

Award Number: W81XWH-04-1-0288

TITLE: A Population-Based Study of Dietary Acrylamide and Prostate Cancer Risk

PRINCIPLE INVESTIGATOR: Hans-Olov Adami, M.D., Ph.D.

CONTRACTING ORGANIZATION: Karolinska Institutet
SE-171 77 Stockholm, Sweden

REPORT DATE: June 2007

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE 01-06-2007			2. REPORT TYPE Final		3. DATES COVERED 2 Feb 2004 – 30 May 2007	
4. TITLE AND SUBTITLE A Population-Based Study of Dietary Acrylamide and Prostate Cancer Risk					5a. CONTRACT NUMBER	
					5b. GRANT NUMBER W81XWH-04-1-0288	
					5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Hans-Olov Adami, M.D., Ph.D.					5d. PROJECT NUMBER	
					5e. TASK NUMBER	
					5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Karolinska Institutet SE-171 77 Stockholm, Sweden					8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012					10. SPONSOR/MONITOR'S ACRONYM(S)	
					11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited						
13. SUPPLEMENTARY NOTES						
14. ABSTRACT Purpose/scope: The aim of this study is to determine whether intake of foods found to have high levels of acrylamide increase the risk of prostate cancer among men. Methods: A population-based case-control study on prostate cancer. The exposure to acrylamide was estimated by using a food frequency questionnaire (FFQ) and by hemoglobin adducts in blood. Results: The intake of acrylamide and adduct levels are in line with previous studies, but there was only a weak correlation between the two estimates. The relative risk of prostate cancer was 0.97 (95% CI 0.75-1.27) for the highest quintile of exposure compare to the lowest quintile. Conclusions: ••There was no evidence of an overall association between exposure to dietary acrylamide and prostate cancer risk in the present study.						
15. SUBJECT TERMS No subject terms provided.						
16. SECURITY CLASSIFICATION OF:				UU	18. NUMBER OF PAGES 24	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U	19b. TELEPHONE NUMBER (include area code)			

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1. Introduction

The substance acrylamide is a chemical that impairs the nervous system and has been classified as a probable carcinogen by the International Agency on Cancer Research (IARC 1995). Recently, the Swedish National Food Administration found high levels of acrylamide in a variety of foodstuffs, including French fries, potato crisps, and crispy bread. It is thought that acrylamide formation occurs during the heating of starch-rich foods to high temperatures. Because the foodstuffs containing acrylamide are commonly consumed both in the US and Sweden, this report created substantial international alarm about the public's health. Several studies on animals exposed to toxic levels of this chemical, which found that acrylamide increases the risk of a variety of cancers in a dose-response relationship. There is, however, scant human data on the carcinogenic effect of acrylamide. The studies on humans thus far have been limited to acrylamide contact in the occupational setting. This project set out to examine the hypothesis that high intake of foods containing acrylamide increases the risk of prostate cancer among men. We addressed the study questions using information from a case-control study of prostate in Sweden. Research in Sweden has a number of advantages, including the use of nationwide cancer registry and the use of national registration numbers uniquely identifying all Swedish residents, which offer opportunities for population-based, cost-effective and reliable studies. The incidence of prostate cancer in Sweden is similar to rates seen in the United States. In addition, both countries have similar consumption of the foods with high levels of acrylamide.

2. Body

We have successfully finished the data collection of CAPS, the population-based case-control study on prostate cancer in Swedish men. In total, 1,895 cases with verified prostate cancer were invited to participate and 1,499 filled out a questionnaire (including a food frequency questionnaire) and 1,400 donated blood samples. Of the 1,684 invited randomly selected controls, 1,130 completed the questionnaire and 879 donated blood. The questionnaire data have been transferred to an electronic format.

The food frequency questionnaire data on foods was translated into a nutrient database, and we developed an algorithm for estimating total dietary acrylamide exposure for each study participant using the Swedish National Food Administration acrylamide database. Quality control of the data was assessed in order to check for data errors and inconsistencies. When errors arose, we consulted the original questionnaires or discussed with the laboratory personnel: we were able to resolve all inconsistencies. We outputted a dataset in SAS which merges together questionnaire data, food and nutrient data, and the record linkage, and includes created variables needed for the analyses.

For the biomarker assays of acrylamide adducts, we selected case-control pairs using a nested case-control design among 377 men. This biomarker has been shown to correlate with levels of occupational exposure to acrylamide (Tornqvist, 2006), and represents acrylamide exposure over the previous four months, or the half life of red blood cells. The blood samples were pulled from the BioRepository at the Karolinska Institutet, and set for analysis at the laboratory of Dr. Margarita Tornqvist. The laboratory work involved Isolation of hemoglobin, detachment of adducts from the hemoglobin, isolation, derivation and analysis of the detached adduct. The samples were processed in four batches of approximately 100 samples each. Laboratory personnel were blinded to the case/control status of the samples. By chance, more case samples were included in batches one and two, and more controls were in batches three and four. Mean acrylamide adduct levels decreased over the four batches. Within each batch, mean adduct levels were similar for cases and controls, suggesting that the difference between batches was due to laboratory drift. Laboratory batch was adjusted for in all analyses. Eleven samples were not processed because the cells were clotted. 34 men who reported using tobacco products (cigarettes, pipes, or snuff) at the time of the questionnaire were excluded from the blood analysis, as tobacco users are exposed to much higher levels of acrylamide through tobacco than through the diet. Mean blood acrylamide in these men was 152 pmol/g globin compared to 54 pmol/g in non-smoking men. After exclusions, 175 cases and 168 controls were used in the analysis of blood acrylamide.

