Serum calcium and breast cancer risk:

Results from a prospective cohort study of 7847 women

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Abbreviations: PTH, parathyroid hormone; pHPT, primary hyperparathyroidism; BMI, body mass index; MPP, Malmö Preventive Project; HRT, hormone replacement therapy; SD, standard deviation; RR, relative risk; CI, confidence interval

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Abstract

Experimental and epidemiological studies suggest that calcium-regulating hormones – parathyroid hormone (PTH) and vitamin D – may be associated with breast cancer risk.

No prospective cohort study has investigated the association between pre-diagnostic calcium levels and subsequent risk of breast cancer. We have examined this in a cohort of 7847 women where serum calcium levels and established risk factors for breast cancer had been assessed at baseline. During a mean follow-up of 17.8 years, 437 incident breast cancer cases were diagnosed. Incidence of breast cancer was calculated in different quartiles of serum calcium levels and a Cox's proportional hazards analysis was used to obtain corresponding relative risks (RR), with a 95% confidence interval (CI), adjusted for potential confounders.

In premenopausal women, serum calcium levels were inversely associated with breast cancer risk in a dose-response manner. The adjusted RR (95% CI) of breast cancer was in the 2nd calcium quartile 0.91 (0.65-1.30), in the 3rd quartile 0.89 (0.60-1.31), and in the 4th quartile 0.56 (0.32-0.98), as compared to the 1st calcium quartile. In postmenopausal overweight women (BMI>25), breast cancer risk was higher in calcium quartiles 2 to 4 as compared to the 1st quartile. Our findings may have implications for primary prevention of breast cancer and for the management of asymptomatic primary hyperparathyroidism.

Introduction

Breast cancer is the most common malignant disease in women. Most established risk factors concern reproductive history, but other factors may be of interest. Experimental studies indicate that calcium levels may affect tumour development [1-4]. The calcium regulating hormones vitamin-D and its metabolites, most notably 1,25 $(OH)_2$ D3, and parathyroid hormone (PTH) have also been suggested to affect breast cancer risk [5, 6].

PTH and vitamin-D regulate the production of each other, and both factors increase serum calcium levels [7]. Experimental studies suggest that PTH has carcinogenic and tumour promoting effects [8-10]. At least three record-linkage studies have found a weak positive correlation between risk of breast cancer and primary hyperparathyroidism (pHPT), a condition with high PTH and often high calcium levels [11-13]. Contrary to this, it has been suggested that high vitamin-D levels may have tumour protective effects [6, 14]. Thus, the serum calcium level, either in itself or as a marker of certain conditions, may be associated with breast cancer risk. This relation is complicated, however, by the fact that potential mechanisms act in opposite directions.

Furthermore, several conditions may modify the relation between the factors mentioned above and the risk of breast cancer. High age and obesity are associated with a high prevalence of pHPT [15, 16] and low levels of vitamin-D, e.g. 25(OH)D [17]. It has also been suggested that pre- and postmenopausal women may have different risk factors for breast cancer [18] and that obesity may modify the association between established risk factors and breast cancer [19].

To our knowledge, there has been no prospective cohort study on serum calcium levels in relation to incidence of breast cancer. Here we report a cohort of 7847 women, with information on total serum calcium and risk factors for breast cancer, followed with regard to breast cancer incidence during an average of 17.8 years.

The aim of the present analysis was to study incidence of breast cancer in women according to prediagnostic levels of serum calcium, with special regard to body mass index (BMI) and menopausal status.

Materials and methods

The Malmö Preventive Project

The Malmö Preventive Project (MPP) in Malmö, Sweden, was established in 1974 for screening with regard to cardiovascular risk factors [20]. Entire birth-year cohorts, men and women, registered as citizens in Malmö were successively invited by mail to a health examination. Approximately 70% of invited women attended [20]. When the department closed in 1992, 10,902 women, born between 1926 and 1949, had been examined. Their mean age at baseline was 49.7 years, and 59.8% were peri-/postmenopausal [21].

