Insulin, leptin, and adiponectin receptors in colon: regulation relative to differing body adiposity independent of diet and in response to dimethylhydrazine

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Although there are well-characterized genetic mutations that predispose individuals to colon cancer, it is clear that the majority of sporadic colon cancers are a complex interplay between multiple genes and environmental factors such as diet. Recently, obesity, a major problem in many countries worldwide, has become a focus of investigations to identify diet and lifestyle factors associated with an increased risk of colon cancer (8, 13, 16, 29). The metabolic stress resulting from obesity is associated with increased oxidative stress (36) and insulin insensitivity with concomitant altered adipokine levels, increases in cytokine production, and inflammatory markers (20, 48). Alterations in metabolism in response to obesity and the associated deregulation of insulin signaling and imbalances in adipokines such as leptin and adiponectin levels have been linked to colon cancer and inflammatory bowel diseases, which increase colon cancer risk (23, 24, 25, 40, 41, 45, 53), promoting speculation on the role of insulin and adipokine signaling in colon pathology.

A number of studies support the influence of aberrant insulin metabolism on the risk of colon cancer (10, 15). The role of insulin in regulating glucose homeostasis and energy supply to cells and tissues and cellular growth and differentiation (18) may be a key link in the promotion of uncontrolled cell growth associated with tumorigenesis. Insulin signaling is initiated via transmembrane receptors to activate intracellular signaling cascades that ultimately influence gene transcription and cellular processes that regulate growth and differentiation (49).

Studies have implicated the adipokines, leptin and adiponectin, in obesity and in the regulation of colonic tissue. Increased leptin levels characteristic of obesity alter immunity and inflammatory responses (12, 41), exert proliferative and antiapoptotic activities, and potentially act as a growth factor in colon tissue (4, 33). Inappropriate signaling and deregulated inflammatory and immune responses perpetuate DNA damage and impair DNA repair systems, increasing cancer risk (5, 32, 41). Leptin receptors exist at least six different isoforms, with the long form Ob-Rb thought to be the major signaling form (43).

Adiponectin is also linked to increased risk of colonic disease associated with obesity (25, 40). Signaling responses in colon epithelial cells in culture indicate that adiponectin receptors may be present on the epithelium (37). Although proposed as an abundant cytokine generated by adipose tissue, adiponectin, unlike leptin, is decreased with increased visceral obesity (21, 52). Adiponectin is reported to be associated with improved insulin regulation, blood glucose, and triglyceride levels (28, 42). Elevated leptin and insulin associated with obesity are correlated with lower adiponectin receptor expression, particularly in skeletal muscle and adipose tissue (14, 52). Two adiponectin receptors have been cloned, ADIPOR1 and ADIPOR2, that mediate the signaling of both full-length adiponectin and the truncated globular portion of adiponectin (51). ADIPOR1 is fairly ubiquitous but is particularly associated with skeletal muscle, whereas ADIPOR2 is highly expressed in liver (51).
The two receptors have distinct affinities to the various forms of adiponectin encountered in vivo.

A number of molecular mechanisms associated with obesity have been studied using monogenic rodent models, which commonly exhibit mutations in leptin signaling pathways and are unsuitable to investigate many aspects of the linkage between obesity, regulation of colonic tissue, and involvement in colon carcinogenesis. Most human obesity is polygenic, representing multiple genetic and environmental interactions. Sprague-Dawley (SD) rats are used as a polygenic model of diet-induced obesity (1). Feeding SD rats a high-energy diet of relatively high fat and energy content, high sugar content, and simple carbohydrates leads to a range of body weight gains and identifies rats that are relatively susceptible to diet-induced obesity (high weight gainers) and others that are relatively resistant (low weight gainers) (1, 30, 31). Rats at opposite ends of the body weight distribution can thus be used as a model to measure differences in sensitivity to treatment with the chemical carcinogen 1,2-dimethylhydrazine (DMH), relative to the level of obesity and associated altered adipokine levels. The colonotrophic chemical DMH promotes precancerous luminal epithelial lesions when administered by subcutaneous injection and is used to investigate colon carcinogenesis (6, 7).

