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Circulating Omentin as a Novel Biomarker for Colorectal Cancer Risk:

Data from the EPIC - Potsdam Cohort Study

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Abbreviations: EPIC, European Prospective Investigation into Cancer and Nutrition; RR, relative risk; CRC, colorectal cancer; CI, confidence interval; BMI, body mass index; HbA$_{1c}$, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein; ICD-10, International Classification of Diseases, Tenth Revision.

Abstract (n= 250)

Omentin is a novel biomarker shown to exert metabolic, inflammatory and immune-related properties, and thereby could be implicated in the risk of colorectal cancer (CRC). So far, the association between omentin and CRC risk has not been evaluated in prospective cohort studies. We investigated the association between pre-diagnostic plasma omentin concentrations and risk of CRC in a case-cohort comprising 251 incident CRC cases diagnosed over a mean follow-up time of 10.4 years and 2,295 persons who remained free of cancer in the European Prospective Investigation into Cancer and Nutrition-Potsdam study. Hazard ratios as a measure of relative risk (RR) and 95% confidence intervals (CI-s) were computed using a Prentice modified Cox regression. In a multivariable model adjusted for age, sex, education, dietary and lifestyle factors, body mass index (BMI) and waist circumference, higher omentin concentrations were associated with a higher CRC risk ($RR_{\text{continuously per doubling of omentin concentrations}}$=1.98, 95%CI: 1.45-2.73). Additional adjustment for metabolic biomarkers, including glycated hemoglobin, high-density lipoprotein cholesterol and C-reactive protein, did not alter the results. In stratified analyses, the positive association between omentin and CRC risk was retained in participants with BMI < 30 ($RR_{\text{continuously per doubling of omentin concentrations}}$=2.26; 95%CI: 1.57-3.27), whereas among participants with BMI $\geq$ 30 no association was revealed ($RR_{\text{continuously per doubling of omentin concentrations}}$ =1.07; 95%CI: 0.63-1.83; $P_{\text{interaction}}$= 0.005). These novel findings provide the first lines of evidence for an independent association between pre-diagnostic omentin concentrations and CRC risk and suggest a potential interaction with the adiposity state of the individual.

Introduction

Omentin - also known as intelectin-1 or intestinal lactoferrin receptor ITLN1 - is a novel 34 kDa protein described to exert metabolic, inflammatory and immune-related properties, which thereby could be implicated in the development of colorectal cancer [CRC] (1-4). There are two genes, omentin-1 and omentin-2, that are adjacent to each other on chromosome 1q band 23 and that share 83% of amino acid sequences, (5); however, omentin-1 is the major circulating isoform in human plasma. Therefore, we refer to omentin-1 as omentin. Omentin is mainly produced in the goblet cells among the epithelial lining of organs, such as the intestines, colon, lung and heart, and is highly abundant in human plasma circulation (6-8). Omentin expression is also observed in the mesothelial cells on adipose tissue (8). Paradoxically, while omentin is highly expressed in visceral fat tissue, circulating plasma omentin concentrations were shown to be down-regulated in obesity-linked metabolic disorders including insulin resistance, glucose intolerance and type 2 diabetes (1,9). In obese and metabolically afflicted people, omentin was positively correlated with adiponectin and high-density lipoprotein cholesterol and inversely correlated with triglyceride and leptin levels (10-14). The underlying regulatory mechanisms mediating omentin dysregulation in obesity are unknown, but could potentially include adipocyte hypertrophy, inflammation and oxidative stress, as well as a failure of transcriptional regulation. Conversely, in cancer models omentin was suggested to promote cancer cell growth via triggering genomic instability (15) and PI3K/Akt (phosphatidylinositol-3 kinase downstream effector) signaling pathways (16,17). These cancer-promoting effects of omentin have been described independent of its abilities to regulate obesity-induced metabolic risk (18). Furthermore, higher omentin levels have been associated to inflammatory bowel disease, immune responses, and infection as potentially predisposing factors to CRC development (19). These controversial findings could reflect dual roles of omentin depending on the adiposity state of

the individual. However, prospective epidemiological studies to evaluate these associations in humans are currently lacking.

Within this context, we aimed to explore the association of circulating omentin concentrations with risk of CRC, and to test whether such an association may be independent of adiposity parameters [body mass index (BMI) and waist circumference] and metabolic biomarkers of relevance for CRC risk [i.e. glycated hemoglobin ($HbA_{1c)}$, high-density lipoprotein cholesterol (HDL-C) and high sensitivity C-reactive protein (hsCRP)] (20-22). In addition, we were interested to explore whether potential associations may differ according to levels of adiposity and associated metabolic risk factors.

Materials and Methods

Study population

The European Prospective Investigation into Cancer and Nutrition (EPIC) -Potsdam study is part of the multicenter prospective cohort study EPIC. In Potsdam, Germany, 27,548 individuals (16,644 women and 10,904 men) were recruited from the general population between 1994 and 1998. The age range was 35–64 years in women and 40–64 years in men (23). The baseline examination included anthropometric measurements and by qualified medical personnel, blood sampling, a self-administered validated food-frequency questionnaire, and a personal interview, including questions about prevalent diseases and sociodemographic and lifestyle characteristics (24). Written informed consent was obtained from all study participants *a priori*, and the study was approved by the ethics committee of the Medical Society of the State of Brandenburg, Germany (25).

Case ascertainment

In the course of the active follow–up, participants were contacted every two years, with response rates ranging between 90% and 96% per follow-up round (26). Follow-up questionnaires were sent out every 2–3 years to identify incident cases of CRC. Cancer cases during follow-up were identified by a combination of methods including: health insurance

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records, cancer and pathology registries, and by active follow-up directly through study participants or through next-of-kin. All incident cases of CRC identified during follow-up were verified by questionnaires that were mailed to the physicians. Thereby, information about the date and the type of diagnosis, about the diagnostic tests, and about the treatment was obtained. Cancer incidence data were coded according to the 10th revision of the *International Statistical Classification of Diseases, Injuries and Causes of Death* (27) and the second revision of the *International Classification of Diseases for Oncology* (28). According to the *International Classification of Diseases*, Tenth Revision (ICD-10), proximal colon tumors include those in the cecum, appendix, ascending colon, hepatic flexure, transverse colon, and splenic flexure (ICD-10 codes C18.0–18.5); distal colon tumors include those in the descending colon (ICD-10 code C18.6) and sigmoid colon (ICD-10 code C18.7); and rectal tumors are those occurring at the rectosigmoid junction (ICD-10 code C19) or in the rectum (ICD-10 code C20). Only participants having had a diagnosis of CRC that was confirmed by a physician and a diagnosis date after the baseline examination were considered as incident CRC cases. Non-melanoma skin cancers were excluded from the analysis. For the current analysis, we considered follow-up as the time between date of enrollment and date of diagnosis of colorectal cancer, death, or last complete follow-up. Within a mean follow-up time of 10.4 (±2.1) years, 279 participants developed incident CRC.

