

**The defensive effects of retinoids in the gastrointestinal tract  
(animal experiments and human observations)**

**Ph.D. thesis**

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## 1. INTRODUCTION

Retinoids are a class of compounds consisting of four isoprenoid units bound in a head-to-tail manner. Vitamin A is a fat-soluble substance found in animal foods and dairy products. Vitamin A is available as preformed vitamin A, contained in liver, cod liver oil, butter, eggs, or as pro-vitamin A carotenoids, as found in dark green, red and yellow vegetables. The dietary carotenoids and preformed retinoids undergo a series of metabolic conversions, extracellularly in the lumen of the intestine and intracellularly in the intestinal mucosa, which result in the preponderance of the absorbed dietary retinoid being converted to retinol. Although hundreds of carotenoids have been identified, only few have been found to exist in appreciable concentrations in human serum: lutein, zeaxanthin,  $\alpha$ - and  $\beta$ -carotene,  $\alpha$ - and  $\beta$ -cryptoxanthin. Retinol is esterified in the intestinal mucosa, packaged as retinyl ester into chylomicra, and carried to the liver via the lymphatic circulation. Approximately 90% of vitamin A in the body is stored in the liver as retinyl esters. The liver has the capacity to store enough vitamin A to last for several months, with longer storage capacity among adults than children. Retinol is released from the liver in combination with plasma retinol-binding protein and transthyretin. Retinol seems to enter cells via specific receptors, although it is unclear whether all cells contain these receptors. Vitamin A exerts its effects through retinoid receptors, which are found in the nucleus of cells. These receptors resemble steroid and thyroid hormone receptors and support the idea that vitamin A acts much like a hormone. Retinoids and carotenoids have numerous biological functions such as regulating of growth, morphogenesis and differentiation of cells, they have a variety of effects on the cell membrane, they can influence the activity of different enzymes furthermore these compounds exert also immunomodulatory effects. Carotenoids are non-enzymatic antioxidants, therefore they are able to prevent genetic changes by preventing DNA damage caused by free radicals. They modulate membrane functions and stabilise initiated cells in promotional phase of carcinogenesis.

The protective role of retinoids in gastrointestinal mucosa prevention has been investigated in our department intensively in the last two decades. Vitamin A and beta-carotene were shown to prevent the experimentally induced gastric mucosal lesions in animals, and these compounds were found to be effective in the treatment of patients with gastric peptic ulcer. Carotenoids have no inhibitory effects on gastric acid secretion neither in animals nor in humans, however  $\beta$ -carotene was able to prevent the gastric mucosal damage in different experimental models such as ethanol (96 %) -, hydrochloric acid (0.6 M) - or indomethacin-induced mucosal damage. Furthermore the ulcer healing effect of vitamin A was proven in randomized, multicenter clinical studies. The gastroprotection induced by carotenoids does not depend on the presence of vitamin A activity,  $\beta$ -ionone ring, number of unsaturated links

or chemical structure of terminal part of molecules. The  $\beta$ -carotene-induced gastric gastroprotection was completely abolished by acute bilateral surgical vagotomy.

A suboptimal diet might be related to approximately 30-60% of all cancer cases the major part of which is possibly preventable by adequate dietary modifications. Chemopreventive potentialis are conceivable for antioxidant micronutrients. Particularly intriguing are those with polyene structure, i.e. vitamin A-type retinoids and carotenoids such as  $\beta$ -carotene.

The effectiveness of retinoids as inhibitors of chemical carcinogenesis in the epithelia of the digestive tract is somewhat contradictory, although positive results have been obtained in animal experiments of stomach, esophageal, liver, pancreatic and colorectal carcinogenesis.

The effect of carotenoid supplementation on precancerous lesions and cancer incidence has been investigated in numerous clinical trials in the last two decades. Smaller trials in human suggest that antioxidants can reduce colorectal epithelial cell proliferation. Vitamin A, combined with ascorbic acid,  $\alpha$ -tocopherol, furthermore selenium caused a significant reduction in the labeling index of the upper colonic crypts and the decrease in polyp recurrence in colorectal adenoma patients. Polyp formation could not be inhibited in larger full-scale trials.

Prospective studies, including the particularly conclusive Basle study, Finnish cohort study, demonstrated that lower levels of retinol and carotene at entry were associated with a significantly increased relative risk for bronchus cancer and all other cancers.