Obesity potentially promotes an imbalance in gut metabolic homeostasis with associated altered insulin and adipokine signaling, increased oxidative stress and subclinical chronic inflammation, and altered immunity. Immunohistochemical investigation has revealed that insulin (27) and leptin (19) receptors are present on colon epithelial cells. Studies in cell culture systems also indicate that adiponectin receptors may also be present in colon epithelial cells (37). This study was initiated to investigate whether the altered biochemistry associated with obesity contributes to colon carcinogenesis, by identifying microanatomical location of cells within colon tissue, subject to regulation by insulin, leptin, and adiponectin; assess alterations in regulation of insulin, leptin, and adiponectin receptors; and examine sensitivity of the colon to procarcinogenic activity in response to the colonotrophic chemical carcinogen DMH in rats with differing levels of adiposity.

MATERIALS AND METHODS

Animals and experimental protocol. All procedures were licensed according to the Animals (Scientific Procedures) Act UK of 1986 and approved by the Rowett Research Institute’s Ethical Review Committee. Male outbred SD rats (n = 208) weighing ~230 g (Charles River Laboratories, Kent, UK) were fed normal SDS stock pellet ad libitum at 22–23°C on a 12:12-h light-dark cycle for 2 wk to acclimatize. Body weights (in g) are given as means ± SD of upper quartile (1) (DIO, n = 52), middle quartiles (2 and 3), and lower quartile (4) (DR, n = 52) of weight-gaining Sprague-Dawley (SD) rats fed on high-energy (HE) diet ad libitum for 2 wk. B: allocation of the groups, upper 25% and lower 25% of body weight was based on weight gain at 2 wk on HE diet from a pool of 208 rats. C: body weights of experimental subgroups during the study period. Means are ± SD body weights (g) of diet-induced DR and DIO rats maintained on the HE diet with and without 1,2-dimethylhydrazine (DMH) injection. DR and DIO groups were subdivided to generate two subgroups with equal body weight distribution after 2 wk on HE diet. The subgroups, saline-injected and DMH, are indicated. Body weights of DR and DIO rats were significantly different throughout the experiment (P < 0.05).

Table 1. Terminal blood metabolites and hormones in DR and DIO Sprague-Dawley rats with and without 1,2-dimethylhydrazine treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DR saline</th>
<th>DR DMH</th>
<th>DIO saline</th>
<th>DIO DMH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>11.03±0.35</td>
<td>10.53±0.25</td>
<td>10.13±0.52</td>
<td>10.41±0.32</td>
</tr>
<tr>
<td>NEFA, mmol/l</td>
<td>0.44±0.02</td>
<td>0.46±0.04</td>
<td>0.62±0.11</td>
<td>0.56±0.07</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>2.00±0.33 †</td>
<td>1.55±0.15†</td>
<td>2.69±0.32*</td>
<td>1.96±0.16†</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>1.35±0.23†</td>
<td>1.38±0.16†</td>
<td>2.08±0.33*</td>
<td>1.89±0.14*</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>14.41±1.53†</td>
<td>14.60±1.16†</td>
<td>19.71±1.06*</td>
<td>19.73±1.05*</td>
</tr>
<tr>
<td>Adiponectin, ng/ml</td>
<td>3.14±0.17</td>
<td>3.64±0.21</td>
<td>3.54±0.17</td>
<td>3.55±0.12</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 12 (control groups), 20 (DMH groups). * is significantly different from † at 0.05 level. DMH, 1,2-dimethylhydrazine treatment; DR, diet-induced obese resistant; DIO, diet-induced obese sensitive.
acclimatize. Body weight was recorded twice weekly throughout the experiment. During the first week, they were group housed. The rats were housed individually at the beginning of week 2. This initial pool of rats was then introduced to a high-energy diet, with 15% energy as protein, 33% as fat, and 52% as carbohydrate (Research Diet D12266B; Research Diets, New Brunswick, NJ). Two weeks later, rats were selected from the lowest and highest quartile of body weight. All of the rats selected for the study continued to be maintained on the D12266B high-energy diet until the end of the experiment. After 10 wk of feeding the high-energy diet, rats were treated with the colontropic chemical carcinogen DMH (two subcutaneous injections 25 and 125 mg/kg body wt 1 wk apart) or saline injection. Fat mass was measured by Magnetic Resonance Imaging using the EchoMRI 2004 Whole Body Composition Analyzer System (Echo Medical Systems, Houston, TX) before injection with the carcinogen, DMH, and again before death. 12–14 wk after the first injection, rats were euthanized with isoflurane to provide colons for histological determination of early markers of colon cancer, aberrant crypt foci (ACF) and mucin-depleted foci (MDF), and also for regional colon analysis of receptor localization and RNA extraction for semi-quantitative real-time PCR. Rats were not fasted before death. Colons were excised from the ileocecal valve and rinsed with sucrose-Tris buffer to remove contents. Colon length measurements were recorded and proximal, and transverse and distal segments were snap frozen separately for regional colon analysis. Alternatively, the colons were opened longitudinally, stretched, pinned flat, and immersed in neutral buffered formalin for microscopical examination of luminal epithelial abnormalities (ACF and MDF).

