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Rye, lignans and human health

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Rye bran contains a high content not only of dietary fibre, but also of plant lignans and other bioactive compounds in the so-called dietary fibre complex. Blood concentrations of lignans such as enterolactone have been used as biomarkers of intake of lignan-rich plant food. At present, evidence from studies in human subjects does not warrant the conclusion that rye, whole grains or phyto-oestrogens protect against cancer. Some studies, however, have pointed in that direction, especially in relation to cancers of the upper digestive tract. A number of prospective epidemiological studies have clearly shown a protective effect of wholegrain cereals against myocardial infarctions. A corresponding protective effect against diabetes and ischaemic stroke (brain infarct) has also been demonstrated. It seems reasonable to assume that these protective effects are associated with one or more factors in the dietary fibre complex.

Rye: Lignans: Dietary fibre: Cancer: Cardiovascular disease: Diabetes mellitus

Lignans and rye

Lignans and isoflavones are two groups of diphenolic substances that are structurally similar to oestradiol, an oestrogen, and are included in the family of phyto-oestrogens. The isoflavones are more oestrogenic than lignans but are still about 1000 times less active than oestradiol. Plant lignans probably have no or very low oestrogenic activity. Lignans are present in the normal diet, e.g. in vegetables and fruits. Among the grains, rye has a high content of plant lignans. In the mammalian gut plant lignans such as secoisolariciresinol and matairesinol are converted to mammalian lignans, enterolactone (Enl) and enterodiol (End) by the microflora. After this conversion, End is oxidized to Enl. Enl and End are primarily formed in the large intestine, and it is assumed that they are absorbed by passive diffusion in a manner similar to that of short-chain fatty acids.

The biotransformation of plant lignans to mammalian lignans has been studied in both human and animal experiments and in the laboratory. Investigations in patients with ileostomies have shown that, for a complete bioconversion of rye lignans secoisolariciresinol and matairesinol to mammalian lignans Enl and End, the entire enzymic and fermentative activity of the intestine is needed. Thus, subjects with ileostomies are not able to complete the bioconversion (Petterson *et al*. 1996). In healthy human subjects markedly higher plasma and urinary Enl levels were observed for subjects consuming a diet containing wholegrain rye bread compared with white wheat bread (Juntunen *et al*. 2000). After a course of antibiotics, it may take more than 1 year before the microflora is again producing Enl normally. The biotransformation of plant lignans to mammalian lignans also depends on the substrates in the gut. In an experiment in which rye bran was fed to mice it was observed that fat decreased the urinary excretion of Enl (Bylund *et al*. 2000), indicating that fat interfered with the formation of Enl in the gut. Rowland *et al*. (2000) reported similar results in a human experimental study of soyabean isoflavones. In this case there was a negative correlation between fat in the diet and the urinary equol. Although blood lignans may be affected by many factors (Table 1), the intake of lignan-rich food and the function of the intestinal microflora are still the most important of these factors. Serum Enl has been used as a crude biomarker of lignan intake (Stumpf *et al*. 2000).

Abbreviations: End, enterodiol; Enl, enterolactone.
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Rye is a cereal grown almost exclusively in the north-western part of the Eastern Hemisphere. Approximately 95 % of the global production occurs in the area between the Ural Mountains and the Nordic Sea. Among grains, rye has the highest content of lignans, while soyabean and other legumes are rich in isoflavones (Mazur *et al*. 1996; Mazur, 1998). Flaxseed has been found to be by far the richest source of lignans. The lignans secoisolariciresinol and matairesinol have been shown to be concentrated in the outer layers of the rye kernel (Nilsson *et al*. 1997*a,b*). Recently, high concentrations of some new lignans have been identified in rye, e.g. pinoresinol, lariciresinol, isolariciresinol and syringaresinol. To date, published quantitative results are available only for secoisolariciresinol and matairesinol. Wheat and barley are devoid of matairesinol. All the new lignans, except isolariciresinol, are converted to Enl and End (Heinonen *et al*. 2001). Rye grain also contains other phenols, e.g. tannins and cinnamic acids, as well as vitamins and minerals (Adlercreutz & Mazur, 1997).

Cancer

The incidences of breast and prostate cancer, the major forms of cancer in women and men respectively, vary considerably between countries, and migration studies have clearly shown that the Western lifestyle has an adverse effect on the incidence of these cancers. It is also evident for prostate cancer that the variation in incidence reflects primarily clinical cancer, while the differences in the incidence of latent cancer are much less pronounced. There is reason to suspect that, to some extent, breast cancer may behave in a similar way, although that behaviour is more difficult to study because of the complex interactions with pregnancies, onset of menarche and other factors related to reproduction.

