

# Correlation between the Content of Albumin and Carotenoids in Human Vitreous Body during Prenatal Development

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The dynamics of the content albumin and carotenoids in human fetal vitreous body during weeks 16-31 of gestation was studied. The maximum values of total albumin (1.42 mg) and carotenoids (276 ng) during the studied period were recorded on weeks 20-22. Albumin concentration peaked during week 17 ( $2.11 \times 10^{-4}$  mol/liter) and carotenoids during weeks 16-17 (about  $0.045 \times 10^{-4}$  mol/liter) of prenatal development. By week 31, the concentrations and total content of albumin and carotenoids in the vitreous body decreased. The physiological role of the studied components of the vitreous body for prenatal development of human eye is discussed.

**Key Words:** *albumin; carotenoids; vitreous body; human fetus*

Carotenoids lutein and zeaxanthine are pigments characteristic of primate and human eyes. They are present in tissues of mature human eye in the retina, lens, ciliary body, iris, choroid/pigmented epithelium. No carotenoids were detected in the vitreous body. The highest concentration of these pigments (mainly zeaxanthine) in the retina was found in the macular area (macula lutea), including the fovea, where the maximum number of cones is located. Outside the macula, lutein is distributed in the entire retina, the gradient of its distribution decreasing towards the periphery [4,5]. Lutein and zeaxanthine perform an important protective function: they act as optic filters protecting visual cells from the destructive effect of blue light and as effective antioxidants. Age-associated macular degenerations of the retina and senile cataracts correlate with impaired protective functions of carotenoids resulting from the decrease in their content [6,8,9].

In our previous study we detected carotenoids in human fetal vitreous body by HPLC and by the absorption spectra of native vitreous body [2]. In addition, it was shown that human vitreous body contained sufficiently high amounts of albumin during prenatal development [1,10]. Albumin is known as a carotenoid carrier protein [11], which attests to a correlations between carotenoids and albumin levels in human fetal vitreous body. In order to prove it, the quantitative characteristics of carotenoids and albumin in the vitreous body should be measured.

Based on the analysis of carotenoids and albumin in the vitreous body, we characterized the dynamics of their concentrations and total content in human fetal vitreous body during weeks 16-31 of gestation.

## MATERIALS AND METHODS

The vitreous bodies were removed from the eyes of human aborted fetuses (weeks 16-31 of prenatal development). The fetuses were obtained from Research Center of Obstetrics, Gynecology, and Perinatology from licensed institutions of the Ministry of Health of the Russian Federation, working with-

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in the framework of legislation on health protection and in accordance with the approved list of medical indications. Fetal age was determined by the obstetrician. The cornea was resected under a binocular lens (MBK-9) and the vitreous body was removed together with the lens; the lens was then accurately removed and the vitreous body was purified from the remnants of the retina. The resultant samples were stored at  $-20^{\circ}\text{C}$ . Before measurements the sample was defrosted, the volume of the vitreous body was measured, and the sample was centrifuged (12,500 rpm, Ependorf centrifuge 5417R,  $4^{\circ}\text{C}$ , 30 min). After centrifugation the supernatants were collected and used for measuring albumin and carotenoid concentrations.

For measuring the concentration of carotenoids in the vitreous body at different terms of human prenatal development, the supernatant (50  $\mu\text{l}$ ) was placed into the measuring cuvette (1 mm) and the absorption spectra were recorded on a UV-3101PC spectrophotometer (Shimadzu) at  $\lambda=300-600$  nm. Carotenoid concentrations were evaluated by extinction coefficient for lutein equal to 137 000 liter/(mol $\times$ cm) at  $\lambda=450$  nm [7]. The extinction coefficient for lutein was chosen because lutein is the main retinal carotenoid in humans up to the age of 2 years, while in adults the main carotenoid is zeaxanthine [4]. The total content of carotenoids in the vitreous body at different terms of gestation was measured.

