tamoxifen and flaxseed alter angiogenesis regulators in normal human breast tissue in vivo. PLoS One, **6**(9): e25720. doi:10.1371/journal.pone.0025720. PMID:21984941.

Abrahamsson, A., Morad, V., Saarinen, N.M., and Dabrosin, C. 2012. Estradiol, tamoxifen, and flaxseed alter IL-1beta and IL-1Ra levels in normal human breast tissue in vivo. J. Clin. Endocrinol. Metab. **97**(11): E2044–E2054. doi:10.1210/jc.2012-2288. PMID:22930784.

Adlercreutz, H. 2007. Lignans and human health. Crit. Rev. Clin. Lab. Sci. **44**(5–6): 483–525. doi:10.1080/10408360701612942. PMID:17943494.

Adlercreutz, H., Bannwart, C., Wahala, K., Makela, T., Brunow, G., Hase, T., et al. 1993. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. J. Steroid Biochem. Mol. Biol. **44**(2): 147–153. doi:10.1016/0960-0760(93)90022-O. PMID:8382517.

American Cancer Society. 2013. Cancer Facts & Figures 2013. Atlanta, Ga., USA.

Anderson, B.M., and Ma, D.W. 2009. Are all n-3 polyunsaturated fatty acids created equal? Lipids Health Dis. **8**: 33. doi:10.1186/1476-511X-8-33. PMID:19664246.

Berger, J., and Moller, D.E. 2002. The mechanisms of action of PPARs. Annu. Rev. Med. **53**: 409–435. doi:10.1146/annurev.med.53.082901.104018. PMID:11818483.

Bergman Jungestrom, M., Thompson, L.U., and Dabrosin, C. 2007. Flaxseed and its lignans inhibit estradiol-induced growth, angiogenesis, and secretion of vascular endothelial growth factor in human breast cancer xenografts in vivo. Clin. Cancer Res. **13**(3): 1061–1067. doi:10.1158/1078-0432.CCR-06-1651. PMID:17289903.

Birkved, F.K., Mortensen, A., Penalvo, J.L., Lindecrona, R.H., and Sorensen, I.K. 2011. Investigation into the cancer protective effect of flaxseed in Tg.NK (MMTV/c-neu) mice, a murine mammary tumor model. Genes Nutr. **6**(4): 403–411. doi:10.1007/s12263-011-0214-1. PMID:21484161.

Boon, H.S., Olatunde, F., and Zick, S.M. 2007. Trends in complementary/alternative medicine use by breast cancer survivors: comparing survey data from 1998 and 2005. BMC Womens Health, **7**: 4. doi:10.1186/1472-6874-7-4. PMID:17397542.

Boucher, B.A., Cotterchio, M., Curca, I.A., Kreiger, N., Harris, S.A., Kirsh, V.A., et al. 2012. Intake of phytoestrogen foods and supplements among women recently diagnosed with breast cancer in Ontario, Canada. Nutr. Cancer, **64**(5): 695–703. doi:10.1080/01635581.2012.687426. PMID:22642930.

Bougnoux, P., and Chajes, V. 2003. Alpha-linolenic acid and cancer. *In* Flaxseed in human nutrition. *Edited by* L.U. Thompson and S.C. Cunnane. AOCS Press, Champaign, Ill., USA. pp. 233–244.

Bougnoux, P., Koscielny, S., Chajes, V., Descamps, P., Couet, C., and Calais, G. 1994. alpha-Linolenic acid content of adipose breast tissue: a host determinant of the risk of early metastasis in breast cancer. Br. J. Cancer, **70**(2): 330–334. doi:10.1038/bjc.1994.302. PMID:7914425.

Bradlow, H.L., Telang, N.T., Sepkovic, D.W., and Osborne, M.P. 1996. 2-hydroxyestrone: the 'good' estrogen. J. Endocrinol. **150**(Suppl.): S259–S265. doi:10.2147/IJWH.S7595. PMID:8943806.

Brenna, J.T., Salem, N., Jr., Sinclair, A.J., and Cunnane, S.C. 2009. alpha-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. Prostaglandins Leukot. Essent. Fatty Acids, **80**(2–3): 85–91. doi:10.1016/j.plefa.2009.01.004. PMID:19269799.

Brooks, J.D., and Thompson, L.U. 2005. Mammalian lignans and genistein decrease the activities of aromatase and 17beta-hydroxysteroid dehydrogenase in MCF-7 cells. J. Steroid Biochem. Mol. Biol. **94**(5): 461–467. doi:10.1016/j.jsbmb.2005.02.002. PMID:15876411.

Brooks, J.D., Ward, W.E., Lewis, J.E., Hilditch, J., Nickell, L., Wong, E., et al. 2004. Supplementation with flaxseed alters estrogen metabolism in postmenopausal women to a greater extent than does supplementation with an equal amount of soy. Am. J. Clin. Nutr. **79**(2): 318–325. PMID:14749240.

