Leptin Concentrations, Leptin Receptor Polymorphisms, and Colorectal Adenoma Risk

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Abstract

Obesity has been shown to be associated with an increased risk of both colorectal cancer and adenomatous polyps. One mechanism underlying this relationship may involve the growth-promoting effects of the circulating hormones associated with obesity, such as leptin. We conducted a gastroenterology clinic-based, case-control study to evaluate the relationship between circulating leptin concentrations and colorectal adenoma risk; in addition, we evaluated the relationship between *leptin receptor* polymorphisms and adenoma risk. Individuals with adenomas (n = 157) and colonoscopy-negative controls (n = 191), who had a clinically indicated colonoscopy, were recruited from a large health maintenance organization in the Seattle metropolitan area from 1999 to 2003. Odds ratios and 95% confidence intervals were obtained using logistic regression, adjusting for age at diagnosis, body mass index, family history of colorectal cancer, smoking history, nonsteroidal anti-inflammatory drug use, physical activity, and, among women, menopausal status and postmenopausal hormone use. Among men, those in the highest tertile of leptin concentrations had a 3.3-fold (95% confidence interval, 1.2-8.7) increased adenoma risk compared with those in the lowest tertile (P trend = 0.01). There were no associations between leptin concentrations and adenoma risk in women. There were no associations of *leptin receptor* genotypes or haplotypes and adenoma risk. The results of this study suggest that, in men, leptin may be associated with risk of colorectal adenomas. (Cancer Epidemiol Biomarkers Prev 2007;16(12):2697–703)

Introduction

Obesity, especially among men, has been shown in epidemiologic studies to be associated with an increased risk of colorectal cancer (1) and its common precursor lesion, adenomatous polyps (2-4). One hypothesized mechanism for this association may be the growth-promoting effects of adipokines, such as leptin, a biologically active polypeptide produced by adipose tissue (5). Leptin, which is highly correlated with body mass index (BMI) in humans (6-8), is involved in the regulation of body weight, appetite, and metabolism (9). It has also been implicated in cell proliferation and angiogenesis as well as in apoptotic inhibition (10-13). Several epidemiologic studies have examined the association between leptin and risk of breast (14, 15) and prostate cancers (16, 17); there have also been three studies of colorectal cancer that suggest that high leptin concentrations were associated with increased risk (18-20) but no studies of adenomas.

In addition, genetic differences responsible for circulating leptin levels may confer differential risk of colorectal neoplasia. Studies have indicated that polymorphisms in the gene encoding leptin that affect obesity are very rare (21). However, saturation of the leptin receptor (LEPR) may instead be the reason for high leptin concentrations in obese individuals (22). Several polymorphisms in the *LEPR* gene (23) may result in both altered circulating leptin concentrations (24) and possibly changes in risk of colorectal neoplasia. However, no prior study has examined these associations. We conducted a clinic-based, case-control study of adult men and women, ages 30 to 79 years, to assess the role of circulating leptin concentrations and genetic variation in *LEPR* on risk of colorectal adenomas, established precursor lesions of colorectal cancer (25).

Materials and Methods

Study Population. Subjects came from a clinic-based study seeking screening markers for colorectal cancer; participants included 738 male and female members of Group Health, a large nonprofit health care system in Washington State. Recruitment for the parent study was based on a sequential sample of individuals, ages 30 to 79 years, who underwent colonoscopy for any indication at the Group Health Central Gastroenterology Clinic between September 1998 and March 2003. All participants provided written informed consent, and the Group Health and Fred Hutchinson Cancer Research

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Center institutional review boards approved all study procedures. Based on pathology review of colonoscopy-obtained lesions, participants in the sample who were diagnosed with ulcerative colitis, colorectal cancer, hyperplastic polyps without additional findings at colonoscopy, or other nonadenoma pathologies were excluded from this current analysis; in addition, individuals who were previously diagnosed with colorectal cancer were also excluded. Eligible cases for this study included individuals with at least one pathologically confirmed adenomatous polyp (n = 192). Eligible controls were participants who also underwent colonoscopy and had normal colon and rectum pathology and who had no history of colorectal polyps (n = 218).

