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Review

Carotenoids and their conversion products in the control of adipocyte function, adiposity and obesity

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ABSTRACT

A novel perspective of the function of carotenoids and carotenoid-derived products – including, but not restricted to, the retinoids – is emerging in recent years which connects these compounds to the control of adipocyte biology and body fat accumulation, with implications for the management of obesity, diabetes and cardiovascular disease. Cell and animal studies indicate that carotenoids and carotenoids derivatives can reduce adiposity and impact key aspects of adipose tissue biology including adipocyte differentiation, hypertrophy, capacity for fatty acid oxidation and thermogenesis (including *browning* of white adipose tissue) and secretory function. Epidemiological studies in humans associate higher dietary intakes and serum levels of carotenoids with decreased adiposity. Specifically designed human intervention studies, though still sparse, indicate a beneficial effect of carotenoid supplementation in the accrual of abdominal adiposity. The objective of this review is to summarize recent findings in this area, place them in physiological contexts, and provide likely regulatory schemes whenever possible. The focus will be on the effects of carotenoids as nutritional regulators of adipose tissue biology and both animal and human studies, which support a role of carotenoids and retinoids in the prevention of abdominal adiposity.

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Introduction

Carotenoids are lipophilic C-40-based isoprenoid pigments usually red, orange or yellow in color which are produced by plants and certain photosynthetic microorganisms. Primary dietary sources are fruits and vegetables, though they can also be obtained from bread, eggs, milk, beverages, fats, and oils. Of ~600 carotenoids in nature, ~50 are present in the human diet, but only six are ubiquitous in human serum, namely β-carotene (BC),[1] α-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin [1].

The main known function of carotenoids in humans is to serve as precursors of vitamin A related retinoids such as retinol, retinal and retinoic acid that play important roles in the visual cycle and in gene regulation linked to many developmental and physiologic processes [2]. Intact carotenoid molecules and carotenoid cleavage products other than the retinoids may have additional biological activities whose relevance for human health is still uncertain, including acting as antioxidants and blue light filters for photoprotection [3,4]. BC is the main provitamin A carotenoid in the human diet. Key to retinoid production from BC in mammals is the activity of β-carotene-15,15′-oxygenase (BCO1), a cytosolic enzyme that cleaves BC centrally into two molecules of retinal (also named retinaldehyde, Rald), which can be oxidized irreversibly to retinoic acid or reduced reversibly to retinol [5] (Fig. 1). BCO1 is specific

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[1] *Abbreviations used:* ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; AMPK, AMP-dependent protein kinase; atRA, all trans retinoic acid; BAT, brown adipose tissue; BC, β-carotene; BCO1, β-carotene-15,15′-oxygenase; BCO2, β-carotene-9′,10′-oxygenase; BMI, body mass index; C/EBP, CCAAT-enhancer binding protein; CD36, cluster of differentiation 36; CRABP, cellular retinoic acid binding protein; CRBP, cellular retinol binding protein; FABP, fatty acid binding protein; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; ISX, intestine-specific homeobox; LRAT, lecithin:retinol acyltransferase; LDLr, low density lipoprotein receptor; LPL, lipoprotein lipase; NF-κB, nuclear factor κB; Nrf2, nuclear factor erythroid 2-related factor 2; PPAR, peroxisome proliferator activated receptor; Rald, retinaldehyde; RAR, retinoic acid receptor; RBP (or RBP4), retinol binding protein; RBPR2, RBP receptor 2; RDH, retinol dehydrogenase; REH, retinyl ester hydrolase; ROS, reactive oxygen species; RXR, retinoid X receptor; SAT, subcutaneous adipose tissue; SR-B1, scavenger receptor class B, member 1; STRA6, stimulated retinoic acid 6; UCP1, uncoupling protein 1; VAT, visceral adipose tissue; WAT, white adipose.

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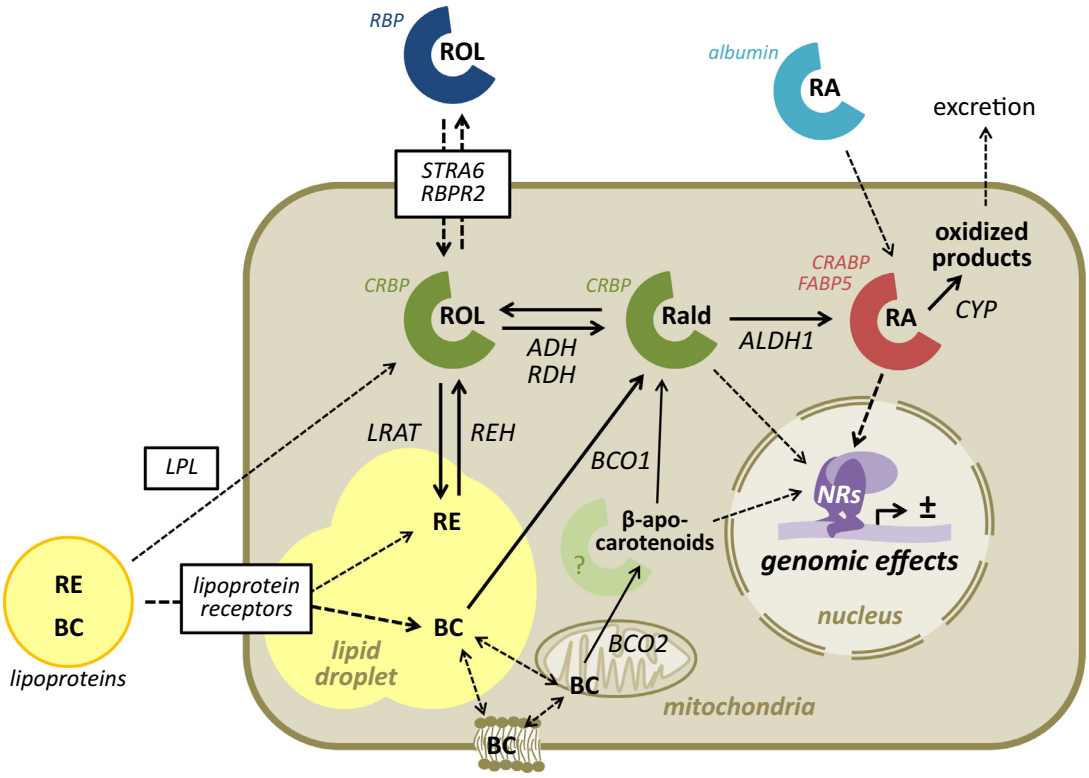

Fig. 1. Overview of cellular retinoid and β-carotene metabolism. Circulating retinol (ROL) bound to retinol binding protein (RBP) is internalized in peripheral cells through the action of specific surface receptors (STRA6, RBPR2) or by diffusion across the plasma membrane. Efficient ROL uptake depends on its binding to the cellular retinol binding protein (CRBP) and the activity of lecithin:retinol acyltransferase (LRAT) in esterifying ROL to fatty acids to form retinyl esters (RE) which incorporate into lipid droplets. RE associated with circulating lipoproteins can be hydrolyzed to ROL by lipoprotein lipase (LPL) and taken up by the cells. Alternatively, circulating lipoproteins containing RE and carotenoids such as β-carotene (BC) can be internalized in cells whole by endocytosis, mediated by lipoprotein receptors. Within the cell, RE is hydrolyzed by RE hydrolases (REH) to ROL, which can be reversibly oxidized to retinaldehyde (Rald) by short-chain dehydrogenase/reductases with retinol dehydrogenase (RDH) activity or medium-chain alcohol dehydrogenases (ADH) able to use retinol as a substrate. Rald can also be produced from BC, through symmetric cleavage catalyzed by β-carotene-15,15′-oxygenase (BCO1). BC (and other carotenoids, not shown) can be asymmetrically cleaved through the action of mitochondrial β-carotene-9′,10′-oxygenase (BCO2). Some BCO2 products can be converted into Rald, with the participation of BCO1. Rald is irreversibly oxidized to retinoic acid (RA) by the action of aldehyde dehydrogenases (ALDH1). RA can be taken up from the circulation where it is found bound to albumin. RA is transferred from the cytoplasm to the nucleus in association with specific intracellular lipid binding proteins (CRABP and FABP5). RA, Rald and other BC derivatives exert genomic effects by modulating the activity of distinct nuclear receptors (NR) and other transcription factors (not shown) in direct and indirect manners. These compounds exert as well non-genomic effects in cells (not shown). RA is catabolized by the cytochrome enzymes (CYP) to more oxidized products that are eliminated from the cell.

for provitamin A carotenoids containing at least one nonsubstituted β-ionone ring, such as BC, α-carotene and β-cryptoxanthin. Mammals express a second BC cleavage enzyme, β-carotene-9′,10′-oxygenase (BCO2), which localizes to mitochondria, cleaves BC asymmetrically to generate diverse β-apocarotenals and β-apocarotenones, and has a broad substrate specificity as it metabolizes, for instance, the acyclic carotene lycopene and oxygenated carotenoids (i.e. xanthophylls, such as lutein and zeaxanthin) besides cyclic carotenes [5]. BCO1 and BCO2 are broadly expressed in mammalian tissues; such widespread expression, together with the wide distribution of carotenoids in animal tissues, has suggested that local, tissue-specific conversion of carotenoids may contribute to the *in situ* generation of retinoids and other apocarotenoids that impact tissue metabolism [5].

