**Supplementary figures and tables**, belonging to

# “*Prolonged niacin treatment leads to increased adipose tissue poly-unsaturated fatty acid synthesis and an anti-inflammatory lipid and oxylipin plasma profile*”

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Table S I: qPCR primer sequences for the household genes *Prlp0* & *Cyclo* and for the target genes *Elovl5*, *Elovl6* &*Fads2*.

RNA was isolated from gWAT using the Nucleospin RNA/Protein kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). Subsequently, 1µg of RNA was used for cDNA synthesis by iScript (BioRad, Hercules, CA, USA), which was purified by the Nucleospin Gel and PCR clean-up kit (Machery Nagel). Real-Time PCR was carried out on the IQ5 PCR machine (BioRad) using the Sensimix SYBR Green RT-PCR mix (Quantace, London, UK) and QuantiTect SYBR Green RT-PCR mix (Qiagen, Venlo, the Netherlands). Target mRNA levels were normalized to *Rplp0* & *Ppia* mRNA levels.

| **Gen** | **FW primer** | **RV primer** | **Temperature** |
|---|---|---|---|
| *Rplp0* | GGACCCGAGAAGACCTCCTT | GCACATCACTCAGAATTTCAATGG | 60 |
| *Ppia* | ACTGAATGGCTGGATGGCAA | TGTCCACAGTCGGAAATGGT | 61 |
| *Elovl5* | CAGCTTGCTTCTGTTCCCG | TCCATTTTAAAACCTCTCTGCCT | 61 |
| *Elovl6* | GCACTAAGACCGCAAGGCAT | CTACGTGTTCTCTGCGCCTC | 61 |
| *Fads2* | CTGGTGGAACCACCGACATT | TCTTGCCATACTCAAGGGGC | 61 |

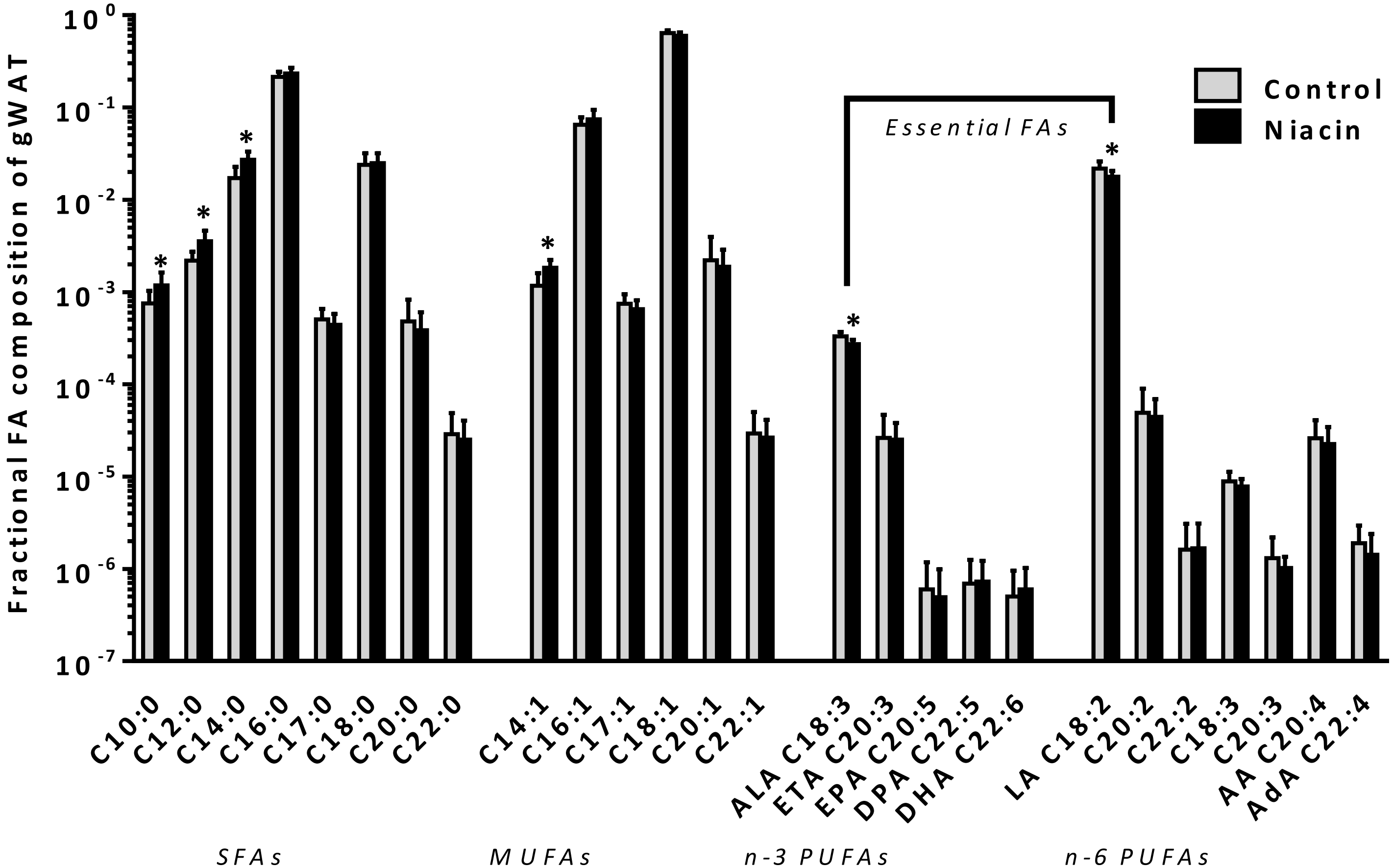



Figure S I: Adipose tissue fatty acid composition of gWAT from APOE*3-Leiden.CETP mice fed a western type diet with 0.1% cholesterol with and without niacin. Mean±SD, N=14 for Control/N=13 for Niacin, *p<0.05 compared to control gWAT after false discovery rate correction.

