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Associations between dietary acrylamide intake and plasma sex hormone levels

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Abstract

Background—The rodent carcinogen acrylamide was discovered in 2002 in commonly consumed foods. Epidemiological studies have observed positive associations between acrylamide intake and endometrial, ovarian and breast cancer risks, which suggests that acrylamide may have sex-hormonal effects.

Methods—We cross-sectionally investigated the relationship between acrylamide intake and plasma levels of sex hormones and SHBG among 687 postmenopausal and 1300 premenopausal controls from nested case-control studies within the Nurses' Health Studies.

Results—There were no associations between acrylamide and sex hormones or SHBG among premenopausal women overall or among never-smokers. Among normal-weight premenopausal women, acrylamide intake was statistically significantly positively associated with luteal total and free estradiol levels. Among postmenopausal women overall and among never-smokers, acrylamide was borderline statistically significantly associated with lower estrone sulfate levels but not with other estrogens, androgens, prolactin or SHBG. Among normal weight women, (borderline) statistically significant inverse associations were noted for estrone, free estradiol, estrone sulfate, DHEA, and prolactin, while statistically significant positive associations for testosterone and androstenedione were observed among overweight women.

Conclusions—Overall, this study did not show conclusive associations between acrylamide intake and sex hormones that would lend unequivocal biological plausibility to the observed increased risks of endometrial, ovarian and breast cancer. The association between acrylamide and sex hormones may differ by menopausal and overweight status. We recommend other studies investigate the relationship between acrylamide and sex hormones in women, specifically using acrylamide biomarkers.

Impact—The present study showed some interesting associations between acrylamide intake and sex hormones that urgently need confirmation.

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Introduction

Acrylamide is classified as a probable human carcinogen (class 2A) by the International Agency for Research on Cancer(1). In 2002, acrylamide was observed to form during cooking of commonly consumed foods, mainly carbohydrate-rich foods heated to temperatures >120; °C, such as French fries and cookies, as well as in coffee (2, 3). Before 2002, exposure was already known to occur in occupational settings and through tobacco smoking(4, 5).

In 2-year carcinogenicity assays, rats exposed to acrylamide developed scrotal mesothelioma and tumors in the thyroid and mammary glands, among other tissues (6, 7). Recently, it was suggested that these tumors may be caused, at least in part, by effects of acrylamide on hormones (8, 9). Although acrylamide can cause genotoxic damage *in vitro* and *in vivo* (10-12), there are indications that genotoxicity alone cannot explain the specific tumor patterns seen in rats (8, 9, 13). In rats, acrylamide exposure led to reduced levels of estradiol and progesterone in females and reduced levels of testosterone and prolactin in males (14-16). However, these animal studies were performed with acrylamide doses several fold higher than human dietary doses, which makes extrapolation to human dietary doses problematic. Very little is known on how acrylamide might exert its effect on sex hormone levels. The only data that exist to biologically underpin a possible effect of acrylamide on sex hormones are gene expression studies in mice and human cell lines that show up or down-regulation of genes involved in sex hormone metabolism, such as enzymes of the cytochrome P450, sulfotransferase and aldo-keto reductase families upon acrylamide or glycidamide exposure (17-19). In humans, positive associations between acrylamide intake and endometrial and ovarian cancer risks were observed in two well-established prospective cohort studies, the Nurses' Health Study (NHS) and the Netherlands Cohort Study (NLCS) (20, 21), although for endometrial cancer not in another prospective cohort study nor a case-control study (22), and for ovarian cancer not in another prospective cohort study (23), a case-control study (24), and a nested case-control study from the Nurses' Health Study using acrylamide biomarkers to asses dietary acrylamide exposure (25). As for breast cancer, most studies observed no association with dietary acrylamide intake (21,24, 26-28), whereas two prospective studies did observe a positive association, specifically for estrogen receptor-positive breast cancer. One of those two studies used acrylamide and glycidamide to hemoglobin adducts as a biomarker of acrylamide exposure, and found a stronger positive association in smokers for whom smoking is the most important source of acrylamide exposure. This complicates the interpretation of this finding (29). In the other study, the positive association with estrogen and progesterone receptor-positive breast cancer was not statistically significant (30). In addition, another prospective study showed that prediagnostic acrylamide intake was associated with increased breast cancer mortality, particularly estrogen receptor-positive breast cancer mortality (31).

Sex hormones play an important role in the etiology of endometrial, ovarian and breast cancer, particularly estrogen receptor-positive breast cancer (32). To investigate whether acrylamide may impact circulating levels of sex hormones in humans, we studied the cross-sectional association between dietary acrylamide intake, as assessed by a food frequency questionnaire, and plasma concentrations of sex hormones in pre- and postmenopausal women from the NHS and NHSII.

Materials and Methods

Study population

In 1976, the NHS enrolled 121,700 female registered nurses from the United States of America, aged 30 to 55 years, and in 1989, the NHSII enrolled 116,430 female registered

nurses (age 25 to 42 years). All women filled out an initial questionnaire on medical history, diet, and lifestyle factors. After that, they have been followed every two years through questionnaires in order to update data on exposure status and disease diagnosis.

