Effects of Partial or Total Replacement of Dietary Fish Oil with Soybean Oil on Intestinal Morphology and Gut Microbiota Structure in Chinese Stripe-Necked Turtles: Postprint

Authors: Ding Li, Li Jiangyue, Xue Shan, Haitao Shi, Hong Meiling

Date: 2017-10-23T00:00:00+00:00

Abstract

This study investigated the effects of partial or complete replacement of dietary fish oil with soybean oil on intestinal morphology and gut microbiota structure in Chinese stripe-necked turtles (Mauremys sinensis) through histological sectioning and 16S rRNA V3-V4 region sequencing. Twenty-four female Chinese stripe-necked turtles [4-year-old, with an average body weight of (1,557±307) g] were selected and randomly divided into 4 groups (n=6 per group). Group I (control group) was fed a formulated diet containing 1% fish oil (full fish oil diet, soybean oil to fish oil ratio of 0:3), while the three experimental groups were fed diets in which 33% (Group II, soybean oil to fish oil ratio of 1:2), 67% (Group III, soybean oil to fish oil ratio of 2:1), and 100% (Group IV, soybean oil to fish oil ratio of 3:0) of the fish oil in the full fish oil diet was replaced by soybean oil. The turtles were fed twice weekly for an experimental period of 10 months. The results showed: 1) The duodenal villus height/crypt depth (VH/CD) ratio in Groups I and III was significantly higher than that in Groups II and IV ($P<0.05$). 2) Based on 97% sequence similarity, the total number of OTUs in Groups I, II, III, and IV was 396, 321, 347, and 331, respectively, with 43, 3, 4, and 5 unique OTUs, respectively, and 139, 117, 128, and 120 genera were identified, respectively. The dominant bacterial phyla in the intestine mainly included four phyla: Bacteroidetes, Firmicutes, Fusobacteria, and Proteobacteria. The relative abundances of Fusobacteria and Proteobacteria increased, while those of Bacteroidetes and Firmicutes decreased in Groups II, III, and IV, with Group III showing the smallest difference in the relative abundances of Firmicutes and Bacteroidetes compared with Group I. The bacterial Ace index and Shannon index were highest in Group I, followed by Groups III and IV, and lowest in Group II; the bacterial Simpson index was highest in Group II, followed by Groups III and IV, and lowest in Group I. It can be concluded that

Chinese stripe-necked turtles fed a diet in which 67% of fish oil was replaced by soybean oil exhibited intestinal cell development maturity and gut microbiota structure similar to those fed the full fish oil diet.

Full Text

Effects of Partial or Total Replacement of Fish Oil by Soybean Oil on Intestinal Morphology and Microbiota Structure of Chinese Striped-Neck Turtle (Mauremys sinensis)

DING Li[1], LI Jiangyue[1], XUE Shan[1], SHI Haitao[1,2], HONG Meiling[1]*

(1. College of Life Science, Hainan Normal University, Haikou 571158, China; 2. Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China)

Abstract: This study investigated the effects of partial or total replacement of fish oil by soybean oil on intestinal morphology and microbiota structure in Chinese striped-neck turtle (*Mauremys sinensis*) using histological staining and 16S rRNA V3-V4 region sequencing. Twenty-four adult female Chinese striped-neck turtles [4 years old, average body weight (1,557±307) g] were randomly divided into four groups (n=6). Group I (control) was fed a formula diet containing 1% fish oil (whole fish oil diet, soybean oil to fish oil ratio of 0:3), while three experimental groups were fed diets in which 33% (Group II, soybean oil to fish oil ratio of 1:2), 67% (Group III, soybean oil to fish oil ratio of 2:1), and 100% (Group IV, soybean oil to fish oil ratio of 3:0) of the fish oil was replaced by soybean oil. Turtles were fed twice weekly for 10 months. The results showed: 1) The villus height to crypt depth ratio (VH/CD) in the duodenum of Groups I and III was significantly higher than that of Groups II and IV ($P<0.05$). 2) At 97% sequence similarity, the total operational taxonomic units (OTUs) in Groups I, II, III, and IV were 396, 321, 347, and 331, respectively, with unique OTUs numbering 43, 3, 4, and 5, and 139, 117, 128, and 120 genera identified, respectively. The dominant phyla in the duodenum were Bacteroidetes, Firmicutes, Fusobacteria, and Proteobacteria. The proportions of Fusobacteria and Proteobacteria increased while those of Bacteroidetes and Firmicutes decreased in Groups II, III, and IV, though Group III showed the smallest difference from Group I in Firmicutes and Bacteroidetes proportions. The bacterial Ace index and Shannon index were highest in Group I, followed by Groups III and IV, and lowest in Group II; the Simpson index was highest in Group II, followed by Groups III and IV, and lowest in Group I. These findings indicate that Chinese striped-neck turtles fed diets with 67% fish oil replacement by soybean oil exhibit similar intestinal cell development maturity and microbiota structure to those fed whole fish oil diets.

