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Is Intestinal Biopsy Always Needed for Diagnosis of Celiac Disease?

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OBJECTIVE: Intestinal biopsy is required for a diagnosis of celiac disease (CD). The aim of this study was to assess diagnostic accuracy of transglutaminase antibodies (TGA) in comparison and in association with that of antiemdomysial antibodies (AEA), calculating the post-test odds of having the disease, to verify whether some patients might avoid undergoing intestinal biopsy for a diagnosis of CD.

METHODS: A total of 181 consecutive patients (131 < 18 yr), referred to our celiac clinic by primary care physicians for suspect CD. Overall diagnostic accuracy, negative predictive value, and likelihood ratio (LR) were calculated both for each serological test and for serial testing (TGA and after AEA, assuming the post-test probability of TGA as pretest probability of AEA). Both serological determination and histological evaluation were blindly performed. Histology of duodenal mucosa was considered the gold standard.

RESULTS: The overall accuracy of TGA and of AEA were 92.8% (89.1–96.6) and 93.4% (89.7–97.0), respectively. The negative predictive value of TGA and AEA were 97.2% (91.9–102.6) and 87.2% (77.7–96.8), respectively. Positive likelihood ratios for TGA and AEA were 3.89 (3.40–4.38) and 7.48 (6.73–8.23), respectively. Serial testing, in groups of patients with prevalence of CD estimated higher than 75%, such as those with classic symptoms of CD, would provide a post-test probability of more than 99%.

CONCLUSIONS: Our results suggest that serial testing with TGA and AEA might allow, in some cases, the avoidance of intestinal biopsy to confirm the diagnosis of CD. (Am J Gastroenterol 2003;98:1325–1331. © 2003 by Am. Coll. of Gastroenterology)

INTRODUCTION

Celiac disease (CD) is a permanent intolerance to gluten of some cereals, mediated by an autoimmune mechanism, in genetically predisposed individuals (1). According to the original diagnostic criteria of the European Society for Pediatric Gastroenterology and Nutrition, to establish the diagnosis definitively one needed to obtain an initial biopsy along with abnormal small intestinal mucosa (usually flat), a second one after a clinical response to a gluten-free diet to show histological response, and a third one to show clinical and/or histological relapse after gluten challenge (2). These criteria have been modified and new or revised European Society for Pediatric Gastroenterology and Nutrition criteria produced (3), which do not regard serial biopsy and challenge as necessary for all children diagnosed as having CD except in certain situations. These include age at presentation of under 2 yr, atypical biopsy or clinical features, no previous biopsy, and teenagers who plan to return to a normal gluten-containing diet despite advice to the contrary.

With the introduction and widespread use of serological tests to detect antigliadin and antiendomysial (AEA) antibodies, it was clear that CD may have been underdiagnosed. The "asymptomatic" form of CD, with typical serological and histological features despite the absence of symptoms, may be five to seven times more common than the symptomatic form of CD (4). More recently, tissue transglutaminase (tTG) was identified as the unknown endomysial autoantigen of CD (5), and ELISAs were established to measure IgA and IgG antitTG titers in serum samples (6). These antitransglutaminase antibodies (TGA), both against guinea pig and human tTG, were demonstrated to have very high diagnostic accuracy for CD, expressed as sensitivity and specificity of the serological test (7, 8). Unfortunately, most studies are biased because the reference or gold standard (intestinal biopsy) was not performed, regardless of the results of the test under evaluation, and the serological test was performed on sera of already known celiac and control patients. Moreover, the whole point of a diagnostic test is to use it to make a diagnosis, so one needs to know the probability that the test will give the correct diagnosis. Sensitivity and specificity do not give us this information; instead, predictive values are recommended. Positive and negative predictive values are known as posterior probabilities. The difference between the probability of having a disease before the test is carried out (pretest probability) and post-test probability is one way of assessing the usefulness of the test. For any test result, the probability of getting that result if the patient truly had the condition of interest with the corresponding probability if he or she were healthy (the ratio of these probabilities is called the likelihood ratio, LR) indicates the value of the test for increasing certainty about a positive diagnosis (9). In the present study, we assessed diagnostic accuracy of TGA in comparison and in associa-

Table 1. Symptoms and Clinical Conditions of the Patients Enrolled in the Study

	All Patients	Pediatric Patients	Adult Patients
GI symptoms (%)	107 (59.1)	86 (65.6)	21 (42)
Extra-GI symptoms or clinical conditions (%)	47 (26)	28 (21.4)	19 (38)
Iron-deficiency anemia	20	10	10
Short stature	9	8	1
Diabetes	4	2	2
Recurrent aphtous stomatitis	2	1	1
Alopecia	2	1	1
Dermatitis herpetiformis	4	1	3
Atopic dermatitis	2	2	0
Down syndrome	1	1	0
IgA nephropathy	1	1	0
Connective tissue diseases	1	1	0
Recurrent spontaneous abortion	1	0	1
Screening (%)	27 (14.9)	17 (13)	10 (20)
Total	181	131	50

tion with AEA, in consecutive patients referred to undergo intestinal biopsy for suspect CD, calculating the post-test odds of having the disease, that is the pretest odds multiplied by the LR. In this way we aimed at verifying whether intestinal biopsy is always necessary for diagnosis of CD. This study suggests that in some cases it is possible to avoid an intestinal biopsy.

PATIENTS AND METHODS

A total of 181 consecutive patients, 131 < 18 yr (86 women, 45 men; mean age 7.6 yr, range 1–17 yr), 50 adults (37 women, 13 men; mean age 30.4 yr, range 18–69 yr), without any known GI disorder, were enrolled in the study from October 7, 1999, to July 20, 2000. They were referred to our celiac clinic by primary care physicians to undergo intestinal biopsy for positive serological tests for CD (AEA or guinea pig-TGA or both) and/or a suspect CD, based on GI symptoms (chronic diarrhea, weight loss or failure to thrive, abdominal pain, abdominal distension, dyspepsia), extra-GI symptoms or clinical conditions associated with CD (sideropenic anemia refractory to therapy with iron, short stature, recurrent stomatitis, insulin-dependent diabetes mellitus, autoimmune diseases, alopecia areata, reproductive disorders, IgA nephropathy, Down syndrome), and familial or scholar screening for CD. Symptoms and clinical conditions of the patients enrolled in the study are shown in Table 1. After drawing a blood sample and storing frozen serum for AEA and TGA determination, all patients underwent esophagogastroduodenoscopy, to provide at least three biopsy samples taken from the third part of the duodenum. Marsh's modified classification was used for histological examination (10).

On the basis of this original standardized scheme, five different histological pictures can be described, from type 0 to type 4, which are grouped in three different diagnostic classes: normal (type 0), borderline including type 1–2, diagnostic including type 3 (a, b, c), and 4.

Briefly, type 0 is a normal mucosa with less than 40 intraepithelial lymphocytes (IEL)/100 epithelial cells (EC); type 1 is the infiltrative type, which is characterized by a normal villous architecture, a normal height of the crypts and an increase in IEL numbers up to more than 40 IEL/100 EC; type 2 is the hyperplastic type, characterized by a normal villous architecture, an increase in IEL numbers up to more than 40 IEL/100 EC and crypt hyperplasia.

The original Marsh's classification type 3 lesions, the destructive ones, which represent diagnostic lesions, are here subdivided into subgroups a–c: type 3a, characterized by a mild villous flattening, an increase in crypt height, and an increase in IEL numbers up to more than 40 IEL/100 EC; type 3b, characterized by a marked villous flattening, an increase in crypt height, and an increase in IEL numbers up to more than 40 IEL/100 EC; type 3c, characterized by a flat mucosa (total villous flattening), an increase in crypt height, and an increase in IEL numbers up to more than 40 IEL/100 EC; type 4 is the very rare hypoplastic lesion. According to this classification even a mild villous atrophy may represent a diagnostic finding.

Forty-one patients with Crohn's disease (mean age 20.4 yr, range 4–65 yr, 21 women) were also enrolled as pathological controls. Total serum IgA were preliminarily determined in all the patients to exclude IgA deficiency. Serum IgA AEA were measured by means of indirect immunofluorescence using cryostat sections of human umbilical cord from a commercial kit (IPR, Catania, Italy). Serum IgA GP-TGA were measured by ELISA method established in our laboratory, preliminarily validated on sera of 43 patients known to have CD, and on sera of 56 patients in whom CD had been ruled out, showing a 100% sensitivity and a 98% specificity (11).

Microtiter plates, high-binding capacity (96-wells kovalink Nunc, Roskilde, Denmark) were coated overnight at 4°C with 100 μl/well of 10 μg/ml of Guinea pig tTG (Sigma T5398, Chemical, St. Louis, MO) and 2 μg/ml of an alcoholic gliadin solution, in coating buffer (50 mmol/L

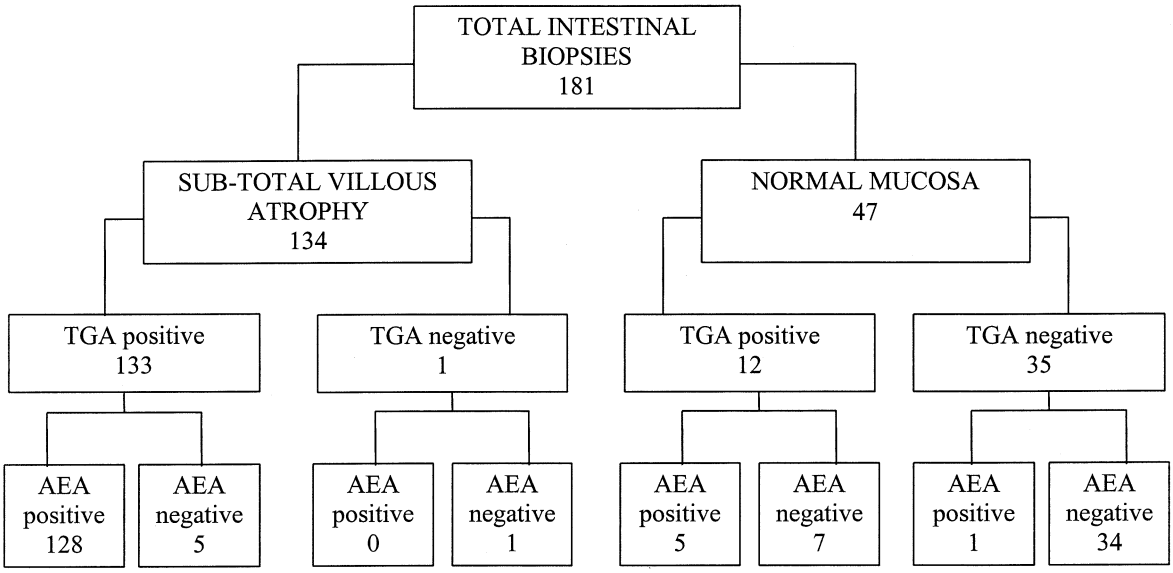

Figure 1. TGA and AEA in 181 consecutive patients who underwent intestinal biopsy.

Tris-HCI, 5 mmol/L $CaCl_2$, 150 mmol/L NaCl, pH 8.2). The plates were washed four times with 50 mmol/L Tris-HCI, 150 mmol/L NaCl, 0.05% triton x-100 pH 8.2, and overcoated for 2 h at room temperature with 200 μl/well of 1% hydrolysated casein, 0.05 polivinilpirrolidone, and 0.05% triton x-100 (MW 10.000, Sigma) in Tris buffer, pH 8.2

Also, 100 μl of serum samples diluted 1:50 in sample buffer (50 mmol/L Tris-HCI, 150 mmol/L NaCl, 0.05% triton x-100, 0.25% casein, pH 7.4) were added to the wells and incubated overnight at 4°C. Then, the plates were washed four times and incubated for 2 h at room temperature with 100 μl of rabbit ALP antihuman IgA (Sigma) and diluted 1:2000 in the sample buffer. The plates were washed four times to remove unbound antibodies. The color was developed by addition of 100 μl/well of dietanolamine buffer, pH 9.7, paranitro-phenilphosphate 1 mg/ml, at room temperature for 30 min. The reaction was stopped with 100/well of NaOH 1N solution, and absorbance was read on an ELISA reader (LP 300 Pasteur) at 405 nm. Two replicates were used for each serum sample. Antibody concentrations were expressed in arbitrary units (AU), that is, as percentages of the positive reference serum.

The intrassay coefficient of variation for IgA class tTGA ELISA was 2.16% at a 1:2,500 dilution (40 AU) (n = 10), 2.53% at a 1:5,000 dilution (20 AU) (n = 10), 1.96% at a 1:10,000 dilution (10 AU) (n = 10), and 3.1% at a 1:20.000 dilution (n = 10). The interassay coefficient of variation was 2.6% (n = 8). The results of the serological tests given by the laboratory were not known by the pathologists who interpreted the reference test and vice versa.

Considering the result of histology as gold standard for diagnosis of CD, we calculated diagnostic accuracy for each test and for serial testing assuming the post-test probability of the test (TGA) with the highest negative predictive value as pretest probability of AEA. Diagnostic accuracy and post-test probability were calculated using the all-purpose 4-fold Table Analyzer and the interactive nomogram for post-test probabilities offered by the home page of the Center for Evidence-Based Medicine (http://cebm.jr2.ox.ac.uk/). This calculation was applied in different clinical conditions, such as an infant with a classic clinical picture of CD, assuming a pretest probability of at least 75%, an asymptomatic first-grade relative of a celiac patient, assuming a pretest probability of 10% (12), and asymptomatic people from the general pediatric and adult population, assuming a pretest probability of 0.5–0.4% (4, 13).

RESULTS

Of all 181 patients enrolled in the study, 134 had a histology that was compatible (type 3a–3c), and 47 not compatible, with CD (type 0–2). All patients with a histology compatible with CD had a clinical and serological response to a gluten-free diet. The results of TGA and AEA in patients with and without histology compatible with CD, regardless of age, and for the two groups of age, are shown in Figures 1, 2, and 3.

Among the 134 patients (100 < 18 yr) with a subtotal villous atrophy, TGA and AEA were negative in only one pediatric case. Among the 47 patients (31 < 18 yr) with a normal mucosa, TGA was positive in 12 (nine children), five (four children) of whom were AEA positive, too. All the four children with positive TGA and AEA had celiac-type human leukocyte antigen (HLA) DQ2. Only one adult patient with normal mucosa and negative TGA had positive AEA.

