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Mediation of Associations Between Adiposity and Colorectal Cancer Risk by Inflammatory and Metabolic Biomarkers

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Abstract

Inflammation and hyperinsulinemia may drive associations between adiposity and colorectal cancer (CRC) risk, but few studies have examined this hypothesis using mediation analysis. We used inverse odds ratio weighting and logistic regression to calculate odds ratios (OR) and 95% confidence intervals (CI) for estimated total effects (OR^{TE}) of body mass index, waist circumference, and adult weight gain on CRC risk, and estimated effects operating through seven inflammatory and metabolic biomarkers (natural indirect effect; OR^{NIE}) or through paths independent of these biomarkers (natural direct effect; OR^{NDE}) among 209 CRC cases and 382 matched controls nested within the Health Professionals Follow-up Study, a prospective cohort of male health professionals. A one-interquartile range (IQR) increase in body mass index (3.6 kg/m^2) was associated with an OR^{TE} of 1.40 (95% CI: 1.13, 1.73), which decomposed into an OR^{NIE} of 1.26 (95% CI: 0.97, 1.52) and an OR^{NDE} of 1.11 (0.87, 1.42), with possibly stronger mediation by these biomarkers for adult weight gain (IQR = 10.4kg; OR^{TE} = 1.32 [95% CI: 1.06, 1.64]; OR^{NIE} = 1.47 [95% CI: 1.01, 1.81]; OR^{NDE} = 0.89 [95% CI: 0.72, 1.11]), but no mediation

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for waist circumference. Mediation appeared to be stronger for the metabolic biomarkers than the inflammatory biomarkers. Inflammatory and metabolic mechanisms may mediate associations between both body mass index and adult weight gain with CRC risk.

Keywords

Adiposity; colorectal cancer; inflammatory biomarkers; mediation analysis; metabolic biomarkers

INTRODUCTION

Adiposity has been identified as one of the strongest and most consistent modifiable risk factors for colorectal cancer (CRC), whether defined as body mass index (BMI)[1], waist circumference (WC)[2], adult weight gain (WG)[3], or body shape trajectory[4]. Adiposity also has well-documented effects on insulin resistance, and is associated with low-grade, chronic inflammation[5], both of which have been suggested as possible mechanisms through which adiposity is associated with CRC risk[6]. However, few studies have formally investigated these potential mechanisms using mediation analysis[7–9].

In recent years, the causal inference literature has expanded to include new methods for mediation analysis[10, 11] that improve upon conventional methods[12] by identifying relevant mediating effects within the counterfactual framework. These methods have been particularly useful in instances of exposure-mediator interaction and mediation by multiple correlated variables, such as biomarkers[11, 13]. Therefore, they may be better suited for studies that attempt to clarify the role of biological factors underlying exposure-disease relationships than traditional methods. However, despite these advantages, application of these methods is underused in investigations of nutritional exposures (including adiposity) and cancer incidence.

The goal of the present study was to examine whether associations between several adiposity measures (i.e. BMI, WC, and WG) and CRC risk are mediated by seven inflammatory and metabolic biomarkers using the inverse odds ratio weighted estimation method (IORW) developed by Tchetgen Tchetgen[14, 15]. This method decomposes the association between adiposity and CRC risk into two separate associations: 1) the estimated effect of adiposity on CRC risk that is mediated by inflammatory and metabolic biomarkers (referred to as the natural indirect effect [NIE]), and 2) the estimated effect of adiposity on CRC risk that is not mediated by these biomarkers (referred to as the natural direct effect [NDE]). We implemented this method in a nested case-control study within the Health Professionals Follow-up Study (HPFS).

METHODS

Study population.

The HPFS is an ongoing cohort of 51 529 male health professionals aged 40 to 75 years at the time of initiation in 1986. Updated data about lifestyle factors, medication use, and other health-related information are collected from participants via biennial questionnaires (average questionnaire response rate >90%). Between 1993 and 1995, 18 225 participants

provided blood samples, which were collected in tubes containing sodium EDTA shipped on ice packs by overnight courier, and, upon receipt, immediately centrifuged, aliquoted, and stored in liquid nitrogen freezers (≤−130°C). More than 95% of blood samples arrived in our laboratory within 26 hours of phlebotomy.

Selection of cases and controls.

