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Dietary cadmium and risk of breast cancer subtypes defined by hormone receptor status: A prospective cohort study

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Diet is the primary source of cadmium—a proven Group 1 human carcinogen—for non-smokers. Observational studies investigating the effect of cadmium from food sources on breast cancer risk have produced inconsistent results. We examined the association between dietary cadmium and risk of breast cancer defined by estrogen receptor (ER), progesterone receptor (PR) and HER2 status, in 8924 women recruited to a prospective study between 1987 and 1992. Dietary cadmium intake was estimated using a semi-quantitative food frequency questionnaire at baseline. During a median of 22 years of follow-up, 451 incident cases of breast cancer were identified through the Varese Cancer Registry. Multivariable-adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) for breast cancer and receptor-defined breast cancer subtypes were estimated for quintiles of dietary cadmium intake, adjusting for confounding factors. Mean dietary cadmium intake was 7.8 (standard deviation 1.4) μg/day. Women with highest quintile of cadmium intake had a greater risk of breast cancer (HR 1.54; 95% CI, 1.06–2.22; *p* trend = 0.028) than those with lowest quintile of intake. Women premenopausal at recruitment had HR = 1.73 (95% CI, 1.10–2.71, highest *vs.* lowest quintile); postmenopausal women had HR = 1.32 (95% CI, 1.05–1.66 for each standard deviation increase in cadmium). Cadmium-related risk of breast cancer did not vary with ER, PR or HER2 status (p-heterogeneity not significant). These findings support the hypothesis that dietary cadmium is a risk factor for breast cancer.

Introduction

Cadmium (Cd) is a proven human carcinogen (group I of International Agency for Research on Cancer classification).[1] Cadmium is absorbed into the body from dietary sources, cigarette smoke, and by inhalation in industrial or polluted environments. Cadmium levels increase with age since the elimination half-life is long (10–30 years).[2–4] The metal accumulates mainly in liver and kidney because these tissues synthesize cadmium-inducible proteins called metallothioneins that protect cells by binding toxic Cd^{2+}. In addition circulating Cd-metallothionein is taken up by proximal tubular cells of kidney resulting in cadmium accumulation in kidney cortex.[2]

In non-smokers, diet is the main source of cadmium.[5,6] In smokers, inhaled tobacco smoke is usually the main source of cadmium. One cigarette contains about 1–2 μg cadmium, and about 10% of inhaled cadmium in tobacco smoke is absorbed.[2]

The Joint FAO/WHO Expert Committee on Food Additives[7] recommends 25 μg/kg body weight as the maximum tolerable monthly intake of cadmium. Concentrations of cadmium in most foods are usually less than 0.15 mg/kg. Notable exceptions are shellfish and kidneys, which contain 1–2 mg/kg and 0.5 mg/kg, respectively.[8] Cadmium in vegetables and cereals mainly derives from the soil. Cadmium in soil is usually derived from cadmium-rich industrial or urban wastes, but may be present naturally in geological terrains characterized by the presence of zinc- or lead-rich minerals.[8] Phosphate fertilizer and sewage sludge, used agriculturally, are important contributors to cadmium in the environment and human foods.[9]

About 5% of the cadmium ingested by adults is absorbed, with marked between-individual variation.[10] Cadmium absorption from dietary sources is reduced if the nutritional

Key words: cadmium, breast cancer, estrogen receptors, progesterone receptors, HER2

Abbreviations: ER: estrogen receptor; PR: progesterone receptor; HRs: hazard ratios; CIs: confidence intervals; Cd: Cadmium; FFQ: food frequency questionnaire; BMI: body mass index; SD: standard deviation

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What's new?

Diet is the primary source of cadmium – a proven Group 1 human carcinogen – for non-smokers. Observational studies investigating the effect of cadmium from food sources on breast cancer risk have produced inconsistent results, however. This first cohort study to investigate the effects of dietary cadmium on the risk of breast cancer and breast cancer subtypes defined by the expression of ER, PR, and HER2 provides further evidence that dietary cadmium increases breast cancer risk. The lack of significant heterogeneity in risk estimates between different ER and PR status neither supports nor refutes the hypothesis that cadmium acts as a metalloestrogen.

status of zinc, iron or calcium is high, and enhanced if the nutritional status of these important micronutrients is low.[11]

