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Review Article

Health effects with consumption of the flax lignan secoisolariciresinol diglucoside

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Flaxseed is the richest source of the lignan secoisolariciresinol diglucoside (SDG). After ingestion, SDG is converted to secoisolariciresinol, which is further metabolised to the mammalian lignans enterodiol and enterolactone. A growing body of evidence suggests that SDG metabolites may provide health benefits due to their weak oestrogenic or anti-oestrogenic effects, antioxidant activity, ability to induce phase 2 proteins and/or inhibit the activity of certain enzymes, or by mechanisms yet unidentified. Human and animal studies identify the benefits of SDG consumption. SDG metabolites may protect against CVD and the metabolic syndrome by reducing lipid and glucose concentrations, lowering blood pressure, and decreasing oxidative stress and inflammation. Flax lignans may also reduce cancer risk by preventing pre-cancerous cellular changes and by reducing angiogenesis and metastasis. Thus, dietary SDG has the potential to decrease the incidence of several chronic diseases that result in significant morbidity and mortality in industrialised countries. The available literature, though, makes it difficult to clearly identify SDG health effects because of the wide variability in study methods. However, the current evidence suggests that a dose of at least 500 mg SDG/d for approximately 8 weeks is needed to observe positive effects on cardiovascular risk factors in human patients. Flaxseed and its lignan extracts appear to be safe for most adult populations, though animal studies suggest that pregnant women should limit their exposure. The present review discusses the potential health benefits of SDG in humans, with supporting evidence from animal studies, and offers suggestions for future research.

Secoisolariciresinol diglucoside: Flax lignans: Health benefits

Investigations into the health effects of whole flaxseed or flaxseed products (for example, defatted flaxseed meal, flaxseed extracts) in human clinical trials and animal models have shown beneficial changes in blood lipid profiles[1,2] and protection against some types of cancer[3,4]. However, such studies cannot reveal to which flaxseed component(s) the benefits can be attributed, as flaxseed contains at least three components that are of health interest: soluble fibres or mucilage (about 6 % of dry weight)[5]; high amounts of α-linolenic acid, an *n*-3 PUFA (about 20 % of dry weight)[6]; and the plant lignan secoisolariciresinol diglucoside (SDG, about 1 % of dry weight)[7]. Flaxseed also contains small amounts of other lignans, namely pinoresinol, lariciresinol and matairesinol[8], and although SDG is the predominant lignan, the others may also contribute to health effects.

Flaxseed is the richest source of SDG[9]; however, the amount of SDG in flaxseed varies between different cultivars and in most studies that examined the health effects of flaxseed or its products, the concentration of lignans was not determined. With the development of HPLC technology, SDG can be extracted from flaxseed and its SDG content determined[7]. Subsequent studies have shown that in defatted flaxseed extracts SDG exists in oligomeric form largely in ester linkages to 3-hydroxy-3-methylglutaric acid[10] and cinnamic acid[11] and with other phenolic compounds also present in glucosidic form[12]. A recent investigation reported the composition of SDG oligomers in defatted flaxseed powder at 38·5 mg/g DM, which corresponded to an SDG content of 15·4 mg/g DM[12]. Furthermore, in commercially available flaxseed lignan complex the levels of free SDG and SDG oligomer were about 381 and about 363 mg/g DM, respectively[12]. The ability to quantify SDG content in flaxseed extract sources enables an association to be drawn between SDG amounts and the putative health effects of flaxseed lignans.

After ingestion, the plant lignan SDG is converted to mammalian lignans by bacteria in the human colon[4]. SDG first undergoes hydrolysis to yield the aglycone plant lignan secoisolariciresinol (SECO). SECO is then converted to enterodiol (ED) and enterolactone (EL), first by dehydroxylation

Abbreviations: CRP, C-reactive protein; ED, enterodiol; EL, enterolactone; SDG, secoisolariciresinol diglucoside; SECO, secoisolariciresinol.

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and demethylation to yield ED, which can then be oxidised to form EL[13]. ED and EL can undergo further phase I and phase II biotransformation with extensive formation of glucuronide and sulfate conjugates[14,15], though the role of these metabolites in inducing biological effects is presently unknown.

