Short-term high-fat diet impacts bone material properties and metabolism for adult and aged C57BL/6JN mice

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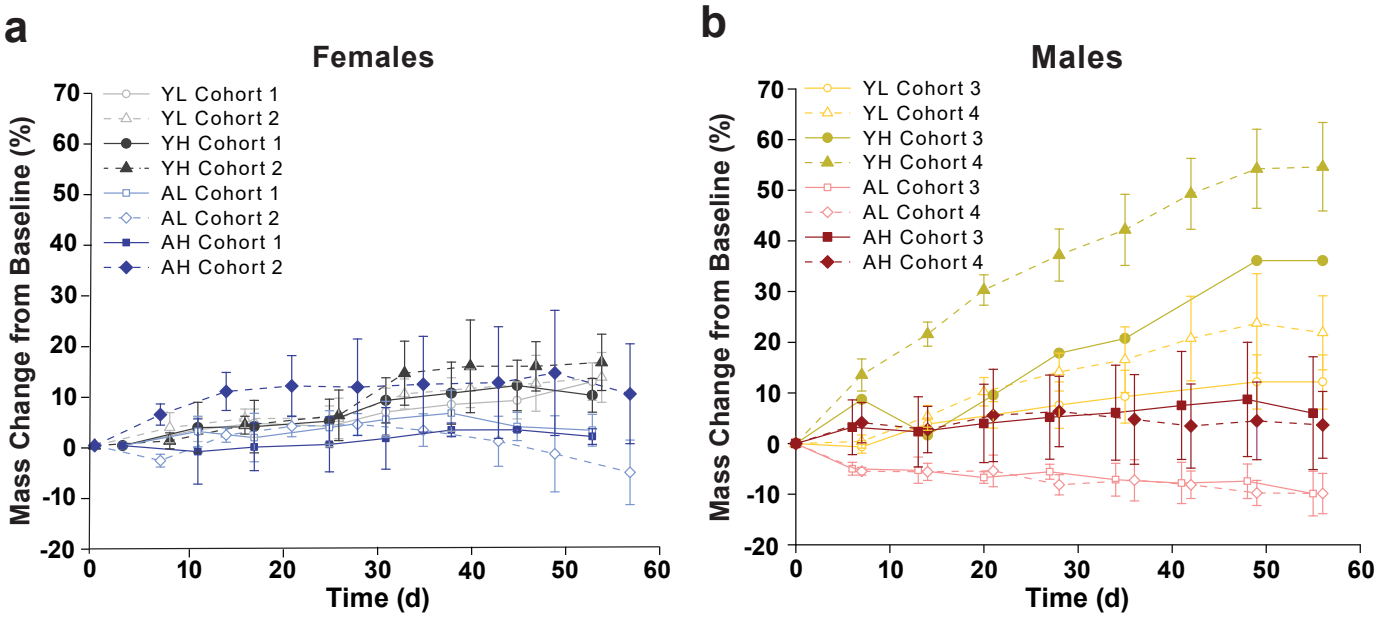


Figure S1. Female cohort mass gain trajectories over the course of the diet intervention were less variable than male cohorts on high fat diet. Mass gain trajectories for the cohorts during the diet intervention of (a) female and (b) male C57BL/6JN mice. Line plots are presented as mean ± 1 SD. YL = Young (5-month) low-fat diet (LFD), YH = Young (5-month) high-fat diet (HFD), AL = Aged (22-month) low-fat diet, AH = Aged (22-month) high-fat diet. Colors indicate groups where light gray is young LFD females, dark gray is young HFD females, light blue is aged LFD females, dark blue is aged HFD females, light yellow is young LFD males, dark yellow is young HFD males, light red is aged LFD males, and dark red is aged HFD males.