Baseline examination

A self-administered questionnaire was used for a comprehensive interview on lifestyle habits, medical history, marital status, education and use of medications [20]. The questionnaire was revised several times. Information on reproductive history was included in the questionnaire for women screened from April 1983 and onwards. Factors that have been associated with breast cancer risk were available from the questionnaire: age at menarche, menopausal status, parity, use of oral contraceptives and hormonal replacement therapy (HRT), educational level and marital status. Information necessary for calculation of body mass index (BMI) (kg/m^2) was assessed by a trained nurse on baseline examination; height was measured to the nearest

centimeter and weight was recorded with the subject wearing no shoes, on a beam scale at intervals of 0.1 kg. In the morning, after an overnight fast, all subjects gave a blood sample, which was analysed immediately. Calcium was measured photometrically by the laboratory at the Dept. of Clinical Chemistry, University Hospital of Malmö, on a PRISMA multichannel autoanalyser (Clinicon AB, Bromma, Sweden) [22, 23]. The coefficient of variation was 1.52% [22]. The reference value for serum calcium during this period was 2.20-2.60 mmol/L. Women were classified as peri/postmenopausal if they affirmed that their menstruations had ceased, that they had menopausal symptoms or that they were taking any "female hormonal medication" because of such symptoms.

Study cohort

Out of 10,902 women, information on reproductive factors including menopausal status was known for 8051 women. Serum calcium had been measured in 8004 of these. A total of 157 women with prevalent invasive breast cancer at baseline were excluded. Thus, the present study is based on 7847 women.

Ethical clearance for this study was obtained from Ethical committee in Lund, LU 639-03.

Follow-up

Breast cancer cases, invasive and *in situ,* were retrieved by record linkage with the Swedish Cancer Registry and the Southern Swedish Regional Cancer Registry. There were in all 437 incident cases. Stage at diagnosis was retrieved from clinical notes and from a clinical registry run by the South Swedish Breast Cancer Group. Stage according to the UICC was given from the TNM classification [24]. Information on vital status was retrieved from the Swedish Cause of Death Registry. Each woman was followed until end of follow-up, 31st December 2003, or until she got breast cancer or died. The average follow-up was 17.8 years (SD: 5.8 years).

Statistical methods

Quartile cut-points for total serum calcium were based on the distribution for women in the study cohort excluding those with prevalent cancer of any site (not including cervical cancer *in situ*). The incidence of breast cancer per 100 000 person-years was calculated in relation to serum calcium quartiles. Cox's proportional hazards analysis was used to estimate corresponding relative risks (RRs), with a confidence interval (CI) of 95%. In a second analysis, potential confounders were introduced as covariates. BMI and age were treated as continuous variables, whereas all other covariates were entered as categorical variables. Test for trend over quartiles were calculated and a p-value<0.05 was considered statistically significant.

Separate analyses were made in pre- *vs.* peri-/postmenopausal women and in different strata of BMI, i.e. BMI < 25 *vs.* BMI ≥ 25 (overweight and obese women). All analyses were repeated excluding cases diagnosed within two years following baseline examination. Analyses were also done using separate quartile cut-points based on serum calcium levels in pre- vs. peri/postmenopausal women.

Stage distribution was assessed in relation to serum calcium quartiles, BMI and postmenopausal status. The chi-square test was used in order to assess heterogeneity between groups, and a p-value less than 0.05 was considered statistically significant.

Results

Use of oral contraceptives and hormone replacement therapy, HRT, were more common in lower calcium quartiles and there was a higher percentage of peri/postmenopausal women in higher calcium quartiles, table 1. Mean calcium levels (range) were in premenopausal women 2.32 (1.89-2.76) mmol/L and in peri/postmenopausal women 2.36 (2.03-2.80) mmol/L. This

is in line with previous studies that have shown serum calcium to rise with menopause [25, 26].

There was no overall association between serum calcium levels and breast cancer, table 2, crude and age-adjusted RR:s were similar to those obtained in the full model.
Serum calcium levels were inversely associated with incidence of breast cancer in premenopausal women in a dose-response manner, but p for trend did not reach statistical significance, p=0.254. In postmenopausal women, the p-value for trend over quartiles was 0.45. There was a weak, non-significant, association between serum calcium levels and breast cancer incidence in peri-/postmenopausal women.

