High-fat Diet Accelerates Intestinal Tumorigenesis Through Disrupting Intestinal Cell Membrane Integrity

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Background: Excess energy supply induces chronic low-grade inflammation in association with oxidative stress in various tissues including intestinal epithelium. The objective of this study was to investigate the effect of high-fat diet (HFD) on intestinal cell membrane integrity and intestinal tumorigenesis in ApcMin/+ mice.

Methods: Mice were fed with either normal diet (ND) or HFD for 12 weeks. The number of intestinal tumors were counted and biomarkers of endotoxemia, oxidative stress, and inflammation were determined. Changes in intestinal integrity was measured by fluorescein isothiocyanate (FITC)-dextran penetration and membrane gap junction protein expression.

Results: HFD group had significantly higher number of tumors compared to ND group (P < 0.05). Blood total antioxidant capacity was lower in HFD group, while colonic 8-hydroxy-2’-deoxyguanosine level, a marker of oxidative damage, was higher in HFD group compared to that of ND group (P < 0.05). The penetration of FITC-dextran was substantially increased in HFD group (P < 0.05) while the expressions of membrane gap junction proteins including zonula occludens-1, claudin-1, and occludin were lower in HFD group (P < 0.05) compared to those in ND group. Serum concentration of lipopolysaccharide (LPS) receptor (CD14) and colonic toll-like receptor 4 (a LPS receptor) mRNA expression were significantly higher in HFD group than in ND group (P < 0.05), suggesting that significant endotoxemia may occur in HFD group due to the increased membrane permeability. Serum interleukin-6 concentration and myeloperoxidase activity were also higher in HFD group compared to those of ND group (P < 0.05).

Conclusions: HFD increases oxidative stress disrupting intestinal gap junction proteins, thereby accelerating membrane permeability endotoxemia, inflammation, and intestinal tumorigenesis.

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Key Words: Apc, Mice, Colon neoplasms, High-fat diet, Permeability, Oxidative stress

INTRODUCTION

Colon cancer is the fourth leading cause of cancer deaths worldwide.1 Epidemiological studies provided evidence that environmental factors are the prime importance in the etiology of colon cancer.2 One of the most important risk factors for colon cancer is body fatness which is often resulted from imbalance between energy intake and energy expenditure.3,4 Therefore, excess dietary fat intake in association with body fat deposition has been regarded as a convincing risk factor for tumorigenesis in the colon. In fact, a chronic fat-rich diet, a typical high energy diet, could results in increased endotoxemia which is a triggering factor for inflammatory responses promoting carcinogenesis.5

Excess reactive oxygen species (ROS) generation in the obese state results in increased oxidative damage which accelerate inflammatory responses.6 Several human studies have shown that obese subjects exhibit lower systemic antioxidant defense activity than normal weight counterparts. Serum 8-hydroxy-2’-de-
Table 1. Composition of the experimental diets

| Variable                      | ND    | HFD   |
|-------------------------------|-------|-------|
| Carbohydrate (% of energy)    | 65.700| 35.700|
| Protein (% of energy)         | 19.300| 19.300|
| Fat (% of energy)             | 15.000| 45.000|
| Ingredient (g/kg)             |       |       |
| Cornstarch                    | 404.000| 266.500|
| Dextrin                       | 134.200| 88.500 |
| Sucrose                       | 101.600| 67.100 |
| Fiber                         | 50.000 | 50.000 |
| Casein                        | 34.600 | 42.100 |
| Corn oil                      | 12.440 |        |
| Lard                          | 45.360 |        |
| Mineral mix \(x\)             | 9.900  | 12.000 |
| Vitamin mix \(x\)             | 198.000| 240.400|
| Tert-butylhydroquinone        | 0.014  | 0.017  |
| Total calories/100 g          | 372.800| 452.800|

ND, normal diet; HFD, high-fat diet. \(x\)Diets were prepared according to the AIN-93 G diet with slight modifications. Mineral mixture and vitamin mixture were prepared according to AIN-93 G diet.
3. Total antioxidant capacity

