Human nutritional relevance and suggested nutritional guidelines for vitamin A5/X and provitamin A5/X

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Abstract

In the last century, vitamin A was identified that included the nutritional relevant vitamin A1 / provitamin A1, as well as the vitamin A2 pathway concept. Globally, nutritional guidelines have focused on vitamin A1 with simplified recommendations and calculations based solely on vitamin A. The vitamin A / provitamin A terminology described vitamin A with respect to acting as a precursor of 11-*cis*-retinal, the chromophore of the visual pigment, as well as retinoic acid(s), being ligand(s) of the nuclear hormone receptors retinoic acid receptors (RARs) α, β and γ. All-*trans*-retinoic acid was conclusively shown to be the endogenous RAR ligand, while the concept of its isomer 9-*cis*-retinoic acid, being "the" endogenous ligand of the retinoid-X receptors (RXRs), remained inconclusive. Recently, 9-*cis*-13,14-dihydroretinoic acid was conclusively reported as an endogenous RXR ligand, and a direct nutritional precursor was postulated in 2018 and further confirmed by Rühl, Krezel and de Lera in 2021. This was further termed vitamin A5/X / provitamin A5/X. In this review, a new vitamin A5/X / provitamin A5/X concept is conceptualized in parallel to the vitamin A(1) / provitamin A(1) concept for daily dietary intake and towards dietary guidelines, with a focus on the existing national and international regulations for the physiological and nutritional relevance of vitamin A5/X. The aim of this review is to summarize available evidence and to emphasize gaps of knowledge regarding vitamin A5/X, based on new and older studies and proposed future directions as well as to stimulate and propose adapted nutritional regulations.

Keywords Nuclear receptors, Beta-carotene oxygenase, Recommended dietary allowance, Apo-carotenoids, Visual cycle

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The vitamin A concept

In the last century, the term vitamin has been used to describe and define essential micronutrients. As one of the first identified vitamins, all-*trans*-retinol and all-*trans*-β,β-carotene were named vitamin A and provitamin A, respectively. The initial importance of vitamin A in health was the prevention of ocular diseases mediated via the visual chromophore 11-*cis*-retinal [1–3]. Later on, vitamin A regained momentum because of its role in nuclear hormone signalling pathways mediated via all-*trans*-retinoic acid (ATRA) [4, 5]. The retinoic acid receptor (RAR)-mediated signalling was initially identified with ATRA (Fig. 1A), being a metabolite dependent on the nutritional

intake of vitamin A / provitamin A [6]. These important investigations were awarded with various Nobel prizes. Frederick Hopkins received in 1929 the Nobel prize in Physiology and Medicine for the importance of growth-stimulating vitamins, with a focus on vitamin A. Paul Karrer won the Nobel prize in Chemistry for the structural identification of vitamin A in 1937 and George Wald followed with the Nobel prize in Physiology and Medicine awarded for demonstrating the importance of vitamin A in ocular health in 1967. In addition, in 2004 Pierre Chambon and Ron Evans received the Albert Lasker Award for Basic Medical Research for the identification of vitamin A / ligand activated nuclear hormone receptor mediated signalling, as reviewed previously [7, 8].

More recently, the detailed molecular signalling concept for specific disease prevention has been studied using "up to date" molecular-biological techniques [9–12]. Gene knock-out (KO)-mouse model studies confirmed the crucial retinoic acid receptor (RAR)-mediated vitamin concept [13, 14]. The primary application of this knowledge was the nutritional supplementation of human food or livestock feed with vitamin A / provitamin A to prevent vitamin A deficiency [15–18].

Further RAR-activators either of natural origin such as ATRA, as well as natural precursors, including retinol, retinyl-palmitate and retinal, were additionally included in pharmaceutical or cosmetic applications, while synthetic RAR-ligands such as Tigason™, Neotigason™, Adapalene™, Tazarotene™ and Trifarotene™ have been employed for systemic and/or local treatment of cancer or dermatological disorders [19–23]. β-Carotene is the most abundant provitamin A carotenoid in the human diet, and is used as a coloring agent, as an antioxidant and as a provitamin A carotenoid for human and animal food supplementation [24–26]. Provitamin A carotenoids are the major sources of dietary vitamin A in low- and middle-income countries and in populations following plant-based diets (including Western vegans and vegetarians) [24, 26].

The traditional focus on vitamin A research and nutritional recommendations has been put on the study of vitamin A1 derivatives. These included e.g. "all-*trans*"-retinol as vitamin A1 alcohol, "all-*trans*"-retinyl esters as the major vitamin A1 derivatives present in animal-derived food sources, the non-nutritional relevant "all-*trans*"-retinal as vitamin A1 aldehyde, the precursor of 11-*cis*-retinal, the active visual pigment in the human organism, as well as various provitamin A1 carotenoids present in vegetable-derived food sources (Fig. 1) [24, 26]. Surprisingly, the vitamin A2 cluster (namely 3,4-dehydroretinol) has been much less investigated, likely because of a lower physiological and nutritional relevance for humans [27–31]. By contrast, in birds and fish, vitamin A2 seems to be of major important physiological and nutritional relevance [28, 32]. Vitamin A3 and A4 are retinal analogues serving as visual pigments for arthropods or crustaceans, solely of relevance for non-mammalian species [33]. The current vitamin terminology was exclusively established with a focus on human relevance [34], which poses the question of whether the vitamin A terminology is appropriate, as vitamin A3 and A4 have apparently no human relevance and one can question if they should be thus termed vitamins.

Recently, a new vitamin A cluster was identified, and as the terms vitamin A3 and A4 were already used, suggested and termed vitamin A5 / provitamin A5 [35]. The term "vitamin A5" was used and based on subordination in the existing vitamin A cluster, due to a familiar retinoid / carotenoid background and slightly overlapping metabolism and mechanisms of signalling. Unfortunately, the subordination of vitamin A5 only under the vitamin A category would partly result in non-conclusive and uncertain nutritional regulatory recommendations, overlapping with existing vitamin A / vitamin

(See figure on next page.)

Fig. 1 **A** The current vitamin A concept (the vitamin A1 concept): Summarized is the metabolic conversion starting from animal derived food products (at the left side with a reddish background), rich in vitamin A derivatives such as all-*trans*-retinyl esters (ATROL-ES) and all-*trans*-retinol (ATROL), which can be metabolized to the intermediate all-*trans*-retinal (ATRAL, a precursor of the visual pigment 11-*cis*-retinal, which performs important functions in the visual cycle) towards the active all-*trans*-retinoic acid (ATRA) as the bioactive metabolite of the vitamin A/vitamin A1 pathway. Besides (on the right side with a greenish background), the plant-derived food sources rich in provitamin A(1) are displayed with the double sided vitamin A(1)-precursor all-*trans*-β,β-carotene (ATBC) and additional carotenoids with a half-sided vitamin A(1) function such as all-*trans*-α,β-carotene (ACAR), all-*trans*-β,β-cryptoxanthin (CRYPT), 13-*cis*-β,β-carotene (13CBC) and 9-*cis*-β,β-carotene (9CBC), which are metabolized via the intermediate ATRAL to ATRA. In addition, in dashed lines, indicating a high uncertainty of this pathway, starting from 9CBC and ATRA to the potential as well as possibly to the endogenous RXR-agonist 9-*cis*-retinoic acid (9CRA). **B** The vitamin A5/X concept: Summarized is the metabolic pathway of the novel vitamin A5/X / provitamin A5/X pathway, starting from nutritional derived retinoids and carotenoids (on the left side with a grey blue background the bioactive metabolites of the vitamin A1 cluster pathway). On the right side (with a yellow background) the vitamin A5/X pathway with 9-*cis*-13,14-dihydroretinyl esters (9CDHROL-ES), as well as the direct nutritional precursor vitamin A5/X alcohol 9-*cis*-13,14-dihydroretinol (9CDHROL) and the provitamin A5/X carotenoids 9-*cis*-13,14-dihydro-β,β-carotene (9CDHBC) and 9-*cis*-β,β-carotene (9CBC), to vitamin A5/X acid, the lipid hormone, the endogenous RXR-ligand 9-*cis*-13,14-dihydroretinoic acid (9CDHRA) as the resulting bioactive ligand enabling RXR-mediated signalling

