Minireview

Carotenoid supplementation and retinoic acid in immunoglobulin A regulation of the gut microbiota dysbiosis

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Impact statement

The concept of carotenoid metabolism in the gut health has not been well established in the literature. Here, we review and discuss the roles of retinoic acid and carotenoids, including pro-vitamin A carotenoids and xanthophylls in the maturation of the gut immune system and IgA production. This is the first review article about the carotenoid supplements and the metabolites in the regulation of the gut microbiome. We hope this review would provide a new direction for the management of the gut microbiota dysbiosis by application of bioactive carotenoids and the metabolites.

Abstract

Dysbiosis, a broad spectrum of imbalance of the gut microbiota, may progress to microbiota dysfunction. Dysbiosis is linked to some human diseases, such as inflammation-related disorders and metabolic syndromes. However, the underlying mechanisms of the pathogenesis of dysbiosis remain elusive. Recent findings suggest that the microbiome and gut immune responses, like immunoglobulin A production, play critical roles in the gut homeostasis and function, and the progression of dysbiosis. In the past two decades, much progress has been made in better understanding of production of immunoglobulin A and its association with commensal microbiota. The present minireview summarizes the recent findings in the gut microbiota dysbiosis and dysfunction of immunoglobulin A induced by the imbalance of pathogenic bacteria and commensal microbiota. We also propose the potentials of dietary carotenoids, such as β-carotene and astaxanthin, in the improvement of the gut immune system maturation and immunoglobulin A production, and the consequent promotion of the gut health.

Keywords: Astaxanthin, beta-carotene oxygenase 2, immune system, gut microbiome, vitamin A

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Introduction

Gut microbiota is the microbes coevolve with the host in the environment. Every individual has a unique composition of the microbiota. The phyla *Bacteroidetes* and *Firmicutes* are most abundant bacterial species in the adult human gut, accounting for more than 90% of the bacterial populations.[1] Recently, gut microbiota dysbiosis has been shown to be associated with weaknesses in gut barrier function, dysregulated innate, and adaptive immune responses and also with the increased incidence of the inflammatory bowel disease (IBD) and other metabolic disorders.[2,3] Studies show that antibiotics play vital roles in the progression of dysbiosis especially in the overgrowth of the resistant opportunistic bacterial pathogen (OBP).[4] Another major factor is a diet which can be directly utilized by microbiota, and in turn causes changes in the composition of microbiota. Dysbiosis is accompanied by increased OBP and decreased commensal microbiota like *Lactobacillales* and *Bifidobacterium*.[5] However, the initiation and progression of dysbiosis still need further studies.

The relationship between gut microbiota and the host is based on the gut immune response and tolerance.[6] Immunoglobulin A (IgA) is one of the critical regulators in protecting the mucosal balance via recognizing and coating particular microbiota to prevent the microbiota from crossing the gut epithelial cell barrier.[7] Commensal microbiotas could be beneficial to the host health through regulating the production of reactive oxygen species (ROS) and short chain fatty acid (SCFA).[8] However, in the dysbiosis condition, inhibition of commensal microbiotas by OBP, like *Gammaproteobacteria* and the relationship between the OBP and production of IgA in dysbiosis are yet exclusive.

Recently, researchers found some bioactive molecules that would be beneficial to improve microbiota composition, and in turn reduce the risk of diseases caused by microbiota dysbiosis. Carotenoid supplementation shows promising improvements in the immune system maturation and IgA production.[9]

Here, we discuss the roles of OBP and commensal microbiota in the pathogenesis of dysbiosis via regulation of the IgA function and immune maturation, and discuss the potential of carotenoid supplementation in dysbiosis prevention and treatment.

Gut microbiota dysbiosis causes diseases

Dysbiosis can be understood with the loss of beneficial microbiota, expansion of pathogenic or potentially harmful microbiota, and a decrease of overall microbiota diversity.[10] There are multiple ways to influence the composition of the microbiota, including the genetics of the host, diet, infection and medical interventions, like antibiotics.[11] Dysbiosis is associated with several pathological processes, including obesity, non-alcoholic fatty liver disease (NAFLD), and IBD (Table 1).[12] The overgrowth of OBP can cause pathoprogression of some diseases. For example, increased abundance of *Escherichia* (alcohol-producing bacteria) was correlated with the elevated blood alcohol concentration in patients with non-alcoholic steatohepatitis (NASH), suggestive of involvement of imbalanced gut microbiome in the pathogenesis of NASH.[13] The bacterial microbiota in IBD has also been investigated. An increase in bacteria in the *Proteobacteria* phylum, such as *Escherichia coli* was observed in the IBD patients.[14] Also, fungal microbiota dysbiosis was also found in patients with IBD.[15] Another study demonstrated that the gut microbiota dysbiosis determined the development of NAFLD.[16] Dysbiosis causes the decrease in commensal microbiota, like *Faecalibacterium prausnitzii*, a dominant butyrate-producing bacteria in the intestine. Butyrate is a SCFA which could be used as an energy source for intestinal epithelial cells and microbiomes. It helps protect the intestinal epithelial barrier integrity and make them resistant to potential pathogens.[17] Similar to IBD, a specific individual microbiota signature seems to be related to the development of obesity. The overall intestinal bacterial diversity was decreased in obese individuals.[18] Lean germ-free (GF) mice would be obese after receiving the transplant of microbiota from obese mice.[19] It seems apparent that changes in microbiota are closely associated with some human diseases. However, whether microbiota dysbiosis is a causal factor or an effect of the disease is yet to be proved. A list of examples of microbiota dysbiosis-associated diseases is provided in Table 1.

