

# Body Fatness, Adipose Tissue Compartments, and Biomarkers of Inflammation and Angiogenesis in Colorectal Cancer: The ColoCare Study



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## Abstract

**Background:** Adiposity has been linked to both risk and prognosis of colorectal cancer; however, the impact of different fat areas [visceral (VFA) vs. subcutaneous fat area (SFA)] is unclear. We investigated associations between adiposity and biomarkers of inflammation and angiogenesis among patients with colorectal cancer.

**Methods:** Preoperative serum samples and computed tomography scans were obtained from 188 patients diagnosed with primary invasive stage I–IV colorectal cancer enrolled in the ColoCare Study. Adiposity was assessed by area-based quantification of VFA, SFA, and VFA:SFA ratio on spinal levels L3/L4 and L4/L5. Circulating levels of inflammation (CRP, SAA, sICAM-1, and sVCAM-1) and angiogenesis (VEGF-A and VEGF-D) were assessed from patient sera on the Meso Scale Discovery platform. Partial correlations and regression analyses, adjusted for age, sex, and tumor stage, were performed.

**Results:** VFA was moderately correlated with CRP and SAA (CRP: L3/L4 and L4/L5:  $r = 0.21$ ,  $P = 0.01$ ; SAA: L3/L4:  $r = 0.17$ ,  $P = 0.04$ ). The correlation between SFA and the measured biomarkers were weak ( $r \leq 0.13$ , not significant). The ratio of VFA:SFA at L3/L4 was moderately correlated with VEGF-A ( $r = 0.28$ ,  $P = 0.0008$ ) and SAA ( $r = 0.24$ ,  $P = 0.006$ ), and less so with CRP ( $r = 0.18$ ,  $P = 0.04$ ) and sICAM-1 ( $r = 0.18$ ,  $P = 0.04$ ). Similar correlations were found for the VFA:SFA ratio at L4/L5.

**Conclusions:** We observed an association between visceral adiposity and biomarkers of inflammation and angiogenesis in colorectal cancer. In particular, the VFA:SFA ratio was correlated with circulating levels of the proangiogenic biomarker VEGF-A.

**Impact:** Our findings support a direct association of visceral adipose tissue with inflammatory and angiogenic processes, which play fundamental roles in the development and progression of colorectal cancer.

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## Introduction

The prevalence of obesity [defined as body mass index (BMI)  $\geq 30$  kg/m<sup>2</sup>] among American adults ages 20 to 74 years has more than doubled since 1979 (1), and is estimated to increase by 65 million more obese adults in the United States alone from 2011 to 2030 (2). Accumulating evidence identifies obesity as a factor of colorectal cancer risk and prognosis (3, 4). The International Agency for Cancer Research (IARC) has reported that individuals with a BMI greater than or equal to 25 kg/m<sup>2</sup> have an increased risk of developing colorectal cancer relative to those with a normal BMI (defined as 18.5–24.9 kg/m<sup>2</sup>; ref. 5). The recently published report of the World Cancer Research Fund (WCRF) on nutrition, physical activity, and colorectal cancer shows that this association is nonlinear with a stronger observed risk increase above a BMI of 27 kg/m<sup>2</sup> (6). A review of the literature has further demonstrated poorer clinical outcomes (e.g., survival rates) for obese patients with colorectal cancer compared with nonobese patients (7). However, the term "obesity-paradox" has risen from accumulating evidence that shows improved survival among overweight or early obese patients compared with patients with a BMI below 22.5 kg/m<sup>2</sup> or over 30 kg/m<sup>2</sup> (8). Given that the prevalence of

obesity among individuals with a history of colorectal cancer increases annually by about 3.5% (9), there is an increasing need to define the biological mechanisms underlying the obesity-colorectal cancer link.

In the obese host–tumor microenvironment, adipocytes and secreted mediators, inflammatory cells, and colonocytes generate a quartet that promotes carcinogenesis (10). In particular, non-tumor cells such as macrophages and adipocytes are suggested to increase inflammatory processes (e.g., production and secretion of inflammatory biomarkers, recruitment of inflammatory cells) that lead to a reprogramming of cancer cell metabolism, as well as to perturbation of additional cancer hallmarks, including invasion, metastasis, and immune clearance (4, 10).

