Dietary acrylamide intake and estrogen and progesterone receptor-defined postmenopausal breast cancer risk

Grete S. Pedersen, Janneke G. F. Hogervorst, Leo J. Schouten, Erik J. M. Konings, R. Alexandra Goldbohm, Piet A. Brandt

Breast Cancer Res Treat (2010) 122:199–210
DOI 10.1007/s10549-009-0642-4

EPIDEMIOLOGY

Dietary acrylamide intake and estrogen and progesterone receptor-defined postmenopausal breast cancer risk

Grete S. Pedersen · Janneke G. F. Hogervorst · Leo J. Schouten · Erik J. M. Konings · R. Alexandra Goldbohm · Piet A. van den Brandt

Received: 24 August 2009 / Accepted: 10 November 2009 / Published online: 1 December 2009

Abstract Acrylamide, a potential human carcinogen, has been discovered in a variety of heat-treated carbohydrate-rich food products. Previously, dietary acrylamide intake was shown to be associated with endocrine-related cancers in humans. We assessed the association between dietary acrylamide intake and risk of postmenopausal breast cancer stratified by estrogen and progesterone receptor status. This study was embedded within the Netherlands Cohort Study on diet and cancer, which was initiated in 1986 enrolling 62,573 women aged 55–69 years at baseline. After 13.3 years of follow-up, 2225 incident breast cancer cases were ascertained, with hormone receptor status information for 43%. Cox proportional hazards analysis was applied to determine hazard ratios in quintiles of dietary acrylamide intake stratifying on estrogen receptor (ER) and progesterone receptor (PR) and smoking status. No association was observed for overall breast cancer or receptor-negative breast cancer risk, irrespective of smoking status. A statistically non-significantly increased risk of ER positive, PR positive and joint receptor-positive breast cancer was found in never-smoking women. The multivariable-adjusted hazard ratios were 1.31 (95% CI: 0.87–1.97, $P_{trend} = 0.26$) for ER+, 1.47 (0.86–2.51, $P_{trend} = 0.14$) for PR+, and 1.43 (0.83–2.46, $P_{trend} = 0.16$) for ER+PR+, when comparing women in the highest quintile of acrylamide intake (median 36.8 μg/day) to women in the lowest (median 9.5 μg/day). This study showed some indications of a positive association between dietary acrylamide intake and receptor-positive breast cancer risk in postmenopausal never-smoking women. Further studies are needed to confirm or refute our observations.

Keywords Acrylamide · Diet · Breast cancer · Estrogen receptor · Progesterone receptor

G. S. Pedersen · J. G. F. Hogervorst (✉) · L. J. Schouten · P. A. van den Brandt
Department of Epidemiology, GROW-School for Oncology and Developmental Biology, Maastricht University, P.O. Box 616, 6200 MD Maastricht, The Netherlands
e-mail: jgf.hogervorst@epid.unimaas.nl

G. S. Pedersen
Department of Epidemiology, Institute of Public Health, University of Southern Denmark, Odense, Denmark

E. J. M. Konings
Department Research & Development, The Food and Consumer Product Safety Authority, region South, Eindhoven, The Netherlands

R. A. Goldbohm
Department of Prevention and Health, TNO Quality of Life, Leiden, The Netherlands

Introduction

In the western world, breast cancer is the most common malignant disease in women, and one out of every eight women will develop the disease during her lifetime [1]. Important causes are genetic defects, reproductive factors, alcohol, overweight, and physical inactivity, but these factors do not account for the total number of incident breast cancer cases [2]. Moreover, risk factors differ according to estrogen receptor (ER) and progesterone receptor (PR) status of the tumors [3], underscoring the importance of stratifying on receptor status when investigating breast cancer etiology.

Acrylamide, an industrial chemical classified as a probable human carcinogen, was discovered in 2002 in various carbohydrate-rich foods such as bread, crisps, and

cookies [4]. Dietary acrylamide forms naturally when amino acids, mainly asparagine, and reducing sugars combine at temperatures above 120°C (Maillard browning) [5]. Acrylamide exposure also occurs with smoking and smokers have on average four times higher acrylamide–hemoglobin adduct (AA–Hb) levels, an internal dose marker, than non-smokers [6].

It is generally thought that acrylamide is carcinogenic through a genotoxic pathway [7], after conversion to glycidamide, a DNA-reactive epoxide. This mechanism has been the focus of most of the toxicological acrylamide research. Recently, some, but not all, epidemiological studies found a positive association between dietary acrylamide exposure and the risk of strongly endocrine-related cancers (ovarian, endometrial, and ER positive breast cancer) [8, 9], which may suggest a different carcinogenic pathway. One of the hypothesized mechanisms behind acrylamide-induced carcinogenicity, other than genotoxicity, is modulation of sex hormone systems [10]. If acrylamide were to affect these systems, it may primarily be a risk factor for ER and PR positive breast cancer, as reproduction-related exposures seem to be more strongly associated with the risk of receptor-positive breast cancer [3]. In a previous analysis, we observed no association with overall breast cancer risk [9]. Here, we aim to investigate the association between dietary acrylamide intake and postmenopausal breast cancer risk in receptor-defined subgroups in a large prospective cohort study.

Materials and methods

Study design and population

This study is embedded within the Netherlands Cohort Study on diet and cancer (NLCS) [11]. The NLCS started in September 1986 with the inclusion of 62,573 women aged 55–69 years, all presumed to be postmenopausal. At baseline, the cohort members completed a self-administered food frequency questionnaire (FFQ) containing 150 foods and questions on lifestyle habits, and medical, and reproductive history.

A case-cohort design was applied for data processing and analysis [11], and to this purpose a random subcohort of 2589 women was sampled from the total cohort shortly after baseline. This subcohort is followed up regularly for migration and vital status to determine the collected person–time experience.

Incident breast cancer cases from the total cohort have been detected by annual record linkage with the nine regional cancer registries and the Netherlands Pathology Registry (PALGA). The completeness of cancer follow-up was estimated to be at least 96% [12], whereas the follow-up of the subcohort was 100% complete at the end of the 13.3 year follow-up period.

Breast cancer tumors were coded according to the [International Classification of Diseases for Oncology (ICD-O)-3: C50]. Receptor status information was obtained from four regional Dutch cancer registries (other cancer registries had not collected this information before 2000) and was assessed by either immunohistochemistry or biochemical assay. During the follow-up, 2225 incident breast cancer cases were identified from the total cohort, with ER status available for 966 (43%) cases. PR status had not been assessed in one of the four cancer registries and was thus only available for 615 cases, corresponding to 28% of the total number of cases. Cases were stratified into separate and concordant ER and PR subgroups to assess potential heterogeneity of the acrylamide-associated risk.

