Abdominal Obesity, Independent from Caloric Intake, Accounts for the Development of Intestinal Tumors in Apc1638N/+ Female Mice

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Abstract
To determine whether visceral fat (VF), independent of other confounders, is causally linked to intestinal tumorigenesis, we surgically removed visceral fat in Apc1638N/+ mice. At 15 weeks of age, male and female Apc1638N/+ mice were randomized to one of three groups: ad libitum, visceral fat removal (VF-) and ad libitum fed, or caloric restriction, and were studied for effects on tumorigenesis and survival. As compared with ad libitum, VF– and caloric restriction reduced macroadenomas to a similar extent (P < 0.05), but only caloric restriction significantly improved survival (P < 0.05). Given that a significant group × gender interaction was observed, we next examined males and females separately. In females, macroadenomas were markedly attenuated by VF– (1.33 ± 0.23 mean ± SE; P < 0.05), but not by caloric restriction (2.35 ± 0.25; P = 0.71), as compared with ad libitum (2.50 ± 0.34). In males, however, caloric restriction (1.71 ± 0.26; P < 0.01), but not VF– (2.94 ± 0.42; P = 0.29), reduced macroadenomas, as compared with ad libitum males (3.47 ± 0.30). In females, both VF– (P = 0.05) and caloric restriction (P < 0.01) improved survival, but not in male mice (P = 0.15). The benefits observed with caloric restriction were consistent with favorable metabolic adaptations, but protection conferred in VF– females was despite lower adiponectin levels (P < 0.05), and failure to reduce body mass, total adiposity, glucose, insulin, leptin, and chemokine (C–X–C motif) ligand 1 (CXCL-1) levels. In conclusion, these data provide the first causal evidence linking visceral fat to intestinal cancer risk, and suggest that factors, other than known metabolic mediators, may impact tumor development. Furthermore, these data emphasize that strategies designed to deplete visceral fat stores in humans should be considered in the prevention of intestinal cancer. Cancer Prev Res; 6(3); 177–87. ©2012 AACR.

Introduction
Obesity rates are at epidemic levels in the United States and other developed countries (1, 2). Obese individuals are at increased risk for developing metabolic syndrome, type II diabetes, cardiovascular disease (3), and cancer at many sites, including colon, kidney, thyroid, endometrium, liver, and esophagus (4–10). Excess adiposity poses an even greater risk for cancer mortality, an observation that is nearly universal among the most common forms of cancer (9). For example, obesity dramatically increases the risk of death from colon cancer by 46% in women and 84% in men, breast cancer by 2-fold, liver and kidney cancer by more than 4-fold, and uterine cancer by more than 6-fold (9).

Since the obesity–cancer link first emerged, studies across disciplines have worked toward establishing the biologic underpinnings explaining this association. Epidemiologic studies (4, 5, 9) strongly suggest a relationship via endocrine and other metabolic effects (11, 12), whereas in vitro (13) and in vivo studies (14, 15) have provided evidence with respect to possible mechanisms. The most commonly studied pathways linking obesity to cancer include the ability of excess adipose tissue to promote insulin resistance and contribute to a chronic, low-grade, proinflammatory state through the secretion of adipokines (16). However, establishing the independent contribution of these mediators to site-specific cancer risk has been difficult, as their importance seems to vary by cancer type and stage and is further complicated by interactions with other hormones (13, 17–19). A second unresolved issue is the lack of in vivo evidence causally linking adipose tissue per se to cancer.
predominant method used in obesity–cancer studies uses high-fat feeding in rodents to cause excess gains in weight and adiposity, but this strategy introduces other confounders, including changes in dietary components, nutrient flux, and energy balance. Furthermore, it is known that nutrients can interact with adipose tissue to provoke the expression and secretion of proinflammatory cytokines (20), thus interventions that limit this influx of nutrients, such as caloric restriction or bariatric surgery, for example, may work in part by attenuating this interaction.

Since the relationship between obesity and disease first emerged, a more careful examination of body fat distribution has revealed that the risk posed by obesity in humans to disease and mortality is primarily harbored by the extent of visceral fat (VF) accretion (21). Using a surgical model of visceral fat removal (VF−) in rats, our group first showed that this relationship in regards to insulin resistance, type II diabetes, and lifespan is causal (22–24). Given that abdominal obesity has been shown to more strongly predict cancer risk and mortality, including colon cancer (25, 26), then general obesity, we addressed whether visceral fat is a direct modulator of intestinal tumor development. We specifically used our surgical approach of depleting visceral fat in a mouse model of intestinal cancer (Apc1638/N+), to distinguish the contribution of visceral adiposity to tumorigenesis, independent of other important confounders. Here, we show that VF− independently protects against the development of macroadenomas in female Apc1638/N+ mice.

