Risk of Cardiovascular Disease–Related and All-Cause Death According to Serum Concentrations of Enterolactone

Kuopio Ischaemic Heart Disease Risk Factor Study

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Background: Enterolactone is a plant-derived compound that has been associated with a reduced risk of acute coronary events and cancer. Several studies have suggested that serum enterolactone concentration may play a role as a biomarker of a diet high in fiber and vegetables. Owing to its phenolic structure, enterolactone and its plant lignan precursors, which are converted by intestinal bacteria to enterolactone, are potential antioxidants.

Methods: The associations between serum enterolactone level and the risk of coronary heart disease (CHD)–related, cardiovascular disease (CVD)–related, and all-cause mortality were investigated in the Kuopio Ischaemic Heart Disease Risk Factor Study, which is a prospective population-based study of middle-aged Finnish men. The serum enterolactone concentration and cardiovascular risk factors were determined in 1889 men aged 42 to 60 years. In an average follow-up of 12.2 years, 70 CHD-related, 103 CVD-related, and 242 all-cause deaths occurred in participants free of prior CVD.

Results: Multivariate analyses showed significant associations between elevated serum enterolactone concentration and reduced risk of CHD- and CVD-related mortality, but weaker associations in relation to all-cause mortality. In the Cox proportional hazards regression model adjusting for the most potent confounding factors, the risk of CHD-related ($P=.03$ for trend) and CVD-related ($P=.04$ for trend) death decreased linearly across quartiles of serum enterolactone concentration.

Conclusions: Our data suggest that a high serum enterolactone level is associated with reduced CHD- and CVD-related mortality in middle-aged Finnish men. These results add to the evidence supporting the importance of whole grain foods, fruits, and vegetables in the prevention of premature death from CVD.
To analyze further the role of this diet-derived phenolic substance in the prevention of CVD, we examined the association between serum enterolactone concentration and coronary heart disease (CHD)-related, CVD-related, and all-cause mortality in the prospective follow-up of the Kuopio Ischaemic Heart Disease Risk Factor Study of 1889 Finnish men free of CVD at baseline.

METHODS

STUDY POPULATION

The Kuopio Ischaemic Heart Disease Risk Factor Study was designed to investigate risk factors for CVD, atherosclerosis, and related outcomes in a population-based randomly selected sample of men in eastern Finland. Of the 3433 eligible men aged 42, 48, 54, or 60 years who resided in Kuopio or its surrounding rural communities, 198 were excluded because of death, serious disease, or migration from the area; of the remaining men, 2682 (82.9%) agreed to participate in the study. Baseline examinations were conducted between March 20, 1984, and December 5, 1989.

Serum enterolactone measurements were obtained in 1998 and in 2000 from baseline serum samples that were available for 2557 subjects. Samples had been stored at −20°C on average for 13.4 years (range, 8.4-16.5 years). One subject was withdrawn because of an exceptionally high enterolactone concentration (205.1 nmol/L), and was considered a clear outlier. Of the 2556 remaining subjects, men with prevalent CVD were excluded, which included 638 men with prevalent CHD and 29 men with a history of stroke. Prevalent CHD was defined as a history of acute coronary events or angina pectoris, angina pectoris on effort, or use of nitroglycerin tablets at least once a week.

ASSESSMENT OF VARIABLES

The collection of blood specimens and the measurement of serum lipoproteins, blood pressure, and 24-hour urinary excretion of nicotine metabolites have been described previously. The determination of serum enterolactone concentration was based on a time-resolved fluoroimmunoassay, described previously. Subjects with a systolic blood pressure of 160 mm Hg or higher, or who used antihypertensive drugs were classified as hypertensive. The body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

Dietary intake of nutrients was assessed with an instructed 4-day food recording by household measures at the Kuopio Ischaemic Heart Disease Risk Factor Study baseline examinations. The intake of nutrients and total calorie (energy) intake was calculated with computer software (Nutrica, version 2.5), which is compiled of mainly Finnish values of the nutrient composition of foods. All nutrient intakes tested were adjusted for calorie intake using the residual method, and, when applicable, corrected for losses due to food preparation. Saturated fatty acid intake was applied as percentage of total calories. Alcohol consumption was estimated by a frequency questionnaire and inserted as dummy variables constructed from quartiles. The frequency of constipation and of bronchitis and other infections was assessed by a questionnaire.