32,826 NHS participants gave a blood sample and filled out a short questionnaire about the blood draw (from 1989 to 1990) (33). From 1996 to 1999, 29,611 NHSII participants gave blood samples and also completed a short questionnaire (34). In the NHSII, premenopausal women (n = 18,521) who had not been pregnant, used hormones, or lactated within 6 months before the blood draw gave follicular blood samples on the 3rd to 5th day of their menstrual cycle, and luteal samples 7 to 9 days before the expected start of their next cycle. Other NHSII women (n = 11,090) and all NHS women provided untimed blood samples. Thus, 63% of the premenopausal women provided timed blood samples. Heparin plasma samples have been stored at ≤-130°C in liquid nitrogen since their collection. These studies were approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital/Partners Health Care.

The premenopausal women included in this cross-sectional analysis were controls in several nested case-control studies with several endpoints conducted within the NHSII, including breast cancer (n = 1268) (35), ovarian cancer (n = 46) (36), endometriosis (n = 592), and rheumatoid arthritis (n = 19) (37), or participants in hormone reproducibility studies (n = 109) (38). For the analyses on postmenopausal women, participants (not using postmenopausal hormones, n = 704) in the current analysis were controls from a nested breast cancer case-control study within the NHS (39), who were matched to cases diagnosed with breast cancer after blood collection (through June 1, 2000). For inclusion in the current analysis, the women had to have filled out a food frequency questionnaire (FFQ) in 1990 (NHS) or 1995 (NHSII). We considered a woman to be premenopausal if she provided timed blood samples, her periods had not ceased, or she had had a hysterectomy with at least one ovary remaining and was aged ≤47 years (for non-smokers) or ≤45 years (for smokers). We considered a woman to be postmenopausal if her natural menstrual periods had ceased permanently, she had had a bilateral oophorectomy, or she had had a hysterectomy with at least one ovary remaining and was aged ≥56 years (for non-smokers) or ≥54 years (for smokers) (40). The remaining women, most of whom had had a simple hysterectomy and were aged 48 to 55 years, were of unknown menopausal status and therefore excluded. We included only premenopausal women with ovulatory cycles (luteal progesterone >400 ng/dL). After exclusions, 1300 premenopausal and 687 postmenopausal participants were included in analyses.

Hormone assays

The hormone assay methods that were used in NHS and NHSII have been described elsewhere (39, 41-43). Briefly, estradiol, estrone, testosterone, androstenedione and dehydroepiandrosterone (DHEA) were extracted, separated by column chromatography and measured using radioimmunoassay. Estrone sulfate concentrations were determined by radioimmunoassay of estrone, after enzyme hydrolysis, extraction, and separation by column chromatography. DHEA sulphate (DHEAS), sex hormone binding globulin (SHBG), and progesterone were measured by chemiluminescent immunoassay (Immulite, Diagnostic Products Corp., UK; ARCHITECT^{R}, **Abbott Diagnostics, Chicago, IL**). Prolactin was measured with a microparticle enzyme immunoassay, using the AxSYM Immunoassay system (Abbott Diagnostics, Chicago, IL).

In premenopausal women, estradiol, estrone, estrone sulfate, testosterone, androstenedione, prolactin, and SHBG were measured in both follicular and luteal samples and dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), and progesterone only in luteal samples. Follicular and luteal samples from each woman were measured in the same batch

in up to 9 batches. In postmenopausal women, we measured estradiol, estrone, estrone sulfate, testosterone, androstenedione, DHEA, DHEAS, prolactin and SHBG in up to 6 batches. Samples were assayed in random order.

Free estradiol and free testosterone were calculated using the formula of Södergård (44). We included 10% blinded duplicate samples in each batch to determine the laboratory precision. Within-batch coefficients of variation ranged from 2% and 17% for all analytes except for single batches of estrone, testosterone, progesterone and SHBG (17.0% to 21.6%). When hormone values were lower than the detection limit, we set the value to half the limit. This was done 13 times for estrone, 2 for estradiol, 6 for estrone sulfate, 1 for DHEA, 2 for DHEAS, and 3 for SHBG, all in postmenopausal blood samples. In premenopausal samples, this was done 0-3 times for all analytes except for DHEAS, for which the value of half the limit of detection was used 15 times.

Acrylamide intake assessment

In both the NHS and NHSII, diet was assessed through a validated, self-administered, semiquantitative food frequency questionnaire (FFQ), administered in 1990 for the NHS and in 1995 for the NHSII (45). Respondents were asked to indicate their average frequency of use of more than 100 commonly consumed foods over the previous year, with 9 choices ranging from “never or almost never” to “6 or more times per day.” The creation and validation of an acrylamide food composition database for the FFQ is described in detail elsewhere (46). In brief, based on data from the US Food and Drug Administration and analyses of US foods by the Swedish National Food Administration, 42 food items on the FFQ were assigned a mean acrylamide concentration. We computed daily dietary acrylamide intake for each participant by multiplying the acrylamide content of a serving of food by the frequency of consumption of that food and summing across all 42 food items. Acrylamide intake was adjusted for total energy intake with the residual method (47), and quartiles were created based on these energy-adjusted intakes. The quartile cut-off points that were used were specific to the study populations, so there were separate cut-off points for pre and postmenopausal women.

The correlation between acrylamide intake as measured by the NHSII FFQ and the sum of acrylamide and glycidamide hemoglobin adducts (a biomarker of internal acrylamide exposure) was 0.34 ($P < 0.0001$) among 296 non-smoking women in the NHSII (46). The validity of a similar FFQ for measuring individual foods was assessed by correlating data from the FFQ and from 28-day dietary records in a subset of NHS women (48). The correlation between FFQ and dietary records for the most important acrylamide-contributing foods was 0.73 for French fries, 0.78 for coffee, 0.60 for potato chips, and 0.79 for cold breakfast cereal.

