Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Sizing lipid droplets from adult and geriatric mouse liver tissue via nanoparticle tracking analysis

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Additional file available under „Supplementary material“.

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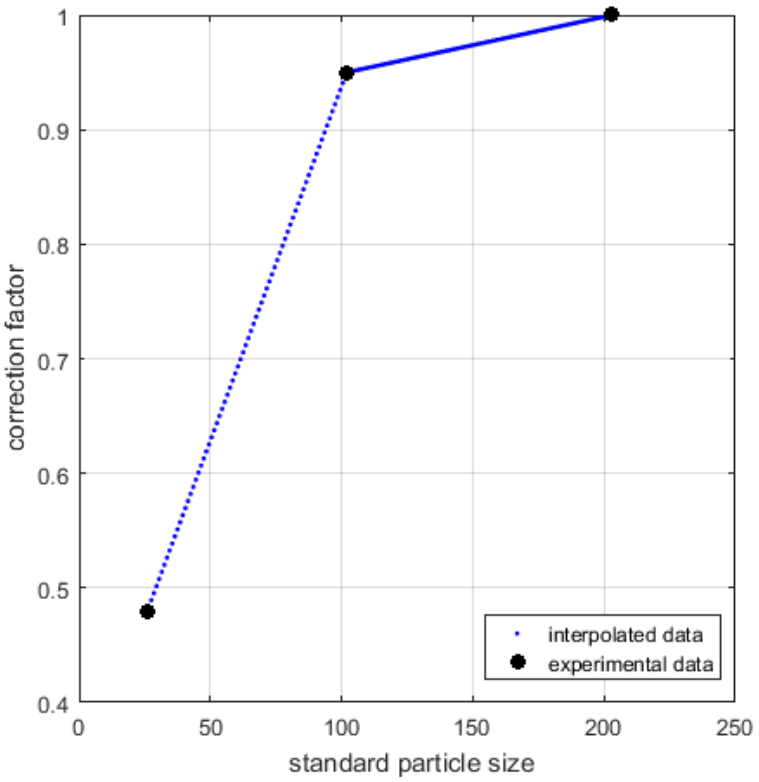
Supplementary Data Analysis

For particle size standards measured by NTA, we report the following % error and correction factors, as defined in the main text:

Size Standard	Nominal Size, nm	Size by TEM, nm	Size by NTA, nm	*e*	*c*
3030A	31	25	38	0.52	0.48
3K-100	102	102	108	0.05	0.95
3K-200	203	203	203	0	1

To determine the correction factor at additional particle sizes in this study, we performed linear interpolation in MATLAB 2016b between the correction factors of the standards. The correction factor for sizes <25 nm was assumed to be 0.48 and the correction for sizes >203nm was assumed to be 1.



A) B)

