REVIEW

Importance of β,β-carotene 15,15′-monooxygenase 1 (BCMO1) and β,β-carotene 9′,10′-dioxygenase 2 (BCDO2) in nutrition and health

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In humans, varying amounts of absorbed β-carotene are oxidatively cleaved by the enzyme β,β-carotene 15,15′-monooxygenase 1 (BCMO1) into two molecules of all-*trans*-retinal. The other carotenoid cleavage enzyme β,β-carotene 9′,10′-dioxygenase (BCDO2) cleaves β-carotene at the 9′,10′ double bond forming β-apo-10′-carotenal and β-ionone. Although the contribution of BCDO2 to vitamin A formation has long been debated, BCMO1 is currently considered the key enzyme for retinoid metabolism. Furthermore, BCMO1 has limited enzyme activity towards carotenoids other than provitamin A carotenoids, whereas BCDO2 exhibits a broader specificity. Both enzymes are located at different sites within the cell, with BCMO1 being a cytosolic protein and BCDO2 being located in the mitochondria. Expression of BCMO1 in tissues other than the intestine has recently revealed its function for tissue-specific retinoid metabolism with importance in embryogenesis and lipid metabolism. On the other hand, biological activity of BCDO2 metabolites has been shown to be important in protecting against carotenoid-induced mitochondrial dysfunction. Single-nucleotide polymorphisms (SNPs) such as R267S and A379V in *BCMO1* can partly explain inter-individual variations observed in carotenoid metabolism. Advancing knowledge about the physiological role of these two enzymes will contribute to understanding the importance of carotenoids in health and disease.

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1 The role of carotenoid cleavage enzymes

Carotenoids are C_{40} lipophilic pigments that can be divided into two groups according to their chemical structure: the carotenes, which are hydrocarbons, and the xanthophylls, which further contain oxygenated functional groups and are therefore more polar than the carotenes. The difference in polarity has consequences on their tissue distribution and also influences repartition and exchange between lipoproteins [1, 2]. After absorption from the gut, carotenoids are either cleaved in a centric or eccentric fashion into retinoids and apo-carotenals or become incorporated into lipoproteins and secreted into the blood circulation [3]. In humans, between 35 and 88% of absorbed β-carotene is oxidatively cleaved by the β,β-carotene 15,15′-monooxygenase 1 (BCMO1; EC 1.14.99.36) into two molecules of all-*trans*-retinal, which subsequently can be oxidised irreversibly into retinoic acid by retinal dehydrogenase or reduced reversibly into retinol by a retinal reductase [4]. The other carotenoid cleavage enzyme β,β-carotene 9′,10′-dioxygenase (BCDO2; (EC 1.14.99.) cleaves β-carotene at the 9′,10′ double bond forming β-apo-10′-carotenal and β-ionone [5]. The

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Abbreviations: BCDO2, β,β-carotene 9′,10′-dioxygenase; **BCMO1,** β,β-carotene 15,15′-monooxygenase 1; **CCD1,** carotenoid cleavage dioxygenase 1; **ISX,** intestine-specific homeobox; **KO,** knock-out; **PPAR-α,** peroxisome proliferator-activated receptor-α; **RA,** retinoic acid; **RAR,** retinoic acid receptor; **SNP,** single-nucleotide polymorphism

