Milk Intake, Circulating Levels of Insulin-Like Growth Factor-I, and Risk of Colorectal Cancer in Men

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Background: **Milk and dietary calcium may have antiproliferative effects against colorectal cancer, but milk intake also raises serum levels of insulin-like growth factor-I (IGF-I). A high ratio of IGF-I to IGF-binding protein-3 (IGFBP-3) has been linked to an increased risk of colorectal cancer.** ***Methods:*** **In a case–control study nested in the Physicians' Health Study, plasma samples were collected from the period 1982 through 1983 from 14 916 men, aged 40–84 years, who also answered dietary questionnaires. Circulating levels of IGF-I and IGFBP-3 were assayed among 193 men who developed colorectal cancer during 13 years of follow-up and 318 age- and smoking-matched cancer-free control men. Conditional logistic regression was used to assess relative risks (RRs) of colorectal cancer for tertiles of IGF-I/IGFBP-3 and dietary factors. Statistical tests were two-sided.** ***Results:*** **Overall, there was a moderate but statistically nonsignificant inverse association between intake of low-fat milk or calcium from dairy food and colorectal cancer risk. Intake of dairy food (especially low-fat milk) was also positively and moderately associated with plasma levels of IGF-I, IGFBP-3, and IGF-I/IGFBP-3 among control men. We observed a statistically significant interaction between low-fat milk intake and IGF-I/IGFBP-3 in association with risk of colorectal cancer ($P_{interaction}$ = .03). Nondrinkers with IGF-I/IGFBP-3 in the highest tertile had a threefold higher risk than nondrinkers with IGF-I/IGFBP-3 in the lowest tertile (RR = 3.05; 95% confidence interval [CI] = 1.29 to 7.24), but no such increase was seen among frequent low-fat milk drinkers (RR = 1.05; 95% CI = 0.41 to 2.69). Conversely, among men with high IGF-I/IGFBP-3, frequent low-fat milk drinkers had a 60% lower risk (95% CI = 0.17 to 0.87; P_{trend} = .02) than nondrinkers.** ***Conclusion:*** **Intake of dairy products was associated with a modest increase in circulating IGF-I levels, but intake of low-fat milk was associated with lower risk of colorectal cancer, particularly among individuals with high IGF-I/IGFBP-3. This subpopulation, which is at increased risk of colorectal cancer, might benefit the most from specific dietary intervention.**

Studies in animals *(1–4)* have suggested that dietary calcium has antiproliferative effects on bile-induced mucosal damage and experimental bowel carcinogenesis. Intervention trials *(5,6)* have also suggested that calcium from low-fat dairy foods reduces human colorectal cell proliferation and restores cellular differentiation among individuals at risk of colorectal neoplasia. A recent intervention study *(7)* reported that calcium supplements produced a moderate but statistically significant reduction in recurrence of adenomatous polyps, precursors of colorectal cancer. In contrast to these findings, however, most prospective observational studies *(8–13)* show only a moderate and not statistically significant decrease in risk of colorectal cancer with increased dietary calcium intake.

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See "Notes" following "References."

In addition to being a major source of dietary calcium, milk may also raise levels of human insulin-like growth factor-I (IGF-I). In a recent randomized intervention study *(14)* among 204 healthy men and women aged 55–85 years, individuals who consumed three servings per day of nonfat or 1% milk for 12 weeks had a statistically significant 10% increase in serum IGF-I levels compared with individuals who consumed no milk. Milk contains IGF-I, whether or not the cows were treated with recombinant bovine somatotropin (rBST) for milk production, but it is not known whether the intact growth factor can be absorbed with milk and, if so, if it is responsible for the increased circulating levels of IGF-I in humans *(15–17)*.

IGF-I is a potent mitogen and inhibitor of apoptosis in both normal and malignant intestinal epithelial cells *(18,19)*. Newborn rat pups fed mother's milk or long arginine3 IGF-I, an IGF-I analogue with high affinity for the IGF-I receptor and low affinity for IGF-binding protein (IGFBP), had increased intestinal cell growth compared with pups fed milk replacer, with the long arginine3 IGF-I-fed pups showing the greatest increase *(20)*. We and others *(21–23)* previously reported that elevated circulating levels of IGF-I are associated with increased risks of colorectal cancer and of large villus colorectal polyps. The highest risk was observed among individuals with high IGF-I and low IGFBP-3 levels (i.e., high IGF-I/IGFBP-3 molar ratio) *(21,22)*. Most circulating IGFs are bound to IGFBP-3, which inhibits cell proliferation by modulating access of IGFs to IGF receptors or by promoting apoptosis via an IGF-independent mechanism *(24)*. Observations from acromegalic patients suggest that high circulating levels of IGF-I relative to IGFBP-3 may be causally related to enhanced colonic epithelial cell proliferation and increased risk of colorectal cancer *(25–29)*.

