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Supplemental information

***AdipoAtlas*: A reference lipidome**

for human white adipose tissue

Mike Lange, Georgia Angelidou, Zhixu Ni, Angela Criscuolo, Jürgen Schiller, Matthias Blüher, and Maria Fedorova

Supplemental Information

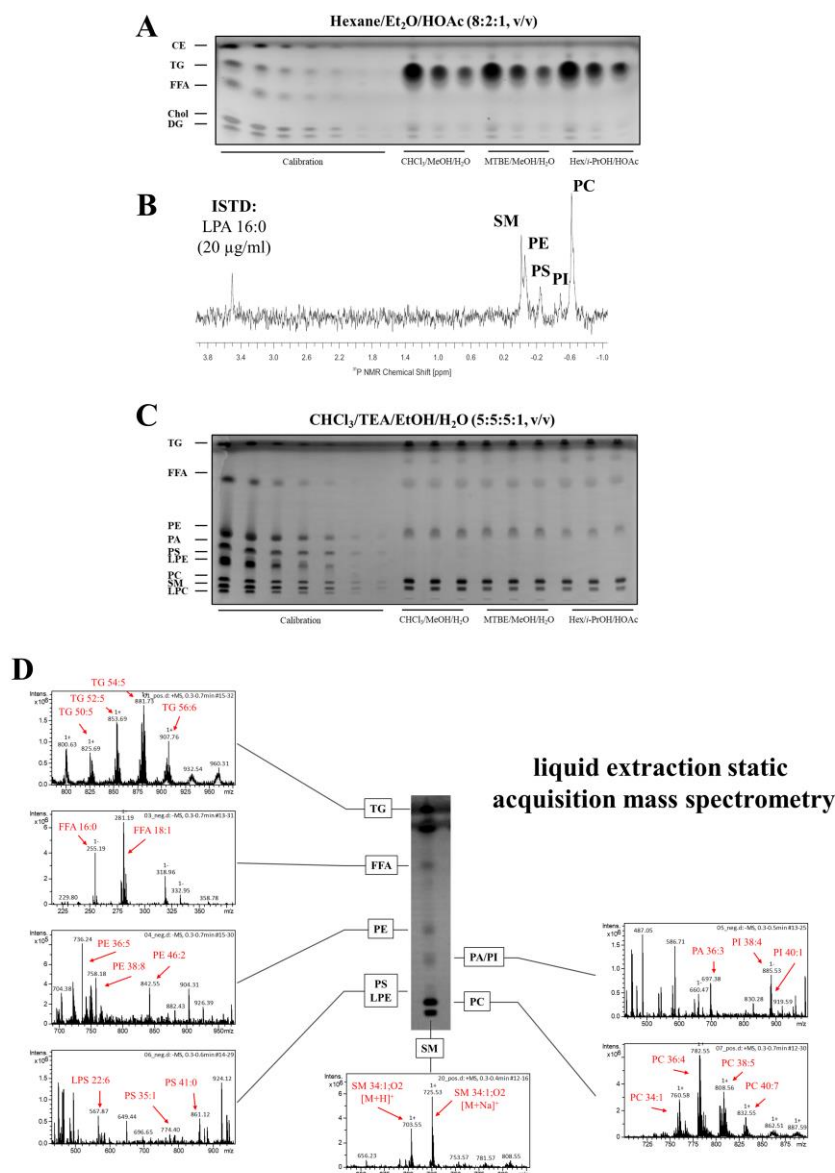


Figure S1, related to Figure 1: Quantification of lipid extraction efficiency of unipolar and polar lipid classes. **A:** Representative image of unipolar lipids extracted using Folch, MTBE, and Hex/IPA methods quantified by high performance thin layer chromatography (qHPTLC). **B:** Representative spectrum of ³¹P-NMR based quantification of phosphate containing lipid classes from a white adipose tissue (WAT) lipid extract. **C:** Representative image of qHPTLC based polar lipid quantification from the enriched polar fraction of WAT lipid extracted by Folch, MTBE, and Hex/IPA methods. **D:** Verification of identity lipid classes separated by qHPTLC using liquid extraction-static acquisition low resolution mass spectrometry.

