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ORIGINAL ARTICLE

Fatty acids in colorectal cancer in adult and aged patients of both sexes

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Summary

Purpose: *Colorectal cancer represents the second most common type of cancer in Serbia. Alteration of lipid metabolism begins early, and can represent a central hallmark in cancer evolution. Fatty acids have various important functions as building components of cell membranes, as signaling molecules in immune responses and also manage the general cancer signaling network. The purpose of this study was to investigate the difference of various fatty acids content between colorectal cancer and adjacent healthy intestinal tissue in adult and aged patients of both sexes.*

Methods: *52 subjects participated in this study. Healthy colon mucosa and tumor tissue samples were obtained from patients previously diagnosed with colorectal carcinoma. Simplified method of Berstad et al was used for direct transesterification of total lipids in tumor and healthy mucosa tissue samples and separations of the methyl esters was carried out using a gas chromatograph equipped with a split/splitless injector and a flame ionization detector.*

Results: *18 0, 18 1 n7, 20 3, 20 4, 20 5, 22 4, 22 5 22 6, SFA, PUFA, n6, n3 and AA/EPA were significantly higher in tumor tissue. On the other hand, 18 1 n9, 18 2, 18 3 n3, MUFA, n6/n3 were significantly higher in healthy tissue.*

Conclusions: *Saturation index (SI) could be a valuable tool to delineate robust immune response and worse prognosis in patients with colorectal cancer. Our study demonstrated significant differences in fatty acid profiles between tumor tissue and healthy mucosa. Parameters, such as gender, age, stage and mucinous component didn't influence altered fatty acid content.*

Key words: *colorectal cancer, fatty acids, healthy tissue, AA/EPA ratio*

Introduction

Incidence and mortality from malignant neoplasms are a constantly growing problem. Estimations for 2018 were 1.8 million new cases of colorectal cancer (CRC), and 881000 deaths worldwide [1]. In Serbia colorectal cancer is the second most common type of cancer in both sexes, with annual incidence of about 4000 and 2500 deaths [2].

Alteration of lipid metabolism begins early, and sometimes can represent a central hallmark in cancer evolution [3]. The ability of cancer cells to synthetize *de novo* cholesterol and fatty acids (FAs), even despite of the plasma lipid levels being high or low, give these cells advantage in growth, survival and drug resistance [4]. When in adequate amounts, FAs have important functions as building components of cell membranes, regulators of their fluidity and as energy reserve, among other important roles [3,5]. There are two kinds of FAs in

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the cell. Exogenous are administered through diet, whereas endogenous require *de novo* synthesis [3]. Cell metabolism and a vast number of signaling cascades that manage various physiological responses can be changed by altered FA composition of cell membrane lipids. It is known the FAs can act as signaling molecules in immune responses, and also manage the general cancer signaling network [6]. Also, *de novo* produced FAs once incorporated into membrane phospholipids changed its permeability and rigidity. The expression of some genes is modulated, in one way or another, by altered FAs metabolism [7]. It was also suggested that n-6 FAs stimulate cancer cell growth, whereas n-3 FAs could possibly inhibit cell growth [7,8]. To determine composition of FAs and their involvement in cell proliferation and apoptosis would be the crucial issue in understanding their role in tumor promotion, progression or regression [3].

Therefore, the purpose of this study was to investigate the difference of various FAs content between CRC tissue and adjacent healthy intestinal tissue, and discuss their possible influence in tumor development and progression. In order to explain the eventual variation in the levels of FAs between cancer and healthy tissue of colon or rectum, we have included patient age, sex and stages of CRC as a potential cause of the mentioned variations.

Methods

Patients and sample collection

Patients included in this study (n=52) had a confirmed colon or rectal cancer diagnosis. They were divided into four groups: middle aged men, older men, middle aged women and older women. All participants went through a set of standard diagnostic procedures and preoperative bowel preparation before collection of healthy and tumor tissue samples during surgery. After resection the bowel was opened and thoroughly rinsed with sterile saline solution. Then, approximately 1g of both tumor tissue and healthy mucosa (at least 15 cm from tumor) were taken and put in sterile 2ml tubes. Sampling of healthy mucosa was conducted in order to confirm the physiological/unchanged status of the tissue with standard hematoxylin & eosin (H&E) staining. Samples were then frozen in liquid nitrogen, transported and stored in -80°C freezer until further processing. Time from surgical devascularization of the colon to emersion of the samples into liquid nitrogen was no longer than 15 min.

