Prevention and Epidemiology

Cancer Research

Leptin and Soluble Leptin Receptor in Risk of Colorectal Cancer in the European Prospective Investigation into Cancer and Nutrition Cohort

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Abstract

Leptin, a peptide hormone produced primarily by the adipocytes, is hypothesized to play a role in the pathogenesis of colorectal cancer (CRC). Soluble leptin receptor (sOB-R) may regulate leptin's physiologic functions; however its relation to CRC risk is unknown. This study explored the association of leptin and sOB-R with risk of CRC in a prospective nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. A total of 1,129 incident CRC cases (713 colon, 416 rectal) were matched within risk sets to 1,129 controls. Conditional logistic regression was used to calculate relative risks (RR) and 95% confidence intervals (CI). After multivariable adjustment including body mass index (BMI), waist circumference, and baseline leptin concentrations, sOB-R was strongly inversely associated with CRC (RR comparing the highest quintile vs. the lowest, 0.55; 95% CI, 0.40–0.76; $P_{trend} = 0.0004$) and colon cancer (RR, 0.42; 95% CI, 0.28–0.63, $P_{trend} = 0.0001$); whereas no association was seen for rectal cancer (RR adjusted for BMI and waist circumference, 0.83; 95% CI, 0.48–1.44, $P_{trend} = 0.38$). In contrast, leptin was not associated with risk of CRC (RR adjusted for BMI and waist circumference, 0.85; 95% CI, 0.56–1.29, $P_{trend} = 0.23$). Additional adjustments for circulating metabolic biomarkers did not attenuate these results. These novel findings suggest a strong inverse association between circulating sOB-R and CRC risk, independent of obesity measures, leptin concentrations, and other metabolic biomarkers. Further research is needed to confirm the potentially important role of sOB-R in CRC pathogenesis. *Cancer Res; 72(20); 5328–37. ©2012 AACR.*

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi: 10.1158/0008-5472.CAN-12-0465

Introduction

Leptin is a 16 kDa protein encoded by the ob gene mostly produced by white adipose tissue that is also secreted into the circulation. Plasma leptin concentration parallels adipose tissue mass and is substantially increased in obesity (1, 2). Originally discovered in 1994 as a long-term regulator of food intake and energy balance acting in the hypothalamus (3), today it is widely recognized that leptin has effects also on energy homeostasis, metabolism, neuroendocrine, and immune function, and, potentially, also on cancer (8). *In vitro* studies suggest that leptin may induce tumor angiogenesis, reduce apoptosis, promote cell growth and migration, and interact with metabolic and growth factors (4, 5). Although leptin may act as a growth factor on colon cancer cells *in vitro* (6–8), it was shown to not promote tumor growth *in vivo* (6), which raises the question whether leptin is directly involved in human carcinogenesis or it is a bystander of the established relation between obesity and colorectal cancer (CRC; 7). Only a limited number of epidemiologic studies have investigated the association between leptin concentrations and CRC risk (8–13). In 2 Scandinavian nested case-control studies (10, 11), leptin was associated with risk for colon cancer in men, but not in women; whereas an association in women was reported in the Japan Collaborative Cohort Study (12) and in the Women's Health Initiative cohort of postmenopausal women (13). Most of these studies were of relatively small sample size and did not fully account for major determinants of serum leptin levels, including lifestyle factors (14), body fat distribution (7, 15), as well as other metabolic markers (16–19). Importantly, leptin actions may be modulated also by other regulatory biologic factors. It is now well established, that the effects of leptin are mediated by membrane protein receptors, which circulate in soluble form in plasma (20–22). The major circulating leptin binding protein, soluble leptin receptor (sOB-R) is a unique form, which consists solely of the extracellular domain of membrane leptin receptor (23, 24). The exact actions of sOB-R are not entirely clear. Binding to sOB-R may on the one hand, delay the clearance of leptin from the circulation and thereby prolong its bioavailability (25), but may on the other hand also neutralize the action of leptin and thereby reduce bioavailability (26). In humans, sOB-R is inversely associated with several important metabolic factors known to be involved in etiology of CRC, such as obesity (27), insulin resistance (28, 29), and diabetes (30), and thus may be also related to CRC risk (31). However, to date no epidemiologic study investigated the association between sOB-R and CRC, as well as the joint effects of sOB-R and leptin on CRC.

We examined the association of leptin and sOB-R with risk of CRC in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. In particular, we investigated if the potential associations are independent of lifestyle and dietary factors, measures of adiposity [body mass index (BMI) and waist circumference (WC)] and circulating metabolic biomarkers.

Materials and Methods

Study population

EPIC is a large, prospective study with approximately 520,000 participants, aged 25 to 70 years at enrollment during the period from 1992 through 2000 and recruited predominantly from the general population residing in a given geographic area (town or province) in 23 centers in 10 European countries. The current study includes subjects from 9 of the participating countries: Denmark, France, Germany, Greece, Italy, Spain, Sweden, the Netherlands, and the United Kingdom.

