Risk of acute coronary events according to serum concentrations of enterolactone: a prospective population-based case-control study

Meri Vanharanta, Sari Voutilainen, Timo A Lakka, Manon van der Lee, Herman Adlercreutz, Jukka T Salonen

Summary

Background The lignan enterolactone, produced by the intestinal microflora from dietary precursors, has been implicated in protection against cancer. We investigated the association of serum enterolactone concentration with the risk of acute coronary events in a prospective nested case-control study in middle-aged men from eastern Finland.

Methods Enterolactone was measured by time-resolved fluoroimmunoassay in serum from 167 men who had an average 7-7 years of follow-up to an acute coronary event and from 167 control men. Both cases and controls were from a cohort of 2005 men who had no clinical coronary heart disease (CHD) at baseline. The controls were matched for age, examination year, and residence. Acute coronary events were registered prospectively.

Findings The mean baseline serum enterolactone concentration was lower among the cases than the controls (18·2 [SD 21·1] vs 23·5 [18·2] nmol/L, p=0·001). The men in the highest quarter of the enterolactone distribution (>30·1 nmol/L) had a 58·8% (95% CI 24·1–77·6, p=0·005) lower risk of acute coronary events than men in the lowest quarter. After adjustment for the nine most strongly predictive risk factors, men in the highest enterolactone quarter had a 65·3% (11·9–86·3, p=0·03) lower risk than men in the lowest quarter.

Interpretation Healthy men with high serum concentrations of enterolactone had a lower risk of acute coronary events than men with lower concentrations. These findings support the hypothesis that plant-dominated fibre-rich food lowers the risk of CHD.

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Introduction

Lignans have been postulated to have a role in protection against both cancer and coronary heart disease (CHD).1 Mammalian lignans are synthesised when plant lignans (phyto-oestrogens) are modified by intestinal bacteria.2 The most abundant mammalian lignan is enterolactone. There is evidence that enterolactone influences metabolism of sex hormones and proliferation of malignant cells, inhibits the human aromatase enzyme, and inhibits the metabolism of growth-promoting steroid hormones.3,4,5 These effects of enterolactone provide potential mechanisms for a postulated preventive influence in CHD.

Many common foodstuffs, such as seeds, cereals (rye, barley, and wheat), berries, and some vegetables, contain plant lignans.1 However, most mammalian lignan precursors may still be unknown.1

We aimed to test the hypothesis that a high serum enterolactone concentration is associated with a low risk of acute coronary events in middle-aged men with no previous atherosclerotic vascular diseases.

Methods

Study population

The Kuopio Ischaemic Heart Disease Risk Factor Study is a population-based study of risk factors for CHD, atherosclerosis, and other related outcomes.6 The study protocol was approved by the Research Ethics Committee of the University of Kuopio. The study population is a random sample of men living in the city of Kuopio or neighbouring rural communities, stratified and balanced in four strata: exactly 42 years, 48 years, 54 years, or 60 years old at the baseline examination. The baseline examinations were carried out between 1984 and 1989. Of 3235 eligible men, 2682 (82·9%) participated. Men with prevalent CHD at baseline (n=677) were excluded. Prevalent CHD was defined as a history of acute coronary events or angina pectoris, angina pectoris on effort, or use of glyceryl trinitrate tablets at least once a week.7 Thus, this study is based on a cohort of 2005 men without CHD.

Design and procedures

Acute coronary events that occurred between 1984 and 1992 were registered as part of the multinational MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) Project.8 Data on coronary events between 1993 and 1996 were obtained by record linkage from the national computerised hospital-discharge registry. The diagnostic classification used was identical to that of the MONICA project. The cohort was followed up on average for 10 years. According to the diagnostic classification of the events,9 there were 81 definite and 55 possible acute myocardial infarctions and 31 typical long episodes of chest pain. The cases were 167 men who had their first acute coronary event by the end of 1996. To ensure the comparability of the controls, one control was matched for each case according to age (42, 48, 54, 60), examination year (1984–1989) and place of residence (the same municipality out of ten), and were drawn from the same cohort as the cases. The follow-up time for the 334 participants varied between 1 month (to event) and 12·8 years. Serum samples
fluoroimmunoassay with a europium chelate as a label, enterolactone. After enzymic hydrolysis and ether extraction, the extractions (each with 1·5 mL). 5. Drawn at baseline were stored at – 20ºC on average for 11·9 years (range 8·4–14·2) from sampling to enterolactone measurements.

