Serum levels of IGF-I, IGFBP-3 and colorectal cancer risk: results from the EPIC cohort, plus a meta-analysis of prospective studies

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Abbreviations: BMI: body mass index; CI: confidence interval; EPIC: European Prospective Investigation into Cancer and nutrition; IGF-I: insulin-like growth factor-I; IGFBP-3: insulin-like growth factor binding protein-3; RR: relative risk; WHR: waist-to-hip ratio

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Several prospective studies have shown a moderate positive association between increasing circulating insulin-like growth factor-I (IGF-I) levels and colorectal cancer risk. However, the associations were often statistically nonsignificant, and the relationship of cancer risk with IGF-I's major binding protein, IGFBP-3, showed major discrepancies between studies. We investigated the association of colorectal cancer risk with serum IGF-I, total and intact IGFBP-3, in a case-control study nested within the EPIC cohort (1,121 cases of colorectal cancer and 1,121 matched controls). Conditional logistic regression was used to adjust for possible confounders. Our present study results were combined in a meta-analysis with those from 9 previous prospective studies to examine the overall evidence for a relationship of prediagnostic serum IGF-I with colorectal cancer risk. In the EPIC study, serum concentrations of IGF-I and IGFBP-3 showed no associations with risk of colorectal cancer overall. Only in subgroup analyses did our study show moderate positive associations of IGF-I levels with risk, either among younger participants only (and only for colon cancer) or among participants whose milk intakes were in the lowest tertile of the population distribution (RR for an increase of 100 ng/ml = 1.43 [95% CI = 1.13–1.93]). Nevertheless, in the meta-analysis a modest positive association remained between serum IGF-I and colorectal cancer risk overall (RR = 1.07 [1.01–1.14] for 1 standard deviation increase in IGF-I). Overall, data from our present study and previous prospective studies combined indicate a relatively modest association of colorectal cancer risk with serum IGF-I.

Insulin-like growth factor-I (IGF-I) is a member of the IGF family of growth factors and related molecules. IGF-I plays an important role in growth and development as a function of available energy and essential nutrients (*e.g.*, amino acids) from body reserves and diet.[1–4] Most circulating IGF-I and IGF-binding protein (IGFBP)-3, the principal IGFBP in blood, originate from the liver.[5] Determinants of circulating and tissue levels of IGF-I and IGFBP-3 include genetic factors,[6,7] but also age,[8] diet (especially intake of proteins, milk and cheese[9,10]) and other lifestyle factors.[11–13]

Experimental studies, both *in vivo* and *in vitro*, have implicated IGF-I in the development of cancer through its stimulatory effects on cell proliferation and inhibitory effects on apoptosis.[3,14–17] Furthermore, epidemiologic studies have associated elevated blood concentrations of IGF-I with increased risk of various forms of cancer, although the strength of the evidence varies by organ site.[14,18] These observations, combined with experimental data, in turn have led to proposals for cancer prevention strategies, *e.g.*, through modification of diet and other lifestyle factors,[19–23] as well as cancer treatment modalities,[24,25] that aim to reduce circulating levels of IGF-I and/or IGF-I receptor signaling.

Also for colorectal cancer, a number of prospective epidemiologic studies have shown a positive association between cancer risk and increasing levels of circulating IGF-I.[26–33] In many of these studies, however, the association was not statistically significant,[28,29,31–33] and in 1 study the association was significant only for colon cancer.[30] Moreover, there have been major discrepancies between studies in the observed relationships between colorectal cancer risk and circulating levels of IGFBP-3, some studies showing a negative association with risk,[27,32] whereas others showed a positive association.[28–30] As a consequence of these inconsistent results, the effect of statistical adjustments for IGFBP-3 levels on the observed relationship of colorectal cancer risk with IGF-I levels differed also between studies. It has been speculated that these discrepancies could be due to different specificities of IGFBP-3 assays used, which might measure either intact IGFBP-3 that is not proteolytically cleaved and which could be decreased among participants who develop cancer, or total IGFBP-3 (intact plus cleaved forms) whose levels actually could be increased among cancer-prone participants.[34,35]

We conducted a large case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC)[36] to examine the relationships of colorectal cancers with serum levels of IGF-I, and with 2 measures of IGFBP-3: one that was designed to measure total IGFBP-3 and one that reflects intact forms of IGFBP-3. With a total of 1,121 colorectal cancer cases (707 cases of colon cancer and 414 cases of rectal cancer), this study is by far the largest prospective study to date, allowing the separate examination of the associations of IGF-I and IGFBP-3 with risks of colon and rectal cancers. In this study, we also examined whether relative risks associated to IGF-I levels were modified by anthropometric and

dietary factors previously shown to be related with circulating IGF-I values[10,37–39] and with colorectal cancer risk.[11,26,40–44] Finally, we performed a meta-analysis combining our study results with those from previously published prospective studies, to assess the overall evidence relating colorectal cancer risk to blood prediagnostic concentrations of IGF-I.

Material and Methods

The European Prospective Investigation into Cancer and Nutrition

The EPIC project is a European network of prospective cohorts that was set up to examine relationships of cancer risk with nutrition and metabolic risk factors. The EPIC cohorts include about half a million men and women, recruited through 23 regional and national research centers located in 10 western European countries: Norway (Tromsø), Sweden (Umeå and Malmö), Denmark (Copenhagen and Åarhus), England (Oxford and Cambridge), The Netherlands (Utrecht and Bilthoven), Germany (Potsdam and Heidelberg), France (Paris), Spain (Oviedo, San Sebastian, Pamplona, Murcia, and Granada), Italy (Turin, Milan, Florence, Naples, and Ragusa) and Greece (Athens).

Baseline questionnaire data and anthropometric measurements (height, weight, waist and hip circumferences) were collected from study participants in the period 1992–1998. The questionnaires included detailed questions about current habitual diet, menstrual and reproductive history, current and past use of oral contraceptives in women, a history of previous illness and surgical operations, lifetime history of tobacco smoking and consumption of alcoholic beverages and physical activity. In addition, blood samples were also collected for most participants. Extensive details about EPIC recruitment procedures, questionnaires and biologic sample collection are given elsewhere.[36,45]

All participants had given their written consent for future analyses of their blood samples, and the Internal Review Boards (IRB) of IARC and all EPIC recruitment centers approved the biochemical analyses on serum samples, performed for the present project.