Diagnostic accuracy of TGA and AEA for the three groups of patients are shown in Tables 2 and 3, respectively. In particular, positive LR of TGA and AEA resulted as 3.89

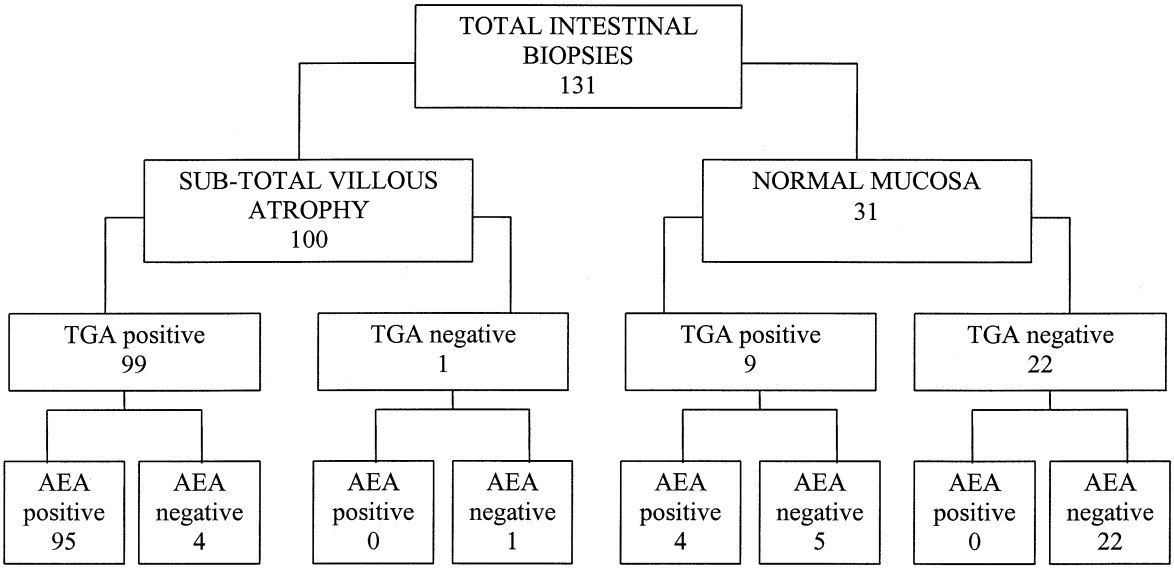

Figure 2. TGA and AEA in 131 consecutive patients <18 yr who underwent intestinal biopsy.

and 7.48, respectively, in patients considered as a whole, and 3.41 and 7.36; 5.33 and 7.76, respectively, in pediatric and adult patients. Positive LR of AEA was higher than that of TGA both in pediatric and adult patients. On the contrary, TGA had a negative predictive value higher than that of AEA, especially in adult patients. In all patients with Crohn's disease TGA and AEA were negative

CALCULATION OF POST-TEST PROBABILITY IN DIFFERENT CLINICAL SITUATIONS

Post-test probability was calculated on the basis of LR obtained for each test, in the corresponding age group, assuming as pretest probability of TGA a probability based on clinical experience or on literature data, and as pretest probability for AEA the post-test probability of TGA.

Pediatric Patients

In infants with a classic clinical picture of CD, the interactive nomogram for post-test probability for TGA is shown in Figure 4. It can be seen that, assuming a 75% pretest probability for these infants, with a 3.41 positive LR, the post-test probability of TGA is more than 90%. Assuming this post-test probability as pretest probability for AEA, it can be seen that with a 7.36 LR, the post-test probability is

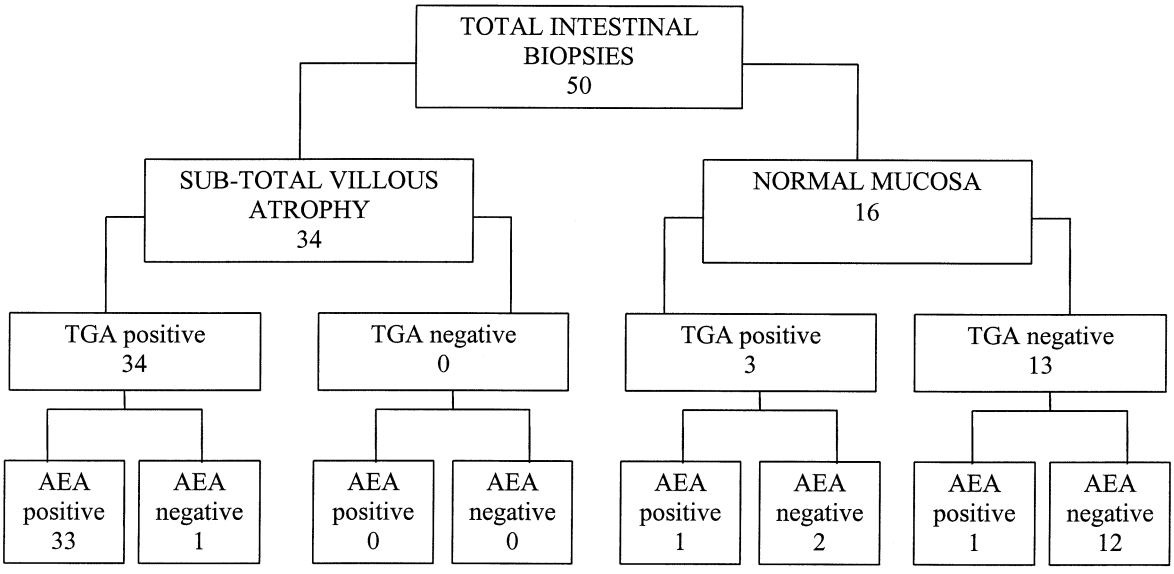

Figure 3. TGA and AEA in 50 consecutive adult patients who underwent intestinal biopsy.

Table 2. Diagnostic Accuracy of TGA Determined in All, Pediatric and Adult, Patients (CI)

	All Patients (n = 181)	Pediatric Patients (n = 131)	Adult Patients (n = 50)
Sensitivity (%)	99.3 (97.8–100.7)	99.0 (97.0–101.0)	100.0 (100.0–100.0)
Specificity (%)	74.5 (62.0–86.9)	71.0 (55.0–86.9)	81.3 (62.1–100.4)
Positive predictive value (%)	91.7 (87.2–96.2)	91.7 (86.5–96.9)	91.9 (83.1–100.7)
Negative predictive value (%)	97.2 (91.9–102.6)	95.7 (87.3–104.0)	100.0 (100.0–100.0)
Overall accuracy (%)	92.8 (89.1–96.6)	92.4 (87.8–96.9)	94.0 (87.4–100.6)
Positive LR	3.89 (3.40–4.38)	3.41 (2.86–3.96)	5.33 (4.31–6.35)
Negative LR	0.01 (−1.95–1.97)	0.01 (−1.95–1.98)	0.00

more than 98%. In these patients, intestinal biopsy may not give any additional probability for diagnosis.

In first-grade relatives of celiac patients, under 18 yr of age, with a 10% pretest probability (12) and a 3.41 positive LR, the post-test probability of TGA is about 25%. Assuming this post-test probability as pretest probability for AEA, with a 7.36 LR, the post-test probability is about 70%. For these patients, an intestinal biopsy is needed to confirm the diagnosis of CD.

In asymptomatic school children screened for CD, with a 0.5% pretest probability (4) and a 3.41 positive LR, the post-test probability of TGA is about 2%. Assuming this post-test probability as pretest probability for AEA, with a 7.36 LR, the post-test probability is only about 12%. For these patients, an intestinal biopsy is mandatory to confirm the diagnosis of CD.

Adult Patients

Keeping in mind the positive LR for TGA and AEA found in patients over 18 yr of age (5.33 and 7.76, respectively), in adults with a symptomatology strongly suggesting CD (pretest probability 75%), intestinal biopsy does not give any additional probability for diagnosis because the post-test probability is at the top of the scale. With respect to adult relatives or asymptomatic people, conclusions similar to those in pediatric patients can be drawn: an intestinal biopsy is needed to confirm the diagnosis of CD, as the post-test probability is 82% and 20%, respectively.

DISCUSSION

After the identification of tTG as the unknown endomysial autoantigen of CD (5), more than 60 articles on TGA as a diagnostic tool for CD can be found with a MEDLINE search. Most of these studies, especially those using human TGA, report a higher diagnostic accuracy of this test in comparison with AEA and antigliadin antibodies. However, intestinal biopsy is still the gold standard for diagnosis of CD (1).

Our data suggest that for adult patients with a classic clinical picture strongly suggesting CD, intestinal biopsy might not be needed to confirm the diagnosis, in the presence of positive antibodies against both transglutaminase and endomysium. However, an endoscopy should be mandatory in case of absence of response to a gluten-free diet to obtain intestinal biopsy samples and to exclude other disorders (*e.g.*, lymphoma).

Reviewing the results of recent studies using the determination of human tTG as substrate of ELISA in children, which has been reported as having a better diagnostic accuracy than that based on guinea pig as substrate, the histology of intestinal biopsy samples does not give the patient any additional probability of having the disease (7).

Keeping into account the 107 patients with GI symptoms strongly suggesting CD, a strategy based on starting a gluten-free diet in patients with both TGA and AEA positive values would have allowed to avoid costs of 72 endoscopic procedures. On the other hand, two of these patients would have a diagnosis of CD in the absence of a compatible histology, but they would undergo endoscopy in case of lack of clinical and/or serological response to a gluten-free diet.

However, this conclusion cannot be generalized for every setting as the diagnostic accuracy of these tests should be validated in each laboratory to which patients are referred, and a positive LR should be calculated to estimate the post-test probability. In the presence of a low positive LR, even in cases with a high pretest probability, the diagnosis

Table 3. Diagnostic Accuracy of AEA Determined in All, Pediatric and Adult, Patients (CI)

	All Patients (n = 181)	Pediatric Patients (n = 131)	Adult Patients (n = 50)
Sensitivity (%)	95.5 (92.0–99.0)	95.0 (90.7–99.3)	97.1 (91.4–102.7)
Specificity (%)	87.2 (77.7–96.8)	87.1 (75.3–98.9)	87.5 (71.3–103.7)
Positive predictive value (%)	95.5 (92.0–99.0)	96.0 (92.1–99.8)	94.3 (86.6–102.0)
Negative predictive value (%)	87.2 (77.7–96.8)	84.4 (71.8–97.0)	93.3 (80.7–106.0)
Overall accuracy (%)	93.4 (89.7–97.0)	93.1 (88.8–97.5)	94.0 (87.4–100.6)
Positive LR	7.48 (6.73–8.23)	7.36 (6.45–8.8)	7.76 (6.47–9.06)
Negative LR	0.05 (−0.74–0.84)	0.06 (−0.81–0.92)	0.03 (−1.91–1.97)

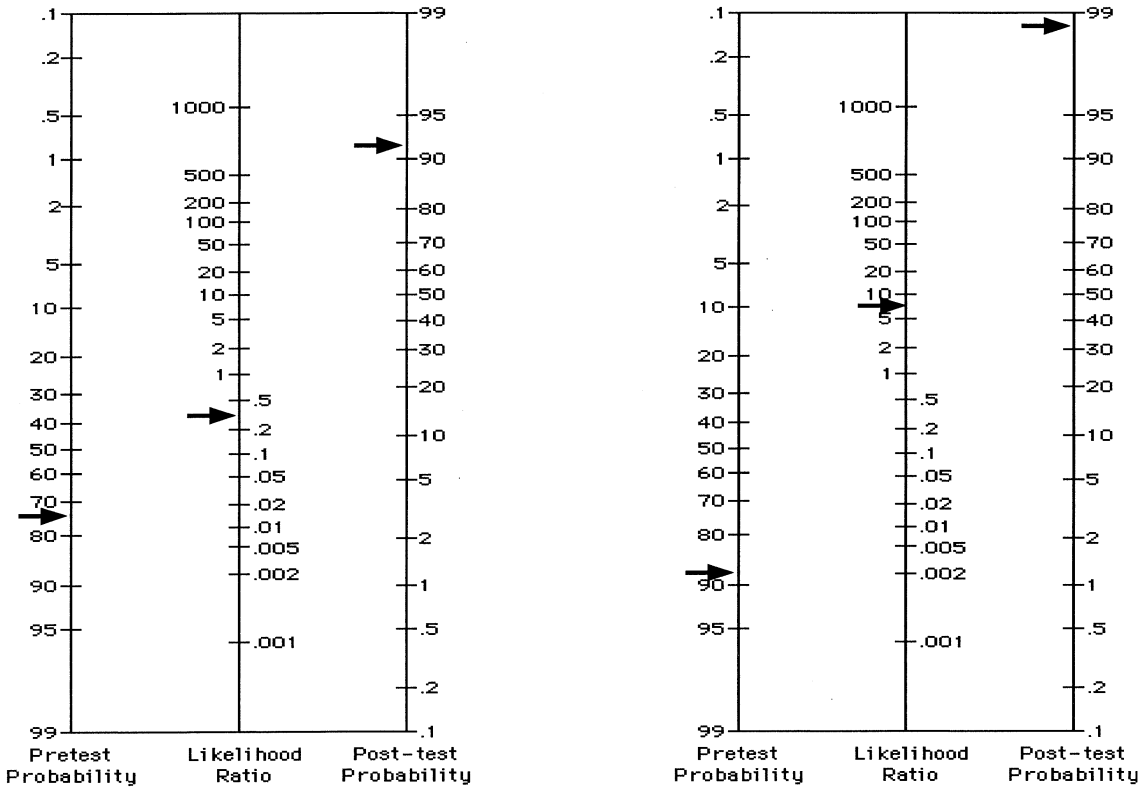

Figure 4. Interactive nomogram for post-test probability for TGA and AEA in infants with a classic clinical picture of CD, assuming the post-test probability of TGA (left) as pretest probability for AEA (right).

of CD should be confirmed by performing intestinal biopsy. On the other hand, an asymptomatic patient found positive for TGA and AEA at a familial or general population screening for CD would not accept the idea of starting a lifelong diet with only a 75% or 15%, respectively, post-test probability of being celiac.

In most studies, both TGA and EMA are performed as diagnostic tests for CD. We carried forward the post-test odds from the last test as the pretest odds for the next test. Only if one wants to use this serial testing to avoid doing further testing would it be cost-effective to perform both tests. It would be better, otherwise, to perform only the test with the highest negative predictive value to select patients to undergo intestinal biopsy.

Intestinal biopsy, however, should be also performed in patients with symptoms strongly suggesting CD, particularly if they have another risk factor for CD, such as familiarity, even though serology is negative (14). Apart from the false negative of a laboratory test, an explanation in these cases may be that tTG is not the only autoantigen of CD (*e.g.*, immune reaction against actin cytoskeleton has been described in both children and adults with CD) (15).

Intestinal biopsy is so far considered as the gold standard for diagnosis of CD. However, a picture of subtotal villous atrophy, next to a normal or subnormal duodenal morphology may be present. Recently, a pattern of patchy duodenal lesions was observed in all untreated CD patients biopsied up to five times (16). For this reason, it would be advisable to take multiple biopsy samples to detect a picture, which is compatible with diagnosis. On the other hand, the observation of a dynamic morphological lesion in CD, as shown by Marsh and Crowe (17), might allow a diagnosis of CD in the presence of minor morphological changes (10). Patients with latent CD who show normal histology and positive AEA while taking a gluten-containing diet, and who later on develop a severe villous atrophy which recovers on a gluten-free diet, have been reported in another study (18). Some observations might suggest considering serology as the gold standard of CD because a gluten-related autoimmune disorder might take place before a severe enteropathy occurs. In this regard, Maki *et al.* showed how reticulin autoantibody negative subjects became antibody positive, followed in several cases by CD (19), in insulin-dependent diabetes mellitus patients. Moreover, in a prospective study, Ventura *et al.* reported that patients with CD have a high prevalence of both insulin-dependent diabetes mellitus and thyroid-related serum autoantibodies and that these autoantibodies would seem to be gluten dependent because they disappear as a result of a gluten-free diet (20). Of our five patients positive for both TGA and AEA without duodenal histology compatible with CD, four had celiac-type HLA. For these patients, a regular surveillance for CD would be advisable, and further investigations (*e.g.*, an increase in $\gamma\delta^+$ intraepi-

thelial lymphocytes) might indicate a CD in its early stage (21).

In conclusion, our study suggests that in some cases intestinal biopsy could be avoided. These cases are those with a classic clinical picture of disease or with possible gluten-related autoimmune disorders with a positive serology for CD in which a severe enteropathy may not develop. The classic example is dermatitis herpetiformis where the gluten-induced skin disease may develop in genetically susceptible subjects without enteropathy, but with a high density of γ/δ T cells in normal mucosa (22).

A situation analogous to that of the skin in dermatitis herpetiformis is the cerebellum or the peripheral nerves in gluten ataxia, where the neurological dysfunction may not only precede CD but may also be its only manifestation (23).

This discovery allows us to shift the emphasis from the gut as the sole protagonist in CD and to adopt Marsh's definition of gluten sensitivity as "a state of heightened immunological responsiveness to ingested gluten in genetically susceptible individuals" (24).