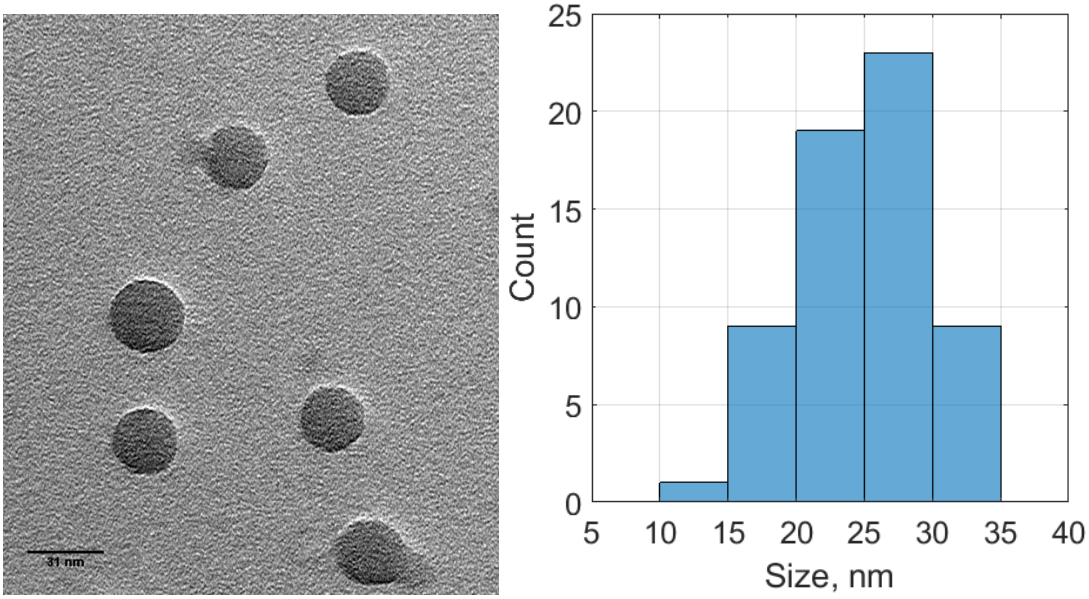


Fig. S1 (A) TEM of 31nm Polystyrene Latex Nanospheres. Two µl of a nanosphere suspension were pipetted onto a copper grid and allowed to air dry. The nanospheres were imaged on a Tecnai T12 transmission electron microscope (FEI, Hillsboro, Oregon). The nanosphere size was determined from the resulting images using ImageJ (NIH, Bethesda, Maryland). The mean particle size was 25nm ± 5nm (average ± SEM, n=60 nanospheres). The above image is representative of several images used to measure 60 nanospheres total. (B) Histogram of 31nm Polystyrene Latex Nanospheres. n=60 nanospheres were measured and included in the histogram

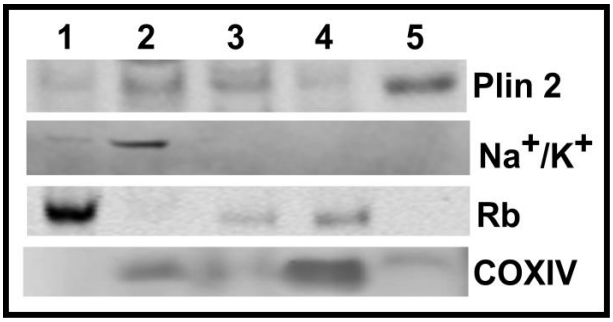


Fig. S2 Isolation of lipid droplets from mouse liver. To determine the purity of the lipid droplets, fractions containing the nucleus, plasma membrane, cytosol, mitochondria, and lipid droplet were collected and subjected to Western blot analysis. The fractions where probed for markers unique to each organelle, and cross contamination assessed. In brief, liver homogenates (10-20 µg protein) were separated on 10% tricine gels using a Mini-Protean II cell (Bio-Rad lab, Hercules, CA) system at constant amperage (40 mA per gel) for about 2 hrs. Proteins were then transferred onto PVDF membranes at constant voltage (90 V) for 2 hrs. Blots were stained with Ponceau S to confirm uniform protein loading before blocking in 5% BSA in TBST (10 mM Tris-HCl, pH 8, 100mM NaCl, 0.05% Tween-20) for 1 hour. Rabbit polycolonal antibodies against Na+/K+ channel (plasma membrane marker), Rb (nuclear marker), and COXIV (mitochondrion marker) were obtained from Cell Signaling Technology (Danvers, MA) and used at 1:1000 dilution in 5% BSA/TBST.Rabbit polyclonal antibody against Plin2 (lipid droplet marker) was prepared in house as previously described [1-2] and used at 1:1000 dilution in 5% BSA/TBST. Blots were incubated with primary antibodies overnight and were developed with IRDye 800CW anti-mouse (LI-COR) or IRDye 680RD antirabbit (LI-COR) secondary antibodies. To visualize the bands of interest, blots were scanned using the LI-COR Odyssey imaging system (Lincoln, NE). Lane 1, nucleus; lane 2, plasma membrane; lane 3, cytosol; lane 4, mitochondria; and lane 5, lipid droplets

Protocol References

1. Atshaves, B. P. *et al.* Expression and intracellular processing of the 58 kDa sterol carrier protein-2/3-oxoacyl-CoA thiolase in transfected mouse L-cell fibroblasts. *J. Lipid Res.* **40,** 610–22 (1999).
2. McIntosh, A. L. *et al.* Direct interaction of Plin2 with lipids on the surface of lipid droplets: a live cell FRET analysis. *AJP Cell Physiol.* **303,** C728–C742 (2012).