Circulating hormones and metabolites. Plasma leptin (Linco RL-83K), insulin (Linco RL-13K), and adiponectin (Linco MADP-60HK) were measured using commercially available rat/mouse-specific radioimmunoassay kits (Linco BioGenesis, Poole, UK). The sensitivity of the assays was 0.5, 0.1, and 1 ng/ml, respectively, with intra-assay coefficients of variation (CVs) of <5%. Plasma glucose, triglycerides, and nonesterified fatty acids were determined as previously (1), by using the fully automated KONE analyzer (35, 39). The sensitivities of the assays were 0.66, 0.09, and 0.14 mmol/l, respectively, with intra-assay CVs of 3.49, 2.82, and 5.88%, respectively.

Microscopical examination of precancerous lesions. The chemical carcinogen, DMH, produces preneoplastic ACF, mucin-depleted lesions (MDF) and tumors in the colon of rodents (6, 7). The level of luminal colon epithelium abnormalities in response to treatment with DMH was assessed by scoring numbers and complexity of ACF and MDF in DIO SD obese (DIO) and nonobese (DR) rats with/without DMH treatment (n = 12), at 10 wk following the first carcinogen administration, essentially applying the procedures described by Bird (6) and Caderni et al. (7). The complexity of ACF and MDF were assessed by examination of aberrant crypts per foci. Precancerous lesions, MDF, are identified as focal lesions characterized by the absence of or very limited production of mucins.

In situ hybridization. Cryostat sections (10 μm) cut from tissue segments dissected adjacent to that used for RNA extraction from each of the proximal, transverse, and distal colon regions (n = 8) were thaw-mounted onto polylysine-coated slides and stored at −80°C until use. In situ hybridization of the three regions was performed essentially as described previously (9). The riboprobe templates were generated by PCR using the following rat specific primer pairs: INSR 5’-CCCCCTCGGACCCCAAATG-3’ and 5’-CAGGCCAGAGATGACCAATGTA-3’; Ob-Rb 5’-AGTTGACACAGTGAACAGTGC-3’ and 5’-YCTGTAGTCACGGAACAGAC-3’; ADIPORI 5’-GGAGGGCGTTCGAGGAGATGA-3’; and ADIPOR2 5’-AGGGCACTTGATGATA-3’ and 5’-TGCA-
GAGGGGCCACAAAAGATA-3'. Plasmids for riboprobe synthesis were prepared from single colony inoculants of *Escherichia coli* transformed with the supplied plasmids using a Wizard 373A DNA purification kit (Promega, Southampton, UK), according to the manufacturer's instructions, and sequences were verified using a Beckman CEQ8000 Genetic Analyzer. Antisense and sense probes were synthesized from the prepared templates by in vitro transcription using RNA T7 and T3 polymerases as appropriate in the presence of 35S-α-thio-UTP (NEN; 1000 Ci/mmol). Tissue sections were hybridized with radiolabeled riboprobes at 58°C and washed to 0.1× SSC at 60°C. Hybridized sections were assessed initially using a Fuji phosphorimager and AIDA Image Analyzer software (Raytest Isotopenmeßgeräte, Straubenhardt, Germany) before coating with LM-1 liquid emulsion (Amersham Pharmacia Biotech, Buckinghamshire, UK) and staining with toluidine blue to facilitate anatomical location by light microscope autoradiography. The sense riboprobe hybridized sections were examined to assess background and nonspecific hybridization and confirm specific silver grain localization.

