A Case-Control Study of Galactose Consumption and Metabolism in Relation to Ovarian Cancer[1]

Daniel W. Cramer,[2] E. Robert Greenberg, Linda Titus-Ernstoff, Rebecca F. Liberman, William R. Welch, Evelyn Li, and Won G. Ng

Ob-Gyn Epidemiology Center, Departments of Obstetrics and Gynecology [D. W. C., R. F. L., E. L.], and Pathology [W. R. W.], Brigham and Women's Hospital, Boston, Massachusetts 02115; Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire 03756 [E. R. G., L. T-E.]; and Biochemical Genetics Laboratory, Children's Hospital of Los Angeles, Los Angeles, California 90027 [W. G. N.]

Abstract

Consumption or metabolism of dairy sugar and ovarian cancer have been linked based on evidence that galactose may be toxic to ovarian germ cells and that ovarian cancer is induced in animals by depletion of oocytes. We assessed consumption of dairy products and obtained blood for biochemical and molecular genetic assessment of galactose metabolism in 563 women with newly diagnosed epithelial ovarian cancer and 523 control women selected either by random digit dialing or through lists of residents in eastern Massachusetts and New Hampshire. We observed no significant differences between cases and controls in usual consumption of various types of dairy products or total daily lactose (the principal source of galactose in the diet); nor did we find that RBC activity of either galactose-1-phosphate uridyl transferase (GALT) or galactokinase differed. The mean (and SE) activity of uridine diphospho-galactose 4′-epimerase (in micromoles per hour per gram of hemoglobin) was, however, significantly lower ($P < 0.005$) in cases compared with controls, 20.32 (0.31) *versus* 21.64 (0.36). Ovarian cancer cases were also more likely to carry the *N314D* polymorphism of the *GALT* gene, generally predisposing to lower GALT activity. The difference was most evident for endometrioid and clear cell types of ovarian cancer, in which 3.9% of cases were found to be homozygous for *N314D* compared with 0.4% of controls, yielding an odds ratio and 95% confidence interval of 14.17 (2.62–76.60). We conclude that, whereas adult consumption of lactose carries no clear risk for the disease, certain genetic or biochemical features of galactose metabolism may influence disease risk for particular types of ovarian cancer.

Introduction

Animal models for ovarian cancer have demonstrated that exposure of ovaries to radiation (1) or chemicals toxic to oocytes (2) disturbs ovarian/pituitary feedback, raises gonadotropins, and eventually induces ovarian neoplasia. On the basis of animal studies showing that a diet high in dairy sugar (galactose) is toxic to oocytes (3) and evidence that galactosemia causes premature ovarian failure in women (4), we proposed that galactose consumption or metabolism might influence risk for human ovarian cancer through mechanisms similar to those in animals. Observations supporting a galactose-ovarian cancer connection include ecological data that ovarian cancer rates worldwide correlate with milk consumption (5) and data from a case-control study (6). Although one subsequent cohort study provided modest support for the hypothesis (7), several subsequent case-control studies have been negative (8–10). Investigators in some of these studies attributed any association between ovarian cancer and dairy products to their fat content (8, 11).

We have completed a large population-based study of ovarian cancer that allows us to address the relationship between galactose consumption/metabolism and ovarian cancer in greater detail. We assessed dairy food consumption and examined RBC activity of the three principal enzymes involved in galactose metabolism including GALK,[3] GALT, and GALE to assess whether any of these factors influenced ovarian cancer risk. In addition, we looked at a common polymorphism of the *GALT* gene, *N314D*, generally associated with lower GALT activity (12).

Materials and Methods

In May 1992, we began a population-based case-control study of newly diagnosed ovarian cancer in MA and NH. Cases were identified from hospital tumor boards and from the Massachusetts and New Hampshire statewide cancer registries. Between May 1992 and March 1997, 1033 cases of ovarian cancer cases were identified (excluding 47 who had a nonovarian primary cancer on the pathology review). Of these, 91 (9%) died before they could be contacted, and 65 (6%) could not be contacted because they had moved out of state (27), had no phone (24), or did not speak English (14). Of the remaining 877 patients, physicians denied contact in 126 instances (14%) and 136 women (16%) declined to participate, and there were 52 cases with nonepithelial ovarian tumors studied but not included in this analysis. This analysis is based on data from 563 cases with

Received 1/15/99; revised 10/11/99; accepted 11/1/99.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by R01 Ca54419 from the National Cancer Institute.
[2] To whom requests for reprints should be addressed, at Ob-Gyn Epidemiology Center, Brigham and Women's Hospital, 221 Longwood Avenue, Boston, MA 02115. Phone: (617) 732-4895; Fax: (617) 732-4899; E-mail: DWCramer@bics.bwh.harvard.edu.

[3] The abbreviations used are: GALK, galactokinase; GALT, galactose-1 phosphate uridyl transferase; GALE, uridine diphospho-galactose 4′-epimerase; MA, (eastern) Massachusetts; NH, (all of) New Hampshire; RDD, random digit dialing; OR, odds ratio; CI, confidence interval; UDPgal, uridine diphosphogalactose; UDPG, uridine diphosphoglucose; OC, oral contraceptive; BMI, body mass index.

epithelial ovarian cancer, including tumors of borderline malignancy.