Keywords: Chinese striped-neck turtle (*Mauremys sinensis*); fish oil replacement; intestinal morphology; intestinal microbiota structure

Introduction

Since the 20th century, rapid advances in molecular biology and sequencing technologies have brought gut microorganisms into scientific focus as an important "functional organ" of the organism. These microbes are intimately associated with host immunity, nutrition, and other life activities, with complexity far beyond initial imagination. Consequently, many countries have launched gut microbiome initiatives. In 2006, the Institute for Genomic Research first studied the genetic functions of gut microbes, revealing that gut microbial genomes are rich in genes involved in carbohydrate, amino acid, and cholesterol metabolism that are largely absent in the human genome [1]. In 2008, the EU, in collaboration with leading international research teams and BGI, launched the "Human Gut Metagenome Project (MetaHIT)" [2]. However, the gut microbial community structure of chelonians—so-called "living fossils" —remains poorly documented, with existing studies limited to the gut microbial structure of red-eared sliders [3-4].

Gut microbiota forms a relatively stable symbiotic system with the host through long-term evolution, yet dietary and environmental factors can alter microbial composition and metabolic activity, thereby affecting nutrient digestion and absorption. Research indicates that high-fat diets substantially change gut microbiota composition and bacterial abundance, leading to intestinal dysbiosis [5-7]. In rats fed high-fat diets, the proportion of Bacteroidetes decreased while Firmicutes and Proteobacteria increased [8]. Dietary polyunsaturated fatty acid (PUFA) content also significantly affects gut microbiota composition in species such as Pacific white shrimp (*Litopenaeus vannamei*) [9] and Shan partridge ducks (*Anas platyrhynchos*) [10], and interactions between gut microbiota and intestinal epithelial cells lead to morphological and functional changes in the intestine [11].

As an emerging sector of aquaculture, turtle farming still relies primarily on fish oil as the dietary fat source. However, dwindling fish oil resources have made production unable to meet demand, and fish oil' s susceptibility to oxidation limits long-term storage [12]. Consequently, research on replacing fish oil with inexpensive and abundant vegetable oils, particularly soybean oil, has attracted considerable attention. Our previous studies demonstrated that replacing fish oil with soybean oil in Chinese striped-neck turtle diets significantly affects lipid absorption and utilization [13], but the effects of different diets on intestinal morphology and microbial community structure remain unexplored. Therefore, this study adjusted dietary soybean oil to fish oil ratios and employed histological staining and Illumina MiSeq high-throughput sequencing to examine changes in intestinal morphology and microbiota structure from histological and metagenomic perspectives, aiming to provide fundamental intestinal data for evaluating soybean oil replacement of fish oil in Chinese striped-neck turtle production.

1.1 Experimental Design and Feed Formulation

Using premium steam fish meal and dehulled soybean meal as protein sources, a formula diet containing 1% fish oil (imported Peruvian fish oil) was formulated as the whole fish oil diet. Based on this diet, three experimental diets were prepared by replacing 33%, 67%, and 100% of the fish oil with soybean oil (Arowana soybean oil from Yihai Kerry Food Marketing Co., Ltd.), yielding four diets with soybean oil to fish oil ratios of 0:3 (Group I, control), 1:2 (Group II), 2:1 (Group III), and 3:0 (Group IV). Feed composition and nutrient levels are shown in Table 1 . Dietary protein and lipid levels were maintained at approximately 45% and 8%, respectively. Feed ingredients were selected to minimize lipid content, passed through a 60-mesh sieve, thoroughly mixed, and processed into wet pellets using a meat grinder (by Dallan Feed Factory, Shunde District, Foshan City), then stored at -20°C.

