Plasma insulin-like growth factor 1, insulin-like growth factor binding protein 3, and risk of colorectal cancer: a prospective study in northern Sweden

R Palmqvist, G Hallmans, S Rinaldi, C Biessy, R Stenling, E Riboli, R Kaaks

Background: Insulin-like growth factor 1 (IGF-1) has antiapoptotic and mitogenic effects on various cell types, and raised IGF-1 levels are increasingly being implicated as potential risk factors for cancer.

Aims: To examine the relationship between IGF-1 and its major plasma binding protein, IGF binding protein 3 (IGFBP-3), and the risk of colorectal cancer.

Methods: We conducted a case-control study nested within the Northern Sweden Health and Disease Cohort. IGF-1 and IGFBP-3 were measured in prediagnostic plasma samples from 168 men and women who developed cancers of the colon (n=110) or rectum (n=58), and from 336 matched controls.

Results: Conditional logistic regression analyses showed an increasing risk of colorectal cancer risk with increasing levels of IGF-1 (odds ratios (ORs) 1.00, 1.89, 2.30, 2.66; p trend=0.03) and IGFBP-3 (ORs 1.00, 0.91, 1.80, 1.93; p trend=0.02). Rectal cancer risk was inversely related to levels of IGF-1 (ORs 1.00, 0.45, 0.33, 0.33; p trend=0.09) and IGFBP-3 (ORs 1.00, 0.75, 0.66, 0.49; p trend=0.21). Mutual adjustments between IGF-1 and IGFBP-3 did not materially alter these relationships.

Conclusions: These results support earlier findings of increased risk of colon cancer in subjects with elevated plasma IGF-1. Our results however do not support the hypothesis that the risk of rectal cancer could also be directly related to IGF-1 levels.

SUBJECTS AND METHODS

Study cohort

Subjects were recruited to the Northern Sweden Health and Disease cohort study through the Västerbotten Intervention Project (VIP), the Northern Sweden part of the WHO study for Monitoring of Trends and Cardiovascular Disease Study (MONICA), and an ongoing Mammary Screening Project (MSP). VIP started as a population based intervention study with the aim of decreasing mortality due to cardiovascular disease by advocating a healthy diet and lifestyle to the general public. In this project, which started in 1986 and which is still recruiting new subjects, all residents in the county of Västerbotten (total population 260 000) are invited to a health survey in the years in which they become 30, 40, 50, or 60 years old. In March 2000, a total of 30 300 men and 31 900 women, aged 30–62 at recruitment, were included. The MONICA study includes 5374 men and 5500 women, recruited in 1986, 1990, 1994, and 1999, as a representative population sample from the counties of Västerbotten and Norrbotten, and who were between 41 and 70 years of age. MSP started in 1995, and in March 2000 included 32 800 women, aged 50–70 years, who were all screened at least once. MSP is currently still recruiting new subjects.

Abbreviations: IGF-1, insulin-like growth factor 1; IGF1-R, IGF-1 receptor; IGFBP-3, IGF binding protein 3; GH, growth hormone; VIP, Västerbotten Intervention Project; MONICA, Monitoring of Trends and Cardiovascular Disease Study; MSP, Mammary Screening Project; BMI, body mass index; OR, odds ratio; IRMA, immunoradiometric assay.
In each of these projects, subjects were asked to complete a self-administered questionnaire to obtain demographic, medical, and lifestyle information. The lifestyle questionnaires included questions on smoking status (current smoker, ex-smoker, non-smoker) and diet. In addition, anthropometric measurements (height, weight) were recorded, and a 20 ml blood sample was obtained. Weight was measured without shoes and in light indoor clothing, and was recorded to the closest 0.2 kg. Height was measured without shoes, and recorded to the closest centimetre. In all three subcohorts, blood was collected in one heparin tube and one EDTA tube, centrifuged, aliquoted as plasma and buffy coat, frozen at -20°C or -80°C, and transferred within one week to a -80°C central storage facility (the Northern Sweden Medical Research Biobank). The procedures for blood donation, processing, and storage as aliquots of plasma and buffy coat were the same in all three subcohorts, except for fasting/non-fasting status. Blood samples from the majority of study subjects, who had been recruited through the VIP and MONICA projects, were obtained in the morning, and fasting time before blood donation was more than eight hours in 55%, 4–8 hours in 32%, and less than four hours in 13%. In MSP however none of the women had been asked to provide blood samples under fasting conditions, and 98% had fasted for less than four hours before blood donation. All participants gave written consent for use of their blood samples in future research projects, and the study was approved by the ethics committee of Umeå University.

