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Does variability in carotenoid composition and concentration in tissues of the breast and reproductive tract in women depend on type of lesion?

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ABSTRACT

Purpose: Vitamin A takes part in many physiological and pathological processes in women's reproductive organs. The study objective was to compare the carotenoid content in benign and malignant lesions of the breast, ovary and uterus, and to demonstrate quantitative and qualitative similarities or differences between the study groups.

Materials and Methods: Materials for analysis were physiological and pathological tissues of breast, ovary and uterus.
The carotenoid pigments were isolated using column chromatography (CC), thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

Results: Sixteen carotenoids were identified in the study material, including those belonging to the provitamin A group. The most common were: β-carotene, β-cryptoxanthin, lutein, mutatoxanthin, violaxanthin, lutein epoxide and zeaxanthin. All the tissues subjected to analysis contained β-carotene, 98% of the tissues had β-cryptoxanthin, whereas α-carotene was detected in about 50% of breast tissue. No differences in carotenoid concentration were found between benign and malignant lesions in the examined tissues, apart from hydroxyechinenone, canthaxanthin, astaxanthin, lutein epoxide, antheraxanthin and neoxanthin. Similarly, no differences in concentration of the provitamin A carotenoids (α-carotene, β-carotene, β-cryptoxanthin, and echinenone) were found between benign and malignant lesions except hydroxyechinenone. The highest total content of carotenoids and the biggest spectrum of predominant carotenoids were found in the breast. Only in tissues of malignant lesions of the uterus, we observed statistically higher total content of carotenoids compared to remaining samples from the uterus ($p<0.001$) and more frequent isolation of some carotenoids (compared to benign lesions).

Conclusion: The results of our study confirmed the presence of a high diversity of carotenoids in the physiologic, benign and malignant tissues of the breast and the reproductive tract in women. The differences observed among the frequency of isolation of some carotenoids do not allow to make straightforward conclusions. The frequent isolation of provitamin A carotenoids in the examined material and the lack of their occurrence as major carotenoids may be connected with using them in the cellular biological processes. However, this requires further investigation.

Key words: carotenoids identification, women's reproductive organ tissues

INTRODUCTION

Vitamin A (carotenoids and retinoids) is a basic factor engaged in the reproductive processes in the female body. Retinoids play a role in steroidogenesis [1], maturation of oocytes and formation of the corpus luteum [2]. The processes taking place in the edometrium and myometrium of the uterus are also associated with the concentration of carotenoid pigments. Retinoic acid takes part in the regulation of the cycles of cell replication and differentiation in the human endometrium [3] and decidualization of fibroblasts [4,5], may regulate leiomyoma growth [6] and may take a role in differentiation of endometrial adenocarcinoma [7]. In some way, carotenoids may prevent development of endometriosis [8, 9]. Furthermore, the vitamin A family compounds are involved in the process of breast development, lactation and pathology. [10-12].

Table 1. **Characteristics of study subjects*.**

Patients (n=280)	The breast (n=40)		The ovary (n=100)	The uterus (n=140)
			Samples collection (n=320)	
	The breast (n=80)		The ovary (n=100)	The uterus (n=140)
	benign lesions (n=20) and fatty tissue (n=20)	malignant lesions (n=20) and fatty tissue (n=20)		
Age (y)**	57,2 ± 3,95		49.3 ± 16.85	50,1 ± 9,7
Wt (kg)**	67,5 ± 6,25		73.5 ± 14.85	74,2 ± 14,72
Age at menarche (y)**	13,3 ± 1,25		14.3 ± 1.85	14,2 ± 1,63
Parity (n)**	2,5 ± 1,3		2.8 ± 1.85	2,1 ± 1,33
Nulliparous (n)	7		20	21
Menopausal status (n)				
Premenopausal	19		64	0
Postmenopausal	21		36	43

* The full characteristic of examined subjects and amount of samples in each group was performed in details in the references 38, 39 and 40.
** Values are means ± SD

Vertebrates do not synthesize carotenoids. Therefore, carotenoids must be supplied with food and converted into compounds that affect both proliferation and differentiation of cells as well as regulation of the physiological and the pathological processes [13,14]. In addition, the non-provitamin A carotenoids may protect cells against mutagenesis and malignant transformation by acting as antioxidants or through the effect on the immune system or formation of gap junctions.

