

Role of PPAR γ in the nutritional and pharmacological actions of carotenoids

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Abstract: Peroxisome proliferator-activated receptor gamma (PPAR γ) has been shown to play an important role in the biological effects of carotenoids. The PPAR γ -signaling pathway is involved in the anticancer effects of carotenoids. Activation of PPAR γ partly contributes to the growth-inhibitory effects of carotenoids (β -carotene, astaxanthin, bixin, capsanthin, lutein, and lycopene) on breast cancer MCF7 cells, leukemia K562 cells, prostate cancer (LNCaP, DU145, and PC3 cells), and esophageal squamous cancer EC109 cells. PPAR γ is the master regulator of adipocyte differentiation and adipogenesis. Downregulated PPAR γ and PPAR γ -target genes have been associated with the suppressive effects of β -carotene and lycopene on 3T3L1 and C3H10T1/2 adipocyte differentiation and adipogenesis. β -Carotene is cleaved centrally into retinaldehyde by BCO1, the encoding gene being a PPAR γ -target gene. Retinaldehyde can be oxidized to retinoic acid and also be reduced to retinol. β -Carotene can also be cleaved asymmetrically into β -apocarotenals and β -apocarotenones by BCO2. The inhibitory effects of β -carotene on the development of adiposity and lipid storage are dependent substantially on BCO1-mediated production of retinoids. The effects of β -carotene on body adiposity were absent in *BCO1*-knockout mice. Retinoid metabolism is connected with the activity of PPAR γ in the control of body-fat reserves. Retinoic acid, retinaldehyde, retinol, and β -apocarotenals exert suppressive effects on preadipocyte differentiation and adipogenesis via downregulation of PPAR γ expression in cell culture. The molecular mechanisms underlying retinoic acid effects on adipose-tissue biology and the development of adiposity remain poorly understood. Adiposity can be affected by retinoids through long-lasting effects at critical developmental stages. Retinol saturase increases PPAR γ -transcriptional activity and adipocyte differentiation. Other carotenoids that have been reported to suppress adipocyte differentiation and lipid accumulation in the main via modulation of PPAR γ and PPAR γ -target genes include astaxanthin, bixin, norbixin, β -cryptoxanthin, fucoxanthin and its metabolites, lycopene, apo-10'-lycopenoic acid, siphonaxanthin, and neoxanthin, except paprika pigments. Lutein, lycopene, and paprika carotenoids reduce proinflammatory cytokine levels by an induction of PPAR γ in immune tissues and cells. Lycopene, apo-10'-lycopenoic acid, and astaxanthin might prevent atherosclerosis through modifying cholesterol metabolism via increasing PPAR γ expression in macrophages.

Keywords: carotenoids, PPAR γ , anti-cancer, anti-obesity, antiatherosclerosis

Introduction

PPAR γ , a member of the nuclear hormone-receptor superfamily, functions as a ligand-activated transcription factor. It participates in numerous physiological and pathological processes, such as lipid metabolism, adipocyte differentiation, insulin sensitivity, the growth of cells in various organs, and the occurrence of several human diseases,

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including obesity, dyslipidemia, diabetes, inflammation, hypertension, atherosclerosis, and cancer.^{1–3} Endogenous PPAR γ ligands include 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂), as well as linoleic, linolenic, and arachidonic acids. Pharmaceutical PPAR γ ligands include the thiazolidinediones (ciglitazone, pioglitazone, rosiglitazone, and troglitazone), which are commonly prescribed medications for the treatment of type 2 diabetes mellitus. The PPAR γ activated by ligands (also known as PPAR γ agonists) forms a heterodimer with RXR α and binds to its specific response elements, PPREs, in the promoter region of various target genes. Emerging information shows that PPAR γ is expressed in many human tissues, such as breast, colon, lung, ovary, prostate, stomach, bladder, and thyroid, where it regulates cell proliferation, differentiation, and apoptosis. This property makes PPAR γ an important target for the development of new and effective anticancer therapies.^{4,5} PPAR γ has been shown to be involved in the important biological effects of carotenoids, such as growth and apoptosis of cancer cells, adipocyte differentiation and adipogenesis, inflammatory responses, and cholesterol metabolism in macrophages.

PPAR γ activation in carotenoid-inhibited cancer-cell proliferation

A growing body of evidence indicates that carotenoids act as potent growth-inhibitory agents in several tumor cells, including breast, colon, prostate, lung, melanoma, and leukemia cells.^{6,7} The PPAR γ -signaling pathway has been shown to be involved in the anticancer effects of carotenoids. Sharoni et al proposed that some carotenoids, such as β -carotene, lycopene, phytoene, and phytofluene, caused the transactivation of PPREs in MCF7 cells cotransfected with PPAR γ . This activity was lower than that achieved with the known PPAR ligands, such as 15d-PGJ₂ and ciglitazone.⁸ Combined treatment of 3.8 μ M fucoxanthin, a carotenoid from the edible seaweed *Undaria pinnatifida*, with 10 μ M troglitazone (a PPAR γ agonist) remarkably induced DNA fragmentation, which led to a reduction in viability of human Caco2 colon cancer cells, but fucoxanthin and troglitazone, individually, did not have significant effects on Caco2-cell viability.⁹ Lycopene at 10–25 μ M showed no effects on the proliferation of prostate cancer PC3 cells. However, lycopene at 25 μ M augmented the antiproliferative effects of PPAR γ agonists (15d-PGJ₂, ciglitazone, and pioglitazone), modulated PPAR γ -induced apoptosis, and inhibited the expression of growth and survival-associated genes in PC3 cells.¹⁰ Effects of lutein on the proliferation of PC3 cells were similar to those of lycopene. Lutein induced a mild decrease in proliferation, improved PPAR γ agonist-

induced cell-cycle arrest and apoptosis, and altered the expression of growth and apoptosis-associated biomarker genes.¹¹