Participants reported incident CRC between baseline and 2012 on biennial questionnaires, and a study physician blinded to exposure reviewed records to confirm diagnoses. Diagnosis of CRC in participants who died from CRC but had not reported a diagnosis was confirmed through various sources, including next of kin, the National Death Index, death certificates, and medical records. For each CRC case, we used risk-set sampling to randomly select up to two controls that had not been diagnosed with CRC by the age of the corresponding case. We additionally matched cases and controls on year of birth and date of blood draw within one month. We excluded individuals with a previous history of cancer (except non-melanoma skin cancer) (n=21) or ulcerative colitis (n=20) prior to blood draw, cases that were diagnosed with CRC within the first two years after the blood draw (n=42), and individuals with missing biomarker data (n=35, <5% missing any biomarker). After applying these criteria, we additionally excluded cases that did not have a matched control (n=4), and controls that did not have a matched case (n=119). There were 209 CRC cases and 382 controls in the final analysis. The study protocol was approved by the Institutional Review Board at the Harvard T.H. Chan School of Public Health.

Laboratory assays.

Details on measurements of included biomarkers (C-reactive protein [CRP], interleukin-6 [IL-6], tumor necrosis factor receptor-2 [TNFR-2], macrophage inhibitory cytokine-1 [MIC-1], adiponectin, C-peptide, and soluble leptin receptor [sOB-R]) have been published elsewhere[16–19]. Cases and matched controls were analyzed in the same batch, and all laboratory personnel were blinded to case-control status. All biomarkers were measured in one batch except for CRP and C-peptide, which were each measured in two batches six years apart. Due to possible assay variation between laboratories, we recalibrated assays of CRP and C-peptide from both batches to have a comparable distribution to an average batch[20]. The intra-assay coefficients of variation from blinded quality control samples were 2.2% (CRP), 10.6% (IL-6), 6.7% (TNFR-2), 9.0% (MIC-1), 8.6% (adiponectin), 11.5% (sOB-R), and 13% (C-peptide).

Exposure and covariate data.

Participants reported their height, weight, and weight at age 21 on the 1986 questionnaire, and additionally reported their current weight on each successive questionnaire (every two years). In 1987, participants recorded WC using a tape measure. We used exposure and covariate information collected in or before 1992 because this was the last questionnaire cycle before the blood draw. We considered three main exposures: 1) 1992 BMI (kg/m^2), 2) WG, which was defined as the difference between weight in 1992 and weight at age 21, and 3) 1987 WC. We collected information on other lifestyle factors including smoking, nonsteroidal anti-inflammatory drug (NSAID) or aspirin use, physical activity, and CRC screening on the biennial questionnaires. Dietary information was collected using validated,

self-administered, semiquantitative food frequency questionnaires provided in 1986 and 1990[21].

We had complete data on BMI for all individuals, but excluded 18 individuals missing weight at age 21 and 107 individuals missing 1987 WC from all WG and WC analyses, respectively. Characteristics of controls with 1987 WC data appeared to be similar to those of controls missing 1987 WC (Supplementary Table 1). If an individual was missing data on any covariate, we imputed the missing value from a previous questionnaire cycle, if available. After this imputation, we observed little missingness for the covariates in the population (<6% missing data on smoking, <3% missing data on screening, and <1% missing data on other covariates). We assigned the missing continuous values to the median value in the population to have the smallest influence on our results, and assumed that those missing data on screening (the only categorical covariate with any missingness) had never undergone screening with a colonoscopy or endoscopy.

Statistical analysis.

We used the IORW method for mediation analysis[14, 15] because this method is advantageous over traditional methods for mediation[11, 12]. Unlike the IORW method, traditional methods for mediation require correct specification of regression models for each mediator (in addition to correct specification of models for outcomes). When examining mediation by multiple correlated variables, as in the present analysis, specifying these models is burdensome and prone to error. Moreover, the IORW method does not assume there are no exposure-mediator interactions, unlike traditional methods. This advantage is particularly important in our study, as we noted statistically significant interactions between BMI with TNFR-2 (P=0.02) and MIC-1 (P=0.05), between WC with MIC-1 (P=0.04) and C-peptide (P=0.05), and between WG with TNFR-2 (P=0.007) and MIC-1 (P=0.005), deeming the traditional methods invalid in our study.

The IORW method circumvents these issues by condensing the relationship between the exposure and all mediators into an odds ratio (OR). Because an OR between two variables is the same regardless of which is considered the dependent variable, an OR for the exposure conditional on all mediators is equivalent to the OR for the mediators conditional on exposure. Applying this OR as a weight to a regression of the outcome on the exposure renders the exposure and mediators independent, thereby deactivating pathways from the exposure to the outcome via the mediators, allowing for calculation of the natural direct effect (NDE) estimate[14, 15]. Because the IORW method does not require modeling of the mediators, it makes no assumptions about their joint densities, and is agnostic about interaction between the mediators and the exposure of interest. It is therefore more flexible (and possibly less biased) than traditional methods for mediation[12], as well as newer methods that use a counterfactual framework and can accommodate interactions between exposure and mediators, but nevertheless make parametric assumptions about the mediators[11, 22]. Like other methods for mediation analysis, the IORW method assumes no unmeasured confounding of the effects of 1) exposure on mediators, 2) mediators on outcome, or 3) exposure on outcome, conditional on pre-exposure covariates, as well as no

confounding variable of the mediator-outcome relationship that is affected by the exposure[15].