With respect to energy storage, white adipose tissue (WAT) is the key adipose tissue compartment (4). WAT and its related inflammatory secretion are together hypothesized to play a key role in the obesity-cancer link (10). Further subdivision of WAT into distinct body fat compartments, visceral (VAT) and subcutaneous adipose tissue (SAT), is based upon anatomical location, cellular structure, molecular composition, and secretome (10). Profiling the metabolome, lipidome, and transcriptome of distinct body fat compartments has further demonstrated that visceral fat area (VFA) consists of higher levels of tumor-promoting molecules (e.g., inflammation-related lipid metabolites, free arachidonic acid, phospholipases, and prostaglandin synthesis-related enzymes) compared with subcutaneous fat area (SFA; ref. 11). Together, these results are consistent with reports that increased visceral adiposity is associated with poorer outcomes, such as postoperative complications, survival, and recurrence, in the short- and long-term (12, 13).

The objective of this study was to investigate associations between different dimensions of body fatness and inflammation-related as well as angiogenesis-related biomarkers. We analyzed the associations between specific fat areas (VFA and SFA), and the (VFA:SFA) ratio, and circulating biomarkers to unravel the impact of VFA and SFA on processes involved in the colorectal carcinogenesis and the progression of colorectal cancer.

## Materials and Methods

### Study population

This study population includes patients from the international prospective ColoCare Study cohort (NCT02328677), that has been described in detail in prior publications (11, 12, 14–16). The ColoCare Study cohort includes men and women ages 18 to 89 years who were diagnosed with a primary invasive colorectal cancer (stages I–IV) undergoing surgery at clinics and sites internationally. Electronic medical charts, including pathologic reports, were reviewed to document other clinical characteristics (e.g., age at surgery, sex, tumor stage and site, treatment regimen). Anthropometric measurements (height, waist, and hip circumference) were taken at the clinic visit, and data on lifestyle (e.g., smoking status) and drug use (e.g., NSAIDs) were obtained from questionnaires collected at baseline, before surgery. Out of 407 patients enrolled in the ColoCare Study site in Heidelberg, Germany, between October 2010 and December 2014, 290 patients had blood draws before undergoing surgery. Patients were excluded if biomarker levels were outside of the detectable range ( $n = 12$ ), computed tomography (CT) scans were not available ( $n = 86$ ), or patients who were classified with stage 0 or "no malignancy" post-surgery ( $n = 4$ ). In total, 188 men and

women were included in this study. The study was approved by the Ethics Committee of the University of Heidelberg, and all subjects provided written informed consent.

### Blood processing and biomarker assays

Non-fasting blood samples were collected from patients before surgery (baseline) at the University Hospital of Heidelberg. Serum was extracted within four hours after blood-draw and stored in aliquots at  $-80^{\circ}\text{C}$  until analysis. 500  $\mu\text{L}$  of each patient's serum was shipped on dry ice to Huntsman Cancer Institute (HCI, Salt Lake City, UT) for analysis.

Serum-based assays for multiplexed vascular endothelial growth factor A (VEGF-A), vascular endothelial growth factor D (VEGF-D), C-reactive protein (CRP), serum amyloid A (SAA), soluble intracellular adhesion molecule 1 (sICAM-1), and soluble vascular cell adhesion molecule 1 (sVCAM-1) have previously been established on the Meso Scale Discovery platform (MSD, Rockville, MD) in the Ulrich laboratory at HCI (17). Biomarkers were selected based upon (i) clinical and epidemiologic relevance in colorectal carcinogenesis and progression, as well as direct links to body fatness (e.g., CRP, SAA), and (ii) high relevance in the stimulation and promotion of angiogenesis and metastases of colorectal cancer (e.g., sICAM-1, sVCAM-1, VEGF-A/D). Blinded patient samples plus three intraplate and interplate quality control samples (QC) were assayed for CRP, SAA, sICAM-1 and sVCAM-1 (V-PLEX Vascular Injury Plate 2), and for VEGF-A and VEGF-D (V-PLEX Angiogenesis Panel 1). Assays were conducted on the Sector 2400A (MSD, Rockville, MD). Blinded serum samples were run at dilutions of 1:1,000 (Vascular Injury panel) and 1:8 (Angiogenesis panel), and the serum was freeze-thawed only once. Data were analyzed with MSD Workbench 4.0 software (MSD). The overall interplate coefficient of variability (CV) was 9.9% and intraplate CV was 4.6%.

