

Supplemental Information

Tissue-specific analysis of lipid species in *Drosophila* during overnutrition by UHPLC-MS/MS and MALDI-MSI

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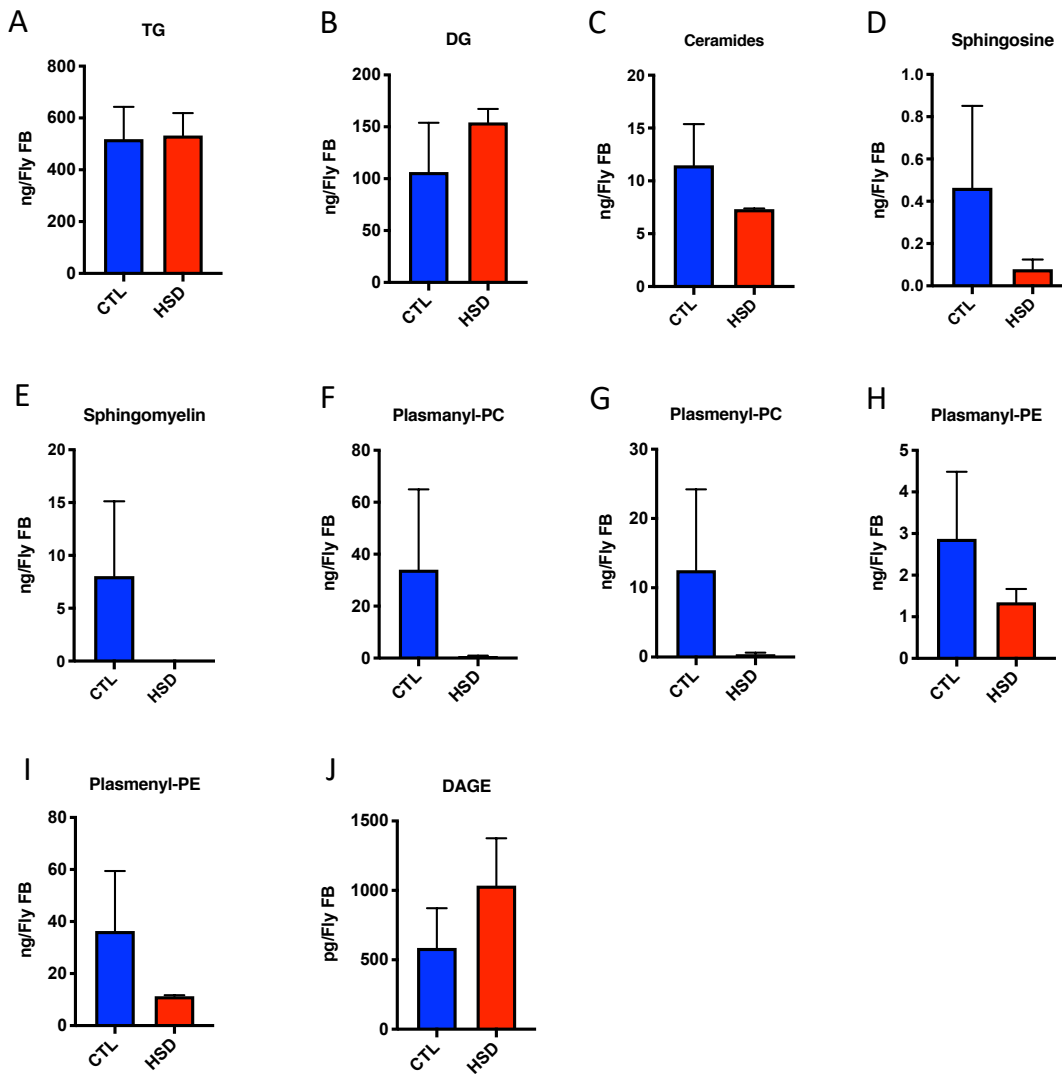
