



## Side-stream smoking reduces intestinal inflammation and increases expression of tight junction proteins

Hui Wang, Jun-Xing Zhao, Nan Hu, Jun Ren, Min Du, Mei-Jun Zhu

Hui Wang, Jun-Xing Zhao, Min Du, Mei-Jun Zhu, Department of Animal Science, University of Wyoming, Laramie, WY 82071, United States

Nan Hu, Jun Ren, School of Pharmacy, University of Wyoming, Laramie, WY 82071, United States

Author contributions: Wang H, Zhao J and Hu N performed the majority of experiments; Ren J, Du M and Zhu MJ designed the study and wrote the manuscript.

Supported by INBRE P20RR016474; USDA-NRI 2008-35203-19084; USDA-AFRI 2009-65203-05716

Correspondence to: Dr. Mei-Jun Zhu, Department of Animal Science, University of Wyoming, Laramie, WY 82071, United States. [meijun@uwyo.edu](mailto:meijun@uwyo.edu)

Telephone: +1-307-7663140 Fax: +1-307-7662355

Received: September 21, 2011 Revised: February 12, 2012

Accepted: April 10, 2012

Published online: May 14, 2012

### Abstract

**AIM:** To investigate the effect of side-stream smoking on gut microflora composition, intestinal inflammation and expression of tight junction proteins.

**METHODS:** C57BL/6 mice were exposed to side-stream cigarette smoking for one hour daily over eight weeks. Cecal contents were collected for microbial composition analysis. Large intestine was collected for immunoblotting and quantitative reverse transcriptase polymerase chain reaction analyses of the inflammatory pathway and tight junction proteins.

**RESULTS:** Side-stream smoking induced significant changes in the gut microbiota with increased mouse intestinal bacteria, *Clostridium* but decreased *Firmicutes* (*Lactococci* and *Ruminococcus*), *Enterobacteriaceae* family and *Segmented filamentous bacteria* compared to the control mice. Meanwhile, side-stream smoking inhibited the nuclear factor- $\kappa$ B pathway with reduced phosphorylation of p65 and I $\kappa$ B $\alpha$ , accompanied with unchanged mRNA expression of tumor necrosis factor- $\alpha$

or interleukin-6. The contents of tight junction proteins, claudin3 and ZO2 were up-regulated in the large intestine of mice exposed side-stream smoking. In addition, side-stream smoking increased c-Jun N-terminal kinase and p38 MAPK kinase signaling, while inhibiting AMP-activated protein kinase in the large intestine.

**CONCLUSION:** Side-stream smoking altered gut microflora composition and reduced the inflammatory response, which was associated with increased expression of tight junction proteins.

© 2012 Baishideng. All rights reserved.

**Key words:** Inflammation; Microbiota; Tight junction protein; Side-stream smoking; Intestine

**Peer reviewer:** Oliver Grundmann, PhD, Clinical Assistant Professor, Department of Medicinal Chemistry, College of Pharmacy, University of Florida, 1600 SW Archer RD, Room P6-20, Gainesville, FL 32610-0484, United States

Wang H, Zhao JX, Hu N, Ren J, Du M, Zhu MJ. Side-stream smoking reduces intestinal inflammation and increases expression of tight junction proteins. *World J Gastroenterol* 2012; 18(18): 2180-2187 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v18/i18/2180.htm> DOI: <http://dx.doi.org/10.3748/wjg.v18.i18.2180>

### INTRODUCTION

Cigarette smoking is a remarkable etiological factor in the pathogenesis of cardiovascular diseases, hypertension, pulmonary diseases and gastroenterological diseases<sup>[1-4]</sup>. Meanwhile, passive smoking (second-hand smoking) is also a contributing factor for the development of coronary artery disease<sup>[5-7]</sup>, lung cancer<sup>[7]</sup> and Crohn's disease<sup>[8]</sup>, which pose a substantial health risk to non-smoking adults and young children worldwide<sup>[9]</sup>. It was estimated in 2004 that more than 600 thousand deaths were due to

second-hand smoke, which accounted for about 1% of worldwide mortality<sup>[9]</sup>. On the other hand, it was reported that smoking had a protective effect in reducing ulcerative colitis mostly based on the epidemiologic studies<sup>[8,10,11]</sup>.

Chronic inflammatory bowel diseases, mainly Crohn's disease and ulcerative colitis, are characterized by chronic inflammation of the intestines<sup>[8]</sup>. Recent studies clearly show that gut epithelial integrity and barrier function are the central predisposing factors in inflammatory bowel diseases, autoimmune and related allergic diseases<sup>[12-16]</sup>. The intestinal epithelium is composed of tightly assembled intestinal epithelial cells which form a protective barrier against pathogenic and commensal bacteria, preventing their penetration from the lumen to initiate inflammatory responses in the mucosal system<sup>[17]</sup>. Impairment of the tight junction barrier is associated with chronic diseases such as inflammatory bowel diseases, obesity and type 1 diabetes<sup>[18-21]</sup>. Epithelial cells form an integrated web through interaction of tight junction proteins including intracellular proteins, zona occludens (ZO)-1, (ZO)-2 and (ZO)-3, cingulin, 7H6 and ZA-1, and membrane proteins, occludin, claudin and junctional adhesion molecules<sup>[22,23]</sup>. The tight junction functions are affected by extracellular stimuli such as the microbial components, pro-inflammatory cytokines and stress<sup>[24,25]</sup>.

Inflammation disrupts tight junctions. Inflammatory cytokines such as interleukin (IL)-13, and IL-6, increase tight junction permeability through increasing claudin 2 expression<sup>[26,27]</sup>. The activation of the inflammatory pathway nuclear factor (NF)- $\kappa$ B by TNF- $\alpha$ , down-regulates ZO-1 gene expression and induces its relocation in Caco-2 cells<sup>[28]</sup>. Therefore, local inflammation impairs the barrier function of gut epithelium.

