1 **Pre-diagnostic plasma enterolactone levels and mortality among women with**
2 **breast cancer**

3
4 Anja Olsen¹, Jane Christensen¹, Knud Erik Bach Knudsen², Nina Føns Johnsen¹, Kim Overvad^{3,4},
5 Anne Tjønneland¹.

6
7 ¹ Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark

8 ² Department of Animal Health and Bioscience, Faculty of Agricultural Sciences, Aarhus
9 University, Tjele, Denmark

10 ³ Department of Epidemiology, School of Public Health, Aarhus University, Aarhus, Denmark

11 ⁴ Department of Cardiology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark
12

13 **Corresponding author:** Anja Olsen, Institute of Cancer Epidemiology, Danish Cancer Society,
14 Strandboulevarden 49, DK-2100 Copenhagen O. Phone: 0045 35257606, fax: 0045 35257731,
15 email: anja@cancer.dk.

16

17

18

19 **Abstract (198 words)**

20 **Purpose:** Experimental and epidemiological studies have suggested that the phytoestrogen
21 enterolactone is associated to biological mechanisms that may have positive effects on breast cancer
22 development. In a recent study based on American breast cancer patients, high intakes of lignans,
23 the precursor of enterolactone, was found related to lower mortality. The aim of the present study
24 was, for the first time, to evaluate if pre-diagnostic plasma levels of enterolactone were associated to
25 mortality in women diagnosed with breast cancer.

26 **Methods:** Among 24,697 postmenopausal women included into a Danish cohort between 1993 and
27 1997, 424 developed breast cancer before December 31, 2000. Enterolactone levels were measured
28 in baseline blood samples and related to cancer recurrence and mortality by Cox proportional hazard
29 models.

30 **Results:** During a median of 10 years after breast cancer diagnosis, 111 women died (80 from breast
31 cancer). When comparing women with enterolactone levels above the median (>20.5 nmol/l) to
32 those with lower levels, decreased hazard rates (HR) were seen for both all cause mortality (HR:
33 0.47 (95% confidence interval: 0.32-0.68)) and breast cancer mortality (HR: 0.56 (95% confidence
34 interval: 0.36-0.87)).

35 **Conclusions:** Higher pre-diagnostic plasma levels of enterolactone were found related to lower
36 mortality among breast cancer patients.

37

38 **Introduction**

39 Women treated for breast cancer contribute a large proportion of cancer survivors[1] and
40 identification of lifestyle factors that might improve prognosis for this patient group has achieved
41 considerable interest[2]. Intake of plant foods (including fruits, vegetables, whole grain cereals and
42 legumes) is one of the factors that have been hypothesized as associated to improved breast cancer
43 outcome[3] and a class of components receiving special interest within this group is
44 phytoestrogens[4]. In Nordic diets, lignans, originating primarily from whole grain cereals and fiber
45 rich vegetables[5], constitute the largest phytoestrogen intake[6]. Lignan products are mainly found
46 as enterolactone in mammalian biofluids from feces, plasma, and urine, and the concentrations are
47 determined primarily by the intake of dietary precursors and the capacity of the intestinal microflora
48 to convert plant lignans to enterolignans [7]. Experimental studies have suggested high intakes of
49 lignans and/or high concentrations of enterolactone to be related to advantageous mechanisms in
50 breast cancer development, including reduction of tumor growth and metastasis[8,9], inhibition of
51 aromatase[10] and modification of steroid hormone metabolism[11]. Dietary intakes of lignans as
52 well as blood or urine levels of enterolactone have previously been related to decreased incidence of
53 breast cancer although the literature is not consistent[12]. The influence of lignans on breast cancer
54 prognosis has not been reported until recently, where a study based on American women found a
55 high dietary intake of lignans to be associated with decreased mortality (both total and breast cancer
56 specific) among postmenopausal but not premenopausal women[13].