Any increase in IGF-I levels attributable to milk ingestion could, therefore, potentially counteract a protective effect of dietary calcium from milk on colorectal cancer. Alternatively, dietary chemoprevention related to calcium ingestion may be particularly important for individuals who have increased colorectal cancer risk associated with a high IGF-I/IGFBP-3 ratio. To assess the interaction between intake of milk or calcium from dairy food and IGF-I/IGFBP-3 molar ratio in relation to colorectal cancer risk, we conducted an analysis among the same men in our previous report of IGFs and colorectal cancer *(21)*.

Subjects and Methods

Subjects

The study subjects and sample collection method have been described in detail elsewhere *(21)*. In brief, this prospective case–control study was nested in the Physicians' Health Study (PHS), a randomized, double-blind, placebo-controlled trial to examine the effect of aspirin and β-carotene on cardiovascular disease and cancer. This trial included 22 071 healthy U.S. male physicians who were 40–84 years of age in 1982 *(30)*. Written informed consent was obtained from all of the PHS participants, and the study was approved by the Human Research Committee at the Brigham and Women's Hospital. We had obtained plasma blood samples from 14 916 of the participants in 1982 or 1983, long before rBST for dairy production was introduced *(21)*. By December 1995, we had confirmed 193 diagnoses of colorectal cancer among men who had adequate baseline plasma samples. For each case subject, we attempted to select two control subjects who had provided blood and had not reported a diagnosis of colorectal cancer at the time the diagnosis was reported by the case subject. Control subjects were matched for age (±1 year) and smoking status (never, past, or current). We could identify a second control subject for only 125 case subjects, and so, in total, 318 men formed the control group.

Dietary Calcium Intake Assessment

At 18 weeks after randomization, participants were asked to report their average intake of 19 food items, including whole and skim/low-fat milk during the past year. Intakes were reported according to seven frequency categories: rarely/never, one to three servings/month, one serving/week, two to four servings/week, five to six servings/week, one serving/day, and two or more servings/day. Intake of hard cheese, ice cream, chicken/turkey, beef as a main dish, beef or ham as a sandwich, hot dogs, tuna fish, dark fish, and other fish was also ascertained on a 12-month questionnaire. Intake of red meat was calculated as the sum of intake of beef as a main dish, beef or ham as a sandwich, and hot dogs. Total fish intake was the sum of all fish intake. We used an 8-oz serving size for analysis of skim and low-fat milk (referred to collectively as low-fat milk). However, because of differences in serving sizes and calcium content in other dairy products, we used the sum of calcium for analyses of milk intake (sum of calcium in low-fat and whole milk) and total dairy product intake (sum of calcium in milk, cheese, and ice cream). For example, calcium from milk was calculated by multiplying the servings per day by the calcium content of whole milk (291 mg/8-oz glass) or low-fat milk (308 mg/8-oz glass) and adding the two. For hard cheese, we used a calcium content of 204 mg per slice or per 1 oz. For ice cream, we used a calcium content of 85 mg per cup.

To assess the adequacy of using the four dairy foods (low-fat milk, whole milk, cheese, and ice cream) to estimate total dietary calcium intake in the PHS, we conducted a validation study using the comprehensive 1986 dietary database from the Health Professionals Follow-up Study (HPFS), a male cohort similar to the PHS cohort. Dietary calcium information was available for the HPFS cohort from 13 dairy products of a total of 131 food items. Using a linear regression model, we demonstrated that the four dairy foods assessed in this study accounted for approximately 82% of the variance of the total dietary calcium consumption (R^2 = .82) in the HPFS. This validation analysis suggests that calcium consumption from these four major dairy foods was an appropriate indicator of total dairy calcium consumption for the PHS cohort**,** thus allowing us to adequately rank individuals by level of dietary calcium intake.

