Lycopene supplementation restores vitamin A deficiency in mice and possesses thereby partial pro-vitamin A activity transmitted via RAR-signaling

Running title: Lycopene and Pro-vitamin A Activity

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Abstract

Scope:

The aim of this study was to compare if lycopene possesses also pro-vitamin A activity comparable to known vitamin A derivatives.

Materials and methods:

We used a transgenic retinoic acid response-element (RARE)-reporter mouse model (n=8, per group) for this study, and after the initial wash out of vitamin A using a vitamin A deficient diet (VAD) for 18 weeks, the animals were supplemented further with a) VAD fed mice, b) VAD fed mice plus retinol (20 mg/kg bw), c) VAD fed mice plus β-carotene (40 mg/kg bw) and d) VAD fed mice plus lycopene (40 mg/kg bw). Using ex-vivo scanning and gene expression analysis of retinoid target and

vitamin A marker gene analysis in various organs of these supplemented mice (b, c, d) we found increased luciferase activity and normalized marker and target gene analysis compared to group a.

Conclusions:

Lycopene can restore vitamin A deficiency and compensate vitamin A for RAR-mediated signaling as the major function of vitamin A in the mammalian organism. Lycopene administration can initiate up-regulation of RAR-mediated signaling in various organs in VAD fed animals via potential novel bioactive lycopene-metabolites. This indicates that lycopene possesses partial pro-vitamin A activity in mice transmitted via RAR-mediated signaling.

Keywords: retinol, carotenoid, retinoic acid receptor, tomato

Introduction:

The term vitamin A characterizes derivatives, which are related to retinol (vitamin A alcohol) and can reverse signs of vitamin A deficiency. Retinol, retinyl esters, retinaldehyde (vitamin A1) as well as 3,4-didehydro-retinol derivatives (vitamin A2) belong to this category, while various carotenoids are pro-vitamin A carotenoids like β-carotene, α-carotene, α- and β-carotene isomers and β-cryptoxanthin ((1, 2) and reviewed in (3)).

In human nutrition and human organism additional carotenoids are present, which have no proven pro-vitamin A activity like the xanthophylls lutein, zeaxanthin, canthaxanthin and astaxanthin as well as open chain carotenoids like lycopene, phytofluene and phytoene (4, 5). Pro-vitamin A carotenoid metabolites like retinoic acids can activate nuclear hormone receptors retinoic acid receptors (RARs)

and the retinoic X receptors (RXRs) which are transmitting major vitamin A mediated pathways via ligand activation (6). In our experiments, we used the retinoic acid response-element (RARE)-mice with a DR5-reporter construct associated with a luciferase reporter construct (7-9), which is activated by liganded RAR-RXR heterodimers to monitor vitamin A mediated signaling via retinoic acid receptors. Latest data by our group identified lycopene as a carotenoid with a potential to initiate RARE-mediated signaling in reporter mouse model (7). In a previous study we identified derivatives which are comparable to the central β-carotene-cleavage metabolites retinoic acids (also called apo-15´-carotenoic acids) derivatives, which may be central cleavage metabolites of lycopene. These found derivatives are indicated by a 2 Da higher molecular weight and with comparable retention times in LC-MS analysis like retinoic acid, but with an unknown structural configuration (10). It was suggested that apo-15´-lycopenoic acid is the link for various of lycopene´s biological transmitted activities, unfortunately it was never identified endogenously (11). A novel study found that lycopene, supplemented as an isomeric mixture, can be cleaved with lower efficiency than β-carotene yielding the central cleavage metabolite apo-15´-lycopenal (acyloretinal) in E. coli overexpressing human beta carotene oxygenase 1 (12). Using HPLC-MS analysis we claimed that *di-/tetra*-hydro-derivatives of apo-15´-lycopenoic acid with an unknown structural configuration may be created and transmit major biological lycopene´s activities (10).

Determining vitamin A activity is a quite complicated task, because various signs of vitamin A deficiency especially in mice are difficult to examine (13-16). McCarthy and Crecedo (16) described that vitamin A deficient animals display high lethality before developing ocular syndromes (16). Various of these ocular syndromes like night-blindness, xerophthalamia, conjunctival xerosis, bitot´s spots, corneal xerosis and corneal scarrings were described and reviewed in (17) as common signs of severe vitamin A deficiency in humans and especially children (18, 19). In addition, internal signs of vitamin A deficiency post mortem were described in humans like changes in internal organs like the

lungs, salivary glands and mainly the pancreas (18, 19). In mice, the commonly described severe vitamin A deficiency signs are tremor, unkempt fur besides eye extrudes; in addition internal and partly irreversible signs, which are just detectable post mortem and are hardly to qualify like characteristic changes of the bladder, kidney, seminal vesicles, salivary glands, testis and trachea (16).