57

58 Estimation of dietary intake of lignans is based on dietary information from food frequency
59 questionnaires (FFQs) and information on lignan content from food databases. The quality of the
60 exposure information is consequently limited due to: incorrect recall among study participants, the
61 ability of the FFQ to estimate intake of lignan containing foods and the ability of the food database
62 to precisely estimate contents of lignans in the standard food items obtained by the FFQ. Usage of

63 plasma levels of enterolactone as a biomarker for lignan intake may be a better alternative for
64 exposure assessment as an objectively assessed biomarker diminish the errors ascribed to recall,
65 FFQ and food databases[14]. The aim of the present study was to evaluate whether pre-diagnostic
66 plasma levels of enterolactone were associated to breast cancer specific and all cause mortality
67 among 424 postmenopausal breast cancer patients originating from a prospective cohort study.

68

69 **Materials and methods**

70 **Cohort**

71 The Diet, Cancer and Health study is a prospective cohort study, established with the primary aim of
72 studying the etiological role of diet on cancer risk. Between 1993 and 1997, 79,729 Danish women
73 aged 50-64 and without previous cancer diagnoses were invited to participate in the study. A total of
74 29,875, corresponding to 37% of those invited, were enrolled into the cohort. A detailed description
75 of the cohort, including socioeconomic factors associated to non-response has been published
76 previously[15]. The mortality rates for the cohort participants has since baseline been considerable
77 lower (approximately 50%) compared to the non-responders, this is, however, mainly due to other
78 causes than breast cancer. Also when compared to the entire Danish population, the age-adjusted
79 mortality rates are lower among cohort participants.

80

81 The Diet, Cancer and Health study and the present substudy were approved by the regional ethical
82 committees on human studies in Copenhagen and Aarhus and by the Danish Data Protection
83 Agency.

84

85 All cohort members attained one of two established study centers, and each participant filled in a
86 FFQ and a life-style questionnaire. The life-style questionnaire included questions about
87 reproductive factors, health status, social factors, and life-style habits. From this questionnaire, we

88 obtained information about school education (short, ≤ 7 years; medium, 8-10 years; or long >
89 10years) and use of hormone replacement therapy (HRT; use at baseline yes/no). Intake of alcohol
90 was obtained from the FFQ. Anthropometric data were obtained by professional staff members.
91 Body mass index (BMI) was calculated as weight (kg) per height (m) squared.
92
93 In the study centers, 20 mL of blood (nonfasting, collected in citrated and plain Venojects) were
94 drawn from each participant. The samples were spun and divided into 1-mL tubes of plasma, serum,
95 red blood cells, and buffy coat. All samples were processed and frozen within 2 hours at -20 °C. At
96 the end of the day of collection, all samples were stored in liquid nitrogen vapor (maximum
97 temperature, -150 °C).
98
99 Of the initial 29,875 women, we excluded 326 who later were reported to the Danish Cancer
100 Registry with a cancer diagnosed before the visit to the study clinic. In addition, eight women were
101 excluded from the study because they did not fill in the life-style questionnaire. Because the present
102 analysis aimed at women who were postmenopausal at study entry, we further excluded 4,844
103 women, including 4,798 who were considered premenopausal because they had reported at least one
104 menstruation <12 months before entry and no use of HRT, nine women who gave a lifetime history
105 of no menstruation, and 37 women who did not answer the questions about current or previous use
106 of HRT, leaving 24,697 postmenopausal women for study.
107
108 Cohort members were identified from their unique personal identification number, which is
109 allocated to every Danish citizen by the Central Population Registry. All the postmenopausal cohort
110 members were linked to the Central Population Registry to obtain information on vital status and
111 emigration. Information on cancer occurrence among cohort members was obtained through record
112 linkage to the Danish Cancer Registry, which collects information on all cases of cancer diagnosed

113 in Denmark [16]. Linkage was done by use of the personal identification number and follow-up was
114 nearly complete (99.8%). Each cohort member was followed up for breast cancer occurrence from
115 date at entry, that is, date of visit to the study center until the date of diagnosis of any cancer (except
116 for nonmelanoma skin cancer), date of death, date of emigration, or December 31, 2000, whichever
117 came first. Incident breast cancer was diagnosed in 434 women during the follow-up period. Of
118 these 10 were excluded due to lack of blood sample, leaving 424 breast cancer cases for study. The
119 424 women diagnosed with breast cancer before end 2000 were linked to the Danish Death
120 Certificate Registry to obtain information about date and cause of death. All cases (100% follow-up)
121 were followed from date of diagnosis (between baseline and end 2000) until death or December 31,
122 2008.

