Neuroendocrinology Letters Volume 39 No. 6 2018
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

Composition of tissue fatty acids of phospholipids depends on tumor localization and disease progression in colorectal patients

Jolanta Bugajska [1], Joanna Berska [1], Diana Hodorowicz-Zaniewska [2], Krystyna Sztefko [1]

1 Clinical Biochemistry Department, IP, Jagiellonian University College of Medicine, Krakow, Poland
2 First Department of Surgery, Jagiellonian University College of Medicine, Krakow, Poland

Correspondence to: Jolanta Bugajska
Clinical Biochemistry Department, IP, Jagiellonian University College of Medicine, Krakow, Wielicka St. 265, Krakow 30-663, Poland
tel./fax: +48126580681 ; e-mail: jola.bugajska@uj.edu.pl

Submitted: 2018-07-02 Accepted: 2018-10-22 Published online: 2018-11-28

Key words: **fatty acids; tissue; phospholipids; colorectal cancer**

Neuroendocrinol Lett 2018; **39**(6):454–458 **PMID:** 30796795 NEL390618A06 © 2018 Neuroendocrinology Letters • **www.nel.edu**

Abstract

OBJECTIVES: Fatty acids play a role in development and progression of colon cancer. The aim of this study was to assess the relation between tissue fatty acids (saturated fatty acids, unsaturated fatty acids, the ratio of C18 to C18:1 - index of fatty acids saturation, SI), colorectal tumor localization and disease progression.
METHODS AND RESULTS: Total of 49 patients (14 with proximal colon, 13 with distal colon and 22 with rectal tumor localization) were studied. One year after surgery 24 patients had the disease progression. Tissue levels of saturated fatty acids and unsaturated fatty acids were measured before surgery by gas-chromatography. These fatty acids were determined in cancerous tissue (CA) and non-cancerous tissue (NCA). The most significant differences in the mean values of fatty acids of phospholipids between CA and NCA in patients with proximal tumor localization were noted. The mean value of C18 was significantly lower while C18:1 was significantly higher in CA as compared to NCA in patients without disease progression ($p<0.02$; $p<0.03$; respectively). SI was significantly lower in CA as compared to NCA only in patients without disease progression ($p<0.02$).
CONCLUSION: Fatty acids of tissue phospholipids' fraction, as well as SI, strongly depend on tumor localization and might be useful as potential markers of the disease progression in colorectal cancer patients.

INTRODUCTION

Colorectal tumors can be located within the proximal or distal part of intestine as well as in the rectum. The risk of developing colon cancer depends on environmental and genetic factors (Bufill 1990). Probability of 5 year survival rate is different for these three locations of the tumor and it is 13% less in patients with tumor located in the proximal part of intestine compared to patients with cancer located in the distal part of intestine (Wong 2010). At present, no tumor marker is known to assess colorectal cancer progression.

Fatty acids play a critical role in several biological processes. The fluidity and permeability of the cell membranes and enzymes activity and membrane receptors activity largely depends on the type and amount of unsaturated fatty acids of phospholipids and esters of cholesterol. The increase of membrane fluidity leads to increase cells metabolism (Cooper 1977). There are many reports in the literature concerning on concentra-

tion the fatty acids level in the blood serum of patients with colorectal cancer, but there is little data about the fatty acids content in colorectal cancer tissue. Proper ratio of saturated and unsaturated fatty acids in membrane phospholipids is essential for permeability and fluidity of the cell membranes. Changes in fatty acids composition may influence on cell structure and function and may have a great impact on the cell proliferation. The levels of C18 and C18:1 in the erythrocytes cell membranes in patients with different types of cancer (breast, prostate, liver, pancreas, colon and lung) were investigated. Lower levels of C18 in patients with cancer and higher levels C18:1 as compared to patients without the cancer, have been demonstrated (Mikirova *et al.* 2004). According to some researchers, the ratio of these two fatty acids (index of fatty acids saturation, SI) in erythrocyte membranes of patients with different types of cancer did not differ significantly from SI for healthy subjects (Mikirova *et al.* 2004; Kelly *et al.* 1990). However, studies Kelly *et al.* (Kelly *et al.* 1990) have shown lower ratio of C18 to C18:1 in the membranes of erythrocytes in patients with colorectal cancer compared with a control group. Very similar data to Kelly in patients with cancer of the gallbladder and patients with cancer of the prostate were observed (Pandey *et al.* 1998; Persad *et al.* 1990). In contrast, Neoptolemos *et al.* (Neoptolemos *et al.* 1991) obtained a higher ratio of C18 to C18:1 in cancerous tissue compared to normal tissue in patients with colorectal cancer.