Statistical analysis

All analyses were conducted separately for pre- and postmenopausal women. For each hormone, women with missing values related to assay difficulties or low plasma volume were excluded. We excluded hormone values (n = 0-11 for postmenopausal and 0-13 for premenopausal women) identified as statistical outliers, based on the extreme studentized residual method by Rosner *et al.* (49). Among premenopausal women, the associations for the estrogens were analyzed separately for follicular and luteal phase measurements. For testosterone, androstenedione, prolactin, and SHBG, the average of follicular and luteal values was used because concentrations did not vary substantially by phase, as described elsewhere (34, 50, 51). Premenopausal hormone levels were adjusted for batch effects using the method of Rosner *et al.* (52), because a batch effect was observed for these analytes. For postmenopausal blood analyses, a batch effect was less clear but nevertheless we did adjust

for batch effect in the multivariable-adjusted models by including variables for batch indicators.

We calculated adjusted geometric means for the natural log-transformed hormones by acrylamide exposure quartile through a generalized linear model. Tests for trend were conducted by modeling the median of the acrylamide quartiles as a continuous variable and calculating the Wald statistic. For premenopausal women, multivariable-adjusted models included age at blood draw (<40, 40-<45, ≥45 years), fasting status at blood draw (≤10, >10 hours), time of day of blood draw (1-8 AM, 9 AM-noon, 1 PM-midnight), month of blood draw, difference between date of luteal draw and date of the next menstrual period (3-7, 8-21 days, unknown), duration of past oral contraceptive use (never, <4, ≥4 years, missing), parity (nulliparous vs parous), body mass index (BMI) at blood draw (continuous), physical activity (<6, 6-<15, 15-<<30, ≥30h/wk mean exercise time), alcohol consumption (0, >0-≤10, >10-≤20, >20-≤30, >30 g/d), energy intake (kcal/d), smoking (never, past, current), and energy-adjusted caffeine intake (quartiles). For postmenopausal women, we adjusted for age at blood draw (<55, 55-60, 60-65, >65 years), batch of hormone analysis (1-6), time of day of blood draw (1-8 AM, 9 AM-noon, 1 PM-4 PM, 5 PM-midnight), age at first birth/parity (nulliparous, age at first birth <25 years/1-4 children, age at first birth 25-29 years/1-4 children, age at first birth ≥30 years/1-4 children, age at first birth <25 years/≥5 children, age at first birth ≥25 years/≥5 children), body mass index (BMI) at blood draw (continuous), physical activity (<5, 5-18, ≥18 h/wk mean exercise time), alcohol consumption (0, >0-≤10, >10-≤20, >20-≤30, >30 g/d), energy intake (kcal/d), smoking (never, past, current), and energy-adjusted caffeine intake (quartiles). Furthermore, in additional analyses, we assessed for confounding by intakes of carbohydrates, protein, dietary fiber, saturated fat, and cholesterol. The data on these covariables were derived from questionnaires completed at blood draw, the closest NHS or NHSII questionnaire in time, or cumulatively from all of the previous NHS and NHSII questionnaires.

In additional analyses, we restricted to never-smokers, because smoking is an important source of acrylamide (5) and could blur the smaller exposures from diet. In addition, smoking may have an antiestrogenic effect (53) and could therefore modify the association, if any, between acrylamide intake and sex hormone concentrations.

We additionally stratified on BMI (< and ≥ 25 kg/m^2). Adipose tissue is a source of sex hormones in postmenopausal women (54), and increased adiposity has been associated with lower hormone levels in premenopausal women (55). In addition, BMI is likely to influence the activity of the cytochrome P4502E1 enzyme that converts acrylamide into its epoxide metabolite glycidamide (56, 57). Glycidamide is often thought to be the central compound involved in acrylamide-related carcinogenesis, due to its observed genotoxicity. For these two reasons, BMI may modify the association, if any, between acrylamide intake and sex hormone concentrations.

Caffeine is correlated with acrylamide intake as coffee is a major contributor to intakes of both. Coffee and caffeine previously were shown to be associated with sex hormone levels in NHS and NHSII (58). Therefore, we performed analyses with and without adjustment for caffeine. All reported p-values are 2-sided and considered statistically significant if ≤0.05.

Results

Among premenopausal women, age at menarche, ever use of oral contraceptives, smoking status and caffeine intake varied linearly over the quartiles of acrylamide intake (table 1). The Spearman correlation between acrylamide and caffeine intake was 0.33 ($p < 0.0001$), in line with the strong contribution of coffee to total acrylamide intake. In postmenopausal

women, there were modest inverse associations between quartiles of acrylamide intake for age at menarche and menopause, total energy intake, and positive associations for smoking status and caffeine intake (table 1). The Spearman correlation between acrylamide and caffeine intake in this group was 0.30 ($p < 0.0001$). In the models to analyze the association between acrylamide intake and sex hormone levels and SHBG, the multivariable-adjusted results were virtually the same as the results adjusted only for age, batch and blood draw conditions (timing, fasting status). Therefore, we reported only the former results.

