Diet-induced weight loss reduces colorectal inflammation: implications for colorectal carcinogenesis[1–3]

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ABSTRACT

Background: Epidemiologic data have shown that obesity independently increases colorectal cancer (CRC) risk, but the mechanisms are poorly understood. Obesity is an inflammatory state, and chronic colonic inflammation induces CRC.

Objective: We conducted this proof-of-principle study to seek evidence of obesity-associated colorectal inflammation and to evaluate effects of diet-induced weight loss.

Design: We measured inflammatory cytokines, gene arrays, and macrophage infiltration in rectosigmoid mucosal biopsies of 10 obese premenopausal women [mean ± SD body mass index (in kg/m^2): 35 ± 3.5] before and after weight loss induced by a very-low-calorie diet.

Results: Subjects lost a mean (±SD) of 10.1 ± 1% of their initial weight. Weight loss significantly reduced fasting blood glucose, total cholesterol, triglycerides, LDL, tumor necrosis factor-α (TNF-α), and interleukin (IL)-8 concentrations ($P < 0.05$). After weight loss, rectosigmoid biopsies showed a 25–57% reduction in TNF-α, IL-1β, IL-8, and monocyte chemotactic protein 1 concentrations ($P < 0.05$). T cell and macrophage counts decreased by 28% and 42%, respectively ($P < 0.05$). Gene arrays showed dramatic down-regulation of proinflammatory cytokine and chemokine pathways, prostaglandin metabolism, and the transcription factors *STAT3* (signal transducer and activator of transcription 3) and nuclear transcription factor κB. Weight loss reduced expression of *FOS* and *JUN* genes and down-regulated oxidative stress pathways and the transcription factors *ATF* (activating transcription factor) and *CREB* (cyclic AMP response element-binding).

Conclusions: Our data show that diet-induced weight loss in obese individuals reduces colorectal inflammation and greatly modulates inflammatory and cancer-related gene pathways. These data imply that obesity is accompanied by inflammation in the colorectal mucosa and that diet-induced weight loss reduces this inflammatory state and may thereby lower CRC risk. *Am J Clin Nutr* 2011;93:234–42.

INTRODUCTION

Epidemiologic evidence suggests obesity as an independent risk factor for the development of several cancers, including colorectal cancer (CRC) (1). Every 5-unit increase in body mass index (BMI) increases risk of CRC by 30% in men and by 13% in women (2). The prevalence of obesity has increased dramatically in adult Americans from 14% in the 1970s to the current level of 34% (3–5). The increased prevalence of obesity indicates that the incidence of CRC and other forms of cancer will become a greater public health problem in the future.

In mouse models of genetic and chemically induced colitis, obesity enhanced the development of colorectal neoplasia (6, 7). Western-style diet consumption in mice caused obesity accompanied by increased oxidative stress, inflammation in the colon, and development of colon tumors without genetic or chemical manipulation (8, 9). Consistent are findings that caloric restriction protected mice from developing aberrant crypt foci and colonic neoplasia (10) and decreased expression of colonic cyclooxygenase-2 (*COX-2*) in the colon (11). In the Iowa Women's Health Study, an intentional weight loss of >20 pounds reduced colon cancer incidence by 9% and obesity-related cancer mortality by 40% (12).

Several factors have been proposed to mediate the relation between obesity and increased CRC risk. Obesity is associated with elevated concentrations of insulin-like growth factor (IGF)-1, which is a known stimulator of epithelial growth (13). Increased plasma concentrations of inflammatory cytokines including tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 occur in obesity and are elevated in subjects with colorectal adenomas (14). Furthermore, antiinflammatory drugs, such as aspirin and celecoxib, reduce colorectal adenoma incidence (15, 16). Subgroup analyses in one study revealed aspirin to have a greater protective effect in obese subjects than in lean subjects (17).

Obesity is associated with chronic low-grade inflammation in the adipose tissue, liver, and coronary endothelium and is accompanied by increased oxidative stress (18), which through intermediary molecules, increase the expression of the proto-

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[2] Supported by a Clinical and Translational Science Award (grant UL1RR024143) to the Rockefeller University from the National Center for Research Resources, a component of the NIH, and an NIH Roadmap for Medical Research.

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Received August 23, 2010. Accepted for publication November 9, 2010.
First published online December 8, 2010; doi: 10.3945/ajcn.110.002683.

oncogenes *FOS* and *JUN* that leads to increased transcription of proinflammatory and cell-cycle regulatory genes that promote carcinogenesis (19). Chronic inflammation can enhance the initiation and progression of CRC (20).

Therefore, we hypothesized that obese humans would show signs of chronic inflammation in their colorectal mucosa. To test this hypothesis, obese women were studied before and after a 10% weight loss induced by a very-low-calorie diet (VLCD), which reduced rectosigmoid mucosal inflammatory cytokine concentrations and inflammatory cell infiltration accompanied by a decreased expression of gene pathways involved in inflammation and cancer, including gene sets regulated by TNF-α, IL-6 and IL-17, prostaglandins, and oxidative stress. Weight loss also decreased the expression of gene sets regulated by transcription factors involved in colorectal inflammation and CRC, such as signal transducer and activator of transcription 3 (STAT3), nuclear transcription factor κB (NF-κB), activating transcription factor (*ATF*), and cyclic AMP response element-binding (*CREB*).