Assays of IGF-I and IGFBP-3

Plasma levels of IGF-I and IGFBP-3 were assayed in the laboratory of Dr. Michael Pollak at the Lady Davis Research Institute of the Jewish General Hospital and McGill University, Montreal, ON, Canada. Samples from case subjects and their matched control subjects were assayed in the same batch to minimize interassay variability, and aliquots from a pool of quality-control plasma were inserted randomly. Laboratory personnel were unable to distinguish among case, control, and quality-control samples. Plasma levels of IGF-I and IGFBP-3 were assayed by enzyme-linked immunosorbent assay with reagents from Diagnostic Systems Laboratory (Webster, TX). The mean intra-assay coefficients of variation for IGF-I and IGFBP-3 from the blinded quality-control samples were 2.9% and 3.2%, respectively. Circulating IGF-I is mainly bound to IGFBP-3, and the molar ratio of IGF-I to IGFBP-3 (referred to collectively as IGF-I/IGFBP-3), which has been suggested to be a marker of bioactive IGF-I, is associated with increased risk of colorectal cancer *(21)*. We, therefore, used IGF-I/IGFBP-3 as an indicator of the bioactive IGF-I. We used the following equivalents for conversion: 1 ng/mL IGF-I = 0.130 n*M* IGF-I, and 1 ng/mL IGFBP-3 = 0.036 n*M* IGFBP-3.

Statistical Analyses

We compared baseline characteristics between case and control subjects by paired *t* tests and chi-squared tests. Analysis of covariance was used to compare the age- and smoking-adjusted levels of IGF-I, IGFBP-3, and IGF-I/IGFBP-3 according to tertiles of dietary intake of milk, dairy food, red meat, poultry, and fish among case and control subjects. Tertiles of dietary intake and IGF-I/IGFBP-3 ratio were categorized on the basis of the distribution among control subjects. Conditional logistic regression was used to assess the age- and smoking-matched relative risks (RRs) and 95% confidence intervals (CIs) of colorectal cancer for the association of tertiles of IGF-I/IGFBP-3 and dietary intake with colorectal cancer. In addition, all models were adjusted for body mass index (BMI) (weight in kg/[height in m]^2), alcohol intake, exercise, multivitamin use, and aspirin use. Tests for trend were conducted by use of median levels of the tertiles, and tests for interaction were conducted with the use of similar models but including the interaction term (i.e., the product of the two main exposures, such as

low-fat milk and IGF-I/IGFBP-3). We assessed the multivariable RRs for colorectal cancer associated with the joint effect of IGF-I/IGFBP-3 and dietary intakes by using the lowest tertiles of both factors as the reference group. We also conducted the analyses after stratifying by tertile of IGF-I/IGFBP-3 or by tertile of dietary intakes. Because such stratification breaks the matching of case–control pairs, we used unconditional logistic regression for stratified analysis as well as for tests for trend within each stratum, controlling for the matching factors and the above covariates. All P values were two-sided, and all of the analyses used the SAS program package *(31,32).*

RESULTS

Age- and smoking-adjusted levels of IGF-I and IGF-I/IGFBP-3 according to intakes of low-fat milk, of calcium from milk or dairy food, and of red meat, poultry, and fish among control subjects are presented in Table 1. Intakes of low-fat milk, of calcium from milk, and of calcium from dairy foods were all associated positively with the levels of IGF-I and IGF-I/IGFBP-3 (Table 1). Among all of the dairy foods (low-fat milk, milk, hard cheese, and ice cream), only low-fat milk was statistically significantly and positively associated with IGFBP-3 levels. To test whether the apparent positive association between intake of milk or dairy food and IGF-I was due to animal protein rather than to something else in milk, we assessed levels of IGF-I, IGFBP-3, and the IGF-I/IGFBP-3 by intakes of red meat, poultry (chicken/turkey), and fish, the major nondairy sources of animal protein. None was statistically significantly associated with IGF-I, IGFBP-3, or IGF-I/IGFBP-3 (Table 1).