Data Collection. Participants were invited to enroll in the study before the scheduled colonoscopy. Questionnaires, administered before colonoscopy, were used to collect data, including current body weight, height, family history of colorectal cancer, usual physical activity per week, smoking history (ever use was defined as at least 100 cigarettes smoked in a lifetime), use of medications (including postmenopausal hormones for at least 6 months and nonsteroidal anti-inflammatory drugs (NSAID) at least once a week for at least 1 year), self-reported history of colorectal polyps, reproductive experiences, and demographics. Fasting blood samples were collected at the time of colonoscopy in EDTA tubes from consenting individuals. Blood samples were processed within 48 h to obtain EDTA plasma and buffy coats and were then stored in aliquots at −80°C until assayed; all samples used for assays had never been previously thawed.

Measurement of Leptin Concentrations. Circulating leptin concentrations were measured in EDTA plasma samples by ELISA using reagents from Diagnostic Systems Laboratories. All samples were run in duplicate and all intraassay coefficients of variation were <10%. Cases and controls were distributed equally throughout the plates. Two pooled control samples were included on each plate; interassay coefficients of variation for leptin were 5.3%. Limits of detection for assays were 0.05 ng/mL.

Identification and Selection of Single Nucleotide Polymorphisms. Based on previous reports, we selected candidate polymorphisms that seemed to have functional relevance, specifically polymorphisms either that were known or suspected to be associated with increased body mass or that alter circulating levels of leptin. In addition, because our population was primarily Caucasian (90%), the single nucleotide polymorphisms (SNP) had to have been previously found in Caucasians and, for this analysis, have a minor allele frequency of at least 5%. Five SNPs in the exonic regions of the *LEPR* gene were chosen: lysine to arginine at codon 109 (A>G; rs1137100), glutamine to arginine at codon 223 (A>G; rs1137101), the synonymous SNP serine at codon 343 (T>C; rs3790419), lysine to asparagine at codon 656 (G>C; rs8179183), and the synonymous SNP proline at codon 1019 (C>T; rs1805096).

Genotyping. DNA was extracted from buffy coats using the Qiagen Midi kit (Qiagen, Inc.). Genotypes were determined by Translational Genomics Research Institute using MassARRAY SNP genotyping. Briefly, SNP-specific PCR and single-base extension primers, designed using SpectroDESIGNER software (Sequenom, Inc.), were used in a multiplex PCR reaction. Multiplex assays were designed by assessing the cross-reaction of primers with each other and minimizing the risk of a primer attaching to a site other than at the position of interest on the PCR product. After the extension reactions, the samples were spotted onto a SpectroCHIP bioarray matrix pad (Sequenom) and then analyzed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (SpectroREADER, Sequenom). Mass spectra were processed and analyzed. In addition to standard quality control procedures, we included blinded duplicates for 10% of samples; concordance for repeats was 100% for all *LEPR* polymorphisms; and 4% to 5% of genotypes were unable to be determined.

Statistical Analysis. Questionnaire data, plasma samples, and buffy coats were available for analysis from 157 of the 192 adenoma cases (82%) and from 191 of the 218 eligible controls (88%). Leptin concentrations were log transformed and geometric means were calculated. Circulating concentrations of leptin were also divided into sex-specific tertiles based on cut points in controls. The leptin concentration for one individual was an extreme outlier (more than four SDs from the mean) and was therefore excluded from analyses. For all genotypes, the reference group was considered to be the one that was homozygous for the common allele.

BMI in kg/m^2 was categorized into sex-specific quartiles based on control values and further categorized as not overweight (BMI, <25 kg/m^2), overweight (BMI, 25-29.9 kg/m^2), and obese (BMI, ≥30 kg/m^2) according to WHO cut points (26). Summary metabolic equivalent-hours/week for recreational physical activity were calculated for each participant using the Compendium of Physical Activities (27) and categorized separately for men and women. Family history of colorectal cancer was defined as having at least one first-degree relative with colorectal cancer. Information from self-report and medical record review about previous polyps allowed the adenoma case group to be stratified into those with a first incident adenoma (n = 72) and those with previous polyps (n = 85).