Buck, K., Zaineddin, A.K., Vrieling, A., Linseisen, J., and Chang-Claude, J. 2010. Meta-analyses of lignans and enterolignans in relation to breast cancer risk. Am. J. Clin. Nutr. **92**(1): 141–153. doi:10.3945/ajcn.2009.28573. PMID:20463043.

Buck, K., Vrieling, A., Zaineddin, A.K., Becker, S., Husing, A., Kaaks, R., et al. 2011*a*. Serum enterolactone and prognosis of postmenopausal breast cancer. J. Clin. Oncol. **29**(28): 3730–3738. doi:10.1200/JCO.2011.34.6478. PMID:21900115.

Buck, K., Zaineddin, A.K., Vrieling, A., Heinz, J., Linseisen, J., Flesch-Janys, D., et al. 2011*b*. Estimated enterolignans, lignan-rich foods, and fibre in relation to survival after postmenopausal breast cancer. Br. J. Cancer, **105**(8): 1151–1157. doi:10.1038/bjc.2011.374. PMID:21915130.

Calder, P.C. 2012. Mechanisms of action of (n-3) fatty acids. J. Nutr. **142**(3): 592S–599S. doi:10.3945/jn.111.155259. PMID:22279140.

Cameron, E., Bland, J., and Marcuson, R. 1989. Divergent effects of omega-6 and omega-3 fatty acids on mammary tumor development in C3H/Heston mice treated with DMBA. Nutr. Res. **9**(4): 383–393. doi:10.1016/S0271-5317(89)80115-0.

Canadian Cancer Society's Advisory Committee on Cancer Statistics. 2013. Canadian Cancer Statistics 2013. Toronto, Ont., Canada.

Carlson, R.W., Allred, D.C., Anderson, B.O., Burstein, H.J., Carter, W.B., Edge, S.B., et al. 2011. Invasive breast cancer. J. Natl. Compr. Canc. Netw. **9**(2): 136–222. PMID:21310842.

Cheeseman, M.A. 2009. Agency response, GRAS Notice No. GRN 000280. United States Food and Drug Administration.

Chen, J., Stavro, P.M., and Thompson, L.U. 2002. Dietary flaxseed inhibits human breast cancer growth and metastasis and downregulates expression of insulin-like growth factor and epidermal growth factor receptor. Nutr. Cancer, **43**(2): 187–192. doi:10.1207/S15327914NC432_9. PMID:12588699.

Chen, J., Tan, K.P., Ward, W.E., and Thompson, L.U. 2003. Exposure to flaxseed or its purified lignan during suckling inhibits chemically induced rat mammary tumorigenesis. Exp. Biol. Med. (Maywood), **228**(8): 951–958. PMID:12968067.

Chen, J., Hui, E., Ip, T., and Thompson, L.U. 2004. Dietary flaxseed enhances the inhibitory effect of tamoxifen on the growth of estrogen-dependent human

breast cancer (mcf-7) in nude mice. Clin. Cancer Res. **10**(22): 7703–7711. doi:10.1158/1078-0432.CCR-04-1130. PMID:15570004.

Chen, J., Power, K.A., Mann, J., Cheng, A., and Thompson, L.U. 2007*a*. Dietary flaxseed interaction with tamoxifen induced tumor regression in athymic mice with MCF-7 xenografts by downregulating the expression of estrogen related gene products and signal transduction pathways. Nutr. Cancer, **58**(2): 162–170. doi:10.1080/01635580701328271. PMID:17640162.

Chen, J., Power, K.A., Mann, J., Cheng, A., and Thompson, L.U. 2007*b*. Flaxseed alone or in combination with tamoxifen inhibits MCF-7 breast tumor growth in ovariectomized athymic mice with high circulating levels of estrogen. Exp. Biol. Med. (Maywood), **232**(8): 1071–1080. doi:10.3181/0702-RM-36. PMID:17720953.

Chen, J., Saggar, J.K., Corey, P., and Thompson, L.U. 2009. Flaxseed and pure secoisolariciresinol diglucoside, but not flaxseed hull, reduce human breast tumor growth (MCF-7) in athymic mice. J. Nutr. **139**(11): 2061–2066. doi:10.3945/jn.109.112508. PMID:19776177.

Chen, J., Saggar, J.K., Corey, P., and Thompson, L.U. 2011. Flaxseed cotyledon fraction reduces tumour growth and sensitises tamoxifen treatment of human breast cancer xenograft (MCF-7) in athymic mice. Br. J. Nutr. **105**(3): 339–347. doi:10.1017/S0007114510003557. PMID:21138602.

Cognault, S., Jourdan, M.L., Germain, E., Pitavy, R., Morel, E., Durand, G., et al. 2000. Effect of an alpha-linolenic acid-rich diet on rat mammary tumor growth depends on the dietary oxidative status. Nutr. Cancer, **36**(1): 33–41. PMID:10798214.