The IORW method requires unconditional logistic regression for a case-control design. Therefore, before using this method, we compared ORs between each exposure and CRC risk from unconditional and conditional logistic regression. Because we obtained similar results for all exposures (results not shown), we proceeded with using unconditional logistic regression.

To implement the IORW method in this study, we first fit a linear regression model for each continuous exposure conditional on all mediators of interest and covariates, including matching factors:

$$E(A/\boldsymbol{M},\boldsymbol{L}) = \beta_0 + \beta\grave{}_1\boldsymbol{M} + \beta\grave{}_2\boldsymbol{L} \quad (1)$$

where A is the exposure of interest, $\boldsymbol{M}$ is a vector of mediators ($\boldsymbol{M}$ =(M_1, M_2, … M_n)), and $\boldsymbol{L}$ is a vector of covariates that includes matching factors (age at blood draw and time of blood draw) and confounders (energy intake, physical activity, regular NSAID or aspirin use, family history of CRC, red and processed meat intake, total folate intake, previous history of CRC screening, smoking, multivitamin use, supplemental calcium use, alcohol intake, height, and Dietary Approaches to Stop Hypertension (DASH) score, an index of healthy eating). Because we did not find evidence of nonlinearity in the relationship between each exposure and each mediator and CRC risk (results not shown), which we examined by comparing models with a linear term for the exposure or mediator to models with restricted cubic splines[26], we modeled all exposures and mediators continuously to increase statistical efficiency. Next, we calculated an inverse odds ratio weight for each individual for the continuous exposure of interest using the following equation provided by Tchetgen Tchetgen[27]:

$$OR(A/\boldsymbol{M},\boldsymbol{L})^{-1} = exp(-A\beta\grave{}_1\boldsymbol{M}/\sigma^2) \quad (2)$$

where σ^2 is the mean squared error of model (1) and $\beta\grave{}_1$ is a vector of regression coefficients for the mediators from model (1).

We next ran an unweighted unconditional logistic regression for the outcome on the exposure of interest and all other covariates (except mediators):

$$logit\ Pr(Y = 1/A,\boldsymbol{L}) = \gamma_0 + \gamma_1 A + \gamma\grave{}_2\boldsymbol{L} \quad (3)$$

This allowed us to calculate the total effect (TE) estimate because the coefficient for the exposure in this regression (γ_1) is equivalent to the log OR relating exposure to outcome through all pathways. We lastly reran model (3), but now weighting the regression with the weights obtained in equation (2), which renders the exposure and mediators independent.

This allowed us to calculate the NDE estimate, since the coefficient for the exposure in the weighted regression is equivalent to the log OR relating exposure to CRC through all pathways other than the mediators of interest. We calculated the natural indirect effect (NIE) estimate by subtracting the NDE estimate from the TE estimate because the NDE and NIE sum to the TE[15], and obtained 95% CI for the NIE estimate by bootstrapping these estimates. We exponentiated the TE, NDE, and NIE estimates to obtain the OR^{TE}, OR^{NDE} and OR^{NIE}, respectively. (Note: we use the terms “total effect”, “natural direct effect”, and “natural indirect effect” to describe the effect estimates of interest. We do this to be consistent with terminology from the mediation literature[23–25], not to imply causality.)

Our primary analyses examined how associations between the adiposity measures and CRC risk were mediated by all inflammatory and metabolic markers jointly. We additionally ran analyses examining mediation by inflammatory markers (i.e. CRP, IL-6, TNFR-2, and MIC-1) and metabolic markers (i.e. adiponectin, C-peptide, and sOB-R) separately, as well as mediation by each individual biomarker. We repeated these analyses for colon cancer (i.e. excluding rectal cancer cases). We additionally explored the possibility of differential mediation within subgroups of regular NSAID/aspirin use (yes vs. no), DASH score (above vs. below median), and physical activity (above vs. below median).

In sensitivity analyses, we reran analyses using WC measured in 1996 and using the average of the 1987 and 1996 WC measures. Although 1996 WC occurred one to three years after the blood draw, it may better represent WC at the time of blood draw than 1987 WC, which occurred six to eight years before the blood draw. For both sensitivity analyses, we excluded individuals diagnosed with CRC in 1996 or before so that measurement of WC always occurred before diagnosis. To further explore the effect that time between exposure and mediator measurements may have had on our results, we reran analyses using BMI from each questionnaire cycle prior to 1996. Finally, we conducted a sensitivity analysis of our primary WC model where we additionally adjusted for BMI in 1986 (roughly the same time WC was measured). WC may better represent central adiposity in this model[28], and its association with CRC risk may be more likely to be mediated by metabolic and inflammatory biomarkers.