Pathological diagnosis and sample preparation and processing

Tissue samples (tumor, healthy mucosa and regional lymph nodes) were fixed in neutral buffered formalin in automated tissue processor for 24 h. After that period, the samples were molded in paraffin blocks, cut into 5μm thick slices and dyed with H&E. All samples were analyzed by an experienced pathologist on Olympus BX41 microscope.

Then, FAs were analyzed: palmitic acid (16 0), palmitoleic acid (16 1), stearic acid (18 0), oleic acid (18 1 n9), vaccenic acid (18 1 n7), linoleic acid (18 2), γ-linoleic acid (18 3 n6), α-linoleic acid (18 3 n3), eicosatrienoic acid (20 3), arachidonic (20 4), eicosapentaenoic acid (EPA) (20 5), adrenic acid (22 4), docosapentaenoic acid (DPA) (22 5), docosahexaenoic acid (DHA) (22 6), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega 6 fatty acids (n6), omega 3 fatty acids (n3), ratio of omega 6 and omega 3 fatty acids (n6/n3), ratio of arachidonic acid and eicosapentaenoic acid (AA/EPA) and stearic to oleic ratio (saturation index, SI).

We used simplified method of Berstad et al [9] for direct transesterification of total lipids in tumor and normal mucosa tissue samples. Approximately 30 mg of tissue sample were directly methylated with 2 ml of 3N MeOH HCl, at 85°C for 1 h. Fatty acid methyl esters (FAME) were extracted with hexane, with subsequent neutralization with KOH in water.

Separations of the methyl esters were carried out using a gas chromatograph (Shimadzu, Kyoto, Japan), equipped with a split/splitless injector and a flame ionization detector. The methyl ester separation was carried out on capillary column RTX 2330 column (60 m x 0.25 mm with a 0.20 μm film) (RESTEK, Bellefonte, PA, USA) using helium as the carrier gas. The injector and detector temperature were set at 220°C and 260°C , respectively. The temperature of the column was initially set at 100°C for 5 min, then increased to 175°C at a rate of 10°C / min, increased one more time at the rate of 3°C /min to 220°C and held at this temperature for 35 min. Analysis was performed in duplicate for each sample. Each FA was identified according to the retention time of that in a PUFA-2 standard mixture (Sigma-Aldrich, St. Louis, MO). The content of FA was expressed as percentage of total fatty acids as previously described [10].

Ethical statement

Prior to study, all participants signed the informed consent form. Blood samples, healthy colon/rectum tissue and tumor tissue were collected in accordance with the Ethics Committee approval (Office for Human Research Protections, University Medical Center Zvezdara, Belgrade, Serbia).

Statistics

Data was analyzed using SPSS software version 21 (IBM Corp. Released 2011. IBM SPSS Statistics for Macintosh, Version 22.0. Armonk, NY: IBM Corp.). Descriptive statistics (frequencies, ranges, means and standard deviations) were used to analyze the patient characteristics. The normality of the data distribution was determined using Kolmogorov- Smirnov test. T-test for independent samples, T-test for paired sample and One-Way ANOVA were used for comparison of normally distributed variables. Wilcoxon Signed Rank Test,

Mann-Whitney U test and Kruskal Wallis nonparametric ANOVA were used for comparison of variables without normal distribution. P value ≤0.05 was considered as the significant level of difference.

Results

Data from 52 patients were analyzed. Twenty-seven (52%) patients were male and 25 (48%) female, with mean age of the entire study group 65.79 ± 9.95 years. According to age we divided all subjects into two groups: middle-aged (≤65 years) and older patients (>65 years). Taking age and sex into consideration, our patients were classified into four groups: middle-aged men (59.21 ± 6.67), older men (73.08 ± 6.22), middle-aged women (58.43 ± 7.61) and older women (74.91 ± 5.28). Histologically all patients had CRC adenocarcinoma. Characteristics of the studied population are shown in Table 1.

Preoperatively, standard blood samples were taken from all patients and concentrations of serum cholesterol, triglycerides, HDL and LDL cholesterol are shown in Table 2.

