Dairy Products, Calcium, Vitamin D, Lactose and Ovarian Cancer: Results from a Pooled Analysis of Cohort Studies

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Ovarian Cancer Survival

Cancer site	Case-fatality rate (%)
Ovary	63
Cervix	37
Breast	19
Endometrium	18

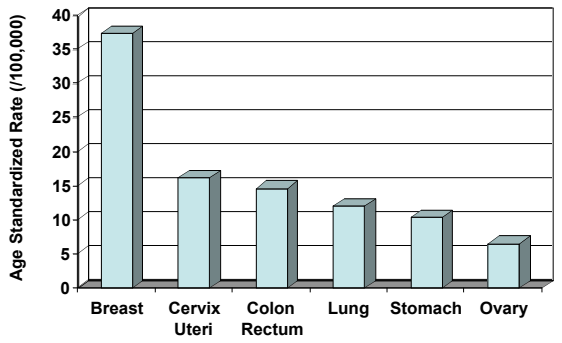
5-year survival: overall ~44%

~70% of cases are diagnosed at advanced stages

American Cancer Society, 2004

I was fortunate to be asked to discuss an analysis that I have been directly involved with, so it was relatively easy to put this together.

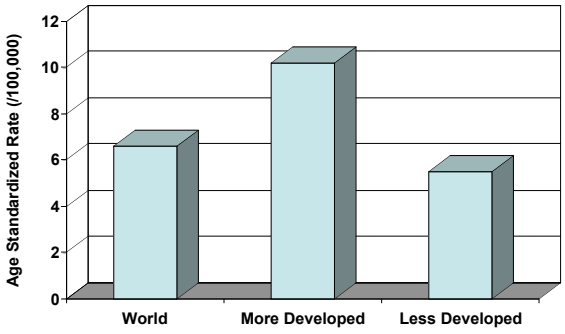
Cancer Incidence Worldwide: Women



GLOBOCAN, 2002

I wasn't sure how familiar people are with the descriptive epidemiology of cancer worldwide. I am going to be talking about ovarian cancer, which is the sixth leading type of cancer in women worldwide. To put it into perspective, ovarian cancer incidence is about 5 times lower than that observed for breast cancer worldwide.

Ovarian Cancer Incidence Rates



GLOBOCAN, 2002

There is a large geographic variation in ovarian cancer incidence rates. The incidence rate in more developed countries is about double that in less developed countries.

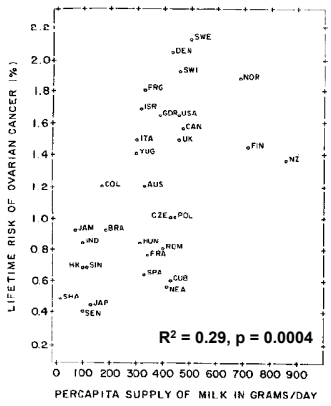
Most ovarian cancers are detected at advanced stages, so the case fatality rate for ovarian cancer is very high, in comparison to cervix, breast, and endometrial cancers. So it is important to identify risk and preventive factors for ovarian cancer.

Risk Factors for Ovarian Cancer

- **Reproductive factors - decrease risk**
  - **high parity**
  - **oral contraceptive use**
  - **increasing duration of lactation**
  - **tubal ligation**
- **Family history: first-degree relative - increase risk**

Some of the risk factors that have been regularly established are reproductive factors. High parity, oral contraceptive use, increasing duration of lactation, and tubal ligation have been found to decrease the risk of ovarian cancer. Whereas, family history has been found to increase the risk.

Ecologic Study: Correlation Between Ovarian Cancer Incidence and Per Capita Milk Consumption



Cramer 1989

Diet has also been looked at in many studies. This is an ecologic study of milk consumption and ovarian cancer incidence rates

worldwide. There was a positive correlation of .29, comparing milk consumption to incidence rates.