contribution of BCDO2 to vitamin A formation has long been debated and led to a critical appraisal of the potential biochemical pathway after eccentric cleavage from β-apo-10′-carotenal to β-apo-10′-carotenoic acid and finally to retinoic acid via a mechanism similar to β-oxidation [6–11]. However, BCMO1 knock-out (KO) mice become vitamin A deficient despite expressing BCDO2 [12]. Furthermore, hepatic BCDO2 expression has been found to be significantly elevated in BCMO1 KO mice compared with wild-type mice, leading to a significant increase in β-apo-12′-carotenal [13] or β-apo-10′-carotenal concentration [14]. Finally, studies in BCMO1 KO mice revealed a large accumulation of β-carotene in tissues (liver, lung, adipose tissue) of animals on a β-carotene-enriched diet [12, 14]. Thus, in agreement with earlier findings [7], BCMO1 is currently considered the key enzyme for β-carotene conversion into vitamin A. Having ruled out a potential rescue pathway for BCMO1 deficiency, the role of BCDO2 has long been shrouded in mystery. However, it was recently shown that BCDO2 plays an important role in protecting against carotenoid-induced mitochondrial dysfunction and has a broader substrate specificity than previously recognized [15]. Indeed, enzyme kinetic analysis indicated that the xanthophylls zeaxanthin and lutein are preferentially cleaved over β-cryptoxanthin, indicating a key role of BCDO2 in non-provitamin A carotenoid metabolism [16]. Since apocarotenoids serve as important signalling molecules in a variety of biological processes, enzymatic cleavage of xanthophylls by mammalian BCDO2 has opened up a new interest in these cleavage enzymes and their biological function [15, 16]. For example, several apo-10′-lycopenoids, BCDO2 cleavage products of lycopene, induce Nrf2 signalling and haeme oxygenase-1 gene expression [17] whereas apo-10′-lycopenoic acid has shown to inhibit lung cancer development [18]. Furthermore, β-apo-14′-carotenal represses retinoid X receptor α (RXRα), peroxisome proliferator-activated receptor-α (PPAR-α), and peroxisome proliferator-activated receptor-γ (PPAR-γ) transcriptional activation and biological responses, supporting the potential significance of asymmetric β-carotene cleavage products as transcriptional modulators of cellular responses such as adipogenesis and inflammation [19].

2 Reaction mechanism and substrate specificity

BCMO1 and BCDO2 are oxygenase enzymes that catalyse the respective symmetric and asymmetric cleavage of carotenoids. Employing β-carotene as a substrate, BCMO1 cleaves at the central 15,15′ position of the polyene chain producing two molecules of retinal while BCDO2 cleaves at the 9′,10′ double bond yielding β-apo-10′-carotenal and β-ionone [11]. Both enzymes require Fe^{2+} as an essential cofactor that binds to four conserved histidine residues in the active centre [20, 21]. These residues were identified as His172, His237, His308 and His514 in human and mouse BCMO1 [21]. Poliakov et al. [21] further demonstrated that mutations in any of the four conserved histidine residues or Glu405 resulted in the total loss of BCMO1 activity. It has been proposed that Fe^{2+} is coordinated by the four histidines, water and molecular oxygen forming an octahedral arrangement with cation-π stabilisation by Y235 and Y326 in the BCMO1 active site [21, 22].

The monooxygenase mechanism of BCMO1 transfers a single oxygen atom from molecular oxygen (O_2) and a second oxygen atom from water, via an epoxide intermediate [23]. This is in contrast to the dioxygenase mechanism of BCDO2 that incoporates both atoms of molecular oxygen via a dioxetane intermediate [24]. Apart from possessing differing oxygenase reaction mechanisms, BCMO1 and BCDO2 are also located at different sites within the cell. BCMO1 is a cytosolic protein with a molecular mass of 60–65 kDa [25–29]. BCDO2 was recently shown to be expressed in mitochondria [15] having a subunit mass of 60–64 kDa [16, 20, 30].

BCMO1 requires at least one unsubstituted β-ionone ring for the cleavage of carotenoid substrates, but also the presence and position of methyl groups on the polyene chain are important [31, 32]. Therefore, activity is limited mainly to α- and β-carotene, β-apo-carotenals and β-cryptoxanthin (Table 1). In the chicken, specificity of BCMO1 towards substrates decreased in the order: β-carotene > β-cryptoxanthin > β-apo-8′-carotenal > β-apo-4′-carotenal > α-carotene > γ-carotene [33]. Enzymes that cleave β-carotene at the 15,15′ position to produce retinal have also been described for zebrafish (bcox) and *Drosophila* (NinaB) with NinaB also possessing retinoid isomerase activity [34–37].