Correlations between circulating levels of leptin and BMI were assessed using the Spearman correlation coefficient. Multivariable-adjusted odds ratios (OR) of adenoma risk and 95% confidence intervals (95% CI) associated with leptin concentrations were obtained using logistic regression adjusting for age, BMI, physical activity, family history of colorectal cancer, smoking history, and NSAID use. For women, additional adjustments were made for menopausal status (premenopausal, postmenopausal, and unknown) and, among postmenopausal women, postmenopausal hormone use. Polytomous logistic regression models, adjusting for the same covariates as above, were used to determine the ORs and corresponding 95% CIs for analysis of participants with adenoma and no previous polyp and those with adenoma and previous polyps compared with controls. Tests for trend were evaluated by including the continuous variable in the regression model. Tests for interaction were assessed by including, in the model, a cross-product term between a continuous leptin concentration variable and the dichotomous effect modifier of interest (sex and

BMI) along with their main effects terms. Similar models were used for genotype associations, adjusting for age and sex. These statistical analyses were done using Statistical Analysis System v8.2 (SAS Institute).

Allele frequencies and haplotypes for *LEPR* were estimated by maximum likelihood using the HPlus software package (version 2.5; Fred Hutchinson Cancer Research Center, Seattle, WA; refs. 28, 29). The most common haplotype was selected as the reference group with all others being compared with it. Associations for haplotypes only with control frequencies >5% are presented. All tests of significance were two sided, and P values of <0.05 were considered statistically significant.

Results

Selected characteristics of study participants are presented in Table 1. Adenomatous polyp cases were more likely to be older than controls, 62.4 versus 57.1 years, respectively. Male cases generally had a higher BMI than controls. Female cases were more likely to be postmenopausal and to have never used postmenopausal hormones. Approximately 90% of cases and controls reported being white/Caucasian.

Table 1. Selected characteristics of colorectal adenoma cases and controls

	Cases (n = 157), n (%)	Controls (n = 191), n (%)
Age (y)		
30-49	19 (12)	49 (26)
50-59	41 (26)	70 (37)
60-69	54 (34)	36 (19)
70-79	43 (27)	36 (19)
Sex		
Male	77 (49)	72 (38)
Female	80 (51)	119 (62)
Race/ethnicity		
Caucasian	141 (90)	169 (88)
Not Caucasian	16 (10)	22 (12)
BMI (kg/m^2) in men (quartiles)		
<24.8	13 (17)	18 (25)
24.8-26.5	8 (11)	18 (25)
26.6-29.1	26 (34)	18 (25)
>29.1	29 (38)	18 (25)
BMI (kg/m^2) in women (quartiles)		
<23.2	20 (25)	30 (25)
23.2-26.6	27 (34)	31 (26)
26.7-30.1	13 (16)	28 (23)
>30.1	20 (25)	30 (25)
Family history of colorectal cancer		
Absent	91 (58)	98 (51)
Present	66 (42)	93 (49)
Smoking status		
Never	65 (41)	83 (43)
Ever	92 (59)	108 (57)
NSAID use		
Never	89 (57)	110 (58)
Ever	68 (43)	81 (42)
Physical activity (MET-h/wk) in men		
0-17.4	45 (58)	35 (49)
≥17.5	32 (42)	37 (51)
Physical activity (MET-h/wk) in women		
0-11.4	45 (56)	59 (50)
≥11.5	35 (44)	60 (50)
Menopausal status (among women)		
Premenopausal	13 (16)	39 (33)
Postmenopausal	67 (84)	78 (67)
Unknown	2 (2)	0 (0)
Postmenopausal hormone use*		
Never	22 (33)	22 (28)
Ever	44 (67)	56 (72)

Abbreviation: MET, metabolic equivalent.
*Among postmenopausal women.

Mean concentrations of leptin were slightly higher for cases than controls and higher in women (cases, 25.5 ± 22.5 ng/mL; controls, 22.7 ± 22.1 ng/mL) than in men (cases, 10.6 ± 8.0 ng/mL; controls, 6.5 ± 5.7 ng/mL). Because mean concentrations of leptin differed between men and women, all subsequent analyses were stratified by sex. For both men and women, there were statistically significant correlations between BMI and leptin concentrations: r = 0.74 in men and r = 0.81 in women.

