Supplementary Information

Hexadecenoic fatty acid positional isomers and *de novo* PUFA synthesis in colon cancer cells

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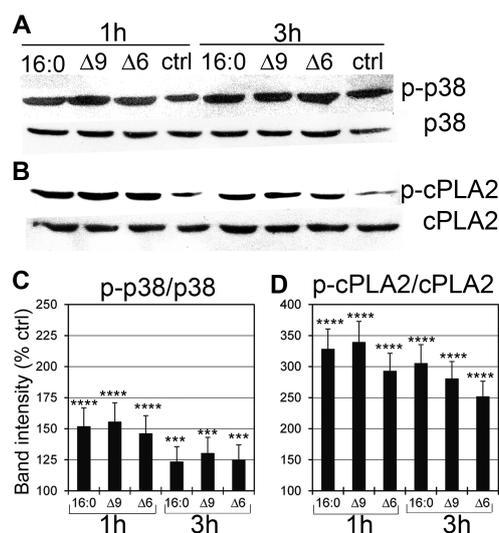*Correspondence: carla.ferreri@isof.cnr.it (C.F.)

Figure S1. Western blot analyses of p38 (A) and cPLA2 (B) total proteins and phosphorylated forms in Caco-2 cells after supplementation with palmitic (16:0), palmitoleic and sapienic acids. Cells were treated for 1 h and 3 h with 150 μM 16:0, 250 μM Δ9 (palmitoleic acid) and 300 μM Δ6 (sapienic acid). Cell lysates were resolved by SDS-PAGE and proteins were blotted and detected with monoclonal antibodies against p38, p-p38, cPLA2 and p-cPLA2. Representative gels are shown. The bar graphs (C and D) represent the band intensity values obtained by Image J analysis, expressed as percentage of the corresponding control. S.D. never exceeds 15% (***, p<0.001; ****, p<0.0001).

S1 Protocol. Gas chromatographic calibration procedures and quantitative analysis

An internal standard of C17:0 was added in the FAME samples at known concentration. 1 μL of the FAME mixture in *n*-hexane solution was injected in the GC equipment using the split mode (50:1) following the run conditions reported in the text. The results of the Caco-2 cell FAME as control and after treatment were expressed as μg/mL (mean± S.E.M).

Here we reported the LOD (Limit of detection) and LOQ (Limit of quantification) values of the GC instrument used in this work, referred to 8*cis*-18:1 and 5*cis*,8*cis*-18:2 preparing a solution of 1 mg/mL of each standard diluted to a series of appropriate concentrations with *n*-hexane: 8*cis*-18:1, LOD: 0.0008 μg/mL, LOQ: 0.0025 μg/mL; 5*cis*,8*cis*-18:2 LOD 0.0009 μg/mL, LOQ 0.0027 μg/mL.

Table S1. Membrane phospholipid fatty acids of Caco- 2 cells expressed as μg/mL (mean ±S.E.M.). The cells were treated with 150 μM palmitoleic acid and processed at the indicated times. The statistics was carried out in comparison with controls grown without supplementation for the same time intervals.

FAME[1]	Control (n=6) μg/mL	0.5 h (n=3) μg/mL	1h (n=3) μg/mL	3h (n=3) μg/mL
14:0	25.6±1.8	26.1±5.9	20.1±6.4	17.9±1.8*
16:0	393.3±14.6	494.5±23.0*	542.6±105.9	414.5±6.4
6*trans*-16:1	1.8±0.4	2.5±0.9	1.1±0.1	0.2±0.0*
9*trans*-16:1	0.4±0.1	1.9±0.1**	0.44±0.1*	0.5±0.1
6*cis*-16:1	27.0±3.5	18.4±4.4	15.4±3.8	14.9±1.3*
9*cis*-16:1	116.7±4.4	251.3±32.6**	187.1±51.1	344.8±15.2***
18:0	148.5±9.0	135.9±13.0	113.7±2.6*	111.3±27.5
9*trans*-18:1	0.7±0.1	3.1±1.2	0.7±0.1	1.0±0.1
8*cis*-18:1	2.5±0.4	14.7±5.1	1.3±0.1*	1.7±0.2
9*cis*-18:1	391.7±68.3	277.1±117.5	500.0±43.6	407.1±12.6
11*cis*-18:1	216.5±45.9	147.3±12.7	112.0±39.1	166.9±2.4
5*cis*,8*cis* 18:2	8.0±0.7	8.0±0.3	3.5±3.1	2.3±0.3**
18:2 ω 6	50.9±6.5	59.1±8.2	62.5±17.3	38.1±5.7
20:0	1.2±0.3	9.4±1.1**	2.8±1.1	2.7±0.7
20:3 ω6	19.9±2.0	26.8±8.8	3.7±3.2*	2.0±0.3***
20:4 ω6	76.9±4.1	53.4±8.3	33.8±8.6*	47.2±5.8*
20:5 ω3	12.7±4.0	13.1±4.0	7.8±1.8	11.2±2.6
22:0	6.1±2.3	24.1±13.5	1.2±0.7	16.7±2.9*
22:5 ω3	31.0±2.5	26.6±2.9	8.7±3.8**	16.2±2.1*
22:6 ω3	61.843±8.6	45.7±7.6	31.0±13.5	31.5±1.0*
SFA	574.7±25.8	690.0±54.7	680.4±95.2	563.2±30.6
MUFA	754.3±28.6	708.8±64.3	815.7±52.5	935.5±29.6*
PUFA	314.2±36.8	232.8±12.0	151.1±43.9*	148.5±2.5*
ω6	147.7±3.0	139.4±3.4	100.0±29.1	87.3±1.8***
ω3	105.6±12.8	85.5±13.8	47.5±11.8*	58.9±1.2*
Total *trans*	5.4±1.5	7.9±5.4	3.0±0.7	2.4±0.3

[1]FAME identified by the standard references and quantified as described in Materials and Methods; the values are obtained from the GC peak areas (>97% of the total peak areas of the chromatogram recognized by appropriate references). Details of the statistical analysis are reported in Materials and Methods. The statistical significance is estimated on the Standard Error of the Mean;* p value ≤0.049;** p value ≤0.009;*** p value ≤0.0001.

