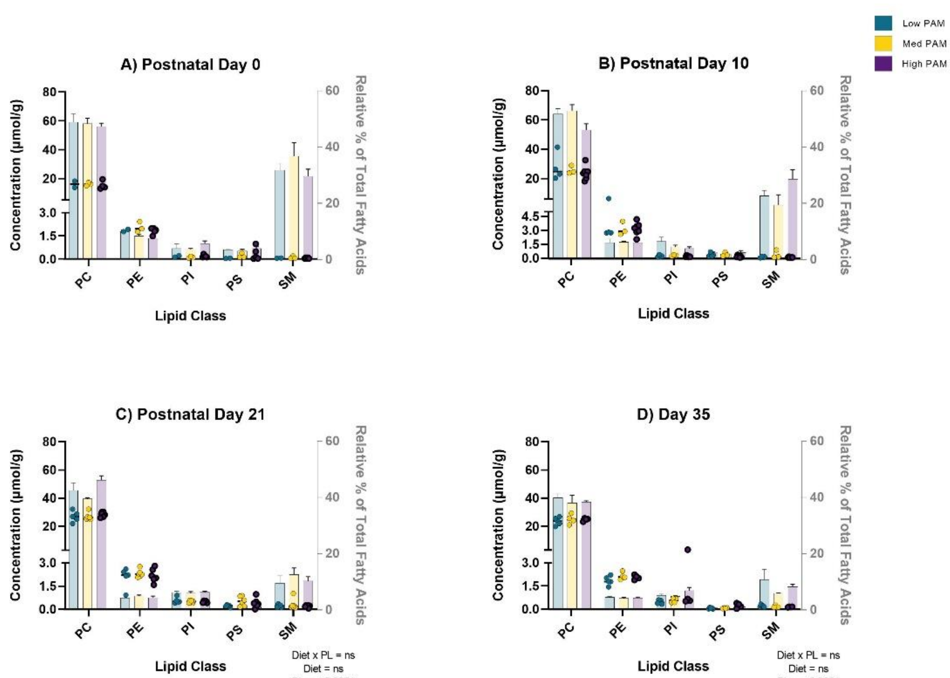
Supplemental Material In: Upregulated Hepatic Lipogenesis from Dietary Sugars in Response to Low Palmitate Feeding Supplies Brain Palmitate

Authors: Mackenzie E Smith[1], Chuck T Chen[1], Chiraag Gohel[2], Giulia Cisbani[1], Daniel K Chen[1], Kimia Rezaei[1], Andrew McCutcheon[1], Richard P. Bazinet*[1]

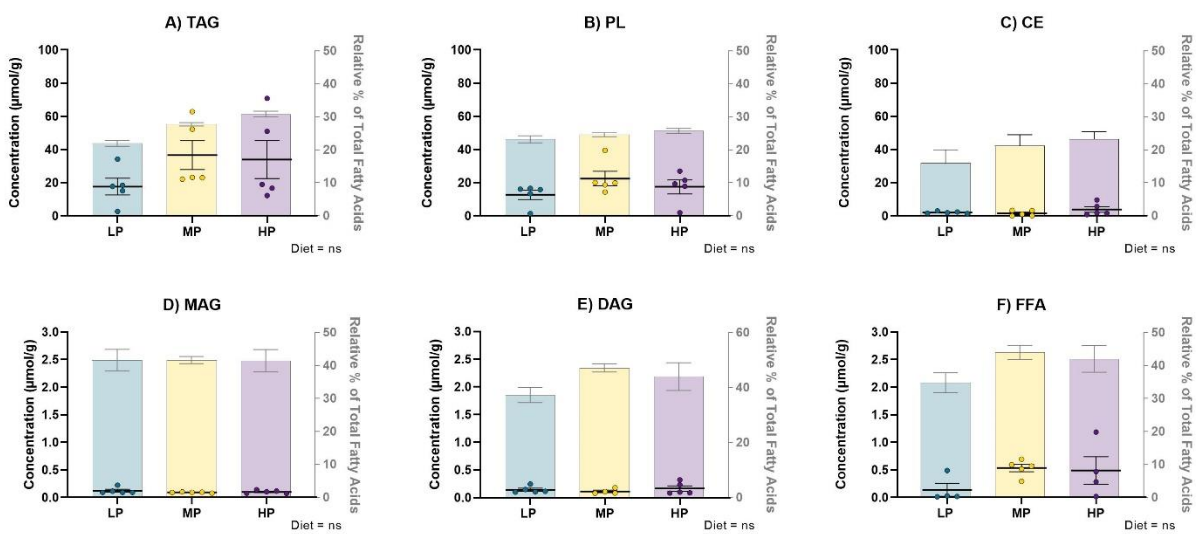
Author Affiliations: [1]Department of Nutritional Sciences, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada, M5S 1A8; [2]Department of Biostatistics and Bioinformatics, George Washington University, 950 New Hampshire Ave, NW, Washington, DC 20052, United States

***Corresponding Author Contact:** richard.bazinet@utoronto.ca

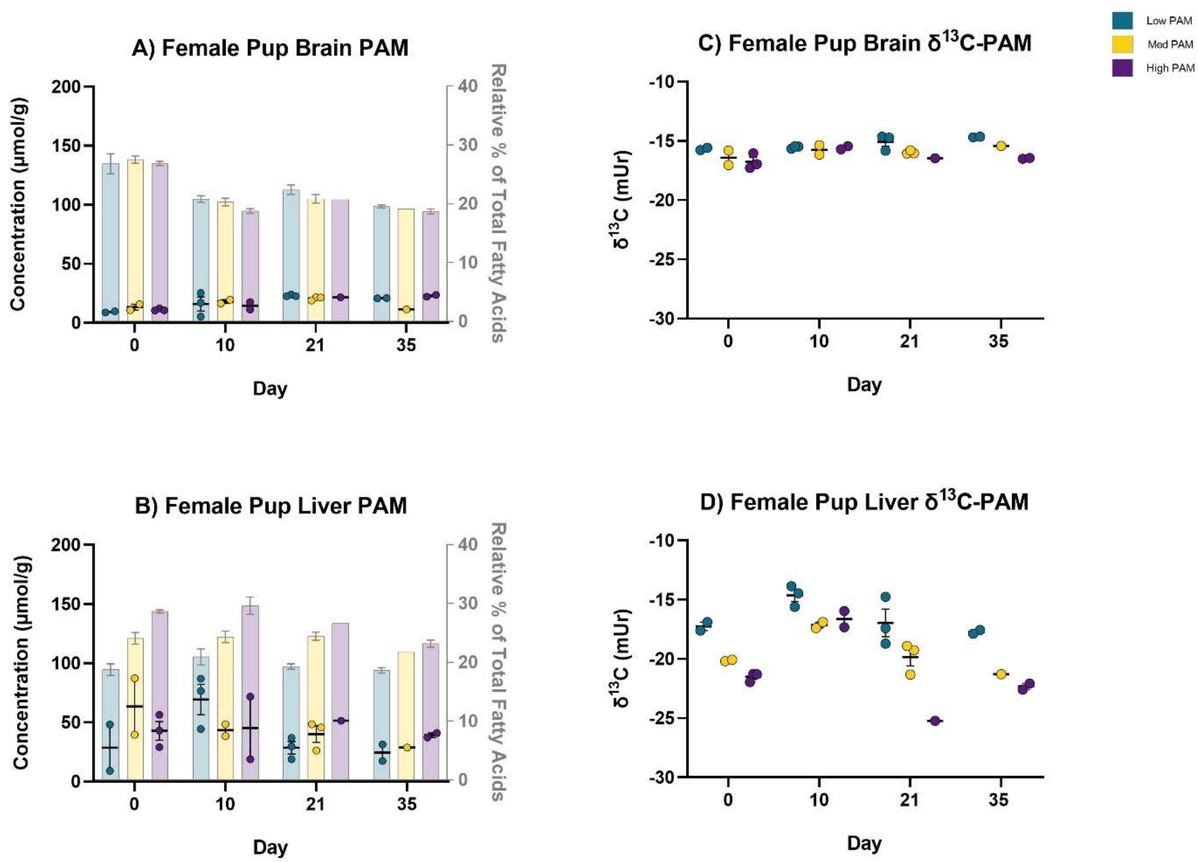
Supplemental Figure 1: Male Pup Brain Palmitic Acid is Maintained in Individual Brain Phospholipid Fractions in Response to Diets. Levels of palmitic acid (PAM) in all individual brain phospholipid (PL) fractions do not appear to be influenced by diet at postnatal day (P) 0 (**A**) and P10 (**B**). There was also not a significant effect of diet at P21 (**C**) and day 35 (**D**), only a significant effect of PL fraction at P21 ($p < 0.0001$) (**C**) and day 35 ($p < 0.0001$) (**D**). Data are biological replicates presented as mean ± SEM analyzed by two-way analysis of variance (ANOVA) (**C, D**). Because of limited sample size at P0 and P10, statistics were not conducted (**A, B**). n = 2, 3, 5 male mice per diet at P0 ethanolamine glycerophospholipids (PE), phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin (SM) (**A**); n = 2, 3, 4 male mice per diet at P0 choline glycerophospholipids (PC) (**A**); n = 4, 3, 7 male mice per diet at P10 PC, PE, PI, PS, SM (**B**); n = 5, 6, 6 male mice per diet at P21 PC, PE, PS, SM (**C**); n = 5, 6, 5 male mice per diet at P21 PI (**C**); n = 5, 4, 4 male mice per diet at P35 PC, PE, PI, PS, SM (**D**). Individual data points represent concentration data in μmol/g (for which figure statistics correspond to) while the bars show relative percentage data. Source data are provided as a Source Data file. Med, medium.



