

Overflow phenomenon in serum lutein after supplementation: a systematic review supported with SNPs analyses

Abdulrahman M Alharbi¹, Mohammed A Kilani², Tos TJM Berendschot³

¹Clinical Biochemistry Unit, Department of Pathology and Laboratory Medicine, College of Medicine, King Saud University, Riyadh 11451, Saudi Arabia

²Public Health Authority of Saudi Arabia, Riyadh 13352, Saudi Arabia

³University Eye Clinic, Maastricht University Medical Center, Maastricht 6229HX, The Netherlands

Correspondence to: Tos TJM Berendschot. University Eye Clinic, Maastricht University Medical Center, Maastricht 6229HX, The Netherlands. t.berendschot@maastrichtuniversity.nl

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Abstract

• Lutein, a type of carotenoids, is found to delay the onset and progression of age-related macular degeneration (AMD). Several lutein supplementation studies showed that after an initial increase, lutein serum levels demonstrated a subsequent decrease despite continuous supplementation. In this systematic literature review, this obscure phenomenon was tried to be explained. The subsequent drop in lutein levels was postulated due to down-regulation of lutein receptors scavenger receptor class B type I (SR-BI) in the gastrointestinal tract, upregulation of lutein degrading enzyme β -carotene dioxygenase (BCDO2), or perhaps a combination of both. Some single nucleotides polymorphisms (SNPs) that could have influence on the occurrence of this phenomenon. To date, an exact scientific explanation for this phenomenon has not been established. Further research is needed to investigate this phenomenon in depth to reach an irrefutable explanation, giving that lutein is proven to be effective in delaying the onset and progression of AMD and its metabolism in the human body becomes of equal importance.

• **KEYWORDS:** lutein; macular degeneration; carotenoids; xanthophylls; β -carotene dioxygenase; scavenger receptor class B type I

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INTRODUCTION

Age-related macular degeneration (AMD) is the most common cause of blindness among elderlies in the developed world^[1]. Blindness typically results from photoreceptors degeneration in the macula^[2]. The condition can be divided into dry and wet form. AMD nearly always starts in the dry form then possibly progress to wet. In the wet type of AMD neovascularisation occurs through fractures of Bruch's membrane^[3]. Photo-toxicity is recognized to be a major contributing factor to the development of AMD. Oxidative stress, particularly lipofuscin-mediated photo-oxidative damage, contributes to the onset and progress of AMD^[4]. The retinal pigmented epithelium (RPE) throughout life accumulates vesicles of undigested materials called residual bodies. In some long-lived cells (*e.g.*, neurones, myocardial cells, RPE, *etc.*), residual bodies can accumulate over time as granules of lipofuscins^[5]. Lipofuscins have some substances that are very reactive to light (*e.g.*, A2E) and can cause serious damage when converted to their triplet state. Subsequently, the free radicle damage to the RPE leads to its thinning and renders its ability to perform its functions, one of which is phagocytosis of the outer segment of the continuously regenerating photoreceptors. That in turns leads to the accumulation of extracellular breakdown products (drusen), which is the first clinical sign of AMD^[6]. Carotenoids are lipophilic pigments that occur widely in plants, fungi, and bacteria. They can be broadly divided into two types, carotenes (also known as provitamin A) and xanthophylls (also known as nonprovitamin A)^[7]. Here we focus on xanthophylls particularly lutein and zeaxanthin, as they are widely found in the human retina. Since they are not produced by humans, they are exogenously obtained from the previously mentioned sources. Lutein and zeaxanthin are both polyisoprenoids containing 40 carbon atoms and cyclic structures at each end of their conjugated chains. Lutein and zeaxanthin are shown to delay the onset and progression of AMD. They can protect the eye and delay AMD by four important functions. First, they act as blue light filters; the conjugated double bonds are responsible for their light absorption property^[8]. Zeaxanthin has a longer chromophore (conjugated chain) when compared to lutein, so it has a longer wave blue-light absorption (only by 4-5 nm). Second, they act

