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# Total and high-molecular weight adiponectin and risk of colorectal cancer: the European Prospective Investigation into Cancer and Nutrition Study

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**Adiponectin—an adipose tissue-derived protein—may provide a molecular link between obesity and colorectal cancer (CRC), but evidence from large prospective studies is limited. In particular, no epidemiological study explored high-molecular weight (HMW) and non-HMW adiponectin fractions in relation to CRC risk, despite them being hypothesized to have differential biological activities, i.e. regulating insulin sensitivity (HMW adiponectin) versus inflammatory response (non-HMW adiponectin). In a prospective, nested case–control study, we investigated whether prediagnostic serum concentrations of total, HMW and non-HMW adiponectin are associated with risk of CRC, independent of obesity and other known CRC risk factors. A total of 1206 incident cases (755 colon and 451 rectal) were matched to 1206 controls using incidence-density sampling. In conditional logistic regression, adjusted for dietary and lifestyle factors, total adiponectin and non-HMW adiponectin concentrations were inversely associated with risk of CRC [relative risk (RR) comparing highest versus lowest quintile = 0.71, 95% confidence interval (CI) = 0.53–0.95, $P_{trend}$ = 0.03 for total adiponectin and RR = 0.45, 95% CI = 0.34–0.61, $P_{trend}$ < 0.0001 for non-HMW adiponectin]. HMW adiponectin concentrations were not associated with CRC risk (RR = 0.91, 95% CI = 0.68–1.22, $P_{trend}$ = 0.55). Non-HMW adiponectin was associated with CRC risk even after adjustment for body mass index and waist circumference (RR = 0.39, 95% CI = 0.26–0.60, $P_{trend}$ < 0.0001), whereas the association with total adiponectin was no longer significant (RR = 0.81, 95% CI = 0.60–1.09, $P_{trend}$ = 0.23). When stratified by cancer site, non-HMW adiponectin was inversely associated with both colon and rectal cancer. These findings suggest an important role of the relative proportion of non-HMW adiponectin in CRC pathogenesis. Future studies are warranted to confirm these results and to elucidate the underlying mechanisms.**

## Introduction

The role of body fatness as a risk factor for colorectal cancer (CRC) has been extensively documented; however, the potential mechanisms behind this relation are largely unknown (1,2). Postulated mechanisms include increased insulin and insulin-like growth factor signaling and chronic inflammation, as well as signaling via adipokines (2,3). Adiponectin is an adipose tissue-derived hormone that—in contrast to most other adipokines—is inversely associated with body weight, insulin resistance and type 2 diabetes (4,5). Adiponectin is involved in the regulation of energy homeostasis, vascular reactivity, inflammation, cell proliferation and tissue remodeling (6,7). Experimental studies suggest that adiponectin may also be inversely associated with cancer, in particular with CRC through direct mechanisms, such as inhibiting

**Abbreviations:** BMI, body mass index; CRC, colorectal cancer; CRP-hs, high-sensitive C-reactive protein; CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; HMW, high-molecular weight; LMW, low-molecular weight; MMW, middle-molecular weight; RR, relative risk; WC, waist circumference.

cancer cell growth (8) and inducing apoptosis (9), as well as indirectly through pathways related to glucose metabolism, insulin resistance and inflammation (10,11).

Despite over the recent years, a number of observational studies explored the association of adiponectin and CRC, the evidence remains inconclusive (12,13). Two recent meta-analyses of 13 studies reported high heterogeneity across the studies mostly explained by the study design and sample size (12,13). The evidence from large-scale prospective studies is particularly scarce with just two studies reporting conflicting results (14,15). Thus, an inverse association with colon cancer was observed in an 8 year follow-up analysis from the Health Professionals Follow-up Study (14), whereas no significant association was found in a 17 year follow-up study from Norway (15). It is important to note that the effects of adiponectin may depend on its complex quaternary structure in plasma. Adiponectin circulates in plasma as a trimer, a hexamer and a high-molecular weight (HMW) form. Experimental evidence suggests that HMW and non-HMW adiponectin fractions may have different biological activities, such that the HMW form may be more closely related to insulin sensitivity, whereas complexes with lower molecular weight (i.e. non-HMW adiponectin) were suggested to have stronger anti-inflammatory actions (16). Although experimental data revealed a potential for differential effect of adiponectin isoforms on CRC risk, no prospective epidemiological study specifically explored the association of both HMW- and/or non-HMW adiponectin fractions with risk of CRC.

We investigated the association between pre-diagnostic concentrations of adiponectin, HMW adiponectin and non-HMW adiponectin with risk of colon and rectal cancer in a nested case–control study within the European Prospective Investigation into Cancer and Nutrition (EPIC). Since obesity is a major determinant of adiponectin concentrations and CRC, we were particularly interested to study the association with and without adjustment for body mass index (BMI) and waist circumference (WC).

## Materials and methods

*Study population*

EPIC is a large prospective study with over 520 000 participants, aged 25–70 years at enrollment during the period from 1992 through 2000 and recruited from 23 centers in 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden and the UK) (17,18). Participants gave written informed consent, underwent anthropometric measurements and completed questionnaires on sociodemographic and lifestyle characteristics, including detailed assessment of diet (18). Approval was obtained from the ethics review board of the International Agency for Research on Cancer, Lyon, France, and the local review boards.

*Cohort follow-up and case ascertainment*

Closure dates for the present study were defined as the latest date of complete follow-up for both cancer incidence and vital status and ranged from December 1999 to June 2003 for centers using registry data and from June 2000 to December 2002 for centers using active follow-up procedures. Colon and rectal cancers were defined according to the 10th Revision of the International Statistical Classification of Diseases, Injuries and Causes of Death (ICD-10).