Gut microbiota dysbiosis leads to IgA dysfunction

There are at least two points of view of the mechanisms in the maintenance of the balance between the host and microbiota via manipulation of the gut immune system. The first is the immune tolerance. The commensal (beneficial) microbiota can induce the mucosal immune cells to recognize them and decrease the inflammatory response. Forkhead box protein P3 (Foxp3) is a protein in regulation of the host immune system response. Induction of Foxp3 triggers regulatory T (Treg) cells, which limit excessive pro-inflammatory responses.[33] A study showed that *Bifidobacteria infantis 35624* induced Foxp3$^+$ Treg cell activation and increased secretion of toll-like receptor 2 (TLR-2) dependent IL-10, production of dendritic cell-specific intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN), and activation of retinoic acid signaling pathways.[33]

The other possible mechanism is the immune ignorance. The lousy microbiota can induce the intestine immune system to recognize them specifically and induce inflammatory responses, while the mucosal immune antibodies ignore other microbiota. IgA production is the primary immune response in the gut.[34] IgA can recognize and coat particular microbiota, and consequently prevent the

Table 1. Examples of microbiota dysbiosis and the bacteria associated with diseases.

Disease	Dysbiosis	Protective or pathogenic microbiotas, effect on host	References
Obesity	1. Ratio of *Firmicutes*: *Bacteroidetes* ↑ 2. *Proteobacteria* ↑ 3. Compositional microbial diversity ↓	*Bifidobacteria* treatment reduced the intestinal lipopolysaccharide levels, and suppressed expression of inflammatory and oxidative stress markers in the liver of ob/ob mice fed a high fat diet.	20–24
Diabetes	Ratio of *Firmicutes*: *Bacteroidetes* ↓	BB-diabetes-resistant rats had higher levels of *Bifidobacterium* and *Lactobacillus* compared to BioBreeding diabetes prone rats (a type 1 diabetic model)	25–27
Inflammatory bowel disease	*Proteobacteria* ↑ *Bifidobacterium* ↓	Increased *Proteobacteria*, and in particular the family of *Enterobacteriaceae*, containing members such as *Escherichia coli*, *Shigella* and *Klebsiella*.	10,28,29
Nonalcoholic fatty liver disease	*Proteobacteria* ↑ *Bacteroidetes* ↓	*Proteobacteria*/*Enterobacteriaceae*/*Escherichia* were similarly represented between microbiomes in healthy and obese mice, but were significantly elevated in nonalcoholic steatohepatitis. Oral administration of *Lactobacillus* rhamnosus PL60 improved liver steatosis in diet-induced obesity mice.	13,30–32

microbiota translocation across the intestinal epithelial barrier, and the destruction of the tight junction in the intestine. Besides the defense and cooperation of microbiota, gut mucosa also can recover from the colitis.[35] Research showed that intestinal interferon regulatory transcription factor 3 (IRF3) could be activated by microbiota-derived nucleic acids and subsequently stimulated the expression of thymic stromal lymphopoietin (TSLP), a cytokine mediated in protection of the large intestine.[36] These gut immune responses to a variety of microbiota depend on the maturation of host immune organs and the recognition ability of IgA. However, the maturation process of the host immune system induced by microbiota remains uncertain. Moreover, how the individual microbiota induces IgA to recognize them specifically is also not clear.

OBP against the commensal microbiota to induce the production of IgA

IgA is the predominant antibody and is thought to provide the first line of the immune protection at mucosal surfaces in the intestine, through coating the microbiota and neutralizing toxins.[37] In addition to the intestine tissue, antigen-specific IgA-secreting cells also distribute in the liver.[38] Additionally, IgA is associated with down-regulation of pro-inflammatory epitopes on commensal bacteria.[39]

Recently, researchers are focusing on the function of the specific IgA-regulated microbiota. Mice with deficiency of or blocked production of IgA contain a higher amount and abundance of *Proteobacteria* especially *Gammaproteobacteria* compared with regular wild-type mice.[40] Interestingly, *Proteobacteria* is thought to exhibit the immaturity status of microbiota in adult mice. *Proteobacteria* is mostly associated with the host inflammation with the elevated expression of interferon gamma (IFN-γ), macrophage inflammatory protein 2 (MIP-2), and tumor necrosis factor alpha (TNF-α).[41] The *Gammaproteobacteria* abundance was increased in the common variable immunodeficiency (CVID) with inhibition of IgA expression.[42] Dysfunction of IgA can lead to the imbalance of the gut microbiota composition, such as increases in the *Gammaproteobacteria* and decreases in the *Bifidobacterium* and *Lactobacilli* in the gut microbiota, which led to the increased production of the pro-inflammatory cytokines.

Dysbiosis is accompanied by a decreased production of IgA or suppression of the ability of IgA to coat the microbiota. A lower fecal IgA amount more likely reflects initiation of dysbiosis in the gut in the alcoholic steatohepatitis mice, compared with healthy mice,[43] though the mechanism by which IgA is inactivated by microbiota needs to be further investigated. Recent research demonstrated that the antibiotic treatment-induced gut dysbiosis led to modification of the immune recognition to the intestinal bacteria.[44] The antibiotic treatments promote the growth of specific pathogens (for example *Neisseria gonorrhoeae* and/or *Corynebacterium diphtheriae*) by a mechanism to which the pathogens can avoid IgA opsonization,[45] but suppress the colonization of beneficial *Lactobacillales* and *Clostridium* cluster IV cells.[44]

The beneficial microbiota is proposed to induce Myd88.[46] Lack of Myd88 resulted in no significant variation in the composition of the adult microbiota, indicative that the microbiota effects on Myd88 are mono-directional without dysbiosis happening.[47] Moreover, this commensal microbiota critically affects the IgA production. Deficiency of IgA led to less abundance of *Lactobacillales* in the gut of mice. When fed the immunocompetent mothers' milk, the IgA deficient mice accepted milk IgA from the foster mothers, and in turn showed an increased population of *Lactobacillales* and then decreased *Proteobacteria* in the gut.[40]

The dysbiosis especially caused by antibiotic treatments is established by the growth of antibiotic-resistant microbiota which is more compatible than commensal microbiota. Bacteriocin generated by microbiota can efficiently influence a strain's ability to compete with other microbiota in the intestine lumen.[48]

Collectively, dysbiosis causes the competition between OBP and commensal microbiota. OBP can inhibit the activity of commensal microbiota by producing bacteriocin and avoid the recognition of IgA through inhibiting the commensal microbiota-induced, Myd88-regulated IgA production.