The structural similarity of EL and ED to the most predominant and active form of oestrogen in the body, oestradiol, allows these lignans to bind to oestrogen receptors and exert weak oestrogenic or anti-oestrogenic effects[16]. However, the micromolar concentrations required to modulate oestrogen receptor activity *in vitro* [17–19], as a result of low binding affinity toward these receptors[20], is much higher than the serum EL and ED levels (nanomolar range) normally measured in the general population[21,22]. Nonetheless, recent studies provide scientific evidence of dietary sources of lignans as modulators of oestrogen receptor signalling *in vivo* [23,24]. EL and ED also possess antioxidant activity[25]. Thus, diets high in plant lignans may provide health benefits by decreasing the risk of hormone-sensitive cancers and diseases associated with increased inflammation and oxidative damage. Furthermore, the ability of ED and EL to inhibit the activity of certain enzymes may also explain the health benefits of flaxseed. For example, inhibition of the enzyme 5α-reductase may help to relieve lower urinary tract symptoms in patients with benign prostatic hyperplasia[26]. Also, the ability of EL to inhibit aromatase[27] may be beneficial in oestrogen-responsive breast cancers[28]. Another mechanism by which SDG may provide health benefits is through induction of phase 2 proteins. Phase 2 enzymes are generally characterised by either promoting the scavenging of oxidants or decreasing the probability of oxidant formation[29]; therefore, inducing phase 2 protein expression decreases oxidative stress. EL is a phase 2 protein inducer[30].

Evidence continues to mount to support the role of SDG, or its metabolites SECO, EL and/or ED, in protection against chronic disease. The mechanism(s) through which lignans mediate the putative health benefits and the actual bioactive lignan form (i.e. SDG, SECO, ED and/or EL) is not known. In the present review SDG is referred to as the lignan source but SDG may not be the final lignan form that induces biological activity. Determining whether or not lignan provided as SDG offers health benefits is a challenge, partly due to the wide variability in study methods used in the literature. The subject characteristics, sources of SDG and lack of product quality assurance, dosage, methods, data analysis and the duration of the study vary greatly between published studies. The purpose of the present review is to provide a summary of the evidence regarding the potential health benefits of the flax lignan, SDG, in humans, with supporting evidence from animal studies. The present review includes studies that report the dosage of SDG provided and that used an enriched lignan product, such as defatted flaxseed, low-α-linolenic flaxseed or flaxseed lignan extracts. Studies that only examined whole flaxseed (i.e. 'regular' flaxseed that has not had its fat or lignan components modified) are not the focus of the present review, as the SDG dosage is not reported in most of these studies and an objective of the present review is to examine the association between SDG dosage and health effects. In addition, any health benefits observed in these studies may be confounded by other components of whole flaxseed.

Cardiovascular health

Major adverse cardiovascular events, such as myocardial infarction and stroke, are the leading causes of mortality among industrialised nations[31]. Oxidative stress, inflammation, obesity, diabetes, dyslipidaemia and hypertension are interrelated and contribute to an atherogenic environment that promotes the development of CVD[31,32]. As discussed later, evidence suggests that SDG and its metabolites possess antioxidant properties and are capable of reducing oxidative stress. This would suggest that SDG consumption may provide protection against CVD.

Animal studies

As shown in Table 1, several studies have shown a beneficial effect of flax lignans on cardiovascular health in animal models. A series of studies from the same laboratory investigated the effects of flax lignan on atherosclerosis in rabbits[33–38]. The researchers first used type II flaxseed, a cultivar of flaxseed that has a similar oil content to regular flaxseed but only 2–3 % α-linolenic acid, to investigate the effects of type II flaxseed on high-cholesterol diet-induced atherosclerosis and serum lipids[38]. After 8 weeks, the 1 % cholesterol diet plus type II flaxseed diet resulted in improvements in the lipid profile and was also shown to be effective in reducing the development of aortic atherosclerosis.

To provide additional evidence that it is the lignan component of the flaxseed offering cardiovascular benefits, the same research group performed three studies using flax lignan complex[34,36,37] and two studies using purified SDG[33,35]. Flax lignan complex contains 34–38 % SDG as well as other potential bioactive components such as 3-hydroxy-3-methylglutaric acid (10–11 %) and cinnamic acids (15–21 %)[36]. Although the methods for each study were similar, they included important variations such as the level of cholesterol in the atherogenic diet (0·25–1·0 %), the dosage (Table 1) and duration of the treatment (2–4 months), and the effects of treatment on an atherogenic *v.* a regular diet. Despite these differences, all of these studies found that both flax lignan complex and SDG were effective in protecting against atherosclerosis.

A study by Felmlee *et al.* [39] and two studies by Penumathsa *et al.* [40,41] have also shown cardioprotective effects of SDG in rat models. Felmlee *et al.* [39] compared the activity of equimolar amounts of purified SDG and SECO on several markers of lipid homeostasis in diet-induced hypercholesterolaemic and hypertriacylglycerolaemic female rats. The results showed that SDG and SECO cause similar dose-dependent reductions in serum and hepatic cholesterol levels. Both lignans also decreased the rate of weight gain and accumulation of hepatic parenchymal fat. The study by Penumathsa *et al.* [40] used *in vitro*, *ex vivo* and *in vivo* models to study the angiogenic properties of SDG and found beneficial effects of SDG in all models. The other study by this laboratory[41] showed that SDG increased the expression of vascular endothelial growth factor, endothelial NO synthase and haeme oxygenase-1 mediated myocardial angiogenesis in male rats.