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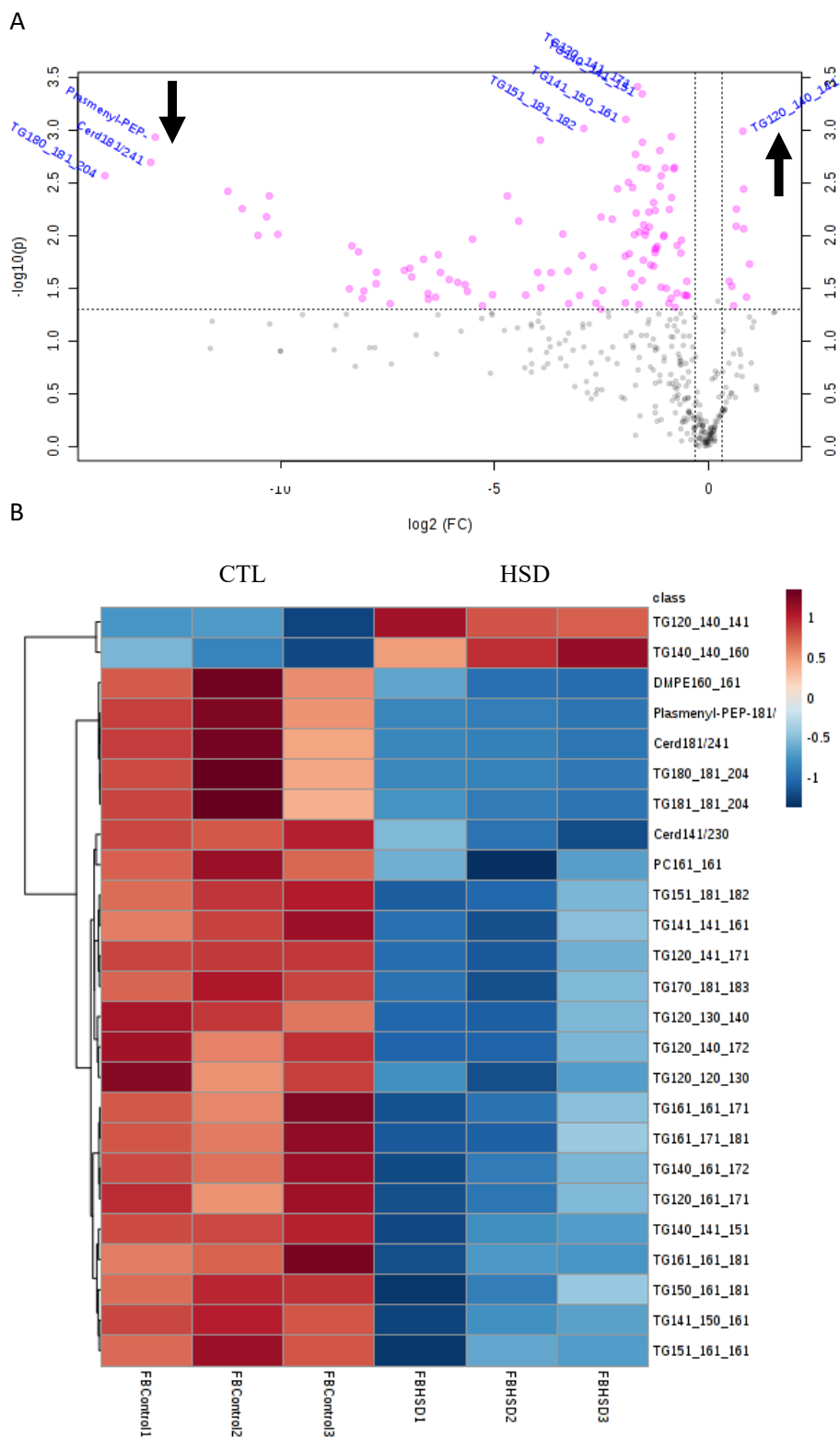
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Running title: Analysis of *Drosophila* lipids during overnutrition

