Poster Reprint

ASMS 2025
Poster number TP 541

Lipid Makeover: How Aging Transforms Adipose and Liver Tissues

Natalia Pascuali[1], Fernando “Ralph” Tobias[2,3], Sofija Jovanovic Gasovic[1], Mark Sartain[3], Karen E. Yannell[3], Almudena Veiga-Lopez[1]

[1]University of Illinois Chicago, [2]Northwestern University, [3]Agilent Technologies, Inc.

Introduction

- Aging alters lipid metabolism across multiple tissues contributing to metabolic inflexibility and disease risk.
- White adipose tissue (WAT), brown adipose tissue (BAT), and the liver are energy reservoirs and metabolic regulators, and lipid remodeling with age is increasingly recognized.
- Untargeted lipidomics was performed on a Revident LC/Q-TOF system to compare lipid alterations across a multi-tissue sample set in young, mature, and old mice (72 samples).
- Over 650 lipid species were identified at the molecular species or sum composition level revealing age-dependent shifts in key lipid classes across tissues

Table 1. Trademark characteristics on how mice can be used as a model for aging and reproductive studies.

Trademark characteristics	*Homo sapiens*	*Mus musculus*
Cycle	Menstrual	Estrous
Cycle Length	25 – 30 day	4 – 5 days
Uterine lining	Shed via menstruation	Reabsorbed
Oopause	Yes	Yes
Reproductive prime	20 – 30 years	15.8 ± 8.3 weeks
Reproductive transition	35 - 49 years	41.3 ± 18.0 weeks
Reproductive cessation	51 – 52 years	70.0 ± 25.7 weeks
Post-reproductive life	Yes	Yes
Lifespan	80.2 years	123.7 weeks

Figure 1. Agilent's omics standardized LC configuration used a 1290 Infinity III Bio LC (left) which is iron free to give best peak shape of metal-sensitive analytes. Coupled to it is a Revident LC/Q-TOF (right) which provides robust and reliable lipidomics analysis.

Experimental

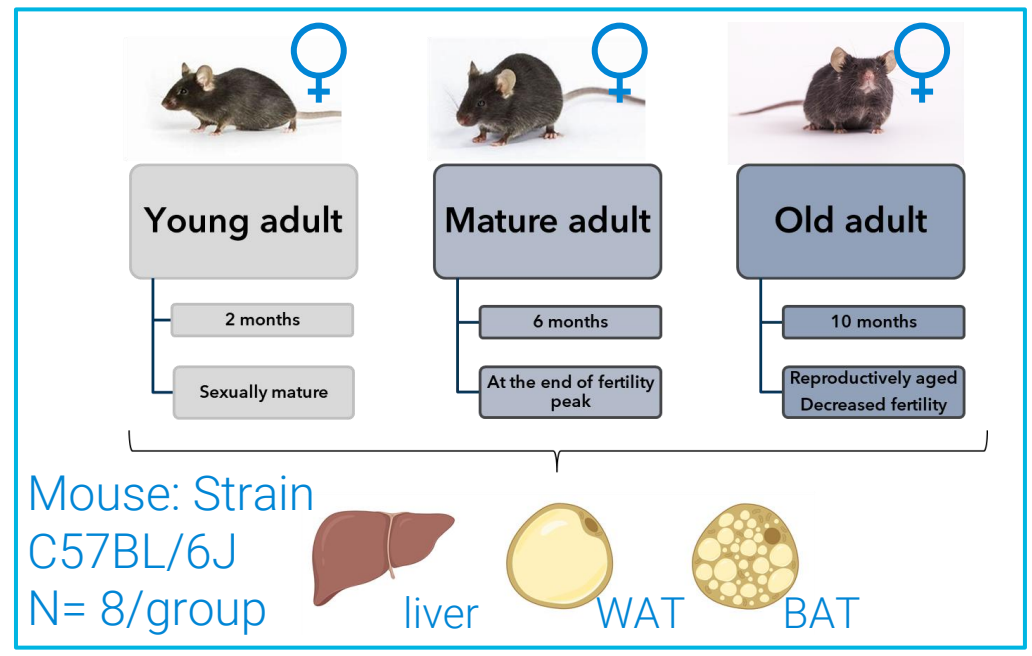


Figure 2. Study design for the aging lipidomics study.