Incident cancer cases were identified through record linkage with regional cancer registries or using a combination of methods, including health insurance records, cancer and pathology registries and active follow-up through study subjects and their next-of-kin. Excluded from the analysis were the subjects from Norway and the Malmö center in Sweden which did not provide blood samples for the current study as well as subjects with missing information on total adiponectin or HMW adiponectin measurements (54 case–control sets). A total of 1206 incident cases of CRC (755 colon and 451 rectum) were included in the present analyses as follows, according to tumor site (colon/rectum): 27/8 from France, 102/40 from Italy, 75/39 from Spain, 147/64 from the UK, 89/45 from the Netherlands, 13/14 from Greece, 82/52 from Germany, 41/25 from Umea, Sweden and 179/164 from Denmark. An incidence-density sampling protocol was used such that for each case, one control subject was chosen at random among appropriate risk sets consisting of all cohort members alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis of the index case. Matching characteristics were the study center, sex, age, time of the day and fasting status at blood collection and among women menopausal status. Premenopausal women were further matched on phase of menstrual cycle at blood collection and postmenopausal women were matched on hormonal replacement therapy.

*Laboratory procedures*

The blood collection and processing protocols are described in detail elsewhere (18). Total and HMW adiponectin serum concentrations were measured by using enzyme-linked immunosorbent assay (Multimeric ELISA) on the principle of a 'sandwich' format (ALPCO Diagnostics, Salem, NH) with a minimum detectable limit of 0.04 ng/ml. To quantify HMW adiponectin, serum samples were pre-treated with a protease that specifically digests low-molecular weight (LMW) and middle-molecular weight (MMW) adiponectin. Based on a subset of quality control samples ($n = 66$), the coefficient of variations for total adiponectin and HMW adiponectin were 8.3 and 9.4%, respectively. The combined fraction of MMW and LMW adiponectin ('non-HMW adiponectin') was calculated by subtracting the individually measured values of HMW adiponectin from the values of total adiponectin. High-sensitive C-reactive protein (CRP-hs) was measured using a high-sensitivity assay as described previously (19).

*Statistical methods*

Participants were divided into quintiles based on the distribution of total, HMW and non-HMW adiponectin concentrations among controls. Conditional logistic regression was used to examine the association of adiponectin with risk of CRC. With risk-set sampling, the odds ratio directly estimates the hazard ratio and thus, we present the results in terms of estimated relative risks (RRs) (20). In multivariable model, we adjusted for possible confounders other than those controlled for by matching, including smoking status, education, physical activity, alcohol consumption and dietary factors. To explore if adiponectin may be associated with CRC through biological pathways that might be independent of adiposity, in an additional model, we also adjusted for BMI and WC individually and in combination. For WC, there were 132 missing values (66 case–control sets), which for the present analysis were substituted with sex-specific median values. To test for linear trend, we used the median concentrations of total adiponectin, HMW adiponectin and non-HMW adiponectin in the controls as a continuous variable. We also estimated the multivariable-adjusted RRs associated with an increase of log-transformed biomarker concentrations by log 2, which corresponds to a doubling of the concentrations on the original scale.

In additional analyses, we also estimated the associations in different population strata and tested for effect modification with sex and age, as well as with various factors that may be relevant for CRC risk and may be also related to adiponectin concentrations (including categories of BMI, WC, alcohol consumption, smoking and physical activity) using interaction terms (log-transformed biomarker concentrations multiplied by the stratum variable). For the matching factors, age at recruitment and sex, we used unconditional logistic regression and included the matching factors except the stratified variables in the multivariable models. Since previously we have observed an increased risk for CRC with increasing CRP levels (19), in a subset of participants with available CRP-hs measurements (1056 case–control sets), we examined whether the associations differ by CRP-hs categories (cut-off point = 3 mg/l).

In the sensitivity analyses, we repeated the main multivariable-adjusted models after excluding cases that occurred in the first 2 years of the follow-up ($n = 301$); participants with extreme values of biomarker concentrations defined as below or above the lower and upper biomarker decile ($n = 242$) and participants with self-reported diabetes or glycated hemoglobin $\geq$6.5% at baseline ($n = 172$).

Two-sided *P*-values of less than 0.05 were considered to indicate statistical significance. All statistical analyses were carried out by using Statistical Analysis System (SAS), Version 9.2, software (SAS Institute, Inc., Cary, NC).

## Results

The mean follow-up time for cases was 3.9 years. Colon cancer cases had higher BMI, WC and CRP-hs concentrations compared with their controls, whereas rectal cancer cases and controls did not differ in these characteristics. Both colon and rectal cancer cases had lower physical activity level and consumed more alcohol and red/processed meat and less fish, fruits and vegetables compared with their control counterparts. The median pre-diagnostic concentrations of total adiponectin and non-HMW adiponectin were lower in cases of both colon and rectal cancer compared with their controls, whereas no statistically significant differences were observed for HMW adiponectin (Table I ).