1A. ANOVA Table

Sources of Variation	*SS*	*df*	*MS*	*F*	*P*
Between Groups	9345	3	3115	65.58	P<0.0001
Within Groups	760	16	47.5		
Total	10110	19			

1B. Tukey's Multiple Comparison Test

	Mean Diff.	**q**	**P value**	**95% CI of diff**
Day 0 vs Day 1	3	0.9733	P > 0.05	-9.471 to 15.47
Day 0 vs Day 2	-10	3.244	P > 0.05	-22.47 to 2.471
Day 0 vs Day 7	-51	16.55	P < 0.001	-63.47 to -38.53
Day 1 vs Day 2	-13	4.218	P < 0.05	-25.47 to -0.5294
Day 1 vs Day 7	-54	17.52	P < 0.001	-66.47 to -41.53
Day 2 vs Day 7	-41	13.3	P < 0.001	-53.47 to -28.53

Table S1 ANOVA and post-hoc Tukey analysis of mean LD size. Rows highlighted in yellow indicate statistical significance

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2A. ANOVA TABLE

Sources of Variation	*SS*	*df*	*MS*	*F*	*P*
Between Groups	2.678	3	0.8928	13.68	0.0001
Within Groups	1.044	16	0.06525		
Total	3.722	19			

2B. Tukey's Multiple Comparison Test

	Mean Diff.	**q**	**P value**	**95% CI of diff**
Day 0 vs Day 1	0.02	0.1751	P > 0.05	-0.4422 to 0.4822
Day 0 vs Day 2	0.75	6.565	P < 0.01	0.2878 to 1.212
Day 0 vs Day 7	-0.22	1.926	P > 0.05	-0.6822 to 0.2422
Day 1 vs Day 2	0.73	6.39	P < 0.01	0.2678 to 1.192
Day 1 vs Day 7	-0.24	2.101	P > 0.05	-0.7022 to 0.2222
Day 2 vs Day 7	-0.97	8.491	P < 0.001	-1.432 to -0.5078

Table S2 ANOVA and post-hoc Tukey analysis of mean LD concentration. Rows highlighted in yellow indicate statistical significance

