

Table 1. Primer sequence for RT-PCR. ACTB – beta-actin, HMGCR - HMG-CoA reductase, CPT1a - carnitine palmitoyltransferase I, CHKA - choline kinase alpha, CHKB - choline kinase beta, PCYT1A- choline-phosphate cytidylyltransferase A, CHPT1 - cholinephosphotransferase 1, PISD - phosphatidylserine decarboxylase, SMS1 - sphingomyelin synthase 1, SMS2- sphingomyelin synthase 2.

Gene name	Forward sequence (from 5` to 3`)	Reverse sequence (from 5` to 3`)
ACTB	AGCACAGAGCCTCGCCTT	CATCATCCATGGTGAGCTGG
CPT1a	ATCAATCGGACTCTGGAAACGG	TCAGGGAGTAGCGCATGGT
HMGCR	TACCATGTCAGGGGTAC	CAAGCCTAGAGACATAAT
CHKA	CGGAAGTATCCCACCAAGAA	TCCCCAGAGGAAATGAGATG
CHKB	TGGTGCTAGAAAGCGTGATG	GCCGACTTGGGATGTACT
PCYT1A	GCAACCAGCTCCTTTCTG	GCAAACCTCCCACAATGAGGT
CHPT1	TCTGCTCTTTATTGGGATGTTG	CAACACAAAGACAATCACTAAAGC
PISD	ATCACTACCGAACCTCAGCGA	TACCTGCTCCACCTCACAGTTC
SMS 1	GCACTTCCCTGTTCGTTCTC	TACAGCGTGCCAATATGC
SMS 2	GCATTCCAGTGTGCTCCAAAGC	GTAACCGTGTGACCGCTGAAGA

Table 2. Analysis of correlations between the ¹H-NMR signal intensities of selected groups of lipids and the levels of fatty acids, and patients BMI, serum triacylglycerols and total cholesterol.

Lipid/lipid group	Correlation with BMI		Correlation with serum triacylglycerols		Correlation with serum total cholesterol	
	R	p	R	p	R	p
Free cholesterol	-0.101	0.662	-0.116	0.588	0.099	0.644
Phosphatidylethanolamines	0.096	0.679	-0.165	0.441	-0.215	0.312
Sphingomyelin	-0.066	0.776	-0.259	0.222	-0.024	0.910
Phosphatidylcholines	-0.043	0.852	-0.260	0.221	-0.013	0.951
Phospholipids	-0.071	0.758	-0.302	0.152	-0.139	0.517
Triacylglycerols	-0.425	0.053	-0.181	0.397	-0.120	0.575
16:0	0.063	0.786	-0.218	0.306	0.269	0.204
18:0	0.024	0.918	0.069	0.750	-0.087	0.685
Total SFA	-0.034	0.884	-0.114	0.597	0.023	0.916
16:1	0.118	0.609	0.121	0.574	0.140	0.516
18:1	0.044	0.850	0.048	0.824	-0.056	0.795
Total MUFA	0.024	0.918	0.060	0.780	-0.044	0.838
18:2 (LA)	0.054	0.815	-0.155	0.470	0.194	0.363
20:4 (ARA)	-0.030	0.899	0.046	0.831	-0.012	0.955
Total n-6 PUFA	-0.028	0.903	-0.005	0.982	0.053	0.806
18:3 (ALA)	-0.127	0.583	-0.124	0.563	-0.350	0.093
20:5 (EPA)	0.208	0.364	-0.246	0.247	0.012	0.956
22:6 (DHA)	-0.021	0.928	-0.050	0.815	0.096	0.655
Total n-3 PUFA	0.079	0.733	-0.109	0.612	0.054	0.803

R - Pearson's correlation coefficient, p - significance.

Table 3. Differences in ^1H -NMR signal intensity of selected lipid groups and fatty acid levels in tumor tissue in various groups of CRC patients.