In men, increasing leptin concentrations were statistically significantly associated with increasing adenoma risk: compared with those in the lowest tertile, men in the second tertile had a 2.7-fold elevated risk (95% CI, 1.0-7.2) and men in the third tertile had a 3.3-fold elevated risk (95% CI, 1.2-8.7; P trend = 0.01; Table 2). Additional adjustment for BMI slightly attenuated these associations. In women, there seemed to be no association between leptin and colorectal adenoma risk. There was a statistically significant interaction between sex and leptin concentrations (P interaction = 0.02). When stratified by previous polyp history, there were no statistically significant differences in the association of leptin concentrations and adenoma risk by polyp history among men or women (data not shown).

To help understand if any of the leptin-adenoma associations we observed may explain (i.e., may be in the causal pathway of) the obesity-adenoma association, we examined the association between obesity and adenoma risk with and without adjustment for leptin concentrations (Table 3). There was an increasing risk of adenoma in men with increasing BMI (P trend = 0.02; OR, 2.4; 95% CI, 0.9-6.5 for the highest quartile compared with the lowest). This association was not present in women; the interaction of BMI by sex was statistically significant (P interaction = 0.03). After additional adjustment for circulating leptin concentrations, the association between BMI and adenoma risk in women remained null; however, the BMI association with adenomas in men was attenuated, with only a nonstatistically significant 1.5-fold increased risk for men in the highest quartile of BMI compared with the lowest.

Allele frequencies of *LEPR* were consistent with those reported for other Caucasian populations, and among controls, the observed genotype frequencies were statistically consistent with Hardy-Weinberg equilibrium, except for the *LEPR* codon 223 variant (χ^2 = 7.31; P = 0.007). The genotype distributions in cases and controls of the *LEPR* polymorphisms are presented in Table 4. There were no statistically significant associations between polymorphisms in the *LEPR* gene and colorectal adenoma risk; results between codominant and dominant models were similar, and thus, only dominant models are presented. Similarly, there were no associations between the inferred *LEPR* haplotypes and colorectal adenoma risk (Table 4). When stratified by history of polyps, there were again no statistically significant associations between adenoma risk and *LEPR* genotype (data not shown).

Table 2. Risk of colorectal adenomas associated with leptin concentrations (ng/mL)

	Cases, *n* (%)	Controls, *n* (%)	OR* (95% CI)	OR† (95% CI)
Men	(*n* = 77)	(*n* = 72)		
1-5	10 (13)	24 (33)	1.0	1.0
6-10	27 (35)	24 (33)	2.7 (1.0-7.2)	2.4 (0.9-6.8)
11-80	40 (52)	24 (33)	3.3 (1.2-8.7)	2.3 (0.7-7.7)
P trend (continuous per ng/mL)			0.01	0.15
Women	(*n* = 80)	(*n* = 119)		
2-16	23 (29)	40 (34)	1.0	1.0
17-43	32 (40)	39 (33)	1.3 (0.7-2.4)	1.7 (0.7-4.1)
44-158	25 (31)	39 (33)	0.8 (0.4-1.6)	1.3 (0.4-3.8)
P trend (continuous per ng/mL)			0.46	0.20

*Adjusted for age, family history of colorectal cancer, NSAID use, cigarette smoking, and physical activity; additional adjustments for menopausal status and postmenopausal hormone use in women.
†Same adjustments as above but additionally adjusted for BMI (in sex-specific quartiles).