Case-cohort study

The current prospective case-cohort study is based on a subcohort of 2,500 individuals randomly sampled from the original cohort of the EPIC-Potsdam study population and 279 CRC cases (including 40 'internal' subcohort cases) (29). Out of the subcohort and the cases, for the current analysis, participants with prevalent cancer at baseline (n=138), incomplete follow–up information (n = 69), and missing measurements for omentin concentrations at study baseline (n = 26) were excluded. Thus, the current analysis is based on 251 incident

CRC cases and 2295 non-case participants (Supplementary Figure 1). We considered recent recommendations for case-cohort study reporting in data analysis and reporting (30).

Baseline anthropometrics and lifestyle characteristics

Weight, height, and waist-circumference were measured by trained interviewers with standardized methods described elsewhere (26). Waist circumference was assessed by midway measurements between the lower rib margin and the superior anterior iliac spine to the nearest 0.5 cm. Body mass index was calculated by weight (kg) divided by squared height (m). Information on educational attainment, smoking, occupational activity level, and leisure time physical activity were assessed with a self-administered questionnaire and a personal interview by trained interviewers using a computer-assisted interview (31). We considered sport activities and cycling as leisure time activities, both calculated as the average time spent per week during the 12 months before the baseline recruitment. Dietary habits including alcohol consumption were assessed by a validated food frequency questionnaires (31).

Measurement of biochemical parameters

At baseline, 30 mL of venous blood was taken from each participant and, after fractionation into serum, plasma (collected on citrate, 10% of total volume), leukocytes, and erythrocytes immediately stored in tanks of liquid nitrogen (approximately -196°C) or deep freezers (-80°C) (23). Plasma omentin concentrations were measured in citrate plasma sample by a sandwich enzyme linked immunosorbent assay (Human Omentin-1 ELISA, BioVendor, Brno, Czech Republic) at the Institute of Clinical Chemistry and Pathobiochemistry, Otto von Guericke University, Magdeburg, Germany. The linear range of the assay was reported to be 50-644 ng/mL. The inter- and intra-assay coefficients of variation varied within 3.2% - 4.1% and 4.4% -4.8%, respectively, with a limit of detection of 0.5 ng/mL according to the manufacturer. Preliminary analyses suggested an excellent reliability of omentin over 4 months period indicated by an intraclass correlation coefficient (ICC) of 0.83 [95% CI 0.78 - 0.87] (32). Plasma levels of glucose, HDL-C, triglycerides, γ-glutamyltransferase, and fetuin-

A and erythrocyte levels of HbA_{1c} were measured with the automatic ADVIA 1650 analyzer (Siemens Medical Solutions, Erlangen, Germany). For determination of fetuin-A, an immunoturbidimetric method was used with specific polyclonal goat anti-human fetuin-A antibodies to human fetuin-A (BioVendor Laboratory Medicine, Modreci, Czech Republic). Adiponectin was determined with an enzyme-linked immunosorbent assay from Linco Research, St Charles, Missouri in 2008. These biomarkers were determined in 2007 in sub-cohort population among 1,222 women and 758 men participating in the EPIC-Potsdam study at the Department of Internal Medicine, University of Tübingen as described elsewhere (33). In order to obtain levels for citrate plasma samples comparable to levels obtainable from EDTA plasma, all biomarker concentrations were multiplied by 1.16 [women] and by 1.17 [men] (34). In order be able to include HbA_{1c}, hsCRP and HDL-C in multivariable-adjustment models, we measured their concentrations in the remaining cases using the same assays, laboratory protocols and in the same laboratory (as described above). We evaluated potential differences in the old and new measurements in a subsample of 40 cases and 40 controls with repeated measurements. The values were corrected using the mean differences in old and new values as correction factors (0.992% for HbA_{1c}, 0.005 mg/L for hsCRP and 2.68 mg/dL for HDL-C, respectively). We used the same approach in order to correct omentin values in a subset of participants (10 cases and 92 non-cases) which laboratory measurements were distorted due to technical reasons in the laboratory. Missing biomarker data for HbA_{1c} (12 cases, 4.7% of all cases/85 non-cases, 3.7% of all non-cases), HDL-C (10 cases, 4% of all cases/1 non-case, 0.04 % of all non-cases) and hsCRP (10 cases, 4% of all cases and 5 non-cases, 0.21% of all non-cases) were imputed with sex- and disease state specific mean values.

Statistical analysis

Differences in cases and non-cases were assessed using Student's paired *t* test and Wilcoxon's signed rank test for continuous variables and by McNemar's test and Bowker's test of symmetry for categorical variables. Omentin concentrations were categorized into quartiles