ASCERTAINMENT OF FOLLOW-UP EVENTS

Deaths were ascertained by computer linkage to the national death registry using the Finnish social security number. There were no losses to follow-up. All deaths that occurred during study enrollment (from March 20, 1984, to December 5, 1989) and to December 31, 1999, were included. Deaths that were coded with the International Classification of Diseases, Ninth Revision (ICD-9), codes 410 to 414 and 390 to 459 were included in the analyses of CHD- and CVD-related deaths, respectively.

DATA ANALYSIS

The associations of serum enterolactone concentration with the risk factors for death were examined using covariate analysis. Serum enterolactone concentration was classified into 4 categories according to quartiles. These categories or serum enterolactone concentrations, as dummy variables, were entered into forced Cox proportional hazards regression models using Statistical Product and Service Solutions 10.0 for Windows (SPSS Inc, Chicago, Ill). Three different sets of covariates were used: the basic model included age, year of serum enterolactone measurement (2 categories), and examination years (1985, 1986, 1987, 1988, and 1989); multivariate model 1 included the basic model, diabetes mellitus, hypertension, urinary excretion of nicotine metabolites, BMI, alcohol consumption, and serum low- and high-density lipoprotein cholesterol levels; multivariate model 2 included multivariate model 1 and dietary intake of fiber, folate, vitamins C and E, and saturated fatty acids. Their confidence intervals were estimated under the assumption of asymptotic normality of the estimates. All tests for statistical significance were 2-sided. A stepwise linear multivariate regression analysis was used to find the strongest determinants of serum enterolactone concentration.

BASELINE CHARACTERISTICS

The mean serum enterolactone concentration for the 1889 participants was 17.1 nmol/L (SD, 14.0 nmol/L). The distribution of the baseline characteristics by quartiles of serum enterolactone concentration is shown in Table 1. Serum enterolactone concentration varied by more than 10-fold between the highest and the lowest quartiles of the study population. Men with a high serum enterolactone level were less often obese and hypertensive, and they smoked less. They also consumed less alcohol and more fruits, berries, and whole grain products; consequently, their intake of water-soluble vitamins was greater than men with low serum enterolactone levels. In addition, the serum enterolactone concentration was higher among men with constipation.

FOLLOW-UP AND CUMULATIVE MORTALITY

During the follow-up of 12.2 years, we documented 70 CHD-related, 103 CVD-related, and 242 all-cause deaths. This equals approximately 23,000 person-years of observation. To illustrate the accumulation of the deaths according to serum enterolactone concentration, we analyzed the data with a Cox proportional hazards model adjusting for age, examination years, and year of enterolactone measurement. In Figures 1, 2, and 3, the cumulative CHD-related, CVD-related, and all-cause mortality, respectively, are presented separately by quartiles of serum enterolactone concentration to illustrate the earlier occurrence of deaths among men in the lowest quartiles of serum enterolactone concentration compared with the others.
In the Cox proportional hazards model, a low serum enterolactone concentration was associated with an increased risk of CHD- and CVD-related mortality (Table 2). When serum enterolactone concentration was analyzed as a continuous variable and adjusted for age and year of examination and of enterolactone measurement, there was a risk reduction of 17% and 13% for each 0.07 nmol/L.

**SERUM ENTEROLACTONE CONCENTRATION AND CHD- AND CVD-RELATED MORTALITY**

In the Cox proportional hazards model, a low serum enterolactone concentration was associated with an increased risk of CHD- and CVD-related mortality (Table 2). When serum enterolactone concentration was analyzed as a continuous variable and adjusted for age and year of examination and of enterolactone measurement, there was a risk reduction of 17% and 13% for each 0.07 nmol/L.

