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Transcriptomic analysis reveals effects of fucoxanthin on intestinal glucose transport☆

Wanxiu Cao[a], Jing Li[a], Yaoxian Chin[a], Robert W. Li[b], Changhu Xue[a], Qingjuan Tang[a,*]

[a] College of Food Science and Engineering, Ocean University of China, Qingdao, China
[b] Animal Genomics and Improvement Laboratory, United States Department of Agriculture, Agriculture Research Service (USDA–ARS), Beltsville, MD, USA

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ABSTRACT

Fucoxanthin, one of carotenoid pigments from plants and algae, is known to regulate blood glucose and insulin levels. The mechanism of its hypoglycemic activity has drawn a lot of scientific interest in recent years. In this study, we investigated the effects of fucoxanthin on intestinal glucose transport using a murine model. Our data demonstrated that fucoxanthin was able to decrease blood glucose level and alleviate insulin resistance significantly. The results from RNA-seq based transcriptomic analysis, suggested that fucoxanthin acted as a key regulator in Insulin/PI3K/AKT/mTOR signaling and PKA/AMPK/mTOR signaling pathways. Moreover, fucoxanthin ingestion resulted in a significant reduction in the protein expression of intestinal glucose transporters, such as SGLT-1, and led to decreased translocation of GLUT-2, which contributed to the regulation of blood glucose level. Together, our findings provided a mechanistic insight into the regulatory effect of fucoxanthin on blood glucose.

1. Introduction

Several recent studies have demonstrated that high-fat high-sugar (HFS) diets can contribute to impaired glucose homeostasis, which results in a metabolic syndrome, such as obesity, hyperglycemia and impairment of insulin sensitivity (Ait-Omar et al., 2011; Ghanim et al., 2009). Despite decades' efforts by medical and scientific communities, long-term results aimed at proper maintenance of glucose homeostasis have proved to be disappointing. As a result, searching for more effective hypoglycemic ingredients, especially those from natural products, has become increasingly important.

Fucoxanthin is a natural xanthophyll produced by plants and algae, such as edible brown seaweeds. Previous experimental findings (Lin, Tsou, Chen, Lu, & Hwang, 2017; Woo et al., 2010) have described that dietary fucoxanthin is a potential compound for decreasing blood glucose levels and mitigating insulin resistance. While it is still not fully understood how fucoxanthin regulates glucose homeostasis, several hypotheses have been proposed recently, including the reduction in the insulin/glucagon ratio, upregulation of glucose transporter 4 translocation and its expression in muscles, or the inhibition of intestinal glucose uptake (Lee & Han, 2012; Miyashita et al., 2011; Miyashita, Mikami, & Hosokawa, 2013).

Intestinal glucose absorption plays a vital role in the regulation of blood sugar concentrations (Ferraris, 2001). Fucoxanthin may regulate blood glucose level partly through its inhibition of intestinal glucose transporters. Besides, several observations suggested that seaweed extracts influence glucose absorption by regulating glucose transporters (Murugan et al., 2015; Zhao et al., 2017). Intestinal glucose absorption involves at least two modes: active transport by sodium glucose transport protein-1 (SGLT-1) and passive transport by glucose transporter-2 (GLUT-2) (Abbasi, Purslow, Tosh, & Bakovic, 2016; Shirazi-Beechey, Moran, Batchelor, Daly, & Al-Rammahi, 2011). Glucose uptake from the gut lumen into the enterocytes is primarily mediated by SGLT1, which is located on the brush border membrane. Further transport from enterocytes into blood across the basolateral membrane is then facilitated by GLUT-2. In addition, GLUT-2 can insert itself into the apical membrane of intestinal epithelial cells in response to lumen high glucose concentrations, a process known as translocation. This part of GLUT-2 also mediates glucose transport into the enterocytes, and is able to transiently increase glucose uptake by 3–5 times through this process. Permanent translocation of GLUT-2 is a characteristic of insulin-resistant state induced by long-term high fat diet (Kellett, Brot-Laroche, Mace, & Leturque, 2008).

In recent years, the advancement in transcriptomic analysis has greatly enhanced the capability of analyzing the alterations of genes expression in nutritional intervention (Wang et al., 2017; Zhou et al.,

☆ For raw data on Mendeley Data, please visit https://doi.org/10.17632/yfm7y22s88.1.

* Corresponding author at: Food Science and Engineering, Ocean University of China, No. 5 Yushan Road, Qingdao, Shandong Province 266003, China.
E-mail address: tangqingjuan@ouc.edu.cn (Q. Tang).

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2012). In this study, we hypothesized that fucoxanthin might decrease blood glucose levels partly through its regulatory effect on intestinal glucose transport. In order to verify our hypothesis and reveal the possible hypoglycemic mechanism of fucoxanthin, we performed RNA-seq based transcriptomic analysis and correlative pathway analysis using a murine model.

2. Material and methods

2.1. Source of fucoxanthin

Fucoxanthin (purity > 98%) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China).

2.2. Animals and treatments

Male C57BL/6J mice (bodyweight: 18–20 g, age: 4-week old) were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The animals were all individually housed in polycarbonate cages at 24 ± 2 °C on a 12 h light/dark cycle.