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based on their distribution in the non-cases in the sub-cohort. Cross-sectional associations between plasma concentrations of omentin and selected CRC risk factors were examined across omentin quartiles. The associations between concentrations of omentin and metabolic biomarkers were evaluated based on age- and sex-adjusted Spearman partial correlation coefficients overall and stratified by level of adiposity. Finally, because omentin is implicated in both ageing and obesity we visually evaluated the associations of omentin with age and obesity parameters using LOESS plots. Hazard ratios as a measure of relative risk (RR) were computed using a weighted Cox proportional hazards model, modified for the case-cohort design according to the Prentice method (35) . This method was suggested to resemble to the highest extent the estimates of the full-cohort estimates. Age was the underlying time variable in the counting processes, with entry defined as the participants' age at the time of recruitment/blood collection and exit defined as age at the cancer diagnosis or censoring. We computed age-adjusted RRs for each quartile of omentin concentrations compared with the lowest quartile. The significance of linear trends across quartiles of omentin concentrations was tested by assigning each participant the median omentin value within quartiles as a continuous variable. Further, we estimated relative risks associated with increases of log-transformed omentin concentrations by log 2, which corresponds to a doubling of omentin concentrations on the original scale. We evaluated these associations in age- and sex-adjusted and multivariable-adjusted models. In the multivariable-adjusted models, in addition to age and sex, we used information on *a-priori* chosen covariates as established risk factors for CRC risk including education (in or no training, vocational training, technical school, or technical college or university degree), sports activity (0, 0.1–4, or >4 h/week), smoking (never, past, or current <20 cigarettes/day or current ≥20 cigarettes/day), alcohol consumption (0, 0.1–5, 5.1–10, 10.1–20, 20.1–40, or >40 g/day), fiber intake (g/day), red and processed meat intake (g/day), fruit and vegetable intake (g/day), fish intake (g/day), BMI (continuous), waist circumference (residually adjusted for BMI). In addition, we also controlled analyses

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for fasting status (<8 hours, ≥8 hours, unknown). In further analyses, we adjusted the associations for metabolic biomarkers previously implicated in higher CRC risk - HbA_{1c}, HDL-C and hsCRP (all continuously) - as well as for reported use of anti-diabetic, anti-hypertensive and anti-inflammatory medication. To test for nonlinearity, we fitted restricted cubic splines, at the 5th, 50th, and 95th percentiles of the omentin distribution, to our multivariable-adjusted regression model and used the likelihood ratio test to evaluate whether a nonlinear term of log-transformed omentin added significant information to the model. We estimated the associations according to different strata and tested for effect modification with factors that may be relevant for CRC risk (including age, sex, BMI, waist circumference, smoking status, alcohol consumption, and according to levels of HbA_{1c}, HDL-C and hsCRP) using interaction terms (log-transformed omentin concentrations multiplied by stratum variables). To explore whether omentin may add statistically significant information for CRC risk assessment beyond established and suspected CRC risk factors (smoking, low physical activity, high alcohol consumption, low fibre intake, fruits and vegetables intake, fish intake, high BMI, and high waist circumference, HbA_{1c}, HDL-C and hsCRP), we compared the fit of the multivariable-adjusted model with a model also including the omentin variable and evaluated whether the difference between the two models is statistically significant using a likelihood ratio test (LRT) as a standard statistical procedure for comparing nested models. Further, we repeated the main multivariable analyses after excluding cases that occurred during the first 3 years of follow-up ($n = 59$) and participants with omentin concentrations below or above first (≥62ngm/L) and last (<550 ng/mL) decile of omentin distribution (37 cases/154 non-cases), as well as participants with corrected biomarker measurements (see above). Finally, we also evaluated the potential influence of prevalent (n=37) and incident (n=4) inflammatory bowel disease (IBD). However, the low number of IBD cases did not justify change in the results. All statistical analyses were performed with SAS release 9.1

(SAS Institute, Cary, NC). All *P* values presented are two-tailed; *P* < 0.05 was considered statistically significant.

Results

Omentin concentrations were higher among CRC cases compared to the controls (median 458.6 (IQR, 378.4 - 569.6 ng/mL) in cases versus 395.6 (IQR, 327.7 − 485.5 ng/mL) in controls, respectively; Table 1). CRC cases were more likely to be men, to be older and to have lower levels of physical activity and fish intake than controls, but higher alcohol consumption, BMI and waist circumference, fruit intake and red and processed meat intakes. Median concentrations of HbA_{1c}, glucose and hsCRP were higher in CRC cases compared to controls whereas HDL-C concentrations were lower (Table 1). Among non-cases, after adjustment for sex and fasting status, omentin concentrations were positively associated with age, alcohol consumption, systolic blood pressure, and fibre intake (Table 2). In contrast, inverse associations were observed for anthropometric indicators and processed meat intake. Among the different biomarkers previously implicated in higher CRC risk, higher omentin concentrations were positively associated with adiponectin and HDL-C and inversely associated with triglycerides in both sexes (Table 3). An inverse association was observed for hsCRP; however it was mostly confined to women. The favorable metabolic profile of omentin seemed to be more enhanced in obese individuals compared to non-obese ones (Supplementary Table 1).

In Cox regression analysis, after adjustment for age, sex, smoking, education, alcohol, physical activity, fiber, fruits and vegetables, red and processed meat, fish, BMI, and waist circumference (residually adjusted for BMI), higher omentin concentrations were associated with a higher CRC risk (RR $_{Q4 \text{ vs } Q1}$= 2.31, 95% CI: 1.48 − 3.58; P_{trend} <0.0001). Based on the log-transformed omentin concentrations, an increase by log 2, which corresponds to a doubling of omentin concentrations on the original scale, was associated with a significant 1.98-fold higher risk (95% CI: 1.45-2.73) without apparent differences according to sex

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($P_{\text{interaction by sex}}$ = 0.98). The strength of the association did not essentially change when HbA_{1c}, HDL-C and hsCRP were added to the multivariable model individually or in combination (Supplementary Figure 2). Further adjustment for antidiabetic, antihypertensive and anti-inflammatory medication did not substantively change the results ($RR_{\text{Q4 vs Q1}}$ =2.23; 95% CI: 1.55-3.04; P_{trend} = 0.0006). These associations did not deviate from linearity as shown in spline regression analysis ($P_{\text{non-linearity}}$ = 0.20). Thereafter, we tested whether omentin variable improves CRC risk assessment beyond established and suspected CRC risk factors by comparing multivariable model with a model also including the omentin variable. In this analysis addition of omentin statistically significantly improved the multivariable-adjusted model beyond established CRC risk factors overall (Figure 1), as well as separately in men and women (Supplementary Figure 3A-B).

