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No changes in serum enterolactone levels after eight weeks' intake of rye-bran products in healthy young men

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Abstract

Background: Enterolactone (ENL) is a mammalian lignan metabolized by the colonic bacterial flora from plant lignan precursors widely distributed in plant foods.
Objective: The aim of the study was to identify the point in time at which the onset of an increase in ENL concentration could be observed, and the point in time at which a stable level of ENL concentration was obtained.
Design: Sixteen young healthy male volunteers, 25.8 ± 4.0 years of age (mean ± SD) and with an average body mass index of 23.4 ± 2.1 kg m^{-2} completed this 8 week intervention study. Subjects were randomly assigned to two groups receiving 315 ± 10 g of enriched bread and muffin products daily, containing either rye bran, rich in ENL precursors, or Vitacel 600®, a purified cellulose fibre, low in ENL precursors. Eleven fasting blood samples from each subject were taken for analysis of serum ENL concentration.
Results: Serum ENL concentration was not affected by the daily supplementation with rye bran and no significant difference in serum ENL concentration was observed between the groups receiving rye bran or Vitacel products after 8 weeks. A significant decrease in ENL concentration was observed in the Vitacel group after 1 day ($p = 0.018$) and 1 week ($p = 0.029$) of dietary intervention.
Conclusions: In an 8 week period no increase in ENL concentration was observed for the rye-bran group and a stable level of ENL concentration was not obtained. The high intake of dietary fibre from the bread products possibly decreased transit time, and thereby reduced microbial fermentation of the plant lignans and absorption of the mammalian lignan ENL.

Keywords: *dietary fibre*; *lignan*; *transit time*

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Introduction

Lignans are diphenolic compounds widely distributed in plants. The first identified plant lignans were secoisolariciresinol (SECO) and matairesinol (MAT), which are metabolized to enterodiol (END) and enterolactone (ENL), respectively, by the colonic microflora (1, 2). Enterodiol is, to some degree, further oxidized to ENL, resulting in ENL being the most important circulating mammalian lignan (1). Other precursors of mammalian lignans, such as lariciresinol, pinoresinol and syringaresinol, were identified later and are likewise metabolized to END and ENL (3).

Large variations in ENL concentrations both within and between population groups were found in a cross-sectional study of the Finnish population (4) and a large intrapersonal variation was found in a small selected Danish population (5). Dietary concentration of plant lignans is suggested to be the main determinant of ENL concentration in serum, plasma and urine (4, 6, 7). Whole grains are rich sources of ENL precursors, and intake of dietary fibre, especially from whole grains, is strongly associated with elevations of ENL concentration in serum (4), plasma (8) and urine (9). Other important determinants of ENL concentration in serum are age, gender, prevalence of constipation and use of antimicrobials (10). A higher prevalence of constipation, female subjects and the elderly compared to younger subjects are associated with higher levels of ENL (4, 8).

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Dietary sources of lignans include whole grains, seeds, vegetables and berries. Of the whole grains, rye has a high concentration of lignans (11). The highest concentration of lignans is localized in the dietary fibre-containing outer parts of the rye kernel, and the short and bran contains approximately 80% of the total content of SECO and MAT (11). An *in vitro* fermentation of rye bran resulted in production of END and ENL exceeding the concentration of SECO and MAT by a factor of 10 (12). This corresponds to findings by Bach Knudsen et al. (13) and Glitsø et al. (14) indicating a large quantity of plant lignans other than SECO and MAT present in rye bran. A 4 week cross-over study on consumption of bread and ENL concentration, including men and women with an average age of 43 years, resulted in significantly higher production of ENL when subjects consumed 160–220 g per day of wholemeal rye bread compared with wheat bread (15).