Among controls, total adiponectin was strongly correlated with HMW adiponectin and less strongly with non-HMW adiponectin (age and sex-adjusted partial Spearman correlation coefficient, $r = 0.96$ and $r = 0.85$, respectively; Supplementary Table I, available at

**Table I.** Baseline characteristics of incident colon and rectal cancer cases and matched controls in the EPIC Cohort

| Characteristics | Colon cancer | | | Rectal cancer | | |
|---|---|---|---|---|---|---|
| | Cases | Controls | $P_{paired}$[a] | Cases | Controls | $P_{paired}$[a] |
| Number | 755 | 755 | | 451 | 451 | |
| Female sex, % | 54.2 | 54.2 | | 45.2 | 45.2 | |
| Age, years, mean (SD) | 58.6 (7.3) | 58.6 (7.3) | 0.64 | 58.0 (6.9) | 58.0 (6.9) | 0.41 |
| Smoking status, % | | | | | | |
| Never-smoker | 42.5 | 45.4 | | 38.4 | 38.6 | |
| Former smoker | 33.5 | 32.9 | 0.82 | 33.0 | 31.5 | 0.40 |
| Current smoker | 23.2 | 20.9 | | 27.9 | 28.8 | |
| Education, % | | | | | | |
| No school degree or primary school | 40.4 | 42.9 | | 36.1 | 41.5 | |
| Technical or professional school | 22.9 | 23.2 | 0.80 | 27.5 | 27.3 | 0.41 |
| Secondary school | 17.0 | 13.5 | | 13.8 | 11.1 | |
| University degree | 16.6 | 17.6 | | 19.5 | 18.4 | |
| Physical activity, % | | | | | | |
| Inactive | 14.4 | 11.4 | | 15.1 | 13.1 | |
| Moderately inactive | 28.7 | 27.8 | 0.25 | 26.4 | 24.2 | 0.04 |
| Moderately active | 41.6 | 43.7 | | 41.7 | 41.0 | |
| Active | 9.3 | 10.9 | | 10.9 | 14.4 | |
| Menopausal status among women, % | | | | | | |
| Premenopausal | 10.8 | 11.0 | | 10.3 | 10.3 | |
| Postmenopausal | 72.4 | 71.4 | 0.75 | 69.6 | 71.1 | 0.81 |
| Everuse of exogeneous hormones, % | 11.6 | 10.3 | 0.59 | 9.5 | 8.6 | 0.32 |
| BMI[b], kg/m$^2$, mean (SD) | 26.9 (4.5) | 26.3 (3.9) | 0.004 | 26.5 (4.1) | 26.4 (3.9) | 0.66 |
| WC, cm, mean (SD) | 90.4 (13.0) | 88.1 (11.8) | <0.0001 | 90.4 (12.8) | 89.9 (12.8) | 0.48 |
| Waist-hip-ratio, mean (SD) | 0.88 (0.10) | 0.87 (0.10) | 0.0003 | 0.89 (0.10) | 0.88 (0.10) | 0.33 |
| Alcohol consumption, g/d, median (IQR) | 8.0 (2.3–18.0) | 7.7 (2.8–17.4) | 0.33 | 11.5 (3.4–26.1) | 10.6 (3.5–21.5) | 0.005 |
| Fiber intake, g/d, median (IQR) | 22.0 (17.0–27.6) | 22.3 (18.1–27.4) | 0.11 | 21.7 (17.4–27.3) | 22.4 (17.7–27.3) | 0.66 |
| Fruit and vegetable intake, g/d, median (IQR) | 370.1 (237.4–538.9) | 391.7 (251.7–555.1) | 0.18 | 352.4 (235.5–507.5) | 361.7 (239.1–522.3) | 0.52 |
| Fish and shellfish intake, g/d, median (IQR) | 25.9 (13.6–44.7) | 28.5 (13.8–49.3) | 0.04 | 26.8 (14.9–47.1) | 28.7 (14.0–50.1) | 0.29 |
| Red meat intake, g/d, median (IQR) | 47.7 (24.8–75.8) | 46.2 (24.2–75.0) | 0.77 | 54.1 (32.7–83.1) | 50.8 (29.9–78.7) | 0.05 |
| Processed meat intake, g/d, median (IQR) | 25.4/13.3–41.3) | 24.0 (12.6–42.3) | 0.45 | 27.5 (14.1–46.0) | 26.6 (13.2–46.4) | 0.35 |
| Total adiponectin, μg/ml, median (IQR) | 6.71 (4.78–9.25) | 6.84 (5.08–9.37) | 0.05 | 6.38 (4.58–8.85) | 6.79 (4.98–9.26) | 0.03 |
| HMW adiponectin, μg/ml, median (IQR) | 3.49 (2.16–5.17) | 3.46 (2.19–5.21) | 0.73 | 3.32 (2.07–4.96) | 3.48 (2.12–5.14) | 0.41 |
| Non-HMW adiponectin, μg/ml, median (IQR) | 3.22 (2.47–4.01) | 3.36 (2.69–4.26) | <0.0001 | 3.03 (2.34–3.97) | 3.38 (2.64–4.13) | <0.0001 |
| CRP-hs, mg/l, median (IQR) | 3.1 (1.2–5.6) | 2.3 (0.1–4.6) | 0.0009 | 2.4 (1.0–4.4) | 2.3 (1.00–4.2) | 0.61 |

Sex, age, menopausal status and hormonal replacement therapy use were among the matching criteria. Mean follow-up time was 3.9 years. SD, standard deviation; IQR, inter-quartile range.

[a]*P*-values for the difference between cases and controls were determined by Student's paired *t*-test for variables expressed as means, by Wilcoxon's signed rank test for variables expressed as medians, by Mc Nemar's test and Bowker's test of symmetry for variables expressed as percentages.

[b]Weight (kilograms)/height (square meter).

*Carcinogenesis* Online). After adjustment for age at study recruitment and sex, total adiponectin, HMW adiponectin and non-HMW adiponectin were similarly inversely correlated with BMI, WC and CRP-hs.

In multivariable-adjusted analysis, including dietary and lifestyle factors, total adiponectin concentrations were inversely associated with risk of CRC [RR of the highest versus the lowest quintile = 0.71, 95% confidence interval (CI) = 0.53–0.95, $P_{trend}$ = 0.03; Table II ). HMW adiponectin concentrations were not statistically significantly associated with risk of CRC (RR = 0.91, 95% CI = 0.68–1.22, $P_{trend}$ = 0.55). In contrast, non-HMW adiponectin concentrations were strongly and statistically significantly inversely associated with CRC risk (RR = 0.45, CI = 0.34–0.61, $P_{trend} < 0.0001$).