Concentrations of FAs in tumor tissue and adjacent healthy tissue are shown in Table 3. 18 0, 18 1 n7, 20 3, 20 4, 22 4, 22 5 22 6, SFA, PUFA, n6, n3 and AA/EPA were significantly higher in tumor tissue (p=0.000). 20 5 was also higher in tumor tissue (p=0.009). On the other hand, 18 1 n9, 18 2, 18 3 n3, MUFA, n6/n3 were significantly higher in healthy tissue (p=0.000).

We also compared FAs content between male and female patients in tumor and healthy tissues (results not shown). Only oleic acid was significantly higher in males' healthy mucosa than in females (p=0.033), and monounsaturated FAs were lower in females' healthy mucosa (p=0.021). All other FAs didn't differ in healthy and tumor tissues between males and females.

When we compared FAs content between middle-aged and older patients in healthy and tumor samples, only palmitoleic, stearic and linoleic acids showed significant difference in healthy mucosa samples. There were no significant differences in other FAs in healthy tissue between middle-aged and older patients; no tumor samples showed significant differences in any examined FA (results not shown). There were no significant differences in FA content between any of the four formed gender-age groups in tumor tissue, whereas in healthy mucosa there were significant differences in 5 FAs: 18 0, 18 2, MUFA, PUFA, n6 (results not shown).

Tumor grade didn't influence significantly the contents of FAs, except for palmitoleic acid, which

Table 1. Characteristics of the studied population

Gender		*n (%)*
Male		27 (52)
Female		25 (48)
Age	*n (%)*	*Mean years ± SD (p value)*
Study group	52 (100)	65.79 ± 9.95
Middle-aged pts	28 (53.8)	58.82 ± 7.03 (0.00)$^{A\varphi}$
Older pts	24 (46.2)	73.92 ± 5.76 (0.00)$^{B\varphi}$
Middle-aged men	14 (26.9)	59.21 ± 6.67 (0.00)^{A¶}
Older men	13 (25)	73.08 ± 6.22 (0.00)^{B¶}
Middle-aged women	14 (26.9)	58.43 ± 7.61 (0.00)^{A¶}
Older women	11 (21.2)	74.91 ± 5.28 (0.00)^{B¶}
T stage		*n (%)*
T1		4 (7.7)
T2		11 (21.2)
T3a		7 (13.5)
T3b		21 (40.4)
T3c		8 (15.4)
T4		1 (1.9)
Tumor localization		*n (%)*
Right colon		18 (34.6)
Left colon		17 (32.7)
Rectum and anal canal		17 (32.7)

$^{\varphi}$ independent samples T-test; ¶ One Way ANOVA; Different superscript letters indicate statistically significant differences among the groups

Table 2. Serum lipid levels

Serum lipids	*Serum level (mean and standard deviation values)*				
	young males	*old males*	*young females*	*old females*	*p value*
Cholesterol (mmol/l)	4.6469 (1.1522)^{AB}	3.9250 (1.0418)^{A}	5.1000 (1.5389)^{B}	4.9424 (1.0677)^{B}	**0.001**¶
Triglycerides (mmol/l)	1.5125 (0.7682)^{A}	1.1778 (0.4043)^{A}	2.2600 (1.4456)^{B}	1.4939 (0.6368)^{A}	**0.004**§
HDL (mmol/l)	1.0491 (0.2376)	1.0178 (0.2871)	1.0460 (0.3178)	1.1864 (0.3561)	>0.05¶
LDL (mmol/l)	2.9188 (1.0275)^{AB}	2.3806 (0.8611)^{A}	2.8300 (1.3211)^{AB}	3.0848 (0.9595)^{B}	**0.032**¶

p values refer to the comparisons among the four gender-age groups within each parameter separately(§ Kruskal Wallis H; ¶ One Way ANOVA). Different superscript letters indicate statistically significant differences among the groups. Bold numbers indicate statistical significance.

showed significant difference (p=0.018) between grade 1 and grade 3, but not between grades 1 and 2 and 2 and 3 (Table 4).

Comparison between FA composition in lower (0-50%) and higher (50-100%) mucinous component groups didn't show significant differences in any measured FA (results not shown). Also, FAs contents didn't differ when compared to modified Astler-Coller stages (results not shown).