Dabrosin, C., Chen, J., Wang, L., and Thompson, L.U. 2002. Flaxseed inhibits metastasis and decreases extracellular vascular endothelial growth factor in human breast cancer xenografts. Cancer Lett. **185**(1): 31–37. doi:10.1016/S0304-3835(02)00239-2. PMID:12142076.

Daun, J.K., Barthet, V.J., Chornick, T.L., and Duguid, S. 2003. Structure, composition, and variety development of flaxseed. *In* Flaxseed in Human Nutrition. *Edited by* L.U. Thompson and S.C. Cunnane. AOCS Press, Champaign, Ill., USA. pp. 1–40.

De Stefani, E., Deneo-Pellegrini, H., Mendilaharsu, M., and Ronco, A. 1998. Essential fatty acids and breast cancer: a case-control study in Uruguay. Int. J. Cancer, **76**(4): 491–494. doi:10.1002/(SICI)1097-0215(19980518)76:4<491::AID-IJC8>3.3.CO;2-I. PMID:9590123.

Demark-Wahnefried, W., Price, D.T., Polascik, T.J., Robertson, C.N., Anderson, E.E., Paulson, D.F., et al. 2001. Pilot study of dietary fat restriction and flaxseed supplementation in men with prostate cancer before surgery: exploring the effects on hormonal levels, prostate-specific antigen, and histopathologic features. Urology, **58**(1): 47–52. doi:10.1016/S0090-4295(01)01014-7. PMID:11445478.

Dowsett, M. 2001. Overexpression of HER-2 as a resistance mechanism to hormonal therapy for breast cancer. Endocr. Relat. Cancer, **8**(3): 191–195. doi:10.1677/erc.0.0080191. PMID:11566610.

Evans, B.A., Griffiths, K., and Morton, M.S. 1995. Inhibition of 5 alpha-reductase in genital skin fibroblasts and prostate tissue by dietary lignans and isoflavonoids. J. Endocrinol. **147**(2): 295–302. doi:10.1677/joe.0.1470295. PMID:7490559.

Fabian, C.J., Kimler, B.F., Zalles, C.M., Klemp, J.R., Petroff, B.K., Khan, Q.J., et al. 2010. Reduction in Ki-67 in benign breast tissue of high-risk women with the lignan secoisolariciresinol diglycoside. Cancer Prev. Res. (Phila.), **3**(10): 1342–1350. doi:10.1158/1940-6207.CAPR-10-0022. PMID:2072470.

Frische, E.J., Hutchins, A.M., Martini, M.C., Thomas, W., and Slavin, J.L. 2003. Effect of flaxseed and wheat bran on serum hormones and lignan excretion in premenopausal women. J. Am. Coll. Nutr. **22**(6): 550–554. doi:10.1080/07315724.2003.10719335. PMID:14684762.

Fritsche, K.L., and Johnston, P.V. 1990. Effect of dietary alpha-linolenic acid on growth, metastasis, fatty acid profile and prostaglandin production of two murine mammary adenocarcinomas. J. Nutr. **120**(12): 1601–1609. PMID:1979807.

Gerber, M. 2012. Omega-3 fatty acids and cancers: a systematic update review of epidemiological studies. Br. J. Nutr. **107**(Suppl .2): S228–S239. doi:10.1017/S0007114512001614. PMID:22591896.

Gonzalez, M.J., Schemmel, R.A., Gray, J.I., Dugan, L., Jr., Sheffield, L.G., and Welsch, C.W. 1991. Effect of dietary fat on growth of MCF-7 and MDA-MB231 human breast carcinomas in athymic nude mice: relationship between carcinoma growth and lipid peroxidation product levels. Carcinogenesis, **12**(7): 1231–1235. doi:10.1093/carcin/12.7.1231. PMID:2070488.

Greenlee, H., Kwan, M.L., Ergas, I.J., Sherman, K.J., Krathwohl, S.E., Bonnell, C., et al. 2009. Complementary and alternative therapy use before and after breast cancer diagnosis: the Pathways Study. Breast Cancer Res. Treat. **117**(3): 653–665. doi:10.1007/s10549-009-0315-3. PMID:19184414.

Guglielmini, P., Rubagotti, A., and Boccardo, F. 2012. Serum enterolactone levels and mortality outcome in women with early breast cancer: a retrospective cohort study. Breast Cancer Res. Treat. **132**(2): 661–668. doi:10.1007/s10549-011-1881-8. PMID:22094938.

Gupta, M., McDougal, A., and Safe, S. 1998. Estrogenic and antiestrogenic activities of 16alpha- and 2-hydroxy metabolites of 17beta-estradiol in MCF-7 and T47D human breast cancer cells. J. Steroid Biochem. Mol. Biol. **67**(5–6): 413–419. doi:10.1016/S0960-0760(98)00135-6. PMID:10030690.