## 2. AIMS

The aims of the study were: 1. To prove further intracellular effects of carotenoids on the membrane-bound ATP-dependent energy systems in a well identified animal experiment and 2. To measure serum levels of carotenoids (vitamin A, lutein, zeaxanthin,  $\alpha$ -,  $\beta$ -carotene,  $\alpha$ -,  $\beta$ -cryptoxanthin) with or without the presence of vitamin A activity in patients with different gastrointestinal acute and chronic inflammations, precancerous and malignant diseases.

2.1. The investigation of NSAID induced gastric mucosal damage in Na-salicylate and IND treated rats

2.1.1. Gastric mucosal damage (number and severity of mucosal lesions) and changes in gastric secretory responses

2.1.2. To check the energetic changes in gastric mucosa

2.1.3. The investigation of the protective effects of vitamin A and  $\beta$ -carotene in IND-induced ulcer model

2.1.3.1. The effect of vitamin A and  $\beta$ -carotene on number, severity of gastric mucosal lesions and gastric secretory responses

2.1.3.2. Vitamin A and  $\beta$ -carotene induced changes in gastric mucosal energy systems

2.2. Changes of serum carotenoid levels in different GI diseases (human observations)

Few and contradictory data can be found in the literature related to the possible changes of serum levels of carotenoids in different human diseases, therefore we checked these parameters in the following GI pathological conditions:

2.2.1. GI inflammatory diseases

2.2.1.1. Gastritis (with and without *Helicobacter pylori* colonisation)

2.2.1.2. Crohn's disease

2.2.1.3. Ulcerative colitis

2.2.2. Patients with hepatitis and cirrhosis (HCV and alcoholic)

2.2.2.1. Patients with alcoholic hepatitis

2.2.2.2. Patients with HCV hepatitis

2.2.2.3. Patients with alcoholic cirrhosis

2.2.3. Colorectal polyps (with different histological classification)

2.2.4. Malignant GI diseases

2.2.4.1. Esophagus carcinoma

2.2.4.2. Gastric adenocarcinoma

2.2.4.3. Pancreas adenocarcinoma

2.2.4.4. Hepatocellular carcinoma

2.2.4.5. Colorectal adenocarcinoma

### 3. METHODS

3.1. The animal experiments were carried out random on both sexes of CFY (Sprague-Dawley) strain rats (LATI, Gödöllő, Hungary), weighing 180 to 210 body weight. The animals were fasted 24 hr before the experiments, but water was allowed ad libitum.

The animals were separated into different groups and went over different surgical procedures and pharmacological treatments. The observation periods were done at 4 h in all groups of animals, meanwhile the different surgical interventions and the intragastric and systemic treatments were done at 0 hr of the observations. The following experimental groups were created:

3.1.1. group A: sham-operation (laparotomy) was carried out (control group);

3.1.2. group B: pyloric ligation was carried out alone;

3.1.3. group C: pyloric ligation was done, the animals received 2 ml saline solution intragastrically (i.g.);

3.1.4. group D: the treatment of animals was the same that in group B, however they received i.g. 200 mg/kg sodium salicylate dissolved in 2 ml of 150 mmol/L HCl;

3.1.5. group E: the rats went over pyloric ligation (as in group B) and received 2 ml saline solution i.g. and furthermore were treated with 20 mg/kg indomethacin s.c. The animals received saline solution+indomethacin treatment immediately after surgery;

3.1.6. group F: animals received 20 mg/kg indomethacin (at the onset of observations) without any surgical intervention;

3.1.7. group H: the treatment of animals was the same as in group F and they received vitamin A or  $\beta$ -carotene (in doses of 0.1-1.0-10 mg/kg) per os.

Animals in all groups received 2 ml saline solution subcutaneously to protect them from dehydration. The animals were sacrificed at 4 h after the surgical procedure and the pharmacological treatments, when the gastric secretory responses (volume and acid output) and gastric mucosal lesions were noted. The biochemical observations were carried out from the homogenate of gastric mucosa at the end (4h) of the observations.

The tissue levels of ATP, ADP, AMP, and lactate were measured enzymatically (Boehringer Ingelheim, Germany), while tissue level of cAMP by RIA (Beckton-Dickinson, Orangeburg, USA). The protein content was assayed by the method of Lowry et al (60). The ratio of ATP/ADP, the values of adenylate pool (ATP+ADP+AMP) and the "energy charge" ((ATP+0.5 ADP) / (ATP+ADP+AMP)) were calculated (88).