| | Control gWAT | | | Niacin gWAT | | | Diet | | | Control vs Niacin |
|---|---|---|---|---|---|---|---|---|---|---|
| **SFA** | **Average** | ***SD*** | | **Average** | ***SD*** | | **Average** | ***SD*** | | **P-value** |
| C10:0 | 0,0008 | *0,0003* | | **0,0012** | *0,0004* | | 0,0013 | *0,0009* | | (*)0,0078 |
| C12:0 | 0,0022 | *0,0005* | | **0,0036** | *0,0011* | | 0,0019 | *0,0013* | | (*)0,0005 |
| C14:0 | 0,0173 | *0,0054* | | **0,0271** | *0,0061* | | 0,0059 | *0,0038* | | (*)0,0003 |
| C16:0 | 0,2153 | *0,0285* | | 0,2339 | *0,0360* | | 0,4351 | *0,0571* | | 0,1679 |
| C17:0 | 0,0005 | *0,0002* | | 0,0004 | *0,0001* | | 0,0014 | *0,0006* | | 0,2804 |
| C18:0 | 0,0239 | *0,0081* | | 0,0250 | *0,0069* | | 0,2162 | *0,0331* | | 0,7049 |
| C20:0 | 0,0005 | *0,0003* | | 0,0004 | *0,0002* | | 0,0030 | *0,0009* | | 0,4066 |
| C22:0 | 2,88E-05 | *1,99E-05* | | 2,51E-05 | *1,55E-05* | | 3,00E-04 | *7,01E-05* | | 0,6070 |
| **MUFA** | | | | | | | | | | |
| C14:1 | 0,0012 | *0,0004* | | **0,0018** | *0,0004* | | 0,0003 | *0,0002* | | (*)0,0006 |
| C16:1 | 0,0652 | *0,0131* | | 0,0745 | *0,0202* | | 0,0055 | *0,0035* | | 0,1773 |
| C17:1 | 0,0007 | *0,0002* | | 0,0007 | *0,0002* | | 0,0002 | *0,0001* | | 0,1951 |
| C18:1 | 0,6400 | *0,0459* | | 0,6007 | *0,0532* | | 0,3036 | *0,0445* | | 0,0593 |
| C20:1 | 0,0022 | *0,0018* | | 0,0019 | *0,0010* | | 0,0004 | *0,0002* | | 0,5577 |
| C22:1 | 2,94E-05 | *2,05E-05* | | 2,65E-05 | *1,47E-05* | | 1,62E-05 | *1,13E-05* | | 0,6729 |
| **PUFA n-3** | | | | | | | | | | |
| ALA C18:3 | 3,32E-04 | *3,89E-05* | | **2,72E-04** | *3,37E-05* | | 4,58E-04 | *9,25E-05* | | (*)0,0003 |
| ETA C20:3 | 2,63E-05 | *2,06E-05* | | 2,51E-05 | *1,31E-05* | | 2,82E-06 | *1,61E-06* | | 0,8598 |
| EPA C20:5 | 6,01E-07 | *5,83E-07* | | 4,93E-07 | *4,93E-07* | | 9,89E-07 | *9,91E-07* | | 0,7985 |
| DPA C22:5 | 6,95E-07 | *5,62E-07* | | 7,28E-07 | *5,00E-07* | | 4,11E-07 | *3,58E-07* | | 0,8961 |
| DHA C22:6 | 5,02E-07 | *4,58E-07* | | 6,00E-07 | *4,28E-07* | | 2,06E-06 | *4,75E-07* | | 0,5940 |
| **PUFA n-6** | | | | | | | | | | |
| LA C18:2 | 2,19E-02 | *4,13E-03* | | **1,78E-02** | *2,82E-03* | | 2,32E-02 | *9,00E-03* | | (*)0,0080 |
| C20:2 | 4,91E-05 | *4,09E-05* | | 4,43E-05 | *2,49E-05* | | 1,15E-05 | *4,28E-06* | | 0,7206 |
| C22:2 | 1,62E-06 | *1,47E-06* | | 1,67E-06 | *1,45E-06* | | 7,95E-06 | *1,70E-06* | | 0,9370 |
| C18:3 | 8,86E-06 | *2,45E-06* | | 7,84E-06 | *1,59E-06* | | 7,16E-07 | *4,56E-07* | | 0,2231 |
| C20:3 | 1,31E-06 | *8,78E-07* | | 1,02E-06 | *3,31E-07* | | 3,55E-07 | *1,66E-07* | | 0,2789 |
| AA C20:4 | 2,60E-05 | *1,47E-05* | | 2,25E-05 | *1,18E-05* | | 1,47E-06 | *9,51E-07* | | 0,5065 |
| AdA C22:4 | 1,90E-06 | *1,07E-06* | | 1,42E-06 | *9,62E-07* | | 2,86E-07 | - | | 0,2540 |

Table S II: Adipose tissue fatty acid composition of gWAT from APOE*3-Leiden.CETP mice fed a western type diet with 0.1% cholesterol with and without niacin and fatty acid composition of the western type diet. Fraction of total area corrected sum. Mean±SD, N=14 for Control/N=13 for Niacin/N=3 for diet.

(*)Significant finding after false discovery rate correction.

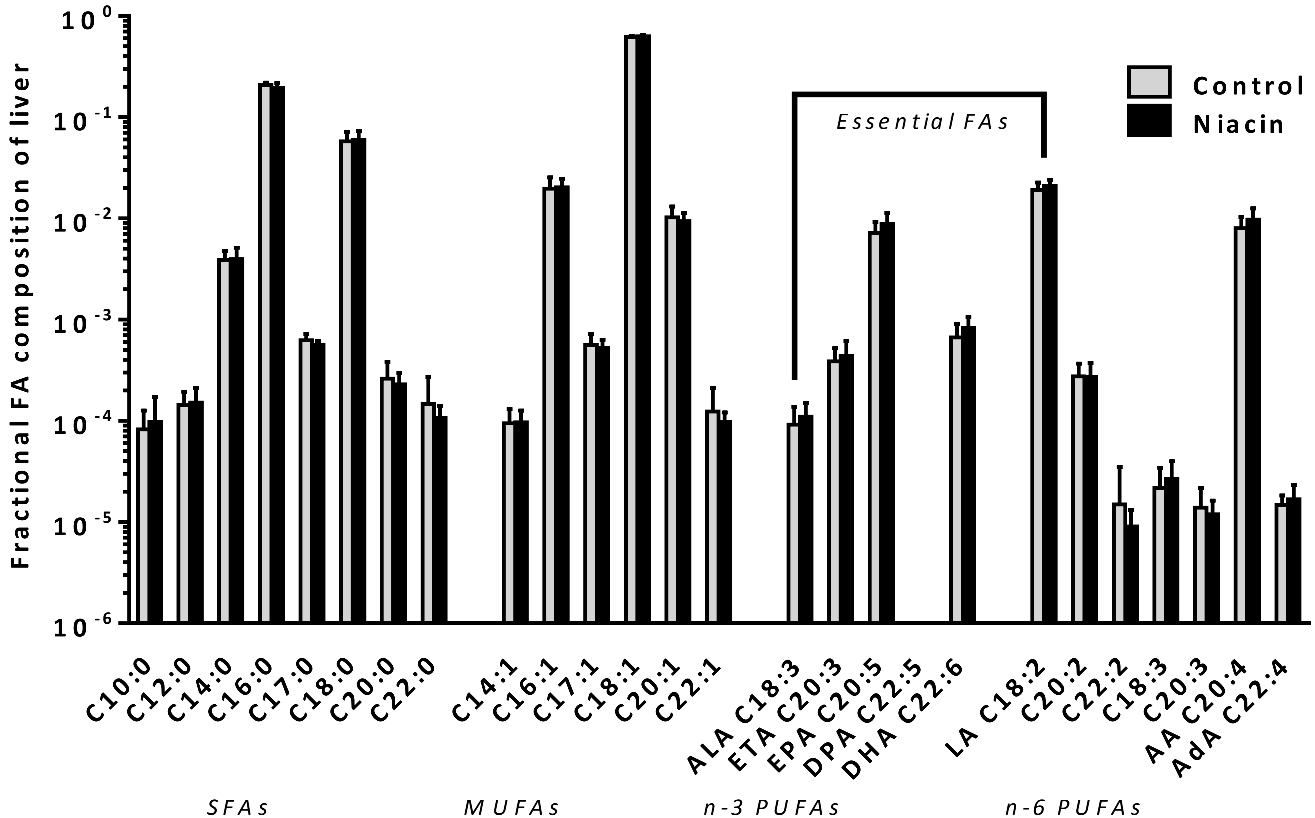



Figure S II: Liver fatty acid composition from APOE*3-Leiden.CETP mice fed a western type diet with 0.1% cholesterol with and without niacin. Fraction of total area corrected sum. Mean±SD, N=14 for Control/N=13 for Niacin.