Identification and selection of colorectal cancer cases

In all EPIC centers, except those in Greece and Germany, data on vital status are collected by record linkage with regional and/or national mortality registries. In Greece and Germany, data on vital status are continuously collected through active follow-up. In all centers except those in Greece, Germany and France, incident cases of cancer are identified through record linkage with regional cancer registries. In France, Germany and Greece, follow-up for cancer incidence is based on a combination of methods, including the use of health insurance records, contacts with cancer and pathology registries and active follow-up through study participants and their next-of-kin. Closure dates for the present study were defined as the latest date of *complete follow-up* for both cancer incidence and vital status. In Denmark, Italy, The Netherlands, Norway, Spain, Sweden and United Kingdom, this corresponded to December 2001, and in France, Germany, Greece to December 2002. Case subjects were selected among men and women who developed colon or rectum cancers after their recruitment into the EPIC study and before the end of the study period (defined for each study centre by the latest end-date of follow-up). For the present study, colon cancers were defined as tumors in the cecum, appendix, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending and sigmoid colon (codes "C18.0" to "C18.7" as per the 10th Revision of the International Statistical Classification of Diseases, Injury and Causes of Death), as well as tumors that were overlapping or unspecified ("C18.8" and "C18.9"). Cancers of the rectum were defined as tumors occurring at the recto-sigmoid junction ("C19") or rectum ("C20"). Anal canal tumors were excluded. Colorectal cancer is defined as a combination of the colon and rectal cancer cases.

Subjects who used any hormone replacement therapy or any exogenous hormones for contraception or medical purposes at blood donation (444 case women) or who had previous diagnosis of cancer (except non-melanoma skin cancer) were excluded from the study. Sweden was not included in the present study because the association between serum levels of IGF-I and IGFBP-3 had already been investigated[30]; Norway was not included because of the short duration of follow-up and small number of incident cases. Twenty-two case sets (cases and matched controls) were excluded because of missing data on either IGF-I or total IGFBP-3 measurements, for either the case or the control subject, leaving a total of 1,121 case sets (4 cases were defined as *in situ*, 333 colon cancer cases were observed in the left colon, 276 in the right colon, 98 defined as "non otherwise specified" and 414 rectal cancers) with IGF-I and total IGFBP-3 measurements in the study. Of these, additional 54 cases and matched controls were excluded from analyses of intact IGFBP-3 because of missing data for intact IGFBP-3 assays.

The distribution of case participants by country was as follows: 126 from Germany, 27 from Greece, 15 from France, 361 from Denmark, 134 from the Netherlands, 197 from the United Kingdom, 119 from Spain and 142 from Italy.

Selection of control subjects

For each case participant with colon or rectum cancer, 1 control participant was selected randomly by incidence density sampling among risk sets consisting of all cohort members alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis of the case patient and, for women, not using exogenous hormones at blood donation. Controls were matched to cases on recruitment center, gender, follow-up time, age at enrollment (±6 months), time of the day at blood collection (±1 hr) and fasting status at blood donation (<3 hr; 3–6 hr, >6 hr). For women, additional matching criteria were menopausal status (premenopausal, postmenopausal, perimenopausal/unknown) and phase of menstrual cycle at blood donation in premenopausal women (5 categories, as described in detail elsewhere[46]).

Blood collection protocol

Blood samples (at least 30 ml) were drawn from participants in the 8 countries contributing to the present study. All blood samples except those from the Oxford recruitment center in the United Kingdom were stored at 5–10°C and protected from light from the time of collection through their transfer to local laboratories, where they were further processed and separated into aliquots. In the Oxford center, blood samples were collected by a network of general practitioners in the United Kingdom and transported to a central laboratory in Norfolk by mail, protected from light and at ambient temperature. The stability of plasma IGF-I and IGFBP-3 measured in blood and kept at ambient temperatures for up to 24 hr has been documented previously.[47] In all recruitment centers contributing to the present study, except the 2 centers in Denmark, 0.5-ml aliquots of the blood fractions (serum, plasma, red cells and buffy coat for DNA extraction) were put in plastic straws, which were heat-sealed and stored under liquid nitrogen (−196°C). In Denmark, 1-ml aliquots of the blood fractions were placed into Nunc tubes and stored in the vapor phase of liquid nitrogen containers (−150°C).

Biochemical assays

All assays of serum IGF-I, total and intact IGFBP-3 were performed at the International Agency for Research on Cancer, Lyon, France, using enzyme-linked immunosorbent assays (ELISA) from Diagnostic Systems Laboratories (DSL, Webster, Texas). All measurements were performed on never thawed serum aliquots. Samples pertaining to matched study participants were always analyzed in the same analytical batch, and the laboratory personnel were blinded to the case-control status of the study participants. The IGF-I assay included an acid–ethanol precipitation step to eliminate IGF-I binding proteins, to avoid their interference with the IGF-I measurement. The mean intra- and interbatch coefficients of variation were 4.5% and 11.8%, respectively, for IGF-I; 2.0% and 7.4% for total IGFBP-3 and 6.4% and 12.9% for intact IGFBP-3. Because of analytical problems with the assays or to a lacking of serum sample, IGF-I could not be measured on 17 subjects, total IGFBP-3 could not be measured on 7 subjects and intact IGFBP-3 could not be measured on 89 subjects.

Statistical analyses

Paired *t*-tests were used to test for case-control differences in means of quantitative variables and paired χ^2 tests (McNemar) were used for categorical variables. Pearson's partial correlation coefficients, adjusted for age, laboratory batch and case-control status, were computed to examine the relationship among serum levels of IGF-I, total and intact IGFBP-3.

Relative risks (RR) for colon and rectal cancer and 95% confidence intervals (CI) were calculated in relation to quintile categories of serum hormone concentrations by conditional logistic regression, using the PHREG procedure of the Statistical Analysis System (SAS Institute, Cary, NC, version 8). Quintile cut-points were based on the distributions in the control participants from all EPIC centers combined.