Microbiota regulates IgA dysfunction via inhibition of the immune organ maturation

Besides competition between the commensal microbiota and pathogens, dysbiosis is also associated with the immune immaturity. The gut microbiota homeostasis is essential for maturation of the immune system. Studies confirmed that lymphoid follicles are immature and IgA production is insufficient in GF mice. However, colonization with commensal bacteria could reverse the above deficiency by induction of the intestinal IgA response and maturation of lymphoid follicles in those mice.[49,50]

The gut microbiota controls the peripheral lymphoid volume expansion and maintenance. In the GF mice, the secondary lymphoid organs are undeveloped. Under normal condition, $CD45^+/CD103^+/RALDH^+$ (retinoic acid dehydrogenases$^+$) immune cells move to the peripheral lymph nodes in healthy mouse pups after birth, driven by commensal microbiome.[51] On the other hand, an unstable structure of gut microbiota composition with high abundance of *Proteobacteria* is also observed in non-disease states in the neonatal period, while the neonatal immune system is still under development to maturation and lacks IgA in the gut mucosa.[52] Given that OPB inhibits the commensal microbiota activity, it is likely that OPB suppresses the maturation of the host immune system, especially in the production of IgA.

Micronutrients in dysbiosis management

The management of dysbiosis should base on global approaches, not only to consider the diet quality and quantity control, but also to include strategies focused on increasing of intakes of foods rich in bioactive components, such as prebiotics, probiotics, and others which could modulate either composition or metabolic/immunological activity of the gut microbiota.[53]

Bioactive compounds to increase the commensal microbiota

Prebiotics exert the beneficial role in the gut via increasing the population of good microbiota like *Lactobacillus* and *Bifidobacterium*. The prebiotics such as fructooligosaccharides, fibers, and polyphenols can be metabolized by particular microbiota and in turn confer health benefits to the host.[54] For instance, polysaccharides can be used to produce SCFA which is beneficial to the gut integrity, host metabolism, and immunological responses. The study showed that SCFA produced by commensal microbiota promotes the peripheral regulatory T cell production and activation, and induces IL-10 production, which could mitigate inflammation in infection tissues.[55] Another study revealed that consumption of the blackcurrant extract powder containing polyphenols increased the amount of *Bifidobacterium* and *Lactobacillus* but decreased the enzymatic activity of β-glucuronidase, a bacterial enzyme considered as a risk factor for colorectal cancer.[56] The IgA production was also improved via prebiotics supplementations. Administration of fructooligosaccharides increased the concentration of IgA and expression of polymeric immunoglobulin receptor (pIgR) in the intestine in mice.[57] It would be interesting to explore the underlying mechanism by which that commensal microbiota metabolizes the fructooligosaccharides and subsequently increases the production of IgA.

Supplementation of carotenoids increases the IgA production and maturation of gut-associated immune tissues

Carotenoid metabolism. Carotenoids are pigments abundant in many fruits, vegetables, and some animal products. Human beings are unable to synthesize carotenoids from endogenous precursors and must acquire them from the diet. The absorption of dietary carotenoids in the intestine is controlled, at least partially by the scavenger receptor class B type 1 (SRB1), which is regulated by beta-carotene oxygenase 1 (BCO1) activity via homeobox transcription factor intestine-specific homeobox (ISX).[58] However, the regulation of SRB1 in the large intestine has not been investigated.

Carotenoids are characterized into two subgroups, e.g. pro-vitamin A carotenoids and non-pro-vitamin A carotenoids, or xanthophylls. Pro-vitamin A carotenoids can be cleaved by BCO1 (at the 15, 15′ double bound) resulting in a production of vitamin A, which can be further converted into retinoic acid in host cells, such as dendritic cells and B cells. All carotenoids can be asymmetrically cleaved by beta-carotene oxygenase 2 (BCO2) to generate apocarotenals.[59,60] As fat-soluble bioactive compounds, the blood carotenoid bioavailability is low 10–40%. Likely, carotenoids may reach the colon and be fermented in the gut by microbiota.[61] However, the utilization of carotenoids by microbiota has not been evidenced, and their biological functions in the gut microbiome are yet well studied.[62]

Carotenoid function. Low levels of carotenoids are considered antioxidants. However, the clinical trials have demonstrated that over-dosed carotenoids are toxic.[63] Functionally, carotenoids play essential roles in all aspects of cell functions, such as in regulation of the host immune responses by activation of macrophages, natural killer cells, T cells, and/or Treg cells (for some recent review articles please visit literature[64–70]). Carotenoids are beneficial for protecting humans against age-related eye diseases,[71] metabolic syndromes,[72] stroke,[73] heart and cardiovascular diseases,[74,75] diabetes,[76] inflammation,[77] and even body composition changes.[78] Unlike retinoic acid (RA), carotenoid metabolism in the gut and their impacts on the gut microbiome remain unknown.

Retinoic acid. RA is a critical regulator for the intestinal immune response. Lack of vitamin A in the mouse diet suppresses pro-inflammatory Th17 cell generation in the gut via reduction of commensal microbes.[79] The primary function of RA in B cells is to induce the IgA production and class switching.[80] Inhibition of the RA pathway in B cells significantly decreases the IgA secretion but increases the *Lachnospiraceae* (Erec482+) and *Lactobacillus*/*Streptococcus* in the fecal samples, compared with the control group, which are thought to be associated with colorectal adenomas. Supplementation of β-carotene increased IgA production and transfer to neonatal mice through maternal milk.[81] Moreover, *Bifidobacteria* infantis 35624 can attenuate the colitis by inducing the CD103+ retinoid acid secreting dendritic cells.[82] However, the direct effect of RA on the microbiota is poorly understood.

