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Use of Oral Antimicrobials Decreases Serum Enterolactone Concentration

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The lignan enterolactone, a phytoestrogen, may protect against hormone-dependent cancers and cardiovascular diseases. It is produced by the intestinal microflora from dietary precursors. Because of the pronounced impact of antimicrobials on the intestinal microflora, the authors examined whether serum enterolactone concentration is affected by previous use of oral antimicrobials. Enterolactone was measured by time-resolved fluoroimmunoassay in 2,753 Finnish men and women aged 25–64 years who participated in a cross-sectional national survey in 1997. Background information was collected by self-administered questionnaire, and data on antimicrobial treatment were gathered from the nationwide prescription database of the Social Insurance Institution. Serum enterolactone concentration was significantly lower in those who had used oral antimicrobials up to 12–16 months before serum sampling than in nonusers (16.4 vs. 19.3 nmol/liter). The concentration was associated with the number of treatments and the time from the last treatment. Modest differences were present between various antimicrobials. The authors' findings support the crucial role of gut microflora in the metabolism of lignans. Furthermore, recent use of antimicrobials should be considered when the association between serum enterolactone concentration and risk of chronic diseases is studied. *Am J Epidemiol* 2002;155:472–7.

antibiotics; bacteria; biological markers; cross-sectional studies; drug therapy; intestines; lignans; metabolism

The lignan enterolactone, a phytoestrogen, is a naturally occurring diphenolic compound that has several biologic properties (1–8). The actions of enterolactone provide potential mechanisms for a postulated preventive influence on hormone-dependent cancers and cardiovascular diseases. In animal models, a diet rich in lignans has been shown to protect against these diseases (9, 10); although human studies are scarce, some evidence suggests that enterolactone may lower the risk of breast cancer (11) and acute coronary events (12).

Precursors of mammalian lignans are highly concentrated in flaxseed, with lesser amounts found in other seeds, nuts, whole grains, berries, fruit, and vegetables (13–15). Recently, however, a number of new mammalian lignan precursors have been identified (16). When plant lignans are consumed, they are converted into biologically active mammalian lignans by bacteria in the gut (17–19). The most abundant mammalian lignan is enterolactone. Once absorbed, mammalian lignans are conjugated with glucuronic acid or sulfate in the liver, reexcreted through the bile duct, deconjugated by the bacteria, and reabsorbed (enterohepatic circulation). Lignans are excreted in the urine and feces mainly as glucuronides.

We previously observed that serum enterolactone concentration varies widely in the population and that consumption of lignan-containing foods and health-related variables explain only a small part of this variation (20). This observation suggests a crucial role for the gut in the metabolism of lignans. Because of the pronounced impact of antimicrobials on the intestinal microflora, we investigated the effects of previous use of oral antimicrobials on serum enterolactone in a large population sample.

MATERIALS AND METHODS

Subjects

This study is part of a cross-sectional health survey carried out in Finland during early spring 1997. The participants were aged 25–64 years and lived in five areas: the Helsinki metropolitan area in the south, the city of Turku and its surrounding rural communities in the southwest, the provinces of North Karelia and Kuopio in the east, and the province of Oulu in the north. The health survey population (n = 10,000) was randomly drawn from the National Population Register stratified by 10-year age groups, regions, and sex. For the dietary survey, a subsample of

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Abbreviations: BMI, body mass index; CI, confidence interval; SD, standard deviation; TR-FIA, time-resolved fluoroimmunoassay.

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4,000 subjects (40 percent) was randomly allocated; of these subjects, 2,862 participated in the dietary survey (21). A serum sample was available for 2,753 subjects, who thus formed the final study population.

All participants were invited to the local health center, where fasting (minimum 4 hours) venous samples were taken. The samples were fractioned and serum aliquots were stored frozen at –20°C. Weight and height were also measured during the examination, and body mass index (BMI) was computed as weight in kilograms divided by the square of height in meters (kg/m^2). Furthermore, the subjects filled in a self-administered questionnaire, which was completed at the examination and contained questions on diet, health, and smoking. Consumption of lignan-containing foods was quantified from the food frequency questionnaire including 38 food items by summing the frequency of consumption, in number of servings per month, of rye bread, crisp bread, porridges, cereals, salad vegetables, roots, legumes, vegetable dishes, fruit, berries, fruit juices, and berry juices.