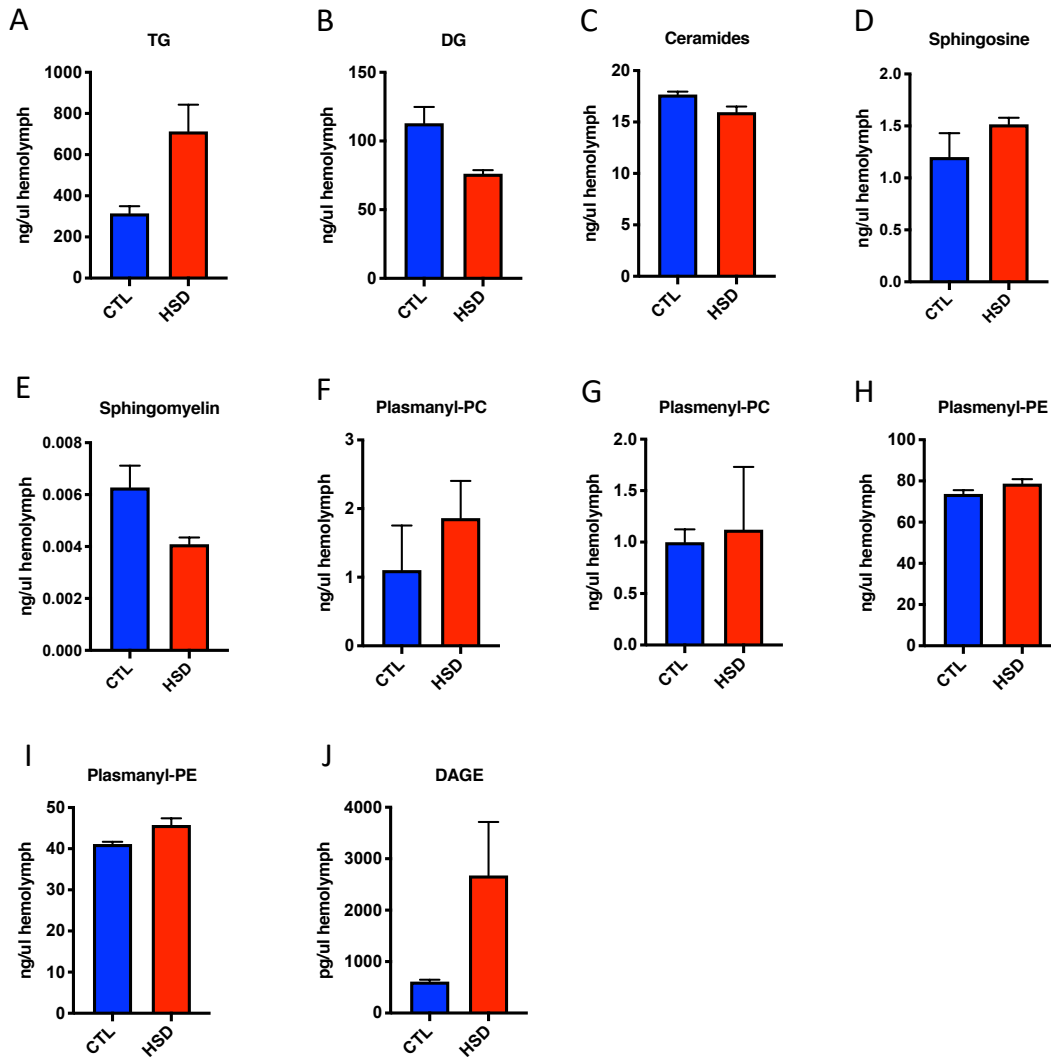


Supplemental Figure S1: Quantitative analysis of fat body lipids. Graphs comparing amount of each lipid class detected per fat body. A: TG, B: DG, C: ceramides, D: SO, E: SM, F: Plasmanyl-PC, G: Plasmenyl-PC, H: Plasmanyl-PE, I: Plasmenyl-PE and J: DAGE. Error bars represent the standard error of the mean. No significant differences between control and HS diets were observed at a cutoff of $p < 0.05$ using a student's t test and Welch's correction.