After additional adjustment for BMI and WC, the association between total adiponectin and CRC was attenuated, so that the trend across quintiles was no longer statistically significant (RR of the highest versus the lowest quintile = 0.81, 95% CI = 0.60–1.09, $P_{trend}$ = 0.23), whereas the association between non-HMW adiponectin and CRC remained unaltered (RR = 0.39, 95% CI = 0.26–0.60, $P_{trend} <$ 0.0001; Table II). Because of the potential for colinearity between BMI and WC, we also evaluated the effect of adding these factors separately in multivariable models and have not seen substantial differences in the effects. Thus, after adjustment for BMI, the RR for total adiponectin was 0.77 (highest versus the lowest quintile, 95% CI = 0.57–1.03; $P_{trend}$ = 0.11) and after adjustment for WC, the risk was 0.82 (95% CI = 0.60–1.10; $P_{trend}$ = 0.25).

When analysed separately by cancer site, total adiponectin was more strongly associated with rectal cancer (RR in multivariable-adjusted model, including dietary and lifestyle factors, of the highest versus the lowest quintile = 0.61, 95% CI = 0.37–1.00, $P_{trend}$ = 0.05) compared with colon cancer (RR = 0.78, 95% CI = 0.54–1.11, $P_{trend}$ = 0.21; Table III ); however, the differences by cancer site were not statistically significant ($P_{difference}$ = 0.74).

### *Stratified and sensitivity analysis*

When stratified by sex, the associations between total adiponectin and non-HMW adiponectin and CRC were stronger in men compared with women; however, these differences were not statistically significant (Table IV ). The multivariable-adjusted RR (including dietary and lifestyle factors) associated with a one unit increase of log-transformed total adiponectin concentrations in men was 0.72, 95% CI = 0.53–0.98, whereas in women, it was 0.79, 95% CI = 0.59–1.05, $P_{difference}$ = 0.62. In stratified analysis, the association between total adiponectin and CRC was statistically significant in people with higher BMI, WC and alcohol consumption and with lower physical activity level, as well as in smokers, but not in the low risk groups; however, there was no statistically significant interaction by any of these factors (Table IV). In a subset of the study population with available CRP-hs measurements (1056 case sets), total adiponectin and HMW adiponectin concentrations were statistically significantly associated with CRC in

**Table II.** RRs (95% CIs) of CRC by quintiles of baseline total adiponectin, HMW adiponectin and non-HMW adiponectin concentrations in the EPIC Cohort

| | Quintiles | | | | | | Continuously per doubling[b] | $P_{value}$[b] |
|---|---|---|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | Q5 | $P_{trend}$[a] | | |
| Total adiponectin | | | | | | | | |
| Median total adiponectin (μg/ml) | 3.7 | 5.3 | 6.8 | 8.8 | 12.2 | | | |
| Number, cases/controls | 297/241 | 227/240 | 239/244 | 223/240 | 220/241 | | | |
| Crude RR[c] (95% CI) | 1.00 (referent) | 0.76 (0.59–0.98) | 0.76 (0.59–0.98) | 0.71 (0.54–0.93) | 0.68 (0.52–0.91) | 0.02 | 0.81 (0.70–0.93) | 0.003 |
| Multivariable RR[d] (95% CI) | 1.00 (referent) | 0.81 (0.63–1.06) | 0.82 (0.63–1.06) | 0.75 (0.57–0.99) | 0.71 (0.53–0.95) | 0.03 | 0.82 (0.71–0.95) | 0.006 |
| Multivariable RR[d] (95% CI) + BMI and WC | 1.00 (referent) | 0.84 (0.65–1.08) | 0.86 (0.66–1.12) | 0.81 (0.61–1.08) | 0.81 (0.60–1.09) | 0.23 | 0.87 (0.75–1.01) | 0.07 |
| HMW adiponectin | | | | | | | | |
| Median HMW adiponectin (μg/ml) | 1.4 | 2.4 | 3.5 | 4.8 | 7.1 | | | |
| Number, cases/controls | 253/241 | 226/241 | 257/241 | 242/242 | 228/241 | | | |
| Crude RR[c] (95% CI) | 1.00 (referent) | 0.89 (0.69–1.15) | 1.01 (0.78–1.31) | 0.94 (0.72–1.23) | 0.89 (0.67–1.18) | 0.50 | 0.94 (0.86–1.04) | 0.24 |
| Multivariable RR[d] (95% CI) | 1.00 (referent) | 0.92 (0.71–1.19) | 1.07 (0.82–1.40) | 0.99 (0.75–1.30) | 0.91 (0.68–1.22) | 0.55 | 0.95 (0.86–1.05) | 0.31 |
| Multivariable RR[d] (95% CI) + BMI and WC | 1.00 (referent) | 0.94 (0.72–1.22) | 1.14 (0.87–1.50) | 1.08 (0.82–1.43) | 1.05 (0.77–1.43) | 0.11 | 1.00 (0.90–1.11) | 0.95 |
| Non-HMW adiponectin | | | | | | | | |
| Median non-HMW adiponectin (μg/ml) | 2.3 | 2.8 | 3.3 | 4.0 | 5.1 | | | |
| Number, cases/controls | 354/242 | 221/240 | 212/242 | 234/241 | 185/241 | | | |
| Crude RR[c] (95% CI) | 1.00 (referent) | 0.61 (0.48–0.78) | 0.57 (0.44–0.73) | 0.60 (0.47–0.78) | 0.45 (0.33–0.60) | <0.0001 | 0.59 (0.50–0.71) | <0.0001 |
| Multivariable RR[d] (95% CI) | 1.00 (referent) | 0.60 (0.47–0.78) | 0.59 (0.46–0.77) | 0.63 (0.48–0.82) | 0.45 (0.34–0.61) | <0.0001 | 0.61 (0.51–0.73) | <0.0001 |
| Multivariable RR[d] (95% CI) + BMI and WC | 1.00 (referent) | 0.59 (0.45–0.77) | 0.57 (0.43–0.75) | 0.58 (0.42–0.80) | 0.39 (0.26–0.60) | <0.0001 | 0.64 (0.53–0.77) | <0.0001 |