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Original Contribution

Chlordecone Exposure, Length of Gestation, and Risk of Preterm Birth

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Persistent organic pollutants have not been conclusively associated with length of gestation or with preterm birth. Chlordecone is an organochlorine pesticide that has been extensively used to control the banana root borer population in the French West Indies. Data from the Timoun Mother–Child Cohort Study conducted in Guadeloupe between 2004 and 2007 were used to examine the associations of chlordecone concentrations in maternal plasma with the length of gestation and the rate preterm birth in 818 pregnant women. Data were analyzed using multivariate linear regression for length of gestation and a Cox model for preterm birth. The median plasma chlordecone concentration was 0.39 μg/L (interquartile range, 0.18–0.83). No correlation was observed with plasma concentrations of p,p′-dichlorodiphenyl dichloroethene ($\rho = 0.017$) or polychlorinated biphenyl 153 ($\rho = -0.016$), the other main organochlorine compounds detected. A 1-$\log_{10}$ increase in chlordecone concentration was associated with a decreased length of gestation (−0.27 weeks; 95% confidence interval: −0.50, −0.03) and an increased risk of preterm birth (60%; 95% confidence interval: 10, 130). These associations may result from the estrogen-like and progestin-like properties of chlordecone. These results are of public health relevance because of the prolonged persistence of chlordecone in the environment and the high background rate of preterm births in this population.

chlordecone; French West Indies; length of gestation; persistent organic pollutants; pesticides; preterm birth

Abbreviations: DDE, p,p′-dichlorodiphenyl dichloroethene; LOD, limit of detection.

Editor's note: *An invited commentary on this article appears on page 545, and the authors' response appears on page 548.*

Organochlorines are manmade chemicals that are persistent and are still present in the environment despite no longer being used. These chemicals are considered to pose a threat to human health, particularly during periods of increased sensitivity, such as gestation (1, 2). A number of studies have shown associations between maternal or cord blood concentrations of p,p′-dichlorodiphenyl dichloroethene (DDE), the main metabolite of dichlorodiphenyl trichloroethane, and decreased duration of gestation and/or increased risk of preterm birth. The largest study conducted to date included 2,380 children from an early pregnancy cohort set up in the United States in the 1960s, and it showed a dose-response relationship between the maternal blood concentration of DDE and preterm birth (3). More contemporary studies with fewer participants and lower DDE concentrations have had conflicting results: Some (4–6), but not all (7–9), showed associations between DDE exposure and adverse outcomes. Several of these studies investigated a number of other organochlorines simultaneously. Increased risks of preterm birth associated with hexachlorobenzene levels have been described in 2 studies (8, 10), and associations with hexachlorocyclohexane (mainly the β isomer) were described in 2 others (4, 8).

Chlordecone (kepone) is an organochlorine insecticide principally used for control of the banana root borer in Central and South America and the Caribbean, including Puerto Rico (11). It was initially manufactured in the United States, but production and use were banned there in 1976. Subsequently, it was produced in Brazil and intensively used in

banana fields in the French West Indies from 1981 to 1993. It is highly persistent in the environment; there is no significant biotic or abiotic degradation. Some soils in current and previous banana fields and some waterways are permanently polluted (12, 13). Currently, human exposure to chlordecone in the French West Indies results mainly from the consumption of contaminated food, especially seafood, root vegetables, and cucurbitaceae, as assessed by studies in the general population (14) and among pregnant women (15).

The toxicity of chlordecone to humans was discovered in 1975 after a poisoning episode in pesticide plant workers in the industrial city of Hopewell, Virginia. Male workers showed evidence of sustained toxicity involving the nervous system, liver, and testes (16–18). Additional studies on animals revealed that pregestational or gestational exposure of rats and mice to chlordecone affected embryo implantation and both prenatal and postnatal development (19). The mechanism of the toxic action of chlordecone is not completely understood. It has been suggested that its hormonal properties may be involved. Chlordecone is an endocrine disruptor with estrogen-like and progestin-like characteristics both in vitro and in vivo (20–24).

Experimental studies have documented the toxic effects of chlordecone exposure during gestation in animals, but there has been no epidemiologic study to determine whether chlordecone has similar consequences in humans at environmentally relevant exposure levels. The Timoun Mother–Child Cohort Study was established to investigate the impact on pregnancy and child development of prenatal exposure to the widespread chlordecone pollution in the French West Indies. Here, we report the relationships between maternal exposure to chlordecone and both the length of gestation and the risk of preterm birth.

METHODS

Study population

The present study was conducted in Guadeloupe (part of the French West Indies), an archipelago situated in the Caribbean Sea with a population of 450,000 inhabitants who are mostly of African descent. From December 2004 to December 2007, there were 1,068 women enrolled in a prospective epidemiologic mother-child cohort study. Women were enrolled during third-trimester check-up visits at public health centers (University Hospital of Pointe-à-Pitre, General Hospital of Basse-Terre, and antenatal care units). To be eligible, participants had to have resided in Guadeloupe for more than 3 years. The proportion of refusal was approximately 7%, and the most common reasons were refusal of the spouse, not wishing to participate in the follow-up, and not wishing to provide biological samples. The study was approved by the Guadeloupean Ethics Committee for studies involving human subjects, and detailed informed consent was obtained from each woman.

At enrollment, the participants answered a standardized questionnaire during a face-to-face interviews with trained midwives. The questionnaire covered sociodemographic characteristics, medical and obstetrical history, and lifestyle factors. Alcohol consumption during pregnancy was ascertained on 2 occasions: during the interview at enrollment and after delivery. After delivery, the medical history of the pregnancy and delivery information were collected from midwives, pediatricians, and hospital medical records. Cases involving multiple births ($n = 29$), terminations of pregnancy for fetal abnormality ($n = 2$), and stillbirths ($n = 4$) were excluded, resulting in a study sample of 1,033 singleton live births. From these eligible cases, maternal blood samples were obtained at delivery for 818 women.

Outcomes

Gestational age in weeks was estimated by the obstetricians in charge of follow-up. It was based on the first day of the last menstrual period and was confirmed or corrected by ultrasound. Data were available for 97% of the pregnancies. Preterm birth was defined as a birth before 37 completed weeks of gestation (25). We distinguished between spontaneous and medically induced births. Induced births included all cases of induced labor and of caesarean delivery before the onset of labor. Elective induced labor was not practiced at the delivery centers involved in this study.

Exposure assessment

Maternal blood samples were collected into spray-coated dipotassium ethylenediaminetetraacetic acid tubes. After centrifugation, plasma samples were stored at −20°C. Samples were identified using only a sample code. They were transferred by airmail on dry ice to Liege, Belgium, for organochlorine and lipid analysis.

Determination of chlordecone was done by the Center for Analytical and Research Technology at Liege University in Belgium. The preparation of samples and the quantification method have been previously described (26–28). Details are provided in Web Appendix 1, available at http://aje.oxfordjournals.org/. The limit of detection (LOD) was set at 3 times the background noise of the chromatogram and was thus 0.06 μg/L. Samples from a subgroup of 358 women who delivered after 37 weeks of gestation and whose children were enrolled for neurodevelopmental follow-up were also assayed to assess DDE and polychlorinated biphenyl 153 levels (26). The LODs for both compounds were 0.05 μg/L. Total cholesterol and triglyceride concentrations in plasma were determined using standard enzymatic procedures (DiaSys Diagnostic Systems GmbH, Holzheim, Germany), and total lipid concentrations were calculated as described previously (29).

Data and statistical analysis

Exposure was defined according to the chlordecone concentration in maternal plasma (modeled on a wet-weight basis) and was categorized in quintiles. A decimal log-transformed continuous exposure variable was also used. Multiple imputations were implemented to impute chlordecone concentrations below the LOD. The likelihood method was used (30) under the assumption that chlordecone is log-normally distributed. Five data sets with imputed concentrations below the LOD were generated. Modeling of associations with the length of gestation and the risk of preterm birth was performed within each set, and the results were combined

Table 1. Characteristics of Study Participants ($n = 818$), Length of Gestation, and Rate of Preterm Births ($n = 115$), Timoun Mother–Child Cohort Study, Guadeloupe, French West Indies, 2004–2007

	Births		Length of Gestation, weeks		Rate of Preterm Birth[a]	
	No.	%	Mean (SD)	*P* Value[b]	%	*P* Value[c]
Maternal age, years				0.01		0.25
<20	62	7.6	38.5 (1.4)		11.3	
20–24	118	14.4	38.5 (1.7)		14.4	
25–29	161	19.7	38.4 (1.8)		10.6	
30–34	214	26.2	38.4 (1.8)		13.5	
≥35	263	32.2	37.9 (2.0)		17.1	
Maternal place of birth				0.05		0.24
French West Indies[d]	634	77.5	38.2 (1.9)		15.0	
Other Caribbean Islands	90	11.0	38.4 (1.4)		11.1	
Europe	94	11.5	38.6 (1.7)		10.6	
Parity				0.10		0.76
0	301	36.8	38.4 (1.7)		13.0	
1	252	30.8	38.3 (1.9)		14.3	
≥2	265	32.4	38.1 (1.9)		15.1	
Marital status				0.09		0.12
Single	206	25.9	38.1 (2.0)		16.0	
Married or in a couple	429	53.8	38.4 (1.7)		11.7	
Living with own family	162	20.3	38.2 (1.9)		16.7	
Maternal education, years				0.69		0.96
<5	47	5.7	38.0 (1.7)		14.9	
5–12	573	70.1	38.3 (1.9)		13.8	
>12	198	24.2	38.3 (1.6)		14.7	
Body mass index[e]				0.02		0.20
<18.5	54	6.7	38.1 (1.4)		11.1	
18.5–24.9	421	52.5	38.5 (1.6)		11.6	
25–29.9	180	22.4	38.0 (2.2)		17.8	
≥30	147	18.3	38.2 (2.0)		15.7	
Smoking during pregnancy				0.23		0.25
Yes	47	5.8	38.6 (1.5)		10.6	
No	771	94.2	38.2 (1.8)		14.3	
Alcohol during pregnancy				0.37		0.88
Yes	23	2.9	38.6 (1.6)		13.0	
No	760	97.1	38.3 (1.7)		13.8	

Table continues

using the Proc MIANALYZE procedure in SAS (SAS Institute, Inc., Cary, North Carolina) according to the methodology described by Rubin (31) and Little and Rubin (32). Because the LOD was below the first quintile of the distribution, the multiple imputation exclusively applied to analyses based on the chlordecone concentration as a continuous predictor.

We collected information on socioeconomic and lifestyle characteristics, including maternal place of birth (French West Indies, other Caribbean islands, or Europe), enrollment site (university hospital (Pointe à Pitre), local hospital (Basse-Terre), or antenatal care units), age (<20, 20–24, 25–29, 30–34, or ≥35 years), years of education (<5, 5–12, or >12 years), smoking during pregnancy (yes or no), and alcohol consumption during pregnancy (yes, defined as at least 1 drink during preceding week or 1 binge drinking session during the previous 3 months reported at enrollment or after delivery, or no). We also collected information on marital status, which we then classified into 3 categories corresponding to family profiles common in the French West Indies: single, living with a partner, and single but living with family (an adult member of her family, typically mother, father, sister, or aunt). Medical and reproductive factors recorded included parity (defined as the number of previous viable pregnancies: 0, 1, or ≥2), number of prior preterm births

Table 1. Continued

	Births		Length of Gestation, weeks		Rate of Preterm Birth[a]	
	No.	%	Mean (SD)	*P* Value[b]	%	*P* Value[c]
Timing of first ultrasound				0.38		0.77
<15 gestational weeks	675	84.7	38.2 (1.8)		13.9	
≥15 gestational weeks	122	15.3	38.4 (2.0)		14.8	
High blood pressure during pregnancy				<0.001		<0.001
Yes	99	12.3	37.1 (2.4)		35.4	
No	705	87.7	38.4 (1.7)		11.1	
Gestational diabetes				0.003		0.06
Yes	74	9.3	37.7 (2.0)		18.9	
No	725	90.7	38.3 (1.8)		13.2	
Urinary tract infection				0.05		0.02
Yes	123	15.6	38.0 (1.9)		20.3	
No	667	84.4	38.3 (1.8)		12.4	
Prior preterm birth				0.01		0.04
Yes	80	9.9	37.7 (1.6)		22.5	
No	725	90.1	38.3 (1.8)		13.4	
Prior miscarriage				0.0001		0.02
Yes	212	25.9	37.8 (1.9)		19.3	
No	605	74.1	38.4 (1.8)		12.2	
Asthma				0.56		0.13
Yes	83	10.2	38.2 (1.9)		19.3	
No	732	89.8	38.3 (1.8)		13.5	
Enrollment site				0.06		0.07
University hospital	558	68.2	38.2 (1.9)		15.6	
Local hospital	186	22.7	38.5 (1.6)		9.7	
Local antenatal care clinic	74	9.1	38.3 (1.4)		13.5	
Sex of the newborn						
Female	404	49.4	38.4 (1.8)	0.05	12.4	0.20
Male	414	50.6	38.1 (1.9)		15.7	

Abbreviation: SD, standard deviation.
[a] Birth before 37 complete weeks of gestation.
[b] Two-sided *P* value for differences between means (*t* test).
[c] Two-sided *P* value for differences between rates (χ^2 test).
[d] Guadeloupe and Martinique.
[e] Weight (kg)/height $(m)^2$.

(0, 1, or ≥2), number of prior miscarriages (0, 1, or ≥2), timing of first ultrasound (before 15 weeks of gestation or at 15 weeks or more), sex of the newborn (male or female), self-reported body mass index (weight (kg)/height $(m)^2$) before pregnancy, and presence of maternal disease (particularly high blood pressure) during pregnancy, including preeclampsia (yes or no), gestational diabetes (yes or no), urinary tract infection during pregnancy (yes or no), and asthma (yes or no). Body mass index was classified into 4 groups: underweight (<18.5), normal weight (18.5–24.9), overweight (25–29.9), and obese (≥30). The rates of missing data for covariates varied from none to 2.6%.

Linear regression models were used to evaluate the relationship between chlordecone exposure and the length of gestation. The regression coefficient (β) represented a difference of 1 week in the length of gestation per chlordecone exposure category or per decimal log unit of the transformed plasma chlordecone concentration. To describe the shape of the dose-response relationship, we examined splines (restricted cubic spline functions with 3 knots at the quartiles of the distribution) using the SAS macro developed by Desquilbet and Mariotti (33). This approach allowed identification of the linear and/or nonlinear components of the relationship. Associations between maternal exposure and the risk of preterm birth were estimated by calculating a hazard ratio using a Cox model that accounted for the left truncation that is a possible consequence of inclusion of participants during late pregnancy (34).

Although many risk factors for preterm births have been clearly established, the potential link between chlordecone exposure and these risk factors has not been thoroughly studied; this is due in part to the very small number of studies of this exposure in humans. Using current knowledge on preterm birth risk factors and the relationship between chlordecone exposure and these factors (Web Table 1), we constructed a directed acyclic graph for preterm birth (Web Figure 1). History of preterm births was not considered as a confounder because it reflects an unmeasured degree of vulnerability and simultaneously may be a consequence of previous exposure (if any association exists); therefore, it appears as a collider. Our data provided no evidence of an association between parity and levels of chlordecone (Web Table 1). This is consistent with the pharmacokinetics of chlordecone: Unlike most other organochlorines, chlordecone is not significantly stored in fat tissues, and plasma levels are therefore not significantly affected by the lipid tissue redistribution that occurs during pregnancy and breastfeeding. The minimally sufficient set of adjustment variables based on the proposed directed acyclic graph included maternal enrollment site, place of birth, marital status, educational level, age, body mass index before pregnancy, and high blood pressure during pregnancy. We conducted sensitivity analyses that included additional adjustment of parity and analyses that included adjustment for gestational diabetes, as well as analyses in which we excluded blood pressure.

