An incident case-referent study on plasma enterolactone and breast cancer risk

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Summary
Objective Using a nested case-referent design, we evaluated the relationship between plasma levels of the lignan enterolactone and the risk of developing breast cancer. Methods 248 cases and 492 referents were selected from three population-based cohorts in northern Sweden. Blood samples were donated at enrolment. All blood samples were stored at –80 °C. Cases and referents were matched for age, date of blood sample and sampling centre. Breast cancer cases were identified through the regional and national cancer registries. Results Plasma enterolactone was lower among smokers in all cohorts and in subjects with BMI<23 and BMI>28 in one of the cohorts. Low plasma concentrations of enterolactone, below the 12.5th percentile (mean plasma enterolactone 2.9 nmol/l), were associated with an increased breast cancer risk. Also, high values of plasma enterolactone, above the 87.5th percentile (mean plasma enterolactone 58.2 nmol/l) were significantly associated with an increased breast cancer risk among women from two cohorts with only incident cases and a higher number of pre-menopausal women. High plasma enterolactone concentrations among older women from a mammmary screening project with mostly prevalent cases were associated with a non-significant slightly reduced breast cancer risk. Conclusion Very low plasma concentrations of enterolactone were associated with an increased breast cancer risk in all three cohorts. In two of the cohorts, with only incident cases, very high plasma concentrations were also associated with an increased breast cancer risk. In the third cohort with mainly screen-detected cases from a mammmary screening program, high plasma enterolactone concentrations were associated with a weak decreased breast cancer risk.

Key words plasma – enterolactone – phyto-oestrogens – breast cancer

Introduction

A consensus meeting on dietary intake of cereals and fibre, and risk for cancer concluded that suggestive evidence exists that cereal fibre may protect against breast cancer [1]. Some studies have shown associations between higher cereal fibre intake and decreased breast cancer risk, although other studies have not demonstrated such associations, particularly not with total fibre intake [2]. The nature of the fibre is different in different countries, which may be one of several explanations for the diverging results. Therefore, insufficient evidence exists for firm conclusion with regard to fibre intake and breast cancer risk. Various mechanisms for an increased risk of breast cancer with lower fibre in-
take have been discussed, e.g., increased concentrations of oestrogens, insulin, and insulin-like growth factor 1 (IGF-1) as well as low concentrations of phyto-oestrogens including lignans [3–6].

The detection [7, 8] and simultaneous identification of the mammalian lignans, later called enterolactone and enterodiol, by two groups [9, 10] initiated research on phyto-oestrogens in man. The urinary lignan excretion was found to correlate positively with fibre intake and plasma sex hormone binding globulin (SHBG) and negatively with plasma levels of free oestriol and testosterone [11, 12]. In case-referent studies the lowest urinary enterolactone excretion was found in breast cancer patients [13, 14, 15] and the highest in vegetarians, particularly macrobiotics [16].

Lignans may influence steroid production, metabolism and biological activity as well as intracellular enzymes, SHBG production, and malignant cell proliferation. These properties make them potential natural cancer protective compounds [5, 17].

Recently, we developed a time-resolved fluoroimmunoassay for the determination of enterolactone in plasma extracts corresponding to 20 µl of plasma [18, 19]. This technique was applied to plasma samples from the Northern Sweden Health and Disease Cohort Study, analyzed within a nested case-referent study on breast cancer risk related to earlier plasma enterolactone values. This report represents the first study with prospective data on exposure measured as enterolactone in plasma and breast cancer risk. Our hypothesis was that low levels of plasma enterolactone are associated with an increased risk of developing breast cancer.

## Subjects and methods

### Study sample

Data were collected within three ongoing cohort studies in northern Sweden. The Västerbotten Intervention Project (VIP) started in 1985 and comprised 30,000 men and 29,000 women by the end of year 2000. The northern Sweden component of the WHO multinational study for Monitoring of Trends and Cardiovascular Disease study (MONICA) included 2,700 men and 28,000 women recruited in 1986, 1990 and 1994. The Mammary Screening Project (MSP) started in 1995 and by the end of year 2000 39,000 women were involved. Two referents for each case were randomly selected from the corresponding cohort, with a few exceptions. In some rare cases, for the MONICA and MSP projects referents were selected from the VIP project. Referents were matched for age (±6 months), date of blood sample (±2 months), and sampling centre. Because of lack of blood samples, 28 subjects were excluded from the study. The final study population with blood samples was thus 740 women (248 cases and 492 referents). The VIP contributed 140 cases and 282 referents, the MONICA 15 cases and 25 referents and the MSP 93 cases and 185 referents. The number of cases with samples collected less than 3 months before diagnosis were 11 in the VIP, none in the MONICA and 61 in the MSP.

### Baseline questionnaire information

In the VIP and MONICA cohorts, information on dietary habits, working conditions and social factors were collected at baseline by questionnaires. For the MSP, questionnaire information was obtained only for reproductive factors. In the VIP and MONICA cohorts, weight was measured with light indoor clothing without shoes, and height was measured by a graded scale fixed to the wall. In the MSP height and weight were self-reported. (Where participation in the MSP cohort overlapped with that of the VIP and the MONICA cohorts, the self-reported anthropometric information from the MSP have shown fair correspondence, r = 0.97 for height and 0.75 for weight).

### Blood sampling

In all the three studies 20 ml of blood was collected at baseline from every subject. 10 ml was collected with heparin and 10 ml with EDTA, as anticoagulants. The blood was thereafter aliquoted into 10 tubes; 6 tubes with plasma, 2 with buffy coat and 2 with erythrocytes. The aliquots were stored at –80 °C. For the VIP and MONICA cohorts 95% (147 cases, 293 referents) of the subjects had fasted at least 4 hours and 57% (84 cases, 175 referents) had fasted more than 8 hours before donating the blood sample. For the MSP cohort it was not required to come in fasting state and only a very small proportion had actually fasted. The study was approved by the Ethical Committee of Umeå University and all study participants have given their informed consent for future use of blood samples for research purposes.

### Follow-up

Incident cases of breast cancer from baseline up to year 2000 were identified through linkage with the regional cancer registry covering the northern region of Sweden, complemented by linkage with the national cancer registry covering the whole of Sweden. The Swedish unique personal identification number was used for linkage. Follow-up for vital status (death), or losses to follow-up due to migration from the country was also carried out for the whole study population through local and national population registries.