We calculated the multivariable-adjusted RR of colorectal cancer according to IGF-I/IGFBP-3 and intakes of low-fat milk, of calcium from milk or dairy food, and of red meat, poultry, and fish (Table 2). Intakes of low-fat milk and of calcium from milk or dairy food were inversely but not statistically significantly associated with colorectal cancer risk after controlling for IGF-I/IGFBP-3, alcohol intake, BMI, exercise, and use of multivitamins and aspirin. In the same multivariable model that was adjusted for intake of low-fat milk, men with IGF-I/IGFBP-3 in the highest tertile had a statistically significant 1.8-fold increased risk (95% CI = 1.12 to 3.01; P_{trend} = .03) compared with men in the lowest tertile, consistent with our previous findings *(21).* Intakes of red meat, poultry, and fish were not associated with risk of colorectal cancer.

Because calcium from low-fat dairy foods may reduce colorectal epithelial cell proliferation, we examined the possibility that the association between dairy food intake and colorectal cancer risk varied with IGF-I/IGFBP-3. IGF-I/IGFBP-3 has been suggested to reflect free, biologically active IGF-I and, thus, may influence the dynamics of colonic epithelial cell renewal *(25,26).* Among men with IGF-I/IGFBP-3 in the highest tertile, intake of low-fat milk was inversely and statistically significantly associated with risk (P_{trend} = .02). Compared with "nondrinkers" of low-fat milk (never/rarely drink low-fat milk), RRs for colorectal cancer were 0.78 (95% CI = 0.36 to 1.70) for moderate milk drinkers (one to three glasses per month to three to four glasses per week) and 0.39 (95% CI = 0.17 to 0.87) for frequent milk drinkers (five or more glasses per week). No suggestion of protection by low-fat milk was observed for men with IGF-I/IGFBP-3 in the lower two tertiles. Similar patterns were observed for calcium intake from milk or dairy foods, with statistically nonsignificant trends.

We also assessed the joint association of low-fat milk or calcium intake from milk or dairy food and IGF-I/IGFBP-3 with risk of colorectal cancer (Table 3). Among nondrinkers of low-fat milk, those in the highest tertile of IGF-I/IGFBP-3 had the highest risk (compared with men in the lowest tertile of IGF-I/IGFBP-3, the RR = 3.05 [95% CI = 1.29 to 7.24]). By contrast, among frequent milk drinkers, there was no increase in risk associated with a high IGF-I/IGFBP-3 as compared with a low molar ratio (RR = 1.05; 95% CI = 0.41 to 2.69). The interaction between low-fat milk intake and IGF-I/IGFBP-3 in association with the risk of colorectal cancer was statistically significant ($P_{interaction}$ = .03). Similar patterns were observed for intake of calcium from milk or dairy foods, although in neither case were the interactions statistically significant. These findings suggest that dairy food, especially low-fat milk, may have a

Table 1. Age- and smoking-adjusted mean plasma levels of insulin-like growth factor-I (IGF-I), insulin-like growth factor-binding protein-3 (IGFBP-3), and the IGF-I/IGFBP-3 molar ratio according to tertiles of intake of skim/low-fat milk, of calcium from milk or dairy food, and of red meat, poultry, and fish among control subjects*

Tertiles of intake	IGF-I ng/mL	IGFBP-3 ng/mL	IGF-I/IGFBP-3 molar ratio
Skim/low-fat milk (8-oz glasses)			
Tertile 1 (never/rarely)	174	2887	0.218
Tertile 2 (1–3/mo to 3–4/wk)	186	3083	0.220
Tertile 3 (5–6/wk to ⩾2/day)	203†	3129‡	0.235§
Calcium from total milk (mg/day)			
Tertile 1 (0–42)	177	2910	0.220
Tertile 2 (43–287)	185	3116	0.217
Tertile 3 (291–906)	195‖	3033	0.233
Calcium from dairy food (mg/day)			
Tertile 1 (0–128)	179	2960	0.218
Tertile 2 (132–334)	181	3028	0.219
Tertile 3 (335–918)	198¶	3057	0.233#
Red meat (servings/day)			
Tertile 1 (0–0.5)	189	3029	0.226
Tertile 2 (0.57–0.87)	195	3057	0.231
Tertile 3 (0.93–2.07)	177	2983	0.214
Poultry (servings/day)			
Tertile 1 (0–0.07)	172	2965	0.211
Tertile 2 (0.14)	189	3021	0.226
Tertile 3 (0.43–0.80)	189	3039	0.225
Fish (servings/day)			
Tertile 1 (0–0.14)	177	2934	0.219
Tertile 2 (0.21–0.28)	188	3082	0.222
Tertile 3 (0.35–2.03)	193	3033	0.230

*Two-sided P values, from analysis of covariance are provided when $P_{high\ versus\ low}$<.05.
†$P_{high\ versus\ low}$ = .0003.
‡$P_{high\ versus\ low}$ = .004.
§$P_{high\ versus\ low}$ = .023.
‖$P_{high\ versus\ low}$ = .021.
¶$P_{high\ versus\ low}$ = .014.
#$P_{high\ versus\ low}$ = .038.