SUBJECTS AND METHODS

Subjects

For this unblinded study, 10 healthy, obese [average (±SD) BMI (in kg/m^2): 34.8 ± 3.5], premenopausal women (mean age: 43.2 ± 8.3 y) were recruited from the community through advertisements. Study volunteers included 6 whites, 1 Hispanic, 2 African Americans, and 1 subject with a mixed racial background (**Table 1**). All subjects underwent a complete medical examination, standard blood and urine tests, and an electrocardiogram. Subjects were weight stable (<2% change) for ≥6 mo before the study. Exclusion criteria included a history of cancer, current treatment of weight loss, previous intestinal surgery, a history suggestive of malabsorption, major medical problems, consumption of medications with antiinflammatory properties (eg, nonsteroidal antiinflammatory drugs, statins, glitazones, and COX-2 inhibitors) or medications that could increase risk of complications associated with consuming a VLCD (eg, lithium), an allergy to soy products or aspartame, or contraindications to undergoing a sigmoidoscopy and biopsy, including any history of excessive bleeding. The study was approved by the Institutional Review Board of The Rockefeller University (New York, NY). Subjects were recruited between September 2007 and May 2008. Written informed consent was obtained from each subject before participation in the study.

Experimental design

Seven subjects chose to complete the weight-loss phase of the study in The Rockefeller University Hospital inpatient unit, and 3 subjects were closely monitored as outpatients. After screening, subjects underwent baseline testing, which included fasting blood determinations, urine analysis, and flexible sigmoidoscopy with multiple mucosal biopsies. Subjects started a VLCD weight-loss program and were monitored weekly by the study physicians. The VLCD was continued until subjects had lost ≥8% of baseline weight, at which time the initial measurements were repeated. This degree of weight loss was chosen because a previous study showed that this would lower rectosigmoid proliferation in obese subjects by ≈40% (21). Subjects were counseled that the study required them to maintain their usual physical activity, and this was emphasized throughout the study.

Subjects consumed a reduced-calorie, Western-style diet that contained 50% of their calculated baseline caloric intake for 3 d before initiation of the VLCD. Subjects were fed a commercially available VLCD product (New Direction Program; Robard Corporation, Mount Laurel, NJ) that provided ≈782 kcal/d with an energy distribution of carbohydrate, fat, and protein of 25%, 25%, and 50%, respectively, and daily requirements of vitamins and minerals (**Table 2**). This VLCD provided a choice of shakes, soups, bars, and pudding in different flavors; subjects had 4 choices/d, consumed one item every 4 h, and drank ≥2 L water daily. Inpatients were monitored daily by the hospital staff, and outpatients were monitored weekly during clinic visits. Weekly blood tests for electrolytes and renal function tests were performed to monitor for complications that can occur with VLCD consumption.

Procedures and sample collection

Fasting electrolytes, liver function tests, renal function tests, lipid profiles, and high-sensitivity C-reactive protein measurements were performed by the clinical chemistry laboratory at

TABLE 1
Baseline characteristics of the 10 obese volunteers[1]

Subject	Age	Weight	Height	BMI	Race
	y	*kg*	*cm*	*kg/m^2*	
1	49	74.8	158	30.2	H
2	43	104	159	41.3	W
3	39	92	171	31.4	W
4	49	95	158	38.3	M
5	52	97	172	32.8	AA
6	24	93	167.5	33.0	W
7	49	120	178	37.9	AA
8	37	98	170	34.0	W
9	47	83	158	33.4	W
10	43	103	170	35.5	W
Combined	43 ± 8[2]	96 ± 12	166 ± 7	34.8 ± 4	6 W, 2 AA, 1 H, and 1 M

[1] H, Hispanic; W, white; M, mixed; AA, African American.
[2] Mean ± SD (all such values).

TABLE 2
Composition of the very-low-calorie diet[1]

Nutrient	Daily intake	Percentage of energy
Energy (kcal)	782	100
Carbohydrates (g)	48	25
Fat (g)	23	26
Protein (g)	96	49

[1] The diet was fortified with vitamins and minerals and provided >100% of the Recommended Dietary Allowance on the basis of a 2000-kcal/d diet.

Memorial Sloan-Kettering Cancer Center. Aliquots of serum samples for cytokine measurements were stored at −80°C. Flexible proctosigmoidoscopy was performed between 0800 and 0900 after a 60-mL tap-water enema, and 12–14 rectosigmoid biopsies were obtained from 4 quadrants between 10 and 20 cm from the anal verge. Four biopsies for mucosal cytokine measurements were immediately frozen in liquid nitrogen; 6 biopsies for gene expression analysis were immersed in RNAlater (catalog no. 7024; Ambion) at 4°C for 24 h and frozen in liquid nitrogen. The remaining biopsies were frozen in optimum cutting temperature compound (OTC; International Medical Equipment, Chico, CA) and stored at −80°C for immunohistochemical analysis.

Sample analyses

Serum cytokines

Serum cytokines were measured in duplicate with the Human Proinflammatory 9-Plex MULTI-SPOT 96-well plate kit (catalog no. N05007A-1; Mesoscale Diagnostics, Gaithersburg, MD) (22) for IL-2, IL-8, IL-12p70, IL-1β, IL-6, and IL-10, TNF-α, and interferon-γ with a intraassay correlation $\leq$10% and interassay variance $\leq$10%.With the use of this multiplex assay, only IL-6 and -8 and TNF-α were detectable with a CV $\leq$10% and recovery rate $\geq$80%. Monocyte chemotactic protein (MCP)-1 was measured with a human Elisa kit (catalog no. DCP00; R&D Systems, Minneapolis, MN) with an intraassay correlation $\leq$8% and interassay variance $\leq$7%.