123

124 A clinical registry exclusively about breast cancer also exist in Denmark and information on
125 estrogen receptor α (ER α) status was obtained by linkage with the Danish Breast Cancer Co-
126 operative Group, which holds records on a range of details for approximately 90% of all breast
127 cancers diagnosed in Denmark [17]. A standardized immunohistochemical method was used in all
128 medical centers. The cutoff level used to define positive ER α status was $\geq 10\%$ positive cells.
129 Information on ER α status was registered for 391 (92%) of the breast cancer cases, of these 302
130 women were diagnosed with an ER α positive tumor and 89 with an ER α negative tumor.

131

132 **Biological analysis**

133 The analytical procedure for time-resolved fluoroimmunoassay of enterolactone in plasma [18,19] is
134 briefly as follows. A 200- μ l aliquot of plasma was mixed with 200 μ l β -glucuronidase and sulfatase
135 in acetate buffer and incubated overnight at 37 °C. Diethyl ether was used to extract unconjugated
136 enterolactone after hydrolysis. The hydrolyzed extract in buffer was then pipetted into prewashed
137 goat anti-rabbit IgG microstrips. Simultaneously, antiserum (dilution 1:250 000) in bovine serum

138 albumin Tris buffer and EU-labeled enterolactone (dilution 1:400 000) were added into micro strips.
139 After incubation and shaking of the strips slowly in a DELFIA plate washer (Wallac, Turku,
140 Finland) at room temperature for 90 min, the strips were washed with a DELFIA plate washer
141 (Wallac). Subsequently, DELFIA enhancement solution (Wallac) was added to each well, and the
142 strips were shaken slowly for an additional 5 min. Fluorescence was read in a DELFIA Victor
143 multilable counter (Wallac). Duplicate control plasma samples were processed through the
144 procedure for each batch and placed at the beginning and end of the plates. The concentration of
145 enterolactone in the control plasma was 19.7 nmol/L, and the intra- and inter-assay coefficients of
146 variation were less than 10 %. The results of a previous study [20] further showed that the assay's
147 coefficients of variation were virtually independent of the concentration of enterolactone in plasma
148 in the range 0-140 nmol/L.

149

150 We used plasma levels of enterolactone to measure exposure, because this biomarker is considered
151 more stable than urinary excretion due to possible enterohepatic recirculation [21]. The plasma
152 concentration is relatively constant within an individual, with a reliability constant of 0.55 over a 2-
153 year period [22].

154

155 **Statistical methods**

156 Analyses of the relations between baseline plasma levels of enterolactone and mortality were based
157 on Cox proportional hazard models using follow-up as the time axis and stratifying by age at
158 diagnosis in 5 year intervals. The associations were evaluated with and without adjustment for
159 baseline values of alcohol intake (continuously) and use of HRT at blood sampling (yes/no). Further
160 tumor grade at diagnosis (I, II, III, or non-ductal carcinoma) was included into the models. Body
161 mass index and education were also evaluated as potential confounders but were found unrelated to
162 the endpoints of interest and therefore not included due to the relatively limited statistical power.

163 Besides tumor grade, the clinical parameters tumor size (mm) and tumor positive axillary lymph
164 nodes (yes/no) were evaluated in the model. In a multivariate model, tumor grade was, however, the
165 only clinical tumor parameter significantly associated to mortality during follow up and to preserve
166 statistical power, only this parameter was included into the model.

167

168 Plasma levels of enterolactone were included into the model as both a linear (per 20 nmol/l) and a
169 dichotomized (below or above the median; 20.5 nmol/l) variable. Before entering plasma levels of
170 enterolactone and alcohol intake linearly into the model, the linearity of the associations were
171 evaluated by linear splines with three boundaries placed at the quartile cutoff points. None of the
172 associations showed signs of deflection or threshold values.

173

174 Unadjusted Kaplan Meier curves were used to examine differences in all cause and breast cancer
175 mortality for enterolactone levels below or above the median. The survival curves were not adjusted
176 for the potential confounders (including age at diagnosis) using the Mean of Covariates Method due
177 to the limitation of having a large number of dichotomous variables [23]. To present unadjusted
178 curves was regarded a minor problem, as the included covariates were found to be essentially
179 without impact on the hazard estimates in the Cox model.

