

Appendix IV:

Summary of literature review of animal and human studies
using flaxseeds or flax lignans

Summary of Key Studies on Flaxseed and Lignans

Area investigated: plasma lipids/heart disease

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments
Lucas, E, J of Clinical Endocrinology and Metabolism 2002; 87: 1527-1532	Flaxseed improves lipid profile without altering biomarkers of bone metabolism in post menopausal women.	Design: randomized control-treatment comparison, parallel study. Treatments: 40g of ground whole flaxseed or a wheat based comparative control. Consumed daily for 3 months	36 Women average 55 y/o.	Serum total cholesterol, triglycerides, HDL, LDL, chylomicrons, Serum IGF-I, IGF-BP3, E2, E1, FSH, SHBG, alkaline phosphatase, and TRAP, BSAP activity, urinary creatinine, urinary Dpd, and urinary helical peptide	Compared to baseline: 6% decrease in total cholesterol and non HDL cholesterol with flaxseed treatment, not seen in controls. No changes observed in bone metabolism measures (IGF-I, IGFBP3, AP, BSAP, TRAP, calcium, urinary Dpd, or helical peptide). No impact of flaxseed treatment on serum hormone levels (E1, E2, FSH, or SHBG)	The findings of this study indicate that flaxseed supplementation mildly improves blood lipid profiles in post menopausal women, but has no impact on reproductive hormones or biomarkers of bone metabolism.
Dodin S, J Clin Endocrinol Metab 2005; 90: 1390-1397	The effects of flaxseed dietary supplement on lipid profile, bone mineral density, and symptoms in menopausal women: a randomized, double blind,	Design: randomized wheat germ control treated comparison group, parallel study. Treatments: 40g/d flaxseed or a wheat germ placebo consumed daily for 12 months. Follow up at 3,6,9, and 12 months.	Post-menopausal women 45 to 65 years old Serum FSH \geq 40 mIU/ml and >6 months amenorrhea	Serum total cholesterol, HDL, LDL, Triglycerides, Blood pressure, blood glucose levels. Also Lumbar and Femoral neck bone mineral density	Compared to control group there was a significant decreases in Total Cholesterol and HDL. There was also a borderline significant reduction in LDL cholesterol. Total cholesterol was reduced - 0.01 mmol/L from baseline. Only in the flaxseed group, compared to baseline, there were significant reductions in systolic and diastolic blood pressure. Systolic BP decreased from baseline (-5.0 mm Hg). Diastolic BP decrease from baseline by -4.1 mm Hg.	This study concludes that flaxseed incorporation into the diet can modify cholesterol levels in post menopausal women. However, there is question as to the clinical strength of these reductions.

Area investigated: plasma lipids/heart disease

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments
Cunnane S., <i>Am J Clin Nutr</i> Jan; 61(1): 62-8, 1995	Nutritional attributes of traditional flaxseed in healthy young adults.	Design: randomized crossover. Treatment: add 50 g/day flaxseed flour in 2 muffins or 2 muffins without flaxseed (control) to ordinary diet for 4 weeks.	10 young human subjects, age 25±3, 5 males and 5 females.	Plasma TC, LDLC, HDLC, TG, PUFA, glucose tolerance, urinary lignan excretion, thiobarbituric acid reaction, and bowel movement.	Plasma TC and LDLC decreased 5.7 and 8.9%, respectively, after 4 weeks flaxseed muffin intake comparing to the values of 0 week ($p < 0.05$), but not different from control values. Bowel movement increased 30% weekly by flaxseed intake. Urinary lignan excretion increased 3.7-5 times. No unfavorable effects on glucose tolerance, plasma peroxide concentrations, and antioxidant vitamin status were noted.	Some moderate beneficial effects of flaxseed consumption on plasma cholesterol levels, PUFA levels, and bowel movement are indicated. No unfavorable influence appears from flaxseed intake. In this study, subjects are very young and have relatively low TC and LDLC levels, therefore, they may not be sensitive subjects for testing the effect of cholesterol lowering factors.
Jenkins DJ. <i>Am J Clin Nutr</i> 69(3):395-402, 1999	Health aspects of partially defatted flaxseed, including effects on serum lipids, oxidative measures, and ex vivo androgen and progestin activity: a controlled crossover trial	Design: controlled crossover. Treatment: muffins containing about 50 g partially defatted flaxseed or wheat bran were added on instructed and self-selected NCEP Step-II diet for 3 weeks.	29 subjects (from 36), 22 males and 7 postmenopausal females, with hyperlipidemia, mean age 57 and BMI 24.9.	Serum TC, LDLC, HDLC, TG, apo-B, apo-AI, protein thiol content, and protein carbonyl content.	Partially defatted flaxseed reduced TC 4.6%, LDLC 7.6%, apo B 5.4%, (all $p \leq 0.001$), and apo A-I 5.8% ($p = 0.005$); had no significant effects on serum HDLC, protein carbonyl content and protein thiol content compared with the values obtained from control phase.	The short period treatment with 50 g of defatted flaxseed moderately decreased serum TC, LDLC, and apo B levels. Although the mild decreased apo A-I is an unfavorable change, HDLC levels are not influenced. (Protein thiol content is an indicator of body oxidative stress increase.)

Area investigated: plasma lipids/heart disease

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments
Vanharanta M, <i>Lancet</i> 1999 Dec 18/25;354 (9196): 2112-5	Risk of acute coronary events according to serum concentrations of enterolactone: a prospective population-based case-control study	Design: cohort case-control. Odds ratios for acute coronary events (ACE) in subjects with different serum enterolactone (ENT) concentrations were determined using logistic regression.	167 Finland men with ACE and 167 paired cases, with mean age 54.2.	Odds ratios (relative risks) of ACE between population group with different serum ENT levels (<7.21, 7.21-15.1, 15.11-30.1, and >30.1 nmol/L).	<ol style="list-style-type: none"> Subjects with ACE had a lower (by 22.3%) serum ENT concentrations than that of controls. The risk of ACE was decreased by 2.3% with per nmol/L increase of serum ENT after adjustment for the other 9 risk factors ($p < 0.01$). Men with high serum ENT (above median) had a 52% lower risk of ACE ($p < 0.05$) than men with low serum ENT (below median). Men in the highest quartile of ENT levels (>30.1) had a 65.3% lower risk of ACE ($p < 0.05$) than men in the lowest quartile (>7.21). 	This study has documented the inverse relationship between ACE and serum ENT concentrations. Briefly, men with serum ENT concentration over 15 nmol/L would be 50% less likely to suffer ACE than ones with serum ENT concentration under 15 nmol/L in the studied cohort.
Prasad, K, <i>Circulation</i> 1999 Mar 16;99(10): 1355-62	Reduction of serum cholesterol and hypercholesterolemic atherosclerosis in rabbits by secoisolariciresinol diglucoside isolated from flaxseed	Design: randomized control-treatment comparison. Rabbits were assigned to 4 groups: group 1, control; group 2, SDG control (15 mg/d/kg body wt); group 3, 1% cholesterol diet; and group 4, same as group 3 but with added SDG (15 mg/d/kg body wt). Study period: 8 weeks.	New Zealand White rabbits, 6-8 weeks old, weight 1.8-2 kg. Control group n=8, n=5 or 6 for other groups.	Plasma TG, TC, VLDL-C, LDL-C, HDL-C. Anti-oxidative activity. Aortic arteriosclerosis lesions. Body weight gain.	Compared to group with 1% chol. diet at the end of study, 1) SDG lowers plasma levels of TC (33%) and LDL-C (35%), both $p < 0.05$, and increases levels of HDL-C ($\approx 90\%$, not sig.) and VLDL-C ($\approx 60\%$, not sig.); 2) SDG lowers malondialdehyde and aortic tissue chemiluminescence (antioxidant activity), $p < 0.05$; and 3) SDG reduces the formation of aortic arteriosclerosis plaques by 73%, $p < 0.05$. Compared to control group, the other groups had lower body weight gain by 21-37%, $p < 0.05$.	<p>The protective effects of SDG on CHD have been noted in this study. It inhibits atherogenesis induced by high cholesterol diet. Favorable changes of plasma lipid profile and antioxidant activity could be the mechanisms of the protective effects.</p> <p>In this study, it seems that outliers of measurement, randomization problem, and interaction effect exist. Repeated procedure is inappropriate for data analysis between groups.</p>