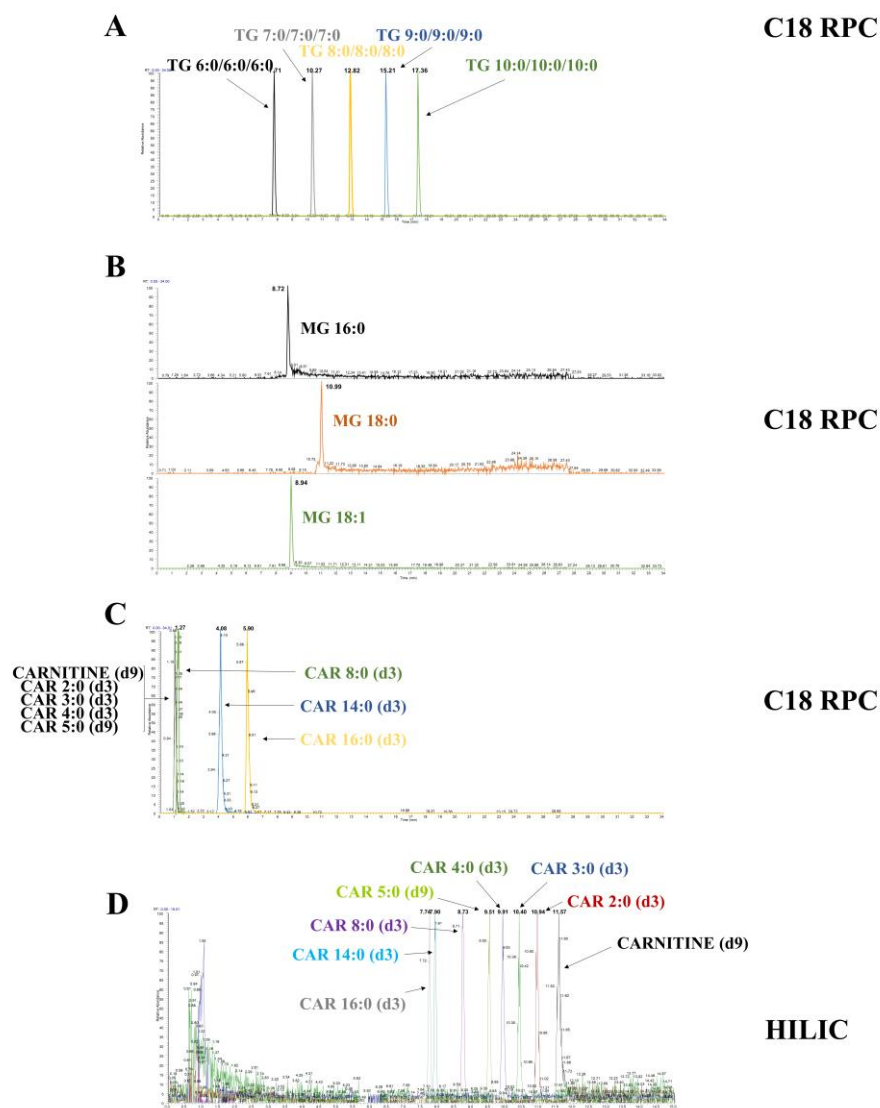


Figure S2, related to Figure 2: Investigation of retention behavior of lipids on various liquid chromatography (LC) platforms coupled to Q-Orbitrap high resolution mass spectrometry (QExactive). **A:** Separation of a mixture of short-chain triacylglycerols (TG) on C18 reversed phase chromatography (RPC). **B:** Separation of a mixture of monoacylglycerols (MG) on C18 RPC. Separation of a mixture of deuterated acylcarnitines (CAR) on **C:** C18 RPC and **D:** hydrophilic interaction chromatography (HILIC).