Haggans, C.J., Hutchins, A.M., Olson, B.A., Thomas, W., Martini, M.C., and Slavin, J.L. 1999. Effect of flaxseed consumption on urinary estrogen metabolites in postmenopausal women. Nutr. Cancer, **33**(2): 188–195. doi:10.1207/S15327914NC330211. PMID:10368815.

Haggans, C.J., Travelli, E.J., Thomas, W., Martini, M.C., and Slavin, J.L. 2000. The effect of flaxseed and wheat bran consumption on urinary estrogen metabolites in premenopausal women. Cancer Epidemiol. Biomarkers Prev. **9**(7): 719–725. PMID:10919743.

Hall, C., III, Tulbek, M.C., and Xu, Y. 2006. Flaxseed. Adv. Food Nutr. Res. **51**: 1–97. doi:10.1016/S1043-4526(06)51001-0. PMID:17011474.

Hassan, A., Ibrahim, A., Mbodji, K., Coeffier, M., Ziegler, F., Bounoure, F., et al. 2010. An alpha-linolenic acid-rich formula reduces oxidative stress and inflammation by regulating NF-kappaB in rats with TNBS-induced colitis. J. Nutr. **140**(10): 1714–1721. doi:10.3945/jn.109.119768. PMID:20724486.

Hilakivi-Clarke, L., Cho, E., deAssis, S., Olivo, S., Ealley, E., Bouker, K.B., et al. 2001. Maternal and prepubertal diet, mammary development and breast cancer risk. J. Nutr. **131**(1): 154S-157S. PMID:11208953.

Hutchins, A.M., Martini, M.C., Olson, B.A., Thomas, W., and Slavin, J.L. 2001. Flaxseed consumption influences endogenous hormone concentrations in postmenopausal women. Nutr. Cancer, **39**(1): 58–65. doi:10.1207/S15327914nc391_8. PMID:11588903.

Johnson, M.D., Kenney, N., Stoica, A., Hilakivi-Clarke, L., Singh, B., Chepko, G., et al. 2003. Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. Nat. Med. **9**(8): 1081–1084. doi:10.1038/nm902. PMID:12858169.

Joint FAO/WHO Expert Committee on Food Additives. 2003. Evaluation of certain food additives and contaminants: sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series. Geneva: 127–132.

Jump, D.B. 2004. Fatty acid regulation of gene transcription. Crit. Rev. Clin. Lab. Sci. **41**(1): 41–78. doi:10.1080/10408360490278341. PMID:15077723.

Khan, G., Penttinen, P., Cabanes, A., Foxworth, A., Chezek, A., Mastropole, K., et al. 2007. Maternal flaxseed diet during pregnancy or lactation increases female rat offspring's susceptibility to carcinogen-induced mammary tumorigenesis. Reprod. Toxicol. **23**(3): 397–406. doi:10.1016/j.reprotox.2007.02.002. PMID:17398067.

Kolodziejczk, P.P., and Fedec, P. 1993. Processing flaxseed for human consumption. *In* Flaxseed in Human Nutrition. *Edited by* S.C. Cunnane and L.U. Thompson. AOCS Press, Champaign, Ill., USA. pp. 261–280.

Kuriki, K., Hirose, K., Wakai, K., Matsuo, K., Ito, H., Suzuki, T., et al. 2007. Breast cancer risk and erythrocyte compositions of n-3 highly unsaturated fatty acids in Japanese. Int. J. Cancer, **121**(2): 377–385. doi:10.1002/ijc.22682. PMID:17354239.

Kymäläinen, H.R., and Sjöberg, A.M. 2006. Cadmium content of Finnish linseed and estimated consumer intake. Agric. Food Sci. **15**: 3–11. doi:10.2137/145960606777245533.

Larsson, S.C., Kumlin, M., Ingelman-Sundberg, M., and Wolk, A. 2004. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am. J. Clin. Nutr. **79**(6): 935–945. PMID:15159222.

Lindahl, G., Saarinen, N., Abrahamsson, A., and Dabrosin, C. 2011. Tamoxifen, flaxseed, and the lignan enterolactone increase stroma- and cancer cell-derived IL-1Ra and decrease tumor angiogenesis in estrogen-dependent breast cancer. Cancer Res. **71**(1): 51–60. doi:10.1158/0008-5472.CAN-10-2289. PMID:21097717.

Lowcock, E.C., Cotterchio, M., and Boucher, B.A. 2013. Consumption of flaxseed, a rich source of lignans, is associated with reduced breast cancer risk. Cancer Causes Control, **24**(4): 813–816. doi:10.1007/s10552-013-0155-7. PMID:23354422.

Luijten, M., Singh, A.V., Bastian, C.A., Westerman, A., Pisano, M.M., Pennings, J.L., et al. 2013. Lasting effects on body weight and mammary gland gene expression in female mice upon early life exposure to n-3 but not n-6 high-fat diets. PLoS One, **8**(2): e55603. doi:10.1371/journal.pone.0055603. PMID:23409006.