In recent years, a novel perspective of the function of carotenoids and carotenoid-derived products – including, but not restricted to, the retinoids – is emerging that connects these compounds to the control of body fat accumulation, with implications for the management of obesity and obesity-related metabolic disturbances [6–10]. Although effects on body fat content can relate to action in different tissues – among them the liver, which constitutes the main reservoir for carotenoids and vitamin A and whose lipid metabolism is indeed affected by retinoids [10] – cell and animal studies indicate that adipose tissue is an important target of carotenoid action. This evidence is the focus of this review. We begin by briefly presenting mechanisms of action that underlie the biological activity of carotenoids and carotenoid cleavage products, and the various lines of evidence that implicate these compounds as players in adipose tissue biology. While BC as the main provitamin A carotenoid is in the center of this review, other carotenoids active in the modulation of adipocyte biology and body adiposity are also given consideration. We review the impact of these compounds on body adiposity and their interaction with key aspects of adipose tissue biology as revealed by cell and animal studies, namely: adipocyte differentiation, adipocyte capacity for fat storage and oxidative metabolism/thermogenesis, and the secretory and inflammatory profile of adipose tissue. Human epidemiological and intervention studies addressing the relationship between carotenoids and vitamin A status and obesity and related metabolic diseases are specifically addressed.

Mechanisms of action of carotenoids and carotenoid derivatives

Collectively, carotenoids and carotenoid conversion products have been shown to impact gene expression and cell (including adipocyte) function through multiple mechanisms, notably by: (a) interacting with several transcription factors of the nuclear receptor superfamily, such as the canonical retinoic acid receptors

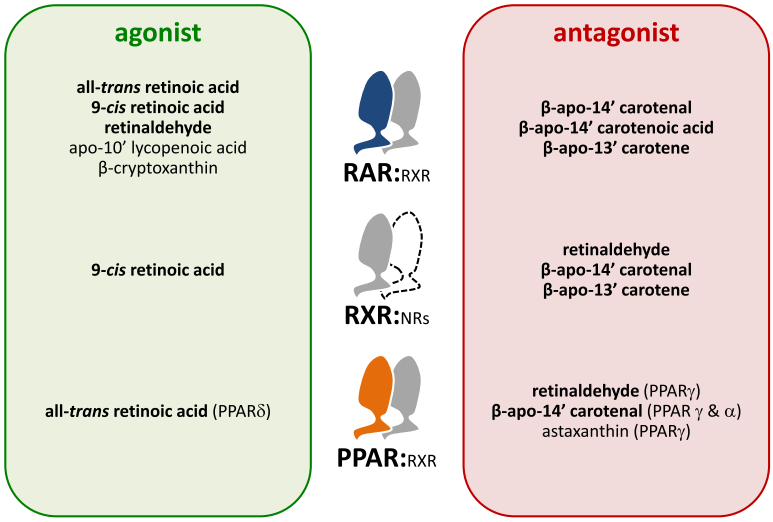

Fig. 2. Carotenoids and carotenoid derivatives as modulators of nuclear hormone receptors. The scheme shows BC derivatives (retinoids and other β-apocarotenoids, in bold) and other carotenoids or carotenoid derivatives that appear to be able to directly interact with the nuclear receptors retinoic acid receptor (RAR), retinoid X receptor (RXR) and peroxisome proliferator activated receptors (PPARs) to function as agonist or antagonist ligands (see text for details and references).

(RARs), the retinoid X receptors (RXRs) and the peroxisome proliferator activated receptors (PPARs), among others; (b) interfering with the activity of other transcription factors, such as activator protein-1, nuclear factor κB (NF-κB) or CCAAT-enhancer binding proteins (C/EBPs); (c) modulating signaling pathways such as the NF-κB and the nuclear factor erythroid 2-related factor 2 (Nrf2) pathways, which are associated with inflammatory and oxidative stress responses, respectively; and (d) through extragenomic actions including scavenging of reactive species, retinoylation (acylation by retinoic acid) of proteins and activation of protein kinase cascades [10–13]. All of these mechanisms contribute to their action in adipocytes, as we shall further see in upcoming sections.

Gene expression control by retinoic acid has been most studied [14]. Retinoic acid isomers are agonist ligands of the RARs (RARα, RARβ and RARγ) and the RXRs (RXRα, RXRβ and RXRγ): all-trans retinoic acid (atRA) binds the RARs, whereas 9-cis retinoic acid binds both the RARs and the RXRs. Heterodimers of RAR with RXR control the expression of typical retinoid-target genes by binding to defined retinoic acid response elements in the gene promoter and modulating transcription in a manner that depends on atRA binding to the RAR moiety and subsequent recruitment of transcriptional co-regulator complexes [14]. RXRs are obligate dimerization partners for many other nuclear receptors, including the PPARs. Some, RXR heterodimers respond to ligands of either partner and are synergistically activated when both ligands are bound, providing a mechanism for widespread effects of retinoids on gene expression [15]. Moreover, besides activating the canonical RARs, evidence has been provided that atRA (but not 9-cis retinoic acid) can behave as an activating agonist ligand of PPARβ/δ (but not the other PPAR isoforms, namely PPARα and PPARγ) [16]. Besides retinoic acid, other retinoids (such as Rald) as well as apocarotenoids different from retinoids (such as β-apo-14′-carotenal, β-apo-13-carotenone and apo-10′-lycopenoic acid) interact with nuclear receptor transcription factors to either antagonize or promote their action on target genes in cells [17–22] (Fig. 2). Even intact carotenoid molecules appear to be able to interact physically with nuclear receptors: studies have suggested that, in differentiating adipocytes, astaxanthin can behave as a PPARγ antagonist ligand [23] and β-cryptoxanthin, as a RAR agonist ligand [24] (Fig. 2).

Carotenoids and carotenoid derivatives as players in adipose tissue biology: lines of evidence

Adipose tissue is an important site of carotenoid storage [25]. Within the adipocyte, carotenoids are mainly stored with triacylglycerol in the lipid droplet, and are also found in cell membranes [26]. In humans, carotenoid levels in abdominal depots show a strong association with both intake and plasma concentrations [27,28]. Total carotenoid concentration is higher in abdominal adipose tissue than adipose tissue from buttock and thigh in humans [27]. However, the mechanisms involved in fat depot-specific uptake of carotenoids are unknown, and a recent report concluded that whole-body adipocyte stores of BC are more or less constant (130 ± 70 (mean ± SD) μmole per person) regardless of different body mass index (BMI) and varied intakes of BC, which raises questions regarding what function it serves and how this constancy is achieved [29]. Adipose tissue is also an important site for retinol storage. An early study estimated that 15–20% of the total body retinol in rats is stored in adipose tissues, in particular in the adipocytes (rather than the stromal vascular cells) and mostly in the form of non-esterified retinol (as opposed to preferential storage as retinyl ester in the liver) [30]. Other retinoids including atRA and Rald have been detected in adipose tissue [18,31]. Importantly, adipose tissue expresses all intracellular binding proteins and enzymes involved in retinol and retinoic acid production and metabolism, including BCO1 and BCO2 [7,8], and it also produces and secretes RBP [30], enabling exportation of retinol to the circulation.

Provitamin A carotenoids and retinol in adipocytes may serve to regulate systemic vitamin A homeostasis. In fact, adipose retinol/retinyl esters stores are readily mobilized under conditions of dietary vitamin A deficiency, as evidenced by decreased levels [9]. Additionally, carotenoids, vitamin A and their derivatives may serve specific functions within the mature adipocytes, including the control of metabolism (relative capacities for fat storage and oxidation), oxidative stress and production of inflammatory mediators (see also the upcoming sections). These aspects may be of special relevance in regard to obesity, as obesity entails the development of hypertrophic adipocytes in which oxidative stress is exacerbated [32] and inflammation of adipose tissue [33].

Systematic analytical studies to show the levels of carotenoids, vitamin A and their metabolites in fat depots in connection with adipose tissue development and function are technically challenging and lacking, yet the importance of carotenoids and retinoids for adipose tissue biology is supported by various lines of evidence:

(i) Studies suggest crosstalk of intracellular retinoid metabolism and lipid droplet dynamics. Hormone sensitive lipase (HSL) hydrolyzes retinyl esters besides triacylglycerol stored in lipid droplets [34]. Furthermore, the proteome of the lipid droplet includes enzymes of retinoid metabolism, such as the retinol dehydrogenase Rdh10, which catalyzes retinol to Rald conversion, and the retinal reductase Dhrs3, which preferentially catalyzes the opposite reaction ([35] and references therein). Interestingly, association of enzymes of retinoid metabolism with the lipid droplet coat seems to be dependent on active acyl ester biosynthesis [35,36]. For instance, in COS7 cells, Rdh10 localizes to mitochondria or mitochondrial-associated membrane and relocates to the surface of the lipid droplets upon active acyl ester biosynthesis [35]. It is conceivable, therefore, that synthesis of Rald and retinoic acid is increased under conditions of net lipid droplet enlargement. It would be interesting to know if relocation events take place as well in adipocytes, and whether BC cleavage enzymes also relocate to lipid droplets (which contain carotenoids) in a regulated manner.