Astaxanthin supplementation. Recently, some nutrition studies have been focused on the health benefits of xanthophylls, such as zeaxanthin, lutein, and astaxanthin. Astaxanthin is an oxycarotenoid found in high amounts largely in some microalgae and marine animals, such as salmon and shrimp.[83] The biological functions of astaxanthin have been reported in protection against inflammation and the associated diseases, including but not limited to ulcers, cancer, neurodegeneration, diabetes, and cardiovascular disease.[84] However, how astaxanthin alters the gut immune system remains exclusive. The treatment with astaxanthin could significantly reduce bacterial loads and attenuate gastric inflammation in mice infected with *H. pylori*.[85] Mice administrated with astaxanthin showed significantly lowered colonization levels and inflammation scores in the stressed rats.[86] Supplementation of astaxanthin increased the production of IgA antibody-secreting cells (ASC) in the small intestine of mice at weanling age.[87]

Astaxanthin also induces the host immune responses, such as activation of T cells, B cells, and NK cells, and production of IFN-γ and IL-6.[88] Astaxanthin activates T cells and NK cells to produce IFN-γ, and in turn mediates B cell differentiation and maturation, resulting in IgA production. Recently, a published human study indicated that the supplementation of astaxanthin increased sIgA levels which may protect against the exercise-induced mucosal immunity dysfunction.[89] Further studies suggested that astaxanthin enhanced the antibody production through the release of IL-1α, which is one of the significant

regulating factors in B cell differentiation.[90] Thus, astaxanthin confers its beneficial effects by regulation of the maturation of B cell and production of IgA. Astaxanthin could have a potential in the prevention or treatment of dysbiosis and its associated diseases.

Astaxanthin alters the gut microbiota. Most recently, we conducted a pilot study to determine the potential role of astaxanthin in the gut microbiome. Six-week-old BCO2 knockout (KO) and the genetic background C57BL/6J mice (WT) (with mixed gender) were fed either AIN-93M (control diet) or AIN-93M supplemented with 0.04% astaxanthin (w/w, astaxanthin diet) for eight weeks (Oklahoma State University Institutional Animal Care and Use Committee Protocol # HS-14–4, approved by the chair of the IACUC). The results showed that astaxanthin administration considerably altered the cecal microbiota at the phylum, which mostly differed by gender and BCO2 gene expression (Figure 1). In particular, *Proteobacteria* and *Bacteroides*, the OBP, were significantly more abundant in both male and female BCO2 KO mice, which could be diminished by application of astaxanthin in female WT and BCO2 KO only. On the contrast, astaxanthin drastically increased the abundance of *Actinobacteria* and *Bifidobacterium*, the commensal microbiota in male WT mice only. There are more than a dozen of commensal microbiota genera with increased abundance at the phylum of *Firmicutes* in mice after astaxanthin treatment (Figure 1, detailed data not shown). So far, the results suggested that astaxanthin administration and the metabolic enzyme, e.g., BCO2 remarkably impact the gut microbiome in mice, although the vast majority of the microbiota identified in the current study in Figure 1 had not been well characterized in literature.

Conclusion

Dysbiosis is one of the most crucial factors in gut dysfunction that can induce many human diseases including obesity, metabolic syndrome, IBD, and NAFLD. The onset and development of the gut dysbiosis are complex and may involve various mechanisms. A better understanding of the pathophysiological mechanisms would be beneficial for the development of new preventative and therapeutic strategies. Impaired IgA production and function exert a

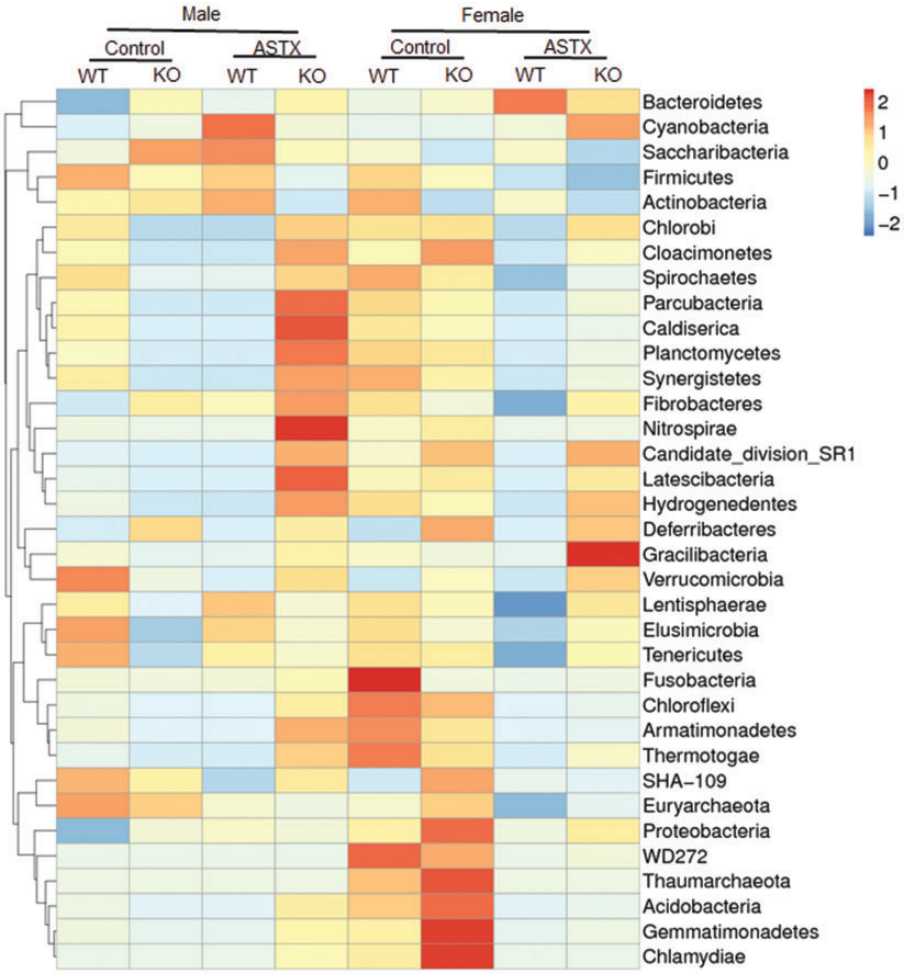

Figure 1. The phylum-level changes of the cecal microbiota in BCO2 knockout (KO) and the wild-type (WT) mice after administration of 0.04% astaxanthin (w/w). Six-week-old mice were fed either the control (AIN-93M) or astaxanthin (ASTX, AIM-93M supplemented with 0.04% astaxanthin (w/w)) diet for eight weeks. The cecal DNA samples were subjected to 16s rRNA sequencing (by Novogene, Inc.). The heatmap of the abundance changes is shown at the phylum level only. (A color version of this figure is available in the online journal.)