The "microflora hypothesis" suggests that gut microflora composition plays an important role in the immunological response of the gut<sup>[29]</sup>. Lactic acid bacteria are known to have an anti-inflammatory effect<sup>[30-34]</sup>, and alteration of microflora composition is linked to the incidence of inflammatory bowel diseases<sup>[35,36]</sup>. Up to now, there is no published studies assessed gut microflora changes due to smoking.

We hypothesized that side stream smoking may possess a potent anti-inflammatory effect on the gut mucosal immune system which promotes the expression of tight junction proteins in the intestine, exerting beneficial effects on the prevention of ulcerative colitis.

## MATERIALS AND METHODS

### Animal care and experiment design

C57BL/6 female mice at 6 mo of age were housed in a temperature-controlled room with a 12 h light and 12 h darkness cycle and were given food and water *ad libitum*. Mice were placed in an exposure box and exposed to side-stream smoke for 1 h daily for 40 d. Commercial cigarettes (golden monkey, tar: 13 mg; nicotine: 1.1 mg; CO: 15 mg) were used at a dose equivalent to one commercial cigarette's smoke per day<sup>[37]</sup>. The animal care procedures

described in this study was approved by the University of Wyoming Institutional Animal Use and Care Committee.

### Tissue collection

On the day of necropsy, mice were anesthetized intraperitoneally with tribromoethanol (250 mg/kg body wt). Blood samples were collected from the orbital sinus while mice were under general anesthesia. Mice were then sacrificed by cervical dislocation. Large intestines were dissected, flushed with phosphate-buffer saline and then frozen in liquid nitrogen for immunoblotting and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analyses. Cecal contents from each mouse were collected and frozen for microflora analyses.

### Reagents and antibodies

Antibodies against ZO1, ZO2, Claudin3 and Occludin were purchased from Invitrogen (Camarillo, CA). Antibodies against phospho- c-Jun N-terminal kinase (SAPK/JNK) (Thr183/Tyr185), SAPK/JNK, phospho-NF- $\kappa$ B p65 (ser536), NF- $\kappa$ B p65, phospho-I $\kappa$ B kinase (IKK)  $\alpha/\beta$  (Ser176/180), IKK $\beta$ , phospho-I $\kappa$ B $\alpha$ , I $\kappa$ B $\alpha$ , phospho-p38 MAP kinase and p38 MAP kinase, phospho-AMP-activated protein kinase (AMPK)  $\alpha$  and AMPK $\alpha$  were purchased from Cell Signaling Technology (Beverly, MA). Antibodies against xanthine oxidase (XO), heat shock protein (HSP) 60 and superoxide dismutase (SOD) 1 were purchased from Santa Cruz Biotech Inc. (Santa Cruz, CA). Anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody was purchased from Affinity BioReagents (Golden, CO).

### Quantitative reverse transcription PCR

Total RNA was extracted from powdered large intestine using Trizol<sup>®</sup> Reagent (Sigma, St. Louis, MO), treated with DNase I (Qiagen, Valencia, CA) and purified with RNeasy Mini kit (Qiagen). cDNA was synthesized with the iScript<sup>™</sup> cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). qRT-PCR was conducted on a Bio-Rad CFX96 machine and SYBR Green Master Mix (Bio-Rad Laboratories, Hercules, CA) was used for all qRT-PCR reactions. Mouse GAPDH was used as the housekeeping gene. Primer sequences are listed in Table 1. The final primer concentration was 200 nmol for each gene. The amplification efficiency was 0.90-0.99. The qRT-PCR conditions were 95 °C, 3 min, and 35 cycles of 95 °C for 10 s, 58 °C for 20 s and elongation step at 72 °C for 20 s. At the end of each run, dissociation melting curve was obtained to confirm the purity of PCR products<sup>[38]</sup>.

### Microflora analyses

The frozen caecal contents (0.1 g) were homogenized and bacterial genomic DNA was extracted using a QIAamp DNA stool mini kit according to the manufacturer's instructions (Qiagen, Valencia, CA). The abundance of specific intestinal bacterial groups was measured by qPCR using Bio-Rad CFX96 machine (Bio-Rad Laboratories, Hercules, CA) as described above. Group specific

Table 1 Primer sets used for quantitative reverse transcriptase polymerase chain reaction of mouse large intestine tissue

Gene name	Accession no.	Product size	Direction	Sequence (5'→3')	Source
IL-6	NM_031168.1	107 bp	Forward	GCTGGTGACAACACGGCCT	This study
			Reverse	AGCCTCCGACTTGTGAAGTGGT	
TNF-α	NM_013693.2	67 bp	Forward	TGGGACAGTGACCTGGACTGT	[58]
			Reverse	TTCGAAAGCCCATTTGAGT	
Claudin 3	NM_009902.4	132 bp	Forward	CAGGGGCAGTCTCTGTGCGAG	This study
			Reverse	GCCGCTGGACCTGGGAATCAAC	
Occludin	NM_008756.2	308 bp	Forward	ATGTCCGGCCGATGCTCTC	[58]
			Reverse	TTTGGCTGCTCTTGGGTCTGTAT	
ZO-1	NM_009386.2	403 bp	Forward	ACCCGAAACTGATGCTGTGGATAG	[58]
			Reverse	AAATGGCCGGGCAGAACTTGTGTA	
ZO-2	AF113005.1	106 bp	Forward	CCCAGCACCAAGCCACCTTTCA	This study
			Reverse	TCGTTAGGGCAGACACACTCCC	
GAPDH	NM_008084.2	132 bp	Forward	AACCTTGGCATTGTGGAAGG	This study
			Reverse	GGATGCAGGGATGATGTCT	

IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-α; ZO: Zona occludens; GPADH: Glyceraldehyde-3-phosphate dehydrogenase.