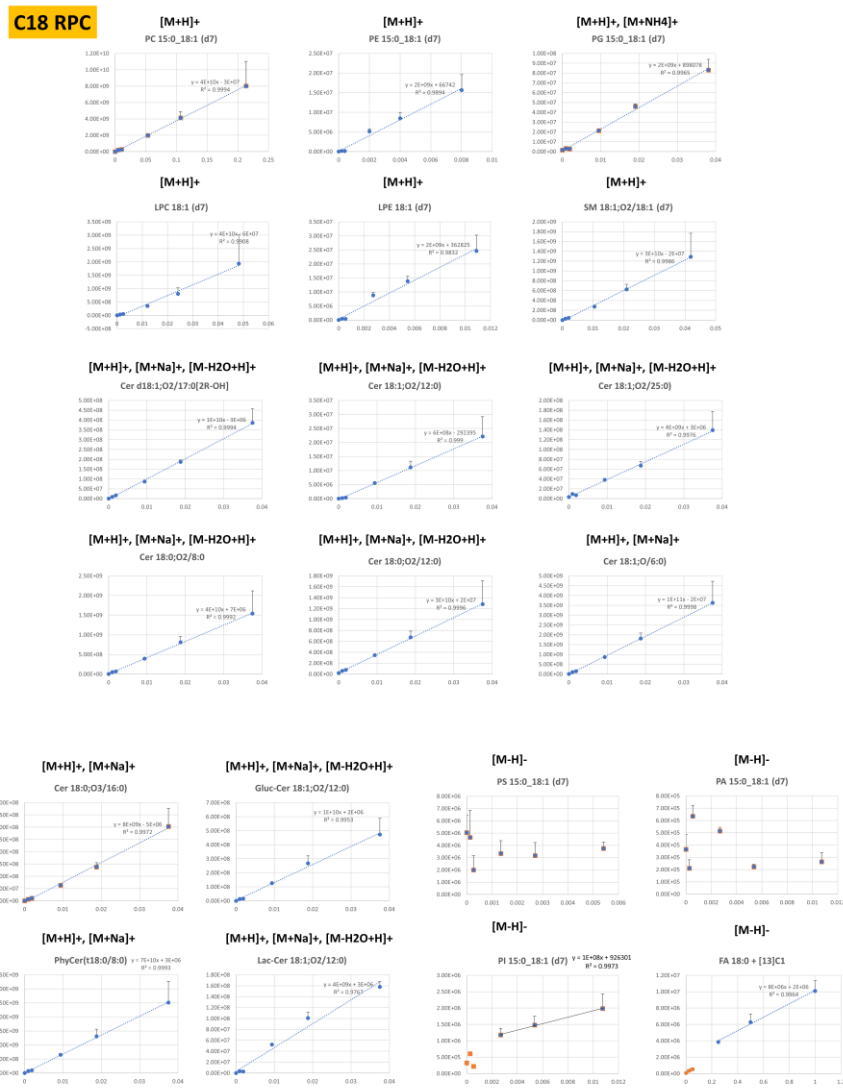
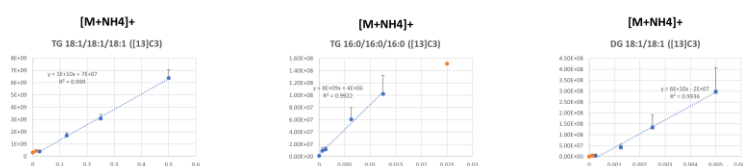


Figure S3, related to Figure 3: Internal calibration curves of spiked internal standards (ISTD) into white adipose tissue (WAT) samples prior to lipid extraction and fractionation. Phospholipid and Sphingolipid ISTD were separated on C18 reversed phase chromatography (C18 RPC). Only calibration points resulting in a linear regression $R > 0.98$ (blue) were used for calibration curve generation. Other calibration points were excluded (orange).

C30 RPC



HILIC

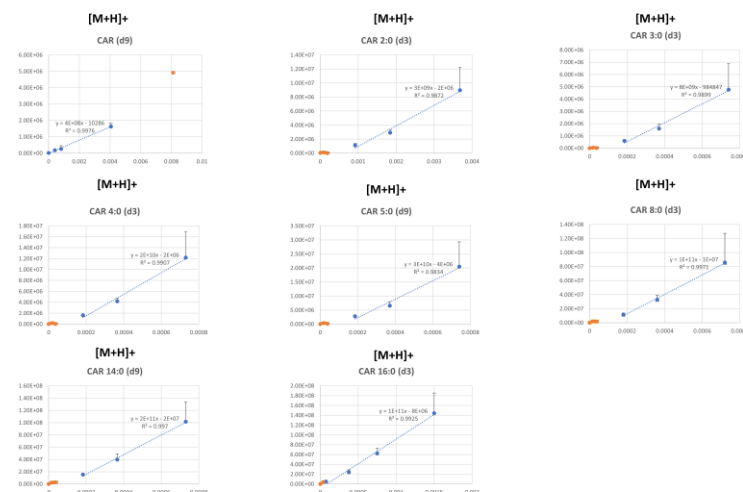


Figure S4, related to Figure 3: Internal calibration curves of spiked internal standards (ISTD) into white adipose tissue (WAT) samples prior to lipid extraction and fractionation. Glycerolipid and acylcarnitine ISTDs were separated on C30 reversed phase chromatography (C18 RPC) or hydrophilic interaction chromatography (HILIC), respectively. Only calibration points resulting in a linear regression $R > 0.98$ (blue) were used for calibration curve generation. Other calibration points were excluded (orange).