Data on the use of antimicrobials were based on the nationwide prescription register of the Social Insurance Institution. All prescriptions reimbursed by the National Sickness Insurance Scheme are registered. The database includes information about the patient, drug, and day of purchase. The medication is classified according to the Anatomical Therapeutic Chemical Classification (ATC) system. In this system, the drugs are divided into different groups according to the organ or system on which they act and/or their therapeutic and chemical characteristics (22). Oral antibacterials include tetracyclines, amoxicillin and other extended-spectrum penicillins, phenoxymethylpenicillin and other beta-lactamase-sensitive penicillins, cloxacillin and other beta-lactamase-resistant penicillins, combined penicillins, cephalosporins, sulfonamides and trimethoprims, macrolides and lincosamides, fluoroquinolone antibacterials, and steroid antibacterials.

The health survey was approved by the Ethics Committee of the National Public Health Institute.

Analytical methods

Serum enterolactone concentration was determined from the frozen serum of 2,753 study subjects (1,301 men and 1,452 women) in spring 1998. The enterolactone analysis was performed by time-resolved fluoroimmunoassay (TR-FIA) (23), with slight modifications (24). In brief, the modified method is as follows: 50 μl of serum were incubated with 50 μl of hydrolysis reagent containing sulfatase (Sigma, St. Louis, Missouri) and β-glucuronidase (Boehringer-Mannheim, Mannheim, Germany) overnight at 37°C. After hydrolysis, 150 μl of 0.5 percent BSA-Tris Buffer (BSA; Merk AG, Darmstadt, Germany), pH 7.8, was added to obtain the optimal pH and protein concentration for analysis. The analyses were performed on anti-rabbit antiserum coated microtitration strips by using 20 μl of sample. Enterolactone concentrations were measured by using the VICTOR 1420 multilabel counter (Wallac Oy, Turku, Finland). Each batch was analyzed with three duplicate quality-control serum samples. The interassay coefficient of variation (percentage) and the concentrations of the samples were 16.8 percent (4 nmol/liter), 10.1 percent (14 nmol/liter), and 13.1 percent (59 nmol/liter). The intra-assay coefficient of variation varied from 6.9 percent to 9.9 percent.

The modified method described above (24) tends to yield 15–30 percent higher results than the standard TR-FIA method with extraction (23). To enable comparison of results with those from previous studies, we derived an equation between the modified TR-FIA method and the standard TR-FIA method by analyzing 92 samples with both methods. The final results were calculated by using the following equation: final concentration in nmol/liter = measured concentration in nmol/liter × 0.934 – 11.03.

Statistical methods

Those subjects who had purchased antimicrobials, according to the Finnish prescription register, from January 1996 to the day of the spring 1997 examination were considered users of antimicrobials; correspondingly, those who had not purchased antimicrobials were categorized as nonusers. Differences in age, BMI, occurrence of constipation, consumption of lignan-containing foods, and smoking habits between users and nonusers were analyzed by using the *t* test for continuous variables and the χ^2 test for dichotomized variables. A linear regression model was used for investigating the dependence of serum enterolactone concentration on the total number of antimicrobials purchased and on time since the last treatment. To examine the association of serum enterolactone with number of treatments and with time from the last treatment, the subjects were divided into the following four groups: 1) time from the last antimicrobial treatment being a maximum of 3 months and at least two treatments received, 2) time from the last antimicrobial treatment being a maximum of 3 months and only one treatment received, 3) time from the last antimicrobial treatment being more than 3 months and at least two treatments received, and 4) time from the last antimicrobial treatment being more than 3 months and a maximum of one treatment received during the study period. By using the last group as the reference, we tested differences in mean serum enterolactone concentrations with an *F*-test statistic from the analysis of variance. To examine differences between various antimicrobials, subjects were divided into groups according to the last antimicrobials used during the 3 months preceding the examination. Differences in mean serum enterolactone concentrations were tested with an *F*-test statistic from the analysis of variance. This analysis was repeated for only those with no prior use of antimicrobials.