Mucosal cytokines

Two frozen mucosal biopsies were homogenized in RIPA buffer [25 mmol tris HCl/L (pH 7.6), 150 mmol NaCl/L, 1% NP-40, 1% sodium deoxycholate, and 0.1% sodium dodecyl sulfate; catalog no. 89900; Pierce, Rockport, IL] with protease and phosphatase inhibitors at 4°C (catalog nos. P8340 and P5726; Sigma-Aldrich, Saint Louis, MO). The homogenate was centrifuged at 15,000 rpm for 20 min at 4°C, and the supernatant fluid was collected and stored at −80°C until further analysis. Mucosal cytokines were measured in duplicate with the Proinflammatory 9-Plex MULTI-SPOT 96-well plate kit (catalog no. N05007A-1; Mesoscale Diagnostics) as described for serum cytokines. Only mucosal IL-6, IL-8, IL-1β, and TNF-α were detectable with a CV $\leq$10% and recovery rate $\geq$80%. MCP-1 concentrations were measured as previously described. Mucosal protein concentrations were measured with the Pierce BCA assay kit (catalog no. 23225; Thermo Fischer, Waltham, MA) with Nanodrop technology (NanoDrop Technologies, Wilmington, DE). Mucosal cytokine concentrations are presented as picograms of each cytokine per milligram of mucosal protein. Samples with cytokine concentrations $\geq$2.5 SD from the mean were considered outliers and excluded from the final analysis.

Immunohistochemistry

Six-micron sections were cut at −20°C onto plus slides (Fisher-Scientific, Waltham, MA) and stored at −80°C until assay. For immunohistochemical analyses, sections were quenched in ice-cold acetone and rehydrated in phosphate buffered saline (PBS). Sections were treated with purified mouse anti-human antibodies to cluster of differentiation (CD) 3 (T cell marker) at a 1:200 dilution (catalog no. 34740; BD Pharminogen, Franklin Lakes, NJ) and to CD163 (macrophage marker) at a 1:500 dilution (catalog no. BM4041; Novus Biologics, Littleton, CO). The secondary antibody, a biotin-labeled horse anti-mouse antibody at a 1:200 dilution was applied and followed by amplification with an avidin-biotin complex (1:100 of avidin and 1:100 of biotin; catalog no. 6102; Vector Laboratories, Burlingame, CA), and the signal was developed with chromogen-3-amino-9-ethylcarbazole (Sigma-Aldrich). The area of the sections and number of positive cells contained were counted manually with the National Institutes of Health Image J software (NIH IMAGE J 1.42; National Institutes of Health, Bethesda, MD) and reported as the number of positive cells per square millimeter. For each subject before and after the weight-loss regimen, all cells were counted in 3 separate sections >100 μm apart at a 10× magnification. Each section was analyzed in a random fashion by a single person blinded to their identity. A subset of sections were analyzed $\geq$3 times in a similar fashion, and the CV was <10%. Mucosal morphology was assessed by hematoxylin (Fisher Scientific, Waltham, MA) and eosin (Shandon, Pittsburg, PA) staining of sections by using a standard protocol. Data from only 8 subjects are presented for CD163 staining because the staining quality was inadequate for analysis in 2 subjects.

Gene expression

Total RNA was extracted from 2 rectosigmoid biopsy specimens by using the Trizol method (Invitrogen, Carlsbad, CA). RNA purification, quality assessment, and hybridization were performed as previously described (23). Biotin-labeled complementary RNA was hybridized onto Human HT-12v3 Ilumina expression chips (Illumina Inc, San Diego, CA). Samples from baseline and post–weight loss for each subject were analyzed on the same chip. The arrays were scanned by the Illumina BeadStation laser scanning imaging system (Illumina).

For determinations of real-time polymerase chain reactions (PCRs), 2 μg total RNA was used as a template for complementary DNA synthesis with a SuperScript-III First-Strand kit (Invitrogen). Complementary DNA was diluted in water, and amounts corresponding to 50 ng original RNA were used for quantitative gene expression by real-time PCR. SYBR Green PCR master mix (Applied Biosystems, Foster City, CA), primers from Sigma-Aldrich, and the ABI Prism7900 real-time PCR system (Applied Biosystems, Carlsbad, CA) were used for the real-time PCR. Six down-regulated and 4 up-regulated genes were assayed, which were normalized to the housekeeping gene *GAPDH* to adjust for the sample loading and efficiency of the reaction. Real-time PCR samples were assessed in duplicate, and messenger RNA concentrations were expressed as the log

ratio of relative expression concentrations before and after treatment (*see* supplemental Table 1 under "Supplemental data" in the online issue for a list of real-time PCRs).

Analysis of the gene-expression microarray data

Gene-expression data from the microarrays were analyzed with the R software (version 2.1) and available packages from Bioconductor (http://www.bioconductor.org). Data were normalized by using robust spline normalization functions from the lumi package (Bioconductor). A classical quality control was performed. Expression concentrations with detection P values ≤ 0.05 were considered detectable. Probes with detectable expression in at least one sample and with SD >0.1 were used in further analysis. Expression values ($\log_2$ transformed) were modeled with the package limma (Bioconductor). Moderated paired t tests were used to test the significance of the effect of weight loss. P values were adjusted for multiple comparisons by using the Benjamini-Hochberg procedure. Genes with fold-changes ≥ 1.2 and $P \leq 0.05$ were considered differentially expressed. Heat maps were generated by hierarchical clustering by using one Pearson r as the distance measure and complete linkage as the agglomeration procedure. The gene-expression data were deposited in the public domain (transcription profiling accession no. GSE20931).