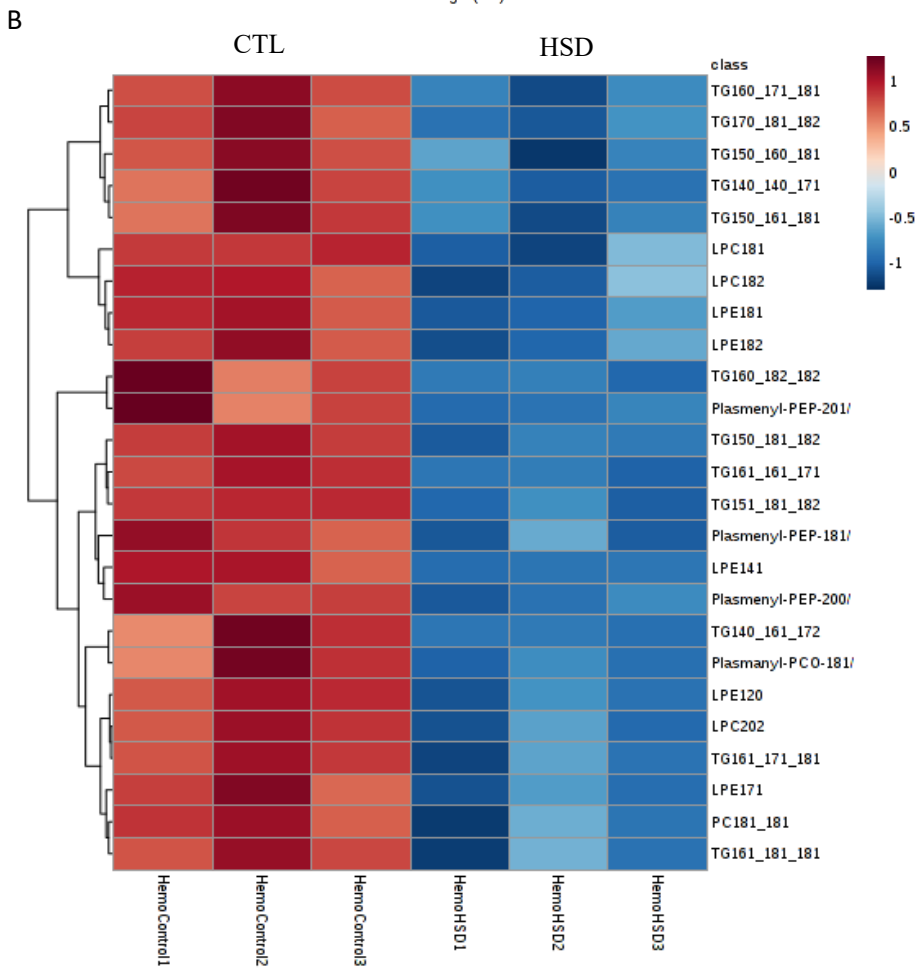
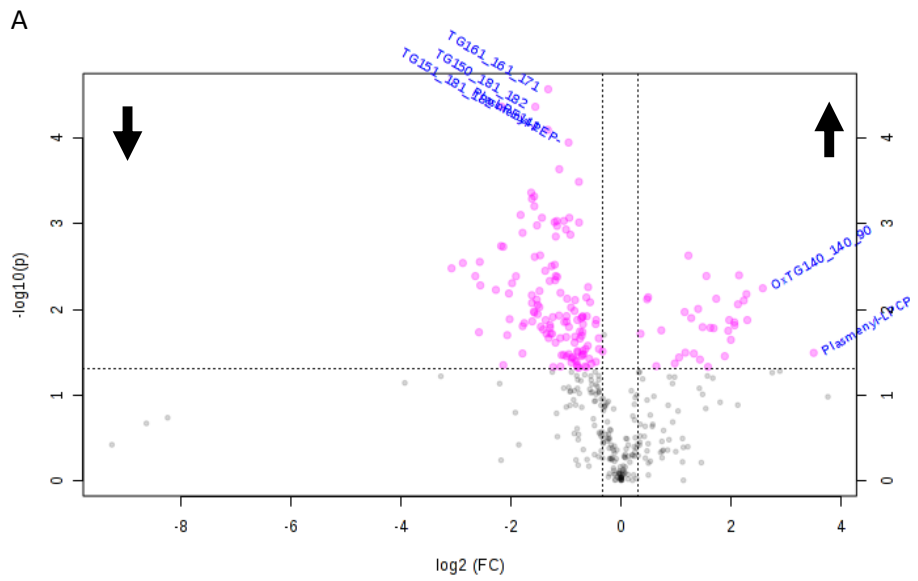


Supplemental Figure S2: Multivariate analysis of fat body lipids. A: Volcano plot with fold change cutoffs of 0.75 and 1.25 and p-value cutoff of $<.05$ (dotted lines). B: Hierarchical clustered heat map indicating the