**Table 1. Distribution of Baseline Characteristics by Quartiles of Serum Enterolactone Concentration**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Quartile of Serum Enterolactone Concentration</th>
<th>P Value for Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Age, y</td>
<td>52 ± 5</td>
<td>52 ± 6</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>137 ± 18</td>
<td>135 ± 16</td>
</tr>
<tr>
<td>Diastolic</td>
<td>91 ± 11</td>
<td>89 ± 10</td>
</tr>
<tr>
<td>Hypertension</td>
<td>43</td>
<td>36</td>
</tr>
<tr>
<td>Cholesterol, mg/dl (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>154 ± 40 (4.0 ± 1.0)</td>
<td>155 ± 39 (4.0 ± 1.0)</td>
</tr>
<tr>
<td>HDL</td>
<td>50 ± 11 (1.3 ± 0.3)</td>
<td>51 ± 11 (1.3 ± 0.3)</td>
</tr>
<tr>
<td>Diabetes mellitus‡</td>
<td>43</td>
<td>36</td>
</tr>
<tr>
<td>BMI</td>
<td>27.6 ± 3.8</td>
<td>26.8 ± 3.4</td>
</tr>
<tr>
<td>Current smokers‡</td>
<td>34</td>
<td>29</td>
</tr>
<tr>
<td>Urinary excretion of nicotine metabolites, mg/24 h</td>
<td>6.7 ± 10.3</td>
<td>6.0 ± 9.7</td>
</tr>
<tr>
<td>Alcohol consumption, g/ wk</td>
<td>95 ± 145</td>
<td>79 ± 116</td>
</tr>
<tr>
<td>Constipation during the previous year‡</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>No. of bronchitis diagnoses during a lifetime‡</td>
<td>1.1 ± 2.4</td>
<td>0.9 ± 2.3</td>
</tr>
<tr>
<td>Dietary intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits and berries, g/d</td>
<td>153 ± 142</td>
<td>160 ± 147</td>
</tr>
<tr>
<td>Whole grain products, g/d</td>
<td>137 ± 68</td>
<td>141 ± 73</td>
</tr>
<tr>
<td>Fiber, g/d</td>
<td>23 ± 7</td>
<td>24 ± 7</td>
</tr>
<tr>
<td>Saturated fat, % of total calories</td>
<td>19 ± 4</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>Vitamin C, mg/d</td>
<td>67 ± 44</td>
<td>71 ± 50</td>
</tr>
<tr>
<td>Vitamin E, mg/d</td>
<td>8.5 ± 2.4</td>
<td>8.8 ± 2.5</td>
</tr>
<tr>
<td>Folate, µg/d</td>
<td>243 ± 54</td>
<td>251 ± 58</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); HDL, high-density lipoprotein; LDL, low-density lipoprotein.

*Data are given as mean ± SD unless otherwise indicated.
†Quartile 1 indicates 0.2 to 6.9 nmol/L; quartile 2, 7.0 to 13.7 nmol/L; quartile 3, 13.8 to 23.8 nmol/L; and quartile 4, 23.9 to 88.7 nmol/L.
‡Data are given as percentage of men.
§A systolic blood pressure of 160 mm Hg or higher and/or a diastolic blood pressure of 95 mm Hg or higher and/or use of an antihypertensive medication.
||From a 4-day food recording.

**Figure 1.** Cumulative coronary heart disease (CHD)–related mortality in men according to quartiles of serum enterolactone concentration, adjusted for age and year of examination and of serum enterolactone measurement. Quartile 1 indicates a serum enterolactone concentration of 0.2 to 6.9 nmol/L; quartile 2, 7.0 to 13.7 nmol/L; quartile 3, 13.8 to 23.8 nmol/L; and quartile 4, 23.9 to 88.7 nmol/L.

**Figure 2.** Cumulative cardiovascular disease (CVD)–related mortality in men according to quartiles of serum enterolactone concentration, adjusted for age and year of examination and of serum enterolactone measurement. Quartiles are described in the legend to Figure 1.
10 nmol/L of serum enterolactone concentration in CHD-related mortality (95% confidence interval, 0.69-0.99) and CVD-related mortality (95% confidence interval, 0.75-1.00), respectively. The trend across serum enterolactone quartiles remained significant after adjustment for diabetes mellitus, hypertension, urinary excretion of nicotine metabolites, BMI, alcohol consumption, and serum low- and high-density lipoprotein cholesterol levels, and after adjustment for dietary factors such as dietary intake of vitamins C and E, folate, fiber, and saturated fatty acids. In this multivariate model, men with a high serum enterolactone level (≥23.9 nmol/L) had a 56% reduced risk of CHD-related death and a 45% reduced risk of CVD-related death, which was borderline significant.