Because of the skewness of serum enterolactone concentration distribution, enterolactone values were log-transformed prior to statistical analyses. On the basis of our previous observations (20) that serum enterolactone concentration is positively associated with constipation, consumption of lignan-containing foods, and normal BMI and is negatively associated with smoking, all of these variables were included in the models.

RESULTS

Altogether, 964 subjects (374 men and 590 women) had purchased antimicrobial agents at least once during the time period between January 1996 and the day of the examination in spring 1997. Thus, the proportion of users among the 2,753 study subjects was 35 percent—29 percent of the men and 41 percent of the women. The characteristics of users and nonusers are shown in table 1. No significant differences between these two groups were observed in age, consumption of lignan-containing foods, occurrence of constipation, or smoking. However, male users had a higher BMI than nonusers.

A total of 1,767 antimicrobial prescriptions were purchased, and the number of administrations of antimicrobials per person varied from one to 10. More than half of the users (62 percent of the men and 55 percent of the women) had purchased antimicrobials only once. Two treatments had been received by 23 percent, three treatments by 10 percent, and four or more treatments by 6 percent of the men. The corresponding values for women were 23 percent, 11 percent, and 12 percent. Nearly two thirds of the prescriptions were purchased by female subjects. The mean number of prescriptions did not differ significantly between categories of age, constipation, or smoking, whereas prescriptions were more frequent among overweight men (BMI = 25–35 kg/m^2) than their normal-weight counterparts.

Subjects who had used antimicrobials up to 12–16 months before serum sampling had a significantly lower serum enterolactone concentration than nonusers: mean, 16.4 (standard deviation (SD), 14.3) nmol/liter versus mean, 19.3 (SD, 16.1) nmol/liter. The difference was similar for men (mean, 14.4 (SD, 13.0) nmol/liter vs. mean, 17.7 (SD, 14.0) nmol/liter) and women (mean, 17.6 (SD, 15.0) nmol/l vs. mean, 20.9 (SD, 18.0) nmol/liter).

A significant linear association between the logarithm of serum enterolactone concentration and the number of antimicrobial administrations was observed for both genders (figure 1). Compared with the previous level, each additional antimicrobial treatment lowered serum enterolactone concentration by 16 percent (95 percent confidence interval (CI): 11, 20) in men and 11 percent (95 percent CI: 7, 14) in women.

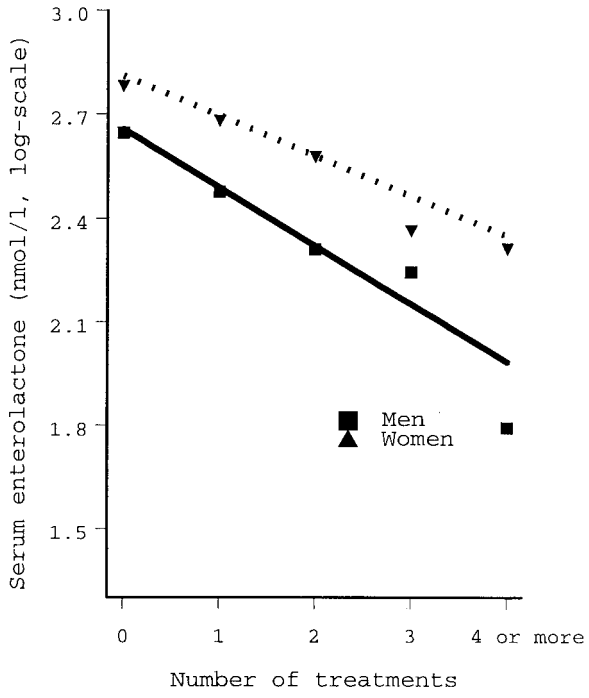

FIGURE 1. Serum enterolactone concentration according to number of antimicrobial treatments, Finland, 1997. Symbols denote the crude mean values and the plotted lines the mean values adjusted for body mass index, occurrence of constipation, consumption of lignan-containing foods, and smoking.