The final acrylamide biomarker dataset was merged together with the final questionnaire dataset into one analysis datafile in SAS. All statistical analyses were undertaken using version SAS 9.1. For the validation of FFQ acrylamide intake, the correlation between calculated acrylamide intake and acrylamide adducts to hemoglobin was calculated in the subset of men with blood measurements. The correlation was adjusted for age, region, and laboratory batch. To measure the association between blood acrylamide and risk of prostate cancer, we used unconditional logistic regression models with indicator variables for quartile of blood acrylamide level. Quartiles were created based on the distribution among the controls. Age group and region, which were matching factors in this study, were included in all models, as was laboratory batch. Fully adjusted models also include variables for BMI (continuous) and former smoking.

To measure the association between dietary acrylamide intake and risk of prostate cancer, we used unconditional logistic regression models with indicator variables for quintiles of calorie-adjusted acrylamide intake. Quintiles were created based on the distribution of intake among the controls. Age group and region were included in all models. Fully adjusted models also include variables for BMI (continuous), former and current smoking, education (four categories), zinc intake (ordinal

quartiles), and total energy intake. Employment status and civil (marital) status were also considered as potential confounders. Several other nutrients and foods were considered as potential confounders, as well, including: alcohol, alpha-linolenic acid, calcium, vitamin D, folate, phytoestrogens, red meat, fish, and tomato. None of these was included in the final models, as they had little effect on the acrylamide effect estimates or precision. Data on these confounders were collected in the self-administered mailed questionnaire. To test for a dose-response trend across quantiles of acrylamide, we modeled acrylamide as a continuous variable using the median intake in each quantile. The p-value of this continuous variable was used to determine the significance of any linear trend across quantiles of intake.

Cases and controls were similar in acrylamide intake calculated from the FFQ and in blood levels of acrylamide (Mean intake controls = 44.5 mcg/day versus cases = 43.8 mcg/day). In the subset of men with blood acrylamide measurements, the mean adduct level was 49.3 pmol/g globin among controls and 51.6 pmol/g globin among cases. Cases were more likely to come from the Northern regions of Sweden. Cases and controls were similar in age, education, BMI, height, smoking status, and diet including daily intakes of dairy, red meat, fish, fruits, and vegetables and total energy intake. Acrylamide intake ranged from 8 to 125 mcg/day, or 0.08 to 1.59 mcg/ kilogram body weight per day. The top food contributors to acrylamide intake were crispbread, coffee, other bread, fried potatoes, and buns and cakes. There was a significant ($p < 0.0001$) correlation between acrylamide intake and intake of carbohydrates, fiber, and zinc (all positive) and alcohol (negative). Acrylamide intake was not correlated with age or height. The partial correlation between dietary acrylamide intake and blood acrylamide (as acrylamide adducts to hemoglobin) was 0.25 ($p < 0.0001$), adjusted for age, region, energy intake, and laboratory batch. Among controls only, the correlation was 0.35 ($p < 0.0001$). Among cases, it was 0.16 ($p = 0.05$).

We found no significant association between quartile of blood acrylamide and prostate cancer risk. Adjusting for age, region, BMI, former smoking, and laboratory batch, the relative risk for the highest versus lowest quartile of blood acrylamide was 1.02 (95% CI: 0.46-2.24). For a 10 pmol/g globin increase in blood acrylamide, the relative risk of prostate cancer was 0.97 (CI: 0.81-1.17, p for trend=0.77). For dietary acrylamide calculated from the FFQ, there was no association between higher intakes and prostate cancer risk. The relative risk for the highest versus lowest quintile of acrylamide intakes was 0.97 (CI: 0.75-1.27), adjusting for age, region, BMI, education, smoking, and zinc and energy intake. For a 10 mcg/day increase in acrylamide intake, the RR of prostate cancer was 0.99 (CI: 0.92-1.06, p for trend=0.67). Similar results were seen when the analysis was limited to non-smokers only. Among non-smokers, a 10 mcg/day increase in acrylamide intake was associated with a relative risk of 1.02 (CI: 0.94-1.09, p for trend=0.68). No association was found between acrylamide intake and specific prostate cancer endpoints including mortality, advanced disease, localized disease, high- or low-grade disease, or high- or low-PSA disease. A manuscript (Appendix 1) summarizing the study results is being circulated to the co-authors.