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A. The current vitamin A(1) based concept
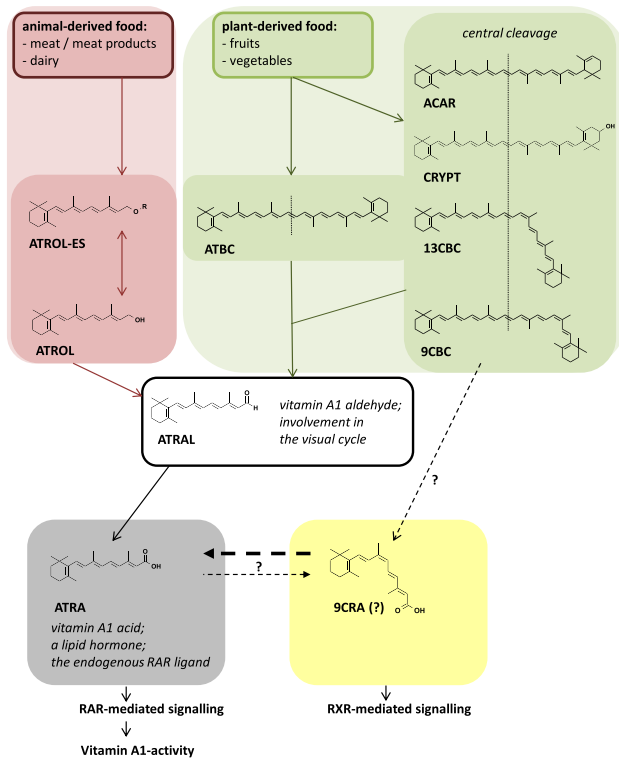
B. The updated vitamin A concept including vitamin A1 and A5/X pathways

Fig. 1 (See legend on previous page.)

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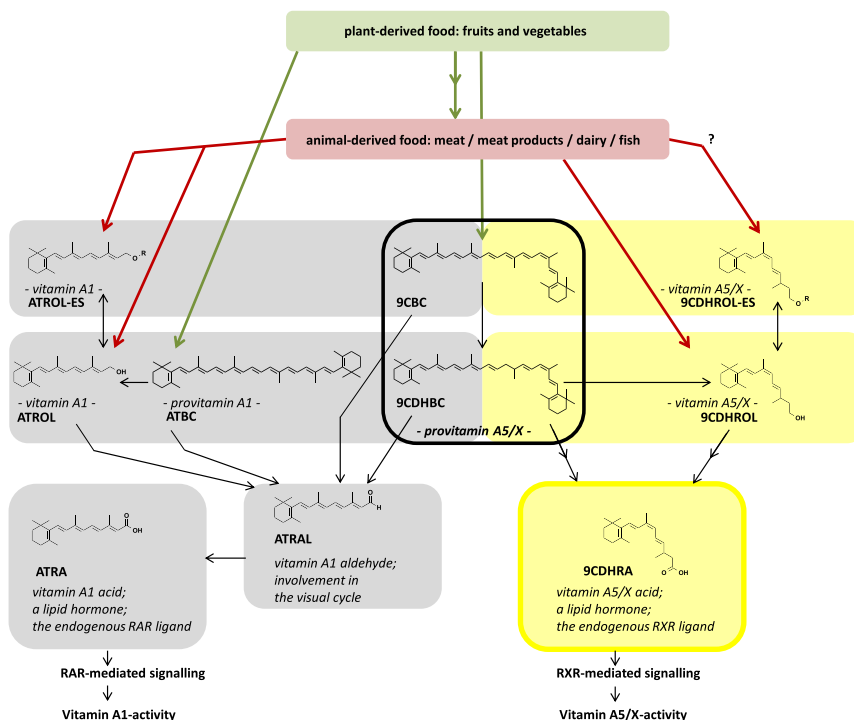Page 4 of 19

A1-guidelines [34, 36, 37]. The currently used vitamin A concept, including dietary recommendations and fortification regulations, do exclusively rely on the vitamin A1 pathway. For practical reasons, its name was replaced by the simplified term vitamin A. Therefore, alternatively, because of functioning as a nutritional precursor of the RXR-activation pathway, the letter "X" was chosen, suggesting a new unique vitamin category, and named "vitamin X".

As reviewed earlier, alternative endogenous and physiologically-relevant RXR-ligands and activators have been described [38]. The two main alternative candidates 9-*cis*-retinoic acid (9CRA) and the non-esterified/free fatty acid docosahexaenoic acid (DHA), failed to demonstrate to act as candidates for RXR-ligands at physiological level. This would comprise the criteria food intake, uptake, metabolism, being present in sufficient concentrations for receptor activation at the cellular level and eliciting nuclear hormone receptor (NHR)-mediated signalling via the RXR-receptor [38]. Based on various gaps and an overall non-conclusive concept, their relevant function as "a" or "the" physiological RXR-ligand is highly unlikely for RXR-mediated signalling at levels as

occurring in food items. However, their relevance cannot be fully excluded. The vitamin A5/X / provitamin A5/X concept proposes novel identified physiologically- and nutritionally-occurring precursors of the recently identified lipid hormone, 9-*cis*-13,14-dihydroretinoic acid (9CDHRA), conclusively identified currently as the only physiologically relevant RXR-ligand (Fig. 1B) [39–43].

The current vitamin A national and international recommendations

At present, several dietary intake recommendations for vitamin A exist. These are generally expressed for preformed vitamin A as retinol-equivalents (RE) or as retinol activity equivalents (RAE) (Table 1 and Additional file 1: Table 1). Among the internationally most important ones are the USDA-DRI (dietary reference intakes) and for Europe EFSA's (European Food Safety Authority) DRVs (dietary reference values), both of which are umbrella terms with a set of recommendations. The EFSA suggested population reference intakes (PRI) as part of the DRVs, which are recommendations of nutrients for the general and healthy population, according to gender and age groups, including vitamin A but not carotenoids.

Table 1 Intake recommendations for vitamin A and provitamin A / β-carotene (**A**) Selected intake recommendations for vitamin A in vitamin A equivalents (VAE), all individual values from international and national organizations are presented in Additional file 1: Table 1. (**B**) Selected intake recommendations for β-carotene

(A)	National RDI's or similar for vitamin A (µg RE/d or µg RAE/d for DACH)	National UL or similar for vitamin A (µg RE/d)
Range		
Adult man	600–900	3000
Adult woman	500–750	3000
Range		
Pregnant woman	700–800	3000
Range		
Lactating woman	850–1300	3000
Range		
Children	300–950	600–2800
Organization **(B)**	**National RDI or similar for β-carotene (mg/d)**	**National UL or similar for β-carotene (mg/d)**
EFSA[5]	N/A	15
UK-EVM-GL & SUL[6]	N/A	7
DGE[7]	2	N/A
DGE-vegetarians[7]	8	N/A

All ranges are based on EFSA[1], USDA[2], WHO-FAO[3] and DACH[4] recommendations listed in Additional file 1: Table 1

References: [1] EFSA: DRV-PRI and UL [145]; [2] DRI-RDA and UL [146]; [3] WHO/FAO: Safe recommended intake [34, 45], [4] DACH Recommended Dietary Intakes [44]; [5] EFSA—safe intake (including smokers from additives & supplements) [46]; [6] UK-Safe upper levels for vitamins and minerals as proposed by the expert group for vitamins and minerals [147]; [7] DGE-RDIs [42]

DACH, German, Austrian and Swiss Recommended Dietary Intakes; DGE, German Nutrition Society; DRI, dietary reference intakes; DRV, dietary reference values; EFSA, European Food Safety Authority; GL, guidance level; PRI, population reference intakes; RDA, recommended dietary allowance; RDI, recommended daily intake; RAE, retinol activity equivalents; RE, retinol equivalents; SUL, safe upper level; UL, tolerable upper intake level; USDA, US Department of Agriculture; WHO-FAO, World Health Organization/Food and Agriculture Organization

These PRIs are intended to cover the needs of a nutrient of approx. 97.5% of persons within a population (i.e. for a specific age and gender group), and are derived from the estimated average requirements (AR) plus adding 2 times the standard deviation of the population's need. The same is true for the USDA derived RDA (recommended dietary allowance) which is obtained from the EAR (estimated average requirement).