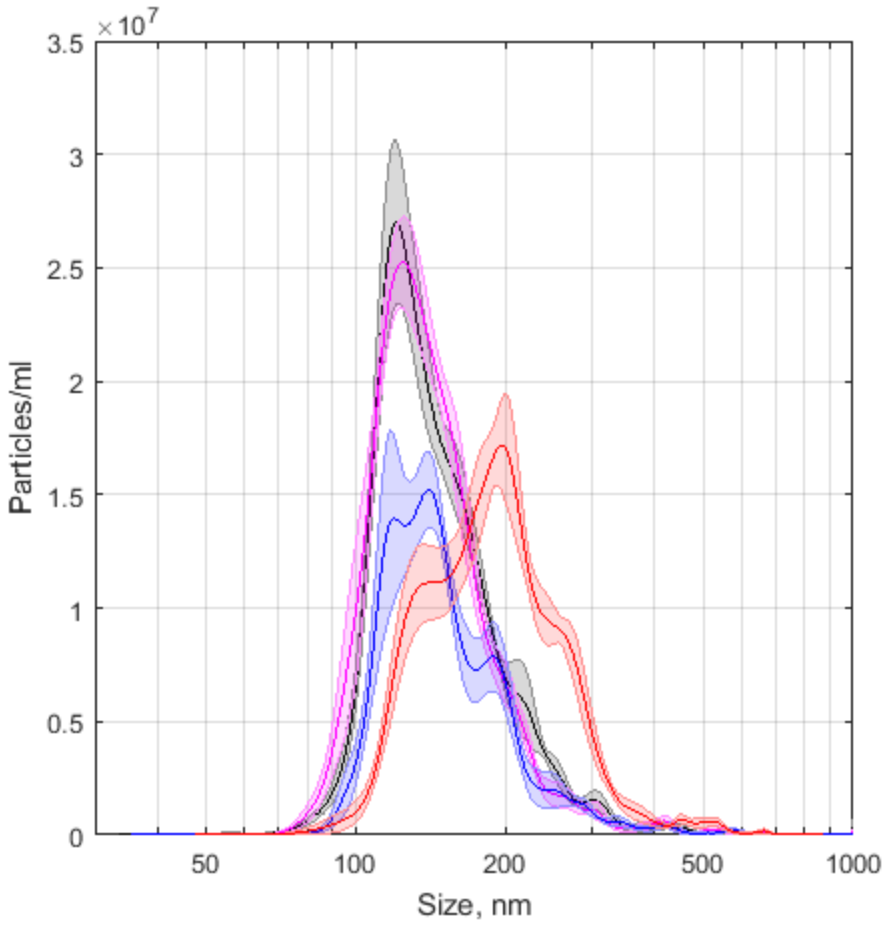

Fig. S3 Histograms for stability study. Day 0 is black, Day 1 is magenta, Day 2 is blue, and Day 7 is red, respectively. Shaded area represents average +/- SEM, n=5 technical replicates