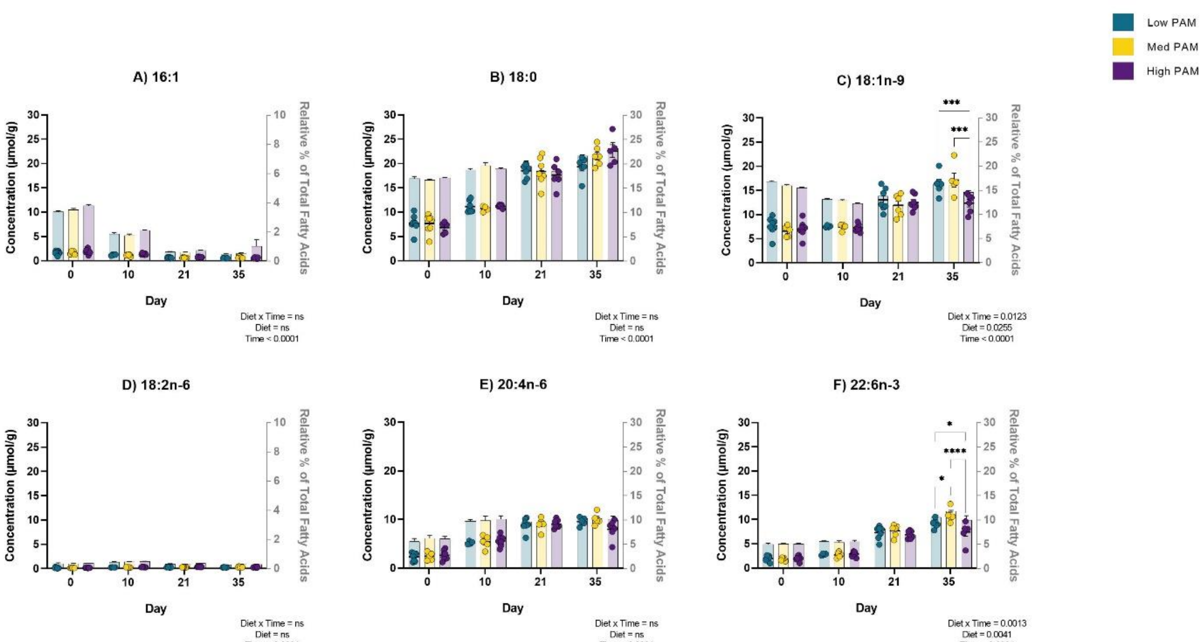
Supplemental Figure 2: The Relative Percentage, but not the Concentration of Palmitic Acid is Reflective of Dietary Palmitic Acid Levels in Separated Male Liver Triacylglyceride and Cholesteryl Ester Fractions at Day 35. There was a significant effect of diet in the relative percentage of palmitic acid (PAM) in separated male liver triacylglyceride (TAG) ($p < 0.0001$) (**A**) and cholesteryl ester (CE) ($p = 0.0135$) (**C**) fractions, however, not in liver phospholipid (PL) (**B**), monoacylglycerol (MAG) (**D**), diacylglycerol (DAG) (**E**) and free fatty acid (FFA) (**F**) fractions. The concentration of PAM in separated male liver neutral lipid fractions was variable overall and there was not a significant effect of diet (**A-F**). Data are biological replicates presented as mean ± SEM analyzed by ordinary one-way analysis of variance (ANOVA) (**C, E**) or Kruskal-Wallis test (**A, B, D, F**) for normally and non-normally distributed data, respectively. n = 5 male pups per diet (**A, B, C, D**), n = 5, 4, 5 mice per diet (**E**), n = 4, 5, 4 mice per diet (**F**). Individual data points represent concentration data in µmol/g (for which figure statistics correspond to) while the bars show relative percentage data. Source data are provided as a Source Data file. HP, high palmitic acid; LP, low palmitic acid; Med, medium; MP, medium palmitic acid.



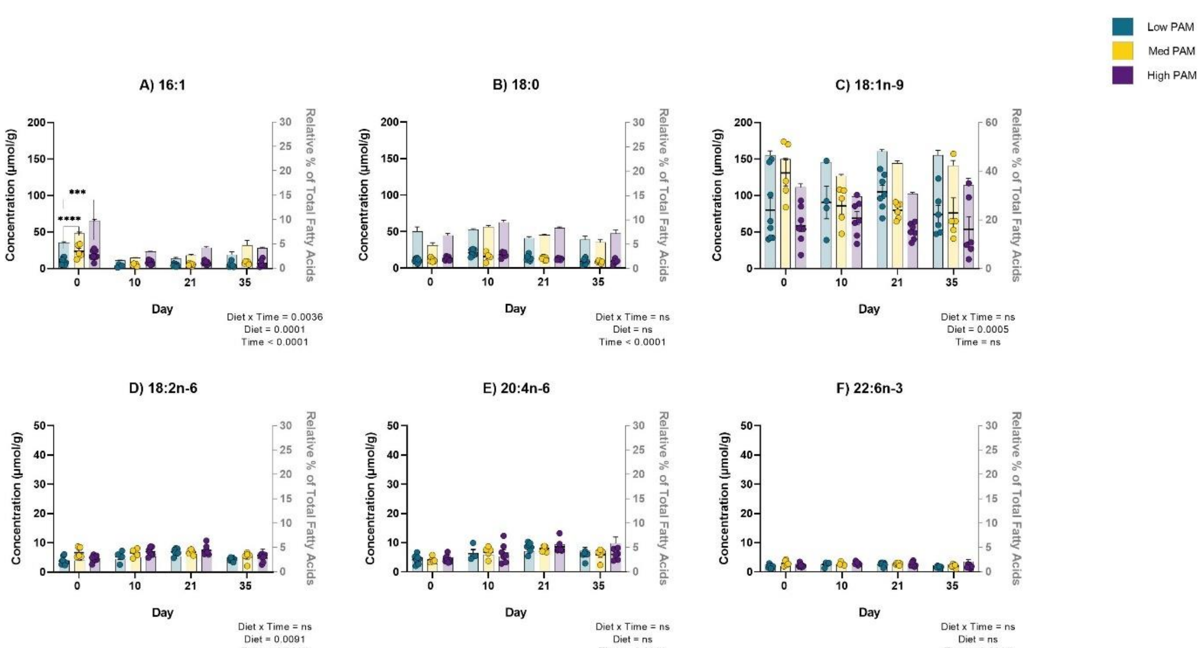
Supplemental Figure 3: Female Pup Brain Palmitic Acid Levels are Maintained Compared to the Liver Primarily by Lipogenesis from Dietary Sugars Augmented in Mice fed Low Palmitic Acid. While levels of palmitic acid (PAM) in the female liver appear impacted by time and diet (**B**), levels of PAM in the female brain do not appear to be impacted by diet, only time (**A**). Female pup tissue δ^{13}C-PAM values are enriched overall and appear augmented in mice fed low compared to medium and high PAM in both the brain (**C**) and liver (**D**). Data are biological replicates presented as mean ± SEM. Because of limited sample size statistics were not conducted. n = 2, 2, 3; 3, 2, 2; 3, 3, 1; 2, 1, 2 female mice per diet per timepoint (**A-D**). Individual data points represent concentration data in µmol/g while the bars show relative percentage data (**A, B**). Source data are provided as a Source Data file. δ^{13}C, ^{13}C/^{12}C; Med, medium.