Serum calcium was associated with a high risk of breast cancer in overweight postmenopausal women, table 3. The RR:s, adjusted for known risk factors for breast cancer, as outlined in table 1, for the 3^{rd} and 4^{th} quartile as compared the 1^{st} were 3.10 and 2.72 respectively. Crude and age-adjusted RR:s were similar. There was no statistically significant trend over quartiles in any of the analyses presented in table 3. In the subgroup of obese, postmenopausal women the distribution of other risk factors was similar in all calcium quartiles.

When the analyses were repeated excluding cases with breast cancer occurring within 2 years following baseline, all results were similar (data not shown). Results were also similar when using separate quartile cut-points based on serum calcium levels in pre- *vs.* postmenopausal women (data not shown); however, results did not reach statistical significance in the 4^{th} calcium quartile among obese peri/postmenopausal women, RR: 1.76 (0.92-3.38).

Stage was known for 422 out of 437 breast cancer cases (96.6%). There was no clear relation between stage distribution and serum calcium quartiles. Thus, 31.5 % of cases in the 1st calcium quartile were diagnosed with a stage II+ tumour (stage II, III or IV), 37.4% in the 2nd, 29.5% in the 3rd and 43.0 % in the 4th serum calcium quartile. These differences corresponded to a p-value of 0.21. Stage distribution in different serum calcium quartiles was also similar in pre- *vs.* postmenopausal and in normal *vs.* overweight/obese women.

Discussion

In this study serum calcium levels were inversely associated with breast cancer risk in premenopausal women in a dose-response manner. This study also indicates that calcium levels are positively associated with breast cancer in overweight peri-/postmenopausal women.

It may be questioned whether it is appropriate to use a single determination for ranking of serum calcium levels. Both short-time [27] and long-time [28] intra-individual variation in total serum calcium are low. Even though serum calcium levels rise with menopause [26, 29] there seems to be significant 'tracking', *i.e.* the ranking of calcium levels between individuals tends to remain the same before and after menopause [25]. Although inter-individual differences in absolute values for serum calcium are low, we still consider these differences important when large groups are compared. Thus, we believe that a single measurement of serum calcium is a useful marker for differences with regard to calcium homeostasis.

It has been argued that free (ionised) calcium provides the best indication of calcium status because it is biologically active and tightly regulated by calcium-regulating hormones. Total calcium levels are affected by plasma protein levels, notably albumin. In our cohort, adjusting

for serum albumin was not possible, since albumin levels were only known for about a quarter (n=2048) of the study population. However, total calcium has been considered a good measure of calcium homeostasis in outpatients and healthy individuals where albumin will be expected to be in the normal range [23]. Albumin was normally distributed among those with known albumin levels, and only seventy-five women (3.7%) had an albumin outside the normal reference range (36-45 g/L). All samples were also collected in a standardised manner, which minimises differences in albumin levels due to fasting status or diurnal variation [30]. Following this, we consider total serum calcium a useful and valid measurement of calcium status in this study population.

Vitamin D and PTH-levels seem to be unaffected by menopause *per se* [25]. The rise of serum calcium with menopause might instead be explained by the fact that bone seems to turn more sensitive to PTH in the absence of estrogen [31, 32]. The associations between some risk factors and breast cancer differ in pre- and postmenopausal women and this was one reason *a priori* to study breast cancer incidence separately in pre- *vs.* peri/ postmenopausal women [33]. Moreover, pHPT is more common in postmenopausal than in premenopausal women [15]. PHPT is often a mild disease with a protracted course, asymptomatic in its early stages, with gradually rising calcium levels. The prevalence of clinical symptomatic pHPT in postmenopausal women has been estimated to be around 3% [34] and the prevalence of asymptomatic pHPT could be even higher. Whether this prevalence is high enough to affect calcium levels overall in this group might be questioned, but we think it is reasonable to assume a higher percentage of women with undiagnosed, asymptomatic pHPT in the higher serum calcium quartiles.