### Area-based CT quantification of abdominal adipose tissue

Abdominal CT scans conducted between August 2010 and December 2014 were assessed retrospectively using Centricity RIS 4.1i and GE PACS (GE Medical Systems). CT scans were predominantly performed before surgery (mean time, before: 42 days, after: 41 days). A prior study that used this data from the same population showed that pre- and postsurgical CT scans were similar, and thus, could be combined for statistical analyses (18). The quantification of VFA and SFA based on diagnostic CT data was performed using a dedicated post-processing software (Syngo Volume tool, MMPW, Siemens Healthcare, Munich, Berlin, Germany).

An area-based quantification of adipose tissue compartments was performed on two spinal levels most representative of the abdominal adipose tissue distribution (L3/L4, L4/L5). The quantity of adipose tissue measured on levels L3/L4 has been reported (e.g., in the Framingham Heart Study) to best reflect the volume-based quantification of abdominal adipose tissue compartments, including in age and sex subgroups (19). Spinal level L4/L5 has been observed to be strongly correlated with diabetes and hypertension (20). By manually tracing specific regions of interest at L3/L4 and L4/L5, total fat area (TFA, whole circumference), VFA (along the fascial plane tracing the abdominal wall; Supplementary Fig. S1) were measured (volumetric quantification of selected slice, divided by slice thickness; ref. 18). Adipose tissue was selected by limiting the measurements to a lower attenuation limit of  $-190$  Hounsfield units (HU) and an upper attenuation

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limit of  $-30$  HU (21, 22). SFA was determined by subtracting VFA from TFA. The VFA:SFA ratio was calculated as VFA:SFA (18).

### Statistical analysis

Mean and standard deviation (SD) values were calculated for continuous variables (age, BMI, VFA, SFA, VFA:SFA ratio, and biomarker measurements) and compared among men and women using the Wilcoxon sign-rank test. Frequencies and percentages were calculated for categorical variables (sex, smoking status, neoadjuvant treatment, tumor stage, and tumor site).

Continuous data were tested for normal distributions by performing the Shapiro–Wilk test and investigating the q–q-plot distributions for each biomarker. All biomarker levels were  $\log^2$ -transformed to prevent heteroscedasticity. Potential confounding by age at surgery (years), sex (male, female), body mass index (BMI,  $\text{kg}/\text{m}^2$ ), tumor stage (I–IV), NSAID use before blood draw (yes, no), and neoadjuvant chemotherapy (yes, no) was assessed. Final analyses were adjusted for patient age, sex, and tumor stage.

Pearson's partial correlation coefficients adjusted for age, sex, and tumor stage, were calculated to address the link between adiposity (including VFA, SFA, and VFA:SFA ratio on both levels L3/L4 and L4/L5, and BMI) and inflammation- and angiogenesis-related biomarkers. We additionally assessed associations between adiposity (exposure) and biomarkers of inflammation and angiogenesis (outcome) computing multiple linear regression models adjusted for age, sex, tumor stage. Sensitivity analyses were performed, excluding i) patients who underwent a CT scan greater than 6 months before or 6 months after the blood draw, and ii) patients with stage IV disease. Statistical analyses were performed using SAS 9.4 (2008, SAS Institute). All tests were considered to be statistically significant at  $P < 0.05$ .

## Results

A total of 188 individuals diagnosed with clinical stage I–IV colorectal cancer with available diagnostic CT scan measurements

**Table 1.** Baseline clinicopathologic and demographic characteristics among 188 patients enrolled in the ColoCare Study

<b>Age at surgery<sup>a</sup> (y)</b>	<b>62.7 ± 12.0</b>
<b>Sex, n (%)</b>	
Female	59 (31.4)
Male	129 (68.6)
<b>BMI<sup>a</sup> (kg/m<sup>2</sup>)</b>	<b>26.2 ± 4.16</b>
<b>Smoking history, n (%)</b>	
Never smoker	61 (32.4)
Ever smoker	116 (61.7)
Unknown	11 (5.9)
<b>Neoadjuvant treatment, n (%)</b>	
None	103 (54.8)
Yes	85 (45.2)
<b>Tumor stage, n (%)</b>	
I	32 (17.0)
II	54 (28.7)
III	51 (27.1)
IV	51 (27.1)
<b>Tumor site, n (%)</b>	
Colon	71 (38)
Rectum	117 (62)

Abbreviations: kg, kilogram; m, meters.