Overall, *LEPR* genotypes did not modify the relationship of BMI and colorectal adenoma nor did they modify the relationship of physical activity and colorectal adenoma (data not shown). Among men with at least one T allele for the Pro[1019]Pro polymorphism, a BMI >26.5 kg/m^2 was associated with a 7-fold increase in adenoma risk (OR, 0.4 for ≤26.5 kg/m^2 versus OR, 2.8 for >26.5 kg/m^2, with each compared with the CC and ≤26.5 kg/m^2 group); among men with a CC genotype, obesity seemed to confer little excess risk (OR, 1.4 for BMI >26.5 kg/m^2 versus BMI ≤26.5 kg/m^2). This interaction of the Pro[1019]Pro genotype with BMI was statistically significant ($P < 0.01$). For several *LEPR* polymorphisms, there was a suggestion that ever having used postmenopausal hormones was associated with a reduced colorectal adenoma risk (Table 5). There was a statistically significant interaction between the *LEPR* Lys[109]Arg genotype and postmenopausal hormone use (P interaction = 0.01). Among women with the AA genotype, there was no association between postmenopausal hormone use and colorectal adenoma risk; however, among women with at least one G allele, there was a suggestion of a decreased risk of adenoma among women who had ever used postmenopausal hormones (OR, 0.6; 95% CI, 0.2-1.5).

Table 3. Risk of colorectal adenomas associated with body mass index (BMI)

	Cases (*n* = 157), *n* (%)	OR* (95% CI)	OR† (95% CI)
Men			
BMI, kg/m^2 (quartiles)			
<24.8	13 (17)	1.0	1.0
24.8-26.5	8 (11)	0.5 (0.2-1.6)	0.4 (0.1-1.3)
26.6-29.1	26 (34)	1.9 (0.7-5.3)	1.3 (0.4-4.1)
>29.1	29 (38)	2.4 (0.9-6.5)	1.5 (0.4-5.3)
P trend (continuous per kg/m^2)		0.02	0.30
BMI‡			
<25.0	16 (21)	1.0	1.0
25.0-29.9	35 (46)	1.0 (0.4-2.4)	0.5 (0.2-1.6)
≥30.0	25 (33)	2.6 (0.9-7.4)	1.2 (0.3-4.6)
Women			
BMI, kg/m^2 (quartiles)			
<23.2	20 (25)	1.0	1.0
23.2-26.6	27 (34)	1.3 (0.6-3.0)	1.0 (0.4-2.6)
26.7-30.1	13 (16)	0.8 (0.3-2.1)	0.6 (0.2-1.9)
>30.1	20 (25)	1.1 (0.4-2.5)	0.8 (0.2-2.8)
P trend (continuous per kg/m^2)		0.56	0.56
BMI‡			
<25.0	32 (40)	1.0	1.0
25.0-29.9	28 (35)	1.2 (0.6-2.4)	1.1 (0.5-2.5)
≥30.0	20 (25)	1.0 (0.5-2.1)	1.0 (0.3-2.8)

*Adjusted for age, family history of colorectal cancer, NSAID use, cigarette smoking, and physical activity; additional adjustments for menopausal status and postmenopausal hormone use in women.
†Same adjustments as above but additionally adjusted for circulating leptin concentrations.
‡WHO cut points in kg/m^2 (not overweight, <25; overweight, 25-29.9; obese, ≥30; ref 26).

Discussion

In our study, increased plasma leptin concentrations were associated with a 2- to 3-fold increased risk of

Table 4. Risk of colorectal adenomas associated with *LEPR* genotypes and haplotypes

	Cases (*n* = 157), %	Controls (*n* = 191), %	OR (95% CI)*
LEPR single polymorphisms			
Lys[109]Arg			
AA	58	54	1.0
AG/GG	42	47	0.8 (0.5-1.2)
Gln[223]Arg			
AA	32	33	1.0
AG/GG	69	67	1.0 (0.6-1.6)
Ser[343]Ser			
TT	62	67	1.0
TC/CC	38	34	1.1 (0.7-1.7)
Lys[656]Asn			
GG	69	68	1.0
GC/CC	31	32	1.0 (0.6-1.6)
Pro[1019]Pro			
CC	42	36	1.0
CT/TT	58	64	0.8 (0.5-1.2)
LEPR haplotypes†			
AATGC	37	35	1.0
GGTGT	17	20	0.8 (0.5-1.2)
AATCT	16	16	0.9 (0.6-1.5)
AGCGC	16	15	1.0 (0.6-1.6)
GGTGC	6	6	1.1 (0.6-2.0)

*Adjusted for age and sex.
†In order of 109, 223, 343, 656, and 1019.