In the Nordic countries rye is often consumed as wholegrain rye bread and is quantitatively the most important dietary source of lignans. In the production of low-extraction rye flour, rye bran is a byproduct that contains high concentrations of dietary fibre and lignans (14), and has been found to be a potential source of mammalian lignans when fed to pigs (13). To the authors' knowledge, no long-term (up to 8 weeks) intervention has previously been conducted to investigate the effects of a high consumption of rye products on the changes in concentration of ENL in serum. In the present study, young healthy male volunteers consumed products based on rye bran for a period of 8 weeks. Only male subjects were included, to minimize the variation between subjects. The aim of the study was to identify the point in time at which the onset of an increase in ENL concentration could be observed, and the point in time at which a stable level of ENL concentration was obtained.

Subjects and methods

Subjects

Twenty healthy men with an age of 25.8 ± 4.0 years (21–34) (mean $\pm$ SD, range) and body mass index of 23.4 ± 2.1 kg m^{-2} were recruited for the study. To be included in the study, subjects had to be omnivorous, and not have donated blood or received antibiotic treatment in the 3 months before or during the study period. Two subjects smoked regularly. Four subjects dropped out of the study, two owing to sickness and two who stated that the amount of bread was too much.

All participants were informed orally and in writing about the study. Written consent to participation, stating that the subject participated voluntarily and was free to withdraw from the study at any time, was obtained before enrolment. The study protocol was approved by the Municipal and Ethics Committee of Copenhagen and Frederiksberg [authorization number (KF) 11-028/02].

Study design

Subjects were randomized into two groups receiving bread baked with either rye bran [rye-bran group (RBG)] or Vitacel 600® [Vitacel group (VG)] for a period of 8 weeks. All subjects consumed a daily portion of bread of 250 g and a muffin of 65 g, weighed out individually on a digital scale and within an accuracy of 5 g, as a substitute for part of their habitual diet. Vitacel 600, a purified wheat fibre, which is highly insoluble and therefore only fermented to a very limited extend in the large intestine, was added to the control bread to obtain an equal amount of dietary fibre in the two types of bread. Subjects were requested not to change their diets apart from replacing the customarily consumed bread products with the supplied test bread products. Subjects were further instructed not to eat any other rye products, wholegrain breads or products containing flaxseeds, sunflower seeds or pumpkin seeds. Compliance was evaluated by feedback from each participant during weekly visits to the department for blood donation and for collecting bread. All subjects were encouraged to inform the staff of any problems or discomfort due to the consumption of bread and muffins.

All visits were made to the Department of Human Nutrition, where all blood samples were taken. Subjects donated a blood sample on the first day of intervention, before any bread consumption, to give a baseline value. During the first week of intervention, blood samples were further donated on days 1 and 3, and one blood sample per week was donated in the remaining 7 weeks. All blood samples were drawn from the cubital vein after 12 h of fasting and an additional 10 min rest in the supine position. At baseline and at the end of the study, the subject's weight was recorded in the fasting state.

Bread

The breads used for the study were non-commercial products provided by the Danish Milling Factory, Valsemøllen, Esbjerg. All bread was stored at −18°C and delivered on a weekly basis to all subjects. Rye bran made up 26% and 15% of the wet ingredients of the rye-bran bread and muffin dough. respectively. Vitacel 600 made up 7% and 4% of the wet ingredients of the Vitacel bread and muffins dough, respectively. The total dietary fibre content (g 100 g^{-1} ready-to-eat product) of the rye-bran bread and rye-bran muffin was 6.5 and 4.4, respectively, and of the corresponding Vitacel bread and muffin was 6.4 and 4.3, respectively. Nutrient values were calculated using Dankost 2000® dietary assessment software (National Food Agency of Denmark, Søborg, Denmark).

Sampling and analysis

Blood samples were collected in dry tubes. After coagulation, samples were centrifuged at 2800 *g* for 15 min at 20°C. Serum was stored at −80°C until analysis, initiated immediately after the end of the intervention period. The analytical method used was based on time-resolved fluoroimmunoassay (TR-FIA) as described previously (16, 17). All serum samples were analysed in duplicate and each subject's samples were analysed within the same run. Within the double sampling the coefficient of variation (CV) was 15% for the RBG and 19% for the VG.

