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Linking inflammation to tumorigenesis in a mouse model of high-fat-diet-enhanced colon cancer

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ABSTRACT

Many observational epidemiologic studies suggest an association between high-fat-diet (HFD) and colon cancer risk. However, the lack of controlled experimental studies that examine this relationship and the mechanisms involved weaken the basis for inferring a causal relationship. Inflammation plays a role in colon cancer progression and HFDs have been reported to increase inflammation; however, the inflammatory effects of HFD in colon cancer have yet to be firmly established. We examined the effects of a novel HFD that closely mimics the standard American diet (12% and 40% of total caloric intake from saturated fat and total fat, respectively) on macrophage markers and inflammatory mediators in a mouse model of intestinal tumorigenesis and relate this to polyp characteristics as well as measures of adiposity. Male $Apc^{Min/+}$ mice (7–8/group) were fed a Control Diet (Con) or novel high-fat-diet (HFD) from 4 to 12 weeks of age. Body weight and body composition were measured weekly and monthly, respectively. Intestinal tissue was analyzed for polyp burden (number and size). Gene expression of macrophage markers and inflammatory mediators were examined in the adipose tissue and polyps. The HFD increased the expression of macrophage markers and inflammatory mediators in the adipose tissue (F4/80, CD11c, TLR-4 and MCP-1) and tumor microenvironment (IL-12, MCP-1, IL-6 and TNF-α). As expected, the HFD increased body weight, body fat percent, fat mass and blood glucose ($P < 0.05$), and was associated with an increase in the number of large polyps ($P < 0.05$) but not total polyps. In summary, consumption of a HFD, similar in macronutrient composition to the standard American diet, altered the expression of macrophage phenotypic markers and inflammatory mediators in adipose tissue and intestinal polyps and this was associated with increased tumorigenesis.

1. Introduction

The etiology of colon cancer is a complex phenomenon that involves the interaction of genetic and environmental factors. However, the vast majority of cases can be ascribed to environmental causes as they account for more than 80% of all incidences [1]. High-fat-diet (HFD) induced obesity has emerged as one of the leading environmental risk factors for the development of colon cancer [2–4] as supported by epidemiological studies as well as controlled experimental studies in mice [5–10].

Animal studies provide evidence for a link between HFD consumption and colon cancer risk [8–11]. For example, Baltgalvis et al. reported a 75% increase in polyp number in the $Apc^{min/+}$ mouse model of intestinal tumorigenesis following treatment with a Western Style diet (40% calories from fat) [8]. Similarly, HFD-induced obesity (60% of total calories from fat) has been shown to increase development of AOM-induced aberrant crypt foci in the colon [10]. In addition, an increase in proliferation and a decrease in apoptosis have been reported in the colonic epithelium following HFD-induced obesity (60% of total calories from fat) in mice [12]. While there is reasonable evidence for an association between HFD-induced obesity and colon cancer risk, there is a fundamental gap in the understanding of underlying mechanisms for such a relationship. Further, many of the HFDs that have been used to test this relationship contain unreasonably high levels of both total fat and saturated fat and typically contain fat from only one food source making it difficult to assess the clinical relevance.

While the pathophysiological mechanisms that link HFD-induced obesity to colon cancer risk are not well understood, it is believed that inflammation plays a key role. Consumption of a HFD can lead to accumulation of excess body fat that is associated with adipose tissue dysfunction and a chronic state of low-grade inflammation [13], which is known to promote tumor

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development and growth [13]. Inflammation associated with HFD-induced obesity is thought to be mediated, at least in part, through numerical and functional alterations in adipose tissue macrophages [13–15]. It has been reported that in HFD-induced obese mice, approximately 45–60% of adipose tissue cells express the macrophage marker EMR-1 (F4/80), whereas only 10–15% of cells from lean mice express this marker [14]. In addition, adipose tissue macrophages in HFD-induced obese mice exhibit a pro-inflammatory classical phenotype (M1) while those from lean mice have an alternatively activated anti-inflammatory phenotype (M2) [15]. While there is an abundance of evidence that implicates inflammation as a link between HFD-induced obesity and cancer, there are few studies that have examined the inflammatory response in a mouse model of colon cancer in the settings of HFD-induced obesity.

The purpose of this study was to examine the effects of a novel HFD closely mimicking the standard American diet [16] (12% and 40% of total caloric intake from saturated and total fat, respectively, a 2:1 monounsaturated: polyunsaturated fatty-acid ratio, and a 20:1 omega-6:omega-3 fatty-acid ratio) on the expression of macrophage markers and inflammatory mediators in adipose tissue and the tumor microenvironment in a mouse model of intestinal tumorigenesis and to relate this to polyp characteristics as well as measures of adiposity. We used the *Apc*$^{min/+}$ mouse, the most widely used genetic mouse model for cancer studies that involve the gastro-intestinal tract [17]. Since the *Apc* gene is mutated in a large percentage of human colon cancer cases, this is a common model for studying factors that may influence progression of colon cancer. A novel HFD, designed by our laboratory, was used to mimic a typical American diet. We hypothesized that this novel HFD would alter the expression of macrophage phenotypic markers and increase the expression of inflammatory meditators in the adipose tissue and tumor microenvironment in association with increased adiposity and enhanced tumorigenesis.