**RNA extraction.** RNA was extracted from 0.5-cm colon segments dissected at the midpoint of each region of proximal, transverse, and distal colon, respectively, using an RNeasy Midi Kit (Qiagen, Crawley, UK) and incorporating a DNase digestion. All of the extracted RNA samples were subjected to analysis using the Agilent Bioanalyzer (Agilent Technologies, Bracknell, UK).

**Real-time PCR.** Real-time PCR analysis was performed, according to the manufacturer's instructions, using Superarray Bioscience SYBR green master mix (Tebu-Bio, Peterborough, UK). Specific rat primer pairs for reference and target genes were obtained from Superarray Bioscience (Tebu-Bio, UK). GAPDH was used as the reference gene for relative quantitation. All PCR products were sequenced to confirm appropriate identities. This established that the Superarray primer pairs for Ob-R real-time assay amplify both the long and short form Ob-R transcripts. The threshold cycle number (Ct) was measured using the iCycler (Bio-Rad, Hertfordshire, UK) and associated software (Bio-Rad, UK). Standard curves for GAPDH and target genes for all real-time analyses were generated from a single template prepared from rat colon. Relative transcript levels were calculated for each reaction by determining ratios of equivalent amounts calculated from the standard curves for targets (INSR, Ob-R, ADIPOR1, or ADIPOR2 as appropriate) and reference gene (GAPDH). Technical replicates were performed in at least duplicate on at least eight biological replicates. Pairwise comparisons were conducted using Student’s *t*-test, and ANOVA was applied for multiple comparisons. *P* values < 0.05 were considered significant.

**RESULTS**

**Body weight gain and fat mass analysis.** Body weights diverged significantly (*P* < 0.0001) over the first 2 wk on the high-energy diet, permitting selection of rats in the lower (DR, 378 ± 18 g) and upper (DIO, 454 ± 16 g) quartiles of body weight for subsequent experiments (Fig. 1, A and B). Rats were further subdivided into two DR and DIO subgroups of evenly distributed body weight and assigned to the saline injected control group or DMH injected group (Fig. 1C). Body weight gain was significantly different between the DR and DIO groups throughout the experiment (Fig. 1). Fat mass was also significantly different (*P* < 0.05) between the DR and DIO groups pre-DMH treatment (57.84 ± 5.41 and 119.51 ± 5.49 g, respectively). The sense riboprobe hybridized sections were examined to assess background and nonspecific hybridization and confirm specific silver grain localization.

**Fig. 4.** In situ hybridization of ADIPOR1 transcripts in rat colon. Emulsion autoradiographs showing expression of ADIPOR1 over ep (A and C), lymphatic tissue (lt) (C and E), and muscularis (m) (A) in rat transverse colon. Sense riboprobe hybridized to adjacent sections to assess nonspecific signal levels (B, D, and F). Bar, 20 μm.
respectively) and at the end of the experimental period (129.82 ±
11.81 and 192.72 ± 12.34 g, respectively). Body weight was
significantly correlated with fat mass prior to DMH treatment
\((R = 0.908)\) and at the end of the experiment \((R = 0.808)\) at the
0.01 level (2 tail test). At death and dissection abdominal fat
and fat wrapping associated with the colon and colonic mes-
entery were observed to be greater in the DIO group compared
with the DR group (unpublished observations). A slight reduc-
ton in the rate of weight gain was observed post-DMH
treatment, but the weight gain trajectory was subsequently
restored; DMH-treated rats continued to gain weight and fat
mass with no significant differences with saline controls within
the corresponding DR or DIO groups. There were no signifi-
cant differences in colon length associated with body weight or
DMH treatment.