(ii) Animal and human studies reveal a differential expression of genes for carotenoid/retinoid metabolizing enzymes in visceral (VAT) and subcutaneous (SAT) adipose tissue [17,37–41]. This further suggests a role of endogenous retinoid metabolism in the control of adipose tissue function and dynamics, since VAT and SAT display important differences regarding genetic origin, metabolism, endocrinology, capacity for adipogenesis and the health risk they entail; VAT is generally considered to be more strongly correlated with elevated risk for metabolic dysfunction and cardiovascular disease, while SAT may even be protective [42]. Recently, VAT obtained from the omental apron of patients with extreme obesity (mean BMI: 56.8 kg/m^2) undergoing open Roux-en-Y gastric bypass showed BCO2 as one of 66 uniquely expressed genes in this depot as compared to subcutaneous or epigastric fat by microarray analysis [39]. Because, as already stated (Fig. 2), BCO2 specific products can antagonize the activation of RARs [19], RXR [20,21] and PPARs [20] in cells including adipocytes, increased BCO2 activity on stored target carotenoids might result in decreased signaling through RAR:RXR and PPAR:RXR in VAT as compared to SAT. As we shall see next, this could contribute to a relatively lower capacity for oxidative metabolism and adipogenesis and changes in adipokine secretion in VAT, all of which could be of pathophysiological significance.

Increased BCO2 signaling in VAT *vs* SAT may accompany increased retinoid metabolism in the visceral fat depots. Comparable levels of retinol were reported in VAT and SAT depots in rodents [30,37], but murine VAT has higher expression levels of various retinoid homeostatic genes compared to SAT [37]. Accordingly, studies in humans (including two non-targeted proteomic studies) showed increased mRNA and protein levels of a main enzyme in the conversion of Rald to atRA – Aldh1a1, also known as Raldh1 – in VAT as compared to SAT [17,40,41]. One study reported a direct association between Aldh1a1 expression in human VAT and BMI ($r^2 = 0.39$, $P < 0.001$) [17]. The latter result might be in keeping with the somewhat reciprocal finding of decreased visceral fat and resistance to diet-induced obesity and associated insulin resistance in knockout mice lacking Aldh1a1 [17,18,38]. These results would suggest a detrimental effect of adipose Aldh1a1 activity in regard to obesity and its sequela. Another human proteomic study, however, found decreased levels of cytosolic Aldh1a1 protein in VAT of pre-obese diabetic subjects as compared with their non-diabetic counterparts [43], suggesting a beneficial effect of adipose Aldh1a1 activity.

(iii) Genetic ablation of various carotenoid/retinoid-metabolizing enzymes and transport proteins – such as BCO1, Aldh1a1, Rdh1, retinol saturase and cellular retinol binding proteins I and III – results in alterations of adipose tissue in mice (reviewed in [9]). This supports a role of endogenous carotenoid/retinoid metabolism in the control of adipose tissue development and function, although caution should be made since substrate specificity of some of the enzymes ablated (Aldh1a1, Rdh1 and retinol saturase) is broad, and therefore action on other targets might contribute or even be responsible for the observed adipose phenotype. The phenotype of most of these mutant models has been reviewed elsewhere [9]. To be highlighted is that BCO1$^{-/-}$ mice are more susceptible to diet-induced obesity and show increased expression of PPARγ-induced genes in VAT compared with their wild-type counterparts [44], which already suggested an anti-adiposity action of BCO1 conversion products.

(iv) Finally, the connection of carotenoids and retinoids with adipose tissue biology is sustained by treatment/supplementation studies, most of them in animals, which indicate an impact on body adiposity together with major effects on the adipose tissue capacities for fat storage, fat burning and secretory function, even if for some of these compounds changes in energy and lipid metabolism in other tissues, such as skeletal muscle [45–47] and the liver [48–50] are also involved. This line of evidence is developed in detail in next sections.

Animal studies linking BC and vitamin A to body adiposity

An adiposity-reducing effect of dietary BC supplementation (~35 mg/kg bw/day, 14 weeks), not accompanied by changes in body weight or energy intake, has been demonstrated in mice [51]. Importantly, this effect is dependent on BC conversion to retinoids via BCO1, as it was not seen in BCO1$^{-/-}$ mice, despite these mutants accumulated large amounts of BC and of the BCO2 product β-10′-apocarotenol (derived from BC) in white fat following supplementation [51]. These results are in keeping with studies in 3T3-L1 white adipocytes showing reduction of accumulated lipids following BC treatment (2 μM) [52]. In seeming disagreement, 6-months BC supplementation (3.2 mg/kg bw/day) resulted in increased body weight (by 14%) and subcutaneous (inguinal) fat mass (by 16%) in ferrets [53,54]. Since the anti-adiposity action of BC requires its conversion to retinoids [51], the difference may relate to previous findings that ferrets are not as efficient as humans and rodents in converting BC to vitamin A [55]; in fact, in the same study, treatment with atRA decreased body fat and adipocyte size in ferrets [54].

Further sustaining an anti-adiposity effect of BC-derived retinoids, work of different groups including ours has shown that atRA treatment (from 0.25 to 100 μg/g bw/day, through various routes of administration and for different duration) reduces adiposity and enhances glucose tolerance and insulin sensitivity in both lean and obese rodents [34,47,48,56–63]. Treatment with atRA has also been shown to counteract the development of dietary obesity in obesity-prone mice [64]. The anti-adiposity action of atRA has been traced to increased oxidative metabolism and energy expenditure in different tissues including white adipose tissue (WAT) and to decreased PPARγ expression and activity in WAT depots, with the involvement of both RAR and PPARβ/δ activation (see Section Impact of BC and BC derivatives on the metabolic profile of adipocytes). The immediate precursor of atRA, Rald, also appears to have an anti-adiposity effect *per se in vivo*, since mice lacking Aldh1a1 had increased Rald levels in WAT and resisted diet-induced obesity [18], *in vivo* knockdown of Aldh1a1 in WAT

(through antisense oligonucleotide) limited diet-induced obesity [17] and administrating Rald or a Aldh1 inhibitor decreased subcutaneous fat mass in obese ob/ob mice [18]. Aldh1a1 is the main aldehyde dehydrogenase involved in atRA production from Rald in adipocytes/adipose tissue [37,38]. Similar to atRA treatment, the anti-adiposity effect of Aldh1a1 knockout/knockdown involves up-regulation of energy expenditure in WAT [17] (see Section Impact on adipocyte capacity for oxidative metabolism/thermogenesis).

Additional dietary studies in animals link vitamin A status to adiposity. Diets low in carotenoids and vitamin A are used traditionally to favor the development of adipose tissue and the formation of intramuscular fat in the cattle [65,66], the so-called bovine marbling. In good concordance, chronic vitamin A-deficient diet feeding increases adiposity in rodents [58,67] this correlating with enhanced PPARγ expression in WAT depots [58]. Reciprocally, chronic dietary vitamin A supplementation (as retinol or retinyl palmitate, at 40- to 50-fold the control dose) led to decreased WAT mass in both lean rats [68] and genetically obese WNIN/Ob rats [69–71] – although in one of the studies at the expense of increased serum and liver lipids [70]. In the WNIN/Ob rats, vitamin A supplementation resulted in decreased 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) activity in liver and visceral WAT, suggesting that decreased glucocorticoid signaling might be another mechanism by which vitamin A modulates adiposity [71]. 11β-HSD1 catalyzes the intracellular regeneration of active glucocorticoids from inert derivatives, thus amplifying local glucocorticoid action, which is known to promote preadipocyte differentiation. However, vitamin A supplementation had only a modest effect counterbalancing the development of diet-induced obesity in obesity-prone mice [45], and it did not affect body weight and adiposity gain in response to a cafeteria diet in rats [72]. Moreover, there are studies pointing to a pro-obesogenic effect of excess intake of preformed vitamin A (retinol) in critical periods in early life, through mechanisms that may relate to changes in WAT development [73,74] (see Section Impact of BC and BC derivatives on adipogenesis next).

Impact of BC and BC derivatives on adipogenesis

Adipose tissue is highly plastic and can adapt to facilitate greater energy storage through the hypertrophic expansion of terminally-differentiated mature adipocytes as well as the hyperplastic growth and differentiation of precursor cells resident in the stroma. The latter process (adipogenesis) is triggered by nutritional and hormonal signals that activate a cascade of transcription factors that includes various C/EBPs and PPARγ, which is considered the master regulator of adipogenesis (reviewed in [75]). Even in (human) adult adipose tissue, about 10% of adipocytes turn over every year [76] and adipogenesis can be induced by environmental cues such as consumption of a high fat diet, both in rodents [77] and humans [78]. Proper adipogenesis is critical for maintaining health, as impairments in the face of a positive energy balance (i.e. more energy ingested than expended per day) may result in ectopic fat deposition and lipotoxicity, leading to insulin resistance, diabetes and vascular complications [79]. However, many obese humans have more than the average number of adipocytes and weight loss decreases the volume of adipocytes but not adipocyte number [80]. This may facilitate body weight regain. In fact, studies in rodents have suggested that increased adipocyte number might *per se* lead to obesity [81]. In this context, the control of adipogenesis emerges as a potential co-adjuvant therapeutic target in obesity when coupled to strategies enhancing a negative energy balance. Agents capable of tipping the adipocyte birth–death balance in favor of reducing the number of fat cells become of potential interest [80].