Among premenopausal women, acrylamide intake was not statistically significantly associated with any of the plasma hormones (table 2), nor were there associations among the never-smoking premenopausal women. Among normal-weight women (BMI <25 kg/m^2), acrylamide intake was statistically significantly and modestly positively associated with luteal total estradiol (geometric mean (GM) of Q1 vs Q4: 138 vs 154 pg/mL; p for trend = 0.02), and with luteal free estradiol (GM of Q1 vs Q4: 1.60 vs 1.76 pg/mL; p for trend = 0.03) (table 3). In premenopausal overweight women (BMI ≥25 kg/m^2), acrylamide intake was not associated with any of the sex hormones (table 3). For luteal total estradiol, the interaction between acrylamide intake and overweight status was statistically significant (p = 0.01), whereas it was not for luteal free estradiol (p = 0.33).

Among postmenopausal women, acrylamide intake was associated with a borderline statistically significant decrease in estrone sulfate levels (GM of Q1 vs Q4: 232 vs 199 pg/mL; p for trend = 0.06) (table 4), but with no consistent dose-response across the quartiles of acrylamide intake. In never-smoking postmenopausal women, the association was similar. No other postmenopausal hormones were associated with acrylamide intake. In postmenopausal normal-weight women, there were statistically significant or borderline statistically significant inverse associations between acrylamide intake and levels of estrone (GM of Q1 vs Q4: 24 vs 20 pg/mL; p for trend = 0.02), free estradiol (GM of Q1 vs Q4: 0.08 vs 0.06 pg/mL; p for trend = 0.04), estrone sulfate (GM of Q1 vs Q4: 185 vs 150 pg/mL; p for trend = 0.07) with no consistent dose-response across the quartiles of acrylamide intake, DHEA (GM of Q1 vs Q4: 241 vs 194 ng/dL; p for trend = 0.06), and prolactin (GM of Q1 vs Q4: 8.7 vs 7.4 ng/mL; p for trend = 0.01) (table 4), the latter with no consistent dose-response across the quartiles of acrylamide intake. In postmenopausal overweight women, acrylamide intake was statistically significantly positively associated with the levels of testosterone (GM of Q1 vs Q4: 19 vs 23 ng/dL; p for trend = 0.03), and androstenedione (GM of Q1 vs Q4: 51 vs 69 ng/dL; p for trend = 0.002), the latter with no consistent dose-response across the quartiles of acrylamide intake (table 4). The interaction between acrylamide intake and overweight status was statistically significant or borderline statistically significant for free estradiol (p = 0.004), estrone sulfate (p = 0.03), and testosterone (p = 0.09), not for estrone (p = 0.66), prolactin (p = 0.19), and DHEA (p = 0.99), and androstenedione (p = 0.91).

Results did not change qualitatively when intakes of carbohydrates, protein, dietary fiber, saturated fat, and cholesterol were included or when caffeine was not included in the multivariable-adjusted models. As an example, the geometric mean luteal estradiol level in normal-weight premenopausal women in Q1 was 137 vs 155 in Q4 (p trend 0.008) when adjusted for carbohydrates, protein, dietary fiber, saturated fat, and cholesterol, and 139 vs 153 (p trend 0.03) when not adjusted for caffeine, as compared to the results in Table 3: 138 vs 154 (p trend 0.02).

Discussion

In this cross-sectional study, we found no associations between acrylamide and plasma hormones among premenopausal women overall, but among premenopausal normal-weight

women, acrylamide intake was associated with an increase in luteal total and free estradiol levels. Among postmenopausal women overall and among never-smoking postmenopausal women, acrylamide was associated with a non-linear decrease in estrone sulfate concentrations. Some inverse associations were noted for acrylamide and estrogens among postmenopausal normal-weight women, while suggestive positive associations for some androgens were observed among overweight postmenopausal women.

In some epidemiological studies, but not all, positive associations have been observed between dietary acrylamide intake and endometrial, ovarian and estrogen receptor-positive breast cancer risks and breast cancer mortality (20-25, 31), which are cancers where sex hormones are thought to play an important role.(59, 60)

For endometrial cancer, exposure to estrogens, unopposed by progesterone, has been associated consistently with an increased risk (59, 61, 62). For ovarian cancer, the role of estrogens is less clear (63). There is ample evidence that higher endogenous estrogen levels are associated with higher breast cancer risk, particularly postmenopausal breast cancer (64, 65). Exposure to androgens is hypothesized to increase ovarian cancer risk (63) since androgen receptors are present in the ovaries and several risk factors for ovarian cancer alter androgen levels. However, prospective epidemiological studies have not observed clear positive associations between circulating androgens and ovarian cancer risk (36, 66), although a positive association was observed for androstenedione and DHEA in one study (67). Androgens might increase endometrial cancer risk as well (61, 62, 68), and also for breast cancer, positive associations with androgen levels have been observed (64, 65). Progesterone reduces endometrial cancer risk (54), and possibly also ovarian cancer risk (69), whereas its role in breast cancer etiology is still unclear (70). SHBG, thought to reduce risk by limiting free circulating testosterone and estradiol, has been associated with a decrease in risk for endometrial, ovarian and breast cancer (62, 64, 66). Prolactin has been shown to increase breast cancer risk (71) and is hypothesized to increase endometrial and ovarian cancer risk and, although data are limited, increased levels of prolactin have been observed in endometrial and ovarian cancer cases compared to controls (72).