Potential confounders considered for adjustment, other than the matching criteria, were body mass index (BMI, calculated as weight, in kilograms, divided by the height, in meters, squared), the ratio of waist- to hip-circumferences (WHR), height (both as continuous variables), smoking status (current, former, never), educational attainment (5 categories), physical activity index (5 categories), alcohol intake (ethanol in g/day as a continuous variable), intake of red and processed meat, fish, dairy products, fruit and vegetables and fiber intake (as continuous variable). Adjustments for these factors did not materially change any of the results and therefore were dropped from final statistical models. Thus, only the matching criteria (age, gender, center, follow-up time, time of the day of blood extraction, fasting status, menopausal status in women and phase of menstrual cycle in premenopausal women) remained as implicit adjustments controlled for through the conditional regression models. Further analyses were conducted with mutual adjustments for IGF-I and IGFBP-3 levels. In addition, we cross-classified tertiles of IGF-I and IGFBP-3 levels and evaluated the statistical interaction by fitting a logistic regression model with the 2 individual variables on a continuous scale along with a term created by multiplying the 2 variables.

We examined heterogeneity of relative risk estimates by age at blood collection and by time interval between dates of diagnosis and date of blood sampling, based on χ^2 statistics calculated as the deviations of logistic beta-coefficients observed in each of the subgroups, relative to the overall beta-coefficient. Tests for heterogeneity among countries of recruitment were based on the same approach.

We assessed the potential effect modification of the relationship of IGF-I and colorectal cancer by the following variables: body mass index, waist- to hip-ratio, intake of milk, dairy calcium, calcium from non-dairy foods, fruit and vegetables, red and processed meat and the ratio of total intake of red and processed meat with respect to total intake of poultry and fish, in analyses stratified by tertiles of each of these variables, using unconditional logistic regression adjusted for the matching variables. In the calculation of the ratio of red to white meat and fish, we excluded all nonconsumers of red and processed meat, fish and poultry. These heterogeneity tests were also performed as described earlier.

To quantitatively summarize the epidemiological evidence from case-control studies nested in prospective cohorts on the association between circulating IGF-I and colorectal cancer risk, we searched published studies in English in the PubMed database up to December 2008 and in the reference lists from relevant articles. The keywords used for the search were a combination of the following: "colorectal" OR "colorectum" OR "colon" OR "rectum"; "cancer"; and "IGF*" OR

"insulin-like." We extracted from the publications relative risks estimates and 95% confidence intervals maximally adjusted for confounders, but not adjusted for IGFBP-3, as it seems likely that assays with different specificities for intact or proteolytically cleaved forms of IGFBP-3 may have been used.[34] Studies that presented only IGFBP-3-adjusted IGF-I data were excluded from the analyses.[31] For the study published by Otani *et al.*,[33] crude relative risks estimates and 95% confidence intervals were provided by the journal that published the article (International Journal of Cancer, Heidelberg, Germany), where the data had been submitted by the authors as supplementary materials. We computed a meta-analysis of the published data following a method by Greenland and Longnecker[48] that consists in estimating a logistic β-coefficient corresponding to the change of 1 standard deviation in the (log-) relative risk estimate in each of the studies included in the meta-analysis, obtaining the overall risk by the combination of the different β coefficients, each weighted inversely by its variance. Tests of heterogeneity between the ORs in different studies were based on χ^2 statistics, calculated as the deviations of logistic beta-coefficients observed in each of the studies, relative to the overall beta-coefficient. For comparison, a meta-analysis of the same data was also performed with a different approach, as suggested by Der Simonian *et al.*, computing the weighted average of the logarithm of the relative risk estimates associated to the highest versus the lowest category of IGF-I originally reported in each study, weighted by the inverse of its variance using random-effect models.[49]

All statistical analyses were computed using the Statistical Analysis System (SAS) software package, version 8.

Results

Baseline characteristics of cases and controls

Baseline characteristics for cases and controls are listed in Table 1. Overall, the mean age of the study participants at blood donation was 59 years and cancer diagnosis occurred, on average, 3.6 years after blood donation. Mean IGF-I levels were slightly higher in men than in women, whereas total and intact IGFBP-3 levels were lower in men than in women.

There were few statistically significant differences in baseline characteristics between cases and matched controls (Table 1). In men, mean serum levels of IGF-I and total IGFBP-3 were slightly higher in colorectal cancer cases compared to controls. Colorectal cancer cases had larger means of waist circumference ($p = 0.02$ in men, $p = 0.01$ in women) and waist to hip ratio ($p = 0.05$ in men, $p = 0.02$ in women) compared to the controls. Among men, case subjects had higher mean alcohol consumption ($p = 0.02$) and higher proportion of smokers and ex-smokers ($p = 0.01$) compared to controls.

After adjustment for age at blood donation, laboratory batch and case-control status, serum IGF-I was positively and strongly correlated with total IGFBP-3 in both men ($r = 0.61$ [95% CI: 0.57–0.65]) and women ($r = 0.62$ [95% CI: 0.58–0.66]), and weakly correlated with intact IGFBP-3 ($r = 0.28$ [95% CI: 0.23–0.34] in men, and $r = 0.16$ [95% CI: 0.10–0.22] in women). There was no statistically significant linear correlation between IGF-I and BMI. Correlation coefficients between intact IGFBP-3 and total IGFBP-3 were 0.42 [95% CI: 0.37–0.47] in men and 0.29 [95% CI: 0.24–0.35] in women.

IGF-I, IGFBP-3 and risk of colon and rectal cancers

Prediagnostic levels of serum IGF-I were not significantly associated with the risk of cancer of the colorectum nor of the colon or of the rectum separately (Table 2). Likewise, neither total IGFBP-3 nor intact IGFBP-3 showed any significant relationship with risk of colorectal cancer, or for colon and rectum cancers separately (Table 2). Adjusting for levels of either total or intact IGFBP-3 did not materially change relationships of IGF-I with risk of colon or rectal cancers. There was no significant interaction between IGF-I and either total IGFBP-3 ($p_{\text{interaction}} = 0.40$) or intact IGFBP-3 ($p_{\text{interaction}} = 0.46$). The same lack of association was observed when analyses were stratified by gender. When analyses were conducted separately for right and colon cancer, no statistically significant associations were observed. Only for IGF-I adjusted for total IGFBP-3 was the association with right colon cancer somehow stronger, although not linear, than for overall colon cancer (RR top *vs.* bottom quintile = 1.98 [1.01–3.89]).

For none of the 3 peptides, and neither for colorectal nor for colon and rectal cancers separately, was there any significant heterogeneity of association with cancer risk by years of follow up (less *vs.* more than 2years and less *vs.* more than 5 years; *p*-values for heterogeneity tests [$p_{\text{heterogeneity}}$] ranging from 0.27 to 0.95) or by country where the participants were recruited ($p_{\text{heterogeneity}} = 0.39$ to 0.88).

