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Orlistat, a competitive lipase inhibitor used as an antiobesity remedy, enhances inflammatory reactions in the intestine

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Abstract

Alterations in secondary gut metabolites derived from the microbial fermentation of food in the gut have significant effects on various aspects of host physiology. Our recent studies on obese mice treated with Orlistat, an antiobesity treatment, revealed a significantly altered gut microbial profile marked by an over-abundance of Proteobacteria and alterations in secondary gut metabolites. In this study, we determined effect of fecal metabolites from high-fat diet fed mice treated with Orlistat (HFDOrl) on colonic epithelial cells in relation to inflammation, barrier function, mitochondrial activity, reactive oxygen species (ROS) levels, and oxidative stress. Quantitative PCR was used to measure intestinal mRNA expression of oxidative stress, inflammation, apoptosis, and gut barrier function genes in mice on a high fat diet with and without Orlistat treatment versus those fed a low-fat diet (HFDOrl, HFD, Normal diet-fed [ND] respectively). Alterations to antioxidant function in HCT-116-ARE-luciferase stable cell line and mitochondrial function in Caco-2 cells was analyzed under oxidative stress with exposure to aqueous fecal extracts from HFDOrl, HFD, and ND groups. The results of this study indicate that a significant increase in anti-oxidative response was observed based on the luciferase activity of HCT-116-ARE-luciferase stable cells. Increased maximal respiration and mitochondrial ROS under oxidative stress was also detected in confluent Caco-2 cells resulting from exposure to fecal extracts from the HFDOrl group compared with the HFD group and pure Orlistat. Furthermore, mice from the HFDOrl group exhibited a significant increase in colonic epithelial expression of oxidative markers (Nrf-2 and SOD-2), inflammation-related markers (IL-6 and TNF-α), and gut barrier function markers (Muc-2 and Occludin). Taken together, the results suggest that Orlistat treatment in the HFD group causes changes in secondary gut metabolites which affect the colonic redox state and may eventually lead to the development of inflammatory, oxidative, and mitochondrial dysfunction at the cellular level.

Keywords: Oxidative stress, Mitochondrial dysfunction, Inflammation, Orlistat

Introduction

Obesity is defined by the International Classification of Diseases 11 (ICD-11) [1] as a chronic complex disease characterized by excessive adiposity that impairs health. Tetrahydrolipstatin, more commonly known as Orlistat and sold under the name Xenical (Roche Pharmaceuticals, Nutley, NJ), was approved by the US Food and Drug Administration for use as a treatment for obesity [2]. Orlistat is a chemically synthesized hydrogenated derivative of lipstatin, a natural product of *Streptomyces toxytrycini*. Orlistat inhibits digestion by gastric and pancreatic lipases by competitively binding to their active sites, thus reducing intraluminal hydrolysis of triglycerides and absorption of dietary fat by approximately 30% [3].

Mild to moderate gastrointestinal adverse events are associated with Orlistat, which include abdominal

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discomfort, liquid stools, oily stools, oily rectal spotting, flatulence and flatus discharge, fecal urgency, fatty or oily stools, increased defecation, and fecal incontinence [4–7]. Recent reports suggests that Orlistat therapy induces significant modifications to gut microbe population [8] with Actinobacteria and Proteobacteria groups shown to be the most affected. However, some studies suggest these changes were limited to Proteobacteria, a potential marker diagnostic marker of ecological imbalance and disease risk [9], and other bacterial populations of low relative abundance [10, 11]. In our previous study, obese mice treated with a HFD underwent two different interventions [12]. Mice that were administered Orlistat at 200 mg/kg of diet, exhibited reduced body weight gain and increased fecal triglycerides. They had a significantly altered gut microbe profile marked by an over-abundance of Proteobacteria. Recent studies have also shown a similar trend in mice together with reduced microbial diversity and richness [8]. Evidence of increased *Proteobacteria* and decreased *Alloprevotella* associated with Orlistat therapy in humans has been reported [11].

Secondary gut metabolites derived from microbial fermentation of food in the gut have broad effects on host physiology, which suggests that they have nutraceutical properties. Of these, short-chain fatty acids (SCFAs), which are derived from the microbial fermentation of dietary fibers, have been a major focus of gut metabolome-based research [13]. The molecular mechanisms responsible for these effects involve mitochondrial function [14]. Changes in gut microbial composition during Orlistat treatment was accompanied by a significant reduction in SCFAs in the gut [12]. A human study on the impact of long-term Orlistat treatment revealed a reduction in the abundance of *Alloprevotella*, an acetic acid-producing bacteria belonging to the *Bacteroidetes* phylum [11]. These changes suggest an alteration in secondary gut metabolites.

Based on these findings, we hypothesized that the gut metabolome of Orlistat-treated, HFD-fed subjects induces mitochondrial dysfunction and results in an altered redox state, inflammation, and barrier integrity of colonic epithelial cells. In the present study, we evaluated the effects on the fecal metabolome of mice fed with a HFD containing Orlistat (HFDOrl) on mitochondrial energy production and reactive oxygen species (ROS) generation in a colon epithelial cell model. RNA sequencing of oxidative stress, inflammation, apoptosis, and gut barrier function markers in the intestine of the HFDOrl group were analyzed and compared with that of untreated mice. To analyze the effects of fecal extracts collected from the HFDOrl group on mitochondrial energy production in healthy Caco-2 cells and those under metabolic stress, a Seahorse cell mitochondrial stress kit was used (Seahorse Bioscience, North Billerica, MA, USA). HCT-116-ARE-luciferase stable cells were analyzed for ROS production using a luciferase assay after inducing oxidative stress by *tert*-butylhydroxy peroxide (*t*-BHP) exposure in the presence or absence of the fecal extracts from the aforementioned groups.