The association we observed between acrylamide intake and luteal estradiol levels in premenopausal normal-weight women is consistent with the hypothesis that acrylamide could increase the risk of endometrial, ovarian and estrogen receptor-positive breast cancers through increased estrogen exposure. Intriguingly, in the NHS, acrylamide intake was associated most strongly with endometrial and ovarian cancer risk in normal-weight women. However, it is not clear why only estradiol during the luteal phase would be associated with acrylamide intake, and it is possible that given the number of comparisons conducted in premenopausal women that this association was observed by chance.

In postmenopausal women overall, we did not observe associations between acrylamide intake and sex hormone levels that might explain associations of acrylamide intake with endometrial, ovarian and estrogen receptor-positive breast cancer risk. Further, in never-smoking postmenopausal women, which is the group in which the strongest association between acrylamide intake and endometrial, ovarian and estrogen and progesterone receptor-positive breast cancer was observed in the NLCS study, no associations between acrylamide intake and hormones were observed. When stratifying by overweight status, inverse associations with several estrogens, prolactin and DHEA were seen in postmenopausal normal-weight women, which are not consistent with the observed positive associations between acrylamide intake and postmenopausal endometrial, ovarian and estrogen receptor-positive breast cancer risk (20). In overweight postmenopausal women, statistically significant positive trends were observed for testosterone and androstenedione levels with increasing acrylamide intake, which would be consistent with the observed

associations between acrylamide intake and postmenopausal endometrial, ovarian and estrogen receptor-positive breast cancer risk. At a first glance, the positive association with estradiol in normal-weight premenopausal women and the inverse association with estrogens in normal-weight postmenopausal women seem incompatible. We currently have no biological explanation for this contradiction, although chance may explain the apparent discrepancy. It would be of interest to know if and how acrylamide influences the expression and activity of enzymes involved in sex steroid metabolism. But more importantly, further research in premenopausal and postmenopausal women is needed to corroborate or refute the observed associations between acrylamide intake and sex hormones, and to assess the interaction of acrylamide with overweight. We additionally stratified our analyses based on waist-to-hip ratio (<0.80 or ≥0.80). The associations described above were not observed in the strata of waist-to-hip ratio (results not shown). The explanation for this could be that BMI is more strongly related to subcutaneous fat mass and waist-to hip ratio is more strongly related to visceral fat mass (73). It has been shown that these different fat deposits differ in the activity of sex steroid metabolizing enzymes, such as aromatase (74, 75). Previously in the NHS, no differences in sex steroid levels were observed in women based on central obesity status as measured by waist-to-hip ratio, whereas clear differences were observed with general obesity as measured by BMI(55). It is conceivable that acrylamide has effects on sex hormones only in a high or low sex hormone environment.

Caffeine is correlated with acrylamide intake, as coffee is a major source of both. Coffee and caffeine previously were shown to be associated with sex hormone levels in NHS and NHSII (58). Therefore, we adjusted our analyses for caffeine. This is likely to render some overadjustment of the acrylamide intake analysis. However, not adjusting for caffeine gave similar results.

A limitation of this study is the measurement error that may occur when assessing acrylamide intake through an FFQ. There have been a few studies that investigated the correlation between dietary acrylamide intake as assessed by FFQ and acrylamide or glycidamide hemoglobin adducts, biomarkers of acrylamide body burden. In these studies, the correlations between FFQ data and hemoglobin adduct levels ranged from absent or low (76, 77) to modest correlations (46, 78, 79). The fact that these correlations are modest at best is probably due to issues relating to both the FFQ and the biomarker, as discussed by Hogervorst *et al.* (80). No association with ovarian cancer was observed using acrylamide biomarkers in a nested case-control study from the NHS, involving some of the same study participants as the current analysis (25). This absence of association would decrease the likelihood that the positive associations in the studies using data from a food frequency questionnaire reflect causal associations if the acrylamide biomarker was the gold standard to assess long-term dietary acrylamide exposure. However, as stated above, both acrylamide biomarkers and food frequency questionnaires have their drawbacks for assessing long-term dietary acrylamide intake to study the association between acrylamide intake and cancer.

Measurement error in the acrylamide intake assessment will have led to underestimation of true associations or even missing true associations, if any, in this study. A further limitation of this study is that it is not possible to establish a temporal relationship between acrylamide exposure and hormone levels based on the cross-sectional analyses, but it is unlikely that endogenous sex hormones would influence the consumption of acrylamide-containing foods. Another limitation is that the time between the completion of the questionnaire and the blood draw was variable. This introduces random variation and decreases the power to detect associations. Both acrylamide intake and hormone levels vary somewhat over time and this too introduces misclassification, diminishing the power to detect associations. However, the intraclass correlation coefficients for within-person repeated measures of these hormones over time are moderate to high (38, 81), suggesting that a single blood sample

reflects long-term values. Acrylamide intake, as measured by hemoglobin adducts of acrylamide and glycidamide, has been shown to be reasonably stable over time, as indicated by an intraclass correlation coefficient of 0.77 over a period of 1-3 years (46). Furthermore, we performed multiple analyses because of the many subgroups and therefore some or all of the observed associations might be chance findings. Therefore, additional assessments of these associations are needed.