Besides being different between national institutions, these intake recommendations also differ according to gender and age groups (Table 1 and Additional file 1: Table 1). For preformed vitamin A, EFSA's PRI values vary between 250 μg/d RE for infants and 1300 μg/d for lactating women. The USDA-RDA values are very similar; though tend to be slightly higher. The German/Austrian/Swiss recommendations (RAE) are similar, up to 1300 μg/d for lactating women. Lowest recommendations generally exist for infants, and highest for lactating followed by pregnant women. Special recommendations for elderly are generally not defined.

For carotenoids, including β-carotene, there are no umbrella recommendations such as DRI or DRV. For provitamin A in the form solely of all-*trans*-β,β-carotene, an intake of 6 mg/d was suggested for men older than 19 years and 4.8 mg/d for women older than 19 years [44]. These take into consideration the equivalence of 1 mg of retinol with 6 mg of β-carotene and 12 mg of other provitamin A carotenoids.

Based on adverse effects of various supplementation trials in smokers, a safe intake of < 15 mg/d from supplements and food additives was recommended by the EFSA. These considered both smokers and the general population [45, 46]. This was established after an increased cancer incidence was found in two major supplementation trials with doses of 20–30 mg/d of β-carotene over several years in human smokers [47, 48]. These studies demonstrated the cancerogenic effects of smoking and the risk of supplementation in heavy smokers. Consequently, it became clear that β-carotene is not an anti-cancer protection dietary supplement reverting and preventing detrimental effects of smoking. Ferret based animal models showed reduced ATRA-levels in the lung of smoke exposed ferrets supplemented orally with high doses of β-carotene in corn oil [49, 50], likely due to a strong bio-feedback mechanism [51].

Even high supplemented amounts of β-carotene have been safely given for a semi-therapeutic application of up to 240 mg/person/d during 6 months for a potential treatment of cystic fibrosis [52]. Some clinics such as the US Mayo clinic recommend 30–300 mg/d for the prevention of negative reactions to sun in subjects with erythropoietic protoporphyria [53, 54]. The only side-effect noted for high carotenoid intake (above ca. 30 mg/d) was carotenodermia, i.e. a reversible orange discoloration of the skin [55]. This discoloration is partly even seen positive, as a healthy sun tanning coloration of the skin by large parts of the population.

In general, vitamin A can be sub-divided into two nutritionally-relevant precursor pathways; preformed vitamin A (retinol and retinyl esters) and provitamin A (all-*trans*-β,β-carotene, previously simplified as all-*trans*-β-carotene and even more simply as β-carotene, and alternative provitamin A carotenoids such as α-carotene, 13-*cis*-/9-*cis*-β,β-carotene and β-cryptoxanthin) (Fig. 1A) [56]. Based on this vitamin A concept, EFSA provides specific recommendations for reaching vitamin A PRI levels for retinol, i.e. 0.65–0.75 mg/d for normal adults (Table 1 and Additional file 1: Table 1), and based on conversion ratios for provitamin A carotenoids. Conversion rates vary due to matrix interactions and host factors, thus various equivalence doses may be calculated [25]. Due to half the equivalence of β-carotene from a bioavailable source, such as for β-carotene in oil of 1.3–1.5 mg/d for this provitamin A carotenoid [57], rather low doses would cover vitamin A needs. Due to lower bioavailability from other food matrices, and equivalences of up to 28:1 for β-carotene [58], equivalent doses of 18.2–21 mg/d would be required. Based on an often-employed equivalence of 1:12 proposed by the US-Institute of Medicine (IOM), for pre-formed vitamin A vs. β-carotene, a dose of 10.8 mg/d would be needed for adult males to meet the USDA requirements if all vitamin A would be derived from this provitamin A carotenoid. Again, these are average values for the general population, with a large uncertainty range, and should be considered with care at the individual level.

When comparing these recommendations with intake data, the latter is typically lower. The average daily intakes of β-carotene for the general population were estimated as: (a) β-carotene from food additives as 1–2 mg/d (we assume pure all-*trans*-β,β-carotene is meant), (b) β-carotene from natural food sources as 2–5 mg/d and, thus resulting in a total β-carotene daily intake of 3–7 mg and up to 10 mg/d, taking into account seasonal variations [59]. In a recent publication summarizing various large-scale observational studies reporting carotenoid intake, an average intake of 4.8 mg/d for β-carotene was reported [26], as summarized in Fig. 2.

When comparing the β-carotene recommendations of 7 mg/d for a safe intake with an estimated real intake of approx. 5–10 mg/d, the recommendation "window" for supplementation remains rather small, at least for smokers. This is based on calculations with additional dietary supplements to the maximum of 15 mg (from supplements and food additives as proposed by EFSA) and is further based on the negative effects of 20 mg/d observed

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General EFSA recommendations for BC

1. Proposed upper limit for BC
- ATBC   15 mg/day *
                                no upper limit **
- 9CBC   no upper limit

2. Current EFSA recommendations
- natural BC within diet   2 - 5 mg/day
- as food additives   1 - 2 mg/day
                                up to 15 mg/day
-> total BC in food   3 - 7, 10 mg/day ***
                                18 - 25 mg/day

-> for 9CBC   0.5 - 1.8 mg/day
                                up to 3.2 - 4.5 mg/day

(extrapolated based on calculations present in Table 2B)

3. Suggestions for 9CBC based on extrapolations
- 9CBC (total)   0.5 – 1.8 mg/day as RDI
- 9CBC (additional)   0.2 – 0.9 mg/day

 # calculated based on missing 9CBC, because of exclusive pure ATBC added within food additives (~50%)

4. Reported supplementation without noticed adverse effects
- ATBC   300 mg/day
- 9CBC   78 mg/day ****

Fig. 2 Current EFSA recommendations for β-carotene (BC) with a focus on all-*trans*-β,β-carotene (ATBC) and our suggestions for 9-*cis*-β,β-carotene (9CBC). # based on our own calculations/ percentile amounts based on calculations originating from Table 2b, * relevant only when the individual is a tobacco smoker (no proposed upper limit for general population) and only a recommendations for people who are tobacco smoking in parallel to taking high-dose BC supplementation/consumption, ** relevant, when the individual is not a smoker; *** based on 10 mg/d as upper value due to EFSA suggestion [43, 70]; **** volunteers with single treatment, Stahl et al. [136] (Table 3)

in smokers in the ATBC study. Alternatively, considering a low average intake of fruits and vegetables and being a non-smoker, then the "window" for supplementation is wide open.

Problems with the current vitamin A recommendation

In chemical terms, the vitamin A1 umbrella terminology includes "all-*trans*-β"-retinol / "all-*trans*-β"-retinyl esters, "all-*trans*-β,β-carotene" and further likely nutritional minor important provitamin A1 precursor carotenoids, however only the term "vitamin A" is currently explicitly mentioned. There is an assumption that the "all-*trans*"-configuration is always the point of reference [34, 36, 37].

The concept of vitamin A2 [29–31] was originally identified in the nineteenth century alongside vitamin A1 [16–18, 60–62], but has received little to no scientific interest. Neither the nutritional relevance nor the molecular signalling mechanisms were further examined. Due to this, we speculate that omitting the vitamin A1 and A2 concept as sub-categories of vitamin A was suggested and executed because of a previously and currently non-felt importance. Therefore, no further specific A1 and A2 nutritional guidance and individual recommendations were given and executed [34, 36, 45].