All incident cases of colorectal cancer and all deaths were identified through linkage with regional and national cancer registries, and with regional and national registries for all-cause mortality. Linkage was done using a national individual identification number as the identity link, and with a high degree of completeness of case ascertainment. We identified 168 incident cases of colorectal cancer, diagnosed after blood donation, up to March 1999. All cancer cases were verified historically by reviewing medical records. Six cases were excluded because their pathology records did not confirm the cancer diagnosis indicated by the cancer registries. Two further cases were excluded because their plasma samples had been used for other research projects. We thus retained 168 colorectal cancer cases for the study. Of the 168 cancer cases retained for the study, 93 were women and 75 were men; of these, 113 had been recruited in the VIP 41 cases in the MSP, and 14 in the MONICA subcohorts. The subzsite distribution of the 168 colorectal cancers was as follows: 58 in the right colon (caecum, ascending colon and hepatic flexure, transverse colon), 52 in the left colon (splenic flexure, descending colon, and sigmoid colon), and 58 in the rectum (rectosigmoid junction, rectum). Twenty three cases were classified by the pathologist as Dukes' stage A, 58 as Dukes' B, and 55 as Dukes' C, and 32 cases had unspecified Dukes' stage.

Controls were selected randomly from all cohort members alive and free of cancer at the time of diagnosis of a given index case, and by matching the case for sex, subcohort, age (±6 months) and date (±2 months) at blood sampling, and fasting time (<4 hours, 4–8 hours, or >8 hours).

Time intervals between blood donation and cancer diagnosis ranged from less than 1.5 months up to 10 years (median 3.35 years). Of the 168 cases, 146 (87%) were diagnosed more than one year, 118 (70%) more than two years, and 68 (40%) more than four years after blood donation.

**Laboratory analyses**

Stored, never thawed plasma samples (from originally heparinised blood samples) of the cancer cases and their matched controls were identified, packed on dry ice, and shipped to the International Agency for Research on Cancer (Lyon, France) for hormone measurements. IGF-1 and IGFBP-3 were measured by double antibody immunoradiometric assays (IRMA) using reagents from Immunotech (Marseille, France). The protocol for the IGF-1 assay included an acetic ethanol extraction step to release IGF-1 from its binding proteins. The laboratory personnel were blinded as to the case-control status of the plasma samples. Samples pertaining to matched study subjects were always analysed together in the same batch (that is, on the same day and within the same immunoassay kit). To control the quality of each type of peptide measurement, analytical batches systematically included three standard sera. The average intra-batch coefficients of variation calculated from these standard sera were 10.9% and 4.5%, respectively, for IGF-1 and IGFBP-3.

**Statistical analyses**

Pearson's coefficients of correlation adjusted for age, sex, and case-control status were used to examine the cross sectional relationships between IGF-1, IGFBP-3, height, and body mass index (BMI=height/weight²). A pairwise t test was used to test for mean differences between anthropometric indices or hormone levels of the cases, and the average value for the two matched controls. Odds ratios (ORs) for disease were calculated by conditional logistic regression for quartile levels of IGF-1 and IGFBP-3. Quartile cut off points were determined on variable distributions of cases and controls combined. Confidence intervals (95%) were computed using the standard errors of the pertinent regression coefficients, and assuming a normal probability distribution for the estimated coefficients. Likelihood ratio tests for linear trends in risk with increasing peptide concentrations were performed using scores 1, 2, 3, and 4 for the four quartile levels. All statistical tests and corresponding p values were two sided.

Multivariate logistic regression was used to estimate ORs adjusted for possible confounding factors other than those controlled for by matching. Potential confounding factors included smoking status at the time of blood donation, BMI, and height. In addition, associations of risk with levels of IGF-1 were estimated with adjustment for levels of IGFBP-3. All logistic regression analyses were performed using the “PHREG” procedure for proportional hazards regression of the Statistical Analysis System (SAS).10

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**Table 1 Anthropometry indices, smoking status, and mean serum hormone measurements in cases with colorectal cancer and control subjects**