The main mechanisms of cadmium carcinogenesis are thought to be promotion of oxidative stress and inflammation, induction of DNA damage, alteration of DNA repair mechanisms, and interference with apoptosis.[12] Cadmium also has estrogen-like effects that include stimulation of human breast cancer cell proliferation,[12,13] increased expression of estrogen-regulated genes,[14] activation of estrogen receptor (ER) alpha,[15] and increased progesterone receptor (PR) levels in breast cancer cells.[14]

Few epidemiological studies have assessed associations between dietary cadmium and breast cancer risk with conflicting results. A meta-analysis of cohort and case–control studies reported a significant positive association between dietary cadmium intake and breast cancer,[16] while a subsequent update which included two additional cohort studies, found that the positive association was no longer statistically significant.[17] A recent meta-analysis of studies on postmenopausal women found that dietary cadmium was unrelated to breast cancer risk.[18] Few studies have investigated associations between dietary cadmium and the ER/PR status of breast cancers that develop[19–23] again with discordant results. As far as we are aware no studies have investigated dietary cadmium exposure and risk of breast cancer according to HER2 status. In the present study, we investigated a cohort of Italian women to ascertain whether dietary cadmium intake was associated with risk of breast cancer, and also with the risk of breast cancer subtypes defined by ER, PR, and HER2 status.

Materials and Methods

Participants were women recruited to the study on hormones, diet, and the etiology of breast cancer (ORDET), which was designed to prospectively investigate associations of hormones and diet with breast cancer risk. Study methods are available elsewhere.[24,25] Briefly, between June 1987 and June 1992, 10,786 healthy women aged 34–70 years, resident in Varese province, northern Italy, were recruited from the general population. Women taking hormone therapy in the 3 months before recruitment, with a history of cancer or liver disease, or who had undergone bilateral ovariectomy, were excluded. The study protocol was approved by the ethics committee of the National Cancer Institute of Milan. The study complied with the Helsinki Declaration, and participants gave written informed consent to use their clinical data for research.

At baseline participants completed a lifestyle questionnaire (that included questions on reproductive history, menstruation, hormone therapy, smoking, and education) and a self-reporting semi-quantitative food frequency questionnaire (FFQ) designed to capture the dietary habits of northern Italians.[24] Anthropometric measurements were taken using a standardized protocol. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Women were considered postmenopausal at recruitment if they reported no menses over the preceding 12 months. All other women were considered premenopausal.

The FFQ investigated the quantities and types of foods and drinks consumed in the year up to reporting, from which average daily diet—food items and portion sizes—was estimated for each person. Nutrient values of food items were obtained from Italian food composition tables.[26] Chemical analyses of foods eaten in northern Italy were performed at the University of Modena and Reggio Emilia. Cadmium levels in foods were determined by inductively coupled plasma mass spectrometry after wet-ashing with 50% aqueous nitric acid in a microwave digestion system. Quantification limit was 0.02 μg/kg, and detection limit was 0.007 μg/kg.[27] The cadmium levels thus obtained were added to the Italian food composition tables to thereby afford estimates of dietary cadmium intake (μg/day) for each participant.

Cancer incidence, available from the Varese Cancer Registry, was linked to the ORDET database to identify breast cancer cases incident up to the end of December 2012. The ORDET database was also linked to the regional file of Varese residents to check vital status at December 31st 2012.

Fifty-one women who moved out of the area, or who were lost to follow-up immediately after recruitment were excluded. An additional 1552 women were excluded because the FFQ was not available. We also decided to exclude women diagnosed with breast cancer in the first 90 days of follow-up. The cohort was further reduced by excluding women with missing values for covariates included in the fully adjusted risk models, or for whom the ratio of total energy intake (determined from the FFQ) to basal metabolic rate, determined by Herris-Benedict equation,[28] was at either extreme (first and last half-percentiles) of the distribution. The analyses were conducted on 8924 women.

ER, PR and HER2 status was assessed from pathology records or determined (where possible) from *ad hoc* analyses of slides or tissue blocks, as described elsewhere.[29] Specimens examined immunohistochemically were considered to be hormone receptor-positive when ≥10% of tumor cell nuclei were

stained, and negative when staining was <10%. HER2 was considered overexpressed (positive) when >30% of cancer Cells exhibited complete intense membrane staining: i.e. score 3+ according to ASCO 2007 guidelines.[30] The determinations were performed at the Molecular Biology Unit Laboratories of the National Cancer Institute, Milan.