3. Key research accomplishments

- Completed collection of questionnaire data and finalized food frequency questionnaire databases from 1,489 cases and 1,111 controls
- Generated nutrient database and estimates of acrylamide exposure through diet for each individual using data from the Swedish National Food Administration
- Analyzed levels of Hb adducts in stored blood specimens from 175 cases and 168 controls in the laboratory of Dr. Tornqvist
- Generated final analysis dataset in SAS, and completed statistical analyses for all aims of the project
- Produced manuscript summarizing study results

4. Reportable outcomes

- An abstract with the title “Acrylamide and prostate cancer risk “ was presented at the DoD IMPACT Meeting in September 2007.
- A manuscript has been circulated to the co-authors, entitled “Exposure to acrylamide and prostate cancer – Swedish population-based case-control study.”
- Data generated from this project provides thesis data for one doctoral student at Harvard School of Public Health
- Co-investigator was promoted to Assistant Professor of Medicine at Harvard Medical School and Assistant Professor of Epidemiology at Harvard School of Public Health based on experience supported by this award

5. Conclusions

We found that the intake of acrylamide from diet in this male population is in line with recent reports by us about the intake of acrylamide in other Swedish populations (Mucci 2003; 2005). The levels of Hb adducts from acrylamide in blood is in line with recent reports by us about the intake of acrylamide in other Swedish populations (Hagmar 2005). The level of exposure to dietary acrylamide is much lower than was anticipated by the National Food Administration when the presence of acrylamide in food products was first discovered in the year of 2002. High exposure to acrylamide, measured by questionnaire or biomarkers, is not associated with an increased risk of prostate cancer in the present study.

6. References

1. IARC Monographs on the evaluation of carcinogen risk to humans: some industrial chemicals, No. 60, International Agency for Research on Cancer, Lyon, 1994.
2. Mucci L, Dickman P, Steineck G, Adam HOi, Augustsson K. Dietary acrylamide and risk of cancer of the large bowel, kidney and bladder: Absence of an association in a population-based study in Sweden. *British Journal of Cancer* 2003;88:84-89.
3. Mucci L, Sandin S, Bälter K, Magnusson C, Weiderpass E, Adami HA. Acrylamide intake and breast cancer risk in a large, prospective study among Swedish women. *JAMA* 2005 Mar 16;293(11):1326-7.
4. Hagmar L, Wirfält E, Paulsson B, Törnqvist M. Differences in hemoglobin adduct levels of acrylamide in the general population with respect to dietary intake, smoking habits and gender. *Mutation Research* 580; 157-165: 2005.

7. Appendices

Manuscript: "Acrylamide exposure and prostate cancer risk in the Cancer of the Prostate in Sweden Study: a validation and case-control analysis"

Acrylamide exposure and prostate cancer risk in the Cancer of the Prostate in Sweden Study: a validation and case-control analysis

Kathryn M. Wilson, Katarina Bälter, Hans-Olov Adami, Henrik Grönberg, Anna Vikström, Birgit Paulsson, Margareta Törnqvist, Lorelei A. Mucci
Harvard School of Public Health and Harvard Medical School, Boston, MA, Stockholm University, Sweden, and Karolinska Institute, Sweden

Abstract

Introduction

In 2002 researchers from the Swedish National Food Administration reported the detection of high levels of acrylamide in commonly consumed baked and fried foods. (SNFA,2002) Acrylamide is classified as a probable human carcinogen (IARC, 1994), so the discovery that the compound is formed during the preparation of many foods caused alarm. Prior to 2002 sources of non-occupational acrylamide exposure were thought to be limited to tobacco products and drinking water.

The data establishing acrylamide as a carcinogen comes from animal studies, which show increased cancer rates in rats given acrylamide in water. (Johnson, 1986; Friedman, 1995) It is unclear whether human cancer risk is affected by chronic, low-level exposure to acrylamide through foods. Several epidemiological studies have examined the association between dietary acrylamide intake, as measured by a food-frequency questionnaire (FFQ), and cancer risk at various sites. (Mucci, 2003a and b, 2004, 2005, 2006; Pelucchi 2003) To date, no published report has found any significant increase in risk associated with higher acrylamide intake. However, the ability of FFQs to measure dietary acrylamide intake has not been established. One study reported significant correlations between acrylamide adducts to hemoglobin and questionnaire-calculated acrylamide intakes in men and in smoking women, but not in non-smoking women. (Wirfalt, 2007) In addition, no study has examined blood levels of acrylamide, a well-established biomarker for exposure in occupational settings, and cancer risk in humans.

In this study, we address these limitations using data from a population-based case-control study of prostate cancer in Sweden. First, we measured blood acrylamide in a subset of men to examine the correlation between this biomarker of exposure and intake measured by a food-frequency questionnaire. Second, we used both the blood data and the FFQ data on acrylamide to conduct case-control analyses of acrylamide exposure and prostate cancer risk.

Methods

Study population. The Cancer of the Prostate in Sweden (CAPS) study is a population-based case-control study of prostate cancer. Cases were drawn from four of the six national cancer registries in Sweden between 2001 and 2002. Participants from the northern and central regions were between 35 and 79 years old, and those from the southern regions were between 35 and 65 years. Cases were incident cases of pathologically or cytologically verified prostate cancer. Cases were informed about the study and asked to participate through their treatment physicians. Clinical data on TNM (tumor, nodes, and metastasis) stage, Gleason score, and serum prostate-specific antigen (PSA) level at diagnosis were obtained from linkage to the National Prostate Cancer Registry. This data was available for 95% of cases in the study.

Controls were randomly selected from the Swedish Population Registry and were frequency matched to cases by five-year age groups and region of residence. Controls were contacted by mail and received the same information as cases.

Overall, 1895 prostate cancer cases were invited to participate. Of those, 1499 (79%) completed the questionnaire and 1400 (74%) donated a blood sample. Of 1684 invited controls, 1130 (67%) completed the questionnaire and 879 (52%) donated blood.