<sup>a</sup>Mean ± SD.

**Table 2.** Summary of adiposity and inflammation- and angiogenesis-related biomarker measurements (geometric mean ± SD) among 188 patients with colorectal cancer

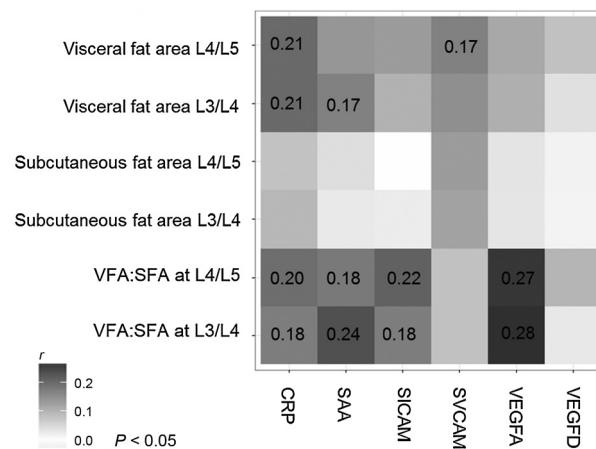
<b>Adiposity (mean ± SD)</b>	
SFA, L3/L4 ( $\text{cm}^2$ )	203 ± 89.5
SFA, L4/L5 ( $\text{cm}^2$ )	237 ± 93.3
VFA, L3/L4 ( $\text{cm}^2$ )	173 ± 100
VFA, L4/L5 ( $\text{cm}^2$ )	148 ± 78.9
Ratio 1: VFA:SFA at L3/L4	0.91 ± 0.54
Ratio 2: VFA:SFA at L4/L5	0.66 ± 0.35
<b>Inflammation and angiogenesis-related biomarkers (mean ± SD)</b>	
VEGF-A (pg/mL)	827 ± 583
VEGF-D (pg/mL)	859 ± 304
CRP (mg/L)	12.0 ± 20.6
SAA (mg/L)	18.4 ± 34.7
sICAM-1 (mg/L)	0.50 ± 0.22
sVCAM-1 (mg/L)	066 ± 0.34

Abbreviations: cm, centimeters; L, liters; mg, milligrams.

and preoperative blood samples were identified from the ColoCare Study over the 4-year study period (December 2010 to May 2014; Table 1). Mean age at surgery among individuals was 62 years. Sixty-eight percent of individuals were men. Over 50% of the population was overweight with mean BMI at  $26.2 \text{ kg}/\text{m}^2$  (23). Approximately two thirds of individuals reported being ever smokers. Among all cases, 62% of cancers were located in the rectum. Assessment of treatment history demonstrated that 45% of study participants had received neoadjuvant therapy. Overall, 27% of patients with colorectal cancer were diagnosed with advanced stage IV disease (Table 1).

The geometric mean concentrations of inflammation and angiogenesis-related biomarkers and adiposity measurements are summarized in Table 2. Mean VEGF-A ( $827 \pm 583 \text{ pg}/\text{mL}$ ) and VEGF-D ( $859 \pm 304 \text{ pg}/\text{mL}$ ) levels were increased compared with reference levels ( $\sim 500 \text{ pg}/\text{mL}$  and  $\sim 300 \text{ pg}/\text{mL}$ , respectively; refs. 24, 25). Elevated mean CRP ( $12 \pm 20 \text{ mg}/\text{L}$ ) and SAA ( $18 \pm 34 \text{ mg}/\text{L}$ ) biomarker levels were indicative of activated systemic inflammatory processes (26). VFA (L3/L4):  $112.1 \pm 73.18 \text{ cm}^2$  vs.  $198.2 \pm 73.18 \text{ cm}^2$ ,  $P < 0.001$ ; VFA (L4/L5):  $114.8 \pm 68.21 \text{ cm}^2$  vs.  $159.6 \pm 70.71 \text{ cm}^2$ ,  $P < 0.001$ ) statistically significantly differed between men and women. We also observed sex-specific differences of VFA:SFA ratios on L3/L4 and L4/L5 (VFA:SFA (L3/L4):  $0.5 \pm 0.35$  vs.  $1.0 \pm 0.47$ ,  $P < 0.001$ ; VFA:SFA (L4/L5):  $0.4 \pm 0.24$  vs.  $0.7 \pm 0.32$ ,  $P < 0.001$ , respectively; Supplementary Table S1; ref. 18). SFA measured on L4/L5 was statistically significantly larger in men compared with women ( $260.8 \pm 97.57 \text{ cm}^2$  vs.  $224.8 \pm 84.26 \text{ cm}^2$ ,  $P = 0.011$ , respectively; ref. 18).