The study period was from date of recruitment (variable) to closure on December 31, 2012, with participants censored at date of cancer diagnosis, death, or loss to follow-up, whichever came first.

Statistical Methods

Dietary cadmium was adjusted for energy intake using the regression-residual method.[31] Cadmium intake was categorized into quintiles based on the distribution of intake in the whole cohort, with lowest quartile as reference. Hazard ratios (HRs) with 95% confidence intervals (CIs) for developing breast cancer in relation to quintiles of dietary cadmium were estimated from multivariable Cox proportional hazard models, with age as primary time variable. HRs were also calculated for 1 standard deviation increases in cadmium intake as a continuous variable. We ran a minimally adjusted model, with age and energy intake (continuous, kcal/day) as covariates. We also ran models additionally adjusted for menopausal status (pre- or postmenopausal), age at menarche (> or ≤ 13 years), height (continuous), BMI (continuous), age at first birth (nulliparous, ≤30, >30 years), years of education (≤5, 8–12, >12 years), smoking status (never, current, former), alcohol intake (continuous, g/day), vegetable intake (excluding potatoes and pulses, g/day). We also ran fully adjusted models, additionally adjusted for dietary zinc, iron and calcium (continuous, mg/day) (these micronutrients may interfere with the intestinal absorption of cadmium). Linear trends were tested using median cadmium values within quintiles as a continuous variable. We also assessed whether the effect of cadmium exposure was influenced by menopausal status, smoking status, and BMI (all at recruitment) using a likelihood ratio test that compared a model that included the cross-product term with one that did not include it. We also performed analyses stratified by menopausal status at recruitment (pre or postmenopausal). Competing risks Cox regression models were used to analyze the risk of developing one breast cancer subtype with other subtypes considered as competing risks. Women who developed a competing breast cancer subtype were censored at the time of diagnosis. The heterogeneity of associations of cadmium intake with different subtypes was assessed after applying data augmentation, as described by Lunn and McNeil.[32] All analyses were performed with STATA software (version 14.0; Stata Corp, College Station, TX).

Results

During a median 22.1 years of follow-up (179,540 person-years), 481 incident breast cancer cases (451 invasive and 30 *in situ*) were identified among the 8924 women. ER status was available for 456 cases (361 ER^+ and 95 ER^-); PR status was available for 435 cases (293 PR^+ and 142 PR^-); HER2 status was available for 421 cases (351 $HER2^-$ and 70 $HER2^+$).

Estimated dietary energy-adjusted cadmium intake ranged from 0.5 to 16.1 μg/day, mean 7.8 (standard deviation, SD, 1.4) μg/day, or 0.89 (SD 0.31) μg/week/kg body weight. Women in the highest quintile of dietary cadmium consumed more vegetables on average (Table 1), less alcohol, were less educated, more likely to be never-smokers, older at menarche, less likely to be premenopausal, and reported older age at first birth, than women in the lowest quintile. Women in the highest quintile also had lower mean dietary calcium intake, and higher mean dietary iron intake, than women in the lowest quintile. In each quintile, mean estimated dietary iron intake was under 18 mg/day—below the level recommended for premenopausal women by the Italian Human Nutrition Society.[33] The main contributors to dietary cadmium (data not shown in tables) were grain-based foods (46.7%), vegetables (22.8%), wine (11.8%), and potatoes (8.4%). Meat, fish and offal products contributed least to cadmium intake (2.3%, 1.4% and 0.7% respectively).

Table 2 shows HRs (with 95% CIs) for breast cancer by quintiles of energy-adjusted dietary cadmium. By the additionally adjusted model women in the highest intake quintile had a significantly greater risk of breast cancer (HR = 1.43; 95% CI, 1.02–2.01; p trend = 0.047) than those in the lowest quintile (reference). In the fully adjusted model, the risk was slightly higher (HR = 1.54, 95% CI, 1.06–2.22; p trend = 0.028). In the fully adjusted model with cadmium as a continuous variable (1.40 μg/day increments), increasing cadmium was associated with an HR of 1.20 (95% CI, 1.05–1.37) for breast cancer. This association persisted after excluding current and former smokers (data not shown). For women premenopausal at recruitment risks were somewhat higher: by the additionally adjusted model those in the highest quintile of intake had a significantly greater risk of breast cancer than those in the lowest quintile (HR = 1.71; 95% CI, 1.12–2.59, p trend = 0.046); the corresponding HR for the fully adjusted model was 1.73 (95% CI, 1.10–2.71, p trend = 0.063).