Area investigated: plasma lipids/heart disease

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments
Prasad K, <i>Atherosclerosis</i> 1998 Feb;136 (2):367-75	Reduction of hyper-cholesterolemic atherosclerosis by CDC-flaxseed with very low alpha-linolenic acid	Design: randomized control-treatment comparison. Rabbits were assigned to four groups: Group I, Control; Group II, Type II flaxseed diet (7.5 g/d/kg BW); Group III, 1% cholesterol diet; Group IV, 1% cholesterol diet plus Type II flaxseed (7.5 g/d/kg BW). Study period: 8 weeks.	New Zealand White rabbits, 6-8 weeks old, weight 1.8-2 kg. Control group n=8, other groups n=5 or 6.	Plasma TG, TC, VLDL-C, LDL-C, HDL-C. Aortic arteriosclerosis lesions.	Compared to group III (1% chol.) at the end of study, 1) flaxseed II lowered plasma levels of TC (14-31%) and LDL-C (17-32%), increases levels of TG (122-157%) and VLDL-C (121-485%); 2) flaxseed II reduced the formation of aortic arteriosclerosis plaques by 69%, all p<0.05. The groups consuming diet with cholesterol had significantly less body weight gain than did the groups without cholesterol., p<0.05.	This study is identical as the above one except replacing SDG with flaxseed. The similar effects of lowering TC and LDL-C and reducing arteriosclerosis plaque formation were observed. The authors could not interpret some observed outcomes, such as flaxseed + cholesterol diet increased VLDL-C levels and diets with 1% cholesterol reduced body weight gain.
Prasad K, <i>Atherosclerosis</i> 1997 July, 11;132(1): 69-76	Dietary flax seed in prevention of hypercholesterolemic atherosclerosis	Design: randomized control-treatment comparison. Rabbits were divided into 4 groups: group I, control; group II, flax seed diet (7.5 g/d/kg BW orally); group III, 1% cholesterol diet; and group IV, same as group III but received flax seed (7.5 g/d/kg BW orally). Study period: 8 weeks.	New Zealand White rabbits, 6-8 weeks old, weight 1.8-2 kg. Sample size: group I, II, IV n=8, and group III n= 6.	Plasma TG and TC levels, formation of arteriosclerosis plaques, oxygen free radical producing activity in leucocytes, and body weight gain.	Compared to the group consuming 1% chol. diet, the group consuming flaxseed+chol. diet had less development of arteriosclerosis plaques (by 46%) and reduced oxygen free radical producing activity, both p<0.05. Control group significantly decreased plasma TG about 39%, while other groups slightly increased TG levels. The groups consuming diet with 1% chol. had significant higher plasma chol. levels than did the groups not consuming high chol. diet.	1. The effect of flaxseed diet on anti-oxidation and reducing formation of aortic plaques were noted in this study. 2. Statistical method of repeated procedure was inappropriately used.

Area investigated: plasma lipids/heart disease

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments
Prasad K Atherosclerosis	Hypocholesterolemic and antiatherosclerotic effect of flax lignan complex isolated from flaxseed.	Four treatment groups (6-16 per group completed the trial). The groups were: 1. Control 2. Lignan Treatment 3. Cholesterol added in diet 4. Cholesterol plus Lignan Treatment.	New Zealand White Female Rabbits, (1.2 – 1.5 kg) 6-8 weeks old at start of study.	Triglycerides, total cholesterol, LDL, HDL, and MDA from serum. Also aortas were assessed for atherosclerotic changes, aortic MDA and antioxidant reserve.	Serum total cholesterol and MDA were decreased in the high cholesterol diet group treated with lignans. There was a 35.7 % decrease in MDA after 2 months of lignan treatment compared with the high cholesterol diet without treatment. Lignan treatment decreased atherosclerosis by 34.37% compared to no treatment in the high cholesterol diet groups. There was also a 69% decrease in aortic MDA.	These results suggest under the conditions of a high cholesterol containing diet, lignans isolated from flaxseed can reduce oxidative stress and the extent of atherosclerosis induced by hypercholesterolemia. Lignans can also lower total cholesterol and raise HDL in hypercholesterolemic conditions.
Stuglin C and Prasad K J Cardiovasc Pharmacol Ther 2005	Effect of flaxseed consumption on blood pressure, serum lipids, hemopoietic system and liver and kidney enzymes in healthy humans.	Consumed flaxseed containing muffins for 4 weeks in addition to their normal diet.	15 Healthy men aged 22-47 y.	Triglycerides, total cholesterol, LDL, HDL, serum chemistry and complete blood count. Also blood pressure was monitored.	Serum cholesterol, HDL, LDL, and VLDL remained unchanged when compared to baseline. Serum triglycerides were elevated. Most serum chemistry remained normal (total bilirubin, AST, ALT, protein, albumin, glucose and urea) while serum creatinine decreased.	Four weeks of dietary flaxseed intervention does not have adverse impacts on the hemopoietic system, renal, or hepatic functions. It did not lower blood pressure or serum lipid levels.

Area investigated: anti-tumor/breast cancer

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments															
David Ingram, <i>Lancet</i> 1997 Oct 4; 350(9083): 990-4	Case-control study of phyto-oestrogens and breast cancer	Design: case-control. Cases are breast cancer patients. (3 consecutive 24-hour urine samples were collected, pooled/mixed, and analyzed).	144 pairs of breast cancer patients and matched controls, mean age 54.	The links of breast cancer risk and urinary concentrations of phyto-estrogens (enterodiol, enterolactone, matairesinol, daidzein, genistein, and equol). The relative risk (odds ratio) of breast cancer was reported upon quartiles of urinary phyto-estrogen concentrations.	The 24-hour urinary excretion of enterolactone and equol are inversely correlated with the relative risk of breast cancer. Compared to quartile 1 (lowest concentration), the relative risks for quartile 2, 3 and 4 are 0.91, 0.65, and 0.36 for enterolactone, respectively; and 0.45, 0.52, and 0.27 for equol, respectively.	This study, with careful case-control design and relatively large sample size, indicates that higher dietary lignan (SDG) and isoflavones (daidzein-equol) intake can significantly lower the risk of breast cancer in the studied cohort, but not the other measured phyto-estrogens.															
Thompson L. <i>Nutr Cancer</i> 26(2):159-65, 1996	Antitumorogenic effect of a mammalian lignan precursor from flaxseed	Design: control-treatment comparison. 1 week after gavage of carcinogen dimethyl-benzanthracene (5 mg/kg bw, DMBA), rats were fed control diet or control diet + SDG (1.5 mg/day) by gavage for 20 weeks.	60 female Spague-Dawley rats, 50 days of age, randomly assigned into 2 groups.	Mammary tumor incidence (TI, %), mean tumor volume (TV, cm ³), tumor numbers (TN) per rat,	At the end of the study: <table border="1"> <thead> <tr> <th>Treat</th> <th>n</th> <th>TI</th> <th>TN</th> <th>TV</th> </tr> </thead> <tbody> <tr> <td>Control</td> <td>30</td> <td>86.6</td> <td>4.3</td> <td>2.2</td> </tr> <tr> <td>SDG</td> <td>30</td> <td>76.6</td> <td>2.7</td> <td>2.1</td> </tr> </tbody> </table> <p>Compared to control, SDG group has 10% smaller TI (not significant) and 37% smaller TN ($p < 0.05$).</p>	Treat	n	TI	TN	TV	Control	30	86.6	4.3	2.2	SDG	30	76.6	2.7	2.1	The anti-mammary tumor effects of SDG are noticed (low TI and TN) in this study. However, the anti-tumor effects of SDG may be masked at some degree by the relatively high dose of carcinogen (DMBA) used. The TI and TN in the studied rats are very high.
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Area investigated: anti-tumor