Table S2. Membrane phospholipid fatty acids of Caco- 2 cells expressed as μg/mL (mean ±S.E.M.). The cells were treated with 300 μM palmitoleic acid and processed at the indicated times. The statistics was carried out in comparison with controls grown without supplementation for the same time intervals.

FAME[1]	Control (n=6) μg/mL	0.5 h (n=3) μg/mL	1h (n=3) μg/mL	3h (n=3) μg/mL
14:0	25.6±1.8	10.7±1.4*	24.1±2.6	26.1±15.1
16:0	393.3±14.6	355.9±30.2	370.6±37.6	410.7±72.7
6*trans*-16:1	1.8±0.4	2.7±0.5	2.4±0.4	1.1±0.4
9*trans*-16:1	0.4±0.1	2.7±0.5*	2.3±0.3**	1.1±0.4
6*cis*-16:1	27.0±3.5	14.6±2.7*	18.2±3.6	9.6±3.0*
9*cis*-16:1	116.7±4.4	313.0±51.5**	381.0±49.2**	369.1±74.6*
18:0	148.5±9.0	124.1±17.4	124.5±23.1	102.6±16.3
9*trans*-18:1	0.7±0.1	4.9±2.6	1.7±0.6	3.5±1.2
8*cis*-18:1	2.5±0.4	4.9±1.0	9.3±3.4	5.9±2.1
9*cis*-18:1	391.7±68.3	410.8±11.9	323.8±33.2	418.9±59.2
11*cis*-18:1	216.5±45.9	159.2±7.2	129.6±16.58	124.9±17.3
5*cis,8cis*-18:2	8.0±0.7	12.6±2.2	12.1±5.8	4.1±1.4
18:2 ω 6	50.9±6.5	83.5±30.2	27.2±5.3*	37.9±8.3
20:0	1.2±0.3	13.4±3.5*	7.6±3.2	24.7±13.3
20:3 ω6	19.9±2.0	24.3±0.4	19.6±11.3	8.9±3.1*
20:4 ω6	76.9±4.1	45.1±6.3*	46.9±3.8**	38.4±5.5**
20:5 ω3	12.7±4.0	8.8±2.0	15.0±1.1	7.1±3.2
22:0	6.1±2.3	9.5±1.8	49.9±30.1	6.7±3.6
22:5 ω3	31.0±2.5	16.0±2.5*	19.4±3.1*	20.2±4.9
22:6 ω3	61.8±8.6	26.2±4.0*	58.3±9.0	25.7±7.1*
SFA	574.7±25.8	513.4±46.2	576.8±77.4	574.1±60.1
MUFA	754.3±28.6	901.8±44.2*	861.9±98.9	925.0±41.8*
PUFA	314.2±36.8	216.6±21.2	198.7±20.8	142.4±29.5*
ω6	147.7±3.0	152.9±25.4	93.8±15.4*	85.2±16.0*
ω3	105.6±12.8	51.0±7.8*	119.4±26.5	53.1±12.5*
Total *trans*	5.4±1.5	17.4±4.1*	12.1±3.1	8.1±2.9

[1]FAME identified by the standard references and quantified as described in Materials and Methods; the values are obtained from the main GC peak areas (>97% of the total peak areas of the chromatogram). Details of the statistical analysis are reported in Materials and Methods. The statistical significance is estimated on Standard Error of the Mean. * p value ≤0.049; ** p value ≤0.0096; *** p value ≤0.0009

Table S3. Membrane phospholipid fatty acids of Caco- 2 cells expressed as μg/mL (mean ±S.E.M.). The cells were treated with 150 μM sapienic acid and processed at the indicated times. The statistics was carried out in comparison with controls grown without supplementation for the same time intervals.

FAME[1]	Control (n=6) μg/mL	0.5 h (n=3) μg/mL	1h (n=3) μg/mL	3h (n=3) μg/mL
14:0	25.6±1.8	21.0±2.9	20.2±2.1	20.1±0.3*
16:0	393.3±14.6	361.2±60.7	327.6±5.2*	303.2±20.0*
6*trans*-16:1	1.8±0.4	2.4±0.8	2.0±0.1	10.4±7.1
9*trans*-16:1	0.4±0.1	1.3±0.3	0.7±0.2	4.9±4.0
6*cis*-16:1	27.0±3.5	57.8±20.8	209.2±1.3***	247.1±10.7***
9*cis*-16:1	116.7±4.4	141.2±55.1	90.5±1.1**	117.4±4.8
18:0	148.5±9.0	209.0±55.7	134.1±3.7	120.0±2.9*
9*trans*-18:1	0.7±0.1	3.3±1.3	1.4±0.3	3.6±0.8*
8*cis*-18:1	2.5±0.4	21.9±4.4*	26.2±3.9**	102.8±11.7**
9*cis*18:1	391.7±68.3	335.4±31.3	380.4±11.1	332.2±8.0
11*cis*-18:1	216.5±45.9	158.3±5.9	148.3±3.0	151.5±7.7
5*cis*,8*cis*-18:2	8.0±0.7	16.7±5.6	12.9±0.3**	13.1±0.3**
18:2 ω 6	50.9±6.5	60.0±3.9	37.6±0.7	26.9±1.4*
20:0	1.2±0.3	9.3±0.5***	2.7±0.3*	12.3±5.6
20:3 ω6	19.9±2.0	28.7±3.0	22.3±1.5	23.0±5.2
20:4 ω6	76.9±4.1	84.0±15.5	96.2±5.4*	59.4±2.6*
20:5 ω3	12.7±4.0	15.2±1.1	17.9±1.4	13.9±5.8
22:0	6.1±2.3	13.8±6.5	11.0±1.1	10.1±3.4
22:5 ω3	31.0±2.5	43.2±16.8	38.5±2.9	34.0±7.6
22:6 ω3	61.8±8.6	58.3±17.4	66.7±1.2	36.6±7.1
SFA	574.7±25.8	614.3±36.4	495.7±5.9*	465.8±15.5*
MUFA	754.3±28.6	714.7±19.8	854.5±6.0*	951.1±28.0**
PUFA	314.2±36.8	306.1±26.7	292.1±11.3	207.0±28.2*
ω6	147.7±3.0	172.7±16.8	156.1±7.6	109.3±8.6*
ω3	105.6±12.8	116.7±15.6	123.1±4.7	84.5±20.3
Total *trans*	5.4±1.5	14.5±3.2	6.6±0.37	10.0±3.3