Magnesium (g/day) 324 0·44 (0·11) 0·44 (0·10) 0·98

Blood glucose (mmol/L) 334 5·03 (1·38) 4·73 (0·68) 0·38

Iron (mg/day) 324 18·7 (5·9) 18·5 (5·5) 0·83

Systolic blood pressure (per 10 mm Hg) ·· ·· 1·26 (1·02–1·54) 0·03

Diabetes (yes vs no) ·· ·· 2·80 (1·40–5·46) 0·004

Serum HDL cholesterol (mmol/L) 326 1·21 (0·27) 1·31 (0·30) 0·003

Smokers 334 72 (43%) 45 (27%) 0·005

Table 2: Strongest risk factors for acute coronary events in conditional multivariate logistic regression models

<table>
<thead>
<tr>
<th>Model 1*</th>
<th>Odds ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterolactone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fourth quarter (&gt;30·10 nmol/L)</td>
<td>0·33 (0·14–0·76)</td>
<td>0·01</td>
</tr>
<tr>
<td>Third quarter (15·11–30·10 nmol/L)</td>
<td>0·69 (0·31–1·57)</td>
<td>0·38</td>
</tr>
<tr>
<td>Second quarter (7·21–15·10 nmol/L)</td>
<td>1·11 (0·50–2·45)</td>
<td>0·80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2†</th>
<th>Odds ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterolactone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fourth quarter (&gt;30·10 nmol/L)</td>
<td>0·35 (0·14–0·88)</td>
<td>0·03</td>
</tr>
<tr>
<td>Third quarter (15·11–30·10 nmol/L)</td>
<td>0·75 (0·31–1·81)</td>
<td>0·53</td>
</tr>
<tr>
<td>Second quarter (7·21–15·10 nmol/L)</td>
<td>1·22 (0·51–2·96)</td>
<td>0·51</td>
</tr>
</tbody>
</table>

Other risk factors

<table>
<thead>
<tr>
<th>Odds ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum apolipoprotein B (per 100 mg/L)</td>
<td></td>
</tr>
<tr>
<td>Dietary iron intake (mg/day)</td>
<td></td>
</tr>
<tr>
<td>Family history of coronary heart disease (yes vs no)</td>
<td></td>
</tr>
<tr>
<td>Ischaemic findings on exercise test (yes vs no)</td>
<td></td>
</tr>
<tr>
<td>Dietary calcium intake (per 100 mg/day)</td>
<td></td>
</tr>
<tr>
<td>Urinary excretion of nicotine metabolites (mg/day)</td>
<td></td>
</tr>
<tr>
<td>Diabetes (yes vs no)</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (per 10 mm Hg)</td>
<td></td>
</tr>
<tr>
<td>Maximum oxygen uptake (L kg⁻¹ min⁻¹)</td>
<td></td>
</tr>
</tbody>
</table>

A logistic model including three highest enterolactone quarters.
†A stepwise model (p for entry 0·05); the second and third enterolactone quarters are forced into the model.

Statistical analysis

Differences in risk factors between the cases and controls were tested for significance with Wilcoxon's signed-rank test or with M cN e a r ' s test for two related samples; those between quarters of the serum enterolactone distribution were tested with K r u s k a l – W a l l i s A N O V A . Odds ratios for acute coronary events, adjusted for risk factors, were estimated by stepwise and forced conditional multivariate logistic-regression modelling in paired data. 95% C I were estimated on the assumption of asymptotic normality of estimates of the E g r e t for W indows software (version 1·0). Missing values in urinary nicotine excretion were imputed by means of linear regression on the basis of the self-reported number of cigarettes smoked daily. The self-reported number of cigarettes daily had a correlation with the urinary nicotine excretion of 0·57 before imputation and 0·72 after the imputation. M issing values in other covariates were replaced by means, separately for cases and controls.