Cases and subcohort members were excluded if they had a baseline diagnosis of cancer other than skin cancer and if their dietary data were incomplete or inconsistent. Furthermore, cases were excluded if they had non-epithelial or non-invasive tumors. Discordant (ER+ PR− and ER− PR+) cases were excluded due to small number of cases. Figure 1 depicts the number of cases in each separate and joint ER and PR stratum.

Acrylamide exposure assessment

Acrylamide intake was assessed from the FFQ, with questions on frequency and portion size of consumption of 150 foods, combined with the mean acrylamide concentration of each food determined from chemical analysis of several Dutch samples per food. The acrylamide intake assessment is comprehensively described in a previous paper by our group [9].

Data analysis

The accumulated person-years of the entire cohort are estimated using the subcohort, while cases are enumerated for the entire cohort. The case–cohort analysis is based on 13.3 years of follow-up (September 1986–December 1999).

Cases with known receptor status were compared to cases with unknown receptor status according to dietary and non-dietary variables, and tumor characteristics by the Wilcoxon–Mann–Whitney *U*-test and Pearson χ^2 test to investigate heterogeneity between the case groups and hence potential selection bias.

Baseline characteristics of the subcohort were compared across quintiles of acrylamide intake by use of the Kruskal–Wallis test and Pearson χ^2 test.

Cox proportional hazards models were used to examine the relationship between dietary acrylamide exposure and

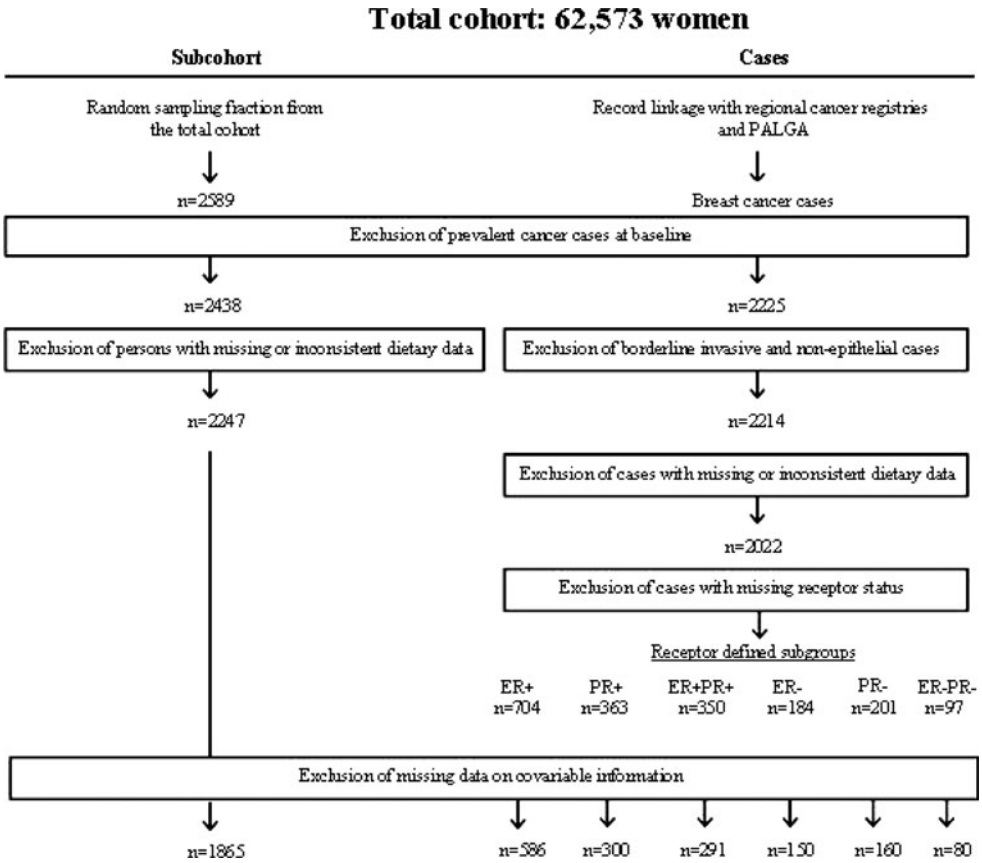

Fig. 1 Flow chart of the cases and subcohort members finally included in the multivariable-adjusted Cox regression analyses

overall breast cancer risk and the risk in receptor-defined subgroups. Hazard ratios (HR) and 95% confidence intervals (CI) were estimated per 10 μg/day increment in acrylamide intake and for quintiles of intake using the lowest as the reference category. For the receptor-negative endpoints, analyses were conducted with acrylamide divided into tertiles due to the small number of cases. Tests for linear trend were performed by assigning the median acrylamide intake in each intake category to the categories, and fitting the ordinal exposure categories as a continuous variable. The proportional hazards assumption was tested through scaled Schoenfeld residuals [13]. The extra variance introduced by sampling from the cohort was accounted for using the robust Huber–White sandwich estimator.

A priori, age at menarche, menopause and first childbirth, parity, body mass index (BMI), family history of breast cancer, personal history of benign breast disease, oral contraceptives use, and postmenopausal hormone use were selected for inclusion in the multivariable-adjusted model [14]. Height, non-occupational physical activity, education level, alcohol, carbohydrate, fiber, saturated fat, trans-unsaturated fat, fruit, and vegetable intake were tested for confounding potential. These variables were included only if they changed the age-adjusted HR, between the 10th and the 90th percentile of daily intake, by more than 10%. Smoking status, smoking years, and cigarettes per day were included in the model, because smoking is an important source of acrylamide exposure [6], and thus has the potential to obscure the effect of dietary acrylamide exposure. Furthermore, smoking can influence estrogen levels in the body [15].

Smokers have been shown to have on average three to four times higher levels of acrylamide–hemoglobin adducts, which is a marker of internal dose of acrylamide, than non-smokers [6]. For this reason and because of the fact that smoking is such an important risk factor for many cancers, subgroup analyses were performed for never smokers.

Interaction with smoking, non-occupational physical activity, obesity, and alcohol intake was tested because of the ability to modify the activity of the enzyme CYP2E1 that has been shown to convert acrylamide into glycidamide. Interaction with age at menarche, menopause and first childbirth, parity, oral contraceptives, and postmenopausal hormone use was tested based on the hypothesis that acrylamide modulates hormone systems.