as quenchers of singlet oxygen^[9]. Some molecules can act as sensitizers by absorbing light, which converts them to their triplet state, then they can pass the excess energy to a normal oxygen molecule converting it to singlet reactive oxygen. Xanthophylls interfere with this process by either quenching the energy of the sensitizers and preventing the formation of singlet O₂, or by absorbing the excess energy of the singlet oxygen after it has been formed, both produce a triplet state carotenoid that is harmless and losses the energy harmlessly. Third, in the presence of oxidizing free radicals, unsaturated lipids are destroyed by chain reactions involving peroxy radicals, carotenoids can initiate reactions that consume peroxy radicals so that they act as a chain-breaking antioxidant^[10]. Finally, AMD is now recognized as a condition of over activation of complement system alternative pathway. Factor D (FD) is the rate-limiting enzyme of the alternative pathway, and found to be high in the serum of individuals with AMD^[11]. FD is synthesized by adipose tissue, which is also the main storage site of lutein. In one study, it has been found that daily lutein supplement in individuals with early signs of AMD lowers the levels of serum FD, which could be considered as the fourth function of xanthophylls in delaying the onset and progression of AMD^[12-14]. Now that the importance of lutein and zeaxanthin in the human retina is established, knowledge regarding their metabolism in the human body becomes of equal importance. β -carotene oxygenases 1 and 2 (BCO1 and BCO2) are the only two carotenoid cleavage enzymes found in animals. They are non-heme iron-dependent enzymes. BCO1 is a monooxygenase and known more accurately as β -carotene monooxygenase (BCMO1), while BCO2 is a dioxygenase, also known as β -carotene dioxygenase (BCDO2). BCMO1 can cleave provitamin A carotenes (*e.g.*, beta-carotene), but not xanthophylls. In contrast, BCDO2 has a very broad substrate specificity and can cleave both carotenes and xanthophyll^[15]. In different lutein supplementation studies it has been shown that after an initial rise the lutein serum concentration demonstrated a subsequent decrease^[16]. This review is an attempt to uncover the obscure mechanism by which this phenomenon occurs.

METHODOLOGY

A comprehensive systematic review was conducted in order to address the research objective. The research was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). A protocol, describing the objectives, methods, inclusion criteria, and approach to assessing study quality, was developed prior to the search.

Several databases were searched, such as PubMed/MEDLINE, EMBASE, Cochrane Library, and Google Scholar. Inclusion and exclusion criteria in this research were English language. Keywords used in the online search included a combination

of the following terms: lutein, carotenoids, xanthophylls, β -carotene dioxygenase, and scavenger receptor class B type I (SR-BI).

Additionally, the “cited by” function in Web of Science and Google Scholar and use of “related articles” option in PubMed was used to expand the search and guarantee that all possible studies were found.

RESULTS AND DISCUSSION

Drop in Serum Concentration As mentioned above, according to different supplementation studies lutein concentration in the serum of the subjects recruited in the various studies reached a maximum before undergoing a sharp decrease, despite continuous supplementation^[16]. To date the exact cause is not clearly understood, however different hypotheses can be postulated to explain this phenomenon. Here, we consider a number of possibilities. This phenomenon may be the result of a decrease in the absorption of lutein from the GI tract, the consequence of an enzymatic degradation post-absorption, or perhaps a combination of both.

Absorption of Xanthophylls and Their Transport in the Bloodstream Dietary carotenoids, including xanthophylls, are released from ingested foodstuff and incorporated into lipid globules in the stomach. The role of the stomach in the absorption of carotenoids is transferring them from the food matrix to the lipid portion of the meal^[17]. This lipid-carotenoid emulsion then enters the duodenum, where the lipid content induces the secretion of bile acids from the gallbladder and lipases from the pancreas. The bile acids act to reduce the size of the lipid droplets, resulting in the formation of mixed micelles. SR-BI is a single-chain transmembrane glycoprotein found on the brush border microvilli of enterocytes, as well as in the adrenals, ovaries, placenta, kidneys, prostate, RPE and liver^[18]. SR-BI plays a major role in the uptake of xanthophylls by enterocytes^[19]. In the Golgi apparatus of the enterocytes, carotenoids and lipids are processed into chylomicrons, which cross the basal layer of enterocytes to reach the lacteal lymphatic system. Once in the lymphatic system, the carotenoid-containing chylomicrons are delivered to the circulation through the thoracic duct. Chylomicrons in the bloodstream are degraded by lipoprotein lipase at the vicinity of adipocytes, leaving chylomicron remnants which are quickly taken up by the liver. Polar carotenoids such as xanthophylls are transported mainly by high density lipoprotein (HDL) to the various organ systems that display SR-BI on their surfaces^[20]. There is a feedback regulatory mechanism to the absorption of xanthophylls, exerted by vitamin A and carotenoids themselves. For instance, retinoic acid produced from dietary precursors induces the expression of the intestinal transcription factor intestine-specific homeobox (ISX) that represses the expression of SR-BI on enterocytes^[21].