Table 2 Primer sets used for quantitative polymerase chain reaction of 16S rRNA of specific bacterial species or genus

Target organism	Primer set	Sequence (5' to 3')	Product size	Annealing temp (°C)	Reference
<i>Bacteroides</i>	BactF285	GGTCTGAGAGGAGGTCCC	53	61	[59]
	UniR338	GCTGCCTCCCGTAGGAGT		61	
<i>Clostridium butyricum</i>	Cbut825F	GTGCCGCCGTAACGCATTAAGTAT	213	72	[60]
	Cbut1038R	ACCATGCACCACCTGTCTTCTGCGC		72	
<i>Clostridium clostridioforme</i>	Cclos99F	AATCTTGATTGACTGAGTGGCGGAC	148	62	[60]
	Cclos247R	CCATCTCACACTACCGAGTTTTTC		62	
<i>Clostridium perfringens</i>	Cperf165F	CGCATAACGTTGAAAGATGG	104	61	[59]
	Cperf269R	CCTTGGTAGGCCGTTACCC		61	
<i>Enterobacteriaceae</i>	Eco1457F	CATTGACGTTACCCGAGAAGAAGC	195	63	[60]
	Eco1652R	CTCTACGAGACTCAAGCTTGC		63	
<i>Enterococcus</i>	Ec-ssu1F	GGATAACACTTGGAAACAGG	115	60	[61]
	Ec-ssu1R	TCCTTGTCTCTCTAACA		60	
Eubacteria	UniF340	ACTCCTACGGGAGGCAGCAGT	210	63	[62]
	UniR514	ATTACCGCGCTGCTGGC		63	
<i>Faecalibacterium prausnitzii</i>	Fprau223F	GATGGCCTCGGTCGCGATTAG	199	58	[60]
	Fprau420R	CCGAAGACCTCTCTCTCC		58	
<i>Lactococci</i>	LabF362	AGCAGTAGGGAATCTTCCA	315	56	[59]
	LabR677	CACCGCTACACATGGAG		56	
Mouse intestinal Bacteria	Uni516F	CCAGAGCCGCGTAATA	161	58	[59]
	MIBR677	CGCATTCCGCATCTTCTC		58	
<i>Segmented filamentous bacteria</i>	SFB736F	GACGCTGAGGCATGAGAGCAT	108	58	[59]
	SFB844R	GACGCAACGGATTGTTATTCA		58	
<i>Ruminococcus albus</i>	Ralb561F	CAGGTGTGAAATTTAGGGGC	246	63	[60]
	Ralb807R	GTCAGTCCCCCACACCTAG		63	

or kingdom specific 16S rRNA gene primers were listed in Table 2. Eubacteria 16S rRNA was used as the house-keeping gene.

Immunoblotting analyses

Immunoblotting analyses were conducted as previously described<sup>[39,40]</sup>. Briefly, protein extracts from the mouse large intestine were separated by 5%-15% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) gradient gels and transferred to nitrocellulose membranes for immunoblotting analyses. Band density was normalized according to the GAPDH content<sup>[39,40]</sup>.

Statistical analysis

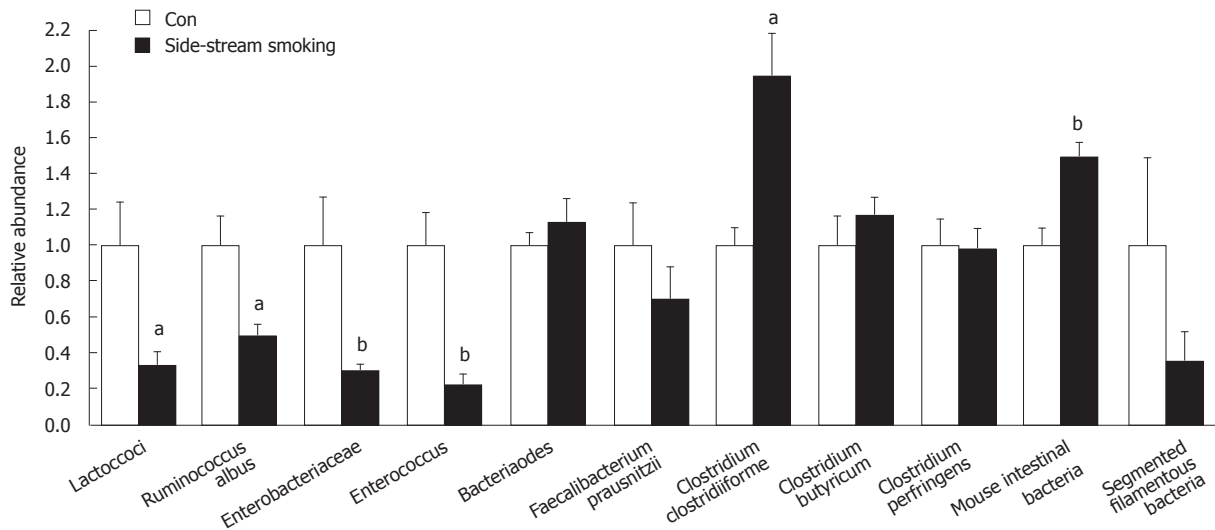
Statistical analyses were conducted as previously described<sup>[41-43]</sup>. Data were analyzed as a complete random-

ized design using General Linear Model of Statistical Analysis System (2000). Mean ± SEM are reported. Mean difference was separated by a least significant difference multiple comparison test. Statistical significance is considered as *P* < 0.05.