Albumin concentration was evaluated by its reaction with cyanine stain (3,3'-di-( $\gamma$ -sulfopropyl)-4,5,4',5'-dibenzo-9-ethylthiacarbocyanine betaine pyridine salt; Khimfotoproekt Institute) effectively reacting with albumin [12]. Calibration curve was plotted by adding albumin in certain concentrations

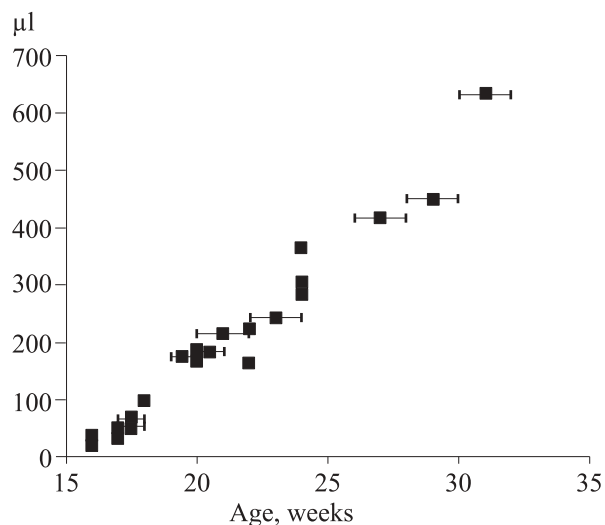


Fig. 1. Increase in the volume of the vitreous body of the eye between weeks 16 and 31 of human prenatal development.

to the dye solution. The intensity of absorption band with the maximum at  $\lambda=612$  corresponding to *trans*-form of the stain forming in albumin reaction with the stain was measured [1,10,12]. The absorption spectra were measured in a 1-cm cuvette on a UV-3101PC spectrophotometer (Shimadzu) at  $\lambda=400-800$  nm.

## RESULTS

The volume of the vitreous body increased incessantly throughout the studied period from week 16 to 31 of gestation (22-fold; Fig. 1).

During week 17 albumin concentration reached the maximum ( $2.11 \times 10^{-4}$  mol/liter; Fig. 2,

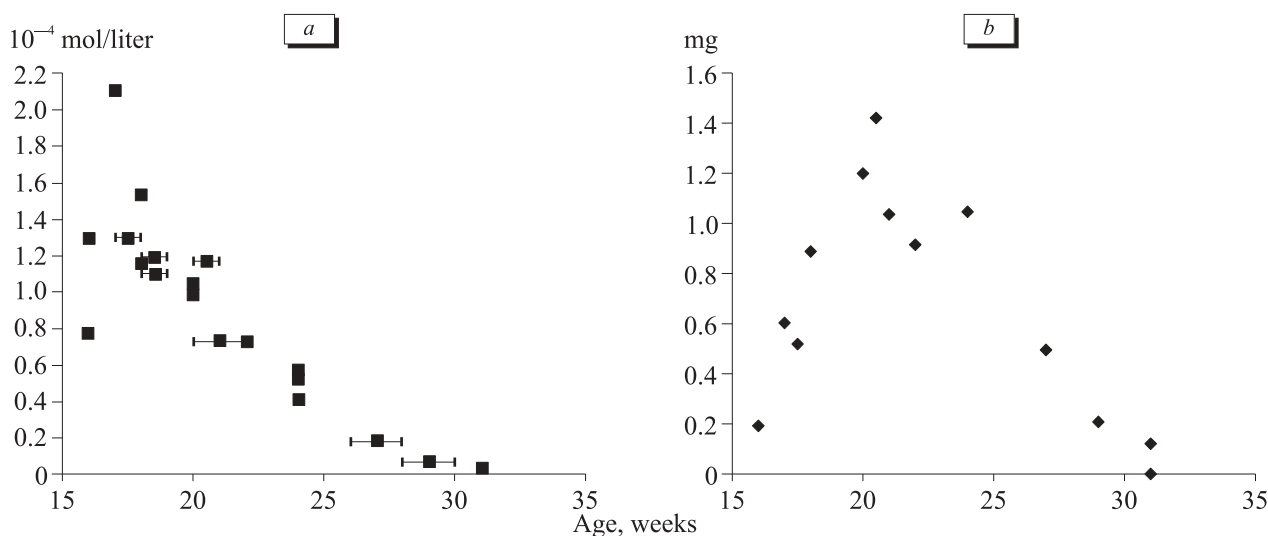
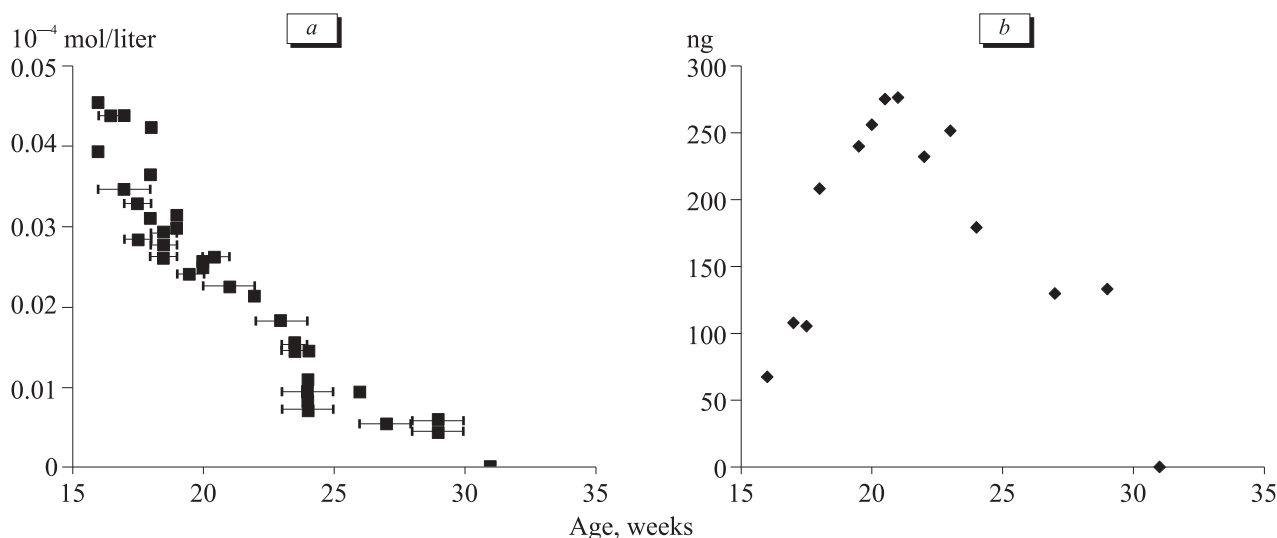