Table 1. Some factors which influence the biotransformation of plant lignans to mammalian lignans and the concentration of enterolactone in blood

	Reference
Amount and type of plant lignan in the diet	Mazur & Adlercreutz (1998) Heinonen *et al.* (2001)
Other dietary factors, e.g. fat content or increased fat content, will give decreased biotransformation of lignan in the gut	Bylund *et al.* (2000) Rowland *et al.* (2000)
Amount, type and activity of intestinal bacteria	Adlercreutz & Mazur (1997)
Other intestinal factors such as the function of the small and/or large intestine	Hallmans *et al.* (1999)
Constipation is associated with increased enterolactone concentration	Kilkkinen *et al.* (2001)
High BMI is associated with decreased enterolactone concentration	Kilkkinen *et al.* (2001) Hultén *et al.* (2002)
Smoking is associated with decreased enterolactone concentration	Kilkkinen *et al.* (2001) Hultén *et al.* (2002)

Breast cancer

Table 2 shows some results of case–control studies of the association between phyto-oestrogens, measured as biomarkers, and breast cancer risk. In three studies urinary phyto-oestrogens were measured quantitatively, and a low excretion of phyto-oestrogens was associated with an increased risk of breast cancer (Ingram *et al*. 1997; Zheng *et al*. 1999). Recently, a case–control study of serum Enl levels and breast cancer risk was performed in eastern Finland, using data from 194 breast cancer cases and 208 community-based controls. An association was found between low Enl concentration and increased risk of breast cancer (Pietinen *et al*. 2001) in both premenopausal and post-menopausal women. Thus, some evidence for a protective effect of both isoflavone- and lignan-rich diets has been found in the three classical case–control studies listed in Table 2. In the classical case–control studies the urinary or blood samples are taken after diagnosis, which may result in a change in Enl concentrations that is related to changes made in diet because of the disease. The study of Pietinen *et al*. (2001) was, in this respect, on the borderline. The cases had suspected breast tumours, but blood samples were taken before diagnosis. In the first nested case–control study, no association was observed between the urinary concentration of Enl and risk of breast cancer (den Tonkelaar *et al*. 2001). In our own nested case–control study (248 cases and 492 controls), however, a low plasma Enl concentration, below the 12·5th percentile (mean plasma Enl 2·8 nmol/l), was associated with a significantly increased risk of breast cancer. The adjusted relative risk and 95 % CI at the lowest percentile were 1·6 (95 % CI 1·0, 2·6). In the same study an increased risk was also found with high plasma Enl values (Hultén *et al*. 2002), particularly in two sub-cohorts with only incident cases. In a sub-cohort

Table 2. Case–control studies (classical or nested) of the association between the concentration of phyto-oestrogens in blood or urine and breast cancer (BC) risk

Reference	Country	Measurement method and study design	Findings
Ingram *et al.* (1997)	Australia	Urinary isoflavonoids and lignans (classical)	High enterolactone and equol excretion associated with decreased risk of BC
Zheng *et al.* (1999)	China, Shanghai	Urinary excretion of isoflavonoids (classical)	Low excretion of isoflavonoids associated with increased BC risk
den Tonkelaar *et al.* (2001)	The Netherlands	Urinary enterolactone : creatinine and genistein : creatinine (nested)	No association between phyto-oestrogen excretion and BC risk in post-menopausal women
Pietinen *et al.* (2001)	Finland	Serum enterolactone (classical)	Serum enterolactone inversely associated with risk of BC
Hultén *et al.* (2002)	Sweden	Plasma enterolactone (nested)	High and low concentrations of plasma enterolactone associated with increased BC risk

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from a mammary screening programme with a high proportion of screening-detected cases, a non-significant decreased risk was found for subjects in the highest percentile for plasma Enl concentration.

The following hypotheses are presented for the observed contradictory results of epidemiological studies of breast cancer:

1. only classical case–control studies have provided a clear indication of an inhibitory effect of lignan-rich food on tumour growth with delayed onset of clinical cancer, while only one of the two existing prospective studies partly supports this relationship. Is it possible that the effect can only be monitored at diagnosis or in cases with a short latency time?
2. could the results obtained in case–control studies reflect secondary effects of the tumour or host defence on the biomarkers themselves?
3. heterogeneity of the material investigated, methodology and different sources of Enl precursors in different countries. The latter may be the most important factors.