For women postmenopausal at recruitment the increased risk of breast cancer was significant only in the model in which cadmium was a continuous variable (HR = 1.32; 95% CI, 1.05–1.66, for 1.40 μg/day increments in cadmium intake). Interactions of risk estimates with menopausal status and other potential effect modifiers (data not shown for BMI and smoking status) were not significant.

We performed competing risks analyses of risk of breast cancer defined by ER, PR and HER2 expression (Table 3). For ER^+ disease, fully adjusted model, the HR was 1.64 (95% CI, 1.06–2.54; highest *vs.* lowest quintile). Associations were weaker and not significant for ER^- disease (HR = 1.30; 95% CI, 0.60–2.83). There was no significant heterogeneity in risk estimates between ER^+ and ER^- disease.

While for PR^- disease there was no evidence that cadmium intake influenced risk, for PR^+ disease, risks were significantly

Table 1. Summary baseline characteristics of 8924 ORDET women by quintiles of estimated dietary cadmium intake

	Quintiles of cadmium intake (energy-adjusted, residual method)				
	Q1	Q2	Q3	Q4	Q5
Cadmium (μg/day)	0.45–6.72	6.73–7.40	7.41–8.02	8.03–8.81	8.82–16.10
N women	1785	1785	1785	1785	1784
Age (years); mean (SD)	47.8 (8.3)	48.3 (8.6)	48.7 (8.6)	48.9 (8.8)	49.0 (8.6)
Body mass index (kg/m^2); mean (SD)	25.0 (4.0)	25.3 (4.3)	25.3 (4.3)	25.4 (4.2)	25.6 (4.4)
Height (m); mean (SD)	1.58 (0.1)	1.58 (0.1)	1.58 (0.1)	1.58 (0.1)	1.58 (0.1)
>30 years at first birth %	11.9	12.9	12.7	12	13.3
>13 years at menarche %	31.2	30.7	34.9	34.2	35.7
Premenopausal at baseline %	70.5	68.8	67.1	65.4	64.1
Never used oral contraceptive %	64.5	66.4	65.2	66.8	69.1
≤5 years of schooling %	42.5	47.5	47.6	51.4	52.4
Never smokers %	60.9	65.0	64.2	67.7	66.3
Energy intake (kcal/day); mean (SD)	1873 (510.7)	1707 (468.2)	1687 (467.2)	1734 (468.2)	1847 (484.5)
Vegetable intake[1] (g/day); mean (SD)	157.0 (76.0)	166.4 (78.6)	189.3 (79.0)	216.6 (83.0)	303.0 (118.9)
>12 g/day alcohol %	52.3	42.5	35.9	31.3	25.3
Zinc (mg/day); mean (SD)	12.0 (3.8)	10.9 (3.0)	10.8 (3.3)	11.2 (3.3)	11.9 (3.6)
Iron (mg/day); mean (SD)	11.8 (3.3)	11.1 (3.0)	11.2 (3.1)	11.8 (3.2)	13.5 (3.5)
Calcium (mg/day); mean (SD)	877.5 (412.7)	704.0 (306.4)	656 (286.5)	652.8 (272.6)	710.4 (287.0)

[1]Leafy vegetables, tomatoes, carrots, fruit and fruiting vegetables.

higher in the 2nd to 5th quintiles of intake compared to the 1st, in both the additionally adjusted and fully adjusted models. For the fully adjusted model HRs were 1.67 (95% CI, 1.12–2.49), 1.83 (95% CI, 1.21–2.76), 2.01 (95% CI, 1.30–3.09), and 1.90 (95% CI, 1.15–3.13). Nevertheless there was no significant heterogeneity in risk estimates between the PR$^+$ and PR$^-$ subtypes.

No significant association of cadmium intake with risk of HER2$^+$ or HER2$^-$ disease was found, with the single exception that, with cadmium as a continuous variable, the HR for HER2$^-$ disease was 1.18 (95% CI, 1.01–1.37). Again there was no significant heterogeneity of risk estimates between the two HER2 subtypes.

Discussion

In our study we found that increasing dietary cadmium intake was significantly associated with increasing risk of breast cancer. Analyses stratified by menopausal status showed that this association persisted in premenopausal women but almost disappeared in postmenopausal women, nevertheless interactions of risk estimates with menopausal status were not significant.