Pearson's partial correlation coefficients, adjusted for age at surgery, sex, and tumor stage, are presented in Fig. 1 and Table 3. Moderately positive correlations were observed between CRP-VFA and SAA-VFA measured on both, L3/L4 and L4/L5 ( $r = 0.21$ ,  $P = 0.01$ ;  $r = 0.17$ ,  $P = 0.04$ , respectively). No significant correlations were observed between SFA and inflammation- or angiogenesis-related biomarkers. The ratio of VFA:SFA on L3/L4 showed a moderate positive correlation with SAA ( $r = 0.24$ ,  $P = 0.006$ ) and VEGF-A ( $r = 0.28$ ,  $P = 0.0008$ ). The correlations of VFA:SFA with CRP ( $r = 0.18$ ,  $P = 0.04$ ) and sICAM-1 ( $r = 0.18$ ,  $P = 0.04$ ) were modest (Fig. 1). The correlations of VFA:SFA ratio with measured biomarkers at L4/L5 were similar, with the strongest correlation with



**Figure 1.**

Heat map of partial Pearson correlation coefficients between adiposity and inflammation- and angiogenesis-related biomarker measurements. Correlation coefficients are presented for associations that reached the significance threshold of  $P < 0.05$ . Analyses were adjusted for age, sex, and tumor stage. L, level.

VEGF-A ( $r = 0.27$ ,  $P = 0.001$ ). Similar results were observed in the multiple linear regression analyses (Table 4). Given the noted differences of body compositions between men and women (Supplementary Table S1), we performed correlation and regression analyses stratified by sex (Supplementary Table S2 and S3). We observed consistent results among men and women across CRP, SAA, sICAM-1, and sVCAM-1 levels, with exception of the correlation between SFA on level L3/L4 and SAA among women, which presented a strong correlation ( $r = 0.41$ ,  $P = 0.02$ ) compared with no observed correlation among men. However, there were marked differences for VEGF-A levels in correlation with VFA on level L4/L5 within the sex-specific subgroups. The positive moderate correlation was limited to men ( $r = 0.20$ ,  $P = 0.04$ ) versus an inverse moderate correlation noted among women ( $r = -0.20$ ,  $P = 0.19$ ). The analyses also identified an interaction between VFA on L4/L5 and sex ( $P < 0.03$ ).

Results from sensitivity analyses excluding patients who underwent a CT scan over 6 months before or 6 months post-blood draw ( $n = 8$ , 4.3%) remained unchanged. Sensitivity analyses excluding stage IV patients with colorectal cancer ( $n = 51$ , 27.1%) resulted in stronger correlations across all biomarker levels (Supplementary Table S4).

## Discussion

Our study of 188 patients diagnosed with colorectal cancer enrolled in the prospective, international ColoCare Study quantified area-based adipose tissue compartments via diagnostic CT scans and systemic biomarkers from patient sera to elucidate associations between the distribution of adipose tissue and circulating inflammation- and angiogenesis-related biomarker levels in patients with colorectal cancer. Serum-based levels of CRP and SAA were modestly correlated with VFA and showed moderate correlations with the VFA:SFA ratio, but not with SFA. Correlations with sICAM-1 and VEGF-A were uniquely associated with the VFA:SFA ratio. This study is the first to investigate correlations between CT-based adipose tissue measurements and systemic levels of inflammatory and angiogenesis biomarkers among patients diagnosed with colorectal cancer.

Evidence from clinical and translational studies has led to the consensus that 13 types of cancer are convincingly associated with body fatness, including cancers of the colon and rectum (5, 27–29). In particular, visceral fat has been identified as the main driver of the obesity–cancer link. Compared with BMI, visceral adiposity has been reported to be a clinically significant predictor of short-term postoperative surgical complications, as well as long-term clinical outcomes (including recurrence and survival) among patients with colorectal cancer (12, 30, 31). Increased metabolic processes leading to the production and secretion of tumor-promoting markers in VAT (11), corroborates the distinct role of visceral fatness as a substantial component of the obesity–cancer link (11, 12, 30–34). Our results show a weaker correlation of pro-inflammatory biomarkers with BMI than with VAT. This observation supports the quantification of adipose tissue compartments as an improved predictor of tumor-promoting processes relative to BMI metrics.