Carotenoids contained in green parts of plants and retinyl esters derived from animal foods are the source of vitamin A. The human diet shows the predominance of β-carotene (the most common and most active carotenoid in nature, giving two molecules of vitamin A), which together with lycopene, lutein, zeaxanthin, β-cryptoxanthin and α-carotene accounts for approximately 90% of the circulating carotenoids [15]. In the human body, carotenoids derived from foods are absorbed in the intestines and carried with blood to various tissues. Some carotenoids (carotenes and nonesterified xantophylls) are absorbed and in the unchanged form appear in the blood, whereas others (esterified xantophylls) have to be cleaved before uptake [15,16]. Then, they bind to lipoproteins and in this form reach tissues [15,17]. It has been shown that provitamin A carotenoids, e.g. β-carotene, can be converted into retinal in the intestinal mucosa and later, depending on body demand for vitamin A, can be reduced to retinol and exploited [18].

The commonly known phenomenon of xantophyll production via the oxidation of hydrocarbon carotenoids (i.e. from carotenes), observed in lower vertebrates, has an opposite mechanism. Such xantophylls as lutein, zeaxanthin (hydroxy carotenoids), cantaxanthin and astaxanthin (ketocarotenoids) via reduction are changed into β-carotene and in all the three forms of vitamin A [19-21]. Such conversions are also likely to occur in higher vertebrates, including humans.

The respective tissues are characterized by non-selective (e.g. skin) or selective (yellow spot) concentration of carotenoids [17]. The mechanism of the selective phenomenon is probably associated with the presence of carotenoid-binding proteins [22,23], about whose potential role in the human body little is still known.

It has been revealed that β-carotene may bind directly to the retinoid acid receptors RARs and retinoid X receptors RXRs. The biological effect is exerted by an active natural metabolite of provitamin A carotenoids – retinoic acid and 9-*cis* and 13-*cis* isomers (retinoids). It has turned out that many various carotenoids can be metabolized to retinoids [24,25]. Studies in vitro have shown that signalling to cells via retinoids may inhibit the process of carcinogenesis in breast cancer cells, whereas impaired retinoid receptor expression (RAR and RXR) can induce their malignant transformation [26].

Taking under consideration the role of carotenoids in physiological and pathological processes in the breast and the female reproductive tract, the study objective was to compare the carotenoid content in benign and malignant lesions of the breast, ovary and uterus, and to demonstrate quantitative and qualitative similarities or differences between the study groups.

MATERIALS AND METHODS

Subject and sample collection

Material for analysis was obtained from 280 women (the total amount of samples were 320) operated in the Oncology Centre in Białystok (breasts), or during the planned curettage or operated on for various tumours of the uterine corpus and ovary in the Department of Gynaecology, the Medical University of Białystok. (*Tab. 1*). Two samples, fatty tissue and lesion, were

Table 2. **List of carotenoids from investigated materials.**

Carotenoid	Summary formula	Structure (see Fig. 1)	Semisystematic name
1. α-carotene	$C_{40}H_{56}$	A-R-B	β,ε-Carotene
2. β-carotene	$C_{40}H_{56}$	A-R-A	β,β-Carotene
3. β-cryptoxanthin	$C_{40}H_{56}O$	A-R-C	β,β-Caroten-3-ol
4. lutein	$C_{40}H_{56}O_2$	C-R-D	β,ε,Carotene-3,3´-diol
5. 3'-lutein	$C_{40}H_{56}O_2$	C-R-D	β,ε,Carotene-3,3´-diol (stereoisomer)
6. zeaxanthin	$C_{40}H_{56}O_2$	C-R-C	β,β-Carotene-3,3´-diol
7. echinenone	$C_{40}H_{54}O$	A-R-E	β1,β-Caroten-4-one
8. hydroxyechinenone	$C_{40}H_{54}O_2$	A-R-F	3-Hydroxy-β,β-caroten-4-one
9. canthaxanthin	$C_{40}H_{52}O_2$	E-R-E	β,β-Carotene-4,4´-dione
10. astaxanthin	$C_{40}H_{52}O_4$	F-R-F	3,3´-Dihydroxy-β,β-carotene-4,4´-dione
11. lutein epoxide	$C_{40}H_{56}O_3$	B-R-G	5,6-Epoxy-5,6-dihydro-β,ε-carotene-3,3´-diol
12. antheraxanthin	$C_{40}H_{56}O_3$	C-R-G	5,6-Epoxy-5,6-dihydro-β,β-carotene-3,3´-diol
13. neoxanthin	$C_{40}H_{56}O_4$	G-R1-H	5´,6´-Epoxy-6,7-didehydro-5,6,5´,6´tetrahydro-β,β- carotene-3,5,3´-triol
14. violaxanthin	$C_{40}H_{56}O_4$	G-R-G	5,6,5´,6´-Diepoxy-5,6,5´,6´-tetrahydro-β,β-carotene-3,3´-diol
15. mutatoxanthin	$C_{40}H_{56}O_3$	C-R1-I	5,8-Epoxy-5,8-dihydro-β,β-carotene-3,3´-diol
16. capsanthin	$C_{40}H_{56}O_3$	C-R-K	3,3´-Dihydroxy-β,x-carotene-6´-one