Potential Carcinogenic Mechanisms

- **Lactose → galactose**
  - **Increases gonadotropin levels**
  - **Toxic to oocytes**
- **Vitamin D → decreases cell proliferation**
- **Calcium**
  - **Downregulates vitamin D absorption**
  - **Decreases parathyroid hormone**
- **Fat → increases estrogen levels**

There are several potential mechanisms as to why milk may be associated with ovarian cancer risk. Milk is a rich source of lactose, which is cleaved to form galactose. Galactose has been found to increase gonadotropin levels, and to be toxic to the oocytes; thereby it might increase the risk of ovarian cancer. Dairy products are also a source of Vitamin D, particularly milk. Vitamin D might decrease cell proliferation so it could decrease the risk of ovarian cancer. Calcium intake has been found to down-regulate Vitamin D absorption, so it could increase ovarian cancer risk. However, calcium also decreases parathyroid hormone, and by decreasing parathyroid hormone, it could result in a decrease in ovarian cancer risk because it decreases IGF-1 levels. Diary products are also a source of fat intake and the hypothesis with fat is that fat intake might increase estrogen levels.

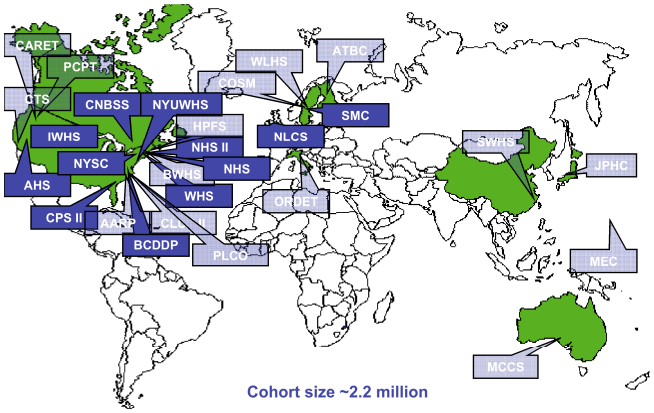
Pooling Project of Prospective Studies of Diet and Cancer

- International consortium of cohort studies to evaluate diet and cancer associations using standardized analytic criteria
  - Meta-analysis of individual data
- Established in 1991

Because ovarian cancer is a less common cancer, many prospective cohort studies have not been able to look at this association on their own. So we decided to look at this within the Pooling Project of Prospective Studies on Diet and Cancer. Because I work on this study, I decided to describe it in a little more detail. This is an international consortium of cohort studies, with the goal to evaluate diet and cancer associations using standardized analytical criteria across all studies. So it's an example of a meta-analysis of the individual data as opposed to a meta-analysis of the published literature where you can only extract the risk estimates from the published literature.

Currently, there are 28 studies in the Pooling Project, with an overall cohort size of about 2.2 million.

Studies in the Pooling Project

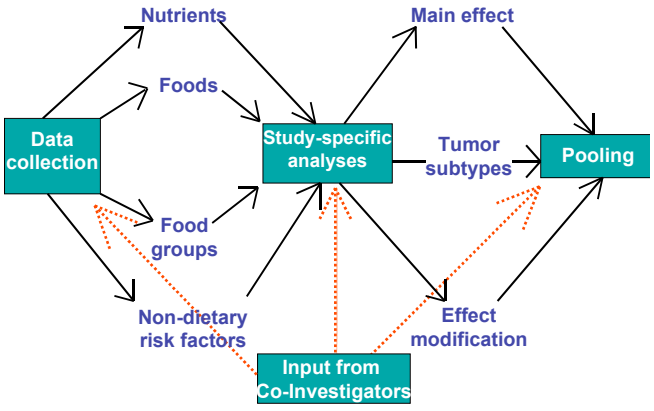


But when we conducted the ovarian cancer analyses, there were only 12 studies that met our inclusion criteria. So the baseline cohort size is over 500,000 with over 2,000 ovarian cancer cases that were detected during follow-up of these studies.

In the Pooling Project we collect the primary data from each of the studies and use the food data to create food groups, such as dairy products. We also collect non-dietary risk factors with which to adjust our estimates. We conduct study specific analyses, looking at the overall effect between, for example, milk and ovarian cancer risk. Because of the large sample size with over 2,000 cases, we can also look at associations for tumor subtypes and evaluate whether the association varies by other cancer risk factors. After we conduct the study specific analyses, we evaluate whether there is heterogeneity between the study specific results and then we pool those estimates.