Although these studies suggested the involvement of the PPAR γ pathway, they did not investigate PPAR γ directly or the contribution of the PPAR γ pathway to the inhibition of cancer-cell growth by carotenoids. Recently, our experimental results showed that β -carotene enhanced the expression of PPAR γ in breast cancer MCF7 cells. GW9662, an irreversible antagonist of PPAR γ , can partly attenuate the growth inhibition and apoptosis caused by β -carotene. In addition, β -carotene induced reactive oxygen species production and cytochrome C release. The results suggested that the synergistic effect of PPAR γ expression and reactive oxygen species production may have accounted for β -carotene-mediated anticancer activities.¹² Our other results demonstrated that pretreatment with GW9662 partly attenuated the inhibition of cell proliferation, apoptosis, and interference in cell-cycle progression induced by β -carotene, astaxanthin, capsanthin, and bixin in leukemia K562 cells. These carotenoids upregulated the expression of PPAR γ and cyclin-dependent kinase inhibitor p21 and downregulated the expression of cyclin D1.¹³ Moreover, rosiglitazone augmented the inhibitory effects of carotenoids on K562-cell proliferation. Specific PPAR γ inhibition by GW9662 and PPAR γ small interfering RNA (siRNA) weakened cell-growth suppression by the carotenoid and rosiglitazone combination greatly. GW9662 attenuated the enhanced upregulation of PPAR γ expression caused by the combination treatment. GW9662 and PPAR γ siRNA also significantly attenuated the upregulation of p21 and downregulation of cyclin D1 caused by carotenoids and rosiglitazone.¹⁴ These data suggest that the PPAR γ -signaling pathway is involved in the suppression of tumor-cell proliferation by carotenoids. Carotenoids suppress the cell growth through activating PPAR γ , in turn via stimulating p21 and inhibiting cyclin D1. A similar role of PPAR γ has been observed in the antiproliferative effects of β -carotene and lycopene on the human esophageal squamous cancer-cell line EC109 (Nguyen et al, unpublished).

Yang et al reported that lycopene treatment significantly inhibited the proliferation of androgen-dependent human prostate LNCaP cancer cells, increased the expression of PPAR γ and LXR α at 24 and 48 hours, and decreased cellular total cholesterol levels and increased the expression of the ABCA1 and ApoA1 proteins at 96 hours. Incubation of LNCaP cells with lycopene in the presence of GW9662 and the LXR α antagonist GGPP restored cell proliferation to the control levels and significantly suppressed protein expression of PPAR γ and LXR α , as well as increased cellular

total cholesterol levels. LXR α knockdown by siRNA against LXR α significantly enhanced the proliferation of LNCaP cells, whereas siLXR α knockdown followed by incubation with lycopene restored the proliferation to the control level. The authors proposed that the antiproliferative effect of lycopene on LNCaP cells involved the activation of the PPAR γ -LXR α -ABCA1 pathway, leading to reduced cellular total cholesterol levels.¹⁵ They observed similar results in androgen-independent prostate cancer DU145 cells, and came to the same conclusion.¹⁶

It has been well known that PPAR γ agonists have anti-cancer activity.^{17,18} Taking these aforementioned findings together, it is obvious that upregulation of PPAR γ expression is associated with the antiproliferative effects of carotenoids on cancer cells. However, it remains unclear whether they have the capacity to function as PPAR γ agonists and thus can inhibit the proliferation of cancer cells. The results tested using the PPAR γ 2 CALUX reporter cell line showed that the carotenoids violaxanthin, phytofluene, neurosporene, lycopene, β -carotene, γ -carotene, and δ -carotene from extracts of tomato were able to induce PPAR γ 2-mediated luciferase expression.¹⁹ It appears that carotenoids might have some PPAR γ -agonist potential.

Repression of PPAR γ expression in the suppressive effects of carotenoids on adipocyte differentiation and adipogenesis

β -Carotene and its metabolite retinoids (vitamin A and its derivatives)

Obesity caused by excess accumulation of adipose tissue has become a medical problem worldwide, because it may increase the risk of various diseases, such as heart disease, type 2 diabetes, and obstructive sleep apnea. Adipose tissue mass is determined by processes governing adipocyte number and size. Proliferation and differentiation lead to increases in adipocyte number, and increasing storage of triacylglycerols causes the expansion of adipocyte size. Adipocyte differentiation is a complex process, in which a variety of transcription factors are involved. One of the early events is the upregulation of C/EBP β expression. C/EBP β then acts to upregulate the expression of adipogenic factors, such as C/EBP α , which control the late stage of adipogenesis. PPAR γ is considered the master regulator of adipogenesis, and in its absence adipocyte differentiation cannot proceed.^{20,21}