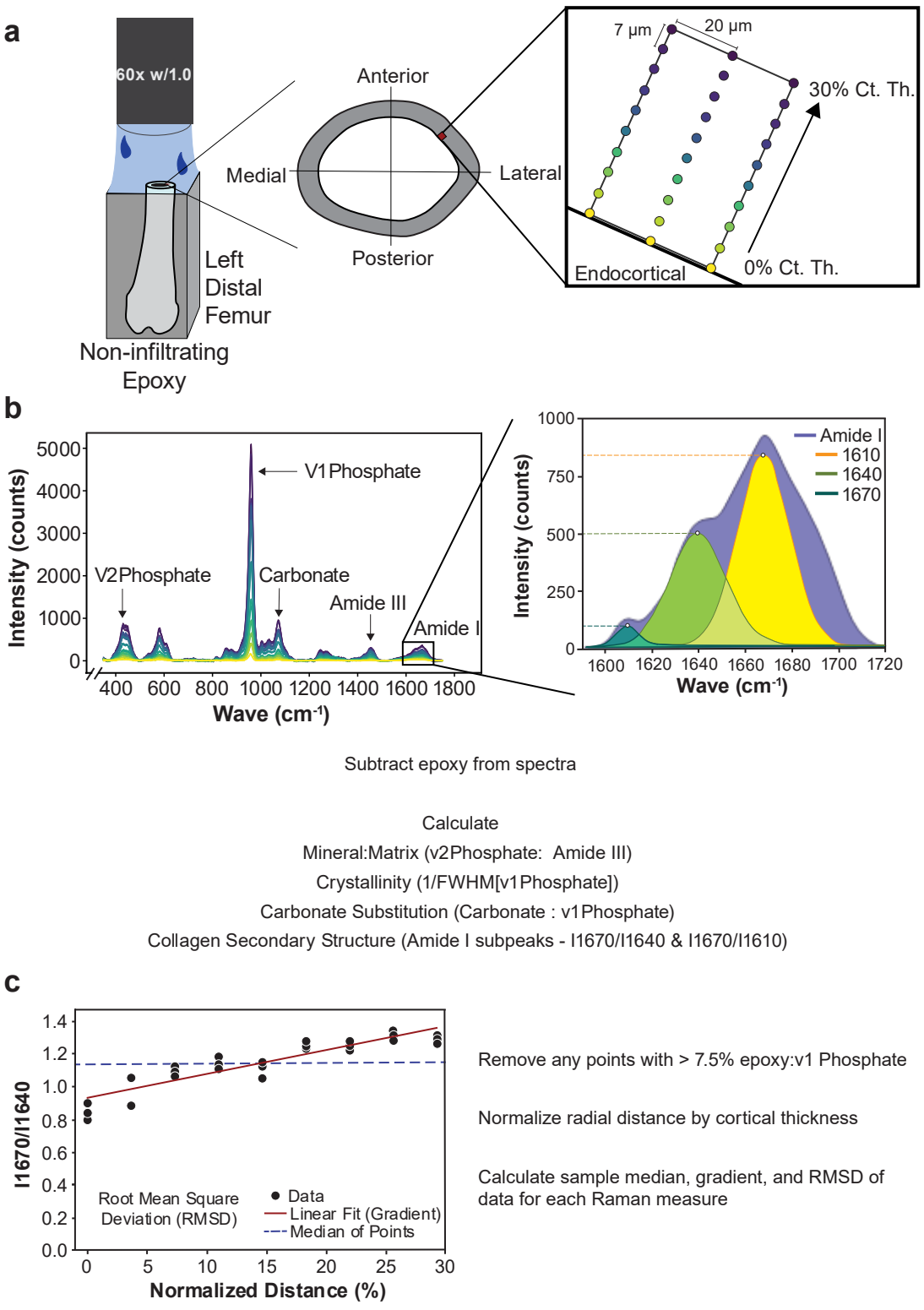
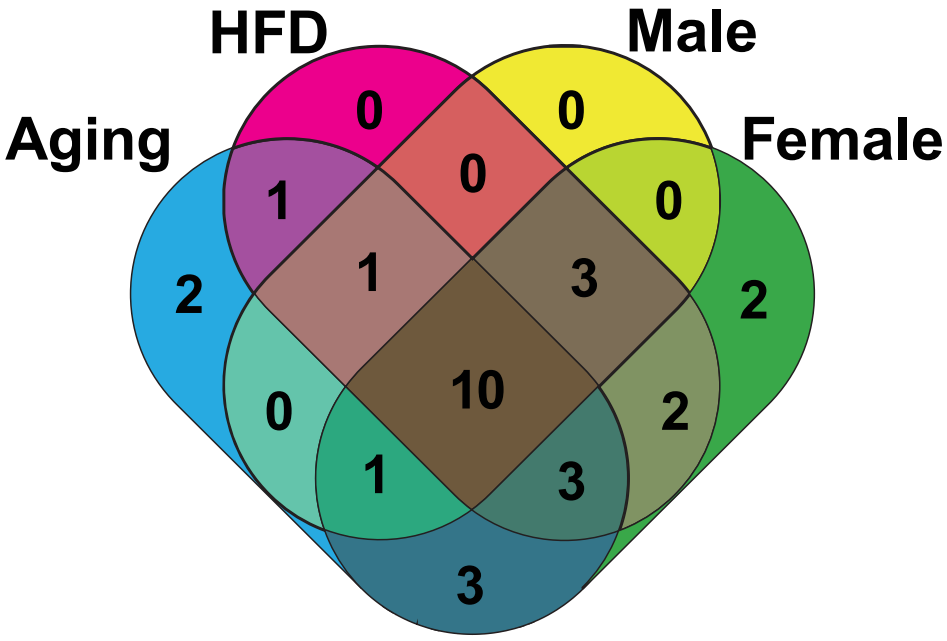