*Circulating hormones and metabolites.* Significant differ-
ences in circulating hormones (insulin and leptin) and triglyc-
erides were measured in DR and DIO groups (Table 1). There
was no significant difference in adiponectin, glucose, or non-
esterified fatty acids between DR and DIO groups, or in
response to DMH treatment.

*Localization of INSR, Ob-R, and ADIPOR1 and 2 receptor
gene expression in rat colon.* In situ hybridization was per-
formed on cryostat sections cut from each of the colon regions,
proximal, transverse, and distal, revealing similar localization
patterns of INSR (Fig. 2), Ob-Rb (Fig. 3), ADIPOR1 (Fig. 4),
and ADIPOR2 (Fig. 5) expression in each region. All the
receptors exhibited epithelial (ep) expression. However, 
OB-Rb (Fig. 3) expression was observed on epithelial cells
lining the colon crypts, rather than the luminal epithelial
surface. In contrast, INSR (Fig. 2A and ADIPOR) (Figs. 4, A
and C; 5, A and C) receptor isoforms were expressed through-
out the luminal epithelium, both within the colon crypts and on
the luminal epithelial surface and at higher levels, as evidenced
by shorter exposure times for visualization of silver grains by
liquid emulsion autoradiography. Evidence of expression of
ADIPOR receptors was observed within lymphatic tissue (Figs. 4,
C and E; 5E). Although expression in the muscularis was confined
to ADIPOR1 (Fig. 4A), expression of the ADIPOR2 isoform was
absent or below detection level in the same tissue. Adjacent
sections probed with the appropriate sense riboprobes are shown
for comparison (Figs. 2B; 3C; 4B, D, and F; and 5B, D, and F).

Expression of insulin, leptin and, adiponectin receptors in
colon with differential body adiposity. Real-time PCR con-
firmed the observed expression of insulin (INSR) (Fig. 6);
leptin (Ob-R) (Fig. 7), and adiponectin (ADIPOR1 and ADI-
POR2) (Fig. 8 and 9) receptors in proximal, transverse, and
distal regions of the colon. There was no change in INSR
expression in DIO rats in response to body weight/composition
or DMH (Fig. 6A). Levels of Ob-R expression revealed a
tendency to exhibit greater variation in the experimental rats
than INSR receptors. A differential response to DMH treatment
was revealed using ANOVA and Fishers least significant difference post hoc test with Ob-R expression significantly reduced in DMH-treated DIO rats compared with DR rats (Fig. 7A). DMH treatment led to an increase in Ob-R expression compared with that observed for DIO rats in transverse colon (Fig. 7B). Regulation of ADIPOR1 was not markedly altered in DR compared with DIO rat colon (Fig. 8), although the slight (22%) upregulation of ADIPOR1 in proximal colon of DIO rats was significant (P = 0.02) (Fig. 8A). This may have consequences for adiponectin signaling since the ADIPOR isoforms are reported to regulate different signal transduction pathways (47). It is speculated that adiponectin may protect against pathogenesis linked to obesity (46). ADIPOR2 was not significantly differentially regulated in DR compared with DIO rats in proximal (Fig. 9A), transverse (Fig. 9B), or distal (Fig. 9C) colon. ADIPOR1 and ADIPOR2 gene expression was not measured in colon post-DMH treatment since adiponectin levels were not significantly altered in plasma in response to differences in the level of obesity.

Microscopical analysis of colon tissue. ACF of varying complexity were observed in the transverse and distal region of all DMH-treated rats but were absent in the proximal region (Table 2). MDF were observed in two rats in the DR group and one rat in the DIO group with one putative adenoma observed in the DIO group (Table 3). There were no significant differences in ACF or MDF complexity, total number per colon, or in sulfomucin or sialomucin secretion (Table 4) in response to body weight or fat mass. Distal colon crypts secrete sulfomucins predominantly with lesions often exhibiting altered mucin secretion. No ACF or MDF were observed in the saline-injected control rats (Tables 2 and 3).