Total antioxidant activity was determined by the ferric reducing antioxidant power (FRAP) assay. The FRAP reagent was prepared from 10 mmol/L 2,4,6-tri-(2-pyridyl)-1,3,5-triazine (TPTZ) solution in HCl 40 mmol/L plus FeCl₃ (20 mmol/L) and acetate buffer (0.3 mol/L, pH 3.6) in a 1:1:10 ratio. Freshly prepared FRAP reagent was warmed at 37°C for 5 minutes. Serum sample or standard (50 μL) was mixed with 1.5 mL of FRAP reagent in a test tube and incubated at 37°C for 10 minutes. Then, the absorbance of the colored products (ferrous TPTZ complex) was measured at 593 nm and compared to the blank.

4. Colonic 8-hydroxy-2’-deoxyguanosine concentration

Colons were homogenized and DNA was extracted using DNA extraction kit (Nirthwest Life Science Specialities, Vancouver, BC, Canada) following the manufacturer’s instruction. Extracted DNA (200 μg) was dissolved in 135 μL of water, and 15 μL of 200 mM sodium acetate and 6 units of nuclease P1 were added to the DNA solution and incubated for 30 to 60 minutes at 37°C. Tris-HCl buffer (1 M, pH 7.4) and 2 unit of alkaline phosphatase were added and incubated for 30 to 60 minutes at 37°C. After filtering the solution, the supernatants were used for the assay.

5. Determination of intestinal permeability

This measure is based on the intestinal permeability to 4,000-Da fluorescent-dextran (Sigma-Aldrich, St. Louis, MO, USA) as described previously. Briefly, 6-hour fasted mice were injected with fluorescein isothiocyanate (FITC)-dextran by gavage (600 mg/kg body weight, 125 mg/mL) and 120 μL blood was collected from the heart 1 hour after the gavage. The blood was centrifuged at 4°C, 4,000 rpm, for 3 minutes. Plasma was diluted in an equal volume of PBS (pH 7.4) and analyzed for FITC-dextran concentration with a fluorescence spectrophotometer at the excitation wavelength of 485 nm and the emission wavelength of 535 nm. Standard curve for calculating the FITC-dextran concentration in the samples were obtained by diluting FITC-dextran in non-treated plasma diluted with PBS (1:2).

6. Western blot analysis

For the immunoblotting analysis of zonula occludens-1 (ZO-1), claudin-1, and occludin, sample proteins (30 μg) were electrophoresed through 7.5% SDS-PAGE and transferred to polyvinylidene difluoride membranes. The transferred membrane was blocked using 2% skim milk to inhibit non-specific proteins, and treated with primary antibodies against ZO-1 (Invitrogen, Carlsbad, CA, USA), claudin-1 (Invitrogen), occludin (Invitrogen), and β-actin (Sigma-Aldrich). Anti-mouse immunoglobulin G conjugated with alkaline phosphatase was used as the secondary antibody. Each protein band was then confirmed and quantified using an enhanced chemiluminescence system (Amersham, Arlington Heights, IL, USA). The integrity of band was quantified by Versa Doc Quantity one program (BioRad, Mississauga, ON, Canada).

