Association between low serum enterolactone and increased plasma F₂-isoprostanes, a measure of lipid peroxidation

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Abstract

Evidence suggests that low serum enterolactone concentration might be an independent risk factor for acute coronary events. Enterolactone is a lignan, which is formed by intestinal bacteria from precursors in plant foods. Due to the biphenolic structure of enterolactone, it could act as an antioxidant and through this contribute to cardiovascular health. The aim of this study was to test the hypothesis that a low serum enterolactone concentration is associated with increased in vivo lipid peroxidation, assessed by plasma F₂-isoprostane concentrations. We investigated this association in a subset of participants in 'The Antioxidant Supplementation in Atherosclerosis Prevention' (ASAP) study. Out of 256 male participants a subsample of 100 consecutive men from baseline was selected for F₂-isoprostane assays. The mean serum enterolactone concentration was 16.6 nmol/l and that of F₂-isoprostanes 29.6 ng/l. The correlation coefficient for association between serum enterolactone and F₂-isoprostane concentrations was −0.30 (P < 0.003). Plasma F₂-isoprostane levels decreased linearly across quintiles of serum enterolactone concentration (P = 0.008 for a linear trend). In a multivariate model, enterolactone persisted as a significant predictor after adjustment for vitamins and other variables, with the strongest associations with F₂-isoprostanes. Our present data suggest that low serum enterolactone concentration is associated with enhanced in vivo lipid peroxidation in men. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

A large body of evidence has supported the view that oxidative damage to lipids is the onset of the atherogenic progress which can advance to cardiovascular disease [1]. Antioxidants are presumed to have an important role in preventing this process [2]. Diets rich in whole grain and fruit and vegetable fibre have been shown to protect against coronary heart disease in large populations studies [3,4]. These diets contain a variety of vitamins, complex carbohydrates and phytochemicals such as lignans, flavonoids and isoflavonoids, which can account for the protective effect. Nevertheless, quantification of these possible antioxidants, other than vitamins, in human samples has become available only recently. Due to the challenges faced also in assessing oxidative stress status reliably [5], studies of the associations between oxidative stress and a diet-derived compound measured from human sample are scarce.

Lignans are a group of polyphenolic compounds widely distributed in plants. Good dietary sources of
lignans are whole grain products, berries, fruits and tea [6]. Plant lignans are converted by intestinal bacteria to mammalian lignans [7] of which enterolactone represents the most abundant end product. The development of a time-resolved fluoroimmunoassay method for quantifying enterolactone in plasma [8,9] has enabled enterolactone analysis in large epidemiologic studies.

Dietary compounds with polyphenolic structure might be able to act as antioxidants by virtue of their hydrogen-donating capacity [10]. For this reason enterolactone has been a subject of interest in the prevention of cancer and CHD [11]. In our prospective population-based case-control study, high serum enterolactone concentration was associated with reduced risk of acute coronary events [12]. The protective effect persisted in a multivariate model adjusting for the established risk factors, indicating an independent protective effect of enterolactone or of other associated compounds.

Plasma F$_2$-isoprostane concentration is currently being considered as one of the few reliable biomarkers of in vivo lipid peroxidation [5,13]. F$_2$-isoprostanes are oxidative modifications of arachidonic acid that result from the free-radical attack of cell membrane phospholipids or circulating LDLs [5].

To our knowledge, the association of serum enterolactone concentration and a measure of in vivo lipid peroxidation has not been studied earlier. In this study we examined whether serum enterolactone concentration is related to F$_2$-isoprostane levels in 100 mildly hypercholesterolemic men and which are the dietary characteristics associated with serum enterolactone level.

2. Methods

We studied the association between serum enterolactone and plasma F$_2$-isoprostanes in a subset of participants in the ‘Antioxidant Supplementation in Atherosclerosis Prevention’ (ASAP) study [14]. The ASAP study is a balanced 2 × 2 factorial double-masked placebo-controlled randomized clinical trial to study the effects of supplementary vitamin E and slow-release vitamin C and their combination (CellaVie®) on oxidative stress, lipid peroxidation, atherosclerotic progression and cardiovascular events in high-risk men and women. The study protocol was approved by the Research Ethics Committee of the University of Kuopio and all subjects gave written consent. For the current study a subsample of 100 consecutive men out of 256 male participants was selected. The age of these men was 58.6 ± 6.5 years (mean ± S.D.).

For the present study, samples for all measurements were taken at the baseline visits between April and October 1995, prior to the initiation of vitamin or placebo supplementation. Thus the present study is a cross-sectional investigation. Subjects were instructed to abstain from eating for 12 h and from ingesting alcohol for a week. Plasma F$_2$-isoprostane concentrations were determined at the Vanderbilt University Medical Center, Nashville, USA, in 1997 as described earlier [15]. Samples were kept stored at −70 °C until the measurement. Determination of serum enterolactone concentration was based on time-resolved fluoroimmunoassay (TR-FIA), described earlier [8,9]. Measurements of urinary nicotine metabolite excretion [16], plasma total homocysteine concentration [15], plasma α-tocopherol and β-carotene [17] were conducted as described earlier.