Prostate cancer

Changing from the soyabean-rich low-fat low-animal-protein diet to a Western diet increases the risk of prostate cancer in Japanese men. The exact nature of the inhibitory factors present in Asian diets is unknown. Since Japanese men have very high urinary excretion of isoflavonoids, it has been suggested that phyto-oestrogens may make a large contribution to the inhibition of the growth of latent cancers (Adlercreutz *et al.* 1991, 1993). There is less variation between populations in latent cancers than in clinical cancer, which suggests that differences in diet may be important for the transformation and growth of prostate cancer to its clinically-detectable form. A decreased risk of prostate cancer was found in Adventist men (Mills *et al.* 1989) who have a high consumption of beans, lentils (*Lens culinaris*), peas (*Pisum sativum*) and some dried fruits (all dietary sources of phyto-oestrogens), and in men of Japanese ancestry in Hawaii (Severson *et al.* 1989) who consume large quantities of rice and tofu, a soyabean product containing isoflavonoids. In Finland the lowest incidence of prostate cancer occurs in the northeast regions, where there is a high consumption of rye bread (Pukkala *et al.* 1987; Kleemola *et al.* 1994).

In experiments on rats and nude mice it has been shown repeatedly that a high intake of rye bran and soyabean protein delays the early growth of hormone-sensitive prostate cancer (Landström *et al.* 1998), with increased tumour cell apoptosis (Bylund *et al.* 2000). The active component(s) in rye bran may be lignans and isoflavonoids, but other substances and/or mechanisms may also be involved, such as effects on insulin and insulin-like growth factor-1. Increased content of fat in a rye diet abolishes the beneficial effects on tumour acquisition, tumour cell apoptosis and tumour growth (Bylund *et al.* 2000). Dietary fat also decreased the urinary excretion of Enl, suggesting that it interfered with the formation of Enl in the gut, possibly by a reduced rate of microbial fermentation in the intestine. The observation that the cancer-protecting effects of components of traditional diets can be reduced simply by adding fat may be of considerable importance for the understanding of the geographical and temporal variation in the incidence of clinical prostate cancer (World Cancer Research Fund and the American Institute for Cancer Research, 1997). The intake of saturated fat may also be related to the continued existence of the disease, as was noted in a recent report (Meyer *et al.* 1999).

The experimental studies also clearly show that the effect of diet on prostate tumour growth is transient and that no effect is seen on aggressively-growing tumours. These results are supported by the observations made by Thompson's group in Canada of the effects of purified lignan extracts on experimental breast and colo-rectal cancers (Thompson, 1995).

Conflicting results have been found in the case–control studies completed to date, and the evidence is insufficient to permit any conclusion of a possible protective effect of a high intake of phyto-oestrogens against prostate cancer (Hirayama, 1979; Oishi *et al.* 1988). Some recent studies have, however, supported the concept that consumption of phyto-oestrogen-rich food is associated with a decreased risk (Key *et al.* 1997; Strom *et al.* 1999) and may affect prostate cancer biology (Demark-Wahnefried *et al.* 2001). A recent large prospective study on circulating Enl and risk of prostate cancer did not support the hypothesis that high circulating Enl is protective against prostate cancer (Stattin *et al.* 2002). The possible effects of diet on prostate cancer growth are most probably very complex, and the results of experimental studies suggest that adjustment for fat and energy intake should be made when studying the effects of lignans. For this purpose, large data sources are needed, such as those collected within the European Prospective Investigation into Cancer and Nutrition study (Riboli & Kaaks, 1997).

Colo-rectal cancer

It has long been accepted that dietary factors, such as dietary fibre-rich food, may be protective against cancer of the colon and rectum (World Cancer Research Fund and the American Institute for Cancer Research, 1997). Recently, the concept of a protective effect of fruits, vegetables and wholegrain cereals has been challenged, since no protective effect has been observed in some recent prospective studies using dietary questionnaires (Fuchs *et al.* 1999; Pietinen *et al.* 1999). A lack of association has also been observed between colo-rectal cancer risk and intake of plant sterols, with wholegrain bread as an important source (Normén *et al.* 2001), or plasma Enl used as a biomarker for intake of dietary fibre-rich food (Lundin *et al.* 2001*a*).

To further increase the uncertainty, a recent prospective study provided new support for a protective effect of fibre-rich food against colo-rectal cancer. In a study by Terry *et al.* (2001*a*), in a cohort of over 60 000 women, individuals with a very low intake of fruit and vegetables had an increased risk.

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The conflicting findings emphasise the potential usefulness of intervention studies, identification of critical phytochemicals with adverse or protective effects on cancer and identification of biomarkers of food intake. Intervention trials to date have been inconclusive or negative, and in one study using a purified fibre fraction, ispaghula fibre, the treatment was even associated with an increased recurrence of colo-rectal adenomas. In another large-scale intervention study it was also found that a low-fat high-fibre diet was ineffective in preventing recurrence of colo-rectal adenomas (Schatzkin *et al*. 2000).