*p<0.05 comparing control gWAT to niacin gWAT after false discovery rate correction.

Table S III: Liver fatty acid composition from APOE*3-Leiden.CETP mice fed a western type diet with 0.1% cholesterol with and without niacin. Fraction of total area corrected sum. Mean±SD, N=14 for Control/N=13 for Niacin.

(*)Significant finding after false discovery rate correction.

| | **Control Liver** | | | **Niacin Liver** | | | **Control vs Niacin** |
|---|---|---|---|---|---|---|---|
| **SFA** | **Average** | **SD** | | **Average** | **SD** | | **P-value** |
| C10:0 | 8,23E-05 | *4,42E-05* | | 9,74E-05 | *7,38E-05* | | 0,5248 |
| C12:0 | 0,000143 | *5,17E-05* | | 0,000152 | *5,92E-05* | | 0,6948 |
| C14:0 | 0,003877 | *0,000907* | | 0,003968 | *0,001167* | | 0,8245 |
| C16:0 | 0,20753 | *0,013181* | | 0,196168 | *0,020302* | | 0,0989 |
| C17:0 | 0,000625 | *9,85E-05* | | 0,000565 | *5,39E-05* | | 0,0739 |
| C18:0 | 0,057714 | *0,014338* | | 0,059939 | *0,013182* | | 0,6860 |
| C20:0 | 0,000262 | *0,000122* | | 0,000229 | *6,67E-05* | | 0,4218 |
| C22:0 | 0,000147 | *0,000125* | | 0,000107 | *3,45E-05* | | 0,2909 |
| **MUFA** | | | | | | | |
| C14:1 | 9,46E-05 | *3,57E-05* | | 9,7E-05 | *2,96E-05* | | 0,8528 |
| C16:1 | 0,019737 | *0,00563* | | 0,020314 | *0,004366* | | 0,7759 |
| C17:1 | 0,00056 | *0,000161* | | 0,000524 | *0,000112* | | 0,5268 |
| C18:1 | 0,621529 | *0,018559* | | 0,628717 | *0,023243* | | 0,3893 |
| C20:1 | 0,010283 | *0,002869* | | 0,009425 | *0,001852* | | 0,3834 |
| C22:1 | 0,000124 | *8,64E-05* | | 9,79E-05 | *2,31E-05* | | 0,3213 |
| **PUFA n-3** | | | | | | | |
| ALA C18:3 | 9,17E-05 | *4,6E-05* | | 0,00011 | *3,92E-05* | | 0,2796 |
| ETA C20:3 | 0,000388 | *0,000133* | | 0,000438 | *0,000173* | | 0,4111 |
| EPA C20:5 | 0,00721 | *0,00204* | | 0,00886 | *0,002503* | | 0,0763 |
| DPA C22:5 | - | - | | - | - | | |
| DHA C22:6 | 0,000669 | *0,000237* | | 0,000828 | *0,000228* | | 0,0945 |
| **PUFA n-6** | | | | | | | |
| LA C18:2 | 0,019119 | *0,003479* | | 0,020887 | *0,003278* | | 0,1973 |
| C20:2 | 0,000276 | *9,29E-05* | | 0,000271 | *0,000102* | | 0,9115 |
| C22:2 | 1,49E-05 | *2,02E-05* | | 9,08E-06 | *4,02E-06* | | 0,3334 |
| C18:3 | 2,17E-05 | *1,29E-05* | | 2,69E-05 | *1,31E-05* | | 0,3204 |
| C20:3 | 1,39E-05 | *7,87E-06* | | 1,19E-05 | *4,41E-06* | | 0,4437 |
| AA C20:4 | 0,008023 | *0,002318* | | 0,009784 | *0,002791* | | 0,0916 |
| AdA C22:4 | 1,47E-05 | *3,65E-06* | | 1,69E-05 | *6,41E-06* | | 0,3014 |

| | Control | | Niacin | | Control vs Niacin |
|---|---|---|---|---|---|
| **PUFA** | **Average (ng/mL)** | **SD** | **Average (ng/mL)** | **SD** | **P-value** |
| ALA | 1139,98 | *69,76* | **784,61** | *61,24* | (*)0,0007 |
| EPA | 91,51 | *7,10* | 82,03 | *6,51* | 0,3342 |
| DPA | 1373,47 | *118,48* | 1133,20 | *81,11* | 0,0996 |
| DHA | 1012,96 | *52,65* | 1194,23 | *52,00* | 0,0226 |
| LA | 9033,60 | *427,04* | 8875,20 | *273,66* | 0,7614 |
| AA | 5833,59 | *417,18* | 5342,29 | *192,80* | 0,2949 |
| AdA | 139,25 | *11,33* | 119,86 | *7,21* | 0,1608 |
| **Oxylipins** | | | | | |
| 12-HETE | 140,60 | *100,95* | 138,77 | *121,74* | 0,9666 |
| Leukotriene $E_4$ | 0,125 | *0,020* | 0,112 | *0,005* | 0,0324 |
| Prostaglandin $D_2$ | 0,545 | *0,073* | 0,528 | *0,097* | 0,6066 |
| Thromboxane $B_2$ | 3,25 | *0,95* | 4,67 | *2,00* | 0,0252 |
| 14,15-diHETE | 0,247 | *0,075* | 0,291 | *0,127* | 0,2891 |
| 19,20-diHDPA | 0,696 | *0,131* | **1,011** | *0,345* | (*)0,0065 |

Table S IV: Unfasted plasma PUFA and oxylipin concentrations of APOE*3-Leiden.CETP mice fed a western type diet with 0.1% cholesterol with and without niacin. Mean±SD, N=14 for Control/N=13 for Niacin.

(*)Significant finding after false discovery rate correction.

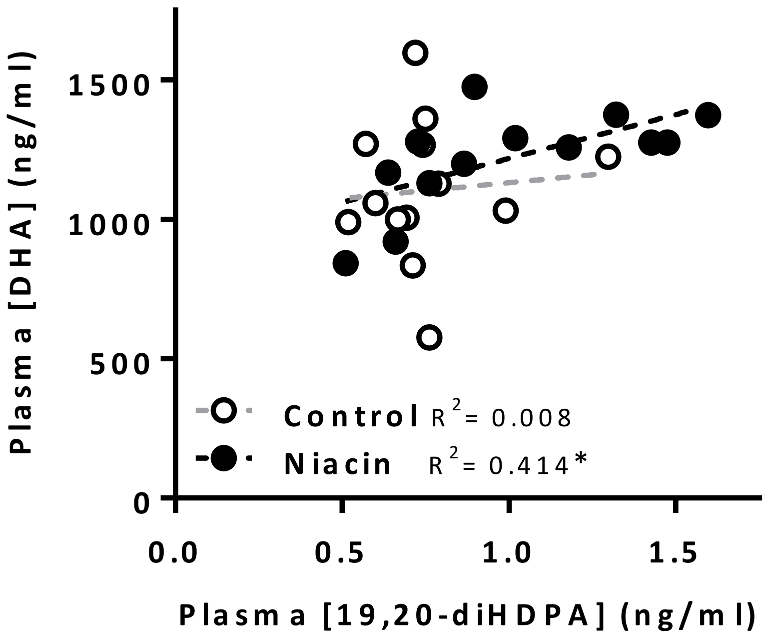



Figure S III: Correlation between the plasma concentrations of 19,20-dihydroxydocosapentaenoic acid and docosahexaenoic acid. N=14 mice per group, *p<0.05 compared to a slope of zero.