In additional analyses, the HRs of acrylamide in never-smokers were one at a time adjusted for the five most important acrylamide-contributing foods (Dutch spiced cake, cookies, coffee, potato crisps, and French fries) in the NLCS cohort to determine whether observed associations

could be ascribed to acrylamide or to other constituents of these foods. Also the independent relationships between these five foods and breast cancer risk were tested. Finally, to check for the influence of changes in diet due to pre-clinical disease, analyses were done excluding the first 2 years of follow-up. Two sided *P*-values of $\leq$0.05 were used as cut-off point for statistical significance.

Results

Figure 2 shows the absolute and relative contribution of various foods to the total acrylamide intake of the subcohort. Although coffee was overall the most important contributor to acrylamide intake, Fig. 2 shows that not coffee, but Dutch spiced cake was most responsible for the variation in acrylamide intake in this population, followed by coffee, French fries, potato crisps, and cookies.

Table 1 shows the covariable distribution between cases with known and unknown ER or PR status. In general, no striking dissimilarities were noticeable between the two case groups, although cases with known receptor status had a slightly higher intake of French fries, and cases with unknown receptor status had a slightly higher intake of cookies. No differences were seen in the distributions of tumor size, stage, and grade, when comparing cases with known and cases with unknown receptor status (Table 2).

Based on the subcohort distribution, the median acrylamide intake per quintile was; 9.5, 14.0, 17.9, 24.3, and 36.8 μg/day, equivalent to 0.14, 0.20, 0.26, 0.36, 0.57 μg/kg bw per day. All dietary variables, except fruits, vegetables, and alcohol intake differed considerably between the quintiles. A linear relationship between acrylamide, carbohydrate, total energy, saturated fat, and fiber intake was seen across the quintiles. Statistically significant differences were observed for age, BMI, parity, percentage current cigarette smokers, number of smoking years, and educational level, but with no linear trend across the quintiles of acrylamide intake (Table 3).

There was no association between dietary acrylamide intake and overall breast cancer risk for acrylamide as a continuous variable or when comparing women in the highest quintile of intake to the lowest, irrespective of smoking status (Table 4). When looking at smokers and non-smokers combined, we found no evidence that acrylamide was related to an increased ER+, PR+, or ER+PR+

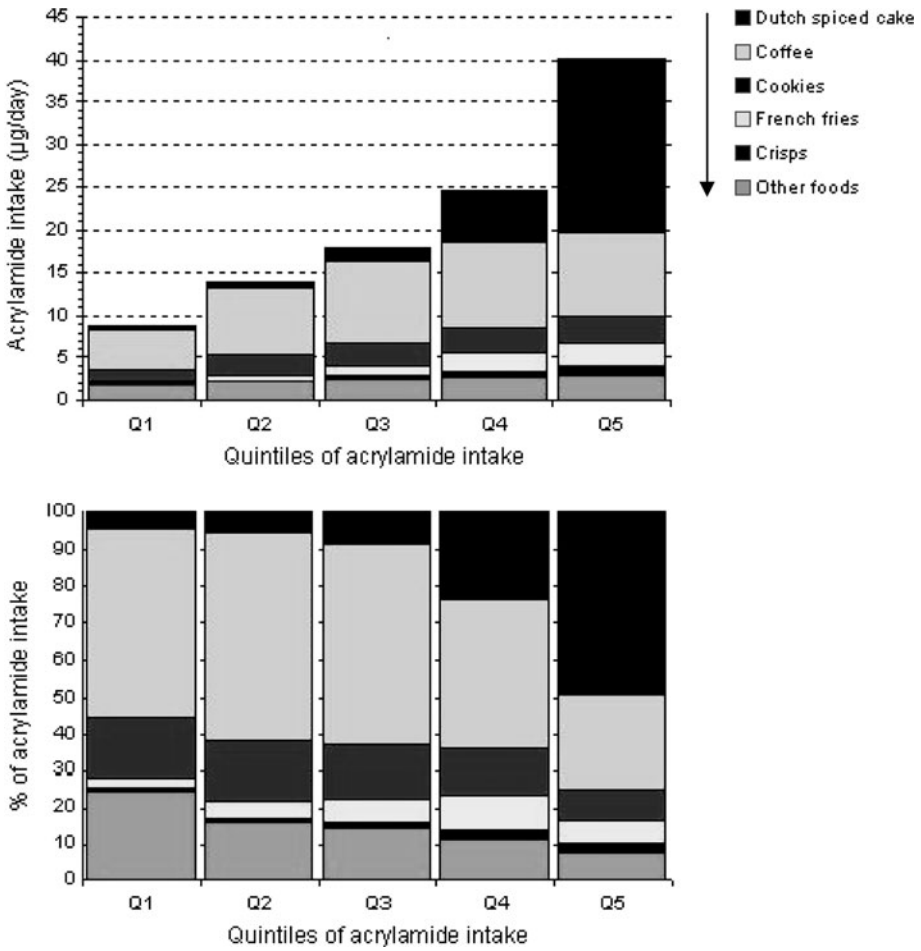

Fig. 2 Absolute and relative contribution of foods to the mean daily dietary acrylamide intake of the NLCS subcohort

Table 1 Characteristics of breast cancer cases with known receptor status compared to breast cancer cases with unknown receptor status; NLCS, 1986–1999