Analysis by disease subtype showed that cadmium intake was associated with increased risk of both ER$^+$ and PR$^+$ breast cancer. The associations were stronger for the fully adjusted model (which also adjusted for dietary iron, calcium and zinc). To our knowledge this is the first prospective study to investigate associations between dietary cadmium and breast cancer defined by hormone receptor expression in an Italian population.

Several cohort and case–control studies found no significant association between dietary cadmium and breast cancer risk,[19–22,34] while the cohort study of Julin *et al.*[23] found a significant positive association, with relative risk (RR) of 1.21 (95% CI, 1.06–1.37) for highest *vs.* lowest tertile of cadmium intake. These six studies (321,315 women, 11,978 breast cancer cases) formed the data for a recent meta-analysis to evaluate the combined RR using a random-effects model. our study did not find a significant association between dietary cadmium and overall breast cancer risk with a combined RR of 1.01 (95% CI, 0.88–1.14) for highest *vs.* lowest category of dietary cadmium.[17] Similarly, a recent meta-analysis of studies on postmenopausal women found no relation between dietary cadmium and breast cancer risk.[18] Thus literature findings are conflicting and suggest the need for further studies on the role of cadmium in breast cancer.

Cadmium has long half-life (10–30 years) and accumulates in tissues, including breast tissue.[35] *In vivo* and *in vitro* studies show that cadmium stimulates the proliferation of breast cancer cells,[36,37] activates and increases the expression of estrogen-regulated genes,[14] activates ER alpha,[14,15,37] and upregulates PR expression in breast cancer cells.[14] These findings suggest that cadmium is an estrogen mimetic and hence may promote the development of estrogen-dependent breast cancer.[23,38,39] We found that ER$^+$ disease was significantly associated high dietary cadmium while ER$^-$ disease was not, however there was no significant heterogeneity in risk estimates between ER$^+$ and ER$^-$ subtypes. Furthermore PR$^+$ disease was significantly associated with high cadmium, while PR$^-$ disease was not, but once again there was no significant heterogeneity of risk between the PR subtypes. A Danish prospective cohort, which found that dietary cadmium was not associated with breast cancer, also found no evidence of an

Table 2. Hazard ratios (HR) with 95% confidence intervals (CIs) for breast cancer in all ORDET women, and according to menopausal status at recruitment, by quintiles of dietary cadmium

	Quintiles of dietary cadmium (energy-adjusted, residual method)						
	Q1	Q2	Q3	Q4	Q5	*p* trend[4]	Continuous
Cadmium intake (μg/day)	0.45–6.72	6.73–7.40	7.41–8.02	8.03–8.81	8.82–16.10		
All women							
N cases	92	100	105	91	93		
Minimally adjusted HR (95% CI)[1]	1.00	1.08 (0.81–1.43)	1.12 (0.84–1.48)	0.99 (0.74–1.32)	1.01 (0.76–1.35)	0.869	0.99 (0.91–1.09)
Additionally adjusted HR (95% CI)[2]	1.00	1.15 (0.86–1.53)	1.26 (0.94–1.68)	1.20 (0.88–1.63)	1.43 (1.02–2.01)	0.047	1.15 (1.02–1.29)
Fully adjusted HR (95% CI)[3]	1.00	1.17 (0.88–1.57)	1.30 (0.96–1.76)	1.25 (0.90–1.74)	1.54 (1.06–2.22)	0.028	1.20 (1.05–1.37)
Premenopausal women							
N cases	60	74	70	51	62		
Minimally adjusted HR (95% CI)[1]	1	1.25 (0.89–1.76)	1.21 (0.85–1.71)	0.91 (0.63–1.33)	1.15 (0.81–1.64)	0.938	0.99 (0.88–1.10)
Additionally adjusted HR (95% CI)[2]	1	1.36 (0.96–1.92)	1.40 (0.98–2.00)	1.13 (0.77–1.69)	1.71 (1.12–2.59)	0.046	1.14 (0.99–1.32)
Fully adjusted HR (95% CI)[3]	1	1.36 (0.96–1.94)	1.40 (0.97–2.04)	1.15 (0.76–1.74)	1.73 (1.10–2.71)	0.063	1.14 (0.97–1.34)
Postmenopausal women							
N cases	32	26	35	40	31		
Minimally adjusted HR (95% CI)[1]	1	0.77 (0.45–1.29)	0.95 (0.59–1.53)	1.08 (0.68–1.72)	0.80 (0.49–1.31)	0.718	1.01 (0.86–1.19)
Additionally adjusted HR (95% CI)[2]	1	0.80 (0.47–1.35)	1.03 (0.63–1.69)	1.25 (0.76–2.05)	1.03 (0.58–1.85)	0.485	1.17 (0.95–1.43)
Fully adjusted HR (95% CI)[3]	1	0.85 (0.50–1.46)	1.14 (0.68–1.92)	1.43 (0.84–2.44)	1.29 (0.68–2.44)	0.190	1.32 (1.05–1.66)
p Interaction[5] Premenopausal *vs.* Postmenopausal = 0.769							