Effect modification of age at blood donation and anthropometric characteristics in the relationship of IGF-I and IGFBP-3 with colorectal cancer risk

Subgroup analyses suggested a positive association of serum IGF-I with risk specifically of cancer of the colon only among study participants who were less than 55 years at blood collection (RR per quintile increase = 1.18 [95% CI: 1.00–1.39]), whereas no significant association was observed among the older participants (RR per quintile increase = 0.99 [95% CI: 0.91–1.089]) ($p_{\text{heterogeneity}}$ between the 2 age groups = 0.07). By contrast, age at blood donation was not found to modify the relationship of IGF-I with rectal cancer, and the relationships of either total or intact IGFBP-3 with risks of colorectal, colon or rectal cancers also did not show any difference according to age at either blood donation or cancer diagnosis (results not shown).

Although BMI and WHR were significantly positively associated with the risk of colorectal cancer (RR = 1.10 [95% CI: 1.01–1.20] and RR = 1.23 [95% CI: 1. 08–1.40], respectively, per a standard deviation increase on a continuous scale in BMI and WHR), there was no evidence for effect

Table 1. Baseline characteristics of cases with colorectal cancer and matched controls in the European Prospective Investigation into Cancer and Nutrition

Characteristic	Colorectal cancer cases Mean[1] (95% CI)	Controls Mean[1] (95% CI)	p_{diff}[2]
Men	($N = 596$)	($N = 596$)	
Age at blood donation (years)	58.9 (47.7–71.4)	58.9 (48.0–71.4)	0.77
Age at diagnosis (years)	62.0 (51.0–74.0)	–	–
Years between blood collection and diagnosis	3.6 (0.5–7.1)	–	–
IGF-I (ng/ml)	211.0 (121.8–330.9)	207.0 (113.8–334.9)	
IGFBP-3 (ng/ml)	4099.0 (2787.3–5580.2)	4026.6 (2844.5–5464.1)	
IGFBP-3 intact (ng/ml)	1134.6 (708.8–1868.9)	1142.3 (725.1–1924.0)	
BMI (kg/m^2)	27.4 (21.6–34.3)	27.1 (22.4–33.0)	0.17
Waist to hip ratio	0.96 (0.87–1.06)	0.95 (0.85–1.05)	0.05
Waist circumference (cm)	98.1 (83.0–117.0)	96.7 (82.0–113.0)	0.02
Height (cm)	174.2 (162.2–185.4)	173.8 (162.0–184.5)	0.22
Recreational physical activity (Met-hours)	67.0 (12.0–157.3)	64.4 (13.1–141.7)	0.91
Alcohol intake (g/day)	28.2 (0.3–83.7)	24.5 (0.1–73.0)	0.02
Smoking	(%)	(%)	0.01
Never	22%	28%	
With technical or professional school	25.2%	26.7%	0.42
Women	($N = 525$)	($N = 525$)	
Age at blood donation (years)	59.2 (46.1-70.8)	59.2 (45.9-71.1)	0.75
Age at diagnosis (years)	62.4 (50.0–74.0)	–	–
Years between blood collection and diagnosis	3.6 (0.42–7.3)	–	–
IGF-I (ng/ml)	193.2 (104.8–318.3)	194.1 (107.6–334.5)	
IGFBP-3 (ng/ml)	4525.6 (3276.1–6154.1)	4527.9 (3325.4–5907.0)	
IGFBP-3 intact (ng/ml)	1476.8 (964.6–2326.7)	1449.2 (926.3–2372.1)	
BMI (kg/m^2)	26.8 (20.7–35.5)	26.4 (20.8–34.4)	0.09
Waist to hip ratio	0.82 (0.71–0.94)	0.81 (0.70–0.93)	0.02
Waist circumference (cm)	85.3 (68.6–109.0)	83.5 (67.0–104.0)	0.01
Height (cm)	161.3 (150.0–171.5)	160.8 (150.5–172.0)	0.17
Recreational physical activity (Met-hours)	102.6 (25.3–206.6)	102.3 (24.4–196.3)	0.91
Alcohol intake (g/day)	9.0 (0.32.6)	8.3 (0–32.0)	0.37
Post-menopausal	78.7%	79.2%	0.71
Smoking			0.80
Never	58%	57%	
With technical or professional school	24.4%	25.3%	0.57

[1]IGF-I and IGFBP-3 values are geometric means (5–95% percentile range). Other values are mean (5–95% percentile range) or percentage of study population. [2]p values are from paired t test for continuous variables and from χ^2 test for categorical variables.

modification by these anthropometric indices of the estimated relationships of colorectal cancer risk with IGF-I or IGFBP-3. However, for rectal cancer only, a significant positive association of IGF-I levels and risk was observed among participants whose BMI was in the lowest tertile of the distribution (BMI < 25; RR $= 1.06$ [95% CI: 1.01–1.12] for an increase of 10 ng/ml of IGF-I concentrations). The statistical tests for this heterogeneity did not reach statistical significance ($p_{heterogeneity} = 0.08$), indicating a certain likelihood that the observations for this subgroup could have been due to chance.

Effect modification by diet

Of all dietary variables investigated, only milk intake appeared to modify the relationship of IGF-I with colorectal cancer risk ($p_{heterogeneity} = 0.03$; Table 3). Higher levels of IGF-I were positively associated with risk of colorectal cancer (RR for an increase of 100 ng/ml $= 1.43$ [95% CI: 1.10–

Table 2. Relative risk and 95% confidence interval [RR (95% CI)][1] of colorectal, colon and rectal cancers for quintiles of serum levels of IGF-I, total and intact IGFBP3 in the nested case-control study in the European Prospective Investigation into Cancer and Nutrition