Stearic to oleic ratio (Saturation Index, SI) was analyzed. There were no significant differences between tumor and healthy tissue, when they were analyzed for gender, age, tumor grade, mucinus component and modified Astler-Coller stage. However, when compared to peritumor lymphocytic response, there was significant difference in SI value between groups with no response and marked response, with higher values in the latter group (p=0.024) (Table 5). Also, SI was higher in the group with no venous invasion compared to the group with marked venous invasion (p=0.002) (Table 6).

Discussion

Saturated fatty acids (SFAs)

Enhanced lipogenesis is associated with development of tumors [11, 12]. Increased activity of fatty acid synthase (FASN) leads to an abundancy of saturated fatty acids (SFAs), which are then incorporated into the membrane phospholipids (PHLs). This accumulation of SFAs lowers the cell membrane's fluidity, making them less susceptible to free radicals and penetration of therapeutics [13]. In 2013 Zhang et al showed elevated presence of stearic acid in cancer tissue, but without changes in shorter chain SFAs (myristic and palmitic acid) [14]. These results are in accordance with our findings.

Palmitic acid, a long chain FA, which role is in energy storage, didn't differ between healthy and tumor tissue in our study. This matches with results of previous research [15], which studied FA profile in azoxymethane induced colorectal tumor

Table 3. Differences in FA levels between tumor and adjacent healthy tissue

Fatty acid	*Tissue sample*				
	Healthy mucosa (n=52)		*Tumor mucosa (n=52)*		*p value*
	Mean (%)	*Standard deviation (%)*	*Mean (%)*	*Standard deviation (%)*	
16 0	21,9992	1,9022	22,2856	1,6396	>0.05¶
16 1	2,8590	1,7488	2,9334	1,5199	>0.05¶
18 0	8,2536	3,2279	10,9093	2,2786	**0.000¶**
18 1 n9	32,4104	6,2650	25,8700	3,9382	**0.000¶**
18 1 n7	2,7491	2,7203	3,0897	1,8469	**0.011§**
18 2	21,9036	3,3823	19,1348	2,9437	**0.000¶**
18 3 n6	0,1000	0,2117	0,1160	0,2044	>0.05§
18 3 n3	0,2498	0,1304	0,1340	0,1004	**0.000¶**
20 3	0,8825	0,3452	1,7051	0,9955	**0.000§**
20 4	6,3274	3,1998	10,3003	2,7010	**0.000¶**
20 5	0,1980	0,1578	0,2463	0,1362	**0.009¶**
22 4	0,7162	0,3038	1,2023	0,443	**0.000¶**
22 5	0,3422	0,1340	0,5697	0,6810	**0.000§**
22 6	0,7988	0,4217	1,5073	0,6535	**0.000¶**
SFA	30,2528	2,7269	33,1948	2,1142	**0.000¶**
MUFA	38,0184	6,7331	31,8932	4,1679	**0.000¶**
PUFA	31,5185	5,5299	34,9158	3,3675	**0.000¶**
n6	29,9298	5,1622	32,4585	3,1233	**0.000¶**
n3	1,5887	0,5938	2,4573	1,1054	**0.000§**
n6/n3	20,6419	6,5965	15,1415	5,9387	**0.000¶**
AA/EPA	43,4658	31,7646	51,4524	22,3807	**0.000§**

p values refer to the comparisons between the two groups within each fatty acid separately. (§Wilcoxon sign rank test; ¶T test for paired samples). Bold numbers denote statistical significance.

in experimental animals. Also, other authors who investigated palmitic acid intake in food and its content in erythrocytes and plasma, didn't find any relationship with CRC [16-19]. When we took age, gender, tumor grade or stage, or mucinous component of the tumor into consideration, none of these factors influenced the concentration of palmitic acid. We only found significant difference in palmitic level concertation between right colon and rectal tumor samples, where right colon tumors had significantly higher levels; despite being saturated FA, which content are increased in tumor