[1]Adjusted for age and energy intake.
[2]Additionally adjusted for menopausal status (pre-, post-menopausal in model including all women), age at menarche (≤13, >13 years), height, BMI, age at first childbirth (nulliparous, ≤30, >30 years), smoking status (current, former, never smoker), years of education (≤5, 8-12, >12 years), alcohol (g/day ethanol), vegetable intake (g/day leafy vegetables, tomatoes, carrots, fruits, fruiting vegetables).
[3]Additionally adjusted for dietary iron (mg/day), dietary calcium (mg/day) and dietary zinc (mg/day).
[4]Test for linear trend performed using median intake of each quintile.
[5]Test for interaction was performed using the likelihood ratio test.

Table 3. Hazard ratios (HRs) with 95% confidence intervals (CIs) for receptor-defined breast cancer in ORDET women by quintiles of dietary cadmium

	Quintiles of dietary cadmium (energy-adjusted, residual method)							
	Q1	Q2	Q3	Q4	Q5	*p* trend[7]	Continuous	*p* heterogeneity[8]
Cadmium intake (μg/day)	0.45–6.72	6.73–7.40	7.41–8.02	8.03–8.81	8.82–16.10			
ER⁻disease[4]								
N cases	21	18	20	15	21			
Minimally adjusted HR (95% CI)[1]	1.00	0.88 (0.47–1.65)	0.97 (0.52–1.80)	0.74 (0.38–1.44)	1.03 (0.56–1.88)	0.952	1.04 (0.85–1.27)	
Additionally adjusted HR (95% CI)[2]	1.00	0.93 (0.49–1.76)	1.08 (0.57–2.04)	0.88 (0.44–1.78)	1.44 (0.71–2.96)	0.408	1.22 (0.94–1.57)	
Fully adjusted HR (95% CI)[3]	1.00	0.91 (0.47 -1.73)	1.03 (0.54–2.00)	0.83 (0.40–1.73)	1.30 (0.60–2.83)	0.600	1.19 (0.90- 1.59)	
ER⁺disease[4]								
N cases	63	78	81	74	65			
Minimally adjusted HR (95% CI)[1]	1.00	1.22 (0.87–1.71)	1.24 (0.89–1.74)	1.16 (0.83–1.63)	1.03 (0.73–1.46)	0.988	0.99 (0.89–1.10)	
Additionally adjusted HR (95% CI)[2]	1.00	1.29 (0.92–1.81)	1.40 (1.00–1.97)	1.41 (0.99–2.01)	1.44 (0.96–2.16)	0.067	1.14 (1.00–1.31)	
Fully adjusted HR (95% CI)[3]	1.00	1.35 (0.96–1.90)	1.49 (1.04–2.12)	1.53 (1.05–2.23)	1.64 (1.06–2.54)	0.025	1.22 (1.04–1.42)	
ER⁺ vs. ER⁻								0.9218
PR⁻disease[5]								
N cases	35	28	31	19	29			
Minimally adjusted HR (95% CI)[1]	1.00	0.77 (0.46–1.27)	0.83 (0.51–1.35)	0.53 (0.30–0.92)	0.82 (0.50–1.35)	0.269	0.95 (0.81–1.13)	
Additionally adjusted HR (95% CI)[2]	1.00	0.85 (0.51–1.39)	0.98 (0.59–1.61)	0.68 (0.38–1.22)	1.29 (0.71–2.33)	0.656	1.16 (0.93–1.45)	
Fully adjusted HR (95% CI)[3]	1.00	0.83 (0.50–1.39)	0.96 (0.57–1.62)	0.66 (0.36–1.23)	1.22 (0.64–2.32)	0.793	1.17 (0.91–1.48)	
PR⁺disease[5]								
N cases	43	65	67	66	52			
Minimally adjusted HR (95% CI)[1]	1.00	1.53 (1.04–2.25)	1.55 (1.06–2.28)	1.56 (1.06–2.29)	1.22 (0.81–1.82)	0.462	1.03 (0.92–1.15)	
Additionally adjusted HR (95% CI)[2]	1.00	1.59 (1.07–2.34)	1.70 (1.15–2.52)	1.82 (1.21–2.72)	1.63 (1.02–2.59)	0.031	1.16 (1.01 -1.35)	
Fully adjusted HR (95% CI)[3]	1.00	1.67 (1.12–2.49)	1.83 (1.21–2.76)	2.01 (1.30–3.09)	1.90 (1.15–3.13)	0.011	1.24 (1.05–1.47)	