Figure S2. Steps for hydrated cross-sectional Raman spectroscopy. (a) The general setup for left distal femurs to map the anterior-lateral region of interest in hydrated conditions. Each point in the mapping region had (b) a corresponding spectrum. The epoxy signal was subtracted from each spectra before the five measures were calculated. The amide I peak was analyzed for subpeaks for 1610, 1640, and 1670 cm^{-1}. The mineral:matrix ratio was calculated by the ratio of the v_2 phosphate and amide III peaks, crystallinity was calculated with the inverse of the full width half maximum intensity of the v_1 phosphate peak, carbonate substitutions in the mineral was calculated as the ratio of the carbonate and v_1 phosphate peak, collagen secondary structure ratios were estimated as the intensities ratios of the amide I subpeaks I1670/I1610 and

I1670/I1640. (c) The measures were plotted with the normalized distance to find a linear fit. The slope of the linear fit was used as the gradient for a measure. The gradient represents the slope of change for a measure with respect to the radial distance from the endocortical surface. The root mean square deviation (RMSD) was calculated from the residual sum of squares about the linear fit and the number of data points in the 0-30% region. The RMSD is an estimate for the spatial variance of a measure. The median of all the points in the 0-30% region was used as the median.



HFD Aging	Tryptophan Metabolism
Male HFD Aging	Steroid Hormone Synthesis
Female HFD Aging	Pyruvate Metabolism Beta-Alanine Metabolism Metabolism of Xenobiotics by Cytochrome P450
Male Female HFD Aging	N-Glycan Biosynthesis GPI-Anchor Biosynthesis Glycosphingolipid Biosynthesis Sphingolipid Metabolism Alpha-Lenolenic Acid Metabolism Arachidonic Acid Metabolism Glycerolipid Metabolism Pyrimidine Metabolism Purine Metabolism Cysteine and Methionine Metabolism

Figure S3. High-fat diet and aging metabolic signatures are shared between female and male signatures. A summary of shared and distinct metabolic pathways for high-fat diet (HFD), aging, male, and female signatures.

Effect of Bone Shape on bone strength

ANOVA on I_{min}/c

There was a significant interaction between sex and age ($p = 0.014$) but no significant effects of diet. Both aged females and males had higher I_{min}/c than their young counterparts (females: +25.0%, males: +36.2%, adjusted p for both < 0.001). There was no difference between sexes when they were young but aged males had significantly rounder bone than aged females (+14.6%, $p < 0.001$).

ANCOVA on ultimate strength with a covariate of I_{min}/c

I_{min}/c was not a significant covariate for ultimate stress.