[1]FAME identified by the standard references and quantified as described in Materials and Methods; the values are obtained from the main GC peak areas (>97% of the total peak areas of the chromatogram). Details of the statistical analysis are reported in Materials and Methods. The statistical significance is estimated on Standard Error of the Mean. * p value ≤0.049;** p value ≤0.009;*** p value ≤0.0001.

Table S4. Membrane phospholipid fatty acids of Caco- 2 cells expressed as µg/mL (mean ±S.E.M.). The cells were treated with 300 µM sapienic acid and processed at the indicated times. The statistics was carried out in comparison with controls grown without supplementation for the same time intervals.

FAME[1]	Control (n=6) µg/mL	0.5 h (n=3) µg/mL	1h (n=3) µg/mL	3h (n=3) µg/mL
14:0	25.6±1.8	11.1±0.7*	6.3±3.0**	19.7±1.2
16:0	393.3±14.6	388.3±28.0	304.5±29.0*	299.6±0.5**
6*trans*-16:1	1.8±0.4	1.7±0.5	2.2±0.2	1.7±0.5
9*trans*-16:1	0.4±0.1	1.2±0.1*	0.8±0.1	1.1±0.1*
6*cis*-16:1	27.0±3.5	137.1±13.0*	208.7±24.2**	417.8±7.9***
9*cis*-16:1	116.7±4.4	80.6±6.9**	66.2±3.4**	110.8±4.9
18:0	148.5±9.0	157.9±3.8	173.6±8.4	102.9±4.9*
9*trans*-18:1	0.7±0.1	7.1±4.4	2.0±0.4*	1.6±0.6
8*cis*-18:1	2.5±0.4	62.0±15.8*	49.5±9.9**	72.3±2.3***
9*cis*-18:1	391.7±68.3	247.0±101.7	361.1±22.0	305.7±4.7
11*cis*-18:1	216.5±45.9	202.5±52.4	164.8±14.0	115.4±7.5
5*cis*,8*cis*-18:2	8.0±0.7	13.0±0.8**	11.0±1.4	12.2±1.7
18:2 ω 6	50.9±6.5	34.8±11.9	30.2±9.6	28.7±3.2*
20:0	1.2±0.3	19.8±16.7*	2.4±0.6	7.5±1.4*
20:3 ω6	19.9±2.0	22.4±6.8	18.8±1.9	21.7±0.7
20:4 ω6	76.9±4.1	94.4±3.7*	87.2±2.3	60.2±2.5*
20:5 ω3	12.7±4.0	8.4±5.3	11.9±1.7	10.6±2.1
22:0	6.1±2.3	13.8±7.4	11.0±2.5	3.7±2.1
22:5 ω3	31.0±2.5	47.8±6.2*	28.1±1.9	22.7±3.7
22:6 ω3	61.8±8.6	79.6±13.4*	109.0±44.7	31.5±0.8*
SFA	574.7±25.8	549.1±13.5	490.1±30.8	433.5±5.0**
MUFA	754.3±28.6	771.2±4.0*	850.3±21.7	1022.0±10.7***
PUFA	314.2±36.8	300.4±19.5	293.1±51.5	187.6±7.3*
ω6	147.7±3.0	151.6±21.0	133.0±11.2	110.6±1.4***
ω3	105.6±12.8	135.8±14.4*	149.0±45.5	64.8±5.8*
Total *trans*	5.4±1.5	18.8±8.5	8.4±0.9	6.4±0.5

[1]FAME identified by the standard references and quantified as described in Materials and Methods; the values are obtained from the main GC peak areas (>97% of the total peak areas of the chromatogram). Details of the statistical analysis are reported in Materials and Methods. The statistical significance is estimated on Standard Error of the Mean. * p value ≤0.049;** p value ≤0.0096;*** p value ≤0.0009

Table S5. Membrane phospholipid fatty acids of Caco- 2 cells expressed as μg/mL (mean ±S.E.M.). The cells were treated with 150 μM palmitic acid and processed at the indicated times. The statistics was carried out in comparison with controls grown without supplementation for the same time intervals.