The consumption of foods was assessed at the time of blood sampling with an instructed 4-day food recording by household measures. The intake of nutrients and total energy intake was assessed with Nutrica® software. All nutrient intakes tested were adjusted for energy intake using the residual method [18] and were applicable corrected for losses due to food preparation.

The statistical significance of linear trend in plasma F$_2$-isoprostane over quintiles of serum enterolactone was tested using one-way analysis of variance (ANOVA). A stepwise linear multivariate regression analysis was used to find the strongest determinants of plasma F$_2$-isoprostane level. Those were used as covariates in analysis of covariance to estimate the independent association of serum enterolactone with F$_2$-isoprostanes. As the distribution on serum enterolactone values was skewed towards higher values, enterolactone values were log-transformed to improve normality. All statistical analyses were repeated with the log-transformed enterolactone values but it had a minimal effect on the results. The R square for the dietary constituents, accounting for the variation of enterolactone was halved. SPSS for Windows 10.0 was used for all statistical analyses.

3. Results

The mean serum enterolactone concentration was 16.6 nmol/l (range 1.1–70.8 nmol/l) and that of F$_2$-isoprostanes 29.6 ng/l, ranging from 7.0 to 70.0 ng/l. The simple correlation coefficient for association between serum enterolactone and F$_2$-isoprostanes was −0.30 (P = 0.003). In a linear regression model, the variables with the strongest associations with F$_2$-isoprostanes selected by stepwise analysis (P in 0.05, P out 0.10) were alcohol consumption (standardized coefficient) 0.29, selenium intake −0.27, serum enterolactone concentration −0.24 and plasma total homocysteine concentration 0.20 (Table 1). Traditional risk factors such as LDL cholesterol, blood pressure, body mass index and smoking assessed by 24 h urinary excretion of
nicotine metabolites were tested for entry to the model but they had no residual association with F2-isoprostanes and were not selected. In the second model we forced the antioxidative vitamins with the strongest correlations with F2-isoprostanes to the model (Table 1). These were plasma alpha-tocopherol, beta-carotene, ascorbic acid and dietary folic acid (energy standardized). Consequently the R square of the model rose from 37 to 39%. Serum enterolactone and other variables remained significant after this further adjustment with the antioxidant vitamins.

The strongest determinants of F2-isoprostanes were also tested in a regression analysis separately in smokers (>1 cigarettes per day) (n = 49) and in non-smokers (n = 51). In smokers only plasma total homocysteine concentration of the tested F2-isoprostanes determinants had a significant association with F2-isoprostanes (0.35, P = 0.02). In non-smokers, the associations of alcohol consumption (0.42, P = 0.001), serum enterolactone concentration (−0.38, P = 0.001) and selenium intake (−0.36, P = 0.002) with F2-isoprostanes were stronger, whereas plasma total homocysteine concentration (−0.03, P = 0.8) showed no association.

Plasma F2-isoprostane levels increased linearly across quintiles of serum enterolactone concentration (P = 0.008 for a linear trend) (Fig. 1). The unadjusted mean (95% confidence interval) F2-isoprostane was 37.4% greater in the lowest enterolactone fifth (20.2–28.4 ng/l) than in the highest fifth (26.7–40.1 ng/l).

The dietary constituents with the strongest univariate associations with serum enterolactone were water soluble fibre (r = 0.39, P < 0.001), water insoluble fibre (r = 0.28, P = 0.005), intake of fruits and berries (r = 0.25, P = 0.01), vegetables (r = 0.24, P = 0.02) and cereals (r = 0.19, P = 0.06). Fibre components together in a regression analysis explained 17% of the variation of serum enterolactone and whereas the other food groups mentioned explained 15% together. Of the dietary factors tested, only the intake of folate had a significant correlation with plasma F2-isoprostanes (r = −0.21, P = 0.04).

4. Discussion

This study shows that high serum enterolactone concentrations are associated with decreased in vivo lipid peroxidation, measured as plasma F2-isoprostane concentration. The concentration of serum enterolactone is determined by both the lignan content of the diet and the bacterial activity in the proximal colon.

Earlier studies do not provide firm evidence of enterolactone’s antioxidative properties. In both lipid and aqueous in vitro model systems’ enterolactone has demonstrated some potential to act as an antioxidant in concentrations of 10–100 μM [19]. These concentrations seem high in comparison with concentrations of 89.1 nmol/l and 19.4 μmol/24 h detected in plasma of vegetarian women and in urine of macrobiotic women, respectively [20,21]. The exceptional stability of enterolactone and the tendency to circulate in conjugated form, leaving none or only one free hydroxyl to act as an antioxidant [20] have led us to assume that enterolactone is unlikely to be an active compound. However, the wide range of enterolactone precursors, some newly identified [22], are more likely responsible for the associations found between body fluid enterolactone concentration and risk of disease. The most studied enterolactone precursors are plant lignans matairesinol and secoisolariciresinol but the evidence supporting their antioxidative properties is scarce and limited to in vitro evidence [19].