BCDO2 exhibits a broader specificity towards substrates which includes β-carotene, β-cryptoxanthin, lycopene, zeaxanthin and lutein (Table 1) to produce apo-carotenoids. In the ferret, zeaxanthin and lutein were the preferred substrates to β-cryptoxanthin [16]. Interestingly, when lycopene is the substrate, BCDO2 has only been reported to display an activity towards *cis*- but not all-*trans* isomers, yielding β-apo-10′-lycopenal [20]. Furthermore, BCDO2 can cleave β-cryptoxanthin, zeaxanthin and lutein at both 9′,10′ and 9,10 positions producing a variety of volatile and non-volatile products such as: β-apo-10′-carotenal, 3-OH-β-apo-10′-carotenal, 3-OH-α-apo-10′-carotenal, β-ionone, 3-OH-β-ionone, and 3-OH-α-ionone [16]. Like BCDO2, plant carotenoid cleavage dioxygenase 1 (CCD1) also cleaves a variety of cyclic and acyclic carotenoids at 9′,10′ and 9,10 positions [38, 39]. Most carotenoid substrates, including β-carotene, lutein, zeaxanthin and *trans*-violaxanthin, appear to be simultaneously cleaved at either end of the molecule to produce a C14 dialdehyde and two variable end group-derived C13 ketones. However, with 9-*cis*-violaxanthin and 9-*cis*-neoxanthin, the C27 apo-carotenal was the major product [38]. Interestingly, CCD1 from *Arabidopsis thaliana* and *Zea mays* is capable of additional cleavage at the 5,6 or 5′6′ positions of lycopene to generate the volatile 6-methyl-5-hepten-2-one (MHO) [39].

Table 1. Substrate specificity for BCMO1 (1) and BCDO2 (2)

Substrate	*Arabidopsis* [38, 39, 78]	Chicken [32, 33, 79]	Cow [80]	*Drosophila* [36]	Ferret [16, 20]	Human [42, 81]	Maize [39]	Mouse [15, 44, 48, 82]	Rabbit [83, 84]	Rat [83, 85, 86]	Zebrafish [34]
α-Carotene		1							1	1	
β-Carotene	2	1	1	1	2	1	2	1,2	1	1	1
δ-Carotene							2				
γ-Carotene		1									
ζ-Carotene							2				
9-/13-*cis*-β-Carotene									1	1	
β-Cryptoxanthin		1			2	1		2	1	1	
β-Apo-4′-carotenal/-carotenol		1							1		
β-Apo-8′-carotenal/-carotenol		1							1	1	
β-Apo-10′-carotenal/-carotenol									1	1	
β-Apo-12′-carotenal/-carotenol										1	
3′,4′-Dehydro-18′-oxo-γ-carotene									1		
Lycopene	2						2	2			
5-/13-*cis*-Lycopene					2						
Zeaxanthin	2				2		2	2			
Lutein	2				2			2			
Neoxanthin											
9-*cis*-Neoxanthin	2										
Violaxanthin	2										
9-*cis*-Violaxanthin	2										

3 Species- and tissue-specific expression

Expression and activity of BCMO1, as well as BCDO2, has been demonstrated in humans and various other species (for review see [4, 40]). BCMO1 activity was detected in the intestinal mucosa from human and other mammals such as mouse, rat, ferret, guinea pig, monkey and rabbit [41–47].

Unlike initial expectations, β-carotene cleavage is not limited to the digestive tract. In a variety of human tissues, the presence of BCMO1 was documented by RNA blotting and immunostaining methods [42, 43]. As summarized by von Lintig et al. [40], BCMO1 is expressed in the mucosal and glandular cells of the stomach, small intestine and colon as well as in hepatocytes and cells comprising the exocrine glands in pancreas, glandular cells in prostate, endometrium and mammary tissue, kidney cells and keratinocytes of skin squamous epithelium. Moreover, skeletal muscle cells as well as cells of testis, ovary and adrenal gland with steroidogenic properties express BCMO1. In addition, BCMO1 expression was found in the retinal pigment epithelium and in the ciliary body pigment epithelia of the eye [43].

