High-fat Diet Induced Dysbiosis & Amelioration by Astaxanthin

Kyle Haasbroek[1], Wakako Takabe[1], Masayuki Yagi[1], Yoshikazu Yonei[1]

[1] Anti-Aging Medical Research Center and Glycation Stress Research Center, Graduate School of Life and Medical Sciences, Doshisha University, Japan

Abstract:

Astaxanthin is a carotenoid that is present in high quantities in the meat of fish like salmon and the shells of shrimp and crab. It exhibits free radical scavenging antioxidant activity when consumed dietarily. Astaxanthin is absorbed by the small intestine before exerting its antioxidant effect; however, a portion of dietary intake remains unabsorbed in the digestive tract and reaches the large intestines . We hypothesized that astaxanthin may exert its antioxidant action in the large intestine to influence the gut microbiota. In this review we introduce the results of two studies of astaxanthin. Firstly, a clinical trial targeting post-menopausal women screened for high oxidative stress burden. Astaxanthin was administered orally for eight weeks in order to examine its effects and safety, and subjects were surveyed for any changes in subjective symptoms. Secondly, in a mouse model, real time PCR (polymerase chain reaction) was used to examine the ability of astaxanthin to prevent changes in the enteric flora induced by a high-fat diet. When fat intake increases due to changes in diet, the equilibrium between the various species that constitute the intestinal flora is altered. As a result, degenerative changes in lifestyle-related disease and aging of the host are promoted. Here we find that the intake of astaxanthin was able to inhibit these changes in the gut microbiota of mice induced by a high-fat diet. Even in humans, it is highly probably that the unabsorbed astaxanthin that remains in the intestinal tract exerts a positive effect against disturbance of the intestinal flora caused by a high-fat diet.

Keywords: Astaxanthin; Gut microbiota; Antioxidant; High fat diet; Dysbiosis

Sažetak:

Disbioza inducirana dijetom bogatom mastima & Amelioracija pomoću astaksantina

Astaksantin je karotenoid prisutan u velikom količinama u mesu riba poput lososa i oklopima kozica i rakova. Kada se uzima kao hrana ispoljava svoju sposobnost hvatanja slobodnih radikala i antioksidativnog djelovanja. Prije no što započne svoje antioksidativno djelovanje apsorbira se preko tankog crijeva, međutim , dio ostaje neapsorbiran i dospijeva u debelo crijevo. Razmatrali smo mogućnost da astaksantin pomoću svojih antioksidativnih svojstava može utjecati na crijevnu mikrobiotu. U okviru ovog preglednog rada osvrnut ćemo se na rezultate dvaju istraživanja. Prvo je kliničko ispitivanje usmjereno na postmenopauzalne žene koje su prošle screening na opterećenost oksidativnim stresom. Astaksantin su dobivale oralno kroz 8 tjedana kako bi se procijenili njegovi učinci i sigurnost, a ispitanice su anketirane vezano za promjene u subjektivnim simptomima. Drugi, mišji model, koristio je PCR (Lančanu reakciju polimeraze) u pravom vremenu kako bi ispitao potencijal astaksantina u sprječavanju promjena u crijevnoj flori induciranih dijetom bogatom mastima. Pri dijeti bogatoj mastima povećava se unos masti što narušava ravnotežu raznih vrsta koje sačinjavaju intestinalnu floru. Posljedična neravnoteža rezultira degenerativnim promjenama i bolestima vezanima za način života. U ovom istraživanju nalazimo da unos astaksantina može spriječiti promjena u mikrobioti miševa izazvane prehranom bogatom mastima. Čak i u ljudi je za vjerovati da neapsorbirani astaksantin koji ostaje u debelom crijevu ima pozitivan učinak protiv poremećaja uzrokovanih prehranom bogatom mastima.

Ključne riječi: Astaksantin; Crijevna mikrobiota; Antioksidans; Prehrana bogata mastima; Disbioza

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Correspondence:
Yoshikazu Yonei
yyonei@mail.doshisha.ac.jp
orcid.org/0000-0002-2682-752X

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Introduction

Astaxanthin is a carotenoid that is present in high quantities in the meat of fish like salmon and the shells of shrimp and crab[1]. It has attracted much attention in the field of preventative medicine for its effects against various lifestyle-related illnesses, its anti-fatigue action, and general health improving properties[2]. Astaxanthin is also garnering attention as an ingredient in supplements, for which it is naturally produced by the microalgae Haematococcus pluvialis. Astaxanthin exhibits free radical scavenging antioxidant activity when consumed dietarily[3,4]. Previously we performed an open-label non-controlled study examining the safety and efficacy of eight-week oral astaxanthin intake in post-menopausal women with high oxidative stress burden, demonstrating increased antioxidant capacity, lower blood pressure, and improvement of constipation and menopause symptoms[5]. Astaxanthin is absorbed by the small intestine before exerting its antioxidant effect; however, a portion of dietary intake remains unabsorbed in the digestive tract and reaches the large intestines[6-8]. We hypothesized that astaxanthin may exert its antioxidant action in the large intestine to influence the gut microbiota (enteric flora), and are continuing research into the phenomenon.