Results

The distributions of the major known risk coronary factors in the cases and controls are shown in table 1. T he mean serum enterolactone concentration was 25·1% lower among the cases than among controls (95% C I 4·1–46·2), p=0·001 for difference). In a univariate logistic model, the risk of acute coronary events was decreased on average by 1·4% per unit (nmol/L) of serum enterolactone (0·2–2·6, p=0·016). T he risk of acute coronary events was decreased on average by 1·4% per unit (nmol/L) of serum enterolactone (0·2–2·6, p=0·016). T here was a decrease in acute coronary events of 4·1–46·2% for each 1·4% per unit (nmol/L) of serum enterolactone (0·2–2·6, p=0·016). T he risk of acute coronary events was decreased on average by 1·4% per unit (nmol/L) of serum enterolactone (0·2–2·6, p=0·016). T here was a decrease in acute coronary events of 4·1–46·2% for each 1·4% per unit (nmol/L) of serum enterolactone (0·2–2·6, p=0·016). T he risk of acute coronary events was decreased on average by 1·4% per unit (nmol/L) of serum enterolactone (0·2–2·6, p=0·016). T here was a decrease in acute coronary events of 4·1–46·2% for each 1·4% per unit (nmol/L) of serum enterolactone (0·2–2·6, p=0·016). T he risk of acute coronary events was decreased on average by 1·4% per unit (nmol/L) of serum enterolactone (0·2–2·6, p=0·016). T here was a decrease in acute coronary events of 4·1–46·2%.
Men with high serum enterolactone (above 6.5% (11.9–86.3, p=0.03) lower risk of acute coronary events than men in the lowest quarter. To examine the dose-response relation, we grouped the participants into quarters of the serum enterolactone distribution. Men in the highest quartile (≥30.10 nmol/L) had a 2.6% (24.1–77.6, p=0.005) lower risk of acute coronary events than men in the lowest quartile. After adjustment for the nine most strongly predictive risk factors (table 2, model 2), men in the highest quartile had a 65.3% (11.9–86.3, p=0.03) lower risk of acute coronary events than men in the lowest quartile. The linear trend in risk of acute coronary events across quarters of the enterolactone distribution was significant (p=0.01).

To test the association between enterolactone and the risk of acute coronary events in men with neither clinical nor subclinical CHD, we excluded 37 pairs because electrocardiography showed ischaemia on the exercise test for either the case or the control. The association of enterolactone with the risk of acute coronary events was stronger than for all pairs. The unadjusted odds ratio for men in the highest quarter of enterolactone was 0.33 (0.14–0.76, p=0.01) compared with those in the lowest quarter.

Since smokers are under increased oxidative stress, the impact of antioxidative nutrients on CHD is expected to be greater in smokers. In an unadjusted unpaired logistic regression analysis, the relative benefit in the highest quarter was 79.3% (37.8–93.1, p=0.005) among smokers and 47.0% among non-smokers (−11.5 to 75.9, p=0.11). The difference was not significant.

The mean (SE) blood pressure and serum lipid concentrations in quarters of the distribution of serum enterolactone concentrations from ANOVA

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>First quarter</th>
<th>Second quarter</th>
<th>Third quarter</th>
<th>Fourth quarter</th>
<th>p for heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mm Hg)*</td>
<td>142 (2)</td>
<td>136 (2)</td>
<td>134 (2)</td>
<td>137 (2)</td>
<td>0.026</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)*</td>
<td>93 (1)</td>
<td>89 (1)</td>
<td>88 (1)</td>
<td>90 (1)</td>
<td>0.017</td>
</tr>
<tr>
<td>Serum total cholesterol (mmol/L)</td>
<td>6.13 (0.13)</td>
<td>6.13 (0.13)</td>
<td>6.27 (0.13)</td>
<td>6.22 (0.13)</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum LDL cholesterol (mmol/L)</td>
<td>4.25 (1.13)</td>
<td>4.34 (1.03)</td>
<td>4.39 (1.02)</td>
<td>4.19 (0.99)</td>
<td>0.52</td>
</tr>
<tr>
<td>Serum HDL cholesterol (mmol/L)</td>
<td>1.24 (0.03)</td>
<td>1.28 (0.03)</td>
<td>1.26 (0.03)</td>
<td>1.28 (0.03)</td>
<td>0.74</td>
</tr>
<tr>
<td>Serum apolipoprotein B (mg/L)</td>
<td>478 (59)</td>
<td>350 (59)</td>
<td>447 (58)</td>
<td>519 (59)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*Adjusted for age, examination year, alcohol intake, 24 h urinary sodium excretion, and dietary magnesium intake.