	Quintiles				
	1 (referent)	2	3 RR (95%CI)	4	5
IGF-I (ng/ml)	<155.2	155.2–190.9	190.9–221.0	221.0–265.9	>265.9
Colorectal cancer					
Cases/Controls	227/224	211/224	198/224	247/224	238/225
Model 1	1.00	0.94 (0.72–1.22)	0.88 (0.68–1.15)	1.11 (0.85–1.45)	1.07 (0.81–1.40)
Additionally adjusted for total IGFBP-3	1.00	0.90 (0.68–1.18)	0.84 (0.63–1.11)	1.04 (0.77–1.40)	1.01 (0.73–1.40)
Additionally adjusted for intact IGFBP-3	1.00	0.95 (0.72–1.25)	0.91 (0.69–1.21)	1.17 (0.88–1.57)	1.11 (0.83–1.48)
Colon cancer					
Cases/controls	141/144	141/144	119/141	161/138	145/140
Model 1	1.00	1.02 (0.73–1.42)	0.88 (0.63–1.23)	1.24 (0.88–1.76)	1.10 (0.77–1.56)
Additionally adjusted for total IGFBP-3	1.00	0.97 (0.69–1.37)	0.83 (0.58–1.18)	1.15 (0.79–1.68)	1.00 (0.66–1.50)
Additionally adjusted for intact IGFBP-3	1.00	1.00 (0.71–1.42)	0.87 (0.61–1.24)	1.23 (0.85–1.78)	1.07 (0.73–1.55)
Rectal cancer					
Cases/controls	86/80	70/80	79/83	86/86	93/85
Model 1	1.00	0.81 (0.52–1.27)	0.89 (0.57–1.38)	0.93 (0.60–1.44)	1.02 (0.66–1.59)
Additionally adjusted for total IGFBP-3	1.00	0.78 (0.48–1.24)	0.85 (0.52–1.36)	0.89 (0.54–1.47)	1.04 (0.59–1.81)
Additionally adjusted for intact IGFBP-3	1.00	0.85 (0.53–1.36)	0.96 (0.61–1.52)	1.04 (0.65–1.66)	1.13 (0.71–1.79)
Total IGFBP-3 (ng/ml)	<3,613	3,613–4,095	4,095–4,541	4,541–5,078	>5,078
Colorectal cancer					
Cases/controls	193/223	241/224	228/225	235/224	224/225
Model 1	1.00	1.24 (0.96–1.62)	1.18 (0.90–1.56)	1.24 (0.93–1.64)	1.17 (0.87–1.56)
Additionally adjusted for IGF-I	1.00	1.26 (0.96–1.65)	1.19 (0.88–1.60)	1.23 (0.90–1.69)	1.14 (0.80–1.61)
Colon cancer					
Cases/controls	123/136	140/148	144/143	154/139	146/141
Model 1	1.00	1.06 (0.76–1.47)	1.14 (0.81–1.62)	1.28 (0.90–1.84)	1.21 (0.83–1.76)
Additionally adjusted for IGF-I	1.00	1.05 (0.74–1.48)	1.14 (0.79–1.65)	1.27 (0.85–1.88)	1.18 (0.76–1.83)
Rectal cancer					
Cases/controls	70/87	101/76	84/82	81/85	78/84
Model 1	1.00	1.62 (1.06–2.50)	1.27 (0.81–1.99)	1.21 (0.76–1.92)	1.11 (0.69–1.78)
Additionally adjusted for IGF-I	1.00	1.66 (1.06–2.62)	1.28 (0.77–2.12)	1.21 (0.72–2.04)	1.06 (0.59–1.92)
Intact IGFBP-3 (ng/ml)	<986.4	986.4–1168.8	1168.8–1391.4	1391.4–1662.2	>1662.2
Colorectal cancer					
Cases/Controls	224/212	192/213	226/212	190/216	235/214
Model 1	1.00	0.86 (0.65–1.13)	1.00 (0.75–1.33)	0.83 (0.60–1.13)	1.05 (0.76–1.46)
Additionally adjusted for IGF-I	1.00	0.84 (0.64–1.11)	0.97 (0.72–1.30)	0.78 (0.57–1.08)	1.00 (0.71–1.40)
Colon cancer					
Cases/controls	134/119	116/137	136/145	122/132	163/138
Model 1	1.00	0.76 (0.53–1.09)	0.83 (0.57–1.20)	0.83 (0.55–1.25)	1.10 (0.72–1.67)
Additionally adjusted for IGF-I	1.00	0.75 (0.52–1.08)	0.80 (0.55–1.17)	0.78 (0.51–1.19)	1.04 (0.67–1.61)
Rectal cancer					
Cases/controls	90/93	76/76	90/67	68/84	72/76
Model 1	1.00	1.02 (0.66–1.58)	1.37 (0.86–2.19)	0.82 (0.50–1.35)	0.96 (0.56–1.64)
Additionally adjusted for IGF-I	1.00	1.02 (0.65–1.57)	1.34 (0.83–2.18)	0.79 (0.48–1.32)	0.93 (0.53–1.62)

[1]Quintile cut-points based on the distribution in control subjects. RRs (95% CI) from conditional logistic regressions. Matching variables in Model 1 are gender, age, study center, time of blood collection, fasting status and menopausal status in women and day of menstrual cycle [in premenopausal women].

Table 3. Relative risk and 95% confidence interval [RR (95% CI)][1] of colorectal cancer for an increase of 100 ng/ml of serum IGF-I according to tertiles of dietary variables in the nested case-control study in the European Prospective Investigation into Cancer and Nutrition

	Milk		Dairy calcium		Non-dairy calcium		Dairy proteins		Non-Dairy proteins	
Tertiles[2]	Cases/controls	RR (95%CI)	Cases/controls	RR (95% CI)	Cases/controls	RR (95% CI)	Cases/controls	RR (95%CI)	Cases/controls	RR (95%CI)
1	378/370	1.43 (1.10–1.85)	404/359	1.33 (1.02–1.73)	359/359	1.22 (0.95–1.57)	411/360	1.25 (0.96–1.63)	340/360	1.17 (0.89–1.54)
2	399/366	1.00 (0.77–1.30)	346/360	1.05 (0.81–1.36)	331/360	1.15 (0.87–1.53)	342/360	1.11 (0.85–1.44)	381/359	0.91 (0.71–1.18)
3	343/384	0.90 (0.70–1.16)	341/371	0.90 (0.70–1.16)	401/371	0.87 (0.68–1.12)	338/370	0.90 (0.70–1.16)	370/371	1.04 (0.81–1.34)
$p_{heterogeneity}$		0.03		0.12		0.13		0.20		0.43