Although much evidence has accumulated in support of a protective effect of fibre-rich food against risk of colo-rectal cancer, follow-up studies may be warranted, employing extensive prospective data on colo-rectal cancers and using biomarkers for the dietary fibre complex such as Enl, and perhaps in the future more specific biomarkers, e.g. alkyresorcinols (for rye; Ross *et al*. 2001; Linko *et al*. 2002) and avenantramides (for oats).

Stomach cancer (including cardia)

A large number of case–control studies strongly point in the direction of a protective effect of foods rich in fibre (World Cancer Research Fund and the American Institute for Cancer Research, 1997). The results are highly consistent with a protective effect associated with wholegrain cereals. Further support is provided by a recent study on cardia cancer performed on a population-based case–control study (Terry *et al*. 2001*b*), where the protective association was strong for cardia cancer and somewhat weaker for adenocarcinomas of the lower oesophagus. Importantly, no indication of a protective effect was observed for oesophageal squamous cell carcinoma, which strongly supports the conclusion that recall bias has not been an important factor leading to bias in this study. One exciting possible mechanism for a protective effect of wholegrain cereals is connected with their binding properties in relation to N-nitroso compounds (Kurtz & Zhang, 2001). Some preliminary data were presented during a whole grain and human health conference in Finland in June 2001 by Jacobs (2001) based on results of prospective studies, which again indicate a protective effect of whole grains in the upper digestive tract.

General conclusion regarding cancer studies in human subjects

Based on the aggregated results from studies in human subjects performed to date, to conclude that rye, whole grains or phyto-oestrogens are protective against cancer is not possible. Some studies, however, have shown promising results, especially in relation to the upper digestive tract. A delay in clinical cancer may be very important from a public health perspective. Dietary intervention studies in human subjects are therefore needed that use various forms of end points, such as apoptosis in tumours and changes in prostate-specific antigen concentration. Unfortunately, dietary intervention trials are both costly and very time-consuming. Nevertheless, they are needed for definitive evidence of the effects of the diets.

Cardiovascular disease, stroke and myocardial infarction

Morris *et al*. (1977) demonstrated a striking association between a high intake of cereal fibre and reduced incidence of CHD in men followed for 16–20 years. Since then, a number of large-scale epidemiological studies have strongly supported this finding of a protective effect of foods rich in dietary fibre, especially wholegrain products, against myocardial infarctions (Willett, 1998; Truswell, 2002). In relation to wholegrain rye, a study involving approximately 22 000 middle-aged Finnish men found an inverse association between the amount of dietary fibre in the diet and the incidence of CHD. This group of men were followed over 6 years, and it was observed that the intake of cereal fibre, derived largely from rye, was more strongly negatively associated with the risk of myocardial infarction than was vegetable or fruit fibre. The protective effect of fibre was strongest for fatal events. The relative risk for fatal myocardial infarction was 0·45 for men with the highest intake of fibre (median, 28·9 g/d) compared with men with lowest intake of fibre (median, 12·4 g/d; Pietinen *et al*. 1996). Other recent studies have yielded similar results, showing cereal fibre to be strongly associated with a protective effect against myocardial infarction (Wolk *et al*. 1999; Liu *et al*. 2002). A similar association has also been observed for post-menopausal women, in this case between wholegrain food and ischaemic stroke, independent of other known cardiovascular disease risk factors (Liu *et al*. 2000). There are probably several mechanisms involved in the protective process, including effects on lipids, insulin metabolism and fibrinolysis. The major protective effects seem, however, to be unrelated to changes in blood cholesterol concentration. Components in wholegrain cereals, such as alkylresorcinols, phenols and lignans, can act as antioxidants (Jacobs *et al*. 1998; Vanharanta *et al*. 2002) and their antioxidant and anti-inflammatory properties may be related to disease-preventing mechanisms.

Antioxidant or anti-inflammatory factors related to the whole-grain complex

There are a large number of potential candidates in the whole-grain complex that have antioxidant and anti-inflammatory properties. The alkylresorcinols have this potential and they are present at very high concentration in wholegrain rye and wheat. Theoretically, the presence in the vessel wall of a factor with anti-inflammatory activity may prevent ‘plaque rupture’ and subsequent thrombosis formation. A study on the absorption of alkylresorcinols in human subjects has recently been performed. Ten subjects who had undergone ileostomy were fed diets containing either whole-rye bread rich in alkylresorcinols or sifted wheat bread with no alkylresorcinols. Diets and ileostomy samples were analysed for alkylresorcinols, and the apparent digestibility was calculated. The average absorption of alkylresorcinols in human subjects was between 50 and 70 % for all major homologues (Ross *et al*. 2001).