All study participants gave informed consent at the time of enrollment in the study. The study was approved by the ethics committees at Karolinska Institute and Umeå University.

Dietary assessment. Participants completed a self-administered 261-item food frequency questionnaire that assessed usual intake of foods over the previous 12 months. Data from the Swedish National Food Administration on energy and nutrient content of foods was used to calculate total energy intake and intake of nutrients.

Data on the acrylamide content of foods was collected from the Swedish National Food Administration and used to calculate usual intake of acrylamide. The acrylamide content of 18 foods was used in the calculation of acrylamide intake. Acrylamide intake was calculated by multiplying the acrylamide content of a serving of the food by the frequency of consumption of that food and

summing across all acrylamide-containing items on the FFQ. The resulting acrylamide intake is in micrograms per day. Acrylamide and other intakes were calorie-adjusted using the residual method. (something by Willett)

2617 men completed the FFQ component of the questionnaire. Sixteen were excluded because of unreasonably high or low energy intakes, and one was excluded because of missing acrylamide data. After exclusions, we had 1499 cases and 1118 controls with dietary acrylamide information.

Measurement of blood acrylamide.

As a biomarker of acrylamide exposure, acrylamide adducts to hemoglobin were measured in blood samples from a random sample of 377 men in the CAPS study. This biomarker has been shown to correlate with levels of occupational exposure to acrylamide (Tornqvist, 2006). It represents acrylamide exposure over the previous four months, or the half life of red blood cells.

Participants received a blood sampling kit along with the questionnaire and informed consent form. They were given four tubes (heparin, plasma and EDTA-treated) and were instructed to donate blood at the nearest clinic. Unprocessed blood samples were sent by overnight mail to the Umeå Biobank, where samples were divided into plasma, serum, white and red blood cell components and stored in a freezer at -80°C until the time of analysis.

Red blood cell samples were analyzed for acrylamide adducts to hemoglobin as described elsewhere (Tornqvist, 1986; Bergmark, 1997)

The samples were processed in four batches of approximately 100 samples each. Laboratory personnel were blinded to the case/control status of the samples. By chance, more case samples were included in batches one and two, and more controls were in batches three and four. Mean acrylamide adduct levels decreased over the four batches. Within each batch, mean adduct levels were similar for cases and controls, suggesting that the difference between batches was due to laboratory drift. Laboratory batch was adjusted for in all analyses.

Eleven samples were not processed because the cells were clotted. 34 men who reported using tobacco products (cigarettes, pipes, or snuff) at the time of the questionnaire were excluded from the blood analysis, as tobacco users are exposed to much higher levels of acrylamide through tobacco than through the diet. Mean blood acrylamide in these men was 152 pmol/g globin compared to 54 pmol/g in non-smoking men. After exclusions, 175 cases and 168 controls were used in the analysis of blood acrylamide.

Statistical analysis.

For the validation of FFQ acrylamide intake, the correlation between calculated acrylamide intake and acrylamide adducts to hemoglobin was calculated in the subset of men with blood measurements. The correlation was adjusted for age, region, and laboratory batch.

To measure the association between blood acrylamide and risk of prostate cancer, we used unconditional logistic regression models with indicator variables for quartile of blood acrylamide level. Quartiles were created based on the distribution among the controls. Age group and region, which were matching factors in this study, were included in all models, as was laboratory batch. Fully adjusted models also include variables for BMI (continuous) and former smoking.

To measure the association between dietary acrylamide intake and risk of prostate cancer, we used unconditional logistic regression models with indicator variables for quintiles of calorie-adjusted acrylamide intake. Quintiles were created based on the distribution of intake among the controls. Age group and region were included in all models. Fully adjusted models also include variables for BMI (continuous), former and current smoking, education (four categories), zinc intake (ordinal quartiles), and total energy intake. Employment status and civil (marital) status were also considered as potential confounders. Several other nutrients and foods were considered as potential confounders, as well, including: alcohol, alpha-linolenic acid, calcium, vitamin D, folate, phytoestrogens, red meat, fish, and tomato. None of these was included in the final models, as they had little effect on the acrylamide effect estimates or precision. Data on these confounders were collected in the self-administered mailed questionnaire.

To test for a dose-response trend across quantiles of acrylamide, we modeled acrylamide as a continuous variable using the median intake in each quantile. The p-value of this continuous variable was used to determine the significance of any linear trend across quantiles of intake. All statistical analysis was done using SAS 9.1.

Results

Characteristics of the study population.

Cases and controls were similar in acrylamide intake calculated from the FFQ and in blood levels of acrylamide (Table 1). Mean intake was 44.5 mcg/day among controls and 43.8 mcg/day among cases. In the subset of men with blood acrylamide measurements, the mean adduct level was 49.3 pmol/g globin among controls and 51.6 pmol/g globin among cases. Cases were more likely to come from the Northern regions of Sweden. Cases and controls were similar in age, education, BMI, height, smoking status, and diet including daily intakes of dairy, red meat, fish, fruits, and vegetables and total energy intake.

Acrylamide intake ranged from 8 to 125 mcg/day, or 0.08 to 1.59 mcg/ kilogram body weight per day. The top food contributors to acrylamide intake were crispbread, coffee, other bread, fried potatoes, and buns and cakes (Figure 1). There was a significant ($p < 0.0001$) correlation between acrylamide intake and intake of carbohydrates, fiber, and zinc (all positive) and alcohol (negative). Acrylamide intake was not correlated with age or height and was mildly correlated with BMI ($r = 0.05$, $p = 0.01$).