Follow-up for cancer incidence

The mean follow-up time of cases was 3.9 years (from 4 months to 11.5 years). Incident cancer cases were identified through record linkage with regional cancer registries or based on a combination of methods, including health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next-of-kin. Closure dates for the present study were defined as the latest date of complete follow-up for both cancer incidence and vital status, 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.

Selection of case and control subjects

Case subjects were men and women who developed CRC after recruitment and before the end of the study period (defined for each study center by the latest enddate of follow-up). Colon cancers were defined as tumors in the cecum, appendix, ascending colon, hepatic flexure, transverse colon, splenic flexure, and descending and sigmoid colon (C18.0–C18.7); rectal cancers included tumors in the recto-sigmoid junction (C19) or rectum (C20), following the 10th Revision of the International Statistical Classification of Diseases, Injury and Causes of Death (32). The present study comprises of 1,129 incident cases of CRC (713 colon, 416 rectum). According to tumor stage, there were 5 cases with carcinoma *in situ*, 230 localized (Stage I), 215 localized with invasion (Stage II), 339 metastatic regional (Stage III), and 120 metastatic distal (Stage IV). Tumor stage data were missing for 220 (16.5%) of the CRC cases. For each case one control subject was chosen at random among risk sets consisting of all cohort members alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the index case. An incidence density sampling protocol for control selection was used, such that controls could include subjects who became a case later in time, while each control subject could also be sampled more than once. Matching characteristics were: study center, sex, age, time at blood collection, fasting status; and among women, menopausal status. Premenopausal women were matched on phase of menstrual cycle and postmenopausal women were matched on current hormonal replacement therapy (HRT) use. Our analysis includes 5 participants who were selected as controls and subsequently had CRC during follow-up. In addition, 5 participants were selected twice as controls during the random selection process. For these participants, leptin and sOB-R

were measured from one of the individuals' aliquots and assigned to each set the individuals appeared in.

Blood collection and laboratory procedures

In EPIC, blood samples were collected, processed, divided into heat-sealed straws, and stored in liquid nitrogen freezers (−196°C). Storage protocols differed in Denmark and Sweden, where tubes were stored in the vapor phase of liquid nitrogen (−150°C) or in −80°C freezers, respectively. Approval was obtained from the ethics review board of the International Agency for Research on Cancer and the local review boards pertaining to the participating institutions. Single measurements of leptin and sOB-R concentrations were conducted using ELISA from Biovendor. The minimum detectable levels were 0.17 ng/mL for leptin, and 0.40 ng/mL for sOB-R. According to the manufacturer, the intraassay (within-run) and interassay (run-to-run) coefficients of variation (CV) were 5.9% ($n = 8$) and 5.6% ($n = 6$) for leptin, and 8.4% ($n = 8$) and 6.7% ($n = 7$) for sOB-R, respectively. To verify assay performance, the laboratory additionally conducted internal quality-control analyses across the analytical range of the assay, such as low, medium, and high controls. From each of these samples, the laboratory created 33 aliquots and put 1 aliquot from each sample on each of the 33 plates. The assays were run on a weekly basis over a 5-month period (from September 14, 2009 until March 1, 2010). In this analysis, the interassay CVs for leptin were 4.5% for low (mean, 5.3 ng/mL); 7.9% for medium (mean, 11.0 ng/mL); and 5.9% for high levels (mean, 26.1 ng/mL); for sOB-R the respective CVs were 10.0% (mean, 10.9 ng/mL); 9.7% (mean, 11.0 ng/mL); and 9.4% (mean, 27.2 ng/mL). In reproducibility studies reported intraclass correlation coefficients for leptin were 0.74, over 4 years in the Health Professionals Follow-up Study (33), 0.83, over 1 year in the Janus cohort study (10), 0.84 over 4 seasons in MONICA and Västerbotten Intervention Study (34), and for sOB-R, 0.82 over 3 years in the Nurses' Health Study (35).

The measurement of other biomarkers included in the analysis: C-peptide, insulin-like growth factor I (IGF-I), glycated haemoglobin (HbA1c), triglycerides (TG), high-density lipoprotein cholesterol HDL-C and high-sensitivity C-reactive protein (hsCRP) has been described elsewhere (16–19). Excluded from the study were subjects with missing information on leptin or sOB-R measurements (131 case sets).

Lifestyle and dietary assessment

Participants completed questionnaires on sociodemographic and lifestyle characteristics according to standardized EPIC protocols (36). Body weight and WC were measured in all centers with the exception of a subgroup of participants from the health conscious in Oxford (United Kingdom). For those participants comprising of 28 case sets in our analysis, prediction equations have been applied to predict sex- and age-specific anthropometric values from subjects with self-reported body measures (37, 38). Data was missing in 65 case sets (5.8%) for the measurement of WC. For each of the variables, fiber, alcohol, fish and shellfish, fruits and vegetables, red and processed meat, there were 2 missing values. Under the assumption that these values are missing at random and after proving that the pattern of missing data was arbitrary, we applied multiple imputation technique (39). All variables (exposure, outcome, and covariates) included in the regression model were also included in the procedure. Ten duplicate data sets were sampled from their predictive distribution based on the observed data with the missing values replaced by imputed values. In a sensitivity analysis, the main associations were compared with estimates from a complete-case analysis ($n = 2{,}126$) to investigate whether missing observations of the covariates influenced the effect estimates.