Just since recent times, starting in late 90ies highly sensitive analytical methodologies enable us to detect low levels of retinoids in animal organs (20-22), which describe that serum vitamin A level is just a weak marker of vitamin A deficiency (23-25). In the last century, many experiments were performed using outdated and moderate sensitive analytical methods or even no analytical approaches to conclusively claim vitamin A deficiency (14-16). These older studies were the major studies performed in mice establishing vitamin A deficiency and vitamin A deficiency related effects. Quantification of major players of vitamin A-transmitted effects like retinoic acids (26) and nuclear hormone retinoid receptors as well as RAR-target genes (6) could not be performed, because these mechanisms were not identified at this time. In addition, no commonly used analytical techniques like sensitive HPLC (27) and PCR (28) were available at this time. Therefore, no comprehensive and conclusive validation of vitamin A deficiency was performed. Nuclear receptor mediated signaling and determination of the vitamin A status using analytical determination in serum and liver were not performed and compared. The validity of these older experiments is therefore partly questionable because with highly sensitive HPLC-MS analysis low but sufficient vitamin A could still be identified, especially in the liver (21, 23-25). In general, vitamin A deficiency was reached using a vitamin A deficient diet to the dam and offspring and we reached very low retinol and retinoic acid levels in serum and liver (16, 24).

The aim of our experiments was to identify if lycopene may transmit pro-vitamin A activity via RAR-mediated pathways compared to retinol and β-carotene using reporter animal bioimaging, PCR-analysis for retinoid target genes and vitamin A deficiency marker genes as well as HPLC-MS analysis for retinoid quantification.

Materials and methods:

Chemicals

Lycopene (a gift from Conesa, Badajoz, ES) and β-carotene (a gift from BASF AG, Ludwigshafen, D) and retinol (a gift from BASF AG, Ludwigshafen, D) were suspended in 25% cremophore solution. The 25% cremophore solution was subsequently used as the control vehicle. Partly isomerized pure lycopene was additionally purified from potential other carotenoids and other lycopene isomers using preparative HPLC (29). A chromatogram of the purified lycopene used is displayed in supplementary figure 1.

Mice and treatments

Retinoic acid response element luciferase construct (RARE-LUC) mice with a CD1 background (7-9) genetically modified to express firefly luciferase under the control of RARE (30) were kindly provided by Cgene AS (Oslo, N). Validation of the RARE-LUC system was based on a previous study (7, 30). The mice were housed in standard plastic cages at room temperature (20 ± 2°C) and they had free access to both food and water.

Pregnant mice were fed a standard pelleted laboratory mouse diet (Altromin, type VRF 1, Charles River, Budapest, H) with the following diet composition: crude nutrients 19%, crude ash 7%, crude fat 4.5%. Shortly before birth, a vitamin A deficient diet (VAD) with the following composition: fat 5%, protein 20%, fiber 8.5%, mineral mix 4%, vitamin mix 1%, carbohydrates 61.5%, was used to feed the mice and the litters during weaning until the mice were 4 weeks old. By the end of the forth week, male mice were selected and were fed by a low fat VAD for 13 weeks until mice were 17 weeks old. Low fat vitamin A deficiency diet composition was as follows: fat 2%, protein 20%, fiber 5%, mineral mix 4%, vitamin mix 1%, carbohydrates 68%. The experimental set up is displayed in Figure 1.

The preparation of a vitamin A free diet is a difficult or even impossible task. Based on these difficulties our diet was just vitamin A deficient and especially the added fat (sunflower oil) and carbohydrates (starch) in the vitamin A deficient diet might have contained traces of known vitamin A sources in form of retinoids or pro-vitamin A carotenoids. In addition, based on our study the presence of traces of additional retinoids or carotenoids with non-proven vitamin A or pro-vitamin A activity may also not be excluded.

The two diets were designed so that intake in grams of all nutrients was be the same except for carbohydrates and fat (isocaloric exchange). Energy per gram of food was calculated to be 3.75 kcal for both of the VAD.