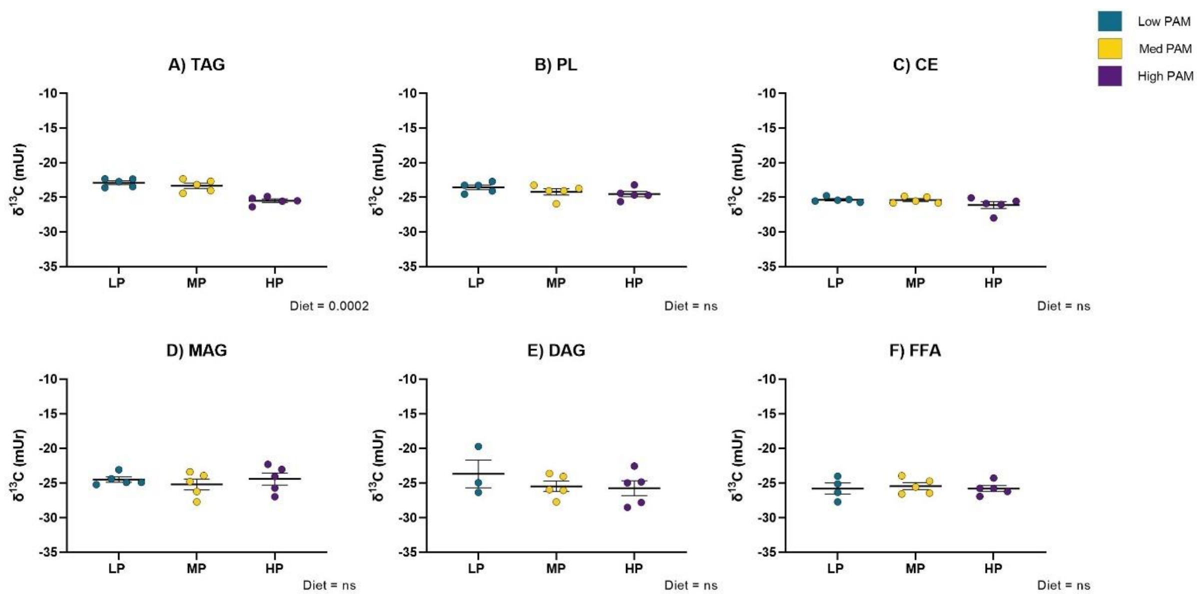
Supplemental Figure 4: Levels of Lipogenic and Other Fatty Acids are Maintained in the Male Pup Brain in Response to Diet. The concentration of lipogenic fatty acids including palmitoleic acid (POA) (**A**) and stearic acid (STA) (**B**) as well as essential fatty acids linoleic acid (LNA) (**D**) and arachidonic acid (ARA) (**E**) were maintained in the brain at all timepoints in response to diet. Interestingly, there was a significant interaction between diet and time in the concentration of brain oleic acid (OLA) (**C**) and docosahexaenoic acid (DHA) (**F**). Data are biological replicates presented as mean ± SEM analyzed by two-way analysis of variance (ANOVA) (**A-F**) and Tukey's multiple comparison test (**C, F**); for visualization purposes only means significantly different by diet at each timepoint are displayed; * < 0.03, **** < 0.0001. n = 8, 5, 8; 4, 5, 7; 7, 6, 7; 6, 5, 6 male pups per diet per timepoint (**A-F**). Individual data points represent concentration data in µmol/g (for which figure statistics correspond to) while the bars show relative percentage data. Source data are provided as a Source Data file. Med, medium.