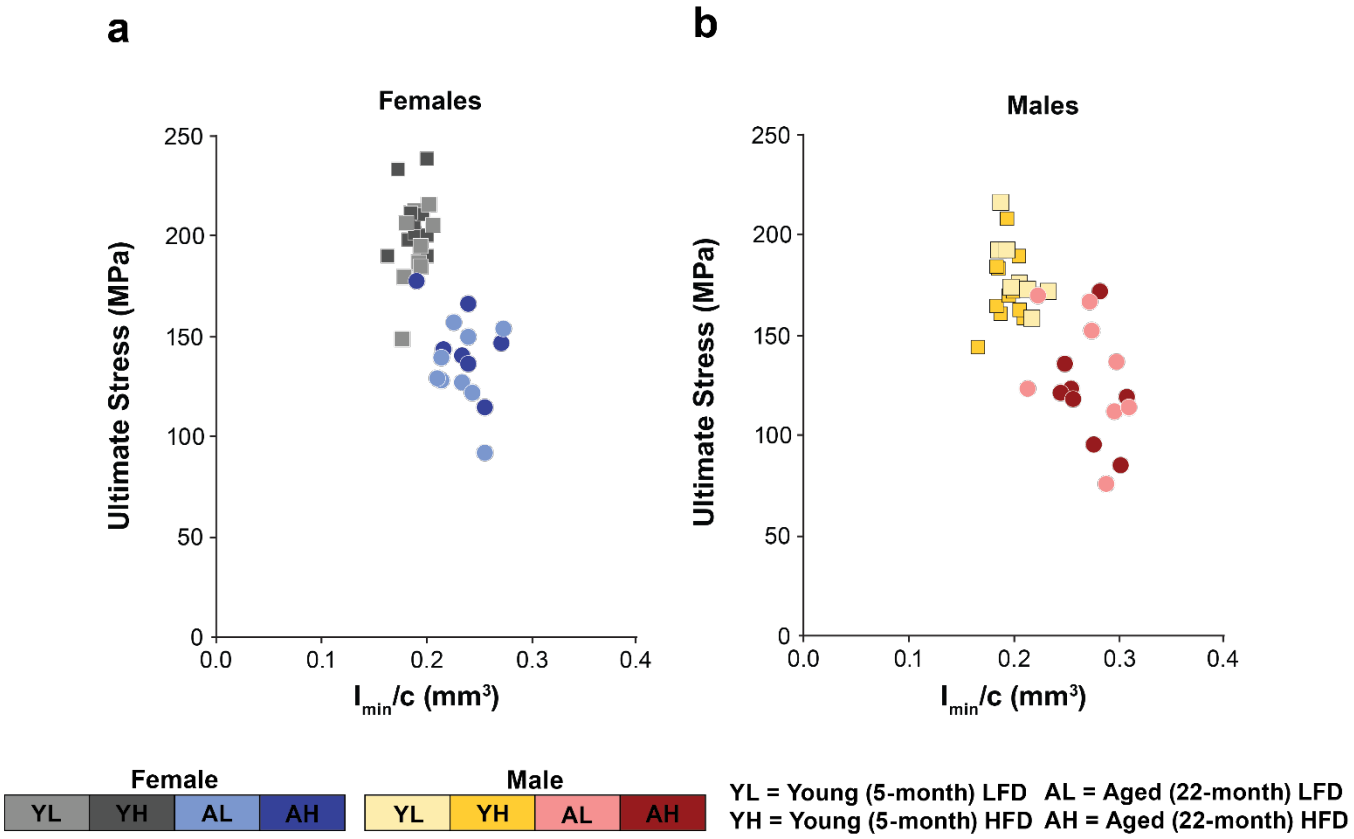


Figure S4. I_{min}/c (section modulus) did not change with high fat diet. Bone shape versus ultimate stress for (a) females and (b) males. Colors indicate groups where light gray is young LFD females, dark gray is young HFD females, light blue is aged LFD females, dark blue is aged HFD females, light yellow is young LFD males, dark yellow is young HFD males, light red is aged LFD males, and dark red is aged HFD males.