Materials and Methods

Animals

Male Apc1638/N+ mice on a C57BL/6 background were bred with C57BL6/J female mice (Jackson Laboratory). Genotyping of Apc1638/N+ offspring was conducted as described (27). At weaning (3-weeks old), mice were group housed in same-sex cages and fed a purified 45% high-fat diet (cat#D12451, Research Diets Inc.) to induce weight gain. Animals were housed at standard temperature (≈22°C) and humidity-controlled conditions under a standard light/dark photoperiod (14L:10D). All experiments were approved by the Institutional Animal Care and Use Committee at the Albert Einstein College of Medicine (Bronx, NY).

Experimental design

At 15 weeks of age, which we found was an age that fat can be ablated without significant regrowth, approximately two thirds of mice were randomly assigned to sham abdominal surgery and approximately one third to VF−. Following a 1-week recovery, sham operated and ad libitum-fed animals were further randomized to either ad libitum or caloric-restricted groups, resulting in 3 experimental groups (ad libitum; n = 45 total, males = 21, females = 24), VF− and ad libitum fed (VF−; n = 40 total, males = 21, females = 19), or ad libitum and 40% caloric restriction (n = 38 total, males = 18, females = 20). Mice were then monitored daily for up to 12 weeks (26 weeks of age) for tumor development. Animals were removed early from the study and sacrificed if evidence of decreased food intake and weight loss (>20%), coupled with signs of sickness and lethargy were observed. In all cases, mice removed early from the experiment due to these criteria were found to harbor intestinal tumors.

Sham and fat removal surgery

For each surgery, mice were anesthetized with isoflurane (2% induction and maintenance) and a small midline incision was made in the lower abdomen under aseptic conditions, as previously described (24). For VF− mice, all visible gonadal (epididymal in males, periovarian in females) and perinephric adipose tissue was carefully removed by blunt dissection, weighed, and recorded. For the sham operation, a similar incision was made and soft tissues were disturbed in a similar manner as VF−, but no fat was removed. Analgesic was given (buprenorphine, 0.1 mg/kg s.c.) perioperatively and as needed for up to 72 hours postoperatively. Recovery was more than 90% for both the sham and fat removal procedures.

Food intake, body weight, and body composition

Following surgery, mice were singly housed for the duration of the experiment. Body weight and food intake was monitored weekly. Animals assigned to the caloric-restricted group were provided a weighed food pellet daily between 12:00 and 17:00 hours. Because ad libitum male mice consumed slightly more food than ad libitum female mice, the amount of food provided to male and female caloric-restricted mice, respectively, was calculated as approximately 60% of the intake consumed by same-sex ad libitum controls. Body composition was assessed by quantitative magnetic resonance (qMR) for determination of fat and lean mass (Echo Medical Systems) at baseline (−1 week), 4, 8, and 12 weeks in study. At sacrifice, visceral fat pads (perinephric, mesenteric, and epididymal/periovarian) were removed, weighed, and recorded.

Necropsy and tissue processing

For determination of tumors, mice were sacrificed and the gastrointestinal tract was separated from the mesenteric fat depot and divided into 4 segments: duodenum, ileum, jejunum, and colon. Each segment was opened longitudinally, rinsed in PBS, and laid out flat for examination of tumor multiplicity with the aid of a dissecting magnifying lens. Macroadenomas (≈>0.5-mm diameter) were counted in the proximal and distal region of each segment of intestinal tissue and recorded. Tissues were then rolled and fixed overnight in 10% neutral-buffered formalin at 4°C, processed through a series of alcohols and xylene, and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and cosin and assessed by a pathologist for histologic changes following consensus recommendations for assessing intestinal tumors in rodents (28).

Four-week cohort for insulin-tolerance tests and serum collection

To minimize the confounding effects of cancer morbidity on metabolic outcomes, a separate cohort of mice were
included and used for insulin-tolerance tests (ITT) at 3 weeks in study and sacrificed for serum collection at 4 weeks in study (i.e., 20 weeks of age). ITTs were conducted in random-fed mice, early in their light cycle (~07:00–08:00 hours), as described (29). Briefly, following a baseline glucose measurement (One Touch Ultra, LifeScan, Inc.), mice were intraperitoneally injected with 0.75 U/kg insulin and blood glucose was measured at 15, 30, 45, and 60 minutes later. For serum collection, food was removed at approximately 06:00 hours and mice were killed 5- to 6 hours later by decapitation without anesthesia. Blood was allowed to clot at room temperature, and serum was separated by centrifugation and stored at −80°C until analysis.