Statistical analysis

Spearman partial correlation coefficients, adjusted for age at study recruitment and sex, were estimated to assess the correlations between baseline leptin and sOB-R concentrations, anthropometric measures, and metabolic markers in the controls. Relative risks (RR), estimated from ORs as derived from the risk set sampling design (40) and 95% confidence intervals (CI) were computed from conditional logistic regression models. Participants were divided into quintiles based on the biomarker distributions among controls and *P* values for trends across quintiles were calculated using the median biomarker levels within quintiles as a metric variable. These associations were also assessed on continuous scale, using log-transformed levels of leptin and sOB-R. Multivariable-adjusted models were constructed with *a priori* chosen covariates: smoking status, education, sex-specific categories of physical activity, alcohol, fiber, red and processed meat, fruits and vegetables, fish and shellfish, BMI, and WC. The final multivariable model also accounted for mutual adjustment of leptin and sOB-R. To examine whether leptin interacts with sOB-R to modify its association with colon cancer, RRs and 95% CIs were calculated for 9 combinations of tertiles of leptin and sOB-R, with reference to the combination of the lowest tertile of leptin and sOB-R. Regression splines (with 3 knots at the 5th, 50th, and 95th percentiles of the biomarker distribution) and likelihood ratio test were used to check whether a nonlinear term of each biomarker added significant information to the model. We have repeated the main analyses also for the ratio of leptin to sOB-R (free leptin index, FLI).

In additional analyses, based on a subset of 566 case sets with available measurements for C-peptide, IGF-I, HbA1c, TG, HDL-C, and hsCRP levels, the association of leptin and sOB-R with CRC was adjusted for each of these biomarkers all included as continuous variables. The associations were further examined by sex, age, cancer stage (I and II vs. III and IV), and different strata of CRC risk factors. Effect modification on the multiplicative scale was tested using interaction terms of log-transformed biomarker concentrations multiplied by stratum variables. The main multivariable analyses were repeated after excluding individuals with extreme biomarker levels, nonfasting participants and those with self-reported diabetes at baseline. To account for potential reverse causality, the main analyses were repeated also after excluding cases that occurred in the first 2 years of follow-up. Two-sided *P* values of less than 0.05 were considered to indicate statistical significance. All statistical analyses were conducted using Statistical Analysis System, Version 9.2, software (SAS Institute, Inc.).

Results

Table 1 shows the baseline characteristics of the study population. Colon cancer cases had higher leptin concentrations and lower sOB-R concentrations than controls. Among controls, after adjustment for age and sex, leptin concentrations were inversely correlated with sOB-R ($r = -0.54$) and positively correlated with BMI ($r = 0.60$) and WC ($r = 0.54$). In contrast, sOB-R concentrations were inversely related to BMI ($r = -0.43$) and WC ($r = -0.38$; Table 2). Median concentrations of leptin and sOB-R did not differ substantially according to tumor stage (Supplementary Table S1).

In conditional logistic regression, in the crude model, leptin was not statistically significantly associated with CRC (RR for the highest vs. the lowest quintile, 1.20; 95% CI, 0.86–1.67; $P_{trend} = 0.68$; Table 3). When the associations were explored by cancer site, individuals in the 4th quintile had a higher risk of colon cancer compared with those in the lowest quintile, although the trend across quintiles was not statistically significant (RR, 1.79; 95% CI, 1.19–2.69; $P_{trend} = 0.46$; $P_{nonlinearity} = 0.04$). No association was observed for rectal cancer ($P_{for\ difference\ by\ cancer\ site} = 0.23$). Adjustment for BMI and WC strongly attenuated the association with colon cancer (RR for the highest vs. the lowest quintile, 0.93; 95% CI, 0.54–1.60; $P_{trend} = 0.27$; Table 3). When sOB-R was additionally included in the multivariable model, leptin concentrations were inversely, although not significantly associated with colon cancer risk

Table 1. Selected baseline characteristics of incident colon and rectal cancer cases and their matched controls, the EPIC, 1992 to 2003