	Red and processed meat		Red and processed meat: poultry and fish		Fruits and vegetables		Fiber	
Tertiles[2]	Cases/controls	RR (95% CI)	Cases/controls	RR (95% CI)	Cases/controls	RR (95% CI)	Cases/controls	RR (95% CI)
1	321/369	1.26 (0.96–1.64)	282/363	0.99 (0.94–1.31)	388/370	1.26 (0.97–1.63)	422/370	1.13 (0.88–1.45)
2	398/371	0.82 (0.64–1.06)	417/365	1.11 (0.87–1.40)	384/370	1.18 (0.92–1.51)	320/370	1.08 (0.81–1.43)
3	401/380	1.19 (0.93–1.51)	403/374	1.19 (0.92–1.54)	348/380	0.85 (0.66–1.10)	378/380	0.91 (0.71–1.16)
$p_{heterogeneity}$		0.05		0.64		0.08		0.43

[1]RRs (95% CI) from unconditional logistic regressions adjusted by matching variables (gender, age at blood donation, study center, length of follow-up, laboratory batch, time of blood collection, fasting status and in women, menopausal status and day of menstrual cycle [among premenopausal women]). [2]Cut-off points (g/day): milk: <84.7, [84.7–280.0], ≥280.0; dairy calcium: <420, [420.6–697.1[,≥697.1; non-dairy calcium: <328, [328.2–422.4], ≥422.4; dairy proteins: <13.8, [13.8–22.5[, ≥22.5; non-dairy proteins: <56.9, [56.9–76.1], ≥76.1; red and processed meat: <60.4, [60.4–103.7], ≥103.7; ratio of red and processed meat: poultry and fish: <1.3, [1.3–2.7], ≥2.7; fruits and vegetables: <287.7, [287.7–484.7], ≥484.7; fiber: <19.7, [19.7–25.2], ≥25.2.

1.85]) among participants whose milk intake was in the lowest tertile of the distribution. This statistically significant association was observed both for colorectal cancer diagnosed less than 2 years after recruitment (RR for an increase of 100 ng/ml = 1.93 [95% CI: 1.02–3.64]) or after a longer time of follow-up (RR for an increase of 100 ng/ml = 1.44 [95% CI:1.05–1.98]). IGF-I levels were not associated with colorectal cancer in study participants whose milk intakes were in the second and third tertiles of the distribution. Similar results were observed for cancers of the colon ($p_{heterogeneity}$ = 0.08) and rectum ($p_{heterogeneity}$ = 0.11) separately.

To assess whether the apparent effect of milk could be due to its content in calcium, we examined the relationship of serum IGF-I with risk of colorectal cancer in analysis stratified by dietary intake of calcium from dairy and from non-dairy foods. In the study population, the percentages of dairy calcium with respect to total dietary calcium ranged from 49.7% to 67.9%, depending on the country where the participants were recruited. The correlation coefficient between dairy calcium and calcium from non-dairy foods was −0.14 (95% CI: −0.18; −0.10). We observed a pattern of association for dairy calcium similar to that observed for non-dairy calcium, although the formal level of statistical significance was not attained (for colorectal cancer, $p_{heterogeneity}$ for tertiles of dairy calcium was 0.12 and 0.13 for non-dairy calcium; Table 3). Regarding protein intakes from either dairy or non-dairy foods, no significant effect modification emerged (for colorectal cancer; $p_{heterogeneity}$ = 0.20 for dairy protein; $p_{heterogeneity}$ = 0.43 for non-dairy protein).

The p-value of the test for effect modification of red and processed meat on the relationship of IGF-I and colorectal cancer was 0.05. Higher IGF-I levels appeared to be related with risk of rectal cancer in study participants with the highest ratio of red to white meat (RR for an increase of 100 ng/ml = 1.47 [95% CI: 0.93–2.33], $p_{heterogeneity}$ = 0.04).

Results from meta-analyses

A total of 10 prospective studies (comprising the present analysis) on colorectal cancer risk and blood IGF-I levels were included in our meta-analysis (Fig. 1). Table 4 provides an overview of key characteristics of each of these studies. All studies combined, there was a total of 2,862 case subjects and 4,966 control participants. When computing the meta-analysis following the Greenland and Longnecker method, these studies showed only an overall very moderately positive association between IGF-I levels and colorectal cancer risk, with a RR of 1.07 [95% CI: 1.01–1.14] for an increase in IGF-I levels corresponding to 1 standard deviation of the average IGF-I distribution of all studies combined. There was no statistically significant between-study heterogeneity in the relationship of IGF-I with colorectal cancer risk ($p_{heterogeneity}$ = 0.88).The addition of the results from the EPIC cohort, by far the largest study included, did not substantially alter the risk estimate.

The meta-analysis as proposed by DerSimonian *et al.*, comparing highest *vs.* lowest reported categories of blood IGF-I levels (top *vs.* bottom quartiles or quintiles, in most studies), and including also our present results from EPIC, also indicated only a very moderate positive association between IGF-I levels and colorectal cancer risk (RR = 1.13 [95% CI: 0.97–1.32]).

Discussion

Our study in the prospective EPIC cohort overall showed no clear evidence for an increased risk of either colorectal cancer or cancers of the colon and rectum separately, among women or men who had higher circulating IGF-I levels. Furthermore, our study showed no associations of colorectal cancer risk with serum measures of either total or intact IGFBP-3 nor was there any effect of IGFBP-3 adjustments on estimated relationships of IGF-I with colorectal cancer risk. In the subgroup of younger study participants with age at blood donation below 55, our study did suggest a possible, albeit very modest increase in the risk specifically of colon cancer. We also observed a positive association between colorectal cancer and increasing IGF-I concentrations in subjects in the lowest tertile of milk. These marginally significant findings in subgroups, however, could have been chance findings, in view of the larger number of significance tests performed.