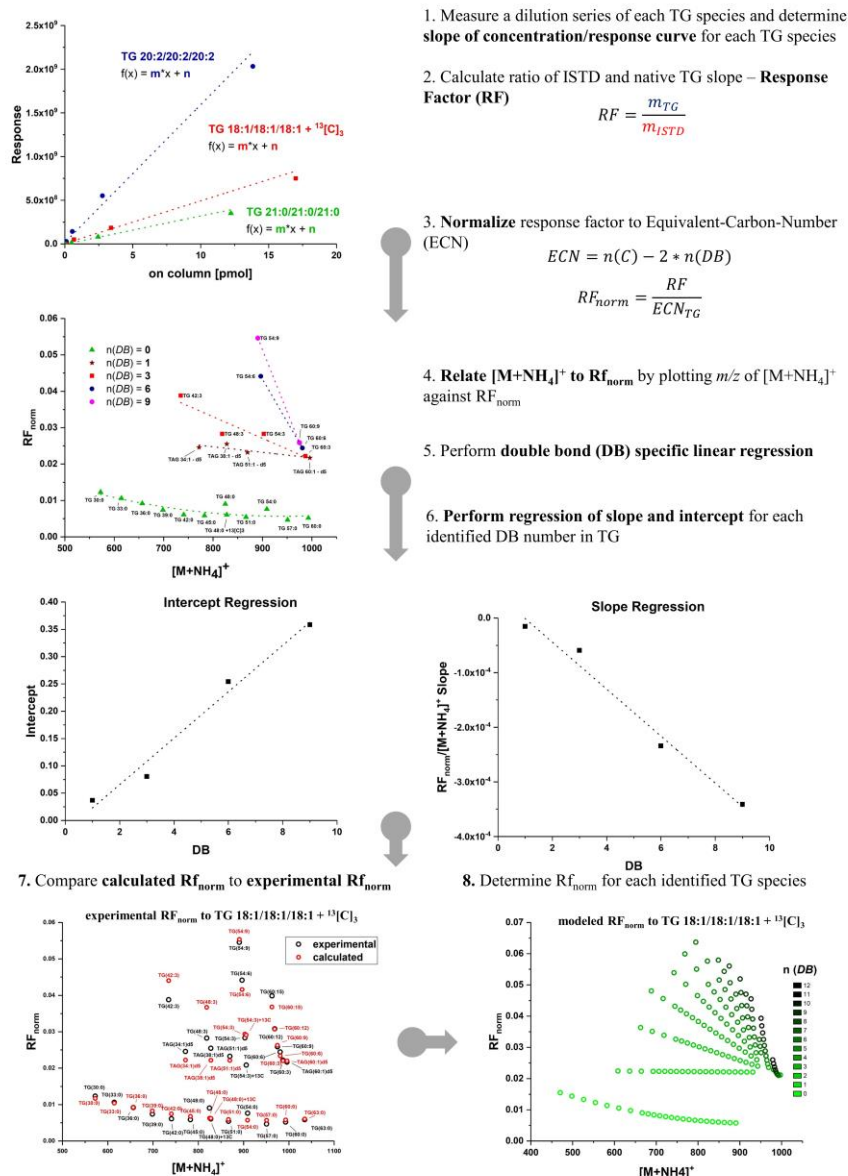


Figure S5, related to Figure 3: Generation of response factors (RF) for triacylglycerols (TG) on C30 reversed phase chromatography. The slope of external calibration curves for 33 native and deuterated TG molecular species with varying degrees of fatty acid unsaturation and length was determined. Each slope was related to the slope of ^{13}C labeled TG used as ISTD during lipid extraction, i.e. a response factor (RF) was generated. The dependence of fatty acid chain length and unsaturation was plotted and used to determine RFs for all TG species identified from WAT. The resulting TG RFs were used to increase TG quantification accuracy.

Table S2, related to Figure 3. Composition of in-house designed WAT Lipid Standards Mixture spiked (100 μ L; in CHCl₃/MeOH (2:1, v/v)) into 50 mg of AT (in 1 mL of MeOH) before homogenization.