	Colon cancer		Rectal cancer	
Characteristics	**Cases**	**Controls**	**Cases**	**Controls**
N	713	713	416	416
Female sex, %	55.1	55.1	45.4	45.4
Mean age, years	58.6	58.6	57.9	57.9
Smoking status, %				
Never smoker	43.2	45.4	38.9	39.9
Former smoker	33.1	32.7	33.2	31.0
Current smoker	22.3	21.0	27.2	27.9
Physical activity, %				
Inactive	14.3	11.2	15.6	13.0
Moderately inactive	28.3	27.8	26.7	23.8
Moderately active	41.5	43.3	40.6	41.4
Active	9.5	11.1	10.8	14.2
Premenopausal women, %	10.4	10.7	10.1	10.6
Hormonal replacement therapy (HRT) use, %	12.0	11.0	10.4	9.5
BMI, kg/m^{2a}	26.8	26.2	26.3	26.4
WC, cm[a]	90.3	88.0	89.8	89.7
Leptin, ng/mL[b]	9.7 (4.9–18.5)	8.9 (4.2–18.7)	7.5 (3.6–15.1)	7.6 (3.6–14.6)
sOB-R, ng/mL[b]	20.2 (16.1–24.1)	21.2 (17.4–26.3)	20.5 (16.8–26.0)	21.2 (17.2–25.1)
Free leptin index, median (IQR)	0.45 (0.21–1.06)	0.41 (0.17–1.02)	0.35 (0.16–0.79)	0.36 (0.15–0.85)
C-peptide, ng/mL[b],	3.9 (2.8–5.9)	3.8 (2.7–5.8)	4.0 (2.8–6.3)	3.9 (2.6–5.7)
HbA1c, %[b]	5.8 (5.5–6.1)	5.7 (5.5–6.0)	5.7 (5.5–6.0)	5.7 (5.5–6.0)
HDL-C, mmol/L[b]	1.37 (1.1–1. 7)	1.42 (1.2–1.7)	1.43 (1.2–1.7)	1.39 (1.1–1.6)
TG, mmol/L[b]	1.41 (1.01–2.18)	1.40 (0.96–2.00	1.51 (1.03–2.13)	1.49 (1.01–2.19)
IGF-I, ng/mL[b]	208.7 (166.8–253.0)	205.1 (162.8–257.8)	212.0 (168.2–262.1)	206.1 (169.6–249.2)
CRP-hs, mg/L[b]	3.05 (1.20–5.5)	2.4 (1.1–4.7)	2.3 (0.9–4.3)	2.3 (0.9–4.1)
Alcohol, g/d[b]	8.0 (2.3–18.1)	7.4 (2.8–16.6)	11.3 (3.3–26.1)	10.0 (3.4–21.3)
Fiber, g/d[b]	22.1 (17.1–27.7)	22.3 (18.1–27.4)	21.7 (17.5–27.3)	22.4 (17.7–27.5)
Fruit and vegetable, g/d[b]	379.1 (241.7–542.2)	398.3 (255.8–558.3)	355.2 (236.9–508.5)	369.2 (242.9–533.5)
Fish and shellfish, g/d[b]	25.3 (13.2–43.8)	28.2 (13.6–49.0)	26.5 (13.9–44.5)	28.2 (13.5–50.0)
Red meat, g/d[b]	45.0 (24.2–73.8)	46.3 (24.3–73.9)	53.3 (32.2–82.4)	48.8 (29.6–76.7)
Processed meat, g/d[b]	25.1 (13.1–41.1)	23.6 (12.6–41.5)	27.2 (13.7–47.4)	26.7 (12.8–48.1)

NOTE: Sex, age, menopausal status, and HRT use were among the matching criteria.

[a]Values expressed as means (SD).

[b]Values expressed as medians (interquartile range).

Table 2. Age- and sex-adjusted Spearman partial correlation coefficients of baseline concentrations of leptin and sOB-R with anthropometric measures and biomarkers among controls, the EPIC, 1992 to 2003

	Leptin		sOB-R	
	R	***P* value**	***R***	***P* value**
sOB-R, ng/mL	−0.54	<0.0001	—	—
BMI, kg/m^2	0.60	0.0001	−0.43	<0.0001
WC, cm	0.54	<0.00001	−0.38	<0.0001
C-peptide, ng/mL	0.31	<0.0001	−0.35	<0.0001
HbA1c, %	0.15	0.0001	−0.14	0.0004
HDL-C, mmol/L	–0.15	<0.0001	0.27	<0.0001
TG	0.22	<0.0001	−0.24	<0.0001
IGF-I	0.03	0.49	−0.18	<0.0001
CRP-hs, mg/L	0.28	<0.0001	−0.19	<0.0001

NOTE: This analysis is based on a subset of control participants with available measurement of C-peptide ($n = 713$); HbA1c ($n = 667$); HDL-C and TG ($n = 1{,}127$); IGF-I ($n = 1696$), and CRP-hs ($n = 1{,}052$).

(RR, 0.71; 95% CI, 0.40–1.26, $P_{trend} = 0.05$; Table 3). sOB-R was strongly inversely associated with CRC risk in the crude model as well as in the final multivariable model adjusted also for BMI, WC, and baseline leptin concentrations (RR, 0.55; 95% CI, 0.40–0.76; $P_{trend} = 0.0004$; Table 4). When analyzed by cancer site, sOB-R was inversely associated with colon cancer (RR, 0.42; 95% CI, 0.28–0.63; $P_{trend} = 0.0001$), but not with rectal cancer (RR, 0.83; 95% CI, 0.48–1.44; $P_{trend} = 0.38$; $P_{for\ difference\ by\ cancer\ site} = 0.03$). The FLI was not statistically significantly associated with CRC after adjustment for BMI and WC (RR, 1.21; 95% CI, 0.81–1.80; $P_{trend} = 0.97$; Supplementary Table S2).