The previously used nutritional RXR-ligand precursor concept was in the first instance associated with the general vitamin A / vitamin A1 category. This postulated a simple isomerization of the active ligand all-*trans*-retinoic acid (ATRA) to 9-*cis*-retinoic acid (9CRA) [40, 63–67]. In reverse, an isomerization of the relatively unstable 9CRA back to ATRA is more likely [68], as shown in Fig. 1A. 9CRA might further bind and activate the RXR [63, 64] to enable further RXR-mediated signalling. While this concept was published in highly ranked prestigious journals and was always well cited with > 2000 citations until August 2022, various experts in retinoid lipidomics could not conclusively confirm the physiological presence of 9CRA in the human organism, as reviewed recently [40]. Additionally, a substantial nutritional-metabolic conversion and activation cascade starting from all-*trans*-retinol / all-*trans*-β,β-carotene towards physiologically- and nutritionally-relevant levels of "the endogenous RXR ligand" 9CRA was neither shown nor conclusively confirmed [67–70]. These concepts were mainly based on identification techniques available in the 80´ies for which the 9CRA detection capacity was limited.

Furthermore, a study using an *ex vivo* human intestinal mucosa model "identified", via simple LC-UV co-elution analytical determination, that 9CBC can be cleaved to "9CRA" [70]. The analytical system used a methodology with strong shifting retention times for ATRA, ranging between 5.8 and 6.8 min. and for 9CRA the range was between 5.9 and 6.5 min. (calculated based on Fig. 1A, [70]). As previously discussed in detail [40], this study used outdated methodologies that lacked robust parallel identification techniques for a conclusive identification of 9CRA.

In summary, based on the methodological issues surrounding the identification of 9CRA and its direct upstream nutritional precursors, a novel alternative 9CDHRA / vitamin A5/X concept was recently suggested [40] and later even confirmed [35]. This is important because a conclusive and irrefutable identification of 9CDHRA has physiological and nutritional implications

for general vitamin A science. The current vitamin A concept and this current recommendation status should now be optimized and better explained, by sub-categorizing this current vitamin A concept into specific vitamin A1 as well as vitamin A5/X concepts.

Vitamin A2 is currently not sub-categorized and nutritional recommendations are not suggested. In addition, no further deeper investigation about food sources and mechanisms of action are clearly known, as there is a large overlap between the vitamin A1 and vitamin A2 pathways regarding RAR-ligands precursors [40] and a likely non-relevant function of vitamin A2 as a human chromophore. Based on food intake of vitamin A2 and impact on vitamin A2-induced signalling in the human body, we believe that there is no strong need for further targeted investigations into this direction.

On the other hand, vitamin A5/X seems to be a highly important new vitamin A concept. Vitamin A5/X is a novel precursor concept for the physiologically- and nutritionally-relevant RXR-ligand, 9CDHRA, for a further highly important initiation, maintenance and a general regulation of RXR-mediated signalling in the human organism [7–9, 39].

The new player and the new concept of a new vitamin; vitamin A5/X / provitamin A5/X

The major importance of vitamin A5/X / provitamin A5/X is to act as a nutritional precursor of the endogenous RXR-ligand 9CDHRA. The precursor concept is similar to the well-established role of retinol and retinyl esters acting as vitamin A1 for further bioactive vitamin A1 derivatives, such as retinal and ATRA (Fig. 1A). In the case of vitamin A5/X, the alcohol form 9-*cis*-13,14-dihydroretinol (9CDHROL) and the ester form both act as precursors for 9-*cis*-13,14-dihydroretinoic acid (9CDHRA) (Fig. 1B) [35]. Similar to vitamin A1 precursors, 9CDHROL precursors are also likely to originate mainly from dietary ingestion of foods from animal origin such as meat, fish and dairy products (Fig. 1A) [35]. No in-depth analysis of these food sources was carried out to date but are the topic of ongoing examination.

In parallel, for vitamin A5/X, similar carotenoid precursors, namely β-carotene and alternative provitamin A1 precursors exist. These were identified as the provitamin A5/X precursor 9-*cis*-β,β-carotene (9CBC) with its proximate intermediate provitamin A5/X precursor 9-*cis*-13,14-dihydro-β,β-carotene (9CDHBC) [35]. 9CBC is present in the human organism in serum, in various organs and in human breast milk (Table 2A). It is also found in different isomer ratios with all-*trans*-β,β-carotene (ATBC) in different concentrations in a variety of vegetable-derived food sources (Table 2B). Here, a special nutritional focus seems to be placed on leafy and root vegetables, which are rich in this compound.

The invisible provitamin, 9-*cis*-β,β-carotene: from a "substitute" to the "most valuable player" in the vitamin A-team

Vitamin A5/X comprises 9CDHROL plus additional potential 9CDHROL-esters. Currently, only the vitamin A5/X alcohol (9CDHROL) was identified in beef liver (8 ng/g) [35], representing a human food source. Our group is working to further determine the vitamin A5/X content in additional animal derived foods, including different meat sources, meat products, marine-/freshwater fish as well as different sources of milk and dairy products.

Additionally, 9CBC acts as a double-edged sword being a unique precursor for enabling further vitamin A1- and A5/X-mediated signalling, functioning as a precursor for the RAR ligand ATRA on one side and as a precursor for the RXR ligand 9CDHRA on the other side. Based on its provitamin A5/X precursor, 9CBC is claimed to be "the" nutritionally relevant provitamin. The contents of 9CBC in human blood serum, tissues and human breast milk, as well as in various food items, are shown in Tables 2A and B. Both tables also present the contents of ATBC. Using these data, the relative portion of 9CBC was calculated in relation to ATBC. Human blood serum only contained very low amounts (0–1% expressed as a percentage of total β-carotene) of 9CBC, while in organs such as liver, kidney and testes high relative proportions (22–28%) of 9CBC were found and lower amounts of approx. 3% 9CBC in breast milk (Table 2A).

This indicates also that 9CBC is an almost "invisible" derivative, as blood serum/plasma levels are very low compared to organ levels and seem to reflect just a minor proportion of the nutritional intake of 9CBC. We speculate that after ingestion of food high in 9CBC there is a quick rise of 9CBC in plasma or serum levels and further 9CBC is either stored in tissues and/or quickly metabolized to 9-*cis*-13,14-dihydro-retinoids in the human organism. Due to this problem, other options for detecting a healthy vitamin A5/X / provitamin A5/X dietary intake and status must be chosen, such as alternatively monitoring the active vitamin A5/X derivative, 9CDHRA, in the easily accessible serum/plasma compartment or choosing harder accessible compartments of the human organism such as white blood cells or organ biopsies for direct provitamin A5/X analysis.

Table 2 (**A**) Concentrations of ATBC and 9CBC in human serum, breast milk and further analyzed organs. (**B**) Selected examples of ATBC and 9CBC as present in fruits and vegetables

(*A*)	ATBC (nM)	9CBC (nM)	% of 9CBC[a]
Serum	131/275/703[b]	UDL[b]	–
	420 (120–890)[c]	0–4 [c,d]	0–1[c,d]
Liver	1400/1600/7300[b]	400/600/2100[b]	22/27/22
	3020 (160–8620)[c]	1162[c,d]	28 [c,d]
Kidney	550 (80–2031)[c]	177[c,d]	24 [c,d]
Adrenals	1800/2000/4400[b]	100/100/300[b]	5/5/6
	5600 (680–31,830)[c]	988[c,d]	15 [c,d]
Testes	2680 (750–4770)[c]	670[c,d]	29[c,d]
Prostate	480 ± 0.06[e]	380 ± 0.06[e]	~44
Breast milk	916[f]	24[f]	~3

(*B*)	Dry weight in µg/g		Fresh wet weight in µg/g		% 9CBC of total BC fresh weight[m]
	ATBC	9CBC	ATBC	9CBC	
Fruits					
Peach[o]		2.2	0.3	12	
Apricots[n]	16.0[g]	4.4[g]	2.0	0.6	23
Papaya[n]	10.6[h]	7.0	1.9	1.3	41
Vegetables/root vegetables					
Pumpkin[n]	9.7[i]	2.7	0.6	0.2	28
Sweet potato[p]			38.2	1.5	4
Tomato[o]			71.0	4.8	6
Carrots[n]	1030[j]	57.1[j]	117	6.5	5
Leafy vegetables					
Lettuce[n]	104[k]	41.0[k]	5.0	2.0	29
Broccoli[o]			29.2	5.0	15
Kale[q]			59.4	14.1	19
Spinach[o]			311.9	38.6	11
Calculated total average for fruits and vegetable					**18**
Algae with no direct food relevance					
Dunaliella salina[n]	38,500	37,800	l	l	50