Validation of FFQ acrylamide measurement.

The partial correlation between dietary acrylamide intake and blood acrylamide (as acrylamide adducts to hemoglobin) was 0.25 ($p < 0.0001$), adjusted for age, region, energy intake, and laboratory batch (Table 2). Among controls only, the correlation was 0.35 ($p < 0.0001$). Among cases, it was 0.16 ($p = 0.05$). Correlations between blood acrylamide and acrylamide intake measured in mcg/kg body weight per day were almost identical to the correlations with acrylamide intake in mcg/day (data not shown). Adjustment for energy intake improved the correlations by reducing within-person measurement error. Without adjusting for calories, the correlation between FFQ and blood acrylamide was 0.18 ($p = 0.0009$), adjusted for age, region, and batch.

Association between blood acrylamide and CaP risk.

As shown in Table 3, no significant association was seen between quartile of blood acrylamide and prostate cancer risk. Adjusting for age, region, BMI, former smoking, and laboratory batch, the relative risk for the highest versus lowest quartile of blood acrylamide was 1.02 (95% CI: 0.46-2.24). For a 10 pmol/g globin increase in blood acrylamide, the relative risk of prostate cancer was 0.97 (CI: 0.81-1.17, p for trend=0.77).

No association was found between blood acrylamide and specific prostate cancer endpoints including advanced disease, localized disease, high- or low-grade disease, or high- or low-PSA disease (Table 4).

Association between dietary acrylamide and CaP risk.

For dietary acrylamide calculated from the FFQ, there was no association between higher intakes and prostate cancer risk (Table 5). The relative risk for the highest versus lowest quintile of acrylamide intakes was 0.97 (CI: 0.75-1.27), adjusting for age, region, BMI, education, smoking, and zinc and energy intake. For a 10 mcg/day increase in acrylamide intake, the RR of prostate cancer was 0.99 (CI: 0.92-1.06, p for trend=0.67). Similar results were seen when the analysis was limited to non-smokers only. Among non-smokers, a 10 mcg/day increase in acrylamide intake was associated with a relative risk of 1.02 (CI: 0.94-1.09, p for trend=0.68).

No association was found between acrylamide intake and specific prostate cancer endpoints including mortality, advanced disease, localized disease, high- or low-grade disease, or high- or low-PSA disease (Table 6). Again, these results were similar when restricted to non-smokers only.

Intake of the five foods that contribute most to acrylamide intake in this study population was examined (Table 7). There was no association between crispbread, other bread, or coffee intake and prostate cancer risk. There was a suggestion of increased risk for men in the highest tertile of intake of fried potatoes and buns/cakes. However, this increased risk was unchanged when dietary acrylamide was also included in the models, suggesting that any association between these foods and prostate cancer risk is due to chance or components other than acrylamide.

Discussion

Validation of FFQ Measurement.

The correlation between blood acrylamide and FFQ acrylamide was highly significant. It was notably higher among controls than among cases. This difference may be due to less accurate reporting of diet among cases, or to recent changes in the diets of cases after diagnosis. Our findings are in line with the other published report comparing FFQ-calculated acrylamide intake and blood levels of acrylamide adducts. In the Malmo Diet and Cancer Cohort, Wirfalt, et al. (2007) found a correlation of 0.36 in smokers and 0.43 in non-smokers.

Our correlations are highly statistically significant, and they are in line with the magnitude of correlations seen for intake of some nutrients when compared to biomarkers of intake. In addition, the observed correlations compare favorably to the correlation of 0.47 observed between intensity of tobacco use and acrylamide adducts in the Malmo Diet and Cancer Cohort. This is reassuring, given that self-reported smoking habits are considered a valid assessment of exposure, and blood acrylamide adducts have been shown to vary with smoking intensity. (Bergmark 2007; Schettgen 2004)

It is also worth noting that acrylamide adducts to hemoglobin have not been definitively established as a valid biomarker of dietary acrylamide exposure. Acrylamide adducts are established as a valid marker of occupational levels of exposure, but it is not clear how responsive adduct levels are to the much lower levels of acrylamide found in the diet. It is known that the level of variation in acrylamide adducts in non-smoking, non-occupationally exposed humans is lower than the estimated variation in dietary acrylamide content. (Hagmar, 2005) One small pilot feeding study (Vesper, 2005) showed mixed results in assessing change in adduct levels after increasing intake of acrylamide; however, the study was too small and too short to draw conclusions. To fully evaluate the results of FFQ validation studies such as ours, it will be necessary to see how a larger and longer-term feeding study affects acrylamide adduct levels.

Our results are promising considering that only acrylamide adducts were measured, and not adducts of glycidamide, the major metabolite of acrylamide. The degree of conversion to glycidamide varies between individuals and by acrylamide intake (Boettcher, 2005; Schettgen, 2004), so a combined measure of acrylamide and glycidamide adducts to hemoglobin is probably the best biomarker of acrylamide exposure. Therefore, our correlation likely underestimates the true validity of the FFQ.

Blood acrylamide and prostate cancer risk.