The enteric flora changes with age and lifestyle/environment[9,10], and is associated with overall health and the incidence of carcinogenesis. Every individual's intestinal tract is inhabited by a characteristic ecosystem of bacteria that collectively forms the gut microbiome. Normally there is equilibrium between the gut microbiota and host, as well as between bacterial species. When this balance is disrupted, dysbiosis[11] and opportunistic infections occur, greatly influencing aging[12], nutrition[13], the efficacy of medication[14,15], physiological function[16], immune function[17], and carcinogenesis[18-20] of the host.

In this review we introduce the results of two studies of astaxanthin. Firstly, a clinical trial[5] targeting post-menopausal women screened for high oxidative stress burden. Astaxanthin was administered orally for eight weeks in order to examine its effects and safety, and subjects were surveyed for any changes in subjective symptoms. Secondly, in a mouse model, real time PCR (polymerase chain reaction)[21-23] was used to examine the ability of astaxanthin to prevent changes in the enteric flora induced by a high-fat diet[24].

Clinical Study Methods

In this study, participants were screened in order to select those with high oxidative stress. The screening test measured diacron-reactive oxygen metabolites (d-ROM) as an indicator of the intensity of oxidative stress burden. From an initial group of 35 healthy post-menopausal women (d-ROM = 362.3 ± 51.4 CARR U), 14 with low d-ROM scores were excluded, and the remaining group of 21 participants (d-ROM = 387.4 ± 32.4 CARR U) with high oxidative stress burden comprised the participants of this study. Participants took two 6 mg astaxanthin soft capsules ([AstaReal ACT] Fuji Chemical Industry Co.) per day for 8 weeks.

For the evaluation, symptoms were divided into "physical symptoms" and "psychological symptoms" in the Anti-Aging QOL Common Questionnaire 25). Participants were asked to rank their severity on a scale of one to five.

Table 1. Symptom Scores from Anti-Aging QOL Common Questionnaire

	Baseline	Week 8
(Physical Symptoms)		
Eye Fatigue	3.1±0.8	2.4±0.8 **
Stiff Shoulders	3.4±1.0	2.9±1.1 **
Weight Gain	2.9±1.4	2.5±1.4 *
Skin Problems	2.5±0.8	2.1±0.8 **
Constipation	2.4±1.2	2.0±1.0 *
Hair Loss	2.3±0.9	1.9±0.8 *
Gray Hair	3.6±0.9	3.1±0.9 *
Cold Sensitivity	2.8±1.0	2.3±1.1 **
(Psychological Symptoms)		
Difficulty Falling Asleep	2.1±0.89	1.8±0.8 *

Symptom Score: 1 not at all, 2 a little, 3 moderately, 4 quite a lot, 5 very much.
** $p < 0.05$, ** $p < 0.01$ vs. baseline score, paired t-test. Cited from reference 5).*

Of the 34 surveyed physical symptoms, eight symptoms significantly improved after 8 weeks of taking the supplement: "eye fatigue," "stiff shoulders," "weight gain," "skin problems," "constipation," "hair loss," "grey hair," and "cold sensitivity" (Table 1). Of 21 psychological symptoms, "difficulty falling asleep" improved significantly after 8 weeks. There was no significant change in the lifestyle habits of the participants.

Improving "constipation" is one of the known benefits of astaxanthin, and is commonly reported in post marketing surveillance. Astaxanthin also relieves loose stool and diarrhea associated with abnormal intestinal fermentation. Its bioavailability is low at 2~5%, with the greater majority remaining and acting within the digestive tract. Astaxanthin may be exerting antioxidant action, clearing free radicals derived from bacterial toxins produced by non-beneficial species, improving the intestinal environment and increasing motility as a result.

Experimental Animal Study Methods

The experimental method is as follows[24]. 20 4-week-old ICR mice were raised in individual cages for one week and acclimatized. After acclimatization, the mice were weighed and sorted into the following 4 groups consisting of 5 mice each: ordinary diet (Group C), ordinary diet + astaxanthin (Group CA), high-fat diet (Group H), high fat diet + astaxanthin (Group HA).

Labo MR Stock (Nosan Corporation) was used for the ordinary diet, and HFD-60 Oriental Yeast Co.) for the high-fat diet. Both types of feed were prepared with added astaxanthin (Fuji Chemical Industry Co., Ltd.) to achieve a final concentration of 0.02%. The nutritional contents of the ordinary diet consisted of 3.9% fat, 18.8% protein, 54.7% carbohydrates, and 6.6% dietary fiber; the high fat diet contained 35% fat, 23% protein, 25.3% carbohydrates, and 6.6% dietary fiber. Feces were collected over 24 hours on the final acclimatization day, hereafter designated Day 0. The collected feces were stored at −20 °C.