FAME[1]	Control (n=6) μg/mL	0.5 h (n=3) μg/mL	1h (n=3) μg/mL	3h (n=3) μg/mL
14:0	25.6±1.8	38.4±6.6**	13.3±1.6	19.7±8.6
16:0	393.3±14.6	497.8±56.2	456.1±42.0	535.1±42.0*
6*trans*-16:1	1.8±0.4	3.3±1.4	10.2±6.4	1.5±0.0
9*trans*-16:1	0.4±0.1	2.4±0.9	6.8±5.1	0.8±0.2
6*cis*-16:1	27.0±3.5	44.8±9.5	20.9±1.5	26.2±4.9
9*cis*-16:1	116.7±4.4	130.7±21.5	114.1±9.5	101.4±41.4
18:0	148.5±9.0	115.6±12.3	155.0±7.9	117.2±1.3*
9*trans*-18:1	0.7±0.1	3.8±0.1***	4.1±2.3	3.8±1.6
8*cis*-18:1	2.5±0.4	2.9±0.8	10.0±5.2	2.0±1.2
9*cis*-18:1	391.7±68.3	405.6±31.5	420.7±9.1	424.5±5.3
11*cis* 18:1	216.5±45.9	167.4±15.3	172.5±4.3	175.3±6.5
5*cis,8cis*-18:2	8.0±0.7	3.3±1.9	4.4±1.4	3.5±0.6**
18:2 ω 6	50.9±6.5	35.2±8.0	44.2±3.1	25.7±1.1*
20:0	1.2±0.3	20.6±9.2	8.2±2.3*	4.4±1.9
20:3 ω6	19.9±2.0	12.9±4.0	17.7±3.1	17.1±1.8
20:4 ω6	76.9±4.1	51.3±19.9	65.0±3.8	71.5±9.0
20:5 ω3	12.7±4.0	8.8±1.5	9.7±3.8	12.6±3.0
22:0	6.1±2.3	11.1±4.1	12.6±5.6	5.3±1.0
22:5 ω3	31.0±2.5	36.7±4.5	35.1±3.6	37.3±1.2
22:6 ω3	61.8±8.6	51.3±11.5	63.2±10.3	58.9±9.3
SFA	574.7±25.8	683.6±74.5	645.4±34.5	681.7±49.9
MUFA	754.3±28.6	751.4±59.8	738.3±4.4	729.5±27.7
PUFA	314.2±36.8	199.5±23.7	239.4±15.8	226.6±24.7
ω6	147.7±3.0	99.3±17.4	127.0±6.1*	114.3±11.1*
ω3	105.6±12.8	96.8±15.8	108.0±12.0	108.8±13.5
Total *trans*	5.4±1.5	15.1±3.9	26.5±16.0	9.3±2.2

[1]FAME identified by the standard references and quantified as described in Materials and Methods; the values are obtained from the main GC peak areas (>97% of the total peak areas of the chromatogram). Details of the statistical analysis are reported in Materials and Methods. The statistical significance is estimated on Standard Error of the Mean. * p value ≤0.049; ** p value ≤0.009; *** p value ≤0.0001

Table S6. Membrane phospholipid fatty acids of Caco- 2 cells expressed as μg/mL (mean ±S.E.M.). The cells were treated with 300 μM palmitic acid and processed at the indicated times. The statistics was carried out in comparison with controls grown without supplementation for the same time intervals.

FAME[1]	Control (n=6) μg/mL	0.5 h (n=3) μg/mL	1h (n=3) μg/mL	3h (n=3) μg/mL
14:0	25.6±1.8	24.8±7.0	34.0±8.3	24.4±0.8
16:0	393.3±14.6	521.0±75.2	490.5±27.3*	648.2±26.2**
6*trans*-16:1	1.8±0.4	2.7±0.9	4.2±0.3*	1.9±1.0
9*trans*-16:1	0.4±0.1	2.1±0.6	2.0±1.0	1.6±0.9
6*cis*-16:1	27.0±3.5	16.3±3.6*	23.8±8.6	14.8±0.9*
9*cis*-16:1	116.7±4.4	154.8±33	159.9±36.5	126.3±9.4
18:0	148.5±9.0	140.1±9.9	142.4±11.5	118.8±23.3
9*trans*-18:1	0.7±0.1	5.6±3.2	4.5±1.3	1.3±0.5*
8*cis*-18:1	2.5±0.4	9.5±4.5	8.7±2.4	5.0±1.1
9*cis*-18:1	391.7±68.3	369.2±22.5	293.0±115.8	319.8±9.2
11*cis*-18:1	216.5±45.9	138.3±7.8	183.3±64.2	115.8±5.6
5*cis*,8*cis*-18:2	8.0±0.7	2.7±1.3*	2.7±0.7**	2.6±0.1**
18:2 ω 6	50.9±6.5	80.8±10.4	63.7±37.2	54.5±13.7
20:0	1.2±0.3	18.7±8.8	15.8±4.9*	25.0±10.3
20:3 ω6	19.9±2.0	18.4±5.4	13.4±1.8	9.4±1.6*
20:4 ω6	76.9±4.1	47.9±4.9*	72.3±12.0	57.2±4.1*
20:5 ω3	12.7±4.0	16.2±1.4	16.5±4.6	16.9±2.8
22:0	6.1±2.3	15.3±5.4	17.9±1.8*	29.3±9.3
22:5 ω3	31.0±2.5	23.0±0.9*	32.3±10.9	22.7±0.9*
22:6 ω3	61.8±8.6	34.3±4.4*	61.6±6.5	49.8±3.4
SFA	574.7±25.8	720.0±60.7	700.5±47.5	845.8±23.1**
MUFA	754.3±28.6	688.3±53.2	668.7±75.7	581.7±7.0*
PUFA	314.2±36.8	223.3±11.1	262.5±43.0	213.2±13.8
ω6	147.7±3.0	147.1±10.6	149.4±38.5	121.1±15.9
ω3	105.6±12.8	73.5±5.6	110.4±18.5	89.5±5.3
Total *trans*	5.4±1.5	18.0±7.0	17.7±1.6*	8.8±2.9

[1]FAME identified by the standard references and quantified as described in Materials and Methods; the values are obtained from the main GC peak areas (>97% of the total peak areas of the chromatogram). Details of the statistical analysis are reported in Materials and Methods. The statistical significance is estimated on Standard Error of the Mean. * p value ≤0.049;** p value ≤0.0096;*** p value ≤0.0009.

Table S7. Membrane phospholipid fatty acids of Caco-2 cells expressed as μg/mL(mean ±S.E.M.) treated for 24 h with A (palmitoleic acid 150 μM) or B (sapienic acid 150 μM) or C (palmitic acid 150 μM), compared to controls grown in the same time without supplementation.