Seventeen-week old mice (n=8, per group) were placed into each of the following experimental groups: a) untreated mice fed with vitamin A sufficient diet (control, CTRL), b) VAD fed mice

treated with vehicle (VAD), c) VAD fed mice treated with retinol (ROL), d) VAD fed mice treated with β-carotene (BC), e) VAD fed mice treated with lycopene (LYC). Single dose oral *gavage* of control vehicle, retinol, β-carotene and lycopene were applied by sterilized stainless steel feeding needles. Mice were treated once every other day for a total of five doses to ensure the ingestion of the calculated dosages. Lycopene and β-carotene were given at a ratio of 40 mg/kg body weight, retinol was given at a ratio of 20 mg/kg body weight and these administered dosages were given to the animals in supra-physiological range. The compounds were suspended in 25% aqueous cremophore solution. This solution was given at a ratio of 5 ml/kg body weight. The last treatment was given 16 hours before the luciferin injections and the subsequent bioluminescence imaging analyses. All mouse experiments were approved and conducted under the guidelines and with ethical approval for the use and care of laboratory animals at the University of Debrecen, Hungary.

Bioluminescence Imaging

Mice were sacrificed by cervical dislocation 20 minutes after luciferin injection. Organs were collected and bioluminescence signal was acquired. An Andor IQ imaging system (Andor, Belfast, GB), consisting of an Andor-ixon cooled charged coupled device (CCD) camera, housed in unit-one (Birkerod, DK) black box and connected to the Andor IQ 1.6. program, which was utilized for bioluminescence data acquisition and quantitative analyses.

Quantitative real time- PCR (QRT-PCR)

mRNA expression of certain retinoid metabolizing enzymes (LRAT, RDH10, RPE65), retinoid receptor (RARβ), retinoid target genes (RARβ, LRAT) and vitamin A deficiency marker genes (RBP4, aSMC, COL4, FN1, RPE65 and RDH10) was analyzed by QRT-PCR. The mouse organs

including: liver, brain, eyes and kidneys were used for QRT-PCR analysis. Total RNA was isolated by Trizol® method according to the manufacturer's directions (Invitrogen, Life Technologies Magyarorszag Kft., Budapest, H). Prior to PCR, total RNA samples were reverse transcribed into cDNA by enzyme according to supplier's protocol under the following conditions: 10 min at 25°C, 120 min at 42°C, 5 min at 72°C and 10 min at 4°C (Applied Biosystems, 2720 Thermal Cycler, H). qRT-PCR was performed by ABI PRISM 7900 sequence detection system (Applied Biosystems) as follows: 1 min at 94°C, followed by 40 cycles of 12 s at 94°C and 30 s at 60°C. Primers were ordered from ABI (Life Technologies). mRNA levels were normalized to the level of cyclophilin expression which served as an internal control for the amount of RNA used in each reaction. Cycle threshold values above 40 were scored as under the limit of detection (UDL).

Retinoid analysis

Concentrations of retinol and retinoic acids (RAs) were determined in mouse serum and liver samples by our HPLC-MS-MS method (31). In summary, 100 mg of the liver sample (if samples were under 100 mg, water was added up to the used standard weight: 100 mg) or 100 µl serum was diluted with a threefold volume of isopropanol, the tissues were minced by a scissor, vortexed for 10 seconds, put in a ultra sonic bath for 5 minutes, shaken for 6 minutes and centrifuged at 13000 rpm (16060 x g) in a Heraeus BIOFUGE Fresco at +4°C. After centrifugation, the supernatants were dried in an Eppendorf concentrator 5301 (Eppendorf, D) at 30°C. The dried extracts were resuspended with 60 µl of methanol, vortexed, shaken, diluted with 40 µl of 60 mM aqueous ammonium acetate solution and transferred into the autosampler and subsequently analysed.

Statistical Analysis

Statistical tests for comparison of means were performed using GraphPad Prism version 5 using a liner average comparison for organ-specific expression of luciferase, QRT-PCR and HPLC. Values are represented as mean ± SEM.

Results:

A. VA deficiency

Vitamin A deficiency was aimed by a long wash out diet with vitamin A deficient diet (VAD) to the mothers of the later studied young offsprings, starting one day before birth to ensure sufficient vitamin A for optimal vitamin A dependent embryogenesis (32). This VAD diet was also followed on to the lactating mothers as well as further after weaning to the selected young male neonates (13).

After 17 weeks of feeding the male young mice the VAD fed animals, these mice were considered to be highly vitamin A deficient identified by reduced weight gain and confirmed by reduced serum and liver retinol and retinoic acid levels (Table 1). Longer time supplementation of VAD resulted in serum and liver retinoid levels under our detection limit in selected animals in addition with severe health problems of the animals involving severe pain. These health effects were non-reversible, because the animal could not eat and move anymore. We did not further examine these effects, but we postulate that they were mainly concerning malfunction of kidney, mental, bone, muscular and cardio-vascular system. These strong deficiency effects were not in accordance with our personal as well as local ethical standards.