Sample Preparation

Tissue samples from each animal were weighed (10 – 15 mg) in microcentrifuge tubes. Lipid extraction was conducted using a modified methanol/MTBE/water extraction method. Briefly, 300 µL of methanol with EquiSPLASH internal standard mix was added prior to tissue pulverization using a BeadBug homogenizer. Samples were centrifuged at 14,000 rpm and transfer into new tubes. Afterwards, 1000 µL of MTBE was added, and sample tubes were vortexed and placed in a bath sonicator. Next, 250 µL of LC-MS grade water was added, and sample tubes were vortexed placed in a bath sonicator once again. All sample tubes were then centrifuged at 14,000 rpm for 5 minutes. The top liquid layer was transferred into a new LC-MS vial and was evaporated under a nitrogen gas stream manifold. Samples were resuspended in 9/1 methanol/chloroform prior to LC-MS/MS analysis.

Instrumentation

Lipid separation was performed using a 16-minute reversed-phase method on a ZORBAX Eclipse Plus C18 column with previously established chromatography[1]. Samples were analyzed on the Revident LC/Q-TOF, tuned in *m/z* 1700 stable (+/-) ion modes. A pooled QC sample was used for both system suitability and for Iterative MS/MS acquisition (n = 6) per polarity for in-depth lipid identification coverage. MS1 was used for individual sample acquisition. The high resolution, isotopic fidelity and dynamic range of Revident enabled confident identification of >650 lipid species, including low-abundant TGs and Ether TGs.

Data Analysis

Sample data were normalized by the corresponding EQUISPLASH internal standard and tissue weight and were analyzed in MS-DIAL 5 and visualized in lipidR[2,3]. Lipid IDs were confirmed by Lipid Annotator 1.0 and MassHunter Explorer 1.0 using a custom database.

Results and Discussion

Principal Component Analysis reveals clustering by tissue type, indicating distinct tissue-specific profiles

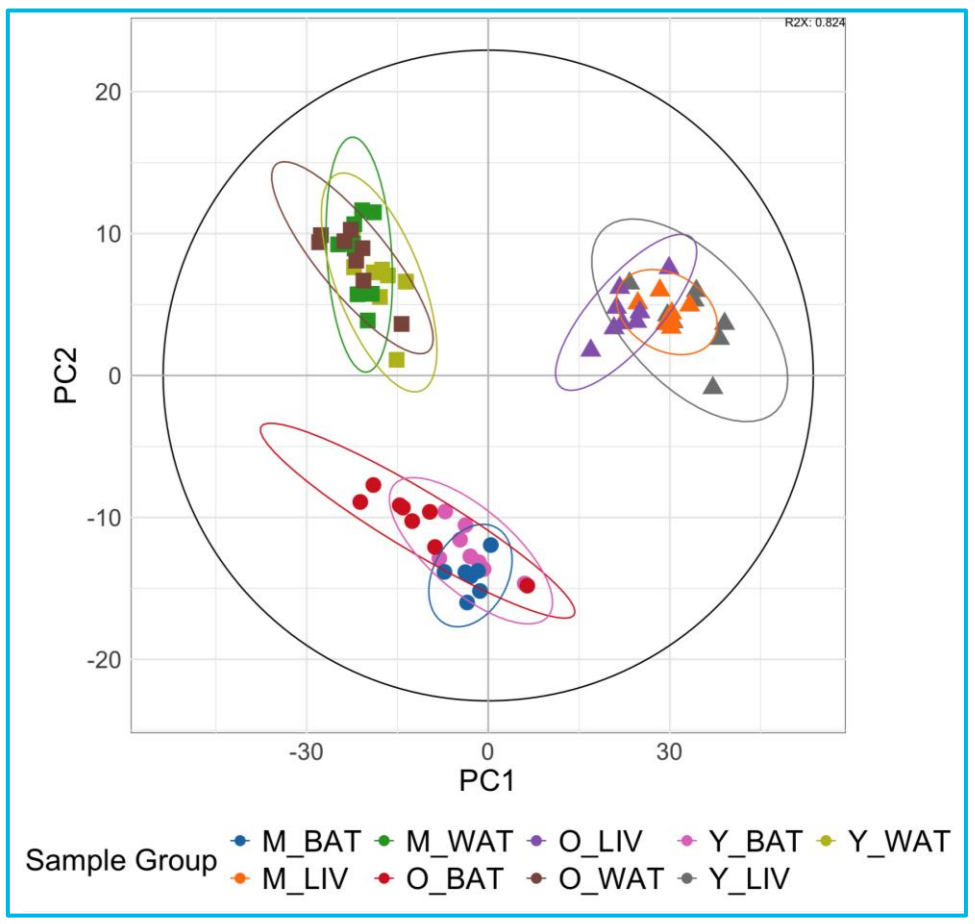


Figure 3. Unsupervised PCA of all lipids annotated by MS/MS reveals tissue-specific clustering.