In the subgroup of 358 women in whom DDE and polychlorinated biphenyl 153 were measured, the Spearman's rank correlations coefficient between chlordecone and DDE was 0.017 ($P = 0.75$), and that between chlordecone and polychlorinated biphenyl 153 was −0.016 ($P = 0.76$). Therefore, DDE and polychlorinated biphenyl 153 were not considered to be confounders.

Lipophilic molecule concentrations are routinely expressed on a per-unit serum lipid basis, but this approach is prone to bias (35). Furthermore, a substantial portion of the chlordecone in blood is associated with proteins (mainly albumin) and high-density lipoproteins (36), whereas most other organochlorine compounds are associated with low-density lipoproteins and very high-density lipoproteins; consequently, expressing the chlordecone concentration on a per-unit serum lipid basis may be misleading. Nevertheless, to account for the residual chlordecone fraction transported by lipids, we considered total lipids in the adjusted models.

We also considered a possible interaction between chlordecone exposure and the sex of the newborn in relation to length of gestation and the risk of preterm birth. All statistical analyses were performed using SAS, version 9.3.

RESULTS

Most of the women in our study were born on Caribbean islands (primarily the French West Indies) where most inhabitants are of African descent, and most were multiparous (Table 1). Approximately two thirds were enrolled into the study at the University Hospital, and approximately two fifths were overweight or obese before pregnancy. Very few women reported smoking (5.8%) or alcohol consumption (2.9%) during pregnancy. The mean duration of gestation was 38.3 weeks, and 14.1% of the children ($n = 115$) were born preterm. Among the preterm births, 55 (47.8%) were spontaneous preterm births and 59 (51.3%) were medically induced preterm births; for 1 woman, the type of preterm birth could not be traced. Gestational age at enrollment (mean, 27.2 weeks) did not differ between spontaneous and induced preterm births and term births. Rates of detection and concentrations of organochlorine compounds in the plasma of a subsample of the study population are presented in Table 2.

We studied the shape of the dose-response relationship using splines. It appeared that the shape of the curve presented a significant decreasing linear component ($P = 0.03$), with the nonlinear component not being significant ($P = 0.39$) (Web Figure 2).

Table 3 shows the results of linear regression analysis for maternal chlordecone levels in relation to length of gestation. In the adjusted model, there was a statistically significant shorter length of gestation in the 2 highest quintiles of exposure. When studying exposure as a continuous variable, we found that higher maternal chlordecone levels were statistically significantly associated with a shorter length of gestation in adjusted models.

Table 2. Detection and Concentration of Organochlorine Compounds in Maternal Plasma Among Timoun Mother–Child Cohort Study Participants, Guadeloupe, French West Indies, 2004–2007

Type of Organochlorine by Group	No. of Births	Detection Frequency, %	Geometric Mean, µg/L	Minimum, µg/L	Percentile, µg/L					Maximum, µg/L
					5th	25th	50th	75th	95th	
All women										
Chlordecone	818	88.4	0.35	<LOD	<LOD	0.18	0.39	0.83	2.50	19.7
Subgroup[a]										
Chlordecone	358	92.2	0.34	<LOD	<LOD	0.19	0.40	0.88	2.42	19.3
DDE	358	86.0	0.63	<LOD	<LOD	0.22	0.59	1.26	4.10	24.5
PCB 153	358	87.7	0.18	<LOD	<LOD	0.09	0.18	0.34	0.94	1.95

Abbreviations: DDE, p,p′-dichlorodiphenyl dichloroethene; LOD, limit of detection; PCB 153, polychlorinated biphenyl 153.

[a] Subgroup of 358 women who delivered after 37 weeks of gestation and whose children were enrolled for neurodevelopmental follow-up.

Table 3. Crude and Adjusted Regression Coefficients for Length of Gestation According to Chlordecone Concentration in Maternal Blood Among Timoun Mother–Child Cohort Study Participants, Guadeloupe, French West Indies, 2004–2007

Chlordecone Level, µg/L	No. of Births	Crude		Adjusted[a]	
		β	95% CI	β	95% CI
<0.14	163	0.0	Referent	0.00	Referent
0.14–0.28	165	−0.10	−0.50, 0.30	−0.26	−0.66, 0.13
0.29–0.51	162	−0.10	−0.50, 0.30	−0.23	−0.62, 0.16
0.52–0.97	165	−0.31	−0.71, 0.30	−0.60	−0.99, −0.20
>0.98	163	−0.25	−0.65, 0.15	−0.48	−0.88, −0.07
Log_{10} chlordecone	818	−0.11	−0.34, 0.11	−0.27	−0.50, −0.03

Abbreviation: CI, confidence interval.

[a] The covariates for which we adjusted were maternal age, place of birth, enrollment site, marital status, educational level, body mass index, high blood pressure during pregnancy, and total plasma lipid level (g/L). The analysis was conducted on 767 complete cases.

When all births were considered, the adjusted hazard ratio of preterm birth appeared to be statistically significantly increased for the fourth (hazard ratio = 3.1, 95% confidence interval: 1.6, 6.0) and fifth (hazard ratio = 2.2, 95% confidence interval: 1.1, 4.5) quintiles (Table 4). Expressing exposure as a continuous variable led to maternal chlordecone levels being statistically significantly associated with a higher hazard ratio for preterm birth in the adjusted model. Similar associations were observed when the analyses were done according to the mode of onset of labor (Table 4).

The findings remained unchanged when parity or gestational diabetes was included in the full model (Web Table 2). However, the strength of the associations decreased when high blood pressure was excluded from the model (Web Table 2). Women with high blood pressure during pregnancy appeared to have lower chlordecone blood concentrations than did other women (Web Table 1).

Women for whom we were not able to measure chlordecone concentration ($n = 215$) were more often recruited in the local hospital of Basse-Terre, which is in the most contaminated area in Guadeloupe. They tended to have shorter lengths of gestation (38.0 weeks vs. 38.3 weeks; $P = 0.04$) and a higher prevalence of preterm births (19.1% vs. 14.1%; $P = 0.07$) than did women for whom blood samples were available. Sensitivity analyses imputing expected median values of chlordecone according to major determinants of chlordecone exposure had only small impact on risk estimates (Web Table 3). Despite the interaction between chlordecone exposure and the sex of the newborn not being statistically significant ($P > 0.2$) in relation to length of gestation and the risk of preterm birth, the stratified results suggest a higher level of association among girls than boys (Web Table 4).

DISCUSSION

We examined the association between levels of chlordecone in maternal plasma and the duration of pregnancy in a population of pregnant women in Guadeloupe. We observed that greater maternal exposure to chlordecone was associated with shorter gestation. In addition, higher levels of exposure were associated with an increased risk of preterm birth in a dose-response–type association for all preterm births and for all modes of labor.

The present epidemiologic study is one of the few in which the association between chlordecone at environmentally relevant exposure levels and human health was examined (27, 37, 38). Local soil pollution by chlordecone (20% of agricultural lands) caused by its use in banana plantations between 1973 and 1993 has led to the contamination of root and cucurbit vegetables and of livestock and poultry that graze on these contaminated areas; costal fish and seafood are also contaminated. These contaminated foodstuffs represent only a fraction of what is consumed in the French West Indies, but they explain the chronic contamination of the population. Regulatory measures to restrict commercialization of those food products that exceed threshold limit values of contamination have been implemented to reduce human exposure, but these measures only apply to licensed farms and fishermen. Indeed, there is an extensive informal network of production, distribution, and sale of foodstuffs, as well as families and individuals who produce food for their own consumption. These chains of supply are not adequately controlled.

Chlordecone has not been measured in populations other than those in the French West Indies (27, 37, 38) and Hopewell, Virginia (16, 17). The poisoning episode that affected pesticide plant workers in Hopewell (most of whom were men) during the mid-1970s led to a very high exposure rate, resulting in blood concentrations 100 to 1,000 times greater than those currently observed in the population of the French West Indies. Chlordecone exposure was also measured in adult Hopewell residents during the mid-1970s, and the serum concentrations in subjects with detectable values (at that time the LOD was around 1.5 µg/L) was between 5–32 µg/L (16), a range similar to those currently observed in the population of the French West Indies.

The strengths of the present study include its prospective design, the evaluation of exposure based on determinations of the chlordecone concentration in maternal plasma, and the consideration of co-exposures to other organochlorine compounds (DDE and polychlorinated biphenyl 153). Single

Table 4. Crude and Adjusted Hazard Ratios for Risk of Preterm Birth According to Chlordecone Concentration in Maternal Blood and Mode of Onset of Labor, Timoun Mother–Child Cohort Study, Guadeloupe, French West Indies, 2004–2007

Chlordecone Level by Birth Category, μg/L	No. of Births	No. of PTB[b]	Crude		Adjusted[a]	
			HR	95% CI	HR	95% CI
All births						
<0.14	163	16	1.0	Referent	1.0	Referent
0.14–0.28	165	21	1.5	0.8, 2.8	1.5	0.7, 3.1
0.29–0.51	162	23	1.6	0.8, 3.0	1.6	0.8, 3.1
0.52–0.97	165	30	2.1	1.1, 4.0	3.1	1.6, 6.0
>0.98	163	25	1.7	0.9, 3.3	2.2	1.1, 4.5
Log_{10} chlordecone	818	115	1.3	0.9, 1.9	1.6	1.1, 2.3
Spontaneous preterm and term births						
<0.14	154	7	1.0	Referent	1.0	Referent
0.14–0.28	151	10	1.5	0.6, 4.1	1.4	0.5, 4.0
0.29–0.51	145	6	1.0	0.3, 2.9	1.1	0.3, 3.5
0.52–0.97	154	16	2.6	1.1, 6.6	3.5	1.3, 9.8
>0.98	154	16	2.6	1.1, 6.6	2.7	0.9, 7.8
Log_{10} chlordecone	758	55	1.7	1.1, 2.8	1.8	1.0, 3.3
Induced preterm and term births						
<0.14	147	8	1.0	Referent	1.0	Referent
0.14–0.28	149	11	1.7	0.7, 4.3	2.2	0.7, 6.2
0.29–0.51	156	17	2.3	1.0, 5.6	2.6	1.0, 6.5
0.52–0.97	155	14	2.1	0.9, 5.2	3.3	1.3, 8.7
>0.98	155	9	1.3	0.5, 3.6	2.2	0.8, 6.4
Log10 chlordecone	762	59	1.1	0.7, 1.8	1.5	0.9, 2.6

Abbreviations: CI, confidence interval; HR, hazard ratio; PTB, preterm birth.

[a] The covariates for which we adjusted were maternal age, place of birth, enrollment site, marital status, educational level, body mass index, high blood pressure during pregnancy, and total plasma lipid level (g/L). The analysis was conducted on 750 complete cases for all births, 694 spontaneous preterm births, and 703 induced preterm births.

[b] For one woman, the type of preterm birth was unknown.

determinations of plasma chlordecone concentration provide an accurate reflection of the load of this compound in the body (17, 39). Its half-life in blood is approximately 6 months (17), so a single measure at the end of pregnancy can be considered to be reasonably representative of exposure throughout the pregnancy.

Our study also has some limitations. The prevalence of preterm birth in our study population was 14.1%, consistent with the high rate of preterm births among populations of African descent irrespective of their geographic location (40, 41). Nevertheless, we may have overestimated this rate because recruitment was mainly at the University Hospital, and this favors inclusion of pregnant women at risk of preterm birth. This also explains the high proportion of induced preterm births compared with spontaneous preterm births. However, any such oversampling should not impair internal comparisons. Also, the models for linear and logistic regression were adjusted for site of enrollment. There were small differences in some population descriptors between the subgroups with and without plasma chlordecone determinations, but sensitivity analyses indicated that these missing data were only weakly associated with risk (Web Table 3). In our study population, high blood pressure during pregnancy is associated with lower chlordecone blood concentrations, and we have no immediate explanation for this finding. The mechanisms through which chlordecone may lower the risk of high blood pressure during pregnancy, the possible influence of high blood pressure on chlordecone concentration in blood, or the influence of unknown confounders in this association remain to be studied. Inclusion of high blood pressure during pregnancy as a variable in the adjusted model reinforces the association between chlordecone exposure and risk of preterm birth.

We are aware that estimates of gestational age based on first trimester ultrasound may be biased if environmental exposure affects early fetal growth (42), although in practice the consequences of this problem are generally limited (43). Moreover, toxicological studies in rats and mice have indicated that chlordecone exposure during gestation, at doses that do not cause maternal toxicity, was not associated with low fetal growth (44).

Parturition is triggered by the shortening and dilatation of the cervix associated with uterine contractions. Progesterone plays a key role in maintaining pregnancy. In humans,

progesterone-receptor–dependent events, rather than circulating progesterone levels, appear to be critical (45). For example, treatment of pregnant women with progesterone-receptor antagonists induces labor at any stage of pregnancy (46). Chlordecone binds estrogen receptors α and β, acting as an agonist of estrogen receptor α and an antagonist of estrogen receptor β (47, 48). It also stimulates the synthesis of the progesterone receptors in rat uterine tissues in vivo (21), and this process is mediated by estrogen receptors. Chlordecone can inhibit the binding of the progestin agonist R5020 to the form A of the progesterone receptor in vitro (22). These observations suggest that the observed associations between exposure to chlordecone and both decreased gestational length and increased risk of preterm birth are due to the estrogenic and/or progestin activities of chlordecone.

In summary, the data that we report provide some evidence that chlordecone, a chemical with well-established estrogen-like and progestin-like properties, may affect the duration of gestation (with gestation at least 3 days shorter for the 40% of women with blood chlordecone levels >0.52 μg/L) and thus increase the risk of preterm birth. These findings were observed in a population with a high baseline risk of preterm birth. The association was detectable even though the concentrations of chlordecone in the blood were in the order of magnitude of 1 part per billion. Further efforts are required to protect pregnant women against exposure to chlordecone, particularly from its major dietary sources.

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ARTICLE

Coffee Consumption and Prostate Cancer Risk and Progression in the Health Professionals Follow-up Study

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Background Coffee contains many biologically active compounds, including caffeine and phenolic acids, that have potent antioxidant activity and can affect glucose metabolism and sex hormone levels. Because of these biological activities, coffee may be associated with a reduced risk of prostate cancer.

Methods We conducted a prospective analysis of 47 911 men in the Health Professionals Follow-up Study who reported intake of regular and decaffeinated coffee in 1986 and every 4 years thereafter. From 1986 to 2006, 5035 patients with prostate cancer were identified, including 642 patients with lethal prostate cancers, defined as fatal or metastatic. We used Cox proportional hazards models to assess the association between coffee and prostate cancer, adjusting for potential confounding by smoking, obesity, and other variables. All *P* values were from two-sided tests.

Results The average intake of coffee in 1986 was 1.9 cups per day. Men who consumed six or more cups per day had a lower adjusted relative risk for overall prostate cancer compared with nondrinkers (RR = 0.82, 95% confidence interval [CI] = 0.68 to 0.98, P_{trend} = .10). The association was stronger for lethal prostate cancer (consumers of more than six cups of coffee per day: RR = 0.40, 95% CI = 0.22 to 0.75, P_{trend} = .03). Coffee consumption was not associated with the risk of nonadvanced or low-grade cancers and was only weakly inversely associated with high-grade cancer. The inverse association with lethal cancer was similar for regular and decaffeinated coffee (each one cup per day increment: RR = 0.94, 95% CI = 0.88 to 1.01, *P* = .08 for regular coffee and RR = 0.91, 95% CI = 0.83 to 1.00, *P* = .05 for decaffeinated coffee). The age-adjusted incidence rates for men who had the highest (≥6 cups per day) and lowest (no coffee) coffee consumption were 425 and 519 total prostate cancers, respectively, per 100 000 person-years and 34 and 79 lethal prostate cancers, respectively, per 100 000 person-years.