This is an example of a collaborative project where we have input from the co-investigators at data collection, at the analysis of the individual studies and also at the pooling of the results and in the interpretation of those results. We have been meeting annually since the project started in 1991 to discuss the results so that we have consensus on the results that we observe and in the writing of those manuscripts.

Pooling Project: Analytic Strategy



Pooling Project: Statistical Analysis

- Study-specific relative risks (RRs) calculated using the Cox proportional hazards model
- Multivariate RRs adjusted for

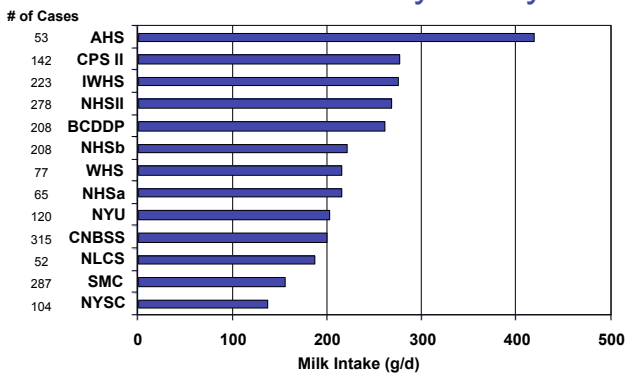
Age	HRT use
Age at menarche	Physical activity
Oral contraceptive use	Body mass index
Parity	Smoking status
Menopausal status	Energy intake

- Study-specific RRs pooled using a random effects model
  - Weighted by inverse of variance

For the ovarian cancer analyses, we adjusted for these risk factors. The age-adjusted results were very similar to the multivariate results, so I'm only presenting the multivariate results. In the pooled analysis, we weight the individual studies by the inverse of the variance of their estimates.

I want to preface the following slides by saying that Jeanine Genkinger, a post-doctoral fellow who is working on the Pooling Project, is the one that conducted these analyses and she is sitting in the back.

Mean Milk Intake by Study

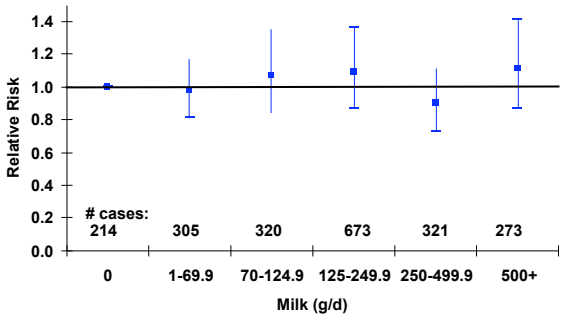


8 oz = 245g

Genkinger et al 2006

Another advantage of the pooled analysis is that we have wide variation in intake. Looking at mean milk consumption across the 12 studies included in the Pooling Project, there is about a two-fold difference between those with the lowest and highest intakes. Also shown for reference are the number of cases within each study, the smallest studies having about 50 cases, and the largest study, which is the Nurses' Health Study, having about 300 cases as with the Canadian National Breast Screening Study.

Pooled RRs for Milk and Ovarian Cancer

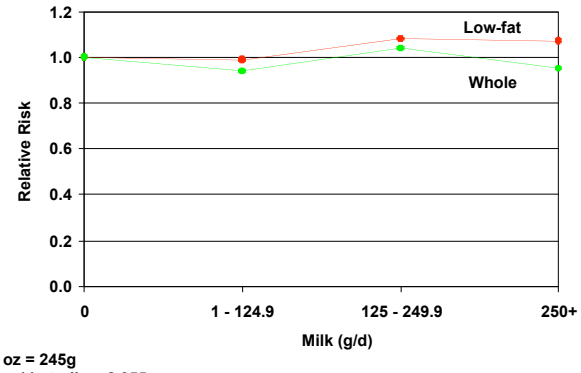


8 oz = 245 g
n = 12 studies; 2,132 cases

Genkinger et al, 2006

We categorized milk consumption into categories, using identical categories across the studies. We compared 500 or more grams of milk consumed per day, which is more than two cups of milk a day, to no consumption, and we really found no evidence of an association for milk consumption.

Pooled RRs for Whole and Low-fat Milk and Ovarian Cancer



8 oz = 245g
n = 11 studies; 2,055 cases

Genkinger et al 2006

We were interested in whether this differed by the fat content of the milk, and, again, when we looked at low fat milk and whole milk separately, the associations still were null.