180

181 Two-sided 95% confidence intervals (95% CI) for the hazard rates (HR) were calculated on the log
182 HR scale. SAS (release 9.1) was used for statistical analyses.

183

184 The association between plasma levels of enterolactone and incidence of breast cancer has
185 previously been reported based on these 424 breast cancer cases and a corresponding number of
186 controls [24].

187

188 **Results**

189 The 424 breast cancer cases were followed for a median of 10 years during which 111 died and of
190 those 80 had breast cancer as the primary cause of death.

191

192 **Table 1** shows the descriptive characteristics of the breast cancer cases at cohort baseline and breast
193 cancer diagnosis according to vital status. Compared to women that died during the follow-up
194 period, women alive by 31/12 2008 seemed to have higher plasma levels of enterolactone at
195 baseline, more often have low (grade I) than high (grade III) tumor grade, more often be diagnosed
196 with an estrogen receptor positive tumor, were more often HRT users at baseline, had a smaller
197 tumor and were more likely to be free of positive lymph nodes at diagnosis. No clear differences
198 were seen for age at diagnosis, alcohol intake, BMI, or length of school education.

199

200 When assessed as a linear variable, a higher level of enterolactone was associated to lower all cause
201 mortality (HR (95%CI): 0.82 (0.70-0.95)) per 20 nmol/l increment (**Table 2**). The tendency was the
202 same for breast cancer specific mortality (HR (95% CI): 0.87 (0.75-1.03)) per 20 nmol/l increment,
203 although the HR did not reach statistical significance. Adjustment for covariates barely affected the
204 associations.

205

206 When stratifying the women according to median levels of enterolactone, the hazard of both breast
207 cancer specific and total mortality was about 50% lower among the breast cancer patients with
208 enterolactone levels above the median (P=0.01 and P<0.0001, respectively) when compared to
209 women with enterolactone levels below the median. Stratifying according to estrogen receptor status
210 of the breast tumor did not indicate that the effect of enterolactone differed between these two
211 clinical subtypes. The statistical strength for evaluating especially ER negative breast cancer was,
212 however, very limited.

213 **Figure 1** shows Kaplan Meier plots for women above or below the median enterolactone level. The
214 plots demonstrate, that the lower mortality, both breast cancer specific and all-cause, among those
215 with the highest enterolactone levels was evident during the entire follow-up period.

216

217 **Discussion**

218 In summary, higher pre-diagnostic plasma levels of enterolactone were found associated to lower
219 mortality (both breast cancer specific and all-cause) among postmenopausal women.

220 Strengths of the present study include: its prospective design, relatively long follow-up period and
221 objective assessments of both exposure and endpoint. The major limitations concern the limited
222 statistical power and potential imprecision of the biomarker [25].

223

224 That high plasma levels of enterolactone may be related to improved survival among breast cancer
225 patients, is in accordance with the only comparable previously published study[13]. The study by
226 McCann and colleagues found that postmenopausal women with high pre-diagnostic dietary intakes
227 of lignans, the precursor of enterolactone, had lower risk of dying during approximately 7 years of
228 follow-up. No association was seen among pre-menopausal women [13]. Also evidence from
229 experimental studies supports that high intake of lignans resulting in high plasma levels of
230 enterolactone may be associated to improved prognosis among breast cancer patients[8,9,10,11].

231

232 Plasma levels of enterolactone are primarily determined by two factors: lignan intake and the
233 efficiency of intestinal bacteria to convert plant lignans to enterolignans [26]. In the previously
234 published study [13] only lignan intake was considered and anticipating that enterolactone is the
235 biologically active form, plasma levels of enterolactone may be considered as superior to lignan
236 intake as exposure measurement. Still, the sources of lignans are very relevant to consider. In the
237 study by McCann and colleagues, dark bread, peaches, coffee, broccoli and winter squash were the

238 main sources [13], and in the present study, whole grain rye bread, oatmeal and cabbages were the
239 primary dietary sources associated to plasma levels of enterolactone[27]. The primary lignan
240 sources in both studies are whole grain and it is highly interesting if this food group, that has been
241 ascribed many other positive health effects[28,29,30], may also be related to survival among breast
242 cancer patients.