PR⁺ vs. PR⁻								0.6551
HER2⁻ disease[6]								
N cases	62	79	74	67	69			
Minimally adjusted HR (95% CI)[1]	1.00	1.27 (0.91–1.78)	1.16 (0.83–1.63)	1.08 (0.76–1.52)	1.11 (0.79–1.57)	0.860	1.02 (0.91–1.13)	
Additionally adjusted HR (95% CI)[2]	1.00	1.32 (0.95–1.86)	1.25 (0.89–1.78)	1.23 (0.85–1.77)	1.40 (0.94–2.10)	0.172	1.12 (0.98–1.29)	
Fully adjusted HR (95% CI)[3]	1.00	1.36 (0.97–1.92)	1.31 (0.91–1.88)	1.30 (0.88–1.91)	1.53 (0.99–2.37)	0.102	1.18 (1.01–1.37)	
HER2⁺disease[6]								
N cases	17	11	15	13	14			
Minimally adjusted HR (95% CI)[1]	1.00	0.67 (0.31–1.44)	0.92 (0.46–1.85)	0.81 (0.39–1.67)	0.86 (0.42–1.75)	0.811	0.95 (0.75–1.20)	
Additionally adjusted HR (95% CI)[2]	1.00	0.80 (0.37–1.73)	1.29 (0.62–2.67)	1.33 (0.61 -2.89)	2.05 (0.88–4.77)	0.073	1.32 (0.97–1.79)	
Fully adjusted HR (95% CI)[3]	1.00	0.79 (0.36–1.73)	1.25 (0.58–2.67)	1.31 (0.58–2.99)	2.02 (0.81–5.05)	0.097	1.34 (0.95–1.89)	
HER2⁺ vs. HER2⁻								0.5075

[1]Competing risks Cox model adjusted for age and energy intake.
[2]Competing risks Cox model additionally adjusted for menopausal status, age at menarche, height, BMI, age at first child, smoking status, years of education, alcohol, and vegetables.
[3]Competing risks Cox model additionally adjusted for dietary iron (mg/day), dietary calcium (mg/day), and dietary zinc (mg/day).
[4]Excluding 25 cases with no information on ER status.
[5]Excluding 46 cases with no information on PR status.
[6]Excluding 60 cases with no information on HER2 status.
[7]Test for linear trend performed using median intake of each quintile.
[8]Test for heterogeneity performed using data augmentation technique, as described by Lunn and McNeil.

association between cadmium and specific PR or ER subtypes.[21] A case–control study on Japanese women, which found no overall association between dietary cadmium and breast cancer risk, and weak or absent associations with PR^+ and PR^- disease, found that high cadmium intake was associated with significantly increased risk of ER^+ disease risk (p trend = 0.032) among postmenopausal women.[22] A large Swedish prospective study on postmenopausal women, found that dietary cadmium was significantly associated with risk of invasive breast cancer overall and also ER^+ disease, but not ER^- disease.[23]

Thus, literature findings on the risk of breast cancer defined by ER and PR status are contrasting, as they are for risk of breast cancer as a whole. Our data suggest that cadmium does have a breast-cancer-promoting effect, but the evidence is insufficient to suggest whether this effect occurs occur *via* estrogen-mimetic mechanisms. Furthermore a potential estrogenic effect of cadmium might be partly masked by the effect of endogenous estrogens in premenopausal women in our cohort.