Serum measures
At sacrifice, glucose was measured in whole blood with a handheld glucose analyzer (One Touch Ultra). Serum insulin was measured in duplicate using a high-sensitivity mouse ELISA (Alpco Inc.). 17β-Estradiol levels in serum were measured in duplicate by enzyme-linked immunosorbent assay (ELISA) in female mice (Cayman Inc.). Serum leptin and adiponectin were measured in duplicate by ELISA (Alpco Inc.) as described (15). Other cytokines and chemokines, including interleukin (IL)-1β, IL-10, IL-12-p70, IL-6, IFN-γ, and chemokine (C–X–C motif) ligand 1 (CXCL-1) were measured in duplicate using an electrochemiluminescence assay with sensitivities of 0.75, 11.0, 35.0, 4.5, 0.38, and 3.3 pg/mL, respectively (Meso Scale Discovery).

Statistics
Tumor multiplicity was analyzed by two-way ANOVA (group × gender) and post hoc comparisons were conducted when appropriate. A total of 5 statistical outliers (>2 SD from the group mean) were identified and excluded from the macroadenoma dataset (1 each from ad libitum males, VF−, caloric-restricted and ad libitum female and VF− groups, respectively). Histologic outcomes were analyzed by the Kruskal–Wallis non-parametric test when appropriate. Survival to 12 weeks in study was conducted using the Kaplan–Meier method and significant differences in survival distribution among groups was tested by a log-rank test. Longitudinal measures were assessed by repeated-measures ANOVA and cross-sectional measures were assessed by one-way ANOVA. When significance was detected for the main effect, planned contrasts were carried out when appropriate. All analyses were conducted using either SPSS (SPSS Inc.) or JMP software version 9 (SAS Institute Inc.). A P ≤ 0.05 was considered statistically significant.

Results
Caloric restriction or visceral fat removal resulted in a similar and significant reduction in macroadenomas
At sacrifice, tumor multiplicity was determined in mice by counting macroadenomas in the small and large intestine. Macroadenomas were primarily confined to the duodenum and jejunum, less frequently in the ileum, and only rarely observed in the colon. No differences in macroadenoma number by intestinal region (i.e., proximal or distal duodenum, ileum, jejunum, and colon) were detected among groups (data not shown), but a significant group effect was observed for total macroadenomas (P < 0.01). Comparisons among groups revealed a similar and significant reduction in tumor multiplicity in VF− and caloric-restricted mice, as compared with ad libitum mice (Fig. 1A; P < 0.05).

Caloric restriction significantly improved survival in Apc1638/N−/− mice
Using criteria described in Materials and Methods, we next examined the effect of interventions on survival to 12 weeks in study (28 weeks of age). Analysis of survivorship revealed that caloric restriction significantly improved survival (89.5% remaining; P < 0.05), but no significant difference was observed between VF− and ad libitum animals (80.0% vs. 62.2% remaining, respectively; Fig. 1B; P = 0.10). In addition, a significant main effect for gender (P < 0.05) and a group × gender interaction (P < 0.01) were detected for tumor multiplicity, leading to a secondary analysis within female and male groups, respectively.

Visceral fat removal, but not caloric restriction, reduced the number of macroadenomas in Apc1638/N−/− female mice
In females, macroadenoma numbers were significantly reduced by VF− (P < 0.05), but not by caloric-restriction (P = 0.71), as compared with ad libitum females (Fig. 1C). When intestinal tissue sections were evaluated histologically by a pathologist, no differences were observed in the severity of crypt hyperplasia or the frequency of dysplasia among female groups, but a marked increase in microadenomas was observed in VF− female mice, as compared with ad libitum females (Table 1; P < 0.05), whereas caloric-restricted female mice had an intermediary level, relative to ad libitum and VF− females. Taken together, these findings suggest that in VF− females, but not in caloric-restricted females, the progression of microadenomas to macroadenomas was significantly attenuated.

Caloric restriction and visceral fat removal significantly improved survival in Apc1638/N−/− female mice
When a survival analysis, truncated at 12 weeks, was conducted in female mice, the percentage of both caloric-restricted (100% remaining; P < 0.01) and VF− female mice (94.7% remaining; P = 0.05) surviving to 12 weeks, was significantly greater than ad libitum females (79.2% remaining; Fig. 1D).