All analyses were done using SAS version 9.4 for UNIX (Cary, NC). We calculated two-sided 95% CIs for all statistical tests.

RESULTS

At the time of blood draw, we observed that CRC cases were more likely to have a slightly higher BMI (and were more likely to be overweight) and have a family history of CRC than controls, while controls were more likely to have undergone previous screening for CRC and be regular users of NSAIDs or aspirin than cases (Table 1). When comparing the distributions of biomarkers, we noted slightly higher median concentrations of inflammatory biomarkers (except for TNFR-2), slightly lower concentrations of adiponectin and sOB-R, and slightly higher concentrations of C-peptide in cases compared to controls. In general, the biomarkers were weakly to moderately correlated with one another and with the adiposity measures (Supplementary Table 2).

In multivariable-adjusted models, we found that an increase in BMI equivalent to one interquartile range (IQR) (3.6 kg/m^2) was associated with an OR^{TE} of 1.40 (95% CI: 1.13, 1.73), which decomposed into an OR^{NDE} of 1.11 (95% CI: 0.87, 1.42) and an OR^{NIE} of 1.26 (95% CI: 0.97, 1.52). This is interpreted as a suggestive 26% increased odds of CRC due to BMI through the biomarkers, and an 11% increased odds through other pathways. We observed stronger mediation for analyses of WG (OR^{TE} for a 1-IQR increase [10.4kg] = 1.32 [95% CI: 1.06, 1.64]; OR^{NDE} = 0.89 [95% CI: 0.72, 1.11]; OR^{NIE} = 1.47 [95% CI: 1.01, 1.81]), suggesting that the biomarkers or pathways represented by these biomarkers may explain most or all of the association between WG and CRC risk. We did not find evidence of mediation for WC (OR^{TE} for a 1-IQR increase [11.4cm] = 1.71 [95% CI: 1.30, 2.25]; OR^{NDE} = 1.90 [95% CI: 1.35, 2.66]; OR^{NIE} = 0.90 [95% CI: 0.70, 1.16]) (Table 2). We did not observe any material differences in our results when adjusting this WC model for 1986 BMI (OR^{TE} = 1.78 [95% CI: 1.10, 2.86]; OR^{NDE} = 1.95 [95% CI: 1.14, 3.34]; OR^{NIE} = 0.91 [95% CI: 0.65–1.22]).

When investigating inflammatory and metabolic biomarkers separately, we observed qualitatively stronger results for metabolic biomarkers than inflammatory biomarkers for both BMI and WG. Mediation results were null for both inflammatory and metabolic biomarkers for WC (Table 2). We also examined mediation by individual biomarkers, and found generally no mediation by any one biomarker for all adiposity measures (although NIEs of adiponectin and SOB-R were qualitatively higher for BMI and WG analyses) (Supplementary Table 3). For all exposures, we did not observe material differences in mediation by the biomarkers when excluding rectal cancer cases (49 cases excluded, Supplementary Table 4).

In exploratory analyses of mediation within subgroups of participant characteristics, we observed suggestively stronger mediation for analyses of WG and BMI in nonusers of NSAIDs or aspirin compared to regular users. However, we had a low number of cases and wide 95% CIs for all associations within these subgroups (Table 3).

We did not observe evidence of mediation when examining the average of 1987 and 1996 WC measures, as well as when examining just the 1996 WC measure, although the results were qualitatively stronger for the 1996 analysis (Table 4). When we evaluated BMI from roughly the same time point as the primary WC analysis (i.e. 1986 questionnaire), we observed weaker overall mediation (OR^{NIE} = 1.04, 95% CI: 0.88, 1.20) than for BMI measured in 1992, with stronger mediation occurring as the time between BMI and biomarker measurements decreased (Supplementary Table 5), suggesting that time between exposure and mediator measurement may be an important factor to consider in these analyses.

DISCUSSION

Results from this nested case-control study suggest that associations between BMI and WG with CRC risk may be jointly mediated by biomarkers of inflammation and metabolism in men. Results from analyses of WC did not suggest mediation by these biomarkers. Previous analyses of BMI and WG have also observed mediation by some metabolic and

inflammatory biomarkers (especially sOB-R and adiponectin)[7, 9], but, unlike our study, other studies observed mediation for analyses of WC[8, 9]. However, one of these studies was conducted only in women[8], and both used different analytic methods than what we used in the current study.