7. Determination of colonic toll-like receptor 4 mRNA expression

Total RNA was isolated from the mouse colon using Trizol reagent (Invitrogen) following the manufacturer’s recommendation. Reverse transcription of total RNA samples and PCR were accomplished using the premix RNA PCR kit (Invitrogen) according to the manufacturer’s instruction. PCR primers were designed using nucleotide sequence for mouse toll-like receptor 4 (TLR4; Bioneer, Daejeon, Korea). The following primers were used: TLR4, forward 5’-AAT TCC TGC AGT GGG TCA AG-3’ and reverse 5’-AGG CGA TAC AAT TCC ACC TG-3’; GAPDH, forward 5’-ACC TCT ATG CCA ACA CAG TGC-3’ and reverse 5’-ACC TCT ATG CCA ACA CAG TGC-3’. The cycling conditions were 3 minutes at 94°C, 30 cycles of 30 seconds at 94°C, 30 seconds at 56°C, 1 minute at 72°C, and 10 minutes at 72°C. PCR products were confirmed by 2% agarose gel electrophoresis and visualized by UV transillumination (Bio-Rad Laboratories Inc., Hercules, CA, USA). For quantitative analysis, Versa Doc Image analyzer (Amersham Biosciences, Piscataway, NJ, USA) was used. All signals were normalized to the mRNA levels of the housekeeping gene β-actin and expressed as ratio.

8. Biochemical measurement

Serum CD14 level was determined using commercially available ELISA kit (Cell Sciences Inc., Canton, MA, USA) according to the manufacturer’s instruction. Interleukin (IL)-6 level was evaluated in plasma using commercially available ELISA kit (BD Bioscience Co., Franklin Lakes, NJ, USA) according to the manufacturer’s instruction. Plasma myeloperoxidase (MPO) activity was determined using commercially available ELISA kit (EIAab Science Co., Wuhan, China) according to the manufacturer’s instruction.

9. Statistical analysis

Statistical analysis was performed by using the SAS package (release 8.01; SAS Institute, Cary, NC, USA). All data from the experiment were expressed as mean ± SD. Data were analyzed by
Student’s t-test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

1. Effect of high-fat diet on body weight, epididymal fat weight, liver weight, and tumor number

The body weight of HFD group was significantly higher than that of ND group between experimental week 2 and 5; however, the difference disappeared thereafter possibly due to faster tumor development in HFD group (Fig. 1A). No difference was found in liver weight and epididymal fat weight (Fig. 1B and 1C). However, the average tumor number was significantly higher in HFD mice compared to that of the ND mice ($P < 0.05$; Fig. 1D).

2. Effect of high-fat diet on blood total antioxidant capacity and colonic 8-hydroxy-2'-deoxyguanosine level

The total antioxidant capacity (TAC) is an indicator of oxidative stress. The level of 8-OHdG is one of the predominant forms of
free radical-induced oxidative lesions in DNA which has been widely used as a biomarker for systemic oxidative stress possibly associated with carcinogenesis. Study results indicated that $APC^{Min/+}$ HFD group possessed a half TAC compared with $APC^{Min/+}$ ND group, indicating that oxidative stress is significantly increased in HFD group ($P < 0.05$, Fig. 2A). In addition, 8-OHdG level in colon tissue was dramatically increased in HFD fed $APC^{Min/+}$ mice compared with tissue 8-OHdG level in ND fed $APC^{Min/+}$ mice ($P < 0.05$; Fig. 2B). These data indicated that a substantial colonic DNA damage is induced by HFD-associated oxidative stress.

3. Effect of high-fat diet on intestinal integrity

Study results showed approximately a four-fold increase in serum FITC-dextran in HFD fed group, demonstrating accelerated transepithelial passage of FITC-dextran in this group ($P < 0.05$; Fig. 3A). The TJ complex is a cluster of proteins that forms a physiologically active barrier at the level of the intestinal epithelial cell, changing its permeability based on the cellular environment. Key TJ proteins include ZO-1, occludin, and claudins. Since the altered paracellular permeability involves a disrupted intestinal barrier, TJ protein expressions were measured by Western blot analysis. Results showed that protein expressions of ZO-1, occludin, and claudin-1 were significantly decreased in HFD fed $APC^{Min/+}$ mice compared with those of ND $APC^{Min/+}$ mice ($P < 0.05$; Fig. 3B–3D). These data indicate that increased intestinal permeability in HFD $APC^{Min/+}$ mice group might be due to the HFD-induced disruption of TJ proteins.