(A) ([a]) The percentage of ATBC and 9CBC of total BC; ([b]) Stahl et al. [148]; ([c]) Stahl et al. [149]; with ([d]) approx. calculations of 9CBC concentrations based on the displayed figures; ([e]) Clinton et al. [150]; ([f]) Johnson et al. [151]; UDL - under detection limit. (B) Individual dry weight listed as ([g]) 12.6% ([n]), ([h]) 18.3% ([n]), ([i]) 6.2% ([n]); ([j]) 11.3% ([n]), ([k]) 4.8% ([n]), ([l]) not calculated because the wet algae and thereby the weight of these algae are not of nutritional relevance. ([m]) total BC = ATBC + 9CBC; ([n]) Ben-Amotz and Fishler [152], ([o]) Khoo et al. [153], ([p]) Berni et al. [154], ([q]) Murador et al. [155]

Regarding dietary intake, fruits, papaya (41%) and apricots (23%) showed the highest relative portions of 9CBC (expressed as a fraction of total β-carotene) in fruits, while being low in total concentration of 1.9–2.0 µg/g of wet weight (Table 2A). Lettuce (29%), pumpkin (28%) and kale (19%) were the vegetables with the highest relative portions of 9CBC. Highest concentrations of 9CBC were found in leafy vegetables including spinach (312 µg/g) and kale (59 µg/g), as well as root vegetables such as carrots (117 µg/g), while levels in fruits were relative low, for instance in papaya (2 µg/g) and apricots (2 µg/g). The selected fruits and vegetables shown in Table 2B were used to calculate a mean content of 9CBC of 18% used for further calculations in Table 2B and Fig. 2/7.

One major question remains, namely at which levels does this specific all-*trans*- to 9-*cis*-isomerization takes place? The first step is a targeted synthesis of 9-*cis*-carotenoids in plants/algae. In *Dunaliella salina,* rich in 9CBC, specific β-carotene isomerases [71] and

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Table 3 Supplementation trials with preparations high in 9CBC

Author	Description/dose	Isomer ratio and 9CBC amount	Purpose of study and beneficial activity outcome
Stahl et al. [156]	5.6 µmol BC/kg bw as a **single dose** of Betatene™; ~70 kg bw and ~5.6 µmol BC (~210 mg BC)	54% ATBC: 37% 9CBC **-> ~77.7 mg 9CBC/day**	Absorption/accumulation study
Johnson et al. [151]	**Single treatment** with dried algae powder	64 mg ATBC (48%): 69 mg 9CBC (52%) **-> ~69 mg 9CBC/day**	Carotenoid nutri-kinetic study
Shaish et al. [157]	Natural derived extract rich in BC with 60 mg/BC day for 6 weeks **(42 days)**	~50% ATBC: 50% 9CBC **-> ~30 mg 9CBC/day**	Increase of HDL-cholesterol in fibrate treated patients
Rotenstreich et al. [158]	Treatment for **90 days** with capsules containing 300 mg 9CBC-rich dried algae containing ~20 mg BC	~50% ATBC: 50% 9CBC **-> ~10 mg 9CBC/day**	Increased retinal functions in patients with Retinitis Pigmentosa
Meshi et al. [159]	1200 mg BC rich powder with ~20 mg BC content for 3 months **(~90 days)**	~50% ATBC: 50% 9CBC **-> ~10 mg 9CBC/day**	Improvement and partly recovery of vision loss after ocular quintine toxicity

breeding conditions with specific environmental conditions/stimuli, such as illumination with red light and avoidance of energy rich purple/blue light illumination [72], strongly promote 9-*cis*-isomerization and accumulation. However, at which specific stage of algae 9CBC-synthesis this 9-*cis*-isomerization occurs is still unclear. If similar mechanisms occur in plants, especially in plants that constitute food for humans, is not clear. In general, these environmental stimuli such as light irradiation and physiologically-relevant thermal interference might also be of relevance in plants under certain still not observed and investigated conditions. Recently, an enzyme (DWARF27) was identified [73] and associated with a specific 9-*cis*-isomerization of all-*trans*-β-carotene to 9-*cis*-β-carotene [74]. Again, its relevance in plants with food-relevance for humans remains unclear.

As a second option, a specific isomerization of all-*trans*-carotenoids to 9-*cis*-carotenoids in mammals has, to the best of our knowledge based on available literature and our own experiments, not been observed.

In our view, the most important mechanism of human relevance is an unspecific isomerization of ATBC to 9CBC during food processing such as cooking (>100 °C) [75]. It was shown that 9CBC increased in total amounts and expressed as a fraction [76] and it was shown to be 3–4 higher after cooking, depending among other on time and temperature [77].

Further food supplementation trials were carried out with preparations rich in 9CBC and were mainly based on carotenoids derived from algae (Table 3), ranging from ~10 to 78 mg 9CBC/d. In all these supplementation trials, no adverse effects were associated with the supplementation of β-carotene.

Current governmental regulations focusing specifically on provitamin A1, β-carotene and biological relevant extracts rich in β-carotene for human nutrition

Based on the general regulation and the current applied terminology, the term provitamin A describes derivatives that can be transformed via metabolic activation to vitamin A [34]. This provitamin A1 term comprises all-*trans*-β,β-carotene and its isomers, such as 13-*cis*-β,β-carotene, 9-*cis*-β,β-carotene and minor potential endogenously occurring mono- or even di-*cis*-isomers. Besides, α-carotene (correctly all-*trans*-α,β-carotene) and β-cryptoxanthin are also included, while no geometric isomers of nutritional relevance have been described [34]. 9CBC is thereby a known provitamin A / provitamin A1 carotenoid with a low average presence in human food ingredients when expressed as the fraction of total β-carotene [35, 45, 78]. This may mean in consequence that besides ATBC, also 9CBC can be considered as a safe food compound with provitamin A activity as described in EFSA regulations about mixed carotenes [45]. β-Carotene is present on the food ingredient market in various forms of applications such as E160a in the sub-categories E160a(i, ii, iii, iv), as described in codex-alimentarius [36]. Surprisingly, the EFSA nomenclature was different, indicating mixed carotenes as E160a(i) and synthetic β-carotene as E160a(ii) [45]. In a correct summary based on the codex alimentarius guidelines; E160a(i) describes chemically synthesized all-*trans*-β,β-carotene, E160a(ii) describes vegetable-derived β-carotene, E160a(iii) describes *Blakeslea trispora* (a fungi)-derived β-carotene and E160a(iv) describes "algal-derived β-carotene" [36]. This nomenclature was later

corrected and implemented and amended by the ESFA [59]. This E160a(iv) definition was recently altered in the codex alimentarius to "β-carotene-rich extracts from *Dunaliella salina*" [79]. In summary, these non-synthetic carotenoids here focused on algae origin (*Dunaliella salina)* are safe and legally approved for food supplementation within specific frames and guidelines in the EU [80] plus further annual amendments [81], as well as by the USA-FDA (GRAS000351) for *Dunaliella (bardawil) salina* [82].

These mixed carotenoids are usually used as general provitamin A as well as mainly yellowish/orange food colorants. This suggests that 9CBC is already present at low amounts as a food ingredient in the EU and US and is approved by various national and international governmental authorities. E160a(iv) is currently only used in minor amounts as a food ingredient and colorant due to a much higher price compared to E160a(i).

In summary, 9CBC is present in low percentage amounts in natural foods as well as in food ingredients and as such is a well-known, safe and approved and governmental licensed food ingredient.

Importance of RXR-mediated signalling in the human organism

The RXR is the crucial binding partner for other heterodimer-partners of the nuclear hormone receptor group [9, 10, 83, 84]. There is a large variety of these nuclear hormone receptors (NHR), while we mainly focus here on the major known ones with a direct "health" and "food"-application potential, i.e. the retinoic acid receptors (RARs), peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), farnesoid X receptors (FXR), vitamin D receptor (VDR) and nuclear receptor subfamily 4 group A member 2 (NR4A2) (Fig. 3), which are known to be involved in various nuclear hormone receptor-signalling pathways with relevance for the human health (Fig. 4).