We identified controls using RDD, in which the sampling unit for an interviewed case comprised the 99 telephone numbers generated from the first five digits of the case's telephone number plus all of the remaining combinations of the last two digits (excluding the case's own number). These numbers were listed in random order and were called to screen households for potential controls who were within 4 years of the case's age. Approximately 5400 calls (excluding those to businesses or nonworking numbers) yielded 10% of households in which the person answering declined to provide a household census and 80% of households in which an age and sex match to the case could not be made or in which a potential match was ineligible by virtue of a prior oophorectomy. In the remaining 10% of households, there was a potentially eligible control; and 72% of these agreed to participate. RDD proved inefficient for identifying controls over age 60 in MA because a substantially greater number of households needed to be screened to obtain an older control. Because of this, we identified older controls in MA by randomly selecting women through the use of lists (townbooks) of all of the residents in towns by name, age, and address according to precinct. We matched older controls to cases by community and age within 4 years based on the townbooks. Of 328 potential controls sampled from townbooks, 21% could not be reached, 18% were ineligible, and 30% declined to participate. The present analysis includes a total of 523 RDD and townbook controls with completed interviews as of May 1997.

We conducted an in-person interview to assess demographic information, menstrual and reproductive history, medical and family history, and personal habits. Exposures were assessed as of a reference date defined as 1 year before the date of diagnosis for the cases and before the date of interview for the controls. Dietary variables were assessed using the Nurses Health Study self-administered food frequency questionnaire (13). Estimates of galactose consumption were based on consumption of its principal dietary source, lactose (a double sugar composed of galactose and glucose), which itself was assessed from the reported frequency of the use of milk, yogurt, ice cream, and cheeses. We asked women to focus on their typical diet in the 5 years before their reference date. Supplemental information was obtained through the general questionnaire about the use of a limited number of dairy products including milk and yogurt at earlier ages. Food frequency questionnaires were missing in eight cases and two controls.

We also collected a heparinized specimen of blood, which was separated into plasma, red cell, and white cell components and which were then aliquoted and frozen at −70°C. Time between the blood draw and processing was recorded. The washed, frozen red cells were used for the biochemical assessment of GALK, GALT, and GALE activity. RBC kinase activity was measured by quantifying the amount of galactose-1-^{14}C converted to labeled galactose-1-phosphate per hour per gram of hemoglobin (14). RBC transferase activity was measured by quantifying the amount of gal (U) ^{14}C-1-P converted to labeled UDPgal per hour per gram of hemoglobin. RBC epimerase activity is measured by an indirect fluorometric method that determines the amount of NADH formed from UDPgal after incubation with UDPG dehydrogenase and NAD (15). Assays were performed in batches of about 30–40 subjects including both cases and controls whose status was unknown to the technician.

GALT mutations associated with lower activity, including Q188R and N314D, were examined by means of PCR amplification and enzyme restriction assays. Germ line DNA was amplified with primers specific for exons 6 and 10, and the products were digested with the restriction enzymes *Ava*II for N314D and *Hpa*II for Q188R, as described by Lin *et al.* and Reichardt *et al.* (12, 16). The restriction products were electrophoresed in 12% native PAGE in 0.5× tris boric acid EDTA at 1000 v for 45 min, which allowed clear separation of wild-type, heterozygote, and homozygote bands.

We requested and reviewed pathology reports on all of the cases and sought slides for any case in which there was a discrepancy between the histological description and final diagnosis. Only 3.5% of initially identified cases were excluded because of a nonovarian primary cancer, and the majority of these were evident from the pathology reports. We requested slides for 20 cases and confirmed the initial report in 18. After completing the review, the following histological categories were established: (*a*) serous cancers (including serous cystadenocarcinomas and surface papillary carcinomas); (*b*) mucinous cancers; (*c*) endometrioid and clear cell cancers (including mixed mesodermal or mixed epithelial with an endometrioid or clear cell component); and (*d*) undifferentiated/other cancers. The serous cancers were further subdivided into borderline and invasive grades, but this distinction was not made for the mucinous tumors, in which there is evidence for overlap in grades within mucinous tumor specimens (17).

Because matching was performed as the most convenient means for selecting controls comparable to cases in age and geographic locale and not as the principal means of controlling for confounding, matching was not preserved in the analysis. We analyzed our data by constructing frequency counts of cases and controls by study variables and by calculating crude ORs or by comparing continuous variables between cases and controls with nonpaired *t* tests. For the calculations of risks associated with quartiles of continuous variables, the control distribution was used as the standard. We then used unconditional logistic regression to adjust for the matching variables, age (continuous), and study site (MA, NH), and for variables strongly linked to ovarian cancer risk such as parity (0, ≥1), oral contraceptive use (never or <3 months, ≥3 months), and family history of breast or ovarian cancer (no, yes). Tests for linear trends were performed using the likelihood ratio test with continuous forms of the variables. All of the analyses were performed using the SAS system (SAS Institute, Cary, NC).

Results

As assessed from the food frequency questionnaire and based on recent diet before diagnosis, the consumption of dairy foods, which are the principal sources of lactose (and, ultimately, galactose), was not related to ovarian cancer risk (Table 1). Specifically, no elevated risks were found for the consumption of any particular type of dairy product including skim milk, whole milk, or yogurt; nor was there any trend for increasing risk seen with increasing frequency of use of any type of milk product.