The current study has the advantage of having a substantial number of colorectal cancer cases, which allowed separate analyses by anatomical subsite (colon and rectum), age at diagnosis, anthropometric characteristic and differences in diet, with sufficient statistical power to detect significant associations by subgroups. Other important strengths of the study are the standardized collection of questionnaire data about dietary habits, and its prospective design, which greatly reduces the likelihood of "inverse causation" biases that may result when latent disease (or disease diagnosis) affects circulating IGF-I levels or the reporting of habitual diet. A limitation of the current study, as of most other prospective studies, is that only 1 single blood sample has been collected per subject. However, it has already been shown that even a single measurement of IGF-I and IGFBP-3 in serum or plasma can correctly rank subjects for their longer-term exposure to these hormones.[52]

Although a substantial body of epidemiologic evidence supports a possible role for circulating IGF-I in the etiology of several forms of cancer, the strength of the evidence varies by cancer type.[18] Regarding colorectal cancer, several previous prospective studies showed a positive relationship of colorectal cancer risk with IGF-I, measured either as absolute levels or relative to concentrations of IGFBP-3.[26–30] In the majority of these studies, however, this association was of a relatively modest strength, and in several of these studies individually the association did not reach statistical significance. Nonetheless, a meta-analysis in 2004 of the first 5 prospective studies published,[26–30] covering a total of 677 incident cases of colorectal cancer and 1,673 control subjects,

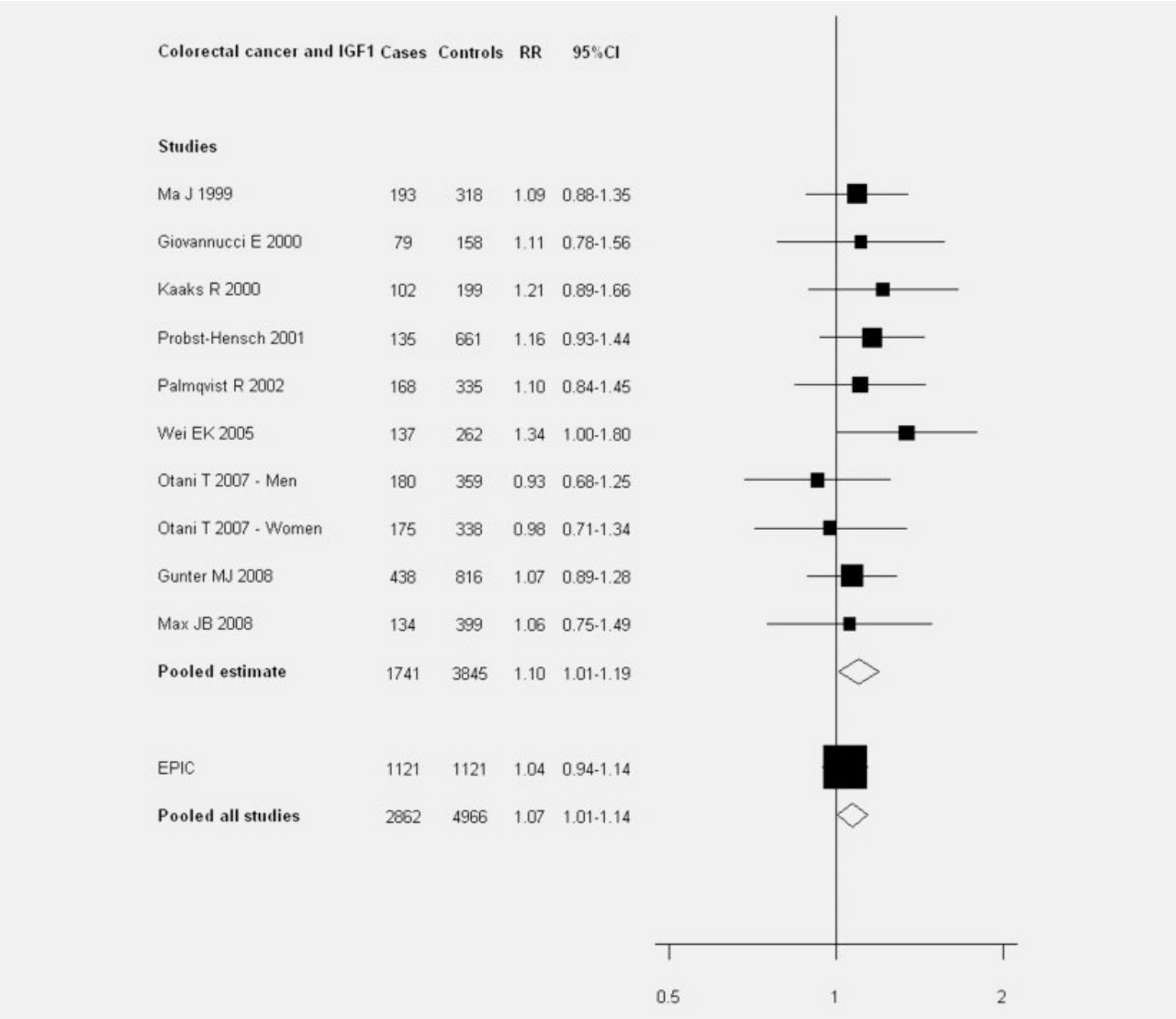

Figure 1. Relative risks and 95% confidence intervals of colorectal cancer for one standard deviation increase in blood IGF-I concentrations, by study.

indicated a relative risk of 1.58 [95% CI: 1.11–2.27] for the highest *vs.* lowest categories of IGF-I levels used in these studies.[18] As this meta-analysis, at least 3 further studies, within the Japan Public Health Center-based Prospective Study,[33] the Women's Health Initiative (WHI)[50] and Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) cohorts[51] which included 375, 438 and 134 incident cases of colorectal cancer, respectively, and at least twice as many control subjects, have been published. Each of these studies showed a lack of association between serum IGF-I concentrations and colorectal cancer risk, with relative risk estimates close to 1.0. With 1,121 incident colorectal cancer cases and 1,121 control subjects our study is by far the largest to date and, in line with these latter and several earlier studies, also showed no clear association of serum total IGF-I levels with colorectal cancer risk. In our meta-analysis, including a total 2,862 case and 4,966 control participants, only a weak association of IGF-I levels with risk of colorectal cancer is observed. For comparison, it is of interest that the recent pooled reanalysis of prospective cohort studies of prostate cancer (3,700 case subjects and 5,200 control participants), which showed an odds ratio of 1.38 [95% CI: 1.19 to 1.60] for the highest *vs.* lowest quintile of IGF-I.[53]

In some previous studies, the association of IGF-I with risk of colorectal cancer,[26,31] and also of colorectal adenomas,[54] was strengthened when IGF-I levels were statistically adjusted for levels of IGFBP-3, or when the molar IGF-I/IGFBP-3 ratio was considered, suggesting that levels of IGF-I relative to IGFBP-3 (which has been proposed to more closely represent bio-available circulating IGF-I) could be a biologically more relevant risk factor. In these same studies, high circulating levels of IGFBP-3 were found to be inversely