The gastric secretory responses (volumes, acid output) were measured in the 4h pyloric ligated rats. The volume of gastric secretion was expressed in ml/100 g body weight / 4 h while the gastric acid secretion in  $\mu$ E/100 g body weight / 4 h. The number of acute

haemorrhagic mucosal lesions was counted, while the severity was evaluated by a semiquantitative scoring system, described previously (88) in a blinded fashion without knowledge of previous treatment. A computerized planimetric method was also used in part of the study to confirm the evaluation of lesions and a close correlation ( $r = 0.92$ ,  $p < 0.01$ ) was found between these two methods (118). The gastric mucosal lesions were expressed as number and severity (means $\pm$  SEM). The parametric results were mathematically evaluated by Anova test, while semiquantitative results (ulcer severity) by Mann-Whitney's test.

3.2. Human observations: carotenoids in human serum samples were measured with high pressure liquid chromatography (HPLC) method (25). We measured the serum level of vitamin A, lutein, zeaxanthin,  $\alpha$ - and  $\beta$ -cryptoxanthin,  $\alpha$ - and  $\beta$ -carotene.

The results were given in  $\mu$ mol/liter. The results of 47 healthy persons (24 males, age: 50 $\pm$ 12 years, 20 females, age: 49 $\pm$ 10 years) were used as control values in the statistical analysis. The results were expressed as means $\pm$ SEM. Anova test was used for the statistical evaluation. The serum levels of patients with the following GI diseases were checked:

3.2.1. Patients with GI inflammatory diseases:

3.2.1.1. 35 patients with gastritis (among them 24 with *Helicobacter pylori* colonisation) (20 males, age: 37 $\pm$ 15 years, 15 females 36 $\pm$ 12 years);

3.2.1.2. 49 patients with an established diagnosis of CD (21 males, 28 females, their age ranged from 17 to 51 years; means  $\pm$  SD: 27.4 $\pm$ 13.4 years) were included in this study. 12 newly diagnosed patients were followed up through one year. The duration between the onset of the symptoms and the measurement was 4.7 $\pm$ 3.8 years. The site of involvement was the small bowel only in 20 patients, the large bowel only in 7 and both in 22. There were no patients with any previous history of intestinal resection. All of the patients were free of any nutritional treatment, non of them was receiving vitamin A supplements. The Crohn's disease activity (CDAI) index was higher than 150 in 22 patients and lower in 28 patients (CDAI: Best et al, Gastroenterology 70:439-44, 1970). The majority of them were outpatients and maintenance therapy was given them.

3.2.1.3. 35 patients years with ulcerative colitis (17 males, age: 35 $\pm$ 15 years, 18 females age: 33 $\pm$ 12);

3.2.2. Patients with hepatitis and cirrhosis

3.2.2.1. 8 patients with alcoholic hepatitis (8 males, age 38 $\pm$ 10 years);

3.2.2.2. 75 patients with chronic C hepatitis (45 males, age 42±14 years, 30 females age: 38±12 years);

3.2.2.3. 25 patients with alcoholic liver cirrhosis (17 males, age 40±15 years, 8 females age: 38±12 years);

3.2.3. Patients with adenomatous colorectal polyps (59 patients, 35 males, age: 59±15 years, 24 females, age 62±13 years): 39 patients had tubular adenoma (10 with mild, 16 with moderate, 13 with severe dysplasia), 11 patients had villous adenoma (4 with mild, 4 with moderate, 3 with severe dysplasia) and carcinoma was found in the removed polyp in 9 cases. The patients were separated into 4 groups depending on the grade of dysplasia.

3.2.4. 98 patients with different types of gastrointestinal malignancy were checked. The majority of them were in a good general condition. The patients were divided into smaller groups depending on the localisation of their disease.

3.2.4.1. 8 patients with esophagus carcinoma (cc. planocellulare) (8 males, age: 60±10 years);

3.2.4.2. 21 with gastric cancer (adenoc.) (16 males, age 64±12 years, 5 females age: 68±10 years);

3.2.4.3. 10 patients with pancreas adenocarcinoma (6 males, age 56±11 years, 4 females age: 63±9 years);

3.2.4.4. 15 with hepatocellular carcinoma (8 males, age 60±8 years, 7 females age: 57±13 years);

3.2.4.5. 44 patients with colon adenocarcinoma (26 males, age 66±16 years, 18 females age: 65±14 years);

The diagnosis was based on clinical, laboratory, endoscopic, radiographic and histological findings. The histopathological diagnosis was based on the World Health Organisation (WHO) classification (47), the histopathologists in the two medical centres were blinded to the serum results.