<table>
<thead>
<tr>
<th>Index</th>
<th>Controls</th>
<th>Colorectal cancer</th>
<th>p for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of individuals</td>
<td>336</td>
<td>168</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>169.4 (9.3)</td>
<td>169.7 (8.3)</td>
<td>0.76</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.7 (13.5)</td>
<td>76.0 (13.4)</td>
<td>0.32</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0 (3.8)</td>
<td>26.3 (3.8)</td>
<td>0.50</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>20.5</td>
<td>16.7</td>
<td>0.30</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>200.4 [192.6-208.2]</td>
<td>198.7 [188.1-209.3]</td>
<td>0.78</td>
</tr>
<tr>
<td>IGFBP3 (ng/ml)</td>
<td>2585 [2504-2666]</td>
<td>2593 [2490-2696]</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Values are mean (population standard deviations) or mean [95% confidence intervals (CI)].
RESULTS

Baseline characteristics of cases and controls are shown in table 1. Colon cancers tended to be more advanced (nine Dukes’ A, 44 Dukes’ B, 39 Dukes’ C, 18 unspecified) than cancers of the rectum (14 Dukes’ A, 14 Dukes’ B, 16, Dukes’ C, 14 unspecified), with an almost fourfold higher probability for colon cancers compared with rectal cancers to be Dukes’ stage C instead of stage A (OR 3.8). Mean values for height, weight, BMI, and the two peptides did not differ significantly between cases (colon and rectum combined) and controls, and there was there no significant difference in the percentage of current smokers. Nevertheless, cases had a significantly larger percentage of ex-smokers than controls (OR 1.70; ptrend=0.048). Cases of rectal cancer had a significantly lower mean IGF-1 level (192.3 (174.2–210.4)) than their matched controls (211.6 (199.7–223.5)) while values for colon cancer cases (202 (189.0–215.1)) were slightly higher than their respective controls (194.5 (184.5–204.6)). BMI had a moderate but non-significant direct association with the risk of colon cancer (ORs 1.00, 1.43, 1.32, 1.53; ptrend=0.33) but not of rectal cancer (ORs 1.00, 0.43, 0.61, 0.85; ptrend=0.97).

Combining the data for cases and controls, but adjusting for age, sex, and case-control status, we observed positive correlations between IGF-1 and IGFBP-3 (r=0.41, p<0.001) but no significant associations for IGF-1 or IGFBP-3 with either height (r=0.11, p=0.01) or BMI (r=0.06, p=0.19). There were no significant associations for IGF-1 or IGFBP-3 levels with smoking status.

Logistic regression analyses for colon and rectal cancers combined, and adjusting for smoking status at the time of blood donation, showed no significant associations between risk and levels of IGF-1 or IGFBP-3 (table 2). Nevertheless, when the analyses were performed separately for the colon and rectum, the risk of colon cancer was found to be positively and significantly related to plasma levels of IGF-1 and IGFBP-3 whereas the risk of rectal cancer was found to be inversely related to levels of these two peptides (although this was not significant). When we divided the analyses for tumours with Dukes’ stages A, B, and C (colon and rectum combined), none of the tumour subcategories showed any risk association with IGF-1 levels (data not shown).

The associations of IGF-1 and IGFBP-3 with risk of colon cancer were unaffected by adjustments for BMI or height. Mutual adjustments between IGF-1 and IGFBP-3 also did not alter the associations (table 3). Finally, the associations also remained unaffected when analyses were restricted to cancer cases diagnosed more than one year after blood donation.