[a]*P*-value for trend (two-sided) across quintiles is based on the median biomarker concentrations within quintiles as a continuous variable using the Wald chi-square statistic.
[b]Estimated multivariable-adjusted RR associated with an increase in continuous log-transformed biomarker concentrations by log 2 and *P*-value for continuous log-transformed biomarker concentrations by log 2.
[c]Crude model controlled for matching factors: age, sex, study centre, follow-up time since blood collection, time of the day at blood collection and fasting status; women were further matched by menopausal status, phase of menstrual cycle at blood collection and postmenopausal women were matched by hormonal replacement therapy use.
[d]Multivariable models were based on the crude model with additional adjustment for smoking status (never, past, current or unknown), education (no school degree or primary school, technical or professional school, secondary school, university degree or unknown), alcohol intake (continuous), physical activity (inactive, moderately inactive, moderately active, active or missing), fiber intake (continuous) and consumption of red and processed meat (continuous), fish and shellfish (continuous) and fruits and vegetables (continuous).

individuals with elevated CRP-hs concentrations (≥3 mg/l), whereas no statistically significant association was seen in the group with CRP <3 mg/l ($P_{difference}$ = 0.02 for total adiponectin; $P_{difference}$ = 0.02 for HMW adiponectin).

When in a sensitivity analysis we excluded individuals diagnosed with cancer within the first 2 years of follow-up, people with diabetes at baseline or those with extreme adiponectin concentrations, the associations remained unaltered. For example, the multivariable-adjusted RR of CRC associated with a unit increase of log-transformed total adiponectin concentrations were 0.78, 95% CI = 0.62–0.98, when CRC cases diagnosed in first 2 years of follow-up were excluded; 0.74, 95% CI = 0.60–0.91, when the people with diabetes were excluded and 0.75, 95% CI = 0.61–0.92, when people with extreme total adiponectin concentrations were excluded.

## Discussion

In this large prospective study, circulating pre-diagnostic concentrations of total adiponectin and non-HMW adiponectin were inversely associated with risk of CRC, independent of dietary and lifestyle factors. In contrast, HMW adiponectin concentrations were not statistically significantly related with CRC risk. Adjustment for BMI and WC attenuated the association with total adiponectin but did not alter the association with non-HMW adiponectin. These data suggest that adiponectin is inversely associated with risk of CRC and that this association is largely accounted for by non-HMW adiponectin.

Our findings of an inverse association between total adiponectin and CRC are generally in line with the results from the Health Professionals Follow-up Study (14), including 179 CRC cases; although in that study, the association was independent of BMI, whereas in our study, the association for total adiponectin was attenuated and no longer significant after adjustment for BMI and WC. However, we relied on measured anthropometric data, whereas in the Health Professionals Cohort, the information on BMI was based on self-reports and residual confounding may not be ruled out in that study. A Norwegian prospective study among 381 CRC cases did not find a statistically significant association between total adiponectin and CRC (15). Importantly, neither the Health Professionals Follow-up Study nor the Norwegian study measured HMW adiponectin concentrations. The results could be affected by the type of blood samples, serum versus plasma, used for the analytical measurement of adiponectin concentrations. Among prior studies on the association between adiponectin and CRC, five studies used plasma samples and six studies used serum samples. Of those studies using plasma, three reported non-significant associations; similarly among studies that used serum also three reported non-significant associations. Therefore, the differences in the type of blood used for the adiponectin analysis are unlikely to account for the differences in the results.

Because HMW adiponectin was suggested to be more closely related to insulin sensitivity than total adiponectin (22,23) and more strongly related to lower risk for incident diabetes (24), our initial hypothesis was that HMW adiponectin may be more closely related to CRC risk than total adiponectin. Surprisingly, in our analysis, HMW adiponectin concentrations were not related to CRC risk. On the contrary, we found that the remaining non-HMW adiponectin form was strongly inversely related to risk of both colon and rectal cancer.

**Table III.** RRs (95% CIs) of colon and rectal cancer according to quintiles of baseline total adiponectin, HMW adiponectin and non-HMW adiponectin concentrations in the EPIC Cohort

