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 (n=246)

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 physiological functions; however its relation to CRC risk is unknown. This study explored the association of leptin and sOB-R with risk of colorectal cancer in a prospective nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. 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 (RRs) and 95% confidence intervals (95% CIs). After multivariable adjustment including body mass index, waist circumference and baseline leptin concentrations, sOB-R was strongly inversely associated with CRC (RR comparing the highest quintile versus the lowest,0.55; 95% CI, 0.40-0.76; \( P_{\text{trend}} = 0.0004 \)) and colon cancer (RR, 0.42; 95% CI,0.28-0.63, \( P_{\text{trend}} = 0.0001 \)); whereas no association was seen for rectal cancer (RR adjusted for body mass index and waist circumference, 0.83; 95% CI, 0.48-1.44, \( P_{\text{trend}} = 0.38 \)). In contrast, leptin was not associated with risk of CRC (RR adjusted for body mass index and waist circumference, 0.85; 95% CI, 0.56-1.29, \( P_{\text{trend}} = 0.23 \)). 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.
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 tumour 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 epidemiological studies have investigated the association between leptin concentrations and CRC risk (8-13). In two 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 biological 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 epidemiological 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 colorectal cancer 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 over 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 nine 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 end-date 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 1129 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 case.
For each case one control subject was chosen at random among risk sets consisting of all
cohort members alive and free of cancer (except non-melanoma skin cancer) at the time of
diagnosis of the 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 vapour 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 performed using enzyme linked immunosorbent assays (ELISA) from Biovendor (Im Neuenheimer Feld 583, 69120 Heidelberg). The minimum detectable level of leptin was 0.17 ng/mL, while the minimum detectable level of sOB-R was 0.40 ng/mL. Single measurements of leptin and sOB-R concentrations were performed using enzyme linked immunosorbent assays (ELISA) from Biovendor (Im Neuenheimer Feld 583, 69120 Heidelberg). The minimum detectable levels were 0.17 ng/mL for leptin, and 0.40 ng/mL for sOB-R. According to the manufacturer, the intra-assay (within-run) and inter-assay (run-to-run) coefficients of variation (CVs) 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 performed internal quality-control analyses across the analytical range of the assay, such as low, medium and high controls. From each of these samples, the lab 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 14.09.2009 until 1.03.2010). In this analysis, the inter-assay 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 intra-class correlation coefficients (ICCs) 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-1 (IGF-1), glycated haemoglobin (HbA1c), triglycerides (TG), HDL-C, 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 socio-demographic 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 (UK). 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, fibre, 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 datasets 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=2126) 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 (RRs), estimated from odds ratios as derived from the risk set sampling design (40) and 95% confidence intervals (CI-s) 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 nine 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 non-linear 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, IGF1, HbA1c, TG, HDL-C and hs-CRP 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-II vs. III-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, non-fasting 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 performed using Statistical Analysis System (SAS), Version 9.2, software (SAS Institute, Inc., Cary, North Carolina).

**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 1).

In conditional logistic regression, in the crude model, leptin was not statistically significantly associated with CRC (RR for the highest versus the lowest quintile, 1.20; 95% CI, 0.86-1.67; $P_{\text{trend}} = 0.68$; 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, though the trend across quintiles was not statistically significant (RR, 1.79; 95% CI, 1.19-2.69; $P_{\text{trend}} = 0.46$; $P_{\text{non-linearity}} = 0.04$). No association was observed for rectal cancer ($P_{\text{for difference by cancer site}} = 0.23$). Adjustment for BMI and WC strongly attenuated the
association with colon cancer (RR for the highest versus the lowest quintile, 0.93; 95% CI, 0.54-1.60; \(P_{\text{trend}} = 0.27\); Table 3). When sOB-R was additionally included in the multivariable model, leptin concentrations were inversely, although not significantly associated with colon cancer risk (RR, 0.71; 95% CI, 0.40-1.26; \(P_{\text{trend}} = 0.05\); 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; \(P_{\text{trend}} = 0.0004\); Table 4). When analysed by cancer site, sOB-R was associated with colon cancer (RR, 0.42; 95% CI, 0.28-0.63; \(P_{\text{trend}} = 0.0001\)), but not with rectal cancer (RR, 0.83; 95% CI, 0.48-1.44; \(P_{\text{trend}} = 0.38\); \(P_{\text{for difference by cancer site}} = 0.03\)). The free leptin index (FLI) was not statistically significantly associated with CRC after adjustment for BMI and WC (RR, 1.21; 95% CI, 0.81-1.80; \(P_{\text{trend}} = 0.97\); Supplementary Table 2).