The crosstalk between a tumor and its adipose tissue microenvironment is a complex interplay that includes heterogeneous cells as well as local and systemic secreted mediators (10). These adiposity-driven inflammatory processes can enhance carcinogenesis and tumor progression, including via the provision of cytokines to the tumor microenvironment (35). VAT is directly adjacent to the colon and thus, part of the developing tumor microenvironment (10). In our study, we demonstrated moderately strong correlations between VFA and VFA:SFA, and inflammation-related biomarkers CRP and SAA.

Inflammatory processes driven by adipose tissue also sustain proangiogenic signals to the tumor microenvironment, as the oxygen and nutrient needs of tumor cells are supplied via the establishment of tumor-associated angiogenesis (35, 36). Key

**Table 3.** Pearson partial correlation coefficients between adiposity and inflammation- and angiogenesis-related biomarker measurements adjusted for age, sex, and tumor stage

	VEGF-A (pg/mL)		VEGF-D (pg/mL)		CRP (mg/L)		SAA (mg/L)		sICAM-1 (mg/L)		sVCAM-1 (mg/L)	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
BMI (kg/m <sup>2</sup> )	−0.09	0.25	−0.03	0.69	0.12	0.16	0.00	0.98	0.09	0.32	<b>0.23</b>	<b>0.006</b>
SFA, L3/L4 (cm <sup>2</sup> )	−0.04	0.66	0.02	0.86	0.10	0.27	0.03	0.74	0.03	0.78	0.13	0.15
SFA, L4/L5 (cm <sup>2</sup> )	−0.04	0.64	−0.02	0.84	0.08	0.36	0.04	0.62	−0.00	0.99	0.13	0.12
VFA, L3/L4 (cm <sup>2</sup> )	0.11	0.17	0.04	0.62	<b>0.21</b>	<b>0.01</b>	<b>0.17</b>	<b>0.037</b>	0.10	0.21	0.15	0.07
VFA, L4/L5 (cm <sup>2</sup> )	0.12	0.14	0.08	0.31	<b>0.21</b>	<b>0.01</b>	0.14	0.09	0.114	0.10	<b>0.17</b>	<b>0.036</b>
Ratio 1: VFA:SFA at L3/L4	<b>0.28</b>	<b>0.0008</b>	0.030	0.72	<b>0.18</b>	<b>0.04</b>	<b>0.24</b>	<b>0.006</b>	<b>0.18</b>	<b>0.04</b>	0.08	0.34
Ratio 2: VFA:SFA at L4/L5	<b>0.27</b>	<b>0.001</b>	0.10	0.23	<b>0.20</b>	<b>0.02</b>	<b>0.18</b>	<b>0.036</b>	<b>0.22</b>	<b>0.01</b>	0.08	0.34

Abbreviations: cm, centimeters; L, liters; mg, milligrams.

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**Table 4.** Multiple regression analyses between adiposity and inflammation- and angiogenesis-related biomarker measurements adjusted for age, sex, and tumor stage

	VEGF-A (pg/mL)		VEGF-D (pg/mL)		CRP (mg/L)		SAA (mg/L)		sICAM-1 (mg/L)		sVCAM-1 (mg/L)	
	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>
<b>BMI (kg/m<sup>2</sup>)</b>	-0.02	0.28	-0.002	0.73	0.04	0.18	-0.0001	1.00	0.01	0.32	<b>0.03</b>	<b>0.006</b>
<b>SFA, L3/L4 (cm<sup>2</sup>)</b>	-0.0003	0.65	0.000007	0.82	0.001	0.31	0.0004	0.79	0.0001	0.78	0.005	0.15
<b>SFA, L4/L5 (cm<sup>2</sup>)</b>	-0.0003	0.64	-0.00003	0.92	0.001	0.38	0.001	0.66	0.000001	1.00	0.005	0.13
<b>VFA, L3/L4 (cm<sup>2</sup>)</b>	0.0001	0.16	0.001	0.68	<b>0.003</b>	<b>0.02</b>	<b>0.00</b>	<b>0.04</b>	0.10	0.21	0.15	0.07
<b>VFA, L4/L5 (cm<sup>2</sup>)</b>	0.001	0.13	0.0004	0.35	<b>0.0039</b>	<b>0.02</b>	0.003	0.09	0.001	0.10	<b>0.001</b>	<b>0.036</b>
<b>Ratio 1: VFA:SFA at L3/L4</b>	<b>0.43</b>	<b>0.0006</b>	0.015	0.80	<b>0.53</b>	<b>0.053</b>	<b>0.66</b>	<b>0.007</b>	<b>0.15</b>	<b>0.04</b>	0.06	0.33
<b>Ratio 2: VFA:SFA at L4/L5</b>	<b>0.62</b>	<b>0.001</b>	0.09	0.30	<b>0.89</b>	<b>0.41</b>	<b>0.76</b>	<b>0.04</b>	<b>0.27</b>	<b>0.01</b>	0.10	0.32