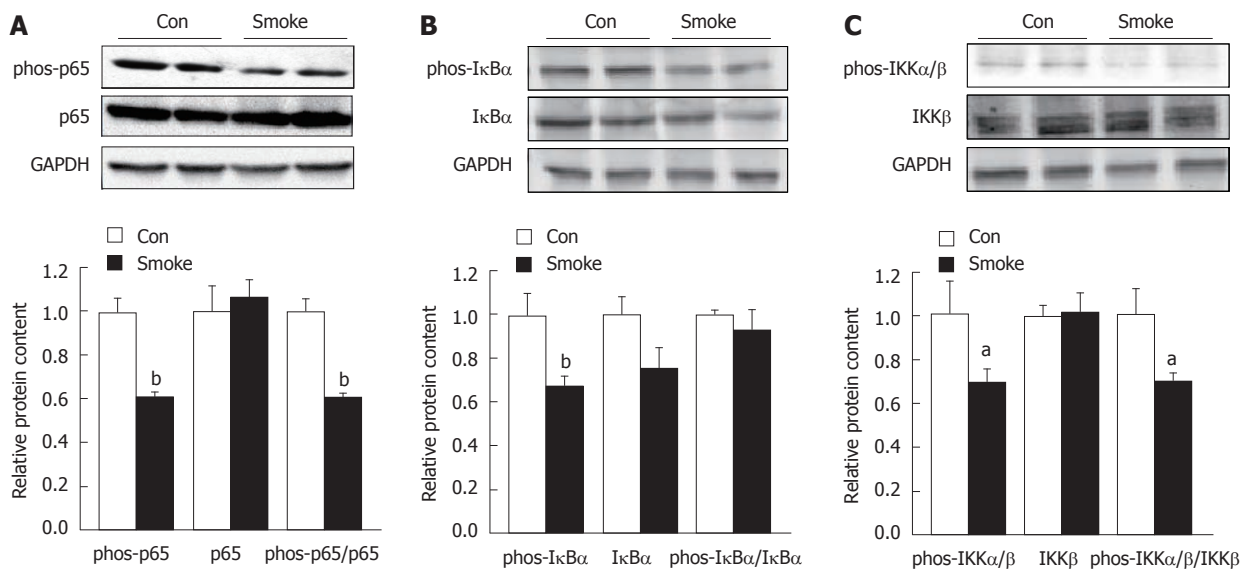
RESULTS

Effect of side-stream cigarette smoking on the gut microflora composition

Quantitative PCR analysis of 16S rRNA showed that exposure of C57BL6 mice to side-stream cigarette smoking increased the amount of *Clostridium clostridioforme* and mouse intestinal bacteria (MIB) in the cecal microflora, while decreasing the content of *Lactococci*, *Ruminococcus albus*, *Enterobacteriaceae* and segmented filamentous bacteria



**Figure 1** Cecal microflora composition of Con and side-stream smoking mice. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs control group (mean  $\pm$  SEM;  $n = 6$  per group).



**Figure 2** NF- $\kappa$ B signaling pathway in large intestine of Con and side-stream smoking mice. A: Phos-p65 and p65; B: Phos-I $\kappa$ B $\alpha$  and I $\kappa$ B $\alpha$ ; C: Phos-IKK $\alpha$ / $\beta$  and IKK $\beta$ . <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs control group (mean  $\pm$  SEM;  $n = 6$  per group).

(SFB) compared with those of control mice (Figure 1).

### Intestinal inflammatory responses of gut to side stream smoking

Side-stream smoking decreased phosphorylation of NF- $\kappa$ B p65, a key mediator of the NF- $\kappa$ B inflammatory signaling pathway. Consistently, phosphorylation of I $\kappa$ B $\alpha$  and IKK $\alpha$ / $\beta$  were also down-regulated in mice exposed to side-stream smoking, indicating that smoking is capable of reducing inflammation in the gut (Figure 2). qRT-PCR analysis indicated that mRNA expression of the two main inflammatory cytokines, TNF $\alpha$  and IL-6, were not changed (data not shown).

### Side-stream smoking induced oxidative stress in large intestine

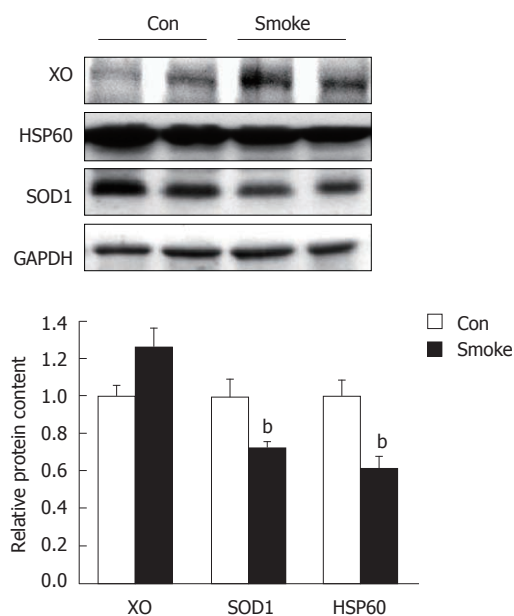
There was an enhanced oxidative stress in side-stream

smoking mice compared to that of control mice, as indicated by increased XO ( $P = 0.06$ ) and decreased SOD1 ( $P < 0.01$ ) protein content in the side-stream smoking mice (Figure 3). Meanwhile, the heat shock protein 60 (HSP60) decreased in the side-stream smoking mouse large intestine when compared to that of control mice (Figure 3). Consistently, the phosphorylation of stress signaling mediators, JNK and p38 MAP kinase, were increased in the large intestine of side-stream smoking mice (Figure 4). However, the phosphorylation of another kinase related to stress, AMPK, was reduced in response to side-stream smoking (Figure 5).

### Tight junction protein expression

Both mRNA expression and protein content of selected tight junction proteins were further analyzed. Protein content of claudin3 ( $P < 0.01$ ) and ZO2 ( $P < 0.05$ ) were





**Figure 3** Xanthine oxidase, superoxide dismutase 1 and heat shock protein 60 content in large intestine of Con and side-stream smoking mice. <sup>b</sup>*P* < 0.01 vs control group (mean ± SEM; *n* = 6 per group). XO: Xanthine oxidase; SOD1: Superoxide dismutase 1; HSP60: Heat shock protein 60.