Marshall, L.A., and Johnston, P.V. 1982. Modulation of tissue prostaglandin synthesizing capacity by increased ratios of dietary alpha-linolenic acid to linoleic acid. Lipids, **17**(12): 905–913. doi:10.1007/BF02534586. PMID:6298554.

Mason, J.K., Chen, J., and Thompson, L.U. 2010*a*. Flaxseed oil-trastuzumab interaction in breast cancer. Food Chem. Toxicol. **48**(8–9): 2223–2226. doi:10.1016/j.fct.2010.05.052. PMID:20580760.

Mason, J.K., Chen, J., and Thompson, L.U. 2010*b*. Flaxseed oil can increase the effectiveness of the drug trastuzumab (herceptin) in breast cancer overexpressing HER2 (BT-474). *In* Proceedings of the 63rd Flax Institute. *Edited by* J.J. Hammond. Flax Institute of the United States, Fargo, N.D., USA. pp. 10–15.

Mason, J.K., Fu, M.H., Chen, J., Yu, Z., and Thompson, L.U. 2013. Dietary flaxseed-trastuzumab interactive effects on the growth of HER2-overexpressing human breast tumors (BT-474). Nutr. Cancer, **65**(3): 451–459. doi:10.1080/01635581.2013.756921. PMID:23530645.

McCann, S.E., Wactawski-Wende, J., Kufel, K., Olson, J., Ovando, B., Kadlubar, S.N., et al. 2007. Changes in 2-hydroxyestrone and 16alpha-hydroxyestrone metabolism with flaxseed consumption: modification by COMT and CYP1B1 genotype. Cancer Epidemiol. Biomarkers Prev. **16**(2): 256–262. doi:10.1158/1055-9965.EPI-06-0633. PMID:17301257.

McCann, S.E., Thompson, L.U., Nie, J., Dorn, J., Trevisan, M., Shields, P.G., et al. 2010. Dietary lignan intakes in relation to survival among women with breast cancer: The Western New York Exposures and Breast Cancer (WEB)

Study. Breast Cancer Res. Treat. **122**(1): 229–235. doi:10.1007/s10549-009-0681-x. PMID:20033482.

Menendez, J.A., Vazquez-Martin, A., Ropero, S., Colomer, R., and Lupu, R. 2006. HER2 (erbB-2)-targeted effects of the omega-3 polyunsaturated fatty acid, alpha-linolenic acid (ALA; 18:3n-3), in breast cancer cells: the "fat features" of the "Mediterranean diet" as an "anti-HER2 cocktail". Clin. Transl. Oncol. **8**(11): 812–820. doi:10.1007/s12094-006-0137-2. PMID:17134970.

Nkondjock, A., Shatenstein, B., and Ghadirian, P. 2003. A case-control study of breast cancer and dietary intake of individual fatty acids and antioxidants in Montreal, Canada. Breast, **12**(2): 128–135. doi:10.1016/S0960-9776(02)00284-9. PMID:14659342.

Olsen, A., Christensen, J., Knudsen, K.E., Johnsen, N.F., Overvad, K., and Tjonneland, A. 2011. Prediagnostic plasma enterolactone levels and mortality among women with breast cancer. Breast Cancer Res. Treat. **128**(3): 883–889. doi:10.1007/s10549-011-1397-2. PMID:21340481.

Osborne, M.P., Bradlow, H.L., Wong, G.Y., and Telang, N.T. 1993. Upregulation of estradiol C16 alpha-hydroxylation in human breast tissue: a potential biomarker of breast cancer risk. J. Natl. Cancer Inst. **85**(23): 1917–1920. doi:10.1093/jnci/85.23.1917. PMID:8230281.

Patterson, R.E. 2011. Flaxseed and breast cancer: what should we tell our patients? J. Clin. Oncol. **29**(28): 3723–3724. doi:10.1200/JCO.2011.37.9669. PMID:21900107.

Penttinen, P., Jaehrling, J., Damdimopoulos, A.E., Inzunza, J., Lemmen, J.G., van der Saag, P., et al. 2007. Diet-derived polyphenol metabolite enterolactone is a tissue-specific estrogen receptor activator. Endocrinology, **148**(10): 4875–4886. doi:10.1210/en.2007-0289. PMID:17628008.

Pitot, H.C. 1993. The molecular biology of carcinogenesis. Cancer, **72**(3 Suppl.): 962–970. doi:10.1002/1097-0142(19930801)72:3+<962::AID-CNCR2820721303>3.0.CO;2-H. PMID:8334671.

Power, K.A., Chen, J.M., Saarinen, N.M., and Thompson, L.U. 2008. Changes in biomarkers of estrogen receptor and growth factor signaling pathways in MCF-7 tumors after short- and long-term treatment with soy and flaxseed. J. Steroid Biochem. Mol. Biol. **112**(1–3): 13–19. doi:10.1016/j.jsbmb.2008.07.003. PMID:18703142.