The aim of this study was to assess the relation between tissue fatty acids (saturated fatty acids, unsaturated fatty acids, SI), colorectal tumor localization and disease progression.

MATERIAL AND METHODS

Forty nine patients with colorectal cancer (26 men and 23 women, mean age 63.5 ± 10.3 years) were selected from patients attending a tertiary care in University Hospital in Krakow, Poland. The clinical diagnosis was based on physical examination, colonoscopy, endorectal USG, abdominal CT, and histopathology of tissue specimens according to Astler-Coller classification. Each patient underwent open surgery in order to remove the tumor. The patients with diabetes mellitus or glucose intolerance assessed based on fasting plasma glucose level and oral glucose tolerance test (OGTT) were excluded from the study. The study was approved by the local Ethical Committee (KBET/14/B/2006, resumed and expanded in 2013). According to UICC/AJCC classification, 2 patients (4.1%) were in stage 0 of the disease, 16 patients (32.7%) in stage I, 5 patients (10.2%) in stage II, 8 patients (16.3%) in stage III and 18 patients (36.7%) in stage IV. In 14 patients (28.6%) the tumor was located in proximal colon, in 13 patients (26.5%) in distal colon and in 22 patients (44.9%) the tumor localization was confined to the rectum. After one year of follow up 25 patients (51%) were in stable condition and the remaining 24 patients (49%) had the disease progression.

19 (38.8%) patients were normal weight (18.5≤BMI<24.99), 22 (44.9%) patients were overweight (25≤BMI<29.99) and 8 (16.3%) patients were obese I (30≤BMI<34.99).

Mucosa samples from malignant and adjacent unchanged tissue were taken from each patient intraoperatively. Samples were frozen at -70°C. Measurement of fatty acids of phospholipids fraction was performed by following steps: 1) extraction of total lipids from tissues homogenate by Folch *et al.* method (Folch, Lees, and Sloane Stanley 1957), 2) separation of lipid fractions using Sep-Pak NH2 columns (Waters, Milford, Mass., USA) (Kaluzny *et al.* 1985), 3) methylation of fatty acids (Kang and Wang 2005; Morrison and Smith 1964) and 4) determination of the fatty acids methyl esters using gas chromatography with Flame Ionization Detector – FID (Agilent Technologies 6890 Network GC System, Wilmington, De., USA) equipped with Agilent J&W HP-88 capillary column (100m, 0.250 mm, 0.20μm) (Bugajska *et al.* 2010). Tissue levels of saturated fatty acids (C12-lauric, C14-myristic, C16-palmitic, C18-stearic) and unsaturated fatty acids (C16:1(n-7)-palmitooleic, C18:1(n-9)-oleic, C18:2(n-6)-linoleic, C20:4(n-6)-arachidonic) were measured. These fatty acids were determined in cancerous tissue (CA) and non-cancerous tissue (NCA). The method was calibrated using the calibration mixture (all fatty acids - Sigma-Aldrich, Steinheim, Germany). The results were expressed as the percentage of total tissue fatty acids.

The sum of saturated fatty acids, the ratio of C18 to C18:1 (SI) and the ratio of C16 to C16:1 were calculated. The data are presented in the form of typical descriptive statistics (mean, SE). The Shapiro-Wilk's test was used to determine the normality of data distribution. To evaluate the differences within each group for every parameter, a repeated measurement one-way ANOVA test was performed. The Tukey test was used to compare groups. Statistical analyses were performed with Statistica 10. The level of statistical significance was established at $p<0.05$.

RESULTS

Among the fatty acids in all colorectal cancer patients without tumor progression the mean value of C18 in NCA was significantly higher as compared to patients with tumor progression (20.9±1.25; 16.0±1.28; respectively; $p<0.04$). In contrast, the mean value of C18:1 in NCA was significantly lower in patients without tumor progression as compared to those with tumor progression (17.5±1.06; 22.08±1.09; respectively; p<0.006). The mean values of calculated parameter (C18/C18:1) in patients without tumor progression were significantly higher as compared to those with tumor progression both in NCA and CA ($p<0.005$; $p<0.02$; respectively) (Fig. 1).