Caloric restriction, but not visceral fat removal, reduced the number of macroadenomas in Apc1638/N−/− male mice
In contrast to females, caloric-restricted male mice (P < 0.01), but not VF− male mice (P = 0.29), had markedly fewer total macroadenomas than ad libitum males (Fig. 1E). In addition, caloric-restricted males had significantly fewer macroadenomas than VF− mice (P < 0.05). Histopathology
revealed that the severity of crypt hyperplasia was lowest in VF− male mice (Table 2; \( P < 0.05 \)), whereas caloric-restrict ed males had the lowest incidence of microadenomas, but the greatest occurrence of crypt dysplasia (Table 2; \( P < 0.05 \)). Collectively, these findings suggest that the progression of dysplastic cells toward adenoma formation was abrogated only in caloric-restricted male mice, leading to development of fewer micro- and macroadenomas in this group. When assessing survivorship in males, the percentage of ad libitum male mice still remaining at 12 weeks was 42.9%, whereas the percentage of VF− and caloric-restricted male mice surviving to 12 weeks were 66.7% and 77.8%, respectively, but no significant model effect was observed (Fig. 1F; \( P = 0.15 \)).

**Caloric restriction, but not visceral fat removal, significantly altered phenotypic characteristics in Apc1638/N− female mice**

On average, 730.4 ± 61.7 mg periovarian fat and 331.5 ± 61.7 mg perinephric fat (1,061.9 mg total) was surgically removed from VF− female mice. As expected, no significant differences were detected for body weight (Fig. 2A), food intake (Fig. 2B), lean mass (Fig. 2C), or total adiposity (Fig. 2D) over the course of the study between ad libitum...
and VF– females. In contrast, caloric-restricted females consumed nearly 40% fewer calories, weighed significantly less, and had reduced amounts of lean and fat mass, as compared with ad libitum and VF– females (Fig. 2A–D; \( P < 0.001 \)). At 12 weeks in study, individual fat pads were weighed, which confirmed both the successful surgical ablation of visceral fat pads in VF– females, and the significant depletion in these same depots by caloric restriction, as compared with ad libitum female mice (Fig. 2E; \( P < 0.05 \)).

### Visceral fat removal resulted in hyperinsulinemia, hyperleptinemia, and lower adiponectin levels in Apc\(^{1638/N} \) female mice

Serum measures in females at 4 weeks in study are presented in Table 3 and Fig. 2F. Glucose and leptin levels were significantly reduced in caloric-restricted females, as compared with ad libitum and VF– females (Table 3; \( P < 0.01 \)). In contrast, VF– females were hyperinsulinemic, hyperleptinemic, and had significantly lower levels of adiponectin, as compared with ad libitum and caloric-restricted female mice (Table 3; \( P < 0.05 \)). Insulin sensitivity, as determined by ITTs, was greatest in caloric-restricted female mice, but no distinguishable difference was observed between ad libitum and VF– females (Fig. 2F). In addition, CXCL-1, which is thought to play a role in angiogenesis and metastasis, was markedly reduced in caloric-restricted females (Table 3; \( P < 0.01 \)), but no significant differences were observed among groups for other serum cytokines and chemokines, including IFN-\( \gamma \), IL-6, IL-10, IL-12-p70, and IL-1\( \beta \). Furthermore, no differences were observed in estradiol levels among female groups (Table 3).

### Caloric restriction, but not visceral fat removal, significantly altered phenotypic characteristics in Apc\(^{1638/N} \) male mice

Similar to females, VF– males had an average of 1,237.9 ± 120.0 mg of epididymal fat and 377.0 ± 38.1 mg of perinephric fat surgically removed. Despite removing approximately 1.6 g of fat, no significant differences were observed over the course of the study for body weight (Fig. 3A), food intake (Fig. 3B), lean mass (Fig. 3C), or total adiposity (Fig. 3D) between ad libitum and VF– male mice. As in females, males caloric restricted by approximately 40% weighed significantly less (Fig. 3A) and had reduced amounts of lean and fat mass (Fig. 3C and D), as compared with ad libitum and VF– males (\( P < 0.001 \)). We confirmed at 12 weeks that epididymal and perinephric fat pads did not return in VF– males, and these fat pads were markedly lower in both VF– and caloric-restricted males, as compared with ad libitum male mice (Fig. 3E; \( P < 0.05 \)).