Rao, G.N., Ney, E., and Herbert, R.A. 2000. Effect of melatonin and linolenic acid on mammary cancer in transgenic mice with c-neu breast cancer oncogene. Breast Cancer Res. Treat. **64**(3): 287–296. doi:10.1023/A:1026552405042. PMID:11200779.

Ratnayake, W.M.N., Behrens, W.A., Fischer, P.W.F., L'Abbé, M.R., Mongeau, R., and Beare-Rogers, J.L. 1992. Chemical and nutritional studies of flaxseed (variety Linott) in rats. J. Nutr. Biochem. **3**(5): 232–240. doi:10.1016/0955-2863(92)90045-K.

Rausch, S.M., Winegardner, F., Kruk, K.M., Phatak, V., Wahner-Roedler, D.L., Bauer, B., et al. 2011. Complementary and alternative medicine: use and disclosure in radiation oncology community practice. Support Care Cancer, **19**(4): 521–529. doi:10.1007/s00520-010-0846-5. PMID:20336329.

Rickard, S.E., Yuan, Y.V., Chen, J., and Thompson, L.U. 1999. Dose effects of flaxseed and its lignan on N-methyl-N-nitrosourea-induced mammary tumorigenesis in rats. Nutr. Cancer, **35**(1): 50–57. doi:10.1207/S1532791450-57. PMID:10624706.

Russo, J., and Russo, I.H. 2004. Development of the human breast. Maturitas, **49**(1): 2–15. doi:10.1016/j.maturitas.2004.04.011. PMID:15351091.

Russo, J., Tay, L.K., and Russo, I.H. 1982. Differentiation of the mammary gland and susceptibility to carcinogenesis. Breast Cancer Res. Treat. **2**(1): 5–73. doi:10.1007/BF01805718. PMID:6216933.

Russo, J., Hu, Y.F., Yang, X., and Russo, I.H. 2000. Developmental, cellular, and molecular basis of human breast cancer. J. Natl. Cancer Inst. Monogr. **2000**(27): 17–37. doi:10.1093/oxfordjournals.jncimonographs.a024241. PMID:10963618.

Saadatian-Elahi, M., Norat, T., Goudable, J., and Riboli, E. 2004. Biomarkers of dietary fatty acid intake and the risk of breast cancer: a meta-analysis. Int. J. Cancer, **111**(4): 584–591. doi:10.1002/ijc.20284. PMID:15239137.

Saarinen, N.M., Huovinen, R., Warri, A., Makela, S.I., Valentin-Blasini, L., Sjoholm, R., et al. 2002. Enterolactone inhibits the growth of 7,12-dimethylbenz(a)anthracene-induced mammary carcinomas in the rat. Mol. Cancer Ther. **1**(10): 869–876. PMID:12492120.

Saarinen, N.M., Power, K., Chen, J., and Thompson, L.U. 2006. Flaxseed attenuates the tumor growth stimulating effect of soy protein in ovariectomized athymic mice with MCF-7 human breast cancer xenografts. Int. J. Cancer, **119**(4): 925–931. doi:10.1002/ijc.21898. PMID:16557589.

Saarinen, N.M., Power, K.A., Chen, J., and Thompson, L.U. 2008. Lignans are accessible to human breast cancer xenografts in athymic mice. Nutr. Cancer, **60**(2): 245–250. doi:10.1080/01635580701649669. PMID:18444157.

Saarinen, N.M., Abrahamsson, A., and Dabrosin, C. 2010*a*. Estrogen-induced angiogenic factors derived from stromal and cancer cells are differently regulated by enterolactone and genistein in human breast cancer in vivo. Int. J. Cancer, **127**(3): 737–745. doi:10.1002/ijc.25052. PMID:19924815.

Saarinen, N.M., Smeds, A.I., Penalvo, J.L., Nurmi, T., Adlercreutz, H., and Makela, S. 2010*b*. Flaxseed ingestion alters ratio of enterolactone enantiomers in human serum. J. Nutr. Metab. **2010**. doi:10.1155/2010/403076. PMID:20721350.

Saggar, J.K., Chen, J., Corey, P., and Thompson, L.U. 2010*a*. Dietary flaxseed lignan or oil combined with tamoxifen treatment affects MCF-7 tumor growth through estrogen receptor- and growth factor-signaling pathways. Mol. Nutr. Food Res. **54**(3): 415–425. doi:10.1002/mnfr.200900068. PMID:19904759.

Saggar, J.K., Chen, J., Corey, P., and Thompson, L.U. 2010*b*. The effect of secoisolariciresinol diglucoside and flaxseed oil, alone and in combination, on MCF-7 tumor growth and signaling pathways. Nutr. Cancer, **62**(4): 533–542. doi:10.1080/01635580903532440. PMID:20432175.