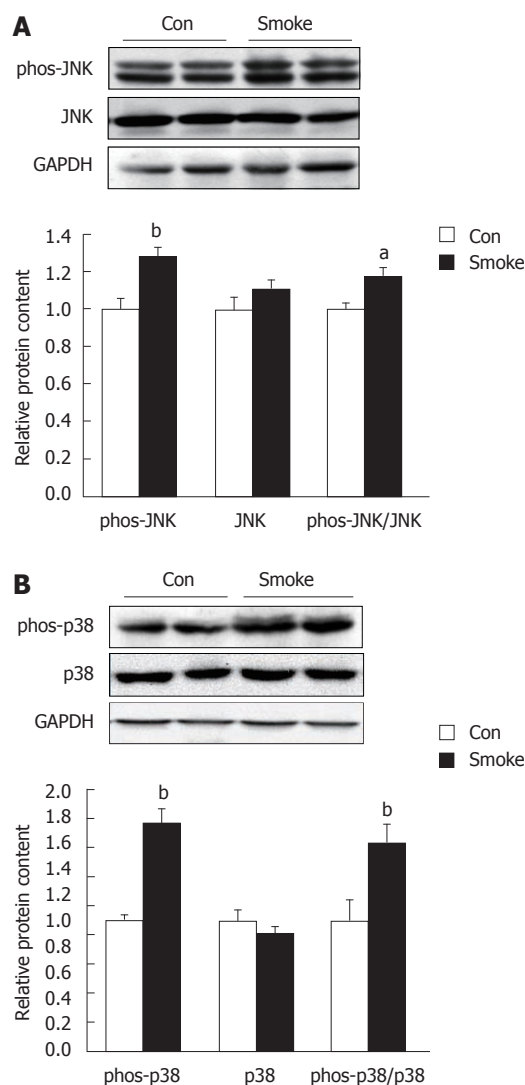
increased in the large intestine of side-stream smoking mice (Figure 6B), while there is no difference in their mRNA expression (Figure 6A).

## DISCUSSION

Epidemiology studies have shown that smoking, including passive smoke inhalation, reduces the incidence of ulcerative colitis, which may be due to the reduction of epithelial permeability<sup>[44]</sup>. Intestinal permeability was reduced in healthy smokers compared to the non-smokers<sup>[45,46]</sup>.

Mechanisms by which side-stream smoking improves intestinal tight junctions are not well understood. Previous studies suggest that activation of NF-κB signaling increases intestinal permeability<sup>[47]</sup>. In this study, we observed that the NF-κB signaling was down-regulated in mice exposed to side-stream smoking. This indicates that side-stream smoking negatively regulates NF-κB signaling which might be a contributing factor to the reduction of intestinal permeability. We also observed that side-stream smoking increased Claudin3 and ZO-2 content without affecting Occludin and ZO-1. In summary, our data revealed that side-stream smoking up-regulated the expression of tight junction proteins and inhibited NF-κB signaling, which may be responsible for the preventive effect of smoking on ulcerative colitis.

Smoking generates reactive oxygen species and nitrogen species in blood, resulting in oxidative stress<sup>[48-50]</sup>. In this study, we also observed that oxidative stress related enzymes such as xanthine oxidase and superoxide dismutase 1 were altered in the large intestine due to side-stream smoking. Consistent with altered oxidative stress, two pivotal stress signaling mediators, the activation of JNK and p38 signaling were enhanced in the large intestine of mice exposed to side stream smoking.

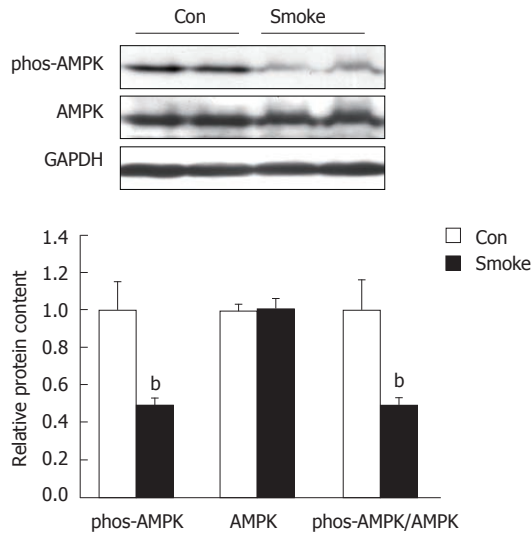


**Figure 4** MAP kinase signaling pathways in large intestine of Con and side-stream smoking mice. A: JNK; B: MAP kinase p38. <sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01 vs control group (mean ± SEM; *n* = 6 per group).

Previously, it was reported that oxidative stress related signaling promotes tight junction protein claudin1 expression in hepatocytes and Sertoli cells<sup>[51,52]</sup>.

A recent published study in gut epithelial cells shows that AMPK is related to the impairment of tight junction and barrier properties of gut induced by inflammation<sup>[53]</sup>. Our data showed that AMPK activity was dramatically inhibited in the gut tissue of side-stream smoking mice, which may provide an additional mechanism for the association between passive smoking and gut epithelial barrier function.

Furthermore, we found that microflora were altered due to the side-stream smoking. The “microflora hypothesis” suggests that gut microflora composition plays an important role in the immunological response of the gut<sup>[29]</sup>. Up to now, there have been no published studies assessing changes in gut microflora due to smoking. Our data showed that exposure to side-stream smoking altered the composition of cecal microflora, reducing *Firmicutes*



**Figure 5** Total AMP-activated protein kinase  $\alpha$  subunit content and its phosphorylation at Thr 172 in large intestine of Con and side-stream smoking mice. <sup>b</sup> $P < 0.01$  vs control group (mean  $\pm$  SEM;  $n = 6$  per group). AMPK: Total AMP-activated protein kinase.