## 4. RESULTS

### 4.1. Animal experiments

The gastric secretory responses were unchanged in pylorus-ligated rats without vs. with intragastrically applied 2 ml saline solution (group B vs. group C). When the animals received sodium-salicylate (group D) intragastrically then the volume of gastric secretory volume increased ( $p<0.001$ ) meanwhile the gastric acid output decreased significantly ( $p<0.001$ ). No significant changes were found in gastric secretion in pylorus ligated plus indomethacin (group E) vs. only pylorus-ligated rats (group B), however, gastric ulceration was found in group E. The gastric secretory response did not modify the indomethacin induced gastric ulceration (group E vs. group F) with or without pyloric ligation.

Vitamin A and  $\beta$ -carotene did not influence the gastric secretory responses. After the co-treatment of IND+vitamin A or  $\beta$ -carotene (group H) the number and severity of IND-induced mucosal injury decreased dose dependently.

These results clearly indicate both sodium salicylate and indomethacin produce gastric mucosal injury in the same extent, however the changes in the gastric secretory responses (volume, acid output) are completely different. Furthermore the gastric secretory responses (volume and acid output) are the same in only pylorus-ligated (group B), pylorus-ligated plus intragastrically 2 ml saline solution treated (group C) rats than those in pylorus-ligated plus intragastrically saline plus indomethacin treated group (group E). These observations prove that the changes in the gastric secretory responses (volume, acid output) can be separated from the development of drug-induced mucosal damage (at least under these experimental circumstances).

The gastric mucosal ATP decreased significantly in the group B ( $p<0.001$ ), C ( $p<0.001$ ), D ( $p<0.001$ ), E ( $p<0.001$ ) and F ( $p<0.001$ ). The gastric mucosal ADP increased in the groups B ( $p<0.001$ ), C ( $p<0.001$ ) and F ( $p<0.001$ ), while it's value decreased in groups D ( $p<0.001$ ) and E ( $p<0.001$ ). The gastric mucosal AMP was decreased in all (B,C,D,E,F) groups. The gastric mucosal cAMP was also decreased in all (B,C,D,E,F) groups. The values of adenylate pool (ATP+ADP+AMP) was decreased in all (B,C,D,F) groups. The values of energy charge are between 0.4 and 0.5. The ratio of ATP/ADP decreased significantly in group B ( $p<0.001$ ), C ( $p<0.001$ ), E ( $p<0.001$ ) and F ( $p<0.001$ ), meanwhile it's value was increased significantly in group D.

After the co-treatment of IND+vitamin A (0.01-0.1-1.0-10.0 mg/kg body weight) or  $\beta$ -carotene (0.01-0.1-1.0-10.0 mg/kg) (group H), the tissue level of ADP decreased dose-dependently, meanwhile the tissue level of ATP, ATP/ADP, cAMP, AMP increased significantly and dose-dependently, however the adenylate pool showed an increasing tendency by the

administration of increasing dosage of vitamin A and beta-carotene. The value of energy charge did not change significantly by the application of vitamin A and beta-carotene.

#### 4.2. Human observations

##### 4.2.1. Patients with inflammatory GI diseases.

Significant changes were not found between the results of healthy volunteers and patients suffering from gastritis with or without Helicobacter pylori colonisation.

Five of the seven measured carotenoids were depleted in CD patients, the serum levels of vitamin A, lutein, zeaxanthin,  $\alpha$ - and  $\beta$ -carotene were significantly lower in patients than in the control group (zeaxanthin:  $p < 0.001$ ; vitamin A, lutein,  $\alpha$ - and  $\beta$ -carotene:  $p < 0.01$ ).

The serum level of  $\alpha$ - and  $\beta$ -cryptoxanthin showed a slight, but not significant decrease.

A strong correlation was found between vitamin A level and CDAI ( $r = 0.7$ ). There was no correlation between the duration of symptoms and carotenoid status. We did not find significant differences in the serum levels of carotenoids depending on the site of involvement.

The patients had lower mean serum albumin and cholesterol levels than the controls (not significant), but there was no correlation between these parameters and vitamin A level.