Table 2 examines average estimated daily lactose consumption (in grams) totaled from all of the food sources among all of the cases and controls and within various subgroups. Among controls, there was nonsignificantly higher lactose consumption in women with more than 12 years of schooling (P = 0.38) and significantly higher lactose consumption for women less than 50 years of age and for women who did not report lactose intolerance. Of potential interest, there was an excess of controls who reported lactose intolerance that was particularly evident for intolerance beginning at an early age. There were 7

Table 1 ORs for ovarian cancer associated with frequency of consumption of various types of dairy products

Dairy product	No. of cases (%) (n = 563)	No. of controls (%) (n = 523)	Adjusted[a] OR	(95% CI)
Cream cheese (1 oz)				
Never or < monthly	244 (44.4)	228 (45.0)	1.0	
1–4 times/mon	244 (44.4)	234 (46.2)	1.06	(0.82–1.38)
2–7 times/wk	60 (10.9)	44 (8.7)	1.36	(0.87–2.13)
> once/day	1 (0.2)	1 (0.2)	0.79	(0.04–14.92)
Cottage or ricotta cheese (1/2 cup)				
Never or < monthly	289 (52.3)	248 (48.6)	1.0	
1–4 times/mon	220 (39.8)	219 (42.9)	0.92	(0.71–1.19)
2–7 times/wk	44 (8.0)	43 (8.4)	1.04	(0.65–1.66)
Ice cream (1/2 cup)				
Never or < monthly	157 (28.6)	143 (27.9)	1.0	
1–4 times/mon	295 (53.6)	290 (56.5)	0.93	(0.70–1.24)
2–7 times/wk	93 (16.9)	78 (15.2)	1.06	(0.72–1.56)
> once/day	5 (0.9)	2 (0.4)	2.14	(0.39–11.70)
Sherbet or ice milk (1/2 cup)				
Never or < monthly	378 (69.4)	364 (71.2)	1.0	
1–4 times/mon	141 (25.9)	125 (24.5)	1.09	(0.82–1.46)
2–7 times/wk	25 (4.6)	22 (4.3)	1.14	(0.63–2.09)
Skim or low fat milk (8-oz glass)				
Never or < monthly	174 (31.8)	153 (29.8)	1.0	
1–4 times/mon	92 (16.8)	72 (14.0)	1.21	(0.82–1.78)
2–7 times/wk	200 (36.6)	220 (42.8)	0.81	(0.60–1.09)
> once/day	81 (14.8)	69 (13.4)	1.10	(0.74–1.64)
Whole milk (8 oz glass)				
Never or < monthly	364 (67.0)	344 (67.7)	1.0	
1–4 times/mon	78 (14.4)	71 (14.0)	0.98	(0.68–1.41)
2–7 times/wk	84 (15.5)	71 (14.0)	1.05	(0.73–1.51)
> once/day	17 (3.1)	22 (4.3)	0.64	(0.32–1.26)
Yogurt (1 cup)				
Never or < monthly	264 (48.0)	215 (42.0)	1.0	
1–4 times/mon	171 (31.1)	168 (32.8)	0.87	(0.65–1.17)
2–7 times/wk	112 (20.4)	125 (24.4)	0.75	(0.54–1.04)
> once/day	3 (0.6)	4 (0.8)	0.85	(0.18–4.0)

[a] Adjusted for age (<50, ≥50), parity (0, 1–2, >2), education (≤12, >12), OC use (<3 mon, ≥3 mon), religion (Jewish, non-Jewish), and total calories.

cases compared with 14 controls (including one control without a diet survey) who reported lactose intolerance beginning before age 20. Relative to subjects with no lactose intolerance, this translated into an OR of 0.45 (95% CI, 0.18–1.16) and a P of 0.09 after adjustment for age, parity, and OC use.

Table 3 displays the results of a multivariate logistic regression model for risk of ovarian cancer with daily lactose in quartiles and adjusting for matching and relevant dietary variables including total calories, saturated fat, and protein. No trend for increasing risk for ovarian cancers associated with increasing quartiles of lactose consumption was observed. When examined as a continuous variable in the model, saturated fat was not related to risk, although protein consumption was significantly and inversely related to ovarian cancer risk.

To address the hypotheses that decreasing enzyme activity or increasing ratios of lactose consumption to enzyme activity are associated with ovarian cancer risk, we examined the risk of ovarian cancer by quartiles of activity for the various galactose enzymes and by the ratios of lactose consumption to these enzymes (Table 4). No trend for ovarian cancer risk was associated with RBC activity of GALT or GALK, but a significant trend for increasing risk with decreasing GALE activity was observed. No significant trends for any of the ratios of lactose consumption to enzyme activity were observed. (See Table 4 footnotes for definition of the L:T, L:K, and L:E ratios).

We assessed mean epimerase levels for cases and controls, stratified by age, parity, site of study, BMI, and quality control variables such as the time between blood draw and processing and the year of assay (Table 5). Within each demographic subgroup, cases had lower epimerase levels, with the differences most apparent for cases 50 years or older. Epimerase activity was somewhat higher for parous women and those with greater BMI. Activity was also affected by the length of time between blood draw and processing, but this factor did not explain the lower measured activity in cases. No similar effects of processing time were observed for transferase or kinase (data not shown). Epimerase activity also seemed to be lower for assays performed during the early years of the study. Adjustment for these variables in a multivariate model did not negate the association between epimerase activity and ovarian cancer (data not shown). Among the various histological types of ovarian cancer, mean epimerase was lowest for the borderline and invasive serous cancers and highest for the undifferentiated cancers. No tendency for cases with recent chemotherapy to have lower or higher epimerase activity was observed. The mean epimerase activity in cases with recent chemotherapy did not differ significantly from cases without recent chemotherapy (P = 0.169).