FAME[1]	Control (n=6) μg/mL	A (n=3) μg/mL	B (n=3) μg/mL	C (n=3) μg/mL
14:0	26.6±1.6	17.7±2.3*	19.0±2.5	21.7±1.1
16:0	473.6±33.5	396.8±101.2	242.4±15.9**	642.8±11.3**
6*trans*-16:1	1.4±0.2	0.6±0.1*	2.1±0.5	1.2±0.6
9*trans*-16:1	1.2±0.2	1.0±0.2	1.0±0.3	1.3±0.7
6*cis*-16:1	27.5±5.5	11.9±0.3*	256.2±26.3**	24.1±4.5
9*cis*-16:1	141.7±17.2	245.3±58.0	63.3±4.8*	167.3±16.7
18:0	128.8±10.8	103.0±8.6	150.6±11.5	110.2±2.9
9 *trans*-18:1	1.8±0.6	2.4±0.5	1.2±0.6	4.7±1.8
8*cis*-18:1	5.0±1.7	6.7±3.9	327.2±10.5***	9.5±3.1
9*cis*-18:1	466.6±19.6	450.7±104.6	320.7±89.6	336.5±18.2**
11*cis*-18:1	173.6±9.8	235.4±70.4	129.1±11.1	172.9±10.8
5*cis*,8*cis*-18:2	5.1±1.6	13.3±6.0	16.8±3.2**	3.8±0.7
18:2 ω 6	33.9±0.7	41.0±17.1	29.3±7.2	35.1±6.0
20:0	3.7±2.8	5.5±1.2	3.1±1.8	5.8±1.1
20:3 ω6	18.5±2.7	33.8±8.4	16.0±3.3	15.1±3.2
20:4 ω6	55.2±8.5	28.8±8.5	76.0±7.9	30.8±7.3
20:5 ω3	17.4±6.6	6.5±1.3	12.2±2.8	5.9±2.4
22:0	1.5±0.5	3.9±0.8	8.7±3.9	12.6±8.2
22:5 ω3	25.5±4.1	17.0±0.5	28.8±2.8	13.3±5.6
22:6 ω3	37.4±3.8	24.5±3.7	42.1±6.0	29.6±1.4
SFA	634.1±42.6	527.0±107.3	423.8±8.0*	793.2±16.2*
MUFA	814.5±53.5	950.0±107.2	1096.5±86.0	710.4±16.5
PUFA	193.1±11.6	165.1±6.5	221.3±23.9	133.7±12.1
ω6	107.7±9.2	103.6±15.7	121.4±17.9	81.0±6.0*
ω3	80.3±1.4	48.1±5.6*	83.0±9.2	48.9±7.1*
Total *trans*	7.9±0.7	10.6±4.6	7.9±1.0	12.3±1.9

[1]FAME identified by the standard references and quantified as described in Materials and Methods; the values are obtained from the main GC peak areas (>97% of the total peak areas of the chromatogram). Details of the statistical analysis are reported in Materials and Methods. The statistical significance is estimated on Standard Error of the Mean. * p value ≤0.049;** p value ≤0.009;*** p value ≤0.0001

Table S8. Membrane phospholipid fatty acids of Caco-2 cells expressed as μg/mL(mean ±S.E.M.) treated

for 24 h with A (palmitoleic acid 300 μM) or B (sapienic acid 300 μM) or C (palmitic acid 300 μM),

compared to controls grown in the same time without supplementation.

FAME[1]	Control (n=6) μg/mL	A (n=3) μg/mL	B (n=3) μg/mL	C (n=3) μg/mL
14:0	26.6±1.6	11.8±2.6**	18.8±0.5*	21.0±7.8
16:0	473.6±33.5	263.3±28.9**	276.8±13.1**	777.4±81.3*
6*trans*-16:1	1.4±0.2	0.8±0.3	2.5±0.4	0.5±0.1*
9*trans*-16:1	1.2±0.2	0.7±0.3	2.3±0.5	0.7±0.1
6*cis*-16:1	27.5±5.5	7.3±1.2*	397.5±10.1***	11.9±4.6
9*cis*-16:1	141.7±17.2	357.6±113.2	56.4±1.8**	86.4±22.9
18:0	128.8±10.8	87.0±1.0*	115.4±1.6	179.8±41.3
9*trans*-18:1	1.8±0.6	2.5±1.0	4.3±1.5	1.6±0.4
8*cis*-18:1	5.0±1.7	6.0±1.6	268.7±22.5***	3.7±1.2
9*cis*-18:1	466.6±19.6	505.1±167.9	209.1±24.4**	384.8±108.4
11*cis*-18:1	173.6±9.8	229.4±17.3*	79.3±5.8**	62.7±13.1**
5*cis*,8*cis*-18:2	5.1±1.6	3.7±1.0	15.4±1.2**	1.6±0.5
18:2 ω 6	33.9±0.7	55.0±13.3	27.3±2.0*	35.7±7.4
20:0	3.7±2.8	29.1±15.3	12.2±0.5*	13.8±2.1*
20:3 ω6	18.5±2.7	12.0±4.2	14.8±4.8	5.1±1.0**
20:4 ω6	55.2±8.5	28.8±6.3	69.9±5.8	30.2±0.6*
20:5 ω3	17.4±6.6	6.6±2.3	11.6±3.1	3.0±0.5
22:0	1.5±0.5	7.3±3.0	11.1±2.3*	5.7±0.8*
22:5 ω3	25.5±4.1	13.9±2.3	24.6±2.2	11.6±3.0
22:6 ω3	37.4±3.8	19.2±4.6*	26.5±2.1	10.9±3.0**
SFA	634.1±42.6	398.5±45.6*	434.4±14.6*	997.8±50.2**
MUFA	814.5±53.5	1105.4±38.2*	1010.9±5.5*	549.5±66.8*
PUFA	193.1±11.6	139.2±18.0	190.2±12.3	98.2±15.8**
ω6	107.7±9.2	95.8±10.3	112.0±6.6	71.1±9.0*
ω3	80.3±1.4	39.7±7.9**	62.8±7.4	25.5±6.5**
Total *trans*	7.9±0.7	6.5±2.0	14.1±1.0**	4.0±1.0*

[1]FAME identified by the standard references and quantified as described in Materials and Methods; the

values are obtained from the main GC peak areas (>97% of the total peak areas of the chromatogram).