Fig. 6. Expression of INSR relative to GAPDH in response to diet-induced obesity in proximal, transverse, and distal colon. Diet-induced obese DR and DIO rats were saline-injected (control) or injected with DMH. A: proximal colon. B: transverse colon. C: distal colon. Results are shown as means ± SE of relative expression ratios (n = 8).

Fig. 7. Expression of OB-R relative to GAPDH in response to diet-induced obesity in proximal, transverse, and distal colon. Diet-induced obese DR and DIO rats treated with saline or DMH. A: proximal colon. B: transverse colon. C: distal colon. Results are shown as means ± SE of relative expression ratios (n = 8). *P < 0.05 by LSD post hoc test.

Fig. 8. ADIPOR1 gene expression in proximal, transverse, and distal colon.

Fig. 9. ADIPOR2 gene expression in proximal, transverse, and distal colon.
DISCUSSION

The recognition of adipose tissue as an endocrine organ secreting a number of adipokines has directed research focus on the role of these adipokines in chronic disease risks associated with obesity (26). Epidemiology studies implicate visceral adipose tissue in the increased risk of intestinal and mesenteric diseases, including cancer (13, 38, 40, 50). The DR and DIO rats fed a high-energy diet have significant differences in body weight and fat mass. Increased visceral and mesenteric fat distribution in the abdominal cavity of DIO compared with DR rats was also apparent (unpublished observations). The colon and mesentry appear wrapped in adipose tissue. The increased fat mass in DIO rats was associated with obesity markers, with insulin, leptin, and triglyceride stress levels, all linked to an increased risk of colon cancer, being significantly elevated (8, 13, 22, 34, 44). However, the elevated fat mass in DIO rats did not result in significantly altered plasma adiponectin levels, counter to reports of reduced plasma adiponectin with increased obesity (21, 51, 52). Further investigation will be required to determine correlations between regulation of adiponectin levels and insulin and leptin.

The localization of INSR, OB-R, ADIPOR1, and ADIPOR2 gene expression throughout the colonic epithelium is intriguing, supports a role in regulation of cellular processes in this tissue layer, and is pertinent to evidence linking obesity to colon pathologies. The colonic epithelium is a dynamic tissue layer that requires tight regulation to maintain a balance be-

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**Fig. 8.** Expression of ADIPOR1 relative to GAPDH in DR and DIO proximal (A), transverse (B), and distal colon (C). Expression levels for each biological replicate (n = 8) and adjacent mean relative expression ratios of ADIPOR1 gene expression (n = 8) are shown. *P < 0.05.

**Fig. 9.** Expression of ADIPOR2 relative to GAPDH in DR and DIO proximal (A), transverse (B), and distal colon (C). Expression levels for each biological replicate (n = 8) and adjacent mean relative expression ratios of ADIPOR1 gene expression (n = 8) are shown.
tween proliferation, differentiation, and apoptosis, permitting constant and controlled renewal of this tissue layer (11). These processes are deregulated in pathologies such as IBD and cancer. Common location of the receptors in the colonic epithelium also indicates a potential for cross talk in regulating colonic tissue. This study indicates potential in vivo regulation of gut epithelium by adiponectin, as implied by recent studies showing adiponectin stimulation of proliferation and cytokine secretion in colon cells in vitro (37). The localization of ADIPOR1 and ADIPOR2 gene expression throughout the colonic epithelium supports a role for adiponectin signaling in regulating cellular processes in this tissue layer and is pertinent to evidence linking adiponectin to colon pathologies. Previous reports of adiponectin signaling in the HT-29 human colon cancer epithelial cell line have revealed potential pro-proliferative and proinflammatory actions (37). Ogunwobi and Beales (37) did not establish the receptor subtypes involved in the associated adiponectin-stimulated phosphorylation of kinase signaling and cytokine secretion. The present study indicates that both receptors may be involved in regulating colon epithelial cells in vivo.