Based on the 17 weeks VAD, further four different groups were formed and four different dietary administrations were given to each eight male young mice (Figure 1). External signs of vitamin A

deficiency like alopecia and eye diseases (13-16) were not observed in our examined and used group of mice.

After feeding the animals with the following dietary administrations each for the following ten days, VAD (vitamin A deficient fed animals plus vehicle), LYC (vitamin A deficient diet fed animals plus lycopene administration), BC (vitamin A deficient diet fed animals plus β-carotene administration) and ROL (vitamin A deficient diet fed animals plus retinol administration) the animals were bioimaged, killed and the organs were stored for further analysis.

External signs of vitamin A deficiency were not conclusively observed, but a marker of vitamin A deficiency, which is diarrhea, was present in 70% of the vitamin A deficient diet fed animals; administration of LYC (25%), BC (25%) and ROL (0%) reduced this marker of vitamin A deficiency.

B. Retinoid concentrations

The concentrations of vitamin A alcohol (retinol) and vitamin A acid (retinoic acid) were determined using HPLC-MS in liver and serum of the five different groups and put in comparison to the VAD fed group. In serum, all-*trans*-retinoic acid (ATRA) and ROL concentrations were higher in CTRL, LYC, BC and ROL groups compared to the VAD fed group. In the liver, ATRA concentrations were just significantly increased in the ROL administered group, while in the liver concentrations of ROL were all significantly higher in CTRL, LYC, BC and ROL groups compared to the VAD fed group.

C. RARE signaling, retinoid target genes and vitamin A deficiency marker gene expression

RARE bioimaging was performed in CTRL as well as for the four VAD fed groups with or without added retinol or carotenoids. Significant reduction of RARE signaling of VAD fed animals was observed in liver, kidney, eye, brain and WAT (Figure 2). Supplementation of VAD fed animals with carotenoids or retinol significantly increased RARE-mediated signaling in all examined organs (Figure 2).

Surprisingly, in the eye significantly increased RARE-mediated signaling was observed when comparing supplementation with the well-known pro-vitamin A carotenoid β-carotene (29 ± 16) compared to the "non-pro-vitamin A carotenoid" lycopene (81 ± 18; $p<0.01$).

Further confirmation of vitamin A signaling using QRT-PCR analysis was performed in liver, kidney and eye for known retinoid target genes RARβ and LRAT, which were significantly decreased in VAD fed animals vs. CTRL animals. Treatment with carotenoids (β-carotene and lycopene) and retinol significantly increased retinoid target gene expression in liver, kidney and eye. Comparable to the increased RARE-mediated signaling of lycopene vs β-carotene, an increased expression of the retinoid target gene LRAT in eye was observed (Figure 3).

Further, we focused on vitamin A deficiency markers retinol binding protein (RBP4 / (33)), α-smooth muscle cell actin (aSMC / (34)), collagen 4 (COL4 / (35)), fibronectin (FN1 / (36)), retinal pigment epithelium-specific 65kDa protein (RPE65 / (37)) and retinol-dehydrogenase (RDH10 / (38, 39)) in liver, kidney and eye. RBP4, RPE65 and RDH10 were significantly lower expressed in VAD fed animals vs CTRL diet fed animals and significantly increased in the LYC-, BC- and ROL-supplementation groups. The positive vitamin A deficiency markers aSMC, COL4 and FN1 were

significantly increased or with a tendency of significant increase p=0.07 / 0.08. Treatment with the carotenoids lycopene, β-carotene or the retinoid retinol significantly reduced (except a tendency of p=0.07 for COL4 VAD vs BC) this expression of "positive" vitamin A deficiency markers.

Discussion:

Lycopene, the major carotenoid in Western human nutrition and in the human organism, has been shown to have multiple beneficial activities (40-42). Besides lycopene´s antioxidant activity (43), which might be of minor physiological relevance (44), lycopene-metabolite mediated activations of nuclear hormone receptor pathways are discussed as a potential mechanism of action (7, 10, 11, 45, 46). Especially the retinoid receptors, the retinoic acid receptors (RARs) and retinoid X receptors (RXRs) as main mediators of vitamin A-mediated effects like reviewed in (47), were in the current focus of observations. We determined in this study that lycopene can also act as a partial pro-vitamin A carotenoid with a still unknown mechanism of action as a potential precursor of ligands activating retinoid receptors. The human physiological relevance of these novel data remains unknown. An indirect prove was obtained when supplementing vitamin A deficient diet (VAD) fed mice with vitamin A alcohol (retinol) or pro-vitamin A (β-carotene) in comparison to lycopene yielding in comparable RARE-mediated signaling and recovery of retinoid target gene expression and reversal of vitamin A deficiency marker gene expression.