The role of β -carotene in adipocyte biology has received more and more attention. The modulation of PPAR γ has

been reported to be associated with the inhibitory effects of β -carotene on adipocyte differentiation and adipogenesis. Kawada et al reported previously that β -carotene, β -apo-8'-carotenal, and retinal markedly suppressed the differentiation of 3T3L1 preadipocytes to adipocytes through RAR upregulation and suppression of PPAR γ 2.²² Murine C3H10T1/2 adipocyte differentiation was conducted with rosiglitazone in the presence of β -carotene, (all-*E*)-lycopene and other dietary constituents for 7 days. β -Carotene inhibited rosiglitazone-stimulated lipid formation and expression of the adipocyte-differentiation markers PPAR γ , PPAR α , and C/EBP α , and of genes related to lipid metabolism, such as *LPL*, *AP2*, and *CPT1B*. (all-*E*)-lycopene showed moderate inhibition. The observed inhibitory effects of β -carotene correlated with the modulation of genes involved in adipocyte differentiation.²³ However, it was observed that β -carotene treatment at a concentration of 20 μ M but not 10 μ M in 3T3L1 adipocytes during differentiation for 4 days enhanced the expression of genes related to insulin sensitivity, including adiponectin, *ALBP*, and *GLUT4* (involved in glucose incorporation), *PPAR γ 1*, *PPAR γ 2*, *C/EBP α* , and *C/EBP β* in the medium. The expression of the *C/EBP δ* gene tended to be reduced. β -Carotene accumulation inhibited TNF α -mediated reduction in the expression of these genes related to insulin sensitivity.²⁴ It is very likely that the expression of *PPAR γ* and other adipogenic and lipogenic genes is suppressed by carotenoid treatment at the early stage of adipocyte differentiation and stimulated at the late stage. In addition, a 9-*cis* β -carotene-enriched diet inhibited atherogenesis and fatty-liver formation in low-density lipoprotein receptor-knockout mice.²⁵

Evidence from cell-culture and animal-model studies indicates that substantial actions of β -carotene in adipose tissue biology are mediated by its metabolites. β -Carotene is the natural precursor for apocarotenoid molecules, including retinoids (vitamin A and its derivatives, such as retinaldehyde [Rald] and retinoic acid [RA]).²⁶ It is cleaved centrally into two molecules of Rald by BCO1, a cytosolic enzyme present in various tissues, including the intestine, liver, testes, and adipose tissue.^{27,28} Rald can be oxidized to RA and also be reduced to retinol.²⁹ β -Carotene can also undergo asymmetric cleavage to yield β -apocarotenals and β -apocarotenones by BCO2, which localizes to mitochondria.^{30,31} BCO1 activity is robust in the intestine and liver. The *BCO1* gene has been shown to be transcriptionally regulated by the action of PPARs and RXRs in both mice and humans^{32,33} and also by feedback inhibition of RA.³⁴ After *BCO1*-knockout mice were fed with chow containing β -carotene as a major

vitamin A precursor, vitamin A levels fell dramatically and the β -carotene accumulated in large quantities in several tissues examined. The mice developed dyslipidemia, such as fatty liver and elevated serum free fatty acids, and were more susceptible to high-fat diet-induced obesity compared to wild mice. The expression of PPAR γ -regulated genes also increased in fat depots. Therefore, BCO1 was identified as the key enzyme for vitamin A production.³⁵ It was reported that BCO1 expression was induced during NIH3T3L1 adipocyte differentiation. β -Carotene but not all-*trans*-retinol decreased lipid content of mature adipocytes and was metabolized to RA in mature adipocytes. RA decreased the expression of PPAR γ and C/EBP α , and reduced the lipid content of mature adipocytes. Dietary β -carotene decreased PPAR γ expression in white adipose tissue of vitamin A-deficient mice. However, *BCO1*, as a PPAR γ -target gene, is highly expressed in mature adipocytes, allowing for β -carotene to be converted to RA.³⁶ Recently, von Lintig et al reported that *BCO1*-knockout mice showed increased expression of BCO2 in adipocytes, and β -10'-apocarotenol accumulated as the major β -carotene derivative. In wild-type mice, dietary β -carotene significantly reduced body adiposity (by 28%), leptinemia, and adipocyte size, resulted in a general downregulation of gene expression in adipose tissue, and downregulated PPAR γ and PPAR γ -target genes. These effects of β -carotene were absent in *BCO1*-knockout mice despite β -10'-apocarotenoid production, demonstrating that these effects were dependent on the BCO1-mediated production of retinoids. The study showed clearly that β -carotene plays an important role in the control of body-fat reserves in mice and that BCO1 is a critical molecular player for the regulation of PPAR γ activity in adipocytes.³⁷ A study in human pulmonary alveolar epithelial cells showed that β -carotene downregulated BCO1 expression through suppression of PPAR γ /RXR α binding to the BCO1 promoter, which decreased retinoid production. Systemic vitamin A insufficiency in the lung may be associated with an increase of lung cancer risk upon β -carotene supplementation in smokers.³⁸ On the other hand, chronic alcohol intake was reported to decrease hepatic retinoid levels but increase messenger RNA (mRNA) concentrations of BCO1, BCO2, PPAR γ , PPAR α , and TR β and upregulate protein levels of CMO2, PPAR γ , and PPAR α in Fischer 344 rats.³⁹