Table 2. Relative risk (RR)* of colorectal cancer according to tertiles of molar ratio of insulin-like growth factor-I (IGF-I) to insulin-like growth factor-binding protein-3 (IGFBP-3) and intake of skim/low-fat milk, of calcium from total milk or dairy food, and of red meat, poultry, and fish in a prospective study of men

	Tertile 1	Tertile 2	Tertile 3	P_{trend}†
IGF-I/IGFBP-3, ng/mL				
Median (range)	0.18 (0.12–0.20)	0.22 (0.20–0.24)	0.27 (0.24–0.42)	
No. of case subjects‡	50	54	77	
No. of control subjects‡	98	100	98	
RR§ (95% confidence interval [CI])	1.00 (referent)	1.21 (0.73 to 1.98)	1.84 (1.12 to 3.01)	.01
Skim/low-fat milk, 8-oz glasses				
Range	Never/rarely	1–3/mo to 3–4/wk	5–6/wk to 2+/d	
No. of case subjects	73	63	45	
No. of control subjects	97	114	85	
RR (95% CI)	1.00 (referent)	0.72 (0.45 to 1.13)	0.66 (0.38 to 1.13)	.22
Calcium from total milk, mg/day				
Median (range)	0 (0–42)	132 (43–287)	308 (291–906)	
No. of case subjects	69	71	50	
No. of control subjects	102	104	105	
RR (95% CI)	1.00 (referent)	0.95 (0.60 to 1.51)	0.66 (0.40 to 1.09)	.09
Calcium from dairy food, mg/day				
Median (range)	70 (0–132)	223 (136–335)	432 (340–918)	
No. of case subjects	66	81	46	
No. of control subjects	106	106	106	
RR (95% CI)	100 (referent)	1.25 (0.80 to 1.94)	0.62 (0.38 to 1.02)	.06
Red meat, servings/day				
Median (range)	0.28 (0–0.5)	0.64 (0.6–0.9)	1.14 (0.9–2.1)	
No. of case subjects	54	66	70	
No. of control subjects	91	104	118	
RR (95% CI)	1.00 (referent)	1.12 (0.68 to 1.83)	0.98 (0.60 to 1.60)	.98
Poultry, servings/day				
Median (range)	0.07 (0–0.07)	0.14	0.43 (0.43–0.8)	
No. of case subjects	29	70	91	
No. of control subjects	40	136	137	
RR (95% CI)	100 (referent)	0.67 (0.37 to 1.21)	0.93 (0.52 to 1.68)	.36
Fish, servings/day				
Median (range)	0.14 (0–0.14)	0.21 (0.21–0.28)	0.57 (0.35–2.03)	
No. of case subjects	54	80	57	
No. of control subjects	94	115	105	
RR (95% CI)	1.00 (referent)	1.20 (0.75 to 1.93)	0.92 (0.56 to 1.51)	.47

*Unless otherwise noted, RRs were adjusted for age, cigarette smoking, body mass index (weight in kg/[height in m]^2), alcohol intake, multivitamin use, aspirin use, exercise, and molar ratio of IGF-I to IGFBP-3.

†All *P* values were two-sided.

‡Numbers of case and control subjects do not always add up to the total (193 case subjects and 318 control subjects) because of missing dietary data.

§RR for IGF-I/IGFBP-3 molar ratio was adjusted for all of the covariates listed above as well as for intake of skim/low-fat milk. Similar RRs were seen in models adjusted for other dietary factors.

protective effect specifically among men with high IGF-I/IGFBP-3.