1.2 Experimental Animals and Management

In early November 2014, 24 adult female Chinese striped-neck turtles [4 years old, average body weight (1,557±307) g] were purchased from Hongwang Agricultural Breeding Co., Ltd. in Wenchang City, Hainan Province. After a 2-week acclimation period in the breeding room on the 6th floor of the Biology Building at Hainan Normal University, turtles were randomly divided into four groups (n=6) and fed diets with fish oil to soybean oil ratios of 3:0 (Group I, control), 1:2 (Group II), 2:1 (Group III), and 3:0 (Group IV). Each group was housed in separate tanks in the same breeding room with regular water changes and disinfection using identical disinfectants and water sources. Turtles were fed twice weekly at 1% of body weight for 10 months before sampling.

1.3 Sample Processing

At the end of the experiment, turtles were anesthetized at low temperature and dissected. The duodenum was collected for histological observation, and colonic contents were gathered for microbial diversity analysis. Duodenal samples were washed with physiological saline, fixed in 4% paraformaldehyde, and processed through dehydration, embedding, sectioning, and staining for morphological observation. Five non-consecutive sections per sample were examined using a Motic digital microscope (BA310). Villus number was counted in four orthogonal fields per section; villus length, crypt depth, and muscularis thickness were measured at five different fields per section, and villus height to crypt depth ratio (VH/CD) was calculated.

Colonic contents were washed with phosphate-buffered saline (PBS) into 50 mL tubes, mixed with glycerol at a 1:4 volume ratio, and stored at -80°C. Samples were sent to Shanghai Majorbio Bio-Pharm Technology Co., Ltd. for metagenomic DNA extraction, which was verified by 1% agarose gel electrophoresis. DNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer; OD260/280 ratios of all four DNA samples ranged from 1.8 to 2.0. Extracted gut microbiota metagenomic DNA served as PCR template. Barcoded specific primers were synthesized for the target sequencing region, PCR amplification was performed, and Illumina libraries were constructed. The V3-V5 region of the 16S rRNA gene was sequenced using the Illumina MiSeq PE250 platform.

1.4 Data Processing

Duodenal histological data are expressed as mean ± standard deviation. Statistical analysis employed one-way ANOVA; when significant differences were detected ($P<0.05$), Duncan' s multiple range test was applied using SPSS 16.0 software.

Raw data from the Illumina MiSeq PE250 platform underwent quality control, with low-quality sequences removed (tail base quality <20, post-QC read length <50 bp). Operational taxonomic units (OTUs) were defined at 97% 16S rRNA sequence similarity, with each OTU representing 120 effective bases and <3% base differences (i.e., <3 base differences per sequence cluster), treated as one bacterial species in analysis. OTUs were aligned against the RDP database (Release 11.1, http://rdp.cme.msu.edu) for microbial classification. Richness index (Ace index) and -diversity indices (Shannon and Simpson indices) were calculated using Mothur 1.30.1 software.

Results

2.1 Effects of Partial or Total Fish Oil Replacement on Duodenal Histology

As shown in Table 2 , after 10 months of feeding, the duodenal VH/CD ratio in Group III did not differ significantly from Group I ($P>0.05$) but was significantly higher than in Groups II and IV ($P<0.05$). Villus length showed no significant differences among Groups I, II, and III ($P>0.05$) but was significantly lower in Group IV ($P<0.05$). Muscularis thickness in Group I was significantly lower than in Groups II and IV ($P<0.05$) but similar to Group III ($P>0.05$). Villus number did not differ significantly among groups ($P>0.05$).

2.2 Effects of Partial or Total Fish Oil Replacement on Intestinal Bacterial Diversity

Rarefaction curve analysis (Figure 1 [Figure 1: see original paper]) showed that OTU coverage plateaued with increasing sequencing depth, indicating saturation and that the sequencing results accurately reflected gut microbiota diversity in Chinese striped-neck turtles.

Table 3 presents bacterial diversity analysis based on 16S rRNA gene sequences. At 97% similarity, total OTUs in Groups I, II, III, and IV were 396, 321, 347, and 331, respectively, representing four dominant phyla: Firmicutes, Bacteroidetes, Fusobacteria, and Proteobacteria. Group I showed the highest Ace index. For - diversity, Group I exhibited the highest Shannon index (4.3) and lowest Simpson index (0.0445), while Group II showed the lowest Shannon index (2.91) and highest Simpson index (0.2367), indicating highest bacterial diversity in Group I and lowest in Group II.