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Serraino <i>M. Nutr Cancer</i> 17(2):153-9, 1992	The effect of flaxseed supplementation on the initiation and promotional stages of mammary tumorigenesis	Design: control-treatment comparison. 1 month after feeding basal Diet (BD) or BD+5% flaxseed, 5 mg dimethyl-benzanthracene (DMBA) administered by gavage into rats, then the rats either kept consuming their original diet or switched to the other one for 20 weeks.	120 female Spague-Dawley rats, 21 days of age, randomly assigned into 2 groups. After one month BD or BD+5% flaxseed, each group was divided into 3 subgroups and received 3 treatments separately.	Mammary tumor incidence (TI), mean tumor volume (TV, cm ³), tumor numbers (TN) per rat and per group, weight gain (WG, g) and food intake (FI, g/day).	At the end of the study: <table border="1"> <thead> <tr> <th>Treat</th> <th>n</th> <th>TI</th> <th>TV</th> <th>TN</th> </tr> </thead> <tbody> <tr> <td>BD</td> <td>22</td> <td>14/22</td> <td>0.9</td> <td>2.2</td> </tr> <tr> <td>5% F</td> <td>22</td> <td>14/22</td> <td>0.6</td> <td>1.8</td> </tr> <tr> <td>BD-5% F</td> <td>22</td> <td>11/22</td> <td>0.3</td> <td>3.4</td> </tr> <tr> <td>5% F-BD</td> <td>21</td> <td>9/21</td> <td>1.2</td> <td>2.0</td> </tr> </tbody> </table> <table border="1"> <thead> <tr> <th>Treat</th> <th>n</th> <th>WG</th> <th>FI</th> </tr> </thead> <tbody> <tr> <td>BD</td> <td>22</td> <td>309.7</td> <td>12.6</td> </tr> <tr> <td>5% F</td> <td>22</td> <td>305.8</td> <td>12.5</td> </tr> <tr> <td>BD-5% F</td> <td>22</td> <td>319.2</td> <td>12.6</td> </tr> <tr> <td>5% F-BD</td> <td>21</td> <td>350.6</td> <td>12.9</td> </tr> </tbody> </table> Rats without DMBA treatment <table border="1"> <tbody> <tr> <td>BD</td> <td>16</td> <td>326.7</td> <td>12.8</td> </tr> <tr> <td>5% F</td> <td>16</td> <td>312.7</td> <td>12.3</td> </tr> </tbody> </table>	Treat	n	TI	TV	TN	BD	22	14/22	0.9	2.2	5% F	22	14/22	0.6	1.8	BD-5% F	22	11/22	0.3	3.4	5% F-BD	21	9/21	1.2	2.0	Treat	n	WG	FI	BD	22	309.7	12.6	5% F	22	305.8	12.5	BD-5% F	22	319.2	12.6	5% F-BD	21	350.6	12.9	BD	16	326.7	12.8	5% F	16	312.7	12.3	As concluded by the authors, 'the effects of flaxseed on mammary tumorigenesis is not discernable' in this study. In 5% F group, TN is the smallest but TI is the highest. In BD-5% F group, TV is the lowest but TN is the biggest. In 5% F-BD group, TI is the lowest but TV is the highest. It is hard to speculate what cause the results.
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Serraino <i>M. Cancer Lett</i> 15:63(2): 159-65, 1992	Flaxseed supplementation and early markers of colon carcinogenesis	Design: control-treatment comparison. Treatment: one week after a carcinogen injection, rats were fed with basal diet (BD), BD+5% flaxseed flour (FF), 10% FF, and BD+5% defatted flaxseed meal (FM), and BD+10% FM. Feeding period: 4 weeks.	35 male Sprague-Dawley rats, mean initial body weight 244.8 grams, randomized into BD, 5% or 10% FF or FM groups.	Number of aberrant crypts in colon, proliferation index of colon cells, and urinary enterolactone and enterodiol excretion.	In the descending colon of FF and FM groups, the total number of aberrant crypts and foci were significantly reduced by 41-53% and 48-57% (all p<0.05), respectively. The labeling index (LI) was also 10-22% lower in these groups (p<0.05), except for the 5% FM group. FF and FM supplementation significantly increased the urinary enterolactone and enterodiol excretion.	The increase in the number of aberrant crypts and proliferation index is used as a risk marker of colon cancer. Thus, the reduction of them by FF and FM supplementation indicates some protective effects of FF and FM on colon tumor. For counted data, it is inappropriate to perform statistical analysis using one-way ANOVA.																																																					

Area investigated: anti-tumor/DNA damage

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments
Lin Yan, <i>Cancer Lett</i> 27, 124(2): 181-6, 1998	Dietary flaxseed supplementation and experimental metastasis of melanoma cells in mice	Design: control-treatment comparison. Treatment: flaxseed was added into diet at 3 levels (2.5, 5 and 10%) and used to feed the experimental mice those were injected melanoma cells.	Male C57BL/6 mice, 3-week old, randomly assigned into 4 groups, 15 mice in each group. Study period was 2 weeks.	Numbers of lung tumors, tumor cross-sectional area, and tumor volume.	The median numbers of lung tumor for control, 2.5%, 5% and 10% of flaxseed groups are 155, 106, 72 and 57, respectively; the median tumor cross-sectional area are 0.31, 0.26, 0.22, and 0.13 (mm ²), respectively; and the median tumor volumes are 0.21, 0.16, 0.12, and 0.05 (mm ³), respectively. All 3 variables in 5% and 10% flaxseed groups are significantly lower than the control.	Effect of dietary flaxseed on inhibiting pulmonary transplantation of injected melanoma cells with a dose-dependent manner was clearly noted in studied mice.
Li D, <i>Cancer Lett</i> 19, 142(1): 91-6, 1999	Dietary supplementation with secoisolariciresinol diglycoside (SDG) reduces experimental metastasis of melanoma cells in mice	Design: control-treatment comparison. Treatment: SDG was added into diet at 3 levels (74, 147 and 293 μ mol/kg) and used to feed the experimental mice those were injected melanoma cells.	Male C57BL/6 mice, 3-week old, randomly assigned into 4 groups, 15 mice in each group. Study period was 2 weeks.	Numbers of lung tumors, tumor cross-sectional area, and tumor volume.	The median numbers of lung tumor for control, 73, 147, and 293 μ mol/kg of SDG groups are 62, 38, 36 and 29 ($p < 0.01$ for the highest dose group), respectively. A significant lowering effect is noted. Dietary SDG also decreases tumor size in a dose-response manner.	This study is an identical experiment as above except replacing flaxseed with SDG. The similar effect of dietary SDG on inhibiting pulmonary transplantation of injected melanoma cells was noted. This implies that SDG is the major functional compound of flaxseed in inhibiting tumor cell transplantation. It is a wonder that the tumor numbers were so different between the 2 control groups.

Area investigated: anti-tumor

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments
Serraino M. <i>Cancer Lett</i> Nov; 60(2): 135-42, 1991	The effect of flaxseed supplementation on early risk markers for mammary carcinogenesis	Design: control-treatment comparison. Treatment: female rats were fed with basal diet (BD), BD+5% flaxseed flour (FF), BD+10% FF, BD+5% defatted flaxseed meal (FM), and BD+10% FM after carcinogen DMBA treatment. Feeding period: 4 weeks.	70 female Sprague-Dawley rats, 28 days of age, randomized into each group.	Mitotic index (MI), labeling index (LI), and nuclear aberrations (NA) of mammary gland cells.	Supplementation of a high-fat diet with FF or FM (5% or 10%) reduced the epithelial cell proliferation by 38.8-55.4% and NA by 58.8-65.9% in female rat mammary gland ($p < 0.05$), with optimum effects seen with the 5% FF. A significant correlation relationship between NA and urinary enterolactone and enterodiols excretion was noted ($r = 0.94$, $p < 0.025$).	The protective effects of FF and FM on risk of mammary carcinogenesis were observed. The correlation relationship between NA and urinary lignan excretion indicates that the effects may be related to lignan precursors in flaxseed.
Jenab M., <i>Carcinogenesis</i> Jun;17(6): 1343-8, 1996	The influence of flaxseed and lignans on colon carcinogenesis and beta-glucuronidase activity	Design: control-treatment comparison. Treatment: baseline diet (BD), BD+1.5 mg SDG/day, BS+2.5 or 5% flaxseed (F), and BD+2.5 or 5% defatted flaxseed (DF). Experiment period: 100 days.	61 male Sprague-Dawley rats, 40 days old, randomized into 6 groups, 16 in control and 9 in each treatment group.	Aberrant crypt formation (ACF), β -glucuronidase activity (β -GA), and urinary mammalian lignans (enterolactone and enterodiols, EL and ET).	Compared with control, 1. total numbers of ACF per focus decreased in all treatment groups (15-20%); 2. the total numbers of ACF decreased in all treatment groups (about 30%); 3. the total β -GA increased in F and DF groups (about 50-80%); 4. the total numbers of ACF were negatively correlated with the total β -GA ($r = 0.33$); and 5. the total EL+ED excretion was (nmol/day): 1600 for 5% DF group, 1280 for 5% F group, 900 for 2.5% DF group, 500 for 2.5% F group, 100 for SDG group, and < 10 for control group.	ACF are considered as valid putative preneoplastic markers of colon carcinogenesis. The data of this study indicate that F, DF, and SDG diets can inhibit ACF. The colon cancer protective effects are most likely due to the function of SDG. The reasons for the differences of urinary lignan excretion (between SDG and flaxseed groups) remain to be cleared.