Details of the statistical analysis are reported in Materials and Methods. The statistical significance is

estimated on Standard Error of the Mean. * p value ≤0.049; ** p value ≤0.0096; *** p value ≤0.0009

Table S9. Triglyceride fatty acids of Caco-2 cells expressed as µg/mL (mean ±S.E.M.) treated for 24 hours with 150µM (A), 300 µM (B) palmitoleic acid and 150µM (C), 300 µM (D) palmitic acid

FAME[1]	A (n=3) µg/mL	B (n=3) µg/mL	C (n=3) µg/mL	D (n=3) µg/mL
14:0	7.4±1.2	4.1±0.6	4.3±1.1	6.0±1.1
16:0	80.2±4.9	60.3±16.7	143.9±27.8	258.7±31.4
6trans-16:1	0.2±0.2	n.d.	0.1±0.1	0.2±0.1
9trans-16:1	0.1±0.1	n.d.	0.3±0.1	0.3±0.2
6cis-16:1	2.4±0.5	2.1±0.8	3.4±1.8	3.5±0.9
9cis-16:1	103.9±10.5	147.6±37.6	34.6±19.7	23.1±13.0
18:0	24.6±1.5	21.3±10.2	45.3±12.1	35.5±4.5
9trans-18:1	0.3±0.3	n.d.	0.3±0.2	0.5±0.1
8cis-18:1	0.5±0.5	0.5±0.5	2.1±0.4	0.9±0.4
9cis-18:1	60.8±1.5	101.9±45.9	115.9±27.1	64.2±8.2
11cis-18:1	131.9±17.5	99.7±22.9	47.4±3.4	38.3±8.7
5cis,8cis-18:2	1.0±0.2	0.1±0.1	1.5±0.7	0.5±0.2
18:2 ω 6	26.5±16.2	14.5±5.2	6.6±3.4	6.8±2.7
20:0	2.8±1.1	0.6±0.1	5.9±4.2	2.6±1.1
20:3 ω6	2.9±0.8	0.8±0.2	4.4±1.2	3.8±0.9
20:4 ω6	3.3±1.0	2.7±1.0	22.7±12.0	5.0±0.7
20:5 ω3	2.5±0.5	1.0±0.4	6.9±3.8	1.6±0.2
22:0	2.1±0.4	0.6±0.2	3.2±0.6	1.3±0.3
22:5 ω3	2.3±0.8	1.1±0.1	3.7±1.8	3.3±0.8
22:6 ω3	4.5±0.7	1.7±0.2	7.7±2.9	4.1±1.8
SFA	117.2±8.2	87.0±26.4	202.7±26.4	304.2±34.6
MUFA	299.5±8.0	351.9±30.5	203.5±28.6	130.0±30.1
PUFA	43.0±14.9	22.0±5.1	53.4±10.3	25.2±5.1
ω6	32.8±14.7	18.1±5.4	33.6±14.8	15.6±2.4
ω3	9.3±0.7	3.8±0.3	18.3±5.3	9.1±2.9
Total *trans*	1.3±1.3	0.1±0.1	1.5±0.8	1.6±0.2

[1]FAME identified by the standard references and quantified as described in Materials and Methods; the values are obtained from the main GC peak areas (>97% of the total peak areas of the chromatogram); nd: not detected.

Table S10. Triglyceride fatty acids of Caco-2 cells expressed as μg/mL(mean ±S.E.M.) treated for the indicated times with 150 μM sapienic acid.

FAME[1]	0.5 h (n=3) μg /mL	1h (n=3) μg /mL	3h (n=3) μg /mL	24h (n=3) μg /mL
14:0	4.4±0.1	2.9±0.1	9.2±0.2	4.1±0.1
16:0	72.2 ± 0.7	47.7±1.1	77.6±1.3	76.9±0.3
6*trans*-16:1	n.d.	n.d.	n.d.	n.d.
9*trans*-16:1	n.d.	n.d.	n.d.	n.d.
6*cis*-16:1	72.8±1.0	174.9±1.5	111.9±1.8	62.1±0.3
9*cis*-16:1	13.4 ± 0.2	8.4±0.2	15.0±0.7	15.1±0.3
18:0	30.1±1.2	17.5±0.6	35.0±0.9	40.4±0.7
9*trans*-18:1	n.d.	0.3±0.1	0.3±0.3	n.d.
8*cis*-18:1	21.1±1.3	10.9±0.7	18.2±2.2	57.9±7.7
9*cis*-18:1	156.1±6.3	138.5±4.0	108.5±4.8	100.8±7.5
11*cis*-18:1	53.0±1.4	24.7±1.6	38.5±4.5	40.4±1.4
5*cis,8cis*-18:2	2.7±0.3	2.7±0.1	8.6±2.2	4.4±1.1
18:2 ω 6	8.2±0.5	8.7±0.4	8.4±0.6	9.7±0.2
20:0	0.9±0.0	1.8±0.3	3.2±0.9	4.7±1.5
20:3 ω6	3.1±0.2	2.7±0.9	2.6±0.1	4.4±1.1
20:4 ω6	4.1±0.2	3.8±1.5	6.8±0.6	8.6±0.1
20:5 ω3	1.7±0.2	1.2±0.1	2.5±0.6	4.0±0.5
22:0	0.7±0.1	0.7±0.1	2.4±0.9	3.3±0.6
22:5 ω3	2.6±1.1	3.3±0.9	4.3±0.5	7.2±1.4
22:6 ω3	13.8±1.4	10.4±1.8	7.8±0.9	16.9±1.2
SFA	108.3±1.5	70.5±1.1	127.4±2.2	129.4±1.4
MUFA	316.5±4.1	357.4±4.1	292.2±1.7	276.3±1.1
PUFA	36.3±2.7	32.8±4.5	40.9±2.5	55.4±1.9
ω6	15.4±0.6	15.2±2.0	17.8±0.1	22.8±0.9
ω3	18.2±2.5	14.9±2.5	14.6±0.5	28.1±1.5
Total *trans*	n.d.	0.3±0.1	0.5±0.5	n.d.