Gene set enrichment analysis

Gene set enrichment analysis (GSEA) was conducted with the GSEA desktop application (http://www.broadinstitute.org/gsea) (24). Our analysis of gene expression before and after weight loss in each subject was performed in a paired fashion, and genes were ranked by their effect size (ie, the mean fold change for each subject). This rank-order list was used as the input to the GSEA. For each gene set, the enrichment score (ES) was calculated by using weighted Kolmogorov-Smirnov statistics to measure the proximity of the gene set to the top of the weight-loss effect ranked list. A high and positive ES indicated that the gene set or pathway was collectively up-regulated by the weight-loss intervention, whereas a negative ES indicated down-regulation. The significance (P value) of the observed ES was calculated by using simulations. Normalized ESs were used to compare analysis results across gene sets. The molecular signature database available at the Broad Institute, MIT (http://www.broadinstitute.org/gsea/msigdb), and gene sets created at the Rockefeller University from the data generated by IL-17 stimulation of keratinocytes (25, 26) were used to query the data. We used sets from 3 different collections from the GSEA website [ie, the curated canonical pathway database (C2), the Gene Ontology database (C5), and the transcription factor database (C3)]. Gene sets that were positively or negatively enriched with $P < 0.05$ and a false discovery rate (FDR) $<5\%$ were considered significant, except for the transcription factor gene sets for which an FDR $<10\%$ was considered significant.

Statistical analyses

Two-tailed paired t tests were used to compare anthropometric measurements, biochemical variables, serum and mucosal cytokine concentrations, and CD3 and CD163 cell counts at baseline and after weight loss. $P \leq 0.05$ was considered significant. S-Plus (S+) version 8.1 for Windows software package (TIBCO Software Inc, Somerville, MA) was used for statistical analyses.

RESULTS

Anthropometric and biochemical measurements

All 10 subjects successfully completed the study without significant adverse events in a mean of 46.5 ± 9.3 d. The VLCD resulted in a mean (±SD) decrease in body weight of 10.1 ± 1% ($P < 0.01$), decreased BMI of 10.2 ± 1.4% ($P < 0.01$), and reduction in waist circumference of 8.3 ± 4%, ($P < 0.01$). The weight-loss regimen also induced improvements in systolic and diastolic blood pressures, fasting blood sugar concentrations, total and LDL serum cholesterol and triglyceride concentrations, and total white blood cell counts (**Table 3**). Serum electrolyte concentrations, liver function tests, and renal function tests were not significantly altered.

Serum and mucosal cytokines

Diet-induced weight loss resulted in mean reductions in serum TNF-α concentrations of 15% and in IL-8 concentrations of 30%

TABLE 3
Effect of diet-induced weight loss on anthropometric and biochemical measurements[1]

Measurement	Baseline	After weight loss	Percentage change	P
Weight (kg)	96 ± 12	86 ± 11	10 ± 1.4	<0.01
BMI (kg/m^2)	35 ± 3.5	31 ± 3	10 ± 1.4	<0.01
Waist circumference (cm)	107 ± 11	97 ± 12	8.3 ± 4.4	<0.01
Systolic blood pressure (mm Hg)	121 ± 8	107 ± 12	11.6 ± 7.7	<0.01
Diastolic blood pressure (mm Hg)	79 ± 4	70 ± 6	11 ± 9.3	<0.01
Fasting blood glucose (mg/dL)	95 ± 10	85 ± 6	9.7 ± 13.2	0.03
Total cholesterol (mg/dL)	187 ± 36	157 ± 26	15 ± 8.8	<0.01
LDL (mg/dL)	110 ± 28	94 ± 19	13 ± 11	0.01
Triglycerides (mg/dL)	122 ± 45	93 ± 36	22 ± 18	<0.01
HDL (mg/dL)	52 ± 9	45 ± 6	12 ± 14	0.02
hs-CRP (mg/dL)	0.8 ± 0.5	0.9 ± 0.7	1.4 ± 0.5	0.85
WBC (1000/mm^3)	6.7 ± 1.3	5.6 ± 1.4	15.2 ± 18.1	0.02

[1] All values are mean ± SDs; n = 10. hs-CRP, high-sensitivity C-reactive protein; WBC, peripheral white blood cell count.

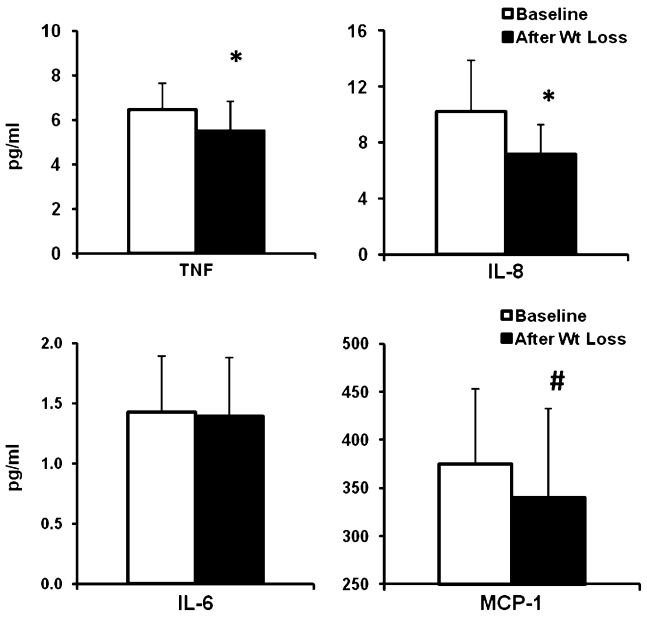

FIGURE 1. Mean (±SD) changes (pg/mL) in serum cytokine and chemokine concentrations in 10 subjects before and after diet-induced weight loss. $^{*}P < 0.05$, $^{\#}P < 0.1$. TNF, tumor necrosis factor; IL, interleukin; MCP, monocyte chemotactic protein.

(both $P < 0.05$). There was also a trend toward decreased serum contents by 9% ($P = 0.097$) (**Figure 1**) but no change in concentrations of IL-6 and high-sensitivity C-reactive protein. Baseline serum MCP-1 concentrations were similar to those previously reported in obese individuals (27). In the rectosigmoid mucosa, concentrations of mucosal TNF-α decreased by 57% ($P < 0.05$), concentrations of IL-8 decreased by 44% ($P < 0.05$), concentrations of IL-1β decreased by 38% ($P < 0.05$), and concentrations of MCP-1 decreased by 25% ($P < 0.05$), and there was a trend toward decreased IL-6 concentrations by 45% ($P = 0.06$) (**Figure 2**). Data from one subject were excluded from the analysis because concentrations of all cytokines from her biopsies were >3 SDs from the mean of all subjects.