### Caloric restriction, but not visceral fat removal, led to beneficial metabolic adaptations in Apc\(^{1638/N} \) male mice

Serum measures of hormones, cytokines, chemokines, and metabolites in male mice from the 4-week cohort are

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**Table 1.** Histopathology of the gastrointestinal tract in ad libitum, VF–, and caloric-restricted female mice

<table>
<thead>
<tr>
<th></th>
<th>Ad libitum</th>
<th>VF–</th>
<th>Caloric restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperplasia, crypt epithelial(^c)</td>
<td>0.20 ± 0.13</td>
<td>0.18 ± 0.13</td>
<td>0.07 ± 0.07</td>
</tr>
<tr>
<td>Dysplasia(^d)</td>
<td>0.20 ± 0.13</td>
<td>0.27 ± 0.15</td>
<td>0.21 ± 0.11</td>
</tr>
<tr>
<td>Microadenomas(^d)</td>
<td>0.30 ± 0.15(^a)</td>
<td>1.27 ± 0.30(^b)</td>
<td>0.77 ± 0.21(^ab)</td>
</tr>
<tr>
<td>Carcinomas(^d)</td>
<td>0.30 ± 0.15</td>
<td>0.36 ± 0.15</td>
<td>0.46 ± 0.17</td>
</tr>
</tbody>
</table>

**Note:** Data are mean ± SE. Different letters (a, b) denote a significant difference between groups, \( P < 0.05 \).

\(^a\)Value indicates the number of identified foci per section.

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**Table 2.** Histopathology of the gastrointestinal tract in ad libitum, VF–, and caloric-restricted male mice

<table>
<thead>
<tr>
<th></th>
<th>Ad libitum</th>
<th>VF–</th>
<th>Caloric restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperplasia, crypt epithelial(^c)</td>
<td>0.66 ± 0.19(^a)</td>
<td>0.11 ± 0.11(^a)</td>
<td>0.63 ± 0.30(^b)</td>
</tr>
<tr>
<td>Dysplasia(^d)</td>
<td>0.33 ± 0.14(^a)</td>
<td>0.67 ± 0.37(^a)</td>
<td>1.13 ± 0.52(^b)</td>
</tr>
<tr>
<td>Microadenomas(^d)</td>
<td>2.08 ± 0.31(^b)</td>
<td>2.44 ± 0.50(^b)</td>
<td>0.75 ± 0.25(^a)</td>
</tr>
<tr>
<td>Carcinomas(^d)</td>
<td>0.25 ± 0.18</td>
<td>0.78 ± 0.32</td>
<td>0.46 ± 0.17</td>
</tr>
</tbody>
</table>

**Note:** Data are mean ± SE. Different letters (a, b) denote a significant difference between groups, \( P < 0.05 \).

\(^a\)Denotes the severity score, based on a 1–4 scale (4 being most severe).

\(^b\)Denotes the number of foci per section.
Caloric-restricted male mice had lower glucose, insulin, and leptin levels \((P < 0.05)\), whereas adiponectin levels tended to be greater in this group, as compared with ad libitum \((P = 0.27)\) and VF/M/C0 males \((P = 0.03)\;\text{Table 4}\). In contrast to the observed trends for VF/M/C0 female mice (see Table 3 for female data), VF/M/C0 in male mice did not significantly alter insulin \((P = 0.55)\) or leptin levels \((P = 0.45)\), as compared with ad

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**Table 3. Serum measures in ad libitum, VF−, and caloric-restricted female mice at 4 weeks in study**