Although most animal model studies suggest that flax lignans provide cardiovascular benefits, not all of the results are positive. A study by Sano *et al.* [42] compared the effect

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Table 1. Comparison of secoisolariciresinol diglucoside (SDG) dosages and summary of significant findings related to CVD in studies using animal models

Reference	Animal model	Dosage	Summary of significant study findings
Sano *et al.* (2003)[42]	Mouse	5 % partially defatted flaxseed meal 0·06 % SDG (100 mg/kg per d)	Partially defatted flaxseed meal reduced rate of thrombus formation and atherosclerosis; SDG had no effect on either outcome
Felmlee *et al.* (2009)[39]	Rat	0, 3 or 6 mg SDG/kg per d 0, 1·6 or 3·2 mg SECO/kg per d	Administration of equimolar amounts of SDG and SECO caused similar dose-dependent reductions in serum and hepatic cholesterol levels in hyperlipidaemic rats
Penumathsa *et al.* (2007)[40]; Penumathsa *et al.* (2008)[41]	Rat	20 mg SDG/kg per d	SDG increased expression of vascular endothelial function, endothelial NO synthase and haeme oxygenase-1-mediated myocardial angiogenesis[41] *In vitro*, *ex vivo* and *in vivo* studies demonstrated that SDG has angiogenic and anti-apoptotic properties[40]
Prasad (2005)[34]; Prasad (2009)[36]; Prasad (2007)[37]	Rabbit	40 mg flax lignan complex/kg per d (containing 34–38 % SDG)	Compared with the group that received a 0·25 % cholesterol diet for 4 months, the group receiving a 0·25 % cholesterol diet for 2 months followed by the same diet plus SDG for another 2 months had fewer atherosclerotic lesions[36] After consuming a 0·25 % cholesterol diet for 2 months, flax lignan complex with a regular diet prevented the progression of atherosclerosis[37] Flax lignan complex decreased the extent of atherosclerosis, reduced total and LDL-cholesterol, increased HDL-cholesterol and reduced malondialdehyde, a lipid peroxidation product[34]
Prasad (2008)[33]	Rabbit	0·04 % SDG (20 mg/kg per d)	SDG treatment prevented the progression of atherosclerosis on a regular diet following a high-cholesterol diet
Prasad (1999)[35]	Rabbit	15 mg SDG/kg per d	SDG decreased hypercholesterolaemic atherosclerosis, total and LDL-cholesterol and malondialdehyde
Prasad *et al.* (1998)[38]	Rabbit	7·5 g low-α-linolenic acid flaxseed/kg per d (66 mg SDG/kg per d)	Low-α-linolenic acid flaxseed reduced levels of total cholesterol, LDL-cholesterol and the development of atherosclerosis

SECO, secoisolariciresinol.

of partially defatted flaxseed meal and SDG on the rate of thrombus formation and atherosclerosis in male mice. Partially defatted flaxseed meal significantly reduced both outcomes whereas SDG had no effect on either. One explanation for this inconsistency is that this study used the lowest dose of SDG of all of the studies discussed in the present review that used mouse models. Overall, the majority of studies that used purified SDG found improvements in markers of CVD.

Human studies

Similar to most animal studies, several human studies have shown cardiovascular benefits from flax lignans (Table 2). A randomised double-blind placebo-controlled trial was conducted in China to investigate the effects of SDG on total cholesterol, LDL-cholesterol, HDL-cholesterol, TAG and glucose concentrations[9]. The study used an SDG-rich flaxseed extract consisting of 33 % SDG, in contrast to defatted flaxseed meal which has an SDG concentration between 0·97 and 3·09 % (w/w)[9]. All subjects were hypercholesterolaemic, of which seventeen subjects received the placebo tablets containing 0 mg SDG, eighteen subjects received 300 mg SDG per d, and twenty subjects received 600 mg SDG per d. After 8 weeks, significant reductions in total cholesterol, LDL-cholesterol and glucose concentrations were found among those receiving 600 mg SDG per d compared with the placebo group. Significant differences were found for total cholesterol and LDL-cholesterol in the 300 mg SDG per d group when treatment values were compared with baseline but not when compared with the placebo group. Plasma concentrations of SECO, ED and EL were also measured and the observed cholesterol-lowering values were correlated with SECO and ED concentrations. The authors suggest that SDG appears to decrease plasma cholesterol and glucose concentrations in a dose-dependent manner, with SDG at 600 mg/d and not 300 mg/d being effective. Partially defatted flaxseed meal has also been shown to lower total and LDL-cholesterol levels, though it increased concentrations of TAG and lowered serum protein thiol groups, suggesting increased oxidative stress[43]. A limitation of this study is that the amount of SDG in the defatted flaxseed is unknown but would be significantly lower than the concentration in an SDG-enriched product.