Statistics

All analyses were performed using the Statistical Analysing System software package, version 8.2 (SAS Institute, Cary, NC, USA). Univariate mixed model analysis of covariance (ANCOVA) was performed in the procedure MIXED.

In the statistical model, ENL measurements from day 2 to day 56 were evaluated as the dependent variable; treatment (RBG or VG) was included as an independent fixed variable and baseline values as a covariate. Subjects were included as a random effect with a covariance structure of spatial power, as the measurements were unequally spaced in time. Homogeneity of variance and normal distribution among random effects were investigated by plots of residuals. Shapiro–Wilk's test for normal distribution was performed. Paired *t*-tests were used to test changes in ENL levels from baseline until 7 days of intervention for both groups.

Results

The rye-bran and Vitacel bread products contained the same amounts of energy and dietary fibre, and the bread and muffin provided a daily intake of 3.0 MJ and 19 g of dietary fibre. All subjects remained weight stable throughout the 8 week study period. Compliance with the dietary intervention, judged by the feedback by the subjects during their weekly visits to the department, was high.

Substantial variation in ENL concentration was observed both between and within subjects in both groups, but was more pronounced in the RBG. The range of subjects' ENL concentration at baseline was 5–74 and 2–44 nmol l^{-1} for the RBG and VG, respectively. Subjects with a low concentration at baseline tended to stay low throughout the study. No significant difference in baseline values was observed between the two groups.

No significant difference in ENL concentration was observed between subjects consuming rye-bran bread products compared with Vitacel bread products in an 8 week period based on repeated measurements (Fig. 1). When analysis was based on the individual groups, no effect of consuming rye-bran bread on the ENL concentration was observed after 24 h, but a tendency for an increase in ENL concentration was observed after 7 days for the RBG ($p = 0.088$) (Table 1). Although the increase in ENL concentration was non-significant, seven out of 10 subjects in the RBG had a higher ENL concentration in serum after the first 7 days of bread intake compared with baseline. In the VG, a decrease in ENL concentration was observed after 24 h ($p = 0.018$) and after 1 week ($p = 0.029$). All subjects in the VG had decreased ENL in serum after 7 days of Vitacel bread consumption.

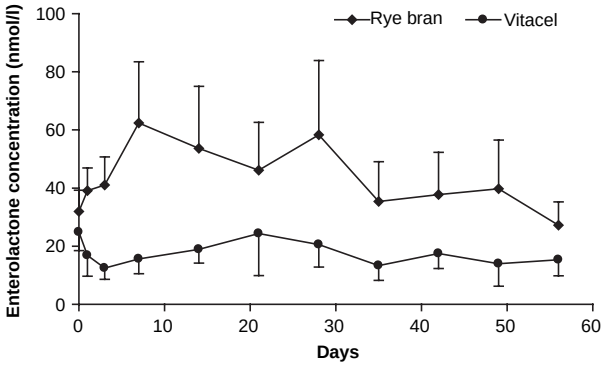

Fig. 1. Enterolactone concentration (nmol l^{-1}) throughout the study for the rye-bran group ($n = 10$) and the Vitacel group ($n = 6$) (mean ± SE).

Table 1. Serum enterolactone concentration (nmol l^{-1}) for the rye-bran group and the Vitacel group: results at baseline and after 24 h, 1 week and 8 weeks of bread consumption (mean ± SE)

	n	Baseline	24 h	1 week	8 weeks
Rye-bran group	10	31.9 ± 7.3	39.1 ± 7.7	62.4 ± 21.0	27.2 ± 8.1
Vitacel group	6	24.8^{a} ± 6.3	16.8^{b} ± 6.1	15.6^{b} ± 5.1	15.3^{ab} ± 5.5

Numbers followed by different superscript letters within the same row are significantly different ($p < 0.05$).