Figure 1 shows the joint associations between leptin and sOB-R. There was no statistically significant interaction between the 2 biomarkers ($P_{interaction} = 0.40$). When the participants were cross-classified, such that those with low sOB-R and low leptin levels were taken as a reference group, within each tertile of leptin levels, the individuals with high sOB-R concentrations had the lowest risk of colon cancer.

Among participants who had biomarker information available for all case-control sets (566 case sets), the associations of leptin and sOBR with CRC risk did not essentially change when C-peptide, HbA1c, TG, HDL-cholesterol, IGF-I, and CRP-hs, were included in the multivariable model individually or in combination. For example, for leptin the multivariable-adjusted RR of CRC for the highest quintile compared with the lowest was 0.87 (95% CI, 0.49–1.62; $P_{trend} = 0.56$) before adjustment for all biomarkers and 0.84 (95% CI, 0.44–1.57; $P_{trend} = 0.43$) after adjustment; the respective risk estimates for sOB-R were 0.56 (95% CI, 0.40–0.84; $P_{trend} = 0.007$) and 0.52 (95% CI, 0.33–0.83; $P_{trend} = 0.02$).

In sensitivity analyses, the risk estimates were not substantially changed after excluding individuals diagnosed with cancer within the first 2 years of follow-up, nonfasting people, diabetics, or those with extreme leptin and sOB-R concentrations (Supplementary Table S3). In analysis stratified according to BMI and WC, sOB-R was associated with CRC risk only in the group with low BMI, albeit the difference was not statistically significant ($P_{interaction} = 0.08$; Supplementary Table S3). There were no substantial differences by sex in the associations of CRC with leptin ($P_{for\ difference\ by\ sex} = 0.60$; Supplementary Table S4) or with sOB-R ($P_{for\ difference\ by\ sex} = 0.58$; Supplementary Table S5). Results from the complete-case analysis were comparable with those derived from the multiple imputation procedure (data not shown).

Discussion

In this large prospective study, sOB-R concentrations were strongly inversely associated with CRC risk, independent of adiposity measures, baseline leptin concentrations, and metabolic markers. In contrast, adiposity-adjusted leptin concentrations were not related to CRC risk. These data suggest that sOB-R may be a protein that is important for the development of CRC.

To our knowledge, we are the first epidemiologic study on the relation between circulating sOB-R concentrations and CRC risk. The interpretation of our findings is challenging because in humans the action of sOB-R that might be relevant for colorectal tumorigenesis is not entirely clear. Experimental data suggests that *in vivo* sOB-R may suppress leptin action through inhibition of specific leptin binding to membrane-bound receptors (25). Thus, *in vivo* sOB-R was suggested to delay leptin clearance and increase leptin bioavailability in the circulation (25). On the other hand, sOB-R may represent the activities of the short form of leptin receptor (OB-Rs) that is expressed primarily in peripheral tissues (41). This might be an important detail of understanding sOB-R–CRC association, because OB-Rs may mediate the effects of insulin sensitivity and other peripheral effects that might be relevant for CRC (42). Moreover, serum sOB-R was suggested as a marker reflecting the impairment of leptin action in type 2 diabetes (43). Interestingly, our findings are similar to those reported with regards to risk of type 2 diabetes. In particular, a recent nested case-control study of 1,054 cases and 1,054 controls, embedded in the Nurses Health Study Cohort, Sun and colleagues (30) observed a strong inverse association between circulating sOB-R and diabetes, independent of obesity and

Table 3. RRs and 95% CIs of colon and rectal cancer according to quintiles of baseline leptin concentrations, the EPIC, 1992 to 2003