In an observational study, the association between serum EL (produced from plant lignans naturally available in the subjects' diets) and acute coronary events was investigated in a prospective nested case–control study in Finland[44]. The study included 167 males who had had an average of 7·7 years of follow-up to an acute coronary event and 167 controls. Both cases and controls were from a cohort of 2005 men in the Kuopio Ischaemic Heart Disease Risk Factor Study. Subjects who had had an acute coronary event had mean serum EL concentrations that were 25·1 % lower

British Journal of Nutrition

Table 2. Comparison of secoisolariciresinol diglucoside (SDG) dosages used in human trials and summary of significant study findings related to health benefits

Reference	SDG dosage	Summary of significant study findings
Cornish *et al.* (2009)[50]	543 mg/d from flax lignan complex	Compared with placebo, supplementation decreased metabolic syndrome composite score in males and reduced diastolic blood pressure among all males as well as males and females with the metabolic syndrome at baseline
Zhang *et al.* (2008)[9]; Zhang *et al.* (2008)[26]	300 or 600 mg/d from flax lignan extract	Hypercholesterolaemic participants receiving 600 mg SDG/d had decreased plasma total cholesterol, LDL-cholesterol, and fasting glucose concentrations compared with placebo[9] Men with benign prostatic hyperplasia receiving 600 mg SDG/d had an improved quality of life score compared with placebo[26]
Pan *et al.* (2007)[53]; Pan *et al.* (2009)[54]	360 mg/d from lignan capsules	Supplementation reduced HbA1C compared with placebo in participants with type 2 diabetes[53] Compared with placebo, increases in CRP from baseline to follow-up were lower with supplementation[54]
Hallund *et al.* (2006)[47]; Hallund *et al.* (2008)[48]; Hallund *et al.* (2006)[49]	500 mg/d from flax lignan complex	No significant effects were seen on plasma lipids, glucose or markers of oxidative stress among the healthy participants[47,49] except that increases in CRP from baseline to follow-up were lower with supplementation compared with placebo[48]
Spence *et al.* (2003)[46]	222 mg/d from low-lignan, high-α-linolenic acid flaxseed 270 mg/d from flaxseed intermediate in SDG and α-linolenic acid 450 mg/d from high-lignan, low-α-linolenic acid flaxseed	Compared with baseline, all dosages reduced blood pressure during mental stress. The highest dosage was associated with the least increase in peripheral resistance, greatest reduction in cortisol and smallest increase in fibrinogen during stress

CRP, C-reactive protein.

than control subjects. Men in the highest quartile of the EL concentration distribution had a 58·8 % lower risk of acute coronary events compared with the lowest quartile. This value increased to 65·3 % after adjustment for the nine most strongly predictive risk factors. In a more recent study that also used data from the Kuopio Ischaemic Heart Disease Risk Factor Study, the association between serum EL concentration and CHD-related mortality, CVD-related mortality and all-cause mortality was examined[45]. The study consisted of the prospective follow-up for an average of 12·2 years of 1889 men free of CVD at baseline. Significant associations were found between elevated serum EL concentrations and reduced risk of CHD-related and CVD-related mortality. Weaker associations were found between serum EL levels and all-cause mortality. Since flaxseed is a rich source of plant lignan precursors for EL, the results of these studies suggest that flaxseed might provide cardiovascular benefits.

Individuals who are happy and socially connected generally have improved cardiovascular health compared with those who have high levels of psychosocial stress and are depressed, lonely and anxious[31]. Psychosocial stress may increase cardiovascular risk by activating the sympathetic nervous system and increasing cortisol, blood glucose and lipid levels as well as elevating blood pressure[31]. A comparison of three flax cultivars with different amounts of SDG was performed to determine their effects on responses to mental stress[46]. Using a three-way cross-over study design, postmenopausal women with vascular disease consumed 30 g of each of the flaxseed cultivars daily for 3 months with a 1-month washout period between treatments. As shown in Table 2, all three strains of flax were found to provide cardiovascular benefits during mental stress.