Conclusions We observed a strong inverse association between coffee consumption and risk of lethal prostate cancer. The association appears to be related to non-caffeine components of coffee.

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Coffee contains diverse biologically active compounds that include caffeine, minerals, and phytochemicals. Long-term coffee drinking has been associated with improved glucose metabolism and insulin secretion in observational and animal studies (1). Coffee is also a potent antioxidant (2–4) and may be associated with sex hormone levels (5–7).

Coffee consumption has been consistently associated with a reduced risk of type 2 diabetes (8), and its effects on insulin, sex hormones, and antioxidants may also be relevant to prostate cancer. We hypothesized that coffee may be associated with lower risk of more advanced prostate cancers because the associations of insulin, antioxidants, and androgens with incidence of prostate cancer are stronger for advanced disease than for overall disease (9–15).

Epidemiological studies of coffee consumption and prostate cancer have generally reported null results (16–30), although most lacked a wide range of coffee intakes and a large number of case subjects and none specifically examined advanced disease. The two studies of coffee consumption and prostate cancer mortality (31,32) found no statistically significant associations, but these were limited by a narrow range of intake, small number of cancer deaths, and inadequate adjustment for potential confounding.

We investigated the relationship between coffee intake and risk of overall prostate cancer and of aggressive disease, defined as lethal, advanced, or high-grade cancer, in the Health Professionals Follow-up Study.

Methods

The Health Professionals Follow-up Study is a prospective cohort study of 51 529 male health professionals in the United States aged 40–75 years at baseline in 1986. The men are followed through biennial questionnaires to update information on lifestyle and health outcomes, and usual diet has been assessed every 4 years.

Men who completed the baseline food frequency questionnaire (FFQ) in 1986 form the study population for this analysis (N = 49 911). We excluded men who had an implausible energy intake (<800 or >4200 kcal/day) or who left more than 70 food items blank on the baseline FFQ. We also excluded men who reported a diagnosis of cancer (except nonmelanoma skin cancer) before baseline (N = 2000). This left a total of 47 911 men who were followed prospectively for cancer incidence until 2006 and for metastases and mortality outcomes until 2008. The Health Professionals Follow-up Study is approved by the Human Subjects Committee at the Harvard School of Public Health.

Assessment of Coffee Intake

Updated dietary data, including coffee consumption, was available from FFQs, which reported on intake of over 130 food items at baseline in 1986, and again in 1990, 1994, 1998, and 2002. Participants were asked how frequently they had consumed a specified portion size of each item over the previous year, with nine possible responses ranging from "never or less than once a month" to "six or more times per day." The FFQ included questions concerning cups of decaffeinated and regular coffee intake. A validation study in this cohort found a high correlation (r = 0.93) between participants' reports of coffee intake on the FFQ compared with two week-long diet records (33).

Ascertainment and Classification of Subjects Who Developed Prostate Cancer

Prostate cancer diagnoses were initially identified by self-reports from the participants or their next of kin on the biennial questionnaires and then confirmed by review of medical records and pathology reports. Deaths in the cohort were ascertained through reports from family members and searches of the National Death Index. Underlying cause of death was assigned by an endpoints committee based on all available data including medical history, medical records, registry information, and death certificates. Approximately 90% of prostate cancer patients were documented by medical records; the remaining 10% of men with prostate cancer, based on self-reports or death certificates, were included because the reporting of prostate cancer was highly accurate (>98%) among men with available medical records. We followed men with prostate cancer starting in 2000 with an additional prostate cancer–specific questionnaire separate from the regular Health Professionals Follow-up Study questionnaire every year to ascertain disease progression and diagnosis of metastases.

We studied total prostate cancer incidence excluding stage T1a cancers, which are discovered incidentally during treatment for benign prostatic hypertrophy. Because of the considerable heterogeneity in the biological potential of prostate cancer, we also examined the data for men with advanced, lethal, or nonadvanced cancers separately to distinguish those patients in whom the cancer was likely to progress clinically. Advanced cancers were those that had spread beyond the prostate, including to the seminal vesicle, lymph nodes, or bone. This category included men with stage T3b, T4, N1, or M1 prostate cancer at diagnosis, men who developed lymph node or distant metastases, and men who died of prostate cancer before the end of follow-up. Lethal cancers, a subset of advanced cancers, were those that caused death or metastasis to bone before the end of follow-up. Nonadvanced cancers were stage T1 or T2 and N0 and M0 at diagnosis and did not progress to lymph node or distant metastases or death during the follow-up period. (Some cancers that were diagnosed near the end of the follow-up period will be misclassified as nonadvanced because they had less time to progress before the end of follow-up). Cancers were also categorized as high grade (Gleason sum at diagnosis 8–10), grade 7, or low grade (Gleason sum 2–6) at diagnosis based on prostatectomy or biopsy pathology reports; Gleason grade was not available for all men with prostate cancer, particularly for those who were diagnosed earlier in the follow-up period.

Statistical Analysis

Each participant contributed person-time from the date on which he returned the baseline questionnaire in 1986 until prostate cancer diagnosis, death, or the end of the study period, January 31, 2006. Participants were followed for prostate cancer incidence until January 31, 2006, and for death and metastases until January

CONTEXT AND CAVEATS

Prior knowledge

Previous epidemiological studies have generally found no association between coffee consumption and risk of prostate cancer, but all such studies had limitations and none focused on advanced disease.

Study design

The association between coffee intake and risk of prostate cancer was prospectively analyzed among 47 911 men of the Health Professionals Follow-Up Study, who had repeatedly provided nutritional data since 1986. Cancer incidence was followed until 2006 and metastases and mortality until 2008. Relative risks were computed and adjusted for age, smoking, obesity, and other variables.

Contribution

Men who drank six or more cups of coffee per day had a slightly lower adjusted relative risk of prostate cancer and a substantially lower adjusted relative risk of lethal prostate cancer compared with nondrinkers. Both caffeinated and decaffeinated coffee consumption were associated with similarly reduced risks.

Implications

There may be biologically active compounds in coffee that protect against risk of lethal prostate cancer.

Limitations

The results depend on self-reported nutritional data and correction for multiple possible confounders. Coffee consumption was not found to be associated with risk of lower grade prostate cancers.

From the Editors

31, 2008. Participants' data were divided according to levels of total (regular and decaffeinated) coffee intake, and relative risks of prostate cancer were calculated as the incidence rate in a given category of intake divided by the rate in the lowest category, adjusted for age and calendar time.

Because coffee intake may affect carcinogenesis over an extended period, we used the cumulative average intake of coffee to represent long-term dietary intake as our primary measure of exposure. That is, the coffee intake reported by particpants in 1986 was used to compute exposure for the 1986–1990 follow-up period, the average of the intakes reported in 1986 and 1990 was used for the 1990–1994 follow-up period, the average of intakes reported in 1986, 1990, and 1994 was used for the 1994–1998 follow-up period, and so on. In a secondary analysis, we used baseline (1986) coffee intake only. In addition, we used our repeated measures to analyze the effect of latency time (time from exposure to cancer diagnosis) by relating each measure of coffee intake to prostate cancer incidence during specific time periods: 0–4, 4–8, 8–12, and 12–16 years after exposure. Finally, to assess the potential for symptoms of subclinical advanced disease to affect coffee intake (reverse causation), we conducted a secondary analysis using cumulative average intake with a lag of 4 years to avoid using data on coffee consumption from FFQs completed immediately before diagnosis.

We used Cox proportional hazards regression to adjust for potential confounding by prostate cancer risk factors previously identified in this cohort and in other studies. Scaled Schoenfeld residuals were used to test the proportional hazards assumption. Multivariable models were adjusted for race (White, African American, Asian American, other), height (quartiles), body mass index at age 21 (<20, 20 to <22.5, 22.5 to <25, ≥25 kg/m^2), current body mass index (<21, 21 to <23, 23 to <25, 25 to <27.5, 27.5 to <30, ≥30 kg/m^2), vigorous physical activity (quintiles, metabolic equivalents-hours/week), smoking (never, former quit >10 years ago, former quit <10 years ago, current), diabetes (type I or II, yes or no), family history of prostate cancer in father or brother (yes or no), multivitamin use (yes or no), history of prostate-specific antigen (PSA) testing (yes or no, lagged by one period to avoid counting diagnostic PSA tests as screening; collected from 1994 onwards), and intakes of processed meat, tomato sauce, calcium, alpha linolenic acid, supplemental vitamin E, alcohol intake (all quintiles), and energy intake (continuous). All covariates except race, height, and body mass index at age 21 were updated in each questionnaire cycle. To test for a linear trend across categories of intake, we modeled coffee intake as a continuous variable using the median intake for each category.

Because we found associations for total coffee intake, we repeated our analyses for regular and decaffeinated coffee separately, and for caffeine intake to see if the observed associations were related to caffeine or other components of coffee. To investigate possible confounding due to differences in PSA testing, we stratified by time period to determine whether the association between coffee and prostate cancer risk differed in the pre-PSA (1986–1994) and PSA screening eras (1994–2006). All P values were two-sided, with a P value less than .05 considered to be statistically significant. Analyses were performed using SAS version 9.1 (SAS Institute, Inc; Cary, NC).

Results

During 20 years (816 130 person-years) of follow-up, 5035 of 47 911 men were confirmed to have developed prostate cancer. Of these cancers, 642 were lethal, 896 were advanced (642 lethal plus 254 additional extraprostatic cancers), and 3221 were nonadvanced prostate cancers. Two-thirds of all cohort participants consumed at least one cup of coffee per day in 1986, and 5% reported drinking six or more cups daily (Table 1). Men who consumed the most coffee were more likely to be ever-smokers and were less likely to engage in vigorous physical activity (Table 1). Frequency of PSA testing was similar among high and low coffee drinkers, whereas men in the middle categories reported somewhat more testing. High coffee consumption was associated with higher intakes of energy, alcohol, and processed meat, and slightly lower intake of calcium.

We observed a weak inverse association between total coffee intake and incidence of prostate cancer (Table 2). Men who consumed six or more cups per day had an 18% lower risk of prostate cancer compared with men who did not drink coffee (relative risk [RR] = 0.82, 95% confidence interval [CI] = 0.68 to 0.98, $P_{\text{linear trend}}$ = .10). The age-adjusted incidence rates of prostate cancer for the highest (≥6 cups per day) and lowest (no coffee) coffee categories were 425 and 529 cancers per 100 000 person-years, respectively. The data suggested an inverse association between coffee intake and risk of high-grade cancers, although the trends were not statistically significant. Coffee was not associated with low-grade cancer.

The strongest associations were for lethal and advanced prostate cancer (men in the highest intake category vs nondrinkers: RR= 0.40, 95% CI = 0.22 to 0.75, P_{trend} = .03 for lethal cancer; RR = 0.47, 95% CI = 0.28 to 0.77, P_{trend} = .004 for advanced cancer). The age-adjusted incidence rates of lethal prostate cancer for the highest and lowest intake categories were 34 and 79 per 100 000 person-years, respectively. Coffee consumption was not associated with risk of nonadvanced cancers that never progressed beyond the prostate.

For comparison with other studies without repeated measures of diet, we repeated the analyses using baseline rather than cumulative coffee intake. With baseline intake the associations of coffee consumption with lethal and advanced cancers remained statistically significant but were somewhat attenuated (the highest vs lowest coffee consumers in 1986: RR = 0.50, 95% CI = 0.30 to 0.84, P_{trend} = .05 for lethal prostate cancer; RR = 0.54, 95% CI = 0.36 to 0.83, P_{trend} = .03 for advanced disease).

To examine whether urinary symptoms affected coffee intake before diagnosis (reverse causation), we looked cross-sectionally at coffee and lower urinary tract symptoms in 1998. We found that coffee intake did not differ in men with and without urinary symptoms. Men were asked to report the frequency of seven urinary symptoms, and these were combined to create four categories of lower urinary tract symptoms scores that ranged from no or low levels of symptoms to severe levels of symptoms (34). Mean coffee intake in the lowest symptom group was 1.59 cups per day (SD = 1.53) and mean coffee intake in the severe symptoms group was 1.57 (SD = 1.58, P for difference in means = .73). There was also no statistically significant trend in total coffee intake across the four symptom groups ($P_{\text{linear trend}}$ = .20).

Table 1. Age-adjusted characteristics of the Health Professionals Follow-up Study population at baseline in 1986, by coffee consumption*

Characteristic	Category of total coffee intake				
	None (n = 7890)	<1 cup per day (n = 9533)	1–3 cups per day (n = 21 261)	4–5 cups per day (n = 6735)	≥6 cups day (n = 2492)
Mean age, y	52	55	55	54	53
White race, %	95	94	96	98	98
Mean BMI, kg/m^2	25	25	26	26	26
Mean BMI at age 21, kg/m^2	23	23	23	23	23
Mean height, inches	70	70	70	70	70
Former smoker, quit >10 y ago, %	20	28	32	34	31
Former smoker, quit ≤10 y ago, %	6	10	14	17	17
Current smokers, %	4	6	10	16	25
Vigorous activity (% highest quintile)	17	17	15	15	11
Diabetes, %	3	3	3	3	3
Family history of prostate cancer, %	13	11	12	12	12
PSA test, 1994, %	35	39	38	38	33
PSA test, 2004, %	60	64	66	66	58
Mean dietary intakes					
Energy, kcal/d	1960	1895	1990	2069	2159
Alcohol, g/d	6	9	13	14	15
Calcium, mg/d	973	931	866	881	873
Alpha-linolenic acid, g/d	1.1	1.1	1.1	1.1	1.1
Supplemental vitamin E, mg/day	41.5	45.1	36.1	33.3	33.0
Multivitamin use, %	42	44	41	40	38
Processed meat, servings per week	2.1	2.1	2.6	2.9	3.4
Tomato sauce, servings per week	0.9	0.9	0.9	0.9	0.9
Total coffee, servings per day	0.0	0.5	1.9	4.2	6.3
Regular coffee, servings per day	0.0	0.2	1.3	2.9	4.2
Decaffeinated coffee, servings per day	0.0	0.2	0.6	1.3	2.0

* All variables (except age) are standardized to the age distribution of the cohort at baseline. BMI = body mass index; PSA = prostate-specific antigen.

In addition, we conducted several other analyses to assess possible reverse causation. Results for lethal and advanced cancers were similar when men with distant metastases or unknown metastasis status at diagnosis were excluded (data not shown). We also studied the subset of men with advanced cancers that were localized at diagnosis (stage <T3b) and spread only later (n = 294), because these cancers were less likely to be symptomatic before diagnosis than cancers that were diagnosed at an advanced stage. The risk was similar to that seen for all men with advanced cancers (RR = 0.20, 95% CI = 0.06 to 0.65, P_{trend} = .02). We also examined coffee intake with a 4-year lag period between exposure and outcome to avoid using the FFQ data that were collected immediately before diagnosis. The associations with lethal and advanced cancers were somewhat attenuated but remained statistically significant using this exposure measure (RR = 0.58, 95% CI = 0.33 to 1.01, P_{trend} = .02 for lethal cancer; RR = 0.59, 95% CI = 037 to 0.94, P_{trend} = .008 for advanced cancer).

To examine whether confounding by PSA testing might explain our findings, we stratified by time period and evaluated the pre-PSA and PSA eras separately. The association of coffee and advanced disease was similar in both time periods (for 1986–1994, RR of advanced cancer for the highest vs lowest categories of intake = 0.41 [95% CI = 0.20 to 0.85, P_{trend} = .06]; for 1994–2006, RR = 0.53 [95% CI = 0.26 to 1.05, P_{trend} = .03]). We also examined a subcohort of men who reported PSA testing in 1994, with follow-up from 1994 until 2006. We observed inverse associations between coffee intake and risk of lethal and advanced cancers in this subcohort that were of similar magnitude to those in the main analyses, but the estimates were not statistically significant, perhaps due to limited power, given the small numbers of lethal and advanced cancers in this highly screened group (in the screened subcohort, RR for advanced cancer [n = 199] = 0.39 [95% CI = 0.11 to 1.34, P_{trend} = .29] for the highest vs lowest category of intake). Coffee consumption was not associated with nonadvanced cancer in this subcohort.