BCDO2 on the other hand is generally considered to be present in most tissues at lower levels compared with BCMO1 [30]. BCDO2 activity could be found in almost all cell types that are known to express BCMO1, except skin, ovary cells and colon epithelial cells. Interestingly, BCDO2 was only found in cardiac and skeletal muscle cells, prostate and endometrial connective tissue and the endocrine pancreas [30]. Tissue-specific expression of BCDO2 in animals such as the mouse, rat and ferret has been described recently [20, 47, 48].

The expression of both BCMO1 and BCDO2 in the liver is different to other tissues, since both enzymes occur in separate hepatic cell types. The liver is the major site of retinoid metabolism and storage in the body. Two important hepatic cell types are involved in retinoid metabolism, namely hepatocytes (also known as parenchymal cells) and stellate cells (also known as fat-storing cells, lipocytes or Itoh cells). In hepatocytes, which are centrally involved in uptake and processing of retinol, high levels of BCDO2 can be found, whereas in stellate cells, which play a fundamental role in hepatic retinoid storage, high BCMO1 levels are present [13]. On a subcellular level, BCMO1 is located in the cytoplasm [42] whereas BCDO2 is a mitochondrial enzyme [15].

4 Importance of extra-intestinal expression of both oxygenases

The recent research and observations in KO animals have shifted the focus away from the traditional function of these two enzymes in the intestine towards other organs

as well as their importance in human health and disease. The expression/activity of BCMO1 in extra-intestinal tissues has been linked to the capacity of these tissues to directly convert locally stored carotenoids into vitamin A [43]. This has recently been shown to be of importance in embryonic retinoid metabolism, since β-carotene was shown to serve as an alternative vitamin A source for the in situ synthesis of retinoids in developing tissues [49]. Furthermore, Kim et al. [49] demonstrated that BCMO1 exerts an additional function on retinoid metabolism by influencing retinyl ester formation via modulation of lecithin:retinol acyltransferase (LRAT) activity, thus confirming the critical role of BCMO1 for embryonic retinoid metabolism.

Another important observation is that BCMO1 has been implicated as a regulator of lipid metabolism. Knockout of BCMO1 (BCMO1 KO) altered serum lipid levels and led to the development of liver steatosis in mice [12]. Steatosis development in BCMO1 KO mice was shown to be independent of the vitamin A status of the animal, indicating that local de novo synthesis of retinal and/or retinoic acid could be the key regulatory molecules in the regulation of liver lipid homeostasis [12]. Furthermore, adipogenic transcription factors, such as CCAAT/enhancer-binding protein, PPAR-γ, liver X receptor-α and sterol regulatory element-binding protein 1c, are required to maintain vitamin A-storing hepatic stellate cells in a ''quiescent state'', therefore preventing transdifferentiation and participation in liver fibrogenesis [50]. More importantly, retinoic acid receptor-α (RAR-α) KO mice developed microvesicular steatosis and spotty focal necrosis [51].

The increased susceptibility of BCMO1 KO mice to diet induced obesity, coupled with increased expression of PPAR-γ induced genes in adipocytes, is in agreement with the observation that retinaldehyde can inhibit PPAR-γ-induced adipogenesis and that retinaldehyde dehydrogenase 1 (RALDH1)-deficient mice are protected against diet-induced obesity and insulin resistance [12, 52] (Fig. 1). In agreement with these observations, a BCMO1-dependent decrease in PPAR-γ target gene expression was observed during β-carotene supplementation in inguinal white adipose tissue [14]. Moreover, body fat, adipose depot mass, iWAT adipocyte size and leptin serum levels were all significantly reduced after β-carotene supplementation in mice with an active BCMO1 enzyme, but not in BCMO1 KO mice [14]. It is of interest to note that BCMO1 deficiency increases both β-apo-12′-carotenal [13] and β-apo-10′-carotenal concentrations [14] combined with increased PPAR-γ target gene expression in hepatic stellate cells, but not in hepatocytes [13]. Although the eccentric cleavage product β-apo-14′-carotenal was shown to inhibit PPAR-γ target gene expression and adipogenesis [19], this eccentric cleavage product was not found in livers of either BCMO1 or BCDO2 KO mice [13]. More importantly, the BCDO2 cleavage product β-apo-10′-carotenal did not affect PPAR-γ target gene expression in adipocytes [14]. However, BCDO2 deficiency can also lead to increased triglyceride concentrations