Methodology

Animal study

The in vivo experiments were performed in compliance with the guidelines of the Ethics and Animal Welfare of Kyungpook National University and were approved by the Institutional Review Board (KNU-2019-0034-1). Five-week-old male C57BL/6 mice were obtained from Orient-Bio Inc. (Seongnam-si, Gyeonggi-do, Korea). The animal diets were procured from Research Diet (New Brunswick, NJ, USA). The mice were provided access to water and chow ad libitum during the 1st week of acclimatization. They were maintained under controlled conditions of 23 °C ± 1 °C and a light–dark cycle of 12 h. They were then divided into four groups of 10 animals per group and treated as follows: Low-fat diet (ND) (D12450J, 10% kcal from fat) and HFD (D12492, 60% kcal from fat) and HFD with Orlistat. Orlistat-treated mice received 200 mg of Orlistat per kg of diet for 8 weeks. The mice were then euthanized by cervical dislocation followed by excision of the whole intestine, which was immediately frozen at − 80 °C. Fecal samples were collected weekly for each group.

RNA isolation from the intestine and quantitative real-time PCR

Total RNA was isolated from the jejunum to the colon using the Takara MiniBEST universal RNA extraction kit (Takara, Kusatsu, Japan). To quantify the amount of mRNA, cDNA was synthesized from 1 μg of total RNA in a final volume of 20 μL using the Maxima H Minus First Strand cDNA Synthesis Kit (K1681; Thermo Fisher Scientific, MA). Next, quantitative real-time PCR (qRT-PCR) was done using the TOPreal™ qPCR 2× pre-mix (SYBR-Green with low ROX, Daejon, South Korea) with the Light Cycler® 96 software version 1.1 PCR Detection System (F. Hoffman-La Roche AG. Basel, Switzerland) following the manufacturer's instructions. The results were analyzed using the $2^{-\Delta\Delta Ct}$ method with GAPDH and β-actin as internal standards. All primer sequences are listed in Additional file 1: Table S1.

Extraction of aqueous secondary metabolites from fecal matter

The fecal matter from the three mouse groups was collected during the final 2 weeks of treatment and pooled. From each pooled sample, 100 mg was placed in sterile

1.5 mL conical Eppendorf tube. Then, 1 mL of phosphate buffered saline (PBS) was added to the tube and the mixture was kept on ice and vortexed every 5 min for 1 h. The final slurry was centrifuged at 10,000*g* for 10 min at 4 °C. The aqueous supernatants were separated and stored at − 80 °C.

Cell culture

The human gut epithelial cancer cell line, Caco-2, was used for cell mitochondrial stress analysis. Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin (Welgene, South Korea). A stable human gut epithelial cell line, HCT-116, was transfected with the pGL4.37[*luc2P*/ARE/Hygro] vector (Promega, Madison, WI, USA) [15]. The resulting HCT-116-ARE-luciferase cell line was maintained in the aforementioned culture medium and included 0.4 mM hygromycin (Sigma-Aldrich, St. Louis, MO, USA). All cultures were maintained in a humidified CO_2-incubator (37 °C and 5% CO_2) for the designated period.

Cell mitochondrial stress analysis

Subconfluent Caco-2 cells were harvested and seeded at 1.6×10^4 cells per well in an 8-well plate for the Agilent Seahorse XFp Cell Mito-Stress assay (Agilent Technologies, DE, USA). Each well contained 100 µL of growth medium and the cells were incubated until 80% confluence was achieved. The cells were either incubated for 3 or 12 h with fecal extract from the HFDOrl and HFD group with or without a subsequent 3 h *t*-BHP challenge by adding 9 µL of 100 µM *t*-BHP. Untreated cells were used as controls. Prior to the assay, the growth medium from each well was removed, the cells were washed twice with 180 µL of pre-warmed assay medium (XF base medium supplemented with 25 mM glucose, 2 mM glutamine, and 1 mM sodium pyruvate; pH 7.4), and pre-incubated with 160 µL of the assay medium at 37 °C without CO_2 for 1 h.

A pre-warmed sensor cartridge containing XF calibrant and the energy substrates, which included oligomycin, FCCP, and rotenone/antimycin A, were loaded into injector ports A, B, and C, respectively, of the Agilent Seahorse XFp analyzer (Seahorse Bioscience, Billerica, MA, USA). The assay was primed with the cartridge calibration using pre-warmed XF calibrant and was continued for the samples using the cell mito-stress test assay protocol provided by the manufacturer (Seahorse Bioscience, Billerica, MA, USA). The oxygen consumption rate was measured under basal conditions followed by the sequential addition of oligomycin, FCCP, and rotenone/antimycin A. This allowed for an estimation of the contribution of individual parameters for basal respiration, proton leak, maximal respiration, spare respiratory capacity, non-mitochondrial respiration, and ATP production using Wave software sourced from Agilent Technologies (Agilent Technologies, DE, USA). This data was then exported to Microsoft Excel for further analysis.

Luciferase activity

A luciferase reporter assay was conducted on the stable HCT-116-ARE-luciferase cells. The cells were treated with fecal extracts for 12 h, then challenged with 300 µM *t*-BHP for 3 h prior to harvest and lysis. Treatment with *t*-BHP activates apoptosis because of changes in the ion channels of the mitochondrial membrane and activation of lipid peroxidation. The luciferase activity, which corresponds to ARE activity, was measured using a luciferase assay system (Promega) according to the manufacturer's instructions. Sulforaphane (Sigma-Aldrich, St. Louis, MO, USA), an isothiocyanate, was used as a control ARE activator. The luminescence of the assay was detected and the values were normalized based on total cell count. The values were then converted to fold-change using the blank as the baseline value.

Measurement of mitochondrial ROS generation using a superoxide indicator

Cells were seeded (5×10^3 cells) on cover slips and placed into 24-well plates (SPL Life Sciences Co., Ltd.) and treated with fecal extracts alone for 12 h or in combination with *t*-BHP for the final hour. Afterwards, the media was discarded. Immediately after treatment, mitochondrial ROS was measured using the MitoSOX TM Red superoxide indicator (Invitrogen, Carlsbad, CA, USA) followed by counterstaining with 4′,6-diamidino-2-phenylindole (DAPI, 1 µg/mL). The cells were washed twice with PBS and then mounted with a drop of mounting medium (Vector Laboratories Inc., California, USA). Fluorescence was assessed using a fluorescence microscope (Eclipse 80i, Nikon, Tokyo, Japan) at 400× magnification and quantified densitrometrically (ImageJ Software, US National Institute of Health, Bethesda, Maryland, USA) from at least three random fields selected for each coverslip. Representative images for all conditions were selected for illustration.