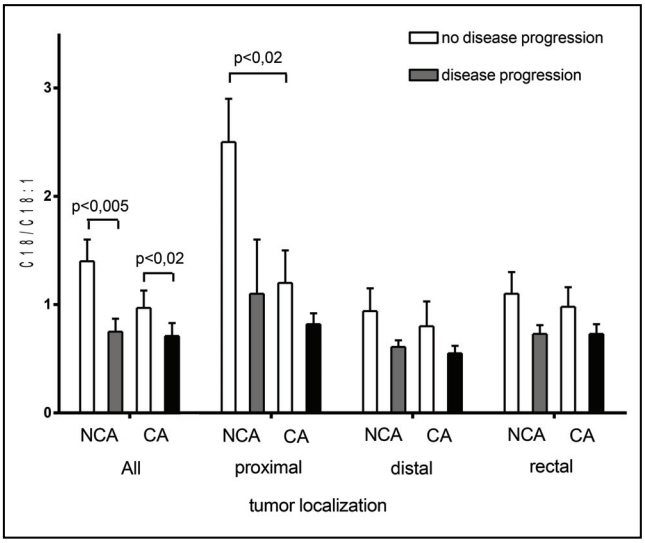

Fig. 1. C18/C18:1 (saturation index – SI) in cancer tissue (CA) and non-cancerous tissue (NCA) in relation to tumor localization and cancer progression.

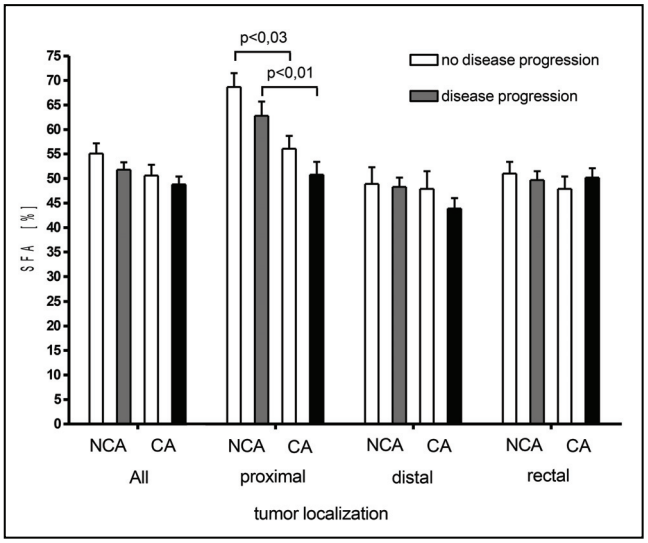

Fig. 2. The sum of saturated fatty acids (SFA= C14+C16+C18), in cancer tissue (CA) and non-cancerous tissue (NCA) in relation to tumor localization and cancer progression.

The most significant differences in the mean values of fatty acids of phospholipids between cancer tissue and non-cancerous tissue in patients with proximal tumor localization were noted. Among saturated fatty acids the mean value of C16 was significantly lower in CA as compared to NCA in patients with disease progression (29.7±1.8; 36.9±2.0; respectively; $p<0.05$), the mean value of C18 was significantly lower in CA as compared to NCA in patients without disease progression (19.9±1.8; 28.0±2.0; respectively; $p<0.02$) and the mean values of SFA were significantly lower in CA as compared to NCA in patients with and without disease progression ($p<0.01$; $p<0.03$; respectively) (Fig.2). In contrast, the mean value of C16:1 was significantly higher in CA as compared to NCA in patients with disease progression (1.5±0.2; 0.5±0.2; respectively; $p<0.01$) and C18:1 was significantly higher in CA as compared to NCA without disease progression (18.2±1.4; 12.9±1.5; respectively; p<0.03) in patients with proximal tumor localization. For distal colon and rectal tumor localization, there were no differences for C18:1, but the mean value of C16:1 was significantly higher in CA as compared to NCA both: in patients with disease progression as well as in patients without disease progression ($p<0.02$-0,04).

SI was significantly lower in CA as compared to NCA only in patients with proximal tumor localization without disease progression ($p<0.02$) (Fig.1). The ratio of C16 to C16:1 was lower in CA as compared to NCA regardless of the tumor localization, but significant difference was observed in patients with disease progression for proximal and distal tumor localization ($p<0.007$; $p<0.002$; respectively) and in patients without disease progression for distal tumor localization ($p<0.04$) (Fig.3).