Sala-Vila, A., and Calder, P.C. 2011. Update on the relationship of fish intake with prostate, breast, and colorectal cancers. Crit. Rev. Food Sci. Nutr. **51**(9): 855–871. doi:10.1080/10408398.2010.483527. PMID:21888535.

Schley, P.D., Brindley, D.N., and Field, C.J. 2007. (n-3) PUFA alter raft lipid composition and decrease epidermal growth factor receptor levels in lipid rafts of human breast cancer cells. J. Nutr. **137**(3): 548–553. PMID:17311938.

Schneider, J., Kinne, D., Fracchia, A., Pierce, V., Anderson, K.E., Bradlow, H.L., et al. 1982. Abnormal oxidative metabolism of estradiol in women with breast cancer. Proc. Natl. Acad. Sci. U.S.A. **79**(9): 3047–3051. doi:10.1073/pnas.79.9.3047. PMID:6953448.

Serraino, M., and Thompson, L.U. 1991. The effect of flaxseed supplementation on early risk markers for mammary carcinogenesis. Cancer Lett. **60**(2): 135–142. doi:10.1016/0304-3835(91)90220-C. PMID:1657368.

Serraino, M., and Thompson, L.U. 1992. The effect of flaxseed supplementation on the initiation and promotional stages of mammary tumorigenesis. Nutr. Cancer, **17**(2): 153–159. doi:10.1080/01635589209514182. PMID:1584708.

Shannon, J., King, I.B., Moshofsky, R., Lampe, J.W., Gao, D.L., Ray, R.M., et al. 2007. Erythrocyte fatty acids and breast cancer risk: a case-control study in Shanghai, China. Am. J. Clin. Nutr. **85**(4): 1090–1097. PMID:17413110.

Sorlie, T., Tibshirani, R., Parker, J., Hastie, T., Marron, J.S., Nobel, A., et al. 2003. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc. Natl. Acad. Sci. U.S.A. **100**(14): 8418–8423. doi:10.1073/pnas.0932692100. PMID:12829800.

Sturgeon, S.R., Heersink, J.L., Volpe, S.L., Bertone-Johnson, E.R., Puleo, E., Stanczyk, F.Z., et al. 2008. Effect of dietary flaxseed on serum levels of estrogens and androgens in postmenopausal women. Nutr. Cancer, **60**(5): 612–618. doi:10.1080/01635580801971864. PMID:18791924.

Sturgeon, S.R., Volpe, S.L., Puleo, E., Bertone-Johnson, E.R., Heersink, J., Sabelawski, S., et al. 2010. Effect of flaxseed consumption on urinary levels of estrogen metabolites in postmenopausal women. Nutr. Cancer, **62**(2): 175–180. doi:10.1080/01635580903305342. PMID:20099191.

Tan, K.P., Chen, J., Ward, W.E., and Thompson, L.U. 2004. Mammary gland morphogenesis is enhanced by exposure to flaxseed or its major lignan during suckling in rats. Exp. Biol. Med. (Maywood), **229**(2): 147–157. PMID:14734793.

Theriault, R.L., Carlson, R.W., Allred, C., Anderson, B.O., Burstein, H.J., Edge, S.B., et al. 2013. Breast Cancer, Version 3.2013. J. Natl. Compr. Canc. Netw. **11**(7): 753–761. PMID:23847214.

Thiebaut, A.C., Chajes, V., Gerber, M., Boutron-Ruault, M.C., Joulin, V., Lenoir, G., et al. 2009. Dietary intakes of omega-6 and omega-3 polyunsaturated fatty acids and the risk of breast cancer. Int. J. Cancer, **124**(4): 924–931. doi:10.1002/ijc.23980. PMID:19035453.

Thompson, L.U. 2003. Analysis and bioavailability of lignans. *In* Flaxseed in Human Nutrition. *Edited by* L.U. Thompson and S.C. Cunnane. AOCS Press, Champaign, Ill., USA. pp. 92–116.

Thompson, L.U., Rickard, S.E., Orcheson, L.J., and Seidl, M.M. 1996*a*. Flaxseed and its lignan and oil components reduce mammary tumor growth at a late stage of carcinogenesis. Carcinogenesis, **17**(6): 1373–1376. doi:10.1093/carcin/17.6.1373. PMID:8681458.

Thompson, L.U., Seidl, M.M., Rickard, S.E., Orcheson, L.J., and Fong, H.H. 1996*b*. Antitumorigenic effect of a mammalian lignan precursor from flaxseed. Nutr. Cancer, **26**(2): 159–165. doi:10.1080/01635589609514472. PMID:8875553.

Thompson, L.U., Chen, J.M., Li, T., Strasser-Weippl, K., and Goss, P.E. 2005. Dietary flaxseed alters tumor biological markers in postmenopausal breast cancer. Clin. Cancer Res. **11**(10): 3828–3835. doi:10.1158/1078-0432.CCR-04-2326. PMID:15897583.