Mediation of associations between BMI and WG with CRC risk appeared to be largely driven by the metabolic biomarkers C-peptide, an indicator of insulin secretion with a longer half-life than insulin[29], sOB-R, a circulating binding protein that regulates leptin bioavailability[30], and adiponectin, which decreases in response to obesity and has a role in insulin regulation[31]. These biomarkers have independently been associated with incident CRC in previous studies[18, 32, 33], suggesting the importance of metabolism on colorectal carcinogenesis. This is supported by the fact that abnormalities in metabolism have been linked to increased CRC risk, including type 2 diabetes[34], as well as high levels of HbA1c (a marker of glycemic control)[35], fasting plasma glucose[36], and HOMA-IR (a marker of insulin resistance)[33]. However, the biological mechanisms behind metabolism and CRC are complex, and may involve several pathways, such as energy sensing pathways (e.g. AMPK, Sirt1, and mTOR signaling) and the gut microbiome[37, 38]. Therefore, any mediation we observed does not necessarily identify C-peptide, sOB-R, and adiponectin as the causal agents driving associations between adiposity and CRC risk, but rather more likely represents the possible importance of metabolism and insulin response as key pathways linking adiposity with CRC risk. Notably, we observed stronger results for the joint mediating effects of the metabolic biomarkers than when each of these biomarkers was considered alone, suggesting that the role of metabolism in CRC may be explained by more than one distinct mechanism (though these mechanisms are interrelated). Moreover, these results suggest that consideration of individuals' metabolic states, as defined by several biomarkers, may be more informative for establishing the biology behind CRC, rather than analysis of individual metabolic biomarkers.

Results for mediation by inflammatory biomarkers were weaker for all analyses, despite the fact that adiposity is associated with low-grade systemic inflammation[5], and that systemic inflammation has been linked to CRC risk[39], though inconsistently. Several studies[40–42], have observed null or weak associations between many of these circulating inflammatory biomarkers and CRC risk, while other studies have observed positive associations[43–45]. Notably, in exploratory subgroup analyses, we observed qualitatively stronger mediation by the biomarkers among nonusers of NSAIDs/aspirin compared to regular users, although these analyses were low in power. Nevertheless, because regular NSAID/aspirin use may reduce chronic inflammation[46], it is worth investigating in greater detail whether adiposity affects CRC risk via inflammatory pathways in individuals who do not receive the anti-inflammatory benefits of NSAIDs/aspirin.

Although all adiposity measures were associated with increased CRC risk, we only observed mediation for analyses of WG, with weaker results for BMI. BMI captures both lean body mass and fat mass[47], whereas weight change since age 21 may better capture changes in metabolic activity in adulthood[48], possibly explaining the stronger mediation results for WG. However, analyses of WC, which is also predictive of insulin response and incident metabolic disease[49], surprisingly did not suggest mediation by the biomarkers. One possible

reason for this may be that while we had measures of BMI and weight in 1992 (one to three years before the blood draw), the only measures of WC we had were in 1987 (six to eight years before the blood draw), and 1996 (one to three years after the blood draw). Thus, these results may be most limited by the fact that we did not have WC measures soon before the blood draw.

One of the major strengths of this study is our use of the IORW approach, which estimated the joint mediating effects of multiple biomarkers (accounting for correlation between biomarkers), allowing us to possibly capture relevant underlying biological pathways. The IORW method estimates this mediation without making assumptions about either the joint densities of the mediators or any possible interactions between exposures and mediators, unlike most other methods[11, 12, 22]. Moreover, the IORW method provides an OR and 95% CI for each relevant pathway, whereas other methods typically do not provide estimates of precision of the amount mediated (i.e. 95% CIs). Other strengths of this study include its prospective design and exclusion of CRC cases diagnosed within two years of the blood draw, which minimized the possibility of reverse causation and selection bias due to control selection. We also had detailed information on lifestyle, diet, medication use, and screening, which allowed us to adjust for major confounders of associations between adiposity, biomarkers, and CRC risk.

Our study also has several limitations. First, while the semi-parametric nature of the IORW method makes it less prone to bias via model specification, it may also make it less statistically efficient than fully parametric methods. This, along with our relatively small sample size, may have limited our power to detect weak mediation by the biomarkers. Second, we only had a single measure of plasma biomarkers, which may not be representative of long-term concentrations. However, these biomarkers have been shown to be generally stable over time[50–52]. Third, there is likely to be random error in the biomarker measurements; however, the intra-assay coefficients of variation for all biomarkers were good to excellent. Fourth, we were unable to determine if WG primarily occurred in early or late adulthood, despite possible differential effects on biomarker concentrations[53]. Fifth, it is possible that other metabolic or inflammatory biomarkers not included in this analysis are related to CRC risk. However, given the diversity of biomarkers we included and the general interrelatedness of metabolic and inflammatory biomarkers, we were likely able to capture much of the participants' metabolic and inflammatory states. Lastly, HPFS mainly consists of older, white, male health professionals, possibly reducing the generalizability of our results.