We tested 545 cases and 497 controls for *GALT N314D* alleles (Table 6). Among cases, 106 (19.4%) were heterozygous and 9 (1.6%) were homozygous for *N314D* compared with 85 (17.1%) and 2 (0.4%) in 497 controls tested (P = 0.08). The excess of *N314D* heterozygotes and homozygotes was even more apparent among women with endometrioid and clear cell

Table 2 Mean daily lactose consumption (in grams) in ovarian cancer cases and controls

Variable	All cases			All controls		
	n	Mean	(SE)	*n*	Mean	(SE)
Age						
<50	263	15.55	(0.78)	260	17.57	(0.84)
≥50	292	17.26	(0.85)	261	15.59	(0.77)
Study center						
MA	428	16.10	(0.66)	410	16.22	(0.63)
NH	127	17.60	(1.23)	111	17.92	(1.34)
Religion						
Jewish	50	12.64	(1.32)	44	16.13	(1.75)
Non-Jewish	505	16.82	(0.62)	477	16.62	(0.60)
Parity						
0	185	16.61	(1.06)	106	15.77	(0.95)
≥1	370	16.36	(0.69)	415	16.79	(0.67)
OC use						
Never or <3 mon	329	16.61	(0.78)	245	16.00	(0.74)
≥3 mon	226	16.21	(0.87)	276	17.09	(0.85)
Lactose intolerance						
No	493	16.80	(0.62)	451	17.35	(0.60)
Yes	62	13.68	(1.47)	70	11.65	(1.54)
Onset of lactose intolerance						
< age 20	7	21.06	(5.57)	13	10.16	(1.64)
≥ age 20	55	12.74	(1.49)	57	12.00	(1.86)
Education						
≤12 yr	215	15.30	(0.93)	169	15.85	(1.03)
>12 yr	340	17.17	(0.74)	352	16.93	(0.68)
All	555	16.45	(0.58)	521	16.58	(0.57)

Table 3 Adjusted risk for ovarian cancer by quartiles of lactose consumption and other dietary variables[a]

Variable	Parameter estimate	OR	(95% CI)	*P*
Lactose Q2[b]	−0.0159	0.98	(0.69–1.40)	0.929
Lactose Q3	−0.00237	1.00	(0.70–1.43)	0.990
Lactose Q4	−0.0820	0.92	(0.62–1.37)	0.686
Lactose intolerance (no, yes)	−0.2019	0.82	(0.56–1.20)	0.301
Total calories	0.000299	1.00	(1.00–1.00)	0.105
Total protein	−0.0116	0.99	(0.98–1.00)	0.002
Saturated fat	0.0112	1.01	(0.99–1.03)	0.226

[a] Adjusted for age, OC use, parity, first-degree relative with breast or ovarian cancer, study site, and education.
[b] Q, quartile.

cancer, among whom 21.1% were carriers and 3.9% were homozygous for *N314D* ($P = 0.002$). After adjustment for potential confounders, including age, study site, parity, oral contraceptive use, education, BMI, and epimerase activity, the OR (and 95% C.I.) associated with homozygosity for *N314D* was 4.51 (0.96–21.20) for all of the ovarian cancers and 14.17 (2.62–76.60) for endometrioid and clear cell cancer. Only 1 (0.2%) of 540 cases and 2 (0.4%) of 494 controls in this study carried a Q188R mutation. In subjects with both the *N314D* and transferase measurement available, the mean transferase activity in micromoles of hexose conversion/h/g hemoglobin (and SD) for the *N314D* genotypes were (*a*) 24.0 (2.88) and 23.7 (2.93) for *N314D* −/− cases and controls, respectively; (*b*) 20.1 (4.32) and 20.1 (5.02) for *N314D* +/− cases and controls, respectively; and (*c*) 15.5 (4.21) and 18.5 (14.64) for *N314D* +/+ cases and controls, respectively.

Table 4 Adjusted[a] risks by quartiles of biochemical activity[b] of major galactose enzymes, alone and relative to estimated lactose consumption