Figure 1 shows the joint associations between leptin and sOB-R. There was no statistically significant interaction between the two biomarkers (\(P_{\text{interaction}} = 0.40\)). 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 sOB with CRC risk did not essentially change when C-peptide, HbA1c, triglycerides, HDL-cholesterol, IGF-1 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 to the lowest was 0.87 (95% CI, 0.49-1.62; \(P_{\text{trend}} = 0.56\)) before adjustment for all biomarkers and 0.84 (95% CI, 0.44-1.57; \(P_{\text{trend}} = 0.43\)) after adjustment; the respective risk estimates for sOB-R were 0.56 (95% CI, 0.40-0.84; \(P_{\text{trend}} = 0.007\)) and 0.52 (95% CI, 0.33-0.83; \(P_{\text{trend}} = 0.02\)).
In sensitivity analyses, the risk estimates were not substantially changed after excluding individuals diagnosed with cancer within the first 2 years of follow-up, non-fasting people, diabetics, or those with extreme leptin and sOB-R concentrations (Supplementary Table 3). 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 ($P_{\text{interaction}} = 0.08$; Supplementary Table 3). There were no substantial differences by sex in the associations of CRC with leptin ($P_{\text{for difference by sex}} = 0.60$; Supplementary Table 4) or with sOB-R ($P_{\text{for difference by sex}} = 0.58$; Supplementary Table 5). Results from the complete-case analysis were comparable to 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 epidemiological 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 1054 cases and 1054 controls, embedded in the Nurses Health Study Cohort, Sun et al. (30) observed a strong inverse association between circulating sOB-R and diabetes, independent of obesity and 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 two 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 two-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. Further, 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 to BMI (7). Recently, Women's Health Initiative cohort reported that in postmenopausal women leptin concentrations were associated with colorectal cancer risk even after adjustment for measured WC (RR for the highest quartile versus 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). Due to the close biological relationship between adiposity and leptin it is difficult to separate independent effects and a possible co-linearity between these two 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 (co-linearity) rather than biological reasons.

Our findings suggest that sOB-R concentrations were associated with risk of colon cancer, but not with rectal cancer; though, 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). Since 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 non-obese individuals compared to their obese counterparts, though 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 performing 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, we restricted our analysis only to fasting participants and
could not detect significant changes in results. 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.
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### Table 1: Selected baseline characteristics of incident colon and rectal cancer cases and their matched controls, the European Prospective Investigation into Cancer and Nutrition (EPIC), 1992-2003