The results of a series of publications by Hallund *et al.* [47–49] suggest that SDG may not improve markers of CVD in healthy individuals compared with those with pre-existing hyperlipidaemia or CVD. In these studies, the effects of a flax lignan complex providing 500 mg SDG per d on plasma lipids, endothelial function, antioxidant capacity and C-reactive protein (CRP) were investigated among twenty-two healthy postmenopausal women. The flax lignan complex was comprised of 32·9 % SDG, 13·9 % cinnamic acids, 11·8 % protein, 10·0 % 3-hydroxy-3-methylglutaric acid, 3·5 % fat, 3·3 % moisture and 1·0 % ash. Using a cross-over study design, the women consumed daily a low-fat muffin, with or without the flax lignan complex, for 6 weeks, separated by a 6-week washout period. As shown in Table 2, the only significant change was that CRP concentrations were lower in the intervention group compared with the placebo group in spite of CRP concentrations increasing in both groups[48]. There were no changes observed for other markers of inflammation[48]. However, it is not surprising that significant reductions in biomarkers for CVD were not observed in these studies, since only healthy subjects were included.

Diabetes and the metabolic syndrome

Diabetes and the metabolic syndrome are risk factors for CVD. The metabolic syndrome is characterised by a combination of risk factors (increases in central adiposity, serum TAG, serum glucose, blood pressure and inflammation; decreases in HDL-cholesterol) that increases the risk of developing insulin resistance and CVD[50]. Thus, dietary interventions that lower the risk of diabetes and the metabolic syndrome will also help to decrease the incidence of CVD.

Animal studies

A study by Fukumitsu *et al.* [51] assessed the effect of SDG on the development of diet-induced obesity in C57BL/6 mice. Compared with a high-fat diet without SDG, a high-fat diet

Table 3. Comparison of secoisolariciresinol diglucoside (SDG) dosages and summary of significant findings related to diabetes and the metabolic syndrome in studies using animal models

Reference	Animal model	Dosage	Summary of significant study findings
Fukumitsu *et al.* (2008)[51]	Mouse	0·5 or 1·0 % SDG	High-fat diet with 0·5 or 1·0 % SDG resulted in reduced visceral fat gain. High-fat diet with 1·0 % SDG also decreased liver TAG content, serum TAG, total cholesterol, and insulin and leptin concentrations
Prasad (2001)[52]	Rat	40 mg SDG/kg per d	SDG administration delayed the onset of diabetes by 80 %

containing 0·5 or 1·0 % SDG resulted in significantly reduced visceral fat gain. The high-fat diet containing 1·0 % SDG also significantly decreased liver TAG content, serum TAG, total cholesterol, and insulin and leptin concentrations compared with the high-fat diet without SDG (Table 3). In addition, a study using female rats found that in the group receiving SDG, only two out of ten rats developed glucosuria at age 72 d whereas all ten rats in the untreated group had glucosuria by this age[52] (Table 3).

Human studies

SDG has been shown to provide benefits among type 2 diabetic patients. A randomised, double-blind, cross-over study performed in China enrolled type 2 diabetic patients to examine the effect of a flaxseed-derived lignan supplement containing 360 mg SDG per d on indices of glycaemic control, insulin resistance and lipid profiles[53]. The lignan supplement was comprised of 20 % SDG, 15·6 % fat, 3·2 % protein, 2·6 % fibre and 30 % carbohydrate. The duration of the intervention and placebo periods was 12 weeks separated by an 8-week washout period. A total of sixty-eight patients completed the trial. Compared with placebo, the lignan supplement significantly reduced HbA1C concentrations, though there was no effect on fasting glucose and insulin concentrations, homeostasis model assessment of insulin resistance (HOMA-IR) and blood lipid profiles. In a secondary analysis of the data, the effects of the lignan supplement on inflammatory factors (CRP and IL-6) were investigated[54]. Retinol-binding protein 4 was also measured, as it has been shown to be associated with insulin resistance, diabetes and inflammation. As in the study by Hallund *et al.* [48], this study also found that CRP levels increased from baseline to follow-up in both the placebo and lignan supplement groups, though increases in CRP were lower with the lignan supplement. However, when stratified by sex, the differences were significant among women but not men.

The effects of flax lignan complex supplementation on the metabolic syndrome were studied in a randomised double-blind placebo-controlled trial[50]. The metabolic syndrome was assessed using a composite score of six risk factors and the supplement provided approximately 543 mg SDG/d. After 6 months of supplementation, the flax lignan complex decreased the metabolic syndrome composite score, compared with placebo, in males but no effects were observed in females. In addition, among a subsample of male and female subjects with the metabolic syndrome at baseline, the flax lignan group had a significant decrease in diastolic blood pressure ($P=0·0085$; 88·7 (SEM 2·8) to 82 (SEM 2·8) mmHg) compared with the placebo group (82·7 (SEM 2·8) to 83·8 (SD 2·8) mmHg).