taken each time from the breast. All together, 80 samples of breast's fatty tissue and lesion were collected. Histologically, the material originated from the breast cancer, benign breast tumour (fibroadenoma) and breast fatty tissues, from normal ovarian tissue and from tumours of ovaries (surface epithelium and stroma tumours, sex cord stroma tumours, germ-cell tumours, soft tissue tumours nonspecific to ovary and tumour-like conditions) and the normal endometrium and myometrium and from tumours of the uterine corpus (epithelial, mesenchymal or mixed tumours). Histological groups were divided according to World Health Organization Classification of Tumours, Pathology and Genetics of Tumours of the Breast and Female Genital Organs. Ed. Tavassoli F.A., Devilee P., IARC Press., Lyon, 2003.

Cancer tissue carotenoid analyses

The carotenoid pigments were isolated using column chromatography (CC), thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Prior to chromatography, the material was homogenised with acetone under nitrogen in dark glass bottles and the extracts were kept in a refrigerator until analysed. Saponification was carried out with 10% KOH in ethanol at 20^0 C for 24 h in the dark under nitrogen. Column and thin-layer chromatography was used to separate the carotenoids, which were identified by comparison with standard compounds (Hoffman-La Roche and Sigma Company) by a) the behaviour on column chromatography; b) their UV-VIS spectra (Beckman 2400); c) their partition between *n*-hexane and 95% ethanol; d) their R_f-values on thin-layer chromatography; e) the presence of allylic OH-group determined by the acid $CHCL_3$ test; f) the epoxide test and g) the mass spectrum [27]. Pigments were determined also by ion – pairing, reverse – phase HPLC. To 1000 µl of the clear extract, 300 µl of ion – pairing reagent was added according to Mantoura and Llewellyn [28]. The HPLC equipment consisted of Shimadzu LC – 6A double – system pump, driven by a gradient programmer Shimadzu SCL-6B and Redone 7125 injector equipped with a 20-µl loop. A Shimadzu SPD –6 AV UV – VIS spectrophotometer detector set detection on 440 nm and Shimadzu RF – 535 fluorescence detectors.

Carotenoids as pigment standards were obtained from Hoffman – La Roche Company, Switzerland, International Agency for ^{14}C Determinations, Denmark and Sigma Chemical Company, USA.

Quantitative determinations were performed by UV, VIS spectroscopy [29]. For the structures of carotenoids see Straub [30] and Czeczuga [31].

Statistical analysis

In each group of examined tissues mean values ± standard deviation (S.D.) were calculated. The Mann-Whitney test was used to perform statistical analysis.

RESULTS

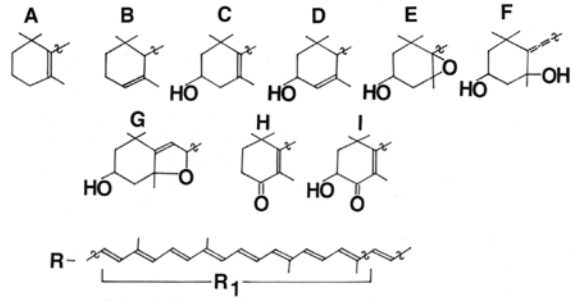
The profiles of female patients, whose tissues were obtained for analysis, have been presented in *Tab. 1*.

The list of carotenoids identified in the material has been presented in *Tab. 2* and *Fig. 1*.