Fecal bacteria were tested using real-time PCR as follows. Feces collected on Days 0, 14, and 28 were thawed and weighed before drying overnight at room temperature. The following day, dry mass was weighed and samples were powderized. Approximately 100 mg of each powdered sample was weighed into a microtest tube, and DNA was extracted using the QIAamp® DNA Stool Mini Kit (Qiagen). DNA solutions were prepared and DNA concentration was measured using NanoDrop.

DNA solutions for total bacteria, Lactobacillus, Streptococcus, Clostridium coccoides group, and Clostridium leptum were diluted 100 times; other targets were adjusted to a final concentration of 20 μg/μL. Primer sequences were determined in accordance with the protocol for the LightCycler® 480 SYBR Green I Master (Roche Diagnostics), and real-time PCR analysis was performed following the

methods of Matsuki et al. or Endo et al.[21-23] Bacteroides, Bifidobacterium, Prevotella, Lactobacillus, Clostridium Coccoides, and Clostridium leptum were targeted for analysis alongside two phyla, Firmicutes and Bacteroidetes, as well as total bacteria. The copy numbers of the targets were standardized as a percentage of total bacterial copies.

Results of the Experimental Study

Change in body weight, feces color and quantity

Mice administered the high-fat diet demonstrated a significant increase in weight ($p < 0.05$) compared to ordinary diet groups (comparing H to C, HA to CA). No significant difference in weight was detected in the astaxanthin fed groups. The color of feces changed to a grayish white in the high-fat diet group mice, and became a wine red after the addition of astaxanthin. The amount of feces collected from the high-fat diet groups on Day 28 was significantly reduced ($p < 0.001$).

Fecal DNA Content

The amounts of DNA recovered from feces as measured by NanoDrop (mean of Day 0, 14, 28) were: Group C: 89.8 ± 21.5 ng/μL; Group CA: 102.7 ± 28.5 ng/μL; Group H: 70.2 ± 21.2 ng/μL; Group HA: 66.9 ± 28.4 ng/μL. When compared to the ordinary diet groups (Group C and Group CA), the high-fat diet groups (Group H and Group HA) tended to have less DNA content ($p = 0.001$).

Genus Streptococcus (lactic acid bacteria)

Streptococcus comprised less than 16% of the total bacterial count (Figure 1). The number of bacteria increased substantially in the ordinary diet, but the change was minor in the high-fat diet ($p = 0.028$). Compared to the ordinary diet, the increase in bacteria in the high-fat diet group was extremely small ($p = 0.008$). With the addition of astaxanthin, the bacterial count of Group HA returned to the same level as the ordinary diet groups (Groups C and CA) on Day 28. The difference with and without astaxanthin in the high-fat diet groups was significant ($p = 0.044$).

The genus Streptococcus is comprised of a variety of gram-positive and generally facultative anaerobic bacteria which belongs to the order Lactobacillales (lactic acid bacteria). While Streptococcus pneumoniae is a well-known pathogenic species (the leading cause of pneumonia), many other species are members of the salivary and gut microbiomes. At the genus level, depletion of Streptococcus has been observed in obese teenagers with high fructose intake[26] and in children with celiac disease[27]. Conversely, Streptococcus is reported to increase in abundance in the guts of infants with a genetic predisposition for type 1 diabetes[28] as well as in colorectal cancer patients[29]. The seemingly contradictory implications of these different shifts in abundance may be due to the specific species which are falling out of balance in each situation.

On the species level, several bacteria are noted in the literature as potential probiotics. Streptococcus salivarius is an inhabitant of both the oral cavity and the gut: it has been demonstrated to possess anti-inflammatory and immuno-regulatory effects[30,31]. Streptococcus thermophilus, another probiotic species, may help to reduce uremic toxins caused by dysbiosis in the colon of patients with chronic kidney diseases[32]. As part of a probiotic cocktail together with other lactic acid bacteria (IRT5), S. thermophilus has shown efficacy in modulating auto-immunity[33-35]. By increasing the abundance of Streptococcus species, astaxanthin may contribute to maintaining the microbiome's homeostasis by promoting the proliferation of these beneficial bacteria.