| | Quintiles | | | | | $P_{trend}$[a] | Continuously per doubling[b] | $P_{value}$[b] |
|---|---|---|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | Q5 | | | |
| Colon cancer | | | | | | | | |
| Total adiponectin | | | | | | | | |
| Number, cases/controls | 177/149 | 136/146 | 160/156 | 137/152 | 145/152 | | | |
| Multivariable RR[c] (95% CI) | 1.00 (referent) | 0.81 (0.58–1.12) | 0.87 (0.63–1.21) | 0.77 (0.54–1.10) | 0.78 (0.54–1.11) | 0.21 | 0.84 (0.71–1.00) | 0.06 |
| Multivariable RR[c] (95% CI) + BMI and WC | 1.00 (referent) | 0.88 (0.63–1.23) | 0.94 (0.67–1.32) | 0.89 (0.62–1.28) | 0.98 (0.67–1.43) | 0.98 | 0.94 (0.28–1.14) | 0.54 |
| HMW adiponectin | | | | | | | | |
| Number, cases/controls | 152/151 | 139/149 | 159/152 | 153/152 | 152/151 | | | |
| Multivariable RR[c] (95% CI) | 1.00 (referent) | 0.97 (0.69–1.36) | 1.14 (0.80–1.62) | 1.10 (0.78–1.57) | 1.06 (0.73–1.54) | 0.71 | 0.96 (0.85–1.099 | 0.54 |
| Multivariable RR[c] (95% CI) + BMI and WC | 1.00 (referent) | 1.02 (0.72–1.44) | 1.25 (0.82–1.28) | 1.27 (0.88–1.83) | 1.35 (0.91–1.99) | 0.11 | 1.04 (0.91–1.19) | 0.56 |
| Non-HMW adiponectin | | | | | | | | |
| Number, cases/controls | 209/150 | 135/150 | 141/151 | 152/145 | 118/159 | | | |
| Multivariable RR[c] (95% CI) | 1.00 (referent) | 0.64 (0.46–0.88) | 0.66 (0.47–0.91) | 0.75 (0.53–1.05) | 0.47 (0.32–0.67) | 0.0006 | 0.64 (0.52–0.80) | <0.0001 |
| Multivariable RR[c] (95% CI) + BMI and WC | 1.00 (referent) | 0.68 (0.49–0.94) | 0.69 (0.50–0.97) | 0.81 (0.58–1.14) | 0.56 (0.39–0.82) | 0.02 | 0.71 (0.57–0.88) | 0.002 |
| Rectal cancer | | | | | | | | |
| Total adiponectin | | | | | | | | |
| Number, cases/controls | 120/92 | 91/94 | 79/88 | 86/88 | 75/89 | | | |
| Multivariable RR[c] (95% CI) | 1.00 (referent) | 0.83 (0.55–1.26) | 0.76 (0.50–1.16) | 0.74 (0.47–1.17) | 0.61 (0.37–1.00) | 0.05 | 0.78 (0.61–0.99) | 0.04 |
| Multivariable RR[c] (95% CI) + BMI and WC | 1.00 (referent) | 0.83 (0.54–1.25) | 0.74 (0.48–1.15) | 0.72 (0.45–1.16) | 0.59 (0.35–0.99) | 0.05 | 0.77 (0.60–0.99) | 0.04 |
| HMW adiponectin | | | | | | | | |
| Number, cases/controls | 101/90 | 87/92 | 98/89 | 89/90 | 76/90 | | | |
| Multivariable RR[c] (95% CI) | 1.00 (referent) | 0.85 (0.56–1.31) | 1.00 (0.65–1.53) | 0.83 (0.52–1.30) | 0.69 (0.42–1.13) | 0.14 | 0.93 (0.79–1.09) | 0.36 |
| Multivariable RR[c] (95% CI) + BMI and WC | 1.00 (referent) | 0.85 (0.55–1.30) | 0.99 (0.64–1.53) | 0.82 (0.52–1.31) | 0.68 (0.41–1.15) | 0.15 | 0.93 (0.78–1.10) | 0.39 |
| Non-HMW-adiponectin | | | | | | | | |
| Number, cases/controls | 145/92 | 86/90 | 71/91 | 82/96 | 67/82 | | | |
| Multivariable RR[c] (95% CI) | 1.00 (referent) | 0.58 (0.38–0.88) | 0.53 (0.35–0.81) | 0.49 (0.31–0.77) | 0.43 (0.25–0.73) | 0.001 | 0.56 (0.41–0.77) | <0.0001 |
| Multivariable RR[c] (95% CI) + BMI and WC | 1.00 (referent) | 0.58 (0.38–0.89) | 0.52 (0.34–0.80) | 0.48 (0.31–0.76) | 0.42 (0.25–0.72) | 0.001 | 0.55 (0.40–0.76) | 0.0003 |

[a]*P*-value for trend (two-sided) across quintiles is based on the median biomarker concentrations within quintiles as a continuous variable using the Wald chi-square statistic.
[b]Estimated multivariable-adjusted RR associated with an increase in continuous log-transformed biomarker concentrations by log 2 and *P*-value for continuous log-transformed biomarker concentrations by log 2.
[c]Multivariable-adjusted models were based on the crude model with additional adjustment for smoking status (never, past, current or unknown), education (no school degree or primary school, technical or professional school, secondary school, university degree or unknown), alcohol intake (continuous), physical activity (inactive, moderately inactive, moderately active, active or missing), fiber intake (continuous) and consumption of red and processed meat (continuous), fish and shellfish (continuous) and fruits and vegetables (continuous).

It is interesting that similar paradoxical findings have been also observed with regards to cardiovascular disease endpoints. For example, despite HMW adiponectin was associated with insulin resistance and other cardiovascular risk factors, no association was seen for incident coronary heart disease and ischemic stroke (25–27). Although the effects of the various multimers of adiponectin on cancer risk are largely uninvestigated, a recent clinically based study reported that higher levels of LMW adiponectin were associated with lower risk the risk of Barrett's oesophagus, a precursor of oesophageal adenocarcinoma, whereas total and HMW adiponectin were not associated with risk (28).

There might be several potential explanations of our findings. Firstly, other effects of adiponectin beyond its beneficial metabolic actions may be more important for CRC risk. Experimental data show that only LMW adiponectin suppresses inflammatory cytokine secretion, whereas HMW adiponectin does not alter the secretion of these cytokines (16,29). Since systemic inflammation is involved in colon cancer pathogenesis (19), the anti-inflammatory effects of non-HMW adiponectin isoforms may specifically underline the observed associations.