Statistical analysis

In vitro and in vivo data are expressed as the mean ± standard error of the mean (SEM). A p-value < 0.05 was considered statistically significant.

Results

Effects of Orlistat treatment on inflammation and barrier function

To determine possible alterations in inflammation and barrier function as a result of Orlistat treatment in the mouse intestinal epithelial layer, RNA was isolated from the intestines from the three groups and analyzed by quantitative real-time PCR (qRT-PCR). Primers for TNF-α and IL-6, as well as occludins and Mucin-2 were used as inflammation and barrier function markers, respectively. The expression of TNF-α and IL-6 mRNA was significantly increased in the HFDOrl group compared with the ND and HFD control groups (Fig. 1A and B). Similarly, reduced levels of both occludins and Mucin-2 (Muc-2) in the HFDOrl group were observed compared with that in the HFD and ND groups (Fig. 1C and D).

ROS detection in Caco-2 cells treated with fecal extracts of the Orlistat-treated group

Ingested materials are a key source of ROS that can activate macrophages and neutrophils to produce inflammatory cytokines that may contribute to intestinal barrier dysfunction in rats [16]. To determine whether increased levels of inflammation resulted from oxidative stress in the gut lumen, the aqueous fraction of fecal matter collected over the final 2 weeks of treatment was extracted from all three groups and used to challenge Caco-2 cells for 12 h or in combination with *t*-BHP for the final hour. Caco-2 cells were pre-treated with 25 µL and 50 µL

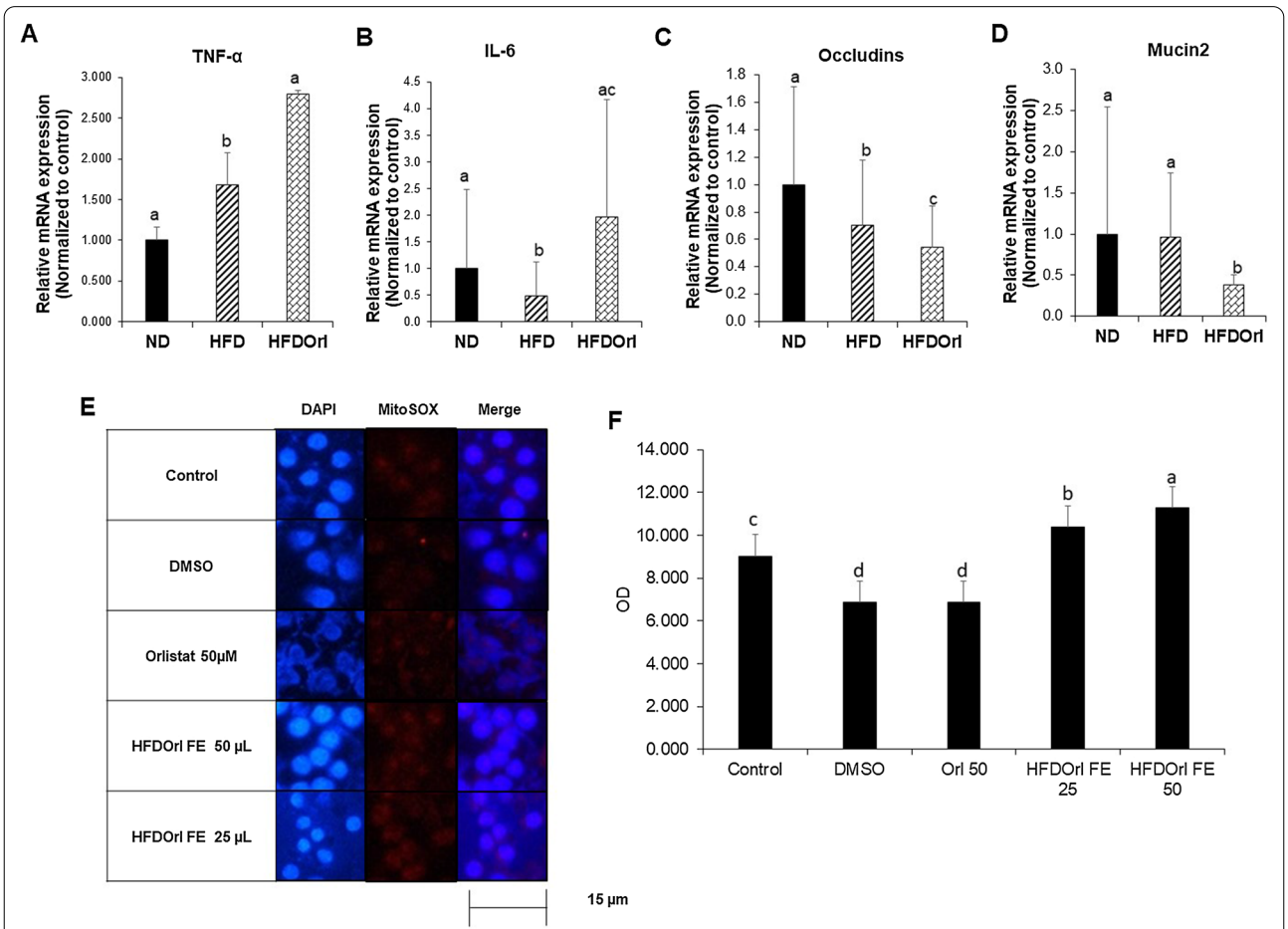

Fig. 1 Intestinal epithelial mRNA expression of **A** TNF-α, **B** IL-6, **C** occludins and **D** Mucin-2 analyzed by qRT-PCR for the three treatment groups; Normal diet-fed control group, High fat diet-fed (HFD) and HFD-fed with Orlistat (HFDOrl) treatment groups. **E** Effect of extracts from 100 mg of fecal matter from mice in the Orlistat treatment (HFDOrl) group and pure Orlistat on ROS production based on the MitoSOX superoxide indicator in Caco-2 cells counter-stained with DAPI (**F**) Mean optical density of randomly selected fields of the representative images. Untreated and DMSO-treated cells were used as controls. Real-time PCR results for data are presented as the mean concentration ± SEM. Different letters denote the significance of the differences compared with the control ($p < 0.05$)