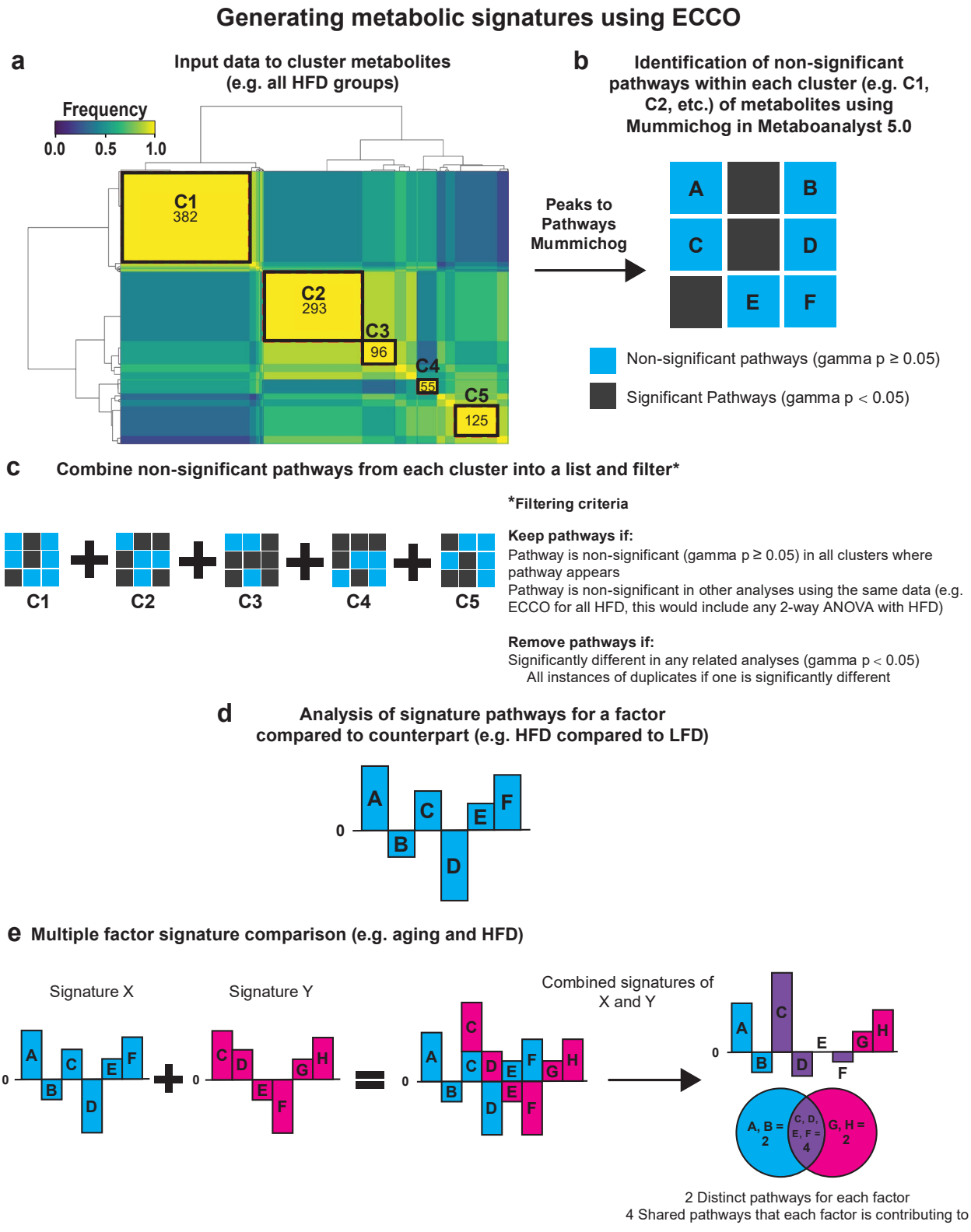


Figure S5. Steps for assessing metabolomic data using ensemble clustering with cluster optimization (ECCO) to identify signatures for multiple factors. (a) Metabolites are clustered according to the frequency the metabolites group together in multiple clustering solutions. (b) Clustered metabolites can be identified using Mummichog. (c) The resulting pathways are grouped into non-significant or significant according to the threshold for the gamma value. The pathways from the clusters are filtered to avoid duplicates and significant pathways from other analyses. (d) The remaining signature pathway metabolites are compared to the factor's control (e.g. high-fat diet (HFD) compared to low-fat diet (LFD)). (e) Multiple factor signatures can be compared, such that distinct and shared signature pathways can be identified.