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Colon cancer</th>
<th>Rectal cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>Female sex, %</td>
<td>55.1</td>
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<tr>
<td>Smoking status, %</td>
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<tr>
<td>Never smoker</td>
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<td>Former smoker</td>
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<td>Current smoker</td>
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<td>Mean age, years</td>
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<td>58.6</td>
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<tr>
<td>Physical activity, %</td>
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<tr>
<td>Inactive</td>
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<tr>
<td>Moderately active</td>
<td>41.5</td>
<td>43.3</td>
</tr>
<tr>
<td>Active</td>
<td>9.5</td>
<td>11.1</td>
</tr>
<tr>
<td>Premenopausal women, %</td>
<td>10.4</td>
<td>10.7</td>
</tr>
<tr>
<td>Ever use of exogenous hormones in women, %</td>
<td>12.0</td>
<td>11.0</td>
</tr>
<tr>
<td>BMI, kg/m²*</td>
<td>26.8</td>
<td>26.2</td>
</tr>
<tr>
<td>WC, cm*</td>
<td>90.3</td>
<td>88.0</td>
</tr>
<tr>
<td>Leptin, ng/mL†</td>
<td>9.7</td>
<td>8.9</td>
</tr>
<tr>
<td>sOB-R, ng/mL†</td>
<td>20.2</td>
<td>21.2</td>
</tr>
<tr>
<td>HOMA-IR, median (IQR)</td>
<td>0.45</td>
<td>0.41</td>
</tr>
<tr>
<td>C-peptide, ng/mL†</td>
<td>3.9</td>
<td>3.8</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.8</td>
<td>5.7</td>
</tr>
<tr>
<td>HDL-C, mmol/L†</td>
<td>1.37</td>
<td>1.42</td>
</tr>
<tr>
<td>Triglycerides, mmol/L†</td>
<td>1.41</td>
<td>1.40</td>
</tr>
<tr>
<td>IGF1, ng/mL†</td>
<td>208.7</td>
<td>205.1</td>
</tr>
<tr>
<td>CRP-hs, mg/L†</td>
<td>3.05</td>
<td>2.4</td>
</tr>
<tr>
<td>Alcohol, g/d†</td>
<td>8.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Fiber, g/d†</td>
<td>22.1</td>
<td>22.3</td>
</tr>
<tr>
<td>Fruit and vegetable, g/d†</td>
<td>379.1</td>
<td>398.3</td>
</tr>
<tr>
<td>Fish and shellfish, g/d†</td>
<td>25.3</td>
<td>28.2</td>
</tr>
<tr>
<td>Red meat, g/d†</td>
<td>45.0</td>
<td>46.3</td>
</tr>
<tr>
<td>Processed meat, g/d†</td>
<td>25.1</td>
<td>23.6</td>
</tr>
</tbody>
</table>

Abbreviations: CRP-hs = high sensitive C-reactive protein; IGF1 = insulin-like growth factor 1; HRT = hormonal replacement therapy; sOB-R = soluble leptin receptor; HDL-C = high-density lipoprotein, HbA1c = glycated hemoglobin, BMI = body mass index; WC = waist circumference.

*Values expressed as means (standard deviation).
†Values expressed as medians (inter-quartile range). Note: Sex, age, menopausal status and HRT use were among the matching criteria.
**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 European Prospective Investigation into Cancer and Nutrition (EPIC), 1992-2003

<table>
<thead>
<tr>
<th></th>
<th>Leptin</th>
<th>sOB-R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P-value</td>
</tr>
<tr>
<td>sOB-R, ng/mL</td>
<td>-0.54</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>0.60</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WC, cm</td>
<td>0.54</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C-peptide, ng/mL</td>
<td>0.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>0.15</td>
<td>0.0001</td>
</tr>
<tr>
<td>HDL-C, mmol/l</td>
<td>-0.15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.22</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IGF-1</td>
<td>0.03</td>
<td>0.49</td>
</tr>
<tr>
<td>CRP-hs, mg/L</td>
<td>0.28</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Abbreviations: BMI = body mass index; WC = waist circumference; sOB-R = soluble leptin receptor; HDL-C = high-density lipoprotein; HbA1c = glycated haemoglobin; IGF1 = insulin-like growth factor 1; CRP-hs = high sensitive C-reactive protein.

*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); IGF1 (n=1696) and CRP-hs (n=1,052).
Table 3: Relative risks* (RR-s) and 95% confidence intervals (CI-s) of colon and rectal cancer according to quintiles of baseline leptin concentrations, the European Prospective Investigation into Cancer and Nutrition (EPIC), 1992-2003