[1]FAME identified by the standard references and quantified as described in Materials and Methods; the values are obtained from the main GC peak areas (>97% of the total peak areas of the chromatogram); nd= not detected.

Table S11. Triglyceride fatty acids of Caco-2 cells expressed as μg/mL(mean ±S.E.M.) treated for the indicated times with 300 μM sapienic acid.

FAME[1]	0.5 h (n=3) μg/mL	1h (n=3) μg/mL	3h (n=3) μg/mL	24h (n=3) μg/mL
14:0	4.0±0.6	7.4±0.7	16.4±0.8	19.1±1.1
16:0	44.9±1.1	63.2±0.1	49.6±2.4	156.7±22.4
6*trans*-16:1	0.9±0.2	1.0±1.0	n.d.	n.d.
9*trans*-16:1	0.1±0.1	n.d.	n.d.	n.d.
6*cis*-16:1	154.0±2.7	212.2±2.2	199.8±9.3	42.6±4.6
9*cis*-16:1	10.3±0.3	8.1±1.1	15.1±1.6	16.8±0.8
18:0	75.1±0.7	26.0±2.2	27.1±2.4	57.2±5.9
9*trans*-18:1	0.2±0.1	n.d.	n.d.	n.d.
8*cis*-18:1	12.9±2.7	22.3±4.0	22.5±5.4	20.8±2.3
9*cis*-18:1	65.6±5.3	67.6±6.0	39.4±6.3	71.2±5.2
11*cis*-18:1	33.8±2.8	14.8±6.1	22.3±7.0	24.6±1.1
5*cis*,8*cis*-18:2	2.1±0.8	2.1±0.1	11.1±3.2	5.4±1.1
18:2 ω 6	15.6±0.8	8.1±2.9	9.6±2.2	8.8±1.1
20:0	1.3±0.3	1.9±0.6	3.9±0.8	4.7±0.5
20:3 ω6	2.7±0.8	3.3±0.8	3.3±0.6	5.0±1.8
20:4 ω6	8.2±0.2	4.7±1.9	11.4±5.6	7.7±1.6
20:5 ω3	1.5±0.5	2.2±0.3	7.8±3.1	4.7±1.0
22:0	1.1±0.3	1.2±0.3	4.7±1.6	2.9±0.5
22:5 ω3	3.8±0.6	3.4±1.2	5.1±1.6	5.4±1.6
22:6 ω3	22.2±1.3	11.3±3.2	11.7±0.7	7.4±1.2
SFA	126.4±0.8	99.7±2.1	101.8±0.8	240.5±17.1
MUFA	276.6±4.4	325.1±11.6	299.2±4.2	176.0±11.7
PUFA	56.3±3.7	35.2±8.5	60.1±4.3	44.5±7.3
ω6	26.6±1.3	16.1±5.6	24.3±5.3	21.5±4.3
ω3	27.6±2.2	16.9±3.9	24.6±5.2	17.6±2.0
Total *trans*	1.8±0.3	1.0±1.0	n.d.	n.d.

[1]FAME identified by the standard references and quantified as described in Materials and methods; the values are obtained from the main GC peak areas (>97% of the total peak areas of the chromatogram); nd = not detected.

Table S12. Cholesteryl ester fatty acids of Caco-2 cells expressed as μg/mL(mean ±S.E.M.) formed after treatment for 1 hour (A) and 3 hours (B) with 150 μM sapienic acid and after 0.5 (C), 1 (D) and 3 hours (E) with 300 μM (B) sapienic acid.

FAME[1]	1h 150μM (n=3)μg/mL	3h 150μM (n=3) μg/mL	0.5h 300μM (n=3) μg/mL	1h 300μM (n=3) μg/mL	3h 300μM (n=3) μg/mL
14:0	8.3±1.5	2.2±0.3	6.1±0.3	4.5±2.1	6.1±0.1
16:0	66.1±0.7	52.3±1.1	62.3±0.7	62.8±1.8	71.1±1.2
6*trans*-16:1	n.d.	n.d.	0.1±0.1	0.2±0.2	0.1±0.1
9*trans*-16:1	n.d.	n.d.	n.d.	n.d.	n.d.
6*cis*-16:1	10.0±6.4	19.1±4.1	16.9±0.8	30.5±1.0	21.4±0.3
9*cis*-16:1	17.0±6.1	8.4±3.6	21.4±0.7	7.3±0.1	3.7±0.0
18:0	21.4±0.6	23.1±9.4	21.4±0.7	20.1±0.1	23.4±0.7
9*trans* 18:1	n.d.	n.d.	n.d.	n.d.	n.d.
8*cis*-18:1	5.3±0.6	7.0±1.7	4.1±1.2	6.3±0.7	6.8±0.8
9*cis*-18:1	20.1±3.0	35.0±3.0	20.0±0.6	20.9±0.7	18.7±1.0
11*cis*-18:1	3.7±0.5	5.8±0.6	3.1±1.1	2.0±0.3	1.6±0.2
5*cis*,8*cis*-18:2	2.2±0.4	2.4±0.2	2.3±0.7	2.0±0.2	2.6±0.9
18:2 ω 6	6.9±3.1	5.8±0.5	3.2±0.5	4.5±0.2	3.7±0.9
20:0	n.d.	n.d.	n.d.	n.d.	15.8±4.9
20:3 ω6	n.d.	n.d.	n.d.	n.d.	n.d.
20:4 ω6	n.d.	n.d.	n.d.	n.d.	n.d.
20:5 ω3	n.d.	n.d.	n.d.	n.d.	n.d.
22:0	n.d.	n.d.	n.d.	n.d.	n.d.
22:5 ω3	n.d.	n.d.	n.d.	n.d.	n.d.
22:6 ω3	n.d.	n.d.	n.d.	n.d.	n.d.
SFA	63.2±31.6	77.6±8.8	89.9±1.3	87.4±0.5	101.2±1.8
MUFA	36.9±18.5	75.2±8.8	65.6±1.6	67.0±0.3	52.2±0.7
PUFA	7.3±4.7	8.3±0.6	5.5±0.3	6.5±0.3	6.3±0.1
ω6	5.6±3.9	5.8±0.5	3.2±0.5	4.5±0.2	3.7±0.9
ω3	n.d.	n.d.	n.d.	n.d.	n.d.
Total *trans*	n.d.	n.d.	0.1±0.1	0.2±0.2	1.3±12