243

244 When comparing the study by McCann to the present study, it must be taken into account that
245 enterolactone is known to reflect other factors than just lignan intake. Lignan intake is associated to
246 enterolactone levels, but other factors like body weight, smoking and frequency of bowel
247 movements are known to modulate the relation between the two [31]. With a reliability constant of
248 0.55 over a two year period [22], a single plasma measurement of enterolactone can be considered a
249 reasonable exposure assessment for use in epidemiological studies, although some degree of
250 impressions will affect the results. Such errors are, however, by definition most likely unsystematic
251 and will thus affect the estimates towards unity

252

253 An important aspect to consider regarding both the current study and the study by McCann and
254 colleagues is that both measure exposure (plasma levels of enterolactone and intake of lignans) prior
255 to breast cancer diagnosis. The studies are, consequently, not able to determine if high plasma levels
256 of enterolactone and/or high intake of lignans after breast cancer diagnosis are related to improved
257 survival. Results from these two studies must therefore primarily be regarded as suggestive of that
258 intake of lignans and/or blood levels of enterolactone may be hold preventive effects with regard to
259 breast cancer survival. Several enterolactone measurements in blood samples obtained before and
260 after diagnosis are needed if a causal association is to be pointed to. Enterolactone levels and/or
261 lignan intake may simply be markers of a generally healthy diet and/or lifestyle that are related to
262 improved general health. Further studies are needed to consider this aspect.

263
264
265
266
267
268
269
270
271
272
273

In conclusion, we found higher pre-diagnostic levels of enterolactone to be related to lower mortality among breast cancer patients. Future studies with better statistical strength are needed to confirm our findings.

Acknowledgement: This work was supported by The Danish Cancer Society; and NordForsk (the Nordic Centres of Excellence HELGA and SysDiet). Neither of the funding agencies has had any influence on: design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review or approval of the manuscript.

Reference List

1. Ferlay J, Bray F, Pisani P, Parkin DM, (2004) GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide.
2. Patterson RE, Cadmus LA, Emond JA, Pierce JP, (2010) Physical activity, diet, adiposity and female breast cancer prognosis: A review of the epidemiologic literature. *Maturitas*
3. Kellen E, Vansant G, Christiaens MR, Neven P, Van LE, (2009) Lifestyle changes and breast cancer prognosis: a review. *Breast Cancer Res Treat* 114:13-22
4. Cornwell T, Cohick W, Raskin I, (2004) Dietary phytoestrogens and health. *Phytochemistry* 65:995-1016
5. Mazur W, (1998) Phytoestrogen content in foods. *Baillieres Clin Endocrinol Metab* 12:729-742
6. Peeters PH, Slimani N, van der Schouw YT, Grace PB, Navarro C, Tjonneland A, Olsen A, Clavel-Chapelon F, Touillaud M, Boutron-Ruault MC, Jenab M, Kaaks R, Linseisen J, Trichopoulou A, Trichopoulos D, Dilis V, Boeing H, Weikert C, Overvad K, Pala V, Palli D, Panico S, Tumino R, Vineis P, Bueno-de-Mesquita HB, van Gils CH, Skeie G, Jakszyn P, Hallmans G, Berglund G, Key TJ, Travis R, Riboli E, Bingham SA, (2007) Variations in plasma phytoestrogen concentrations in European adults. *J Nutr* 137:1294-1300
7. Kilkkinen A, Stumpf K, Pietinen P, Valsta LM, Tapanainen H, Adlercreutz H, (2001) Determinants of serum enterolactone concentration. *Am J Clin Nutr* 73:1094-1100
8. Wang L, Chen J, Thompson LU, (2005) The inhibitory effect of flaxseed on the growth and metastasis of estrogen receptor negative human breast cancer xenografts attributed to both its lignan and oil components. *Int J Cancer* 116:793-798
9. Chen J, Wang L, Thompson LU, (2006) Flaxseed and its components reduce metastasis after surgical excision of solid human breast tumor in nude mice. *Cancer Lett* 234:168-175
10. Brooks JD and Thompson LU, (2005) Mammalian lignans and genistein decrease the activities of aromatase and 17 β -hydroxysteroid dehydrogenase in MCF-7 cells. *J Steroid Biochem Mol Biol* 94:461-467
11. Hutchins AM, Martini MC, Olson BA, Thomas W, Slavin JL, (2001) Flaxseed consumption influences endogenous hormone concentrations in postmenopausal women. *Nutr Cancer* 39:58-65
12. Boccardo F, Puntoni M, Guglielmini P, Rubagotti A, (2006) Enterolactone as a risk factor for breast cancer: a review of the published evidence. *Clin Chim Acta* 365:58-67
13. McCann SE, Thompson LU, Nie J, Dorn J, Trevisan M, Shields PG, Ambrosone CB, Edge SB, Li HF, Kasprzak C, Freudenheim JL, (2010) Dietary lignan intakes in relation to survival among women with breast cancer: the Western New York Exposures and Breast Cancer (WEB) Study. *Breast Cancer Res Treat* 122:229-235