Cancer

Flax lignans may be protective against some cancers (i.e. breast, lung and colon) because of their antioxidant, anti-proliferative, anti-oestrogenic or anti-angiogenic properties or possibly due to their ability to inhibit certain enzymes[4].

Animal models

To examine the effect of flaxseed and its components on colon cancer risk, Jenab & Thompson[4] used a rat model of colon cancer. The treatments used in this study are shown in Table 4. The presence of aberrant crypts and aberrant crypt foci, which are considered early markers of colon cancer risk, was determined after the rats had been on the diets for 100 d. The results showed that flaxseed, defatted flaxseed and SDG supplementation reduced aberrant crypt multiplicity and, thus, may protect against colon cancer. Because the results from the treatment groups were similar, the researchers concluded that it was the lignan component and not the oil content of flaxseed that provided protection.

Li *et al.* [55] examined the effect of SDG supplementation on pulmonary metastasis of melanoma cells in male mice aged 3 weeks. A control diet with or without SDG supplementation was used (Table 4). After 2 weeks on the control or SDG-supplemented diets, each mouse was injected with melanoma cells. The mice were then fed the diets for an additional 2 weeks. In the control group, 59 % of the mice had more than fifty lung tumours, whereas in the SDG-supplemented groups 30, 21 and 22 % of the mice had more than fifty tumours, respectively, with the latter two groups being significantly different from the control. The median number of tumours was also significantly reduced in the 200 mg/kg group compared with control. In addition, SDG reduced tumour cross-sectional area and volume in a dose-dependent manner.

Enhanced early mammary gland differentiation may reduce the risk of mammary carcinogenesis later in adulthood[56]. Terminal end buds are the most undifferentiated terminal ductal structures and are highly susceptible to chemical carcinogenesis[56]. Conversely, alveolar buds and lobules, the products of terminal end bud differentiation, are less vulnerable to carcinogens[56]. Dietary components such as SDG have the potential to promote early enhancement of mammary gland differentiation and, thus, may provide protection against breast cancer[56]. A series of studies have examined the effect of flaxseed and SDG on breast cancer risk using a rat model. A comparison of the treatments used in these studies is found in Table 4. The oldest of these studies[57] showed that flaxseed and SDG appeared to delay the progression of N-methyl-N-nitrosourea-induced mammary tumorigenesis.

Table 4. Comparison of secoisolariciresinol diglucoside (SDG) dosages and summary of significant findings related to cancer in studies using animal models

Reference	Dosage	Summary of significant study findings
Mouse model		
Li *et al.* (1999)[55]	50, 100 or 200 mg SDG/kg per d	Median number of tumours significantly reduced in the 200 mg/kg group compared with control. SDG reduced tumour cross-sectional area and volume in a dose-dependent manner
Rat model		
Tan *et al.* (2004)[56]	10 % flaxseed 200 mg SDG/kg diet (equivalent to 10 % flaxseed)	Exposure to flaxseed and SDG during suckling resulted in a decreased number of terminal end buds and higher ratio of lobules to terminal end buds in early adulthood
Chen *et al.* (2003)[60]	10 % flaxseed 20·1 mg SDG/100 g diet (equivalent to 10 % flaxseed)	Exposure to flaxseed or SDG during suckling reduced mammary tumour incidence in adulthood
Ward *et al.* (2000)[59]	10 % flaxseed SDG equivalent to amount in 10 % flaxseed (4·28 (SEM 2·41) μmol SDG per d)	Exposure to 10 % flaxseed or equivalent amount of purified SDG during lactation only or from lactation to postnatal day 50 reduced terminal end bud structures in the mammary gland
Rickard *et al.* (1999)[57]	2·5 or 5 % flaxseed 0·7 or 1·4 mg SDG/d (equivalent to 2·5 or 5 % flaxseed, respectively)	Proportion of tumours with higher level of invasiveness and grade was greater in control group than all treatment groups. Higher SDG dose lowered tumour multiplicity whereas lower SDG dose increased tumour multiplicity[57]
Tou & Thompson (1999)[58]	5 or 10 % flaxseed 1·82 % flax oil 1·5 mg SDG/d (equivalent to 5 % flaxseed)	Gestation and lactation exposure to purified SDG reduced terminal end bud and alveolar bud density, delayed puberty onset and reduced number of oestrous cycles
Tou *et al.* (1998)[85]	5 or 10 % flaxseed 1·5 mg SDG/d (equivalent to 5 % flaxseed)	SDG altered mammary gland structure by reducing terminal end buds and alveolar buds which may reduce mammary cancer risk
Jenab & Thompson (1996)[4]	2·5 or 5 % flaxseed 2·5 or 5 % defatted flaxseed 1·5 mg SDG/d (equivalent to 5 % flaxseed or defatted flaxseed)	Flaxseed, defatted flaxseed and SDG reduced aberrant crypt multiplicity which may protect against colon cancer

Subsequently, two studies[58,59] found that exposure to SDG during gestation and/or lactation results in beneficial mammary gland structural changes. Further research found that SDG exposure during suckling could result in more differentiated mammary glands[56] which could protect against mammary tumorigenesis later in life[60].