Chemically, the carotenoids identified in the study material can be divided into four groups [30]:

1. Hydrocarbon carotenoids (carotenes), including α- and β-carotene.
2. Hydroxy carotenoids (more numerous group): β-cryptoxanthin, lutein, 3'-epilutein and zeaxanthin.

Figure 1. **Structural features of carotenoids from investigated materials (see Table 2).**



3. Ketocarotenoids: echinenone, hydroxyechinenone, canthaxanthin, astaxanthin and capsanthin.
4. Epoxy carotenoids: lutein epoxide, antheroxanthin, neoxanthin, violaxanthin, mutatoxanthin.

Sixteen carotenoids were identified in the study material, including those belonging to the provitamin A group, namely β-carotene, β-cryptoxanthin, α-carotene, echinenone and hydroxyechinenone. The most common were: β-carotene, β-cryptoxanthin, lutein, mutatoxanthin, violaxanthin, lutein epoxide and zeaxanthin (*Tab.3*). All the tissues subjected to analysis contained β-carotene, 98% of the tissues had β-cryptoxanthin, whereas α-carotene was detected in about 50% of breast tissue. No differences in the carotenoids concentration were found between benign and malignant lesions in the examined tissues, apart from hydroxyechinenone, canthaxanthin, astaxanthin, lutein epoxide, antheraxanthin and neoxanthin (*Tab. 3*). Similarly, no differences in concentration of the provitamin A carotenoids were found between benign and malignant lesions except hydroxyechinenone. The predominant carotenoids in all examined tissues were demonstrated in *Tab. 4*. Breast tissue had the highest carotenoid content and the largest number of predominant carotenoids (the mean content in breast fatty tissue surrounding benign lesions 27.021±13.18 μg/g tissue). The total content of carotenoids in malignant lesions from the uterus was significantly higher in comparison to benign lesions and unchanged tissues ($p<0.001$) (*Fig. 2*). No such differences were observed either in breast or ovarian tissue samples. On the other hand, mean concentration of carotenoids in malignant and benign lesion were statistically lower in samples from the ovary and uterus in comparison to the breast ($p<0.0001$). The differences, in concentration of carotenoids existed between fatty tissues versus benign lesions, and between fatty tissues and cancer tissues in breast samples, confirmed the thesis that the adipose tissue may be considered a major carotenoid depot (*Fig. 2*).

DISCUSSION

The presence of carotenoids in various human tissues has been reported [32-35]. According to literature data, β-carotene, α-carotene, cryptoxanthin, lycopene and lutein have been most commonly identified in human serum [15], zeaxanthin being less common. This seems to suggest that these carotenoids should be most frequently found in the examined samples. However, the processes described above and a number of other factors, including the carotenoid structure, proteins for

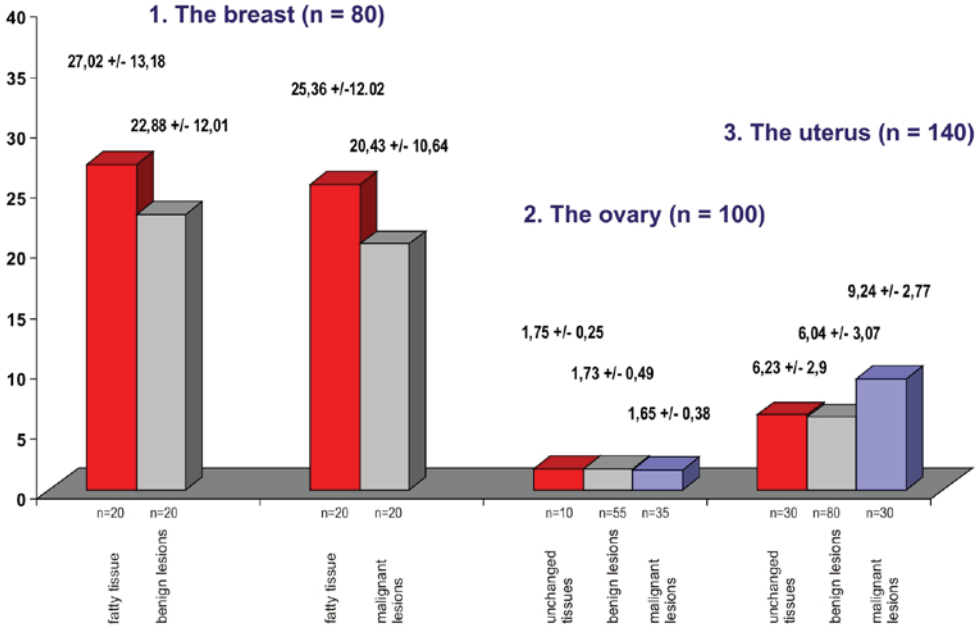
Table 3. **The frequency of carotenoids isolation in the examined material (in%).**