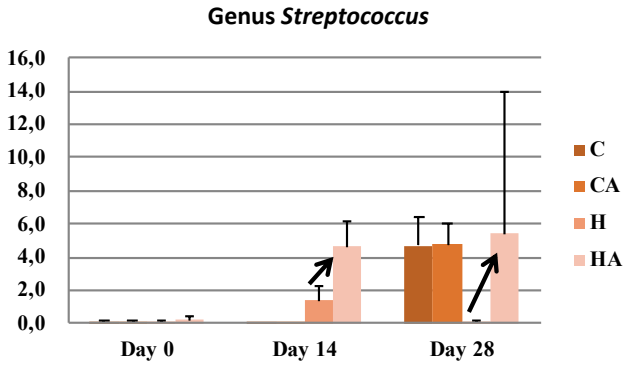

Figure 1. Putative copy numbers of the genus Streptococcus
Results shown as percent of total bacterial copy number, mean ± standard deviation. C: ordinary diet group, CA: ordinary diet + astaxanthin group, H: high-fat diet group, HA: high-fat diet + astaxanthin group, (n = 5 each). The addition of astaxanthin increased bacterial count in the high-fat diet group (indicated by arrows, $p < 0.05$). Groups compared using Tukey's multiple comparison test. Cited from reference 24).

Genus Lactobacillus (lactic acid bacteria)

Lactobacillus comprised less than 3% of total bacterial copy numbers (Figure 2). Bacterial count was increased significantly in the high-fat diet group ($p = 0.004$). The ordinary meal groups (groups C and CA) also showed a trend of increasing bacterial count on days 14 and 28. There was no significant difference in the rate of bacterial count increase between the ordinary and high fat diets ($p = 0.069$). The rate of increase of bacterial count was shown to significantly increase with the addition of astaxanthin ($p = 0.031$).

The genus Lactobacillus is widely known as a prominent member of the lactic acid bacteria and a common source of probiotics. As such, a large amount of research has focused on the beneficial effects of Lactobacillus species on gut health. In the early days of research into the human microbiomes, Lactobacillus was easily cultured from feces and mistakenly thought to constitute a majority of the gut microbiome[36]. With the advent of genomic techniques to detect the countless species that are not easily cultivated in the lab (many of which are anaerobic), it was realized that Lactobacillus species are in actuality a small minority of the total biological diversity of the human gut, and many species are only transitory inhabitants that do not maintain a permanent presence in the intestinal tract. Despite this, the health effects of various Lactobacillus species have been heavily studied and numerous benefits have been reported.

Lactobacillus sakei has demonstrated efficacy in ameliorating obesity

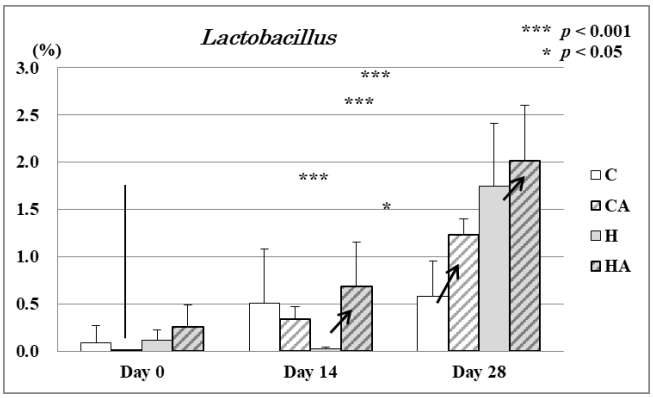

Figure 2. Putative copy numbers of the genus Lactobacillus
Results shown as percent of total bacterial copy number, mean ± standard deviation. C: ordinary diet group, CA: ordinary diet + astaxanthin group, H: high-fat diet group, HA: high-fat diet + astaxanthin group, (n = 5 each). Addition of astaxanthin raised bacterial count (indicated by arrows, p < 0.05). Groups compared using Tukey's multiple comparison test. Cited from reference 24).

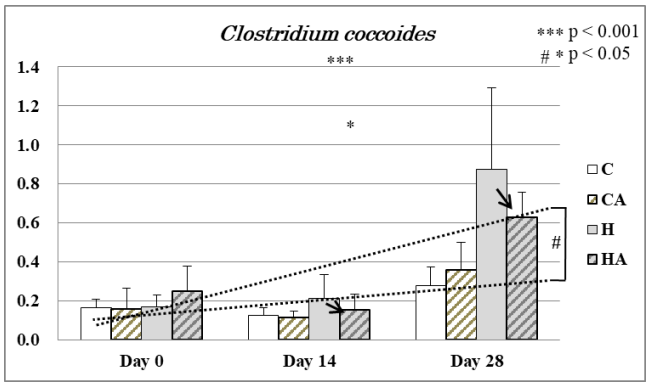

Figure 3. Putative copy numbers of the Clostridium coccoides group
Results shown as percent of total bacterial copy number, mean ± standard deviation. C: ordinary diet group, CA: ordinary diet + astaxanthin group, H: high-fat diet group, HA: high-fat diet + astaxanthin group, (n = 5 each). High-fat diet increased the rate of growth of bacterial count, (indicated by dotted lines), and addition of astaxanthin reduced it (indicated by arrows, p < 0.05). Groups compared using Tukey's multiple comparison test, rate of change by diet compared using Mann-Whitney's U test. Cited from reference 24).

caused by a high-fat diet in mouse models[37,38]. Mice fed L. sakei as a probiotic show reduced weight gain and lower serum levels of cholesterol, triglycerides, and leptin. Serum and fecal levels of short chain fatty acids are also increased after ingestion of L. sakei, counteracting the reduction seen in high-fat diets.