Variable[a]	ER or PR status known	ER or PR status unknown	P[b]
Number of cases	888	1134	
Acrylamide intake (μg/day)[c]	18.3 (14.6)	17.1 (14.3)	0.26
Acrylamide intake (μg/kg body weight per day)	0.27 (0.22)	0.25 (0.21)	0.08
Coffee consumption (g/day)[d]	500 (250)	500 (250)	0.35
Dutch spiced cake consumption (g/day)	0.7 (7.8)	0.0 (7.8)	0.10
Cookie consumption (g/day)	9.3 (20.4)	13.9 (20.4)	0.02
Potato crisp consumption (g/day)	0.0 (0.0)	0.0 (0.0)	0.70
French fries consumption (g/day)	0.0 (1.7)	0.0 (1.2)	0.00
Total energy intake (kcal/day)	1651 (506)	1661 (512)	0.91
Carbohydrate intake (g/day)	174 (64)	172 (59)	0.48
Saturated fat intake (g/day)	29.2 (12.9)	29.1 (12.8)	0.42
Trans-unsaturated fatty acid intake (g/day)	2.3 (1.5)	2.4 (1.5)	0.56
Fiber intake (g/day)	24.5 (9.5)	24.1 (9.2)	0.27
Vegetable intake (g/day)	183 (97)	178 (98)	0.11
Fruit intake (g/day)	180 (148)	171 (144)	0.33
Alcohol intake (g/day)	1.3 (9.1)	1.8 (9.4)	0.14
Age (years)	61 (7.0)	61 (7.0)	0.72
BMI (kg/m^2)	24.8 (4.0)	25.0 (4.4)	0.15
Height (cm)	165 (9)	166 (8)	0.11
Parity (*n* children)	2 (3)	2 (3)	0.26
Age at menarche (years)	13 (3)	13 (3)	0.94
Age at menopause (years)	50 (6)	50 (5)	0.26
Age at first childbirth (years)	27 (6)	27 (5)	0.67
Current cigarette smoking (% yes)[b]	23.7	20.4	0.08
n cigarettes per day among ever-smokers	10 (15)	10 (15)	0.61
n smoking years among ever-smokers	30 (19)	28 (20)	0.07
Non-occupational physical activity (min/day)	49 (58)	47 (51)	0.23
Oral contraceptive use (% ever)[b]	23.1	25.8	0.17
Oral contraceptive use duration among ever-users (years)	7.0 (8.0)	7.0 (8.0)	0.68
Postmenopausal hormone use (% ever)[b]	12.3	13.3	0.77
Postmenopausal hormone use duration among ever users (years)	2 (4)	1 (3)	0.47
Education (%)[b]			
Primary school	33.2	32.4	
Lower vocational school	21.4	21.4	
Intermediate vocational/high school	34.8	36.9	
Higher vocational school/university	9.5	8.8	0.73
Family history of breast cancer (% yes)[b]	13.7	14.6	0.61
History of benign breast disease (% yes)[b]	11.6	13.9	0.12

[a] Data represent medians with (interquartile range) for continuous variables, percentages for categorical

[b] Wilcoxon–Mann–Whitney *U*-test for comparisons of means, or Pearson chi-square test for comparisons of proportions

[c] Microgram per day

[d] Gram per day

breast cancer risk. However, for never-smoking women, increased HRs in the highest quintile compared to the lowest quintile were observed for ER+, PR+ and ER+PR+ tumors, although not statistically significant. A positive association was also observed for acrylamide as a continuous variable for PR+ and ER+PR+ tumors, but again not statistically significant.

For PR-tumors, a statistically significantly decreased risk was seen comparing the second quintile to the first, and for ER-tumors there was a borderline statistically significantly decreased risk, regardless of smoking status. Yet, no clear trends were seen across the acrylamide categories for the receptor-negative endpoints.

Adjustment for potato crisps did not notably change the HRs, while adjustment for Dutch spiced cake and French fries somewhat decreased the HRs (results not shown). Adjustment for coffee slightly increased the HRs, and adjustment for cookies increased the HRs considerably

Table 2 Tumor characteristics of the cases with known receptor status compared to the cases with unknown receptor status; NLCS 1986–1999

	ER or PR status known	ER or PR status unknown	χ^2 test[d]
Size of tumor			
N cases[a]	877	1025	
<2 cm	53.9%	54.5%	
2–5 cm	37.1%	36.8%	
>5 cm	2.7%	3.3%	
Any size with extension to skin or chest wall	6.2%	5.4%	0.78
Stage of tumor[b]			
N cases[a]	880	1031	
Stage I	40.5%	39.6%	
Stage IIA, IIB	48.6%	47.3%	
Stage IIIA, IIIB	6.7%	8.2%	
Stage IV	4.2%	4.8%	0.53
Tumor grade[c]			
N cases[a]	457	446	
Grade 1	12.0%	11.7%	
Grade 2	38.9%	39.5%	
Grade 3	47.7%	46.9%	
Grade 4	1.3%	2.0%	0.86

[a] N cases after elimination of cases with missing information on the tumor characteristics

[b] Staging according to clinical and pathological characteristics, TNM system: tumor, nodes and metastasis

[c] Grading is subdivided into; well, intermediately, poorly or not differentiated

[d] Pearson χ^2-test, comparisons of proportions

when comparing never-smoking women in the highest quintiles of intake to the lowest for ER+, PR+ and ER+PR+ tumors. A statistically significantly increased risk was now also seen for PR+ and ER+PR+ cases with acrylamide as a continuous variable in never-smokers. Adjustment for the five foods did not notably change the HRs for any of the other subgroups.

There were no signs of an independent relationship between coffee, Dutch spiced cake, cookies, potato crisps, or French fries consumption and overall or receptor-negative breast cancer risk, regardless of smoking status (results not shown). A statistically significant inverse relationship between consumption of cookies and risk of breast cancer was observed for ER+ cases and also for never-smoking ER+, PR+ and ER+PR+ cases; HRs (95% CI) were 0.88 (0.80–0.98), 0.83 (0.72–0.96), 0.82 (0.69–0.97) and 0.79 (0.66–0.95) per 10 g of cookies/day, respectively.

None of the interaction terms between acrylamide intake and the selected covariables was statistically significant (results not shown). Exclusion of the first two years of follow-up did not cause any noteworthy changes in the HRs for overall breast cancer or the receptor-defined subgroups (results not shown).

Discussion

This prospective cohort study revealed no association between dietary acrylamide intake and overall breast cancer risk, regardless of smoking status, which confirms our previous analysis with 11.3 years of follow-up [9]. A modestly increased risk of ER and PR positive breast cancer was observed in never-smoking women, but these results were not statistically significant. The significantly decreased risks in the second quintile of acrylamide intake for receptor-negative breast cancers may be chance findings due to random fluctuations in the data.

Recently, a Danish nested case–control study examined the relationship between breast cancer risk and exposure to acrylamide using AA–Hb adducts [8]. The study showed a statistically significant positive smoking-adjusted association between AA–Hb adducts and breast cancer risk, strongest for ER+ tumors. In our study, the positive association was apparent only among never smokers, which is similar to our findings for endometrial and ovarian cancer in a previous study, where stronger effects were observed among never smokers [9]. A tendency towards a stronger smoking-adjusted association among smokers compared to non-smokers was shown in the Danish study, although the heterogeneity was not statistically significant.