If lutein supplementation could also lead to the downregulation of SR-BI in the small bowel, the sudden decrease in serum lutein observed in the previously mentioned studies can be explained.

Xanthophylls and the Enzymatic Degradation by BCDO2

BCDO2 is a non-heme iron dependent dioxygenase that cleaves carotenoids asymmetrically at both the 9,10 and 9',10' carbon-carbon double bond^[22]. The enzyme has a wide range of substrates, including carotenes and xanthophylls, and is located in the inner membrane of the mitochondria in various organs. Several studies in mammals prove that BCDO2 is essential for carotenoids homeostasis^[23-25]. Studies in BCDO2 knocked-out mice show accumulation of xanthophylls in the mitochondria, which interrupts the function of the electron transport chain (ETC), hence generating reactive oxygen species (ROS) and subsequently inducing the intrinsic apoptotic pathway^[26]. ROS production by xanthophylls accumulation in the mitochondria was not only seen in mice cells, but also in human cells. In a study carried out using two human cell lines, human liver carcinoma (HepG2) and human breast carcinoma (T47D) *in vitro*, it was found that HepG2 cells contain no traces of BCDO2, while T47D cells contain the enzyme. Both of the aforementioned cells were treated with a particular amount of carotenoids, as a result, it was found that the cells lacking BCDO2 (*i.e.*, HepG2) produced a substantial amount of ROS, while the cells with the endogenous BCDO2 produced none. The high ROS induced the initiation of the intrinsic apoptotic pathway in HepG2 cells through the release of cytochrome C^[27]. In another study done using ferrets BCDO2 *in vitro*, the enzyme cleaved lutein to form 3-OH- β -apo-10'-carotenal, 3'-OH- α -ionone, 3'-OH- α -10-carotenal, and 3-OH- β -ionone. While, when it acted on zeaxanthin the breakdown products were only 3-OH- β -apo-10'carotenal and 3-OH- β -ionone^[28]. The role of these breakdown products is not very well-understood and it is not the focus of this review. Given the fact that accumulation of lutein and zeaxanthin in the mitochondria could induce apoptosis of the affected cells, the high lutein intake and ROS generation should induce the upregulation of BCDO2 to maintain homeostasis.

Proposed Experiment to Accept or Refute the SR-BI Downregulation Hypothesis In a study by Nolan *et al*^[16], a drop in serum lutein was observed after 6mo of the supplementation of a daily capsule containing 10 mg lutein, 2 mg zeaxanthin, and 10 mg mesozeaxanthin suspended in sunflower oil (Figure 1). Therefore, in an experiment to accept or refute the SR-BI downregulation hypothesis subject should be recruited, that are eligible according to the implemented inclusion criteria in the Nolan study^[16]. In the gastrointestinal tract, the SR-BI receptors are present throughout the small and large intestine, from the duodenum

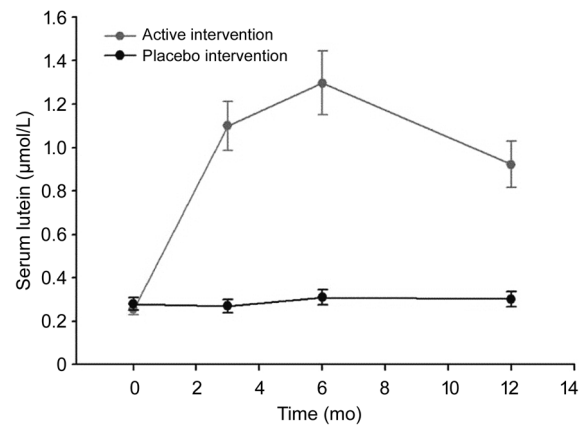


Figure 1 The mean serum levels in subjects recruited in the study by Nolan *et al*^[16] Measurements performed in the 3rd, 6th, and 12th month.

to the rectum^[29]. Most of the lipid in the diet is absorbed in the duodenum, when compared to other GI structures^[30]. Therefore, in this experiment two biopsy specimens from the subject's duodenum must be obtained. The first specimen is collected before the start of the supplementation with the previously mentioned capsule contents, and the second one is collected 6mo after supplementation, with one capsule per day. Immunohistochemistry targeting SR-BI should be done on both samples. Rabbit polyclonal antibody 1336 can be used when performing the immunohistochemistry staining. The hypothesis is supported if there is an obvious decrease in the amount of SR-BI when comparing both specimen and refuted if there is no clear decrease.