Table 4. Differences in FA between tumor grades

Fatty acids	*Tissue samples*			
	Tumor tissue			*p value*
	grade 1 *mean (std. dev.) (%)*	*grade 2* *mean (std. dev.) (%)*	*grade 3* *mean (std. dev.) (%)*	
16 0	21.7694 (0.7803)	22.3587 (1.8007)	22.8593 (1.0444)	>0.05¶
16 1	4.2087 (2.3448)^{A}	2.6790 (1.1724)^{AB}	2.4996 (1.1038)^{B}	**0.018¶**
18 0	11.0383 (1.8615)	11.0319 (2.3640)	8.8870 (1.7471)	>0.05¶
18 1 n9	25.5965 (3.5990)	25.5542 (3.8397)	30.9022 (3.9852)	>0.05¶
18 1 n7	4.1127 (3.5983)	2.9278 (1.2073)	2.1794 (0.5479)	>0.05¶
18 2	17.1763 (1.7248)	19.4369 (3.0321)	20.9815 (2.3665)	>0.05¶
18 3 n6	0.0776 (0.0377)	0.1289 (0.2314)	0.0598 (0.0427)	>0.05§
18 3 n3	0.1128 (0.0772)	0.1370 (0.1082)	0.1577 (0.0413)	>0.05§
20 3	1.6435 (0.4342)	1.7553 (1.1113)	1.2212 (0.1206)	>0.05§
20 4	10.5915 (1.7859)	10.4585 (2.8297)	7.3170 (1.7025)	>0.05¶
20 5	0.2807 (0.1385)	0.2458 (0.1378)	0.1495 (0.0826)	>0.05¶
22 4	1.2213 (0.2728)	1.2041 (0.4913)	1.1213 (0.0970)	>0.05¶
22 5	0.5193 (0.1331)	0.5870 (0.7752)	0.4906 (0.1066)	>0.05§
22 6	1.6514 (0.8236)	1.4949 (0.6303)	1.2405 (0.4755)	>0.05¶
SFA	32.8077 (1.7228)	33.3906 (2.1557)	31.7463 (2.6280)	>0.05¶
MUFA	33.9179 (3.6979)	31.1610 (4.0869)	35.5812 (3.6057)	>0.05¶
PUFA	33.2745 (3.4773)	35.4484 (3.3016)	32.7392 (1.8312)	>0.05¶
n6	30.7103 (2.7479)	32.9837 (3.1453)	30.7008 (1.3192)	>0.05¶
n3	2.5641 (0.9982)	2.4647 (1.1650)	2.0383 (0.6264)	>0.05§
n6/n3	13.5016 (5.6195)	15.4577 (6.1723)	15.8448 (3.9123)	>0.05¶
AA/EPA	44.2071 (17.6360)	52.7881 (23.6174)	55.3802 (18.8931)	>0.05¶

§Kruskal Wallis H; ¶One Way ANOVA. One bold number denotes statistical significance. One bold number denotes statistical significance

Table 5. Values of SI in peritumor lymphocytic response groups

	Peritumor lymphocytic response			
	No response	*Mild to moderate*	*Marked response*	*p value*
SI (mean ± SD)	0.3689 (0.1482)^{A}	0.4467 (0.1111)^{AB}	0.5244 (0.1007)^{B}	0.024¶

SI: saturation index; ¶One Way ANOVA, Tukey B *post hoc* test, Different superscript letters indicate statistically significant differences among the groups.

Table 6. Values of SI in venous infiltration groups

	Venous infiltration		
	Not present	*Present*	*p value*
SI (mean ± SD)	0.4484 (0.1173)	0.1706 (0.0371)	0.002φ

SI: saturation index; φ independent T-test samples

tissue, it seems that palmitic acid content has no influence in etiology or progression of CRC.

Compared to the study of Neoptolemos et al [15], we also found significantly higher stearic acid in CRC tissue than in healthy mucosa (p=0.000), although other authors found no difference in stearic acid concentrations [20].