2.3 Effects of Partial or Total Fish Oil Replacement on Intestinal Microbiota Structure

A total of 13 phyla and 117 genera were identified from Chinese striped-neck turtle intestines, with each sample containing 15,000 bacterial gene sequences; over 98% of bacteria were anaerobic or facultatively anaerobic.

2.3.1 Phylum-Level Taxonomic Analysis Thirteen phyla were identified: Firmicutes, Bacteroidetes, Spirochaetes, Fusobacteria, Proteobacteria, Tenericutes, Synergistetes, Actinobacteria, Planctomycetes, Verrucomicrobia, Lentisphaerae, Cyanobacteria, and SHA-109. As shown in Figure 2 [Figure 2: see original paper], all four groups shared four dominant phyla—Bacteroidetes, Firmicutes, Fusobacteria, and Proteobacteria—though their proportions varied. In Group I, Bacteroidetes (38.45%), Firmicutes (30.91%), Fusobacteria (19.79%), and Proteobacteria (7.51%) constituted the main microbiota structure. Soybean oil replacement increased Fusobacteria and Proteobacteria proportions, with Group II showing the most pronounced increase in Fusobacteria (51.85%), becoming absolutely dominant. As replacement increased further (Groups III and IV), Fusobacteria proportions decreased but remained higher than Group I, stabilizing around 30%. Bacteroidetes and Firmicutes proportions were lowest in Group II (21.67% and 15.94%, respectively). Soybean oil replacement also increased Proteobacteria proportions in Groups II, III, and IV (9.51%, 13.40%, and 10.67%, respectively). Minor phyla accounted for very low proportions, some <0.01%; Cyanobacteria and SHA-109 appeared only in Group I.

2.3.2 Genus-Level Analysis As shown in Figure 3 [Figure 3: see original paper], Groups I, II, III, and IV yielded 139, 117, 128, and 120 genera, respectively. Dominant genera were *Fusobacterium* (17.70%) and *Bacteroides* (24.19%) in Group I; *Fusobacterium* (51.17%) and *Plesiomonas* (6.85%) in Group II; *Fusobacterium* (31.95%) and *Plesiomonas* (9.25%) in Group III; and *Fusobacterium* (32.35%) and *Bacteroides* (15.01%) in Group IV.

2.3.3 Unique OTU Analysis Venn analysis (Figure 4 [Figure 4: see original paper]) revealed 43, 3, 4, and 5 unique OTUs in Groups I, II, III, and IV, respectively. Unique OTUs in Group II belonged solely to Firmicutes; those in Group IV belonged to Firmicutes and Bacteroidetes; those in Group III belonged to Firmicutes, Proteobacteria, and Tenericutes; while Group I contained the most diverse unique OTUs across nearly all phyla, predominantly Firmicutes and Bacteroidetes.

Intestinal bacterial diversity results from strong host-microbe selection and co-evolution, but is also influenced by dietary composition and structure. In this study, the whole fish oil group (Group I) showed the highest Ace and Shannon indices and the greatest number of phyla and genera. Partial or total fish oil replacement by soybean oil decreased bacterial diversity and richness, with the 33% replacement group (Group II) showing the lowest Ace and Shannon indices. Although basal diet composition was identical across groups, fish oil is rich in n-3 PUFAs while soybean oil is rich in n-6 PUFAs, suggesting that PUFA types and n-3/n-6 ratios affect gut bacterial diversity and richness. This aligns with Zhang et al. [9] regarding lipid source effects on shrimp gut microbiota. Different bacteria have distinct nutritional requirements, and nutrient sources largely determine microbiota structure and function. When nutrient sources change, microbiota composition and abundance shift accordingly. Zhang et al. [8] found that high-fat diets decreased Bacteroidetes and Bifidobacteria while increasing Firmicutes and Proteobacteria in rats. Firmicutes and Bacteroidetes dominate the gut microbiota of reptiles (snakes, lizards), humans, and other mammals, possibly due to their roles in food re-digestion and metabolism [14-16]. In this study, Chinese striped-neck turtle gut microbiota was dominated by Firmicutes, Fusobacteria, Bacteroidetes, and Proteobacteria; soybean oil replacement decreased proportions of Bacteroidetes and Firmicutes—key phyla for digestion and absorption—but the 67% replacement group (Group III) showed the smallest decrease, most closely resembling the whole fish oil group.