Proposed Experiment to Accept or Refute the BCDO2 Upregulation Hypothesis

For an experiment to accept or refute the BCDO2 upregulation hypothesis subjects with the same inclusion criteria as mentioned above should be recruited^[16]. In addition, the same capsuled contents from the above-mentioned experiment can be used for the same specified period. BCDO2 is proven to be present in the inner mitochondrial membrane of various human tissues including the liver, heart, RPE and testis^[31]. Here instead of a duodenal biopsy, two liver biopsies must be utilised. The first biopsy should be taken before the start of the supplementation and the second at the end of supplementation. To measure changes in BCDO2 levels in response to xanthophylls intake, commercially available ELISA kit can be used. For higher sensitivity, the ELISA kit with the sandwich principle is preferred. Cells obtained from the biopsy should be subjected to the well plates of the ELISA kit. In the kit the density of the yellow coloration will be proportional to the amount of BCO2 in the sample. Results of both biopsies should be compared. The hypothesis will be supported if there is a significant increase in the amount of BCDO2 in the second biopsy as compared to the one before the supplementation and refuted otherwise.

Certain SNPs Potentially Explain the Observed Maximum and Decreased Phenomenon of Lutein Supplementation

A randomized control trial (RCT) used daily 10 mg of lutein and placebo, and involved two centers the first in Manchester (United Kingdom) and the second in Maastricht (The Netherlands). The study aimed to investigate the effect of daily lutein supplementation on macular pigment optical density (MPOD) and visual acuity (VA)^[32]. Serum data collected from this study demonstrated clearly the overshoot phenomenon of lutein in the serum (Figure 2). In addition, DNA samples were taken from patients in The Netherlands and investigated for several single nucleotide polymorphisms (SNPs). Data of the genotype frequencies and whether patients showed the overshoot were compared using a Chi-square test. Only one SNP was found to be significantly different in people who demonstrated the overshoot phenomenon, namely rs2230199 in the C3 complement gene ($P=0.014$). In addition, two other SNPs, rs675679 and rs2229742, showed a P-value smaller than 0.10. Unfortunately, we have only limited of data. Thus, these observations and the lack of significance may be caused by the rather small size of population and should be treated with caution. Nevertheless, we explored these three in more detail.

Rs2230199 in C3 Gene All 4 out of 6 individuals who had this SNP demonstrated the overshoot phenomenon ($P=0.014$). Rs2230199 is the most common functional SNP in the C3 complement gene. The polymorphism results in C3 protein that has arginine residue at position 80 instead of glycine (Arg80Gly). Substitution of the neutral glycine residue with the positively charged arginine can impair the function of C3^[33]. In all the different complement pathways C3 is considered a major and central protein. The exact biochemical link between this altered C3 and the observed max and decrease phenomenon of lutein levels is yet to be uncovered.

Rs675679 in Glutathione S-Transferase Gene Out of the 6 patients only 3 patients have this SNP who all demonstrated the overshoot phenomenon ($P=0.083$). Rs675679 results in GSTP1 isoform. This enzyme is important for phase II cellular metabolism, by catalysing the transfer of the tripeptide glutathione to a wide range of electrophilic compounds^[34]. It is also found to function as a xanthophyll-binding protein (XBP) in the retina where it binds with high affinity to zeaxanthin, but did not show any high-affinity binding to lutein^[35]. The exact relation between this SNP and the observation of the overshoot lutein levels phenomenon is not very clear.

Rs2229742 in Nuclear Receptor Interacting Protein 1 Gene Three patients out of six had this SNP, which all showed the overshoot phenomenon ($P=0.083$). Nuclear receptor interacting protein 1 (NRIP 1) is a protein that acts as a co-regulator of the various nuclear receptors superfamily, including estrogen, progesterone, glucocorticoids and retinoid acid receptors^[36].