Lipid/lipid group	CRC patients with T stage 1 and 2 Mean \pm SEM	CRC patients with T stage 3 and 4 Mean \pm SEM	p	Male CRC patients Mean \pm SEM	Female CRC patients Mean \pm SEM	p
Free cholesterol ^a	163 \pm 19.1	140 \pm 20.4	0.437	130 \pm 16.4	186 \pm 22.4	0.053
Phosphatidylethanolamines ^a	31.9 \pm 6.20	23.6 \pm 5.07	0.309	23.9 \pm 19.1	33.3 \pm 6.81	0.259
Sphingomyelin ^a	181 \pm 15.5	146 \pm 19.8	0.201	150 \pm 16.9	181 \pm 20.9	0.271
Phosphatidylcholines ^a	563 \pm 47.9	443 \pm 60.3	0.149	461 \pm 50.0	557 \pm 69.6	0.272
Phospholipids ^a	81.1 \pm 10.5	63.2 \pm 10.2	0.240	64.6 \pm 9.21	82.7 \pm 12.3	0.251
Triacylglycerols ^a	45.3 \pm 9.72	38.6 \pm 8.01	0.603	40.6 \pm 6.56	43.4 \pm 12.8	0.837
16:0 ^b	21.0 \pm 0.485	20.5 \pm 0.429	0.466	20.5 \pm 0.462	21.0 \pm 0.340	0.486
18:0 ^b	12.3 \pm 0.825	12.7 \pm 0.777	0.728	12.5 \pm 0.656	12.7 \pm 1.07	0.867
Total SFA ^b	36.7 \pm 0.778	36.9 \pm 0.722	0.857	36.8 \pm 0.593	36.9 \pm 1.04	0.907
16:1 ^b	3.38 \pm 0.330	3.31 \pm 0.259	0.863	3.41 \pm 0.266	3.23 \pm 0.313	0.679
18:1 ^b	35.4 \pm 1.57	35.9 \pm 1.50	0.828	35.8 \pm 1.24	35.5 \pm 2.08	0.896
Total MUFA ^b	40.0 \pm 1.81	40.7 \pm 1.69	0.778	40.5 \pm 1.46	40.0 \pm 2.26	0.851
18:2 (LA) ^b	12.0 \pm 0.651	10.6 \pm 0.453	0.087	11.0 \pm 0.547	11.6 \pm 0.553	0.519
20:4 (ARA) ^b	6.58 \pm 0.579	6.85 \pm 0.787	0.793	6.77 \pm 0.640	6.66 \pm 0.847	0.922
Total n-6 PUFA ^b	21.0 \pm 1.05	20.1 \pm 1.00	0.542	20.4 \pm 0.930	20.8 \pm 1.18	0.765
18:3 (ALA) ^b	0.061 \pm 0.011	0.050 \pm 0.008	0.424	0.060 \pm 0.009	0.046 \pm 0.008	0.304
20:5 (EPA) ^b	0.466 \pm 0.077	0.455 \pm 0.045	0.804	0.499 \pm 0.052	0.376 \pm 0.062	0.156
22:6 (DHA) ^b	1.00 \pm 0.091	1.03 \pm 0.080	0.845	1.00 \pm 0.067	1.05 \pm 0.117	0.678
Total n-3 PUFA ^b	2.11 \pm 0.190	2.10 \pm 0.125	0.957	2.12 \pm 0.126	2.08 \pm 0.204	0.840
Number of samples	11	14		16	9	

^aSignal intensity form ^1H -NMR (relative area), ^b% of total fatty acid content, p - significance from two-tailed t-test.

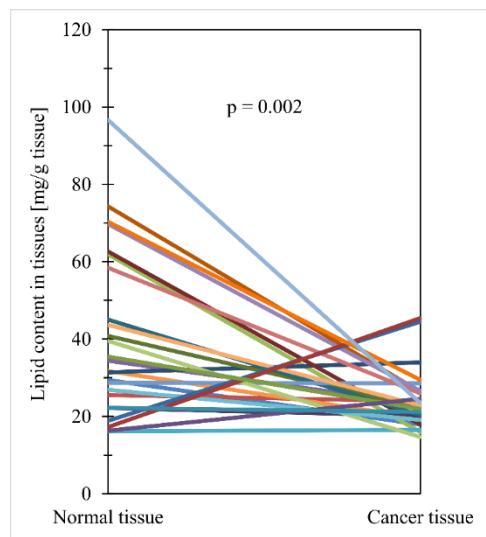


Figure 1. Total lipid content in normal and colorectal cancer tissue. Each line represents individual patient; $n = 25$.