4. Effect of high-fat diet on blood CD14 and colonic toll-like receptor 4 mRNA level

LPS is a component of the cell wall of gram-negative bacteria and is known as endotoxin. In an effort to indirectly determine endotoxemia, CD14, the LPS receptor, was measured in serum. As

![Figure 3](image)
LPS is a ligand of TLR4 in the epithelium, the mRNA expression of TLR4 was determined in colon tissue. Figure 4 showed that APC\(^{min/+}\) mice fed HFD had significantly higher concentration of serum CD14 than mice fed ND (\(P < 0.05\); Fig. 4A). A two-fold increase mRNA expression of TLR4 in colon tissue was also observed in APC\(^{min/+}\) mice fed HFD (\(P < 0.05\); Fig. 4B).

5. Effect of high-fat diet on blood interleukin-6 level and myeloperoxidase activity

Plasma IL-6 level and MPO level are shown in Figure 5. Plasma IL-6 concentration was significantly increased in APC\(^{min/+}\) mice fed HFD (\(P < 0.05\); Fig. 5A). MPO level, an index of granulocytic infiltration, was also significantly increased in the HFD fed APC\(^{min/+}\) mice (\(P < 0.05\); Fig. 5B). These results indicate that HFD significantly accelerates inflammatory response in APC\(^{min/+}\) mice which is possibly linked to tumorigenesis.

**DISCUSSION**

The aim of this study was to provide mechanistic insights for HFD mediated intestinal tumorigenesis. Three weeks after the experimental diet began, animals on HFD started to gain significantly more weight. However, differences in body weight between groups became insignificant starting at week 6. Mice in both experimental groups may have developed polyps as we observed bloody stools. Tumor-induced weight loss is a common feature of cancer and is caused by wasting of muscle and adipose tissue. Depletion of body fat is caused by either the inhibition of the lipoprotein lipase or the stimulation of triglyceride hydrolysis. A decreased rate of protein synthesis and enhanced protein degradation also contributed to protein depletion. These may result in the loss of body weight as they start to bear polyps. Concurrently, HFD itself can remain to directly affect the integrity of intestinal epithelium by generating ROS followed by creating inflammatory environment. Previous studies have also indicated that the increased production of secondary bile acids...
It is reported that increased fat storage is linked with increased generation of ROS. ROS can interact with DNA to produce damage including single and double-stranded DNA breaks and nucleotide modifications. The level of 8-OHdG, the oxidized form of the nucleoside 2'-deoxyguanosine present in DNA, is one of the most reliable and abundant markers for free radical-induced oxidative lesions. Study results indicated that total antioxidant capacity was lower in the HFD group than those of the ND group, while 8-OHdG level was higher in the HFD group. These results show that mice fed HFD were under a heavier oxidative stress and antioxidant defenses might be weakened than mice fed ND.

ROS may also be one of the contributor in gut dysfunction. Increased ROS can rapidly stimulate compartmental redistribution of TJs such as occludin and ZO-1 in Caco-2 cells. The disruption of TJs plays an important role in the pathogenesis of a number of gastrointestinal diseases including inflammatory bowel disease, celiac disease, allergy, and cancer. TLR4-dependent inflammatory response contributed to the secretion of inflammatory cytokines including IL-6 and IL-8, and TLR4/NF-κB signaling causes endotoxia, resulting in increased intestinal permeability possibly accelerating CRC progression. In this study, serum IL-6 concentration and MPO level were used as inflammatory markers. Among several inflammatory cytokines, IL-6 is known as a predictive marker for CRC progression. MPO is a specific marker of neutrophils infiltration, which can be considered as an inflammatory damage index.

As expected, both serum IL-6 and MPO level were elevated in the HFD group compared to the ND group in accordance with increased permeability markers. In summary, HFD induced oxidative stress and endotoxia, leading to disruption of intestinal barrier in APC<sup>min/+</sup> mice. In addition, HFD increased both oxidative stress and LPS-related markers. These results indicate that increased intestinal permeability allows entrance of bacterial pathogens and may cause chronic inflammation which accelerates the formation of intestinal polyps in APC<sup>min/+</sup> mice. This study is one of a few studies suggesting that HFD influences intestinal tumorigenesis by increasing intestinal permeability via oxidative stress and endotoxia.