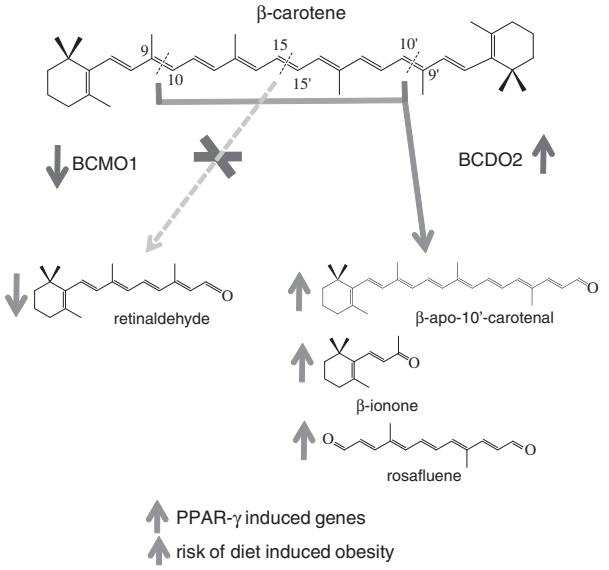

Figure 1. Observed interaction between BCMO1 and BCDO2 in BCMO1 KO mice. Increased susceptibility of BCMO1 KO mice to diet induced obesity, coupled with increased expression of PPAR-γ-induced genes [12] as well as BCDO2 [14].

and the development of liver steatosis combined with impaired mitochondrial respiration and oxidative stress [15]. Since liver steatosis is associated with mitochondrial dysfunction and oxidative stress [53], this is further emphasizing the critical role of BCDO2 in cell protection [15]. Genetic variations in BCMO1 and BCDO2 could possibly increase the risk of NAFLD in subjects who are living in an obesogenic environment.

5 Regulation by dietary factors

There is a range of dietary factors such as the amount and composition of fat and protein as well as antioxidants, polyphenols and carotenoids which were found to impact on BCMO1 activity (see review [4]). The uptake of β-carotene is positively correlated with dietary fat content and BCMO1 activity [54, 55], but also dietary fat composition seems to influence BCMO1 activity [56, 57]. The BCMO1 inducing effect of long-chain fatty acids is mediated by PPAR-γ since both murine and human genes for BCMO1 contain functional PPAR-responsive elements (PPRE) [58, 59]. Moreover, the human BCMO1 promoter also contains a functional myocyte enhancer factor 2 (MEF2)-binding element suggesting that MEF2C and PPAR-γ may synergistically interact to transactivate BCMO1 expression [59]. In contrast to BCMO1, no PPRE elements could be found in the BCDO2 promoter region [47]. However, BCDO2 activity leads to enhanced production of apo-carotenoids, derived from β-carotene but also from other substrates, which may interfere with PPAR signalling. Products of asymmetric β-carotene cleavage were found to repress PPAR-γ and

PPAR-α as well as RXR activities in cell culture [19]. Likewise, renal and adrenal gland *PPAR-γ* mRNA levels were significantly lower following lycopene supplementation in rats [47]. Although no alteration of *BCMO1* or *BCDO2* mRNA levels were detected when lycopene was fed to wild-type and BCDO2 KO mice [60], a three-fold increase in *BCDO2* mRNA levels in the lung of ferrets as compared with the non-supplemented control animals was observed [20]. In line with this is the finding that hepatic *BCDO2* mRNA levels increased sevenfold on zeaxanthin supplementation in mice as compared with age-matched animals raised on a control diet [15]. Chronic alcohol intake significantly up-regulated hepatic BCMO1 expression in rats, possibly mediated via PPAR-γ; BCDO2 on the other hand was induced by alcohol to a much lesser extent [61].