To investigate the role of caffeine vs other components of coffee, we studied regular and decaffeinated coffee separately and found similar associations for both with lethal and advanced cancers (Tables 3 and 4). Compared with men who drank no coffee at all (regular or decaf), men who drank six or more cups per day of regular coffee had a lower risk for advanced cancer, adjusting for decaffeinated coffee intake as a continuous variable (RR = 0.69, 95% CI = 0.38 to 1.27, P_{trend} = .01). Compared with nondrinkers, men who drank four or more cups per day of decaffeinated coffee had a lower risk for advanced cancer, adjusting for regular coffee intake as a continuous variable (RR = 0.67, 95% CI = 0.43 to 1.05, P_{trend} = .02). To compare these associations quantitatively, we included caffeinated and decaffeinated coffee as continuous variables in the same model. For advanced cancer, these relative risks were not statistically significantly different from one another (each one cup per day increment: RR = 0.94, 95% CI = 0.89 to 0.99, P = .03 for regular coffee and RR = 0.92, 95% CI = 0.85 to 1.00, P = .04 for decaffeinated coffee; P for difference in coefficients = .68). Similar associations for regular and decaffeinated coffee were seen

Table 2. Relative risk (RR) with 95% confidence interval (95% CI) of prostate cancer by category of total coffee intake, 1986–2006*

Risk of prostate cancer	Category of coffee intake					P_{trend}
	None	<1 cup/d	1–3 cups/d	4–5 cups/d	≥6 cups/d	
All prostate cancers, No.	587	1139	2438	719	152	
Age-adjusted RR (95% CI)	1.00	0.96 (0.87 to 1.06)	0.97 (0.88 to 1.06)	0.95 (0.85 to 1.06)	0.81 (0.67 to 0.96)	.08
Fully adjusted RR (95% CI)	1.00	0.94 (0.85 to 1.05)	0.94 (0.86 to 1.04)	0.93 (0.83 to 1.04)	0.82 (0.68 to 0.98)	.10
Lethal prostate cancers†, No.	89	150	298	93	12	
Age-adjusted RR (95% CI)	1.00	0.76 (0.58 to 0.99)	0.73 (0.58 to 0.93)	0.83 (0.62 to 1.11)	0.43 (0.24 to 0.80)	.08
Fully adjusted RR (95% CI)	1.00	0.76 (0.58 to 1.00)	0.71 (0.55 to 0.92)	0.76 (0.56 to 1.04)	0.40 (0.22 to 0.75)	.03
Advanced prostate cancers†, No.	122	211	422	122	19	
Age-adjusted RR (95% CI)	1.00	0.81 (0.65 to 1.02)	0.78 (0.63 to 0.95)	0.79 (0.61 to 1.01)	0.49 (0.30 to 0.80)	.01
Fully adjusted RR (95% CI)	1.00	0.81 (0.64 to 1.02)	0.75 (0.60 to 0.93)	0.73 (0.56 to 0.95)	0.47 (0.28 to 0.77)	.004
Nonadvanced prostate cancers†, No.	353	729	1554	483	102	
Age-adjusted RR (95% CI)	1.00	1.04 (0.91 to 1.18)	1.04 (0.92 to 1.16)	1.04 (0.91 to 1.20)	0.88 (0.71 to 1.10)	.60
Fully adjusted RR(95% CI)	1.00	1.01 (0.88 to 1.15)	0.99 (0.87 to 1.12)	1.02 (0.88 to 1.18)	0.93 (0.74 to 1.16)	.77
Grade 8–10 cancers, No.	61	111	255	78	11	
Age-adjusted RR (95% CI)	1.00	0.86 (0.63 to 1.18)	0.93 (0.70 to 1.23)	0.96 (0.69 to 1.35)	0.57 (0.30 to 1.09)	.58
Fully adjusted RR (95% CI)	1.00	0.84 (0.61 to 1.16)	0.87 (0.65 to 1.18)	0.88 (0.61 to 1.26)	0.53 (0.27 to 1.02)	.29
Grade 7 cancers, No.	174	295	641	226	41	
Age-adjusted RR (95% CI)	1.00	0.86 (0.72 to 1.04)	0.88 (0.74 to 1.04)	0.98 (0.80 to 1.20)	0.69 (0.49 to 0.97)	.58
Fully adjusted RR (95% CI)	1.00	0.85 (0.70 to 1.04)	0.85 (0.71 to 1.02)	0.94 (0.76 to 1.16)	0.69 (0.49 to 0.99)	.50
Grade 2–6 cancers, No.	232	489	1045	298	70	
Age-adjusted RR (95% CI)	1.00	1.08 (0.92 to 1.26)	1.07 (0.93 to 1.24)	0.99 (0.83 to 1.18)	0.94 (0.72 to 1.23)	.34
Fully adjusted RR (95% CI)	1.00	1.02 (0.87 to 1.20)	1.01 (0.87 to 1.18)	0.96 (0.80 to 1.15)	1.00 (0.75 to 1.31)	.53

* All relative risks are from an age-adjusted model adjusted for age in months and calendar time. The multivariable model was additionally adjusted for: race (White, African American, Asian American, Other), height (quartiles), BMI at age 21 (<20, 20 to <22.5, 22.5 to <25, ≥25), current BMI (<21, 21 to <23, 23 to <25, 25 to <27.5, 27.5 to <30, ≥30 kg/m^2), vigorous physical activity (quintiles), smoking (never, former quit >10 years ago, former quit <10 years ago, current), diabetes (type I or II, yes/no), family history of prostate cancer in father or brother (yes/no), multivitamin use (yes/no), intakes of processed meat, tomato sauce, calcium, alpha linolenic acid, supplemental vitamin E, alcohol intake (all quintiles), and energy intake (continuous), and history of PSA testing (yes/no, lagged by one period to avoid counting diagnostic PSA tests as screening; collected frsom 1994 onwards). All *P* values were from two-sided tests. BMI = body mass index; PSA, prostate-specific antigen.

† Lethal prostate cancer: Prostate cancer death or bone metastases at diagnosis or during follow-up. Advanced: Lethal, or stage T3b, T4, N1, or M1 at diagnosis, or spread to lymph nodes or other metastases during follow-up. Nonadvanced: T1 or T2 and N0/M0 at diagnosis with no spread to lymph nodes or other metastases or death during follow-up.

for lethal cancer (for each one cup per day increment, RR = 0.94 , 95% CI = 0.88 to 1.01, *P* = .08 for regular coffee and RR = 0.91, 95% CI = 0.83 to 1.00, *P* = .05 for decaffeinated coffee).

To further explore whether the association with lethal and advanced disease was related to caffeine or to other components of coffee, we studied total caffeine intake from all sources. Caffeine intake was inversely associated with lethal and advanced cancers (the highest vs lowest quintile: RR = 0.77, 95% CI = 0.61 to 0.96, P_{trend} = .01 for advanced). However, when coffee and caffeine were included in the model together, coffee intake continued to be associated with lethal or advanced disease, whereas caffeine no longer had a statistically significant association with lethal or advanced disease (RR = 0.86, 95% CI = 0.65 to 1.13, P_{trend} = .23 for advanced).

We used the repeated measures of coffee intake over time to study the effect of latency time by relating each measure of coffee intake to prostate cancer incidence during specific time intervals after exposure. In this analysis, coffee intake was most strongly inversely associated with risk of advanced prostate cancer for the shorter latency periods, 0–4 and 4–8 years after exposure, and weaker for 8- to 12-year and 12- to 16-year lags (Table 5). Coffee consumption was not associated with nonadvanced cancer for any latency period.

Discussion

In this large prospective study, coffee intake was weakly inversely associated with overall risk of prostate cancer, but it was associated with statistically significantly lower risk of lethal and advanced prostate cancers, with those who drank the most coffee having less than half the risk of these outcomes as nondrinkers. Coffee was not associated with nonadvanced or low-grade cancer and only weakly inversely associated with high-grade cancers. Inverse associations with lethal and advanced disease were similar for regular and decaffeinated coffee. The associations were stronger for more recent coffee exposure, suggesting possible effects later in the development of advanced prostate cancers.

The characteristics of heavy coffee drinkers make it unlikely that confounding is a major explanation for these findings because coffee drinkers were more likely to smoke and less likely to engage in vigorous exercise, behaviors which may increase advanced prostate cancer risk. Therefore, confounding by smoking and other lifestyle factors would bias the associations toward the null, rather than explaining the inverse associations for coffee that we observed. In addition, PSA testing was similar for high coffee consumers and nonconsumers, and results were similar in pre-PSA and PSA eras. Thus, PSA screening is an unlikely explanation for these associations.

Table 3. Relative risk (RR) and 95% confidence interval (95% CI) of prostate cancer by category of regular coffee intake*

Risk of prostate cancer	Category of regular (with caffeine) coffee intake						
	No coffee at all†	<1 cup per day	1–3 cups per day	4–5 cups per day	≥6 cups per day	P_{trend}	No regular, some decaf
Lethal prostate cancers‡, No.	89	207	209	52	6		79
Fully adjusted RR (95% CI)*	1.00	0.81 (0.61 to 1.07)	0.71 (0.54 to 0.93)	0.77 (0.53 to 1.10)	0.46 (0.20 to 1.08)	.07	0.72 (0.51 to 1.01)
Advanced prostate cancers‡, No.	122	298	293	65	12		106
Fully adjusted RR (95% CI)*	1.00	0.86 (0.69 to 1.09)	0.74 (0.59 to 0.93)	0.70 (0.51 to 0.95)	0.69 (0.38 to 1.27)	.01	0.75 (0.56 to 1.00)
Nonadvanced prostate cancers‡, No.	353	1042	1164	270	43		349
Fully adjusted RR (95% CI)*	1.00	0.98 (0.86 to 1.12)	0.99 (0.87 to 1.12)	1.00 (0.84 to 1.18)	0.93 (0.67 to 1.29)	.97	1.10 (0.93 to 1.29)

* All relative riskss are from a multivariable model adjusted for: age in months, calendar time, race (White, African American, Asian American, Other), height (quartiles), BMI at age 21 (four categories), current BMI (six categories), vigorous physical activity (quintiles), smoking (never, former quit >10 years ago, former quit <10 years ago, current), diabetes (type I or II, yes/no), family history of prostate cancer in father or brother (yes/no), multivitamin use (yes/no), intakes of processed meat, tomato sauce, calcium, alpha linolenic acid, supplemental vitamin E, alcohol intake (all quintiles), energy intake (continuous), and history of prostate-specific antigen testing. Models for regular coffee are also adjusted for decaffeinated coffee intake (continuous). All *P* values are from two-sided tests. BMI = body mass index.

† Reference group is men who drink no regular or decaffeinated coffee.

‡ Lethal prostate cancer: Prostate cancer death or bone metastases at diagnosis or during follow-up. Advanced: Lethal, or stage T3b, T4, N1, or M1 at diagnosis, or spread to lymph nodes or other metastases during follow-up. Nonadvanced: T1 or T2 and N0/M0 at diagnosis with no spread to lymph nodes or other metastases or death during follow-up.

Reverse causation is a possible explanation for these findings. Men with undiagnosed prostate cancer might decrease their consumption of coffee due to urinary symptoms. However prostate cancer often produces no urinary symptoms, because most tumors arise in the peripheral zone of the gland (35). Indeed, we found no association between lower urinary tract symptoms and coffee intake. Moreover, in subanalyses to explore reverse causation, the association between coffee and risk of advanced or lethal prostate cancer remained statistically significant. Thus, reverse causation does not appear to explain the association.

Previous studies of coffee and prostate cancer have generally not reported the striking inverse associations that we observed (16–32). However, all but two studies reported findings only for overall prostate cancer, potentially overlooking inverse associations with advanced disease. Several studies were limited by a narrow range in coffee intakes, small numbers of case subjects, and lack of adjustment for smoking; confounding by smoking could obscure an inverse association because smoking is associated with coffee consumption in many populations and is also associated with prostate cancer–specific mortality (36).

Prostate cancer mortality, an outcome more comparable to our lethal disease, was examined in two cohorts (31,32). Both adjusted only for age as a potential confounder. The Seventh-day Adventists cohort (31) found a relative risk of 0.70 for men drinking two or more cups per day compared with nondrinkers, based on 93 cancer deaths. Hsing et al. (32) found no association between coffee and prostate cancer death in the Lutheran Brotherhood cohort, based on 149 deaths (≥5 cups per day vs reference <3 cups daily: RR = 1.0, 95% CI = 0.6 to 1.6). The results from both studies are compatible with our findings for lethal prostate cancer, given the wide confidence intervals, differing categorizations of intake, and lack of control for smoking. Smoking is likely less of an issue in the Seventh-day Adventists cohort (a population with very low smoking rates), which observed results closer to ours. However, the range of exposures was low in this cohort, with only 5% of men consuming more than two cups of coffee daily.

Three prospective cohort studies found no statistically significant associations between coffee intake and prostate cancer incidence. Two reports from a cohort of 8000 Japanese men in Hawaii found no association, based on 174 incident cancers (16,17). Another Hawaiian cohort (18) also found no association, though coffee intake was low, such that the highest category was greater than 2.5 cups per day. A Norwegian cohort (19) that included 260 men with prostate cancer found a statistically nonsignificant relative risk of 0.74 for men who consumed seven or more cups per day compared with men who consumed two or fewer cups. The Norwegian study (19) was the only prospective cohort to adjust for smoking.

Five population-based and two hospital-based case–control studies found no association of coffee intake and overall prostate cancer incidence (20–24,29,30), whereas two hospital-based studies (25,26) found increased risks of prostate cancer with higher coffee intake. A small retrospective cohort study in Canada (27) and a prospective case–control study in Sweden (28) found positive associations between coffee and prostate cancer, but these were not statistically significant and the confidence intervals were wide in both studies. None of the studies that found positive associations

Table 4. Relative risk (RR) and 95% confidence interval (CI) of prostate cancer by category of decaffeinated coffee intake*

		Category of decaffeinated coffee intake				
	No coffee at all†	<1 cup/d	1–3 cups/d	≥4 cups/d	P_{trend}	No decaf, some regular
Lethal prostate cancers‡, No.	89	264	125	15		149
Fully adjusted RR*	1.00	0.81 (0.62 to 1.06)	0.68 (0.50 to 0.91)	0.53 (0.30 to 0.94)	.01	0.71 (0.52 to 0.98)
Advanced prostate cancers‡, No	122	374	171	25		204
Fully adjusted RR*	1.00	0.85 (0.68 to 1.07)	0.70 (0.54 to 0.89)	0.67 (0.43 to 1.05)	.02	0.77 (0.59 to 1.01)
Nonadvanced prostate cancers‡, No.	353	1455	688	86		639
Fully adjusted RR*	1.00	0.99 (0.87 to 1.13)	1.04 (0.90 to 1.19)	0.94 (0.74 to 1.20)	.88	0.98 (0.85 to 1.14)

* All relative riskss are from a multivariable model adjusted for: age in months, calendar time, race (White, African American, Asian American, Other), height (quartiles), BMI at age 21 (four categories), current BMI (six categories), vigorous physical activity (quintiles), smoking (never, former quit >10 years ago, former quit <10 years ago, current), diabetes (type I or II, yes/no), family history of prostate cancer in father or brother (yes/no), multivitamin use (yes/no), intakes of processed meat, tomato sauce, calcium, alpha linolenic acid, supplemental vitamin E, alcohol intake (all quintiles), and energy intake (continuous), and history of prostate-specific antigen testing. Models for decaffeinated coffee are also adjusted for regular coffee intake (continuous). All *P* values are from two-sided tests. BMI = body mass index.