Strengths of this study are that we had timed blood samples from a large subset of premenopausal women in order to accurately assess sex hormone concentrations during luteal and follicular phases. In addition, we limited our analysis of postmenopausal women to women not using postmenopausal hormone therapy at blood draw. Another strength of the study is that it is the same study population in which associations between acrylamide and endometrial and ovarian cancer risk were observed (21).

Apart from the above-mentioned limitations of this study that may have obscured true associations between acrylamide intake and sex hormones, acrylamide may have endocrine activity through other mechanisms than influencing sex hormone levels, such as directly or indirectly activating sex steroid receptors. To overcome some of the limitations of observational studies, such as confounding, measurement error, and the temporal relationship issue, a human intervention study (e.g. using commercially available potato chips) to investigate the effect of acrylamide on sex hormone levels and on the enzymes involved in sex hormone metabolism would be useful.

In conclusion, this study observed some associations between acrylamide intake and sex hormones. However, not all are in a direction that would explain the positive associations between dietary acrylamide intake and endometrial, ovarian and estrogen receptor-positive breast cancer risk. Consistent with the hypothesis that acrylamide might cause endometrial and ovarian cancer through effects on sex hormones are the observed positive associations between acrylamide intake and luteal estradiol in normal-weight premenopausal women, and the positive associations with testosterone and androstenedione in overweight postmenopausal women. However, the inverse association with several estrogens, prolactin and DHEA in normal-weight postmenopausal women seems to, at a first glance, run counter to this hypothesis. This is the first study to examine these associations in humans; other studies should investigate this question, especially those studies with data on acrylamide and glycidamide to hemoglobin adducts, stratifying both on overweight and menopausal status.

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Table 1

Age-standardized characteristics* of the study population in 1995 (premenopausal women) and 1990 (postmenopausal women)

	Quartiles of energy-adjusted acrylamide intake			
	Q1	**Q2**	**Q3**	**Q4**
Premenopausal women (n = 1300) †				
Acrylamide intake, μg/day	11.6	18.0	23.4	35.0
Age at blood draw, y	42.5	42.1	41.8	41.8
Age at menarche, y	12.5	12.5	12.5	12.4
Parity‡, n	2.3	2.3	2.3	2.3
BMI at blood draw, kg/m^2	24.5	25.7	24.6	25.0
Ever OC use, %	79	86	86	86
Energy intake, kcal/day	1829	1887	1862	1848
Alcohol consumption, g/day	2.6	3.4	4.0	3.3
Total physical activity, MET-h/wk	20.8	17.7	20.1	16.6
Caffeine intake, mg/day	134	199	247	337
Smoking status, %				
Never-smokers	73	75	67	62
Ex-smokers	20	19	24	25
Current smokers	7	6	9	12
Postmenopausal women (n = 687)				
Acrylamide intake, μg/day	8.0	13.5	17.6	26.8
Age at blood draw, y	61.9	62.3	61.5	60.8
Age at menarche, y	12.8	12.6	12.6	12.3
Age at menopause, y	49.7	49.2	48.7	48.2
Parity‡, n	3.6	3.6	3.6	3.6
BMI at blood draw, kg/m^2	26.5	26.0	26.1	25.8
Ever OC use, %	37	26	29	35
Energy intake, kcal/day	1763	1758	1758	1756
Alcohol consumption, g/day	5.8	6.1	4.4	5.5
Total physical activity, MET-h/wk	17.3	16.7	14.7	15.7

	Quartiles of energy-adjusted acrylamide intake			
	Q1	**Q2**	**Q3**	**Q4**
Caffeine intake, mg/day	147	216	266	340
Smoking status, %				
Never-smokers	52	47	47	39
Ex-smokers	41	41	40	42
Current smokers	7	12	13	19

*data represent means or percentages

†this number represents women with an ovulatory cycle only.

‡among parous women: n= 1057 for premenopausal women, n = 651 for postmenopausal women

Table 2

Geometric mean concentrations of estrogens, androgens, progesterone, prolactin and SHBG by quartiles of acrylamide intake in **premenopausal** women

		Quartiles of energy-adjusted acrylamide intake				
	n	**Q1**	**Q2**	**Q3**	**Q4**	**p-trend**
All premenopausal women						
Estrone, pg/mL						
Luteal	1251	84	85	86	86	0.50
Follicular	1112	41	41	40	41	0.83
Estradiol, pg/mL						
Luteal	1217	134	135	136	141	0.11
Follicular	1097	50	48	46	49	0.78
Free estradiol, pg/mL						
Luteal	1209	1.66	1.70	1.70	1.76	0.12
Follicular	1089	0.62	0.61	0.58	0.60	0.60
Estrone sulfate, pg/mL						
Luteal	372	1363	1570	1368	1432	0.91
Follicular	368	677	717	698	646	0.51
Testosterone, ng/dL	1258	22	23	22	23	0.29
Free testosterone, ng/dL	1252	0.18	0.19	0.19	0.19	0.49
Androstenedione, ng/dL	415	100	102	106	103	0.55
DHEA, ng/dL	318	622	635	679	635	0.75
DHEAS, μ/dL	813	84	87	85	90	0.33
Progesterone, ng/dL	1269	1499	1473	1615	1492	0.78
Prolactin, ng/mL	900	14	15	15	15	0.11
SHBG, nmol/L	1265	65	62	64	65	0.63
Never-smoking premenopausal women						
Estrone, pg/mL						
Luteal	864	84	85	87	85	0.64
Follicular	768	42	41	40	41	0.60
Estradiol, pg/mL						
Luteal	839	135	138	140	141	0.30
Follicular	756	50	48	46	48	0.37
Free estradiol, pg/mL						
Luteal	835	1.67	1.74	1.74	1.76	0.23
Follicular	753	0.62	0.61	0.56	0.58	0.20
Estrone sulfate, pg/mL						
Luteal	267	1432	1596	1302	1440	0.68
Follicular	265	650	692	659	616	0.53
Testosterone, ng/dL	866	22	23	22	23	0.45
Free testosterone, ng/dL	864	0.18	0.19	0.18	0.19	0.58
Androstenedione, ng/dL	297	99	103	106	101	0.74