Subgroup and sensitivity analyses

In stratified analysis, no statistically significant interaction was revealed for any of the investigated factors with the exception of BMI. Thus, the positive association of omentin with CRC risk was retained in participants with a BMI < 30 (Multivariable-adjusted $RR_{\text{continuously per doubling of omentin concentrations}}$ = 2.26; 95% CI: 1.57-3.27; P_{trend} <0.0001), whereas among participants with BMI ≥ 30 no association was revealed (Multivariable-adjusted $RR_{\text{continuously per doubling of omentin concentrations}}$ = 1.07; 95% CI: 0.63-1.83; $P_{\text{interaction by BMI}}$ = 0.002; Table 5). In further analyses testing linearity assumptions, no statistical deviation from linearity was observed [$P_{\text{non linearity}}$ = 0.43 for participants with BMI<30 (Figure 2-A) and $P_{\text{non-linearity}}$ = 0.26 for participants with BMI ≥30 (Figure 2-B)]. Of note, having that no interaction by waist circumference and other metabolic factors was observed and the particularly low number of cases in subgroup analyses, these stratified results should be interpreted with caution. After exclusion of cases that occurred during the first 3 years of study follow-up in the main multivariable analysis, the relative risks for omentin concentrations remained unaltered ($RR_{\text{continuously per doubling of omentin concentrations}}$ = 1.89, 95% CI: 1.25 – 2.97). Exclusion of participants

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with omentin concentrations below and above first and last decile of omentin distribution, as well as participants with corrected biomarker measurements (see above) did not markedly change the pattern of the results (data not shown).

Discusssion

In this prospective cohort study, higher circulating concentrations of omentin were statistically significantly associated with a higher CRC risk, independent of CRC risk factors, adiposity and metabolic biomarkers. Furthermore, omentin statistically significantly improved risk assessment of CRC beyond established risk factors. The positive association between omentin and CRC risk was observed only among participants with BMI less than 30, whereas it did not pertain in those individuals whose BMI was equal or above 30. To our knowledge, this is the first prospective cohort study on the association between omentin and CRC risk.

Our overall results for a positive association between omentin and CRC extend those reported by a small case-control study which observed higher omentin concentrations in patients with colon cancer compared to the healthy controls (18). Similar findings for omentin have also been reported with regards to other cancers, such as prostate cancer (36) and mesothelial cancer (8). Exact pathophisiological roles of omentin are not well characterized, but potentially plausible pathways as explaining mechanisms of our findings for a positive association between omentin and CRC risk include (1) acting via inducing chromosomal instability; (2) enhancing Akt phosphorylation/activation signaling pathways; and (3) reflecting immune response to bacterial pathogen infection, intestinal inflammation and inflammatory bowel activity.

Omentin overexpression has been related to complex translocations indicative of chromosomal instability during carcinogenesis (15). Since studies suggest that the majority of CRC cases arise through chromosomal instability (37), this pathway could provide a plausible link; however the exact initiating mechanisms and relationship with tumor progression are still to be elucidated. Novel data has also implicated omentin in the Akt

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phosphorylation/activation (PI3K) signaling pathways (16,17). PI3K activity is known to be associated with the transforming activity of viral oncogenes (38) and to trigger a cascade of tumorigenic responses, from cell growth and proliferation to survival and motility (39). In addition, a downstream target of Akt - endothelial nitric oxide synthase (eNOS) - has been implicated in tumorigenesis, including colorectal carcinogenesis (40). It can be speculated that by promoting activation of the Akt signaling pathway and in turn modulating eNOS, omentin may contribute to the pathogenesis of CRC. Omentin may also exert a number of effects reflecting cellular immune responses. Thus, omentin is largely expressed in paneth cells, goblet cells, endothelial cells, epithelial cells, all of which play a role in immune responses. Furthermore, a body of literature has reported omentin to be increased during parasite and bacterial infection suggesting its roles in innate immunity and inflammation (19). Remarkably, a recent study suggested that omentin selectively binds to pathogenic organisms suggesting its functions in microbial surveillance (41). Experimental studies have shown that omentin secretion is induced in endothelial cells by IL-6 (42), one of the major inflammatory cytokines in response to infection and tissue damage. During infection IL-6 increases omentin secretion whereas it decreases expression of anti-inflammatory proteins such as adiponectin, thus maintaining energy homeostasis of the host organism. Finally, omentin has also been identified as an intestinal lactoferrin receptor able to mediate mucosal immunity functions of lactoferrin in the gastrointestinal tract (3). Polymorphisms in the omentin gene have been associated with the risk of Crohn's disease – a form of inflammatory bowel disease (43), thereby a change of omentin level may indicate altered immune response to infection, facilitating the process of trans-mural inflammation. However, in our data there was no indication of inflammatory bowel disease to explain this association.

Interestingly, the positive association between pre-diagnostic omentin concentrations and CRC in our study was confined to non-obese individuals; conversely, a null association was revealed in obese participants. Previous studies have shown that omentin concentrations are

decreased with obesity and are inversely correlated to insulin resistance (9). Furthermore, randomised control trials have shown that weight loss significantly increases plasma omentin concentrations (44), whereas hyperinsulinemic induction in healthy individuals reduces them (45). So far there is no evidence on the prospective association between omentin and cancer risk in obese individuals. However, a study conducted in obese women, showed that omentin was down-regulated in ovarian cancer patients (45). Collectively, omentin could be down-regulated by insulin and glucose abundance which may partly explain the lower omentin concentrations observed in obese individuals in our study. Future studies are warranted in order to confirm these findings and to shed light on potential mechanisms.

Apart from providing etiological insights, our group is interested in employing novel biomarkers for improved disease risk assessment. We therefore tested whether addition of omentin variable would add statistically significant predictive value to a model based on established lifestyle risk factors for CRC. Our data essentially revealed an ability of omentin in improving model discrimination beyond established lifestyle and suspected metabolic CRC risk factors. Despite these promising results, further work is needed in order to evaluate the predictive value of omentin (i.e. risk reclassification) along with other novel biomarkers in improving the risk assessment of CRC. Such work is largely warranted having that the time course of cancer initiation and progression from adenoma to cancer provides a window of opportunity for prevention.