AA–Hb adduct levels are not specifically a measure of dietary acrylamide exposure, but for acrylamide exposure from all sources, including smoking, which has on average a much stronger effect on AA–Hb levels than dietary acrylamide intake. Thus, results from analyses in a smoking subgroup may be clouded by acrylamide from and other constituents of tobacco smoke. Adjusting the AA–Hb adduct level for smoking may lead to collinearity, because smoking has such a strong positive effect on AA–Hb adduct levels. For these reasons, analyses in non-smokers are preferable when using acrylamide biomarkers. Clarification of the observed differences according to smoking status by other epidemiological studies is necessary.

A Swedish prospective cohort study reported no association between acrylamide intake and ER+ or PR+ breast cancer risk [16]. This study investigated pre- and postmenopausal women combined, thus not taking into account that the etiology of pre- and postmenopausal breast cancer may differ [14]. Furthermore, coffee was the main contributor to the acrylamide exposure and their adjustment for coffee intake could thus have resulted in overcorrection of the acrylamide risk estimate, which cannot be judged from their paper because they do not

Table 3 Subcohort characteristics across quintiles of daily dietary intake; NLCS

Variable[b]	Q1	Q2	Q3	Q4	Q5	P^c
Number of subcohort members[a]	450	461	433	457	446	
Acrylamide intake (μg/d)	9.5 (3.8)	14 (1.6)	17.9 (2.2)	24.3 (4.6)	36.8 (9.8)	
Acrylamide intake (μg/kg body weight per day)	0.14 (0.06)	0.20 (0.05)	0.26 (0.06)	0.36 (0.10)	0.57 (0.20)	
Coffee consumption (g/day)	250 (125)	500 (125)	500 (250)	500 (375)	500 (375)	<0.001
Dutch spiced cake consumption (g/day)	0.0 (0.0)	0.0 (0.7)	0.0 (1.8)	3.1 (7.8)	20.4 (4.7)	<0.001
Cookie consumption (g/day)	3.7 (13)	9.3 (20.4)	13.9 (14.8)	16.7 (14.8)	16.7 (14.8)	<0.001
Potato crisp consumption (g/day)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.2)	0.0 (0.3)	<0.001
French fries consumption (g/day)	0.0 (0.0)	0.0 (0.0)	0.0 (1.7)	0.0 (4.3)	0.0 (3.4)	<0.001
Total energy intake (kcal/day)	1463 (442)	1580 (453)	1657 (4659)	1732 (550)	1822 (545)	<0.001
Carbohydrate intake (g/day)	154 (55)	161 (50)	176 (54)	184 (61)	200 (69)	<0.001
Saturated fat intake (g/day)	25.3 (11.7)	27.9 (12.3)	28.6 (11.4)	29.9 (13.0)	30.9 (12.3)	<0.001
Trans-unsaturated fatty acid intake (g/day)	2.3 (1.0)	2.5 (0.9)	2.4 (1.0)	2.5 (1.19	2.5 (1.1)	<0.001
Fibre intake (g/day)	22.9 (9.0)	23.8 (8.6)	24.5 (9.0)	25.8 (9.5)	26.5 (8.5)	<0.001
Vegetable intake (g/day)	184 (102)	182 (120)	179 (84)	190 (103)	184 (93)	0.41
Fruit intake (g/day)	184 (152)	178 (150)	172 (140)	174 (139)	178 (145)	0.41
Alcohol intake (g/day)	1.0 (8.4)	1.6 (7.8)	2.1 (8.3)	1.9 (6.9)	1.4 (7.4)	0.16
Age (years)	62 (7)	61 (7)	60 (7)	61 (8)	61 (7)	<0.001
BMI (kg/m^2)	24.5 (4.4)	24.8 (4.4)	24.8 (4.2)	24.6 (4.3)	24.3 (4.2)	0.05
Height (cm)	165 (8)	165 (9)	165 (9)	165 (8)	165 (9)	0.32
Parity (*n* children)	2.0 (3)	3.0 (3)	3.0 (2)	3.0 (2)	2.0 (3)	0.002
Age at menarche (y)	13 (3)	13 (2)	14 (3)	14 (2)	14 (3)	0.15
Age at menopause (y)	50 (6)	50 (6)	49 (7)	50 (6)	50 (6)	0.55
Age at first childbirth (y)	27 (5)	26 (5)	27 (5)	26 (5)	26 (5)	0.92
Current cigarette smoking (% yes)	18.0	18.2	26.8	21.7	21.1	0.006
n cigarettes per day among ever-smokers	10 (15)	10 (14)	10 (14)	10 (10)	10 (15)	0.66
n smoking years among ever-smokers	32 (20)	27 (20)	30 (20)	29 (21)	30 (19)	0.02
Non-occupational physical activity (min/day)	53.6 (55.7)	52.1 (49.3)	53.6 (52.9)	53.6 (52.9)	51.4 (58.2)	0.98
Oral contraceptive use (% ever)	22.7	25.3	26.3	26.3	24.4	0.72
Oral contraceptive use duration ever-users (years)	6.0 (9)	6.0 (9)	5.5 (8)	7.0 (10)	8.0 (8)	0.31
Postmenopausal hormone use (% ever)	13.5	15.3	13.4	11.7	11.9	0.53
Postmenopausal hormone use duration ever users (years)	2.0 (5)	1.0 (3)	1.0 (2)	2.0 (3)	2.0 (4)	0.23
Education (%)						<0.001
Primary school	29.6	31.3	35.8	35.2	35.6	
Lower vocational school	18.8	20.4	25.6	26.9	24.3	
Intermediate vocational/high school	38.1	39.8	33.3	29.1	32.2	
Higher vocational school/university	13.5	8.5	5.4	8.8	7.9	
Family history of breast cancer (% yes)	8.4	9.3	7.4	7.4	10.8	0.34
History of benign breast disease (% yes)	7.6	9.1	7.2	7.2	7.4	

[a] Total number of subcohort members $n = 2247$

[b] Data represent medians (interquartile range) for continuous variables, percentages for categorical variables

[c] P value of Kruskal–Wallis test, or of Pearson χ^2-test

show results that were unadjusted for coffee. However, the Swedish study did not stratify on both receptor and smoking status, so their results cannot be compared to the Dutch and Danish study results.

In another prospective cohort study (United States), no association between acrylamide intake and ER+ or PR+ breast cancer risk was observed, but their population consisted of premenopausal women only [17].