<table>
<thead>
<tr>
<th>RR (95% CI)</th>
<th>Quintiles</th>
<th>P-trend†</th>
<th>Continuously per doubling‡</th>
<th>P-value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median leptin (ng/mL)</td>
<td>1 (2.1)</td>
<td>2 (4.7)</td>
<td>3 (8.3)</td>
<td>4 (14.2)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases /Controls, n</td>
<td>198/225</td>
<td>228/226</td>
<td>237/227</td>
<td>247/225</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00 (Referent)</td>
<td>1.17 (0.89 - 1.54)</td>
<td>1.26 (0.94 - 1.69)</td>
<td>1.35 (0.99 - 1.84)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00 (Referent)</td>
<td>1.12 (0.85 - 1.48)</td>
<td>1.23 (0.91 - 1.67)</td>
<td>1.25 (0.91 - 1.73)</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.00 (Referent)</td>
<td>1.03 (0.77 - 1.37)</td>
<td>1.04 (0.75 - 1.43)</td>
<td>1.01 (0.70 - 1.45)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.00 (Referent)</td>
<td>0.97 (0.72 - 1.29)</td>
<td>0.93 (0.67 - 1.30)</td>
<td>0.86 (0.59 - 1.25)</td>
</tr>
<tr>
<td>Colon cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases /Controls, n</td>
<td>103/134</td>
<td>143/137</td>
<td>148/144</td>
<td>168/141</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00 (Referent)</td>
<td>1.43 (1.00 - 2.05)</td>
<td>1.50 (1.02 - 2.21)</td>
<td>1.79 (1.19 - 2.69)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00 (Referent)</td>
<td>1.37 (0.94 - 1.99)</td>
<td>1.48 (0.99 - 2.23)</td>
<td>1.74 (1.14 - 2.66)</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.00 (Referent)</td>
<td>1.20 (0.81 - 1.76)</td>
<td>1.17 (0.76 - 1.79)</td>
<td>1.29 (0.81 - 2.05)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.00 (Referent)</td>
<td>1.10 (0.74 - 1.62)</td>
<td>1.03 (0.66 - 1.59)</td>
<td>1.08 (0.67 - 1.74)</td>
</tr>
<tr>
<td>Rectal cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases /Controls, n</td>
<td>95/91</td>
<td>85/89</td>
<td>89/83</td>
<td>79/84</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00 (Referent)</td>
<td>0.90 (0.59 - 1.38)</td>
<td>1.01 (0.64 - 1.59)</td>
<td>0.88 (0.53 - 1.45)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00 (Referent)</td>
<td>0.87 (0.55 - 1.35)</td>
<td>0.95 (0.60 - 1.56)</td>
<td>0.72 (0.43 - 1.26)</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.00 (Referent)</td>
<td>0.88 (0.56 - 1.39)</td>
<td>0.95 (0.56 - 1.60)</td>
<td>0.72 (0.39 - 1.34)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.00 (Referent)</td>
<td>0.85 (0.54 - 1.35)</td>
<td>0.88 (0.52 - 1.51)</td>
<td>0.64 (0.33 - 1.22)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI = body mass index; WC = waist circumference; sOB-R = soluble leptin receptor *Model 1: Taking into account 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 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.

†P-value for trend, calculated using the median leptin concentrations within categories of leptin as a continuous variable.

‡Multivariable-adjusted RR associated with an increase in continuous log-transformed leptin concentrations by log 2.
Abbreviations: BMI = body mass index; WC = waist circumference; sOB-R = soluble leptin receptor

*Model 1: Taking into account 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 matched by HRT use.

**Model 2: Model 1 + BMI and WC.

***Model 3: Model 2 + leptin concentrations.

†P-value for trend, calculated using the median sOB-R concentrations within categories of sOB-R as a continuous variable.