Recently, the intestine-specific transcription factor intestine-specific homeobox (ISX) was identified in mouse intestine as an important regulator of BCMO1 expression [62]. Severe vitamin A deficiency markedly decreased ISX expression accompanied by an increase in BCMO1 expression in duodenum and jejunum [62]. Lobo et al. [63] could further demonstrate that retinoic acid (RA) is activating ISX expression via RAR that bind to RA-responsive elements within the ISX promoter. Activation of ISX results in repression of SR-BI and BCMO1 expression thereby controlling β-carotene absorption as well as vitamin A production [63]. The vitamin A status is possibly the most important factor that influences BCMO1 activity through this diet-responsive regulatory network. This is supported by the observation of an inverse relationship between hepatic vitamin A stores and intestinal BCMO1 activity [64, 65], indicating that production of excess vitamin A and RA via BCMO1 can be avoided when vitamin A stores are adequate.

6 Enzyme homology and genetic variation

The family of carotenoid-cleaving enzymes characterised by their dependence on ferrous iron for catalytic activity includes BCMO1, BCDO2 and RPE65. BCMO1 and BCDO2 are classified as carotenoid oxygenases, whereas RPE65 is a retinoid isomerase [66–68]. Figure 2 shows amino acid sequence homology among 8 members of BCMO1, 8 members of BCDO2, 6 members of RPE65 and 3 members of other carotenoid oxygenases. Sequence homology was more than 72% in RPE65 proteins among six species. However, sequence homology between species is lower in BCMO1 (>55%) or BCDO2 (>54%) than that for RPE65. Notably, there seems to be a lower conservation of amino acid sequences between species for BCMO1 and BCDO2 compared with RPE65. Sequence homology between cleaving enzymes indicate 34–43% homology between BCMO1 and BCDO2, 35–41% homology between BCMO1 and RPE65 and 38–43% homology between BCDO2 and RPE65. Information about conserved amino acids in RPE65 could shed some light onto structurally important areas in BCMO1 or BCDO2, since more information about clinically associated polymorphisms for RPE65 is available compared with either BCMO1 or BCDO2 [68–70].

Homology analysis among enzymes in the carotenoid oxygenase family indicated four conserved histidine residues (H172, H237, H308 and H514) necessary for the activity in mouse BCMO1 [21]. These amino acids have also been shown to be essential for human RPE65 activity [68, 69].

Single-nucleotide polymorphism (SNP) information about the human *BCMO1* gene has already been reviewed elsewhere [4]. T170 in the human *BCMO1* gene [4] is conserved in three enzymes in vertebrates [71], therefore further indicating the importance of this residue. Likewise, based on functional assays in vitro, it was reported that two tyrosine residues (Y235 and Y326) are important for the activity of mouse BCMO1 [5]. Y235 is completely conserved in three enzymes. Y326 is conserved in all BCMO1 homologues and changed to glutamine and tryptophan in most BCDO2 and all RPE65, respectively [5].

So far 19 different human splice variants of BCDO2 have been identified, out of which 9 are protein coding (http://www.ensembl.org). Additionally, 10 non-synonymous and 3 synonymous SNPs within the human BCDO2 have been identified (http://www.ncbi.nlm.nih.gov/SNP). However, out of 13 identified exonic SNPs within the open reading frame of BCDO2, only 2 (rs10891338 and rs17113607) have minor allele frequencies above 5% according to HapMap (http://hapmap.ncbi.nlm.nih.gov). This indicates that splice variants could potentially be more important in introducing large inter-individual differences in BCDO2 activity. The intronic SNP rs2115763 at the BCDO2 locus for example was associated with IL-18 concentrations, a proinflammatory cytokine that correlates with type 2 diabetes and cardiovascular disease [72]. Furthermore, SNP rs2115763 has a rare allele frequency ranging from 7.1% in Yoruba Nigerians to 61.9% in Gujarati Indians from Texas, USA (http://hapmap.ncbi.nlm.nih.gov).