If breast cancer itself causes hypercalcemia, such as in hypercalcemia of malignancy, this could lead to a spurious association between calcium levels and breast cancer risk. This would be a serious problem in cross-sectional studies, i.e. case-control studies, but it is less of a problem when pre-diagnostic calcium levels are available, as in this analysis. Moreover, hypercalcemia of malignancy usually presents with advanced disease [35], and such patients would probably have been diagnosed with breast cancer at baseline and would, thus, have been excluded. To further exclude cases where hypercalcemia might have been caused by undiagnosed breast cancer, the analyses were repeated excluding cases with breast cancer occurring within two years following baseline. All results were similar and we do not consider that malignancy-related hypercalcemia associated with breast cancer have affected the observations in the present study. Malignancies other than breast cancer can also give rise to hypercalcemia. In these cases, an association between serum calcium and breast cancer could be due to calcium levels, or mediators of calcium homeostasis, affecting breast cancer growth. Hence, we did not exclude other prevalent malignancies from our analysis, but only those with prevalent breast cancer.

Incomplete follow-up may affect the results. However, the Swedish Cancer Registry and the Swedish Cause of Death Registry have been validated and found to have a completeness of about 99% [36].

Another relevant issue is whether the results could have been caused by detection bias. Women in these birth cohorts have regularly been invited to mammographic screening since the end of the 1970s [37]. If calcium levels were associated with factors that affect time of diagnosis, such as participation in mammographic screening or patient's delay, a trend over quartiles with respect to stage at diagnosis would be expected. No such trend was observed and we consider it unlikely that detection bias has influenced our results.

It may be asked whether breast cancer cases in this cohort may be considered representative of the whole breast cancer population. This cohort mainly comprised middle-aged women and 30% of the women invited to the health examination did not attend. As we have no information about exposure to the studied risk factors in women outside this cohort, observed incidence rates may not be applicable to all age groups or to the general population. However, as there was a wide distribution of calcium levels, it was possible to make internal comparisons between subjects with low and high values respectively. We consider that our estimations of relative risks were not considerably affected by selection bias.

It is possible that both high serum calcium levels and breast cancer are caused by a common factor. The results were probably not confounded by most known risk factors for breast cancer though, since information on these were known for the study cohort, and results were similar when statistical analyses were repeated adjusted for these factors. We were not able to adjust our estimates for heredity or physical activity, since we had no information on these variables. It is, however, unlikely that these factors have influenced the results, since, to our knowledge, there is no strong correlation between either physical activity or heredity and serum calcium levels. Other factors that would have been of interest are determinants of calcium homeostasis, such as diet, sunshine exposure, vitamin D and PTH-levels. The inclusion of these factors in future studies would be very valuable.

This is the first prospective cohort study on serum calcium levels and breast cancer risk. In order to explore whether factors that affect PTH and vitamin-D levels, i.e. menopausal status and obesity, modify the relation between calcium levels and breast cancer risk, several subgroup analyses were performed. Some groups had a limited number of cases and our

finding that high calcium levels are associated with breast cancer in overweight peri-/postmenopausal women was based an a low number of cases and CIs were wide. However, the distribution of risk factors in postmenopausal obese women between calcium quartiles does not differ from the whole study population (data not shown) and the results may represent a true threshold-effect in RR between the first and second calcium quartiles. Indeed, it may be that the lowest quartile has a lower than average risk; that low calcium levels in obese postmenopausal women reflects some protective factor. Such factors may be related to parameters that affect calcium homeostasis, i.e. PTH or vitamin-D, but this remains to be evaluated. Given the small number of cases in some subgroups, a chance finding cannot completely be ruled out and our results will have to be confirmed in larger studies.

High levels of calcium *per se* can in experimental models increase cell differentiation, decrease proliferation, and induce apoptosis [1-4]. All of this would have a tumour protective effect. Calcium levels in serum may also be considered a marker for certain conditions, as calcium levels are increased following stimulation by PTH and vitamin-D. Experimental studies have shown that PTH have anti-apoptotic effects and may promote invasiveness [8-10], mechanisms that stimulate tumour growth. Contrary to this, vitamin-D may induce apoptosis, cell cycle arrest, and differentiation. Vitamin-D also inhibit invasiveness and angiogenesis [6, 14, 38], all of which have tumour protective effects. Following these potential biological mechanisms, it is possible to hypothesise that high serum calcium levels may be associated with both tumour protective and tumour promoting effects. To date, no study has investigated the influence of calcium levels as well as PTH and vitamin-D levels on breast cancer risk.