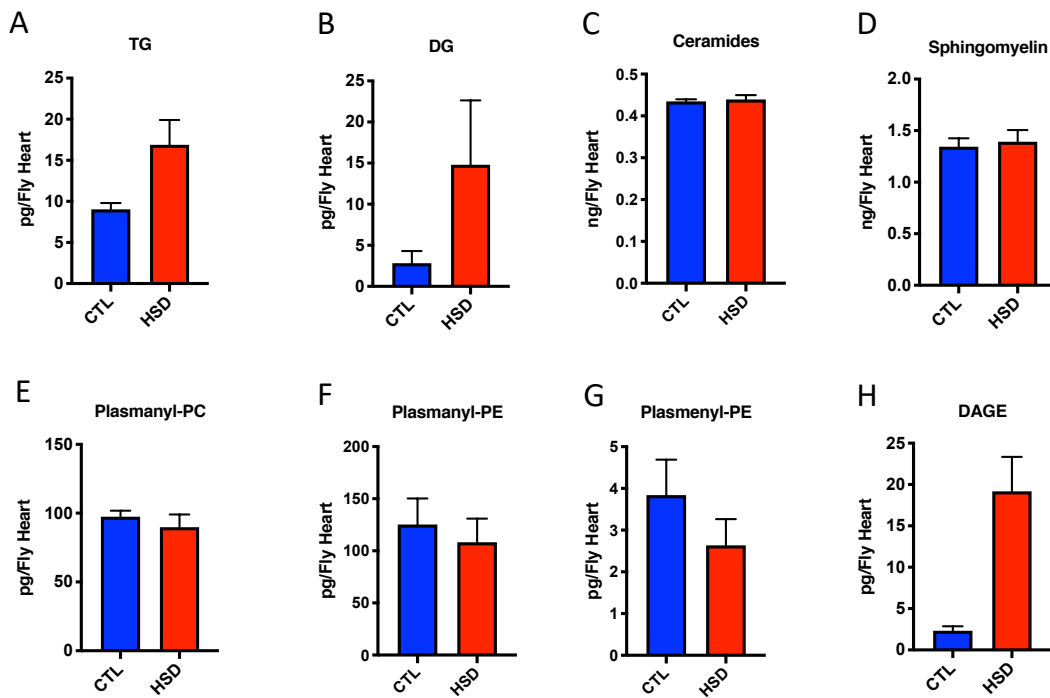
relative abundance of the top 25 most significantly differentially present lipids in fat bodies from flies aged on control vs HS diets.