To date, BCDO2 SNPs in bovine [73, 74], sheep [75] and chicken [76] have been described. Nonsense mutations were reported at W80 or Q66 in bovine BCDO2 [73, 74] and sheep BCDO2 [75], respectively. The nonsense mutation in bovine BCDO2 affects subcutaneous fat colour and β-carotene concentration in plasma, milk and adipose tissues [73, 74]. Våge and Boman [75] reported that the nonsense mutation at Q66 was found in 16 out of 18 yellow fat lambs. Additionally, the authors also showed two potential polymorphisms, which may affect fat colour. One is a homozygous phenylalanine residue at position 315 and the other is a heterozygous isoleucine residue at position 422 (N422I) in sheep BCDO2 [75]. Especially, N422I is interesting as a potential polymorphism which may affect fat colour in lamb since the asparagine (N) residue at position 422 is completely

Amino Acid (n)		Human BCMO1	Ferret BCMO1	Mouse BCMO1	Rat BCMO1	Bovine BCMO1	Sheep BCMO1	Chicken BCMO1	Zebrafish BCMO1
547	Human BCMO1								
488	Ferret BCMO1	85							
566	Mouse BCMO1	85	85						
566	Rat BCMO1	84	85	93					
596	Bovine BCMO1	81	83	77	77				
596	Sheep BCMO1	79	80	76	76	90			
526	Chicken BCMO1	69	68	71	71	66	65		
516	Zebrafish BCMO1	59	56	59	60	56	55	59	
579	Human BCDO2	38	38	37	37	36	34	43	37
541	Ferret BCDO2	40	39	40	39	39	37	42	40
532	Mouse BCDO2	40	40	42	40	41	39	42	38
532	Rat BCDO2	39	40	40	40	40	39	42	38
575	Sheep BCDO2	38	38	37	37	36	35	41	37
575	Bovine BCDO2	36	38	38	36	35	34	39	37
579	Chicken BCDO2	39	40	39	39	37	36	42	38
555	Zebrafish BCDO2	38	39	40	39	38	37	39	39
533	Human RPE65	38	40	39	40	38	36	40	36
533	Bovine RPE65	38	39	39	39	38	36	40	36
533	Rat RPE65	38	40	40	40	39	36	40	35
533	Mouse RPE65	38	40	39	40	38	36	40	36
533	Chicken RPE65	38	39	39	39	38	35	39	36
531	Zebrafish RPE65	40	41	40	41	38	36	39	36
620	Fly NinaB	30	31	30	30	28	27	35	32
538	Arabidopsis	16	16	17	15	15	14	17	18
604	Maize VP14	15	15	15	13	13	12	17	15

	Human BCDO2	Ferret BCDO2	Mouse BCDO2	Rat BCDO2	Sheep BCDO2	Bovine BCDO2	Chicken BCDO2	Zebrafish BCDO2
Ferret BCDO2	84							
Mouse BCDO2	81	80						
Rat BCDO2	80	80	92					
Sheep BCDO2	80	85	82	82				
Bovine BCDO2	78	81	75	75	91			
Chicken BCDO2	62	67	66	64	64	63		
Zebrafish BCDO2	55	58	58	57	56	54	59	
Human RPE65	42	41	41	42	41	39	41	41
Bovine RPE65	41	41	41	42	41	38	41	41
Rat RPE65	42	42	41	43	42	39	41	42
Mouse RPE65	42	42	42	43	42	39	41	42
Chicken RPE65	42	42	42	43	42	39	42	42
Zebrafish RPE65	39	38	40	40	39	36	38	38
Fly NinaB	29	31	33	34	29	30	30	32
Arabidopsis	18	18	17	18	17	13	17	15
Maize VP14	17	17	18	18	17	15	17	15