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**CONFLICTS OF INTEREST**

No potential conflicts of interest were disclosed.

**REFERENCES**

1. World Health Organization (WHO), 2013. Cancer Incidence and Mortality Worldwide: IARC. http://www.who.int/mediacentre/factsheets/fs297/en/. Accessed June 13, 2015.
2. Tárraga López PJ, Albero JS, Rodríguez-Montes JA. Primary and secondary prevention of colorectal cancer. Clin Med Insights Gastroenterol 2014;7:33-46.
3. Ames BN. DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutat Res 2001;475:7-20.
4. Ju J, Liu Y, Hong J, Huang MT, Conney AH, Yang CS. Effects of green tea and high-fat diet on arachidonic acid metabolism and aberrant crypt foci formation in an azoxymethane-induced colon...
carcinogenesis mouse model. Nutr Cancer 2003;46:172-8.
5. Laugerette F, Vors C, Peretti N, Michalski MC. Complex links between dietary lipids, endogenous endotoxins and metabolic inflammation. Biochimie 2011;93:39-45.
6. Dandona P, Ghanim H, Chaudhuri A, Dhindsa S, Kim SS. Macronutrient intake induces oxidative and inflammatory stress: potential relevance to atherosclerosis and insulin resistance. Exp Mol Med 2010;42:245-53.
7. Nikolaidis MG, Kerksick CM, Lamprecht M, McAnulty SR. Hypocaloric diet and regular moderate aerobic exercise is an effective strategy to reduce anthropometric parameters and oxidative stress in obese patients. Obes Facts 2012;5:12-22.
8. Al-Aubaidy HA, Jelinek HF. Oxidative DNA damage and obesity in type 2 diabetes mellitus. Eur J Endocrinol 2011;164:899-904.
9. Mena S, Ortega A, Estrela JM. Oxidative stress in environmental-induced carcinogenesis. Mutat Res 2009;674:36-44.
10. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160:1-40.
11. Delpe L. Oxidative stress in the pathogenesis of colorectal cancer: cause or consequence? Biomed Res Int 2013;2013:725710.
12. Perle M. Oxidative stress in the pathogenesis of colorectal cancer? Int J Mol Sci 2014;15:24501-45.
13. Nikolaidis MG, Kerksick CM, Lamprecht M, McAnulty SR. Hypocaloric diet and regular moderate aerobic exercise is an effective strategy to reduce anthropometric parameters and oxidative stress in obese patients. Obes Facts 2012;5:12-22.
14. Al-Aubaidy HA, Jelinek HF. Oxidative DNA damage and obesity in type 2 diabetes mellitus. Eur J Endocrinol 2011;164:899-904.
15. Mena S, Ortega A, Estrela JM. Oxidative stress in environmental-induced carcinogenesis. Mutat Res 2009;674:36-44.
16. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160:1-40.
17. Delpe L. Oxidative stress in the pathogenesis of colorectal cancer: cause or consequence? Biomed Res Int 2013;2013:725710.
18. Perle M. Oxidative stress in the pathogenesis of colorectal cancer? Int J Mol Sci 2014;15:24501-45.
19. Nikolaidis MG, Kerksick CM, Lamprecht M, McAnulty SR. Hypocaloric diet and regular moderate aerobic exercise is an effective strategy to reduce anthropometric parameters and oxidative stress in obese patients. Obes Facts 2012;5:12-22.
20. Al-Aubaidy HA, Jelinek HF. Oxidative DNA damage and obesity in type 2 diabetes mellitus. Eur J Endocrinol 2011;164:899-904.
21. Mena S, Ortega A, Estrela JM. Oxidative stress in environmental-induced carcinogenesis. Mutat Res 2009;674:36-44.
22. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160:1-40.
23. Delpe L. Oxidative stress in the pathogenesis of colorectal cancer: cause or consequence? Biomed Res Int 2013;2013:725710.