Table 4 Hazard ratios (HR) and 95% confidence intervals for the associations between dietary acrylamide intake and postmenopausal breast cancer risk in receptor-defined subgroups; (NLCS), 1986–1999

Type of breast tumor	Increment per 10 μg AA/day HR (95% CI)	Category (Q = quintile, T is tertile) of dietary acrylamide intake					*P* trend[b]
		Q1/T1 HR (ref)	Q2 HR(95% CI)	Q3/T2 HR (95% CI)	Q4 HR (95% CI)	Q5/T3 HR(95% CI)	
All cases[a]							
All	1690/22,879	359/4487	341/4684	323/4354	318/4653	349/4701	
Age adjusted[c]	0.98 (0.92–1.03)	1.00	0.92 (0.74–1.13)	0.94 (0.76–1.16)	0.86 (0.69–1.06)	0.93 (0.76–1.15)	0.40
Multivariable[d]	0.97 (0.91–1.03)	1.00	0.91 (0.73–1.23)	0.96 (0.76–1.19)	0.89 (0.72–1.12)	0.92 (0.73–1.15)	0.48
Never smokers	953/13760	189/2976	201/2796	184/2453	183/2764	196/2771	
Age adjusted	1.01 (0.94–1.09)	1.00	1.14 (0.87–1.49)	1.23 (0.93–1.63)	1.07 (0.81–1.41)	1.13 (0.86–1.48)	0.57
Multivariable	1.01 (0.93–1.10)	1.00	1.11 (0.84–1.48)	1.28 (0.95–1.72)	1.08 (0.80–1.45)	1.15 (0.86–1.53)	0.48
ER+[a]							
All	586/22,879	122/4487	117/4685	114/4354	111/4653	122/4701	
Age adjusted	0.98 (0.91–1.06)	1.00	0.93 (0.69–1.25)	0.98 (0.73–1.32)	0.89 (0.66–1.20)	0.96 (0.72–1.04)	0.81
Multivariable	0.96 (0.88–1.04)	1.00	0.93 (0.69–1.26)	0.98 (0.72–1.33)	0.92 (0.67–1.26)	0.93 (0.68–1.27)	0.71
Never smokers	321/13760	61/2976	64/2796	60/2453	65/2764	71/2771	
Age adjusted	1.05 (0.94–1.16)	1.00	1.13 (0.76–1.68)	1.26 (0.84–1.91)	1.19 (0.79–1.77)	1.27 (0.86–1.87)	0.28
Multivariable	1.05 (0.94–1.17)	1.00	1.14 (0.75–1.73)	1.32 (0.85–2.04)	1.21 (0.79–1.86)	1.31 (0.87–1.97)	0.26
PR+[a]							
All	300/22,879	58/4487	64/4685	55/4354	56/4653	67/4701	
Age adjusted	1.02 (0.92–1.13)	1.00	1.08 (0.73–1.59)	1.00 (0.67–1.50)	0.95 (0.64–1.42)	1.11 (0.76–1.63)	0.71
Multivariable	0.99 (0.89–1.11)	1.00	1.04 (0.70–1.56)	0.96 (0.63–1.47)	0.94 (0.61–1.44)	1.03 (0.69–1.55)	0.95
Never smokers	169/13760	30/2976	30/2796	34/2453	35/2764	40/2771	
Age adjusted	1.12 (0.98–1.28)	1.00	1.08 (0.63–1.86)	1.47 (0.86–2.53)	1.30 (0.77–2.20)	1.45 (0.87–2.41)	0.14
Multivariable	1.12 (0.97–1.30)	1.00	1.04 (0.59–1.82)	1.46 (0.82–2.60)	1.30 (0.74–2.28)	1.47 (0.86–2.51)	0.14
ER+PR+[a]							
All	291/22,879	56/4487	63/4685	52/4354	54/4653	66/4701	
Age adjusted	1.03 (0.93–1.13)	1.00	1.10 (0.74–1.63)	0.99 (0.65–1.49)	0.95 (0.64–1.43)	1.14 (0.77–1.67)	0.64
Multivariable	0.99 (0.89–1.11)	1.00	1.06 (0.70–1.59)	0.94 (0.61–1.44)	0.93 (0.61–1.44)	1.05 (0.69–1.59)	0.89
Never smokers	164/13760	30/2976	30/2796	31/2453	34/2764	39/2771	
Age adjusted	1.12 (0.98–1.28)	1.00	1.08 (0.63–1.86)	1.35 (0.78–2.34)	1.27 (0.75–2.15)	1.42 (0.85–2.36)	0.17
Multivariable	1.12 (0.97–1.30)	1.00	1.03 (0.59–1.82)	1.32 (0.74–2.38)	1.26 (0.71–2.23)	1.43 (0.83–2.46)	0.16
ER−[a]							
All	150/22,879	33/4487	19/4685	30/4354	36/4653	32/4701	
Age adjusted	1.01 (0.89–1.15)	1.00	0.55 (0.31–0.98)*	0.92 (0.55–1.55)	1.03 (0.63–1.69)	0.91 (0.55–1.52)	0.58
Multivariable	1.01 (0.88–1.16)	1.00	0.56 (0.31–1.00)	1.01 (0.58–1.75)	1.13 (0.67–1.90)	0.93 (0.53–1.62)	0.61

Table 4 continued

Type of breast tumor	Increment per 10 μg AA/day HR (95% CI)	Category (Q = quintile, T is tertile) of dietary acrylamide intake					*P* trend[b]
		Q1/T1 HR (ref)	Q2 HR(95% CI)	Q3/T2 HR (95% CI)	Q4 HR (95% CI)	Q5/T3 HR(95% CI)	
Never smokers	83/13760[e]	29/4795		27/4411		27/4553	
Age adjusted	0.97 (0.81–1.18)	1.00		1.03 (0.60–1.78)		0.98 (0.57–1.69)	0.93
Multivariable	0.95 (0.77–1.18)	1.00		1.11 (0.61–2.02)		0.95 (0.52–1.72)	0.77
PR− [a]							
All	160/22,879	34/4487	20/4685	37/4354	32/4653	37/4701	
Age adjusted	1.04 (0.92–1.17)	1.00	0.55 (0.31–0.97)*	1.07 (0.66–1.76)	0.87 (0.52–1.44)	1.01 (0.62–1.65)	0.46
Multivariable	0.98 (0.85–1.12)	1.00	0.54 (0.30–0.97)*	1.06 (0.62–1.79)	0.83 (0.48–1.42)	0.87 (0.51–1.48)	0.97
Never smokers	83/13760[e]	30/4795		27/4411		26/4553	
Age adjusted	0.94 (0.78–1.15)	1.00		0.99 (0.57–1.72)		0.92 (0.53–1.58)	0.74
Multivariable	0.91 (0.73–1.14)	1.00		0.98 (0.53–1.82)		0.84 (0.63–1.56)	0.56
ER−PR− [a]							
All	80/22,879[e]	28/7602		23/7506		29/7771	
Age adjusted	1.01 (0.84–1.22)	1.00		0.79 (0.45–1.40)		0.98 (0.58–1.67)	0.90
Multivariable	0.96 (0.77–1.19)	1.00		0.86 (0.46–1.58)		0.90 (0.48–1.68)	0.80
Never smokers	43/13760[f]	[f]		[f]		[f]	
Age adjusted	0.87 (0.65–1.16)						
Multivariable	0.81 (0.57–1.14)						