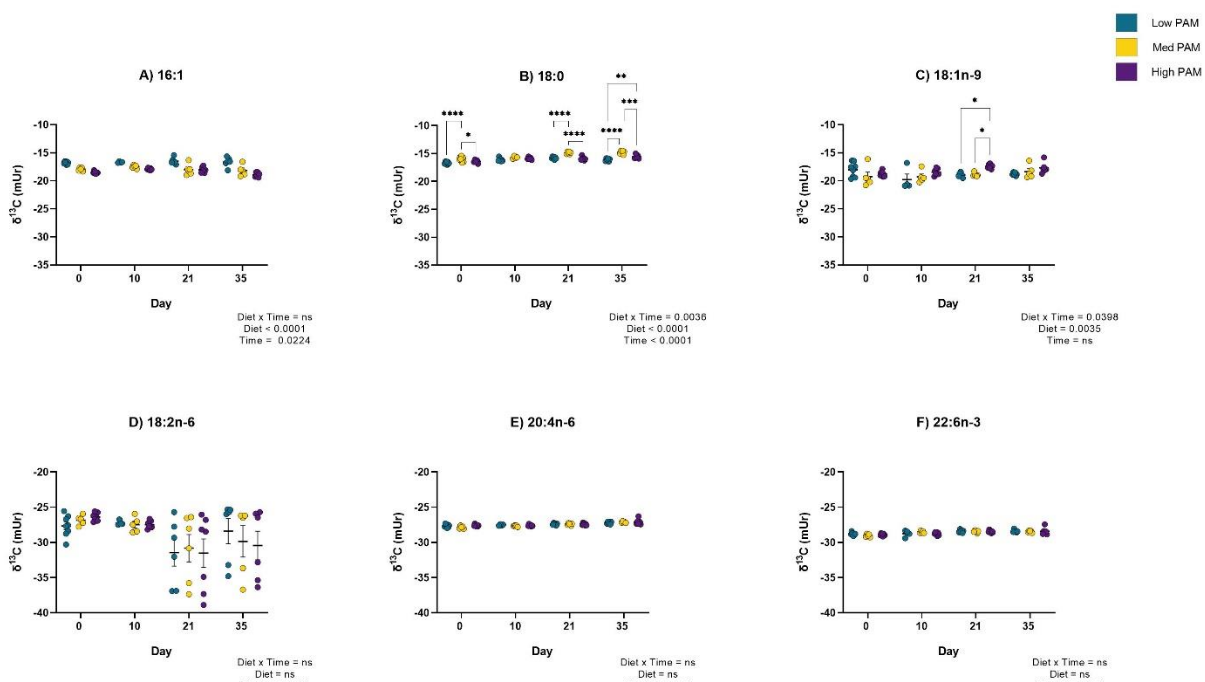
Supplemental Figure 5: Levels of Lipogenic, but not Other Fatty Acids are Responsive to Diet in the Male Pup Liver. There was a significant diet x time interaction in the concentration of lipogenic liver fatty acid palmitoleic acid (POA) (**A**), whereby mice fed low palmitic acid (PAM) had lower levels of liver POA than mice fed medium and high PAM at postnatal day (P) 0, but not P10, P21 and day 35. While liver stearic acid (STA) was not affected by diet (**B**), liver oleic acid (OLA) was significantly affected by diet whereby mice fed low PAM (high OLA) had higher levels of OLA than mice fed the medium, and high PAM diet (low OLA) at all timepoints (**C**). The concentration of liver essential fatty acid linoleic acid (LNA) (**D**), but not arachidonic acid (ARA) (**E**) nor docosahexaenoic acid (DHA) (**F**) was affected by diet. Data are biological replicates presented as mean ± SEM analyzed by two-way analysis of variance (ANOVA) (**A-F**) and Tukey's multiple comparison test (**A**); for visualization purposes only means significantly different by diet at each timepoint are displayed; *** < 0.0002, **** < 0.0001; n = 8, 5, 8; 4, 5, 7; 7, 6, 7; 6, 5, 6 male mice per diet per timepoint (**A-F**). Individual data points represent concentration data in µmol/g (for which figure statistics correspond to) while the bars show relative percentage data. Source data are provided as a Source Data file. Med, medium.



