SUPPLEMENTARY DATA

Novel members of FAHFA lipid class from DHA - Supplementary information

Differential mobility spectrometry

Chromatographic separation of PAHSA isomers, especially 13- and 12-PAHSA, can't be achieved using reverse-phase columns ((1) and Figure 1C and SF1A). Therefore, we tested whether differential mobility spectrometry (DMS) is capable of separating PAHSA isomers based on their mobility in an oscillating electrical field with an asymmetric waveform. Here we show, that ion mobility coupled to LC-MS/MS is capable of differentiating closely related PAHSA isomers partially co-eluting and unresolvable using LC-MS/MS only (Figure SF1B), but due to low levels of PAHSA in biological samples and low sensitivity of DMS operated with isopropanol as a modifier (loss of absolute signal by factor of ~100), this approach was not used to quantify FAHFA. Given the practical impossibility to separate positional isomers of 12- and 13-PAHSA in human samples, 12/13-PAHSA levels are reported together.

Synthesis of DHAHLA standard

Organic synthesis of 13-DHAHLA was performed according to Steglich esterification (2). 13-HODE was prepared enzymatically using soy bean lipoxygenase (3) and DHA bought from Sigma-Aldrich. Briefly, DHA was dissolved in benzene and activated with dicyclohexylcarbodiimide and esterified to 13-HODE. Reaction mixture was extracted with ethyl acetate, dried and applied on SPE columns following the FAHFA extraction protocol. Sample was purified using preparative chromatography on Discovery C18 5 µm 10x250 mm column (Sigma) based on previously published methodology (3), 13-DHAHLA fraction dried and stored at -80 ºC under argon atmosphere. The purified reaction product consisted of 13-DHAHLA (purity >95%) and trace amounts of 9-DHAHLA created via non-enzymatic oxidation of linoleic acid to 9-HODE during preparation.

Cell cultures

Differentiated 3T3-L1 adipocytes growing in 6-well plates were incubated with 100 µM LA and 100 µM DHA complexed to BSA 3:1 for 24 hours and extracted for FAHFA analysis. hMADS cells (adipocytes (4)) were incubated with 10 µM LA and 10 µM $^{2}H_{5}$-DHA complexed to BSA 3:1 for 24 hours. RAW macrophages, growing in 100 mm dishes, were incubated with 300 µM LA and 300 µM DHA complexed to BSA 5:1 to maximize external fatty acid incorporation into cells.

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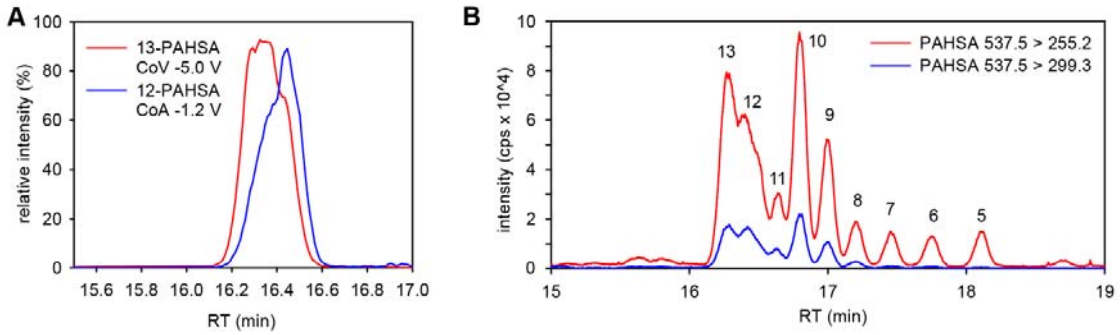
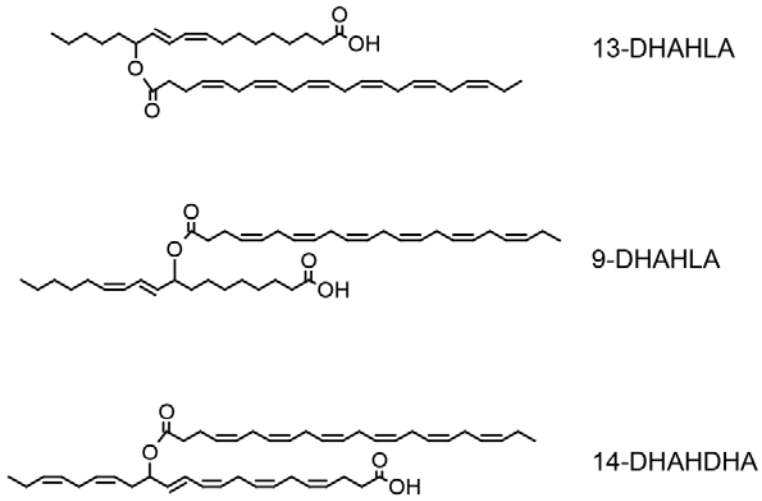
Supplementary Figure 1.
A: MRM tracings of co-eluting 13- and 12-PAHSA separated in ion mobility cell. CoV, compensation voltage specific for 13- and 12-PAHSA, red line,13-PAHSA; blue line, 12-PAHSA. B: Chromatographic profile of PAHSA isomers detected in murine serum. Numbers mark PAHSA isomers. Red line. PAHSA MRM 537.5 > 255.2 tracing; blue line, 537.5 > 299.3 tracing.