We found a significant decrease in the serum level of vitamin A ( $p < 0.001$ ) and  $\beta$ -carotene ( $p < 0.001$ ) in patients with ulcerative colitis, a moderate, but not significant decrease in the serum level of lutein and zeaxanthin, all of the other checked carotenoids were in the normal range. There was no correlation between the duration of symptoms and carotenoid status. We did not find significant differences in the serum levels of carotenoids depending on the site of involvement. Neither in patients with ulcerative colitis, nor in CD patients were found differences depending on gender.

##### 4.2.2. Patients with liver cirrhosis, HCV and alcoholic hepatitis

Significant decrease of vitamin A was found in the sera of patients with alcoholic cirrhosis ( $p < 0.01$ ) and HCV hepatitis ( $p < 0.01$ ) from that of control. The serum levels of other carotenoids did not change significantly. The results of patients with alcoholic hepatitis were similar to the control values.

The serum levels of albumin and cholesterol were significantly ( $p < 0.05$ ) lower in patients with liver cirrhosis, a strong correlation ( $r = 0.75$ ,  $p < 0.01$ ) was found between serum vitamin A and cholesterol level.

##### 4.2.3. Patients with colorectal polyp

The serum levels of vitamin A ( $p < 0.01$ ) and zeaxanthin ( $p < 0.01$ ) were significantly lower in patients with different histological types of colorectal polyp, than in the control group. The most significant decrease was found in patients with adenocarcinoma in the polyp.

The serum level of beta-cryptoxanthin and beta-carotene showed a slight, but not significant decrease.

The serum level of lutein and alpha-cryptoxanthin did not differ significantly from the control values.

Carotenoids are fat soluble therefore the absorption of these materials depends on fat absorption. In case of malnutrition or malabsorption the serum carotenoid levels will be also lower than the normal values. To preclude the possibility of malabsorption and malnutrition, the body mass index (BMI) of the patients, the serum level of cholesterol, albumin, total protein and haemoglobin were checked. Neither in patients with ulcerative colitis nor in patients with colorectal polyp differed these parameters significantly from that of controls, these parameters did not indicate malabsorption or malnutrition.

##### 4.2.4. Patients with GI malignant diseases

The results of patients with colorectal cancer were similar to that of in patients with colorectal polyp, however the serum level of vitamin A was more depleted in these patients than in patients with colorectal polyp. The serum level of vitamin A was significantly lower in patients with gastrointestinal malignancies except of pancreas adenocarcinoma than in the control group. The reduction was different in certain types of GI malignancies. The smallest decrease was found in patients with pancreas adenocarcinoma and gastric cancer, the largest was found in patients with colon adenocarcinoma. The zeaxanthin level was significantly lower in all of these groups. It decreased most significantly in patients with pancreas adenocarcinoma, the smallest decrease was found in patients with gastric cancer. The serum level of  $\beta$ -cryptoxanthin and  $\beta$ -carotene showed a slight, but not significant decrease in patients with pancreas and colorectal adenocarcinoma, significant decrease was found in not one of the checked diseases. The serum level of lutein,  $\alpha$ -carotene and  $\beta$ -cryptoxanthin did not differ considerably from the control values.

Significant differences were not found between the results of males and females, neither in the control group, nor in the patients with adenoma therefore we did not calculate the results of genders separated. The checked serum parameters did not indicate malabsorption or malnutrition.

## 5. DISCUSSION

Vitamin A and  $\beta$ -carotene prevented dose-dependently the IND-induced gastric mucosal damage in animals.

Our results indicated that these compounds produce dose dependent changes of membrane-bound ATP-dependent energy systems.

Our investigations have shown the deficiency of certain serum carotenoids in different GI diseases.

Plasma retinol levels fall only in case of exhausted liver reserves (vitamin A is stored almost entirely in liver), therefore the screening of serum levels is not the most sensitive marker of vitamin stores but it is a simple and informative method to check vitamin A deficiency.

Hypovitaminosis in CD patients may be induced by several mechanisms: poor intake, malabsorption, bacterial overgrowth of the small intestine and increased requirement of the inflammatory process. Previous studies and the other checked serum parameters, furthermore the correlation between serum vitamin A level and CDAI indicate that our finding is unambiguously a consequence of CD.

However, because many factors are related to the serum concentrations of vitamins, we are cautious in drawing any conclusions. We did not evaluate the vitamin intake in this study, therefore it is rather difficult to determine causal relationships - we could not conclude which of the above mentioned factors was more important in decreasing of carotenoid levels.