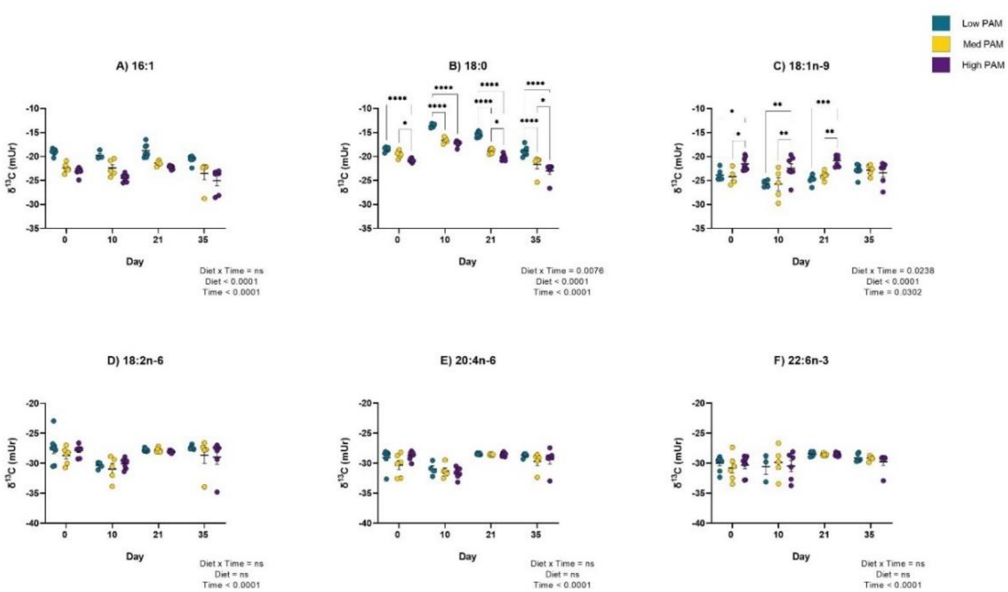
Supplemental Figure 6: Male Liver δ^{13}C-Palmitic Acid in Separated Triacylglyceride, but not Other Separated Liver Fractions Responsive to Diet at Day 35. There was a significant dose-response increase in δ^{13}C-palmitic acid (PAM) in response to diet in separated male liver triacylglyceride (TAG) (**A**) whereby mice fed low PAM had an increased δ^{13}C-PAM value compared to mice fed medium and high PAM. However, there was not a significant dose-response increase in δ^{13}C-PAM in response to diet in male separated liver phospholipid (PL) (**B**), cholesteryl ester (CE) (**C**), monoacylglycerol (MAG) (**D**), diacylglycerol (DAG) (**E**) and free fatty acid (FFA) (**F**) fractions. Data are biological replicates presented as mean ± SEM analyzed by ordinary one-way analysis of variance (ANOVA) (**A-F**). n = 5 male mice per diet (**A-D**), n = 3, 5, 5 male mice per diet (**E**), n = 4, 5, 5 male mice per diet (**F**). Source data are provided as a Source Data file. δ^{13}C, ^{13}C/^{12}C; HP, high palmitic acid; LP, low palmitic acid; Med, medium; MP, medium palmitic acid.



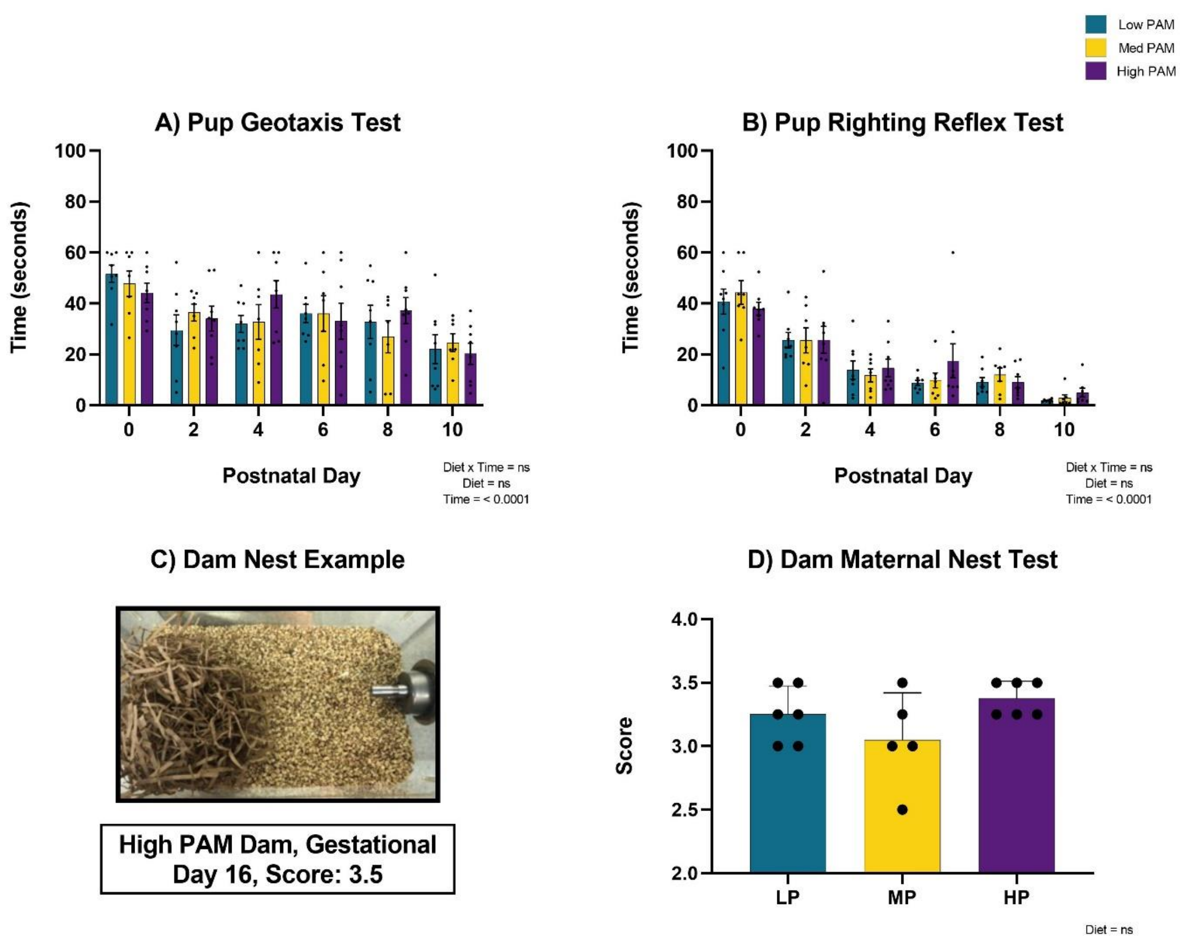
Supplemental Figure 7: Lipogenic, but not Other Brain Fatty Acid δ^{13}C Values Impacted by Diet in Male Pups. Male brain δ^{13}C-palmitoleic acid (POA) is enriched overall and is significantly augmented in mice fed low palmitic acid (PAM) compared to medium and high PAM (**A**). Male brain δ^{13}C-stearic acid (STA) and δ^{13}C-oleic acid (OLA) are enriched overall, whereby the δ^{13}C value depended on time (**B**, **C**). Compared to δ^{13}C values of lipogenic brain fatty acids (**A**, **B**, **C**), δ^{13}C values of essential brain fatty acids; linoleic acid (LNA) (**D**) and arachidonic acid (ARA) (**E**) are more deplete in δ^{13}C and are only significantly affected by time and not diet. Similarly, docosahexaenoic acid (DHA) is more deplete in δ^{13}C and is significantly affected by time and not diet (**F**). Data are biological replicates presented as mean ± SEM analyzed by two-way analysis of variance (ANOVA) (**A-F**) and Tukeys multiple comparison test (**B, C**); for visualization purposes only means significantly different by diet at each timepoint are displayed; * < 0.03, ** < 0.002, *** < 0.0002, **** < 0.0001. n = 8, 5, 8; 4, 5, 7; 6, 6, 7; 6, 5, 6 male mice per diet per timepoint (**A-F**). Source data are provided as a Source Data file. δ^{13}C, ^{13}C/^{12}C; Med, medium.