<table>
<thead>
<tr>
<th></th>
<th>Ad libitum-fed</th>
<th>VF−</th>
<th>Caloric restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>166.8 ± 6.9(^b)</td>
<td>167.9 ± 6.0(^b)</td>
<td>128.3 ± 6.5(^a)</td>
</tr>
<tr>
<td>Insulin, ng/mL</td>
<td>0.61 ± 0.06(^a)</td>
<td>0.86 ± 0.10(^b)</td>
<td>0.64 ± 0.06(^a)</td>
</tr>
<tr>
<td>Leptin</td>
<td>1.75 ± 0.22(^a)</td>
<td>3.76 ± 0.69(^b)</td>
<td>0.53 ± 0.18(^b)</td>
</tr>
<tr>
<td>Adiponectin, μg/mL</td>
<td>56.2 ± 5.4(^b)</td>
<td>42.2 ± 1.7(^a)</td>
<td>56.8 ± 5.3(^a)</td>
</tr>
<tr>
<td>Estradiol, pg/mL</td>
<td>984.5 ± 34.2</td>
<td>1,014.1 ± 18.8</td>
<td>1,018.3 ± 35.1</td>
</tr>
<tr>
<td>IFN-γ, pg/mL</td>
<td>1.25 ± 0.47</td>
<td>2.73 ± 0.72</td>
<td>1.67 ± 0.61</td>
</tr>
<tr>
<td>IL-10, pg/mL</td>
<td>36.2 ± 4.8</td>
<td>56.7 ± 13.6</td>
<td>26.4 ± 9.3</td>
</tr>
<tr>
<td>IL-12-p70, pg/mL</td>
<td>51.5 ± 17.8</td>
<td>130.0 ± 47.4</td>
<td>73.1 ± 34.8</td>
</tr>
<tr>
<td>IL-13, pg/mL</td>
<td>1.55 ± 0.58</td>
<td>0.78 ± 0.21</td>
<td>1.68 ± 0.51</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>12.7 ± 3.8</td>
<td>26.1 ± 8.8</td>
<td>26.4 ± 7.0</td>
</tr>
<tr>
<td>CXCL-1, pg/mL</td>
<td>92.8 ± 6.5(^b)</td>
<td>100.6 ± 6.1(^b)</td>
<td>59.6 ± 3.9(^a)</td>
</tr>
</tbody>
</table>

**NOTE:** Data are mean ± SE. Different letters denote a significant difference between groups, \(P < 0.05\).
libitum male controls, whereas total adiponectin levels were numerically lowest in this group \((P = 0.16)\), similar to what was found in VF\(\text{-}\) females. ITTs in male mice revealed a tendency for improved insulin sensitivity in VF\(\text{-}\) males, as compared with ad libitum mice (Fig. 3F; \(P = 0.09)\), but glucose levels during the ITT were only significantly improved in caloric-restricted male mice (Fig. 3F; \(P < 0.001)\). Similar to female caloric-restricted

<table>
<thead>
<tr>
<th>Table 4. Serum measures in ad libitum, VF(\text{-}), and caloric-restricted male mice at 4 weeks in study</th>
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</thead>
<tbody>
<tr>
<td>Ad libitum-fed</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
</tr>
<tr>
<td>Insulin, ng/mL</td>
</tr>
<tr>
<td>Leptin, (\mu)g/mL</td>
</tr>
<tr>
<td>Adiponectin, (\mu)g/mL</td>
</tr>
<tr>
<td>IFN-(\gamma), pg/mL</td>
</tr>
<tr>
<td>IL-10, pg/mL</td>
</tr>
<tr>
<td>IL-12-(p70), pg/mL</td>
</tr>
<tr>
<td>IL-1(\beta), pg/mL</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
</tr>
<tr>
<td>CXCL-1, pg/mL</td>
</tr>
</tbody>
</table>

NOTE: Data are mean ± SE. Different letters denote a significant difference between groups, \(P < 0.05)\.
mice, CXCL-1 levels were markedly reduced in caloric-restricted males (Table 4; *P* < 0.01), but no significant differences were observed among groups for IFN-γ, IL-1β, IL-6, IL-10, and IL-12-p70 levels (Table 4).

**Discussion**

This study establishes the importance of visceral adiposity in obesity-associated intestinal tumorigenesis in the Apc(1638N)/+ mouse model. Numerous epidemiologic and preclinical studies, including a prior effort in this model (30), have linked the obese state to increased colon cancer risk and/or mortality. However, obesity is a complex phenotype, characterized not only by excess weight gain and adiposity, but also by dietary factors and a sedentary lifestyle (31). Collectively, this has made isolating the individual contribution of adipose tissue to cancer risk challenging for the field. Here, using a surgical approach to deplete visceral fat, we have circumvented these issues, providing causal evidence linking visceral adiposity per se to intestinal tumorigenesis, independent of other important confounders related to energy balance. Similarly, Lu and colleagues found that partial removal of visceral fat protected female SKH-1 mice against UVB-induced skin carcinogenesis (32). Importantly, these data provide yet another line of evidence directly linking visceral fat to the etiology of aging (24) and age-related diseases (22, 23, 32, 33).