Obviously, in our study, stearic acid content contributed to increased level of SFAs in tumor tissue when compared to healthy mucosa. However, previous results concerning the role of SFAs in CRC development are not convincing. Kondo and colleagues [21] found decreased level of stearic acid, while Zhang et al [14] showed a 50% increase in this FA in cancer tissue, without changes in palmitic acid content. On the other hand, The Singapore Chinese Health Study (2017) presented no differences in both mentioned acids in CRC [22]. However, a review has been published which showed that stearic acid downregulates the expression of antiinflammatory genes (IκBα) and increase the plasma concentration of MCP-1 proinflammatory cytokines [23]. Nevertheless, the SFA changes in CRC may suggest that the products of FA synthase activity may serve as substrates for production of other long chain saturated FAs, for which has been shown recently that may serve as biomarkers of CRC [24]. Taken together, our results along with earlier studies support the hypothesis that saturated FAs are probably important in etiology, and with greater certainty, in the development of CRC. Also, cancer cells promote the saturation of their membranes and modulate their biophysical properties. As saturated lipids are less susceptible to lipid peroxidation, this shift may protect cancer cells from lipid peroxidation–mediated cell death. It also alters the membrane dynamics and affects the uptake and efficacy of chemotherapeutics.

Monounsaturated fatty acids (MUFAs): palmitoleic and oleic acids

Our results showed significantly higher amount of MUFAs in healthy tissue compared to cancer tissue.

No significant difference was detected in palmitoleic acid, monounsaturated fatty acid (MUFA), between tumor and healthy tissue. However, interestingly, it was the only one FA that differed between tumor grades. It was significantly lower in grade 2 and 3, when compared to grade 1 CRC. Palmitoleic acid, as well as its elongated product vaccenic acid are markers of lipogenesis [25,26]. Decrease of 16:1 n-7 FA in advanced stages of CRC suggests increased consumption for energy production, so characteristic for higher grades, which will eventually lead to cachexia.

Another MUFA, oleic acid, one of the most abundant FA in human tissues and the major fatty acid in olive oil, responsible for its beneficial effects, significantly decreased in CRC tissue when compared to healthy control. The influence of oleic acid to malignant cells, despite its abundance of evidence, still remains unsatisfactorily unclear. It is well known that oleic acid exerts antiinflammatory effect, thus reducing the risk of cardiovascular disorders, insulin resistance, oxidative stress, atherosclerosis and inflammation, the last being implicated in carcinogenesis [23]. However, one study showed that colon tumor growth is promoted by oleic acid probably by mechanisms that include an increase in FA oxidation and disturbance of membrane enzymes [27]. On the other hand, the majority of research provided evidence that oleic acid specifically regulates cancer-related oncogenes. The work of Colomer and Menendez showed that oleic acid exposure may suppresses HER2 at the transcriptional level by up-regulating the expression of the Ets protein PEA3, a DNA-binding protein that specifically blocks HER2 promoter activity in breast, ovarian and gastric cancer cell lines [28]. Also, it has been proved that oleic acid induces apoptosis and cell differentiation by downregulation of COX-2 which is followed by a reduction in Bcl-2 expression [29]. Bioinformatic analysis of signaling pathways confirms that oleic acid represents a major supplier for full reconversion of cancer cell into healthy intestinal cells in Caco-2 cell line [30]. Taken together, the significant decrease of oleic acid content in CRC tissue in our study supports the hypothesis that cancer cells shift into different biosynthetic pathways to generate a diverse cellular pool of lipid species with distinct functions required for tumor growth, decrease, among other outcomes, its protective role in tumor development [31].

PUFAs, n-6, n-3 and n-6/n-3 ratio

PUFAs represent FAs which contain more than one double bond in their structure. They are present in all membranes in human cells, thus ensuring a hydrophobic boundary between hydrophilic compartments. There are two families of PUFAs (n-3 and n-6), which generally exert opposite effects. It is widely acknowledged that n-3 PUFAs and their products exhibit antiinflammatory properties, while n-6 show proinflammatory characteristics. It does stay for the majority of results, however there are studies showing different outcomes [32]. Our results will probably contribute to this pool of rather complex and not fully clear functions of PUFAs.

Various studies showed that PUFAs are differently metabolized by healthy and tumor cells;

normal cells by metabolizing PUFAs produce protective lipids, while tumor cells generate toxic hydroperoxy FAs [33,34]. Our study demonstrates increased content of PUFAs in CRC patients, previously showed by Berstad and colleagues in young individuals with CRC [9]. We assume that having multiple double bonds makes PUFAs more susceptible to oxidation, so their rise in tumor tissue leads to increased production of highly proinflammatory products. Even though the increase in EPA and DHA content in our study should be viewed as an adaptive and favourable change in PUFA metabolism in tumor tissue, since being highly oxidizable FAs, their increased content can lead to their proinflammatory activity and possible carcinogenic effect.