Vitamin A deficiency was obtained in RARE-mice, supplementing VAD to dams and offspring indicated by reduced weight gain (15). It was confirmed after killing via HPLC-MS analysis of serum and liver retinol and retinoic acid levels (table 1). External signs of vitamin A deficiency observed in rats, like eye diseases (night blindness, xerophthalmia, thick and colorless eye extrudates), tremor and alopecia were in general not observed in mice as well as in our examined animals, which was already

previously described (16). Longer supplementation with VAD resulted in severe and non-reversible health problems of the animals, involving severe pain, and was not in accordance with our personal as well as local ethical standards. Just one external visible sign of vitamin A deficiency was observed, which was increased incidence of diarrhea (13, 15, 16). In our study, VAD induced diarrhea was reversed after administration of retinol, β-carotene or lycopene.

In VAD fed animal's, retinol and retinoic acid levels decreased in serum compared to CTRL diet fed animals. After administration with retinol or comparable equimolar amounts of β-carotene or lycopene to the VAD fed mice the serum ATRA and retinol levels remained higher compared to VAD fed animals. β-Carotene and retinol are well known precursors of retinoic acids, while from lycopene no pathways in mammals is known for conversion to retinol and retinoic acids. Liver ATRA levels were not lower in VAD fed mice, which does not correspond to lower RARE-mediated signaling observed in the liver but indicates that besides ATRA maybe alternative retinoids may also mediate RAR-RXR-mediated signaling (48, 49) or that alternative mechanisms like retinoid-binding proteins may be involved in RARE-mediated signaling (50). Liver retinol levels in retinol- and β-carotene-supplemented VAD fed mice remained higher compared to VAD fed mice or increased from vitamin A or pro-vitamin A supplementation. Surprisingly, in lycopene supplemented VAD fed mice retinol levels in serum and liver also remained higher than in VAD fed mice. Due to still constantly decreasing retinoid levels in serum and liver in further VAD fed mice, this indicates that lycopene may substitute vitamin A usage via still unknown metabolic pathways and result in higher left-over retinol reservoirs in serum and liver.

RARE-mediated signaling in liver, kidney, eye, brain and WAT as well as retinoid target gene expression in liver (RARβ), kidney (RARβ) and eye (LRAT) were increased by supplementation of

retinol, β-carotene or lycopene to VAD fed mice compared to VAD fed mice. In addition, we focused on markers of vitamin A deficiency in mice which can be easily and conclusively qualified and quantified like the positive or negative vitamin A deficiency markers. The validity of these genes as vitamin A markers was validated also in our study (Figure 3). These liver (RBP4, aSMC, COL4), kidney (FN1) and eye (RPE65, RDH10) as vitamin A deficiency markers remained comparable in retinol-, β-carotene- and lycopene-supplemented VAD fed animals to the control diet supplemented animals. These data indicate that lycopene can compensate vitamin A for RAR-mediated signaling as the major function of vitamin A in the mammalian organism and restore vitamin A deficiency. Unfortunately, we were not able to find the responsible lycopene-metabolites enabling RAR- and/or RXR-activation due to the lack in knowledge and availability of identified bioactive lycopene-metabolites. We further postulate that the *di-/tetra*-hydro-apo-15´-lycopenoic acids of unknown structural configuration are mediating these lycopene-mediated effects (10) and our current research is focusing in conclusive identification of these bioactive lycopene-metabolites.

The second important branch of vitamin A activity is functioning as a pigment during the visual process (51, 52). These experiments examining the visual function are a complicated task and we plan and try to perform these experiments soon. Based on previous studies, retinal as a pigment is attached via the chain function of the retinoid core structure to opsin and then further called rhodopsin might also enable potential acyclic retinoids to function as pigments in the visual cycle (52, 53). Additionally, experimental approaches are planned and we will try to prove this theory. Surprisingly, RARE-mediated signaling as well as retinoid target gene expression in the eye was higher in lycopene supplemented VAD fed animals vs β-carotene supplemented VAD fed animals which may indicate that lycopene-metabolites may obtain important functions in the eyes.