Further studies, including the composition of vitamins in the diet and investigation of vitamin absorption are necessary to clarify the vitamin status in CD. These approaches seem to reveal more information to the necessity of intensive nutritional care concerning vitamins and, if necessary, as to the dose of replacement from the clinical point of view.

Although the laboratory parameters of UC patients did not indicate malnutrition, the decrease of carotenoids in the serum of patients is highly likely the consequence of the inflammatory process or poor intake.

The decrease of vitamin A level in patients with HCV hepatitis and liver cirrhosis may be the consequence of impaired storage capacity of the liver.

Significant decrease of vitamin A and zeaxanthin was found dominantly in the serum of patients with different malignant gastrointestinal diseases (except of zeaxanthin in patients with pancreas adenocarcinoma). Similar results were found in cases of colorectal polyps with different histological stages. No significant changes were observed in the serum level of the other carotenoids.

It is rather difficult to determine causal relationships in the present study the based on these results but the decrease in serum level of vitamin A and zeaxanthin may offer relation of cause and effect between their serum levels and development of colorectal polyps and GI cancers in patients. Earlier the decrease of serum level of vitamin A has been emphasised in development of different malignancies (included GI malignancies) in Basle study and other prospective studies. Our study indicated the existence of the same negative correlation between the decrease of vitamin A and zeaxanthin in the patients' sera vs. colorectal polyps and different GI carcinomas.

These observations indicate:

1. The serum level of vitamin A decreases in patients with different histological stages of colorectal polyps, proceeding the progression of colorectal cancers;
2. It was surprising that the serum levels of vitamin A precursors ( $\alpha$ - and  $\beta$ -carotene) were normal in the patients with GI cancer and colorectal polyps, when the serum level of vitamin A decreased significantly. At this moment we have no explanation why these provitamins are not able to transform into vitamin A (disorders of enzymatic transformation or decrease of vitamin A binding capacity, etc.). Probably these events are in the background why vitamin A decreased in the sera of patients with adenoma and GI cancer.
3. Accepting the fact that colorectal polyps represent precancerous state for colon tumor and the same clinical correlation exists between carotenoids vs. colorectal diseases (polyps and cancers), furthermore between carotenoids vs. the other malignant GI diseases, the decrease of vitamin A and zeaxanthin may be one of the nutritional promoting compounds in the development of these diseases;
4. Zeaxanthin does not have vitamin A activity, so surprisingly the supposed preventive effect is not only vitamin A activity's consequence.

## 6. NEW RESULTS

1. Vitamin A and  $\beta$ -carotene decreased dose-dependently the number and severity of gastric mucosal lesions.
2. The gastric cytoprotective effect of retinoids produces a dose dependent inhibition on the extent of ATP transformation to ADP, meanwhile it produces an increase in the transformation of ATP into cAMP, indicating their intracellular metabolic effects.
3. The significant decrease of five serum carotenoids have been proved in CD patients (vitamin A, zeaxanthin, lutein,  $\alpha$ -,  $\beta$ -carotene), furthermore a strong correlation between vitamin A level and CDAI.

4. Our investigations have shown the deficiency of certain serum carotenoids in other inflammatory (ulcerative colitis - vitamin A and  $\beta$ -carotene) and premalignant GI diseases (colorectal polyposis-vitamin A and zeaxanthin).
5. Vitamin A and zeaxanthin were depleted in the serum of patients with different GI malignant diseases (esophagus, gastric, pancreas, hepatocellular, colorectal carcinoma) except of zeaxanthin in patients with pancreas adenocarcinoma.
6. The serum levels of vitamin A precursors decreased only in patients with IBD-s significantly.
7. Furthermore vitamin A deficiency has been proved in patients with HCV hepatitis (the similar result of patients with alcoholic cirrhosis is well known as a consequence of exhausted liver reserves).

Further studies, including the composition of vitamins in the diet and investigation of vitamin absorption are necessary to clarify the vitamin status in IBDs. These approaches seem to reveal more information to the necessity of intensive nutritional care concerning vitamins and, if necessary, as to the dose of replacement from the clinical point of view.

Our data support the importance of micronutrients in colorectal polyposis-cancer sequence. We plan to measure the tissue levels of carotenoids in premalignant and malignant GI diseases.

## 7. PUBLICATION

### Original papers

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#### Book chapters

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