Immunohistochemistry

Standard hematoxylin and eosin staining of mucosal biopsies at the onset and end of the study showed no identifiable changes in total cellular content or mucosal morphology (data not shown). However, immunohistochemical staining showed a 28% decrease in CD3-positive T lymphocytes ($P < 0.01$) ($n = 10$) and a 42% decrease in CD163-positive macrophages ($P < 0.05$) ($n = 8$) (**Figure 3**) at the end of the study.

Microarray analyses

Microarray analyses revealed that diet-induced weight loss was accompanied by 1211 differentially expressed genes (with $P \leq 0.05$) of which 70 genes (56 down-regulated and 14 up-regulated genes) had a fold-change $\geq$1.2. This differential pattern was strikingly shown in the heat map in **Figure 4** (*see* supplemental Table 2 under "Supplemental data" in the online issue for the differential pattern shown in each gene). Genes involved in inflammation and cancer were down-regulated, including many genes associated with cytokines and chemokine signaling such as *IL-8*, *CCL-21*, *CCL-19*, and *CCL4L1*, as well as the proto-oncogenes *JUN* and *FOS*. Genes coding for molecules for which the circulating concentrations decreased with weight loss also were down-regulated, including peptide YY (*PYY*), vasoactive intestinal peptide (*VIP*), and somatostatin (*SST*).Genes that coded for gastrointestinal substrate transport, including ATP-binding cassette, subfamily A members 8 and 9 (*ABCA8* and *ABCA9*), and carnitine palmitoyltransferase-I (*CPT-1A*) were up-regulated. Six down-regulated and 4 up-regulated genes were identified from the differentially expressed gene list for validation by real-time PCR, and gene-fold changes confirmed the microarray data findings (s*ee* supplemental Table 3 under "Supplemental data" in the online issue).

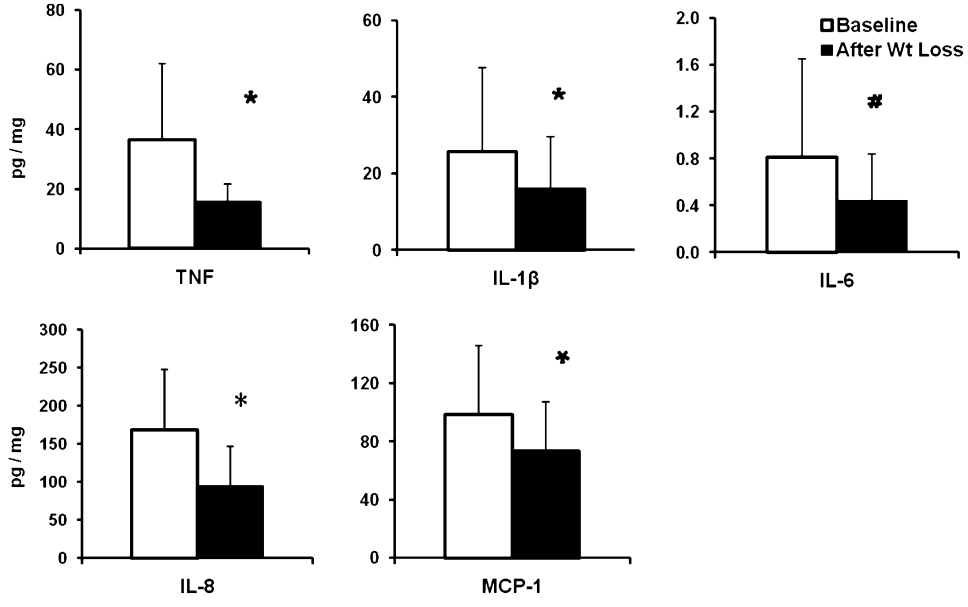

FIGURE 2. Mean (±SD) changes (pg/mg protein) in mucosal cytokines and chemokines in 9 subjects before and after diet-induced weight loss. $^{*}P < 0.05$, $^{\#}P < 0.1$. TNF, tumor necrosis factor; IL, interleukin; MCP, monocyte chemotactic protein.

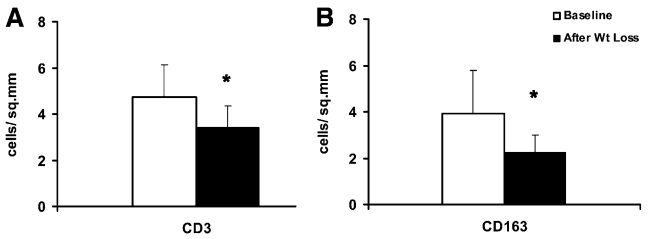

FIGURE 3. Mean (±SD) changes (number of cells/mm^2) in mucosal T cells and macrophages before and after diet-induced weight loss. A: CD3-positive T cells ($n = 10$). B: CD163-positive macrophages ($n = 8$). *$P < 0.05$.