In summary, here we identified using supplementation experiments with vitamin A (retinol), pro-vitamin A (β-carotene) or lycopene to VAD fed mice that lycopene-supplementation can restore vitamin A deficiency induced effects. Using retinoid lipidomics analysis as well as molecular biological methodologies, we conclude that lycopene may act as a carotenoid with partial or even full pro-vitamin A activity in mice.

Author contribution:

GA and YK performed the animal experiments, KF and VB purified the lycopene, EMB and EB performed the PCR analysis, JM and RR planed the study and wrote the article.

Conflict of interest

The authors declare no conflict of interest.

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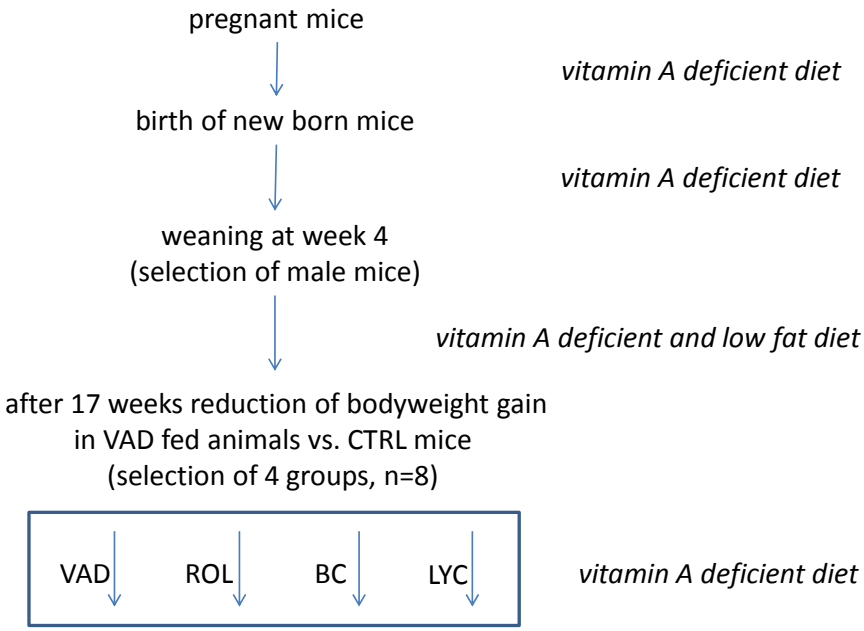
Figure legends:

Figure 1: **Scheme of our experimental study**: VAD – vitamin A deficient diet, BC - vitamin A deficient diet plus β-carotene, LYC - vitamin A deficient diet plus lycopene, ROL - vitamin A deficient diet plus retinol.

Figure 2: **Lycopene displays RAR-activation potential in LYC-supplemented VAD-fed animals**. RARE-LUC bioimaging of liver, kidney, eye, brain and white adipose tissue (WAT), each n=6-8. CTRL - control diet, VAD – vitamin A deficient diet, BC - vitamin A deficient diet plus β-carotene, LYC - vitamin A deficient diet plus lycopene, ROL - vitamin A deficient diet plus retinol. Error bars indicate SEM, *- $p \leq 0.05$ vs. VAD; #- $p \leq 0.05$ vs. BC.

Figure 3: **Lycopene restores RAR-target gene and restores vitamin A deficiency marker gene expression.** QRT-PCR based analysis of retinoid target genes (retinoid target genes; retinoic acid receptor β (RARβ), lecithin retinol-acyltransferase (LRAT)) and various vitamin A deficiency marker genes (retinol binding protein 4 (RBP4), α-smooth muscle cell actin (aSMC), collagen 4 (COL4), fibronectin (FN1), retinal pigment epithelium-specific 65kDa protein (RPE65) and retinol-dehydrogenase (RDH10)). CTRL - control diet, VAD – vitamin A deficient diet, BC - vitamin A deficient diet plus β-carotene, LYC - vitamin A deficient diet plus lycopene, ROL - vitamin A deficient diet plus retinol, each group contained n=6-8 samples analysed. Error bars indicate SEM, *- $p \leq 0.05$ vs. VAD; #- $p \leq 0.05$ vs. BC.