- 313 14. Kaaks RJ, (1997) Biochemical markers as additional measurements in studies of the accuracy
314 of dietary questionnaire measurements: conceptual issues. *Am J Clin Nutr* 65:1232S-1239S
- 315 15. Tjonneland A, Olsen A, Boll K, Stripp C, Christensen J, Engholm G, Overvad K, (2007) Study
316 design, exposure variables, and socioeconomic determinants of participation in Diet, Cancer
317 and Health: a population-based prospective cohort study of 57,053 men and women in
318 Denmark. *Scand J Public Health* 35:432-441
- 319 16. Storm HH, Michelsen EV, Clemmensen IH, Pihl J, (1997) The Danish Cancer Registry--
320 history, content, quality and use. *Dan Med Bull* 44:535-539
- 321 17. Fischerman K and Mouridsen HT, (1988) Danish Breast Cancer Cooperative Group (DBCG).
322 Structure and results of the organization. *Acta Oncol* 27:593-596
- 323 18. Stumpf K, Uehara M, Nurmi T, Adlercreutz H, (2000) Changes in the time-resolved
324 fluoroimmunoassay of plasma enterolactone. *Anal Biochem* 284:153-157
- 325 19. Uehara M, Lapcik O, Hampl R, Al Maharik N, Makela T, Wahala K, Mikola H, Adlercreutz H,
326 (2000) Rapid analysis of phytoestrogens in human urine by time-resolved fluoroimmunoassay.
327 *J Steroid Biochem Mol Biol* 72:273-282
- 328 20. Heinonen S, Nurmi T, Liukkonen K, Poutanen K, Wahala K, Deyama T, Nishibe
329 S, Adlercreutz H, (2001) In vitro metabolism of plant lignans: new precursors of mammalian
330 lignans enterolactone and enterodiol. *J Agric Food Chem* 49:3178-3186
- 331 21. Adlercreutz H, (2002) Phytoestrogens and breast cancer. *J Steroid Biochem Mol Biol* 83:113-
332 118
- 333 22. Zeleniuch-Jacquotte A, Adlercreutz H, Akhmedkhanov A, Toniolo P, (1998) Reliability of
334 serum measurements of lignans and isoflavonoid phytoestrogens over a two-year period.
335 *Cancer Epidemiol Biomarkers Prev* 7:885-889
- 336 23. Nieto FJ and Coresh J, (1996) Adjusting survival curves for confounders: a review and a new
337 method. *Am J Epidemiol* 143:1059-1068
- 338 24. Olsen A, Knudsen KE, Thomsen BL, Loft S, Stripp C, Overvad K, Moller S, Tjonneland A,
339 (2004) Plasma enterolactone and breast cancer incidence by estrogen receptor status. *Cancer*
340 *Epidemiol Biomarkers Prev* 13:2084-2089
- 341 25. Kilkkinen A, Valsta LM, Virtamo J, Stumpf K, Adlercreutz H, Pietinen P, (2003) Intake of
342 lignans is associated with serum enterolactone concentration in Finnish men and women. *J*
343 *Nutr* 133:1830-1833
- 344 26. Adlercreutz H, (2007) Lignans and human health. *Crit Rev Clin Lab Sci* 44:483-525
- 345 27. Johnsen NF, Hausner H, Olsen A, Tetens I, Christensen J, Knudsen KE, Overvad
346 K, Tjonneland A, (2004) Intake of whole grains and vegetables determines the plasma
347 enterolactone concentration of Danish women. *J Nutr* 134:2691-2697
- 348 28. Slavin JL, Jacobs D, Marquart L, Wiemer K, (2001) The role of whole grains in disease
349 prevention. *J Am Diet Assoc* 101:780-785