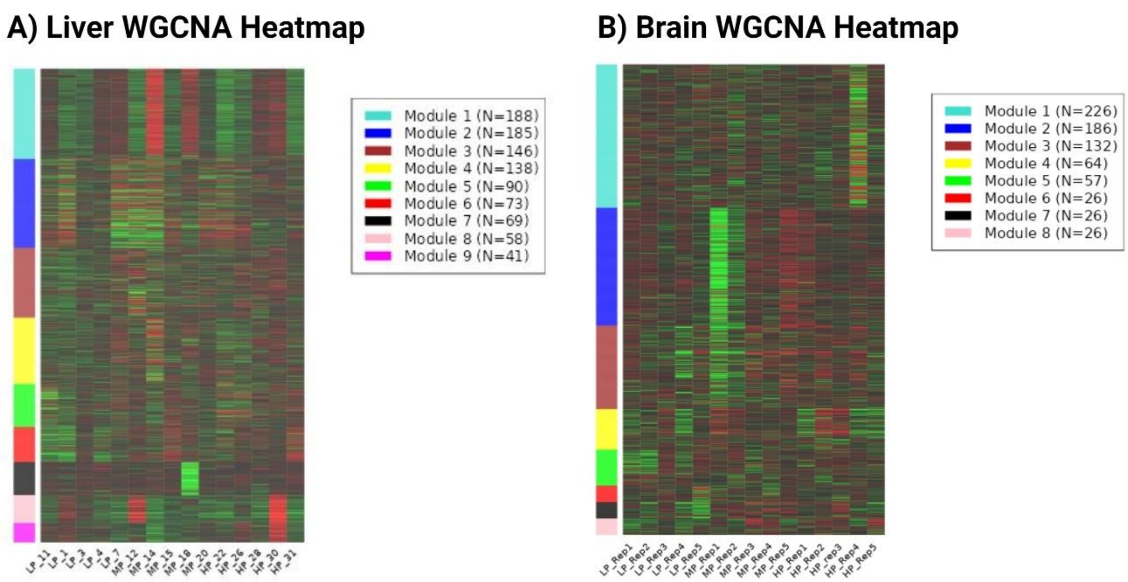
Supplemental Figure 8: Lipogenic, but not Other Liver Fatty Acid δ^{13}C Values Impacted by Diet in Male Pups. Male liver δ^{13}C-palmitoleic acid (POA) is enriched overall and is significantly augmented in mice fed low PAM compared to medium and high palmitic acid (PAM) (**A**), while male liver δ^{13}C-oleic acid (OLA) depends on time but is enriched overall and appears augmented in mice fed medium and high PAM compared to low PAM (**C**). Male liver δ^{13}C-stearic acid (STA) is also enriched overall, whereby the δ^{13}C value depends on time but appears augmented in mice fed low PAM compared to medium and high PAM (**B**). Compared to δ^{13}C values of lipogenic liver fatty acids (**A**, **B**, **C**), δ^{13}C values of essential liver fatty acids; linoleic acid (LNA) (**D**) and arachidonic acid (ARA) (**E**) are more deplete in δ^{13}C and only significantly affected by time, not diet. Similarly, docosahexaenoic acid (DHA), is more deplete in δ^{13}C and significantly affected by time and not diet (**F**). Data are biological replicates presented as mean ± SEM analyzed by two-way analysis of variance (ANOVA) (**A-F**) and Tukeys multiple comparison test (**B**); for visualization purposes only means significantly different by diet at each timepoint are displayed; ** < 0.002, *** < 0.0002, **** < 0.0001. n = 8, 6, 8; 4, 5, 7; 7, 6, 7; 6, 5, 6 male mice per diet per timepoint (**A-B, D-E**), n = 6, 4, 8; 4, 5, 7; 7, 6, 7; 6, 5, 6 (**C**); n = 8, 5, 8; 3, 5, 6; 7, 6, 7; 6, 5, 6 (**F**). Source data are provided as a Source Data file. δ^{13}C, ^{13}C/^{12}C; Med, medium.