of fecal extract from the Orlistat-treated HFD group (HFDOrl FE) and 50 µM Orlistat dissolved in DMSO for 12 h with DMSO-treated and untreated cells as controls. A subsequent *t*-BHP challenge for 1 h prior to the addition of the superoxide indicator was done separately to determine whether ROS generation was enhanced with mitochondrial respiration. Mitochondrial superoxide levels were increased following exposure to the HFDOrl fecal extract, but not by 50 µM Orlistat (Fig. 1E and F). The *t*-BHP challenge resulted in a significant increase in mitochondrial superoxide levels, which showed a minimal increase as a result of pre-treatment with 50 µL fecal extracts from the HFDOrl group (Additional file 1: Figure S1A and B).

Fecal extracts of the Orlistat-treated group enhance oxidative stress induced by t-BHP

The water soluble fecal extract from the HFDOrl group enhanced the oxidative stress caused by *t*-BHP and resulted in a fold-change increase of luminescence from 5.9 to 8.1 (Fig. 2A). Interestingly, the luminescence fold-change measured in the *t*-BHP challenged Caco-2 cells treated with Orlistat increased to 6.8, whereas DMSO alone did not cause any change. Orlistat is a highly hydrophobic substance, whereas the fecal matter from the HFDOrl group was extracted in water and, therefore, had hydrophilic components. Fecal extracts with a hydrophobic solvent, dichloromethane (DCM), was tested for luciferase activity. The extract from DCM did not affect oxidative stress in the presence of *t*-BHP. Similarly, without *t*-BHP treatment, the cells only exhibited slight changes in luciferase activity (Fig. 2A).

Orlistat-induced alterations to oxidative stress and apoptosis markers in the intestine

To determine possible alterations to the oxidative stress response as a result of Orlistat treatment in the mouse intestinal epithelial layer, RNA was isolated form the intestines of the three mouse groups. Primers for Nrf2 and SOD2 were used as oxidative stress markers, whereas caspase-3 was used as an apoptosis-related marker. The expression of SOD2 mRNA in the intestinal epithelial cells was significantly increased in the HFD-fed group compared with the ND-fed group (Fig. 2B). The Orlistat-treated group displayed a large increase in SOD2 mRNA compared with the HFD- and ND-fed group. Nrf2 mRNA was also significantly higher in the HFDOrl-treated group compared with the HFD-fed group, but not significantly different from the ND-fed group (Fig. 2C). Caspase 3 exhibited the highest increase in the ND group, but was very low in both the HFD and HFDOrl groups (Fig. 2D).

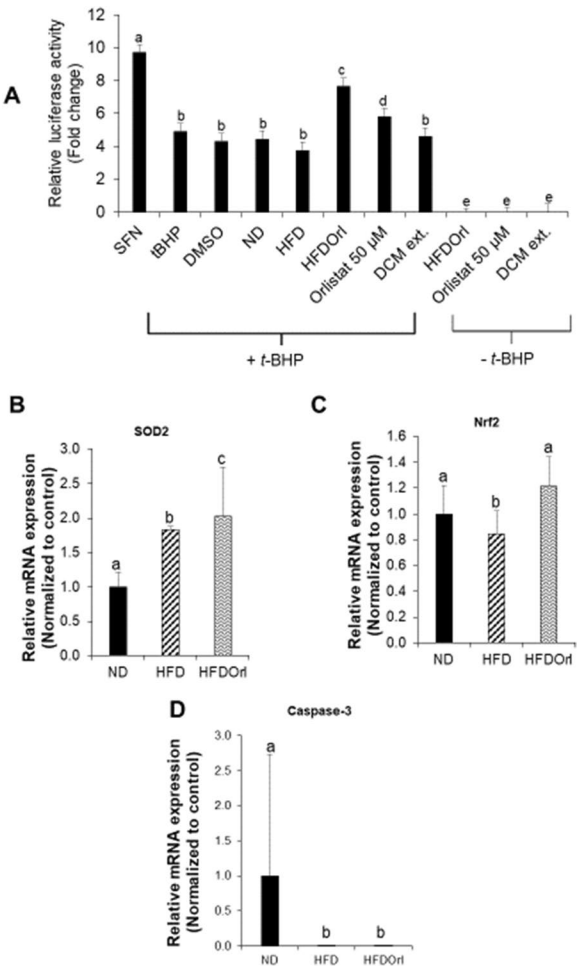

Fig. 2 **A** Effect of extracts from 100 mg of fecal matter from mice in HFDOrl group and its Dichloromethane (DCM) extract versus fecal extracts from ND and HFD-fed mouse groups and pure Orlistat (dissolved in DMSO) on ROS production in HCT-116-ARE cells challenged with and without *t*-BHP. Untreated, DMSO, and *t*-BHP-treated cells were used as controls. Intestinal epithelial mRNA expression of **B** SOD2, **C** Nrf2, and **D** caspase 3 analyzed by qRT-PCR for the three treatment groups; Normal Diet-fed (ND) as the control group, High fat diet-fed (HFD), and HFD-fed with Orlistat (HFDOrl) treatment groups. Data are presented as the mean concentration ± SEM. Different letters denote the significance of differences compared with the control ($p < 0.05$)

Cell mitochondrial stress analysis of Caco-2 cells treated with fecal extracts from the Orlistat-treated group