A significant trend toward a higher logarithm of serum enterolactone concentration with increasing time from the last antimicrobial treatment was also observed for both genders (figure 2). Compared with the previous level, for each 2-month time period, serum enterolactone concentration increased by 8 percent (95 percent CI: 5, 10) in men and 6 percent (95 percent CI: 3, 8) in women. Serum enterolactone concentration was associated with the number of treatments and the time from the last administration (figure 3). The

TABLE 1. Characteristics† of the population studied to determine whether use of oral antimicrobials decreases serum enterolactone concentration, Finland, 1997

Characteristic	Use of antimicrobials			
	Men		Women	
	No (n = 927)	Yes (n = 374)	No (n = 862)	Yes (n = 590)
Serum enterolactone (nmol/liter)	17.7 (14.0)	14.4 (13.0)*	20.9 (18.0)	17.6 (15.0)*
Age (years)	45.4 (11.3)	45.6 (11.2)	45.4 (11.3)	44.3 (11.7)
Body mass index (kg/m^2)	26.7 (4.1)	27.2 (4.0)*	26.1 (5.1)	26.2 (5.1)
Consumption of lignan-containing foods (servings/month)‡	107 (47)	111 (45)	131 (51)	130 (47)
Constipation (%)	8.2	8.2	20.8	25.1
Smoker (%)	30.7	32.2	18.5	23.1

* $p < 0.05$.
† Unless otherwise noted, all values are expressed as mean (standard deviation).
‡ Includes rye bread, crisp bread, porridge, breakfast cereals, roots, salad vegetables, vegetarian dishes, fruit, berries, fruit juices, and berry juices.

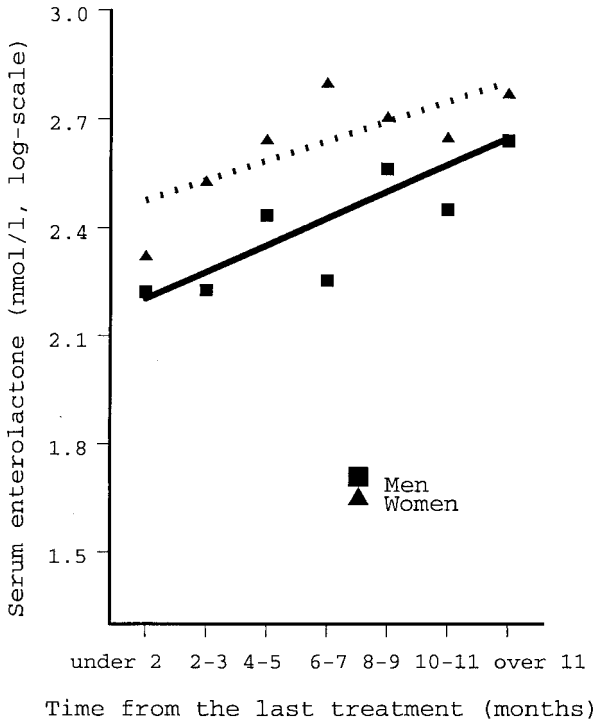

FIGURE 2. Serum enterolactone concentration according to time (number of months) from the last antimicrobial treatment, Finland, 1997. Symbols denote the crude mean values and the plotted lines the mean values adjusted for body mass index, occurrence of constipation, consumption of lignan-containing foods, and smoking.

associations were similar for both men and women, although women had a higher serum enterolactone concentration than men.