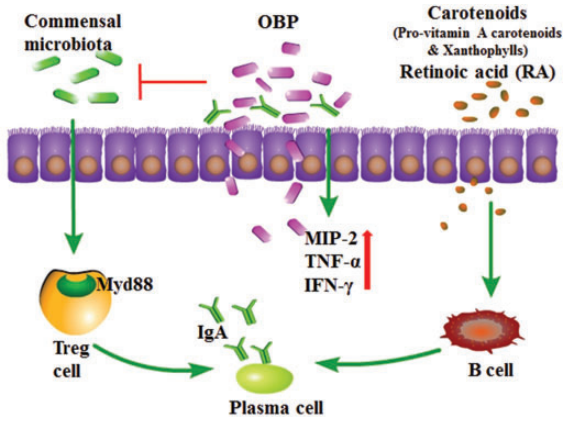

Figure 2. Regulation of microbiota homeostasis by immunoglobulin A (IgA) and carotenoids. Commensal microbiota can induce Treg cells via Myd88 signaling to up-regulate the T Follicular Helper cells, which in turn stimulates the plasma cells to produce IgA. The increased opportunistic bacterial pathogens (OBP) inhibit the commensal microbiota, leading to suppression of IgA production in the plasma cells. OBP also triggers the enhanced expression of macrophage inflammatory protein 2 (MIP-2), tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ), inflammation-associated factors in the gut tissue. Dietary carotenoids (including pro-vitamin A carotenoids and non-pro-vitamin A carotenoids, e.g. xanthophylls) and their supplementation may increase the production of retinoic acid (RA). RA sequentially induces the maturation of gut immune system, e.g. B cell activation, and IgA production. Xanthophylls activate T cells and natural killer cells resulting in the production of IFN-γ. IFN-γ further stimulates the differentiation and maturation of B cells to produce IgA, and in turn promote the gut health. (A color version of this figure is available in the online journal.)

vital role in initiation of the imbalance between OBP and commensal microbiota. Carotenoids, such as β-carotene (in the metabolite form of RA) and astaxanthin may contribute to the gut immune homeostasis by directly regulating IgA production, thereby preventing of and/or delaying the development of dysbiosis (Figure 2). Future investigations are warranted to elucidate the precise mechanisms by which carotenoids are preventive/protective in the gut dysbiosis.

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DECLARATION OF CONFLICTING INTERESTS

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REFERENCES

1. Sekirov I, Russell SL, Antunes LCM, Finlay BB. Gut microbiota in health and disease. *Physiol Rev* 2010;**90**:859–904
2. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I, Kim SG, Li H, Gao Z, Mahana D, Zárate Rodriguez JG, Rogers AB, Robine N, Loke P, Blaser MJ. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell* 2014;**158**:705–21
3. West CE, Renz H, Jenmalm MC, Kozyrskyj AL, Allen KJ, Vuillermin P, Prescott SL; in-FLAME Microbiome Interest Group. The gut microbiota and inflammatory noncommunicable diseases: associations and potentials for gut microbiota therapies. *J Allergy Clin Immunol* 2015;**135**:3–13
4. Asahara T, Nomoto K, Shimizu K, Watanuki M, Tanaka R. Increased resistance of mice to Salmonella enterica serovar Typhimurium infection by synbiotic administration of *Bifidobacteria* and transgalactosylated oligosaccharides. *J Applied Microbiol* 2001;**91**:985–96
5. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. *Nat Rev Immunol* 2013;**13**:790–801
6. Hooper LV, Gordon JI. Commensal host-bacterial relationships in the gut. *Science* 2001;**292**:1115–8
7. Palm NW D, Zoete MR, Cullen TW, Barry NA, Stefanowski J, Hao L, Degnan PH, Hu J, Peter I, Zhang W, Ruggiero E, Cho JH, Goodman AL, Flavell RA. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell*. 2014;**158**:1000–10
8. Lambeth JD, Neish AS. Nox enzymes and new thinking on reactive oxygen: a double-edged sword revisited. *Annu Rev Pathol* 2014;**9**:119–45
9. Ni Y, Nagashimada M, Zhuge F, Zhan L, Nagata N, Tsutsui A, Nakanuma Y, Kaneko S, Ota T. Astaxanthin prevents and reverses diet-induced insulin resistance and steatohepatitis in mice: a comparison with vitamin E. *Sci Rep* 2015;**5**:17192
10. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol* 2014;**16**:1024–33
11. Strachan DP. Family size, infection and atopy: the first decade of the'-hygiene hypothesis. *Thorax* 2000;**55**(Suppl 1):S2–10
12. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. Host-gut microbiota metabolic interactions. *Science* 2012;**336**:1262–7
13. Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, Gill SR. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatol* 2013;**57**:601–9
14. Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, Schwager E, Knights D, Song SJ, Yassour M, Morgan XC, Kostic AD, Luo C, González A, McDonald D, Haberman Y, Walters T, Baker S, Rosh J, Stephens M, Heyman M, Markowitz J, Baldassano R, Griffiths A, Sylvester F, Mack D, Kim S, Crandall W, Hyams J, Huttenhower C, Knight R, Xavier RJ. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* 2014;**15**:382–92
15. Sokol H, Leducq V, Aschard H, Pham H-P, Jegou S, Landman C, Cohen D, Liguori G, Bourrier A, Nion-Larmurier I, Cosnes J, Seksik P, Langella P, Skurnik D, Richard ML, Beaugerie L. Fungal microbiota dysbiosis in IBD. *Gut* 2017;**66**:1039–48
16. Le Roy T, Llopis M, Lepage P, Bruneau A, Rabot S, Bevilacqua C, Martin P, Philippe C, Walker F, Bado A, Perlemuter G, Cassard-Doulcier AM, Gérard P. Intestinal microbiota determines the development of non-alcoholic fatty liver disease in mice. *Gut* 2013;**62**:1787–94
17. Li J, Butcher J, Mack D, Stintzi A. Functional impacts of the intestinal microbiome in the pathogenesis of inflammatory bowel disease. *Inflamm Bowel Dis* 2015;**21**:139–53
18. Arslan N. Obesity, fatty liver disease, and intestinal microbiota. *World J Gastroenterol* 2014;**20**:16452
19. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci Usa U S A* 2007;**104**:979–84