Abbreviations: cm, centimeters; L, liters; mg, milligrams.

mediators involved in angiogenesis, including VEGF and platelet-derived growth factor (PDGF), may also be driven by the obesity-related imbalance of adipokines (37) and are important targets for therapeutic development (38). Consistent with these findings, body fatness has been hypothesized as a predictive marker of anti-VEGF agents' efficacy (e.g. bevacizumab, ramucirumab), particularly among individuals diagnosed with metastatic colorectal cancer (39–41). However, studies have reported conflicting evidence on the predictive role of body fatness in therapeutic efficacy, presenting none or a negative association between excess adiposity and outcomes of anti-VEGF agents (39–42). Intervention studies among cancer-free individuals with excess body fat have also reported a reduction in circulating VEGF levels associated with diet- and/or exercise-induced weight loss (43–46). These results suggest that the weight loss-induced reduction of adipose tissue does lead to alterations in the production and availability of angiogenesis-related mediators. Yet whether adiposity and angiogenesis-related pathways are linked among patients with colorectal cancer remains unknown.

We observed associations between VFA, but not SFA, and angiogenesis biomarkers among patients with colorectal cancer. In particular, systemic levels of VEGF-A were moderately correlated with the VFA:SFA ratio. These correlations were stronger in sensitivity analyses after excluding individuals with advanced colorectal cancer. This observation further lends support to our hypothesis that VFA may be correlated with circulating angiogenesis and inflammation biomarkers, particularly because patients with metastatic disease often have considerable cachexia and extensive depletion of adipose tissue, particularly VFA (47). Given our observed findings from sex stratified analysis, further sex-specific research regarding body fatness and angiogenesis biomarkers is warranted. Together, our results support an important role for visceral adiposity in the activation of inflammation and proangiogenic pathways, and warrant future studies that seek to evaluate the effectiveness of circulating angiogenesis-related biomarkers as predictive markers of targeted therapeutics of angiogenic signals among patients with colorectal cancer.

Our study has several strengths and limitations: To our knowledge, this study is the first to assess correlations between adipose tissue compartments and systemic biomarker levels of inflammation and angiogenesis among patients with colorectal cancer. The use of paired CT scan data to quantify specific adipose tissue compartments, medical records, and sera from patients with colorectal cancer enrolled in the prospective ColoCare Study provided a well-characterized cohort of patients to examine these associations. Our study was limited in that inclusion of patients required available, pre-existing CT scan measurements from standard diagnostics for retrospective evaluation due to radiation

protection. Because we evaluated correlations between biomarkers of inflammation and angiogenesis with adiposity in a cross-sectional design, our results do not allow for temporal interpretations. Finally, our results should be interpreted in the context of our study population of patients with cancer, which additionally comprised a high proportion of smokers and patients with rectal cancer, and are not generalizable to the general population.

In conclusion, visceral adiposity is associated with systemic biomarker levels of inflammation and angiogenesis among patients with colorectal cancer. Although no such associations were reported for SAT, identification of this link between visceral fat and circulating biomarkers supports the impact of visceral adiposity on carcinogenesis and angiogenesis-promoting processes in colorectal cancer. Given the rise of the obesity epidemic among adults, our results emphasize the critical need to evaluate components of body fatness among patients with colorectal cancer at time of cancer diagnosis and to understand the unique contributions of adipose tissue compartments to colorectal carcinogenesis.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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# Cancer Epidemiology, Biomarkers & Prevention

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