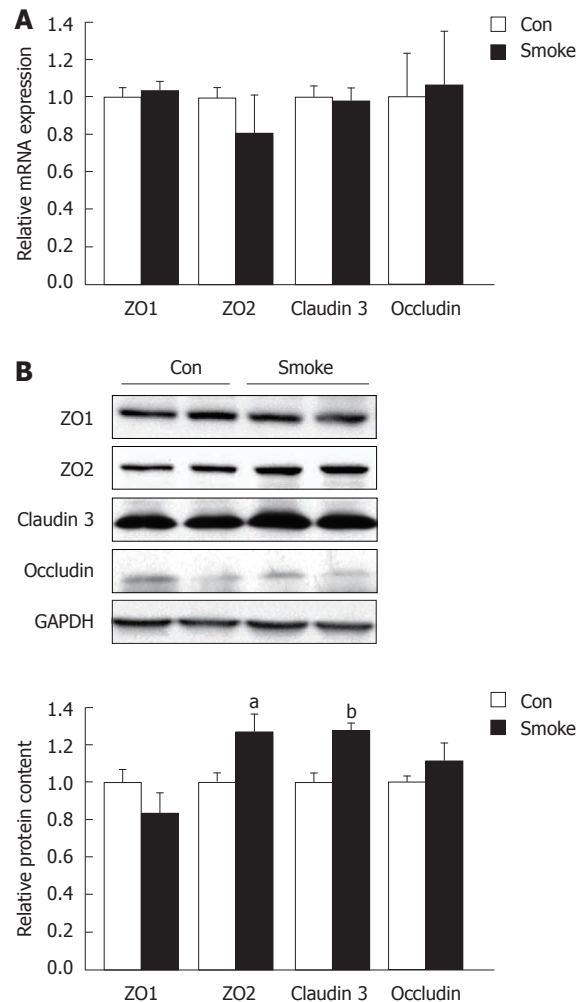
and *Enterobacteriaceae*. Both *Firmicutes* and *Enterobacteriaceae* belong to a group of bacteria contributing to fermentation and nutrient intake. *Lactococci* and other lactic acid bacteria are known to have anti-inflammatory effects<sup>[30-34]</sup>. The dramatic reduction of *Lactococci* in side-stream smoking mice indicates that *Lactococci* might not be responsible for the reduced inflammation in the gut of side-stream smoking mice. The reason for the reduction of *Lactococci* in cecal microflora due to smoking is unclear, but might be related to oxidative stress. Many *Lactococci* lack catalase and are sensitive to oxidative stress<sup>[54]</sup>, which may render them less competitive in the oxidative environment induced by smoking. We also observed that MIB was increased while SFB was decreased in smoking mice. Because SFB is known to have important roles in maturation of the gut immune system, its reduction in smoking mice could be associated with the adverse effect of smoking on Crohn's disease<sup>[55]</sup>. MIB refers to a group of bacteria called *Cytophaga-Flavobacter-Bacteroides* phylum<sup>[56]</sup>, and their abundance in the gut is known to be altered by environmental factors<sup>[57]</sup>. The biological effect of MIB alteration due to smoking is unclear.

In conclusion, data from our present study demonstrated that exposure to side-stream smoking inhibited mucosal inflammation and enhanced the expression of tight junction proteins in the large intestine. Further, side-stream smoking increased oxidative stress and altered gut microflora composition.

## COMMENTS

### Background

Despite its apparent harmful effects, side-stream smoking reduces the risk of inflammatory gastrointestinal diseases. Gut epithelial integrity and barrier function is a central predisposing factor to inflammatory bowel diseases. Local inflammation impairs the barrier function of gut epithelium. We hypothesized that side stream smoking may possess potent anti-inflammatory effects, which promote the expression of tight junction proteins in the intestine, exerting ben-



**Figure 6** Tight junction protein content in large intestine of Con and side-stream smoking mice. A: mRNA expression; B: Protein content. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs control group (mean  $\pm$  SEM;  $n = 6$  per group).

eficial effects on the prevention of ulcerative colitis.

### Research frontiers

Epidemiologic studies indicate that smoking had a protective effect on ulcerative colitis though the underlying mechanisms remain elusive. In this study, we demonstrated that exposure to side-stream smoking inhibited mucosal inflammation, improved gut tight junction protein expression, and altered gut microflora composition in mice, which could partially explain the preventive effects of smoking on ulcerative colitis.

### Innovations and breakthroughs

Recent epidemiologic studies have highlighted the preventive effect of smoking on ulcerative colitis. This is the first study to report that side-stream smoking has anti-inflammatory effect on gut mucosal, improving gut tight junction protein expression and altering gut microflora composition.

### Applications

By understanding how side-stream smoking affects gut mucosal immune response and tight junction protein expression, the authors can develop alternative strategies to reduce the risk of ulcerative colitis and possibly other inflammatory bowel diseases without the harmful effects of smoking.

### Terminology

Inflammatory bowel diseases are characterized by chronic inflammation in the intestine. Side-stream smoking, mimicking secondhand smoking, has anti-inflammatory effect, which may be responsible for its beneficial effects against ulcerative colitis.

### Peer review

The authors address the observation that passive smoking decreases inflammatory response in large intestine. The authors are to be commended for excel-

lent work in performing a very important and informative study. The experimental methods are well summarized and explained. The statistics are appropriate for this study.