20. Ruan X, Shi H, Xia G, Xiao Y, Dong J, Ming F, Wang S. Encapsulated *Bifidobacteria* reduced bacterial translocation in rats following hemorrhagic shock and resuscitation. *Nutrition* 2007;**23**:754–61
21. Griffiths EA, Duffy LC, Schanbacher FL, Qiao H, Dryja D, Leavens A, Rossman J, Rich G, Dirienzo D, Ogra PL. In vivo effects of *Bifidobacteria* and lactoferrin on gut endotoxin concentration and mucosal immunity in Balb/c mice. *Dig Dis Sci* 2004;**49**:579–89
22. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* 2009;**58**:1091–103
23. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. *Nature* 2009;**457**:480–4
24. Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. *J Clin Invest* 2011;**121**:2126–32
25. Brugman S, Klatter F, Visser J, Wildeboer-Veloo A, Harmsen H, Rozing J, Bos NA. Antibiotic treatment partially protects against type 1 diabetes in the Bio-Breeding diabetes-prone rat. Is the gut flora involved in the development of type 1 diabetes? *Diabetologia* 2006;**49**:2105–8
26. Valladares R, Sankar D, Li N, Williams E, Lai K-K, Abdelgeliel AS, Gonzalez CF, Wasserfall CH, Larkin J, Schatz D, Atkinson MA, Triplett EW, Neu J, Lorca GL. *Lactobacillus johnsonii* N6. 2 mitigates the development of type 1 diabetes in BB-DP rats. *Plos One* 2010;**5**:e10507.
27. Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sørensen SJ, Hansen LH, Jakobsen M. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *Plos One* 2010;**5**:e9085
28. Ayres JS, Trinidad NJ, Vance RE. Lethal inflammasome activation by a multidrug-resistant pathobiont upon antibiotic disruption of the microbiota. *Nat Med* 2012;**18**:799–806
29. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, Reyes JA, Shah SA, LeLeiko N, Snapper SB, Bousvaros A, Korzenik J, Sands BE, Xavier RJ, Huttenhower C. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol* 2012;**13**:R79
30. Mouzaki M, Comelli EM, Arendt BM, Bonengel J, Fung SK, Fischer SE, McGilvray ID, Allard JP. Intestinal microbiota in patients with the non-alcoholic fatty liver disease. *Hepatol* 2013;**58**:120–7
31. Lee HY, Park JH, Seok SH, Baek MW, Kim DJ, Lee KE, Paek KS, Lee Y, Park JH. Human originated bacteria, *Lactobacillus* rhamnosus PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. *Biochim Biophys Acta* 2006;**1761**:736–44
32. Iacono A, Raso GM, Canani RB, Calignano A, Meli R. Probiotics as an emerging therapeutic strategy to treat NAFLD: focus on molecular and biochemical mechanisms. *J Nutr Biochem* 2011;**22**:699–711
33. Konieczna P, Groeger D, Ziegler M, Frei R, Ferstl R, Shanahan F, Quigley EM, Kiely B, Akdis CA, O'Mahony L. *Bifidobacterium* infantis 35624 administration induces Foxp3 T regulatory cells in human peripheral blood: potential role for myeloid and plasmacytoid dendritic cells. *Gut* 2012;**61**:354–66
34. Malin M, Suomalainen H, Saxelin M, Isolauri E. Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with *Lactobacillus* GG. *Ann Nutr Metab* 1996;**40**:137–45
35. Mora JR, Iwata M, Eksteen B, Song S-Y, Junt T, Senman B, Otipoby KL, Yokota A, Takeuchi H, Ricciardi-Castagnoli P, Rajewsky K, Adams DH, von Andrian UH. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science* 2006;**314**:1157–60
36. Negishi H, Miki S, Sarashina H, Taguchi-Atarashi N, Nakajima A, Matsuki K, Endo N, Yanai H, Nishio J, Honda K, Taniguchi T. Essential contribution of IRF3 to intestinal homeostasis and microbiota-mediated Tslp gene induction. *Proc Natl Acad Sci U S A* 2012;**109**:21016–21
37. Macpherson A, McCoy K, Johansen F, Brandtzaeg P. The immune geography of IgA induction and function. *Mucosal Immunol* 2008;**1**:11–22
38. Moro-Sibilot L, Blanc P, Taillardet M, Bardel E, Couillault C, Boschetti G, Traverse-Glehen A, Defrance T, Kaiserlian D, Dubois B. Mouse and human liver contain immunoglobulin A-secreting cells originating from Peyer's patches and directed against intestinal antigens. *Gastroenterol* 2016;**151**:311–23