Carotenoid	The sample collection					
	The breast (n=40)		The ovary (n=90)		The uterus (n=110)	
	Benign (n=20)	Malignant (n=20)	Benign (n=55)	Malignant (n=35)	Benign (n=80)	Malignant (n=30)
1. α-carotene	50.0	55.0	(-)	(-)	1.2	(-)
2. β-carotene	100.0	100.0	100.0	100.0	100.0	100.0
3. β-cryptoxanthin	100.0	90.0	100.0	100.0	100.0	100.0
4. lutein	100.0	60.0	100.0	100.0	100.0	100.0
5. 3'-lutein	10.0	25.0	(-)	(-)	(-)	(-)
6. zeaxanthin	100.0	80.0	74.5	60.0	100.0	100.0
7. echinenone	(-)	(-)	9.1	11.4	(-)	(-)
8.hydroxyechinenone	(-)	(-)	7.3	(-)	15.0	26.7
9. canthaxanthin	35.0	15.0	50.9	25.7	3.7	(-)
10. astaxanthin	25.0	10.0	36.4	42.8	63.7	66.7
11. lutein epoxide	100.0	95.0	96.4	100.0	75.0	10.0
12. antheraxanthin	(-)	(-)	34.5	25.7	7.5	26.7
13. neoxanthin	(-)	10.0	87.3	57.1	100.0	100.0
14. violaxanthin	50.0	60.0	100.0	100.0	100.0	100.0
15. mutatoxanthin	90.0	85.0	100.0	100.0	100.0	100.0
16. capsanthin	(-)	(-)	1.8	(-)	(-)	(-)

Table 4. **The major carotenoids in the examined material (in %).**

Carotenoid	The sample collection					
	The breast (n=40)		The ovary (n=90)		The uterus (n=110)	
	Benign (n=20)	Malignant (n=20)	Benign (n=55)	Malignant (n=35)	Benign (n=80)	Malignant (n=30)
β-cryptoxanthin	(-)	(-)	(-)	(-)	11.2	16.6
lutein	10,0	5,0	(-)	(-)	(-)	(-)
3'-lutein	5,0	5,0	(-)	(-)	(-)	(-)
zeaxanthin	15,0	10,0	(-)	(-)	(-)	(-)
canthaxanthin	15,0	5,0	(-)	(-)	(-)	(-)
astaxanthin	(-)	(-)	27.3	28.6	48.7	30.0
lutein epoxide	25,0	40,0	(-)	(-)	(-)	(-)
neoxanthin	(-)	5,0	21.8	25.7	(-)	26.6
violaxanthin	(-)	(-)	50.9	45.7	40,0	26.6
mutatoxanthin	30,0	30,0	(-)	(-)	(-)	(-)

Figure 2. **The total content of carotenoids (in µg/g in tissue) in the examined material (values are means +/- SD).**



absorption (SR-B1), general health condition, type of diet, interfering drugs or compounds, genetic factors and type of the population studied may affect carotenoid distribution. In humans, the respective tissues are known to differ in the carotenoid content. The most common are β-carotene and lycopene [32,33]. However, our study did not confirm the occurrence of lycopene in any of the tissues (320 samples). Maybe this absence may be related to the physiological function of this carotenoid as a radical scavenger (considered most effective) [36] .

Although β-carotene was not a predominant carotenoid (in %), it was found in all the examined tissues. It was revealed that the conversion of β-carotene to retinoids, occurring directly in the ovary [37], indicates that carotenoids may also reach target tissues in an unchanged form. Moreover, lutein and zeaxanthin, which predominate in the breast gland, are also a likely source of β-carotene. The other provitamin A carotenoids (echinenone and hydroxyechinenone) were seldom identified in our study. Also isolation of echinenone was characteristic only for benign and malignant ovary tissues. We observed α-carotene almost exclusively in breast gland tissue and there were no statistically significant differences between benign and malignant lesions. According to literature data, α-carotene has been identified in serum [15] and other tissues [34,35,38]. In our previous study, it was not found in ovarian tissue and only once was detected in the uterus (nonatypical hyperplasia complex) [39,40]. The analysis of the mean β-carotene content in the tissues of the sexual organs revealed its highest levels in the ovary and endometrium, whereas the mean β-carotene values in neoplastic endometrial tissues were statistically