Our study benefits from a well-characterized study population embedded into the EPIC-Potsdam cohort. Data were collected prospectively, thereby eliminating the potential for recall bias and reducing the possibility that biomarker levels change as a result of the outcome. Furthermore, follow-up proportions exceeded 90%; and all self-reports on incident CRC cases were verified through medical records, treating physicians, or death certificates (26). Because we used a case-cohort design, our findings are expected to be generalizable to the source population without the need to assess biomarker levels in the entire cohort; the external

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generalizability though may be still limited to populations with similar characteristics. We relied on a single baseline blood sample from each participant. Thus, random measurement errors may have attenuated the true relation between the biomarkers and the end point. However, our preliminary analyses suggested a good reliability of omentin measurements over a period of several months (32) arguing against potential influences of within-subject variations in biomarker levels over time. Due to the restricted number of cases, in our analyses, we did not stratify according to CRC subtypes [colon (proximal/distal) or rectal cancer], therefore we are limited in our interpretation of findings to overall CRC. Having that previous research has suggested that certain CRC risk factors could be differentially associated according to cancer subtype (46), future studies are needed to evaluate CRC anatomic site-specific associations. Finally, given the observational nature of our study, our results could neither prove nor disprove a causal association between circulating omentin concentrations and CRC risk. Replication of findings in other populations is warranted in the future research.

In conclusion, in this prospective cohort study, we provide first lines of evidence for a positive association between circulating omentin concentrations and risk of CRC independent of established CRC risk factors and a range of metabolic biomarkers. This association was largely confined to non-obese individuals, whereas no association was revealed in those categorized as obese. Whether up-regulation of immune-related or metabolic pathways may underline adiposity specific associations between omentin and CRC risk remains to be addressed by future research.

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Acknowledgements

Figure legends:

Figure 1. Predictive ability of pre-diagnostic omentin concentrations beyond established lifestyle and metabolic risk factors for colorectal cancer: The EPIC Potsdam Case-Cohort Study. The multivariable model is adjusted for age, sex, smoking status, education, alcohol consumption, physical activity, fiber intake, consumption of fruits and vegetables, red and processed meat, fish, fasting status, BMI and waist circumference (residually adjusted for BMI, HbA_{1c}, HDL-C and hsCRP. *P*-values for comparison of the fit of the multivariable-adjusted model with a model also including the omentin variable using a likelihood ratio test (LRT) for comparing nested models.

Figure 2: Association of omentin with colorectal cancer in a spline regression model by level of adiposity, (Panel A) in participants with BMI <30 and (Panel B) in participants with BMI ≥30: The EPIC Potsdam Case-Cohort Study. Restricted cubic splines, at the 5th, 50th, and 95th percentiles of the omentin distribution were fitted to the multivariable-adjusted regression model and likelihood ratio test was used to evaluate whether a nonlinear term of log-transformed omentin added significant information to the model.

Table 1: Baseline Characteristics of Incident Colorectal Cancer Cases: The EPIC Potsdam Case-Cohort Study[a]

Characteristics	Cases	Subcohort[b]
N	251	2295
Socio-demographic factors		
Age, years	56.3 ± 7.3	50.2 ± 9.0
Female sex, %	40.2	60.4
Education, %		
No school degree or primary school	36.3	37.1
Technical or professional school	24.3	24.2
University degree	39.4	38.7
Lifestyle factors		
Sports activities, hours/week, mean ± SD	0.77 ± 1.76	1.02 ± 1.76
Smokers, incl. ex-smokers ≤5 years , %	11.8	7.8
Baseline alcohol intake, grams/ day, mean ± SD	19.1 ± 20.3	14.9 ± 20.9
BMI, kg/m^2, mean (SD)	27.7 ± 4.9	26.1 ± 4.2
Waist circumference, cm	92.3 ± 13.9	85.9 ± 12.8
HRT in postmenopausal women, %	10.2	5.9
Dietary factors		
Fibre intake, grams/ day, mean ± SD	22.4 ± 6.3	22.1 ± 6.8
Fruits, grams/ day, mean ± SD	146.1±98.4	138.3±93.0
Vegetables, grams/ day, mean ± SD	51.4±34.3	56.8±44.9
Red meat, grams/ day, mean ± SD	45.1±30.5	43.1±29.4
Processed meat, grams/ day, mean ± SD	67.7±49.9	61.2±46.5
Fish, grams/ day, mean ± SD	23.3±20.4	24.4±26.8
Biomarkers		
Omentin, ng/mL, median (IQR)	459 (378-570)	396 (328-486)
hsCRP, mg/L, median (IQR)	1.4 (0.5-2.8)	0.7 (0.2-1.9)
HbA_{1c}, [%], median (IQR)	6.59 (6.39-6.79)	6.42 (6.12-6.74)
Glucose, mg/dL, median (IQR)	92.2 (85.2-99.2)	86.8 (80.2-95.1)
HDL-C, mg/dL, median (IQR)	44.7 (37.7-51.7)	46.4 (38.9-54.9)

Abbreviations: EPIC =European Prospective Investigation into Cancer and Nutrition; N = number; BMI = body mass index; SD = standard deviation; HRT = hormonal replacement therapy; IQR = interquartile range; hsCRP = high-sensitivity C-reactive protein; HbA_{1c} = glycated hemoglobin; HDL-C = high-density lipoprotein cholesterol.

[a]The study population in the current analysis is based on participants with available information on circulating omentin concentrations.

[b]*P*-values for the difference between cases and controls were determined by Student's paired t-test for variables expressed as means and by Mc Nemar's test and Bowker's test of symmetry for variables expressed as percentages. Except for education, vegetable intake and glucose levels, all other difference proved statistically significant (P<0.0001).

Table 2. Age- and Sex-adjusted Characteristics, by Quartiles of Omentin within the subcohort (n = 2295): The EPIC Potsdam Case-Cohort Study