Variable/ quartile	Cases		Controls		OR	(95% CI)	*P* for trend
	n	(%)	*n*	(%)			
Transferase							
4th	158	(31.0)	106	(24.8)	1.0		
3rd	122	(24.2)	112	(26.2)	0.70	(0.49–1.01)	
2nd	89	(17.5)	99	(23.1)	0.60	(0.41–0.88)	
1st	139	(27.4)	111	(25.9)	0.82	(0.57–1.17)	0.65
Kinase							
4th	120	(26.4)	97	(25.3)	1.0		
3rd	131	(26.9)	89	(23.2)	1.19	(0.81–1.76)	
2nd	120	(24.6)	100	(26.1)	0.97	(0.66–1.42)	
1st	116	(23.8)	97	(25.3)	1.01	(0.68–1.49)	0.68
Epimerase							
4th	96	(19.7)	91	(23.9)	1.0		
3rd	105	(21.6)	101	(26.5)	1.00	(0.67–1.50)	
2nd	142	(29.2)	93	(24.4)	1.54	(1.03–2.30)	
1st	144	(29.6)	96	(25.2)	1.52	(1.02–2.28)	0.002
L:T[c]							
1st	125	(24.9)	104	(24.4)	1.0		
2nd	143	(28.5)	112	(26.3)	1.12	(0.77–1.62)	
3rd	115	(22.9)	104	(24.4)	0.90	(0.62–1.31)	
4th	119	(23.7)	106	(24.9)	0.93	(0.64–1.36)	0.379
L:K[d]							
1st	115	(23.9)	96	(25.2)	1.0		
2nd	137	(28.5)	94	(24.7)	1.25	(0.85–1.84)	
3rd	112	(23.3)	96	(25.2)	1.01	(0.68–1.50)	
4th	117	(24.3)	95	(24.9)	1.04	(0.70–1.54)	0.17
L:E[e]							
1st	127	(26.4)	93	(24.5)	1.0		
2nd	95	(19.8)	96	(25.3)	0.66	(0.45–0.99)	
3rd	108	(22.4)	95	(25.1)	0.80	(0.54–1.19)	
4th	151	(31.4)	95	(25.1)	1.12	(0.77–1.64)	0.17

[a] Adjusted for age (continuous), BMI (continuous), parity (0, ≥1), OC use (<3 mon, ≥3 mon), first-degree relative with breast or ovarian cancer, site (MA, NH), and education (≤12 yr, >12 yr).
[b] Units of activity are micromoles of hexose conversion/h/g hemoglobin.
[c] L:T, ln (lactose) × 10:transferase (ratio of lactose consumption to transferase enzyme activity).
[d] L:K, ln (lactose) × kinase (ratio of lactose consumption to kinase enzyme activity).
[e] L:E, ln (lactose) × 10:epimerase (ratio of lactose consumption to epimerase enzyme activity).

Discussion

In this study, we found no association between ovarian cancer risk and the estimated adult consumption of various types of dairy products or total lactose. These findings are inconsistent with our previous report of a link between ovarian cancer and increased consumption of dairy sugar (6). One possible explanation for the discrepancy between the two studies may involve differences in control selection between the two studies. In the current study, control women were largely selected by RDD whereas, in the previous study, controls were selected through population rosters. RDD yielded a more educated control group with 68% of controls in the current study going beyond 12 years of schooling compared with 52% of controls in the study conducted about 10 years earlier, whereas the percentage of cases who had gone beyond high school was about 60% in both the current and previous study. This may be relevant because we observed a nonsignificant tendency for greater lactose consumption with higher educational attainment (Table 2).

Another potential study limitation is that, although cases are asked about their diet-before-disease, their recall may be influenced by their current diet. Thus, there might be a tendency

Table 5 Epimerase activity[a] in ovarian cancer cases and controls by potential confounding variables

	Cases			Controls		
	n	Mean	(SE)	*n*	Mean	(SE)
All	487	20.32	(0.31)	381	21.64	(0.36)[b]
Age						
<50	232	20.32	(0.47)	190	20.80	(0.49)
>50	255	20.31	(0.42)	191	22.47	(0.51)[b]
Study center						
MA	386	19.43	(0.33)	310	20.79	(0.38)[b]
NH	101	23.70	(0.72)	71	25.35	(0.77)
Religion						
Jewish	48	21.27	(0.97)	30	20.90	(1.45)
Non-Jewish	439	20.21	(0.33)	351	21.70	(0.36)[b]
BMI						
<20	56	20.09	(0.98)	41	21.16	(1.05)
20–24	181	20.46	(0.50)	149	21.28	(0.59)
>24	250	20.26	(0.44)	190	21.97	(0.49)[b]
Parity						
0	161	19.66	(0.57)	82	21.23	(0.79)
≥1	326	20.64	(0.37)	299	21.75	(0.40)[b]
Sample processing interval						
<1 day	233	21.45	(0.41)	177	23.17	(0.44)[b]
≥1 day	170	16.95	(0.47)	135	17.61	(0.56)
Year of analysis						
1992–1993	101	17.68	(0.60)	87	20.40	(0.66)[b]
1994	129	18.47	(0.59)	90	19.44	(0.80)
1995	127	21.73	(0.58)	95	22.45	(0.69)
1996	46	20.68	(0.83)	40	20.55	(0.88)
Histological type						
Serous borderline	71	19.67	(0.83)	381	21.64	(0.36)[b]
Serous invasive	199	20.12	(0.48)	381	21.64	(0.36)[b]
Mucinous	73	20.82	(0.80)	381	21.64	(0.36)
Endometrioid/Clear cell	110	20.27	(0.68)	381	21.64	(0.36)[b]
Other/Undifferentiated	34	21.86	(1.25)	381	21.64	(0.36)

[a] Units of activity are micromoles of hexose conversion/h/g hemoglobin.
[b] $P \leq 0.05$.