Lactobacillus plantarum has also been studied in mice fed high-fat diets[39]. When fed the probiotic, mice consuming a high-fat diet again experienced reduced weight gain and lower plasma levels of leptin, cholesterol, and triglycerides. It is also able to mitigate liver damage and weakened intestinal permeability of the intestines caused by obesity. Additionally, L. plantarum has been reported to increase the diversity of the enteric flora[39,40], reduce the severity of colitis[41,42], and it has also demonstrated anti-tumor effects[43].

Lactobacillus rhamnosus has been shown to decrease the amount of colon aberrant crypt foci and reduce pro-carcinogenic biomarkers in a rat model[44], lower cholesterol[45], and improve insulin resistance and obesity induced by high-fat diet[46].

Lactobacillus casei has demonstrated immunoregulatory activity, reducing colorectal cancer[46] and breast tumors[48] in mice models.

Finally, Lactobacillus acidophilus has many reported beneficial effects. It has been found to improve gut dysbiosis, intestinal barrier function, and liver pathology in non-alcoholic fatty liver disease[49]. Like several of the previous species, it reduces high-fat diet induced obesity and improves insulin resistance[46]. It also demonstrates an anti-inflammatory effect, protecting mice from induced colitis[50,51]. In humans, L. acidophilus has been observed to be significantly reduced in the feces of type 2 diabetics[52].

Clostridium coccoides Group

The Clostridium coccoides group contributed less than 1.4% of the total bacterial count (Figure 3). Bacterial count significantly increased from the high-fat diet (p = 0.016). The increase was greater in the high-fat diet than the ordinary diet (p = 0.012). The addition of astaxanthin was revealed to significantly lower the increase of bacteria in the high-fat diet (p = 0.029).

Clostridium is a major genus of anaerobic endo-spore forming bacteria[53]. Several species in the genus are known pathogens, such as Clostridium botulinum (the causative pathogen of botulism), Clostridium tetani (tetanus) and former member Clostridium difficile (now Clostridioides difficile). Conversely, the Clostridium coccoides group also contains many bacteria that inhabit the human gut and play a considerable role in the intestinal ecosystem by fermenting dietary plant polysaccharides.

As a group, Clostridium has been reported to shift with age, becoming less abundant in the elderly[53]. This diminishing of Clostridium is associated with complications of aging such as frailty, hospitalization, and antibiotic treatment. However, Clostridium is also found to be elevated in the gut of children with type-1 diabetes, and its abundance is positively correlated with plasma glucose levels[54]. Clostridium coccoides group is also significantly increased in the stool of obese individuals with metabolic syndrome and its abundance is correlated with higher serum levels of triglycerides[55]. Much like the genus Streptococcus, the effects of these shifts in abundance may differ depending on the specific species involved.

At the species level, Clostridium asparagiforme, Clostridium scindens, and Clostridium hylemonae are reported at high frequency in the elderly[53]. C. scindens and C. hylemonae exhibit bile-acid 7-dehydroxylating activity, producing secondary bile acids which are associated with the risk of developing gallstones and colon cancer.

The Clostridium coccoides group also includes other anaerobic endo-spore forming bacteria of the human microbiome that are not necessarily taxonomically related to the genus Clostridium. Some Eubacterium species are also lumped into this group. These are commonly probiotic bacteria that are known for producing short-chain fatty acids such as butyrate, which exhibit anti-inflammatory and immuno-regulatory activity. Generally, Eubacterium abundance follows the opposite trend of the previously named Clostridium species,

being reduced in abundance in the elderly[53].
Depending on which species are involved, the results of changes in Clostridium abundance could be either beneficial or harmful. In the present study, Clostridium was found to increase during the high-fat diet, and was reduced toward normal levels by treatment with astaxanthin. Thus, the specific species affected may be those associated with dysbiosis caused by high fat intake, and not necessarily the beneficial varieties.