24. Perle M. Oxidative stress in the pathogenesis of colorectal cancer? Int J Mol Sci 2014;15:24501-45.
25. Nikolaidis MG, Kerksick CM, Lamprecht M, McAnulty SR. Hypocaloric diet and regular moderate aerobic exercise is an effective strategy to reduce anthropometric parameters and oxidative stress in obese patients. Obes Facts 2012;5:12-22.
26. Al-Aubaidy HA, Jelinek HF. Oxidative DNA damage and obesity in type 2 diabetes mellitus. Eur J Endocrinol 2011;164:899-904.
27. Mena S, Ortega A, Estrela JM. Oxidative stress in environmental-induced carcinogenesis. Mutat Res 2009;674:36-44.
28. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160:1-40.
29. Delpe L. Oxidative stress in the pathogenesis of colorectal cancer: cause or consequence? Biomed Res Int 2013;2013:725710.
30. Perle M. Oxidative stress in the pathogenesis of colorectal cancer? Int J Mol Sci 2014;15:24501-45.
31. Nikolaidis MG, Kerksick CM, Lamprecht M, McAnulty SR. Hypocaloric diet and regular moderate aerobic exercise is an effective strategy to reduce anthropometric parameters and oxidative stress in obese patients. Obes Facts 2012;5:12-22.
32. Al-Aubaidy HA, Jelinek HF. Oxidative DNA damage and obesity in type 2 diabetes mellitus. Eur J Endocrinol 2011;164:899-904.
33. Mena S, Ortega A, Estrela JM. Oxidative stress in environmental-induced carcinogenesis. Mutat Res 2009;674:36-44.
34. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160:1-40.
35. Delpe L. Oxidative stress in the pathogenesis of colorectal cancer: cause or consequence? Biomed Res Int 2013;2013:725710.
36. Perle M. Oxidative stress in the pathogenesis of colorectal cancer? Int J Mol Sci 2014;15:24501-45.
37. Nikolaidis MG, Kerksick CM, Lamprecht M, McAnulty SR. Hypocaloric diet and regular moderate aerobic exercise is an effective strategy to reduce anthropometric parameters and oxidative stress in obese patients. Obes Facts 2012;5:12-22.
38. Al-Aubaidy HA, Jelinek HF. Oxidative DNA damage and obesity in type 2 diabetes mellitus. Eur J Endocrinol 2011;164:899-904.
39. Mena S, Ortega A, Estrela JM. Oxidative stress in environmental-induced carcinogenesis. Mutat Res 2009;674:36-44.
40. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160:1-40.
41. Delpe L. Oxidative stress in the pathogenesis of colorectal cancer: cause or consequence? Biomed Res Int 2013;2013:725710.
42. Perle M. Oxidative stress in the pathogenesis of colorectal cancer? Int J Mol Sci 2014;15:24501-45.
43. Nikolaidis MG, Kerksick CM, Lamprecht M, McAnulty SR. Hypocaloric diet and regular moderate aerobic exercise is an effective strategy to reduce anthropometric parameters and oxidative stress in obese patients. Obes Facts 2012;5:12-22.
44. Al-Aubaidy HA, Jelinek HF. Oxidative DNA damage and obesity in type 2 diabetes mellitus. Eur J Endocrinol 2011;164:899-904.
45. Mena S, Ortega A, Estrela JM. Oxidative stress in environmental-induced carcinogenesis. Mutat Res 2009;674:36-44.
46. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160:1-40.
41. Zheng L, Gao ZQ, Wang SX. A chronic ulcerative colitis model in rats. World J Gastroenterol 2000;6:150-2.

42. Gambero A, Maróstica M, Abdalla Saad Mj, Pedrazzoli J Jr. Mesenteric adipose tissue alterations resulting from experimental reactivated colitis. Inflamm Bowel Dis 2007;13:1957-64.