39. Peterson DA, McNulty NP, Guruge JL, Gordon JI. IgA response to symbiotic bacteria as a mediator of gut homeostasis. *Cell Host Microbe* 2007;**2**:328–39
40. Mortera SL, Del Chierico F, Vernocchi P, Rosado MM, Cavola A, Chierici M, Pieroni L, Urbani A, Carsetti R, Lante I, Dallapiccola B, Putignani L. Monitoring perinatal gut microbiota in mouse models by mass spectrometry approaches: parental genetic background and breastfeeding effects. *Front Microbiol* 2016;**7**:1523
41. Kau AL, Planer JD, Liu J, Rao S, Yatsunenko T, Trehan I, Manary MJ, Liu TC, Stappenbeck TS, Maleta KM, Ashorn P, Dewey KG, Houpt ER, Hsieh CS, Gordon JI. Functional characterization of IgA-targeted bacterial taxa from undernourished Malawian children that produce diet-dependent enteropathy. *Sci Transl Med* 2015;**7**:276ra24
42. Jørgensen S, Trøseid M, Kummen M, Anmarkrud J, Michelsen A, Osnes L, Holm K, Høivik ML, Rashidi A, Dahl CP, Vesterhus M, Halvorsen B, Mollnes TE, Berge RK, Moum B, Lundin KE, Fevang B, Ueland T, Karlsen TH, Aukrust P, Hov JR. Altered gut microbiota profile in common variable immunodeficiency associates with levels of lipopolysaccharide and markers of systemic immune activation. *Mucosal Immunol* 2016;**9**:1455–65
43. Inamine T, Yang AM, Wang L, Lee KC, Llorente C, Schnabl B. Genetic loss of immunoglobulin A does not influence development of alcoholic steatohepatitis in mice. *Alcohol Clin Exp Res* 2016;**40**:2604–13
44. Džunková M, Moya A, Vázquez-Castellanos JF, Artacho A, Chen X, Kelly C, D'Auria G. Active and secretory IgA-coated bacterial fractions elucidate dysbiosis in Clostridium difficile infection. *mSphere* 2016;**1**:e00101–16
45. Ng KM, Ferreyra JA, Higginbottom SK, Lynch JB, Kashyap PC, Gopinath S, Naidu N, Choudhury B, Weimer BC, Monack DM, Sonnenburg JL. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. *Nature* 2013;**502**:96–9
46. Wang S, Charbonnier L-M, Rivas MN, Georgiev P, Li N, Gerber G, Bry L, Chatila TA. MyD88 adaptor-dependent microbial sensing by regulatory T cells promotes mucosal tolerance and enforces commensalism. *Immunity* 2015;**43**:289–303
47. Mirpuri J, Raetz M, Sturge CR, Wilhelm CL, Benson A, Savani RC, Hooper LV, Yarovinsky F. *Proteobacteria*-specific IgA regulates maturation of the intestinal microbiota. *Gut Microbes* 2014;**5**:28–39
48. Majeed H, Gillor O, Kerr B, Riley MA. Competitive interactions in *Escherichia coli* populations: the role of bacteriocins. *ISME J* 2011;**5**:71–81
49. Sutherland DB, Fagarasan S. IgA synthesis: a form of functional immune adaptation extending beyond gut. *Curr Opin Immunol* 2012;**24**:261–8
50. Macpherson AJ, Köller Y, McCoy KD. The bilateral responsiveness between intestinal microbes and IgA. *Trends Immunol* 2015;**36**:460–70
51. Zhang Z, Li J, Zheng W, Zhao G, Zhang H, Wang X, Guo Y, Qin C, Shi Y. Peripheral lymphoid volume expansion and maintenance are controlled by gut microbiota via RALDH+ dendritic cells. *Immunity* 2016;**44**:330–42
52. Guaraldi F, Salvatori G. Effect of breast and formula feeding on gut microbiot a shaping in newborns. *Front Cell Infect Microbiol* 2012;**2**:94
53. Etxeberria U, Hijona E, Aguirre L, Milagro FI, Bujanda L, Rimando AM, Martínez JA, Portillo MP. Pterostilbene-induced changes in gut microbiota composition in relation to obesity. *Mol Nutr Food Res* 2017;**61**:1500906.
54. Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, Gibson GR, Delzenne NM. Selective increases of *Bifidobacteria* in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* 2007;**50**:2374–83
55. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K. Treg induction by a rationally selected