BC (50 μM) was shown to inhibit adipose conversion of 3T3-L1 preadipocytes, the prototypical model for studies of adipogenesis [82]. This is most likely due to BC conversion into atRA, for which an inhibitory effect on adipogenesis is well known [83,84]. Inhibition of adipogenesis by atRA is explained by several non-mutually exclusive mechanisms which translate into repression of PPARγ. One is the interference of liganded RAR with the activity of the early adipogenic transcription factor C/EBPβ on its downstream target genes in the adipogenic program [84], through the RAR-dependent induction of the C/EBPβ inhibitory protein Smad3 [85]. Additionally, atRA works upstream C/EBPβ in preadipocytes by inducing – at the transcriptional level and through an RAR pathway – specific proteins that inhibit adipogenesis, namely Pref-1, Sox9 and KLF2 [64]. Retinoylation of regulatory proteins leading to decreased PPARγ availability for transcription regulation may also play a role, this one independent of RAR and RXR [86]. Promotion of apoptosis of primary rat preadipocytes and clonal preadipocytes by atRA has also been reported [87,88]. Inhibition of adipogenesis is unlikely to contribute to the body fat reduction elicited by short-term atRA treatment in animals, but it has been shown to contribute to the protective effect of chronic atRA treatment against the development of high fat diet-induced obesity in mice [64].

BC metabolites other than atRA have been shown to repress adipogenesis of preadipocyte cell lines, in particular Rald [18,82] and β-apo-carotenals resulting from asymmetric cleavage of BC, such as β-apo-8′-carotenal [82] and, especially, β-apo-14′-carotenal [20]. Both Rald and β-apo-14′-carotenal behave as weak RAR agonists [18,20], yet their inhibitory effect on adipogenesis has been traced to their ability to suppress PPARγ- and RXR-mediated responses through RAR-independent mechanisms, possibly following direct physical binding to these receptors [18,20].

Because the transcriptional landscape changes dynamically during adipogenesis and RXR activation can affect signaling by many nuclear receptors, it is not surprising that atRA effects on adipogenesis are complex and both differentiation stage- and concentration-dependent events. Adding atRA to preadipocytes in high doses (0.1–10 μM) at the early stages of adipogenic differentiation has an inhibitory effect [83,84]. In contrast, adding atRA at lower doses (1 pM to 10 nM range) promotes adipogenic conversion [89]. Moreover, stem cell commitment into the adipocyte lineage requires a time-defined treatment with atRA [90], and endogenous atRA production appears to be required for efficient adipogenesis of 3T3-L1 fibroblast cell lines, by favoring PPARγ expression through zinc-finger protein 423-dependent mechanisms [38]. Therefore, it is possible that increased adiposity seen in animals chronically fed diets low in vitamin A [58,65] relates to proadipogenic effects of low atRA doses. Studies in HSL deficient mice support the concept that atRA can be pro- or anti-adipogenic *in vivo* depending on the dose [34]. HSL-null mice display decreased atRA levels in WAT (reflecting the lack of the retinyl ester hydrolase activity of HSL) and have defective adipogenesis in response to a high fat diet; dietary atRA supplementation partly restored WAT in these mutants, in which it would have served to restore atRA levels back to normal or near-normal levels, whereas it lowered WAT in the wild-type animals [34].

Nutritional clues at critical stages in early life can affect the susceptibility to metabolic alterations and diseases including obesity later in life (reviewed in [91]). In this context, the ultimate impact of vitamin A supplements on body adiposity might be developmental stage-dependent. For instance, in line with an anti-adipogenic action of retinoids, we recently reported that treatment with a moderate, threefold excess vitamin A (as retinyl ester) during the suckling period – which is a critical period in the development of adipose tissue in the rat – favors the accumulation of immature adipocytes, smaller and with less expression of PPARγ

and more of proliferating cell nuclear antigen, a classical marker of proliferative status [74]. These changes apparently boosted the hyperplasic component of fat expansion upon an obesogenic stimulus, since vitamin A-treated animals gained more adiposity – but not body weight – than their controls on a high fat diet, particularly subcutaneous fat, paralleling a higher increase in WAT DNA content [74]. A previous report already evidenced a synergic effect of exposure to a fourfold excess vitamin A (retinol) and high fat diet on WAT mass expansion in young (3-weeks-old) rats, which paralleled a higher proliferation competence of precursor cells isolated from the animals' fat depots [73]. These studies point, therefore, to a pro-obesogenic effect of excess vitamin A intake (even if moderate) in early life, likely by influencing the proliferative status of adipocytes. Interestingly, different from preformed vitamin A intake, a threefold excess vitamin A intake as BC during the suckling period did not elicit changes in the developing WAT of rats at weaning, even though BC was readily absorbed and partially metabolized by the suckling rats, as indicated by BC accumulation in serum and liver and enhanced atRA-dependent responses in intestine and liver [92]. This latter work establishes a new potential model in studies of BC action, and has suggested that BC supplementation may serve to replenish liver retinol stores in infants/children while avoiding eventual unwanted effects of early-life supplementation with pre-formed vitamin A on the susceptibility to obesity later in life [92].

Impact of BC and BC derivatives on the metabolic profile of adipocytes

Impact on adipocyte fat storage capacity

Accumulating evidence links adipose BC and BC-conversion products to the control of fat storage capacity of mature adipocytes through suppression of PPARγ. This nuclear receptor is required to maintain the adipocyte phenotype [93] and to mediate high fat diet-induced adipocyte hypertrophy [94] besides its essential role in adipocyte differentiation. Characterized PPARγ target genes code for proteins that facilitate uptake, cytosolic binding and activation of fatty acids for triacylglycerol synthesis and lipid droplet formation and maintenance ([95] and references therein). Reduction of PPARγ activity in mature adipocytes results in fat mobilization [93].

In mice, changes of adiposity in response to vitamin A status correlate with changes of PPARγ expression in WAT depots: both parameters are decreased after atRA treatment and increased after chronic vitamin A deficient diet feeding [58]. In mature 3T3-L1 adipocytes, exposure to BC (2 μM) decreases lipid content and the expression of PPARγ and PPARγ target genes while boosting the production of atRA; these effects are dependent on BCO1 and RAR, as they were abrogated by either a BCO1 inhibitor or a pan-RAR antagonist [52]. Likewise, reduction of adiposity following dietary BC supplementation in mice is linked to the suppression of PPARγ and PPARγ target genes in WAT, as indicated by both transcriptomic and targeted gene and protein expression analysis, and BCO1-dependent (not seen in $BCO1^{-/-}$ mice) [51]. Importantly, BC effects in 3T3-L1 adipocytes were not reproduced upon incubation of the cells with vitamin A (retinol), suggesting that BC is a key precursor for retinoid production in mature adipocytes [52], and, in the animal study, evidence was provided of BC accumulation in serum and WAT of BC-supplemented animals [51]. Considered together, these results support the notion that a BCO1-dependent, local production of retinoids from BC contributes to the regulation of fat storage capacity in adipocytes (Fig. 3). Remarkably, while BCO1-derived products repress PPARγ, BCO1 itself is encoded in a PPARγ target gene [96] that is induced during adipocyte differentiation [52], which is suggestive of a negative feed-back loop to keep control of PPARγ. How retinoid-activated RAR inhibits PPARγ in mature adipocytes is not known, but mechanisms similar to those involved in atRA inhibition of adipogenesis may be involved (see Section Impact of BC and BC derivatives on adipogenesis).

White adipocytes are cells specialized in the storage of excess dietary energy as triacylglycerol. Agents that repress adipogenesis and/or reduce fat storage capacity of mature white adipocytes are not necessarily beneficial; on the contrary, they might be detrimental, especially under conditions of positive energy balance, as they may boost ectopic fat deposition and lipotoxicity. This is not the case for several carotenoids and carotenoid-derived products with anti-adiposity action which, besides repressing PPARγ, are able to promote lipid oxidation in white adipocytes and other tissues (see next).