Discussion

A daily intake of 315 g of rye-bran products, a rich source of ENL precursors, was expected to lead to an increase in serum ENL concentration in young men when consumed over an 8 week period. A non-significant increase in serum was observed for the RBG after 1 week, but after 8 weeks' intervention with rye-bran products, no changes in serum ENL concentrations were observed. Considering the high intake of plant lignans from the rye bran, the results obtained contradicted results from both cross-sectional studies (4, 6, 8, 9) and intervention studies (15, 18–20) on whole grains and rye products. The studies all identified positive associations between intake of lignan-rich precursors and ENL concentration in serum, plasma and urine. The variation in ENL concentration between the individual subjects, especially regarding the RBG, was rather large, but a large interpersonal variation in ENL concentration is often observed and can be due to habitual diet, prevalence of constipation (4) and colonic fermentation patterns. In the present study, the remaining diet of the study population was not recorded, but subjects were instructed not to consume any other rye products, wholegrain breads or products containing flaxseeds, sunflower seeds or pumpkin seeds, which are known to be rich sources of plant lignans.

Dietary intake of whole grains is one of the strongest determinants for serum ENL concentration, but only explains a small part of the variation in ENL levels measured in a population (4). The composition and activity of the colonic microflora are important factors in the utilization of available plant lignans. Adaptation of the microflora to a high-fibre diet, rich in plant lignans, could increase metabolism of plant lignans and absorption of mammalian lignans, but there is no agreement on the appropriate adaptation time. In the present study it was anticipated that 8 weeks would be sufficient to ensure appropriate adaptation to a diet rich in plant lignans.

A high intake of dietary fibre, young age, high physical activity and male gender are all determinants of faster intestinal transit time and a lower prevalence of constipation (21–23). Subjects in the present study had a relatively high intake of dietary fibre since the portion of bread products provided 19 g of dietary fibre daily, in addition to the subject's remaining habitual diet. In comparison, a representative Danish population has an average daily intake of 22 g per day (24). Intestinal transit time was not measured in the present study, but considering the dietary fibre intake, age and gender of the study population, it is assumed that the intestinal transit time was fast, in line with the decrease in transit time in the study by Gråsten et al., where an increased amount of dietary fibre was consumed on a daily basis (21). Metabolism of plant lignans and absorption of mammalian lignans occur in the colon (2) and a faster transit time may result in an incomplete biotransformation of the plant lignans present in the rye bran and in the additional diet to mammalian lignans, and therefore a reduced absorption of mammalian lignans. In a cross-sectional study, the prevalence of constipation was estimated to be a strong determinant of the ENL concentration in serum (4).

Other studies have observed increased serum ENL concentration 8–9 h after consuming diets rich in ENL precursors, such as flaxseed and strawberries (25–27). In the present study a non-significant increase in average ENL concentration was observed after 24 h and 1 week. It could be speculated that a higher rate of fermentation of the available plant lignans would occur within the first week of supplementation, before the effect of the high-fibre diet on the intestinal transit time was induced, resulting in the non-significant increase in ENL concentration.

The content of dietary fibre in the Vitacel bread products was equivalent to that in the rye-bran bread products, but only contained a limited amount of plant lignan precursors. In addition, all

subjects were instructed not to consume any other rye or wholegrain products, and the combination of a low intake of plant lignans and a reduced transit time probably resulted in the markedly decreased ENL concentration. This is in line with results from the study by Juntunen et al., who observed a decrease in serum ENL concentration from 28 to 12 nmol l^{-1} when subjects received wheat bread as a substitute for part of their habitual diet (15).

Three previous intervention studies found increased serum or plasma ENL levels after the consumption of lignan-rich diets for a period of 3–4 weeks (18, 19, 28). In spite of high dietary fibre contents, the intervention diets led to an increase in serum ENL concentration after 3–4 weeks, in contradiction to results of the present study. The study populations consisted of men who were considerably older than those in the present study, which probably resulted in a slower average transit time and therefore increased metabolism of available lignans and absorption of mammalian lignans in comparison to a smaller relative absorption observed in the present study, including younger men.

The onset of an increase in ENL concentration occurred within a few days of the dietary intervention, but over an 8 week period no increase in ENL concentration was observed for the RBG, and it was not possible to obtain a stable level of ENL concentration in this group of young men.

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