Pooled RRs of Dairy Products and Ovarian Cancer

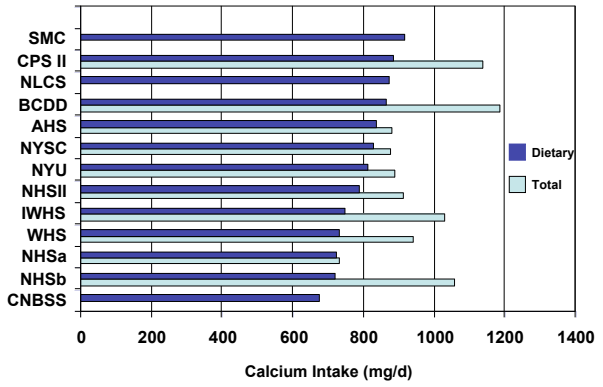
Food Item	n	RR (95% CI)	Exposure (ref=0 g/d)
Milk	12	1.11 (0.87-1.41)	≥ 500 (16 oz) g/d
Cheese	11	1.30 (0.96-1.78)	≥ 114 (_ cup) g/d
Cottage Cheese	9	0.88 (0.63-1.23)	≥ 50 (~2 oz) g/d
Yogurt	9	1.04 (0.86-1.24)	≥ 53 (_ cup) g/d
Ice Cream	9	0.91 (0.63-1.32)	≥ 66 (_ cup) g/d

p for trend > 0.3

Genkinger et al 2006

We also looked at a variety of other dairy products, and in the interest of time, I show only the relative risk for the highest category in comparison to the reference. And in all cases, the reference was no consumption of that product. The results for milk were a non-significant, 10 or 11 percent elevation in risk. None of the results for these dairy products was statistically significant. And the only one where there was a suggestion of a positive association was cheese. For cheese, that positive association was really limited to the highest category, which was approximately 2 ounces of hard cheese a day compared to none.

Mean Calcium Intake by Study



Genkinger et al 2006

Shown here is the variation in calcium intake by study. Calcium intake from food is referred to as dietary, and from both foods and supplements, as total calcium intake.

Pooled RRs for Calcium and Ovarian Cancer

Intake (mg/d)	Dietary	Total
< 500	1.00	1.00
500 - < 700	1.02	1.05
700 - < 900	1.05	1.06
900 - < 1100	1.00	1.04
1100 - <1300	0.98	1.16
≥ 1300	1.17 (0.93 – 1.47)	1.08 (0.84 – 1.38)
p for trend	0.38	0.49

n = 12 studies; 2,132 cases

Genkinger et al, 2006

We categorized the calcium intake, and, again, for both dietary and total calcium intake, we see really no evidence of an association.

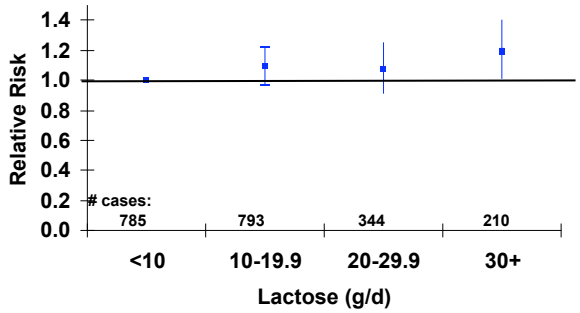
Pooled RRs for Vitamin D and Ovarian Cancer

Intake (IU/d)	Dietary	Total
< 100	1.00	1.00
100 - < 200	1.05	1.20
200 - < 300	1.10	1.26
300 - < 400	1.05	1.09
400 - < 500	1.56*	1.27
≥ 500	1.37 (0.78 – 2.40)	1.12 (0.90 – 1.38)
p for trend	0.04	0.60

n = 12 studies; 2,132 cases *p<0.05 Genkinger et al, 2006

When we also looked at Vitamin D, both dietary and dietary plus supplemental intake we saw this peculiar finding of an elevation in risk for the high categories of Vitamin D from food sources only, which was really not observed when we looked at total Vitamin D intake. We also looked at other dietary sources of Vitamin D, such as from fish and cereals, and we saw no evidence of a positive association for those sources. So we think it is unlikely that this association is causal.