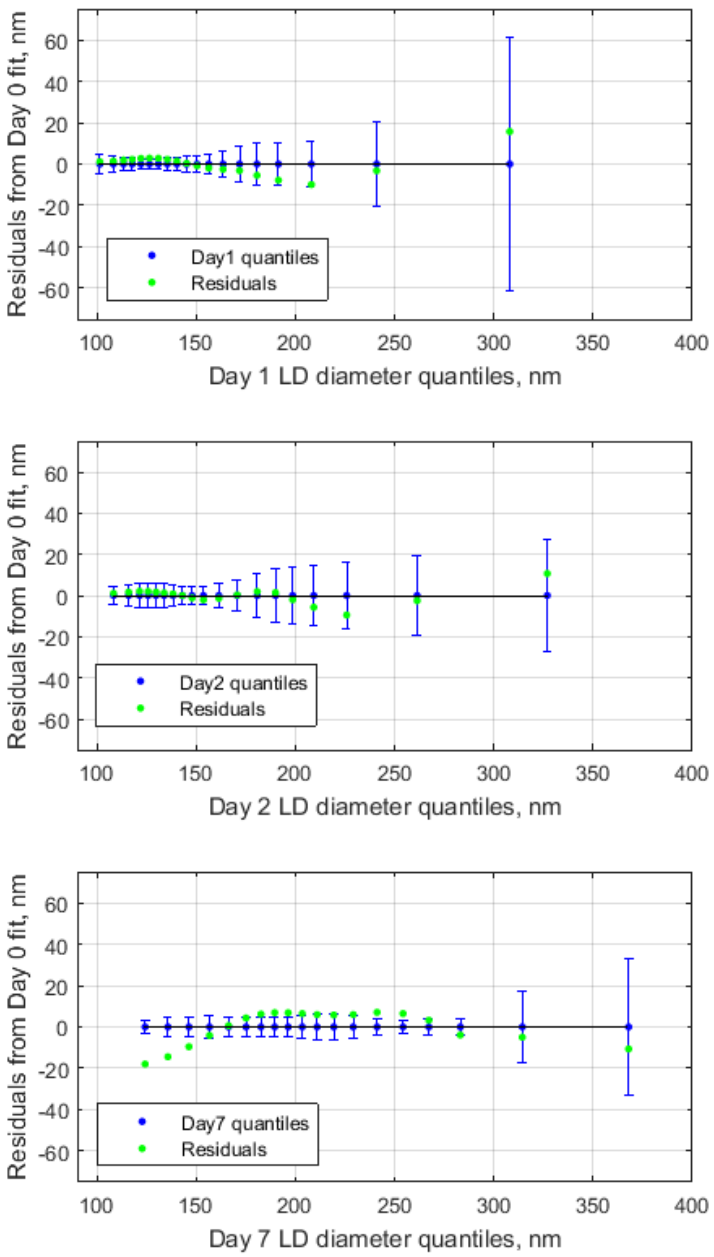

Fig. S4 Residuals of linear regression analyses of QQ plots. Each marker represents percentiles from 5th to 95th quantile, increasing by 5%. Their values (blue) are average ± SEM, n=5 technical replicates. The residuals of linear fits of percentile values in QQ plots are graphically represented on top of the respective quantile. If a majority of residuals fall within the error bars, the linear fit predicted by the Day 0 distribution is a suitable model. When the residuals for the Day 1 vs Day 0 and Day 2 vs Day 0 are graphed, they are within the range of SEM for each day, respectively. When the residuals are graphed for Day 7 vs Day 0, the majority of the residuals fall outside the SEM. This strengthens the evidence for a statistically significant difference in distribution shape between Day 0 and Day 7