To assess whether this apparent protective effect of milk and dairy foods could be due to protein intake, we assessed the joint association of red meat, poultry, or fish—the major sources of nondairy animal protein—and IGF-I/IGFBP-3 with risk of colorectal cancer. Elevated IGF-I/IGFBP-3 was associated with increased risk of colorectal cancer, whatever the level of intake of these high-protein animal foods (Table 3). Moreover, in contrast to men with high milk intake and a high IGF-I/IGFBP-3, who had no increase in their risk of colorectal cancer compared with men in the lowest tertile of both, men in the highest tertiles of both intake of red meat (the major contributor of animal protein) and IGF-I/IGFBP-3 had a more than threefold increase in risk (RR = 3.12; 95% CI = 1.30 to 7.49) compared with men in the lowest tertile of both (Table 3). These results suggest that intake of milk or dietary calcium, and not of dietary animal protein, may protect men with high IGF-I/IGFBP-3 from colorectal cancer.

Discussion

Milk and dietary calcium have been suggested to protect against the development of colorectal cancer, perhaps by precipitating fatty acids and bile acids that are potentially toxic to the colorectal epithelium *(1)*. In experimental animals, supplemental calcium in the diet or drinking water decreases the colonic epithelial hyperproliferation induced by bile and fatty acids, enteric resection, or nutritional stress *(2)*. Calcium or low-fat milk can also suppress induction of the tumor-promoting enzyme ornithine decarboxylase and inhibit experimental colon carcinogenesis induced by azoxymethane or 1,2-dimethylhydrazine *(2,4,33,34)*. Several intervention studies *(2,5,6)* have suggested that supplemental calcium inhibits epithelial cell proliferation in the human colon, although the findings are not entirely consistent. A randomized, single-blinded study *(5)* found that high intake of low-fat dairy food (equivalent to a daily calcium intake of 1200 mg) reduced colonic epithelial cell proliferation and restored differentiation among individuals at risk for colonic neoplasia. Furthermore, administration of calcium modestly reduced the incidence of recurrent adenomatous polyps in individuals with prior colon adenoma *(7)*.

Our finding of weak and not statistically significant inverse overall associations between the intake of low-fat milk or dietary calcium and risk of colorectal cancer is consistent with epidemiologic data from prospective studies *(8–10,12)*. It has been suggested that the consistently weak and not statistically significant association from epidemiologic studies compared with the apparent protective effect of calcium from animal studies and clinical trials may be due partly to misclassification of dietary calcium. Indeed, one major limitation of the current analysis is the restricted list of dietary items that were ascertained. Although we determined that the four major dairy foods (skim/low-fat milk, whole milk, hard cheese, and ice cream) assessed in the PHS account for about 82% of the variation in dietary calcium consumption, we could not calculate intake of energy, protein, and calcium supplements; therefore, we were unable to assess the role of these factors or control for them in these analyses. Our observed statistically nonsignificant association between milk and colorectal cancer risk may be attributed, in part, to such measurement error and limited statistical power. We also cannot exclude the possibility of measurement error in the assessment of the risk factors, the

Table 3. Relative risk (RR)* of colorectal cancer according to molar ratio of insulin-like growth factor-I (IGF-I) to insulin-like growth factor-binding protein-3 (IGFBP-3) and intake of skim/low-fat milk, of calcium from total milk or dairy food, and of red meat, poultry, and fish in a prospective study of men