The importance of vitamin A and its derivatives, Rald and RA from β -carotene, in adipose tissue biology and the development of adiposity has received close attention in recent years. It has long been established that RA is an inhibitor of preadipocyte differentiation in cell culture.^{40–44} RA inhibits

adipogenesis early in adipocyte differentiation by repressing C/EBP β -mediated transcription and decreasing PPAR γ expression through binding to and activating RA receptors.^{45–48} RA can no longer prevent adipocyte differentiation if added to the cell culture after early differentiation is completed.^{36,49} Results from preadipocytes and mesenchymal stem cell models have shown that RA specifically blocks the occupancy of C/EBP β of the *CEBP α* promoter and does not act directly on C/EBP β . RA stimulates the expression of the TGF β effector protein Smad3, which can interact with C/EBP β via its Mad homology 1 domain and can interfere with C/EBP β DNA binding. In the absence of Smad3, RA is not able to inhibit adipocyte differentiation or to elicit a decrease in C/EBP β DNA occupancy. The data suggest that Smad3 is required for the inhibition effects of RA on adipogenesis.⁵⁰ RA inhibits pig preadipocyte differentiation, activating RAR and downregulating PPAR γ , RXR α , and SREBP1C.^{45,51} Recently Berry and Noy reported that in mature adipocytes, RA activated both RARs and PPAR β/δ , thereby enhancing lipolysis and depleting lipid stores. RA treatment of obese mice induced expression of PPAR β/δ and RAR-target genes involved in regulation of lipid homeostasis, leading to weight loss and improved insulin responsiveness. The results indicated that suppression of obesity and insulin resistance by RA was largely mediated by PPAR β/δ and further enhanced by activation of RARs.⁵² Berry et al also showed that RA inhibited adipocyte differentiation by activating the CRABP2–RAR γ path in preadipose cells, thereby upregulating the expression of the adipogenesis inhibitors Pref1, Sox9, and KLF2, resulting in inhibition of the expression of the adipogenic proteins CEBP and PPAR γ and SREBP1C. Therefore, they pointed out that RA suppresses adipogenesis in vivo.⁵³ Interestingly, Dave et al reported that stem bromelain (SBM) inhibited adipogenesis irreversibly. SBM and all-*trans*-retinoic acid (atRA) treatment together inhibited adipocyte differentiation more effectively than either alone. SBM, together with atRA, may be a potent modulator of obesity by repressing the PPAR γ -regulated adipogenesis pathway at all stages and by augmenting TNF α -induced lipolysis and apoptosis in mature adipocytes.⁵⁴ In addition, Lee et al reported that RA inhibited BMP4-induced C3H10T1/2 mesenchymal stem cell adipogenic commitment and differentiation, as observed by reductions in key adipogenic genes/transcription factors (C/EBP α , PPAR γ , aP2), lipogenic genes (*LPL*, *FAS*, *GLUT4*), and lipid accumulation. RA significantly suppressed the BMP4-triggered phosphorylation of both Smad1/5/8 and p38MAPK. They suggested that RA inhibited the BMP4

induction of C3H10T1/2 adipocyte commitment via down-regulating Smad/p38MAPK signaling.⁵⁵

Although a growing body of evidence indicates that RA represses adipogenesis and reduces body-fat reserves in tissues, including brown adipose tissue, white adipose tissue (WAT), skeletal muscle, and the liver,^{56–59} controversial data have been reported, including retinoid effects on hepatic lipid and blood-lipid profile.^{60,61} García-Rojas et al indicated that 9-*cis*-RA and atRA are powerful agents for the promotion of bovine adipogenesis. The 9-*cis*-RA and atRA increased PGC1 α mRNA expression in bovine adipogenic cultures, with 9-*cis*-RA being a better activator. Expression of PPAR γ mRNA was increased by 30 μ M β -carotene, though 10 μ M β -carotene decreased expression compared with a differentiation medium. However, it is not conclusive that activation of the PPAR system contributes to the ability of carotenoids to promote adipose tissue differentiation.⁶²

Another *in vivo* trial showed that PPAR γ mRNA was significantly increased in rat adipose tissue by 13-*cis*-RA treatment.⁶³ It was reported that adipogenesis in 3T3L1 preadipocytes was accompanied by RA production generated primarily by Aldh1a1. Aldh1 enzymes and RA regulated PPAR γ expression by mechanisms involving ZnF423 expression.⁶⁴ Villarroya et al held that the final effect of RA on preadipocyte differentiation depends on the concentration and isomer availability, as well as on the relative RAR and RXR availability in the cells.⁶⁵ A new pathway of RA inhibition of adipocyte differentiation was proposed recently. Retinoylated CRM1 disrupts the export of MEK1 from the nucleus. Rich MEK1 in the nucleus decreases PPAR γ availability and thus inhibits adipogenesis, while also disrupting the MEK1–ERK signaling cascade.⁶⁶ This shows that the molecular mechanisms underlying RA effects on adipocyte differentiation and adipogenesis are complex and remain poorly understood. Furthermore, it is proposed that there is a high turnover of adipocytes at all adult ages in humans. Adiposity can be affected by retinoids throughout life, and particularly through long-lasting effects at critical developmental stages.^{59,67} The following experimental results are pertinent to this comment. In WAT of retinyl palmitate-fed young rats during the suckling period (day 21), which is a critical period in the development of adipose tissue, small adipocytes and proliferating cell nuclear antigen increased and expression of adipogenic markers (PPAR γ and LPL) reduced. Vitamin A-treated rats fed a high-fat diet for 16 weeks after vitamin A being stopped developed higher adiposity gain (particularly subcutaneous fat) than control rats through mechanisms that may relate to changes in adipose tissue development, likely mediated by RA.⁶⁸

Rald is also an inhibitor of adipogenesis. Ziouzenkova et al showed that Rald was present in rodent fat, inhibited PPAR γ -induced adipogenesis, and increased energy dissipation. *In vivo*, mice lacking the Rald-catabolizing enzyme Raldh1 resisted diet-induced obesity and insulin resistance. *In vitro*, Rald inhibited RXR and PPAR γ activation. They identified Rald as a distinct transcriptional regulator of metabolic responses to a high-fat diet.^{69,70} It has been shown that Rald induced UCP1 levels in white adipocytes by selectively activating RAR, recruiting the coactivator PGC1 α , and inducing UCP1-promoter activity. Raldh1 is expressed predominately in WAT, including visceral depots in mice and humans. Raldh1 and Rald have been established as the determinants of adipocyte plasticity and adaptive thermogenesis.⁷¹