Area investigated: anti-tumor

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments
Tou, Janet, <i>Carcinogenesis</i> 20(9): 1831-5, 1999	Exposure to flaxseed or its lignan component during different developmental stages influences rat mammary gland structures	Design: control-treatment comparison. Treatment: baseline diet (BD), BD+5% ground flaxseed (F), BD+10% F, BD+1.82% flaxseed oil (FO), or BD+1.5 mg SDG. The female offspring were also fed with the same diets as dams consumed (6 in each group). Experiment period: through lifetime, during gestation, lactation, or after weaning.	28 pregnant Sprague-Dawley rat dams and their offspring randomized into BD, 5% F, 10% F, FO, and SDG groups.	Mammary gland structures (terminal end buds-TEBs, alveolar buds-ABs, and lobule structure), puberty onset, estrous cycle, relative ovarian weight, and serum estradiol of offspring	Compared to BD group on post-natal day 50, TEBs reduced in the groups consuming BD+5% F, +10% F, and +SDG; ABs increased in the group consuming BD+10% F; and ABs decreased in the group consuming BD+SDG. Exposure to 5 or 10% flaxseed starting at weaning had no significant influence on TEBs and ABs. BD+10% flaxseed resulted in a younger age of puberty onset and longer estrous cycles, but BD+5% flaxseed and BD+SDG resulted in an older age of puberty onset and less number of estrous cycles. BD+10% flaxseed also resulted in relative ovarian weight and serum estradiol increase on post-natal day 50.	The observed variables are used to evaluate mammary gland structure and responses to sex hormones. A higher mammary tumor incidence is related to a higher density of TEBs. The effect of dietary flaxseed on reduction of TEBs suggests its protective potential against mammary tumors, although 5% and 10% flaxseed probably act with different way (antiestrogenic or estrogenic). In this study, 5% flaxseed diet contains a similar level SDG as BD+SDG. Their similar activity indicates that SDG is the major functional component of flaxseed. There are no obvious effects on testing variables noted from the exposure to flaxseed after weaning.
Thompson L., <i>Carcinogenesis</i> Jun;17(6):1 373-6, 1996	Flaxseed and its lignan and oil components reduce mammary tumor growth at a late stage of carcinogenesis	Design: control-treatment comparison. Treatment: baseline diet (BD), BD+2.5 or 5% ground flaxseed, and BD+1.82% flaxseed oil or 2200 nmol/day SDG. Feeding period: 7 weeks.	Female Sprague-Dawley rats, with tumors (diameter 1-2 cm) induced by carcinogen DMBA, randomized into BD, 5% and 10% flaxseed, oil, and SDG groups 19-21 in each group).	Mammary tumor volume, urinary enterolactone and enterodiol excretion, new tumor incidence, and dietary fatty acid composition.	Compared with BD group, tumor volumes were reduced at least 50% for all treatment groups; new tumor volume was reduced more than 70% in SDG group; total tumor volumes were reduced more than 50% in SDG, 2.5 and 5% flaxseed groups; and SDG and 2.5% flaxseed groups had significantly lower incidences of new tumors, Established tumor volumes, not new tumor volumes, were negatively correlated with urinary lignan excretions (r=0.997). No treatment toxicity was noted.	The inhibiting effects of dietary flaxseed and SDG on the growth of established tumors and development of new tumors were documented. Why 5% flaxseed supplementation did not significantly lower the tumor incidence remains to be explained. The inhibitory effects may be related to the repression of aromatase and angiogenesis by lignans.

Area investigated: anti-tumor

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments																																																												
Sharon E. Richard, <i>Nutr Cancer</i> 35(1), 50-57, 1999	Dose effects of flaxseed and its lignan on N-methyl-N-nitroso-urea-induced mammary tumorigenesis in rats	Design: control-treatment comparison. 2-day after injection of carcinogen N-methyl-N-nitrosourea (50 mg/kg bw, MNU), rats were fed basal diet (BD), BD supplemented with 2.5, 5% flaxseed (F), or SDG by gavage, SDG doses matched with the SDG contents of 2.5% (LSDG) and 5% (HSDG) F. The study lasted for 22 weeks.	155 female Spague-Dawley rats, 50 days of age, randomly assigned into 5 groups.	Tumor incidence (TI, %), mean tumor volume (TV, cm ³), tumor numbers (TN) per rat, tumor multiplicity (TM), mean tumor weight (TW, g), and SDG daily consumption (SDG-C, mg).	<p>At the end of the study:</p> <table border="1"> <thead> <tr> <th>Treat</th> <th>n</th> <th>TI</th> <th>TN</th> <th>TM</th> <th>TW</th> </tr> </thead> <tbody> <tr> <td>BD</td> <td>30</td> <td>93</td> <td>142</td> <td>5.1</td> <td>1.34</td> </tr> <tr> <td>2.5 F</td> <td>31</td> <td>90</td> <td>144</td> <td>4.9</td> <td>1.3</td> </tr> <tr> <td>5 F</td> <td>31</td> <td>87</td> <td>173</td> <td>6.3</td> <td>0.96</td> </tr> <tr> <td>LSDG</td> <td>31</td> <td>94</td> <td>213</td> <td>7.0</td> <td>1.14</td> </tr> <tr> <td>HSDG</td> <td>32</td> <td>100</td> <td>127</td> <td>3.8</td> <td>1.34</td> </tr> </tbody> </table> <table border="1"> <thead> <tr> <th>Treat</th> <th>n</th> <th>TV</th> <th>SDG-C</th> </tr> </thead> <tbody> <tr> <td>BD</td> <td>30</td> <td>0.83</td> <td>-</td> </tr> <tr> <td>2.5 F</td> <td>31</td> <td>0.84</td> <td>0.71</td> </tr> <tr> <td>5 F</td> <td>31</td> <td>0.62</td> <td>1.41</td> </tr> <tr> <td>LSDG</td> <td>31</td> <td>0.76</td> <td>0.70</td> </tr> <tr> <td>HSDG</td> <td>32</td> <td>0.82</td> <td>1.43</td> </tr> </tbody> </table>	Treat	n	TI	TN	TM	TW	BD	30	93	142	5.1	1.34	2.5 F	31	90	144	4.9	1.3	5 F	31	87	173	6.3	0.96	LSDG	31	94	213	7.0	1.14	HSDG	32	100	127	3.8	1.34	Treat	n	TV	SDG-C	BD	30	0.83	-	2.5 F	31	0.84	0.71	5 F	31	0.62	1.41	LSDG	31	0.76	0.70	HSDG	32	0.82	1.43	In this study, no clear conclusion can be drawn. In HSDG group, TN and TM are the lowest but TI is the highest. In LSDG group, TN and TM are the highest but TW is the second lowest. In 5 F group, TW and TV are the lowest but TN is the second highest. The authors mentioned potential reasons for the study results that are not consistent with other studies. We believe that the dose of the carcinogen (MNU) used in this study is too high. The high dose MNU caused more than 90% of rats suffered from tumors and each tumor-bearing rat at least grew 4 tumors (mean value). The potent tumor induction can erase all possible effects of an anti tumor factor.
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Demark-Wahnefried, W. <i>Urology</i> 58:47-52, 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.	All subjects were fed 30g flaxseed/d	25 patients with prostate cancer.	PSA, testosterone, androgen index, Total serum cholesterol, tumor histopathologic findings by Apoptotic index (Tunel), and MIB-1 proliferation index.	<p>At the end of the study:</p> <table border="1"> <thead> <tr> <th>Measure</th> <th>PSA</th> <th>T</th> <th>Andro</th> <th>Choles</th> </tr> </thead> <tbody> <tr> <td>Baseline</td> <td>8.14</td> <td>422</td> <td>36.3</td> <td>200.8</td> </tr> <tr> <td>Follow-up</td> <td>8.50</td> <td>360</td> <td>29.3</td> <td>174.3</td> </tr> </tbody> </table> <p>Cholesterol was reduced (-26.5 mg/dl, P<0.001) with treatment compared to baseline measure.</p> <p>TUNEL apoptotic index was significantly lowered with flaxseed treatment (P=0.01) compared to historical controls.</p> <p>MIB-1 proliferation index also decreased (P=0.05).</p>	Measure	PSA	T	Andro	Choles	Baseline	8.14	422	36.3	200.8	Follow-up	8.50	360	29.3	174.3	The data from this preliminary study suggest that flaxseed supplementation, on a fat-restricted diet appeared to lower proliferation index and increase apoptosis in prostate cancer.																																													
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Area investigated: anti-tumor