## REFERENCES

- Singer MV, Feick P, Gerloff A. Alcohol and smoking. *Dig Dis* 2011; **29**: 177-183
- Frey P, Waters DD. Tobacco smoke and cardiovascular risk: a call for continued efforts to reduce exposure. *Curr Opin Cardiol* 2011; **26**: 424-428
- Birrenbach T, Bocker U. Inflammatory bowel disease and smoking: a review of epidemiology, pathophysiology, and therapeutic implications. *Inflamm Bowel Dis* 2004; **10**: 848-859
- Virdis A, Giannarelli C, Neves MF, Taddei S, Ghiadoni L. Cigarette smoking and hypertension. *Curr Pharm Des* 2010; **16**: 2518-2525
- Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. *J Am Coll Cardiol* 2004; **43**: 1731-1737
- Glantz SA, Parmley WW. Passive smoking and heart disease. Mechanisms and risk. *JAMA* 1995; **273**: 1047-1053
- Glantz SA, Parmley WW. Passive and active smoking. A problem for adults. *Circulation* 1996; **94**: 596-598
- van der Heide F, Dijkstra A, Weersma RK, Albersnagel FA, van der Logt EM, Faber KN, Sluiter WJ, Kleibeuker JH, Dijkstra G. Effects of active and passive smoking on disease course of Crohn's disease and ulcerative colitis. *Inflamm Bowel Dis* 2009; **15**: 1199-1207
- Oberg M, Jaakkola MS, Woodward A, Peruga A, Pruss-Ustun A. Worldwide burden of disease from exposure to second-hand smoke: a retrospective analysis of data from 192 countries. *Lancet* 2011; **377**: 139-146
- Mahid SS, Minor KS, Soto RE, Hornung CA, Galandiuk S. Smoking and inflammatory bowel disease: a meta-analysis. *Mayo Clin Proc* 2006; **81**: 1462-1471
- Mokbel M, Carbonnel F, Beaugier L, Gendre JP, Cosnes J. [Effect of smoking on the long-term course of ulcerative colitis]. *Gastroenterol Clin Biol* 1998; **22**: 858-862
- Barreau F, Ferrier L, Fioramonti J, Bueno L. Neonatal maternal deprivation triggers long term alterations in colonic epithelial barrier and mucosal immunity in rats. *Gut* 2004; **53**: 501-506
- Bischoff SC, Krämer S. Human mast cells, bacteria, and intestinal immunity. *Immunol Rev* 2007; **217**: 329-337
- Demaude J, Salvador-Cartier C, Fioramonti J, Ferrier L, Bueno L. Phenotypic changes in colonocytes following acute stress or activation of mast cells in mice: implications for delayed epithelial barrier dysfunction. *Gut* 2006; **55**: 655-661
- Groschwitz KR, Ahrens R, Osterfeld H, Gurish MF, Han X, Abrink M, Finkelman FD, Pejler G, Hogan SP. Mast cells regulate homeostatic intestinal epithelial migration and barrier function by a chymase/Mcpt4-dependent mechanism. *Proc Natl Acad Sci USA* 2009; **106**: 22381-22386
- Yu LC. The epithelial gatekeeper against food allergy. *Pediatr Neonatol* 2009; **50**: 247-254
- Ohman L, Simren M. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 163-173
- Canli PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* 2009; **58**: 1091-1103
- Lee AS, Gibson DL, Zhang Y, Sham HP, Vallance BA, Dutz JP. Gut barrier disruption by an enteric bacterial pathogen accelerates insulinitis in NOD mice. *Diabetologia* 2010; **53**: 741-748
- Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA, Gordon JI, Chervonsky AV. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature* 2008; **455**: 1109-1113
- Nell S, Suerbaum S, Josenhans C. The impact of the microbiota on the pathogenesis of IBD: lessons from mouse infection models. *Nat Rev Microbiol* 2010; **8**: 564-577
- Hossain Z, Hirata T. Molecular mechanism of intestinal permeability: interaction at tight junctions. *Mol Biosyst* 2008; **4**: 1181-1185
- Liu Z, Li N, Neu J. Tight junctions, leaky intestines, and pediatric diseases. *Acta Paediatr* 2005; **94**: 386-393
- Lewis K, McKay DM. Metabolic stress evokes decreases in epithelial barrier function. *Ann N Y Acad Sci* 2009; **1165**: 327-337
- Husebye E. The pathogenesis of gastrointestinal bacterial overgrowth. *Chemotherapy* 2005; **51** Suppl 1: 1-22
- Suzuki T, Yoshinaga N, Tanabe S. Interleukin-6 (IL-6) regulates claudin-2 expression and tight junction permeability in intestinal epithelium. *J Biol Chem* 2011; **286**: 31263-31271
- Weber CR, Raleigh DR, Su L, Shen L, Sullivan EA, Wang Y, Turner JR. Epithelial myosin light chain kinase activation induces mucosal interleukin-13 expression to alter tight junction ion selectivity. *J Biol Chem* 2010; **285**: 12037-12046
- Ma TY, Iwamoto GK, Hoa NT, Akotia V, Pedram A, Boivin MA, Said HM. TNF-alpha-induced increase in intestinal epithelial tight junction permeability requires NF-kappa B activation. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G367-G376
- Round JL, O'Connell RM, Mazmanian SK. Coordination of tolerogenic immune responses by the commensal microbiota. *J Autoimmun* 2010; **34**: J220-J225
- Turdi S, Fan X, Li J, Zhao J, Huff AF, Du M, Ren J. AMP-activated protein kinase deficiency exacerbates aging-induced myocardial contractile dysfunction. *Aging Cell* 2010; **9**: 592-606
- Li SY, Gomelsky M, Duan J, Zhang Z, Gomelsky L, Zhang X, Epstein PN, Ren J. Overexpression of aldehyde dehydrogenase-2 (ALDH2) transgene prevents acetaldehyde-induced cell injury in human umbilical vein endothelial cells: role of ERK and p38 mitogen-activated protein kinase. *J Biol Chem* 2004; **279**: 11244-11252
- Kleessen B, Hartmann L, Blaut M. Fructans in the diet cause alterations of intestinal mucosal architecture, released mucins and mucosa-associated bifidobacteria in gnotobiotic rats. *Br J Nutr* 2003; **89**: 597-606
- Karczewski J, Troost FJ, Konings I, Dekker J, Kleerebezem M, Brummer RJ, Wells JM. Regulation of human epithelial tight junction proteins by *Lactobacillus plantarum* in vivo and protective effects on the epithelial barrier. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G851-G859
- Grimoud J, Durand H, de Souza S, Monsan P, Ouarné F, Theodorou V, Roques C. In vitro screening of probiotics and synbiotics according to anti-inflammatory and anti-proliferative effects. *Int J Food Microbiol* 2010; **144**: 42-50
- Bruzzese E, Canani RB, De Marco G, Guarino A. Microflora in inflammatory bowel diseases: a pediatric perspective. *J Clin Gastroenterol* 2004; **38**: S91-S93
- Sartor RB. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. *Gastroenterology* 2004; **126**: 1620-1633
- Miller LM, Foster WM, Dambach DM, Doebler D, McKinnon M, Killar L, Longphre M. A murine model of cigarette smoke-induced pulmonary inflammation using intranasally administered smoke-conditioned medium. *Exp Lung Res* 2002; **28**: 435-455
- Yan X, Huang Y, Wang H, Du M, Hess BW, Ford SP, Nathanielsz PW, Zhu MJ. Maternal obesity induces sustained inflammation in both fetal and offspring large intestine of sheep. *Inflamm Bowel Dis* 2011; **17**: 1513-1522
- Zhu MJ, Du M, Hess BW, Means WJ, Nathanielsz PW, Ford