‡Multivariable-adjusted RR associated with an increase in continuous log-transformed sOB-R concentrations by log 2

Table 4: Relative risks* (RR-s) and 95% confidence intervals (CI-s) 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

<table>
<thead>
<tr>
<th>RR (95% CI)</th>
<th>Quintiles</th>
<th>P-trend</th>
<th>Continuously per doubling‡</th>
<th>P-value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median sOB-R (ng/mL)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases /Controls, n</td>
<td>290/228</td>
<td>217/219</td>
<td>250/233</td>
<td>192/223</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00 (Referent)</td>
<td>0.74 (0.57 - 0.97)</td>
<td>0.80 (0.62 - 1.04)</td>
<td>0.65 (0.49 - 0.85)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00 (Referent)</td>
<td>0.73 (0.55 - 0.95)</td>
<td>0.77 (0.59 - 1.00)</td>
<td>0.64 (0.48 - 0.85)</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.00 (Referent)</td>
<td>0.74 (0.56 - 0.97)</td>
<td>0.79 (0.59 - 1.04)</td>
<td>0.66 (0.49 - 0.88)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.00 (Referent)</td>
<td>0.70 (0.53 - 0.93)</td>
<td>0.74 (0.55 - 0.98)</td>
<td>0.60 (0.44 - 0.81)</td>
</tr>
<tr>
<td>Colon cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases /Controls, n</td>
<td>195/140</td>
<td>132/147</td>
<td>169/141</td>
<td>119/138</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00 (Referent)</td>
<td>0.60 (0.43 - 0.84)</td>
<td>0.79 (0.56 - 1.11)</td>
<td>0.57 (0.40 - 0.81)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00 (Referent)</td>
<td>0.59 (0.42 - 0.84)</td>
<td>0.75 (0.53 - 1.06)</td>
<td>0.55 (0.38 - 0.79)</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.00 (Referent)</td>
<td>0.62 (0.43 - 0.88)</td>
<td>0.79 (0.55 - 1.13)</td>
<td>0.59 (0.40 - 0.87)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.00 (Referent)</td>
<td>0.57 (0.39 - 0.82)</td>
<td>0.72 (0.50 - 1.04)</td>
<td>0.51 (0.35 - 0.77)</td>
</tr>
<tr>
<td>Rectal cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases /Controls, n</td>
<td>95/88</td>
<td>85/72</td>
<td>81/92</td>
<td>73/85</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00 (Referent)</td>
<td>1.07 (0.69 - 1.67)</td>
<td>0.81 (0.53 - 1.23)</td>
<td>0.79 (0.51 - 1.23)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00 (Referent)</td>
<td>1.01 (0.64 - 1.60)</td>
<td>0.79 (0.50 - 1.24)</td>
<td>0.77 (0.49 - 1.23)</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.00 (Referent)</td>
<td>0.98 (0.62 - 1.57)</td>
<td>0.75 (0.47 - 1.21)</td>
<td>0.72 (0.43 - 1.17)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.00 (Referent)</td>
<td>0.98 (0.61 - 1.58)</td>
<td>0.75 (0.46 - 1.23)</td>
<td>0.71 (0.43 - 1.19)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI = body mass index; WC = waist circumference; sOB-R = soluble leptin receptor

*Model 1: Taking into account 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 HRT use.

†P-value for trend, calculated using the median sOB-R concentrations within categories of sOB-R as a continuous variable.

‡Multivariable-adjusted RR associated with an increase in continuous log-transformed sOB-R concentrations by log 2
FIGURE LEGENDS

Figure 1

Legend: Relative risks and 95% confidence intervals for the joint effects of leptin and soluble leptin receptor (sOB-R) on colon cancer risk, the European Prospective Investigation into Cancer and Nutrition (EPIC), 1992-2003

Footnote: RRs and 95% CIs for nine combinations of tertiles of leptin and sOB-R, with reference to the combination of the lowest tertile of leptin and sOB-R. Relative risks 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.

Note: 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.

Abbreviations: BMI = body mass index; WC = waist circumference; sOB-R = soluble leptin receptor
Figure 1. Relative risks and 95% confidence intervals for the joint effects of leptin and sOB-R on colon cancer risk, the European Prospective Investigation into Cancer and Nutrition (EPIC), 1992-2003.
Leptin and Soluble Leptin Receptor in Risk of Colorectal Cancer in the European Prospective Investigation into Cancer and Nutrition Cohort

Krasimira Aleksandrova, Heiner Boeing, Mazda Jenab, et al.

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