Variables	Quartiles of circulating omentin concentrations (median, ng/mL)				P-trend
	Q1 (286.7)	Q2 (364.2)	Q3 (437.3)	Q4 (562.6)	
Cases/ sub-cohort, N	573	577	573	572	
Demographic and lifestyle factors					
Age, years, mean [a]	47.5	48.5	51.2	53.4	<0.0001
Female sex, % [b]	59	63	60	60	0.98
University degree, %	43	41	40	40	0.23
Sports, mean hours per week	0.96	1.00	0.95	1.15	0.07
Smokers, incl. ex-smokers ≤5 years, %	17	16	15	11	0.007
Smokers ≥ 20 cigarettes per day, %	7	7	5	6	0.11
Alcohol intake, g/day	15.1	16.4	16.5	18.1	0.006
HRT use, %	16	18	22	23	0.02
Anthropometric indicators					
BMI, kg/m^2, mean	26.7	26.2	26.3	25.7	0.0002
Waist circumference, cm, mean	88.4	87.3	87.3	86.5	0.006
Hip, cm, mean	101.6	100.8	100.5	99.6	<0.0001
Height, cm, mean	169.4	169.5	168.3	168.3	0.001
Waist- to- hip ratio, mean	0.87	0.86	0.86	0.86	0.64
Waist-to height ratio, mean	0.52	0.51	0.51	0.51	0.08
Fat mass, kg, mean	23.5	22.6	22.1	21.2	<0.0001
Body fat percent,%	30.5	29.7	29.6	28.7	<0.0001
Systolic blood pressure, mmHg, mean	127.8	129.8	131.3	133.3	<0.0001
Diastolic blood pressure, mmHg, mean	83.4	84.2	84.9	84.5	0.06
Dietary factors					
Fibre intake, g /day, mean	22.2	22.1	22.4	23.0	0.04
Fruit intake, g/day, mean	129.8	130.3	138.0	141.4	0.01
Vegetable intake, g/day, mean	54.6	53.5	55.7	55.7	0.56
Red meat intake, g/day, mean	46.2	44.0	46.0	44.9	0.54
Processed meat intake, g/day, mean	68.4	62.6	63.3	63.2	0.04
Fat intake, g/day, mean	86.4	85.5	85.3	84.2	0.15
Fish, g/day, mean	24.3	25.8	24.5	26.2	0.42

Abbreviations: EPIC =European Prospective Investigation into Cancer and Nutrition; Q = quartile; N = number; BMI = body mass index; g = grams; SD = standard deviation; HRT = hormonal replacement therapy.

[a]Adjustment only for sex .

[b]Adjustment only for age at study recruitment.

[c]*P* for trend from a linear model, calculated using the median omentin concentrations within qaurtiles as a continuous variable, adjusted for age and sex.

Table 3. Spearman Partial Correlations[a] of Omentin with Metabolic Biomarkers within the subcohort overall and by adiposity status (n = 2295): The EPIC Potsdam Case-Cohort Study

Biomarkers	All	Men	Women
	R (95% CI)	R (95% CI)	R (95% CI)
hsCRP, mg/L	-0.10 (-0.14; -0.06)	0.02 (-0.04; 0.09)	-0.18 (-0.22; -0.13)
HbA$_{1c}$, [%]	-0.00 (-0.04; 0.03)	0.02 (-0.04; 0.08)	-0.01 (-0.07; 0.03)
HDL-C, mg/dL	0.15 (0.11-0.20)	0.13 (0.06; 0.19)	0.17 (0.12; 0.22)
Triglycerides, mg/dL	-0.10 (-0.14; -0.06)	-0.05 (-0.11; 0.02)	-0.14 (-0.19;-0.09)
Adiponectin, mg/dL	0.22 (0.18-0.26)	0.17 (0.10; 0.23)	0.23 (0.20; 0.30)
Fetuin A, g/L	-0.02 (-0.07; 0.01)	0.008 (-0.05; 0.07)	0.05 (-0.10; 0.005)

Abbreviations: EPIC =European Prospective Investigation into Cancer and Nutrition; CI= confidence interval; hsCRP = high sensitivity C-reactive protein; HbA$_{1c}$ = glycated hemoglobin; HDL-C = high-density lipoprotein cholesterol
[a]Adjusted for age at study recruitment and sex.

Table 4. Relative risks and 95% confidence intervals of colorectal cancer across quartiles of omentin levels: The EPIC Potsdam Case-Cohort Study

Model	Quartiles of circulating omentin levels (median, ng/mL)					Continuously per doubling of biomarker concentrations	*P*-value
	Q1 (286.6)	Q2 (364.2)	Q3 (437.3)	Q4 (562.7)	*P* for trend		
All participants							
No. of Cases/Controls	34/573	43/577	59/573	115/572			
Age and sex-adjusted RR (95% CI)[a]	1 [Ref.]	1.10 (0.69-1.78)	1.30 (0.83-2.03)	2.18 (1.43-3.31)	<0.0001	1.96 (1.44-2.66)	<0.0001
Multivariable RR (95% CI)[b]	1 [Ref.]	1.14 (0.70-1.86)	1.33 (0.85-2.10)	2.31 (1.48-3.58)	<0.0001	1.98 (1.45-2.73)	<0.0001
Men							
No. of Cases/Controls	22/230	27/208	38/238	63/234			
Age and sex-adjusted RR (95% CI)[a]	1 [Ref.]	1.03 (0.55-1.91)	1.33 (0.75-2.35)	1.86 (1.09-3.18)	0.005	1.69 (1.13-2.54)	0.01
Multivariable RR (95% CI)[b]	1 [Ref.]	1.00 (0.53-1.88)	1.27 (0.70-2.28)	1.80 (1.01-3.17)	0.01	1.70 (1.10-2.62)	0.02
Women							
No. of Cases/Controls	12/343	16/369	21/335	52/338			
Age and sex-adjusted RR (95% CI)[a]	1 [Ref.]	1.05 (0.49-2.25)	1.11 (0.53-2.33)	2.49 (1.30-4.82)	0.0005	2.20 (1.39-3.50)	0.0009
Multivariable RR (95% CI)[b]	1 [Ref.]	1.20 (0.53-2.70)	1.27 (0.58-2.75)	3.00 (1.45-6.19)	0.0002	2.36 (1.47-3.77)	0.0004

Abbreviations: Q =quartile, EPIC =European Prospective Investigation into Cancer and Nutrition; Q = quartile

[a]Age- and sex-adjusted models were calculated using Cox proportional-hazard regression modified according to the Prentice method. In the counting processes age was the underlying time variable with "entry time" defined as age at baseline and "exit time" as age at cancer event or censoring.

[b]The multivariable model is adjusted for age, sex, smoking status, education, alcohol consumption, physical activity, fiber intake, consumption of fruits and vegetables, red and processed meat, fish, fasting status, BMI and waist circumference (residually adjusted for BMI).