Pooled RRs for Lactose and Ovarian Cancer

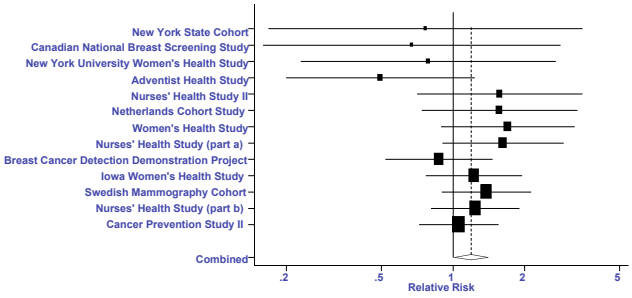


8 oz. milk contains ~10g of lactose
n = 12 studies; 2132 cases

Genkinger et al, 2006

Finally, we looked at the association between lactose and ovarian cancer risk. For these analyses Jeanine calculated the lactose intake for each of the studies because we had the dairy product intake data. We found a 20 percent elevation in the risk of ovarian cancer that was marginally significant for individuals who consumed ≥30 grams of lactose a day, about the amount of lactose in 3 cups of milk, compared to individuals who consumed less than 10 grams of lactose a day, about the amount of lactose in 1 cup.

Study-Specific RRs for Lactose and Ovarian Cancer (≥30 vs <10g/day)



p for heterogeneity = 0.58

n = 2,132

8 oz. milk contains ~10g of lactose

Genkinger et al, 2006

I also want to show you the study specific results for lactose. Since ovarian cancer is less common, none of the studies have that many cases, and none of these associations was statistically significant. The squares are the risk estimates for the individual studies. The square size is the weight that the study was given in the pooled analysis, and is proportional to the inverse of the variance. Studies that had about 200 cases are at the bottom of the graph and studies that had fewer than 100 cases are at the top of the graph.

Many of the individual estimates were on the right side of one, suggesting a positive association, but we did have some that were on the inverse side, but, again, those were not statistically significant. It's only when we pool them that we see this marginally significant 20 percent elevation in risk. I want to point out that the Iowa Women's Health Study, The Swedish Mammography cohort and the Nurses' Health Study, which are the ones near the bottom of the graph, are more heavily weighted in the analysis. Each published previously on the association between lactose and ovarian cancer, probably because they had more cases than these other individual studies near the top of the graph. In addition, they had lactose data available in their databases.

Pooled RRs for Lactose and Ovarian Cancer by Histologic Type

	n	RR (95% CI)
All Cancers	2132	1.04 (0.99-1.08)
Serous	1025	1.06 (0.97-1.16)
Endometrioid	261	1.07 (0.95 -1.20)
Mucinous	122	0.97 (0.81-1.16)

P for common effect by type = 0.63
Increment = 10 g/d

Genkinger et al 2006

We also looked at whether the association varied by ovarian cancer subtype, because previous prospective studies suggested that maybe the association was limited to serous ovarian cancer, which is the most common type of ovarian cancer. We did not see evidence that the association differed by histological subtype, although we did have small numbers of endometrioid and mucinous ovarian cancers in this pooled analysis.

Additional Results

- Results similar for serous, endometrioid, and mucinous cancers
- Results similar when the first 2 years of follow-up were excluded
- No effect modification by
  - Age at diagnosis
  - Parity
  - OC use
  - ♦ HRT use
  - ♦ Menopausal status
  - ♦ Fat intake

Genkinger et al 2006

We also looked at the other diary products to see whether the association varied by histological subtype, and for none of those did we find a statistically significant difference between the subtypes. We also evaluated whether the results were similar when the first two years of follow-up were excluded, just in case individuals who had undiagnosed ovarian cancer maybe had changed their diets so that their baseline diet was not reflective of their true adult diet, and we found the results were similar. We found no effect modification by a variety of ovarian cancer risk factors

Dairy Product and Ovarian Cancer Associations from a Pooled Analysis and a Meta-analysis