Thompson, L.U., Boucher, B.A., Liu, Z., Cotterchio, M., and Kreiger, N. 2006. Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. Nutr. Cancer, **54**(2): 184–201. doi:10.1207/s15327914nc5402_5. PMID:16898863.

Thompson, L.U., Truan, J., and Chen, J. 2010. Flaxseed oil is more effective than lignan in reducing the growth of estrogen sensitive human breast cancer (MCF-7) at high circulating estrogen level. *In* Proceedings of the 63rd Meeting of the Flax Institute of the United States, Doublewood Inn, Fargo, North Dakota, 25–26 March 2010. Flax Institute of the United States.

Tou, J.C., and Thompson, L.U. 1999. Exposure to flaxseed or its lignan component during different developmental stages influences rat mammary gland structures. Carcinogenesis, **20**(9): 1831–1835. doi:10.1093/carcin/20.9.1831. PMID:10469631.

Truan, J.S., Chen, J.M., and Thompson, L.U. 2010. Flaxseed oil reduces the growth of human breast tumors (MCF-7) at high levels of circulating estrogen. Mol. Nutr. Food Res. **54**(10): 1414–1421. doi:10.1002/mnfr.200900521. PMID:20425756.

Truan, J.S., Chen, J.M., and Thompson, L.U. 2012. Comparative effects of sesame seed lignan and flaxseed lignan in reducing the growth of human breast

tumors (MCF-7) at high levels of circulating estrogen in athymic mice. Nutr. Cancer, **64**(1): 65–71. doi:10.1080/01635581.2012.630165. PMID:22136581.

Velentzis, L.S., Cantwell, M.M., Cardwell, C., Keshtgar, M.R., Leathem, A.J., and Woodside, J.V. 2009. Lignans and breast cancer risk in pre- and postmenopausal women: meta-analyses of observational studies. Br. J. Cancer, **100**(9): 1492–1498. doi:10.1038/sj.bjc.6605003. PMID:19337250.

Wang, D., and Dubois, R.N. 2010. Eicosanoids and cancer. Nat. Rev. Cancer, **10**(3): 181–193. doi:10.1038/nrc2809. PMID:20168319.

Wang, C., Makela, T., Hase, T., Adlercreutz, H., and Kurzer, M.S. 1994. Lignans and flavonoids inhibit aromatase enzyme in human preadipocytes. J. Steroid Biochem. Mol. Biol. **50**(3–4): 205–212. doi:10.1016/0960-0760(94)90030-2. PMID:8049151.

Ward, W.E., Jiang, F.O., and Thompson, L.U. 2000. Exposure to flaxseed or purified lignan during lactation influences rat mammary gland structures. Nutr. Cancer, **37**(2): 187–192. doi:10.1207/S15327914NC372_11. PMID:11142092.

Welsh, J. 2007. Steroid hormones and their receptors in breast cancer. *In* The World of Hormones: Molecular and Physiological Insights. *Edited by* H. Henry and A. Norman. Henry Stewart Talks Ltd., London, UK.

Wiggins, A.K., Mason, J.K., and Thompson, L.U. 2013. Beneficial influence of diets enriched with flaxseed and flaxseed oil on cancer. *In* Cancer Chemoprevention and Treatment by Diet Therapy. *Edited by* W.C.S. Cho. Springer Science + Business Media Dordrecht, Dordrecht, the Netherlands. pp. 55–90.

World Cancer Research Fund/American Institute for Cancer Research 2007. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington DC, USA.

Yang, X.R., Sherman, M.E., Rimm, D.L., Lissowska, J., Brinton, L.A., Peplonska, B., et al. 2007. Differences in risk factors for breast cancer molecular subtypes in a population-based study. Cancer Epidemiol. Biomarkers Prev. **16**(3): 439–443. doi:10.1158/1055-9965.EPI-06-0806. PMID:17372238.

Yu, B., Khan, G., Foxworth, A., Huang, K., and Hilakivi-Clarke, L. 2006. Maternal dietary exposure to fiber during pregnancy and mammary tumorigenesis among rat offspring. Int. J. Cancer, **119**(10): 2279–2286. doi:10.1002/ijc.22201. PMID:16921499.

Zaineddin, A.K., Vrieling, A., Buck, K., Becker, S., Linseisen, J., Flesch-Janys, D., et al. 2012. Serum enterolactone and postmenopausal breast cancer risk by estrogen, progesterone and herceptin 2 receptor status. Int. J. Cancer, **130**(6): 1401–1410. doi:10.1002/ijc.26157. PMID:21544804.

Zheng, J.S., Hu, X.J., Zhao, Y.M., Yang, J., and Li, D. 2013. Intake of fish and marine n-3 polyunsaturated fatty acids and risk of breast cancer: meta-analysis of data from 21 independent prospective cohort studies. BMJ, **346**: 3706. doi:10.1136/bmj.f3706. PMID:23814120.