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments
Thompson, L.U. Clin Cancer Res 2005; 11(10): 3828-3835	Dietary flaxseed alters tumor biological markers in postmenopausal breast cancer.	Muffins containing 25 g flaxseed or control muffins were fed for 32-39 days.	Treated (N=19) Control (N=13) Patients were randomized to treatment or control group. Menopausal women (>6 m). Ages ranged from 50-88 yr. Average age ~67 years. Patients had a positively diagnosed breast carcinoma, confirmed by breast core biopsy.	Cell proliferation, Ki-67 labeling index, apoptosis, c-erb-2 expression and ER and Progesterone receptor (PR) expression.	Changes compared to baseline for flaxseed treatment: (P<0.01) <u>Ki-76 index</u> -5.2 % <u>Apoptosis index</u> -0.27 % <u>C-erb2 score</u> -0.22 There were no significant changes in the control group There were no changes in ER expression or PR expression. The percentage change in the flaxseed group compared to the placebo group was significantly higher for the apoptotic index and c-erb2 score.	This study shows that daily intake of 25 g of flaxseed can significantly reduce tumor cell proliferation, increase apoptosis, and affect tumor cell signaling by decreasing c-erb2 expression in breast cancer cells. These improvements were significantly correlated with the total amount of flaxseed consumed. In addition, the expression of c-erb2 has been associated with more aggressive breast cancer phenotypes, as this molecule plays a role in cell differentiation, adhesion, and motility.
Pietinen, P. Cancer Epi Biomark Prevent 2001; 10 339-344.	Serum enterolactone and risk of breast cancer: a case-control study in Eastern Finland.	Breast cancer patients and comparative population controls were examined for serum enterolactone levels.	194 breast cancer patients participants of the Kuopio Breast Cancer Study. Patients had a suspected lump or breast symptom referred for study. Comparative population controls were selected from the same geographical area and matched by age to the patients.	Serum enterolactone, food frequency questionnaire, anthropometric measures, and lifestyle questionnaire	For all women examined, serum enterolactone (EL) results were grouped into quintiles. Compared to women that had less than 6.19 nmol EL/l, women in the highest level of EL quintile (>34.8 nmol/l) had an odds ratio (OR) of 0.38. The overall trend is that there is a significant reduction in the risk of breast cancer (P=0.01) with increasing levels of circulating EL. This trend remained when adjusted for lifestyle variables (P=0.03).	This shows the daily intake of lignans in the diet, from a variety of sources commonly consumed, does correlate with a decreased risk of breast cancer. This study shows that serum enterolactone is associated with a substantial reduction in breast cancer risk.

Area investigated: kidney disease

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments
Ogborn M, <i>Kidney International</i> . 55(2):417-23, 1999 Feb.	Flaxseed ameliorates interstitial nephritis in rat polycystic kidney disease.	Design: control-treatment comparison. Treatment: 10% flaxseed + control chow diet. Study period: 8 weeks.	Male Han: SPRD-cy rats were fed a 10% flaxseed or control rat chow diet for eight weeks from weaning.	Tissue was harvested for analysis of cystic change, apoptosis, cell proliferation, and fibrosis. Tissue was also harvested for lipid analysis using gas chromatography	Noted effects of flaxseed diet are: 1. modest reduction of kidney cystic change; 2. reduction of renal fibrous volume; 3. decrease of the numbers of macrophages infiltrating the kidney; 4. decrease of serum creatinine levels; and 5. no effect on lowering blood cholesterol concentrations.	Amelioration of poly-cystic renal disease by flaxseed consumption was noted in the studied rats. The lignans and PUFA in flaxseed could be the functional compounds related to the amelioration.
Clark et al, <i>United States Patent, #5837256, Lupus</i> 2000;9(6):429-36	Method for Treatment of Lupus Nephritis	SDG used at doses up to 4.8 mg/mouse (about 150 mg/kg BW).	Mice	Renal function-proteinuria and glomerular filter rate (GFR)	Renal function improved. Changes in proteinuria, GFR and renal size showed a time- and dose-dependent protection for the lignan precursor.	This patent was based on limited data obtained from mouse studies. Very high dose of SDG was used (up to 150 mg/kg, orally). The purity of SDG was about 90%. The tolerance of high dose used may indicate that the toxicity of SDG is low.
Clark WF, <i>Kidney Int</i> 48:475-80, 1995	Flaxseed: a potential treatment for lupus nephritis	Design: self-controlled repeated measurement. After the baseline studies, 8 patients were given 15, 30, and 45 g of flaxseed/day sequentially at four week intervals.	Patients with lupus nephritis	Compliance, disease activity, blood pressure, plasma lipids, rheology, platelet activating factor (PAF)-induced platelet aggregation, renal function, and serum immunology.	TC and LDLC, and blood viscosity were significantly reduced with 30 g and to a lesser extent with 45 g doses. PAF- induced platelet aggregation was inhibited by all doses. There was a significant decline in serum creatinine with 30 and 45 g, and a concomitant increase in creatinine clearance with increasing flaxseed dose. Proteinuria was reduced with 30 g and to a lesser extent with 45 g of flaxseed. Complement C3 was significantly elevated by all three doses.	The authors concluded: 30 g flaxseed/day was well tolerated and conferred benefit in terms of renal function as well as inflammatory and atherogenic mechanisms. <i>*this summary was based on abstract.</i>

Area investigated: anti-oxidation

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments
Uttara Pattanaik, <i>J Cardiovas. Pharmacol. Therapeutics</i> Vol 3 No. 4, 305-18, 1998	Oxygen free radicals and endotoxic shock: effect of flaxseed	Design: control-treatment comparison. Treatment: 5 mg/kg bw of endotoxin (from <i>Escherichia Coli</i>) injected intravenously to induce endotoxic shock in dogs consuming flaxseed (2 g/kg/day) for 6-days or not.	Mongrel dogs, 15-30 kg of body weight, in control group, n=5; in flaxseed group, n=8; and in non-flaxseed group, n=10.	Plasma creatine kinase activity, lactate concentration, oxyradical-producing activity, antioxidant reserve, antioxidant enzyme activity, cardiac malondialdehyde concentration, lipid peroxidation product, and hemodynamics.	It was shown that flaxseed offered some protection in early stage of endotoxic shock. This favorable effect mainly exhibited in hemodynamic parameters, plasma creatine kinase activity, and lactate levels.	Pretreatment with flaxseed attenuated endotoxin-induced cardiac dysfunction and cellular damage in the studied dogs. The protective effect of flaxseed may be due to its anti-oxidation activity.