This is the first study to look at the association between blood acrylamide and cancer risk in humans. We found no association between blood levels of acrylamide and risk of overall prostate cancer or specific prostate cancer endpoints. Our ability to study specific endpoints such as advanced or localized disease was limited by low sample sizes in the subgroup with blood acrylamide data.

As discussed above, our ability to study this association was limited by the lack of glycidamide adduct data. In addition, we have only one blood sample per participant, so we cannot accurately measure levels of acrylamide exposure over time. These limitations would likely result in non-differential misclassification of blood acrylamide, reducing our ability to see an increased risk of cancer associated with blood acrylamide levels. Finally, for cases the blood sample was collected after diagnosis, so it may not reflect typical levels before the cancer. It is unclear how this source of error might affect our estimates.

Dietary acrylamide and prostate cancer risk.

We found no association between FFQ-measured acrylamide intake and risk of overall prostate cancer or specific prostate cancer endpoints. We also found no association between intake of the major acrylamide-contributing foods and prostate cancer risk. This is the first study to examine prostate cancer risk and acrylamide intake. Several other cancer sites have been examined, and all reports to date have been null. (Mucci, 2003a and b, 2004, 2005, 2006)

Random measurement error in acrylamide intake is likely. The FFQ was not originally designed to measure acrylamide intake, so food items with different cooking methods and quite different acrylamide content are sometimes grouped into a single FFQ question. This makes it more difficult to measure intake accurately and likely biases our results towards the null.

Differential measurement error is also a possibility. The difference between cases and controls in the validation component of this study suggest that FFQ accuracy may vary by disease status. If so, it is not clear how such recall bias might affect our results. Further study of acrylamide intake in prospective cohorts will be necessary to eliminate the possibility of recall bias.

In conclusion, we find no association between acrylamide exposure as measured in blood or by FFQ and risk of prostate cancer. Epidemiological studies do not have the power to rule out very small increases in risk associated with high acrylamide intake. However, the concordance of the blood and FFQ results, along with previous epidemiological studies, suggest that the levels of acrylamide taken in the diet are not responsible for a significant increase in cancer risk in humans.

References

Swedish National Food Administration. Acrylamide in Food. 2002.

IARC. IARC Monographs on the evaluation of carcinogen risk to humans: Some industrial chemicals. vol 60. Lyon: IARC, 1994

Friedman, M.A. et al. (1995) A lifetime oncogenicity study in rats with acrylamide. *Fundam. Appl. Toxicol.* 27:95-105.

Johnson K.A. et al. (1986) Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fisher 344 rats. *Toxicol. Appl. Pharmacol.* 85:154-168.

Mucci, L.A. et al. (2003a) Dietary acrylamide and cancer of the large bowel, kidney, and bladder: absence of an association in a population-based study in Sweden. *Br. J. Cancer* 88:84-89.

Mucci, L.A. et al. (2003b) Dietary acrylamide and cancer risk: additional data on coffee [Letter]. *Br. J. Cancer* 89:775-776.

Mucci, L.F. et al. (2004) Dietary acrylamide and risk of renal cell cancer. *Int. J. Cancer.* 109:774-776.

Mucci, L.A. et al. (2005) Acrylamide intake and breast cancer risk in Swedish women. *JAMA* 293 1326-1327.

Mucci, L.A. et al. Prospective study of dietary acrylamide and risk of colorectal cancer among women. *Int. J. Cancer* 2006; 118:169-173.

Pelucchi, C. et al. (2003) Fried potatoes and human cancer. *Int. J. Cancer* 105:558-560.

Tornqvist M., et al. (2006) Biomonitoring of acrylamide and its metabolites. In: Skog K, Alexander J (eds). *Acrylamide and other hazardous compounds in heat-treated foods*. Woodhead Publishing Limited: Cambridge, pp. 163-194.

Tornqvist M, et al. (1986). Monitoring of environmental cancer initiators through hemoglobin adducts by a modified Edman degradation method. *Ann Biochem* 154, 255-266.

Bergmark E (1997). Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers, and nonsmokers. *Chem Res Toxicol* 10, 78-84.

Wirfalt E, et al. (in press) Associations between estimated acrylamide intakes and hemoglobin AA adducts in a sample from the Malmo Diet and Cancer cohort. *Eur J Clin Nutr.* Epub ahead of print, 14 March 2007.

Kutting B et al. (2005) Influence of diet on exposure to acrylamide - reflections on the validity of a questionnaire. *Ann Nutr Metabol* 49, 173-177.

Boettcher MI et al. (2005) Mercapturic acids of acrylamide and glycidamide as biomarkers of the internal exposure to acrylamide in the general population. *Mutat Res* 580, 167-176.

Schettgen T, et al. (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, 531-539.

Hagmar, L. et al. (2005) Differences in hemoglobin adduct levels of acrylamide in the general population with respect to dietary intakes, smoking habits, and gender. *Mutation Research* 580:157-165.

Vesper HW, et al. (2005) Pilot study on the impact of potato chips consumption on biomarkers of acrylamide exposure. In: Mottram D, Freidman MA (eds). *Acrylamide in Food Products: Identification, Formation and Analytical Methodology*. Stockholm University: Stockholm.

Tables and Figures

Table 1. Characteristics of the study population by disease status.