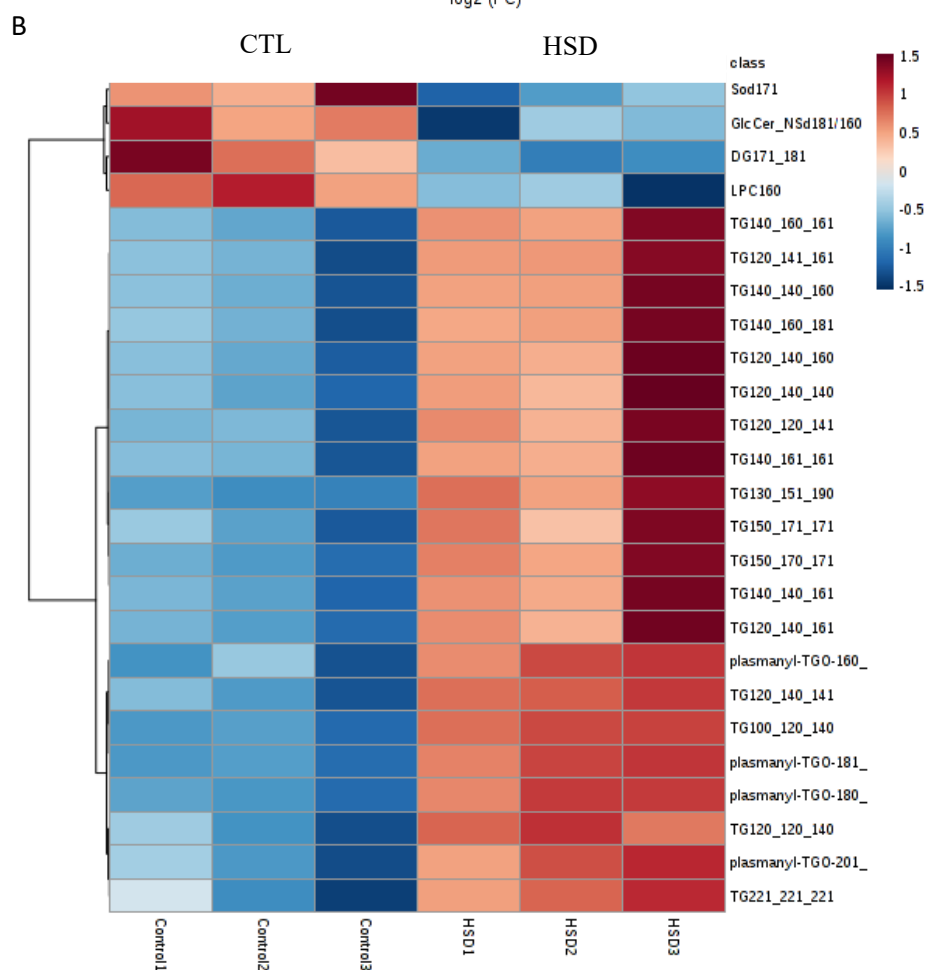
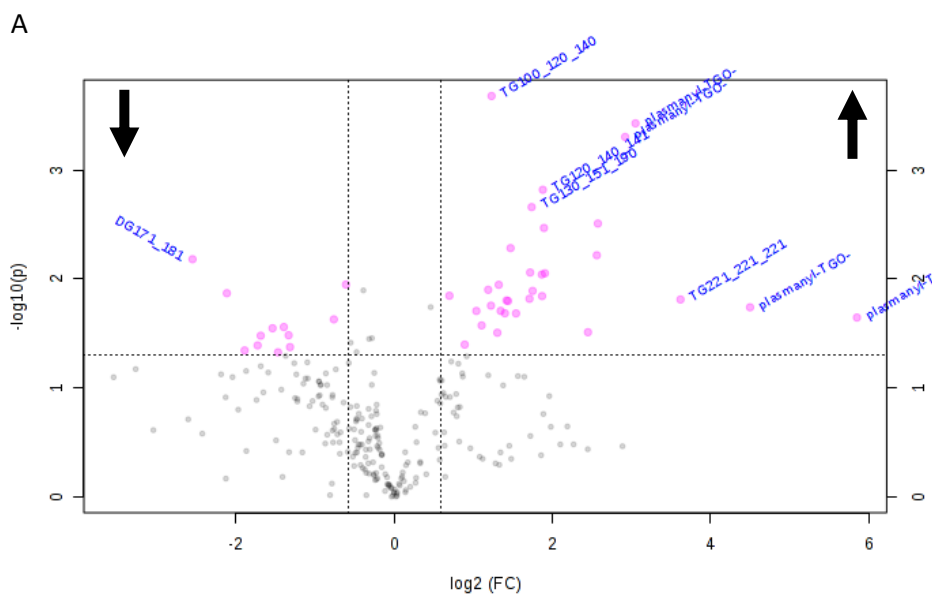
Supplemental Figure S3: Quantitative analysis of hemolymph lipids. Graphs comparing amount of each lipid class detected in each microliter of hemolymph. A: TG, B: DG, C: ceramides, D: SO, E: SM, F: Plasmanyl-PC, G: Plasmenyl-PC, H: Plasmanyl-PE, I: Plasmenyl-PE and J: DAGE. Error bars represent the standard error of the mean. No significant differences between control and HS diets were observed at a cutoff of $p < 0.05$ using a student's t test and Welch's correction.