Impact on adipocyte capacity for oxidative metabolism/thermogenesis

Mammals possess different types of adipocytes, with important differences among them regarding energy metabolism ([97] and references therein). Brown adipocytes in typical brown adipose tissue (BAT) depots are rich in mitochondria, have a high oxidative capacity and are specialized in the regulated production of heat (nonshivering adaptive thermogenesis) through oxidation of fatty acids and

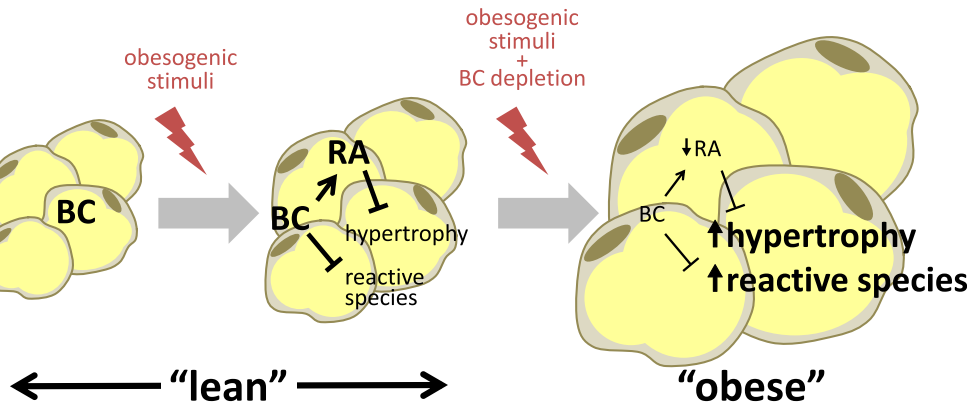

Fig. 3. Proposed action of local β-carotene (BC) adipose stores in obesity. BC and possibly other provitamin A carotenoids in adipocytes may serve to scavenge reactive species and (through the action of BCO1, not shown) as the precursors of retinoids such as retinoic acid (RA) capable of repressing adipocyte hypertrophy by suppressing PPARγ and enhancing energy utilization. These activities may help counteracting adipose tissue pathological expansion under obesogenic conditions and keeping the individual lean. However, BC is consumed in the course of these activities. If the obesogenic conditions persist and the diet is defective in carotenoids, depletion of adipose BC stores would result in a reduced capacity to scavenge reactive species and for anti-adiposity retinoid signaling, thus contributing to the development of pathological obesity.

other fuels linked to the subsequent dissipation of the proton electrochemical gradient generated by the respiratory chain via uncoupling protein 1 (UCP1), an inner mitochondrial membrane protein that behaves functionally as a proton transporter. Typical white adipocytes in WAT depots are poor in mitochondria, have a low oxidative capacity, do not express UCP1 and are specialized in the storage and release of energy, according to biological needs. A third type are the so-called "brite" (from brown-in-white) or "beige" adipocytes: these are brown-like, UCP1-expressing adipose cells which can be induced in WAT depots in response to cold and a variety of nutritional and pharmacological factors, in a process known as *browning* [97,98]. Brite adipocytes in WAT derive from precursor cells that are different from those in classical BAT and are closer to the white adipocyte cell lineage [97]. Brite cells may also arise from white-to-brown transdifferentiation of white adipocytes [97].

Until recently, BAT was believed to play a negligible role in the adult human and thought to be only present in neonates. However, it has gained substantial interest since active BAT has been shown to be present in adults and BAT activity is negatively associated with increasing BMI in humans [99]. Increasing BAT activity and inducing beige adipocytes in WAT both represent attractive strategies to counteract obesity [100,101]. Moreover, in spite of the widely neglected contribution of WAT to whole-body energy metabolism, pharmacological and nutrient-dependent stimulation of mitochondrial oxidative capacity and fatty acid oxidation in WAT independent of UCP1 has been documented which may also contribute to a lean phenotype [102]. Carotenoids and retinoids impact on BAT thermogenic function, and on oxidative capacity, mitochondrial genes and browning of WAT.

An interaction of carotenoids and retinoids with BAT function is known for some time. Early work showed that BC and other provitamin A carotenoids can induce UCP1 expression in cultured brown adipocytes [103], possibly reflecting their local conversion to atRA, which is a potent signal for UCP1 gene transcription in brown adipocytes [56,104]. The retinoid responsiveness of the UCP1 gene is explained by the presence in its promoter of both a noncanonical retinoic acid response element and a PPAR response element [105], and is mediated by RARs and RXRs [106] and also by p38 mitogen-activated protein kinase (p38 MAPK) [107]. atRA treatment in rodents leads to activation of BAT thermogenesis including induction of UCP1 accompanying body weight and body fat loss [56,57,108]. Moreover, feeding studies in rodents showed the thermogenic capacity of BAT to be dependent on vitamin A status, being decreased following a vitamin A-deficient diet and increased following a vitamin A (retinyl ester)-supplemented diet [45,57,68], thus supporting a role of vitamin A as a physiological regulator of the BAT thermogenic system.

More recently, retinoids have been shown to stimulate features of WAT browning. Our pioneer work in this area demonstrated this activity for atRA in mice [61] and in mature murine 3T3-L1 white adipocytes in culture [109]. In the latter cells, atRA treatment led to increased basal lipolysis and fatty acid oxidation rate, changes in gene expression consistent with increased fatty acid mobilization, oxidation and turnover and, upon prolonged treatment, decreased intracellular lipid content [109]. Even if atRA treatment failed to induce UCP1 in mature 3T3-L1 adipocytes [110,111], it potently induced UCP1 in other murine models of white adipocytes, such as adipocytes derived from primary mouse embryo fibroblasts (MEFs) and mature C3H10T1/2 adipocytes [110,111], in a p38 MAPK-dependent [110], RAR-dependent [111] and PPARγ coactivator 1α-independent manner [110,111]. In line with a browning effect *in vivo*, atRA treatment in mice resulted in increased expression of mitochondrial, thermogenic and fatty acid oxidation genes in WAT – including UCP1 protein in the subcutaneous (inguinal) fat depot – and appearance in WAT of adipocytes with a multilocular distribution of intracellular fat [61], the latter also a feature of brown adipocytes. atRA treatment has been shown to have comparable effects in ferrets, in which acute treatment led to decreased adiposity and increased multilocularity and UCP1 content in the retroperitoneal adipose depot [54]. However, atRA failed to induce UCP1 expression in human adipocyte cell lines and primary human white adipocytes [111].

Not only atRA but also Rald has been implicated as an agent promoting WAT browning. Rald was shown to induce UCP1 expression in mature primary MEFs-derived adipocytes, though at higher effective doses than atRA (10 μM *vs* 1 μM), as it can be expected for a Rald effect following its intracellular conversion to atRA [110]. More recently, Rald *per se* has been implicated as a browning agent, largely based on work in Aldh1a1$^{-/-}$ mice. Deficiency of Aldh1a1 enhanced cold-induced thermogenesis and induced a BAT-like transcriptional program in visceral WAT of the knockout mice, without changes in BAT [17]. Aldh1a1$^{-/-}$ mice were previously shown to resist high fat diet-induced obesity due to hypermetabolism [18]. Moreover, WAT-selective Aldh1a1 knockdown in adult obese mice triggered WAT browning while limiting weight gain and improving glucose homeostasis [17]. The browning effect of Aldh1a1 deficiency has been attributed to increased levels of its substrate, Rald, as evidence was obtained of increased Rald levels in WAT of Aldh1a1$^{-/-}$ mice [18] and that Rald can induce UCP1 expression in cultured white adipocytes independently of its conversion to atRA, following binding to and activation of RAR [17].

The similar phenotype of Aldh1a1$^{-/-}$ mice, deficient in the major atRA generating enzyme, and atRA-treated mice is striking and not completely understood [112]. Some considerations are pertinent. First, it cannot be excluded that Rald-independent effects contribute to the phenotype of Aldh1a1$^{-/-}$ mice [17], since Aldh1a1 can use other aldehyde substrates besides Rald [113]. Second, since atRA levels in WAT were not reported in the studies by Ziouzenkova et al. 2007 [18] and Kiefer et al. 2012 [17], it is unknown if decreased atRA levels accompanied the increased Rald levels reported in WAT of Aldh1a1$^{-/-}$ mice. Besides there are enzymes redundant to Aldh1a1 for atRA generation in adipocytes [38], atRA degrading enzymes such as CYP26A1 are expected to be inhibited under conditions of Aldh1a1 deficiency (and thus decreased atRA production) [114]. Notwithstanding other possible explanations [112], it is quite possible that atRA and Rald have somewhat redundant effects on adipose tissue metabolism.

Whereas browning effects of retinoids have been demonstrated in white adipocytes in culture, rodents and ferrets (see above), evidence for such an effect is lacking for BC. In mature 3T3-L1 adipocytes, BC-derived atRA decreased lipid content and PPARγ action, but potential effects on browning-related genes were not investigated [52]. In mice supplemented with BC, BC-derived retinoids led to decreased adiposity and PPARγ in WAT, but no evidence of WAT browning was apparent from transcriptomic and morphological analysis of inguinal WAT [51]. Furthermore, in ferrets, chronic intake of BC (3.2 mg/kg bw/day, 6 months) had opposite effects than short-term atRA treatment and resulted in a decreased presence of brown-like multilocular adipocytes and UCP1 in the retroperitoneal depot [54]. This inhibitory effect cannot be explained solely on the basis of inefficient BC conversion into vitamin A in the ferret [115], as this would have led to a neutral effect. Differences in the potential of retinoids and BC as browning agents and mechanisms involved deserve further investigation.

Other carotenoids active in the modulation of adiposity

β-Cryptoxanthin

β-cryptoxanthin is a provitamin A carotenoid which displays both structural and functional similarities to BC. Oral

supplementation with β-cryptoxanthin (0.8 mg/kg bw/day contained in 400 mg of a powder derived from Satsuma mandarins, *Citrus unshiu* Marc., 8 weeks) lowered body weight, visceral adipose tissue mass, adipocyte hypertrophy and serum lipid concentrations in a genetic obese mouse model (Tsumura Suzuki obese diabetic), independent of changes in food intake [116]. Anti-obesity effects in rodents have also been reported for mango (*Mangifera indica* L.) pulp (10% w/w) – which is a rich source of β-cryptoxanthin, violaxanthin and BC [117].