Genus Bifidobacterium

Bifidobacterium made up an extremely small amount of the total bacterial at less than 0.000006% (Figure 4). There was no significant difference between groups or before and after astaxanthin treatment. Since the standard deviation among individuals was so large, this result is only included for reference purposes.
Bifidobacterium is a genus of gram-positive anaerobic bacteria that is the first major colonizer of the human gastrointestinal tract and plays a significant role in maintaining intestinal health by preventing constipation, diarrhea, and colorectal cancer[56]. An abundance of the genus is closely correlated with the aging process[57]. Bifidobacterium colonizes newborns from birth and makes up a majority of infant microbiomes during their breast-feeding years, with species such as Bifidobacterium longum, B. breve, and B. bifidum being the most abundant. Their abundance falls to lower levels by adulthood (2-14% of total bacteria), and is significantly reduced in the elderly. Low levels of Bifidobacterium are associated with obesity and diabetes (types 1 and 2)[54,57].
Bifidobacterium species have demonstrated numerous positive health effects and see common use as probiotics. B. longum and B. breve have both shown anti-obesity effects in a high-fat diet mouse model 58): oral intake of both species resulted in a reduction in weight gain, reduced triglyceride accumulation in the liver, and increased cecal abundance of lactic acid bacteria; B. longum intake additionally reduced serum triglycerides. Similar effects are seen in B. bifidum[59] as well as various other strains[60].
B. animalis subsp. lactis (AKA B. lactis), in addition to reducing weight gain, glycemic response, and fasting insulin levels in a high-fat diet mouse model, is also reported to reduce inflammation by down-regulating tumor-necrosis factor expression in adipose and hepatic tissues[61]. B. lactis also mediates the effects of metabolic syndrome by improving glucose sensitivity, modulating the enteric flora, and producing a high amount of the short chain fatty-acid acetate[62].
Bifidobacterium may also impact anxiety and stress (via the gut-brain axis). In a mouse model of chronic stress, mice that were resistant to social stress from exposure to aggression had a higher abundance of Bifidobacterium, and intake of a probiotic containing B. longum and B. infantis increased resilience to stress and mitigated its symptoms[63]. B. bifidum has also been reported to reduce signs of stress in a maternal separation mouse model: mice ingesting the probiotic showed significantly reduced susceptibility to stress, with lower serum corticosterone and improved intestinal permeability[64].
The lack of any observed change from astaxanthin intake in contrast

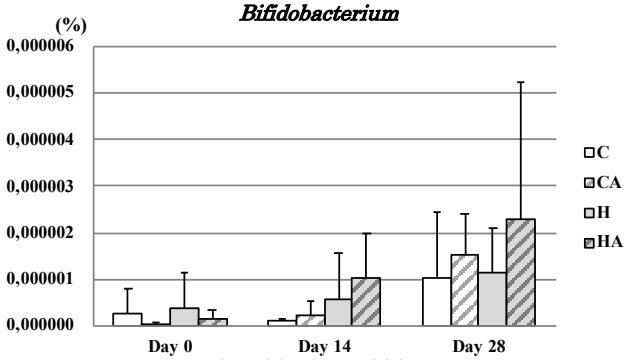

Figure 4. Putative copy numbers of the genus Bifidobacterium
Results shown as percent of total bacterial copy number, mean ± standard deviation. C: ordinary diet group, CA: ordinary diet + astaxanthin group, H: high-fat diet group, HA: high-fat diet + astaxanthin group, (n = 5 each). Cited from reference 24).

to the other genera examined may simply be due to the very low initial abundance, or perhaps indicate that a difference in metabolic pathways of Bifidobacterium prevents the positive effect of the carotenoid on its physiology.

Other bacteria: Bacteroides, Prevotella, Clostridium leptum

Of the total bacterial, Bacteroides comprised less than 0.003%, a very small amount. The count was significantly increased by the high fat diet ($p = 0.006$). Addition of astaxanthin resulted in no significant difference.
Prevotella copies were also few, at 0.06%. The high fat diet markedly reduced bacterial count ($p < 0.001$). There was no significant change with the addition of astaxanthin.
Clostridium leptum copies comprised less than 1.6% of the total bacteria. It was significantly increased by the high fat diet ($p = 0.017$). There was no significant difference with astaxanthin.

Phylum level analysis

The results of phylum level analysis are represented as the ratios of Firmicutes, Bacteroidetes, and other copy numbers, as shown in Figure 5.
Firmicutes accounted for 20% of total copies on Day 0, but increased to 40% on both Day 14 and Day 28 in the high-fat diet group ($p < 0.001$, Figure 6). There was no difference with the addition of astaxanthin.
Bacteroidetes made up 45-50% of total copies on Day 0, but fell to 20% on both Day 14 and Day 28 ($p < 0.001$ (Figure 7). There was no difference with the addition of astaxanthin.

Changes in enteric flora due to ingestion of high-fat diet

Consumption of a high-fat diet increases the secretion of bile acids[65], and causes various changes to the intestinal microbiota[66]. These changes are more pronounced in the large intestine than the small intestine[67]. High-fat diets weaken intestinal immunity, disrupt mucosal barrier function, and cause abnormal fermentation to occur in the intestines[68]. As a result, carcinogenesis is also increased[69].