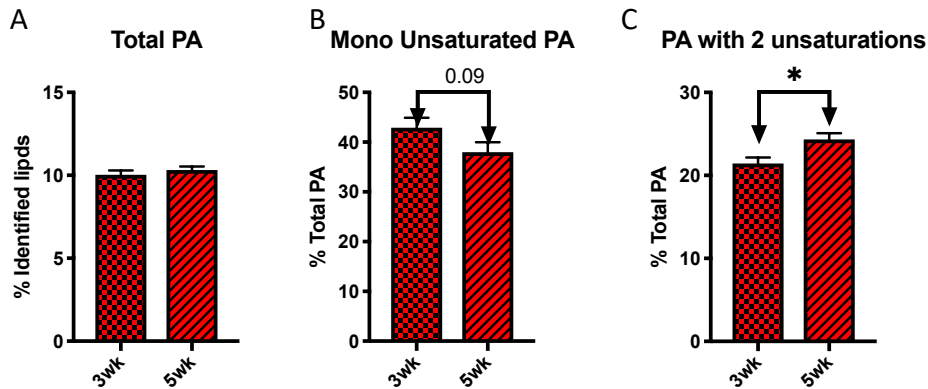
Supplemental Figure S4: Multivariate analysis of hemolymph lipids. A: Volcano plot with fold change cutoffs of 0.75 and 1.25 and p-value of $<.05$ (dotted lines). B: Hierarchical clustered heat map indicating the relative abundance of the top 25 most significantly differentially present lipids in flies aged on control vs HS diets.



Supplemental Figure S5: Quantitative analysis of cardiac lipids. Graphs comparing amount of each lipid class detected in each heart. A: TG, B: DG, C: Ceramides, D: SM, E: Plasmanyl-PC, F: Plasmanyl-PE, G: Plasmeyl-PE and H: DAGE. Error bars represent the standard error of the mean. No significant differences between control and HS diets were observed at a cutoff of $p < 0.05$ using a student's t test and Welch's correction.



Supplemental Figure S6: Multivariate analysis of heart lipids. A: Volcano plot with fold change cutoffs of 0.75 and 1.25 and p-value of <.05 (dotted lines). B: Hierarchical clustered heat map indicating the abundance of the top 25 most significantly differentially present lipids in flies aged on control vs HS diets.



Supplemental Figure S7: Quantification of phosphatidic acid using MALDI-MSI. A: Total detected PA, B: Monounsaturated PA, C: polyunsaturated PA detected at 3 and 5 weeks by MALDI-MSI. * $p < .05$.

Supplemental File S1: Volcano table comparing control to HS 5-week FB by UHPLC-MS/MS. All significantly differentially present compounds identified meeting the criteria of p-value less than 0.05 and a fold change (HS/ctrl) of >25%.

Supplemental File S2: Volcano table comparing control to HS 5-week hemolymph by UHPLC-MS/MS. All significantly differentially present compounds identified meeting the criteria of p-value less than 0.05 and a fold change (HS/ctrl) of >25%.

Supplemental File S3: Volcano table comparing control to HS 5-week heart by UHPLC-MS/MS. All significantly differentially present compounds identified meeting the criteria of p-value less than 0.05 and a fold change (HS/ctrl) of >25%.

Supplemental File S4: Volcano table of 3-week FB by MALDI-MSI. All significantly differentially present compounds identified that had a p-value of less than 0.05 and a fold change (HS/ctrl) of >25%.

Supplemental File S5: Volcano table of 5-week FB by MALDI-MSI. All significantly differentially present compounds identified that had a p-value of less than 0.05 and a fold change (HS/ctrl) of >25%.

Supplemental File S6: Volcano table of 3-week to 5-week control diet comparison of FB by MALDI-MSI. All significantly differentially present compounds identified that had a p-value of less than 0.05 and a fold change (5 week/3 week) of >25%.

Supplemental File S7: Volcano table of 3-week to 5-week HS comparison of FB by MALDI-MSI. All significantly differentially present compounds identified that had a p-value of less than 0.05 and a fold change (5 week/3 week) of >25%.

Supplemental File S8: Volcano table of 3-week heart by MALDI-MSI. All significantly differentially present compounds identified that had a p-value of less than 0.05 and a fold change (HS/ctrl) of >25%.

Supplemental File S9: Volcano table of 5-week heart by MALDI-MSI. All significantly differentially present compounds identified that had a p-value of less than 0.05 and a fold change (HS/ctrl) of >25%.

Supplemental File S10: Volcano table of 3-week to 5-week control diet comparison of heart by MALDI-MSI. All significantly differentially present compounds identified that had a p-value of less than 0.05 and a fold change (5 week/3 week) of >25%.

Supplemental File S11: Volcano table of 3-week to 5-week HS comparison of heart by MALDI-MSI. All significantly differentially present compounds identified that had a p-value of less than 0.05 and a fold change (5 week/3 week) of >25%.