This RXR-mediated signalling is of general importance for cell differentiation, general development, embryogenesis, cell cycle regulation, apoptosis, general inflammation and immune response, as well as general lipid metabolism as outlined in Figs. 3 and 4 (reviewed in [9, 83, 84]). These summarized physiological pathways can be regulated by RXR-ligand (9CDHRA) – RXR - "plus other NHR" (i.e. LXRs, FXR, PPARs, NR4A2 and VDR)- mediated pathways, but also via RAR - ligand (ATRA) – RAR – RXR – RXR-ligand (9CDHRA) - RXR-regulated pathways and are thereby likely directly dependent on sufficient nutritional vitamin A1 and vitamin A5/X supply.

Many of these physiological pathways rely on complex co-regulatory pathways, involving multiple RXR-LXRs, -PPARs, -NR4A2, -FXR and -VDR-mediated signalling pathways, including RAR - RXR-mediated signalling [11, 12, 85–92]. These parallel signalling pathways require RAR- and RXR-ligands and in consequence the dietary intake of their nutritional precursors. In summary, all these previously mentioned pathways thereby depend on sufficient external nutritional vitamin A1 and vitamin A5/X supply, while nothing is known on further homeostatic pathways to control intracellular concentrations.

Elucidating vitamin A1 ligand - RAR-mediated signalling-independent pathways is necessary to claim and
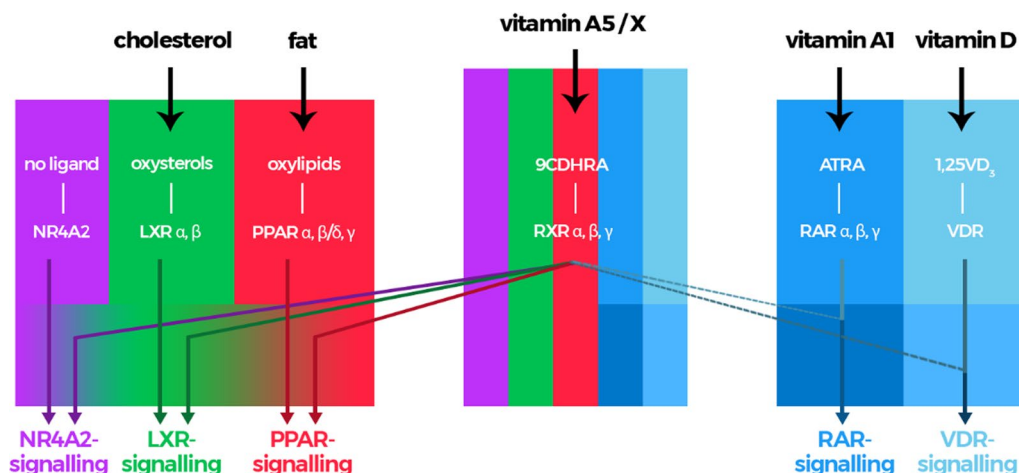

Fig. 3 Nuclear hormone receptor mediated signalling with ligand activation by RXR-heterodimers with permissive partners including nuclear receptor subfamily 4 group A member 2 (NR4A2/Nurr1), peroxisome proliferator-activated receptor (PPARs) and liver X receptor (LXRs) and non-permissive partners such as vitamin D receptor (VDR) and retinoic acid receptors (RARs). Abbreviations; 9-*cis*-13,14-dihydroretinoic acid (9CDHRA), all-*trans*-retinoic acid (ATRA) and 1,25-dihydroxy-vitamin D$_3$ (1,25VD$_3$)

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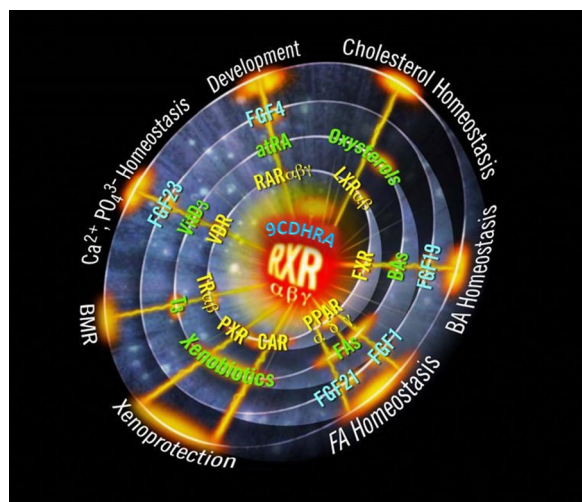
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Fig. 4 Summarized relevance of various nuclear hormone receptor mediated signalling pathways ranging from retinoic acid receptor (RARα,β,γ), liver X receptor (LXRα,β), farnesoid receptor (FXR), pregnane X receptor (PXR), constitutive androstane receptor (CAR), thyroid receptor (TRα,β), vitamin D receptor (VDR) and peroxisome proliferator-activated receptor (PPAR) interacting with the RXRs α,β,γ to mediated described physiological response pathways in the mammalian organism. Based on Evans and Mangelsdorf [9], with adaptations

identify a vitamin A5/X-specific vitamin deficiency. These vitamin A5/X-ligand – RXR —"plus other NHR"-NHR-ligand mediated-signalling pathways may be implicated in insulin/glucose regulation [11, 12, 85–92], cholesterol metabolism [93–95] with a large impact on the cardiovascular system and especially in the nerve system/brain area such as neurogenesis [96], neuroprotection [97, 98], working memory [99, 100], appetite regulation [101], sleep–wake cycle regulation [102], myelination / re-myelination [103, 104] and dopaminergic signalling [105–108] via dopamine 2 receptor (D2R) expressional control and are all mainly PPARs-, FXRs- and LXRs - RXR-mediated pathways. In the majority of all these listed complex physiological regulations a vitamin A1 - RAR-mediated signalling co-occurs, while for myelination / re-myelination [103, 104], this singular mechanisms is clearly non-vitamin A1 - RAR-co-mediated but exclusively dependent on vitamin A5/X - RXR-mediated signalling [103–106]. In consequence, this singular physiological mechanism thereby presents an exclusive vitamin A5/X-dependent physiological mechanism, as displayed in Fig. 5. These summarized vitamin A5/X-dependent physiological mechanisms, in addition to a non-sufficient daily vitamin A5/X intake, may result

in a vitamin A5/X-deficiency syndrome, which we will focus in detail on in a follow-up review.

This puts vitamin A5/X in the center as the major switch enabling nuclear hormone receptor-mediated signalling via activation of one side, the RXR-side, of the NHR-heterodimer and thereby enabling a larger array of NHR-mediated signalling pathways, ranging from RARs-, PPARs-, LXRs-, VDR-, FXR- to NR4A2-mediated signalling pathways [7, 8]. The RXR-mediated pathways were excellently summarized by Evans and Mangelsdorf [9], even claiming RXR-mediated signalling starting by RXR-activation, with 9CRA being the "Big Bang" of molecular endocrinology. As summarized in various review articles [24, 40, 67, 83], and based on analytical data, the physiological-relevant existence of 9CRA in the mammalian organism is highly questionable. Therefore, the vitamin A5/X - 9CDHRA - RXR-connection is a new proven theory, putting vitamin A5/X as a nutritional-dependent spark for a real "Big Bang" in human life [9]. In addition to vitamin A5/X - 9CDHRA, also further possible RXR ligands have been described recently [109], such as synthetic analogues including e.g. bexarotene (and related compounds), an anti-cancer drug, could be potentially acting on RXR mediated pathways. Thus, a large array of physiological processes is thereby enabled by vitamin A5/X, summarized in a slightly modified and "corrected" Fig. 4. Physiological processes including cholesterol homeostasis, bile acid homeostasis, fatty acid homeostasis, xenoprotection, basal metabolic rate, calcium- and phosphate-homeostasis and development are vitamin A5/X - RXR-co-regulated or even VA5/X-specific regulated physiological pathways that are important for a large array of crucial life-maintaining functions within the mammalian organism [9].