Table 5. Risk of colorectal adenomas associated with postmenopausal hormone use, stratified by *LEPR* genotype, among postmenopausal women

	Postmenopausal hormone use			
	Never		Ever	
	Cases/controls	OR (95% CI)*	Cases/controls	OR (95% CI)*
LEPR				
$Lys^{109}Arg$				
AA	11/16	1.0	29/26	1.7 (0.7-4.3)
AG/GG	10/6	2.1 (0.6-7.6)	13/29	0.6 (0.2-1.5)
P interaction				0.01
$Gln^{223}Arg$				
AA	4/11	1.0	17/12	3.3 (0.9-11.4)
AG/GG	17/11	2.3 (0.7-8.1)	25/43	0.9 (0.3-2.6)
P interaction				<0.01
$Ser^{343}Ser$				
TT	13/14	1.0	27/35	0.8 (0.3-1.9)
TC/CC	8/8	0.7 (0.2-2.4)	15/20	0.6 (0.2-1.8)
P interaction				0.99
$Lys^{656}Asn$				
GG	17/13	1.0	28/44	0.5 (0.2-1.1)
GC/CC	4/9	0.3 (0.1-1.2)	14/11	1.1 (0.4-3.2)
P interaction				0.03
$Pro^{1019}Pro$				
CC	11/9	1.0	18/22	0.7 (0.2-2.0)
CT/TT	10/13	0.5 (0.2-1.8)	24/33	0.6 (0.2-1.5)
P interaction				0.79

*Adjusted for age, BMI, family history of colorectal cancer, NSAID use, cigarette smoking, and physical activity.

colorectal adenoma in men but not women. To discern whether the associations of leptin with colorectal adenoma risk were due to differences in BMI, we considered models with and without BMI adjustment. For circulating leptin concentrations, there was a modest attenuation in adenoma risk in men after adjusting for BMI, suggesting that other factors associated with BMI, perhaps elevated levels of other growth hormones, may at least partially explain the relationship between leptin and colorectal adenoma risk. For the BMI-adenoma association in men, adjustment for leptin resulted in a marked attenuation of risk. Overall, we did not find any associations between *LEPR* genotype and colorectal adenoma risk. Additionally, there were no statistically significant changes in risk when examining the most common *LEPR* haplotype with the other haplotypes.

To our knowledge, we are the first to report on the association between circulating leptin and colorectal adenoma risk. However, our finding of a 2- to 3-fold increased risk of colorectal adenomas in men with the highest tertiles of leptin is consistent with two studies of colorectal cancer. Two nested case-control studies observed 2.2-fold (18) and 2.7-fold (19) increases in colorectal cancer risk in men with the highest quartile of leptin compared with the lowest. Adjustment for BMI (18) did not alter these results. We found no associations between leptin concentrations and adenoma risk in women; elsewhere, findings have been inconsistent. In a study of northern Europeans, with multivariate adjustments similar to ours, Stattin et al. (18) found no association between leptin concentrations and colorectal cancer risk among women (n_{cases} = 93), whereas Tamakoshi et al. (20) observed almost a 4-fold increase in risk (OR, 3.9; 95% CI, 1.0-14.9) among Japanese women with the highest quintile of leptin concentrations compared with the lowest (n_{cases} = 58).

It is reasonable to hypothesize that because circulating levels of leptin have been associated with risk of colorectal cancer, and in our study with adenoma risk, we might expect genetic variation to also be associated with disease risk as it may lead to alterations in circulating levels of leptin (8, 24). We found no association between genetic variation in *LEPR* and colorectal adenoma risk, and it is likely that these polymorphisms did not influence the circulating levels enough to create differences in bioavailable leptin. To our knowledge, there have been no prior studies of the relationship of *LEPR* polymorphisms with colorectal cancer or adenoma risk.

Despite our limited power to detect gene-environment interactions, we observed that the relation between BMI and adenoma risk in men and between postmenopausal hormone use and risk in women was modified by *LEPR* genotypes. These results may suggest that heterogeneity in certain *LEPR* genotypes may be partially responsible for the observed sex differences in the relationship between obesity and colorectal adenoma risk. To our knowledge, there have been no studies reporting the association between the $Pro^{1019}Pro$ polymorphism and circulating leptin levels, and no studies have examined the association between obesity and adenoma risk modified by *LEPR* genotype. Although the $Pro^{1019}Pro$ polymorphism does not result in an amino acid change, it is plausible that it may affect mRNA stability or may be in linkage disequilibrium with a polymorphism that does alter circulating leptin levels.