There is typically a weak, non-significant, inverse association between calcium intake and risk of breast cancer as reported by at least eight case-control studies and three cohort studies (referred to in [39, 40]). However, calcium homeostasis is kept very tight in humans, and dietary intake is a poor predictor of calcium levels in blood. No prospective cohort study has investigated the association between serum calcium levels in blood and breast cancer incidence. Three epidemiological record-linkage studies have evaluated primary hyperparathyroidism (pHPT) and risk of breast cancer [11-13]. Two of these studies found a statistically significant positive association between pHPT and subsequent breast cancer. Two studies have examined vitamin-D levels in pre-diagnostic blood samples in relation to breast cancer risk. Hiatt et al. did not find any significant association between the vitamin-D metabolite $1,25(OH)_2D$ and breast cancer, but included only 96 cases [41]. Bertone-Johnson et al. found that the vitamin-D metabolites 25(OH)D and $1,25(OH)_2D$ were associated with a small, non-significantly, decreased risk of breast cancer [42]. None of these studies included information on calcium levels or PTH.

Studies on the relation between dietary intake of vitamin-D, or dairy products, and risk of breast cancer, do not however provide consistent evidence for an association [4, 40, 42, 43].

High calcium levels may reflect different conditions in specific groups as pHPT and low vitamin-D-levels are more common in postmenopausal [15, 17] and obese women [16]. It is possible to hypothesise that calcium levels in postmenopausal and obese women may reflect PTH levels rather than vitamin-D levels and that calcium levels in premenopausal women may mainly be a marker of vitamin-D levels. Obese are known to have an altered endocrine metabolism [44] and possibly factors as insulin or insulin-like growth factor (IGF) could be related to our observations [45].

Our findings will have to be tested in future studies, which must include information on PTH and vitamin-D levels as well as on serum calcium. Further research will give guidance on primary prevention for breast cancer, and can be important when deciding whether or not to treat individuals with mild or asymptomatic hyperparathyroidism.

We conclude that in this cohort of 7847 women, serum calcium levels were inversely associated with breast cancer risk in premenopausal women in a dose-response manner. This study also indicates that high calcium levels may increase breast cancer risk in overweight peri-/postmenopausal women.

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TABLE 1 - DISTRIBUTION OF POTENTIAL RISK FACTORS FOR BREAST CANCER ACCORDING TO SERUM CALCIUM LEVEL

Factor*	Serum calcium quartile [mmol/L]				
	1 n=1880 [<2.29]	2 n=2109 [2.29-2.34]	3 n=2034 [2.35-2.40]	4 n=1824 [>2.40]	All n=7847
	(column percent; *mean (SD) in italics*)				
Age at baseline (years)	*51.4 (4.5)*	*52.0 (4.6)*	*52.5 (4.5)*	*53.5 (4.0)*	*52.3 (4.5)*
BMI (kg/m^2)	*24.7 (4.2)*	*24.6 (4.2)*	*24.9 (4.2)*	*25.1 (4.4)*	*24.8 (4.2)*
<12 years at menarche (vs. ≥ 12 years)	11.8	13.1	12.0	13.7	12.6
Oral contraception (yes vs. no)	8.4	5.3	3.8	2.0	4.9
Peri-/postmenopausal (vs. premenopausal)	46.6	59.4	69.6	78.8	63.5
Number of children					
0	13.9	14.6	17.4	17.2	15.8
1-2	59.5	59.6	56.6	56.4	58.0
3-4	25.2	24.5	24.3	24.8	24.7
>4	0.9	1.1	1.0	1.2	1.1
HRT among peri-/postmenopausal (n=4980) (vs. no-use)	24.7	16.0	10.1	8.3	13.6
Smoking status					
Never	47.7	46.6	46.5	49.0	47.4
Ex	20.5	20.7	19.2	18.7	19.8
Current	31.9	32.7	34.3	32.3	32.8
Married					
Yes	51.2	4.8.7	50.6	50.3	50.2
No	44.9	44.9	42.1	41.2	43.3
Missing	3.9	6.4	7.3	8.6	6.5
Alcohol consumption					
None	15.6	15.3	16.9	18.1	16.5
Less than every week	59.4	61.0	58.8	58.7	59.5
Every week	25.0	23.7	24.3	23.2	24.1
≥12 years of education	34.4	32.4	32.1	32.0	32.7