Caco-2 cells were treated with fecal matter extracts from the HFDOrl group and *t*-BHP (HFDOrl FE + *t*-BHP) for 3 h prior to cell mitochondrial stress analysis, which showed a higher maximal respiration and ATP production compared with cells treated only with *t*-BHP (Fig. 3A). Similarly, Caco-2 cells pre-treated for 12 h with the fecal extract from the HFDOrl group (HFDOrl FE) prior to a 3 h *t*-BHP challenge

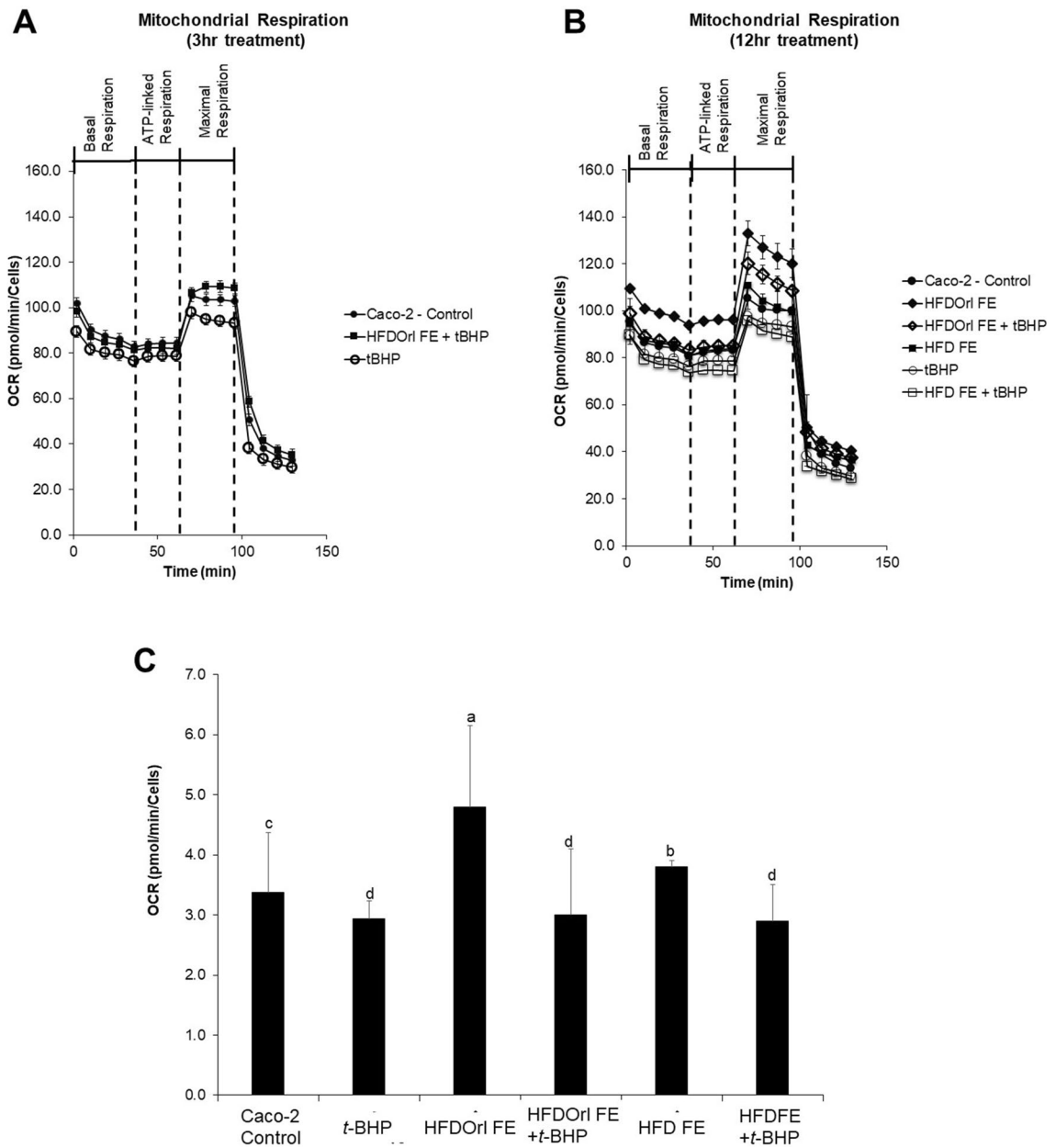

Fig. 3 **A** Cell mitochondrial stress profile of Caco-2 cells treated with extracts of fecal matter from HFDOrl group for 3 h with *t*-BHP challenge versus cells only challenged with *t*-BHP. **B** Effect of a 12-h treatment with HFD and HFDOrl fecal extracts with or without *t*-BHP challenge for an additional 3 h; cells treated with *t*-BHP and untreated cells were used as controls. **C** ATP produced in the aforementioned 12-h treatment with fecal extracts followed by *t*-BHP challenge. *t*-BHP-challenged and untreated cells served as controls. Data are presented as the oxygen consumption rate ± SEM. Different letters denote the significance of differences compared with the control ($p < 0.05$)

exhibited higher maximal respiration compared with fecal extracts from the HFD group (HFD FE) without Orlistat treatment (Fig. 3B). *t*-BHP caused a significant reduction in ATP production for all treatment conditions, which suggests a negative effect on mitochondrial energy production capacity. In contrast, the high-fat diet fecal extract (HFD FE) caused an increase in ATP production compared with untreated cells, suggesting the presence of increased substrates for energy production (Fig. 3C). Despite the increase in mitochondrial respiration, the fecal extracts from Orlistat-treated mice did not induce a significant increase in ATP production.

Discussion

The aim of the current study was to assess the effect of fecal metabolites of HFD-fed mice on Orlistat treatment of colonic epithelial cells with respect to inflammation, barrier function, mitochondrial activity, ROS levels, and oxidative stress. Our previous study showed that Orlistat-induced alterations to the intestinal ecosystem and gut microbe populations as a result of increased fat content and possible alterations in gut secondary metabolites [12]. We hypothesized that these changes may result from increased proinflammatory signaling and increased oxidative stress resulting from mitochondrial dysfunction in the colonic epithelia. To evaluate this possibility, C57BL/6J mice were fed with a HFD with Orlistat treatment for 8 weeks and the whole intestine was excised for qRT-PCR analysis of oxidative stress, inflammation, apoptosis, and barrier function-related genes. The fecal matter from the final 2 weeks of treatment was collected and pooled for both the treatment and control groups for cell-based studies on oxidative stress and mitochondrial function.