Fig. 5 Physiological relevance of summarized overlapping and non-overlapping physiological biochemical pathways mediated via RXR-RAR-mediated signalling and/or RXR-"alternative nuclear hormone receptor (NHR)"-mediated signalling in the mammalian organism

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Disease specific dysfunctions based on RXR-mediated pathways with a focus on neurological diseases

Retinoid signalling, particularly RXR-mediated pathways, plays a crucial role not only during the development of the central and peripheral nervous system (CNS/PNS), but is also involved in various maintenance functions of the adult CNS. Besides the pivotal involvement of RXR-mediated signalling in the modulation of immune-mediated processes [110–115] and in the cardio-vascular system [9, 10, 83, 116], RXR-mediated signalling has been demonstrated to be critically involved in neuronal homeostasis at numerous levels, as reviewed [117–123]. These various physiological events in the CNS and PNS that depend on RXR-mediated signalling are thus likely dependent on a nutritional supply of vitamin A5/X compounds.

Key processes that are both RXR-mediated and found to be also dysregulated in neurological disorders include cholesterol metabolism [93–95], immune-mediated mechanisms [110–115], myelination / remyelination [103, 104] and dopamine signalling [105–108]. Here, we assume and propose for the first time that a non-sufficient nutritional supply with vitamin A5/X / provitamin A5/X, which are present mainly in fruits and vegetables, as shown in Table 2b and Fig. 6/7, might contribute, in consequence, via their RXR-ligand precursor function, to the comparably large prevalence of neurological disorders in the Western society [124–127].

These neurological disorders represent a growing socioeconomic burden [128, 129] and are expected to become one of the leading causes of disability worldwide along with the projected demographical changes [130, 131]. Current commercial data confirm this importance
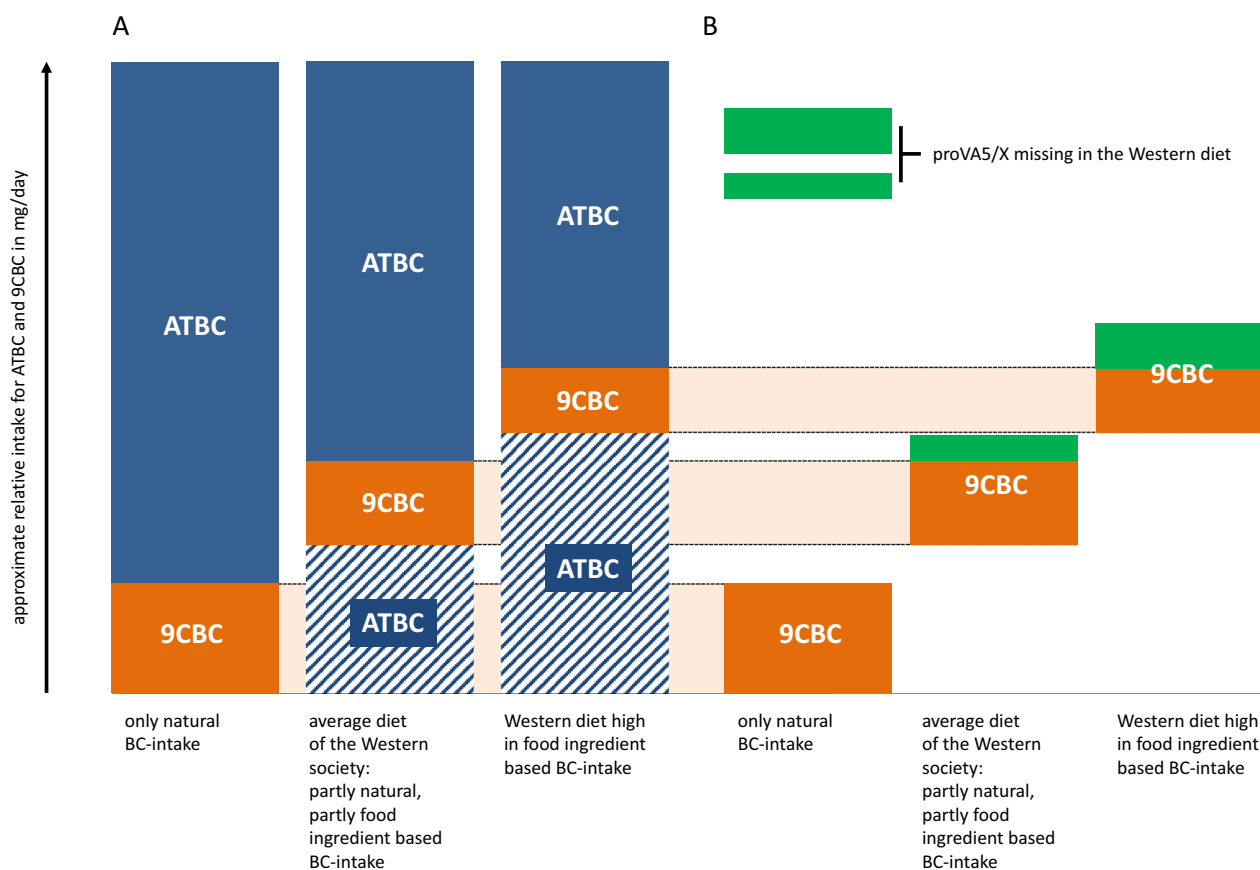

Fig. 6 A Outlined is the missing micronutrient / vitamin concept based on only natural β-carotene (BC) derived intake of 9-*cis*-β,β-carotene (9CBC) and all-*trans*-β,β-carotene (ATBC), with further increased amounts of food ingredient-based BC-intake present in Western-based diets. **B** Summarized are the missing amounts of 9CBC based on only natural β-carotene (BC) derived intake of 9CBC and ATBC, with further increased amounts of food-ingredient based BC-intake marked with orange bars. Resulting missing 9CBC amounts / provitamin A5 (proVA5/X) in the Western diet are represented by the green bars. For approximate intake recommendations, see Fig. 2

A. Food products   **Calculated amount of food to be eaten daily in g (wet weight) / day**

		calculated range	calculated average
Food high in 9CBC			
	spinach	1) 13 * – 47 **	28
		2) 5 * – 23 **	15
	carrots	1) 78 * – 277 **	171
		2) 31 * – 139 **	93
Food medium high in 9CBC			
	lettuce	1) 250 * – 900 **	550
		2) 100 * – 450 **	300
Food moderate high in 9CBC			
	apricots	1) 834 * – 3000 **	1837
		2) 334 * – 1667 **	1002
	peach	1) 1333 * – 6000 **	3663
		2) 666 * – 3000 **	1998

* - low in food ingredients; ** - high in feed ingredients

B. Suggested calculated average daily intake of provitamin A5 in the form of 9-cis-β,β-carotene can be achieved by:

raw food

spinach	lettuce	apricots	carrots

~30 g/d	~600 g/d (~ 300 g / picture)	~1800 g/d	~171 g/d

cooked food

cooked carrots   stew with vegetables and carrots

~50 g/d   unknown amounts

Fig. 7 A Examples of individual food products needed: (1) To fulfill the total 9-*cis*-β,β-carotene (9CBC) RDI demand of 0.5–1.8 (calculated and further used average of 1.1) mg/day, based on our calculations from Table 1/Fig. 2. (2) Missing 9CBC in the human food chain due to pure all-*trans*-β,β-carotene (ATBC) used in food fortification of 0.2–0.9 (calculated and further used average of 0.6) mg/day, based on calculations from Table 1/Fig. 2. **B** Summary of food items and amounts as examples of a balanced and recommended diet rich in provitamin A5/X in the form of 9CBC based on our current suggested recommendations are based on Table 1/Figs. 2/7A. Selected pictures contain a mm/cm grid for precise size adjustment of the precise food amount described

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of neurological diseases, while a large share of all pharma sales in the Western world relies on neuro-pharmaceuticals [132, 133].