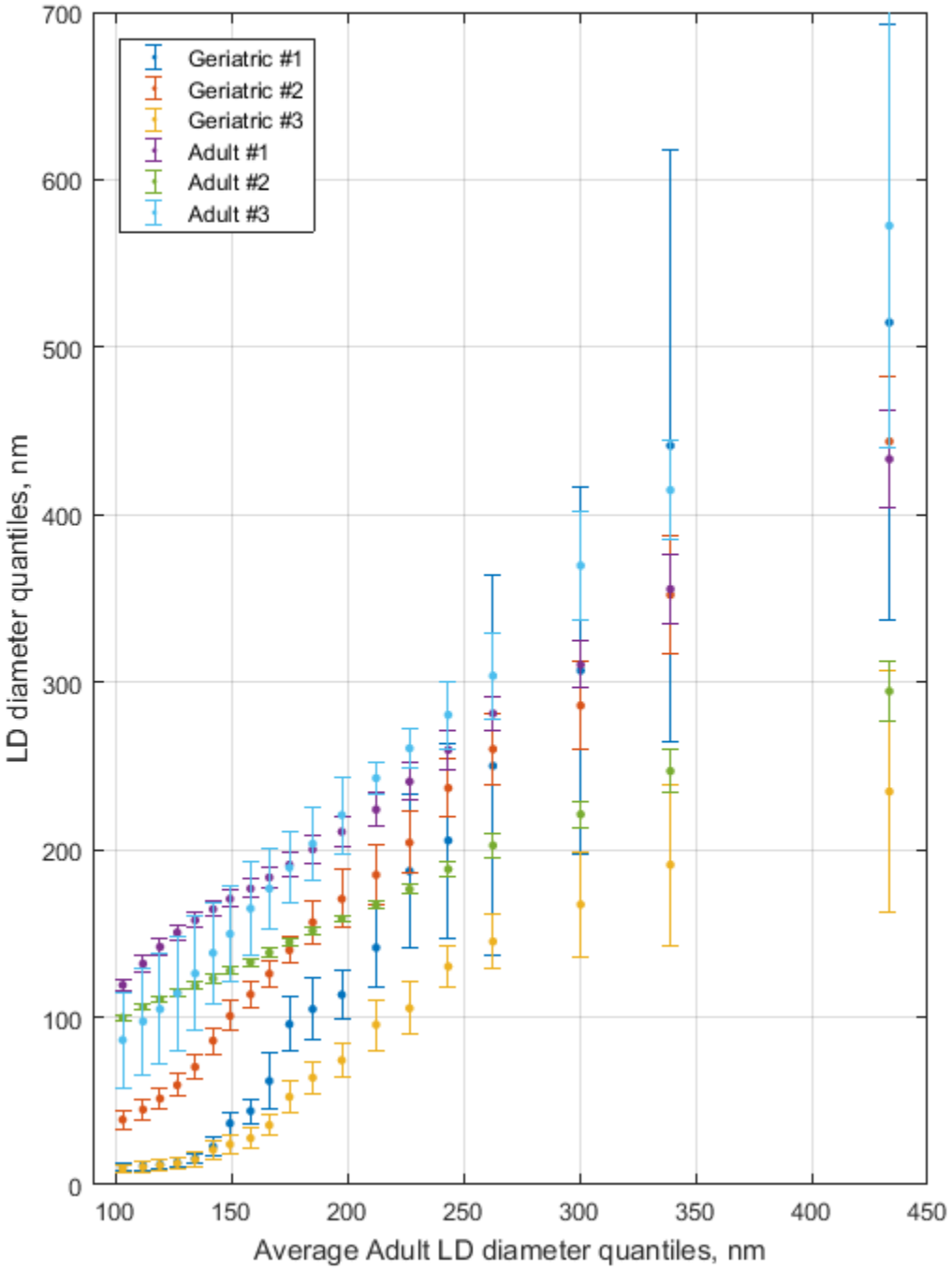

Fig. S5 Comparison of the lipid droplet size distributions of individual mice. Geriatric and adult mice were ~ 115 and ~ 35 weeks old, respectively. Markers represent percentiles ranging from 5^{th} to 95^{th} percentile at 5% increments. Each percentile is represented as average ± SEM, n = 3 mice

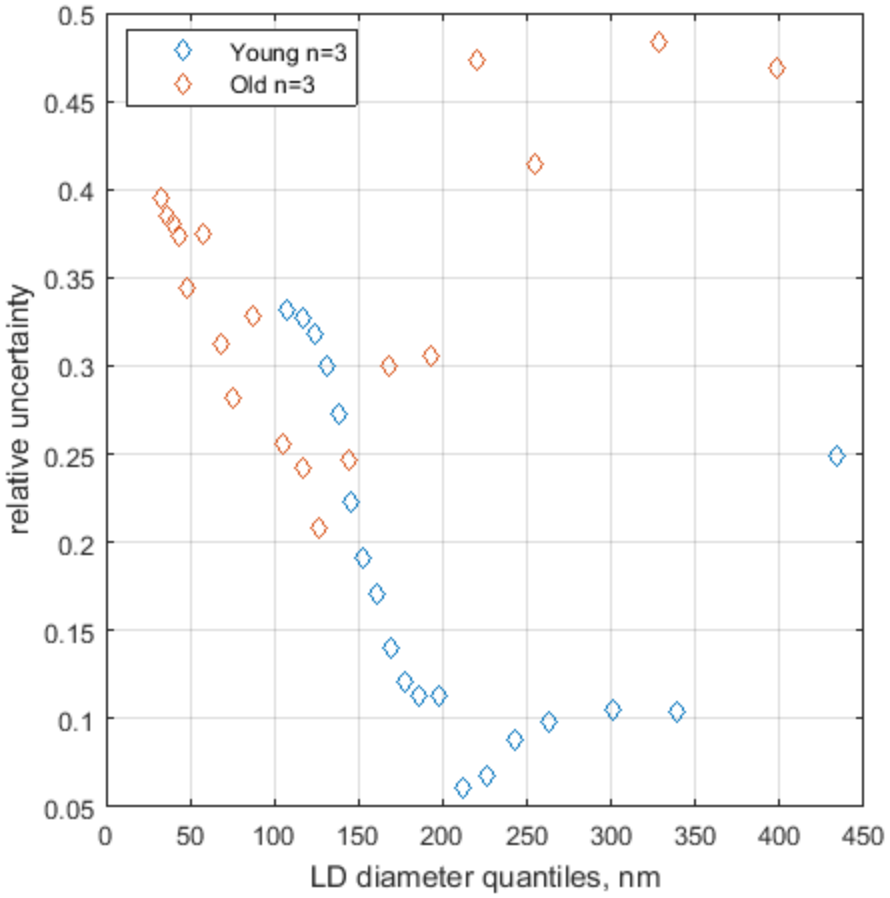

Fig. S6 Scatter plot of relative uncertainty. Relative uncertainty (y-axis) is defined as SEM/average. Each marker represents a percentile in the range from 5^{th} to 95^{th} percentile (x-axis)