The location of adiponectin receptors in lymphatic tissue also supports a role in immune function in the gut. Altered signaling via the deregulated epithelium and gut lymphatic tissue subsequently also disrupts interactions with the colonic microflora. Considering the role of the epithelium in regulating epithelial renewal (11) and gut barrier function (17), altered signaling of hormones and receptors linked to obesity is potentially important with regard to colon pathogenesis. This may be another contributing factor in links between obesity, altered adipokine levels, and colon pathology.

Homeostatic regulation of the as yet unknown functions regulated by insulin, leptin, and adiponectin are potentially disrupted in the colon as a consequence of altered adipose tissue mass and distribution. In the current study this was not reflected in marked deregulation of hormone receptors in the colon or sensitivity to the colonotropic chemical DMH. Although elevated insulin, leptin, and triglycerides are associated with increased risk of colon cancer (8, 13, 22, 34, 44), this was not reflected in an increased sensitivity to DMH treatment. However, Aparicio et al. (3, 4) also reported that leptin was not observed to increase precancerous lesions despite the observation of stimulation of colon epithelial cells in vitro. Additionally, plasma levels of hormones may not directly reflect local exposure to adipokines in organs and tissues in the obesogenic environment, particularly in view of the fat wrapping associated with organs in the abdominal cavity. Hence, the influence of insulin, and adiponectin on signaling in colon tissues needs to be assessed locally. Alternatively, perhaps a threshold level of body fat, visceral abdominal fat, and time of exposure to disrupted adipokine signaling is required to initiate promotion of increased colon cancer risk. Additionally, administration of increased doses of DMH may potentially uncover differences in sensitivity to the carcinogen associated with body adiposity. Investigation of the role of insulin and adipokine signaling in the colon is necessarily complex with hormone receptor expression in three (epithelium, lymphatic tissue, and muscularis) different locations within colon tissue. This study is the first to investigate insulin, leptin, and adiponectin receptors in the colon and regulation relative to different levels of body adiposity, independent of diet, and in response to DMH. Establishing the distribution of colon insulin, leptin and, adiponectin receptor expression is a necessary requisite to further investigations to explain the biological effects of altered hormone levels on colon tissue and potential influences on colon pathogenesis relative to body adiposity. The SD model permits studies that are particularly relevant to common human obesity with the range of responses more relevant to that of susceptible and resistant individuals in the human population. This contrasts with previous studies using monogenic mutant rodent models and different diet compositions to investigate obesity and colon cancer risk. The current study is therefore more likely to accurately reflect associations that will have a meaningful impact on the human condition.

ACKNOWLEDGMENTS

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Table 2. Aberrant crypt foci in DR and DIO rats treated with saline or DMH

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>ACF</th>
<th>&gt;7</th>
<th>Total ACF/Colon</th>
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<tbody>
<tr>
<td>DR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DMH</td>
<td>95±6.8</td>
<td>6.7±1.3</td>
<td>1.25±0.5</td>
</tr>
<tr>
<td>DIO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DMH</td>
<td>107.1±12.1</td>
<td>8.25±2.9</td>
<td>1.9±1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 4 (saline groups), 12 (DMH groups). No significant differences. ACF, aberrant crypt foci.

Table 4. Mucin secretion scores in DR and DIO rats treated with saline or DMH

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>Sialomucin</th>
<th>Sulomucin</th>
<th>Mixed Mucin</th>
<th>Low Mucin</th>
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<tbody>
<tr>
<td>DR</td>
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<td></td>
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<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DMH</td>
<td>112.0±9.6</td>
<td>68.3±8.4</td>
<td>7.4±1.1</td>
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<tr>
<td>DIO</td>
<td></td>
<td></td>
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<tr>
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<tr>
<td>DMH</td>
<td>113.9±9.1</td>
<td>67.4±10.4</td>
<td>8.5±1.6</td>
<td>1.6±0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 4 (saline groups), 12 (DMH groups). No significant differences.
GRANTS

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REFERENCES