Plasma F2-isoprostanes is a measure of oxidative modification of arachidonic acid in situ on phospho-

Table 1
Associations of serum enterolactone concentration (nmol/l) with plasma F2-isoprostane concentration (ng/l) in two multivariate models

<table>
<thead>
<tr>
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<th>4-variable model</th>
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<th>8-variable model</th>
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<td>Unstandardized</td>
<td>Standardized</td>
<td>Unstandardized</td>
<td>Standardized</td>
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<tr>
<td></td>
<td>regression coefficient</td>
<td>regression coefficient</td>
<td>P value</td>
<td>regression coefficient</td>
</tr>
<tr>
<td>Serum enterolactone,</td>
<td>−0.20 (−0.34−0.06)</td>
<td>−0.24</td>
<td>0.007</td>
<td>−0.18 (−0.33−0.03)</td>
</tr>
<tr>
<td>nmol/l</td>
<td>95% CI</td>
<td></td>
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<td>95% CI</td>
</tr>
<tr>
<td>Energy adjusted</td>
<td>−0.19 (−0.31−0.07)</td>
<td>−0.27</td>
<td>0.003</td>
<td>−0.17 (−0.29−0.04)</td>
</tr>
<tr>
<td>selenium intake, μg</td>
<td>95% CI</td>
<td></td>
<td></td>
<td>95% CI</td>
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<tr>
<td>per day</td>
<td>0.034 (0.01−0.05)</td>
<td>0.29</td>
<td>0.001</td>
<td>0.03 (0.009−0.50)</td>
</tr>
<tr>
<td>Alcohol consumption,</td>
<td>0.79 (0.05−1.52)</td>
<td>0.20</td>
<td>0.04</td>
<td>0.85 (0.10−1.60)</td>
</tr>
<tr>
<td>g/week</td>
<td>95% CI</td>
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<td>95% CI</td>
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a Variables with the strongest associations with F2-isoprostanes selected by stepwise analysis.

b Additional adjustment for plasma alpha-tocopherol, beta-carotene, ascorbic acid and dietary folate.
lipids which has raised the question whether the amount of free arachidonic acid in serum, which can be affected by the diet, would affect the measure of F2-isoprostanes. However, it has been shown that the measurement of plasma F2-isoprostanes does not appear to be confounded by the lipid content of the diet [23].

In our stepwise analysis, selenium intake was the second most predictive variable for plasma F2-isoprostanes. Consistent results have been reported earlier. For example, a study in mice reported that F2-isoprostane formation was accelerated by selenium deficiency in the liver and it was concluded that selenium-dependent cellular glutathione peroxidases, which are important antioxidant enzymes, protect mice against a pro-oxidant-induced oxidation of inter alia lipids [24]. Also, the associations between plasma F2-isoprostanes and alcohol consumption and plasma total homocysteine have been reported earlier [5].

The strongest determinant of serum enterolactone level of the nutrients assessed by food records was the energy adjusted water-soluble fibre. This is comprehensible, considering that good sources of soluble fibre like fruits, berries, grains and vegetables are also very apparent contributors to lignan intake. Dietary fibre assessed using five-day diet records has been reported to have a correlation of 0.36 with the sum of urinary lignans [25]. Similarly, our finding is in agreement with a study in which subjects’ excretion of enterolactone increased by a third on a nine-day controlled diet high in apples, pears, potatoes and carrots [26], all sources of soluble fibre.

The second strongest dietary determinant of serum enterolactone was the energy-adjusted water-insoluble fibre. In grains, plant lignans are localized in the aleuronic layer, which is hard to separate from the bran, and lignan concentration in the milled fractions of rye seems to follow closely the changes in fibre content [27]. As it is estimated that rye bread, usually made of whole grain flour, contributes approximately 40% to fibre intake in Finland [28], the association between enterolactone and energy-adjusted water-insoluble fibre is plausible.

Assuming that fibre plays an important role in the colonic metabolism and consequently most likely affects lignan transformation, it is difficult to deduce from our dietary data whether the sources of soluble fibre contain the major part of enterolactone precursors or whether the soluble fibre just provides the optimal circumstances in the colon for enterolactone production. These dietary associations underline the earlier shown importance of fibre rich diet for enterolactone levels. As discussed earlier [11], enterolactone measurement might provide a useful biomarker of fibre consumption and, therefore, possibly serve as a more reliable and precise measure of dietary exposure than one obtained using traditional dietary assessment tools. Interestingly, however, water-soluble noncellular fibre, the strongest dietary determinant for enterolactone, had no association with F2-isoprostanes. This suggests that enterolactone itself or its precursors, rather than fibre generally, possibly contributes to the antioxidative defence system. High levels of serum enterolactone could also be indicative of a certain kind of bacterial function in the colon or just denote the presence of other beneficial substances.

Our study showing an association between high serum enterolactone levels and reduced in vivo lipid peroxidation has provided new information suggesting that enterolactone obtained through fibre rich food could be one of the substances that have a role in explaining the protective effect of fruits, vegetables and grain in oxidative stress related diseases such as coronary heart disease and cancer.

Acknowledgements

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References