DISCUSSION

In this prospective cohort study, we observed an increase in the risk of colon cancer but a decrease in the risk of rectal cancer with rising levels of circulating IGF-1 and IGFBP-3.
One of the strengths of the prospective design was that for the majority of cases insulin, IGF-1, and IGFBP-3 were measured in blood samples collected well before cancer diagnosis. Thus as none of the cases was aware of their disease at the time of blood donation, blood peptide levels could not have been affected by metabolic alterations related to psychological stress or cancer treatment. Furthermore, the prospective design also makes it unlikely that case-control differences in peptide levels were a consequence, rather than a possible cause, of tumour development. The latter is corroborated by the fact that associations between cancer risk and peptide levels remained unaltered when cases with tumours diagnosed at the time of blood collection were excluded from the statistical analyses. Our finding of an increased risk of colon cancer with increasing levels of circulating IGF-1 concurs with observations in one small case control study, and in two previous cohort studies, one in US male physicians and one in US nurses. In each of these studies however the increase in risk was reported for cancers of the colon and rectum combined, and in the two previous cohort studies risk was significantly related to IGF-1 levels only after adjustment for IGFBP-3. The latter findings led to the hypothesis that, more specifically, risk was related to IGF-1 levels only after adjustment for IGFBP-3. All previous studies where IGFBP-3 was related inversely to the risk of cancer after adjustment for IGF-1 (also for cancers of the prostate and breast) used an ELISA method from Diagnostic Systems Laboratories (DSL, Webster, Texas, USA). The other studies used either an IRMA from Immunotech (Marseille, France) or a radioimmunoassay after purification. IGFBP-3 in blood plasma and within tissues undergoes proteolytic cleavage by specific enzymes, including prostate specific antigen and other proteases. It may be that the DSL-ELISA measures more specifically the intact forms of IGFBP-3 whereas the other assays measure the combination of intact and proteolytically cleaved forms. Thus, conceivably, cancer cases could have higher levels of total IGFBP-3 (intact plus proteolytically cleaved forms) which would be reflected by the Immunotech-IRMA method, but also have more proteolytic cleavage of IGFBP-3 (and hence lower levels of intact IGFBP-3) which would be reflected more by the DSL-ELISA method. We recently tested this hypothesis in a study of 102 cases of colorectal cancer and 200 matched controls nested within the New York University Women's Health Study comparing IGFBP-3 measurements obtained by the Immunotech-IRMA and DSL-ELISA. We found that the two types of IGFBP-3 measurements were highly correlated (Spearman's correlations 0.82), and that neither IGFBP-3 assay demonstrated any clear increase in risk with increasing levels of IGF-1 adjusting for IGFBP-3, or any inverse association of risk with IGFBP-3 adjusting for IGF-1. These results thus argue against the hypothesis of different assay specificities, but should be confirmed by further studies.

GH is a principal regulator of plasma and tissue levels of both IGF-1 and IGFBP-3, and increases in pituitary GH secretion during the pubertal growth spurt or in pathological conditions such as acromegaly are generally accompanied by rises in circulating IGF-1 and IGFBP-3. However, the rise in IGF-1 is generally stronger than that in IGFBP-3 and hence the IGF-1/IGFBP-3 ratio is usually also increased under conditions of elevated GH secretion. We therefore speculate that the observations in this and other studies relating elevated circulating IGF-1 levels to an increased risk of colon

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Odds ratios of cancer of the colorectum, colon, or rectum for quartiles of serum IGF-1 and IGFBP-3, mutually adjusted.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile</td>
<td>1</td>
</tr>
<tr>
<td>IGF-1 adjusted for IGFBP-3</td>
<td></td>
</tr>
<tr>
<td>Colorectum</td>
<td>1.00</td>
</tr>
<tr>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Colon</td>
<td>1.00</td>
</tr>
<tr>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Rectum</td>
<td>1.00</td>
</tr>
<tr>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>IGFBP-3 adjusted for IGF-1</td>
<td></td>
</tr>
<tr>
<td>Colorectum</td>
<td>1.00</td>
</tr>
<tr>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Colon</td>
<td>1.00</td>
</tr>
<tr>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Rectum</td>
<td>1.00</td>
</tr>
<tr>
<td>OR</td>
<td>95% CI</td>
</tr>
</tbody>
</table>

Conditional logistic regression analyses were matched for age, sex, date of blood sampling, fasting status, and time of last food consumption and further adjusted for smoking status.
cancer, either as absolute concentrations or as concentrations relative to IGFBP-3, might reflect a relative increase in pituitary GH secretion. The inverse (but non-significant) associations of both plasma IGF-1 and IGFBP-3 with the risk of rectal cancer in the present study is puzzling, and does not fit our hypothesis that elevated IGF-1 levels would generally enhance tumour development. This observation is even more striking in view of the positive association of IGF-1 levels with the risk of colon cancer within the same study. The inverse association of IGF-1 with rectal cancer risk could not be explained by confounding by smoking, height, BMI, or IGFBP-3 levels. The total number of rectal cancer cases was small however and further studies are needed to confirm whether this inverse association reflects a true relationship or is due to chance. It has been suggested that IGF-1 may predict more strongly the risk of advanced disease status at presentation. For example, in a large prospective study of prostate cancer risk in relation to prediagnostic serum IGF-1 levels, the associations of IGF-1 and IGFBP-3 were limited to the presentation of more advanced disease.ём 11 Our data confirmed the general observation that rectal cancers tend to present at an earlier stage than colon cancers, and this may have masked associations with IGF-1 and IGFBP-3. In colon cancers, and this may have masked associations with IGF-1 and IGFBP-3 levels. The total number of rectal cancer cases was small however and further studies are needed to confirm whether this inverse association reflects a true relationship or is due to chance.

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