Table 6 Adjusted[a] odds of ovarian cancer with *N314D*

	Cases		Controls		Adjusted OR	(95% CI)
	n	(%)	*n*	(%)		
All cases						
N314D −/−	430	(78.9)	410	(82.5)	1.0	
N314D −/+	106	(19.4)	85	(17.1)	1.17	(0.84–1.61)
N314D +/+	9	(1.6)	2	(0.4)	4.51	(0.96–21.20)
Endometrioid/Clear cell cases						
N314D −/−	96	(75.0)	410	(82.5)	1.0	
N314D −/+	27	(21.1)	85	(17.1)	1.40	(0.84–2.32)
N314D +/+	5	(3.9)	2	(0.4)	14.17	(2.62–76.60)

[a] Adjusted for age (continuous), lactose consumption (continuous), OC use (<3 mon, ≥3 mon), parity (0, ≥1), BMI (continuous), first-degree relative with breast or ovarian cancer (no, yes), study site (MA, NH), and epimerase activity (continuous).

for cases, who may be recovering from surgery or undergoing chemotherapy, to underreport food consumption. This is, perhaps, illustrated by the lower total protein consumption among the cases in this study. For this reason, cohort studies may be preferable for assessing diet in relation to ovarian cancer. In a recent study (7), a modest trend for an increasing risk of ovarian cancer with increasing lactose consumption was found ($P = 0.12$). However, considered in the context of the results reported here and other negative case-control studies (8–10), there is only limited support for the proposition that adult lactose consumption has an appreciable influence on ovarian cancer risk. Our study does not address the possibility that dietary patterns earlier in life may be important, which may be suggested by our observation that lactose intolerance beginning before age 20 was protective ($P = 0.09$). A recent Italian study also reported a protective effect of lactose intolerance based on hydrogen breath tests in cases and controls (18). Saturated fat consumption, proposed as another key dietary element, was not significantly related to ovarian cancer risk in our current study.

Our current study also did not confirm our previous finding (6) that women with ovarian cancer have lower biochemical activity of a key enzyme involved in galactose metabolism known as galactose-1-phosphate uridyl transferase (GALT). In our previous study, GALT was measured in heparinized whole blood specimens with a 1- or 2-week delay, whereas, in this study, the blood was first processed, and a frozen red cell pellet was used for the analysis. Average transferase activity was about the same for controls but was about 5% higher for cases compared with the previous study.

Although ovarian cancer cases and controls in the current study did not differ in GALT activity levels, they were found to differ in frequency of possession of a polymorphic variant of *GALT* known as *N314D* which is associated with lower GALT activity. Almost 2% of women with all types of ovarian cancer and about 4% of women with endometrioid and clear cell cancers were homozygous for *N314D* compared with a control frequency of 0.4%. This difference represents a 4-fold increase in risk for all types of ovarian cancer and a 14-fold increase for endometrioid and clear cell types. Because of the rarity of homozygosity for *N314D*, this finding carries a very low attributable risk (less than 2%) for ovarian cancer overall. However, the association may be of interest because its basis may involve a common condition in women known as endometriosis, which is believed to arise from the growth of endometrial cells contained in menstrual fluid that may spill into the pelvic cavity during menstruation (19).

Endometrioid and clear cell cancers of the ovary are histologically similar to cancers of the endometrium and frequently occur in the setting of endometriosis (20). In turn, the risk for ovarian cancer is increased among women with endometriosis (21). The link between endometriosis and endometrioid and clear cell cancers could underlie the association between *N314D* and endometrioid and clear cell cancers of the ovary because we have previously described an increased occurrence of *N314D* not only in women with endometriosis (22) but also in women with the Mullerian anomaly, vaginal agenesis (23). We speculated that the latter association was due to decreased galactose metabolism in a mother or fetus carrying *N314D*; this would lead to a higher-than-normal fetal exposure to galactose, which has been shown to affect Mullerian development in animals. Pregnant rodents fed high galactose diets produced female offspring with a reduced number of oocytes and delayed opening of the vagina (24). If high fetal exposure to galactose is capable of causing failure of the vagina to canalize, it may also cause failure of the cervix to canalize properly; this which would lead to a stenotic opening, which would predispose to retrograde menstruation, a likely cause of endometriosis. The possible connection between *N314D* and reproductive tract problems such as vaginal agenesis, endometriosis, and ovarian cancer is further strengthened by case reports of ovarian cancers in women with vaginal agenesis (25–27), including rare endodermal sinus tumors.

These intriguing observations provide support for the proposition that galactose consumption during adult years may be less important in influencing Mullerian development and ovarian cancer risk than fetal galactose exposure, which may be mediated both by maternal and fetal *GALT* genotypes and by maternal galactose consumption. Whereas the latter variable would be difficult to estimate, the decreased metabolism of galactose associated with *N314D* may be expected to cause the greatest fetal exposure to galactose, when both the mother and her fetus carry the allele as would occur in all of the homozygotes and in those heterozygotes with maternal transmission. Another investigator (28) has recently reported a link between *N314D* and all ovarian cancers, although the excess for women with endometrioid and clear cell types was less apparent than observed in our study. Further study of the link between *N314D* and ovarian cancer would be worthwhile, including an assessment of whether maternal rather than paternal transmission of *N314D* carries greater risk.