Retinol is very hydrophobic, and normally sequestered by RBPs.⁷² Intracellularly, CRBPs protect retinol from the cellular milieu and facilitate channeling of retinol toward specific enzymes required either for its oxidation to RA or for its esterification to retinyl esters.^{73–76} Three CRBPs – CRBP1, CRBP2, and CRBP3 – have been identified in murine tissues.^{73,75,77} It was reported that CRBP1 was a cytosolic protein specifically expressed in preadipocytes in adipose tissue that regulated adipocyte differentiation, in part by affecting PPAR γ activity. CRBP1 deficiency led to significantly enhanced adipocyte differentiation and increased intracellular triglyceride accumulation, due to augmented PPAR γ activity.⁷⁸ CRBP3 is present in adipocytes and a direct target of PPAR γ activation. It is expressed during mid- and late-stage differentiation of adipocytes. The lack of CRBP3 is associated with decreased adipose tissue development and markedly downregulated PPAR γ activity. The function of CRBP3 is to facilitate the esterification of retinol to retinyl ester in the mammary gland during lactation.^{17,79,80} It is well established that CRBP2 is expressed primarily in the small intestine and binds both retinol and Rald in the enterocyte. CRBP2 mediates intracellular retinoid transport and metabolism.⁸¹ The effect of vitamin A deficiency on obesity might increase the risk of fat deposition and also the risk of chronic inflammation associated with obesity.⁸² However, a synergistic effect of dietary lipids and retinol on the growth of adipose tissue was observed in young (3 weeks old) rats. Cafeteria diets enriched in retinol (excess vitamin A) increased adiposity due to increased adipocyte hypertrophy, which was concomitant with a lower expression of RAR α and RAR γ mRNA and a higher expression of PPAR γ in subcutaneous WAT and in isolated mature adipocytes. The adipocyte precursors from subcutaneous WAT had higher proliferation and differentiation capacities.⁸³ It

has been reported that β -carotene given orally was readily absorbed intact and partially metabolized by suckling rats, as indicated by intact β -carotene in serum and the liver and increased RA-mediated transcriptional responses in the intestine and liver. Responses in WAT were dependent on retinyl palmitate supplementation. The WAT of β -carotene-supplemented rats was enriched in larger adipocytes with enhanced adipogenic markers (PPAR γ and downstream genes) and reduced markers of proliferative status (proliferating cell nuclear antigen) compared to the WAT of vitamin A-supplemented rats.⁸⁴ It is evident that vitamin A supplementation with β -carotene or retinyl palmitate in early life may affect adipose tissue development differentially.

Retinol saturase (RetSat), an enzyme catalyzing the saturation of all-*trans*-retinol to produce (*R*)-all-*trans*-13,14-dihydroretinol, is proposed to play an important role in the biology of adipocytes. It is induced during adipocyte differentiation of 3T3L1 cells and by PPAR γ activation. PPAR γ is required for RetSat expression in mature adipocytes. Schupp et al showed that RetSat was required for adipocyte differentiation in the 3T3L1 cell-culture model. In adipose tissue, RetSat was expressed in adipocytes but downregulated in obesity, most likely owing to infiltration of macrophages that repressed RetSat expression. RetSat increased PPAR γ transcriptional activity and adipocyte differentiation, depending on its intact FAD/NAD dinucleotide-binding motif. However, RetSat was not required for adipogenesis when 3T3L1 cells were provided with pioglitazone. Thiazolidinedione treatment reversed low RetSat expression in the adipose tissue of obese mice.⁸⁵ Moise et al reported that RetSat-null mice maintained on either low-fat or high-fat diets gained weight and accumulated more fat. This increased adiposity was associated with upregulation of PPAR γ and also its downstream target: FABP4/AP2. They proposed that dihydroretinoids produced by RetSat control physiological processes that influence PPAR γ activity and regulate lipid accumulation in mice.⁸⁶

β -Apocarotenals resulting from asymmetric cleavage of β -carotene, such as β -apo-8'-carotenal and especially β -apo-14'-carotenal, inhibit PPAR γ and PPAR α responses. During adipocyte differentiation, β -apo-14'-carotenal inhibits PPAR γ -target gene expression and adipogenesis.^{22,87} β -Apo-13-carotenone was reported to upregulate expression of the adipocyte marker genes *LPL*, *AP2*, adiponectin, and *PPAR* γ in 3T3L1 cells. β -Apo-10'-carotenoic acid increased expression of *LPL* and *AP2*, but did not have a significant influence on expression levels of adiponectin or PPAR γ .⁸⁸ β -Apo-14'-carotenal, β -apo-14'-carotenoic acid, and β -apo-13-carotenone were demonstrated to be RAR antagonists in

transactivation assays.⁸⁹ However, β -apo-13-carotenone and β -apo-10'-carotenoic acid lack RAR-antagonist activity. They failed to diminish the inhibitory effects of a relatively large dose of exogenous all-*trans*-RA on adipocyte differentiation.⁸⁸

It is evident that β -carotene has a complex influence on PPAR γ -regulated pathways controlling adipogenesis. An adipose tissue-specific conversion of β -carotene via carotenoid oxygenases can influence the activities of key transcription factors. Moreover, different differentiation stages and different organ resources of adipocytes may contribute to changeable consequences of β -carotene derivatives. β -Carotene could suppress PPAR γ expression and activity and decrease lipid content in mature adipocytes, mainly through being metabolized to their derivatives (retinoids and apocarotenoids). Carotenoid derivatives could regulate the expression of direct target genes by serving as ligands of the PPAR and/or the RXR moiety of permissive PPAR:RXR heterodimers, which help control adipogenesis and adipocyte metabolism.