Figure 1:



- treatment at days 1, 3, 5, 7, 9
- killing, bioimaging and storage for further analysis at day 10

Figure 2:

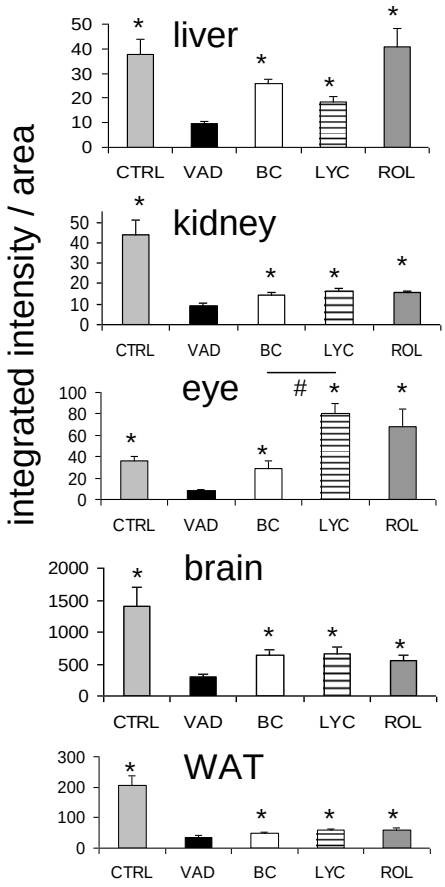


Figure 3:

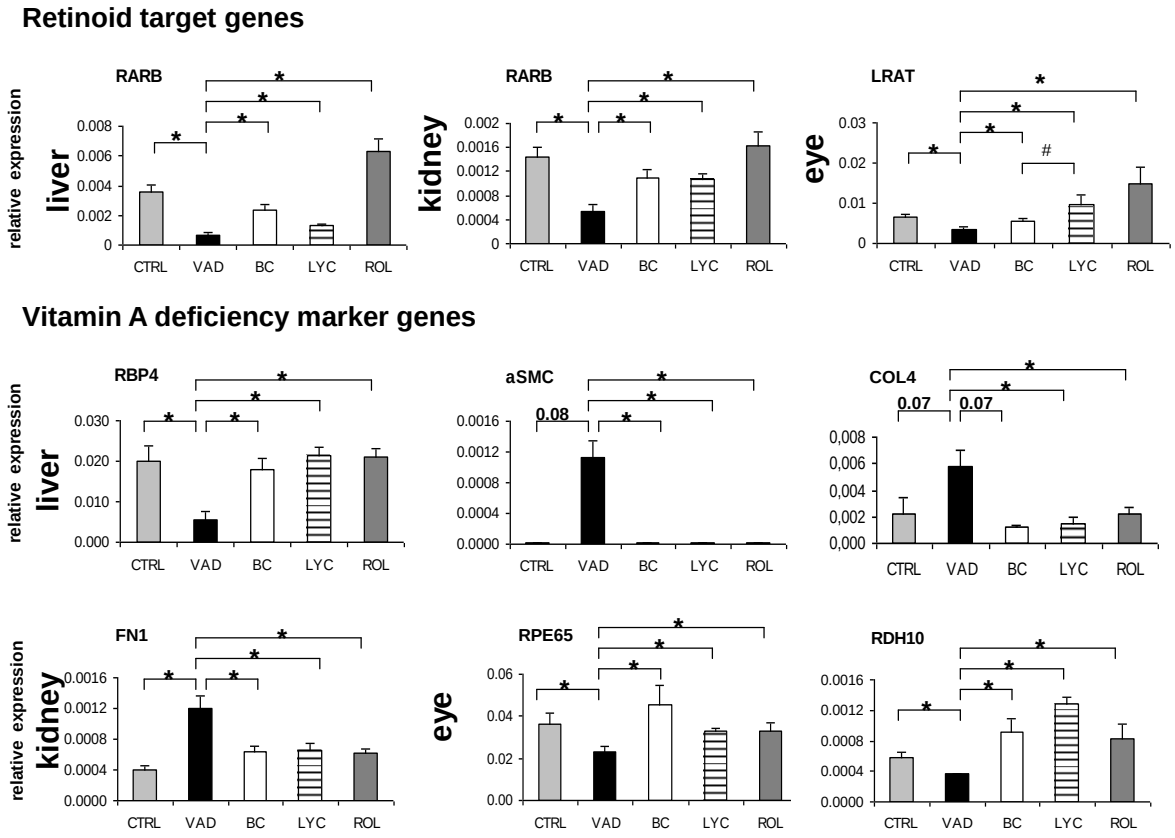


Table 1: Serum and liver concentrations of retinol (ROL) and all-*trans* retinoic acid (ATRA), each group n=6 displayed as (mean ± SEM**)**. CTRL - control diet, VAD – vitamin A deficient diet, BC - vitamin A deficient diet plus β-carotene, LYC - vitamin A deficient diet plus lycopene, ROL - vitamin A deficient diet plus retinol. *- p≤0.05 vs VAD.