In addition to our studies of GALT, we measured the biochemical activity of two other enzymes involved in galactose metabolism known as GALK and GALE. Although we found no association between kinase activity and ovarian cancer, GALE activity was significantly lower in cases compared with controls. In terms of the relative importance of GALK and GALE to human disease, cataract development is the principal manifestation of GALK deficiency. This apparently results from an excess of galactose (and its alcohol, galactitol) rather than from the reduction of any downstream metabolites, which can be measured even in GALK-deficient patients, which suggests residual enzyme activity (29). GALE deficiency may have features similar to GALT deficiency, including newborn feeding problems and retardation (29). However, GALE deficiency is relatively rare; and it has been postulated that a complete GALE deficiency is incompatible with life (30). GALE catabolizes the interconversion of UDPgal to UDP-glucose (31). The biochemical assay for GALE is substantially dependent on the time elapsed between specimen collection and processing; therefore, future epidemiological investigations of epimerase should focus on polymorphisms of the *GALE* gene or its activation region. Interestingly, the *epimerase* gene is located on 1p36 (32) near a region frequently shown to have loss of heterozygosity in various neoplasms, including breast, colon, and endodermal sinus tumors (33–35). Further study is needed to distinguish whether this coincidence suggests a direct role for epimerase or linkage disequilibrium with tumor suppressor gene(s).

A major limitation of our study is that about 15% of cases could not be interviewed in that they had died or could not be located, and 30% of those remaining could not be interviewed because of physician or patient refusal. Thus, associations with any variables influencing survival or willingness to be interviewed may be subject to a selection bias. In addition, not all of the subjects had the biochemical assays for assessing galactose enzymes inasmuch as these were "phased-in." Our assessment of galactose metabolism was based on red cell specimens drawn after the diagnosis of ovarian cancer and, therefore, may not reflect metabolism before the clinical onset of ovarian cancer. The tests themselves, especially for epimerase activity, may be subject to error based on the length of time between drawing and processing the RBCs for testing. Furthermore, the use of RBCs as the most convenient source for the enzyme may not reflect the expression of activity in more important organs including the liver and ovary. For these reasons, we emphasize that molecular genetic assessment of galactose metabolism may be more valid in future studies.

Despite our inability to confirm an association between adult lactose consumption or GALT activity and ovarian cancer risk, findings indicating a protective effect of lactose intolerance at an early age, and the increased risk with *N314D* mutations of *GALT* and lower GALE activity provide some support for an ovarian cancer/galactose connection. We believe that future studies should attempt to address the hypothesis that intrauterine galactose exposure is an important determinant of risk for ovarian cancer.

References

1. Furth, J., and Butterworth, J. S. Neoplastic diseases occurring among mice subjected to general irradiation with X-rays. Am. J. Cancer, *28:* 66–95, 1936.

2. Jull, J. W., Streeter, D. J., and Sutherland, L. The mechanism of induction of ovarian tumors in the mouse by 7,12-dimethyl[*a*]benzanthracene. I. Effect of steroid hormones and carcinogen concentration *in vivo*. J. Natl. Cancer Inst., *37:* 409–420, 1966.

3. Swartz, W. J., and Mattison, D. R. Galactose inhibition of ovulation in mice. Fertil, Steril., *49:* 522–526, 1988.

4. Kaufman, F. R., Kogut, M. D., Donnell, G. N., Goebelsmann, U., March, C., and Koch, R. Hypergonadotropic hypogonadism in female patients with galactosemia. N. Engl. J. Med., *304:* 994–998, 1981.

5. Cramer, D. W. Lactase persistence and milk consumption as determinants of ovarian cancer risk. Am. J. Epidemiol., *130:* 904–910, 1989.

6. Cramer, D. W., Harlow, B. L., Willett, W. C., Welch, W. R., Bell, D. A., Scully, R. E., Ng, W. G., and Knapp, R. C. Galactose consumption and metabolism in relation to the risk of ovarian cancer. Lancet, *2:* 66–71, 1989.

7. Kushi, L. H., Mink, P. J., Folsom, A. R., Anderson, K., Zheng, W., Lazovich, D., and Sellers, T. A. Prospective study of diet and ovarian cancer. Am. J. Epidemiol., *149:* 21–31, 1999.

8. Mettlin, C. J., and Piver, M. S. A case-control study of milk drinking and ovarian cancer risk. Am. J. Epidemiol., *132:* 871–876, 1990.

9. Risch, H. A., Jain, M., Marrett, L. D., and Howe, G. R. Dietary lactose intake, lactose intolerance, and the risk of epithelial ovarian cancer in southern Ontario (Canada). Cancer Causes Control, *5:* 540–548, 1994.

10. Herrinton, L. J., Weiss, N. S., Beresford, S. A., Stanford, J. L., Wolfla, D. M., Feng, Z., and Scott, C. R. Lactose and galactose intake and metabolism in relation to the risk of epithelial ovarian cancer. Am. J. Epidemiol., *141:* 407–416, 1995.

11. Risch, H. A., Jain, M., Marrett, L. D., and Howe, G. R. Dietary fat intake and risk of epithelial ovarian cancer. J. Natl. Cancer Inst., *86:* 1409–1415, 1994.

12. Lin, C. H., Kirby, L. T., Ng, W. G., and Reichardt, J. K. V. On the molecular nature of the Duarte variant of galactose-1-phosphate uridyl transferase (GALT). Hum. Genet., *93:* 167–169, 1994.

13. Willett, W. C., Sampson, L., Stampfer, J. M., Rosner, B., Bain, C., Witschi, J., Hennekens, C. H., and Speizer, F. E. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am. J. Epidemiol. *122:* 51–65, 1985.