| Symbol | Lipid Maps ID | RT (min) | Q1 (m/z) | Q3 (m/z) | DP (Volts) | EP (Volts) | CE (Volts) | CCEP (Volts) |
|---|---|---|---|---|---|---|---|---|
| RvE1 | LMFA03070019 | 4.0 | 349.1 | 195.0 | -95 | -10 | -22 | -13 |
| 20-hydroxy $LTB_4$ | LMFA03020018 | 4.4 | 351.1 | 195.0 | -60 | -10 | -24 | -17 |
| 8-iso-$PGF_2α$ | LMFA03110001 | 5.1 | 353.1 | 193.0 | -135 | -10 | -34 | -11 |
| 15-keto-$PGE_2$ | LMFA03010030 | 5.1 | 349.0 | 234.9 | -65 | -10 | -20 | -13 |
| $TxB_2$ | LMFA03030002 | 5.2 | 369.1 | 169.0 | -55 | -10 | -24 | -15 |
| 8-iso-$PGE_2$ | LMFA03110003 | 5.3 | 351.1 | 271.0 | -5 | -10 | -24 | -19 |
| 13,14-dihydro-15-keto-$PGE_2$ | LMFA03010031 | 5.6 | 351.1 | 235.0 | -45 | -10 | -30 | -13 |
| **$PGE_2$-$d_4$** | LMFA03010008 | 5.6 | 355.1 | 193.0 | -50 | -10 | -26 | -17 |
| $PGE_2$ | LMFA03010003 | 5.7 | 351.2 | 271.1 | -50 | -10 | -22 | -21 |
| $PGD_2$ | LMFA03010004 | 5.8 | 351.1 | 233.0 | -30 | -10 | -16 | -13 |
| $LXB_4$ | LMFA03040002 | 6.0 | 351.1 | 220.9 | -60 | -10 | -22 | -13 |
| $PGF_{2α}$ | LMFA03010002 | 6.1 | 353.1 | 193.0 | -80 | -10 | -34 | -11 |
| RvD2 | LMFA04000007 | 6.2 | 375.1 | 277.1 | -60 | -10 | -18 | -15 |
| $LXA_4$ | LMFA03040001 | 6.5 | 351.1 | 114.8 | -40 | -10 | -20 | -11 |
| 13,14-dihydro-15-keto-$PGF_2α$ | LMFA03010027 | 6.6 | 353.1 | 195.0 | -110 | -10 | -32 | -11 |
| AT-RvD1 | LMFA04000074 | 6.7 | 375.0 | 215.0 | -50 | -10 | -26 | -11 |
| RvD1 | LMFA04000006 | 6.7 | 375.1 | 215.0 | -50 | -10 | -26 | -11 |
| epi-$LXA_4$ | LMFA03040003 | 6.8 | 351.1 | 114.9 | -20 | -10 | -22 | -11 |
| RvE1 | LMFA03070019 | 7.8 | 333.1 | 114.9 | -35 | -10 | -18 | -15 |
| 18S-RvE3 | LMFA03070048 | 8.8 | 333.1 | 245.2 | -25 | -10 | -16 | -17 |
| 6-trans-$LTB_4$ | LMFA03020013 | 8.9 | 335.1 | 194.9 | -105 | -10 | -22 | -11 |
| 8S,15S-diHETE | LMFA03060050 | 8.9 | 335.1 | 207.9 | -55 | -10 | -22 | -17 |
| $LTD_4$ | LMFA03020006 | 9.0 | 495.1 | 177.0 | -70 | -10 | -28 | -19 |
| 6-trans-12-epi-$LTB_4$ | LMFA03020014 | 9.1 | 335.1 | 194.9 | -80 | -10 | -22 | -25 |
| 10S,17S-diHDHA (PDX) | LMFA04000047 | 9.2 | 359.1 | 153.0 | -70 | -10 | -22 | -9 |
| 18R-$RvE_3$ | LMFA03070049 | 9.2 | 333.1 | 245.0 | -55 | -10 | -18 | -23 |
| 7S-MaR1 | n.a. | 9.3 | 359.1 | 249.9 | -20 | -10 | -20 | -19 |
| MaR1 | LMFA04000048 | 9.4 | 359.2 | 250.2 | -65 | -10 | -20 | -13 |
| **$LTB_4$-$d_4$** | LMFA03020030 | 9.4 | 339.1 | 196.9 | -70 | -10 | -22 | -19 |
| $LTB_4$ | LMFA03020001 | 9.4 | 335.1 | 195.0 | -65 | -10 | -22 | -21 |
| 14,15-diHETE | LMFA03060077 | 9.5 | 335.1 | 207.0 | -65 | -10 | -24 | -21 |
| 7,17-diHDPA | n.a. | 9.5 | 361.1 | 198.9 | -45 | -10 | -26 | -23 |
| $LTE_4$ | LMFA03020002 | 9.6 | 438.1 | 333.1 | -55 | -10 | -26 | -15 |
| 19,20-diHDPA | LMFA04000043 | 10.2 | 361.1 | 273.0 | -55 | -10 | -22 | -15 |
| 9-HOTrE | LMFA02000024 | 10.2 | 293.0 | 170.9 | -75 | -10 | -20 | -15 |
| 13-HOTrE | LMFA02000051 | 10.3 | 293.0 | 195.0 | -45 | -10 | -24 | -19 |
| 18-HEPE | LMFA03070038 | 10.4 | 317.1 | 259.0 | -5 | -10 | -16 | -7 |
| 15-HEPE | LMFA03070009 | 10.5 | 317.1 | 219.0 | -65 | -10 | -18 | -19 |
| 13-HODE | LMFA02000228 | 10.8 | 295.0 | 194.9 | -110 | -10 | -24 | -21 |
| 9-HODE | LMFA02000188 | 10.8 | 295.0 | 171.0 | -130 | -10 | -22 | -7 |
| **15-HETE-$d_8$** | LMFA03060080 | 10.9 | 327.2 | 226.0 | -85 | -10 | -18 | -11 |
| 15-HETE | LMFA03060001 | 11.0 | 319.1 | 219.1 | -55 | -10 | -18 | -9 |
| 11-HETE | LMFA03060003 | 11.1 | 319.1 | 167.0 | -70 | -10 | -22 | -15 |
| 17-HDHA | LMFA04000072 | 11.1 | 343.1 | 245.0 | -65 | -10 | -16 | -15 |
| 12-HETE | LMFA03060007 | 11.2 | 319.1 | 179.0 | -65 | -10 | -20 | -23 |
| 8-HETE | LMFA03060006 | 11.2 | 319.1 | 154.9 | -70 | -10 | -20 | -19 |
| 5-HETE | LMFA03060002 | 11.3 | 319.1 | 115.0 | -65 | -10 | -18 | -11 |
| ALA | LMFA01030152 | 12.4 | 277.0 | 233.0 | -90 | -10 | -22 | -29 |
| EPA | LMFA01030759 | 12.4 | 301.0 | 202.9 | -125 | -10 | -18 | -21 |
| **DHA-$d_5$** | LMFA01030762 | 12.4 | 332.0 | 288.1 | -75 | -10 | -16 | -13 |
| DHA | LMFA01030185 | 12.7 | 327.1 | 229.2 | -115 | -10 | -18 | -11 |
| AA | LMFA01030001 | 12.7 | 303.0 | 205.1 | -155 | -10 | -20 | -11 |
| LA | LMFA01030120 | 12.8 | 279.0 | 261.0 | -115 | -10 | -28 | -13 |
| DPA n-3 | LMFA04000044 | 13.0 | 329.1 | 231.1 | -50 | -10 | -20 | -17 |
| AdA | LMFA01030178 | 13.1 | 331.1 | 233.0 | -130 | -10 | -22 | -11 |

Table S V: Multiple Reaction Monitoring setup for ion transitions of the target compounds. Symbols in black refer to internal standards.