Fig. 2. Dynamics of albumin concentration (a) and total content (b) in the vitreous body of the eye in human fetuses on weeks 16-31 of development.



**Fig. 3.** Dynamics of carotenoid concentration (a) and total content (b) in the vitreous body of the eye in human fetuses on weeks 16-31 of development.

a). After week 17, this parameter decreased and by week 31 reached the minimum value ( $0.029 \times 10^{-4}$  mol/liter). Total albumin content in the vitreous body calculated with consideration for the vitreous volume reached the maximum (1.42 mg) during weeks 20-21 of prenatal development, after which it decreased to 0.12 mg during week 31 (Fig. 2, b).

The concentration of carotenoids in the vitreous body was maximum on weeks 16-17 (about  $0.045 \times 10^{-4}$  mol/liter), after which it gradually decreased throughout the entire observation period and reached zero during week 31 of prenatal development (Fig. 3, a). The total content of carotenoids in the vitreous body calculated with consideration for the vitreous volume reached the maximum (about 276 ng) during weeks 20-22 of prenatal development and decreased to 130 ng during weeks 27-29. No carotenoids were detected during week 31 (Fig. 3, b).

Similar age-related dynamics of albumin and carotenoid concentrations (Figs. 2, a; 3, a) and dynamics of their total content and the coincidence of the period of their peak values (Figs. 2, b; 3, b) attest to a correlation between albumin and carotenoid content in the vitreous body, which confirms the hypothesis on carotenoid deposition in the vitreous body realized by albumin for subsequent transfer of carotenoids into the target tissues of developing human eye, specifically, into the retina and, presumably, into the lens [2]. On the other hand, our data indicate that the concentration of albumin is much higher than that of carotenoids. Albumin excess can provide optimal binding of carotenoids to this protein for their preservation and transportation to target tissues of the developing eye. Presumably, albumin is essential for other important

physiological functions during this period of eye development.

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