Aging promotes the accumulation of long-chain lipids across BAT, WAT, and liver tissues.

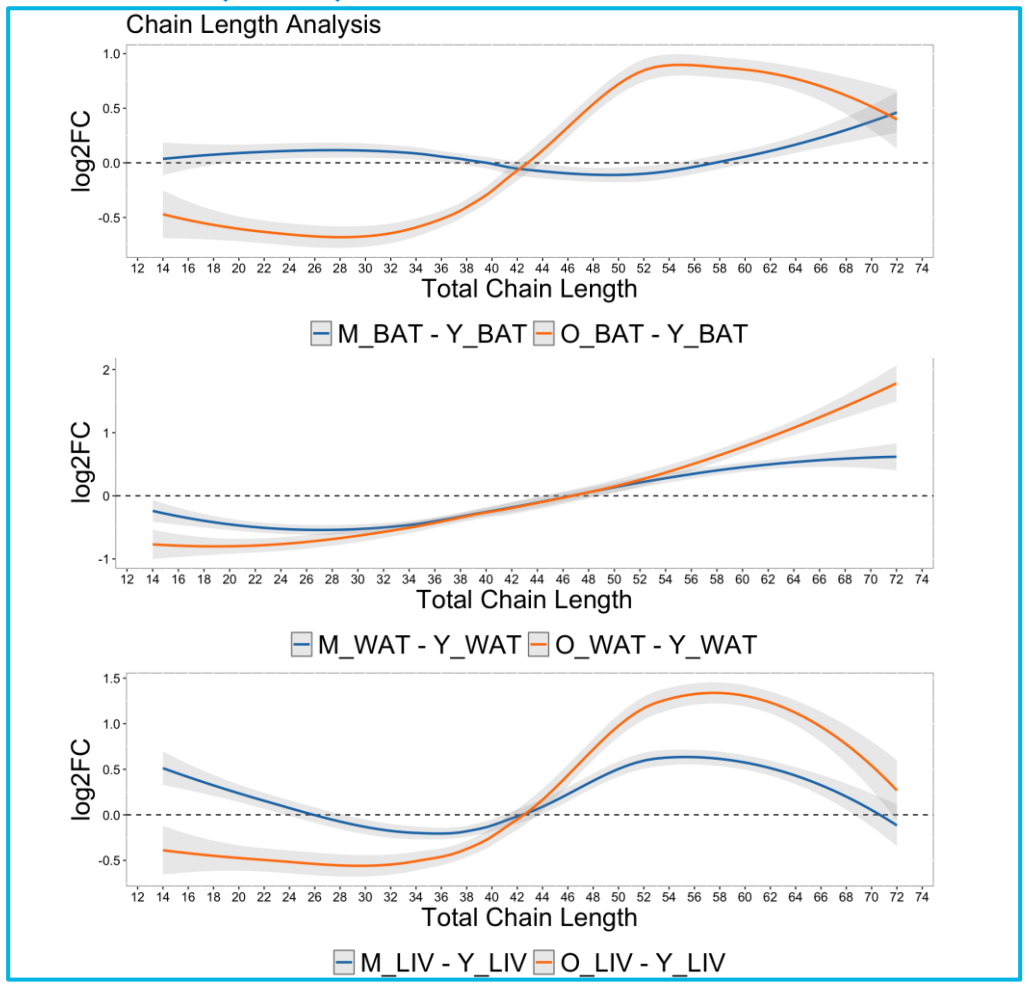
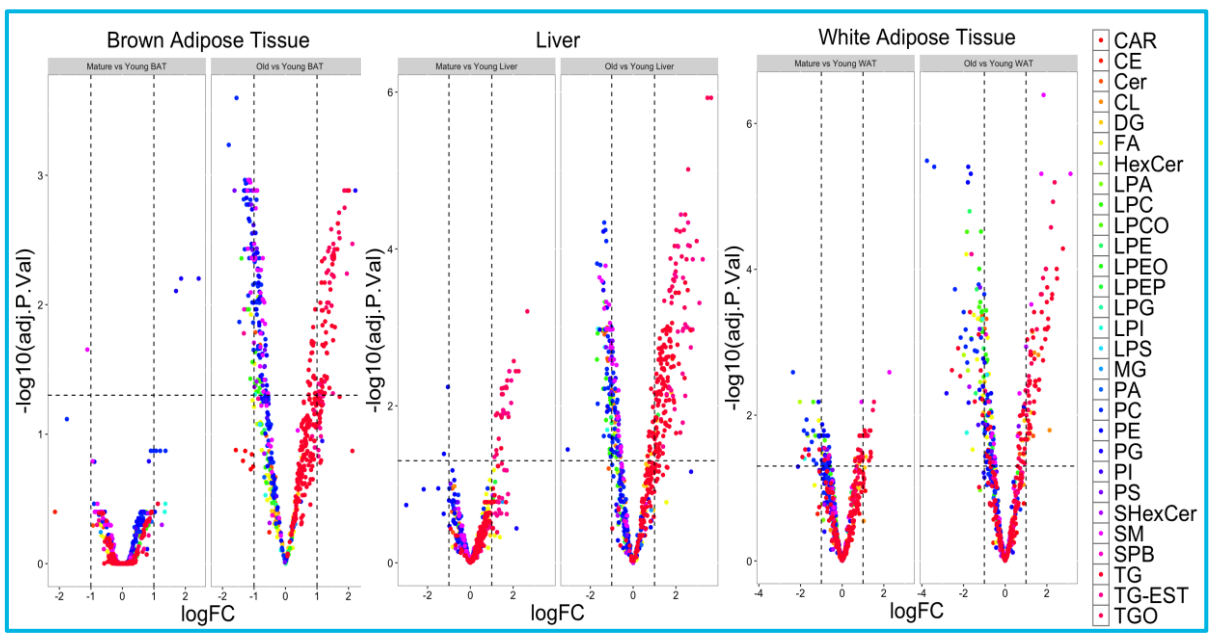