Remarkably, we found that VF− was not only effective at attenuating macroadenoma development, but this reduction was comparable with that observed in caloric-restricted mice. This was accompanied by improved survival in caloric-restricted mice, with a similar, albeit nonsignificant trend observed for increased survival in VF− mice, as compared with ad libitum mice. However, we also observed a clear effect of sex differences in the efficacy of these interventions on tumor initiation, promotion, and survival. Perhaps most striking was the marked protection conferred by VF− for development of macroadenomas in females, but not in males. While it is possible that the inability of VF− to protect against macroadenomas in males was simply due to inherent sex differences, we also observed a marked shift in fat distribution among VF− males that could also explain this difference. Indeed, when we evaluated mesenteric fat mass in females (Fig. 4A) and males (Fig. 4B), we observed a distinct increase in mesenteric fat in VF− males, but not in VF− females. Given the hazardous nature of the mesenteric fat depot, coupled with anatomic location of this depot lying in close proximity to the intestine, it is plausible that mesenteric fat accretion abrogated the benefits of removing the epididymal and perinephric fat depots via endocrine and/or paracrine mechanisms.

Interestingly, although VF− conferred protection against macroadenoma development in female mice, it was unexpectedly accompanied by a greater incidence of microadenomas. We believe the most likely reason for this finding is that VF− led to a systemic change in factor(s) that blocked the progression of tumor development at the microadenoma-macroadenoma transition. In contrast, caloric restriction was associated with increased dysplasia in males but was protective against the development of both micro- and macroadenomas. However, caloric restriction had no apparent effect on tumorigenesis in females. This suggests that energy availability may play a unique role in tumor initiation and early promotion in male mice, but caloric restriction was also beneficial for females, perhaps at later stages, as shown by their improved survival (see Fig. 1D). Future studies are needed to explore the mechanisms whereby abdominal obesity and nutrient availability act independently during stages of initiation, promotion, and progression, and how these interactions are modulated by gender.

We also noted that while both ad libitum males (24.3% fat) and females (26.3% fat) were obese, due to
consuming a 45% high-fat diet, ad libitum females developed fewer tumors \((P < 0.05)\) and had improved survival \((P < 0.05)\), as compared with ad libitum males. Indeed, consistent evidence from human studies shows a stronger risk posed by obesity and visceral obesity to colon cancer incidence and mortality in men, as compared with women \((9, 26)\). The reason for this sex difference is not clear, but may be related to reproductive hormone status, as indicated by protection conferred from oral contraceptive use in women \((34)\), and evidence that ovariectomized female mice injected with colon cancer cells have increased fat mass, insulin resistance, and tumor growth \((29)\). Females in our study also had approximately 50% lower insulin levels than males, and nearly 2-fold greater adiponectin levels, which may also have also contributed to these differences.

Another important observation from this study was the observed hyperinsulinenia, hyperleptinemia, and reduced adiponectin levels with VF in females, which seems to be at odds with the reduction in macroadenomas. Indeed, the modest, but significant increase in fasting insulin was unexpected given that our group has previously shown improved hepatic insulin action with VF in chow-fed male rats \((23)\), whereas Shi and colleagues \((33)\) showed that removing just a single visceral fat depot (periovarian) was sufficient to improve glucose tolerance in high-fat fed female mice. It should be pointed out, however, that any potential difference in insulin action here between ad libitum and VF females was likely limited to basal conditions, as we did not detect any difference in response to an insulin challenge (ITTs).

We also observed that VF in males and females resulted in an approximately 21% reduction in adiponectin, which was not unexpected, given that a significant amount of fat tissue was removed. While there is in vitro and in vivo evidence to support a protective role for increased adiponectin levels in colon cancer \((15)\), rodent studies linking low adiponectin levels with colon cancer risk are mostly derived from models of constitutive adiponectin deficiency (adiponectin knockout mice; ref. 35). Thus, a much more modest 21% reduction in adiponectin may not be sufficient to predispose to tumorigenesis, at least in females, in which levels are 2-fold higher than in male mice. However, an adverse effect in males cannot be ruled out and along with the compensatory increase in mesenteric fat, may explain why VF was ineffective in male mice.

Leptin has also been widely implicated in linking obesity to colon as well as other cancers \((31)\), and we observed that VF females had greater leptin levels, which may be indicative of leptin resistance in these mice. However, this may be a complex effect as evidence for leptin as a stimulator of proliferation is clear only for colon cancer cells in vitro \((31, 36)\). We also measured several other inflammatory mediators in serum and observed a reduction in the chemokine, CXCL-1, which has been linked to angiogenesis and metastasis, in both male and female caloric-restricted mice, but no other consistent patterns emerged. It is important to note that \(Apc^{1638/N-}\) mice presented with splenomegaly, which is commonly observed in these mice \((37)\), and corresponded with elevated cytokine levels in some animals, making it difficult to distinguish the contribution of adipose tissue to the proinflammatory milieu.