Area investigated: reproductive organs of offspring

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments
Janet C Tou, <i>J Nutr</i> Nov;128 (11):1861-8, 1998	Flaxseed and its lignan precursor, secoisolariciresinol diglycoside, affect pregnancy outcome and reproductive development in rats	Design: control-treatment comparison. Treatment: basal diet and basal diet supplemented with 5%, 10% flaxseed, or a daily gavage of 1.5 mg SDG. Study period: whole gestation and lactation.	28 Sprague-Sawley pregnant rats, randomly assigned into 4 groups.	In offspring of the studied rat dams, body weight, reproductive organ weight, puberty onset age, estrous cycle and length and anogenital distance.	1. 10% flaxseed diet lowered birth weight (postnatal day 3) by 10.2% and increased prostate weight by 30% in male offspring; 2. in female offspring, 10% flaxseed diet increased ovarian relative weights by 27%, shortened anogenital distance by 15%, shortened puberty onset 4.6 days, and lengthened estrous cycle 2.4 days; 3. however, 5% flaxseed diet reduced immature ovarian relative weight and delayed puberty onset 4.7 days. SDG had similar effects as 5% flaxseed diet. (For all, $p < 0.05$)	In this rat study, some effects of dietary flaxseed and SDG on offspring reproductive development were noted. How to interpret and use these outcomes to humans is not clear. The authors suggested that caution should be paid consuming flaxseed during pregnancy and lactation.
Sprando RL <i>Food Chem Toxicol</i> 2000 Oct;38(10):887-92	Testing the potential of flaxseed to affect spermatogenesis: morphometry	Design: control-treatment comparison. Maternal and postnatal dietary exposure to flaxseed (20 or 40%), flaxseed meal (13 or 26%) or standard NIH AIN-93 feed (0% flaxseed control), gestation and lactation period for dams and 70 days for pups.	Pregnant rat dams and F1 generation of male pups (5 in each group).	The seminiferous tubules volume and the interstitial space volume for all groups.	The seminiferous tubules comprised 86%, 84%, 84%, 84% and 85% of the total testis volume, while the interstitial space comprised 12%, 14%, 14%, 14%, 13% of the total testis volume for the 0%, 20% flaxseed, 13% flaxseed meal, 40% flaxseed and 26% flaxseed meal groups, respectively. Statistically significant decreases in the absolute volume of the seminiferous tubules were observed in the 20% and 40% flaxseed-treated groups compared to controls. These effects were not considered biologically significant because other parameters of male reproductive function appeared normal.	The authors concluded: "overall, the quantitative information obtained suggests that exposure to flaxseed/flaxseed meal at the doses used in the present study does not adversely affect testis structure or spermatogenesis in the rat".

Area investigated: metabolism/bio-availability

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments
Sharon E. Rickard, <i>J Nutri</i> , 126: 2012-9, 1996	Dose-dependent production of mammalian lignans in rats and in vitro from the purified precursor secoisolariciresinol diglycoside in flaxseed.	Design: in vivo and vitro experimental observation. In vivo, 42 rats were randomly divided into 7 groups, and fed basal diet (BD), BD + 2.5, 5, or 10g ground flaxseed per 100 g BD, or BD+ 1.1, 2.2, or 4.4 $\mu\text{mol/day}$ SDG for 2 weeks. In vitro, 1.1, 2.2 and 4.4 μmol SDG fermented with human fecal inoculum for 24 hrs.	42 female Sprague-Dawley rats, 43-day old, with mean body weight 204.8 grams.	Urinary SDG, enterolactone and enterodiol excretion and their production in fermentation.	1. Urinary excretions of lignans are linearly correlated with the intakes of flaxseed/SDG doses under levels of 5% flaxseed or 2.2 $\mu\text{mol/day}$ SDG (both $r>0.8$, $p<0.001$). 2. Urinary lignan excretions in rats fed SDG are much lower than in rats fed flaxseed (about 26-30%) 3. Enterolactone and enterodiol production by SDG fermentation has similar trend compared to in vivo experiment.	This rat study indicates that urinary lignan excretion is linearly correlated with intake levels of flaxseed or SDG within a dose range. Why rats fed SDG have only 26-30% of lignan (enterolactone, enterodiol and SDG) excretion of rats fed flaxseed in which contains equivalent amount of SDG needs to be revealed.
Adlercreutz, H, <i>J Steroid Biochem Mol Biol</i> 1995 Jan; 52(1):97-103	Lignan and isoflavonoid conjugates in human urine	Design: experimental observation. The pattern and concentrations of conjugation of the phytoestrogens in urine samples were determined.	6 people involved. 4 women, 2 of them consumed soy products, 2 of them were vegetarians, 2 men consumed ordinary Finnish diet.	Seven compounds were determined: enterodiol, enterolactone, matairesinol, diadzein, equol, genistein and O-desmethylangolensin.	The three lignans are excreted mainly as monoglucuronides (MG) by 73-94%, and a small part occurs as monosulfate (MS) by 2-10%. For isoflavones, diadzein is excreted 79-82% as MG and 6-17% as sulphoglucuronide (SG); genistein 53-76% as MG and 12-26% as diglucuronide; equol 32-93% as MG and 0-43% as SG; and O-desmethylangolensin 97% as MG.	In the article, it is indicated that most part of urinary lignans are their monoglucuronides and the forms of urinary isoflavones appear differently.

Area investigated: metabolism/ bioavailability

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments																											
Marton MS, <i>J Endocrinol</i> 1994 Aug;142(2): 251-9	Determination of lignans and isoflavonoids in human female plasma following dietary supplementation.	Design: plasma lignans and isoflavonoids under dietary conditions. Supplementation of soy flour (45g/d), linseed (flaxseed, 25g/d), or clover sprouts (equivalent to 10 g/d dry seed) were added on ordinary diet for 2 weeks, sequentially.	29 postmenopausal women (23 of them completed the study), healthy, non-smoker, not in using of any drugs which could influence sex hormone levels.	Plasma concentrations of enterodiol, enterolactone, daidzein, equol and genistein	Including control and supplemented diets, the concentration ranges of measured compounds are (ng/ml): enterodiol 1.85-390, enterolactone 41.8-244, daidzein 2.74-153, equol 1.28-106.3, and for genistein, there was a standard problem and only part of samples were measured.	This study shows that: 1, dietary flaxseed obviously increases plasma enterodiol and enterolactone concentrations (up to 200 and 48 times, respectively). Without supplementation, enterolactone concentration is 5-20 times higher than enterodiol; 2, dietary soy flour can increase plasma daidzein (up to 46 times); 3, only one third of the subjects (4 from 12) are able to metabolize daidzein into equol. The reason and significance of this is unclear.																											
Lamoe JW, <i>Am J Clin Nutr</i> 1994 Jul; 60(1):122-8	Urinary lignan and isoflavonoid excretion in premenopausal women consuming flaxseed powder	Design: Urinary excretion of lignans and isoflavonoids under supplementation of dietary flaxseed powder at 10g/day, for 3 menstrual cycles.	30 premenopausal women selected, only 18 of them completed the study, mean age 27 and BMI 22.2.	Urinary excretion of enterodiol, enterolactone, daidzein, genistein, equol, and O-desmethy-langolensin	<table border="1"> <thead> <tr> <th></th> <th>Control</th> <th>Flaxseed</th> </tr> <tr> <th></th> <th colspan="2">$\mu\text{mol/day (LSM)}$</th> </tr> </thead> <tbody> <tr> <td>Enterodiol</td> <td>1.09</td> <td>19.48</td> </tr> <tr> <td>Enterolactone</td> <td>3.16</td> <td>27.79</td> </tr> <tr> <td>Total lignans</td> <td>3.74</td> <td>47.9</td> </tr> <tr> <td>Daidzein</td> <td>1.93</td> <td>1.65</td> </tr> <tr> <td>Genistein</td> <td>0.29</td> <td>0.30</td> </tr> <tr> <td>Equol</td> <td>0.11</td> <td>0.12</td> </tr> <tr> <td>Total isoflav.</td> <td>2.56</td> <td>2.17</td> </tr> </tbody> </table>		Control	Flaxseed		$\mu\text{mol/day (LSM)}$		Enterodiol	1.09	19.48	Enterolactone	3.16	27.79	Total lignans	3.74	47.9	Daidzein	1.93	1.65	Genistein	0.29	0.30	Equol	0.11	0.12	Total isoflav.	2.56	2.17	This study shows that 10 g/day flaxseed powder supplement increases urinary lignan excretion about 20 times and has no influence on urinary isoflavone excretion. Phases of menstrual cycles have no influence on both of lignan and isoflavone excretion.
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Area investigated: metabolism/ bioavailability

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments												
Hutchins AM, Cancer Epidemiol Biomarkers Prev 2000 Oct;9(10):113-8	Flaxseed influences urinary lignan excretion in a dose-dependent manner in postmenopausal women	Design: experiment observation. Ground flaxseed, at 0, 5, or 10 grams per day, were added to habitual diets in 31 women during 3 7-week feeding periods.	31 postmenopausal women, with an age range 52-82.	Urinary enterodiol, enterolactone and matairesinol excretion.	Compared to 0g treatment, excretions of lignan metabolites are (nmol/day): <table border="1"> <thead> <tr> <th></th> <th>5g</th> <th>10g</th> </tr> </thead> <tbody> <tr> <td>Enterodiol</td> <td>1009</td> <td>2867</td> </tr> <tr> <td>Enterolactone</td> <td>21242</td> <td>52862</td> </tr> <tr> <td>Total</td> <td>24333</td> <td>60640</td> </tr> </tbody> </table>		5g	10g	Enterodiol	1009	2867	Enterolactone	21242	52862	Total	24333	60640	Excretion of enterodiol and enterolactone increased by consumption of flax in a dose-dependent manner in this group of postmenopausal women. Urinary excretion of lignan metabolites is a dose-dependent biomarker for flaxseed intake within the doses consumed.
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Area investigated: metabolism/ bioavailability