	IGF-I/IGFBP-3 molar ratio					
	Tertile 1		Tertile 2†		Tertile 3	
Dietary intake	No. of case subjects/No. of control subjects	RR (95% confidence interval [CI])	No. of case subjects/No. of control subjects	RR (95% CI)	No. of case subjects/No. of control subjects	RR (95% CI)
Skim/low-fat milk						
Tertile 1	15/37	1.00 (referent)	27/35	1.96 (0.83 to 4.62)	31/25	3.05 (1.29 to 7.24)
Tertile 2	22/44	1.18 (0.48 to 2.93)	11/36	0.84 (0.33 to 2.16)	30/34	2.24 (0.97 to 5.18)
Tertile 3	13/17	1.59 (0.55 to 4.64)	16/29	1.43 (0.59 to 3.51)	16/39	1.05 (0.41 to 2.69)
				$P_{interaction}$ = .03†		
Calcium from total milk						
Tertile 1	18/38	1.00 (referent)	23/36	1.48 (0.65 to 3.39)	28/28	2.24 (1.00 to 5.02)
Tertile 2	22/40	1.02 (0.44 to 2.40)	18/35	1.14 (0.48 to 2.69)	31/29	2.49 (1.09 to 5.68)
Tertile 3	14/25	1.04 (0.41 to 2.64)	15/34	0.99 (0.43 to 2.28)	21/46	1.00 (0.43 to 2.36)
				$P_{interaction}$ = .18		
Calcium from dairy food						
Tertile 1	21/37	1.00 (referent)	18/40	0.80 (0.34 to 1.91)	27/29	2.05 (0.93 to 4.55)
Tertile 2	22/45	0.81 (0.36 to 1.84)	22/32	1.23 (0.54 to 2.77)	37/29	2.78 (1.23 to 6.27)
Tertile 3	12/24	0.75 (0.29 to 1.93)	16/34	0.89 (0.39 to 2.03)	18/48	0.72 (0.31 to 1.67)
				$P_{interaction}$ = .14		
Red meat						
Tertile 1	13/29	1.00 (referent)	19/31	1.83 (0.72 to 4.61)	22/31	2.38 (0.93 to 6.07)
Tertile 2	21/26	2.12 (0.84 to 5.36)	21/35	1.61 (0.66 to 3.92)	24/43	1.91 (0.76 to 4.80)
Tertile 3	21/49	1.14 (0.48 to 2.71)	14/39	0.99 (0.38 to 2.61)	35/30	3.12 (1.30 to 7.49)
				$P_{interaction}$ = .38		
Poultry						
Tertile 1	10/18	1.00 (referent)	11/13	1.86 (0.50 to 6.93)	8/9	1.71 (0.46 to 6.32)
Tertile 2	17/47	0.63 (0.23 to 1.73)	20/41	0.94 (0.35 to 2.55)	33/48	1.61 (0.62 to 4.16)
Tertile 3	28/38	1.45 (0.57 to 3.67)	22/52	0.93 (0.38 to 2.28)	41/47	2.06 (0.81 to 5.19)
				$P_{interaction}$ = .50		
Fish						
Tertile 1	16/34	1.00 (referent)	13/32	1.04 (0.41 to 2.68)	25/28	2.63 (1.08 to 6.39)
Tertile 2	26/40	1.63 (0.70 to 3.78)	24/43	1.46 (0.63 to 3.37)	30/32	2.24 (0.98 to 5.12)
Tertile 3	13/30	0.86 (0.33 to 2.26)	17/31	1.34 (0.53 to 3.39)	27/44	1.90 (0.81 to 4.44)
				$P_{interaction}$ = .93		

*Adjusted for age, cigarette smoking, body mass index (weight in kg/[height in m]^2), alcohol intake, multivitamin use, aspirin use, and exercise.
†All *P* values were two-sided.

extent of which may be similar (i.e., nondifferential measurement error) or different (i.e., differential measurement error) for each milk/dairy item. Because results from multivariable analyses showed associations that were similar to, although slightly stronger than, those in the matched analysis, we believe that nondifferential error remains a possibility; such an error would substantially attenuate the true association between milk intake and colorectal cancer. Moreover, studies *(9, 11,12)* that used validated food-frequency questionnaires and assessed long-term (>6 years) average or consistent dietary calcium intake, with and without calcium supplements, also found weak and not statistically significant inverse associations with colorectal cancer risk.

The inconsistent observations regarding the relationship between dietary calcium and colorectal cancer risk may also be due, in part, to heterogeneity between individuals in responding to the chemoprotective effect of calcium or dairy products. Individuals with elevated levels of IGF-I have an increased risk of cancer at several sites, including breast, prostate, lung, and colorectum *(21–23,35,36).* Elevated IGF-I/IGFBP-3 has been suggested to be a possible surrogate for elevated IGF-I bioactivity in tissues and for increased colonic epithelial cell turnover and proliferation *(37).* This notion is supported by our previous finding that men with high IGF-I/IGFBP-3 are at the highest risk of colorectal cancer *(21)* and by the recent finding of the joint effect of IGF-I and mutagen sensitivity in lung cancer risk *(38).* Our present findings suggest that milk or dietary calcium may be protective specifically among men with a high IGF-I/IGFBP-3. That is, we found that frequent milk drinking (five or more glasses of low-fat milk weekly) was associated with a statistically significant reduction in risk of colorectal cancer only among men with high IGF-I/IGFBP-3. No such protection was observed among men with a low IGF-I/IGFBP-3, and thus presumably lower proliferation potential in their colonic epithelium. Conversely, only among men with little or moderate intake of milk or dietary calcium was an elevated IGF-I/IGFBP-3 associated with an increased risk of colorectal cancer. Among the dairy foods assessed in this analysis, low-fat milk showed the strongest and most linear inverse association. It is possible that intake of low-fat milk was better assessed in the dietary questionnaire than that of other dairy foods because 64% of the men in the study reported that they did not drink whole milk, and low-fat milk was the single largest contributor of dietary calcium.