14. Lee, J. E. S., and Ng, W. G. Semi-micro techniques for the genotyping of galactokinase and galactose-1-phosphate uridyl transferase. Clin. Chim. Acta, *124:* 351–356, 1982.

15. Beutler, E. Red Cell Metabolism. New York: Grume & Stratton, Inc., 1975.

16. Reichardt, J. K. V., Packman, S., and Woo, S. L. C. Molecular characterization of two galactosemia mutations: correlation of mutation with highly conserved domains in galactose-1-phosphate uridyl transferase. Am. J. Hum. Genet., *49:* 860–867, 1991.

17. Scully, R. S. Tumors of the Ovary and Maldeveloped Gonads, pp. 83–84. Washington, DC: Armed Forces Institute of Pathology, 1979.

18. Meloni, G. F., Colombo, C., LaVecchia, C., Ruggiu, G., Mannazzu, M. C., Ambrosini, G., and Cherchi, P. L. Lactose absorption in patients with ovarian cancer. Am. J. Epidemiol. 150: *183–186:* 1999.

19. Sampson, J. A. The development of the implantation theory for the origin of endometriosis. Am. J. Obstet. Gynecol., *40:* 540–557, 1940.

20. Mostoufizadeh, M., and Scully, R. E. Malignant tumors arising in endometriosis. Clin. Obstet. Gynecol., *23:* 951–963, 1980.

21. Brinton, L. A., Gridley, G., Persson, I., Baron, J., and Bergquist, A. Cancer risk following a hospital discharge diagnosis of endometriosis. Am. J. Obstet. Gynecol., *176:* 572–579, 1997.

22. Cramer, D. W., Goldstein, D. P., Fraer, C., and Reichardt, J. K. V. Vaginal agenesis (Mayer-Rokitansky-Kuster-Hauser Syndrome) associated with the N314D mutation of galactose-1-phosphate uridyl transferase (GALT). Mol. Hum. Reprod., *2:* 145–148, 1996.

23. Cramer, D. W., Hornstein, M. D., Ng, W. G., and Barbieri, R. L. Endometriosis associated with the N314D mutation of galactose-1-phosphate uridyl transferase (GALT). Mol. Hum. Reprod., *2:* 149–152, 1996.

24. Chen, Y. T., Mattison, D. R., Feigenbaum, L., Fukui, H., and Schulman, J. D. Reduction in oocyte number following prenatal exposure to a diet high in galactose. Science (Washington DC), *214:* 1145–1147, 1981.

25. Ghirardini G, Magnani A. Mayer Rokintansky Kuster Hauser syndrome and ovarian cancer. Clin. Exp. Obstet Gynecol., *22:* 247–248, 1995.

26. Koonings, P. P., al-Marayati, L., Schlaerth, J. B., and Lobo, R. A. Mayer-Rokitansky-Kuster-Hauser syndrome associated with endodermal sinus tumor of the ovary. Fertil Steril., *56:* 577–578, 1991.

27. Nam, J. H., Chung, D. G., Kim, Y. M., Kim, Y. T., and Mok, J. E. Endodermal sinus tumor arising from a dysgenetic gonad in a 46 XX female combined with mullerian agenesis Gynecol. Oncol., 55:465–468, 1994.

28. Morland, S. J., Jiang, X., Hitchcock, A., Thomas, E. J., and Campbell, I. G. Mutation of galactose-1-phosphate uridyl transferase and its association with ovarian cancer and endometriosis. Int. J. Cancer, *77:* 825–827, 1998.

29. Holton, J. B. Galactose disorders: an overview. J. Inherit. Metab. Dis., *13:* 476–486, 1990.

30. Kalckar, H. M. Galactose metabolism and cell "sociology." Science (Washington DC), *150:* 305–313, 1965.

31. Petry, K. G., and Reichardt, J. K. V. The fundamental importance of galactose metabolism: lessons from genetics and biochemistry. Trends Genet., *14:* 98–102, 1998.

32. Daude, N., Gallaher, T. K., Zeschnigk, M., Starzinski-Powitz, A., Petry, K. G., Haworth, I. S., and Reichardt, J. K. V. Molecular cloning, characterization, and mapping of a full-length cDNA encoding human UDP-galactose 4′-epimerase. Biochem. Mol. Med., *56:* 1–7, 1995.

33. Croce, C. M. Detection and cloning of a common region of loss of heterozygosity at chromosome 1p in breast cancer. Cancer Res., *55:* 1752–1757, 1995.

34. Bromme, L., Bardi, G., Pandis, Fenger, C., Kronborg, O., and Heim, S. Chromosome abnormalities in colorectal adenomas: two cytogenetic subgroups characterized by deletion of 1p and numerical aberrations. Hum. Pathol., *27:* 1192–1197, 1996.

35. Perlman, E. J., Valentine, M. B., and Griffin, C. A. Deletion of 1p36 in childhood endodermal sinus tumors by two-color fluorescence *in situ* hybridization: a pediatric oncology group study. Genes Chromosomes Cancer, *16:* 5–20, 1996.

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

A Case-Control Study of Galactose Consumption and Metabolism in Relation to Ovarian Cancer

Daniel W. Cramer, E. Robert Greenberg, Linda Titus-Ernstoff, et al.

Cancer Epidemiol Biomarkers Prev 2000;9:95-101.