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments																								
Sharon E Rickard, <i>J Nutr</i> Mar;128(3): 615-23, 1998	Chronic exposure to secoisolariciresinol diglycoside alters lignan disposition in rats	Design: experimental observation. Radioactively labeled SDG (tracer) was gavaged into rats either pretreated with 1.5 mg/day SDG for 10 days or without this pretreatment. Then distribution and excretion of SDG were determined.	24 female Sprague-Dawley rats, 70 to 72-day old, assigned into 2 groups. The experiment lasted 48 hours.	Radioactivity of plasma, urine, feces, gastrointestinal contents, muscle, adipose, liver, kidney, uterus, and the other organs.	Within 24 hours, kidney, liver, and uterus have higher radioactivity than the other organs; SDG pre-loaded group has higher tissue tracer levels, higher levels of gastrointestinal residues, and slower fecal and urinary excretion. Dietary SDG were excreted >80% (>50% from feces and about 30% from urine) by 48 hours in both groups.	The study provides some useful parameters regarding to SDG tissue distribution and excretion. It is suggested that continuous administration of SDG do not cause its excretion delayed. This is an evidence of safety for using dietary SDG supplement. In this study, no data before 12 hours was generated. It obviously limits the possibility to obtain more important kinetic parameters, such as absorption rate, tissue specific clearance rate.																								
Morton MS, <i>Prostate</i> July 1;32(2): 122-8, 1997	Lignans and isoflavonoids in plasma and prostatic fluid in men: samples from Portugal, Hong Kong, and the United Kingdom	Design: report of clinical measurement of lignans and isoflavones in plasma and prostatic fluids.	For plasma levels, 53 men from Hong Kong, 50 men from Portugal, and 36 men from British; for prostatic fluid measurement, 20 men from Hong Kong, 22 men from Portugal, and 17 men from British; range of age 31-85.	Plasma (Pla) and prostatic fluid (PF) enterolactone (ENL), enterodiol (END), equol (EQ), and daidzein (D).	<p>Plasma (ng/ml):</p> <table border="1"> <thead> <tr> <th></th> <th>ENL+END</th> <th>EQ+D</th> </tr> </thead> <tbody> <tr> <td>H.K.</td> <td>7.9</td> <td>35.1</td> </tr> <tr> <td>Lisbon</td> <td>4.5</td> <td>1.65</td> </tr> <tr> <td>U.K.</td> <td>3.9 (ENL)</td> <td>8.77</td> </tr> </tbody> </table> <p>Prostatic fluid (ng/ml):</p> <table border="1"> <thead> <tr> <th></th> <th>ENL+END</th> <th>EQ+D</th> </tr> </thead> <tbody> <tr> <td>H.K.</td> <td>78.5</td> <td>32.7</td> </tr> <tr> <td>Lisbon</td> <td>6.32</td> <td>175.5</td> </tr> <tr> <td>U.K.</td> <td>11.8</td> <td>22.9</td> </tr> </tbody> </table> <p>There was a correlation between the levels of Pla and PF daidzein noted ($r = 0.71, p = 0.001$).</p>		ENL+END	EQ+D	H.K.	7.9	35.1	Lisbon	4.5	1.65	U.K.	3.9 (ENL)	8.77		ENL+END	EQ+D	H.K.	78.5	32.7	Lisbon	6.32	175.5	U.K.	11.8	22.9	This is a report of clinical measurements for some lignans and isoflavones in plasma and PF. Because of the small sample sizes, its representative significance is not certain.
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Area investigated: metabolism/bio-availability

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments																																							
Jensb M., <i>Nutr Cancer</i> 33(2):154-8, 1999	Flaxseed and lignans increase cecal beta-glucuronidase activity in rats	Design: observation of experiment. Seven groups rats each were fed, for four weeks, a basal high-fat (20%) diet (BD), BD supplemented with 2.5%, 5.0%, or 10.0% flaxseed, or BD with daily gavage of 0.75, 1.5, or 3.0 mg of SDG (correspond to flaxseed diet levels).	48 female Sprague-Dawley rats, 57-day old, separated into 7 groups, 6 in each. The experiment lasted 4 weeks.	β -glucuronidase specific and total activity of cecal samples. Urinary excretion of enterolactone and enterodiol.	Cecal β -glucuronidase activity was significantly induced by SDG and flaxseed supplementation. The specific and total activities of β -glucuronidase in the cecum were significantly related to the levels of flaxseed ($r = 0.539$, $p < 0.008$ and $r = 0.599$, $p < 0.002$, respectively) and SDG ($r = 0.567$, $p < 0.007$ and $r = 0.435$, $p < 0.04$, respectively). The urinary mammalian lignan excretion also increased with increasing flaxseed or SDG levels and thus was significantly related to the specific activity ($r = 0.38$, $p < 0.017$) and total activity ($r = 0.429$, $p < 0.007$) of β -glucuronidase.	It is clearly shown that SDG and flaxseed intake can induce cecal β -glucuronidase activity and both of them have similar ability and pattern for doing so. It was suggested that increased β -glucuronidase activity might activate conjugated carcinogen or mutagen through hydrolysis. However, studies show SDG or flaxseed are colon cancer protective. It can be explained by that lignans competitively inhibit hydrolysis of conjugated carcinogens, together with other anti-tumor activities of lignans. As reported by other studies, flaxseed intake can increase urinary lignan excretion much more efficiently (about 5-fold in this study) than does SDG intake. This phenomenon remains to be further understood.																																							
Rowland lan, <i>Nutr Cancer</i> . 2000;36(1): 27-32	Interindividual variation in metabolism of soy isoflavones and lignans: influence of habitual diet on equol production by the gut microflora	Design: experimental observation with crossover feeding soy-containing food low (0.9 mg daidzein and 1 mg genistein) or high (21.2 mg daidzein and 34.8 mg genistein) in isoflavones. Each treatment period lasted for 17 days.	24 adults, 19 F and 5 M, mean age 30, and mean BMI 22.5	Plasma and urinary isoflavones and lignans.	<table border="1"> <thead> <tr> <th></th> <th>Low Iso.</th> <th>High Iso.</th> </tr> </thead> <tbody> <tr> <td>Pla levels nmol/L</td> <td></td> <td></td> </tr> <tr> <td>Enterodiol</td> <td>1\pm1</td> <td>2\pm2</td> </tr> <tr> <td>Enterolactone</td> <td>31\pm40</td> <td>27\pm30</td> </tr> <tr> <td>Daidzein</td> <td>14\pm9</td> <td>317\pm247</td> </tr> <tr> <td>Equol</td> <td>2\pm2</td> <td>76\pm137</td> </tr> <tr> <td>Genistein</td> <td>28\pm20</td> <td>779\pm541</td> </tr> <tr> <td>Urine levels nmol/day</td> <td></td> <td></td> </tr> <tr> <td>Enterodiol</td> <td>439\pm594</td> <td>515\pm358</td> </tr> <tr> <td>Enterolactone</td> <td>3898\pm 4485</td> <td>2804\pm 3053</td> </tr> <tr> <td>Daidzein</td> <td>1060\pm 404</td> <td>17969\pm10952</td> </tr> <tr> <td>Equol</td> <td>43\pm41</td> <td>3995\pm 6393</td> </tr> <tr> <td>Genistein</td> <td>384\pm265</td> <td>10326\pm 8346</td> </tr> </tbody> </table>		Low Iso.	High Iso.	Pla levels nmol/L			Enterodiol	1 \pm 1	2 \pm 2	Enterolactone	31 \pm 40	27 \pm 30	Daidzein	14 \pm 9	317 \pm 247	Equol	2 \pm 2	76 \pm 137	Genistein	28 \pm 20	779 \pm 541	Urine levels nmol/day			Enterodiol	439 \pm 594	515 \pm 358	Enterolactone	3898 \pm 4485	2804 \pm 3053	Daidzein	1060 \pm 404	17969 \pm 10952	Equol	43 \pm 41	3995 \pm 6393	Genistein	384 \pm 265	10326 \pm 8346	<p>This study shows that there is a large variation of urinary excretion responding to dietary isoflavones among individuals.</p> <p>Based on the data reported in this paper, urinary excretion of mammalian lignans per day can be 20-100 fold of plasma pools. It implies that plasma lignans can be cleared very efficiently, if the measurement is accurate.</p>
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Area investigated: hormones