† Reference group is men who drink no regular or decaffeinated coffee.

‡ Lethal prostate cancer: Prostate cancer death or bone metastases at diagnosis or during follow-up. Advanced: Lethal, or stage T3b, T4, N1 or M1 at diagnosis, or spread to lymph nodes or other metastases during follow-up. Nonadvanced: T1 or T2 and N0/M0 at diagnosis with no spread to lymph nodes or other metastases or death during follow-up.

Table 5. Relative risk (RR) and 95% confidence interval (95% CI) of prostate cancer by category of total coffee intake for various latency periods between exposure and diagnosis*

	Total prostate cancer		Advanced cancer†		Nonadvanced cancer†	
	N	RR (95% CI)	N	RR (95% CI)	N	RR (95% CI)
0 to 4-year lag, cups per day						
None	810	1.00 (referent)	160	1.00 (referent)	493	1.00 (referent)
<1	1003	.95 (.87 to 1.05)	180	.78 (.62 to .97)	655	1.05 (.93 to 1.18)
1–3	2501	.96 (.89 to 1.05)	436	.77 (.63 to .93)	1592	1.01 (.91 to 1.13)
4–5	587	.97 (.87 to 1.09)	100	.73 (.56 to .95)	389	1.08 (.93 to 1.24)
≥6	134	.81 (.67 to .98)	20	.52 (.33 to .84)	92	.96 (.76 to 1.21)
P_{trend}		.20		.008		.91
4 to 8-year lag, cups per day						
None	745	1.00 (referent)	131	1.00 (referent)	468	1.00 (referent)
<1	886	.92 (.83 to 1.02)	152	.84 (.66 to 1.07)	589	1.00 (.88 to 1.13)
1–3	2267	.93 (.85 to 1.02)	329	.72 (.58 to .89)	1519	1.00 (.89 to 1.11)
4–5	594	.92 (.82 to 1.03)	99	.81 (.61 to 1.07)	397	.97 (.85 to 1.12)
≥6	153	.80 (.67 to .96)	18	.47 (.28 to .78)	102	.88 (.71 to 1.10)
P_{trend}		.06		.008		.34
8 to 12-year lag, cups per day						
None	551	1.00 (referent)	79	1.00 (referent)	360	1.00 (referent)
<1	664	.98 (.87 to 1.10)	91	.85 (.62 to 1.17)	458	1.06 (.92 to 1.22)
1–3	1687	.99 (.89 to 1.10)	181	.68 (.51 to .91)	1175	1.06 (.93 to 1.20)
4–5	465	.91 (.80 to 1.03)	63	.82 (.58 to 1.17)	329	.98 (.83 to 1.14)
≥6	154	.93 (.77 to 1.12)	18	.71 (.42 to 1.21)	111	1.04 (.83 to 1.30)
P_{trend}		.17		.18		.70
12 to 16-year lag, cups per day						
None	389	1.00 (referent)	41	1.00 (referent)	268	1.00 (referent)
<1	448	.97 (.84 to 1.11)	49	.90 (.58 to 1.39)	307	.96 (.81 to 1.14)
1–3	1046	.93 (.82 to 1.06)	93	.72 (.48 to 1.07)	742	.94 (.81 to 1.10)
4–5	353	.97 (.83 to 1.13)	41	.97 (.61 to 1.55)	260	1.02 (.85 to 1.23)
≥6	115	.93 (.75 to 1.16)	8	.59 (.27 to 1.29)	84	.98 (.76 to 1.26)
P_{trend}		.61		.43		.78

* All relative riskss are from multivariable models adjusted for: age in months, calendar time, race (White, African American, Asian American, Other), height (quartiles), BMI at age 21 (<20, 20 to <22.5, 22.5 to <25, ≥25 kg /m^2), current BMI (<21, 21 to <23, 23 to <25, 25 to <27.5, 27.5 to <30, ≥30 kg/m^2), vigorous physical activity (quintiles), smoking (never, former quit >10 years ago, former quit <10 years ago, current), diabetes (type I or II, yes/no), family history of prostate cancer in father or brother (yes/no), multivitamin use (yes/no), intakes of processed meat, tomato sauce, calcium, alpha linolenic acid, supplemental vitamin E, alcohol intake (all quintiles), and energy intake (continuous), and history of PSA testing (yes/no, lagged by one period to avoid counting diagnostic PSA tests as screening; collected from 1994 onwards). All *P* values are from two-sided tests. BMI = body mass index; PSA = prostate-specific antigen.

† Advanced: Lethal, or stage T3b, T4, N1, or M1 at diagnosis, or spread to lymph nodes or other metastases during follow-up. Nonadvanced: T1 or T2 and N0/M0 at diagnosis with no spread to lymph nodes or other metastases or death during follow-up.

were adjusted for smoking, thus confounding is a major concern in these studies. Case–control studies are also prone to selection and recall bias.

An association between coffee and lower risk of advanced prostate cancer is biologically plausible. Coffee improves glucose metabolism, has anti-inflammatory and antioxidant effects, and affects sex hormone levels, all of which play roles in prostate cancer progression.

Coffee contains chlorogenic acids (CGAs), which inhibit glucose absorption in the intestine and may favorably alter levels of gut hormones, which affect insulin response (1). Quinides, the roasting products of CGAs, inhibit liver glucose production in experimental models (1). Coffee also contains lignans, phytoestrogens with potent antioxidant activity, which may have positive effects on glucose handling (37). In humans, coffee drinking has been cross-sectionally associated with lower glucose levels after oral glucose loads and better insulin sensitivity (38–40). A cross-sectional study in women found a negative correlation between coffee consumption and circulating C-peptide, a marker of insulin secretion (41).

Insulin may promote tumor progression through the insulin and insulin-like growth factor 1 (IGF-1) receptors in cancer cells. Insulin levels have been associated with a greater risk of cancer progression or mortality among men diagnosed with prostate cancer (9–11), even though insulin has been unassociated (12,13) or inversely associated (14) with overall incidence.

Coffee is a major source of antioxidants and is estimated to provide half of total antioxidant intake in several populations (2,3). Coffee has been associated with improved markers of inflammation in cross-sectional studies and in a recent trial (4,42,43). Inflammation is hypothesized to play a role in the development of prostate cancer through the generation of proliferative inflammatory atrophy lesions (15). Various dietary antioxidants may reduce inflammation and have been associated with lower risk of advanced prostate cancer (44,45).

Coffee drinking may be associated with increased sex hormone –binding globulin (SHBG) and total testosterone levels (5). One study in Greek men found a positive association with estradiol levels but not with SHBG or testosterone (6), whereas another found no association between coffee and sex hormones in young Greek men (7). Coffee has been consistently associated with higher SHBG levels in women (46–49).

Sex hormones play a role in prostate cancer, though the relationships between circulating levels within normal ranges and risk have been difficult to elucidate. It has been hypothesized that although testosterone is necessary for the initial development of prostate cancer, it may limit progression of the disease (50,51). A pooled analysis of 18 prospective studies found an inverse association between SHBG levels and prostate cancer risk (51).

Strengths of our study include the prospective and updated assessment of coffee, long follow-up, and a large number of incident prostate cancers, which allowed us to study stage- and grade-based subtypes. The range of intakes in this cohort was wide, with 16% of men consuming no coffee and 19% of men consuming four or more cups per day. Coffee was accurately reported on FFQs (33), and any misclassification in coffee intake due to differences in cup size or brewing strength would be expected to bias observed associations toward the null and thus would not explain the inverse associations that we observed. Our use of repeated measures of diet over time captured changes in diet and reduced measurement error (52); however, we were not able to assess coffee intake in young adulthood or total lifetime coffee intake.

This study also has some limitations. First, we relied on self-reported diet, which will inevitably be imperfect. Although coffee is well reported, we assessed usual intake only every 4 years, thus missing shorter-term fluctuations in intakes. In addition, we do not have coffee intake information from earlier periods of life, limiting our ability to determine the most relevant time periods of exposure. Finally, although reverse causation does not appear to explain our findings, we cannot rule it out as a possible source of bias.

In conclusion, men who consumed coffee regularly had a reduced risk of lethal or advanced prostate cancer. It is premature to recommend that men increase coffee intake to reduce advanced prostate cancer risk based on this single study. In addition, the effects of coffee consumption on other aspects of health must be considered in making consumption recommendations. However, our findings are potentially important, given the lack of identified modifiable risk factors for advanced prostate cancer. The association between coffee and prostate cancer should be studied in other prospective cohorts with a wide range of coffee intakes, with control for smoking, and evaluation of lethal or advanced cancers.

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ORIGINAL PAPER

Coffee and risk of prostate cancer incidence and mortality in the Cancer of the Prostate in Sweden Study

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Abstract
Purpose Coffee intake has recently been associated with significantly lower risk of lethal and advanced prostate cancer in a US population.
Methods We studied the association between coffee and prostate cancer risk in the population-based case–control study Cancer of the Prostate in Sweden. Dietary data were available for 1,499 cases and 1,112 controls. We calculated odds ratios (ORs) for the risk of prostate cancer in high versus low categories of coffee intake using logistic regression. We studied overall prostate cancer risk as well as risk of fatal, advanced, localized, high-grade, grade 7, and low-grade disease.
Results Mean coffee intake was 3.1 cups per day among both cases and controls. Coffee intake was not associated with overall prostate cancer risk. Risk of fatal prostate cancer was inversely, but not statistically significantly, associated with coffee intake, with an odds ratio of 0.64 [95 % confidence interval (CI) 0.34–1.19, p value for linear trend = 0.81] for men consuming greater than 5 cups per day compared to men drinking less than 1 cup per day. The highest intake of coffee was associated non-significantly with lower risk of advanced disease (OR = 0.73, 95 % CI 0.41–1.30, p trend = 0.98) and associated significantly with lower risk of high-grade cancer (Gleason 8–10; OR = 0.50, 95 % CI 0.26–0.98, p trend = 0.13). Risk of localized, grade 7, and low-grade cancers was not associated with coffee intake.
Conclusions This study provides some support of an inverse association between coffee and lethal and high-grade prostate cancer.

Keywords Coffee · Diet · Prostate cancer

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Introduction

We recently reported a strong inverse association between coffee intake and risk of lethal and advanced prostate cancer in the large, prospective Health Professionals Follow-up Study [1]. Coffee, a powerful antioxidant [2, 3] with anti-inflammatory effects [4], also has a positive influence on glucose metabolism [5] and perhaps on sex hormone levels [6, 7]. Higher levels of insulin [8–10] and inflammation [11–13] have been associated with risk of prostate cancer progression, while higher levels of testosterone have been hypothesized to prevent prostate cancers from progressing [14, 15]. Therefore, it is plausible that coffee might be associated with a lower risk of more advanced prostate cancers but not slow-growing cancers with limited potential to metastasize.

Most previous studies of coffee intake and prostate cancer risk analyzed overall risk only, not the risk of lethal or advanced prostate cancer specifically [16–30]. One meta-analysis of 8 case–control and 4 cohort studies found

an increased risk of total prostate cancer with higher coffee consumption in the case–control studies, but no association in the cohort studies [31]. Another meta-analysis of 5 cohort studies found a statistically significant 21 % lower risk of total prostate cancer in the highest versus lowest consumers [32].

Two studies of coffee and prostate cancer mortality [33, 34] found no significant associations; however, these results were adjusted only for age and not for other potential confounders such as smoking, which could have attenuated associations. In addition, the range of intakes was low. Two more recent studies have examined coffee intake with respect to prostate tumor grade, with one finding an inverse association with high-grade disease and the other reporting no associations [35, 36].

We examined the association between coffee and prostate cancer using data from a population-based case–control study of prostate cancer in Sweden with a large number of advanced and fatal cancers, as well as a high intake of coffee among controls.

Subjects and methods

Participants

The Cancer of the Prostate in Sweden (CAPS) study is a population-based case–control study of prostate cancer [37]. Cancer cases were men with pathologically or cytologically verified incident prostate cancer diagnosed in 2001 and 2002 identified from four of the six regional cancer registries in Sweden. The men were initially recruited through their treating physicians, and the average time between date of diagnosis and date when the questionnaire was sent was 5 months. Clinical data on tumor, nodes, and metastasis (TNM) stage, Gleason score, and serum prostate-specific antigen (PSA) level at diagnosis were obtained from linkage to the National Prostate Cancer Registry and were available for 95 % of cases in the study. Control subjects were randomly selected from the Swedish Population Registry, which maintains complete coverage of the population, and were frequency matched to cases by 5-year age group and region of residence. Controls were contacted by mail and received the same information about the study as cases.

Of the men invited to participate, 79 % (1,499 of 1,895) prostate cancer cases and 67 % (1,130 of 1,684) controls completed a baseline lifestyle and health questionnaire. All study participants gave informed consent at the time of enrollment in the study. The study was approved by the ethics committees at Karolinska Institutet and Umeå University in Sweden.

Dietary assessment

As part of the baseline questionnaire, all participants completed a self-administered 261-item FFQ that assessed usual intake of foods over the previous 12 months. Participants were asked an open-ended question about coffee consumption and were asked to fill in how many cups of coffee they drank either per day or per week. The specific cup size was not specified. A shorter form of this FFQ was validated against four 7-day weighed diet records in women and found a correlation of 0.61 for coffee [38]. Data from the Swedish National Food Administration were used to calculate total energy intake and intake of nutrients based on questionnaire responses. Nutrient intakes were energy-adjusted using the residual method.

Of the 2,617 men who completed the FFQ component of the questionnaire, 16 were excluded because of unreasonable energy intakes, leaving 1,489 cases and 1,112 controls in the study population.

Statistical analysis

To measure the association between coffee intake and risk of prostate cancer, we used unconditional logistic regression models with indicator variables for category of coffee intake (None, <2 cups/day, etc.). Age group and region, which were matching factors in this study, were included in all models. Fully adjusted models also included: smoking status (never, former, current), body mass index (<20, 20 to <22.5, 22.5 to <25, 25 to <27.5, 27.5 to <30, 30+ kg/m^2), education (0–9 years, secondary education: 10–12 years, higher education: 13+ years), and intake of calcium, zinc, and total energy (all quartiles). Several other variables were considered as potential confounders based on previous findings in this and other studies including: civil/marital status, employment status, and intake of alpha-linolenic acid, vitamin D, phytoestrogens, red meat, and dairy. None of these was included in the final models, as they had little effect on the coffee–prostate cancer effect estimates or precision. To test for a dose–response trend across categories, we modeled coffee as a continuous variable using the median intake in each category.

In addition to overall risk of prostate cancer, we studied the risk of fatal, non-fatal, advanced, localized, high-grade (Gleason sum 8–10), grade 7, and low-grade disease (Gleason sum 2–6). Fatal disease was defined as death from prostate cancer, with follow-up for prostate cancer-specific mortality through June 2009. Non-fatal cases were those who had not died of prostate cancer through June 2009 and who did not have stage M1 disease at diagnosis. Advanced disease was defined as death or stage M1, N1, or T4 at diagnosis. Localized disease was defined as stage T1 or T2 and N0/M0 at diagnosis. Stage T3 cancers were not

included as either advanced or localized disease to keep these categories more clearly defined. Gleason 7 cancers were not included in either high-grade or low-grade disease because of the heterogeneity of these tumors and the divergent outcomes seen for Gleason 3 + 4 compared to Gleason 4 + 3 tumors [39].