The confining of the apparent protective effect of milk or dietary calcium against colorectal cancer to individuals with a high IGF-I/IGFBP-3 is also consistent with findings from animal and human intervention studies *(2,4,5,7,33,34).* In these studies, animals predisposed to high colonic epithelial cell proliferation by carcinogens and humans with previously detected adenomas both experienced reduced risk of colorectal neoplasia from high calcium intake. These results may, in part, explain the inconsistent observations between an apparent protective effect by calcium in animal/human intervention studies and a weak and statistically nonsignificant risk reduction observed in epidemiologic studies in which high-risk populations were not clearly defined. Our findings raise the possibility that IGF-I-related markers may define subpopulations predisposed to high colonic epithelial cell proliferation and for whom specific dietary interventions could be most beneficial. This possibility is further supported by a recent cohort study of Finnish male smokers showing that high intake of dietary calcium or milk products was related to a 40% lower risk of colorectal cancer compared with that in smokers with low intake of these items *(13).* Individuals with long-term exposure to tobacco carcinogens may be at particularly high risk of colorectal adenomas and cancer *(39,40)* and, therefore, might benefit the most from high calcium intake.

The physiologic basis for the positive association between milk intake and circulating IGF-I levels remains unclear. It is well established that malnourishment or fasting in humans is associated with reduction in IGF-I levels, and both protein and energy are needed to restore IGF-I after fasting *(41,42).* However, little is known about how other features of the diet of adequately nourished individuals affect IGF-I levels. We found that frequent consumption of milk and dairy food (at a time before rBST was used for milk production) was associated with a modest increase in circulating IGF-I levels and in IGF-I/IGFBP-3. This observation is consistent with the recent finding from a randomized trial that intake of low-fat milk moderately but statistically significantly increased serum IGF-I levels *(14).* However, increased intake of total energy and protein was also observed in milk drinkers compared with nondrinkers in this study and it is, therefore, unclear whether it was milk or the increased intake of energy and protein that caused the increase in IGF-I levels. Although we could not assess intake of total energy and protein in our study, we believe that the effect we observed is milk specific because a similar pattern was seen for all dairy products but not for major sources of nondairy animal protein. Similar findings were shown in a recent study of lifestyle and dietary determinants of IGF-I in the Nurses' Health Study *(43),* which used a validated and comprehensive food-frequency questionnaire and adjusted for total energy intake.

It is possible that milk, as the major source of protein for infants, is rich in specific amino acids that may have strong influence on IGF-I levels and growth *(41).* Cow milk (even from cows that are not treated with rBST) contains IGF-I that is structurally identical to human IGF-I *(15,16,44).* It is generally believed that IGF-I will not retain bioactivity if delivered orally because of rapid proteolysis in the upper gut *(15,16).* However, an *in vivo* study of ^{125}I-labeled IGF-I in rats indicated that some IGF antibodies and dietary protein casein, the major protein in milk, may protect the IGF-I from degradation in the adult rat gastrointestinal tract *(45).* A recent study of suckling rats *(17)* also showed that milk-borne IGF-I can be absorbed intact and may exert effects on the liver and other peripheral tissues. No human data are available, and it is unclear whether these findings from animal studies can be applied to humans. Nevertheless, our findings suggest that milk is only a modest contributor to high IGF-I and that most of the men with a high IGF-I/IGFBP-3 may have this high ratio for reasons other than milk intake, such as genetics or other nutritional factors. These men predisposed to higher cancer risk might benefit from the high calcium intake to an extent that far outweighs any increase in IGF-I due to milk.

In summary, this prospective study suggests a protective effect of dietary calcium on colorectal cancer incidence among men with a high IGF-I/IGFBP-3, despite the moderate positive influence of milk or dairy food intake on circulating IGF-I levels. The hypothesis that the effect of calcium to reduce colorectal cancer risk varies according to IGF-I levels should be examined further in studies with more detailed dietary assessment and in dietary intervention trials.

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NOTES

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