The anti-adiposity effect of β-cryptoxanthin in rodents might be in keeping with studies in differentiating 3T3-L1 adipocytes showing reduction of lipid accumulation following exposure to β-cryptoxanthin (1–10 μM) [24,118], although there are conflicting results [119]. β-cryptoxanthin effects on adipogenesis entail RAR activation and subsequent PPARγ down-regulation [24]. Results from *in vitro* nuclear receptor binding assays indicated that β-cryptoxanthin can efficiently bind RARs (but not PPARγ), which raises the possibility that β-cryptoxanthin acts *per se* as a RAR agonist to down-regulate PPARγ in adipocytes [24].

Astaxanthin

Astaxanthin is a natural antioxidant, non-provitamin A carotenoid, abundant in marine animals. Astaxanthin supplementation (6–30 mg/kg bw/day) prevented visceral fat accumulation, metabolic syndrome, and insulin resistance induced by high fat diet feeding in mice and rats [120,121].The anti-adiposity action of astaxanthin was not due to changes in food intake and was traced to enhanced systemic fatty acid utilization, as indicated by reduced respiratory quotient in indirect calorimetry tests [120]. Whether enhanced fat catabolism occurred in adipose tissue depots was not addressed. Astaxanthin has also been shown to inhibit rosiglitazone (a PPARγ ligand)-induced adipogenesis of 3T3-L1 cells by antagonizing PPARγ transcriptional activity, possibly upon direct binding, since results of *in vitro* assays indicate that astaxanthin is able to selectively bind PPARγ (but not PPARα or PPARβ/δ) [23].

Fucoxanthin

Fucoxanthin is an orange colored carotenoid present in edible brown seaweeds, such as *Undaria pinnatifida* (Wakame), *Hijikia fusiformis* (Hijiki), *Laminaria japonica* (Ma-Kombu) and *Sargassum fulvellum*. It is a non-provitamin A xanthophyll whose distinct structure includes an unusual allenic bond (reviewed in [122]). Fucoxanthin or fucoxanthin-rich seaweed extract, alone or as part of mixtures with other selected agents, counteracts the development of dietary obesity in obesity-prone mice fed a high fat diet (when added to the diet at 0.05–2%, w/w) and reduces abdominal WAT in genetically obese KK-A^y mice (when added to the diet at 0.2%) but not in normal mice fed a regular diet [122]. Fucoxanthin supplementation decreased WAT mass in obese animals by favoring fatty acid oxidation, UCP1 induction and heat production in abdominal WAT [123]. Notably, fucoxanthin intake promotes WAT browning at doses at which it does not affect UCP1 expression in BAT, suggesting a WAT selective effect [123]. The actual mammalian targets for interaction with fucoxanthin metabolites in fat cells remain to be identified, but enhancement of the sensitivity of white adipocytes to sympathetic nerve stimulation (which favors thermogenic activation) [124] and activation of AMP-dependent protein kinase (AMPK) [125] appear to contribute to the fucoxanthin effects in WAT. In humans, weight and adiposity reducing effects of fucoxanthin and Xanthigen™ – a supplement commercialized for weight management which is a source of fucoxanthin from brown seaweed plus pomegranate seed oil extract rich in punicic acid, a conjugated linolenic acid – have been linked to increases of resting energy expenditure, which may be related to the induction of browning by fucoxanthin demonstrated in rodents [126].

Interaction of fucoxanthin with adipogenesis of preadipose cells can also contribute to its anti-adiposity action, since suppressive effects of fucoxanthin and its metabolite, fucoxanthinol (which is found in WAT of fucoxanthin-treated animals) on adipogenesis of 3T3-L1 preadipocytes have been described when applied at intermediate and late stages of the adipogenic process [127,128]. On the opposite, fucoxanthin enhanced adipogenesis when applied at an early stage (coincident with preadipocyte clonal expansion) [128]. More recently, inhibition of 3T3-L1 preadipocyte differentiation by Xanthigen™ has been reported and traced to down-regulation of PPARγ and C/EBPs, up-regulation of Sirtuin 1, activation of AMPK and modulation of FoxO pathways [129].

Other

Animal studies indicate an anti-adiposity action for some other carotenoids. Dietary supplementation with crocetin (50 mg/kg bw/day) – which is a natural antioxidant carotenoid abundant in saffron, *Crocus sativus* Linn – prevented visceral fat accumulation, metabolic syndrome, and insulin resistance induced by high fat diet feeding in mice and rats, without affecting food intake [49]. These effects were traced to enhanced hepatic fatty acid oxidation [49]. Supplementation with violaxanthin-rich crude thylakoids prepared from spinach leaves reduced body weight and body fat gain in mice on a high fat diet without affecting energy intake, suggestive of the involvement of a metabolic mechanism [130].

Lycopene deserves an special mention since it is the second most abundant carotenoid after BC in human fat tissue and it is found at similar or even higher levels than BC in some other human tissues [131]. Results indicate that lycopene does not exert an anti-adiposity action. For instance, in obese Wistar rats lycopene supplementation (10 mg/kg bw/day during 6 weeks) did not affect body weight or adiposity [132], and cell studies showed no effect of lycopene and apo-10′-lycopenoic acid on adipogenesis [22]. However, apo-10′-lycopenoic acid was shown to activate the RAR and impact the transcription of certain RAR target genes in adipocytes [22]. Moreover, lycopene has anti-inflammatory properties of interest in obesity, including direct anti-inflammatory action in adipocytes and obese WAT tissue (see next Section).

Carotenoids and carotenoid derivatives as modulators of adipose tissue secretory function, inflammation and oxidative stress

Besides storing and releasing energy, WAT has an important endocrine function: it actively participates in the systemic control of energy balance, glucose homeostasis, insulin sensitivity, vascular haemostasis, inflammation and other processes through the secretion of signaling molecules, among them many signaling proteins (collectively named adipokines) produced by the adipocytes and/or cells of the stromal-vascular fraction, often in concert. The two roles of WAT are closely related to one another. Thus, the production of many of these signals, including hormones and immunomodulatory factors, is altered in obesity, which is nowadays recognized as a state of chronic, low-grade local (of WAT) and systemic inflammation, which links central obesity to metabolic disturbances as in the metabolic syndrome [33,133]. Both hypertrophic adipocytes and infiltrating macrophages in WAT are a source of inflammatory mediators in obesity.

Carotenoids and carotenoid derivatives affect the secretory profile of adipose tissue, including the production of specific

adipokines and cytokines, by affecting the activity of target transcription factors and inflammatory pathways. Additionally, the secretory profile may be affected indirectly, i.e. secondarily to effects on adipocyte lipid content and body fat.

Impact on adipokines

Elevated levels of resistin, leptin and RBP4 have been associated with inflammation and insulin resistance in humans and rodents [134]. We and others have shown that exposure to atRA suppresses the adipose production of leptin [57,108,135], resistin [59] and RBP4 [62] *in vivo* and in adipocyte cell models, through transcriptional mechanisms that involve both RAR- and RXR-dependent pathways, as suggested by studies using receptor-specific agonists [59,60,62]. Down-regulation of these adipokines paralleled an enhancement of systemic insulin sensitivity in the atRA-treated mice [59,62]. atRA-induced down-regulation has been demonstrated in humans besides rodents for leptin [135]. Remarkably, hepatic expression of RBP4 was unaffected by atRA treatment that resulted in down-regulation of RBP4 expression in WAT [62], pointing to an adipocyte-specific effect; this is of interest, since it is the RBP4 of adipose origin which has been related to inflammation and insulin resistance (although any physical difference between hepatic and adipose RBP4 remains to be established, to our knowledge). Similar to atRA treatment, dietary vitamin A supplementation to rodents resulted in decreased adipose expression and circulating levels of resistin and leptin to an extent that largely exceeded the reduction of adipose mass [59,60,68], whereas opposite changes of leptin (i.e., up-regulation) were demonstrated in mice fed a vitamin A deficient-diet [57]. Moreover, transcriptome analysis revealed leptin, RBP4 and resistin among the top fifty down-regulated genes in inguinal WAT of BC-supplemented wild-type mice, and these effects were provitamin A-dependent, as they were absent in BC-supplemented BCO1$^{-/-}$ mice [51].

Adiponectin is an adipokine quite specific of adipocytes that is down-regulated in obesity and with well-established insulin-sensitizing, anti-inflammatory and anti-atherogenic action [136]. Several carotenoids enhance adiponectin production in adipocytes, through mechanisms still to be defined. Paprika pigments, which contain large amounts of the carotenoids capsanthin and capsorubin, and crocetin from saffron have been shown to up-regulate adiponectin in 3T3-L1 adipocytes and freshly isolated rat adipocytes, respectively [137,138]. Fucoxanthin supplementation to mice on a high fat diet up-regulated adiponectin expression in visceral WAT together with genes involved in fatty acid mobilization and oxidation, and led to reduced body fat gain [139]. Lycopene supplementation to obese rodents increased adiponectin in blood and mRNA expression of adiponectin in WAT independent of changes in body weight or body fat content [140]. BC treatment (20 μM, from days 4–8 of differentiation) induced adiponectin expression in differentiating 3T3-L1 adipocytes [141], perhaps through a provitamin A-independent effect, since in our hands atRA treatment had no effect on adiponectin expression in 3T3-L1 adipocytes or adipose tissue of mice (Bonet, Ribot and Palou, unpublished results) and even down-regulation of adiponectin expression in WAT following acute atRA treatment to rats (7.5 mg/kg, i.p. single dose) has been described [142]. Interestingly, an independent positive association between BC and adiponectin levels in blood has been reported, both in general population [143] and in non-diabetic obese adult human subjects [144]. Other plasma/serum carotenoids tested in those human studies, among them lycopene, did not show an association with circulating adiponectin.