The evidence implicating obesity and its related sequelae to cancer risk spans a wide array of models and systems. Yet, efforts to elucidate mechanism(s) linking the obese state to site-specific cancers such as breast, colon, prostate, and skin, particularly in vivo, have revealed a more complex biology then perhaps was initially anticipated. For example, studies using the fatless A-ZIP/F-1 mouse model showed that adipokines are not absolutely required for tumor development. Indeed, despite the absence of adipose tissue and adipose-derived peptides, such as leptin, these mice present with several other features of the obese phenotype, including hyperglycemia, hyperinsulinenia, and elevated levels of proinflammatory cytokines. When subjected to a 2-stage skin carcinogenesis procedure, these mice develop more skin papillomas \((38, 39)\), but this effect is not seen in the severely obese, ob/ob mouse model \((39)\).

A-ZIP/F-1 mice also have accelerated development of mammary tumors when crossed with C3(11)/T-Ag transgenic mice \((38)\), whereas a similar effect was observed when MKR mice, which are lean but diabetic, were crossed with MMIV-PyVmT mice \((40)\). In contrast, MMIV-TGF\(_{a}\)/db/db, which are obese and insulin resistant, but leptin-receptor deficient, fail to develop mammary tumors \((19)\), but have increased incidence of intestinal neoplasms \((30, 41)\). Thus, our finding that protection conferred from intestinal tumors in VF females occurs despite a failure to produce favorable changes in factors suggested to play an important role in obesity-associated tumor growth is not without precedent. Collectively, these data show the complexity of the obesity–cancer interface and emphasize the need for continued efforts to delineate how specific perturbations to endocrine and other factors \((42)\) by obesity, contribute to site-specific cancer risk.

In summary, these data provide causal evidence linking visceral fat to intestinal cancer risk. The protection conferred by VF was preferentially seen in female mice, despite a lack of favorable changes in leptin, insulin, adiponectin, and several proinflammatory cytokines and chemokines, suggesting that other unknown mechanisms may underlie the obesity–colon cancer link. However, as the genetic model used here develops tumors predominantly in the small intestine, rather than the colon, further work on the underlying mechanisms will need to focus on a model in which the colon is the principal site of tumor development.

Given that visceral fat accrual and subcutaneous fat depletion represent a common hallmark of aging \((3)\), it is tempting to conclude that the nearly logarithmic increase in cancer incidence and mortality with age is driven in part by these unfavorable changes in fat redistribution. Therefore, strategies designed to deplete visceral fat stores in abdominally obese individuals, including pharmacologic and/or behavioral strategies, such as diet and exercise, may be an
important cancer prevention strategy as well as an adjuvant therapy for improving outcomes following a cancer diagnosis.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: D.M. Huffman, L.H. Augenstein
Development of methodology: D.M. Huffman
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D.M. Huffman, J.J. Lofrese, J.P. Chamberland
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D.M. Huffman, L.H. Augenstein, X. Zhang, G. Atzmon, J.P. Chamberland, C.S. Mantzoros
Writing, review, and/or revision of the manuscript: D.M. Huffman, J.J. Lofrese, J.P. Chamberland, C.S. Mantzoros
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Acknowledgments
The authors thank Dr. Rani Sellers for assistance with the histopathologic evaluation of tissue sections and Youngmei Zhao and Hongqiang Liang for technical support.

Grant Support
This work has been supported by grants from the Prevent Cancer Foundation and National Institute on Aging (AG037547) to D.M. Huffman, and the Einstein Nathan Shock Center Healthy Aging Physiology Core (P30AG038072) and the Diabetes Research and Training Center (DK20541) at the Albert Einstein College of Medicine. C.S. Mantzoros is supported by the National Institute of Diabetes and Digestive and Kidney Diseases grants 58785, 79929, and 81913 as well as Award Number 1H01CX000422-01A1 from the Clinical Science Research and Development Service of the VA Office of Research and Development.

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Received October 2, 2012; revised December 2, 2012; accepted December 3, 2012; published online March 6, 2013.

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Abdominal Obesity, Independent from Caloric Intake, Accounts for the Development of Intestinal Tumors in \(Apc^{1638N/+}\) Female Mice

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