Note: *P* for interaction by sex = 0.85

Table 5. Multivariable-adjusted RRs (95% CIs) of colorectal cancer associated with an increase of continuous log-transformed omentin concentrations, in stratified analyses according to CRC risk factors: The EPIC Potsdam Case-Cohort Study

Subgroups	N cases/ non-cases	Continuously per doubling of biomarker concentrations[b]	*P*-value[c]
Age[a]			
< 60 years	162/1881	1.97 (1.32-2.93)	0.0009
≥ 60 years	89/414	2.36 (1.29-4.31)	0.005
P for interaction[d]			*0.07*
BMI			
< 30 kg/m^2	189/1927	2.27 (1.53-3.35)	<0.0001
≥ 30 kg/m^2	62/368	1.07 (0.63-1.83)	0.78
P for interaction			*0.005*
Waist circumference[e]			
<102 cm in men and <88 cm in women	164/1768	2.25 (1.49-3.39)	0.0001
≥102 cm in men and ≥88 cm in women	87/527	1.51 (0.86-2.66)	0.14
P for interaction[d]			*0.55*
Fibre intake			
< 21.5 g/day	114/1164	2.36 (1.47-3.79)	0.002
≥ 21.5 g/day	137/1131	1.79 (1.09-2.94)	0.02
P for interaction[d]			*0.53*
Alcohol consumption			
<18.5 mL/day in men and <5 mL/day in women	116/1153	1.87 (1.15-3.06)	0.03
≥18.5 mL/day in men and ≥5mL/day in women	135/1142	2.12 (1.38-3.26)	0.02
P for interaction[d]			*0.48*
Smoking status			
Non-smokers	182/1645	2.12 (1.38-3.26)	0.007
Smokers [a]	69/650	2.28 (1.17-4.40)	0.01
P for interaction[d]			*0.53*
HbA$_{1c}$			
<6.5%	110/1285	2.13 (1.20-3.76)	0.009
≥6.5%	141/1010	1.79 (1.20-2.67)	0.04
P for interaction[d]			*0.48*
HDL-C			
<60 mg/dL	231/1939	2.08 (1.47-2.91)	<0.0001
≥60 mg/dL	20/356	0.95 (0.33-2.66)	0.91
P for interaction[d]			*0.35*
hsCRP			
<3 mg/L	187/1905	1.87 (1.28-1.75)	0.001

	64/390	1.86 (0.94-3.67)	0.08
≥3 mg/L			
P for interaction[d]			*0.93*

Abbreviations: EPIC =European Prospective Investigation into Cancer and Nutrition; hsCRP = high sensitivity C-reactive protein; HbA$_{1c}$ = glycated hemoglobin; HDL-C = high-density lipoprotein cholesterol.

[a]The stratified variables were excluded from the multivariable adjusted model.

[b]Models were calculated using Cox proportional-hazard regression modified according to the Prentice method. In the counting processes age was the underlying time variable with "entry time" defined as age at baseline and "exit time" as age at cancer event or censoring. The multivariable model is adjusted for age, sex, smoking status, education, alcohol consumption, physical activity, fiber intake, consumption of fruits and vegetables, red and processed meat, fish, fasting status, BMI and waist circumference (residually adjusted for BMI).

[c]P-value for continuous log-transformed omentin concentrations.

[d]P-value for statistical interaction on the multiplicative scale between log-transformed omentin concentrations and the stratified variables in a multivariable-adjusted Cox-regression analysis.