	Quintiles							
RR[a] (95% CI)	**1**	**2**	**3**	**4**	**5**		**Continuously**	
Median leptin (ng/mL)	**2.1**	**4.7**	**8.3**	**14.2**	**29.8**	***P* trend[b]**	**per doubling[c]**	***P* value[c]**
CRC								
Cases/controls, *n*	198/225	228/226	237/227	247/225	219/226			
Model 1	1.00 (Referent)	1.17 (0.89–1.54)	1.26 (0.94–1.69)	1.35 (0.99–1.84)	1.20 (0.86–1.67)	0.68	1.06 (0.98–1.14)	0.13
Model 2	1.00 (Referent)	1.12 (0.85–1.48)	1.23 (0.91–1.67)	1.25 (0.91–1.73)	1.14 (0.81–1.61)	0.85	1.05 (0.97–1.13)	0.25
Model 3	1.00 (Referent)	1.03 (0.77–1.37)	1.04 (0.75–1.43)	1.01 (0.70–1.45)	0.85 (0.56–1.29)	0.23	0.98 (0.89–1.09)	0.76
Model 4	1.00 (Referent)	0.97 (0.72–1.29)	0.93 (0.67–1.30)	0.86 (0.59–1.25)	0.68 (0.43–1.05)	0.04	0.93 (0.83–1.03)	0.16
Colon cancer								
Cases/controls, *n*	103/134	143/137	148/144	168/141	151/157			
Model 1	1.00 (Referent)	1.43 (1.00–2.05)	1.50 (1.02–2.21)	1.79 (1.19–2.69)	1.49 (0.96–2.31)	0.46	1.10 (1.00–1.21)	0.05
Model 2	1.00 (Referent)	1.37 (0.94–1.99)	1.48 (0.99–2.23)	1.74 (1.14–2.66)	1.45 (0.92–2.29)	0.54	1.09 (0.99–1.21)	0.09
Model 3	1.00 (Referent)	1.20 (0.81–1.76)	1.17 (0.76–1.79)	1.29 (0.81–2.05)	0.93 (0.54–1.60)	0.27	0.99 (0.87–1.13)	0.86
Model 4	1.00 (Referent)	1.10 (0.74–1.62)	1.03 (0.66–1.59)	1.08 (0.67–1.74)	0.71 (0.40–1.26)	0.05	0.92 (0.80–1.05)	0.22
Rectal cancer								
Cases/controls, *n*	95/91	85/89	89/83	79/84	68/69			
Model 1	1.00 (Referent)	0.90 (0.59–1.38)	1.01 (0.64–1.59)	0.88 (0.53–1.45)	0.92 (0.55–1.53)	0.79	1.00 (0.89–1.13)	0.97
Model 2	1.00 (Referent)	0.87 (0.55–1.35)	0.97 (0.60–1.56)	0.74 (0.43–1.26)	0.89 (0.51–1.53)	0.76	0.99 (0.87–1.12)	0.86
Model 3	1.00 (Referent)	0.88 (0.56–1.39)	0.95 (0.56–1.60)	0.72 (0.39–1.34)	0.88 (0.43–1. 78)	0.88	1.00 (0.85–1.18)	0.98
Model 4	1.00 (Referent)	0.85 (0.54–1.35)	0.88 (0.52–1.51)	0.64 (0.33–1.22)	0.76 (0.36–1.61)	0.67	0.97 (0.82–1.16)	0.77

[a]Model 1, taking into account matching factors: age, sex, study center, 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 HRT use.

Model 2, Model 1 + smoking status, education, alcohol consumption, physical activity, fiber intake, consumption of fruits and vegetables, red and processed meat, fish and shellfish.

Model 3, Model 2+ BMI and WC.

Model 4, Model 3 + sOB-R concentrations.

[b]*P* value for trend, calculated using the median leptin concentrations within categories of leptin as a continuous variable.

[c]Multivariable-adjusted RR associated with an increase in continuous log-transformed leptin concentrations by log 2.

Table 4. RRs and 95% CIs of colorectal, colon and rectal cancer according - quintiles of baseline soluble leptin receptor (sOB-R) concentration, the European Prospective Investigation into Cancer and Nutrition (EPIC), 1992–2003

	Quintiles							
RR[a] (95% CI)	**1**	**2**	**3**	**4**	**5**		**Continuously**	
Median sOB-R (ng/mL)	**14.3**	**18.0**	**21.2**	**24.8**	**31.5**	***P* trend**	**per doubling[b]**	***P* value[c]**
CRC								
Cases/controls, *n*	290/228	217/219	250/233	192/223	180/226			
Model 1	1.00 (Referent)	0.74 (0.57–0.97)	0.80 (0.62–1.04)	0.65 (0.49–0.85)	0.60 (0.46–0.79)	0.0002	0.69 (0.58–0.82)	<0.0001
Model 2	1.00 (Referent)	0.73 (0.55–0.95)	0.77 (0.59–1.00)	0.64 (0.48–0.85)	0.58 (0.44–0.77)	0.0002	0.67 (0.56–0.81)	<0.0001
Model 3	1.00 (Referent)	0.74 (0.56–0.97)	0.79 (0.59–1.04)	0.66 (0.49–0.88)	0.61 (0.45–0.83)	0.002	0.69 (0.57–0.84)	0.0002
Model 4	1.00 (Referent)	0.70 (0.53–0.93)	0.74 (0.55–0.98)	0.60 (0.44–0.81)	0.55 (0.40–0.76)	0.0004	0.64 (0.52–0.79)	<0.0001
Colon cancer								
Cases/controls, *n*	195/140	132/147	169/141	119/138	98/147			
Model 1	1.00 (Referent)	0.60 (0.43–0.84)	0.79 (0.56–1.11)	0.57 (0.40–0.81)	0.45 (0.32–0.65)	<0.0001	0.59 (0.47–0.74)	<0.0001
Model 2	1.00 (Referent)	0.59 (0.42–0.84)	0.75 (0.53–1.06)	0.55 (0.38–0.79)	0.43 (0.30–0.63)	<0.0001	0.51 (0.39–0.67)	<0.0001
Model 3	1.00 (Referent)	0.62 (0.43–0.88)	0.79 (0.55–1.13)	0.59 (0.40–0.87)	0.49 (0.33–0.72)	0.0009	0.61 (0.48–0.80)	0.0001
Model 4	1.00 (Referent)	0.57 (0.39–0.82)	0.72 (0.50–1.04)	0.51 (0.35–0.77)	0.42 (0.28–0.63)	0.0001	0.55 (0.42–0.72)	<0.0001
Rectal cancer								
Cases/Controls, n	95/88	85/72	81/92	73/85	82/79			
Model 1	1.00 (Referent)	1.07 (0.69–1.67)	0.81 (0.53–1.23)	0.79 (0.51–1.23)	0.96 (0.62–1.48)	0.54	0.87 (0.66–1.15)	0.33
Model 2	1.00 (Referent)	1.01 (0.64 - 1.60)	0.79 (0.50–1.24)	0.77 (0.49–1.23)	0.91 (0.58–1.45)	0.50	0.86 (0.64–1.15)	0.31
Model 3	1.00 (Referent)	0.98 (0.62–1.57)	0.75 (0.47–1.21)	0.72 (0.43–1.17)	0.83 (0.49–1.41)	0.34	0.80 (0.57–1.12)	0.20
Model 4	1.00 (Referent)	0.98 (0.61–1.58)	0.75 (0.46–1.23)	0.71 (0.43–1.19)	0.83 (0.48–1.44)	0.38	0.80 (0.56–1.14)	0.22