In summary, our results suggest that associations between BMI and WG with CRC risk may be mediated by inflammatory and metabolic biomarkers, although the differing roles of metabolism and inflammation require further investigation. More studies, preferably with repeated measures of exposures and both metabolic and inflammatory biomarkers, are necessary to elucidate these relationships. Our results additionally highlight the utility and advantages of applying methods developed from the causal mediation literature to the study of adiposity, biomarkers, and CRC risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

BMI	Body mass index
CI	Confidence interval
CRC	Colorectal cancer
CRP	C-reactive protein
DASH	Dietary Approaches to Stop Hypertension
IL-6	Interleukin-6
IORW	Inverse odds ratio weighting
IQR	Interquartile range
MIC-1	Macrophage inhibitory cytokine-1
NDE	Natural direct effect estimate
NIE	Natural indirect effect estimate
NSAID	nonsteroidal anti-inflammatory drug
OR	Odds ratio
OR^{NDE}	Odds ratio for the natural direct effect estimate
OR^{NIE}	Odds ratio for the natural indirect effect estimate
OR^{TE}	Odds ratio for the total effect estimate
sOB-R	Soluble leptin receptor
TE	Total effect estimate
TNFR-2	Tumor necrosis factor receptor-2
WC	Waist circumference

WG	Adult weight gain

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What's New:

Insulin response and inflammation are hypothesized to drive associations between adiposity and colorectal cancer risk. Here, the authors used a novel method for mediation analysis to demonstrate that metabolic and inflammatory biomarkers may jointly mediate associations between both BMI and adult weight gain with colorectal cancer risk in men, with stronger results for metabolic biomarkers. These results offer mechanistic and methodologic insights into the underlying biology of adiposity and colorectal cancer risk.

Table 1.

Characteristics of Colorectal Cancer Cases and Controls at Blood Draw (1993-1995)[a]

	Cases (n=209)	Controls (n=382)
Age at blood draw (years)	65.0 (8.3)	64.9 (8.3)
Body mass index (kg/m^2)	26.2 (3.1)	25.4 (2.9)
BMI ≥ 25 (%)	66	52
BMI ≥ 30 (%)	9	7
Waist circumference (cm)	98.2 (8.8)	95.2 (8.6)
Adult weight gain (kg)[b]	10.0 (10.7)	8.8 (9.6)
Young adult body mass index (kg/m^2)	23.0 (2.6)	22.7 (2.7)
Physical activity (MET-hr/week)	32.4 (29.3)	29.7 (27.2)
Energy intake (kcal/day)	1994 (581)	1997(537)
DASH score	24.2 (4.4)	24.3 (4.6)
Supplemental calcium use (mg/day)	109 (219)	108 (239)
Alcohol intake (g/day)	12.6 (15.9)	12.3 (14.8)
Total folate intake (mg/day)	493 (218)	517 (237)
Red and processed meat intake (servings/day)	1.0 (0.8)	0.9 (0.6)
Ever smokers (%)	54	50
Pack-years among ever smokers	23.4 (17.0)	22.8 (17.3)
NSAID/aspirin use (%)	41	49
Family history (%)	19	14
Screened for CRC (%)	51	61
Multivitamin use (%)	42	45
Biomarkers[c]		
CRP (mg/L)	1.2 (0.6-2.2)	1.0 (0.6-1.9)
IL-6 (pg/mL)	1.5 (1.0-2.3)	1.3 (0.9-2.0)
TNFR-2 (ng/mL)	2.7 (2.3-3.2)	2.7 (2.3-3.3)
MIC-1 (pg/mL)	863 (642-1072)	782 (592-1001)
Adiponectin (ng/mL)	5.0 (3.3-6.8)	5.3 (3.8-7.5)
C-peptide (ng/ml)	2.4 (1.7-3.5)	2.1 (1.4-3.3)
sOB-R (ng/ml)	25.4 (20.8-29.5)	26.6 (22.3-31.7)

CRC, colorectal cancer; CRP, C-reactive protein; IL-6, interleukin-6; MIC-1, macrophage inhibitory cytokine-1; NSAID, nonsteroidal antinflammatory drug; sOB-R, soluble leptin receptor; TNFR2, tumor necrosis factor receptor 2

[a]Means (standard deviations) presented unless stated otherwise

[b]Calculated as the difference between weight in 1992 and weight at age 21

[c]Median and interquartile range

Table 2.