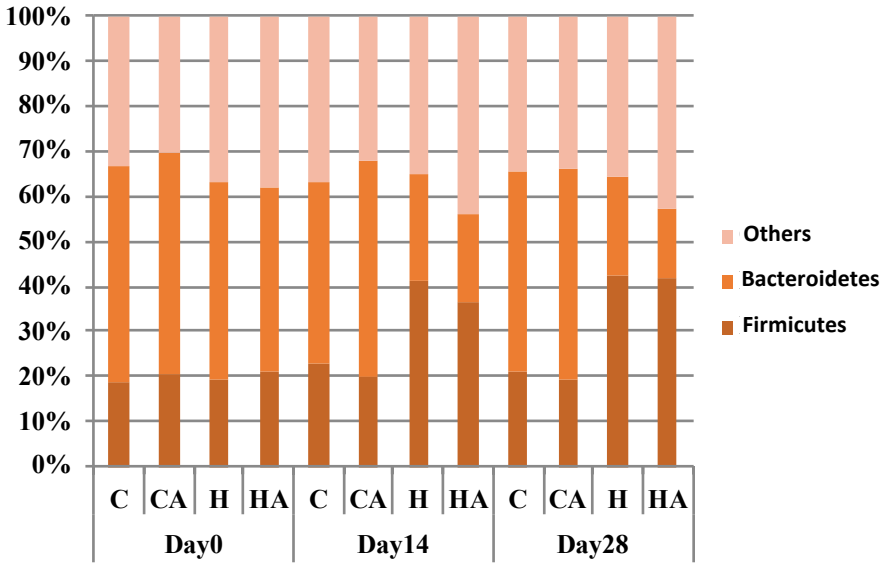

Figure 5. Phylum Level Analysis
Ratios of bacterial abundance of the phyla Bacteroidetes, Firmicutes, and others, relative to total primer measurement for all bacteria. C: ordinary diet group, CA: ordinary diet + astaxanthin group, H: high-fat diet group, HA: high-fat diet + astaxanthin group, (n = 5 each). Cited from reference 24).

Results pertaining to changes in gut microbiota caused by a high-fat diet have been reported in both animal and human studies. In a mouse model of type 2 diabetes (KK-Ay) Bacteroides is reduced and Clostridium coccoides tends to increase in the cecum[70]. In a mouse model of high-fat diet induced obesity, Bifidobacterium abundance in the cecum and feces was decreased[71]. In rats, Bacteroides and Prevotella were increased[72]. In humans, it has been reported that obese subjects likely consuming a high-fat diet carry a reduced amount of Bacteroides[73]. At the phylum level, Firmicutes have been found to increase while Bacteroidetes decrease[73-75].

In this study, the results of the phylum level analysis generally agreed with those previously reported[73-75]. Due to the high-fat diet (Group H), Bacteroides, Clostridium coccoides, and Clostridium leptum amounts increased, while Streptococcus (lactic acid bacteria) decreased. The difference in the change of Bacteroides may be due to differences between host animal (rat vs. mouse), or perhaps differences in testing methodology. However, Bacteroides species have previously been considered non-beneficial bacteria, so the results of the present study seem reliable.

Effects of astaxanthin on enteric flora

The following results regarding the effectiveness of astaxanthin were obtained from the animal study. First, astaxanthin can be expected to inhibit the short-term increase of Bacteroides (gram negative bacteria) caused by high-fat diet. Interestingly, the addition of astaxanthin increased the amount of Lactobacillus (lactic acid bacteria). Similarly, Streptococcus (lactic acid bacteria) was also increased by astaxanthin in the high fat diet. These results suggest that administering astaxanthin may improve enteric flora (inhibiting the overgrowth of gram-negative bacteria, increasing lactic acid bacteria).

For this experiment, astaxanthin was not administered via tube feeding, but instead a fixed amount (0.02% astaxanthin) was mixed into the mice's feed. While tube feeding would have allowed for more consistency in dosage, the resulting damage to the upper gastrointestinal tract and increased stress on the mice would likely affect the intestinal microbiota. Individual differences were also increased: with mixed feed the dosage of astaxanthin depends on feed intake and is not a fixed quantity, however there is little stress on the mice.

Astaxanthin intake was estimated from the feed intake of the mice (mean body weight at 8 weeks: Group C 38~41 g, Group H 44~48 g). The feed intake of Group C mice was about 3 g/day, consisting of 0.2 g/day of fat, 0.9 g/day of protein, and 1.0 mg/day of astaxanthin per mouse. The group H mice received about 5 g/day of feed consisting of 1.1 g/day of fat, 0.7 g/day of protein, and 0.6 mg/day of astaxanthin. The equivalent intake of astaxanthin for a human (average body weight 60 kg) would be 1,500 mg/day for group CA