* Separate missing category reported if > 1% of subjects had no information

TABLE 2- BREAST CANCER INCIDENCE IN PRE-, PERI/POSTMENOPAUSAL AND ALL WOMEN IN RELATION TO SERUM CALCIUM LEVELS

Menopausal status	Serum calcium quartile	Individuals	Breast cancer cases	Person-years	Incidence/ 100000	RR (CI: 95%)	RR* (CI: 95%)
Premenopausal	1	1003	72	17281	417	1.00	1.00
	2	857	56	14763	379	0.91 (0.65-1.30)	0.92 (0.65-1.31)
	3	618	39	10626	367	0.89 (0.60-1.31)	0.88 (0.59-1.30)
	4	386	15	6518	230	0.56 (0.32-0.98)	0.56 (0.32-0.99)
Peri/postmenopausal	1	877	39	14055	277	1.00	1.00
	2	1252	66	20335	324	1.17 (0.79-1.74)	1.20 (0.81-1.79)
	3	1416	80	22185	361	1.31 (0.89-1.92)	1.38 (0.93-2.03)
	4	1438	70	21899	320	1.17 (0.79-1.73)	1.26 (0.84-1.89)
All	1	1880	111	31336	354	1.00	1.00
	2	1987	122	35098	348	0.98 (0.76-1.27)	0.99 (0.76-1.28)
	3	1915	119	32811	363	1.03 (0.80-1.34)	1.05 (0.81-1.36)
	4	1739	85	28417	299	0.86 (0.65-1.14)	0.89 (0.67-1.19)
Total		7847	437	127662	342		

* adjusted for age, BMI, age at menarche, use of oral contraception, number of children, use of hormone-replacement therapy (in peri-/postmenopausal women), smoking status, marital status, alcohol consumption and educational level

TABLE 3- BREAST CANCER INCIDENCE IN PRE- AND PERI/POSTMENOPAUSAL WOMEN IN RELATION TO SERUM CALCIUM IN DIFFERENT STRATA OF BMI

Menopausal status	Serum calcium quartile	BMI < 25			BMI ≥ 25		
		Individuals	Cases	RR* (CI: 95%)	Individuals	Cases	RR* (CI: 95%)
Premenopausal	1	650	45	1.00	353	27	1.00
	2	582	35	0.91 (0.58-1.43)	275	21	0.98 (0.55-1.76)
	3	405	28	1.04 (0.65-1.69)	213	11	0.60 (030-1.22)
	4	228	10	0.68 (0.34-1.35)	158	5	0.44 (0.16-1.15)
Peri/postmeno-pausal	1	518	31	1.00	359	8	1.00
	2	736	35	0.82 (0.50-1.34)	516	31	2.74 (1.25-5.98)
	3	797	40	0.91 (0.57-1.47)	619	40	3.10 (1.44-6.68)
	4	795	37	0.88 (0.54-1.44)	640	33	2.72 (1.24-5.94)
All	1	1168	76	1.00	712	35	1.00
	2	1318	70	0.84 (0.61-1.17)	791	52	1.34 (0.87-2.06)
	3	1202	68	0.95 (0.68-1.32)	832	51	1.26 (0.81-1.94)
	4	1023	47	0.82 (0.56-1.19)	798	38	1.09 (0.68-1.74)
Total		4711	261		3133	176	

*adjusted for age, age at menarche, use of oral contraception, number of children, use of hormone-replacement therapy (in peri-/postmenopausal women), smoking status, marital status, alcohol consumption and educational level