Supplementary Figure 2.
FAHFA structures. 13-DHAHLA, docosahexaenoic acid-13-hydroxylinoleic acid; 9-DHAHLA, docosahexaenoic acid-9-hydroxylinoleic acid; 14-DHAHDHA, docosahexaenoic acid-14-hydroxydocosahexaenoic acid.



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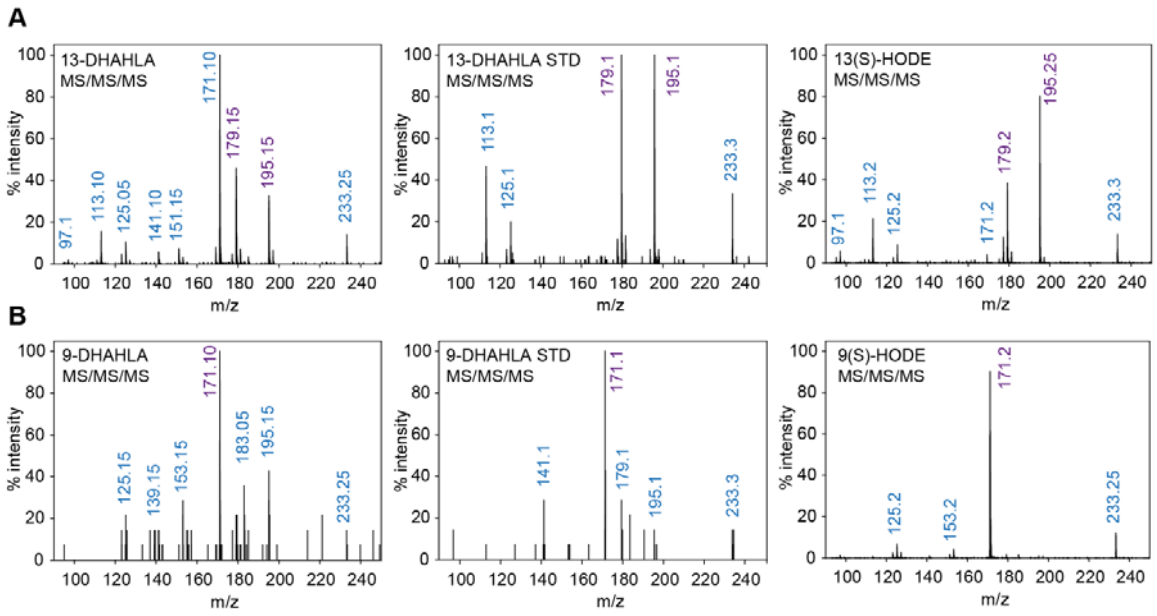
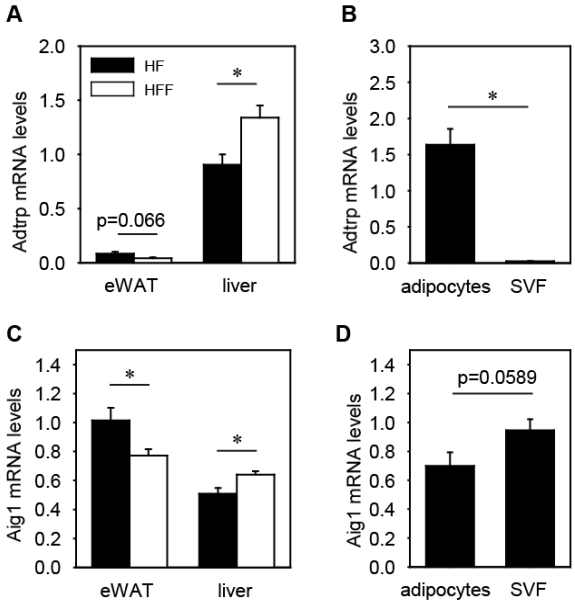
Supplementary Figure 3.
A: Linear ion trap spectra of native 13-DHAHLA, standard of 13-DHAHLA and standard of 13(S)-HODE (HLA) backbone. B: Linear ion trap spectra of native 9-DHAHLA, standard of 9-DHAHLA and standard of 9(S)-HODE (HLA) backbone. Specific fragments highlighted in magenta.