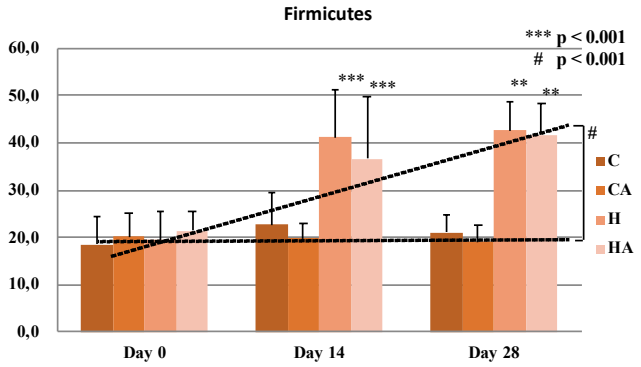

Figure 6. Putative copy number of the phylum Firmicutes
*Results shown as percent of total bacterial copy number, mean ± standard deviation. C: ordinary diet group, CA: ordinary diet + astaxanthin group, H: high-fat diet group, HA: high-fat diet + astaxanthin group, (n = 5 each). Groups compared using Tukey's multiple comparison test (*** vs. Day 0), rate of change by diet compared using Mann-Whitney's U test (#, dotted lines). Cited from reference 24).*

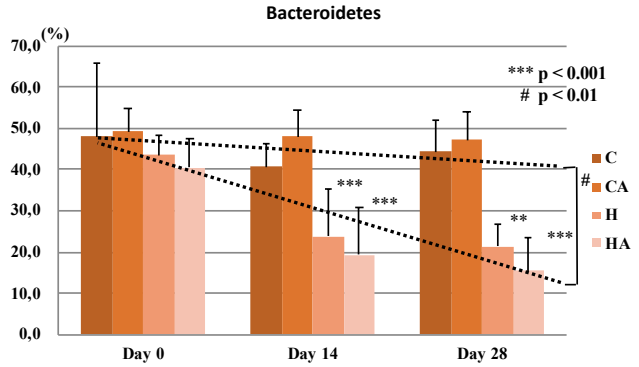

Figure 7. Putative copy numbers of the phylum Bacteroidetes
*Results shown as percent of total bacterial copy number, mean ± standard deviation. C: ordinary diet group, CA: ordinary diet + astaxanthin group, H: high-fat diet group, HA: high-fat diet + astaxanthin group, (n = 5 each). Groups compared using Tukey's multiple comparison test (*** vs. Day 0), rate of change by diet compared using Mann-Whitney's U test (#, dotted lines). Cited from reference 24).*

and 800 mg/day for group HA, significantly higher than the typical supplement amounts of 6-24 mg/day. Thus, from this experiment it is not clear whether such an amount is suitable for human intake. Approximately 95% of the astaxanthin remains in the intestinal tract unabsorbed. Less than 5% is absorbed and enters systemic circulation, only some of which reaches the digestive tract again. Therefore, the majority of the astaxanthin likely acts directly on the intestinal microbiota. Any action by the astaxanthin on the intestinal tract or intestinal immunity has not yet been reported.

Conclusion

The various bacterial cells that live in the human gut and form the intestinal microbiome number in the range of 600 trillion, 10 times the number of native cells that make up the human body itself. The makeup of the enteric flora shifts not only with age, but also due to diet, exercise, alcohol intake, smoking, and other lifestyle/environmental factors. These changes play a role in health, contributing to things such as obesity, skin conditions, atopy, allergies, and carcinogenesis. In order to maintain a youthful and healthy body, it is crucial to protect the health and stability of the enteric flora. Modern diets have led to an increase in the consumption of fats in recent years, and the resulting changes to the intestinal microbiota have been reported[70,71,76,77]. Mitigating these changes is of great importance.

The mechanism by which astaxanthin prevented changes to the bacterial flora from a high-fat diet could not be determined within the scope of the presented studies. However, the following potential mechanisms are proposed:

1. It is possible that the antioxidant action of astaxanthin exerted a beneficial effect on the enteric flora. The change in bacterial composition induced by the high-fat diet, particularly the growth of gram-negative bacteria, created higher levels of oxidative stress. As astaxanthin intake improved the enteric flora, the increased oxidative stress may have been mitigated. This needs to be confirmed.

2. Astaxanthin may be degraded by bacteria, producing low molecular weight substances which may then be responsible for the beneficial changes to the enteric flora in high-fat diets. It is necessary to examine the metabolic pathways of astaxanthin in the intestinal tract.

3. Astaxanthin or its metabolites may have an effect on intestinal immunity. Markers related to intestinal immunity (e.g. IgA, defensin) need to be examined.

The intestinal tract of every individual is inhabited by an assortment of characteristic bacteria, which collectively form the enteric microbiome. When fat intake increases due to changes in diet, the equilibrium between the various species that constitute the intestinal flora is altered. As a result, degenerative changes in lifestyle-related disease and aging of the host are promoted. Here we find that the intake of astaxanthin was able to inhibit these changes in the gut microbiota of mice induced by a high-fat diet. Even in humans, it is highly probably that the unabsorbed astaxanthin that remains in the intestinal tract exerts a positive effect against disturbance of the intestinal flora caused by a high-fat diet.

Author contributions:

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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