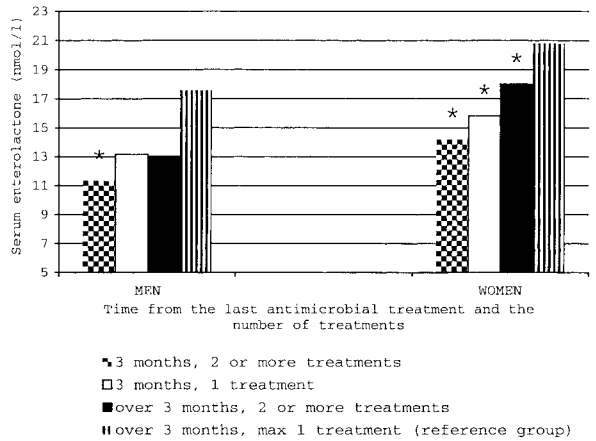

FIGURE 3. Serum enterolactone concentration according to the time (number of months) from the last antimicrobial treatment and the number of treatments, Finland, 1997. Adjusted for body mass index, occurrence of constipation, consumption of lignan-containing foods, and smoking. *, $p < 0.05$ compared with those who had purchased antimicrobials a maximum (max) of once and with time from treatment being at least 3 months.

The most frequently prescribed antimicrobials were cephalosporins (25 percent of the prescriptions), the majority of which consisted of first-generation preparations. The other major groups were tetracyclines (24 percent), macrolides and lincosamides (18 percent), and amoxicillin and other extended-spectrum penicillins (13 percent); the other antimicrobial agents together constituted about 20 percent of the prescriptions. There were modest differences in serum enterolactone concentration between various antimicrobials (figure 4). The strongest suppression in serum enterolactone concentration seemed to be caused by macrolides (10.5 nmol/liter), while phenoxymethylpenicillin and other beta-lactamase-sensitive penicillins (18.3 nmol/liter), cephalosporins (15.6 nmol/liter), and amoxicillin and other extended-spectrum penicillins (15.2 nmol/liter) had minor effects. When only those subjects who had no prior use of antimicrobials were included in the analysis, the impression was still the same, except for amoxicillin and other extended-spectrum penicillins as well as macrolides (17.5 and 12.1 nmol/liter, respectively).

DISCUSSION

Serum enterolactone concentration was significantly lower in subjects who had purchased oral antimicrobials up to 12–16 months before serum sampling than in nonusers. Serum enterolactone level was associated with both number of treatments and length of time from the last treatment. Furthermore, modest differences were found between the various antimicrobials. These findings support our view of the crucial role of gut microflora in the metabolism of lignans.

Previously, a small-scale human study showed that oral administration of metronidazole or wide-spectrum oxytetracycline reduces urinary excretion of lignans (17), whereas another study did not find significant differences in enterolactone levels between antimicrobial users and nonusers (25). Our results are consistent with the findings of the first study;

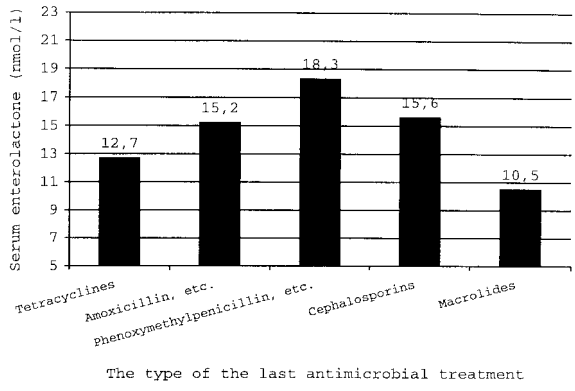

FIGURE 4. Serum enterolactone concentration according to type of the last antimicrobial treatment (time from treatment being a maximum of 3 months), Finland, 1997. Adjusted for body mass index, occurrence of constipation, consumption of lignan-containing foods, and smoking.