- 350 29. Jacobs DR, Jr. and Gallaher DD, (2004) Whole grain intake and cardiovascular disease: a
351 review. *Curr Atheroscler Rep* 6:415-423
- 352 30. Fardet A, (2010) New hypotheses for the health-protective mechanisms of whole-grain
353 cereals: what is beyond fibre? *Nutr Res Rev* 23:65-134
- 354 31. Milder IE, Kuijsten A, Arts IC, Feskens EJ, Kampman E, Hollman PC, Van 't, V, (2007)
355 Relation between plasma enterodiol and enterolactone and dietary intake of lignans in a Dutch
356 endoscopy-based population. *J Nutr* 137:1266-1271
- 357
358
359

360 Table 1. Descriptive characteristics of breast cancer cases at cohort baseline and breast cancer
361 diagnosis by vital status.

Characteristics	Women alive 31/12 2008 (n=313)		Women dead 31/12 2008 (n=111)	
	Median (5 - 95%) or fraction		Median (5 - 95%) or fraction	
Enterolactone (nmol/l)	29	(4-110)	19	(2-89)
Age at cancer diagnosis	60	(53-66)	60	(54-67)
Alcohol intake (g/day)	11	(1-41)	11	(1-55)
Body mass index (kg/m ²)	25	(20-34)	25	(20-34)
Tumor grade at diagnosis				
I (%)	32		16	
II (%)	29		23	
III (%)	11		27	
Non-ductal carcinomas (%)	20		21	
Unknown (%)	8		13	
Estrogen receptor (ER) α status				
Positive (%)	75		60	
Negative (%)	16		35	
Unknown (%)	9		5	
Use of HRT at inclusion (%yes)	57		43	
Schooling (years)				
≤ 7 (%)	28		33	
8-10 (%)	49		46	
≥ 11 (%)	23		21	
Tumor size (mm)				
< 20 (%)	58		30	
20-50 (%)	33		51	
> 50 (%)	1		7	
Unknown (%)	7		12	
Positive lymph nodes				
0 (%)	64		33	
≥ 1 (%)	31		56	
Unknown (%)	5		11	

362
363

364
 365 Table 2. Hazard ratios (HR) and 95% confidence intervals (95% CI) for breast cancer specific
 366 mortality and all cause mortality according to plasma levels of enterolactone (assessed linear or
 367 dichotomized by the median) for all breast cancer cases, cases with estrogen receptor positive breast
 368 cancer and cases with estrogen receptor negative breast cancer.

Enterolactone level	HR (95% CI)			
	All breast cancer cases			Estrogen receptor positive breast cancer (n=302)
	Per 20 nmol/l	Per 20 nmol/l	> median ²	> median ²
Breast cancer mortality (n=80)	0.87 (0.75-1.03)	0.88 (0.75-1.03) ¹	0.56 (0.36-0.87) ¹	0.59 (0.32-1.09) ¹
All cause mortality (n=111)	0.82 (0.70-0.95)	0.82 (0.70-0.96) ¹	0.47 (0.32-0.68) ¹	0.43 (0.26-0.69) ¹

369
 370
 371 ¹ Adjusted for tumor grade at diagnosis, baseline levels of alcohol intake and use of hormone
 372 replacement therapy.
 373 ² Median plasma level of enterolactone were 20.5 nmol/l. The estimates illustrate mortality rates
 374 relative to mortality rates among women with enterolactone levels below the median (reference
 375 category).
 376 ³ Among estrogen receptor positive cases 67 died (43 from breast cancer)
 377 ⁴ Among estrogen receptor negative cases 39 died (34 from breast cancer)
 378

379 **Figure 1**
380 Figure legend: Unadjusted Kaplan Meier curves, illustrating hazard of dying from breast cancer or
381 dying from any cause according to pre-diagnostic plasma levels of enterolactone above or below the
382 median (20.5 nmol/l) in a population of 424 breast cancer cases followed for a median of 10 years.
383
384