Other carotenoids

In addition to β -carotene, other carotenoids also display anti-obesity effects, in the main via downregulation of PPAR γ expression. Astaxanthin has been shown to inhibit increases in body weight and weight of adipose tissue induced by a high-fat diet, reduce liver and plasma triglycerides and total cholesterol in mice,⁹⁰ and promote size reduction in visceral adipocytes, with a remarkable decrease in free fatty acid and a significant increase in blood high-density lipoprotein (HDL)-cholesterol concentrations in Otsuka Long-Evans Tokushima fatty rats.⁹¹ It was found that astaxanthin was able to selectively bind PPAR γ , but unable to activate transcription of PPAR γ reporters. Astaxanthin inhibited rosiglitazone-induced adipogenesis of 3T3L1 cells by antagonizing PPAR γ transcriptional activity.⁹² The reporter-gene assay and ligand-binding domain studies showed that astaxanthin activated PPAR α but inhibited PPAR γ transactivation activity, and had no effect on PPAR δ / β activation. It reduced lipid accumulation and cellular cholesterol and triglyceride concentrations by rewiring the transcriptome in lipid-loaded HepG2 hepatocytes.⁹³ However, astaxanthin improved the adipogenic differentiation potential of mouse neural progenitor cells. Astaxanthin-treated neural progenitor cells showed prominent fat formation and induced significant overexpression of adipogenesis-related AP and PPAR γ mRNA.⁹⁴ This may relate to lipid metabolism in different organs.

Takahashi et al reported that in 3T3L1 adipocytes and HepG2 hepatocytes, the treatment of such isoprenols as farnesol and geranylgeraniol caused the transactivation of the PPAR γ -reporter gene and increased expression of AP2 in 3T3L1 adipocytes. It can be seen in their Figure 2 that, except zeaxanthin, astaxanthin, β -carotene, β -cryptoxanthin, and lycopene at 100 μ M, a concentration hardly achievable in aqueous solutions, induced PPAR γ transactivation.⁹⁵ A determination of PPAR γ activity in monkey CV1 kidney cells by reporter assay showed that astaxanthin, bixin, and lycopene had the potential to activate PPAR γ as novel ligands.⁹⁶

Bixin and norbixin (annatto extracts) activated PPAR γ and induced mRNA expression of PPAR γ -target genes, such as *AP2*, *LPL*, and adiponectin, in differentiated 3T3L1 adipocytes and enhanced insulin-dependent glucose uptake.⁹⁷ Bixin and norbixin activated PPAR α -transactivation activity and induced the mRNA expression of PPAR α -target genes involved in fatty acid oxidation in PPAR α -expressing HepG2 hepatocytes. In obese KK-Ay mice, bixin suppressed hyperlipidemia development and hepatic lipid accumulation and increased the mRNA levels of PPAR α -target genes related to fatty acid oxidation in the livers. Moreover, bixin also improved obesity-induced dysfunctions of carbohydrate metabolism.⁹⁸

Tsuchida et al reported that the oral intake of β -cryptoxanthin exerted anti-obesity effects by lowering visceral fat levels.⁹⁹ Anti-obesity effects of β -cryptoxanthin have also been reported in an obese mouse model.¹⁰⁰ β -Cryptoxanthin inhibited lipid accumulation in 3T3L1 cells, affected cell differentiation through its function as a ligand of RAR α and RAR γ , and downregulated mRNA expression of PPAR γ . Their results indicated that β -cryptoxanthin inhibited 3T3L1 adipogenesis via the downregulation of PPAR γ through RAR activation.¹⁰¹

Fucoxanthin is a major carotenoid present in edible seaweed, such as *Undaria pinnatifida* and *Hizikia fusiformis*. Fucoxanthin and its metabolites, fucoxanthinol and amarouciaxanthin A, inhibited the adipocyte differentiation of 3T3L1 cells through downregulation of PPAR γ and C/EBP α .^{102–104} Interestingly, Kang et al reported that fucoxanthin from the edible brown seaweed *Petalonia binghamiae* promoted 3T3L1 adipocyte differentiation, increased triglyceride accumulation, and increased protein levels of PPAR γ , C/EBP α , SREBP1C, and AP2, and adiponectin mRNA level during the early stage of differentiation (days 0–2). However, it reduced the expression of PPAR γ , C/EBP α , and SREBP1C during the intermediate (days 2–4) and late stages (days 4–7) of differentiation. Their results suggest

that fucoxanthin exerts differing effects on 3T3L1 cells of different differentiation stages.¹⁰⁵ Xanthigen (brown marine algae fucoxanthin + pomegranate-seed oil) was reported to suppress adipocyte differentiation and lipid accumulation in adipocytes and markedly downregulate the protein levels of PPAR γ , C/EBP β , and C/EBP δ , as well as a key enzyme involved in adipogenesis – FAS. It also upregulated NAD⁺-dependent histone deacetylases (Sirt1) and activated AMPK signaling in differentiated 3T3L1 adipocytes.¹⁰⁶ Xanthigen promoted weight loss and reduced body and liver fat in obese women.¹⁰⁷