Secondly, it was shown that the profile of adiponectin multimers secreted by adipose tissue differs from their plasma profile, such that HMW is the predominant form secreted by subcutaneous and visceral fat and the less abundant form circulating in plasma (30). A possible explanation of this phenomenon is that the transportation of the HMW adiponectin from fat tissue across the endothelial barrier into the blood circulation is limited due to its large molecular size (30). Thus, circulating concentrations of HMW adiponectin in plasma may not sufficiently represent the metabolic activities that this form exerts in adipose tissue.

Thirdly, recent evidence suggests that HMW adiponectin as the major active form maybe preferentially utilized by the liver (31). This may result in limiting the metabolic effects of HMW adiponectin in other organs and tissues such as the colon.

Finally, although inflammation and insulin resistance induced by obesity are suggested to be the main molecular mediators of the association between obesity and colon cancer, adiponectin may have a direct effect on CRC development. This hypothesis is supported by the recent advancements in genetic epidemiology showing that adiponectin directly inhibits colon cancer cell proliferation via AdipoR1- and AdipoR2-mediated adenosine monophosphate-activated protein kinase activation (8). To date, two studies have addressed the question of an association between genetic variation in adipokine genes and the risk of CRC and both have reported positive associations (32,33). However, the effects of specific adiponectin isoforms on colon cancer cell growth are largely unclear, and the mechanism by which

**Table IV.** Multivariable-adjusted[a] RRs (95% CIs) of CRC associated with an increase of continuous log-transformed total adiponectin, HMW adiponectin and non-HMW adiponectin concentrations, by subgroups, in the EPIC Cohort

| | Number, cases/controls | Total adiponectin | | HMW adiponectin | | non-HMW adiponectin | |
|---|---|---|---|---|---|---|---|
| | | RR (95% CI) | $P_{value}$[b] | RR (95% CI) | $P_{value}$[b] | RR (95% CI) | $P_{value}$[b] |
| Sex | | | | | | | |
| Men | 593/593 | 0.72 (0.53–0.98) | 0.03 | 0.91 (0.75–1.11) | 0.34 | 0.53 (0.36–0.77) | 0.0008 |
| Women | 613/613 | 0.79 (0.59–1.05) | 0.10 | 0.76 (0.78–1.19) | 0.71 | 0.47 (0.33–0.67) | <0.0001 |
| *P* for interaction[c] | | | *0.62*[c] | | *0.65*[c] | | *0.74*[c] |
| Median age, years | | | | | | | |
| <59 | 603/602 | 0.72 (0.54–0.95) | 0.02 | 0.90 (0.74–1.09) | 0.19 | 0.47 (0.32–0.67) | <0.0001 |
| ≥59 | 603/604 | 0.86 (0.64–1.16) | 0.32 | 0.97 (0.79–1.19) | 0.74 | 0.52 (0.36–0.74) | 0.0003 |
| *P* for interaction[c] | | | *0.64*[c] | | *0.61*[c] | | *0.68*[c] |
| BMI categories, kg/m$^2$ | | | | | | | |
| <25 | 446/481 | 0.86 (0.64–1.17) | 0.33 | 1.04 (0.84–1.29) | 0.23 | 0.48 (0.33–0.71) | <0.0001 |
| ≥25 BMI <30 | 534/175 | 0.68 (0.51–0.92) | 0.07 | 0.90 (0.73–1.10) | 0.57 | 0.43 (0.30–0.63) | <0.0001 |
| ≥30 | 226/175 | 0.86 (0.53–1.41) | 0.54 | 0.96 (0.68–1.34) | 0.60 | 0.77 (0.45–1.32) | 0.34 |
| *P* for interaction[c] | | | *0.18*[c] | | *0.16*[c] | | *0.83*[c] |
| WC[d], cm | | | | | | | |
| <94 in men and < 80 in women | 440/520 | 0.93 (0.68–1.26) | 0.63 | 1.12 (0.90–1.40) | 0.32 | 0.51 (0.34–0.74) | 0.0005 |
| ≥94 cm in men and ≥80 cm in women | 766/686 | 0.75 (0.58–0.98) | 0.03 | 0.92 (0.77–1.10) | 0.40 | 0.54 (0.40–0.74) | 0.0002 |
| *P* for interaction[c] | | | *0.27*[c] | | *0.15*[c] | | *0.80*[c] |
| Sex-specific median alcohol intake, g/day | | | | | | | |
| Low (<13.7 for men and <3.8 for women) | 594/602 | 0.82 (0.63–1.07) | 0.15 | 0.98 (0.82–1.19) | 0.87 | 0.51 (0.37–0.72) | 0.0001 |
| High (≥13.7 for men and ≥3.8 for women) | 612/604 | 0.68 (0.52–0.90) | 0.007 | 0.88 (0.73–1.07) | 0.20 | 0.45 (0.32–0.64) | <0.0001 |
| *P* for interaction[c] | | | *0.31*[c] | | *0.39*[c] | | *0.60*[c] |
| Smoking status | | | | | | | |
| Never-smoker | 503/528 | 0.76 (0.58–1.01) | 0.31 | 0.99 (0.81–1.21) | 0.52 | 0.43 (0.30–0.61) | <0.0001 |
| Ever-smoker | 703/678 | 0.74 (0.57–0.96) | 0.02 | 0.89 (0.74–1.06) | 0.19 | 0.54 (0.39–0.74) | 0.0001 |
| *P* for interaction[c] | | | *0.86*[c] | | *0.39*[c] | | *0.30*[c] |
| Physical activity | | | | | | | |
| Inactive | 615/563 | 0.71 (0.54–0.93) | 0.01 | 0.91 (0.76–1.10) | 0.32 | 0.44 (0.31–0.62) | <0.0001 |
| Active | 591/643 | 0.78 (0.60–1.03) | 0.07 | 0.95 (0.78–1.15) | 0.58 | 0.52 (0.37–0.73) | 0.0002 |
| *P* for interaction[c] | | | *0.58*[c] | | *0.75*[c] | | *0.48*[c] |
| CRP-hs groups, mg/l | | | | | | | |
| CRP-hs<3 | 552/642 | 0.93 (0.70–1.23) | 0.60 | 1.08 (0.89–1.31) | 0.42 | 0.56 (0.39–0.81) | 0.002 |
| CRP-hs ≥3 | 504/414 | 0.59 (0.42–0.81) | 0.001 | 0.77 (0.61–0.97) | 0.02 | 0.45 (0.30–0.66) | <0.0001 |
| *P* for interaction[c] | | | *0.02*[c] | | *0.02*[c] | | *0.37*[c] |