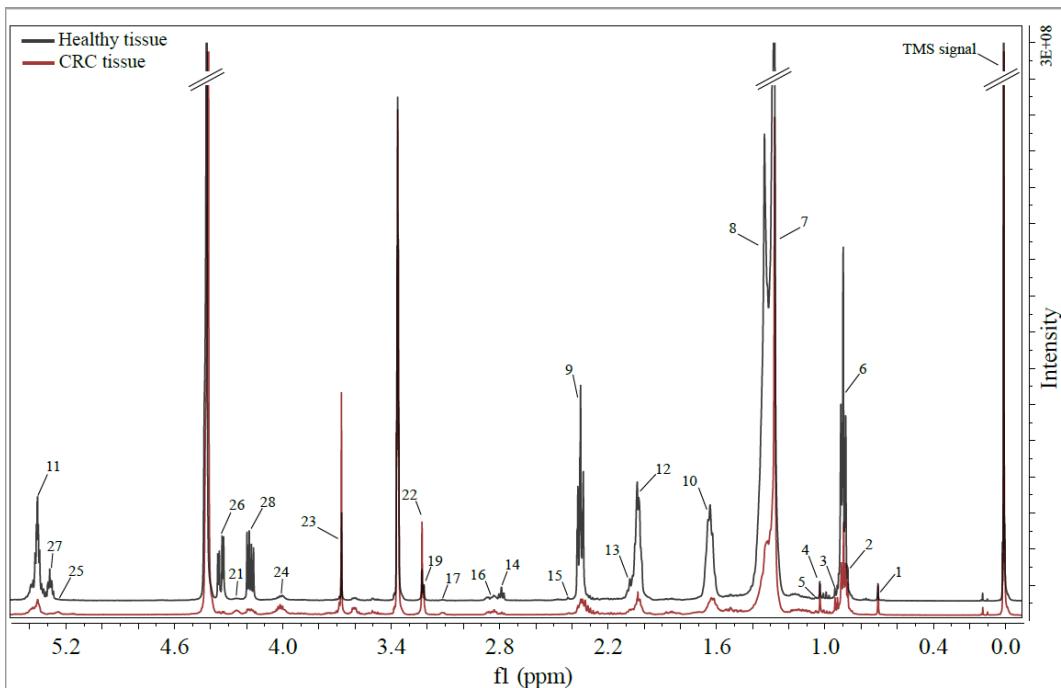


Figure 2. Example of a ^1H -NMR spectrum for lipids isolated from healthy tissue and CRC tissue. 1 - C₁₈H₃ in total cholesterol; 2 - C₂₆H₃-C₂₇H₃ in total cholesterol; 3 - C₂₁H₃ in free cholesterol; 4 - C₁₉H₃ in free cholesterol; 5 - C₁₉H₃ in esterified cholesterol; 6 - -CH₃ in fatty acyl chain; 7 - -(CH₂)_n in fatty acyl chain; 8 - CHCH₂CH₂(CH₂)- in fatty acyl chain; 9 - -CO-CH₂- in fatty acyl chain; 10 - -CO-CH₂CH₂- in fatty acyl chain; 11 -HC=CH- in fatty acyl chain; 12 -CH₂HC=C in fatty acyl chain: 18:1; 13 -CH₂HC= in fatty acyl chain: 18:2n-6/20:4n-6; 14 CHCH₂CH= in fatty acyl chain: 18:2n-6; 15 -CO-CH₂- in fatty acyl chain: 22:6n-3; 16 CHCH₂CH = in fatty acyl chain: 20:4n-6/22:6n-3; 17 -CH₂-CH₂-NH₂ of PE; 18 C₂H in glycerol backbone of PE; 19 -N+(CH₃)₃ in SM head group; 20 -CH₂N+(CH₃)₃ in SM head group; 21 -CH₂CH₂N+(CH₃)₃ in SM head group; 22 -CH₂N+(CH₃)₃ in PC head group; 23 -N+(CH₃)₃ in PC head group; 24 >C₃H₂ in glycerol backbone of PL; 25 -C₂H in glycerol backbone of PL; 26 >C₁H₂/C₃H₂ in glycerol backbone of TG; 27 -C₂H in glycerol backbone of TG; 28 >C₁H₂/C₃H₂ in glycerol backbone of TG and PL.

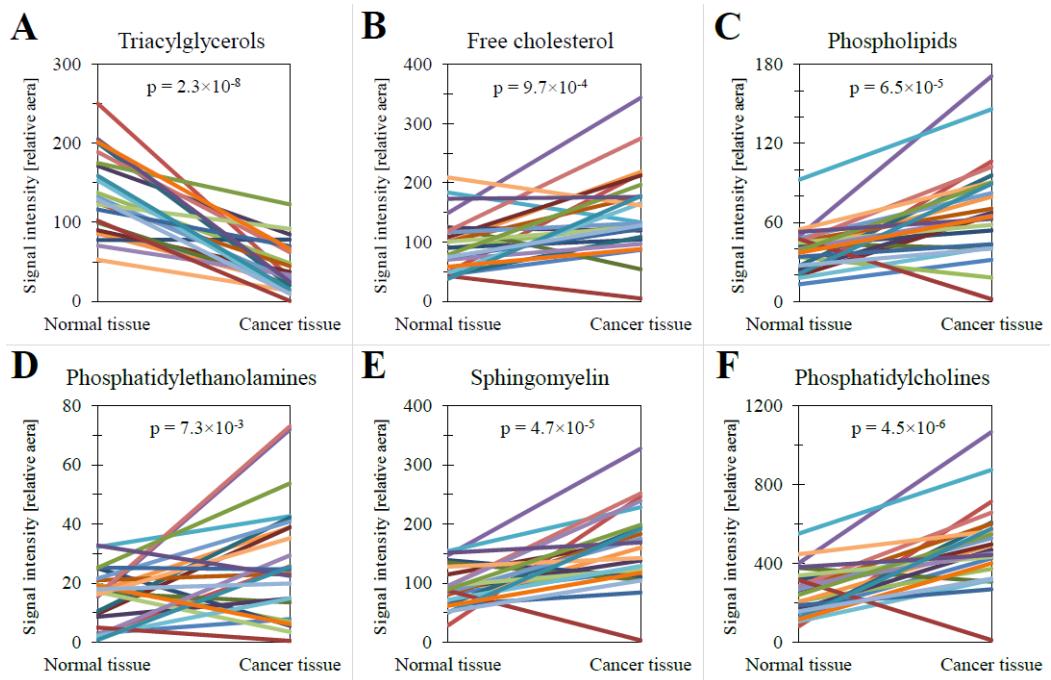


Figure 3. Intensities of ^1H -NMR signal for selected groups of lipids: (A) triacylglycerols; (B) free cholesterol; (C) phospholipids; (D) phosphatidylethanolamines; (E) sphingomyelin; (F) phosphatidylcholines. Each line represents individual patient; n=25.