Dairy Product	Pooled Analysis of Cohort Studies		Meta-analysis of Case-Control Studies	
	n	RR (95% CI)†	n	RR (95% CI)†
Milk	12	1.11 (0.87-1.41)	8	0.87 (0.68-1.10)*
Low-fat	11	1.07 (0.93-1.23)	8	0.80 (0.64-1.01)*
Whole	11	0.95 (0.73-1.24)	7	1.27 (0.97-1.68)*
Cheese	11	1.30 (0.96-1.78)	7	0.93 (0.80-1.09)
Yogurt	9	1.04 (0.86-1.24)	6	1.19 (0.99-1.42)

†highest vs. lowest category
***p for heterogeneity ≤ 0.03**

Genkinger et al 2006, Larsson et al 2006

I want to put these results in perspective as to what is available in the published literature. Shown here are the results that I just presented from the pooled analysis juxtaposed to Alicja Wolk's group that at about the same time published a meta-analysis of case control and cohort studies. I mostly include their case control results because there were only three published cohort studies on dairy products and ovarian cancer and all of them are included in the pooled analysis. I've actually included one cohort study and 7 case control studies in the milk results because the results from the 7 case control studies were not reported separately.

One of the differences between a meta-analysis and a pooled analysis is that in the former you are limited to the comparisons that are in the published literature. There was statistically significant heterogeneity among the associations for milk and by type of milk in the case control studies that was not observed in our pooled analysis where we were able to define the contrast identically across the studies and control for the same risk factors.

But in all cases, the association with any of the dairy products was not significant in the pooled analysis or among the case control studies, except for cheese, where it is marginally significant in the pooled analysis but not suggestive in the case control studies.

Lactose and Ovarian Cancer: Results from a Pooled Analysis and a Meta-analysis

Study Design	Pooled Analysis n	Pooled Analysis RR (95% CI)*	Meta-analysis n	Meta-analysis RR (95% CI)*
Cohort	12	1.04 (0.99-1.08)	3	1.13 (1.05-1.22)
Case-Control			6	0.96 (0.89-1.03)
Both			9	1.02 (0.95-1.09)†

*** Increment = 10 g/d**
† p for heterogeneity = 0.04

Genkinger et al 2006, Larsson et al 2006

Alicja's group also reported results for lactose in their meta-analysis. These results are also shown juxtaposed to our pooled results. The results from the three published cohort studies were stronger than what we observed for the 12 studies in the pooled analysis. In one of these three cohort studies the case number is almost identical, but in the pooled analysis there is at least five more years of follow-up than in the other two cohort studies. In the process of there being more follow-up, the association has actually become weaker. So even if we limit our analysis to these three published studies, the association is weaker than what was reported previously. There was no evidence of an association in the case control studies. I want to also note that there was statistically significant heterogeneity in the meta-analysis between the case control and cohort studies, which is indicated at the bottom of the slide, so that you really shouldn't be pooling the two study types together to come up with a combined estimate.

Conclusions

- Overall
  - No strong or significant associations with dairy products or calcium
  - Non-significant higher risk with vitamin D
  - Modest positive association with lactose
- Associations did not vary by histological type
- Results were not modified by several ovarian cancer risk factors

Genkinger et al 2006

In conclusion, we found that there were no strong or significant associations with dairy products. This result was also observed in the meta-analysis of the case control studies. We also found no association with calcium intake either from foods only or from food and supplemental sources. We found a non-significant higher risk with Vitamin D that was stronger in the analyses from foods only compared to those that included supplemental intake, so we do not believe that this is a causal association. And then we found this modest positive association with lactose when we compared individuals who were consuming three or more servings of milk compared to one serving. So we think that there should be more evaluation of whether this is really true because the current recommendations are to consume at least three servings of dairy products per day.

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I want to again acknowledge Jeanine, who is the one who conducted these analyses as well as the other Pooling Project members at Harvard.

Acknowledgements, cont'd

- Adventist Health Study
- Breast Cancer Detection Demonstration Project
- Canadian National Breast Screening Study
- Cancer Prevention Study II Nutrition Cohort
- Iowa Women's Health Study
- Netherlands Cohort Study
- New York State Cohort
- New York University Women's Health Study
- Nurses' Health Study
- Nurses' Health Study II
- Swedish Mammography Cohort
- Women's Health Study

Whenever I give a talk on the pooled results, I must also acknowledge the investigators for each of the individual studies because without their collaboration this project would not be possible.