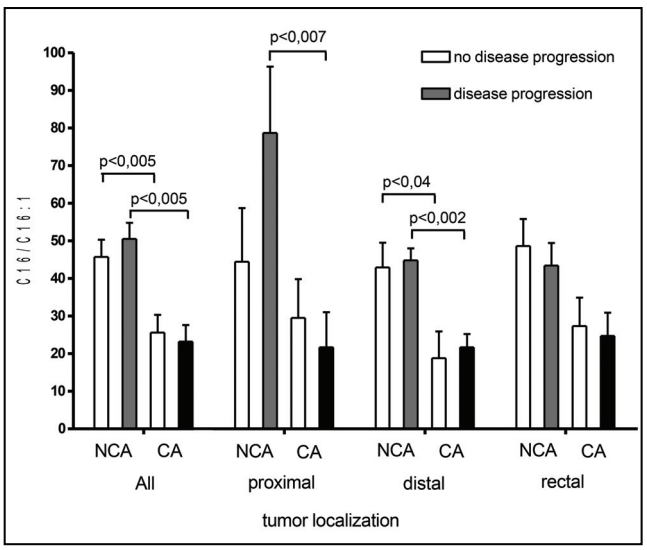

Fig. 3. C16/C16:1 in cancer tissue (CA) and non-cancerous tissue (NCA) in relation to tumor localization and cancer progression.

The mean values of C12, C14, C16 and C18 were similar in CA and NCA for patients with distal colon and rectal tumor localization. There were no significant differences between studied groups regarding the mean values of C18:2 and C20:4, however higher value was noted in CA as compared to NCA in patients with proximal tumor localization independently of the cancer progression (Table 1).

There were no differences in BMI, depending on the tumor localization (proximal tumor localization BMI=25±0.97; distal tumor localization BMI=26.4±0,97; rectum BMI=26.6±0,75).

Tab. 1. Percentage of fatty acids: C18:2; C20:4 in cancer tissue (CA) and non-cancerous tissue (NCA) in relation to tumor localization and cancer progression.

Fatty acid	all patients		Tumor localization					
			proximal colon		distal colon		rectum	
	NCA	CA	NCA	CA	NCA	CA	NCA	CA
	% of total, mean ± SE							
C 18:2	8.5±0.6	8.6±0.6	6.4±1.4	8.2±1.2	10.5±0.7	11.5±0.8	8.1±0.6	7,4±0,6
	9.3±0.8	9.3±0.8	6.4±1.0	8.7±0.9	12.0±1.8	9.0±1.9	9.1±1.0	9,9±1,1
C 20:4	16.5±0.8	16.3±0.8	12.6±1.6	17.4±1.5	16.1±1.8	15.5±1.9	18.1±0.9	16,2±0,9
	16.7±1.0	17.3±1.1	11.0±1.8	15.3±1.6	17.8±1.5	16.1±1.6	19.7±1.3	19.9±1.3

□ – disease progression, ▭ – no disease progression

DISCUSSION

Fatty acids are very important biological compounds which may be associated with different diseases including metabolic diseases such as type 2 diabetes, inflammatory diseases (Calder 2015), depressive disorders (Vareka *et al.* 2012), autism (Sliwinski *et al.* 2006) or are linked with an increased risk of certain cancer. Literature evidences suggest that dietary lipids could play an important role within the context of colorectal cancer tumor localization although inconsistent results are frequently seen (Hodge *et al.* 2015; Mo *et al.* 2018). The diet may contribute to reducing overall colorectal cancer and particularly rectal cancer risk. Dietary saturated fatty acids (stearic acid, palmitic acid) and monounsaturated fatty acids (palmitoleic acid, oleic acid) were more strongly positively associated with rectal than colon cancer (Hodge *et al.* 2015). Dietary polyunsaturated fatty acids may be positively associated with risk early neoplasia in the proximal colon (Mo *et al.* 2018). There are reports in the literature concerning on diet, but there is little data about the fatty acids content in colorectal cancer tissue, therefore the main goal of the present study was to find out the relationship between the fatty acids composition in tissue phospholipids' fraction, tumor localization and disease progression in colorectal cancer patients. Measurement of plasma phospholipids fatty acids is more common and can be considered as the most useful because a correlation exists between dietary fatty acid intake and the proportion of fatty acids in plasma in cancerous diseases and other non-cancerous diseases (Vareka *et al.* 2012; Sliwinski *et al.* 2006). To our knowledge, this is the first report of an association between levels of fatty acids of tissue phospholipids in the cancer tissue and non-cancerous tissue in relation to tumor localization and colorectal cancer progression. Only fatty acids of total lipids from colorectal cancer tissue were investigated (Neoptolemos *et al.* 1991; Fernandez-Banares *et al.* 1996). The levels of cell membrane fatty acids in the colorectal tumors and non-malignant colonic mucosa were compared only in rats (Nicholson *et al.* 1991). In the present study the fatty acids of phospholipids' fraction were studied because they are the most sensitive indicators of fatty acids fluctuations.