Impact on adipose tissue inflammation

Anti-inflammatory properties of carotenoids have been demonstrated in different contexts and tissues/cell types including adipocytes and adipose tissue (reviewed in [13]). Anti-inflammatory action is thought to arise from the ability of carotenoids to reduce oxidative stress (see Section Impact on adipose tissue oxidative stress) and to suppress NF-κB activation, thus inhibiting the downstream production of inflammatory cytokines [13].

At the adipocyte level, an anti-inflammatory action has been best demonstrated for lycopene and fucoxanthin. Lycopene and its metabolite, apo-10′-lycopenoic acid, have been shown to inhibit tumor necrosis factor alpha (TNFα)-mediated induction of pro-inflammatory cytokine and chemokine (macrophage-attractant signals) expression in both murine and human adipocytes [22,145]. Similar results have been reported for the fucoxanthin metabolite, fucoxanthinol [146]. Lycopene, fucoxanthine and fucoxanthinol also attenuate the response of RAW 264.7 macrophages to pro-inflammatory agents such as lipopolysaccharide or palmitic acid [146–148]. This is of interest because in the obese adipose tissue there is a vicious cycle between adipocytes and macrophages contributing to inflammation, which could be interrupted by these compounds. Inhibition of the NF-κB pathway and of mitogen-activated protein kinase pathways (such as the JNK pathway) underlies the anti-inflammatory action of lycopene and fucoxanthin in adipocytes and macrophages [145,148].

Anti-inflammatory action of fucoxanthin and lycopene has also been demonstrated *in vivo* in adipose tissue of obese animals. Genetically obese (KK-A^{y}) mice (but not lean mice) fed a fucoxanthin-supplemented diet (0.2%) displayed a decreased expression of pro-inflammatory factors and less infiltration of macrophages in visceral WAT compared with control animals fed the non-supplemented diet [146]. Likewise, treatment with fucoxanthin led to decreased expression of pro-inflammatory factors, myeloperoxidase activity (which attracts polymorphonuclear blood cells) and macrophage infiltration in the mammary gland of mice on a high fat diet [149]. In these two reports, fucoxanthin supplementation/treatment also elicited an anti-adiposity action [146,149]. As for lycopene, supplementation to obese Wistar rats (10 mg/kg bw/day during 6 weeks) did not affect body weight or adiposity, but decreased leptin, resistin, interleukin 6 and monocyte chemoattractant protein-1 gene expression in gonadal adipose tissue, and plasma concentrations of the former three factors [132].

Impact on adipose tissue oxidative stress

Oxidative stress generated in the obese adipose tissue is an important pathogenic mechanism of obesity-associated metabolic syndrome [32], tightly linked to adipose tissue inflammation and secretion of systemic inflammatory mediators, since reactive species activate inflammatory pathways, such as the NF-κB pathway. Cell and *in vivo* studies have suggested that carotenoids have antioxidant properties that stand from their ability to scavenge reactive species such as singlet oxygen ($^{1}O_2$) and peroxyl radicals and, especially, their ability to interact with and potentiate the Nrf2 pathway, enhancing Nrf2 translocation to the nucleus and the subsequent activation of the expression of a collection of antioxidant and cytoprotective enzymes [13].

Although antioxidant properties of carotenoids have been proven in different tissues and cell types, reports dealing specifically with antioxidant action in adipocytes or adipose tissue are scarce.

Evidence has been provided that crocetin [138] and BC [141] can reduce the production of reactive oxygen species (ROS) in cultured adipocytes exposed to stressful agents such as palmitate or TNFα. In the study of Kameji et al. (2010) [141], pre-treatment with BC suppressed TNFα-induced ROS production, suggesting that intracellular BC accumulation enables elimination of ROS in adipocytes. In an intervention study in mice, astaxanthin treatment (6 mg/kg bw/day) counteracted the development of diet-induced obesity and insulin resistance and associated with decreased levels of oxidative stress markers in adipose tissue and skeletal muscle of the high fat diet-fed mice [121]. In another *in vivo* study, treatment of mice on a high fat diet with fucoxanthin (0.4–0.6%) resulted in lower body weight gain and suppressed maleic dialdehyde (used as an indicator of oxidative stress) production and adipocyte hypertrophy in the mammary gland, although analysis of classical WAT depots was not included in this report [149].

It is known that carotenoids may act as antioxidants or prooxidants depending on their concentration in cells, the cell oxidative environment and other factors [150–153]. Carotenoid breakdown products including highly reactive aldehydes (such as Rald) and epoxides formed in the course of antioxidant action can increase oxidative stress and impair mitochondrial function (including respiration), as shown in isolated rat liver mitochondria [154]. Additionally, studies in BCO2-deficient mice indicate that carotenoids in excess, if not properly detoxified by the activity of BCO2, accumulate in mitochondria and cause oxidative stress leading to mitochondria dysfunction [155] and the activation of the intrinsic apoptotic pathway [156]. The latter studies were conducted mostly in liver tissue and hepatic cells. Whether excess carotenoids or carotenoid breakdown products can contribute to oxidative stress specifically in adipocytes/adipose tissue has not been reported, to our knowledge. However, exposure of immortalized human preadipocytes (Chub-S7) to BC (10–30 μM) resulted in decreased mitochondrial membrane potential, mitochondrial respiration and cellular ATP content, indicative of an impaired mitochondrial function [157]. Most likely, as in other cell types and tissues, carotenoids may protect adipocytes/adipose tissue against oxidative stress or contribute to it, depending on the dose and cell/tissue factors.

Human studies linking carotenoids and vitamin A to body adiposity

Human epidemiological studies

Studies in adults and children/adolescents have consistently shown serum levels of carotenoids including BC to be decreased in overweight and obese subjects (e.g. [158–161]). Moreover, several large population-based, cross-sectional epidemiological studies have reported an inverse association between carotenoid concentrations in blood and BMI and other measures of obesity including adiposity, in some cases even when adjusted for other factors associated with carotenoid concentration, such as intake of fruit and vegetables, fat, fiber, alcohol, supplement use, smoking, gender and lipid concentrations (e.g. [162–169]). Cross-sectional studies have also reported lower serum carotenoid concentrations in adults and children with the metabolic syndrome [170,171]. In fact, independent associations of low serum carotenoids with risk factors/biomarkers of the metabolic syndrome including increased insulin resistance index (HOMA-IR), fasting insulinemia, oxidized LDL, glycosylated hemoglobin, and circulating levels of inflammatory markers such as C-reactive protein have been reported that persisted after adjusting for confounders including BMI or related measures of obesity, and which are generally considered to be related to antioxidant and anti-inflammatory activities of carotenoids [167,172–179]. Cross-sectional and prospective studies fairly consistently show that a higher intake and status of carotenoids is associated with lower levels of low-grade inflammation in relation to overweight, obesity and the metabolic syndrome [180].

The inverse association between carotenoid concentrations in blood and BMI or adiposity may have different explanations. The three more common are the following ([166] and references therein). First, BMI and serum carotenoids may be correlated because of dietary and other lifestyle factors that affect them both. Second, since serum carotenoids are partially fat soluble, adipose tissue may act as a sink for them, so that relatively fewer are located in the blood. However, the concentration of carotenoids in adipose tissue and in isolated adipocytes is also lower in obese people [27,29,181,182]. A recent report showed that isolated adipocytes from obese subjects contain 50% lower concentrations of BC than cells from lean or non-obese subjects [29]. Third, adipose tissue in obesity may generate oxidative stress, and the carotenoids may be lowered because of defending against this stress, and being consumed upon their action as antioxidants. This latter explanation could indeed explain decreased carotenoids levels in both blood and adipose tissue in obesity.

Notwithstanding the aforementioned explanations, we believe a mechanistic link cannot be ruled out in view of data indicating that, in adipocytes, BC rather than retinol may function as the precursor for the local synthesis of retinoids capable of exerting an anti-adiposity action [52]. Decreased BC per adipocyte in obesity could reflect increased BC consumption in antioxidant reactions needed to neutralize increased reactive species, and perhaps also an attempt to counteract adipocyte hypertrophy under obesogenic conditions by increasing retinoid production from BC. Whatever its origin, if not corrected through dietary consumption, once established decreased BC per adipose cell could contribute to the maintenance and further development of pathological obesity by limiting BC-derived, anti-adiposity retinoid signaling (Fig. 3). Moreover, human obese subjects may have a decreased efficiency of BC conversion to retinoids compared to lean subjects which could further contribute to the obese state: an inverse association of BMI with BC conversion efficiency as assessed by a stable-isotope reference method was demonstrated [183], and another study found a slower rate of BC decline in serum after cessation of dietary supplementation in individuals with the highest BMI [184].