	Full Study Population		Blood Cohort	
	Cases	Controls	Cases	Controls
N	1489	1111	173	164
Age (approx)	66	67	67	67
Region (%)				
North	31%	18%	29%	16%
South	69%	82%	71%	84%
Education Level (%)				
9 years of less	46%	46%	43%	48%
10-12 years	40%	42%	45%	42%
13+ years	14%	11%	12%	10%
BMI	26.2	26.2	26.0	26.5
Height (cm)	177	176	176	176
Smoking				
Never Smokers (%)	39%	38%	42%	48%
Former Smokers (%)	49%	48%	57%	52%
Current Smokers (%)	11%	12%	0%	0%
Dietary characteristics				
Acrylamide Intake (mcg/d)	43.8	44.5	45.1	44.0
Acrylamide in mcg/kg/day	0.54	0.56	0.56	0.54
Acryl. Adduct (pmol/g globin)	na	na	51.6	49.3
Total energy intake (kcal/d)	2283	2219	2278	2290
Zinc	11.6	11.7	11.3	11.8
Food Intakes (serv/d)				
Dairy	5.7	5.6	5.6	5.6
Red Meat	1.4	1.3	1.3	1.4
Fish	0.5	0.5	0.5	0.5
Crispbread	2.4	2.6	2.6	2.5
Other bread	2.9	2.8	3.0	2.8
Fruit	1.9	1.7	2.0	2.0
Vegetables	2.7	2.5	2.5	2.9
Baked Goods	1.0	0.9	1.0	0.9
Coffee	3.1	3.1	3.0	3.0
Disease Characteristics among Cases [N (% of cases)]				
	205		23	
CaP Mortality	(14%)	-	(13%)	-
	535		64	
Advanced cases*	(36%)	-	(37%)	-
Localized cancer (T1-T2, N0/M0)	1020		112	
	(69%)	-	(65%)	-
Gleason Grade sum	6.5	-	6.5	-
PSA Level	88.5	-	66.3	-

* Advanced case = death from CaP, or N1, or M1, or T4 or T3.

Figure 1. Contribution of foods to acrylamide intake.

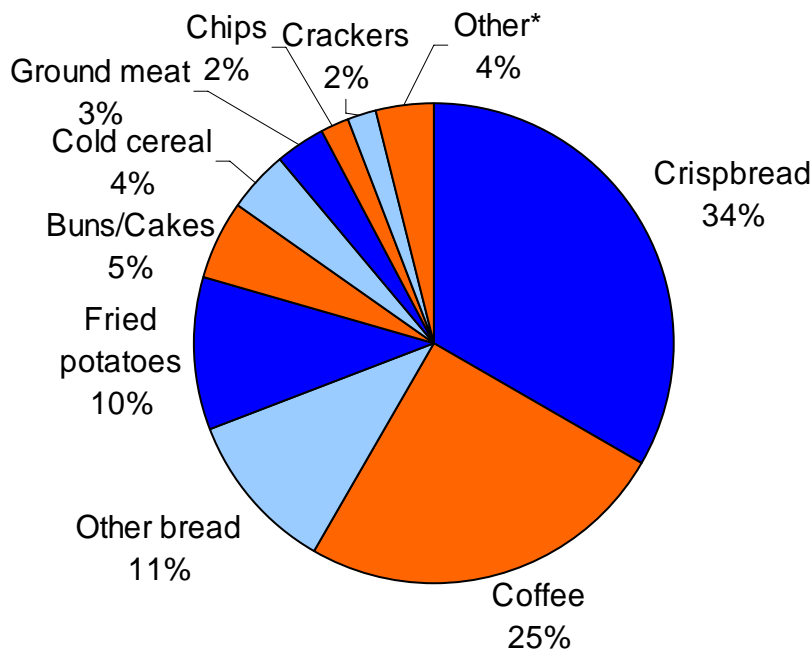


Table 2. Correlation between calorie-adjusted dietary acrylamide intake and blood acrylamide adducts

	Full blood cohort n=336	Controls only n=164	Cases only n=172
Unadjusted	0.25	0.36	0.13
95% CI	0.15 - 0.35	0.22 - 0.48	-0.02 - 0.27
p-value	<0.0001	<0.0001	0.09
Adjusted for age, region, batch	0.25	0.35	0.16
95% CI	0.15 - 0.35	0.21 - 0.48	0.003 - 0.30
p-value	<0.0001	<0.0001	0.05

Table 3. Relative risk of prostate cancer by quartile of acrylamide blood level

	Quartile of blood acrylamide level				Per 10 unit incr in level
	Q1 (Lowest) Median: 33 pmol/g	Q2 44 pmol/g	Q3 57 pmol/g	Q4 (Highest) 72 pmol/g	
Basic Model					
# case/cont	30/42	54/45	45/44	43/33	
OR	1.00	1.50	0.98	1.18	1.00
95% CI	--	0.77-2.92	0.48-1.98	0.55-2.55	0.83-1.19
p-value	--	0.23	0.95	0.68	0.97
Multivariable Model					
# case/cont	30/39	51/44	45/43	43/32	
OR	1.00	1.25	0.90	1.02	0.97
95% CI	--	0.63-2.49	0.44-1.85	0.46-2.24	0.81-1.17
p-value	--	0.51	0.78	0.97	0.77

Basic model: adjusted for age in 5-year intervals, region (north/south), and laboratory batch

Multivariable model: adjusted for age in 5-year intervals, region (north/south), laboratory batch, BMI (continuous) and former smoking