[e]The cut-off points for stratification according to waiust circumference are based on the National Cholesterol Education Program/Adult Treatment Panel III criteria for defining abdominal obesity as a component of the metabolic syndrome definition.
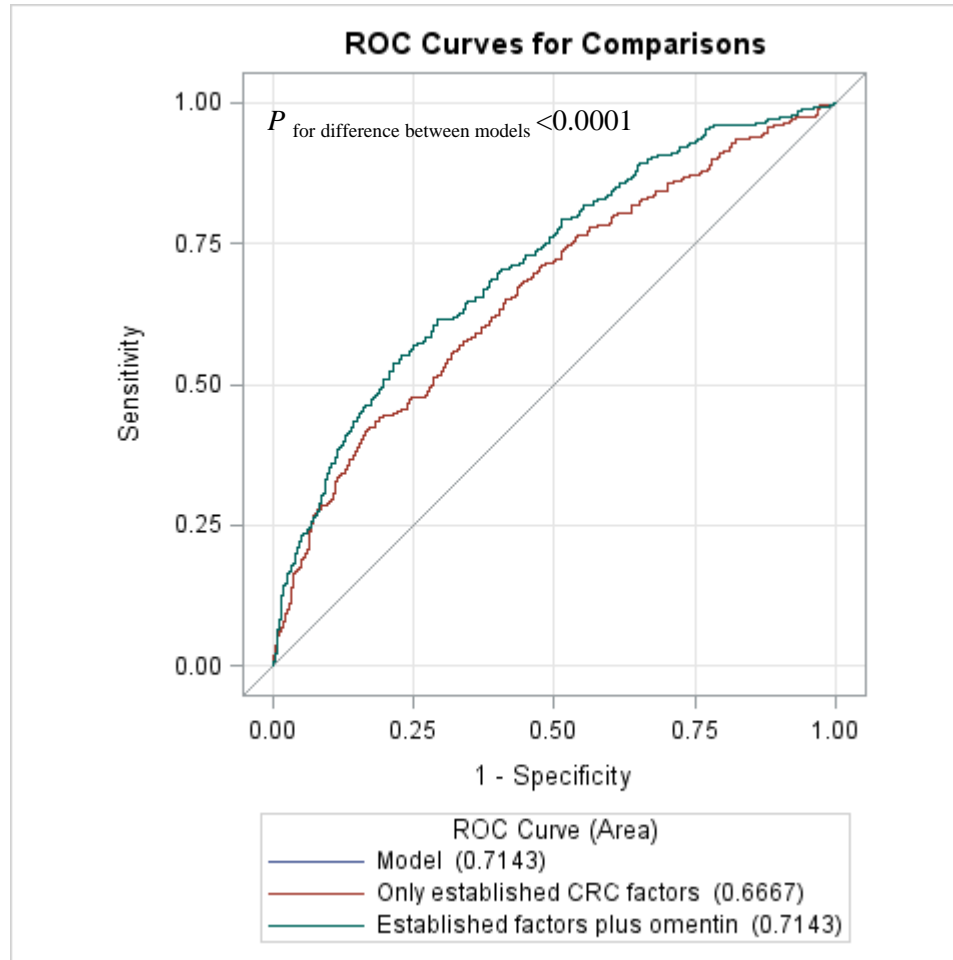
Figure 1. Predictive ability of pre-diagnostic omentin concentrations beyond established lifestyle and metabolic risk factors for colorectal cancer: The EPIC Potsdam Case-Cohort Study
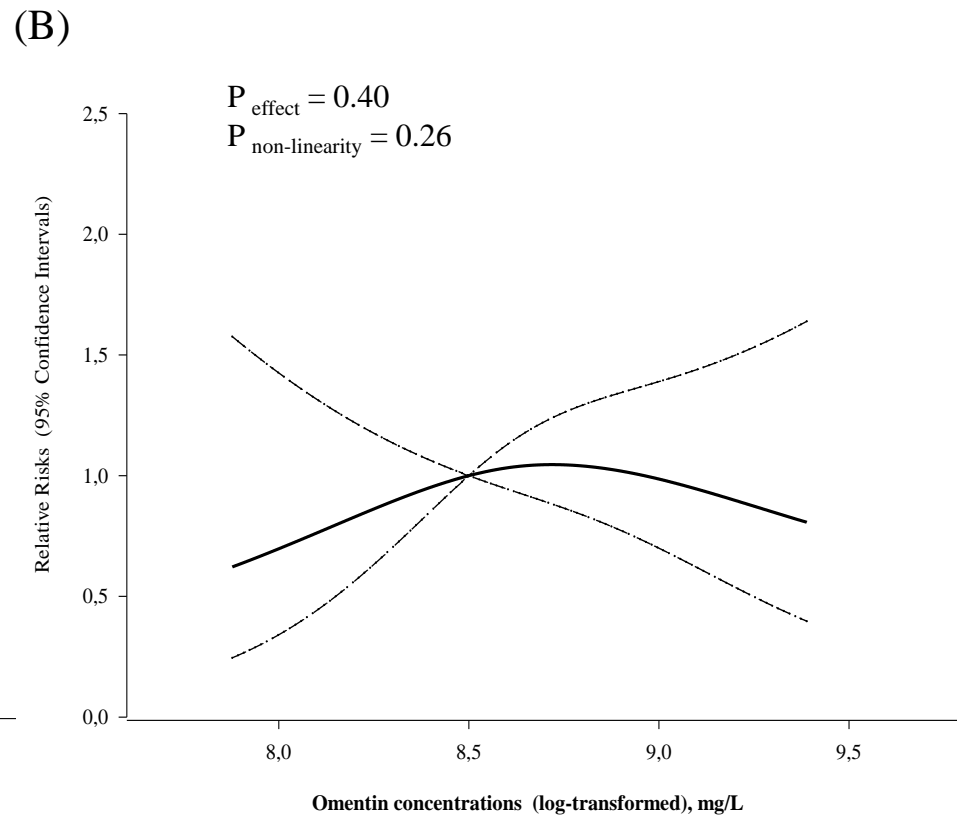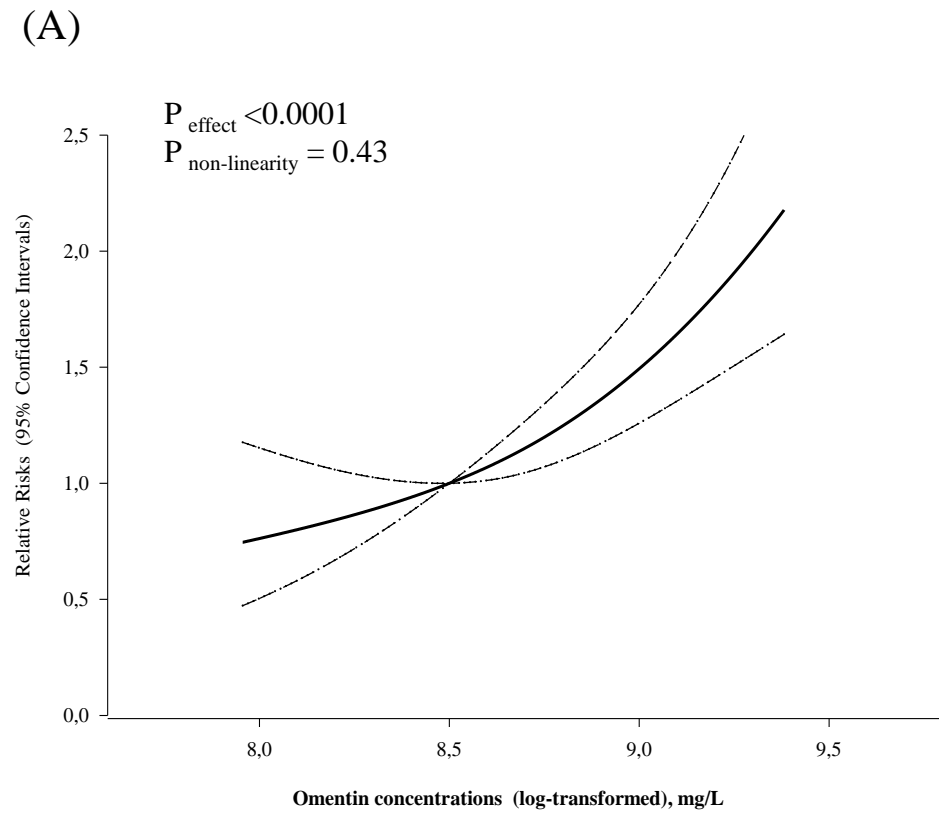
(A)

$P_{effect} < 0.0001$
$P_{non-linearity} = 0.43$

(B)

$P_{effect} = 0.40$
$P_{non-linearity} = 0.26$

Relative Risks (95% Confidence Intervals)

Omentin concentrations (log-transformed), mg/L

Figure 2: Association of omentin with colorectal cancer in a spline regression model by level of adiposity, (Panel A) in participants with BMI <30 and (Panel B) in participants with BMI ≥30: The EPIC Potsdam Case-Cohort Study