The evidence was presented that colorectal cancer cases should be divided into proximal colon cancer, distal colon cancer and rectal cancer by showing differences in physiology and anatomy, environmental carcinogens, genetic mechanism and prognosis between these three cancer location (Li and Lai 2009; Iacopetta 2002). In the large retrospective cohort study in US significantly lower 5-year survival for patients with proximal colon cancer compared to distal colon cancer was demonstrated (Wong 2010). In the present study the biggest changes of fatty acids were observed in patients with proximal tumor localization.

Decreased membrane rigidity is one of the characteristic attribute of malignant cells, resulting in part from the desaturation of stearic acid into oleic acid. The study of Habib *et al.* (Habib *et al.* 1987) demonstrated that treatment with stearic acid inhibits tumor development in rats. The SI in erythrocyte membranes was significantly reduced in the tumor bearing rats, but was normal in tumor-free animals treated with stearic acid. Decrease SI in erythrocyte membranes of patients with prostatic carcinoma and colorectal carcinoma has reported (Kelly *et al.* 1990; Persad *et al.* 1990). Similar findings in the phospholipids' fraction of prostatic tissue of patients with benign and malignant prostatic disease were observed (Chaudry *et al.* 1991). Freeman *et al.* (Freeman *et al.* 2007) and Kositsawat *et al.* (Kositsawat *et al.* 2007) showed that the oleic-to-stearic acid ratio of total lipids in the prostate tissue predicted the risk of biochemical failure following radical prostatectomy for clinically localized prostate cancer.

In our study, in patients with proximal tumor localization without disease progression the significantly decreased value of stearic acid and SI in CA as compared to NCA were noticed. These changes were accompanied by increased the value of oleic acid.

Human organism can synthesize all fatty acids except two: linoleic acid (LA) and α-linolenic acid, known as 'essential fatty acids'. LA is the precursor to the higher unsaturated n-6 series of fatty acids (arachidonic acid,

C20:4, AA). AA is the precursor of putative cancer promoting prostaglandins. Nicholson *et al.* (Nicholson *et al.* 1991) found in rats high values of arachidonic acid in cell membrane in the colorectal tumors compared to colonic mucosa. Also Neoptolemos *et al.* (Neoptolemos *et al.* 1991) showed the increased value of AA of total lipids from colorectal cancer tissue as compared to normal mucosa. However, in other study there were no significant differences in values of AA and LA of total lipids tissue between colorectal cancer tissue and normal mucosa (Fernandez-Banares *et al.* 1996). Our study showed the higher content of AA and LA in CA as compared to NCA in patients with proximal tumor localization.

In conclusion, the results of the study are preliminary, but they suggest that fatty acids of tissue phospholipids' fraction, as well as SI, strongly depend on tumor localization. It should be taken into account while considering these markers as a potential factor of the disease progression in colorectal cancer patients.