As regards the HER2 status of breast cancer, although we found a significant positive association between dietary cadmium as a continuous variable and $HER2^-$ disease (Table 3), in general associations of cadmium with breast cancer defined by HER2 status were not significant. More importantly, there was no significant heterogeneity of risk between HER2 subtypes. To our knowledge no other study has examined the association between dietary cadmium and breast cancer defined by HER2 status. However Strumylaite *et al.* examined urinary cadmium (marker of long-term cadmium exposure) and the HER2 status of breast cancer in a case–control study,[40] finding a significant association (p trend <0.001) between risk of $HER2^-$ breast cancer and urinary cadmium.[38,39]

Mean intake of cadmium from the diet in our cohort was 0.89 (SD 0.31) μg/week/kg body weight, which is well below the intake limit of 7 μg/week/kg considered safe by the WHO.[41] The daily mean intake of dietary cadmium was 7.8 μg (standard deviation, SD, 1.4), which is similar to the intake reported in a recent study in a Northern Italy community (median 5.0 μg/day, interquartile range 3.17–7.65).[27]

Cadmium is absorbed from the intestine and moved into the blood by the same transporters used for essential metals like zinc, iron and calcium,[42] and since these transporters are up-regulated when iron status is low,[43] cadmium absorption is increased when iron status is low. In fact cadmium absorption is increased when calcium, iron or zinc are deficient[11]; and blood levels of cadmium are significantly increased in premenopausal women with low iron status (serum ferritin <20 μg/l).[10] For 26% of our cohort dietary zinc was under the recommended level 9 mg/day; and for 95% of the cohort women who were premenopausal at recruitment, dietary iron intake was below the 18 mg/day recommended.[33] As regards calcium intake, this was estimated from food sources only, but if we assume that women were drinking about 1.5 l of water/day, calcium intake would be deficient in 33% of the cohort. Based on these considerations, we suggest that the increased breast cancer risk, particularly in premenopausal women, is in part be due to increased cadmium absorption due to mild chronic iron deficiency[10] and perhaps also deficient zinc and calcium intake.

Strengths of the present study are its prospective design, availability of information on diet, availability of ER, PR and HER2 status of the cancers in most cases, and availability of abundant additional information making it possible to adjust for potential confounders including smoking. Furthermore breast cancer cases were identified by the local cancer registry, and it is unlikely that any cases were lost.[44] Other strengths are the large number of food samples used for the analytical determinations of cadmium, collected from local groceries and supermarkets in order to mimic the real sources of foodstuffs in the population.[27]

The fact that diet was only investigated on one occasion (at recruitment) is a limitation, since dietary habits may have changed over time. The FFQ assessed diet over the year up to recruitment, permitting assessment of long-term dietary cadmium intake, even though the FFQ was not originally designed to assess cadmium. Although cadmium levels in foods were determined accurately, their levels vary with soil type, water source, fertilizer used, and levels of anthropogenic contamination.[41,45] In addition we had no information on environmental/occupational exposure to cadmium, although data indicate that in most non-smokers diet is the main source of cadmium.[46] Also, and as noted above, cadmium absorption is affected by nutritional status,[11] so dietary intake may not accurately reflect the cadmium absorbed by individuals. All these factors will have resulted in some unquantifiable misclassification of exposure. It is noteworthy however that our estimates of daily cadmium intake were closely similar to those estimated by other Italian studies[47,48] while the ranking of cadmium sources (cereals > vegetables > sweets > fish/seafood) was the same as that found in other Italian,[27,48] and European studies.[45]

Finally, the limited number of breast cancer cases likely resulted in insufficient power to assess whether the cancer-promoting effect of cadmium was due to estrogen-mimetic mechanisms. Similarly we were unable to analyze subtypes such as ER^++PR^+ which might have provided insight in the role of cadmium in cancer subtypes known to differ in etiology and prognosis.

Conclusions

We have found that the highest levels of dietary cadmium are associated with increased risk of breast cancer in this large population-based prospective cohort. The lack of significant heterogeneity in risk estimates between ER^+ and ER^- disease and between PR^+ and PR^- disease, mean that our data neither support nor refute the hypothesis that cadmium acts as a metalloestrogen, mimicking or interfering with the action of physiological

estrogens. This may be because relatively few breast cancer cases were analyzed, resulting in insufficient power. Thus, further studies on the effects of prolonged exposure to low levels of cadmium on breast cancer development are required.

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