	Human RPE65	Bovine RPE65	Rat RPE65	Mouse RPE65	Chicken RPE65	Zebrafish RPE65	Fly NinaB	Arabidopsis
Bovine RPE65	98							
Rat RPE65	94	93						
Mouse RPE65	93	93	97					
Chicken RPE65	90	89	87	88				
Zebrafish RPE65	74	74	72	73	73			
Fly NinaB	33	32	33	33	32	32		
Arabidopsis	12	12	14	11	15	13	13	
Maize VP14	15	15	15	15	14	15	9	35

Figure 2. Homology comparison among BCMO1, BCDO2, RPE65 and other related carotenoid cleavage enzymes by ClastalW2. More than 90%, 70–89%, 40–69% and lower than 39% of homology were shown in white numbers on black background, white numbers on gray background, black numbers on light gray back ground and black numbers on white background, respectively. The following sequences were used: Human BCMO1 (NP_059125); Ferret BCMO1 (ADK26620); Mouse BCMO1 (NP_067461); Rat BCMO1 (NP_446100); Bovine BCMO1 (NP_001019730); Sheep BCMO1 (NP_001153227); Chicken BCMO1 (XP_414163); Zebrafish BCMO1 (NP_571873); Human BCDO2 (NP_114144.3); Ferret BCDO2 (AAS20392); Mouse BCDO2 (NP_573480); Rat BCDO2 (NP_001121184); Sheep BCDO2 (CAX63047); Bovine BCDO2 (NP_001019730); Chicken BCDO2 (XP_417929); Zebrafish BCDO2 (NP_001035402); Human RPE65 (NP_000320); Bovine RPE65 (NP_776878); Rat RPE65 (NP_446014); Mouse RPE65 (NP_084263); Chicken RPE65 (NP_990215); Zebrafish RPE65 (NP_957045); Fly NinaB (NP_650307); *Arabidopsis* CCD1 (NP_191911) and Maize VP14 (AAB62181).

conserved in all of the three enzymes. However, additional experiments are necessary for verification of the hypothesis since other factors may also affect fat colour [75]. In chicken, a relationship between sequences derived from 23.8 kb of the BCDO2 locus and colour of skin has been reported, with observed 115 fixed nucleotide substitutions between the clusters of yellow skin and white skin sequences [76]. Interestingly, the effect of nucleotide substitutions was found to be tissue-specific [76]. Finally, there are indications that female variant allele carriers of a common SNPs in the human *BCDO2* gene have reduced fasting HDL-cholesterol concentrations compared with wild-type carriers [77]. However, the mechanism of the interaction between lipid metabolism and BCDO2 genotype still needs clarification.

7 Summary

The importance of BCMO1 as the key enzyme in retinoid production from β-carotene has been eloquently shown in studies applying knockout mouse models [12, 87]. These studies have indicated that BCDO2 cannot compensate for lost BCMO1 activity. BCMO1 activity has been shown to be regulated via a diet regulatory network involving the intestine-specific homeodomain transcription factor ISX. Although provitamin A carotenoid conversion is regulated via this RA-sensitive gate keeper, genetic variations in the human *BCMO1* gene have also been shown to affect conversion efficiency. What is not known at this stage is to which extent both mechanisms synergistically affect retinoid metabolism in both the intestinal and extra-intestinal tissues.

Maybe the most intriguing discovery is the role of BCMO1 in tissues other than the intestine. In embryos, BCMO1 was shown to maintain normal development under conditions of limited maternal vitamin A supply. In the liver and in adipocytes, BCMO1 has been implicated as an important regulator in lipid metabolism.

Although the role of BCDO2 was until recently uncertain, there is some indication that it could play an important role in protection against mitochondria impaired respiration and oxidative stress. Therefore, the function of this enzyme might be pivotal in preventing adverse health effects observed in intervention studies with high dietary intake of carotenoids. On the other hand, SNPs in BCDO2 have also been shown to influence lipid metabolism in female volunteers, indicating a similar role compared with BCMO1 in lipid metabolism. Indeed, both BCMO1 as well as BCDO2 deficiency have been implicated in the development of liver steatosis. Since BCMO1 deficiency has shown to induce BCDO2 expression levels, more research is clearly needed to fully unravel the importance of both enzymes in human health and disease and to study their interactions in these processes.

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