Detecting changes in gene pathways by altered expression of single genes relies on relatively large changes and may be insensitive to many physiologically significant changes of lesser magnitude. A more sensitive approach has been GSEA, which examines many genes in a pathway for changes that may not, by themselves, be significant but are significant in the aggregate (28). In our analyses, we used gene sets curated in the canonical pathway database, in the Gene Ontology database, IL-17 pathway gene sets, and the transcription factor database from the GSEA website. In the canonical pathway database, 74 gene sets were down-regulated, and 24 gene sets were up-regulated (*see* supplemental Tables 4 and 5 under "Supplemental data" in the online issue); in the Gene Ontology database, 55 gene sets were down-regulated, and none were up-regulated (*see* supplemental Table 6 under "Supplemental data" in the online issue); and in the IL-17 gene sets, 6 gene sets were down-regulated and 2 gene sets were up-regulated (*see* supplemental Table 7 under "Supplemental data" in the online issue). Gene sets related to inflammation and cancer that were down-regulated are highlighted in **Table 4** and include pathways activated by TNF-α, IL-6, IL-1, IL-17, chemokine and cytokine signaling pathways, prostaglandin synthesis pathways, and pathways involved in oxidative stress, glutathione metabolism, and apoptosis. Up-regulated mucosal gene pathways were involved in carbohydrate metabolism (*see* supplemental Table 5 under "Supplemental data" in the online issue). The reduction in oxidative stress pathways was supported by down-regulation of downstream gene targets *FOS* and *JUN*.

Importantly, the analysis of changes in transcription factor genes identified 38 down-regulated and 9 up-regulated transcription factor gene sets (*see* supplemental Tables 8 and 9 under "Supplemental data" in the online issue). These gene sets included down-regulated transcription factor gene sets for *STAT3*,

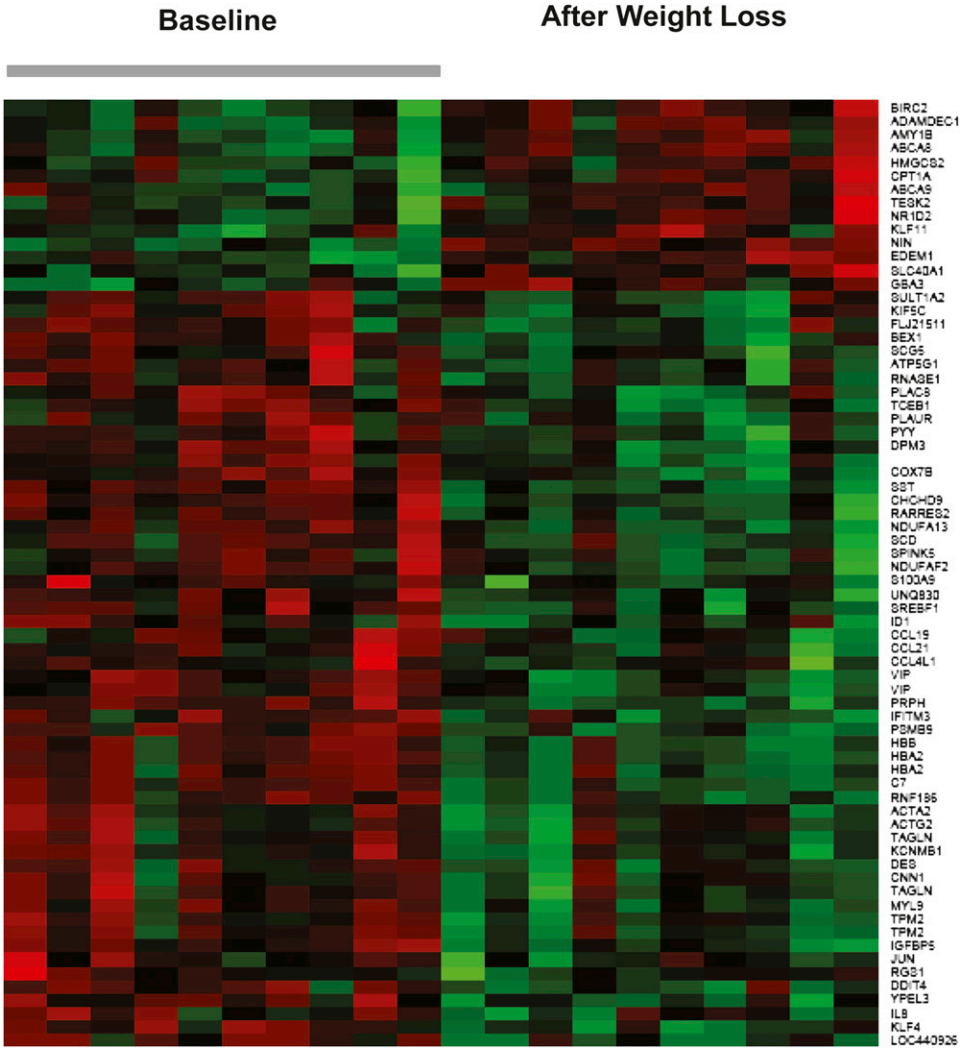

FIGURE 4. Heat map of differentially expressed genes in rectosigmoid biopsies taken at baseline and after diet-induced weight loss in 10 individual subjects. The red color indicates a higher expression, and the green color indicates a lower expression.

TABLE 4
Negatively enriched gene sets related to inflammation and cancer in rectosigmoid biopsies before and after diet-induced weight loss in all 10 subjects[1]