Recently it was shown that alkylresorcinols circulate in blood in the unconjugated form (Linko *et al*. 2002). The alkylresorcinol concentration in blood may therefore be considered a good marker for intake of whole cereal grain and, most probably, better than Enl concentration in blood as a marker for intake of whole-rye grain and wheat. Their biological effects and distribution in the body should certainly be studied further.

In the field of cardiovascular disease, in particular myocardial infarction, there is general agreement between the results of prospective studies that use biomarkers in blood with antioxidant properties, such as Enl (Vanharanta *et al.* 1999) and ascorbic acid (Khaw *et al.* 2001), and those of prospective studies that use dietary questionnaires. In a recent Finnish study a high serum concentration of Enl was clearly associated with a decreased risk of myocardial infarction (Vanharanta *et al.* 1999). Thus, in this study serum Enl was found to be a useful biomarker for the intake of lignan-rich foods. As with ascorbic acid and other anti-oxidants in blood, a negative correlation might be expected to exist between Enl and markers of inflammation in blood, a hypothesis that has not yet been tested. It is, however, unlikely that plasma Enl is confounded by the effect of a low-grade inflammation associated with advanced athero-sclerosis. In fact, in a very recent study by Vanharanta *et al.* (2002), serum Enl was associated with decreased *in vivo* lipid peroxidation. This association remained even after adjustment for plasma antioxidant vitamins (α-tocopherol, β-carotene, ascorbic acid) and dietary folic acid. It was suggested, therefore, that Enl or some of its precursors might contribute to the antioxidant defence system in blood. Other biomarkers or phytochemicals may of course also be the active antioxidants.

Conclusion

There is strong evidence for a protective effect of a high intake of wholegrain products on myocardial infarction, and probably also on diabetes and stroke. It is concluded that the effects of a whole-grain diet, e.g. rye bran, on different risk factors may be associated with the reduced overall risk for myocardial infarction and stroke.

Diabetes

Diabetes is a well-established risk factor for cardiovascular disease. In a number of studies in human subjects and animals intake of rye bran has been associated with improved metabolic status or preventive effects on diabetes. The effect of bread with a high rye-bran content was compared in patients with insulin-dependent diabetes with the effects of either low-bran bread or the patients' usual bread (Nygren *et al.* 1984). When the bread with high rye-bran content was included in the diet it was observed that the glucose profile during the day improved or the insulin doses could be reduced.

Protective effects of rye bran on the diabetes syndrome have also been found in rats and mice (Nygren *et al.* 1981; Lundin *et al.* 2001*b*). Diabetic rats fed a high-fibre bread lost less body weight, and exhibited lower blood glucose levels and lower urinary glucose excretion than the animals fed a low-fibre bread. Another study found that rye bran slightly lowered blood glucose levels and led to slower weight gain in normal rats and mice and prolonged survival of diabetic mice (Berglund *et al.* 1982).

It has long been recognised that a high intake of wheat bran or a mixture of wheat and rye bran improves glucose tolerance in human subjects without diabetes (Brodribb & Humphreys, 1976; Sandman *et al.* 1983) and in individuals with glucose intolerance (Bosello *et al.* 1980). It has recently been observed in an experimental study that increased intake of whole grains is associated with increased insulin sensitivity (Pereira *et al.* 2002). A clear association between a high intake of dietary fibre from cereal grain and a reduced risk of diabetes has also been observed in two large-scale prospective studies in women. In one of the studies a low glycaemic index was concomitantly associated with a decreased risk (Salmeron *et al.* 1997) in younger women. However, the second, more recent study (Meyer *et al.* 2000) on older women did not support this finding. The outcomes of the epidemiological and experimental studies performed to date suggest that substituting refined-grain products with wholegrain products may decrease the risk of both diabetes and cardiovascular disease. It is also reasonable to assume that the protective effect is associated with some factor in the dietary fibre complex. At present, no studies on diabetes have so far been published that use biomarkers such as Enl for food intake.

Conclusion

Experimental evidence suggests that increased fibre intake, with a high intake of whole grains, is related to increased insulin sensitivity. Furthermore, intake of wholegrain products was associated with a decreased risk of diabetes in women in two prospective studies. Thus, the outcome of epidemiological and experimental studies suggests that substitution of refined-grain products with wholegrain products may decrease the risk of, or alternatively delay, the onset of diabetes. It is also reasonable to assume that the protective effect is associated with some factor(s) in the dietary fibre complex.

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