Figure 5. Total chain length analysis reveals age-related trends in mature and old mice.

Volcano plot analysis reveals a thematic alteration between tissue types.



- In BAT, older samples shift from negative to positive log2FC at ~46 total chain length, suggesting enrichment of long-chain lipids with age.
- In WAT, both mature and old groups trend upward with chain length, but old samples have a stronger positive enrichment of very-long-chain lipids.
- In liver, old samples show a marked positive log_2FC peak between 44–66 chain length, compared to young liver.
- Mature groups generally exhibit smaller changes relative to their old counterparts.
- The aging process consistently favors longer lipid chain accumulation in BAT, WAT, and liver tissues.

Figure 4 (above). Volcano plot between age groups relative to the young tissue group. With lipid species color legend on the right.

Table 2 (right). Summary of the lipid changes by tissue and age group. Top 3 up- and downregulated lipid classes listed for each comparison.

Tissue	Comparison	Significant Lipids	Top 3 Up	Top 3 Down
Liver	Mature Vs. Young	34	EtherTG, TG-EST, TG	PE
	Old Vs. Young	351	TG, EtherTG, TG-EST	PC, SM, Cer
BAT	Mature Vs. Young	4	PG	SM
	Old Vs. Young	266	TG, EtherTG, TG-EST	PC, SM, PE
WAT	Mature Vs. Young	101	TG, EtherTG, SM	PC, PE, TG
	Old Vs. Young	256	TG, EtherTG, CL	PC, PE, FA

Results and Discussion

Triacylglycerols and EtherTGs are among the most altered lipid classes with aging.