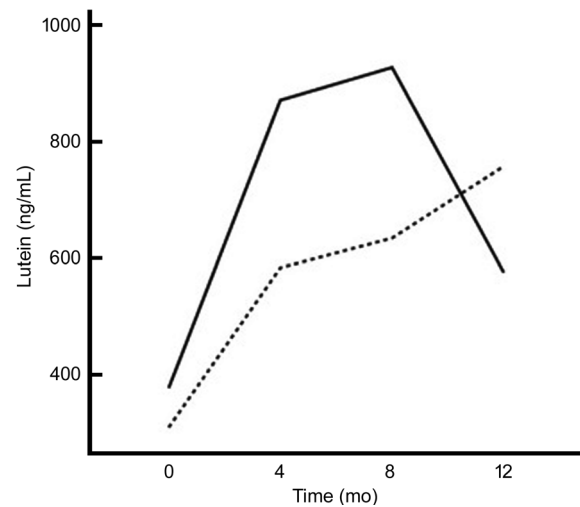


Figure 2 The overshoot phenomenon in data of a study by Murray *et al*^[32] Measurements were obtained at baseline and 4, 8, and 12mo after supplementation. The solid line shows mean serum lutein values of subjects with the overshoot present and the dotted line shows values of subjects in which no overshoot occurred.

NRIP 1 is known for its co-repressive activity in gene transcription^[37]. However, several studies showed that NRIP 1 can also have a co-activator function on the transcription of some genes such as fatty acid synthase (FAS) in hepatocytes and other pro-inflammatory genes in macrophages. Whether NRIP 1 is going to function as a co-repressor or a co-activator depends on its post-translational modifications (PTMs)^[38]. The reason why all the three patients with this SNP demonstrated the overshoot lutein phenomenon is not understood.

Gender Difference in Relation to the Observed Max and Decrease Lutein Phenomenon In the dual centre RCT^[32] 15 out of the 27 individuals (13 females and 14 males) showed the overshoot phenomenon, significantly more in females (10) than in males (5, $P=0.031$). This may be attributed to the fact that females have more adipose tissue, considering that lutein is lipophilic. Adipose tissue is the major body storage site for lutein. Individuals with low body fat, *e.g.*, anorexic patients, have higher circulating carotenoids levels^[39]. In contrast, according to Bovier *et al*^[40], deposition of lutein in target tissues (*e.g.*, retina) was significantly lower in females when compared to males, although serum concentrations showed no differences in relation to gender. Whether differences in body fat percentages between genders is the only cause of the fact that the overshoot occurs more often in women than men is unclear and merits further studies.

CONCLUSION

Lutein is found to delay the onset and progression of AMD by various protective properties. It serves as a blue light absorbing agent, antioxidant, and FD lowering agent. In some lutein supplementation studies, it was found that after an observed increase, lutein levels demonstrated a subsequent decrease

in the serum, despite continuous supplementation. This phenomenon was investigated in this review and a number of hypotheses were postulated in an attempt to explain the causes and the reasons behind it. Two enzymes in the non-heme iron dependent oxygenases were found to work on carotenoids, namely BCOM1 and BCDO2. BCOM1 has a very specific ligand binding site and can only catalyse beta-carotene, but not xanthophylls. In contrast, BCDO2 was found to interact with a wide range of carotenoids including the xanthophylls lutein and zeaxanthin. We suggest that the serum lutein overshoot phenomenon may be the result of either decrease lutein absorption from the GI tract or increase degradation post-absorption. The absorption of xanthophylls from the apical surface of enterocytes is believed to be mediated by SR-BI receptors. Continuous lutein supplementation was suggested to downregulate the expression of SR-BI receptors, hence a possible explanation for the observed phenomenon. Several studies in mammals prove that BCDO2 is essential for carotenoids homeostasis^[23-25]. Some human cancer cell lines were treated with a specific amount of carotenoids, including lutein, the internal pathway of apoptosis was initiated in the cells lacking BCDO2 due to interruption of the ETC^[27]. Therefore, it was postulated that high lutein levels in the serum triggers the upregulation of BCDO2 to maintain homeostasis, which subsequently leads to increase lutein degradation, which could potentially result in the observed max and decrease lutein phenomenon. Using data from Murray *et al*^[32] we noted a significant relation between the observed overshoot phenomenon and the SNP rs2230199 in C3 gene. This phenomenon was observed significantly more in females than males, which might be attributed to the difference in body fat percentages. To date the exact cause of this puzzling phenomenon is not fully understood, and further studies are needed to identify its cause.

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