Discussion

Enterolactone is a diphenolic compound of dietary origin that has been associated with a reduced risk of cancer. A role of enterolactone in protection against CHD has been suggested previously. Although enterolactone may be simply a biomarker of healthy fibre-rich food, there are mechanisms through which enterolactone itself could actively protect against cardiovascular diseases. Plasma enterolactone concentration depends on the intake of lignan-containing food and the activity of the intestinal microflora. For example, antibiotic treatment decreases the production of enterolactone for weeks.

There is an increasing amount of evidence that the initiation of atherosclerosis is related to the oxidative modification of LDL. Lipid peroxidation is associated with accelerated atherosclerotic progression. Like all phenolic compounds, enterolactone is likely to inhibit lipid peroxidation. Cigarette smoke increases lipid peroxidation. Because smokers are under high oxidative stress, they are likely to benefit from additional antioxidants, such as enterolactone. We found higher relative benefits from high serum enterolactone for smokers than for non-smokers.

Intracellular enterolactone could also protect against lipid peroxidation. Because of the interaction between lignans and the sex-hormone-binding globulin (SHBG), enterolactone is carried to the target cells. Enterolactone passes freely into the cell and could protect against oxidative damage due to intracellular free radicals.

Evidence from both basic research and cross-sectional human population studies suggests that increased free-radical stress is associated with hypertension. Also, low plasma concentrations of vitamin C and selenium have been associated with high blood pressure. We found higher blood pressure in men with low serum enterolactone concentrations than in those with higher values.

Lignans have weak oestrogenic activity, as a result of the 2,3-dibenzylbutane structure, which resembles synthetic oestrogens. High amounts of lignans together with other similar compounds entering the portal circulation stimulate SHBG production. This is one mechanism through which lignans could protect against atherosclerosis.
and C H D. Low SHBG concentrations are associated with smaller and denser LDL particles, which in turn have been associated with increased CHD risk. 25 The oestrogenic effect of lignans on liver SHBG production may parallel an effect on LDL synthesis resulting in larger LDL particles that are more resistant to oxidation. In a female population during a 12-year follow-up period, low plasma LDL-BG concentrations were associated with increased CHD mortality, 27 but in men the evidence is controversial. 24

Our finding of high blood pressure in men with low serum enterolactone concentrations needs to be retested. We confirmed the previous finding that fibre intake correlates only weakly with enterolactone concentration in body fluids. 28 Even though a high dietary intake of both total fibre and cereal fibre has been associated with a lower risk of CHD in several prospective studies, 28,29 we found no such association. Nevertheless, we cannot rule out the possibility that there are other compounds in fibre-rich foods that might add to the protective effect of enterolactone or even be the protective nutrients, enterolactone merely being a biomarker of a protective diet.

Our findings must be confirmed by further epidemiological studies, and randomised trials testing the effects of diets and supplements high in plant and mammalian lignans will be warranted. A diet high in enterolactone precursors has no conceivable harmful effects. Eventually, these further studies could justify changes in the current recommendations for a healthy diet.

Contributors

M eri Vanharanta undertook most of the data analyses and drafted the paper. Sari Vuotilainen contributed to the data analyses and checked the food record data. T imo Lakk a classified coronary events. M anon van der Lee carried out the enterolactone assays, E sako Adlercreutz developed and supervised the enterolactone assays, and J ukka Salonen designed the K IH D investigation and initiated this study. All investigators contributed to the writing of the report.

Acknowledgments

We thank Kristiina Nyssonen for supervising part of the chemical analyses; E sako Taikkinen, J uh a Venäl äinen, R iitta Salonen, H annu Lintmanen, and R ainer Rauramaa for supervising exercise tests; J aakko T ummi l eht o, and K aleld Pyörr äl ä for the access to the F in nmoni ca coronary registry data; P lonja K üper for participating in the ent erolactone analyses; a nd S udhir K ur i and H annu M ykk änen for useful comments. T his study was supported by research grants from the N ational H eart, L ung, and B lood Institute of the U SA (grant H L 44199 to G eorge A K aplan) and the A cademy of F inland (grants 41471, 1041086 and 2041022 to J ukka T . Salonen).

References