Name of gene set	Size	ES	NES	*P*	FDR
CYTOKINE_ACTIVITY	72	−0.51	−2.17	<0.001	<0.001
CHEMOKINE_RECEPTOR_BINDING	34	−0.65	−2.30	<0.001	<0.001
CHEMOKINE_ACTIVITY	33	−0.68	−2.36	0.004	<0.001
INFLAMMATORY_RESPONSE	108	−0.46	−2.02	<0.001	<0.001
TNFALPHA_ALL_UP	70	−0.48	−2.2	<0.001	<0.001
TNFALPHA_30MIN_UP	39	−0.55	−2.00	<0.001	0.008
CROONQUIST_IL6_STROMA_UP	36	−0.61	−2.20	0.002	0.001
IL6_FIBRO_UP	42	−0.50	−1.82	<0.001	0.009
IL1 KC UP	38	−0.63	−2.24	<0.001	<0.001
IL17ANDTNF KC UP	28	−0.63	−2.11	<0.001	<0.001
IL17 GAFFEN	30	−0.56	−1.95	<0.001	<0.001
IL17 KC UP	40	−0.51	−1.85	<0.001	0.002
ADDITIVE IL17 AND TNFA KC	181	−0.35	−1.67	<0.001	0.008
EICOSANOID_SYNTHESIS	15	−0.67	−1.88	0.004	0.027
PROSTAGLANDIN_AND_LEUKOTRIENE_METABOLISM	23	−0.58	−1.84	0.004	0.037
ICOSANOID_METABOLIC_PROCESS	15	−0.7	−1.99	<0.001	0.042
HSA00590_ARACHIDONIC_ACID_METABOLISM	42	−0.49	−1.80	<0.001	0.046
GLUTATHIONE_METABOLISM	30	−0.65	−2.24	<0.001	0.001
GLUTATHIONE_TRANSFERASE_ACTIVITY	15	−0.71	−2.06	<0.001	0.007
ELECTRON_TRANSPORT_CHAIN	87	−0.66	−2.86	<0.001	<0.001
HSA00190_OXIDATIVE_PHOSPHORYLATION	105	−0.66	−2.82	<0.001	<0.001
PROTEASOME_DEGRADATION	30	−0.66	−1.81	0.002	0.045
UBIQUINONE_BIOSYNTHESIS	14	−0.66	−2.02	<0.001	0.001
PROTEASOME	17	−0.66	−2.04	<0.001	<0.001

[1] Size, number of genes analyzed for each gene set; ES, enrichment score; NES, normalized ES; *P*, *P* value of ES; FDR, false discovery rate. Gene sets with $P \leq 0.005$ and an FDR $\leq$5% are shown.

STAT5A, *STAT5B*, *NF-κB*, *ATF*, *CREB*, and multiple serum response factors, which are recognized as very important in colorectal inflammation, proliferation, apoptosis, and oncogenesis, which are shown in **Table 5.**

DISCUSSION

Epidemiologic studies indicated that obesity is accompanied by an increased risk of CRC, and intentional weight loss reduced this risk (2, 12). To explore potential mechanisms, we studied effects of diet-induced weight loss on the colorectal mucosa in obese women. A VLCD was used to induce a 10% weight loss, which resulted in reduced rectosigmoid mucosal cytokine and chemokine concentrations and fewer tissue macrophages and T cells. Gene-expression analysis showed down-regulation of multiple genes involved in inflammation and cancer, including genes involved in cytokine and chemokine signaling and the proto-oncogenes *JUN* and *FOS*. The gene-pathway analysis showed down-regulation of pathways activated by TNF-α, IL-6, IL-1, and IL-17 and pathways involved in chemokine and cytokine signaling, prostaglandin synthesis, oxidative stress, and glutathione metabolism. In addition, transcription factor pathways for *STAT3*, *STAT5*, *NF-κB*, *ATF*, *CREB*, and several serum response factors were down-regulated. These results strongly suggested that chronic inflammation was present in the colorectal mucosa of obese individuals and that diet-induced weight loss decreased inflammation and down-regulated inflammatory and cancer gene pathways.

Obesity is accompanied by elevated circulating concentrations of insulin and IGF-1, and changes in the insulin-IGF axis

TABLE 5
Negatively enriched transcription factor gene sets related to inflammation and cancer in rectosigmoid biopsies before and after diet-induced weight loss in all 10 subjects[1]

Name of gene set	Size	ES	NES	*P*	FDR
CCAWWNAAGG_V$SRF_Q4	63	−0.53	−2.13	<0.001	<0.01
V$SRF_01	40	−0.55	−2.00	<0.001	<0.01
V$SRF_Q5_01	165	−0.41	−1.94	<0.001	<0.01
V$STAT3_01	16	−0.62	−1.82	0.005	0.01
V$STAT5A_01	169	−0.37	−1.77	<0.001	0.01
V$STAT5B_01	171	−0.37	−1.74	<0.001	0.02
V$ATF_B	133	−0.35	−1.63	0.001	0.06
V$CREB_Q4_01	150	−0.34	−1.58	0.001	0.07
V$NFKB_Q6_01	149	−0.33	−1.55	0.003	0.07
GGGNNTTTCC_V$NFKB_Q6_01	91	−0.35	−1.52	0.005	0.08

[1] Size, number of genes analyzed for each gene set; ES, enrichment score; NES, normalized ES; *P*, *P* value of ES; FDR, false discovery rate. Gene sets with $P \leq 0.005$ and an FDR $\leq$10% are shown.

are believed to be important in CRC pathogenesis in obesity through the PIK3/Akt pathway (29) Insulin is mitogenic only at supraphysiologic concentrations through the activation of the IGF-1 pathway (30). Physiologic concentrations of IGF-1 are growth promoting and mitogenic, and increased concentrations of IGF-1, when adjusted for concentrations of its binding protein, are associated with a modest increase in CRC risk (31). Leptin concentrations also increase in obesity, and leptin has growth-promoting, mitogenic, and antiapoptotic properties in colon cancer cell lines but not in the normal epithelium (32) In the Adenomatous polyposis coli (APC^{min}) mouse, leptin did not promote CRC or xenograft growth in mice (33). However, obesity is recognized as a chronic inflammatory state with increased serum cytokine concentrations, including those of TNF-α, IL-6 and -8, and MCP-1 (27, 34), and evidence of inflammation in adipose tissue, liver, skeletal muscle, and coronary arteries (18). In our study, weight loss significantly reduced circulating concentrations of TNF-α, IL-8, and MCP-1. Increased concentrations of obesity are accompanied by progressively higher concentrations of serum cytokines and increased risk of colorectal neoplasia (27, 35). This relation is supported by evidence that cytokines have a procarcinogenic effect in colorectal tissues (20). Reducing systemic TNF-α activity (36) and knocking out *NF-κB* in myeloid cells (37) decreased colonic tumors in mouse colitis models. Thus, this diet regimen–induced reduction in systemic cytokines might contribute to lowering colon neoplasia in obese individuals.