RT retention time,

Q1 quadrupole 1 ion selection,

Q3 quadrupole 3 ion selection,

EP entrance potential,

CE, collision energy,

CCEP collision cell exit potential

HODEs, HOTrEs, HETEs, HEPEs, diHETEs and diHDPAs are given without chiral descriptors.

Figure S IVa: MS/MS of 0.1 ng/mL standard sample at Relative RT 1.016 (Leukotriene $E_4$)

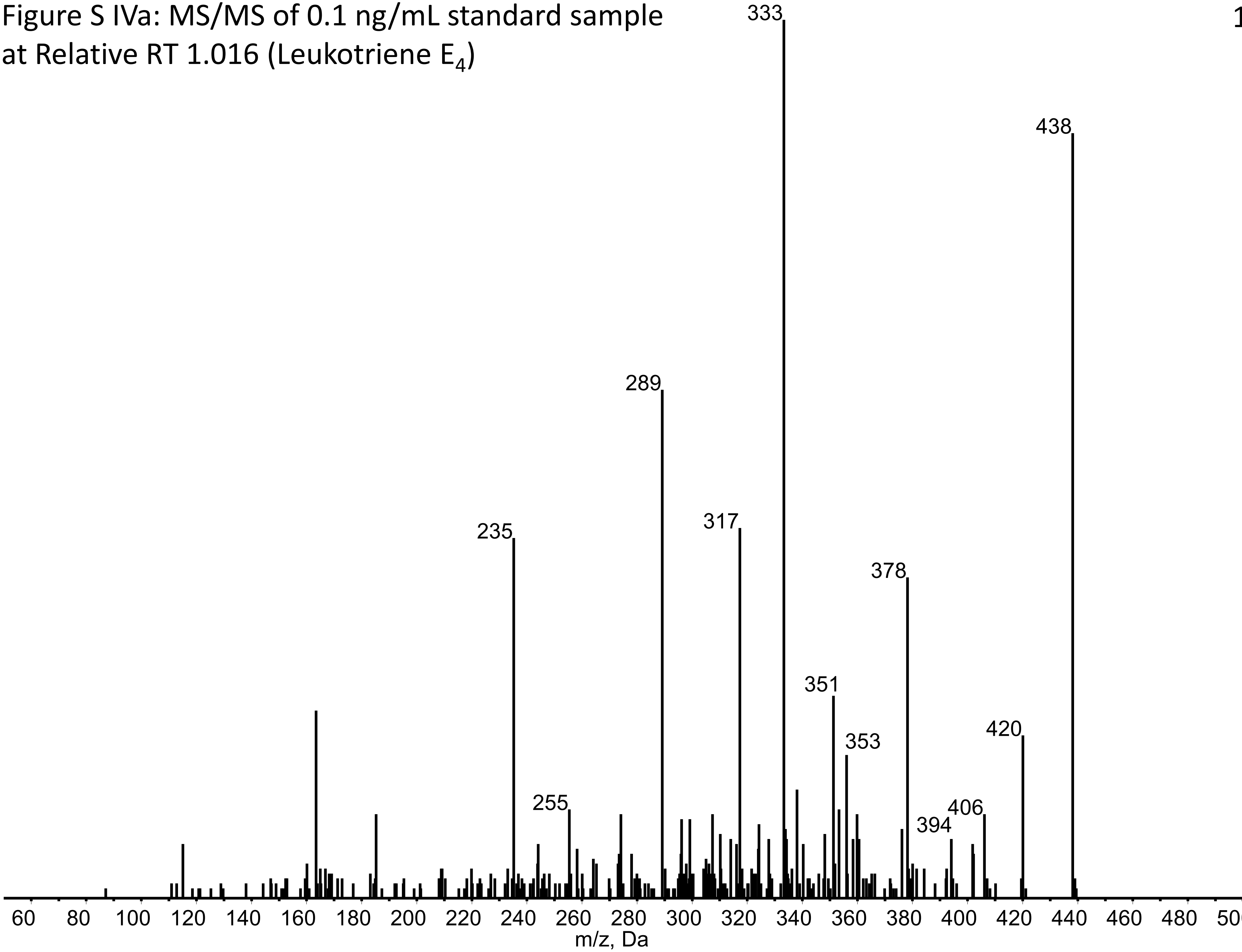


Figure S IVb: MS/MS spectra of representative sample at Relative RT 1.015 (Leukotriene $E_4$)