Several studies have shown that lycopene has an impact on adipose tissue biology. It was reported that a higher intake of lycopene was associated with a smaller waist circumference and lower visceral and subcutaneous fat mass.¹⁰⁸ Lycopene is beneficial in protecting against high-fat diet-induced fatty liver. Dietary lycopene downregulated BCO1, PPAR γ , and FABP3 mRNA expression in rat kidney and adrenal glands. Lycopene may play an important role in the modulation of β -carotene, retinoid, and/or lipid metabolism.¹⁰⁹ Lycopene increased plasma concentration of adiponectin and upregulated mRNA expressions of adiponectin, Sirt1 (a nicotinic adenine dinucleotide-dependent protein deacetylase), (FoxO1, a transcriptional factor involved in the regulation of adipocyte differentiation), and FAT/CD36, but downregulated PPAR γ in the adipose tissue of obese rats.¹¹⁰ Apo-10'-lycopenoic acid (APO10LA), a potential oxidation product of apo-10'-lycopenal generated endogenously by BCO2 cleavage of lycopene, modulated the transcriptome of 3T3L1 adipocytes in a manner similar to atRA. It activated RAR and impacted the transcription of RAR-target genes, downregulating leptin, CEBP α , and RXR α and upregulating PPAR α expression, but showed no effect on adipogenesis, PPAR γ , or adiponectin mRNA-expression levels in 3T3L1 cells.¹¹¹ APO10LA protected against the development of steatosis in *ob/ob* mice by upregulating *SIRT1* gene expression and activity. It decreased acetylated FoxO1 protein levels and the mRNA level of acetyl-CoA carboxylase 1.¹¹² Lycopene and APO10LA inhibited high-saturated-fat diet-induced steatosis in BCO2-knockout male mice through differential mechanisms. Lycopene or APO10LA reduced hepatic total cholesterol and activated Sirt1 signaling, which resulted in reduced fatty acids and triglyceride-synthesis markers and elevated cholesterol-efflux genes. Steatosis inhibition by lycopene induced PPAR α - and PPAR γ -related genes in mesenteric adipose tissue and increased mesenteric adipose tissue fatty acid utilization. Female mice did not develop steatosis.¹¹³ Tomato and lycopene feeding decreased lipid

uptake, hepatic triglyceride concentrations, and PPAR γ expression in both male wild-type and Bco2^{-/-} mice.¹¹⁴ It seems that the effects of lycopene are not dependent on the products cleaved by BCO2.

Paprika pigments containing large amounts of capsanthin and capsorubin were shown to promote 3T3L1 adipocyte differentiation and upregulate PPAR γ mRNA and protein expression, as well as adiponectin mRNA expression and secretion.¹¹⁵ The green algal carotenoid siphonaxanthin was found to suppress adipocyte differentiation and lipid accumulation in 3T3L1 cells. The effects of siphonaxanthin were largely limited to the early stages of adipogenesis. After 8 days of adipocyte differentiation, siphonaxanthin decreased the gene expression of C/EBP α , PPAR γ , FABP4, and Scd1. Siphonaxanthin administration reduced the total weight of WAT, especially the mesenteric WAT in KK-Ay mice, and enhanced fatty acid oxidation in adipose tissue.¹¹⁶

Okada et al¹¹⁷ tested the suppressive effects of 13 naturally occurring carotenoids (lutein, violaxanthin, α -carotene, β -carotene 5,6-epoxide, canthaxanthin, citranaxanthin, rhodoxanthin, β -cryptoxanthin, antheraxanthin, lutein epoxide, neoxanthin, and capsorubin) on 3T3L1 adipocyte differentiation. They demonstrated that neoxanthin reduced intracellular lipid accumulation and the expression of C/EBP α and PPAR γ mRNAs without affecting the expression of C/EBP β or C/EBP γ mRNAs. The other 12 carotenoids used did not show suppressive effects on adipose-cell differentiation. Combined with their results on fucoxanthin and fucoxanthinol, they suggested that carotenoids containing an allene bond and an additional hydroxyl substituent on the side group may show suppressive effects on adipocyte differentiation in 3T3L1 cells.^{102–104,117} It should be noted that their observation is not in agreement with the results obtained by Shirakura et al using β -cryptoxanthin¹⁰¹ or García-Rojas et al using lutein in a bovine adipocyte-differentiation system, in which 30 μ M lutein increased PPAR γ mRNA expression.⁶² Perhaps the hydroxyl group at the third position of the ionone ring is also important for carotenoid effects. Moreover, carotenoid metabolism in adipose tissues has not yet been understood adequately. It is thus unclear whether the intact molecules of carotenoids or their derivatives exert suppressive effects on adipogenesis and PPAR γ activity.