The current study showed that the diet-induced weight loss reduced rectosigmoid mucosal concentrations of the inflammatory mediators TNF-α, IL-6, IL-8, IL-1β, and MCP-1. In confirmation, the expression of gene pathways regulated by TNF-α and IL-6 and -1β also decreased. In mouse models of colitis-induced CRC, the inhibition of activated gene pathways downstream of TNF-α and IL-6 decreased CRC. We also observed a decreased expression of transcription factor pathways implicated in colorectal inflammation, CRC, and other types of cancer, including *STAT3*, *NF-κB*, *ATF*, *CREB*, and *STAT5* (38). STAT3 activation up-regulates inflammatory cytokines such as IL-6, IL-1β, IL-8, MCP-1, and COX-2 (39). NF-κB induces the expression of many inflammatory mediators and is a core transcription factor in the immune response. Both STAT3 and NF-κB signaling are recognized as pathways involved in inflammation-induced carcinogenesis (38). STAT3 and STAT5 can expand regulatory T cells, which inhibit anti-tumor immunity and promote tumor progression (40). An analogous pathway of inflammation that leads to increased risk of liver cancer in obesity was recently published (41). Thus, diet-induced weight reduction down-regulates core transcription factors that are crucial in inflammation and carcinogenesis and thereby may reduce risk of CRC. The expression of gene pathways involved in prostaglandin synthesis were also lower. In human obesity and mouse obesity models, the increased expression of the *COX2* gene is a major regulator of colonic prostaglandin synthesis. Caloric restriction has been shown to reduce colonic *COX2* expression in mouse obesity accompanied by decreased numbers of aberrant crypt foci (11).

The current study showed that our experimental regimen reduced rectosigmoid mucosal T cell and macrophage numbers accompanied by decreased expression of *IL-8*, *CCL19*, *CCL20*, *CCL4L1*, and gene pathways associated with chemokine activity. These gene pathways are involved in mucosal leukocyte accumulation, and their activity is increased in the colonic mucosa of patients with inflammatory bowel disease during acute exacerbations and down-regulated during remissions (42).

Oxygen free-radical formation and increased oxidative stress link colonic inflammation and cancer formation (20). Obesity enhances systemic oxidative stress, which, through intermediary pathways, enhances the expression of proinflammatory and cell cycle genes that can lead to tissue inflammation and DNA damage and result in loss of tumor suppressor functions that are characteristic of CRC (43). Western diet–consuming obese mice have shown enhanced oxidative stress as well as inflammation in the colorectal mucosa, (9) and calorie restriction has reduced oxygen free-radical formation and DNA damage in rodent liver and heart tissue (44, 45). Our study subjects showed down-regulation of pathways involved with oxidative phosphorylation and *FOS* and *JUN* genes that activate the transcription of proinflammatory and cell proliferative mediators and can promote several human cancers (19). Thus, obesity creates an environment in the colon that promotes the initiation and progression of CRC, and calorie restriction has an opposite effect by reducing oxygen free-radical formation and decreasing oxidative stress.

The current experimental model was chosen as a proof-of-principle study for several reasons. Our experience with similar translational studies has shown that endpoint data obtained in the same individual obtained before and after an intervention provided optimum and reproducible information. A weight loss of over 8% of initial weight was used because a previous study showed that this would greatly lower proliferation rates in humans (21). Previous experience permitted us to study only 10 subjects because the research staff of the Rockefeller University Hospital has provided remarkable nutritional and environmental control in intervention studies. One limitation is that the protocol did not include control subjects such as obese individuals fed an isoenergetic diet by using VLCD products. As a result, it is not possible to determine whether the observed improvements in the inflammatory and carcinogenesis pathways were changes in energy balance, adiposity, or diet composition. However, unpublished data (P Holt, 2010) using dramatically differing diets have not shown similar degrees of changes in mucosal cytokines or gene array pathway analysis. Irrespective of the precise factors responsible, our analysis showed a marked reduction in inflammatory and colon carcinogenesis endpoints with the intervention, which indicated that obesity must be accompanied by increases in these pathways. We recognize that the diet can alter luminal microbiota diversity, which might influence epithelial cell signaling (46) and, thus, contribute to some of the changes that we observed.

In conclusion, our study shows that diet-induced weight loss in obese women reduced colorectal mucosal inflammation as shown by decreased inflammatory cytokines, inflammatory cells, down-regulated inflammatory and cancer gene pathways, and transcription factor gene sets that enhance inflammation and CRC risk. Thus, obesity appeared to be accompanied by a low-grade inflammatory state in the colorectal mucosa, and diet-induced weight loss decreased such inflammation. Because colorectal inflammation is an important cofactor for colorectal carcinogenesis, such an inflammatory state may contribute to enhanced CRC risk in obesity; weight loss counteracts this effect and may thereby lower CRC risk.

We thank Jan L Breslow for his advice and support, Jeanne Walker for her close supervision of study volunteers, James G Krueger and Artemis Khatcherian for help with immunohistochemistry, and Juana Gonzalez for technical help with the Mesoscale multiplex assay at the Clinical and Translational Science Center immunology core facility.

The authors' responsibilities were as follows—SP: study concept and design, data acquisition, data analysis and interpretation, and drafting of the manuscript; LMN: study design and VLCD management; MS-F: statistical analyses; and PRH: study concept and design, data interpretation, and drafting of the manuscript. None of the authors had a conflict of interest.

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