Obesity has a causal relationship with gut microbial dysbiosis and is characterized by a significant increase in *Proteobacteria* and a decrease in *Firmicutes*/*Bacteroidetes* [17]. A high content of dietary fat is a key factor in the development of gut microbial dysbiosis in obese individuals [18, 19]. Concentrations of bacterial metabolites in the gut lumen are significantly altered by diet [20], more so by Orlistat treatment as indicated by changes to SCFAs, such as butyrate [21], which is consistent with our previous findings [12].

A study on high-fat-fed mice showed changes in gut microbiota and as a consequence, increased ROS coupled with reduced antioxidant capacity in the colon, which suggests an altered redox state in the intestines of these mice [22]. Free radical formation in human feces was also correlated with increased fat content in the diet [23]. In the present study, mitochondrial superoxide levels were analyzed using the MitoSOX superoxide indicator after exposure to fecal extracts from the HFDOrl group. This resulted in a significant increase in mitochondria superoxide levels with the margin of increase growing concomitantly with the volume of fecal extract (Fig. 1A and B). In contrast, pure Orlistat did not induce a change in mitochondrial superoxide levels, which were equal to that of the DMSO control (Fig. 1B). Together with the insolubility of Orlistat in the water used for fecal extraction, the results indicate that increased ROS production in the colonic epithelia is not directly linked to the presence of Orlistat in the gut lumen. This was supported by the higher luciferase activity observed in HCT-116-ARE-luciferase cells following exposure to fecal extracts from the HFDOrl group and challenged with *t*-BHP (Fig. 2A). Interestingly, there was no significant change in luminescence resulting from fecal extracts of the HFDOrl group in the absence of by *t*-BHP. Conversely, fecal extracts from the ND and HFD control groups caused slight dampening of the luminescence signal, which may be explained by the level of SCFA in these groups, which was higher compared with that in the Orlistat group as shown in our previous study [12]. Pure Orlistat exerted a similar effect to that of fecal extracts from the HFDOrl group, but by a smaller margin, whereas the dichloromethane extract showed no change (Fig. 2A). This indicates that the secondary metabolites responsible for this effect may be more hydrophilic, but may be associated with or be a derivative of Orlistat. These results also indicate that the ROS levels in the presence of fecal metabolites from the HFDOrl group may not only be a result of increased ROS, but also the dysregulation of oxidative stress-related signaling pathways. It is noteworthy that ARE-luciferase activity is known to be increased by Nrf2 activators, such as sulforaphane [24].

Nrf2 is closely related to amount of cellular ROS [25]. Nrf2 is a transcriptional activator that can serve as a sensor of oxidative stress [26]. Through the Nrf2/Keap1 pathway, elevated ROS causes Nrf2 to translocate to the nucleus and bind to ARE sites located within the regulatory regions of genes encoding antioxidant enzymes, including glutathione reductase and glutathione peroxidase [27]. In the present study, the expression of Nrf2, an oxidative stress marker, was lower in the colons of HFD-fed mice compared with ND-fed mice; however, the expression of Nrf2 was highest in HFD-fed mice treated with Orlistat (Fig. 2C). In addition, the expression of mitochondrial SOD mRNA was markedly increased in the intestine of the HFDOrl group compared with the HFD and ND groups (Fig. 2B). These results suggest that increased oxidative stress may cause increased expression of Nrf2 as well as mitochondrial antioxidant enzymes. Additionally, Caco-2 cells challenged with *t*-BHP under acute exposure (3 h) to fecal extracts from the HFDOrl group exhibited increased mitochondrial respiration (Fig. 3A). Constipation is a known adverse effect of Orlistat treatment in obese patients [6]. We hypothesize that as a result, the colonic epithelium is exposed to fecal metabolites for a longer period of time as a result of Orlistat treatment. Therefore, Caco-2 cells were exposed to fecal extracts from HFDOrl group for 12 h and this resulted in increased maximal respiration (Fig. 3B). Similarly, ATP production was significantly increased in Caco-2 cells exposed to fecal extracts from the HFDOrl group. This suggests that the increased levels of ROS may be associated with higher concentrations of energy substrates in the gut of the HFDOrl group available for ATP production and maximal respiration. A human

study revealed an increased pro-oxidant capacity of fecal waters in healthy normal-weight volunteers administered Orlistat as a result of increased fat malabsorption [10]. Moreover, fecal extracts from the HFD group (HFD FE) showed an increase in both ATP production and maximal respiration, but to a lesser degree compared with the Orlistat-treated group. Increased fat content in the gut as a result of treatment with Orlistat in individuals with a high intake of dietary fat may, therefore, alter the metabolic activity of colonocytes, resulting in increased fat metabolism and ROS generation.

Changes in oxidative status and gut microbiota dysbiosis are associated with inflammation and changes in gut barrier function. Orlistat treatment is known to induce fat malabsorption [28] given that high-fat content in the gut independently increases ROS production [22], which causes intestinal barrier dysfunction [29]. Studies on the effects of Orlistat on human gut barrier function are contradictory [30, 31]. The expression of occludin, a known intestinal barrier marker [32], was thereby analyzed. In the present study, Orlistat treatment in mice caused a significant decrease in occludin mRNA levels compared with the HFD-fed mice, whose levels were lower than those of the ND-fed mice (Fig. 1E). This result suggests that a reduction in gut barrier function as a result of a HFD is exacerbated by Orlistat treatment. Furthermore, the expression of Mucin-2 was also significantly decreased by Orlistat treatment compared with the controls (Fig. 1F). A reduction in Mucin-2, the most-abundant gel-forming component of mucus in the colonic epithelium, is also indicative of a reduction in mucosal barrier integrity and the ability of the host to restrict bacteria in the lumen of the intestine [33]. The development of chronic intestinal inflammation may be preceded by increased epithelial permeability [34]. Colonic epithelial levels of TNF-α and IL-6 were increased in the Orlistat-treated group compared with the untreated HFD and ND groups indicating increased inflammation (Fig. 1C and D). However, it is noteworthy that the significant increase in inflammation and reduced gut barrier integrity was not accompanied by increased circulating serum levels of LPS or inflammatory markers [12]. A similar phenomena was observed in another study using Orlistat for antiobesity therapy [10].