The mechanism by which SDG protects against breast cancer is unknown. Insulin-like growth factor I is associated with increased risk for breast cancer and SDG has been shown to lower plasma insulin-like growth factor I concentrations[61]. The concentration of Zn is higher in breast cancer tissues than in normal breast tissues. Thus, another mechanism could be related to the ability of SDG to regulate the expression of Zn transporters[62]. Lastly, vascular endothelial growth factor stimulates the production of new blood vessels (i.e. angiogenesis), which is critical in the progression of cancer[63]. *In vitro* and *in vivo* evidence suggests that ED and EL may provide protection against breast cancer by limiting angiogenesis[63].

Human studies

The human studies that have examined the relationship between flax lignans and cancer have been observational studies that examined the correlation between serum EL concentrations and cancer risk. It has been suggested that EL may provide protection against breast cancer due to its antioxidant activity or ability to inhibit enzyme action, in particular enzymes that are involved in steroid hormone metabolism[64]. Furthermore, EL was shown to suppress cell proliferation and migration and induce apoptosis of prostate cancer cells[65,66].

Studies that have examined the association between serum EL concentrations and breast cancer risk have produced mixed results[64,67,68]. Although these studies simply measured EL concentrations and correlated these concentrations with risk of breast cancer, evidence that serum EL protects against breast cancer would suggest that, as an EL precursor, SDG may also provide benefits. Women with palpable cysts have an increased risk of developing breast cancer. Boccardo *et al.* [67] studied 383 women with palpable cysts to investigate whether a relationship exists between serum EL concentration and breast cancer risk. From the time of their first cyst aspiration to a median follow-up time of 6·5 years, eighteen women developed breast cancer. Women who developed breast cancer were found to have significantly lower median EL concentrations than in those who were cancer-free. Among the risk factors considered in this study (serum EL concentration, age at first cyst aspiration, family history of breast cancer, and cyst type), serum EL concentration was the only variable that had a significant inverse correlation with breast cancer risk. However, the authors acknowledge that limitations of this study include the small sample size and that a single serum EL measurement may not be reliable[67].

Pietinen *et al.* [68] also found an inverse association between serum EL concentration and breast cancer risk in a study that included 194 breast cancer cases (sixty-eight premenopausal and 126 postmenopausal) and 208 controls. Also of interest from this study was that dietary patterns alone did not explain differences in serum EL. The authors propose that other factors such as the amount or type of intestinal flora may affect how much EL is produced from its plant lignan precursors.

British Journal of Nutrition

On the contrary, a nested case–control study performed in Finland that included 206 cases and 215 controls did not find a significant correlation between serum EL concentration and reduced risk of premenopausal breast cancer[64]. In addition, the results showed a modest, non-significant increase in the risk of postmenopausal breast cancer among those with higher levels of serum EL.

Oxidative stress and inflammation

The ability to scavenge oxidants produced by normal cellular metabolism becomes poorer as we age[69]. Individuals who undergo healthy ageing have 'youthful' antioxidant defence systems[70]. Increased oxidative stress promotes: (1) development of hypertension[71] and all its attendant problems including cognitive impairment[72]; and (2) activation of pro-inflammatory genes[73] causing the characteristic generalised inflammatory conditions known as 'inflammaging' seen in many of the elderly[74].

In vitro studies have shown that SDG and its metabolites, SECO, EL and ED, possess antioxidant activity. Early research in this area found that SDG was effective in preventing lipid peroxidation of liver homogenate in a concentration-dependent manner[75]. However, it is the metabolites of SDG (SECO, EL and ED), which are found in the portal circulation, plasma and urine, that are more likely to exert protective effects against oxidative stress systemically. Therefore, using lipid and aqueous *in vitro* model systems, Kitts *et al.* [76] used several assays to demonstrate that ED, EL and SDG have antioxidant activity. All three lignans showed similar activity in lowering lipid peroxidation. However, ED and EL were more effective than SDG in reducing deoxyribose oxidation and DNA strand breakage. Additionally, Prasad[25] found that the antioxidant potency of SECO, ED, EL and SDG is 4·86, 5·02, 4·35 and 1·27, respectively, as compared with vitamin E at a concentration of 2·5 mg/ml. A study by Hosseinian *et al.* [77] also suggested that SECO is a superior antioxidant compared with SDG. However, Hu *et al.* [13] questioned the relevance of the high lignan concentrations used in the study by Prasad[25]. Thus, they performed a study that used concentrations of SDG, SECO, ED and EL more likely to be achieved *in vivo* and concluded that these lignans are likely to be effective against oxidative stress in the colonic lumen and epithelial cells[13]. However, as noted previously, it is the systemic antioxidant properties of SECO, EL and ED that are the most physiologically relevant and additional work is needed in this area[13].