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments																			
Haggans CJ., <i>Nutr Cancer</i> 33(2):188-95, 1999	Effect of flaxseed consumption on urinary estrogen metabolites in postmenopausal women	Design: randomized crossover, with 3 7-week treatments, 0, 5, or 10 g/day ground flaxseed added in usual diet of subjects.	28 (from 34) postmenopausal women, with mean age 68.3 years old. and mean BMI 23.9.	Urinary excretion of the estrogen Metabolites, 2-hydroxyestrogen (2-OHE), 16 α -hydroxyestrone (16 α -OHE1), and 2-OHE/16 α -OHE1 ratios.	<p>Urinary concentrations of 2-OHE, 16-α-OHE1 (ug/24 hr urine), and their ratios:</p> <table border="1"> <thead> <tr> <th rowspan="2"></th> <th colspan="3">Flaxseed (g/day)</th> </tr> <tr> <th>0</th> <th>5</th> <th>10</th> </tr> </thead> <tbody> <tr> <td>2-OHE</td> <td>10.72</td> <td>12.09</td> <td>14.39</td> </tr> <tr> <td>16-α-OHE1</td> <td>2.67</td> <td>2.60</td> <td>2.97</td> </tr> <tr> <td>Ratios</td> <td>4.02</td> <td>4.64</td> <td>4.85</td> </tr> </tbody> </table> <p>Flaxseed supplementation significantly increased urinary 2-OHE excretion ($p < 0.0005$) and the urinary 2/16 α-OHE1 ratio ($p < 0.05$) in a linear, dose-response fashion ($r=0.90$, $p<0.0005$ and $r=0.88$, $p<0.05$, respectively). There were no significant differences in urinary 16 α-OHE1 excretion.</p>		Flaxseed (g/day)			0	5	10	2-OHE	10.72	12.09	14.39	16- α -OHE1	2.67	2.60	2.97	Ratios	4.02	4.64	4.85	16 α -OHE1 metabolite is proposed to be associated with increased risk of breast cancer, while 2-OHE metabolite is proposed to be protective against breast cancer. 2-OHE/16 α -OHE1 ratios are also suggested to be correlated with the risk of breast cancer. Therefore, the observed effects of flaxseed supplementation on the increases of 2-OHE and 2-OHE/16 α -OHE1 ratios indicate a mechanism of anti-breast-cancer function of flaxseed.
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Area investigated: bone loss

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments																														
Kardinaal AFM, <i>Eur J Clinical Nutr</i> 53: 850-55, 1998	Phyto-oestrogen excretion and rate of bone loss in postmenopausal women	Design: secondary analysis of a 10-year follow-up study for bone loss and habitual calcium intake. For each subject, bone loss was measured annually. Phytoestrogen levels were analyzed in an aggregate urine sample (one 24-hr collection each year) 7 years after the end of original study.	32 postmenopausal women with $\leq 0.5\%$ of bone loss (BL) per year, and 35 postmenopausal women with $\geq 2.5\%$ of BL per year were selected from 154 subjects.	Percentage of bone loss, urinary levels of genistein (G), daidzein (D), equol (E), total isoflavones (Tiso) enterolactone (ENL) and BL. Some related factors such as body weight, BMI, vege-protein intake, fiber intake, and Correlation coefficients (β) between BL and urinary phytoestrogen levels were also determined.	Urinary concentrations of phytoestrogens (medians) and β : (1979-89) <table border="1"> <thead> <tr> <th></th> <th>BL$\leq 0.5\%$</th> <th>BL$\geq 2.5\%$</th> </tr> </thead> <tbody> <tr> <td>G</td> <td>124</td> <td>88</td> </tr> <tr> <td>D</td> <td>363</td> <td>282</td> </tr> <tr> <td>E</td> <td>15</td> <td>12</td> </tr> <tr> <td>Tiso</td> <td>410</td> <td>350</td> </tr> <tr> <td>ENL</td> <td>838</td> <td>1108</td> </tr> </tbody> </table> <table border="1"> <thead> <tr> <th></th> <th>Correlation β</th> </tr> </thead> <tbody> <tr> <td>G</td> <td>0.044</td> </tr> <tr> <td>D</td> <td>0.014</td> </tr> <tr> <td>E</td> <td>0.142</td> </tr> <tr> <td>Tiso</td> <td>0.042</td> </tr> <tr> <td>ENL</td> <td>0.009*</td> </tr> </tbody> </table> * $p < 0.05$		BL $\leq 0.5\%$	BL $\geq 2.5\%$	G	124	88	D	363	282	E	15	12	Tiso	410	350	ENL	838	1108		Correlation β	G	0.044	D	0.014	E	0.142	Tiso	0.042	ENL	0.009*	<p>The article is a secondary analysis based on data and samples of another study. The authors concluded: "our results do not support a preventive effect of low, unsupplemented dietary intake of phytoestrogens on postmenopausal bone loss. However, no conclusions can be drawn about effects of high doses of phytoestrogens." Considering the followings, the conclusion is weak.</p> <ol style="list-style-type: none"> 1. Urinary concentrations of isoflavones and mammalian lignans are very sensitive to dietary intakes. Random one day urine sample of each year would very likely have accidental error within or between subjects. The data obtained in this study showed this potential problem with its non-normal distribution and very big range (even though urine samples were combined together). This would make correlation analysis less meaningful and achievable. 2. In the correlation model, only age, BMI, and years since menopausal were used as adjusting factors. It may be not enough. As indicated by the authors, enterolactone was correlated with vege-protein intake, fiber intake, and BW. 3. The reported urinary total isoflavone concentrations are smaller than the values of G+D. This would not be considered as a correct measurement. It needs to be confirmed which part is incorrect.
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Area investigated: diabetes

Author & Journal	Title of article	Design & treatment groups	Characters of subjects	Main Variables observed	Results	Comments
Prasad K., <i>Mol Cell Biochem</i> , 206:141-9, 2000	Protective effect of secoisolariciresinol diglucoside against streptozotocin-induced diabetes and its mechanism	Design: control-treatment comparison. Group I, Control; Group II, SDG (22 mg/kg body wt, orally) for 24 days; Group III, streptozotocin (STZ, 80 mg/kg intraperitoneally); Group IV, SDG days prior to STZ and 21 days thereafter	Rats.	Malondialdehyde (MDA, lipid peroxidation product) Pancreatic antioxidant reserve (pancreatic-CL) Oxygen free radical producing activity of white blood cells (WBC-CL) Incidence of diabetes	Incidence of diabetes was 100% in group III and 25% in group IV. SDG lowered 75%. Development of diabetes was associated with an increase in serum and pancreatic MDA and WBC-CL, and a decrease in pancreatic antioxidant reserve	Streptozotocin-induced diabetes here is type-I diabetes. SDG effectively inhibits its occurrence by a 75% at a relatively high dose.
Prasad K., <i>Mol Cell Biochem</i> , 209:89-96, 2000	Oxidative stress as a mechanism of diabetes in diabetic BB prone rats: effect of secoisolariciresinol diglucoside.	Design: control-treatment comparison. Group I, BioBreeding normal rats (BBn rats) (n = 10); group II, BBdp untreated (n = 11); and group III, BBdp treated with SDG 22 mg/kg body wt, orally) (n = 14)	Diabetic prone BioBreeding rats (BBdp rats) and BioBreeding rats (BBn rats)	Malondialdehyde (MDA, lipid peroxidation product) Pancreatic chemiluminescence (Pancreatic-CL, a measure of antioxidant reserve) Incidence of diabetes	SDG prevented the development of diabetes by approximately 71%. Development of diabetes was associated with an increase in serum and pancreatic MDA and a decrease in antioxidant reserve.	The effect of SDG on diabetes prevention is demonstrated. The dose of SDG used is relatively high (22mg/d/kg body weight).