SERUM ENTEROLACTONE CONCENTRATION AND ALL-CAUSE MORTALITY

Serum enterolactone concentration, adjusted for age and year of the examination and of enterolactone measurement, was inversely associated with all-cause mortality (Table 2). When serum enterolactone concentration was used as a continuous variable and adjusted for the same covariates, there was a risk reduction of 12% (95% confidence interval, 0.80-0.97) for each additional 10 nmol/L of serum enterolactone. After adjustment in model 1, the trend across the quartiles of serum enterolactone concentration remained significant, but the risk reduction in the highest quartile (≥23.9 nmol/L) compared with the lowest (≤6.9 nmol/L) was not significant anymore. Additional adjustment for diet weakened the observed association further.

DETERMINANTS OF SERUM ENTEROLACTONE CONCENTRATION

The dietary constituent with the strongest univariate association with enterolactone concentration was calorie-adjusted fiber, which in a linear regression model explained approximately 6% of the variation in enterolactone concentration. Of the factors that remained significant in the model (vegetable consumption, alcohol intake, saturated fatty acid intake, constipation, BMI, and the number of bronchitis diagnoses during a lifetime), only vegetable consumption and constipation showed a positive association with serum enterolactone concentration. Other infections, such as tonsillitis, sinusitis, or ear infections, were not associated with enterolactone concentration. All of these variables together explained approximately 10% of the variation in serum enterolactone concentration.

In this 12-year prospective study of middle-aged men, we found an association between serum enterolactone concentration and reduced risk of CHD- and CVD-related mortality. In the standard multivariate model, the risk of all-cause mortality was reduced in men with a high serum enterolactone concentration, but this association did not persist after additional adjustment for diet. The rare possibility to measure the level of a diet-derived polyphenol in human samples in a sizable study with a long follow-up adds to the importance of these findings.

Concerning the impact of dietary polyphenols on CHD-related mortality, some evidence from prospective studies is available suggesting that dietary flavonoids have a weak, but protective, effect against CHD-related death in men. In the Mobile Clinic Health Survey (n=2748; 324 deaths), in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (n=25372; 815 deaths), and in the Male Health Professionals Study (n=38036; 140 deaths), the results suggest a reduced CHD-related death risk among men in the highest quartile or quintile of flavonoid intake, although the association weakened significantly after adjustment. In the Zutphen Elderly Study (n=805; 42 deaths), a strong inverse association was observed, despite the small number of deaths that occurred during the follow-up. Flavonoid intake in the Zutphen Elderly Study correlated highly with tea consumption, which also had a significant and inverse association with CHD-related mortality risk. In the Mobile Clinic Health Survey study, the estimated intake of flavonoids mostly derived from apples and onions, both of which presented similar associations with CHD-related mortality.

The advantage of a specific analytical method to determine the concentration of a diet-derived polyphenol in human samples is obvious compared with the inaccuracy derived from the estimations of dietary intake. In the case of polyphenols, in which some need partial degradation by the colon microflora to be absorbed and, thus, bioavailable, having a meaningful biomarker is of even greater value. Although other compounds in connection with dietary lignans and enterolactone might play a role in the observed association, data on human sample concentrations restrict the possible confounding factors compared with dietary surveys.