In contrast, *in vivo* studies of the antioxidant properties of SDG and its metabolites provide uncertain results. A study using rabbits found that a flax lignan complex (34–38 % SDG, 15–21 % cinnamic acid and 9–11 % hydroxymethylglutaric acid by weight) is able to reduce the extent of atherosclerosis by reducing oxidative stress as measured by aortic and serum malondialdehyde (a lipid peroxidation product) and aortic chemiluminescence (a measure of antioxidant reserve)[34]. The flax lignan complex decreased serum malondialdehyde by 35 % and aortic malondialdehyde by 58 % in hypercholesterolaemic rabbits. However, the results were difficult to interpret because normocholesterolaemic rabbits receiving the flax lignan complex had increased aortic malondialdehyde. Nevertheless, the researchers concluded that the flax lignan complex was associated with marked decreases in oxidative stress.

A study involving human participants used partially defatted flaxseed and found a decrease in protein thiol groups, an indicator of increased oxidative stress[43]. In addition, Hallund *et al.* [47] did not find a difference in serum lipoprotein resistance to oxidation, Trolox-equivalent antioxidant capacity, and ferric-reducing ability of plasma between the placebo and SDG intervention groups. However, F2-isoprostane levels have become the 'gold standard' for *in vivo* assessment of oxidative stress[78], so the results of these studies need to be confirmed using this biomarker. One study that did measure F2-isoprostane levels in human subjects found that plasma EL is inversely correlated with plasma F2-isoprostanes[79].

Safety

Both animal and human studies provide evidence that flaxseed and its lignan extracts appear to be safe. A recent study reported that SDG administration at 3 mg/kg body weight for 4 weeks had no adverse health effects in female rats[39]. Dietary supplementation with SDG at levels as high as 200 mg/kg had no adverse effects on growth in mice[55]. Hemmings & Barker[80] reported that flaxseed does not appear to affect growth development or behaviour and does not show signs of toxicity or liver damage in male or female rats. Erythrocyte and leucocyte counts and platelets were not adversely affected after feeding rabbits a flax lignan complex at a dose of 40 mg/kg for 2 months[81]. In addition, SDG does not appear to negatively affect bone strength in young male and female rats[82,83]. Human studies have found that flaxseed[1] and lignan capsules[53] are well tolerated. Participants did not report any adverse events in a study that used SDG supplementation at 300 and 600 mg/d[9].

One possible area for concern is the effect of flaxseed on offspring of animals fed flaxseed during pregnancy. Two studies[84,85] have reported that flaxseed or flaxseed meal did not affect pregnancy in rat dams but did exert reproductive changes in the offspring, such as shortening of the anogenital distance and lengthening of oestrous cycles. However, in rats, maternal consumption of flaxseed or SDG during suckling did not affect reproductive indices in male or female offspring[86]. Though it appears that flaxseed and its components are safe for most people, until further evidence is available, pregnant women may be advised not to consume flaxseed in large quantities.

Conclusion

In summary, animal studies using rat, mice and rabbit models suggest that SDG supplementation protects against the development of chronic diseases such as CVD, cancer and diabetes. However, the outcomes of these studies are complicated by differences in sex, age and species strain[39,87]. Direct comparisons cannot be made between different species and animal doses are almost always greater than human doses due to a variety of factors. The wide variability in the methods used in human trials also complicates the interpretation of results though there is growing evidence that

SDG-enriched flaxseed products offer health benefits. The studies to date suggest that a dose of at least 500 mg SDG per d is needed to observe significant benefits and that this dose appears to be safe for most people, though animal studies suggest that pregnant women should limit their exposure. The actual mechanism by which SDG may provide health benefits is not currently known and more research is needed in this area.

Several of the human studies that have included SDG were performed by the same research group[47–49], so it is important that these results are corroborated by other researchers. More studies are emerging that used a well-described flax lignan complex, which reduces the confounding issue of lack of product quality assurance[9,53]. However, more randomised controlled trials are needed before we can elucidate whether or not SDG supplementation protects against disease in humans.

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British Journal of Nutrition

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