As an internal environmental factor, gut microbiota produces various metabolites that can be beneficial or harmful to the host. Interactions between microbiota and gastrointestinal epithelial cells induce structural and functional changes in the digestive tract. Intestinal digestion and absorption are influenced by morphological indices such as villus length and crypt depth [17-18]. Villus length determines enterocyte number, while crypt depth reflects epithelial cell generation rate; deeper crypts indicate active proliferation but lower maturity [17]. The VH/CD ratio comprehensively reflects duodenal function status, with higher values indicating enhanced digestion and absorption [18]. Reports on dietary nutrient effects on small intestine morphology vary. Zeitz et al. [19] found that dietary oils rich in lauric and myristic acids significantly altered intestinal histology, particularly villus length, in broilers. Chen et al. [20] reported that increased duodenal villus length significantly promoted nutrient absorption. Ngoc et al. [21] observed that cassava residue and brewer' s grains increased jejunal and ileal villus length in pigs without affecting duodenal villus length. Other studies show dietary fiber does not significantly affect porcine small intestine morphology [22]. Du et al. [10] found that 2% dietary fish oil significantly decreased VH/CD ratio and goblet cell number in Shan partridge ducks. In this study, the 67% soybean oil group and whole fish oil group showed similar duodenal VH/CD ratios, both significantly higher than the 33% soybean oil and whole soybean oil groups. This suggests that whole fish oil and 67% soybean oil groups had superior enterocyte numbers and maturity compared to 33% soybean oil and whole soybean oil groups, which may affect digestive enzyme secretion—consistent with Qiu et al. [13] showing higher enzyme activities in Groups I and III.

Gut microbiota influences goblet cell growth and intestinal mucosal integrity through bioactive substances or indirect immune system activation, playing crucial roles in host nutrition and immunity. Thus, microbiota affects lipid digestion and modifies protein and carbohydrate digestion, increasing energy and protein requirements [23]. Osborn and Olefsky [24] demonstrated that gut microbiota can directly regulate host gene expression to control lipid metabolism, enhancing hepatic fatty acid synthase (FAS) activity while suppressing expression of fasting-induced adipose factor, a gene that inhibits fat accumulation. The specific effects of gut microbiota on nutrient digestion, absorption, and immunity in Chinese striped-neck turtles warrant further investigation.

Conclusions

1. The 67% soybean oil replacement group exhibited similar intestinal morphology to the whole fish oil group in Chinese striped-neck turtles.
2. Partial or total replacement of fish oil by soybean oil decreased intestinal bacterial diversity in Chinese striped-neck turtles.
3. The 67% soybean oil replacement group showed proportions of Bacteroidetes and Firmicutes most similar to the whole fish oil group.

References

[1] ECKBURG P B, BIK E M, BERNSTEIN C N, et al. Diversity of the human intestinal microbial flora[J]. Science, 2005, 308(5728):1635-1638.

[2] QIN J J, LI R Q, RAES J, et al. A human gut microbial gene catalogue established by metagenomic sequencing[J]. Nature, 2010, 464(7285):59-65.

[3] SOCCINI C, FERRI V. Bacteriological screening of *Trachemys scripta elegans* and *Emys orbicularis* in the Po plain (Italy)[J]. Biologia, Bratislava, 2004, 59(Suppl.14):201-207.

[4] 杜爽, 张文飞, 史海涛. 基于 16S rRNA 序列分析红耳龟肠道拟杆菌和厚壁杆菌菌群多样性[J]. 基因组学与应用生物学,2013,32(6):700-706.

[5] 陈杏云, 曾本华, 魏泓, 等. 高脂饮食对菌群人源化小鼠肠道菌群结构的影响 [J]. 食品科学,2013,34(17):278-283.

[6] 曹宏芳, 张家超, 王芳, 等. 高脂饮食对雄性 SD 大鼠肠道菌群的影响 [J]. 中国微生态学杂志,2012,24(2):102-108.

[7] 刘雪姬, 陈庆森, 闫亚丽. 高脂饮食对小鼠肠道菌群的影响 [J]. 食品科学,2011,32(23):306-311.