[a]Model 1, Taking into account matching factors: age, sex, study center, 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 HRT use.
Model 2, Model 1 + smoking status, education, alcohol consumption, physical activity, fiber intake, consumption of fruits and vegetables, red and processed meat, fish and shellfish.
Model 3, Model 2 + BMI and WC.
Model 4, Model 3 + leptin concentrations.
[b]*P* value for trend, calculated using the median sOB-R concentrations within categories of sOB-R as a continuous variable.
[c]Multivariable-adjusted RR associated with an increase in continuous log-transformed sOB-R concentrations by log 2.

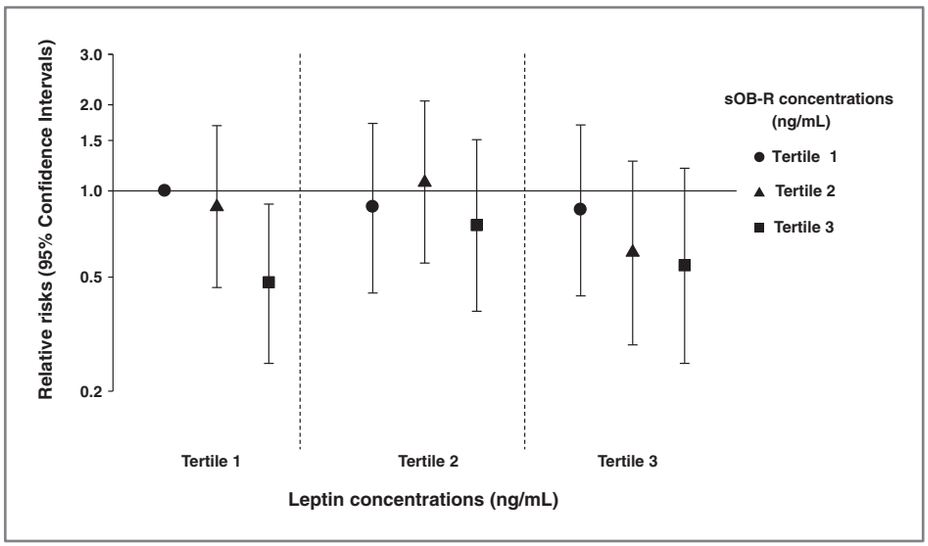

Figure 1. RRs and 95% CIs for the joint effects of leptin and sOB-R on colon cancer risk, the EPIC, 1992 to 2003. NOTE: RRs and 95% CIs for 9 combinations of tertiles of leptin and sOB-R, with reference to the combination of the lowest tertile of leptin and sOB-R. RRs taking into account matching factors, smoking, education, alcohol, physical activity, fiber, fruits and vegetables, red and processed meat, fish and shellfish, BMI, and WC. RRs are plotted on a logarithmic scale. Median concentrations of leptin within first, second, and third tertile were 2.96 ng/mL, 8.31 ng/mL, and 23.4 ng/mL for sOB-R, respectively, 15.8 ng/mL, 21.2 ng/mL, and 28.6 ng/mL.

leptin levels. Although the *in vitro* carcinogenic effects of leptin signaling through sOB-R in CRC cell lines are well characterized, the *in vivo* effects are quite complex and can be affected by multiple factors. Therefore, more studies taking into account leptin regulatory functions in energy balance, inflammation, and insulin signaling as contributors of CRC are needed to shed light on the underlying mechanisms of the observed associations.