Our results showed significantly higher levels of both omega-3 and omega-6 PUFAs in tumor tissue compared to healthy tissue, but omega-6/omega-3 ratio was significantly higher in healthy tissue. Similar results have been reported earlier [9,15,35], however, the importance of n-6/n-3 ratio is not that great, as presumed by many researchers. It is generally believed that lower n-6/n-3 ratio is associated with decreased risk for many disorders, including cancer and this has been confirmed many times. Still, there are studies that challenged this assertion [9,36]. One has to be very cautious when interpreting the results of n-6/n-3 ratio; it may be the source of bias since some n-6 derived oxidation products may in fact have antiinflammatory effects [37]. So, in our opinion, a more reliable indicator of inflammatory status in certain tissue is the AA/EPA ratio. In this study, we showed a significant increase in this ratio which confirms that tumor inflammatory environment is an indispensable factor in the neoplastic process, fostering proliferation, survival and migration of malignant cells.

Another attention should be payed when interpreting changes of distinct FAs in malignant tissue. Humans, like all other mammalians, need essential FAs (linoleic and α-linolenic acids) in order to synthesize other PUFAs. However, PUFAs from these two classes (n-6 and n-3) compete for the same desaturases and elongases, which means that diet can strongly influence the concentration of one or another PUFA classes [38]. This is not the case with arachidonic acid, which is more tightly regulated and thus less varying in tissue. Therefore, the content of arachidonic acid reflects more accurately the proinflammatory status of cancer tissue.

In our study, healthy mucosa had significantly higher levels of linoleic acid compared to tumor tissue. The same result has been shown for α-linolenic acid, which is significantly more present in healthy mucosa than in tumor tissue. The decrease of these essential FAs probably reflects their increased elongation into longer chain FAs in tumor tissue. In n-6 series γ-linolenic acid is unchanged, but the final products, dihomo-γ-linoleic acid, arachidonic acid and adrenic acid, are significantly increased. The same happened in n-3 series; despite the decrease of α-linolenic acid, EPA and DHA are increased in CRC tissue. The proinflammatory status of arachidonic acid is known, but the activity of product of its elongation, adrenic acid, is still largely unknown. A recent study reported that adrenic acid might be an inflammatory enhancer in non-alcoholic fatty liver disease, so in that way, it will boost the inflammation process and subsequently cancer propagation in CRC patients [39].

Interpretation of FA changes in malignant tissue could be rather tricky. The established facts have been revised as the results of various experiments and studies accumulate. α-linolenic acid, a precursor for longer chain PUFAs (EPA and DHA), which are known for their protective effects, showed, in one study using carcinoma cells culture, to induce expression of MEK1 and MEKK1 genes, both drivers of cellular proliferation [40]. Also, n-6 FAs are considered to be proinflammatory. One study showed that linoleic acid metabolite, 13-S HODE, serves as a signaling molecule associated with anticarcinogenic properties [41]. Further on, the study of Das and Madhavi demonstrated that both γ-linolenic acid and arachidonic acid exert tumoricidal action by enhancing free radical generation and lipid peroxidation, and by increasing intracellular concentration of anticancer drugs in human cervical carcinoma cells [33].

Likewise, the changes in concentration of various FAs differ significantly among published results. In one study γ-linolenic acid was proposed as potential biomarker of CRC, because its concentration was altered early in the course of the disease [21]. We have found no changes in content of the same FA. The work of Zhang and colleagues showed marked increase of linoleic n-6 FA in CRC tissue, which content depended on tumor stage [14]. Our study showed no changes in the content of linoleic acid and we established no correlation between its content and tumor stage, just as it had been shown in a previous research [42]. Also, a search for specific biomarker of CRC led to various suggestions. One of the frequently cited, proposed accumulations of palmitic and palmitoleic acids as rather reliable biomarkers of CRC [43]. We found no changes in content of these FAs in CRC tissue of our patients. So, search for a proper and clinically useful lipid biomarker(s) demands consistent and reproducible methodology, in order for the results to be reliably compared.