The content of plant lignans in the diet is often considered the most important determinant of serum enterolactone concentration. In dietary intervention trials, consumed plant lignans have shown a clear dose-dependent response on serum enterolactone concentration and on uric-
determinants. In a cross-sectional study of 100 Finnish men, fiber components together explained 4.5% of the variation in serum enterolactone concentration. Correctly diagnosed bronchitis is rarely treated with antimicrobial agents. In the present study, approximately 10% of the variation in serum enterolactone concentration could be explained by the selected determinants. In a cross-sectional study of 100 Finnish men, fiber components together explained 4.5% of the variation, whereas Horner et al reported that demographic characteristics and total fiber, alcohol, and caffeine intake explained 22% of the variability in plasma enterolactone concentration in 193 volunteers from Seattle, Wash. Nevertheless, the incoherent and rather modest proportions explained in most of the mentioned studies point out the possible existence of other, still unknown, determinants of serum enterolactone concentration. Of these unknown factors, the composition and activity of the intestinal microflora are likely to be key contributors. Considering the effect of dietary changes and bowel movement on serum enterolactone concentration, it is interesting to note that even a single measurement of serum enterolactone concentration can predict relatively well the level during a 2-year period.

Our knowledge, for the first time, alcohol consumption was shown to present a negative association with serum enterolactone concentration, and the mechanism behind this could be hypothesized. There are several lines of evidence indicating that short-term alcohol ingestion affects transit time and induces bacterial overgrowth in the small intestine, which might contribute to diarrhea associated with heavy drinking. Also, colonic bacterial flora might be affected, because orally ingested alcohol might increase the levels of intracolonic ethanol and make them equal to those in the blood. Furthermore, large-bowel ethanol is oxidized by alcohol dehydrogenates of intestinal bacteria, resulting in the accumulation of toxic acetaldehyde.

**Table 2. The RRs for CHD-Related, CVD-Related, and All-Cause Mortality by Quartiles of Serum Enterolactone Concentration**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Quartile of Serum Enterolactone Concentration*</th>
<th>P Value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of deaths</td>
<td>CHD-Related Death</td>
<td>CVD-Related Death</td>
</tr>
<tr>
<td>RR (95% CI)</td>
<td>1†</td>
<td>2</td>
</tr>
<tr>
<td>Adjusted for age and year of examination and of serum enterolactone measurement</td>
<td>1.00</td>
<td>0.89 (0.48-1.63)</td>
</tr>
<tr>
<td>Multivariate model</td>
<td>1.00</td>
<td>1.07 (0.77-1.50)</td>
</tr>
</tbody>
</table>

Abbreviations: CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; RR, relative risk.

*Quartiles are described in the third footnote to Table 1.
†Reference.
‡Adjusted for age, year of examination and of serum enterolactone measurement, diabetes mellitus, hypertension, urinary excretion of nicotine metabolites, body mass index, alcohol consumption, and low- and high-density lipoprotein cholesterol levels.
§In addition, adjusted for dietary intake of fiber, folate, vitamins C and E, and saturated fatty acids.
in the colon. Thus, it can be hypothesized that the effects of alcohol use on intestinal bacterial flora and on transit time result in decreasing enterolactone production. This could also explain the inverse association observed with alcohol consumption and serum enterolactone level in this study. Inconsistently, though, Horner et al reported from the referred cross-sectional study that alcohol had a positive correlation with plasma enterolactone level. However, only participants whose alcohol consumption did not exceed 2 drinks per day were selected for the study, which lessens the speculative value of the observation.

In previous studies, the enterolactone level has been associated with several biological properties, which might be of interest in the prevention of prostate and breast cancers. The structure of dietary polyphenols, which might be an indication of antioxidative function, has also been discussed. Consistently with these speculations, the results from a cross-sectional study associate low serum enterolactone concentration with enhanced in vivo lipid peroxidation, measured as F2-isoprostanes. However, the accumulating data on previously unknown enterolactone precursors have multiplied the number of substances that the effects associated with enterolactone might result from. Thus, with the existing evidence, it is difficult to distinguish the actual active substance, whether it is enterolactone, its precursors, or even another substance related to them. Even after comprehensive adjustment for related factors in the multivariate models, some of the observed associations might possibly be explained by dietary and other lifestyle factors positively associated with higher concentrations of serum enterolactone.

The data presented suggest that a high concentration of serum enterolactone may protect against premature CHD- and CVD-related death. The results support the hypothesis that polyphenols in diets high in fiber can be of importance in the prevention of CVD. Colon bacteria, which are essential in generating enterolactone, may have an important, but yet unknown, role in the protection against CVD.

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