Retinoid-, particularly RXR-mediated signalling, has been linked at multiple levels with both, neurodegenerative diseases such as Alzheimer´s disease [98, 117, 122, 134, 135] and Parkinson's disease [106, 136, 137], inflammation- and myelination-associated disorders, such as the various forms of multiple sclerosis [103, 104] and neurological disorders with a pathophysiological basis in artherosclerosis, including stroke and vascular dementia [117, 122]. Last but not least, there is the group of socio-economically highly relevant psychiatric disorders [121], particularly major depression and schizophrenia [137], which have both been linked to abnormal retinoid signaling [121, 136, 137]. Several of the RXR-mediated mechanisms associated with neurological disorders may be considered "disease-spanning", including neuroinflammation, synaptic plasticity, dopamine signalling, homeostatic maintenance mechanisms within the CNS, and there are also disease-specific alterations and therapeutic benefits of RXR-signaling.

Is there a specific vitamin A5/X deficiency?

As analytical monitoring of provitamin A5/X, vitamin A5/X and active derivatives of vitamin A5/X was only recently established for the human body and food items, it is difficult to conclusively associate specific deficiency syndromes with reduced vitamin A5/X levels in the organisms and even more importantly with reduced vitamin A5/X dietary intakes. As important specific functions were already outlined (Figs. 4 and 5), it would appear logic that in these areas potential deficiency symptoms occur, due to low intake of vitamin A5/X and provitamin A5/X.

Especially vegetables, with a focus on leafy and root vegetables (Table 2B), are high in provitamin A5/X content. A reduced intake of vegetables is co-associated with vitamin A5/X - RXR-mediated signalling dependent diseases that entail an increased incidence of neurological diseases [124–127, 138] as well as with cardio-vascular diseases, cancer and immune-disorders/allergies. In consequence, specific physiological mechanisms within the central and peripheral nervous system (as described earlier and in Fig. 5) and a non-sufficient daily vitamin A5/X intake (as outlined earlier and in Fig. 2), may represent a direct link between a low vegetable intake and dysfunctions of the nervous system found in various studies. Especially dietary intake of food items as described earlier (Table 2B) to be rich in 9CBC such as green leafy vegetables, which are usually consumed low in Western-type diets [139], correlate inversely with cognitive decline [140].

Unfortunately, a direct clear connection as indicated by a step-by-step cascade, starting from lower vegetable intake resulting in reduced endogenous vitamin A5/X-derivatives, reduced vitamin A5/X - RXR-mediated signalling and an increased incidence of specific diseases and other RXR-co-associated physiological dysfunctions was thus far not identified. However, summarising all these arguments, a cascade based on scientific evidence starting from food intake towards physiological/patho-physiological functions, is clearly plausible and evident.

Vitamin A5/X acid and vitamin A5/X / provitamin A5/X as new candidates for food and pharma applications for enabling maintenance and even increased RXR-mediated signalling

Given that all vitamins / provitamins are usually used in food applications to help achieving the recommended daily dietary vitamin intake, this also entails a general suggestion for vitamin A intake. In consequence, this should also be addressed for vitamin A5/X, including provitamin A5/X, and should result in a daily suggested recommendation for their individual intake.

For this reason, the concept of the missing micronutrient has been put forward in this review. We have calculated and proposed recommendations for vitamin A5/X, which are calculated based on natural food sources and provitamin A1 as food ingredients (Fig. 6). For a healthy diet, various national and international health organizations, including the World Health Organization (WHO, [34]), the National Health Service (NHS, [141]) of the United Kingdom and the German Nutrition Society (DGE, [44]) encourage the consumption of at least five portions of fruits and vegetables each day. Unfortunately, based on governmental nutritional surveys, for example in the USA [142], France [143] and Germany [144] a mere 5–25% of our population may reach these daily intake recommendations, resulting in 75–95% of the general population being below the recommended daily dietary suggested intakes. This low daily intake of fruits and vegetables results in postulating that the levels of the newly identified vitamin A5/X / provitamin A5/X, which is mainly present also in fruits and vegetables, are also potentially low in our Western society.

A reduced fruit and vegetable intake results usually in too low intake of vitamin A1 / provitamin A1 and likely also for provitamin A5/X / vitamin A5/X. Unfortunately, only vitamin A1 / provitamin A1 is supplemented to our convenient and readily prepared diet, while the vitamin A5/X / provitamin A5/X is not additionally supplemented. This means that there exists likely a nutritional

gap (represented by the green blocks in Fig. 6), a micronutrient gap of provitamin A5/X / vitamin A5/X daily intake, shown and outlined in Fig. 6. A simple supplementation with vitamin A1 / provitamin A1 (shown by the blue dashed line blocks) may not help to maintain related vitamin A5/X / provitamin A5/X levels, as this "normal" provitamin A1 and vitamin A1 are proven to constitute no direct precursors of vitamin A5/X and provitamin A5/X in humans.

In consequence, this suggests that;

(a) a low additional supplementation of provitamin A5/X / vitamin A5/X is needed when high natural provitamin A(1+5) and low supplemental provitamin A(1) is ingested, and

(b) a high additional supplementation of vitamin A5/X / provitamin A5/X is needed when low natural provitamin A(1+5) / high supplemental provitamin A(1) is ingested.

The daily recommended intake amounts were calculated to be in the range of 0.5–1.8 mg of 9CBC per day as a total and 0.2–0.9 mg of 9CBC per day as a currently missing micronutrient amount in the Western-based diets, as calculated in Fig. 2.

Furthermore, 9CDHROL, 9CDHROL-esters, 9CDHRA and 9CDHRA-esters may be used also in pharmaceutical applications with relevance for the CNS/PNS and the cardio-vascular system (CVS) and applications addressing cancer, such as relevant for alternative synthetic RXR-agonists [97, 117, 122, 134].

We propose a "to do list" for national and international authorities

Due to the fact that a new direct and independent food to ligand to action (physiological relevance) pathway was outlined, national and international authorities should get active in various areas to set up clear governmental regulations and dietary suggestions. We suggest here, summarized in several points, due to the unclear and uncertain general vitamin and especially vitamin A definition and regulation, the following actions:

(A) Define clearly what is vitamin A / provitamin A and define what potential sub-categories such as vitamin A1 / A2 exist and adjust the current nutritional recommendations for DRI and upper limits with relevance for vitamin A, vitamin A1 and vitamin A2.

(B) Define whether vitamin A5/X / provitamin A5/X are fully or partly included in these recommendations, especially considering the double precursor function of 9CBC as provitamin A1 / A5/X.

(C) Define the precursor functions of vitamin A1 / A2 as well as of vitamin A5/X in regard of vitamin A deficiencies, especially under consideration of the RAR- and RXR-ligand precursor concept.

(D) Define gaps and "a to-do list" to clarify main aspects regarding the definition and food regulation aspects for vitamin A1 / A2 and vitamin A5/X.

Based on the summarized data in this review and on existing EFSA-based recommendations regarding BC and vitamin A(1), we propose novel daily recommendations for vitamin A5/X. This would rather constitute a general recommendation for a natural occurring nutrient that is present in natural foods, but could also be included in the diet as a supplement or to a fortified food compound if intakes from natural sources are perceived as low (Table 1, Additional file 1: Table 1, Figs. 2 and 6). This may be especially true due to the exclusive usage of pure ATBC in commonly used food ingredients. Further calculated amounts of natural food products that would cover low, medium or high intakes of provitamin A5/X were additionally proposed based on their content of provitamin A5/X (Fig. 7).

Conclusions

When novel concepts are identified, which partly overlap with existing knowledge, novel guidelines and clear borders and overlaps must be described and defined. We now suggest vitamin A5/X / provitamin A5/X as a new vitamin A sub-category, termed "vitamin A5", or even as a novel independent vitamin category, with the suggested name "vitamin X". In addition, for vitamin A5/X and with a focus on provitamin A5/X, nutritional regulations comparable to DRIs/DRVs applied should be suggested, based on known nutritional and mechanistic pathways for optimal healthy, natural based, as well as further fortification-based recommendations.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12986-023-00750-3.

> **Additional file 1.** Supplementary table 1.

Author contributions
TB wrote parts of the manuscripts and tables and was involved in all corrections. RR conceptualized the manuscript, and was involved in writing the manuscript and figure preparations. ARdL, J H-R and VB wrote specific sections of the article and were involved in its critical revision. All authors read and approved the final manuscript.

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