lower as compared to the unchanged tissues, yet by far higher in the carcinoma of the breast and ovary [41]. Our study confirmed higher mean values of β-carotene were found only in endometrial carcinoma as compared to the unchanged endometrium [40]. Nevertheless, Mühlhöfer et al. confirmed the occurrence of higher levels of carotenoids (α-carotene, β-carotene, lutein, lycopene, zeaxanthin and β-cryptoxanthin) in unchanged tissue as compared to neoplastic tissue [42].

The relationship between β-carotene and cancer are not simple and particularly associated with nutritional aspects and disease prevention. β-carotene shows a protective effect against ovarian cancer, mainly mucinous tumour [37]. This effect seems to depend on the histological type of the tumour, which may affect the profile of nuclear retinoid receptors [43]. Taking into account a complete analysis of carotenoid pigments present in the tissues (16 various carotenoids), no significant differences were found in the mean content of carotenoids between unchanged tissues, benign and malignant lesions of the ovary (*Fig. 2*). In the group of benign mucinous tumours, the mean values were the lowest (1.0±0.12 μg/g), whereas in malignant mucinous tumours, they did not differ from the values obtained for unchanged ovarian tissue [39].

The mean values of carotenoids were by far the highest in the group of benign breast tumours (*Fig.2*). In comparison with ovarian and uterine tumours, the values in breast cancers were over 10-fold and 2-3-fold higher, respectively. The carotenoid content differs between particular types of tissues. As carotenoids do not dissolve in water, they often occur in combination with lipids [44]. The biggest spectrum of predominant carotenoids was found in the breast (7 carotenoids). It is probably connected to the large amount of fatty tissue in this organ. Lutein and zeaxanthin, being predominant in breast tissue, show antimutagenic and anticarcinogenic effects. Xantophylls also reduce the risk of ovarian cancer [45]. However, in mucous cancer, xantophyll depletion during oxidative stress is likely to occur [46]. We did not observe the differences between the frequency of β-carotene, β-cryptoxanthin, lutein, violaxanthin and mutatoxanthin isolation between benign and malignant tissues from the ovary and uterus and neoxanthin and zeaxanthin from the uterus.

Significant differences observed among isolated carotenoids did not demonstrate any regularity. Apart from cantaxanthin, we could not conclude, that the benign samples contained more or less (in%) of one of the carotenoids in comparison to the malignant samples.

In general, only benign samples from the uterus demonstrated less frequent isolation of some carotenoids compared to malignant samples.

The total carotenoid content (in μg/g tissue) in myomas (the most common benign lesion) was lower to that found in leiomyosarcoma (malignant lesions) group. Whereas, a higher percentage of provitamin A carotenoids was observed in the group of myomas [40]. However, data reported by Palan in two different studies [41,47] on β-carotene content in this benign lesions are divergent.

Alsharif et al. postulated that vitamin A may protect against the development of endometriosis [8]. Furthermore, it has been shown that in *in vivo* conditions β-carotene can suppress angiogenesis, which is essential for the ectopic endometrial foci [9]. In our study, the mean values (in μg/g tissue) and the content of provitamin A carotenoids (in %) were only slightly higher in the foci of endometriosis as compared to healthy ovarian tissue (2.185/1.75) [39].

The results of our study confirmed the presence of high diversity of carotenoids in the physiologic, benign and malignant tissues of breast and reproductive tract in women. However, the most intriguing point, the relation between carotenoid concentration, the frequency of their isolation and the type of lesion still remain an unanswered question.

The differences observed among the frequency of isolation of some carotenoids do not allow to make straightforward conclusions.

The frequent isolation of provitamin A carotenoids in the examined material and the lack of their occurrence as major carotenoids may be connected with using them in the cellular biological processes. The carotenoids play an important role in cell proliferation control by lowering growth factor concentration, slowing the rate of cell division and the transcription of DNA [48, 49, 50]. The possible influence of carotenoid composition, concentration as well as admixture of provitamin A group carotenoids on cell proliferation needs further investigation.

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