Results

Mean coffee intake in the previous year was 3.1 cups per day among both cases and controls (Table 1). Among controls, 8.8 % consumed less than one cup of coffee per day (with 4.0 % consuming no coffee), 10.9 % consumed 1 to less than 2 cups/day, 44.2 % consumed 2 to less than 4 cups/day, 26.5 % consumed 4–5 cups/day, and 9.6 % consumed more than 5 cups of coffee per day. Cases and controls were similar in body mass index, smoking, and intake of energy, calcium, and zinc (Table 1). Cases were slightly younger than controls. High-grade cases had slightly lower coffee intake, and fatal cases were somewhat more likely to smoke than non-fatal cases or controls.

Coffee intake was not associated with overall risk of prostate cancer (Table 2). Risk of fatal prostate cancer was inversely, though not statistically significantly, associated with coffee intake, with an adjusted odds ratio of 0.64 [95 % confidence interval (CI) 0.34–1.19] for men consuming 6 or more cups per day compared with non-drinkers. For fatal cancer, there was no significant linear trend across categories of coffee intake (p trend = 0.81), as the risk was lower for all coffee-consuming groups compared to non-drinkers (Fig. 1). Risk of advanced prostate cancer was non-significantly lower among the highest coffee consumers with an adjusted OR of 0.73 (CI 0.41–1.30, p trend = 0.98). Risk of high-grade (Gleason 8–10) prostate cancer was significantly inversely associated with the highest coffee intake, with an OR of 0.50 (0.26–0.98, p trend = 0.13) for high-grade (Table 2; Fig. 1). Coffee intake was not associated with risk of non-fatal, localized, grade 7, or low-grade prostate cancer. Smoking and energy intake, both positively associated with both coffee intake and with fatal disease, were the main confounders accounting for the differences in the age- and region-adjusted models and the fully adjusted models.

Discussion

In this Swedish population, we found a suggestive inverse association between coffee intake and risk of fatal,

Table 1 Characteristics of the study population by case and control status, means (standard deviations) or percents, Cancer of the Prostate Sweden Study

	Controls	Cases	Prostate cancer case types						
			Fatal	Non-fatal	Advanced	Localized	High-grade	Grade 7	Low-grade
n	1,112	1,489	307	1,144	377	1,020	231	406	693
Coffee intake (cups/day)	3.11 (2.01)	3.10 (1.92)	3.07 (1.79)	3.10 (1.95)	3.10 (1.83)	3.06 (1.86)	2.98 (1.88)	3.25 (2.17)	3.08 (1.83)
Age	67.7 (7.5)	66.8 (7.3)	69.0 (7.3)	66.2 (7.1)	68.7 (7.3)	66.0 (7.1)	68.3 (7.4)	67.1 (7.0)	65.5 (7.0)
BMI	26 (3)	26 (3)	26 (3)	26 (3)	26 (3)	26 (3)	26 (3)	26 (3)	26 (4)
Current smokers (%)	12	11	14	10	14	10	12	12	10
Former smokers (%)	48	49	43	52	44	50	46	48	52
0–9 years education (%)	46	46	53	44	52	43	47	47	43
10–12 years education (%)	42	40	37	41	37	40	39	41	41
13+ years education (%)	11	14	10	15	11	17	13	13	16
Energy (kcal/day)	2,218 (655)	2,283 (646)	2,363 (701)	2,257 (628)	2,373 (689)	2,270 (630)	2,387 (655)	2,289 (637)	2,234 (624)
Calcium (mg/day)	1,197 (363)	1,199 (370)	1,241 (406)	1,187 (361)	1,246 (398)	1,188 (363)	1,213 (381)	1,214 (388)	1,193 (363)
Zinc (mg/day)	11.7 (1.8)	11.6 (1.9)	11.5 (1.9)	11.6 (1.9)	11.6 (1.9)	11.6 (1.9)	11.6 (1.9)	11.5 (1.8)	11.7 (1.8)
Gleason sum[a]	–	6.5 (1.2)	7.7 (1.0)	6.3 (1.1)	7.5 (1.1)	6.2 (1.1)	8.5 (1.0)	7.0 (–)	5.6 (1.0)
PSA at diagnosis (ng/mL)[a]	–	89 (360)	264 (573)	26 (79)	276 (665)	39 (241)	167 (364)	106 (411)	22 (136)

Advanced prostate cancer: death, M1, N1, or T4. Localized prostate cancer: T1 or T2 and N0/M0

High-grade prostate cancer: Gleason sum 8–10. Low-grade prostate cancer: Gleason sum 2–6

[a] Gleason sum was available for 1,330/1,489 (89 %) cases. PSA at diagnosis was available for 1,446/1,489 (97 %) cases

Table 2 Odds ratios, OR (and 95 % CI) of prostate cancer by category of coffee intake, Cancer of the Prostate Sweden

	Category of coffee intake					*p* trend
	<1 cup/day	1 to <2 cups/day	2 to <4 cups/day	4–5 cups/day	>5 cups/day	
All prostate cancer (cases/controls)	139/98	150/121	644/491	413/295	143/107	
Age- and region-adjusted OR	1.00	0.89 (0.62–1.26)	0.97 (0.73–1.29)	1.04 (0.77–1.41)	0.97 (0.67–1.39)	0.63
Fully adjusted OR	1.00	0.97 (0.62–1.52)	0.98 (0.65–1.49)	1.06 (0.69–1.62)	0.97 (0.60–1.57)	0.84
Fatal prostate cancer	31/98	24/121	133/491	94/295	25/107	
Age- and region-adjusted OR	1.00	0.58 (0.32–1.07)	0.83 (0.53–1.31)	1.03 (0.64–1.64)	0.77 (0.43–1.41)	0.57
Fully adjusted OR	1.00	0.59 (0.32–1.09)	0.79 (0.49–1.26)	0.93 (0.57–1.51)	0.64 (0.34–1.19)	0.81
Non-fatal prostate cancer	107/98	123/121	494/491	306/295	114/107	
Age- and region-adjusted OR	1.00	0.97 (0.67–1.42)	0.99 (0.73–1.35)	1.03 (0.75–1.43)	1.01 (0.68–1.48)	0.81
Fully adjusted OR	1.00	0.96 (0.66–1.41)	0.96 (0.70–1.32)	1.01 (0.73–1.41)	1.00 (0.67–1.49)	0.85
Advanced prostate cancer	35/98	32/121	159/491	119/295	32/107	
Age- and region-adjusted OR	1.00	0.70 (0.40–1.22)	0.89 (0.58–1.37)	1.15 (0.73–1.79)	0.87 (0.50–1.53)	0.38
Fully adjusted OR	1.00	0.70 (0.40–1.23)	0.83 (0.53–1.29)	1.02 (0.64–1.62)	0.73 (0.41–1.30)	0.98
Localized prostate cancer	98/98	103/121	452/491	273/295	94/107	
Age- and region-adjusted OR	1.00	0.88 (0.59–1.30)	1.01 (0.74–1.38)	1.01 (0.72–1.40)	0.89 (0.60–1.33)	0.96
Fully adjusted OR	1.00	0.88 (0.59–1.31)	0.98 (0.71–1.35)	1.01 (0.72–1.42)	0.89 (0.59–1.35)	0.97
High-grade prostate cancer	30/98	22/121	98/491	62/295	19/107	
Age- and region-adjusted OR	1.00	0.58 (0.31–1.08)	0.67 (0.42–1.07)	0.73 (0.44–1.20)	0.63 (0.33–1.19)	0.43
Fully adjusted OR	1.00	0.54 (0.29–1.01)	0.59 (0.36–1.95)	0.61 (0.36–1.03)	0.50 (0.26–0.98)	0.13
Grade 7 prostate cancer	42/98	36/121	158/491	125/295	45/107	
Age- and region-adjusted OR	1.00	0.70 (0.41–1.18)	0.79 (0.52–1.18)	1.04 (0.68–1.58)	1.04 (0.63–1.72)	0.21
Fully adjusted OR	1.00	0.69 (0.41–1.18)	0.76 (0.50–1.15)	0.98 (0.64–1.52)	0.96 (0.57–1.63)	0.43
Low-grade prostate cancer	55/98	79/121	313/491	177/295	69/107	
Age- and region-adjusted OR	1.00	1.22 (0.78–1.91)	1.22 (0.84–1.76)	1.14 (0.77–1.68)	1.14 (0.72–1.80)	0.87
Fully adjusted OR	1.00	1.26 (0.80–1.99)	1.22 (0.84–1.77)	1.17 (0.78–1.74)	1.17 (0.73–1.87)	0.80

Advanced prostate cancer: death, M1, N1, or T4. Localized prostate cancer: T1 or T2 and N0/M0

High-grade prostate cancer: Gleason sum 8–10. Low-grade prostate cancer: Gleason sum 2–6

Fully adjusted model adjusted for: age, region, smoking (never, former, current), BMI (<20, 20 to <22.5, 22.5 to <25, 25 to <27.5, 27.5 to <30, 30 +), education (0–9, 10–12, 13 +), and intake of calcium, zinc, and total energy (all quartiles)

advanced, and high-grade prostate cancers, but no association with overall or localized prostate cancer.

Our results are similar to those reported from the US Health Professionals Follow-up Study, with relative risks of 0.40 (95 % CI 0.22–0.75, p trend = 0.03) for lethal prostate cancer (fatal or bone metastases), 0.47 (95 % CI 0.28–0.77, p trend = 0.004) for advanced disease, and 0.53 (95 % CI 0.27–1.02, p trend = 0.29) for high-grade disease [1]. Two previous studies of coffee intake and prostate cancer mortality [31, 32] found no significant associations; however, both adjusted only for age as a potential confounder and were limited by narrow ranges of coffee intake. The Seventh Day Adventists cohort [33] found a relative risk of 0.70 for men drinking 2 or more cups per day compared to non-drinkers, based on 93 cancer deaths; while not significant, these results are similar to ours for coffee intake at that level. Hsing et al. [34] found no association between coffee and prostate cancer death in the Lutheran Brotherhood Cohort, based on 149 deaths, with a relative risk of 1.0 (CI 0.6–1.6) for 5 or more cups per day compared to a reference group of less than three cups daily. Given the wide confidence intervals, differing categorizations of intake, and lack of control for smoking, these results are not incompatible with ours. Two recent studies have examined coffee intake with respect to high-grade disease. One small prospective study found a significantly lower risk of high-grade, but not overall disease [35], whereas a population-based case–control study found no association overall or by grade [36].

Indeed, the consistency of the inverse association between coffee intake at any level and risk of more aggressive prostate cancer was striking. These results may be due to chance, due to a threshold or saturation effect of coffee, or because men who do not consume coffee have other characteristics related to higher probability of prostate cancer progression. We attempted to deal with this potential confounding by adjusting for a variety of factors related to coffee intake and prostate cancer risk. In fact, the

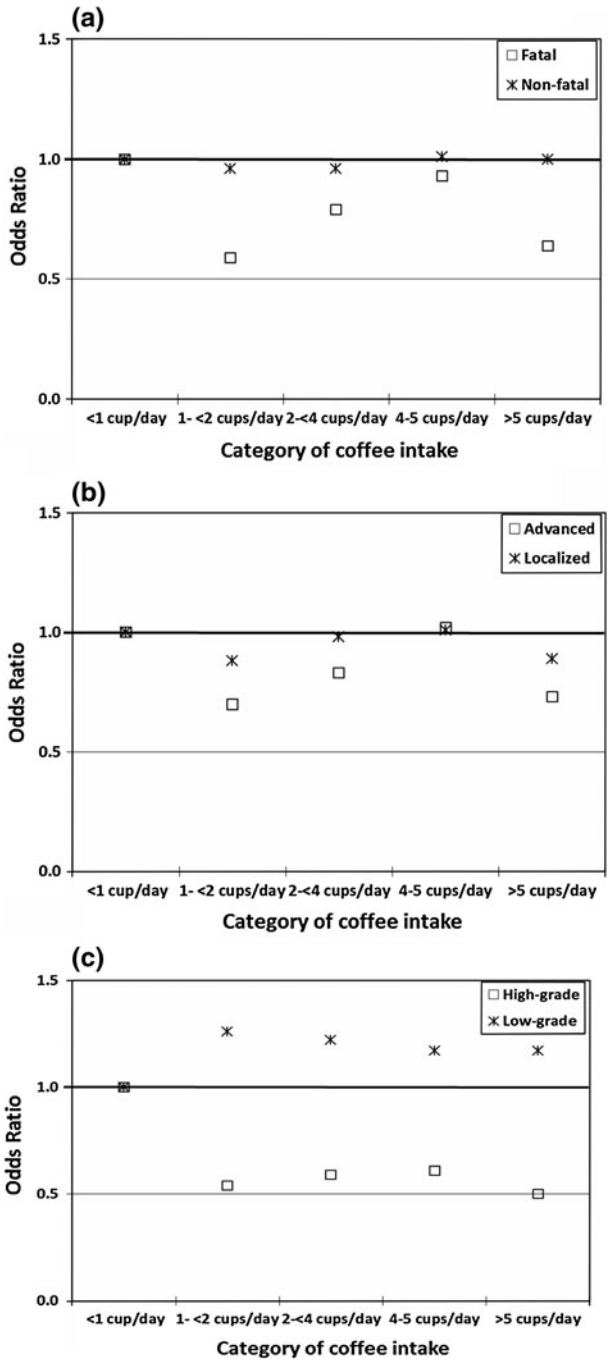

Fig. 1 Odds ratios of prostate cancer by category of coffee intake. **a** Fatal and non-fatal prostate cancer, **b** advanced and localized prostate cancer, **c** high-grade and low-grade prostate cancer

major confounders that we adjusted for actually shifted the odds ratios further from the null. However, it is always possible that our results are due to confounding by other, unknown factors.

Several plausible biological mechanisms linking coffee and risk of fatal prostate cancer exist, including coffee's effects on insulin metabolism [5], its antioxidant content and anti-inflammatory effects [2–4], and its possible effect on sex hormone levels [6, 7]. All of these factors have been associated with risk of more advanced prostate cancer [8–15].

Strengths of our study include the population-based design, large number of cases, and detailed information on subtypes of prostate cancer by stage and grade. The population also has a wide range of intakes, with 27 % of men consuming 4–5 cups per day and 10 % of men consuming 6 or more cups per day. In addition, we were able to control for several possible confounding factors including smoking and various aspects of diet.

The study has several limitations. Cup size was not specified in the questionnaire, so men reported based on their own perceptions of the size of a "cup" of coffee. In addition, we have no information on brewing methods and brew strength. Information on PSA screening is also not available in this study, and screening behavior may be correlated with coffee intake. However, PSA screening is not common in Sweden, particularly during this study period, and is unlikely to play a major role in our findings. Reverse causation is one possible explanation for our findings, as cases may have reduced their coffee consumption prior to diagnosis due to undiagnosed disease causing urinary symptoms. We assessed coffee consumption in the year before cancer diagnosis only, so we have limited ability to explore this question (for instance, by studying lifetime coffee intake or assessing changes in coffee intake in the years prior to diagnosis).

In addition, there are several potential sources of bias in case–control sizes which must be considered. Selection bias is a possible concern, and participation rates were higher in cases than in controls (79 vs. 67 %), which could result in over-sampling of more health-conscious men in the control group. However, it is not clear that over-selection of health-conscious controls would be associated with *increased* coffee consumption in the control group relative to the cases. Recall bias is another possible issue, as diet was assessed after cancer diagnosis, possibly introducing differential misclassification. However, coffee is not generally perceived to be associated with cancer risk, particularly with *reduced* cancer risk, so it is unlikely that cases would attribute their disease to low coffee intake and underreport their typical intake as a result. In addition, differential misclassification due to recall bias would have to be related to disease stage and grade to explain our results, and this seems unlikely.

In conclusion, there was a suggestion that regular coffee consumption was associated with lower risk of lethal and high-grade prostate cancer in this population-based case–control study in Sweden. Given the lack of identified modifiable risk factors for prostate cancer, this association is potentially important and should be studied in other populations.

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Conflict of interest The authors declare that they have no conflict of interest.

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