[8] ZHANG C H, ZHANG M H, PANG X Y, et al. Structural Resilience of the gut microbiota in adult mice under high-fat dietary perturbations[J]. The ISME Journal, 2012, 6(10):1848-1857.

[9] ZHANG M L, SUN Y H, CHEN K, et al. Characterization of the intestinal microbiota in Pacific white shrimp, *Litopenaeus vannamei*, fed diets with different lipid sources[J]. Aquaculture, 2014, 434:449-455.

[10] DU X, XU X Q, TIAN Y, et al. Morphology and microbiota of different section of intestines and their influences induced by dietary fish oil in Shan partridge ducks (*Anas platyrhynchos*)[J]. Journal of Agricultural Biotechnology, 2016, 24(11):1652-1663.

[11] LIU T Y, HOUGEN H, VOLLMER A C, et al. Gut bacteria profiles of *Mus musculus* at the phylum family levels influenced by saturation of dietary fatty acids[J]. Anaerobe, 2012, 18(3):331-337.

[12] PICKOVA J, MØRKØRE T. Alternate oils in fish feeds[J]. European Journal of Lipid Science and Technology, 2007, 109(3):256-263.

[13] 裘正元, 盛成, 洪美玲, 等. 豆油替代鱼油对中华条颈龟血脂代谢及相关酶活的影响 [J]. 水产科学,2015,34(7):453-458.

[14] COSTELLO E K, GORDON J I, SECOR S M, et al. Postprandial remodeling of the gut microbiota in Burmese pythons[J]. The ISME Journal, 2010, 4(11):1375-1385.

[15] HONG P Y, WHEELER E, CANN I K O, et al. Phylogenetic analysis of the fecal microbial community in herbivorous land and marine iguanas of the Galàpagos Islands using 16S rRNA-based pyrosequencing[J]. The ISME Journal, 2011, 5(9):1461-1470.

[16] MACKIE R I, NELSON D M, WHEELER E, et al. Fermentative digestion in herbivorous lizards: bacterial population analysis in the intestinal tract of free-living land (*Conolophus pallidus*) and marine iguanas (*Amblyrhynchus cristatus*) on the Galapagos archipelago[M]//MORRIS S, VOSLOO A. *Molecules to Migration: The Pressures of Life*. Bologna, Italy: Medimond Publishing Company, 2008:193-202.

[17] 刘秋东, 张中文, 刘凤华, 等. 复方白头翁胶囊对腹泻犬小肠绒毛长度和隐窝深度的影响 [J]. 北京农学院学报,2011,26(3):38-40.

[18] 刘晓静, 史彬林, 赵育国, 等. 日粮中添加沙蒿籽粉对肉仔鸡肠绒毛形态及肠道菌群的影响 [J]. 饲料工业,2011,32(5):13-15.

[19] ZEITZ J, FENNHOFF J, KLUGE H, et al. Effects of dietary fats rich in lauric and myristic acid on performance, intestinal morphology, gut microbes, and meat quality broilers[J]. Poultry Science, 2015, 94(10):2404-2413.

[20] 陈丛亮, 杨洪勋, 杨磊, 等. 半胱胺提高畜禽营养物质消化、吸收和利用率的作用机制探讨 [J]. 饲料研究,2008(12):18-21.

[21] NGOC T T B, HONG T T T, LEN N T, et al. Effect of fibre level and fibre source on gut morphology and micro-environment in local (Mong Cai) and exotic (Landrace×Yorkshire) pigs[J]. Asian-Australasian Journal of Animal Sciences, 2012, 25(12):1726-1733.

[22] CHEN H, MAO X B, CHE L Q, et al. Impact of fiber types on gut microbiota, gut environment and gut function fattening pigs[J]. Animal Feed Science and Technology, 2014, 195(9):101-111.

[23] GABRIEL I, LESSIRE M, MALLET S, et al. Microflora of the digestive tract: critical factors and consequences for poultry[J]. World' s Poultry Science Journal, 2006, 62(3):499-511.

[24] OSBORN O, OLEFSKY J M. The cellular and signaling networks linking the immune system and metabolism in disease[J]. Nature Medicine, 2012, 18(3):363-374.

Note: Figure translations are in progress. See original paper for figures.