Mediation of Associations Between Measures of Adiposity[a] on Risk of Colorectal Cancer by Inflammatory and Metabolic Biomarkers

	All Mediators		Inflammatory Mediators[b]		Metabolic Mediators[c]	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Body Mass Index (209 cases/382 controls)[d]						
Total effect estimate	1.40	1.13, 1.73	1.40	1.13, 1.73	1.40	1.13, 1.73
Natural direct effect estimate[e]	1.11	0.87, 1.42	1.34	1.07, 1.68	1.13	0.90, 1.41
Natural indirect effect estimate[f]	1.26	0.97, 1.52	1.05	0.96, 1.14	1.24	0.92, 1.55
Adult Weight Gain (204 cases/369 controls)[d,g]						
Total effect estimate	1.32	1.06, 1.64	1.32	1.06, 1.64	1.32	1.06, 1.64
Natural direct effect estimate[e]	0.89	0.72, 1.11	1.23	0.97, 1.56	0.97	0.81, 1.16
Natural indirect effect estimate[f]	1.47	1.01, 1.81	1.07	0.93, 1.22	1.35	0.88, 1.90
Waist Circumference (166 cases/318 controls)[d]						
Total effect estimate	1.71	1.30, 2.25	1.71	1.30, 2.25	1.71	1.30, 2.25
Natural direct effect estimate[e]	1.90	1.35, 2.66	1.77	1.31, 2.38	1.84	1.32, 2.57
Natural indirect effect estimate[f]	0.90	0.70, 1.16	0.97	0.89, 1.05	0.93	0.72, 1.18

[a] Per one-unit increase in the interquartile range of each adiposity measure (body mass index: 3.6 kg/m^2; adult weight gain: 10.4 kg; waist circumference: 11.4 cm)

[b] Includes C-reactive protein, interleukin-6, tumor necrosis factor receptor 2, and macrophage inhibitory cytokine-1

[c] Includes adiponectin, C-peptide, and soluble leptin receptor

[d] Adjusted for age at blood draw (montds), time of blood draw (montd and year), energy intake (kcal/day, continuous), physical activity (MET-hrs/week, continuous), regular NSAID or aspirin use (<2 vs. ≥2 pills/week), family history of CRC (yes vs. no), red and processed meat intake (servings/day, continuous), total folate intake (mg/day), previous history of CRC screening (yes vs. no), smoking (packyears, continuous), regular multivitamin use (yes vs. no), supplemental calcium use (mg/day, continuous), alcohol intake (g/day, continuous), DASH score (continuous), and height (inches, continuous)

[e] The estimated effect of the exposure on colorectal cancer risk that is not mediated by the biomarkers of interest.

[f] The estimated effect of the exposure on colorectal cancer risk that is mediated by the biomarkers of interest.

[g] Additionally adjusted for BMI at age 21 (kg/m^2, continuous)

Table 3.

Mediation of the Effects of Measures of Adiposity[a] on Risk of Colorectal Cancer by Inflammatory and Metabolic Biomarkers Within Subgroups of Participant Characteristics[b]

	Body Mass Index			Adult Weight Gain[c]			Waist Circumference		
	No. cases/controls	**OR**	**95% CI**	**No. cases/controls**	**OR**	**95% CI**	**No. cases/controls**	**OR**	**95% CI**
Low physical activity									
Total effect estimate	94/200	1.63	1.19, 2.24	92/192	1.64	1.13, 2.38	73/165	1.86	1.25, 2.76
Natural direct effect estimate[d]		1.45	0.97, 2.15		1.06	0.68, 1.66		1.87	1.15, 3.04
Natural indirect effect estimate[e]		1.12	0.92, 1.41		1.55	1.00, 2.56		0.99	0.66, 1.38
High physical activity									
Total effect estimate	115/182	1.25	0.91, 1.72	112/177	1.18	0.87, 1.61	93/153	1.63	1.07, 2.50
Natural direct effect estimate[d]		1.02	0.72, 1.44		1.06	0.78, 1.44		1.93	1.14, 3.25
Natural indirect effect estimate[e]		1.23	0.78, 1.56		1.12	0.66, 1.41		0.85	0.55, 1.27
NSAID/aspirin users									
Total effect estimate	86/188	1.55	1.10, 2.20	84/179	1.39	0.95, 2.03	73/157	1.68	1.10, 2.57
Natural direct effect estimate[d]		1.48	0.97, 2.25		1.12	0.71, 1.75		1.86	1.09, 3.16
Natural indirect effect estimate[e]		1.05	0.83, 1.35		1.24	0.92, 1.62		0.91	0.62, 1.29
NSAID/aspirin nonusers									
Total effect estimate	123/194	1.37	1.02, 1.83	120/190	1.36	0.99, 1.87	93/161	1.90	1.28, 2.82
Natural direct effect estimate[d]		0.97	0.70, 1.35		0.82	0.59, 1.14		2.19	1.37, 3.51
Natural indirect effect estimate[e]		1.41	0.89, 1.85		1.65	0.78, 2.52		0.87	0.51, 1.32
Low DASH score									
Total effect estimate	105/186	1.55	1.14, 2.11	103/180	1.31	0.91, 1.89	84/148	1.89	1.27, 2.83
Natural direct effect estimate[d]		1.31	0.89, 1.91		0.81	0.54, 1.21		2.22	1.28, 3.84
Natural indirect effect estimate[e]		1.19	0.75, 1.63		1.63	0.80, 2.43		0.85	
High DASH score									
Total effect estimate	104/196	1.31	0.96, 1.80	110/187	1.37	1.01, 1.86	91/156	1.83	1.24, 2.70