REFERENCES

1 Bufill J A (1990). Colorectal Cancer: Evidence for Distinct Genetic Categories Based on Proximal or Distal Tumor Location. Ann Intern Med. **113** (10): 779–88.
2 Bugajska J, Berska J, Hodorowicz-Zaniewska D, Sztefko K (2010). Walidacja Metody Oznaczania Kwasów Tłuszczowych Frakcji Fosfolipidów w Surowicy Krwi. Diagn Lab. **46** (2): 125–30.
3 Calder P C (2015). Functional Roles of Fatty Acids and Their Effects on Human Health. JPEN. Jpen-Parenter Enter. **39** (1 Suppl) 18S–32S.
4 Chaudry A, McClinton S, Moffat LE, Wahle KW (1991). Essential Fatty Acid Distribution in the Plasma and Tissue Phospholipids of Patients with Benign and Malignant Prostatic Disease. Brit J Cancer. **64** (6): 1157–60.
5 Cooper R A (1977). Abnormalities of Cell-Membrane Fluidity in the Pathogenesis of Disease. N Engl J Med. **297** (7): 371–77.
6 Fernandez-Banares F, Esteve M, Navarro E, Cabre E, Boix J, Abad-Lacruz A, et al (1996). Changes of the Mucosal N3 and N6 Fatty Acid Status Occur Early in the Colorectal Adenoma-Carcinoma Sequence. Gut **38** (2): 254–59.
7 Folch J, Lees M, Sloane Stanley GH (1957). A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. J Biol Chem. **226** (1): 497–509.
8 Freeman VL, Flanigan RC, Meydani M (2007). Prostatic Fatty Acids and Cancer Recurrence after Radical Prostatectomy for Early-Stage Prostate Cancer. Cancer Causes Control. **18** (2): 211–18.
9 Habib N A, Wood C B, Apostolov K, Barker W, Hershman M J, Aslam M, et al (1987). Stearic Acid and Carcinogenesis. Brit J Cancer. **56** (4): 455–58.
10 Hodge AM, Williamson EJ, Bassett JK, MacInnis RJ, Giles GG, English DR (2015). Dietary and Biomarker Estimates of Fatty Acids and Risk of Colorectal Cancer. Int J Cancer. **137** (5): 1224–34.
11 Iacopetta B (2002). Are There Two Sides to Colorectal Cancer? Int J Cancer. **101** (5): 403–8.
12 Kaluzny MA, Duncan LA, Merritt MV, Epps DE (1985). Rapid Separation of Lipid Classes in High Yield and Purity Using Bonded Phase Columns. J Lipid Res.. **26** (1): 135–40.
13 Kang JX, Wang J (2005). A Simplified Method for Analysis of Polyunsaturated Fatty Acids. BMC Biochem. **6** (March): 5.
14 Kelly SB, Miller J, Wood CB, Williamson RC, Habib NA (1990). Erythrocyte Stearic Acid Desaturation in Patients with Colorectal Carcinoma. Dis Colon Rectum. **33** (12): 1026–30.
15 Kositsawat J, Flanigan RC, Meydani M, Choi YK, Freeman VL (2007). The Ratio of Oleic-to-Stearic Acid in the Prostate Predicts Biochemical Failure after Radical Prostatectomy for Localized Prostate Cancer. J Urol. **178** (6): 2391–6.
16 Li FYi, Lai MD (2009). Colorectal Cancer, One Entity or Three. J Zhejiang Univ Sci B. **10** (3): 219–29.
17 Mikirova N, Riordan HD, Jackson JA, Wong K, Miranda-Massari JR, Gonzalez MJ (2004). Erythrocyte Membrane Fatty Acid Composition in Cancer Patients. P R Health Sci J. **23** (2): 107–13.
18 Mo A, Wu R, Grady JP, Hanley MP, Toro M, Swede H, et al (2018). Associations of Dietary Fat with Risk of Early Neoplasia in the Proximal Colon in a Population-Based Case-Control Study. Cancer Causes Control. **29** (7): 667–74.
19 Morrison WR, Smith LM (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res. **5** (October): 600–608.
20 Neoptolemos JP, Husband D, Imray C, Rowley S, Lawson N (1991). Arachidonic Acid and Docosahexaenoic Acid Are Increased in Human Colorectal Cancer. Gut **32** (3): 278–81.
21 Nicholson ML, Neoptolemos JP, Clayton HA, Talbot IC, Bell PR (1991). Increased Cell Membrane Arachidonic Acid in Experimental Colorectal Tumours. Gut **32** (4): 413–18.
22 Pandey M, Khatri AK, Dubey SS, Gautam A, Shukla VK (1998). Erythrocyte Membrane Stearic to Oleic Acid Ratio in Carcinoma of the Gallbladder: A Preliminary Study. Eur J Surg Oncol. **24** (1): 43–46.
23 Persad RA, Gillatt DA, Heinemann D, Habib NA, Smith PJ (1990). Erythrocyte Stearic to Oleic Acid Ratio in Prostatic Carcinoma. Br J Urol. **65** (3): 268–70.
24 Sliwinski S, Croonenberghs J, Christophe A, Deboutte D, Maes M (2006). Polyunsaturated Fatty Acids: Do They Have a Role in the Pathophysiology of Autism? Neuro Endocrinol Lett. **27** (4): 465–71.
25 Vareka T, Vecka M, Jirak R, Tvrzicka E, Macasek J, Zak A, et al (2012). Plasma Fatty Acid Profile in Depressive Disorder Resembles Insulin Resistance State. Neuro Endocrinol Lett. **33** Suppl 2: 83–86.
26 Wong R (2010). Proximal Tumors Are Associated with Greater Mortality in Colon Cancer. J Gen Intern Med. **25** (11): 1157–63.