ISTD Mix Composition	
Lipid	Spiked in $\approx$ 50 mg WAT [nmol]
SPLASH® Lipidomix®	
PC 15:0_18:1 (d7)	2.134
PE 15:0_18:1 (d7)	0.080
PS 15:0_18:1 (d7)	0.054
PG 15:0_18:1 (d7)	0.381
PI 15:0_18:1 (d7)	0.107
PA 15:0_18:1 (d7)	0.107
LPC 18:1 (d7)	0.482
LPE 18:1 (d7)	0.109
CE 18:1 (d7)	5.411
MG 18:1 (d7)	0.055
DG 15:0_18:1 (d7)	0.160
TG 15:0_18:1_15:0 (d7)	0.705
SM d18:1_18:1 (d9)	0.419
Chol (d7)	2.499
Cer/SpH Mix I	
SPB 17:1;O2	0.375
SPB 17:0;O2	0.375
SPBP 17:1;O2	0.375
SPBP 17:0;O2	0.375
Lac-Cer 18:1;O2/12:0	0.375
Gluc-Cer d18:1;O2/12:0	0.375
SM 18:1;O2/12:0	0.375
Cer 18:1;O2/12:0	0.375
CerP 18:1;O2/12:0	0.375
Cer 18:1;O2/25:0	0.375
Acylcarnitine Mix NSK-B-1	
Free Carnitine (d9)	0.162
CAR 2:0 (d3)	0.037
CAR 3:0 (d3)	0.007
CAR 4:0 (d3)	0.007
CAR 5:0 (d9)	0.007
CAR 8:0 (d3)	0.007
CAR 14:0 (d9)	0.007
CAR 16:0 (d3)	0.015
Individual Lipid ISTD	
FA 18:0 ([13]C1)	10.000
TG 18:1/18:1/18:1 ([13]C3)	1000.000
TG 16:0/16:0/16:0 ([13]C3)	50.000
DG 18:1/18:1/0:0 ([13]C3)	10.000
Cer 18:0;O2/12:0	0.375
Cer 18:0;O2/8:0	0.375
Cer 18:1;O/6:0	0.375
Cer 18:0;O3/16:0	0.375
Cer 18:0;O3/8:0	0.375
Cer 18:1;O2/17:0,O[2R-OH]	0.375

Table S3, related to Figure 3. Lipid class specific adducts and in-source fragments used for quantification of molecular lipid species. Internal standards (ISTD) used for quantification of naturally occurring lipids in lipid extracts of human WAT.

Lipid Class	Adducts/Fragments	Used ISTD
TAG	[M+NH ₄] ⁺ , [M+Na] ⁺ , [M+K] ⁺	TAG 18:1/18:1/18:1 ([13]C3)
DAG	[M+H] ⁺ , [M+Na] ⁺	DAG 18:1/18:1 ([13]C3)
PC	[M+H] ⁺	PC 15:0_18:1 (d7)
PE	[M+H] ⁺	PE 15:0_18:1 (d7)
LPC	[M+H] ⁺	LPC 18:1 (d7)
LPE	[M+H] ⁺	LPE 18:1 (d7)
PG	[M+H] ⁺ , [M+NH ₄] ⁺	PG 15:0_18:1 (d7)
PS	[M-H] ⁻	PS 15:0_18:1 (d7)
PI	[M-H] ⁻	PI 15:0_18:1 (d7)
Cer	[M+H] ⁺ , [M+Na] ⁺ , [M-H ₂ O+H] ⁺	Cer 18:1;O2/12:0 ; Cer 18:1;O2/25:0
DihydroCer	[M+H] ⁺ , [M+Na] ⁺ , [M-H ₂ O+H] ⁺	Cer 18:0;O2/12:0 ; Cer 18:0;O2/8:0
DeoxyCer	[M+H] ⁺ , [M+Na] ⁺	Cer 18:1;O/6:0
PhytoCer	[M+H] ⁺ , [M+Na] ⁺	Cer 18:0;O3/16:0 ; Cer 18:0;O3/8:0
Cer + α-OH FA	[M+H] ⁺ , [M+Na] ⁺ , [M-H ₂ O+H] ⁺	Cer 18:1;O2/17:0;O
HexCer	[M+H] ⁺ , [M+Na] ⁺ , [M-H ₂ O+H] ⁺	Gluc-Cer 18:1;O2/12:0
Hex2Cer	[M+H] ⁺ , [M+Na] ⁺ , [M-H ₂ O+H] ⁺	Lac-Cer 18:1;O2/12:0
SM	[M+H]	SM 18:1;O2/18:1 (d7)
CAR	[M+H] ⁺	CAR 2:0 - CAR 16:0 (d3-d9)
Carnitine	[M+H] ⁺	Free Carnitine (d9)
Cholesteryl Esters	PRM [M+NH ₄] ⁺ --> [Cholesterol-H ₂ O+H] ⁺	CE 18:1 (d7)