	Body Mass Index			Adult Weight Gain[c]			Waist Circumference		
	No. cases/controls	OR	95% CI	No. cases/controls	OR	95% CI	No. cases/controls	OR	95% CI
Natural direct effect estimate[d]		1.16	0.80, 1.67		1.11	0.80, 1.54		2.18	1.28, 3.71
Natural indirect effect estimate[e]		1.13	0.89, 1.39		1.24	0.88, 1.59		0.84	0.54, 1.16

[a] Per one-unit increase in the interquartile range of each adiposity measure (body mass index: 3.6 kg/m^2; adult weight gain: 10.4 kg; waist circumference: 11.4 cm)

[b] Adjusted for age at blood draw (montds), time of blood draw (montd and year), energy intake (kcal/day, continuous), physical activity (MET-hrs/week, continuous), regular NSAID or aspirin use (< vs. ≥2 pills/week), family history of CRC (yes vs. no), red and processed meat intake (servings/day, continuous), total folate intake (mg/day), previous history of CRC screening (yes vs. no), smoking (packyears, continuous), regular multivitamin use (yes vs. no), supplemental calcium use (mg/day, continuous), alcohol intake (g/day, continuous), height (inches, continuous), and DASH score (continuous)

[c] Additionally adjusted for BMI at age 21 (kg/m^2, continuous)

[d] The effect of the exposure on colorectal cancer risk that is not mediated by inflammatory and insulin biomarkers.

[e] The effect of the exposure on colorectal cancer risk that is mediated by inflammatory and insulin biomarkers.

Table 4.

Mediation of Associations Between Waist Circumference[a] and Risk of Colorectal Cancer by Inflammatory and Metabolic Biomarkers Using Alternative Waist Circumference Measures

	All Mediators		Inflammatory Mediators[b]		Metabolic Mediators[c]	
	Average of 1987 and 1996 Waist Circumference Measures					
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Total effect estimate[d]	1.56	1.17, 2.08	1.56	1.17, 2.08	1.56	1.17, 2.08
Natural direct effect estimate[e]	1.55	1.08, 2.23	1.60	1.17, 2.19	1.50	1.05, 2.14
Natural indirect effect estimate[f]	1.01	0.75, 1.35	0.98	0.88, 1.10	1.04	0.75, 1.39
	1996 Waist Circumference Measure					
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Total effect estimate[d]	1.54	1.20, 1.98	1.54	1.20, 1.98	1.54	1.20, 1.98
Natural direct effect estimate[e]	1.34	0.98, 1.82	1.55	1.19, 2.03	1.34	0.99, 1.80
Natural indirect effect estimate[f]	1.15	0.86, 1.44	0.99	0.90, 1.09	1.15	0.87, 1.45

[a] Per one-unit increase in the interquartile range of WC (average of 1987 and 1996 WC: 11.7 cm; 1996 WC: 13.3 cm)

[b] Includes C-reactive protein, interleukin-6, tumor necrosis factor receptor 2, and macrophage inhibitory cytokine-1

[c] Includes adiponectin, C-peptide, and soluble leptin receptor

[d] Adjusted for age at blood draw (months), time of blood draw (month and year), energy intake (kcal/day, continuous), physical activity (MET-hrs/week, continuous), regular NSAID or aspirin use (< vs. ≥2 pills/week), family history of CRC (yes vs. no), red and processed meat intake (servings/day, continuous), total folate intake (mg/day), previous history of CRC screening (yes vs. no), smoking (packyears, continuous), regular multivitamin use (yes vs. no), supplemental calcium use (mg/day, continuous), alcohol intake (g/day, continuous), DASH score (continuous), and height (inches, continuous)

[e] The estimated effect of the exposure on colorectal cancer risk that is not mediated by the biomarkers of interest.

[f] The estimated effect of the exposure on colorectal cancer risk that is mediated by the biomarkers of interest.