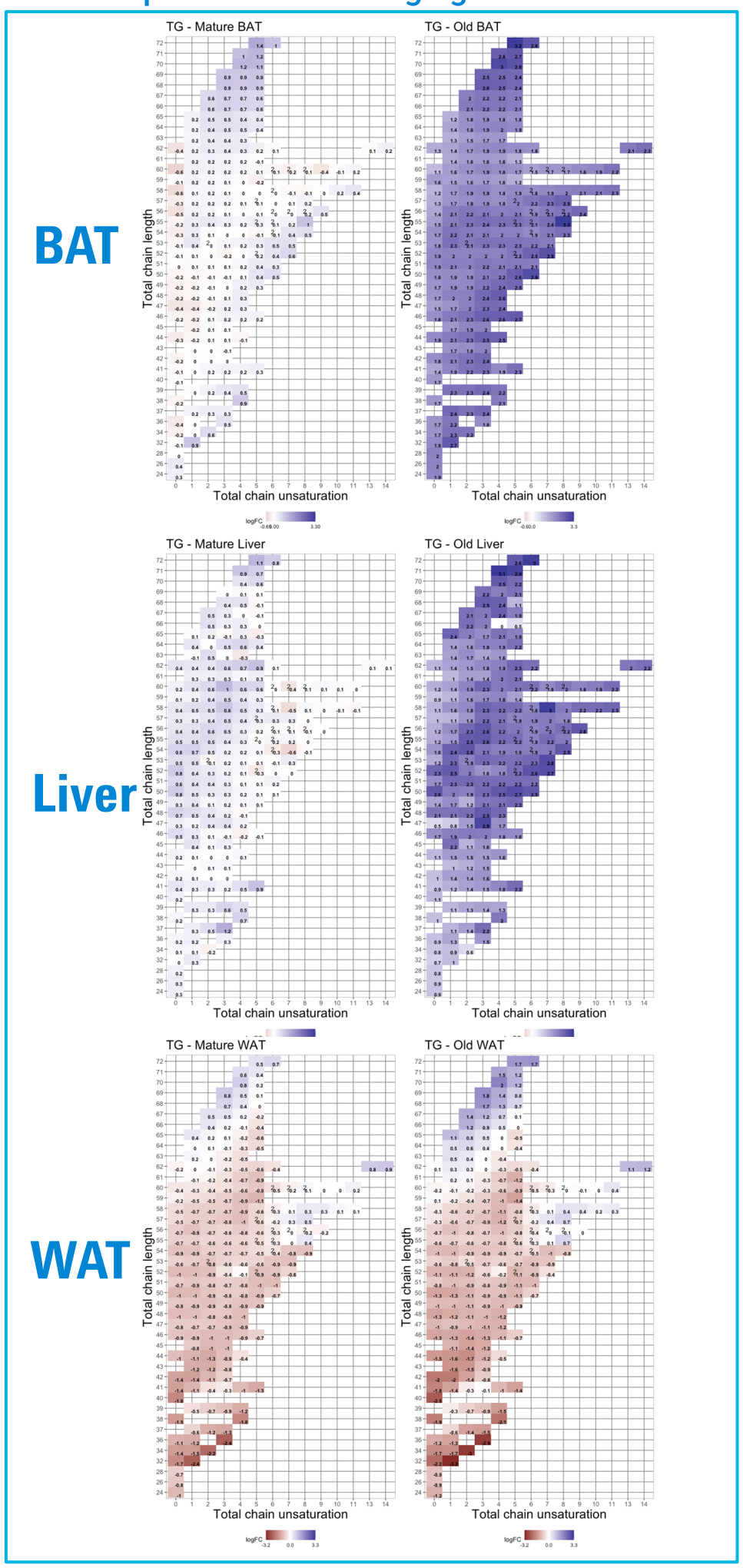


Figure 6. TG lipid profile with respect to their total chain length and total degree of unsaturation for each tissue type and age group. The lipid abundance were visualized as a log2 fold change between mature vs young and old vs young groups. Lipids that have the same total chain and unsaturation have their abundance visualized as an average. Blue denote an increase awhile red denote a decrease.

In silico and Spectral Libraries complement annotation of the adipose and liver lipidome

Table 3. Summary of results using two lipid packages with the Revident MS1 and MS/MS data: MS-DIAL and Agilent's Lipid Annotator and MassHunter Explorer 1.0 workflow.

Experimental Step	MS-DIAL	Lipid Annotator / MH Explorer
Features Extracted	1248 (pos), 416 (neg)	4712 (pos), 860 (neg)
Annotated Lipids by MS/MS and RT order	653 (combined)	441 (pos), 338 (neg)
Existing Spectral library with Retention Time		677
Lipids Annotated	653	309 (pos) , 189 (neg)

Conclusions

- This study demonstrates how untargeted lipidomics on the Revident LC/QTOF platform can reveal insights on lipid remodeling occurring during aging in metabolically active tissues. Iterative MS/MS data acquisition was instrumental in annotating low abundant molecules while enabling comprehensive profiling across a wide dynamic range.
- Across all tissue, aging induced significant changes in lipid composition, with the number of differentially abundant lipids increasing markedly from mature to old age groups, particularly in metabolically active tissues like liver and BAT.
- Aging drives tissue-specific lipid remodeling characterized by increased accumulation of neutral and stress-related lipids, mitochondrial dysfunction, and membrane composition shifts – especially in metabolically active tissues like liver and BAT – highlighting a systemic loss of lipid homeostasis and metabolic flexibility

References

1. Huynh et al., A Comprehensive, Curated, High-Throughput Method for the Detailed Analysis of the Plasma Lipidome. Agilent Application Note 5994-3447EN, 2021.

2. Tsugawa et al., A lipidome atlas in MS-DIAL 4. Nature Biotechnology, 2020.

3. Mohamed et al., lipidr: A Software Tool for Data Mining and Analysis of Lipidomics Datasets. Journal of Proteome Research, 2020.

https://www.agilent.com/en/promotions/asms

DE-006680

Published in USA, May 15, 2025

Agilent
Trusted Answers