Table 4. Relative risk of specific prostate cancer endpoints by 10 pmol/g globin increase in blood acrylamide

	Advanced	High-Grade	High PSA
# case/cont	63/158	68/158	101/158
OR	1.05	1.19	0.99
95% CI	0.82-1.33	0.94-1.51	0.79-1.23
p-value	0.71	0.16	0.90
	Localized	Low-Grade	Low PSA
# case/cont	109/158	83/158	61/158
OR	0.91	0.83	0.96
95% CI	0.73-1.13	0.66-1.05	0.75-1.23
p-value	0.40	0.12	0.75

Results adjusted for age in 5-year intervals, region (north/south), laboratory batch, BMI (continuous), and former smoking

Advanced disease: death, N1, M1 or T4 or T3. Localized disease: T1-T2 with N0 and M0. High-grade disease:

Gleason sum 7-10. Low-grade disease: Gleason sum 2-6. High PSA disease: >10. Low PSA disease: ≤10.

Table 5. Relative risk of prostate cancer by quintile of dietary acrylamide intake

	Quintile of dietary acrylamide intake					Per 10 mcg increase
	Q1 (Low)	Q2	Q3	Q4	Q5 (High)	
	8-33 mcg/d	33-40 mcg/d	40-47 mcg/d	47-56 mcg/d	56-125 mcg/d	
	n=504	n=542	n=483	n=510	n=465	
Basic Model						
OR	1.00	1.17	0.99	1.03	0.94	0.97
95% CI	--	0.91-1.49	0.77-1.27	0.81-1.32	0.73-1.21	0.91-1.04
p-value	--	0.21	0.93	0.81	0.65	0.39
Multivariable Model						
OR	1.00	1.14	0.99	1.06	0.97	0.99
95% CI	--	0.89-1.47	0.76-1.28	0.82-1.37	0.75-1.27	0.92-1.06
p-value	--	0.31	0.92	0.65	0.84	0.67

Basic model: adjusted for age in 5-year intervals and region (north/south)

Multivariable model: adjusted for age in 5-year intervals, region (north/south), education (4 categories), former and current smoking, zinc intake (ordinal quartiles), and energy intake (continuous)

Table 6. Relative risk of specific prostate cancer endpoints for 10 mcg/day increase in dietary acrylamide intake

	Advanced	High-Grade	High PSA
# case/cont	516/1066	612/1066	223/1066
OR	0.98	0.99	0.96
95% CI	0.90-1.07	0.91-1.08	0.88-1.03

p-value	0.64	0.84	0.25
	Localized	Low-Grade	Low PSA
# case/cont	988/1066	675/1066	589/1066
OR	0.98	0.99	1.05
95% CI	0.91-1.06	0.91-1.07	0.96-1.14
p-value	0.66	0.73	0.33

Multivariable model: adjusted for age in 5 year intervals, region (north/south), education (4 categories), former and current smoking, zinc intake (ordinal quartiles), and energy intake (continuous)

Advanced disease: death, N1, M1 or T4 or T3. Localized disease: T1-T2 with N0 and M0. High-grade disease: Gleason sum 7-10. Low-grade disease: Gleason sum 2-6. High PSA disease: >10. Low PSA disease: ≤10.

Table 7. Relative risk of prostate cancer by tertile of intake of high-acrylamide foods

	Tertile of food intake			p-trend*
	T1 (Low)	T2	T3 (High)	
Crispbread				
#				
case/cont	X/X	X/X	X/X	
OR	1.00	0.98	0.94	
95% CI	--	0.81-1.19	0.75-1.18	0.58
Also adjusted for dietary acrylamide				
OR	1.00	0.98	0.96	
95% CI	--	0.80-1.21	0.74-1.25	0.76
Other Bread				
#				
case/cont	X/X	X/X	X/X	
OR	1.00	1.11	1.01	
95% CI	--	0.90-1.36	0.83-1.24	0.97
Also adjusted for dietary acrylamide				
OR	1.00	1.11	1.01	
95% CI	--	0.90-1.36	0.83-1.24	0.96
Coffee				
#				
case/cont	X/X	X/X	X/X	
OR	1.00	0.94	1.01	
95% CI	--	0.76-1.16	0.83-1.22	0.86
Also adjusted for dietary acrylamide				
OR	1.00	0.94	1.04	
95% CI	--	0.75-1.17	0.83-1.30	0.66
Fried Potatoes				
#	240/246	749/511	449/309	

case/cont				
OR	1.00	1.36	1.31	
		1.10-	1.03-	
95% CI	--	1.69	1.66	0.16
Also adjusted for dietary acrylamide				
OR	1.00	1.36	1.32	
		1.09-	1.03-	
95% CI	--	1.69	1.69	0.14

Buns & Cakes

#				
case/cont	356/318	626/434	456/314	
OR	1.00	1.25	1.26	
		1.02-	0.99-	
95% CI	--	1.53	1.59	0.26
Also adjusted for dietary acrylamide				
OR	1.00	1.24	1.25	
		1.01-	0.98-	
95% CI	--	1.52	1.58	0.28

Models adjusted for age in 5 year intervals, region (north/south), education (4 categories), former and current smoking, zinc intake (ordinal quartiles), and energy intake (continuous)

*p-value for food intake as a continuous variable using median intake for each tertile