Serum levels of triacylglycerols, cholesterol, HDL and LDL and tumor tissue FAs

Association between obesity, high level of serum lipids and various malignancies is well established [44]. It also shows that serum lipids have a role in etiology of gastrointestinal cancers [45]. Regarding TG serum levels and CRC, published results are different, with no certain link between TG levels and CRC risk [37]. In our study, only the levels of TG were significantly different between modified Astler-Coller stages A and C, with higher levels in stage A (results not shown). The results possibly reflect tumor consumption of host energy stores. It has also been published that higher low density lipoprotein (LDL) levels enhance intestinal inflammation and CRC progression, possibly through activation of ROS and MAPK signaling pathway [44]. This complies with our findings, where LDL serum levels were significantly higher in M1a stage than in M0 stage (results not shown). We found no correlation between serum TGs and any of the measured FAs, neither in tumor tissue nor in adjacent healthy mucosa; and the other results in our study are not so conclusive.

Stearic to oleic ratio (saturation index, SI)

Cancer development induces profound modifications of the membrane lipid composition, which invariably leads to alterations of membrane physicochemical properties. Among these, changes in membrane lipid composition resulting in membrane fluidity modifications have been widely reported [46]. Membrane fluidity is one of the key parameters for membrane fusion, since it determines the mobility of lipids, proteins and water molecules that cooperate in the reorganization and the assembly required and induced by the membrane fusion. As it is directly dependent on the membrane lipid composition, any modification of the lipid metabolism results in a membrane fluidity change. Membrane fluidity is controlled by multiple factors which include the ratio of saturated to (mono) unsaturated fatty acids in the cell wall, i.e. the saturation index (SI). The stearic to oleic ratio (SI) is known to be reduced in the membranes of some types of neoplastic cells [47,48].

Our results didn't provide differences of SI between examined parameters (gender, age, tumor grade, Astler-Coller stage and mucinous component).

However, to our knowledge this is the first study which investigated the relationship between SI and peritumor lymphocytic response and venous invasion. In our study, we found that the group with marked peritumor lymphocytic response had higher SI values, and the group with absent venous invasion had also higher SI values. Generally, more expressed peritumor lymphocytic response indicates better immune response and better prognosis [49]. Also, venous infiltration is an indicator of more aggressive tumor [50]. When these results are considered, higher SI values in our study groups indicate biologically less aggressive tumor and stronger immune response, leading to possible better prognosis. All things considered, it seems that SI is a promising parameter that needs further investigation in order to establish its diagnostic and prognostic significance.

Conclusion

Our study demonstrated significant differences in FA profiles between tumor tissue and healthy mucosa. Other examined parameters, such as gender, age, tumor grade, tumor stage and mucinous component, didn't correlate with altered FAs content in tumor tissue and healthy mucosa.

Our results are in accordance with some studies and in contrast to others. However, there are certain points which can help clarify the metabolic/lipid changes in CRC tissue and bolster the search for clinically convenient biomarker.

1. Palmitoleic acid didn't differ between tumor tissue and healthy mucosa, however it was the only FA which was significantly different between CRC stages.
2. n-6/n-3 ratio may not be the right indicator of tumor metabolic status and progression; we suggest AA/EPA ratio as more reliable.
3. Arachidonic acid and adrenic acid contents reflect inflammatory status of tumor tissue.
4. Higher SI value indicates biologically less aggressive tumor and stronger immune response.

On the basis of the results from numerous studies conducted so far, and our study, there is sufficient evidence to suggest that changes in FAs profile play a role in CRC development and progression. However, all these studies differ in examined cells (taken from tumor, cell culture, animal models), patients (young/old, male/female), and methodology. A greater sample size with more homogeneous methodology and techniques would result in better understanding of altered lipid metabolism in CRC development, which could lead to improved prevention, diagnosis and treatment of this malignancy.

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Author contributions

This study was designed by S. R. D. L. and A. M. T.; J. T. J. and V. V. Ć. performed the surgery. M. S. M. P. analyzed and described histopathological specimens. T. P. and J. D. M. performed fatty acids analysis. The results were analyzed by J. T. J., V. V. Ć., T. P., M. S. S., A. M. T. and S. R. D. L. The manuscript was written by J. T. J., T. P. and S. R. D. L. All authors approved the final manuscript.

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Conflict of interests

The authors declare no conflict of interests.

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