we found notably lower serum enterolactone concentrations in subjects who had used antimicrobials than in nonusers. This suppression was similar in both genders and was not affected by consumption of lignan-containing foods, smoking, BMI, or constipation. Furthermore, the lowest serum enterolactone concentrations were found in subjects who had recently used antimicrobials. Thus, the contradictory results obtained for the association between use of antimicrobials and enterolactone concentration may be due to the fact that Horn-Ross et al. (25) had no data on how recently the antimicrobials had been used. After antimicrobial use ceased, serum enterolactone levels seemed to increase over at least a 6–7-month period. However, no obvious plateau occurred, suggesting that it might take even longer for concentrations to be fully restored. Intestinal microflora has been reported to return to its normal level within 2 weeks of administration of antimicrobials (26–28). However, our results suggest that the ability of microflora to metabolize lignans normalizes more slowly. In addition, an inverse association was observed between serum enterolactone concentration and number of antimicrobial prescriptions. This finding is in accordance with some earlier observations showing greater disturbances in the intestinal flora after repeated treatments (29).

By affecting the intestinal flora, antimicrobials presumably interfere with formation of enterolactone from its precursors. Antimicrobials might also interfere with enzymatic hydrolysis of enterolactone conjugates excreted in bile, reducing enterolactone reabsorption (enterohepatic circulation) from the gut. As a result of antibiotic-associated diarrhea, metabolism and absorption of lignans may be incomplete. Other mechanisms, such as stimulation of enterolactone-metabolizing enzymes in the liver, cannot be ruled out, however.

Differences between various antimicrobials regarding serum enterolactone concentration were moderate. Serum enterolactone concentration was strongly suppressed by macrolides, whereas amoxicillin and other extended-spectrum penicillins, phenoxymethylpenicillin and other beta-lactamase-sensitive penicillins, and cephalosporins seemed to have minor effects. This finding is in accordance with earlier studies (30–32) showing that macrolides induce remarkable changes in both the aerobic and anaerobic microflora, while amoxicillin, phenoxymethylpenicillin, and cephalosporins cause minor ecologic alterations. The fact that most differences between various antimicrobials regarding serum enterolactone concentration did not reach statistical significance may be due to subjects using several different types of drugs and the small number of subjects in each drug group. Furthermore, it may reflect individual variation in the effect of antimicrobials on gastrointestinal flora.

Epidemiologic studies demonstrate an elevated risk of breast cancer for women with a low urinary enterolactone level (11) and of acute coronary events for men with a low serum enterolactone concentration (12). This finding raises the question of whether long-term or repeated antimicrobial treatments might lead to prolonged lowering of serum enterolactone concentration, thus enhancing the risk of chronic diseases. Thus far, the causality of these events remains speculative, although a recent study reported an elevated risk of breast cancer for women who had a history of medication use for urinary tract infection (33).

We used a nationwide drug register to obtain data on purchases of antimicrobials. The coverage of this register is very good; at the time of our study, more than 95 percent of Finnish pharmacies were covered. The only pharmacies excluded from the register were those without a computer system for handling prescriptions. We also had no data on antimicrobials received in hospitals. However, only 12 percent of all antimicrobials are used in hospitals (34). Information was also lacking on the dosages of these antimicrobials and the length of treatment, as well as antimicrobials not included in the reimbursement system because of their low price (less than 8 euros (US $8)). Most of these inexpensive drugs are phenoxymethylpenicillin, sulfatrimethoprim, and nalidixic acid, which have been suggested to have a minor impact on microflora (31, 32). Another limitation is the fact that purchase of antimicrobials does not necessarily mean actual use. However, this possible error would only attenuate the results.

In conclusion, we found that lowered serum enterolactone concentration was associated with use of antimicrobials. This finding is in line with the known role of gut microflora in formation of mammalian lignans from their precursors. Our findings also suggest that recent use of antimicrobials should be considered when the association between serum enterolactone concentration and risk of chronic diseases is studied. The importance of antimicrobials in the prophylaxis and treatment of infections should not be underestimated. However, extended use of antimicrobials and the trend toward broad-spectrum preparations are alarming, especially since a substantial portion of antibiotic use has questionable therapeutic value (35). Besides being expensive, overuse of broad-spectrum antimicrobials may also have implications for increasing antimicrobial resistance and the risk of chronic diseases. To avoid these serious consequences, more attention should be directed at the appropriate use of antimicrobials.

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