In contrast to the strong independent association for sOB-R, leptin was not related with overall CRC risk and although our data was suggestive of an association with CRC, it was largely explained by the degree of adiposity (as measured by BMI and WC). Our data also did not support the role of FLI, which is considered to better reflect leptin activity (43, 44). These results are in contrast to the evidence from 2 previous prospective studies, which reported that leptin is associated to risk of CRC independently of BMI. In a nested case-control study in Northern Sweden among 168 cases and 327 controls, BMI-adjusted leptin levels were associated with about 2-fold higher risk of colon cancer in men (11); whereas a Japanese nested case-control study among 58 cases and 145 controls reported similar associations for the combined risk of colon and rectal cancer in women (12). Compared with these prior investigations, our study was based on a much larger number of cases. Furthermore, we were able to provide more accurate adjustment for obesity indices, which were measured rather than self-reported in majority of EPIC centers. More importantly, these previous studies did not account for the effect of WC and therefore could not rule out that the reported associations between leptin and CRC were confounded by the presence of abdominal fatness, which is more strongly associated with CRC compared with BMI (7). Recently, Women's Health Initiative cohort reported that in postmenopausal women leptin concentrations were associated with CRC risk even after adjustment for measured WC (RR for the highest quartile vs. the lowest, 1.76; 95% CI, 1.11–2.77, $P_{trend} = 0.07$). However, when insulin was also included in the model the association was no longer statistically significant (RR, 1.57; 95% CI, 0.98–2.51, $P_{trend} = 0.22$), indicating that the association may have been explained by hyperinsulinemia (13). Because of the close biologic relationship between adiposity and leptin it is difficult to separate independent effects and a possible colinearity between these 2 factors should be considered when interpreting the results.

Interestingly, when adjusted for sOB-R, leptin concentrations became inversely related to colon cancer risk, which may be indicative for the strong influence of sOB-R on the association. However, as the correlation between leptin and sOB-R is relatively high this effect might have been due to statistical (colinearity) rather than biologic reasons.

Our findings suggest that sOB-R concentrations were inversely associated with risk of colon cancer, but not with rectal cancer; although, it must be noted that the number of colon cancer cases in this study was higher than the number of rectal cancer cases. Colon versus rectal cancer differences have been previously reported to exist also for associations with physical inactivity (45), hyperinsulinemia (16), hyperglycemia (17), dyslipidemia (18), inflammation (19), and metabolic syndrome (32). Because differences in etiology and risk between colon and rectal cancer are plausible, further studies are needed with adequate power to confirm the observed associations by cancer site.

In the subgroup analyses, sOB-R was associated with lower risk among nonobese individuals compared with their obese counterparts, although these differences were not significant. Obesity is associated with decreasing levels of sOB-R in humans, whereas weight loss increases it (46, 47), which may explain potential coupling of the effect between lean body mass and sOB-R. In practical terms, these findings add to the rationale in reducing body weight as an effective way for CRC prevention.

Strengths of our study include its prospective design and the largest to date number of incident cases, which allowed conducting detailed analyses by cancer site and sex. The study included participants with a broad range of characteristics from Western European countries, therefore presented findings could be generalizable to Western populations.

Some limitations should be taken into account when interpreting the results, as well. A single assessment of leptin and sOB-R concentrations at baseline may be susceptible to short-term variation, which could bias results toward the null. However, previously, leptin and sOB-R levels were reported to be stable over time within individuals, with no evidence of seasonal fluctuation in the population observed, indicating high reliability of single measurements (33–35, 48, 49). Along with these, some studies suggest that plasma leptin levels are influenced by fasting/satiety states (50). However, when we restricted our analysis only to fasting participants no significant changes in results were observed. Despite exclusion of participants with cancer at baseline, we cannot exclude the possibility that some individuals had yet undiagnosed cancer. However, results did not change appreciably after we excluded people with a follow-up time of less than 2 years.

In conclusion, sOB-R was strongly inversely associated with risk of CRC, independent of adiposity measures, leptin and circulating metabolic biomarkers. Adiposity-adjusted leptin was not related to CRC risk. These novel findings suggest potentially important role of sOB-R, as an independent factor for CRC risk. Further research is needed to replicate these findings and to shed light on the underlying mechanisms.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acknowledgments

The authors thank the participants, study and administrative staff, and researchers in the European Prospective Investigation into Cancer and Nutrition Cohort for their outstanding cooperation.

Grant Support

This work has been supported by World Cancer Research Fund International and Wereld Kanker Onderzoek Fonds (WCRF NL). The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by Danish Cancer Society (Denmark); Ligue contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM; France); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum and Federal Ministry of Education and Research (Germany); Hellenic Health Foundation, the Stavros Niarchos Foundation, and the Hellenic Ministry of Health (Greece); Italian Association for Research on Cancer (AIRC) and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); ERC-2009-AdG 232997 and Nordforsk, Nordic Centre of Excellence programme on Food, Nutrition and Health (Norway); Health Research Fund (FIS), Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, ISCIII RETIC (RD06/0020) (Spain); Swedish Cancer Society, Swedish Scientific Council and Regional Government of Skåne and Västerbotten (Sweden); Cancer Research UK, Medical Research Council, Stroke Association, British Heart Foundation, Department of Health, Food Standards Agency, and Wellcome Trust (United Kingdom).

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Received February 7, 2012; revised August 13, 2012; accepted August 19, 2012; published OnlineFirst August 27, 2012.

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