Median acrylamide intake of the subcohort in the quintiles of intake: 9.5; 14.0; 17.9; 24.3; and 36.8

Median acrylamide intake of the subcohort in the tertiles of intake: 11.3; 17.9; and 32.1

[a] Number of cases/person-years, number of cases accumulated in the total cohort/number of person-years at risk in the subcohort. Numbers are after list-wise deletion of observations with missing values on the preselected and tested confounders

[b] Two sided *P*-values for linear trend

[c] Age adjusted (same dataset as multivariable-adjusted analysis)

[d] Adjusted for age (years), age at menarche (years), age at menopause (years), age at first childbirth (categorical), parity (*n* children), body mass index (kg/m^2), family history of breast cancer (yes, no), history of benign breast disease (yes, no), use of oral contraceptive (yes, no), postmenopausal hormone use (yes, no), energy intake (kcal/day), smoking status (never, ever), duration of smoking (n smoking years), quantity of smoking (cigarettes/day)

[e] We considered 100 cases as the minimum number needed to do quintile analyses, 60 for tertile analyses and 20 for analyses with acrylamide as a continuous variable

[f] Insufficient number of cases

* $P < 0.05$

None of the *P*-values for interaction with any of the possible CYP2E1-influencing variables or hormonal factors were significant. This may have been caused by insufficient power. However, it can also be that the mentioned variables do not have a clear effect on CYP2E1, contrary to expectation, and do not affect the risk associated with acrylamide intake in another way than through influence on CYP2E1 activity. We obtained the information of the effect of the mentioned variables on CYP2E1 activity from literature on animal studies and it is unclear if these are relevant for the human situation. In addition, CYP2E1 activity is not only determined by environmental influences, but also by genetic polymorphisms in the gene.

The etiology of receptor-positive and negative breast cancer has been shown to differ [3], providing an explanation for the observed difference in acrylamide-associated risk between receptor-positive and overall breast cancer risk. As previously described, one of the hypotheses for the mechanism of acrylamide-induced carcinogenicity is modulation of sex steroid hormone systems [10]. This could explain the increased receptor-positive breast cancer risk in this study. Previous findings of a positive association with endometrial and ovarian cancer risk [9], known to be induced by changes in sex hormones [18, 19], support the hypothesis of a hormonal pathway. Furthermore, an in vitro study with human breast cells has shown a glycidamide-induced up-regulation of genes that catalyze the conversion of estrogen precursors to active forms, such as 17β-estradiol [20].

Tumors with concordant ER and PR status have been shown to be sensitive to both hormones [21], and therefore both receptors should be taken into account. Furthermore, two variants exist of ERs; ERα and ERβ [22]. ERα and ERβ regulate different genes in response to estrogens, indicating that estrogens can exert different effects in different tissues dependent on the receptor variant [23]. PR is also expressed in two isoforms, A and B. Over-expression of PR-A increases resistance to endocrine therapy compared to over-expression of PR-B [22]. Therefore, future studies should ideally allow for receptor subtype variants.

Variation in acrylamide exposure variation in our study was to a large extent due to Dutch spiced cake. Dutch spiced cake was not independently associated with breast cancer risk, irrespective of receptor status. The association between acrylamide and receptor-positive breast cancer risk is therefore probably not attributable to other substances in Dutch spiced cake than acrylamide. The decrease in acrylamide-associated risks after adjustment for Dutch spiced cake is most likely due to overcorrection caused by the moderate correlation between total acrylamide and Dutch spiced cake intake (Spearman correlation coefficient: 0.68). Adjustment for coffee (Spearman correlation with acrylamide coefficient: 0.43) somewhat increased the HRs, whereas the HRs for the receptor-positive breast cancers became stronger and statistically significant after adjustment for cookies. Cookies (Spearman correlation with acrylamide: 0.33) were independently associated with a statistically significantly decreased receptor-positive breast cancer risk, yet only in never-smoking women. The reason for this inverse association is unknown. Coffee contains antioxidants (e.g. lignans), and caffeine intake is reported to be positively associated with sex hormone binding globulin (SHBG), which lowers levels of free estrogens and androgens. Furthermore, caffeic acid can inhibit key mechanisms for silencing tumor suppressor genes [24]. These observations may explain why the risk estimate of acrylamide was slightly increased after adjustment for coffee.

The prospective design, a considerable follow-up period and the large size of the cohort are important assets of the study. Follow-up of the subcohort members was 100% complete and case ascertainment was estimated to be at least 96% for all cases [12], though lesser for the receptor-defined cases. In this study, we had a power of 80% to detect a HR of 1.55 or higher when comparing the highest quintile of acrylamide intake to the lowest for all ER+ tumors, in case of no misclassification [25]. Breast cancer cases with known and unknown receptor status did not differ importantly according to baseline and tumor characteristics, making selection bias of the cases unlikely. Dietary information was prospectively collected by use of a validated and reproducible FFQ combined with chemical analyses of the acrylamide content in specifically Dutch foods, making the exposure estimation a central quality of this study [26].

The study also has some limitations that call for a cautious interpretation of the results and for replication of the analyses in future studies. There were some differences in acrylamide intake and intake of some acrylamide-containing foods (French fries and cookies) between cases with known and cases with unknown ER/PR status, which may have biased the results to some extent. It is unclear if the distribution over the receptor subtypes in the population of cases with unknown receptor status is the same as in the population of cases with known receptor status. Therefore, it is impossible to tell what the influence of having receptor status information for only a part of the case population on the results is.

Furthermore, chance is always a possible alternative explanation for observed associations from analyses using statistical inference.