PPAR γ in anti-inflammatory actions and modification of cholesterol metabolism by carotenoids

PPAR γ is known to be involved in governing inflammatory response, particularly in macrophages. It may play a role

in anti-inflammatory actions by interfering with proinflammatory transcription factors.¹¹⁸ Immune-cell macrophages induced by lipopolysaccharide (LPS) produce nitric oxide using iNOS. iNOS is an important enzyme that mediates inflammatory processes. Improper upregulation of iNOS is associated with pathophysiology of certain types of human cancers, as well as inflammatory disorders, including atherosclerosis.¹¹⁹ It is well known that PPAR and RXR regulate immune function, including repression of NF κ B signaling and inflammatory cytokine production.¹²⁰ The anti-inflammatory effects of carotenoids have been found to be associated with the suppression of proinflammatory transcription factors through increasing PPAR γ levels. Selvaraj et al reported that lutein may play a role in LPS-induced inflammatory responses. Dietary lutein and fat interacted to modify nitrite production in LPS-stimulated macrophages. High levels of lutein increased nitrite production; however, high levels of fat reversed the stimulatory effect of lutein.¹²¹ They also reported that lutein and dietary fat or eicosapentaenoic acid interact to modulate iNOS mRNA levels through the PPAR γ -RXR pathway in LPS-stimulated chickens and HD11 cell lines. Increasing lutein with high fat (6%) or eicosapentaenoic acid (15 mmol/L) increased PPAR γ and RXR α mRNA levels. Lutein increased PPAR α mRNA levels in both macrophages and HD11 cells and RXR γ mRNA levels in macrophages. GW9662 and LG101208, an RXR antagonist, prevented lutein-induced iNOS mRNA regulation.¹²² The effect of dietary lutein or polyunsaturated fatty acid (PUFA) fat on lutein and lipid content and PPAR and RXR expression in chicken immune tissues during inflammation was investigated to assess the modification of in vivo inflammatory responses by dietary PUFA fat and lutein.¹²³ Lutein in immune tissues was depleted during inflammation, and the depletion extent was dependent on dietary lutein levels.¹²⁴ Similarly, fat levels were modified during inflammation.¹²⁵ Selvaraj et al showed that LPS injection decreased the lutein content and increased the fat content in chicken liver and spleen. Dietary PUFA fat at 6% ameliorated the lutein-depletion effect of LPS. Dietary lutein and PUFA fat content modulated liver and spleen PPAR γ/α and RXR γ/α isomers during the proinflammatory response to LPS. High dietary lutein or high PUFA fat increased all PPAR- and RXR-isomer mRNA levels. Therefore dietary lutein and PUFA fat were anti-inflammatory, due to modification of immune tissue lutein content, PPAR and RXR isomers, and IL-1 β mRNA levels in the liver and spleen, mainly due to modification of immune responses to LPS stimulation.¹²³ Apo-10'-lycopenoic acid exerts anti-inflammatory effects in

adipose tissue and adipocytes. It reduces the production of the proinflammatory markers IL-6 and IL-1 β with upregulation of PPAR α expression and without effect on PPAR γ expression.¹¹¹ Paprika carotenoids have also been suggested to be useful for suppressing the enhanced inflammatory response of adipose tissue. Adipocytokine from adipocyte cells has been known to be related to chronic inflammation in adipocyte cells in obesity. Paprika pigments suppressed mRNA expression of IL-6, MCP1, resistin, and TNF α , as well as NO production in a coculture of 3T3L1 adipocytes and RAW264.7 macrophage system.¹¹⁵

Hypercholesterolemia is one of the most important risk factors for atherosclerosis, in which macrophages play a central role. The accumulation of cholesterol-loaded macrophages in the arterial wall is the key event of the early atherosclerotic lesion. Cholesterol efflux from macrophages, which is mediated by ABCA1 and ABCG1, is thought to be a major process involved in plaque regression when hypercholesterolemia is reversed.^{126,127} Astaxanthin was shown to promote ABCA1 and ABCG1 expression in various macrophages and to have little effect on PPAR γ , LXR α , or LXR β . Astaxanthin enhanced apoA-I/HDL-mediated cholesterol efflux from RAW264.7. It is proposed that the potential cardioprotective properties of astaxanthin might be associated with an enhanced antiatherogenic function of HDL.¹²⁸ In addition, astaxanthin was reported to reduce total cholesterol in mice and the cellular cholesterol in HepG2 hepatocytes and increase HDL-cholesterol concentrations in rats.^{90,91,93} As mentioned earlier, lycopene increased the expression of PPAR γ , LXR α , and ABCA1 and decreased cellular total cholesterol levels in human prostate LNCaP and DU145 cancer cells.^{15,16} Lycopene or APO10LA reduced hepatic total cholesterol and elevated cholesterol-efflux genes in *BCO2*-knockout male mice.¹¹³ It appears that lycopene, apo-10'-lycopenoic acid, and astaxanthin may act as antiatherosclerotic nutrients through modifying cholesterol metabolism. β -Cryptoxanthin was also found to induce ABCA1 and ABCG1 mRNAs and ABCA1 protein in macrophages.¹²⁹ It is also helpful for cholesterol efflux.

Summary

As the roles of carotenoids in relation to health and disease have become progressively understood, molecular mechanisms mediating the effects of carotenoids have received growing attention. In addition to their antioxidant activity, carotenoids may exert their beneficial effects via another way: transcriptional modulation of the important gene expression concerned. As an important transcription factor, PPAR γ has

been proven to be involved in antiproliferation, antiadiposity, anti-inflammation, and cholesterol-homeostasis modulation of carotenoids, as reviewed in this paper. Carotenoids, as well as their products and metabolites, can regulate the expression of direct target genes by serving as ligands of the PPAR and/or the RXR moiety of permissive PPAR:RXR heterodimers or by indirect mechanisms. Furthermore, in recent years new research has emerged, pointing to new molecular pathways, including PPAR γ functioning, by which carotenoids and their derivatives exert extensive beneficial effects. Ongoing studies will offer new insights into their biological activities and molecular mechanisms, and thereby contribute to improved dietary or supplementation-based strategies and future therapeutic interventions using PPAR γ as a potential target.

Disclosure

The authors report no conflicts of interest in this work.

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