After a week of acclimatization, the mice were randomly divided into 4 groups (n = 8 per group), as illustrated in Fig. 1. One group was fed a control diet (10% calories in fat, 7% calories in sugar), and the other groups were fed a high-fat high-sugar diet (HFS; 45% calories in fat, 17% calories in sugar) for 8 weeks. At the end of 8 weeks, mice fed with HFS were regrouped according to their body weights and reassigned into 3 groups. The mice in all groups were continuously fed their respective diets for additional 4 weeks. At the meantime, fucoxanthin (dissolved in 200 μL soybean oil) was fed via oral garage at a daily dose of 150 mg/kg body weight (the F group). Similarly, the equal amount of soybean oil alone (200 μL) was delivered via oral garage to the solvent group (the S group) while the control (the C group) and model (the M group) groups were fed via oral garage of 200 ul of saline. To eliminate the effect of administration of soybean oil by gavage, the equivalent amount of soybean oil was deducted from HFS diets in both F and S groups.

This study complied with the ARRIVE guidelines and was carried out according to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). All experiment protocols were approved by the Committee on the Ethics of Animal Experiments of Ocean University of China (Approved protocol ID SCKK2012-0001).

2.3. Oral Glucose Tolerance Test (OGTT)

OGTT was performed after mice were maintained for 11 weeks. After fasting for 12 h, mice were administered 2 g/kg body weight of glucose by oral gavage, and blood samples were then collected from tail vein at 30-minute intervals for a total of 120 min (i.e., 0, 30, 60, 90 and 120 min). The serum glucose levels were determined using a commercial kit (Biosino glucose assay kit., Beijing, China) following manufacturer's protocol.

2.4. Sample collection

At the end of the experimental period (12 weeks in total), the mice were anesthetized and killed by cervical dislocation after fasting for 12 h. The jejunum tissue was removed and divided into two segments, and immediately frozen in liquid nitrogen. All samples were stored at −80 °C until analysis.

2.5. Biochemical analysis

Serum was prepared, and analyzed for fasting blood glucose (FBG) and fasting insulin (FINS). FBG was analyzed with the same kit used in OGTT. FINS was determined with a commercial ELISA kit (Cloud Clone Corp. ELISA Kit for Insulin, USA) following the instructions of manufacturer. Insulin resistance was estimated by homeostatic model assessment of insulin resistance (HOMA-IR).

2.6. RNA preparation and transcriptomic analysis

Total RNA from 32 jejunum samples was extracted as previously described (Shi et al., 2017). The RNA integrity was tested using Agilent Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA). An Illumina TruSeq RNA sample prep kit (Illumina, San Diego, CA, USA) was used to deal with high quality RNA (RNA Integrity number or RIN > 9.0). After sequencing, quality control procedures, pooling of individual RNA-seq libraries, checking of raw sequence reads and subsequent operation were conducted as previously described (Baldwin et al., 2012).

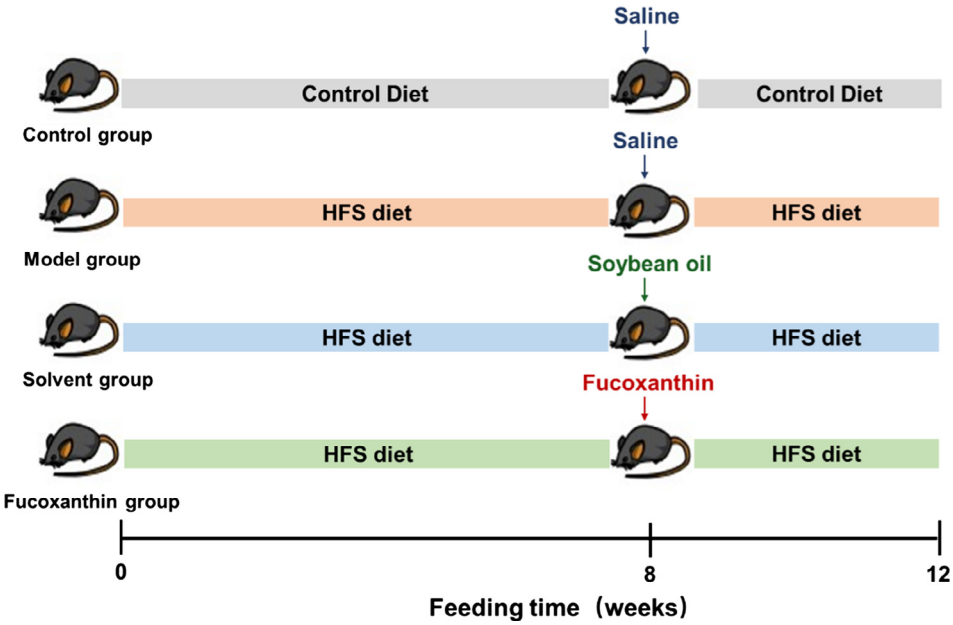

Fig. 1. Schematic of the experimental procedures. Mice were fed control diet or high-fat high-sugar (HFS) diet for a period of 12 weeks. In the final 4 weeks of the experiment, all groups were concomitantly administrated equal amount of saline, soybean oil or fucoxanthin by gavage, respectively.