- SP. Maternal nutrient restriction upregulates growth signaling pathways in the cotyledonary artery of cow placentomes. *Placenta* 2007; **28**: 361-368
- 40 **Zhu MJ**, Du M, Hess BW, Nathanielsz PW, Ford SP. Periconceptional nutrient restriction in the ewe alters MAPK/ERK1/2 and PI3K/Akt growth signaling pathways and vascularity in the placentome. *Placenta* 2007; **28**: 1192-1199
  - 41 **Zhu MJ**, Ford SP, Means WJ, Hess BW, Nathanielsz PW, Du M. Maternal nutrient restriction affects properties of skeletal muscle in offspring. *J Physiol* 2006; **575**: 241-250
  - 42 **Zhu MJ**, Ford SP, Nathanielsz PW, Du M. Effect of maternal nutrient restriction in sheep on the development of fetal skeletal muscle. *Biol Reprod* 2004; **71**: 1968-1973
  - 43 **Zhu MJ**, Han B, Tong J, Ma C, Kimzey JM, Underwood KR, Xiao Y, Hess BW, Ford SP, Nathanielsz PW, Du M. AMP-activated protein kinase signalling pathways are down regulated and skeletal muscle development impaired in fetuses of obese, over-nourished sheep. *J Physiol* 2008; **586**: 2651-2664
  - 44 **Arslan G**, Atasever T, Cindoruk M, Yildirim IS. (51)CrEDTA colonic permeability and therapy response in patients with ulcerative colitis. *Nucl Med Commun* 2001; **22**: 997-1001
  - 45 **McGilligan VE**, Wallace JM, Heavey PM, Ridley DL, Rowland IR. The effect of nicotine in vitro on the integrity of tight junctions in Caco-2 cell monolayers. *Food Chem Toxicol* 2007; **45**: 1593-1598
  - 46 **Prytz H**, Benoni C, Tagesson C. Does smoking tighten the gut? *Scand J Gastroenterol* 1989; **24**: 1084-1088
  - 47 **Aveleira CA**, Lin CM, Abcouwer SF, Ambrósio AF, Antonetti DA. TNF- $\alpha$  signals through PKC $\zeta$ /NF- $\kappa$ B to alter the tight junction complex and increase retinal endothelial cell permeability. *Diabetes* 2010; **59**: 2872-2882
  - 48 **Suits MD**, Jaffer N, Jia Z. Structure of the Escherichia coli O157: H7 heme oxygenase ChuS in complex with heme and enzymatic inactivation by mutation of the heme coordinating residue His-193. *J Biol Chem* 2006; **281**: 36776-36782
  - 49 **Tharappel JC**, Cholewa J, Espandiari P, Spear BT, Gairola CG, Glauert HP. Effects of cigarette smoke on the activation of oxidative stress-related transcription factors in female A/J mouse lung. *J Toxicol Environ Health A* 2010; **73**: 1288-1297
  - 50 **Talukder MA**, Johnson WM, Varadharaj S, Lian J, Kearns PN, El-Mahdy MA, Liu X, Zweier JL. Chronic cigarette smoking causes hypertension, increased oxidative stress, impaired NO bioavailability, endothelial dysfunction, and cardiac remodeling in mice. *Am J Physiol Heart Circ Physiol* 2011; **300**: H388-H396
  - 51 **Yamamoto T**, Kojima T, Murata M, Takano K, Go M, Hatakeyama N, Chiba H, Sawada N. p38 MAP-kinase regulates function of gap and tight junctions during regeneration of rat hepatocytes. *J Hepatol* 2005; **42**: 707-718
  - 52 **Lui WY**, Lee WM, Cheng CY. TGF- $\beta$ s: their role in testicular function and Sertoli cell tight junction dynamics. *Int J Androl* 2003; **26**: 147-160
  - 53 **Scharl M**, Paul G, Barrett KE, McCole DF. AMP-activated protein kinase mediates the interferon-gamma-induced decrease in intestinal epithelial barrier function. *J Biol Chem* 2009; **284**: 27952-27963
  - 54 **Miyoshi A**, Rochat T, Gratadoux JJ, Le Loir Y, Oliveira SC, Langella P, Azevedo V. Oxidative stress in Lactococcus lactis. *Genet Mol Res* 2003; **2**: 348-359
  - 55 **Gaboriau-Routhiau V**, Rakotobe S, Lécuyer E, Mulder I, Lan A, Bridonneau C, Rochet V, Pisi A, De Paepe M, Brandi G, Eberl G, Snel J, Kelly D, Cerf-Bensussan N. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* 2009; **31**: 677-689
  - 56 **Salzman NH**, de Jong H, Paterson Y, Harmsen HJ, Welling GW, Bos NA. Analysis of 16S libraries of mouse gastrointestinal microflora reveals a large new group of mouse intestinal bacteria. *Microbiology* 2002; **148**: 3651-3660
  - 57 **Kibe R**, Sakamoto M, Yokota H, Benno Y. Characterization of the inhabitancy of mouse intestinal bacteria (MIB) in rodents and humans by real-time PCR with group-specific primers. *Microbiol Immunol* 2007; **51**: 349-357
  - 58 **Cani PD**, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 2008; **57**: 1470-1481
  - 59 **Barman M**, Unold D, Shifley K, Amir E, Hung K, Bos N, Salzman N. Enteric salmonellosis disrupts the microbial ecology of the murine gastrointestinal tract. *Infect Immun* 2008; **76**: 907-915
  - 60 **Bartosch S**, Fite A, Macfarlane GT, McMurdo ME. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Appl Environ Microbiol* 2004; **70**: 3575-3581
  - 61 **Matsuda K**, Tsuji H, Asahara T, Kado Y, Nomoto K. Sensitive quantitative detection of commensal bacteria by rRNA-targeted reverse transcription-PCR. *Appl Environ Microbiol* 2007; **73**: 32-39

S- Editor Cheng JX L- Editor A E- Editor Xiong L