		Quartiles of energy-adjusted acrylamide intake				
	n	**Q1**	**Q2**	**Q3**	**Q4**	**p-trend**
DHEA, ng/dL	233	596	645	682	634	0.53
DHEAS, μg/dL	581	83	86	85	90	0.26
Progesterone, ng/dL	875	1479	1441	1681	1484	0.47
Prolactin, ng/mL	636	14	16	15	15	0.39
SHBG, nmol/L	873	65	61	64	65	0.80

Multivariable-adjusted models included: age at blood draw (≤40, >40-45, >45 years), fasting status at blood draw (≤8, >8 hours), time of day of the blood draw (1-4 AM, 5-6 AM, 7 AM-noon), month of blood draw (continuous), difference between luteal draw date and date of the next menstrual period (3-7, 8-21 days, unknown/untimed), duration of past oral contraceptive use (never, <4, ≤4 years, missing), parity (nulliparous vs parous), body mass index at blood draw (continuous), physical activity (<6, 6-<15, 15-<30, ≥30 hours per wk mean exercise time), total energy intake (kcal/d), alcohol consumption (0, >0-≤10, >10-≤20, >20-≤30, >30 g/d), smoking status (never, past, current; only in models in which smoking and non-smoking women were combined), and energy-adjusted caffeine intake (quartiles)

The number of study participants varies in the analyses of the different hormones and SHBG due to the fact that not all hormones or SHBG were measured in each nested case-control study from which the current study population was derived

Table 3

Geometric mean concentrations of estrogens, androgens, progesterone, prolactin and SHBG by quartiles of acrylamide intake in **premenopausal** women stratified by normal versus overweight

		Quartiles of energy-adjusted acrylamide intake				
	n	**Q1**	**Q2**	**Q3**	**Q4**	**p-trend**
Estrone, pg/mL						
Luteal						
BMI<25	784	84	87	85	87	0.56
BMI>=25	467	82	83	88	84	0.57
Follicular						
BMI<25	703	42	40	39	40	0.28
BMI>=25	409	40	41	42	42	0.28
Estradiol, pg/mL						
Luteal						
BMI<25	765	138	143	143	154	0.02
BMI>=25	452	128	123	124	124	0.71
Follicular						
BMI<25	695	55	50	49	52	0.42
BMI>=25	402	43	43	42	45	0.64
Free estradiol, pg/mL						
Luteal						
BMI<25	757	1.60	1.65	1.66	1.76	0.03
BMI>=25	452	1.79	1.79	1.76	1.76	0.72
Follicular						
BMI<25	688	0.62	0.59	0.57	0.59	0.46
BMI>=25	401	0.61	0.64	0.58	0.63	0.99
Estrone sulfate, pg/mL						
Luteal						
BMI<25	236	1382	1626	1337	1480	0.84
BMI>=25	136	1386	1494	1481	1231	0.43
Follicular						
BMI<25	227	693	701	646	667	0.66
BMI>=25	141	650	689	805	633	0.93
Testosterone, ng/dL						
BMI<25	786	22	23	23	23	0.28
BMI>=25	472	22	22	22	23	0.67
Free testosterone, ng/dL						
BMI<25	780	0.17	0.17	0.17	0.17	0.35
BMI>=25	472	0.22	0.23	0.22	0.22	0.72
Androstenedione, ng/dL						
BMI<25	260	106	107	110	106	0.95
BMI>=25	155	87	97	101	95	0.41

		Quartiles of energy-adjusted acrylamide intake				
	n	Q1	Q2	Q3	Q4	p-trend
DHEA, ng/dL						
BMI<25	198	632	625	666	647	0.69
BMI>=25	120	622	643	679	622	0.98
DHEAS, ng/dL						
BMI<25	502	85	87	85	94	0.15
BMI>=25	311	84	86	86	82	0.72
Progesterone, ng/dL						
BMI<25	793	1618	1548	1735	1634	0.54
BMI>=25	476	1327	1345	1421	1292	0.66
Prolactin, ng/mL						
BMI<25	556	14	16	15	15	0.30
BMI>=25	344	16	14	15	16	0.46
SHBG, nmol/L						
BMI<25	791	75	75	73	75	0.91
BMI>=25	474	51	46	51	52	0.21

Multivariable-adjusted models included: age at blood draw (≤40, >40-45, >45 years), fasting status at blood draw (≤8, >8 hours), time of day of the blood draw (1-4 AM, 5-6 AM, 7 AM-noon), month of blood draw (continuous), difference between luteal draw date and date of the next menstrual period (3-7, 8-21 days, unknown/untimed), duration of past oral contraceptive use (never, <4, ≥4 years, missing), parity (nulliparous vs parous), body mass index at blood draw (continuous), physical activity (<6, 6-<15, 15-<30, ≥30 hours per wk mean exercise time), total energy intake (kcal/d), alcohol consumption (0, >0-≤10, >10-≤20, >20-≤30, >30 g/d), smoking status (never, past, current; only in models in which smoking and non-smoking women were combined), and energy-adjusted caffeine intake (quartiles)