SUPPLEMENTARY DATA

Supplementary Figure 4.
A: mRNA levels of murine Adtrp in epididymal adipose tissue (eWAT) and liver. HF, mice on high-fat diet; HFF, mice on HF diet supplemented with omega-3 PUFA. B: mRNA of Adtrp in isolated adipocytes and stromal-vascular fraction (SVF) from mice fed HF diet.C: mRNA levels of murine Aig1 in eWAT and liver. D: mRNA levels of Aig1 in isolated adipocytes and SVF. Data are mean ± SE * p <0.05, n=9-12.



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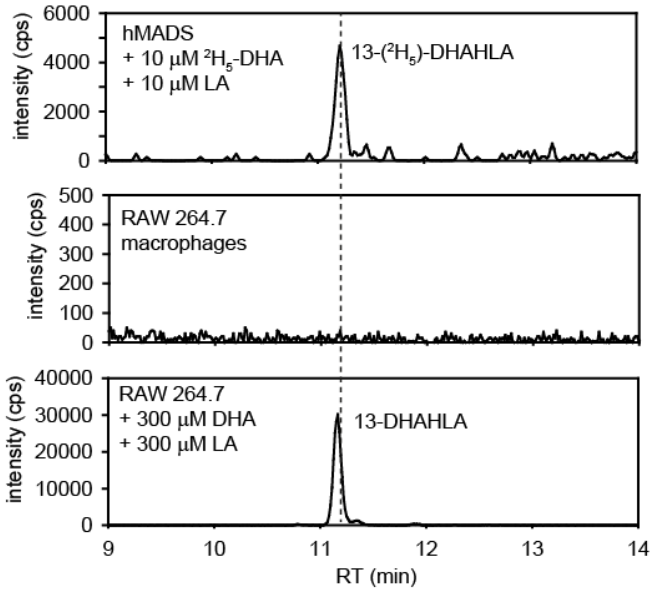
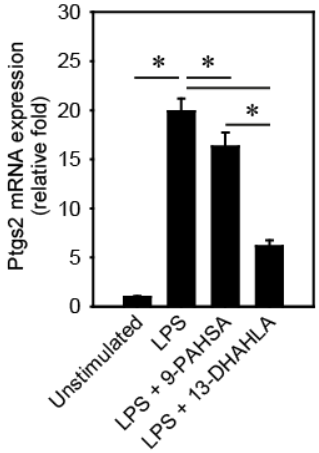
Supplementary Figure 5.
Chromatographic profile of 13-DHAHLA in hMADS (adipocytes) supplemented with 10 μM $^{2}H_{5}$-DHA and LA for 24 hours, naïve RAW 264.7 macrophages and RAW 264.7 macrophages supplemented with 300 μM DHA and LA for 24 hours, as indicated.



Supplementary Figure 6.
A: mRNA levels of murine Ptgs2 (cyclooxygenase-2) in RAW 264.7 macrophages. Complementary to Figure 6A.



SUPPLEMENTARY DATA

Supplementary Table 1.

ID	Direction	Sequence	Length	Tm
mAig1	Forward	AGAAGGTGCATATGGCGGCGAGTC	24	58.0
	Reverse	TGTTTGTTGTTGCGGTGTTCTGGA	24	58.0
mAdtrp	Forward	CCAGGGAAGCCATGACGAAAACA	23	58.4
	Reverse	GGCAGGCGACCCCAAAGAAAA	21	58.4

qPCR primer sequence for mouse Aig1 and mouse Adtrp (5).

Supplementary References

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5. Parsons WH, Kolar MJ, Kamat SS, Iii AB, Hulce JJ, Saez E, Kahn BB, Saghatelian A, Cravatt BF: AIG1 and ADTRP are atypical integral membrane hydrolases that degrade bioactive FAHFAs. Nat Chem Biol 2016;12:367-372