Table 4. Characteristics of the studies included in the meta-analysis

Study	Cohort	Sex	No. of cases/ controls	Samples medium	IGF-I assay (Commercial Brand)	IGFBP-3 test	Variables of matching/ adjustment	Design	Categories
Ma *et al.*[26]	Physicians' Health Study	M	193/318	EDTA plasma	ELISA (DSL)	ELISA (DSL)	Age, smoking, BMI, alcohol intake	Matched case/control	Quintiles
Giovannucci *et al.*[27]	Nurses' Health Study	W	79/158	Heparin plasma	ELISA (DSL)	ELISA (DSL)	Year of birth, time period of endoscopy, routine screening, fasting status, month of blood withdraw, BMI, alcohol intake	Matched case/control	Tertiles
Kaaks *et al.*[28]	New York University Women's Health Study	W	102/199	Serum	IRMA (Immunotech)	IRMA (Immunotech)	Age, menopausal status at recruitment, date of recruitment, number of blood donations overtime, time of day of blood donation, day of menstrual cycle for pre-menopausal women, time since last food, smoking	Matched case/control	Quintiles
Probst-Hensch *et al.*[29]	Shanghai Cohort Study	M	135/661	Serum	RIA (Nichols)	IRMA (DSL)	Age, neighbourhood of residence, year and month of blood collection, smoking, alcohol intake, BMI	Matched case/control	Quintiles
Palmqvist *et al.*[30]	Northern Sweden Health and Disease Study	M/W	168/335	Heparin plasma	IRMA (Immunotech)	IRMA (Immunotech)	Sex, subcohort, age, date at blood sampling, fasting time, smoking	Matched case/control	Quartiles
Wei *et al.*[32]	Nurses' Health Study (follow-up)	W	137/262	Heparin plasma	ELISA (DSL)	ELISA (DSL)	Year of birth, fasting status, month of blood drawn, BMI, physical activity, pack-years smoked, alcohol intake, family history, aspirin use, screening, menopausal status, use of hormone replacement therapy	Matched case/control	Quartiles
Otani *et al.*[33]	Japan Public Health Center-based Prospective Study	M/W	355/697	Heparin plasma	IRMA (Mitsubishi)	IRMA (DSL)	Sex, age, date of blood withdrawn, time since last meal, study location, pack-years smoking, alcohol consumption, BMI, physical exercise, family history	Matched case/control	Quartiles
Gunter *et al.*[50]	Women's Health Initiative	W	438/816	Serum	ELISA (DSL)	ELISA (DSL)	Age	Case/sub-cohort	Quartiles
Max *et al.*[51]	Alpha-Tocopherol, Beta-Carotene Study	M	134/399	Serum	ELISA (DSL)	ELISA (DSL)	Age, pack-years smoked, BMI, fiber intake	Case/sub-cohort	Quartiles

M, men; W, women; ELISA, enzyme-linked immunosorbent assay; IRMA, immounoradiometric assay; DSL, Diagnostic Systems Laboratories, Texas, USA; Immunotech, Marseille, France; Nichols, Nichols Institute, California, USA; Mitsubishi, Mitsubishi Kagaku Iatron, Tokyo, Japan.

associated with colorectal cancer risk, independently of adjustments for IGF-I. Interestingly, in all of these studies IGFBP-3 had been measured by the same ELISA assay from Diagnostic Systems Laboratories (DSL, Webster, TX). In other studies, in which IGFBP-3 had been measured by a variety of other assays,[28,30] elevated IGFBP-3 was more often found to be positively associated with the risk of colon cancer. We thus previously speculated that this heterogeneity of study findings could be the result of different specificities of the IGFBP-3 assays used in different studies.[35]

IGFBP-3 undergoes proteolytic cleavage by specific enzymes and in blood it circulates in different, more or less intact molecular forms. Subjects at increased risk of cancer could have elevated total IGFBP-3 concentrations, intact and proteolytically cleaved forms combined, but simultaneously could also have lower blood levels of intact IGFBP-3 due to increased proteolysis.[34] To address this question, we used different assays for IGFBP-3 measurements: a first one that is supposed to measure total IGFBP-3 (*i.e.*, intact and cleaved forms combined), and a second one that is designed by the manufacturer to measure only intact IGFBP-3. The relatively low correlation between the measurements of intact and total IGFBP-3 clearly indicated that the 2 measurements reflected different molecular entities, and also suggests that IGFBP-3 proteolysis could indeed be a factor influencing measured IGFBP-3 concentrations. However, as for total IGFBP-3, measurements of intact IGFBP-3 in our study showed no relationship to colorectal cancer risk, and neither did the adjustment of IGF-I by intact IGFBP-3 result in any clearer association with cancer risk.

In subgroup analyses, we observed a positive association between colorectal cancer and increasing IGF-I concentrations in subjects in the lowest tertile of milk and dietary calcium intakes, confirming similar observations from the Physicians' Health Study.[55] This suggests that endogenous IGF-I could play a role in the etiology of colorectal cancer only when the intake of dietary milk is low (<84.7 g/day). There is substantial epidemiological data suggesting a reduction in colorectal cancer risk among subjects who have high intakes of milk or dairy products.[11,56] This possible preventive effect has been attributed to a variety of possible mechanisms, including increased binding of secondary bile acids to calcium or calcium phosphate in the gut lumen, or to direct intracellular actions of calcium, inhibiting colon cell proliferation.[11] Paradoxically, however, a number of large cross-sectional studies, including studies in EPIC, have shown increases in serum IGF-I levels with increasing intake levels of milk or dairy products, as well as of dietary calcium.[11,38,55] This paradox may be counter parted by the fact that, as indicated by the current results, the risk for colorectal cancer associated with IGF is modest, as is the effect of milk on IGF-I concentrations, so the overall effect of milk on colorectal cancer risk through the IGF pathway may be so small as to be hardly detectable.

In conclusion, findings from our study show no association of colorectal cancer risk with serum levels of IGF-I or IGFBP-3 (measured as total or intact forms). Results from our meta-analysis, including all prospective studies on circulating IGF-I and colorectal cancer risk published to date, showed only a very mild significant positive association. Overall, the findings from prospective cohort studies suggest only a modest role for elevated circulating IGF-I levels in the development of colorectal cancer.

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