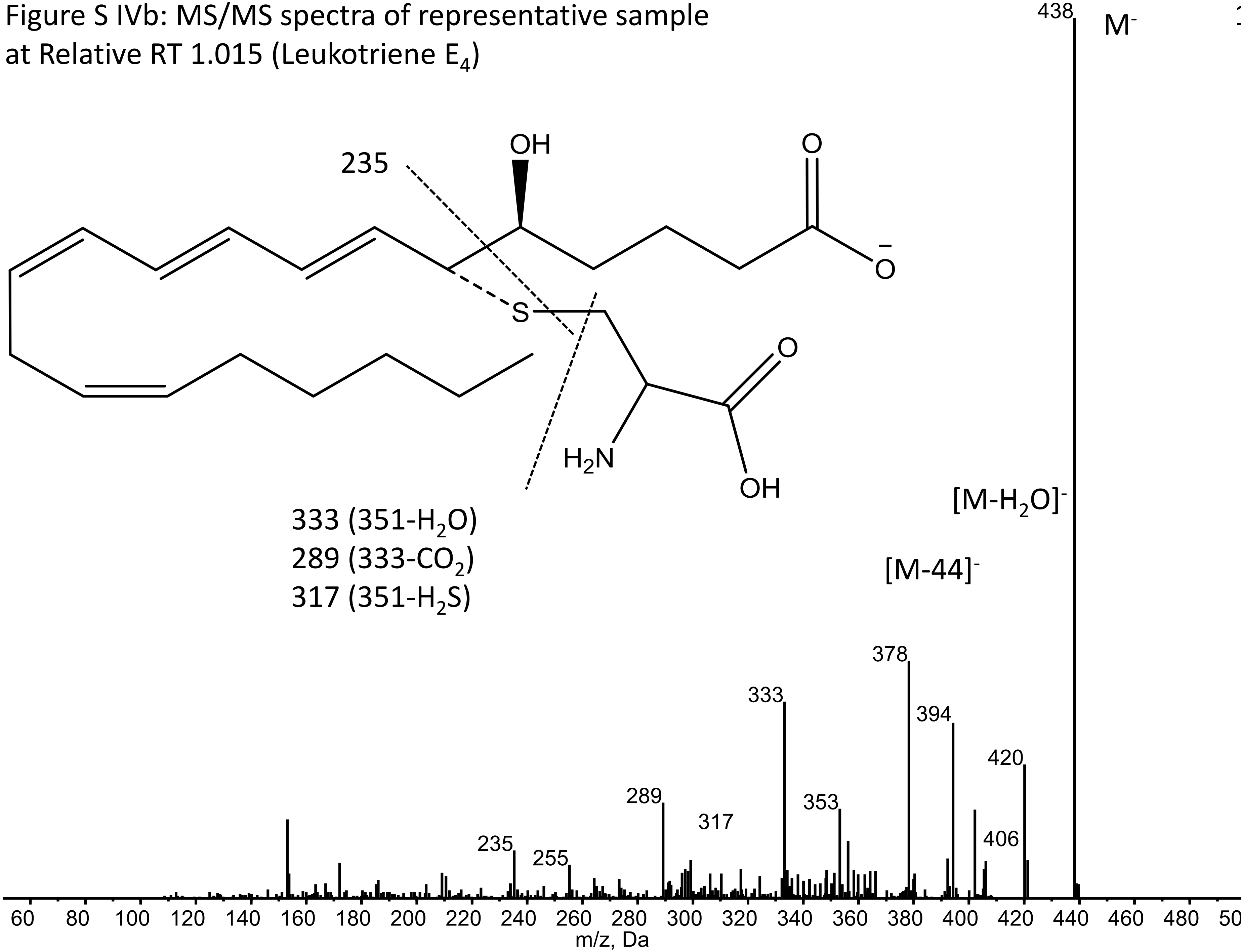


Figure S Va: MS/MS spectra of 0.1 ng/mL standard sample at Relative RT 0.925 (Thromboxane $B_2$)



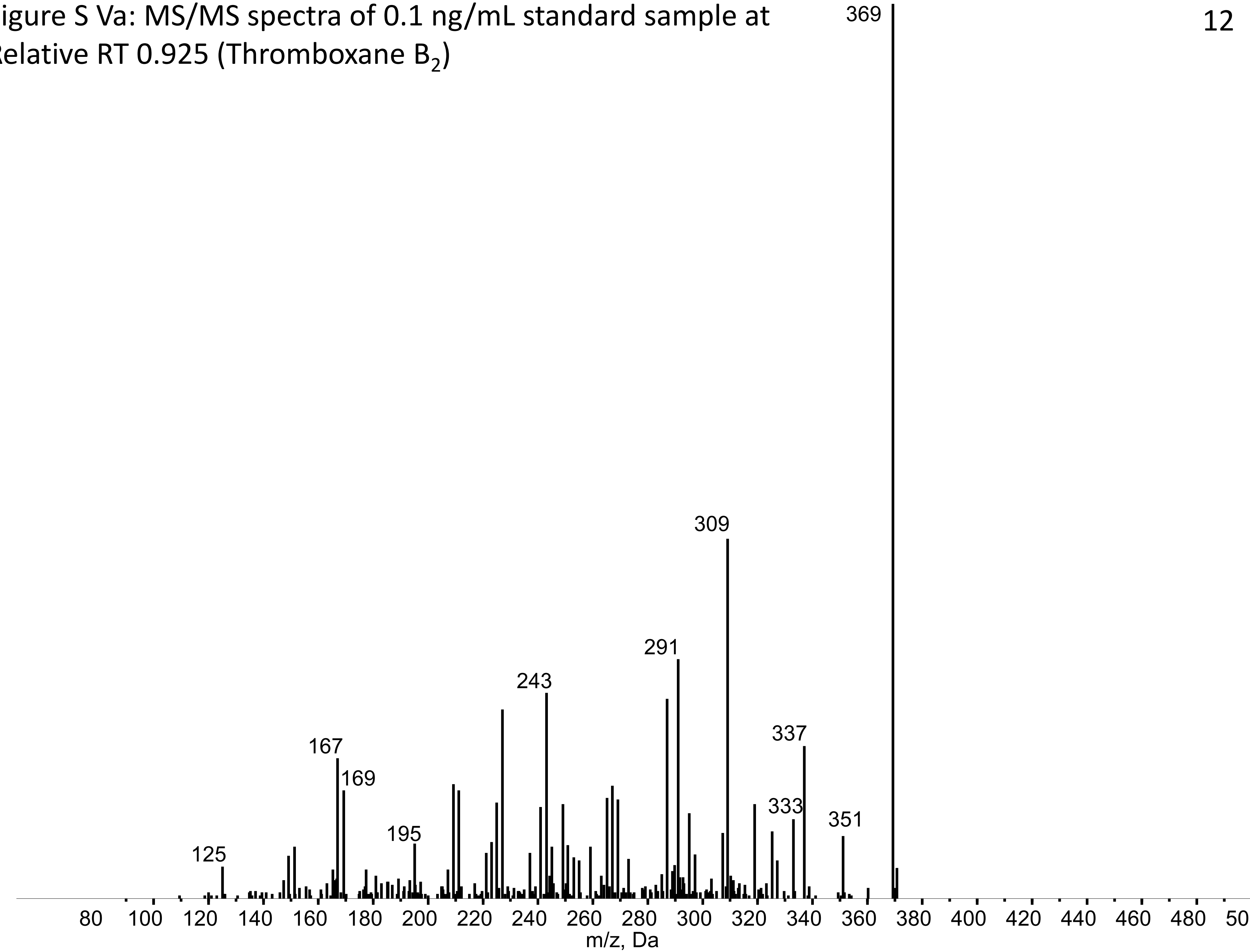


Figure S Vb: MS/MS spectra of representative sample at Relative RT 0.927 (Thromboxane $B_2$)