In the present study, we determined the effect of Orlistat treatment on cellular respiration, inflammation, and oxidative status of colonic epithelia exposed to metabolites found in subjects treated with a HFD. The inhibition of lipase activity by Orlistat significantly reduces the breakdown of lipids, thus altering the metabolic activity of the gut microbiota. These alterations drive changes in the gut microbiota composition and metabolome that interacts with intestinal epithelial cells. Our results provide insight into the effects of metabolome changes that Orlistat treatment may inflict on gut epithelial cells. The fecal extracts of mice in the HFDOrl group increased ATP production and cellular respiration in Caco-2 cells. These increments may explain the increase mitochondrial superoxide levels observed in Caco-2 cells exposed to fecal extracts from the HFDOrl group and the increased levels of oxidative stress markers in the HFDOrl mouse group compared with those not treated with Orlistat. The present study also highlights the potential attenuation of oxidative stress that may result from aggregation of these metabolic changes and increased lipid peroxidation as shown by co-treatment with *t*-BHP. Taken together, the results explain the increase in inflammation-related markers and reduced gut barrier integrity in mice treated with an HFD and Orlistat. Interestingly, these effects appear to be limited to the colonic epithelia and, therefore, further studies are needed to elucidate the possible short and long-term effects of these changes. Additionally, the current study was limited by the small sample size and the inadequate information on fecal metabolome profile [8]. A metabolomics approach may further elucidate the impact and mechanisms of the response to Orlistat treatment in obese subjects. This information will be useful for the careful risk–benefit assessment when prescribing antiobesity drugs.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-022-00712-y.

Additional file 1: Table S1. Sequences of the primers used for quantitative real-time PCR. **Figure S1.** Effect of varied volumes of extracts from 100 mg of fecal matter of mice in Orlistat-treated group (HFDOrl) and *t*-BHP co-treatment on ROS production based on MitoSOX superoxide indicator in Caco-2 cells. (B) Mean Optical density (OD) of randomly selected fields of the representative images. Untreated and *t*-BHP treated cells were used as a control. Data represented as mean concentration ± SEM. Different letters denote the significance of differences compared to the control ($p < 0.05$).

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Author contributions
Animal experiments: DAK, JL. Conceptualization: JL, DAK. Funding acquisition: JL. Investigation: DAK, JO, CHJ. Methods development: DAK, JO, CHJ, JL. Supervision: JL. Writing, review, and editing: DAK, JL. All authors read and approved the final manuscript.

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Availability of data materials
Available upon request.

Declarations

Competing interests
The authors declare no conflicts of interest.