[1]FAME identified by the standard references and quantified as described in Materials and Methods; the values are obtained from the main GC peak areas (>97% of the total peak areas of the chromatogram); nd = not detected.
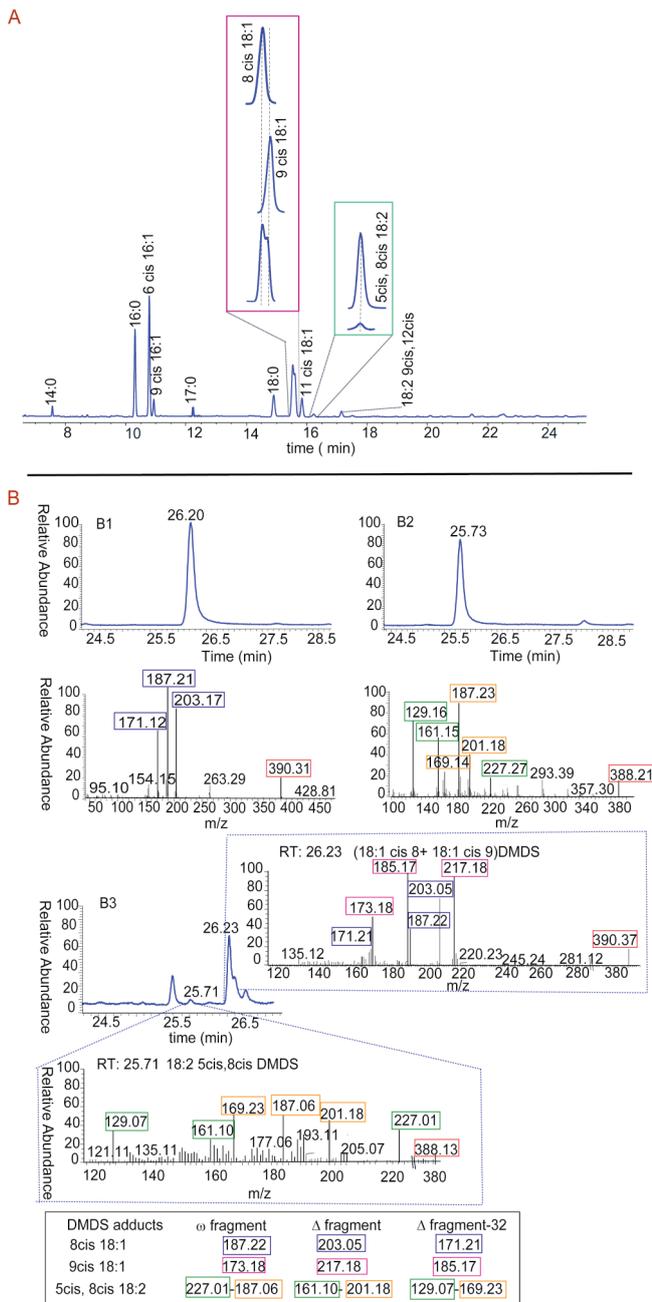
A

8 cis 18:1
9 cis 18:1
5cis, 8cis 18:2

14:0
16:0
6 cis 16:1
9 cis 16:1
17:0
18:0
11 cis 18:1
18:2 9cis,12cis

time (min)

B

B1 26.20

Relative Abundance

Time (min)

187.21
203.17
171.12
95.10 154.15 263.29 390.31 428.81

B2 25.73

Time (min)

187.23
129.16
161.15
169.14 201.18
227.27 293.39 357.30 388.21

m/z

B3 26.23 25.71

time (min)

RT: 26.23 (18:1 cis 8+ 18:1 cis 9)DMDS
185.17 217.18
173.18 203.05
171.21 187.22
135.12 220.23 245.24 281.12 390.37
m/z

RT: 25.71 18:2 5cis,8cis DMDS
129.07
161.10 169.23 187.06 201.18 227.01
121.11 135.11 177.06 193.11 205.07 388.13
m/z

DMDS adducts	ω fragment	Δ fragment	Δ fragment-32
8cis 18:1	187.22	203.05	171.21
9cis 18:1	173.18	217.18	185.17
5cis, 8cis 18:2	227.01-187.06	161.10- 201.18	129.07-169.23

Figure S2. GC analysis (A) and mass spectra (B) of the DMDS adducts of fatty acid methyl esters obtained from membrane phospholipids in the fatty acid supplementation experiments. **A)** Chromatogram region of the fatty acid residues as fatty acid methyl esters (FAME) of membrane phospholipids in Caco-2 cell line treated with sapienic acid; in the purple box, A and B are the peaks of 8*cis*-18:1 and 9*cis*-18:1, as reference standards, while C refers to these FAMEs detected in the a representative cell samples; in the green box, A' and B' are the peaks of 5*cis*,8*cis*-18:2 as standard and the presence in the sample, respectively. **B)** GC-MS of fatty acid methyl esters treated as described in Materials and Methods for the DMDS derivatization. (B1) DMDS adduct of the standard reference 8*cis*-18:1; (B2) DMDS adduct of the standard reference of 5*cis*,8*cis*-

18:2 and (B3) DMDS adducts of FAME mixture obtained from membrane phospholipids of Caco-2 cell line after sapienic acid supplementation; in the box the diagnostic fragmentations of the DMDS adducts recognized in the sample are reported.