mixture of Clostridia strains from the human microbiota. *Nature* 2013;**500**:232–6.
56. Molan AL, Liu Z, Plimmer G. Evaluation of the effect of blackcurrant products on gut microbiota and on markers of risk for colon cancer in humans. *Phytother Res* 2014;**28**:416–22
57. Nakamura Y, Nosaka S, Suzuki M, Nagafuchi S, Takahashi T, Yajima T, Takenouchi-Ohkubo N, Iwase T, Moro I. Dietary fructooligosaccharides up-regulate immunoglobulin A response and polymeric immunoglobulin receptor expression in intestines of infant mice. *Clin Exp Immunol* 2004;**137**:52–8
58. Lobo GP, Amengual J, Baus D, Shivdasani RA, Taylor D, Von Lintig J. Genetics and diet regulate vitamin A production via the homeobox transcription factor ISX. *J Biol Chem* 2013;**288**:9017–27
59. von Lintig J. Colors with functions: elucidating the biochemical and molecular basis of carotenoid metabolism. *Annu Rev Nutr* 2010;**30**:35–56
60. Lobo GP, Amengual J, Palczewski G, Babino D, von Lintig J. Mammalian carotenoid-oxygenases: key players for carotenoid function and homeostasis. *Biochim Biophys Acta* 2012;**1821**:78–87
61. Awad AB, Fink CS. Phytosterols as anticancer dietary components: evidence and mechanism of action. *J Nutr* 2000;**130**:2127–30
62. Palczewski G, Widjaja-Adhi MAK, Amengual J, Golczak M, von Lintig J. Genetic dissection in a mouse model reveals interactions between carotenoids and lipid metabolism. *J Lipid Res* 2016;**57**:1684–95
63. Bohn T. Bioactivity of carotenoids – chasms of knowledge. *Int J Vitam Nutr Res* 2017;**10**:1–5
64. Larange A, Cheroutre H. Retinoic acid and retinoic acid receptors as pleiotropic modulators of the immune system. *Annu Rev Immunol* 2016;**34**:369–94
65. Ni Y, Zhuge F, Nagashimada M, Ota T. Novel action of carotenoids on non-alcoholic fatty liver disease: macrophage polarization and liver homeostasis. *Nutrients* 2016;**8**:pii: E391
66. Bono MR, Tejon G, Flores-Santibañez F, Fernandez D, Rosemblatt M, Sauma D. Retinoic acid as a modulator of T cell immunity. *Nutrients* 2016;**8**:pii: E349
67. Kim YS, Sayers TJ, Colburn NH, Milner JA, Young HA. Impact of dietary components on NK and Treg cell function for cancer prevention. *Mol Carcinog* 2015;**54**:669–78
68. Raverdeau M, Mills KH. Modulation of T cell and innate immune responses by retinoic acid. *J Immunol* 2014;**192**:2953–8
69. Ross AC. Vitamin A and retinoic acid in T cell-related immunity. *Am J Clin Nutr* 2012;**96**:1166S–72S
70. Cicero AFG, Colletti A. Effects of carotenoids on health: are all the same? Results from clinical trials. *Curr Pharm Des* 2017;**23**:2422–7
71. Gorusupudi A, Nelson K, Bernstein PS. The age-related eye disease 2 study: micronutrients in the treatment of macular degeneration. *Adv Nutr* 2017;**8**:40–53
72. Goncalves A, Amiot MJ. Fat-soluble micronutrients and metabolic syndrome. *Curr Opin Clin Nutr Metab Care* 2017;**20**:492–7
73. Bahonar A, Saadatnia M, Khorvash F, Maracy M, Khosravi A. Carotenoids as potential antioxidant agents in stroke prevention: a systematic review. *Int J Prev Med* 2017;**8**:70
74. Zuluaga M, Gueguen V, Letourneur D, Pavon-Djavid G. Astaxanthin-antioxidant impact on excessive reactive oxygen species generation induced by ischemia and reperfusion injury. *Chem Biol Interact* 2018;**279**:145–58
75. Costa-Rodrigues J, Pinho O, Monteiro PRR. Can lycopene be considered an effective protection against cardiovascular disease? *Food Chem* 2018;**245**:1148–15
76. Roohbakhsh A, Karimi G, Iranshahi M. Carotenoids in the treatment of diabetes mellitus and its complications: a mechanistic review. *Biomed Pharmacother* 2017;**91**:31–42
77. Rubin LP, Ross AC, Stephensen CB, Bohn T, Tanumihardjo SA. Metabolic effects of inflammation on vitamin A and carotenoids in humans and animal models. *Adv Nutr* 2017;**8**:197–212
78. Gust JL, Logomarsino JV. The association between cartenoid status and body composition in children 2 - 18 years of age – a systematic review. *Int J Vitam Nutr Res* 2017;**8**:1–24
79. Cha HR, Chang SY, Chang JH, Kim JO, Yang JY, Kim CH, Kweon MN. Downregulation of Th17 cells in the small intestine by disruption of gut flora in the absence of retinoic acid. *J Immunol* 2010;**184**:6799–806
80. Pantazi E, Marks E, Stolarczyk E, Lycke N, Noelle RJ, Elgueta R. Cutting edge: retinoic acid signaling in B cells is essential for oral immunization and microflora composition. *J Immunol* 2015;**195**:1368–71
81. Nishiyama Y, Yasumatsuya K, Kasai K, Sakase M, Nishino O, Akaike M, Nagase T, Sugimoto M, Ikeda S, Kume S. Effects of supplemental β-carotene with whey on IgA transfer from maternal milk and mucosal IgA induction in neonatal mice and calves. *Livestock Sci* 2011;**137**:95–100
82. Konieczna P, Ferstl R, Ziegler M, Frei R, Nehrbass D, Lauener RP, Akdis CA, O'Mahony L. Immunomodulation by *Bifidobacterium* infantis 35624 in the murine lamina propria requires retinoic acid-dependent and independent mechanisms. *Plos One* 2013;**8**:e62617
83. Ambati RR, Phang S-M, Ravi S, Aswathanarayana RG. Astaxanthin: sources, extraction, stability, biological activities and its commercial applications – a review. *Mar Drugs* 2014;**12**:128–52
84. Yuan JP, Peng J, Yin K, Wang JH. Potential health-promoting effects of astaxanthin: a high-value carotenoid mostly from microalgae. *Mol Nutr Food Res* 2011;**55**:150–65
85. Bennedsen M, Wang X, Willén R, Wadström T, Andersen LP. Treatment of *H. pylori* infected mice with antioxidant astaxanthin reduces gastric inflammation, bacterial load and modulates cytokine release by splenocytes. *Immunol Lett* 1999;**70**:185–9
86. Nishikawa Y, Minenaka Y, Ichimura M, Tatsumi K, Nakamoto T, Urabe K. Effects of astaxanthin and vitamin C on the prevention of gastric ulcerations in stressed rats. *J Nutr Sci Vitaminol* 2005;**51**:135–41
87. Nagayama T, Sugimoto M, Ikeda S, Kume S. Effects of astaxanthin-enriched yeast on mucosal IgA induction in the jejunum and ileum of weanling mice. *Anim Sci J* 2014;**85**:449–53
88. Park JS, Chyun JH, Kim YK, Line LL, Chew BP. Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. *Nutr Metab* 2010;**7**:18
89. Baralic I, Andjelkovic M, Djordjevic B77, Dikic N, Radivojevic N, Suzin-Zivkovic V, Radojevic-Skodric S, Pejic S. Effect of astaxanthin supplementation on salivary IgA, oxidative stress, and inflammation in young soccer players. *Evid Based Complement Alternat Med* 2015;**2015**:783761783761
90. Giri J, Kincade P, Mizel S. Interleukin 1-mediated induction of kappa-light chain synthesis and surface immunoglobulin expression on pre-B cells. *J Immunol* 1984;**132**:223–8