We also found that two *LEPR* polymorphisms may increase adenoma risk in women with the variant alleles who never used postmenopausal hormones and decrease

risk in women who were ever users. These findings may be biologically plausible as the Arg^{109} and Arg^{223} alleles have been associated with higher leptin concentrations than the Lys^{109} and Gln^{223} alleles (8, 24). Among women who had never used hormones, the polymorphisms that were associated with higher leptin concentrations could also be increasing cell proliferation in the colon (10). Postmenopausal hormone use has been consistently associated with a decreased risk of colorectal neoplasia (30-33), including the recent Women's Health Initiative report for estrogen plus progestin use (34). Studies in postmenopausal women have shown decreased circulating leptin concentrations associated with postmenopausal hormone use (35, 36). Taken together, these data indicate that postmenopausal hormone use reduces risk of adenomas, even among women with polymorphisms in the *LEPR* that are associated with higher circulating leptin concentrations, by decreasing leptin concentrations. This hypothesis is speculative, as our study had limited power to detect gene-environment interactions; further, the data about *LEPR* polymorphisms and their effects on leptin concentrations have been inconsistent, with one study finding increased leptin levels associated with the Gln^{223} allele (37).

There are several aspects of the study design that must be considered when interpreting these findings. First, we had modest power to assess some associations because of small sample size, especially after stratification by sex, which clearly is an important factor. Limited sample size also prevented us from investigating associations separately by polyp characteristics, such as histology, size, or location. Several studies report that established risk factors for colorectal cancer are also associated with risk of ''advanced'' adenomas (i.e., size $\geq$1 cm and/or villous histology) that are more likely to progress to cancer (38, 39).

Strengths of the study include a well-characterized control group that was known to have a normal colon pathology and our ability to stratify on previous polyp history. Previous clinic-based studies of adenomas characterized controls as individuals with a clean colon at the time of colonoscopy and ignored previous history. By restricting our control group to participants with normal findings at colonoscopy and no prior history of polyps, we maximized our ability to ensure the best comparison possible.

It is biologically plausible that obesity increases risk of colorectal adenoma and cancer, especially among men (1). As our results support, one mechanism may be through leptin concentrations, which have been associated with higher BMI (6-8, 40, 41) and which have also been shown to have actions in the colon that might promote carcinogenesis. *In vitro* studies have found leptin to be a growth factor in colonic epithelial cells (10, 42, 43) and that leptin promotes angiogenesis (12). It has also been shown to promote invasiveness of colonic cells by directly stimulating the Janus-activated kinase 2 pathway (43). Further data suggested that leptin plays a role in the proliferation, migration, and renewal of intestinal cells, and leptin may exert a cumulative adverse effect during tumor progression (43). Other hormones that are elevated with obesity, such as insulin and C-peptide, have also been shown to be associated with an increased risk of colorectal neoplasia (44-47). Thus, it may be difficult to separate out the effects of these hormones from leptin. There are several reasons why women, who have higher leptin concentrations than men, would not have an increased risk of colorectal adenoma. For example, there are differences in body composition between men and women; women have more s.c. fat than men (48) and leptin concentrations have been observed to be much higher in s.c. fat than in visceral fat (49, 50). Further, overweight or obese women may also have higher concentrations of hormones, such as estrogens, which may provide some protection from the deleterious effects of leptin (51).

Observations that circulating leptin concentrations influence colorectal adenoma risk in men may be explained by interindividual variation in circulating leptin concentrations. We did not find such an association in women, which may be explained by differences in endogenous hormones. We found no associations between genetic variation in *LEPR* and colorectal adenoma risk; however, because of the increasing evidence that leptin levels may be associated with colorectal cancer risk, additional larger studies should be done to assess the relationship between *LEPR* polymorphisms and colorectal adenoma and cancer risk, particularly in conjunction with lifestyle and exogenous factors, especially postmenopausal hormone use.

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