Foods analyzed in 2002–2005 may not be completely representative of foods from 1986, because changes may have occurred in acrylamide content, especially after the discovery of acrylamide in food in 2002. Yet, the reduction of the acrylamide content in foods up to 2005 had been

very limited [27]. Furthermore, variations in food due to home cooking were not accounted for. These sources of misclassification are most likely to have been non-differential, reducing the estimated risk towards the null. Different receptor assessment methods have been applied between the cancer registries and PALGA. Nevertheless, high correlations of 92% and 83% between the two methods (immunohistochemistry and biochemical assay) have been shown for ER and PR, respectively [28]. Yet, some misclassification of the receptor status may have occurred, due to differences in receptor status cut-off values, however, most likely unrelated to exposure. Non-differential misclassification of outcome may lead to bias towards the null-value, and thus potentially an underestimation of the true association.

In conclusion, this study revealed no association between dietary acrylamide intake and overall breast cancer risk. A statistically non-significant positive association was seen with risk of receptor-positive breast cancer in never-smoking women. However, the fact that we observed this association in never smokers and a Danish study observed it more strongly in smokers impedes the interpretation of these findings. Further epidemiological studies are required to confirm or refute these observations and to clarify the differences related to smoking.

Acknowledgments We are indebted to the participants of this study and further wish to thank the cancer registries (IKA, IKL, IKMN, IKN, IKO, IKR, IKST, IKW, IKZ and VIKC), and the Netherlands nationwide registry of pathology (PALGA). We thank Arnold Kester, PhD, from Maastricht University for statistical advice; Sacha van de Crommert, Henny Brants, Jolanda Nelissen, Conny de Zwart, Annemie Pisters from Maastricht University, and Willy van Dijk and Martine Jansen from TNO Quality of Life for assistance; Linda van den Bosch from TNO Quality of Life, and Jack Berben and Harry van Montfort from Maastricht University for programming assistance.

References

1. Parkin DM (2001) Global cancer statistics in the year 2000. Lancet Oncol 2(9):533–543
2. Hankinson S, Hunter D (2002) Breast cancer. In: Adami HO, Hunter DJ, Trichopoulos D (eds) Textbook of cancer epidemiology. Oxford University Press, New York
3. Althuis MD, Fergenbaum JH, Garcia-Closas M, Brinton LA, Madigan MP, Sherman ME (2004) Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. Cancer Epidemiol Biomarkers Prev 13(10):1558–1568
4. Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M (2002) Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem 50(17):4998–5006
5. Mottram DS, Wedzicha BL, Dodson AT (2002) Acrylamide is formed in the Maillard reaction. Nature 419(6906):448–449
6. Schettgen T, Rossbach B, Kutting B, Letzel S, Drexler H, Angerer J (2004) Determination of haemoglobin adducts of acrylamide and glycidamide in smoking and non-smoking persons of the general population. Int J Hyg Environ Health 207(6):531–539
7. Dybing E, Farmer PB, Andersen M et al (2005) Human exposure and internal dose assessments of acrylamide in food. Food Chem Toxicol 43(3):365–410
8. Olesen PT, Olsen A, Frandsen H, Frederiksen K, Overvad K, Tjonneland A (2008) Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study. Int J Cancer 122(9):2094–2100
9. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2007) A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. Cancer Epidemiol Biomarkers Prev 16(11):2304–2313
10. Besaratinia A, Pfeifer GP (2007) A review of mechanisms of acrylamide carcinogenicity. Carcinogenesis 28(3):519–528
11. van den Brandt PA, Goldbohm RA, van't Veer P, Volovics A, Hermus RJ, Sturmans F (1990) A large-scale prospective cohort study on diet and cancer in The Netherlands. J Clin Epidemiol 43(3):285–295
12. Goldbohm RA, van den Brandt PA, Dorant E (1994) Estimation of the coverage of Dutch municipalities by cancer registries and PALGA based on hospital discharge data. Tijdschr Soc Gezondheidsz 72:80–84
13. Schoenfeld D (1982) Partial residuals for the proportional hazards regression model. Biometrika 69:239–241
14. Key TJ, Verkasalo PK, Banks E (2001) Epidemiology of breast cancer. Lancet Oncol 2(3):133–140
15. Tanko LB, Christiansen C (2004) An update on the antiestrogenic effect of smoking: a literature review with implications for researchers and practitioners. Menopause 11(1):104–109
16. Larsson SC, Akesson A, Wolk A (2008) Long-term dietary acrylamide intake and breast cancer risk in a prospective cohort of Swedish women. Am J Epidemiol 169(3):376–381
17. Wilson KM, Mucci LA, Cho E, Hunter DJ, Chen WY, Willett WC (2009) Dietary acrylamide intake and risk of premenopausal breast cancer. Am J Epidemiol 169(8):954–961
18. Linkov F, Edwards R, Balk J et al (2008) Endometrial hyperplasia, endometrial cancer and prevention: gaps in existing research of modifiable risk factors. Eur J Cancer 44(12):1632–1644
19. Riman T, Nilsson R, Persson IR (2004) Review of epidemiological evidence for reproductive and hormonal factors in relation to the risk of epithelial ovarian malignancies. Acta Obset Gynecol Scand 83(9):783–795
20. Clement FC, Dip R, Naegeli H (2007) Expression profile of human cells in culture exposed to glycidamide, a reactive metabolite of the heat-induced food carcinogen acrylamide. Toxicology 240 (1–2):111–124
21. Rosenberg LU, Einarsdottir K, Friman EI et al (2006) Risk factors for hormone receptor-defined breast cancer in postmenopausal women. Cancer Epidemiol Biomarkers Prev 15(12): 2482–2488
22. Speirs V, Walker RA (2007) New perspectives into the biological and clinical relevance of oestrogen receptors in the human breast. J Pathol 211(5):499–506
23. Kian TM, Rogatsky I, Tzagarakis-Foster C et al (2004) Estradiol and selective estrogen receptor modulators differentially regulate target genes with estrogen receptors alpha and beta. Mol Biol Cell 15(3):1262–1272
24. Tang N, Zhou B, Wang B, Yu R (2009) Coffee consumption and risk of breast cancer: a metaanalysis. Am J Obstet Gynecol 200(3): 290–299
25. Cai J, Zeng D (2004) Sample size/power calculation for case-cohort studies. Biometrics 60(4):1015–1024
26. Goldbohm RA, van den Brandt PA, Brants HA et al (1994) Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. Eur J Clin Nutr 48(4): 253–265

27. Petersen BJ, Tran N (2005) Exposure to acrylamide: placing exposure in context. Adv Exp Med Biol 561:63–76
28. Zafrani B, Aubriot MH, Mouret E et al (2000) High sensitivity and specificity of immunohistochemistry for the detection of hormone receptors in breast carcinoma: comparison with biochemical determination in a prospective study of 793 cases. Histopathology 37(6):536–545