2. Materials and methods

2.1. Animals

Apc$^{Min/+}$ on a C57BL/6 background and C57BL/6 wild-type mice were originally purchased from Jackson Laboratories (Bar Harbor, ME). *Apc*$^{Min/+}$ male mice were bred with female C57BL/6 mice in the University of South Carolina's Center for Colon Cancer Research (CCCR) mouse core facility to generate *Apc*$^{Min/+}$ mice. Offspring were genotyped as heterozygotes by RT-PCR for the *Apc* gene by taking tail snips at weaning. The primer sequences were sense: 5′-TGAGAAAGACAGAAGTTA-3′; and antisense: 5′-TTCCACTTTGG-CATAAGGC-3′. Mice were maintained on a 12:12 h light–dark cycle in a low-stress environment (22 °C, 50% humidity and low noise) and provided food and water *ad libitum*. All animal experimentation was approved by the University of South Carolina's Institutional Animal Care and Use Committee.

2.2. High-fat-diet treatment

Apc$^{Min/+}$ mice (7–8/group) were assigned to one of two treatment groups: (1) an AIN-76A Control Diet (Con) or (2) a HFD (12% and 40% of total caloric intake from saturated and total fat, respectively) resembling the standard American Diet (HFD) (Bio-Serv, Frenchtown, NJ) (Table 1). Treatments began at 4 weeks of age and continued until 12 weeks of age. The HFD retained the same vitamin and mineral content as the control diet. Food and water was available *ad libitum* and measured on a weekly basis.

Table 1
Diet composition.

	AIN-76A	HFD
Ingredient (g/kg)		
Casein	200	165
DL Methionine	3	3
Lard	0	35.4
Coconut Oil	0	30
Corn Oil	50	49.9
Soybean Oil	0	9.3
Olive Oil	0	78.4
Corn Starch	150	50
Maltodextrin	0	100
Sucrose	500	381.5
Cellulose	50	50
Vitamin Mix (AIN-76A)	10	10
Mineral Mix (AIN-76A)	35	35
Choline Bitartrate	2	2.5
Energy (kcal/g)	3.79	4.57
Energy (% kcal)		
Carbohydrate	68.8	47
Fat	12.2	40
Protein	19.0	13
Fatty Acid Profile (g/kg)		
Caprylic Acid (C8:0)	0	2.3
Capric Acid (C10:0)	0	1.8
Lauric Acid (C12:0)	0	13.5
Myristic Acid (C14:0)	0	5.5
Palmitic Acid (C16:0)	5.3	26
Palmitoleic Acid (C16:1)	0	2
Stearic Acid (C18:0)	0	8.5
Oleic Acid (C18:1)	13.7	88
Linoleic Acid (C18:2)	26.8	43.2
α-Linolenic Acid (C18:3)	0.6	2.2
% of Total Calories from SFAs	1.4%	12%
% of Total Calories from MCFAs (C6:0-C12:0)	-	3.6%
% of Total Calories from LCSFAs (C14:0-C20:0)	1.4%	8.4%
% of Total Calories from USFAs	10.8%	28%
% of Total Calories from MUFAs	3.6%	18.6%
% of Total Calories from PUFAs	7.2%	9.4%
% of Total Calories from n-3 FAs	.15%	.45%
% of Total Calories from n-6 FAs	7.0%	8.9%
Cholesterol (mg/kg)	0	34
Ratio: MUFA:PUFA	1:2	2:1
Ratio: n-6:n-3 FA	45:1	20:1

2.3. Body composition

Body weight was measured weekly and body composition was measured at baseline (4 weeks), midpoint (8 weeks), and at the 12-week endpoint via a Dual Energy X-ray Absorptiometry (DEXA) (Lunar PIXImus, Madison, WI) scan under isofluorane anesthesia.

2.4. Blood collection

At 8 weeks and 12 weeks of age, blood samples were collected from the tip of the tail after a 5-h fast. Blood glucose concentrations were determined in whole blood using a glucometer (Bayer Contour, Michawaka, IN). At sacrifice, blood was collected from the inferior vena cava using a heparinized syringe and was spun in a microcentrifuge at 10,000 relative centrifugal force for 10 min. Plasma was then stored at −80 °C until analysis.

2.5. Tissue collection

Mice were sacrificed for tissue collection by isoflurane overdose. Epididymal, mesenteric, and retroperitoneal fat pads were removed and weighed. The small intestine was carefully dissected distally to the stomach and proximal to the cecum. Sections 1 and 4 of the small intestine and the large intestine (Section 5) were