The number of study participants varies in the analyses of the different hormones and SHBG due to the fact that not all hormones or SHBG were measured in each nested case-control study from which the current study population was derived

Table 4

Adjusted geometric mean concentrations of estrogens, androgens, prolactin and SHBG by quartiles of acrylamide intake in **postmenopausal** women not taking postmenopausal hormones

		Quartiles of energy-adjusted acrylamide intake				
	n	**Q1**	**Q2**	**Q3**	**Q4**	**p-trend**
All postmenopausal women						
Estrone, pg/mL	519	26	25	22	25	0.45
Estradiol, pg/mL	655	7.2	6.7	6.4	7.0	0.50
Free estradiol, pg/mL	627	0.10	0.09	0.09	0.10	0.31
Estrone sulfate, pg/mL	652	232	199	197	199	0.06
Testosterone, ng/dL	660	21	20	20	23	0.15
Free testosterone, ng/dL	647	0.21	0.19	0.20	0.22	0.47
Androstenedione, ng/dL	518	56	55	53	60	0.22
DHEA, ng/dL	504	220	201	183	215	0.83
DHEAS, ng/dL	525	86	74	74	83	0.88
Prolactin, ng/mL	650	9.1	8.8	8.6	8.3	0.09
SHBG, nmol/L	662	46	50	49	49	0.37
Never-smoking postmenopausal women						
Estrone, pg/mL	246	27	23	23	24	0.19
Estradiol, pg/mL	301	7.5	6.3	6.8	7.2	0.82
Free estradiol, pg/mL	286	0.11	0.09	0.10	0.10	0.47
Estrone sulfate, pg/mL	304	253	187	226	196	0.09
Testosterone, ng/dL	308	20	19	20	22	0.38
Free testosterone, ng/dL	299	0.21	0.19	0.20	0.21	0.84
Androstenedione, ng/dL	245	57	53	53	56	0.91
DHEA, ng/dL	240	241	199	192	220	0.51
DHEAS, ng/dL	246	100	68	78	85	0.41
Prolactin, ng/mL	301	8.9	9.2	8.6	8.6	0.46
SHBG, nmol/L	305	45	49	46	49	0.34
All postmenopausal women, stratified by BMI						
Estrone, pg/mL						
BMI<25	254	24	22	20	20	0.02
BMI>=25	265	28	27	24	30	0.57
Estradiol, pg/mL						
BMI<25	315	6.0	5.4	5.4	5.3	0.12
BMI>=25	340	8.6	8.2	7.4	9.2	0.49
Free estradiol, pg/mL						
BMI<25	301	0.08	0.07	0.07	0.06	0.04
BMI>=25	326	0.14	0.12	0.12	0.14	0.58
Estrone sulfate, pg/mL						
BMI<25	313	185	154	170	150	0.07

		Quartiles of energy-adjusted acrylamide intake				
	n	Q1	Q2	Q3	Q4	p-trend
BMI>=25	339	286	247	226	262	0.41
Testosterone, ng/dL						
BMI<25	316	23	21	21	22	0.72
BMI>=25	344	19	20	20	23	0.03
Free testosterone, ng/dL						
BMI<25	311	0.20	0.17	0.17	0.18	0.57
BMI>=25	336	0.22	0.22	0.23	0.25	0.11
Androstenedione, ng/dL						
BMI<25	253	60	57	55	53	0.18
BMI>=25	265	51	52	49	69	0.002
DHEA, ng/dL						
BMI<25	248	241	224	200	194	0.06
BMI>=25	256	205	175	165	243	0.14
DHEAS, ng/dL						
BMI<25	255	91	76	82	77	0.34
BMI>=25	270	82	71	67	90	0.44
Prolactin, ng/mL						
BMI<25	314	8.7	8.9	8.7	7.4	0.01
BMI>=25	336	9.4	8.7	8.4	9.1	0.72
SHBG, nmol/L						
BMI<25	320	58	61	60	61	0.54
BMI>=25	342	38	43	39	40	0.69

Adjusted for assay batch (1-6), age at blood draw (≤55, >55-60, >60-65, >65 years), fasting status at blood draw (≤10, >10 hours), time of day of blood draw (1- 8 AM, 9 AM-noon, 1 PM-4 PM, 5 PM-midnight), age at first birth/parity (nulliparous, age at first birth <25 years/1-4 children, age at first birth 25-29 years/1-4 children, age at first birth 30 years/1-4 children, age at first birth <25 years/≥5 children, age at first birth >25 years/≥5 children), body mass index at blood draw (continuous), physical activity (<5, 5-18, ≥18 h/wk mean exercise time), total energy intake (kcal/d), alcohol consumption (0, >0-≤10, >10-≤20, >20-≤30, >30 g/d), smoking status (never, past, current; only in models in which smoking and non-smoking women were combined), and energy-adjusted caffeine intake (quartiles)

The number of study participants varies in the analyses of the different hormones and SHBG due to the fact that not all hormones or SHBG were measured in each nested case-control study from which the current study population was derived