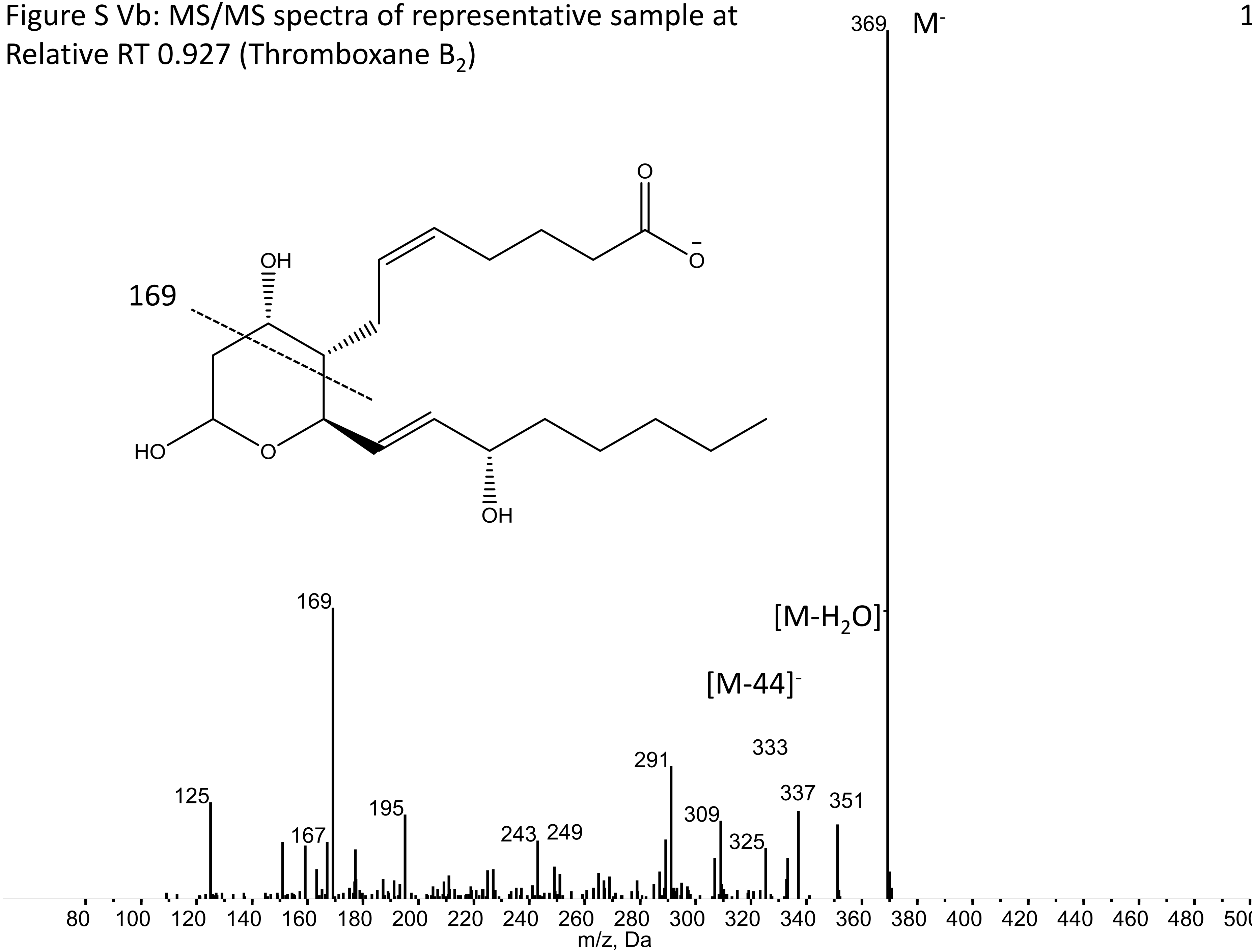


Figure S VIa: MS/MS spectra of 0.1 ng/mL standard sample at Relative RT 1.087 (19,20-diHDPA)



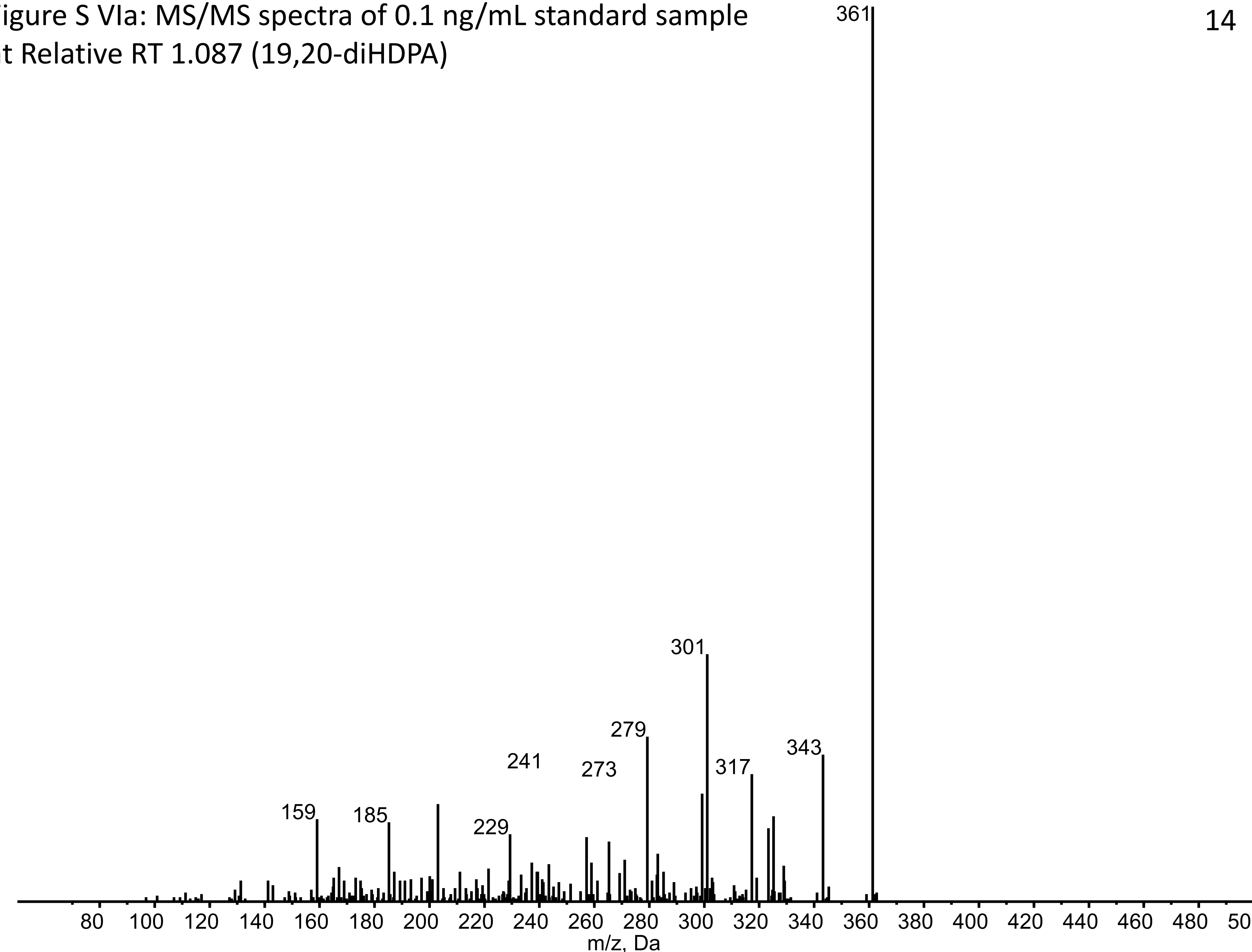
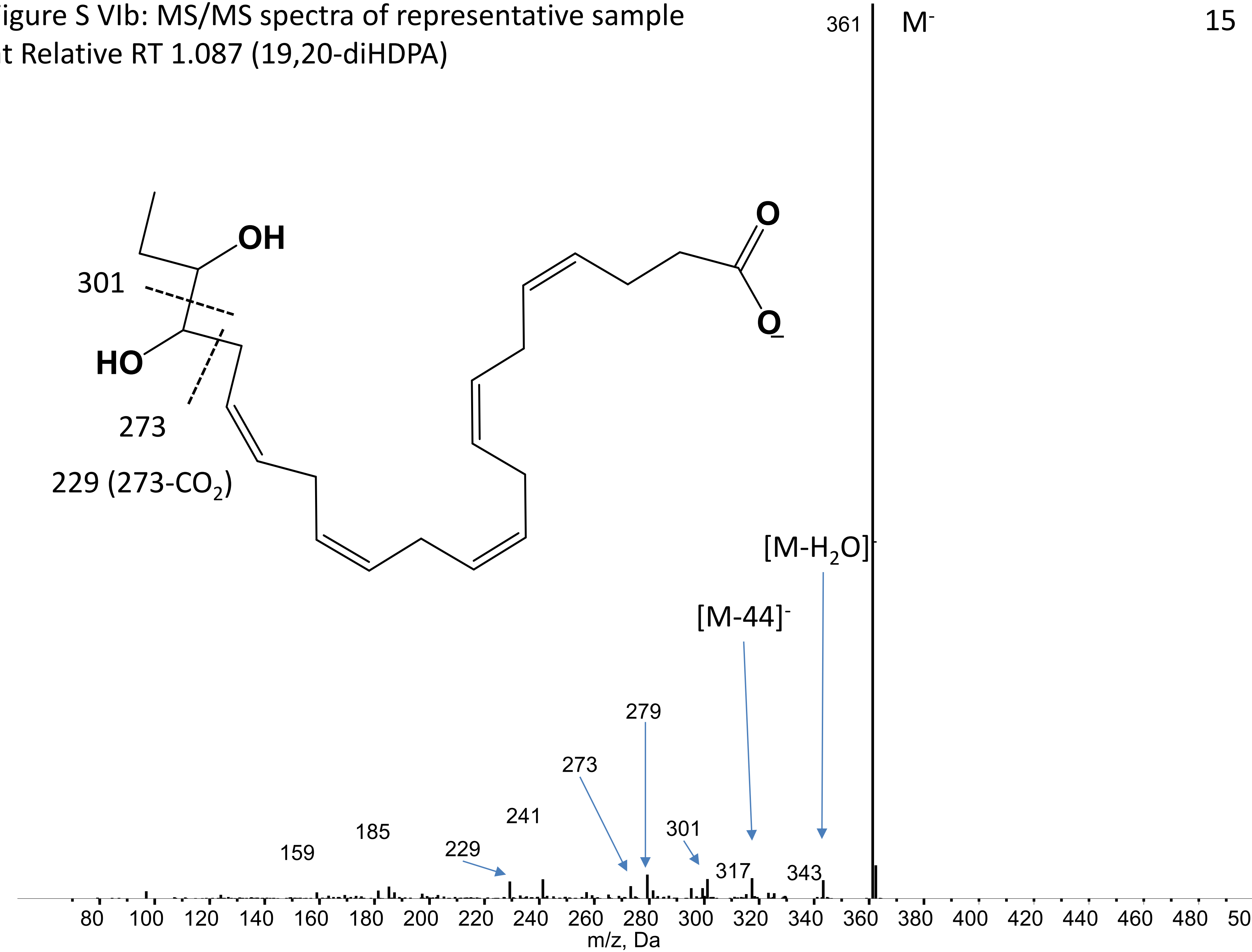