Whereas an inverse association of serum carotenoids with obesity measurements is well established, an association of vitamin A status as serum retinol levels with human obesity is less clear. Some of the studies reporting an inverse association for carotenoids found instead serum retinol to be constant across BMI values or between obese and non-obese groups [158,160,163,168], likely reflecting homeostatic regulation of circulating retinol through controlled storage in and release from the liver. A recent study reported higher concentrations of serum retinol (and lower of serum carotenoids) to be associated with increased probability of overweight and obesity in children [169]. Other studies, in overweight and obese (adult) subjects, found the opposite, i.e. lower serum retinol to be associated with increased BMI [185,186].

Association of dietary intake of vitamin A or carotenoids as evaluated through ad-hoc questionnaires with adiposity has also been investigated. An inverse association between preformed vitamin A intake after adjusting for total energy intake and several measurements of adiposity – such as body weight, BMI, waist circumference and waist-to-hip ratio – has been reported in healthy young adults [187]. Higher total carotenoid intakes, mainly those

Table 1
Beneficial effects of carotenoid supplementation in the accrual of abdominal adiposity in humans.

Carotenoid source (dose)	*n*	Duration	Change in waist circumference	*P*	Change in adiposity	*P*	Refs.
Encapsulated fruit and vegetable juice concentrate Juice Plus® (3.75 mg β-carotene/day)	30 children (lean/obese)	6 months			−1.47% *vs* +11.2% in abdominal fat[b,c]	0.029	[190]
Jarrow Formulas CaroteneAll® (~3 mg mixed carotenoids/day)	21 children (obese)	6 months			−4% in SAT[a,d] −8% in VAT[a,c]	0.016 0.041	[191]
Marine seaweed extract (12 mg fucoxanthin/day)	6 females	8 weeks	−5.5 cm[a]	<0.001			[195]
Pomegranate seed oil plus brown seaweed extract Xanthigen™ (2.4 mg fucoxanthin/day)	36 females (obese)	16 weeks	−5.6 cm[a]	<0.05	−4.4 kg of total body fat[a,c]	<0.001	[126]
Satsuma mandarin-derived drink (0.5 mg β-cryptoxanthin/day)	men (obese)	12 weeks	−1.2 cm[a]	<0.05	−13.5 cm^2 of visceral fat area[a,e]	<0.01	[193]

[a] Relative to baseline values.
[b] Carotenoid supplemented group vs placebo group.
[c] Fat measured using a dual-energy X-ray absorptiometry scan (DEXA).
[d] Fat measured using a cross-sectional multi-slice MRI.
[e] Fat area measured using computed tomography (CT) cross-sectional images.

of BC and lycopene, were found to be associated with lower waist circumferences and visceral and subcutaneous fat mass and lower prevalence of metabolic syndrome in a cross-sectional study involving middle-aged and elderly men ($n = 374$) [188]. A high intake of carotenoids derived from a high consumption of vegetables and fruits, as in the Mediterranean diet, was shown to associate with lower development of metabolic syndrome traits including increased waist circumference in a prospective study involving 3232 subjects [189]. Altogether, the results of these studies point to an inverse association between dietary intake of vitamin A and carotenoids and body adiposity.

Human intervention studies

Despite the many animal studies and human epidemiological studies linking carotenoids and carotenoid conversion products to adiposity, there is a paucity of randomized controlled trials utilizing dietary carotenoid supplementation to assess changes in adiposity, and particularly visceral adiposity in relationship with components of the metabolic syndrome. Such studies have been conducted mainly for BC, β-cryptoxanthin and fucoxanthin (Table 1).

Two small pilot double blind placebo-controlled studies in overweight and obese children using similar doses of BC (3–4 mg/day as part of supplements) have recently reported significant changes in the 6 months rate of accrual in abdominal adiposity [190,191]. In the first of these two studies, lean and overweight children underwent daily supplementation with an encapsulated supplement of fruit and vegetable juice concentrate (providing approximately 3.75 mg of BC, 117 mg of vitamin C, 22.5 IU of vitamin E, 210 mg of folate and 30 mg of calcium per day) or placebo in the presence of nutritional counseling; the supplement led to increased serum BC levels and resulted in a reduction in abdominal fat mass in conjunction with an improvement in insulin resistance in the overweight children [190]. In the second study, obese children completed a 2-weeks intense lifestyle intervention program followed by 6 months of supplementation with Jarrow Formulas CaroteneAll® complex (providing daily 5000 IU of β- and α-carotene, 20 mg of lutein, 4 mg of zeaxanthin, 20 mg of lycopene, 1 mg of astaxanthin and 20 mg of vitamin E) or placebo; in the treatment group, reductions in both subcutaneous and visceral adiposity relative to baseline values were reported together with concomitant increases in serum adiponectin, while these parameters changed in the opposite direction in the placebo group [191].

The administration of β-cryptoxanthin extracted from Satsuma mandarin (0.5 mg/day as part of test drink) to moderately obese Japanese males resulted in increased levels of β-cryptoxanthin in serum and led to reductions in body weight, visceral fat and waist circumference [192,193]. Another 3-weeks long study in seventeen postmenopausal obese women supplemented with a beverage containing β-cryptoxanthin (4.7 mg/day) reported no differences in body weight or BMI but a 4-fold increase in adiponectin serum levels after the treatment, suggestive of a reduction in adiposity (-data on body fat mass were not include in this report) [194].

Regarding fucoxanthin, in a small randomized double blinded placebo-controlled trial involving adult non-seaweed consuming subjects in Ecuador with at least one symptom of metabolic syndrome, researchers showed that 6 g/day of dietary brown seaweed containing fucoxanthin consumed for 2 months resulted in decreased waist circumference in women and improved systolic blood pressure [195]. Another 16 weeks study investigating the effects of Xanthigen™ reported significant reductions in body weight, waist circumference, body fat content and serum triacylglycerol in obese, non-diabetic female volunteers with non-alcoholic fatty liver disease and normal liver fat content compared to baseline, which were associated to increases in resting energy expenditure [126].

Concluding remarks

Carotenoids and carotenoids conversion products seem to play a substantial role in the control of key aspects of adipose tissue biology including the production of novel adipocytes from precursor cells (adipogenesis), the metabolic and secretory capacities of mature adipocytes and local inflammation through well-defined or emerging mechanisms. Because adiposity, particularly visceral adiposity, is an important determinant of disease susceptibility and adipose tissue represents a main reservoir for carotenoids and retinol, it is conceivable that a putative beneficial effect of carotenoids and retinoids on health is tightly linked to their local effects in adipose tissue, counteracting features of the so-called "adiposopathy" or "sick fat" phenomena [196]. Animal studies support an anti-adiposity and anti-inflammatory action of carotenoids and carotenoid derivatives in obesity. Interestingly, some of these compounds – such as fucoxanthin, astaxanthin and the BC-derived retinoids atRA and Rald – exert both suppressive effects on PPARγ activity and adipogenesis and activating effects on lipid oxidation and thermogenesis in mature adipocytes and other cell types. Such compounds might, therefore, help in moderating the formation of new adipocytes under obesogenic conditions while simultaneously favoring the dissipation of excess energy. Human epidemiological studies reveal a low intake and nutritional status of carotenoids among obese subjects, including obese children/adolescents, which needs to be critically addressed. So far, there have been limited human intervention studies with

carotenoid-rich supplements or extracts to prevent or reduce adiposity or obesity-related co-morbidities; even if these studies are insufficient to prove a cause-effect relationship, the scenario they depict is encouraging, since both in children and adults the interventions had beneficial effects on the accrual of body fat, abdominal fat and related risk parameters (Table 1).

Despite epidemiological evidence linking carotenoid intake in the form of fruits and vegetables to decreased risk of certain diseases, a number of intervention trials have shown limited if any preventive benefit of relatively large doses of carotenoids and particularly BC on disease incidence, and even in some cases long-term supplementation has proven to be harmful in people at risk for disease, such as smokers [197]. This might relate to the fact that dual pro- and anti-oxidant effects as well as gene expression modulation by carotenoids and their derivatives may be concentration-dependent phenomena, affected as well by additional factors, including components of the food matrix, cellular conditions, and features of the individual affecting carotenoid bioavailability and requirements, among them genetic variability [198] and, as suggested here, body composition. Optimal upper and lower carotenoid levels to achieve benefit based on body composition and other interacting factors are yet to be clearly defined. Importantly, beneficial effects of BC supplements on adiposity in humans have been achieved under mild BC supplementation, at doses quite lower than the ones that caused concern and controversy in large intervention trials, and in obese people.

There are more than 600 carotenoids in nature and many of them possibly capable of interacting with cellular machineries and signaling pathways to elicit potential beneficial effects. These compounds entail, therefore, a vast potential to tackle health problems, as illustrated here for obesity and some of its co-morbidities. However, more human intervention studies are needed to verify this potential for specific carotenoids, and more functional and mechanistic studies are needed to fully understand the connection of carotenoids with the control of adiposity. At the same time, the identification and characterization of novel sources of carotenoids can help regional economic development through the exploitation of autochthonous edible or cultivatable carotenoid sources.

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