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References
1. International classification of diseases for mortality and morbidity statistics (2020) https://icd.who.int/browse11/l-m/en. Accessed 23 Oct 2020
2. Arzola-Paniagua MA, García-Salgado López ER, Calvo-Vargas CG, Guevara-Cruz M (2016) Efficacy of an orlistat-resveratrol combination for weight loss in subjects with obesity: a randomized controlled trial. Obesity 24:1454–1463. https://doi.org/10.1002/oby.21523
3. Heck AM, Yanovski JA, Calis KA (2000) Orlistat, a new lipase inhibitor for the management of obesity pharmacotherapy. J Hum Pharmacol Drug Ther 20:270–279. https://doi.org/10.1592/phco.20.4.270.34882
4. Hill JO, Hauptman J, Anderson JW, Fujioka K, O'Neil PM, Smith DK, Zavoral JH, Aronne LJ (1999) Orlistat, a lipase inhibitor, for weight maintenance after conventional dieting: a 1-y study. Am J Clin Nutr 69:1108–1116. https://doi.org/10.1093/ajcn/69.6.1108
5. Bakris G, Calhoun D, Egan B, Hellmann C, Dolker M, Kingma I (2002) Orlistat improves blood pressure control in obese subjects with treated but inadequately controlled hypertension. J Hypertens 20:2257–2267
6. Bloch KV, Salles GF, Muxfeldt ES, da Rocha Nogueira A (2003) Orlistat in hypertensive overweight/obese patients: results of a randomized clinical trial. J Hypertens 21:2159–2165
7. Theodosios D, Filippatos CSD, Gazi IF, Nakou ES, Mikhailidis DP, Elisaf MS (2008) Orlistat-associated adverse effects and drug interactions. Drug-Saf 31:53–65. https://doi.org/10.2165/00002018-200831010-00005
8. Ke J, An Y, Cao B, Lang J, Wu N, Zhao D (2020) Orlistat-induced gut microbiota modification in obese mice. Evid Based Complement Altern Med. https://doi.org/10.1155/2020/9818349
9. Shin N-R, Whon TW, Bae J-W (2015) Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol 33:496–503. https://doi.org/10.1016/j.tibtech.2015.06.011
10. Morales P, Fujio S, Navarrete P, Ugalde JA, Magne F, Carrasco-Pozo C, Tralma K, Quezada M, Hurtado C, Covarrubias N, Brignardello J, Henriquez D, Gotteland M (2016) Impact of dietary lipids on colonic function and microbiota: an experimental approach involving Orlistat-induced fat malabsorption in human volunteers clinical and translational. Gastroenterology 7:e161. https://doi.org/10.1038/ctg.2016.20
11. Jin J et al (2021) Distinctive gut microbiota in patients with overweight and obesity with dyslipidemia and its responses to long-term orlistat and ezetimibe intervention: a randomized controlled open-label trial. Front Pharmacol 12:2247. https://doi.org/10.3389/fphar.2021.732541
12. Katimbwa DA, Ma J, Kim CK, Hahn D, Lim J (2021) Potato extract inhibits lipase activity and ameliorates gut microbiome dysbiosis and weight gain in mice fed a high-fat diet. Appl Biol Chem. https://doi.org/10.1186/s13765-021-00590-w
13. Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, Terasawa K, Kashihara D, Hirano K, Tani T, Takahashi T, Miyauchi S, Shioi G, Inoue H, Tsujimoto GZ (2013) The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. Nat Commun 4:1829. https://doi.org/10.1038/ncomms2852
14. Hu S, Kuwabara R, de Haan BJ, Smink AM, de Vos P (2020) Acetate and butyrate improve β-cell metabolism and mitochondrial respiration under oxidative stress. Int J Mol Sci 21:1542. https://doi.org/10.3390/ijms21041542
15. Woo Y, Oh J, Kim J-S (2017) Suppression of Nrf2 activity by chestnut leaf extract increases chemosensitivity of breast cancer stem cells to paclitaxel. Nutrients 9:760
16. Tian R, Tan J, Wang R, Xie H, Qian Y, Yu K (2013) The role of intestinal mucosa oxidative stress in gut barrier dysfunction of severe acute pancreatitis. Eur Rev Med Pharmacol Sci 17:349–355
17. Ley R, Turnbaugh P, Klein S et al (2006) Human gut microbes associated with obesity. Nature 444:1022–1023. https://doi.org/10.1038/4441022a
18. Arumugam MRJ, Pelletier E, Le Paslier D, Yamada T et al (2011) Enterotypes of the human gut microbiome. Nature 473:174–180. https://doi.org/10.1038/nature09944
19. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen Y-Y, Knight R, Ahima RS, Bushman F, Wu GD (2009) High-fat diet determines the composition of the murine gut microbiome independently of obesity. Gastroenterology 137:1716-1724.e1712. https://doi.org/10.1053/j.gastro.2009.08.042
20. Davila AM, Blachier F, Gotteland M, Andriamihaja M, Benetti PH, Sanz Y, Tomé D (2013) Intestinal luminal nitrogen metabolism: role of the gut microbiota and consequences for the host. Pharmacol Res 68:95–107. https://doi.org/10.1016/j.phrs.2012.11.005
21. Brinkworth G, Noakes M, Clifton P, Bird A (2009) Comparative effects of very low-carbohydrate, high-fat and high-carbohydrate, low-fat weight-loss diets on bowel habit and faecal short-chain fatty acids and bacterial populations. Br J Nutr 101:1493–1502. https://doi.org/10.1017/S0007114508094658
22. Qiao Y, Sun J, Ding Y, Le G, Shi Y (2013) Alterations of the gut microbiota in high-fat diet mice is strongly linked to oxidative stress. Appl Microbiol Biotechnol 97:1689–1697. https://doi.org/10.1007/s00253-012-4323-6
23. Erhardt JG, Lim SS, Bode JC, Bode C (1997) A diet rich in fat and poor in dietary fiber increases the in vitro formation of reactive oxygen species in human feces. J Nutr 127:706–709. https://doi.org/10.1093/jn/127.5.706
24. Woo Y, Lee H, Jeong YS, Shin GY, Oh JG, Kim JS, Oh J (2017) Antioxidant potential of selected Korean Edible Plant Extracts. BioMed Res Int. https://doi.org/10.1155/2017/7695605
25. DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, Mangal D, Yu KH, Yeo CJ, Calhoun ES, Scrimieri F, Winter JM, Hruban RH, Donahue CI, Kern SE, Blair IA, Tuveson DA (2011) Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature 475:106–109. https://doi.org/10.1038/nature10189
26. Zhou X, Wang W, Wang C, Zheng C, Xu X, Ni X, Hu S, Cai B, Sun L, Shi K, Chen B, Zhou M, Chen G (2019) DPP4 inhibitor attenuates severe acute pancreatitis-associated intestinal inflammation via Nrf2 signaling. Oxid Med Cell Longev. https://doi.org/10.1155/2019/6181754
27. Calvani M, Subbiani A, Vignoli M, Favre C (2019) Spotlight on ROS and β3-adrenoreceptors fighting in cancer cells. Oxid Med Cell Longev. https://doi.org/10.1155/2019/6346529
28. Cruz-Hernandez C, Destaillats F, Thakkar SK, Goulet L, Wynn E, Grathwohl D, Roessle C, de Giorgi S, Tappy L, Giuffrida F, Giusti V (2016) Monoacylglycerol-enriched oil increases EPA/DHA delivery to circulatory system in humans with induced lipid malabsorption conditions. J Lipid Res 57:2208–2216. https://doi.org/10.1194/jlr.P070144
29. Cani PD et al (2009) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 58:1091–1103. https://doi.org/10.1136/gut.2008.165886
30. Brignardello J, Morales P, Diaz E, Romero J, Brunser O, Gotteland M (2010) Pilot study: alterations of intestinal microbiota in obese humans are not associated with colonic inflammation or disturbances of barrier function. Aliment Pharmacol Ther 32:1307–1314. https://doi.org/10.1111/j.1365-2036.2010.04475.x
31. Gummesson A et al (2011) Intestinal permeability is associated with visceral adiposity in healthy women. Obesity 19:2280–2282. https://doi.org/10.1038/oby.2011.251
32. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R (2008) Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 57:1470–1481

33. Wei X, Yang Z, Rey FE, Ridaura VK, Davidson NO, Gordon JI, Semenkovich CF (2012) Fatty acid synthase modulates intestinal barrier function through palmitoylation of Mucin 2. Cell Host Microbe 11:140–152. https://doi.org/10.1016/j.chom.2011.12.006
34. Olson TS et al (2006) The primary defect in experimental ileitis originates from a nonhematopoietic source. J Exp Med 203:541–552

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