Figure S VIb: MS/MS spectra of representative sample at Relative RT 1.087 (19,20-diHDPA)



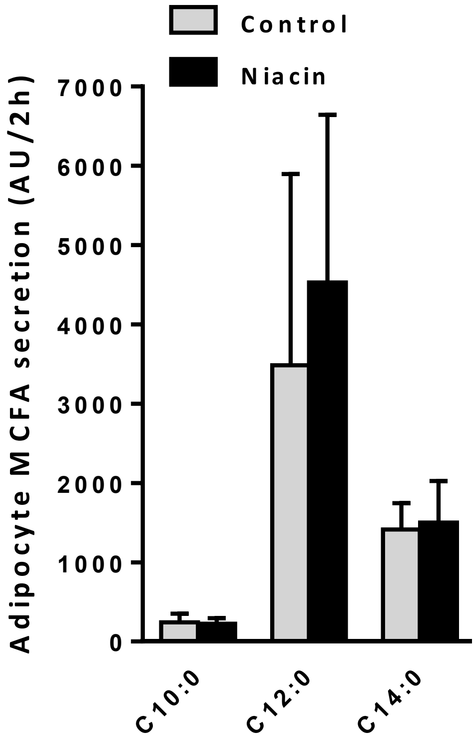



Figure S VII: Release of medium chain saturated fatty acids from adipocytes isolated from APOE*3-Leiden.CETP mice fed a western type diet with 0.1% cholesterol with and without niacin. Fatty acid release in arbitrary units during a 2 hour *ex vivo* basal incubation. Mean±SD, N=14 for Control/N=13 for Niacin.

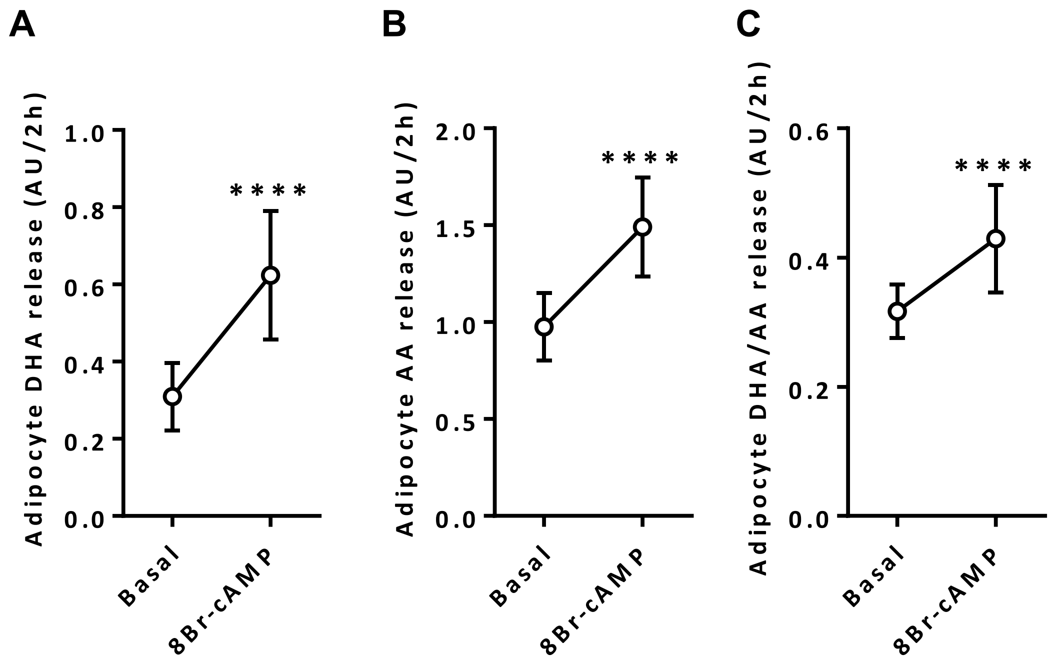



Figure S VIII: Release of DHA and AA from adipocytes isolated from APOE*3-Leiden.CETP mice fed a western type diet with 0.1% cholesterol without niacin. Fatty acid release in arbitrary units during a 2 hour *ex vivo* incubation in basal and 8Bromo-cAMP stimulated conditions. Mean±SD, N=14 for Control/N=13 for Niacin.

**** p<0,0001 for Basal vs 8Br-cAMP

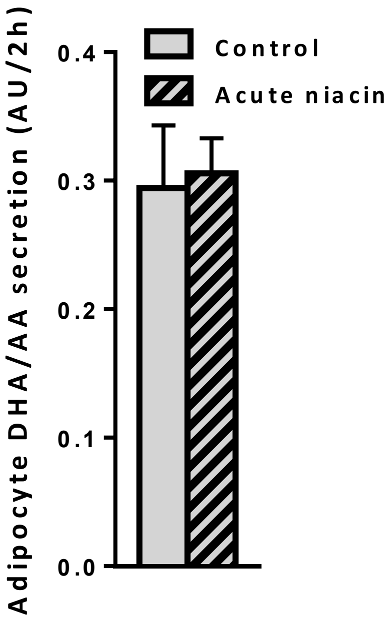



Figure S IX: Ratio of DHA/AA released fatty acids from adipocytes isolated from APOE*3-Leiden.CETP mice fed a western type diet with 0.1% cholesterol without niacin. Fatty acid release in arbitrary units during a 2 hour *ex vivo* incubation under basal and acute niacin conditions. Mean±SD, N=14 for Control/N=10 for Acute niacin.