Note: The stratified variable was excluded from the multivariable adjusted model. The analysis by CRP-hs groups was based on a subset of 1056 case sets with available CRP-hs measurements. The cut-off point 3 mg/l is based on our previous work on CRP-hs and CRC (19).

[a]Multivariable-adjusted models were based on conditional logistic regression controlled for matching factors: age, sex, study center, follow-up time since blood collection, time of blood collection and fasting status, with further adjustment for smoking status, education, alcohol consumption, physical activity, fiber intake, consumption of fruits and vegetables, consumption of red and processed meat and consumption of fish and shellfish. Women were further matched by menopausal status and phase of the menstrual cycle at blood collection; postmenopausal women were matched by use of hormone replacement therapy. The analysis by sex and age was based on unconditional logistic regression where matching factors except for stratified variables were included in the model.

[b]*P*-value for continuous log-transformed adiponectin, HMW adiponectin and non-HMW adiponectin concentrations.

[c]P-value for statistical interaction on multiplicative scale between log-transformed adiponectin, HMW adiponectin and non-HMW adiponectin concentrations and the stratified variables in a multivariable-adjusted logistic regression.

[d]The cut-off points for stratification of WC are based on the criteria for defining abdominal obesity for European populations of the International Diabetes Federation (21).

adiponectin might regulate the proliferation of colon cancer cells remains to be elucidated.

Our findings showing stronger inverse associations of total adiponectin with rectal cancer than with colon cancer were also unexpected, as in EPIC and in other studies, metabolic factors such as abdominal fatness (23), physical inactivity (34), hyperlipidemia (35) and chronic low-grade inflammation (36) were more closely associated with risk of colon cancer than with rectal cancer. Indeed, in EPIC, only hyperglycemia was associated with rectal cancer risk (37). To further understand the differences between these cancers and the potential interactions with metabolic abnormalities, more detailed analyses are needed.

Interestingly, our data suggested a potential effect modification in the association between adiponectin and CRC by CRP-hs levels, such that the inverse association between total adiponectin and CRC was apparent in people with elevated CRP-hs concentrations (≥3 mg/l) but not in those with low CRP-hs levels. As a marker of systemic inflammation, CRP-hs was implicated in colon carcinogenesis (19) and decreased synthesis of adiponectin, such as in obese individuals, was suggested to lead to dysregulation of the production of pro-inflammatory cytokines and increased secretion of pro-inflammatory mediators (38). Further work investigating the potential interaction between site-specific inflammation, adiponectin and CRC is warranted to better understand why chronic inflammation may modify the association between adiponectin and CRC.

Among the strengths of our study are the prospective design and the largest number of cases to date, which allowed analysis by cancer subsite and sex. To the best of our knowledge, we are the first to report on the association of HMW adiponectin and non-HMW adiponectin and risk of CRC. In addition, this is the first report on these associations also in women. The study included participants with a broad range of characteristics from several European countries, and the biologic relations observed in our study may be generalizable for European populations.

Some limitations should be taken into account when interpreting the results. We determined the levels of non-HMW adiponectin based on a subtraction method and were not able to differentiate the effects of LMW- and MMW-adiponectin separately. The use of a single

adiponectin measurement at baseline might have caused regression dilution bias leading to underestimation of the actual associated risk; however, this might be unlikely as previously the intra-individual adiponectin concentrations showed to be stable over time (39). Adjusting for BMI and WC may have resulted in over-adjustment because adipose tissue is the location of adiponectin secretion and therefore, BMI and WC are partial determinants of adiponectin concentrations. In addition, the study was matched on a large number of factors, which could have resulted in overmatching. However, these factors are not likely to be intermediary in the association between adiponectin and CRC, which lowers the potential for overmatching. Despite exclusion of cancer cases at baseline, we cannot exclude the possibility that some subjects had yet-undiagnosed cancer, but our results did not change appreciably after we excluded cases with less than 2 years follow-up. The limited mean follow-up time of our study does not provide information on the strength of the association over longer time periods. Finally, ALPCO ELISA assay which we used for the adipokine measurements was criticized by Blüher *et al.* (40), suggesting that total adiponectin measured by this assay may have weaker association with metabolic traits compared with other commercially available assays. In our study, based on a population-based sample, including 1206 healthy controls, the correlation of total adiponectin and metabolic traits was stronger than reported by Blüher *et al.* in line with the results from other cohort studies (24,41,42). Nevertheless, our findings should be essentially confirmed by future studies in order to allow firm conclusions to be drawn.

In summary, in this large prospective study, circulating pre-diagnostic concentrations of total and non-HMW adiponectin were inversely associated with CRC risk. When stratified by cancer site, non-HMW adiponectin was inversely associated with both colon and rectal cancer. HMW adiponectin was not related to risk of CRC. These findings suggest that adiponectin may be involved in CRC pathogenesis largely due to its non-HMW fraction. If confirmed by future research, non-HMW adiponectin may be a novel marker for CRC risk and may have important implications in the targeted prevention of CRC.

## Supplementary material

Supplementary Table 1 can be found at http://carcin.oxfordjournals.org/.

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