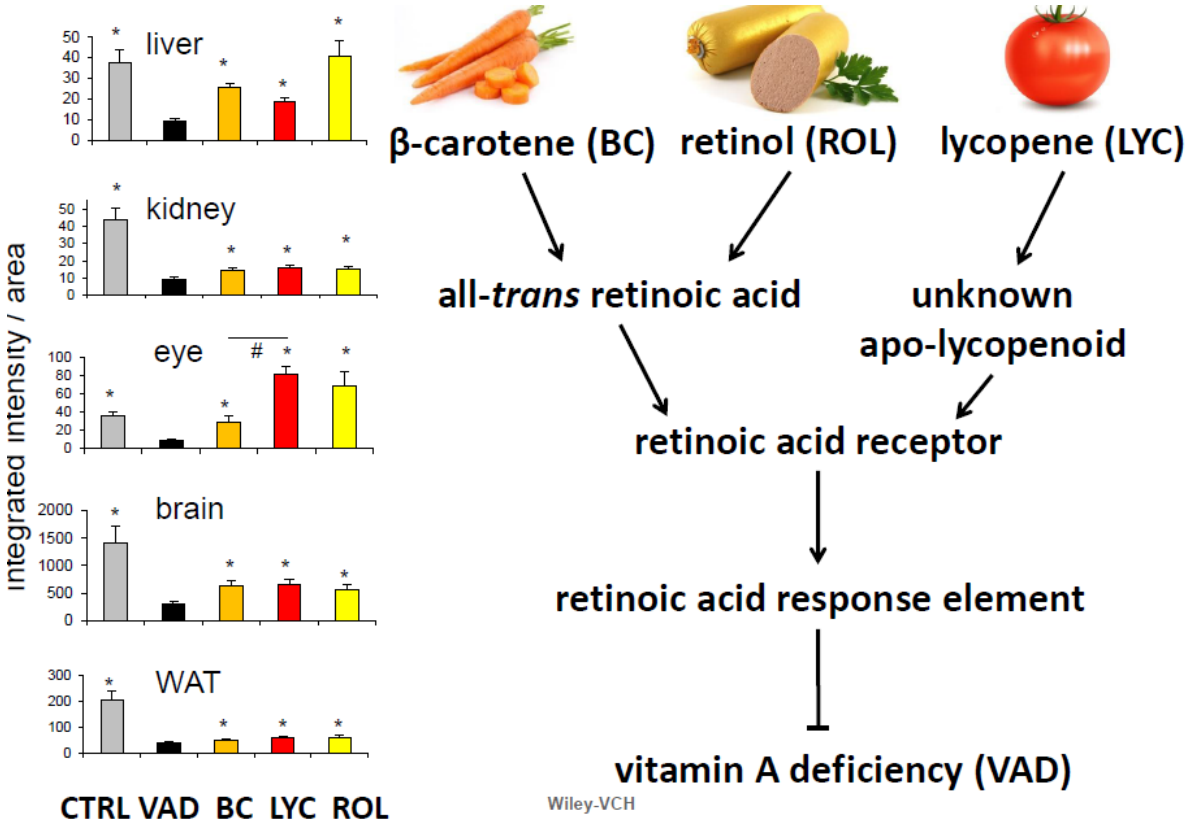
SERUM

	ROL in ng/ml		**ATRA in ng/ml**	
CTRL	83	± 29 *	0.6	± 0.2 *
VAD	14	± 9	0.4	± 0.1
BC	68	± 29 *	0.6	± 0.2 *
LYC	46	± 51 *	0.6	± 0.1 *
ROL	84	± 40 *	0.6	± 0.2 *

LIVER

	ROL in ng/g		**ATRA in ng/g**	
CTRL	6374	± 6299 *	1.5	± 1.3
VAD	96	± 145	2.4	± 1.1
BC	970	± 1091 *	2.0	± 1.7
LYC	214	± 243 *	1.5	± 1.3
ROL	19920	± 4453 *	8.7	± 4.6 *

The aim of this study was to compare if lycopene possesses also pro-vitamin A activity comparable to known vitamin A derivatives. We found, that lycopene can restore vitamin A deficiency and compensate vitamin A for RAR-mediated signaling as the major function of vitamin A in the mammalian organism. Lycopene administration can initiate up-regulation of RAR-mediated signaling in various organs in VAD fed animals via potential novel bioactive lycopene-metabolites. This indicates that lycopene possesses partial pro-vitamin A activity in mice transmitted via RAR-mediated



signaling.