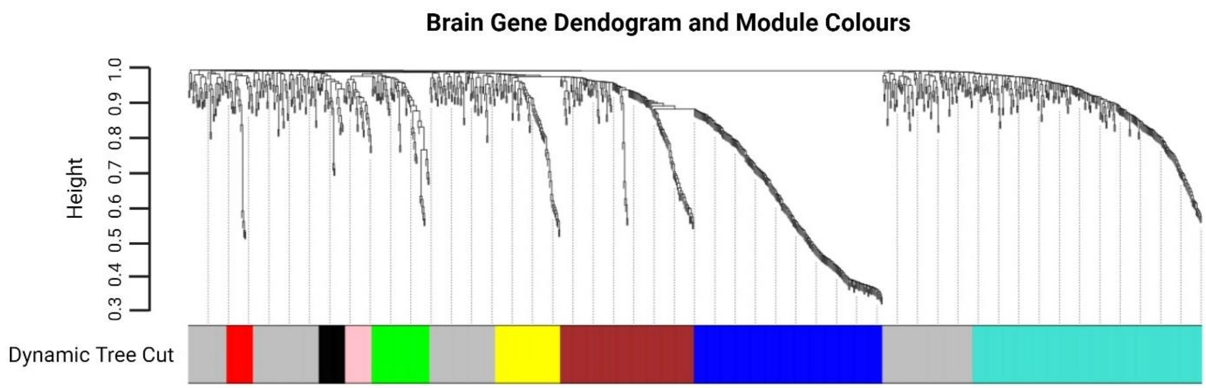
Supplemental Figure 9: Maternal Behaviour and Pup Sensorimotor Development are Not Impacted by Diets. There were no differences by diet in average pup litter scores for either the geotaxis test (**A**) nor the righting reflex test (**B**), however, pup scores for both tests improved with time (p < 0.0001 for both). Similarly, there were no differences by diet in dam nest scores between gestational days 15 to 18 (**D**). Data are biological replicates presented as mean ± SEM analyzed by two-way analysis of variance (ANOVA) (**A, B**) and ordinary one-way ANOVA (**D**). n = 8, 7, 8 average litter scores per diet (**A, B**); n = 6, 5, 6 dams per diet (**D**). One dam was excluded from the maternal next test in the MP group due to late impregnation. Source data are provided as a Source Data file. HP, high palmitic acid; LP, low palmitic acid; Med, medium; MP, medium palmitic acid; PAM, palmitic acid.



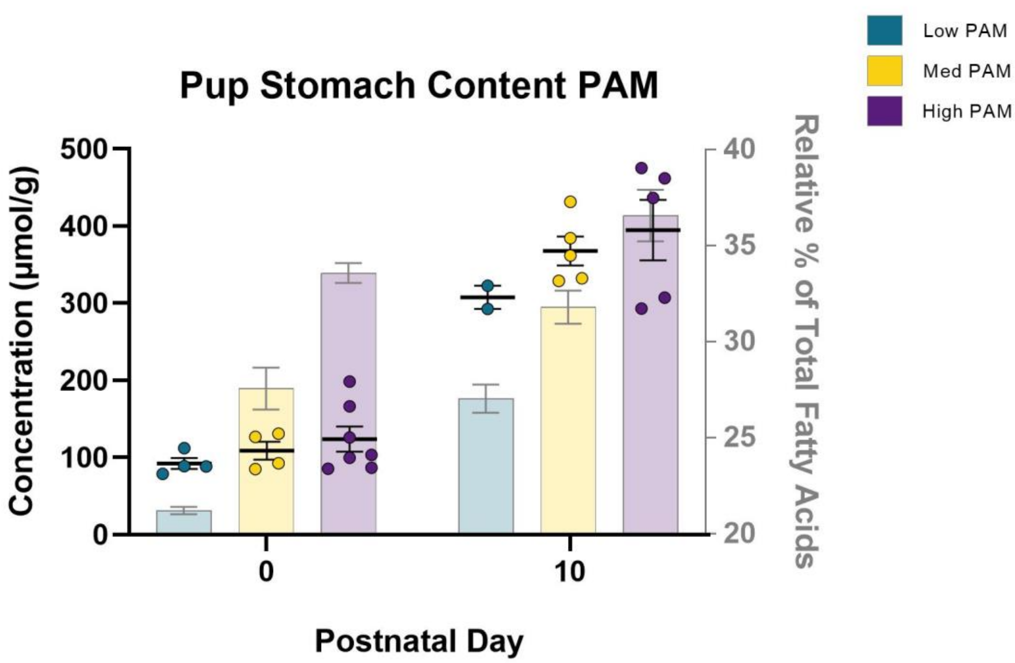
Supplemental Figure 10: Heatmaps for Male Pup Liver and Brain Weighted Gene Co-Expression Network Analysis at Day 35. Modules of highly correlated genes were explored by weighted gene co-expression network analysis (WGCNA) on the top 1000 most variable genes in the liver (**A**) and brain (**B**). n = 5 male mice per diet (biological replicates). Soft threshold was set to 6 and a minimum module size of 20 was used.



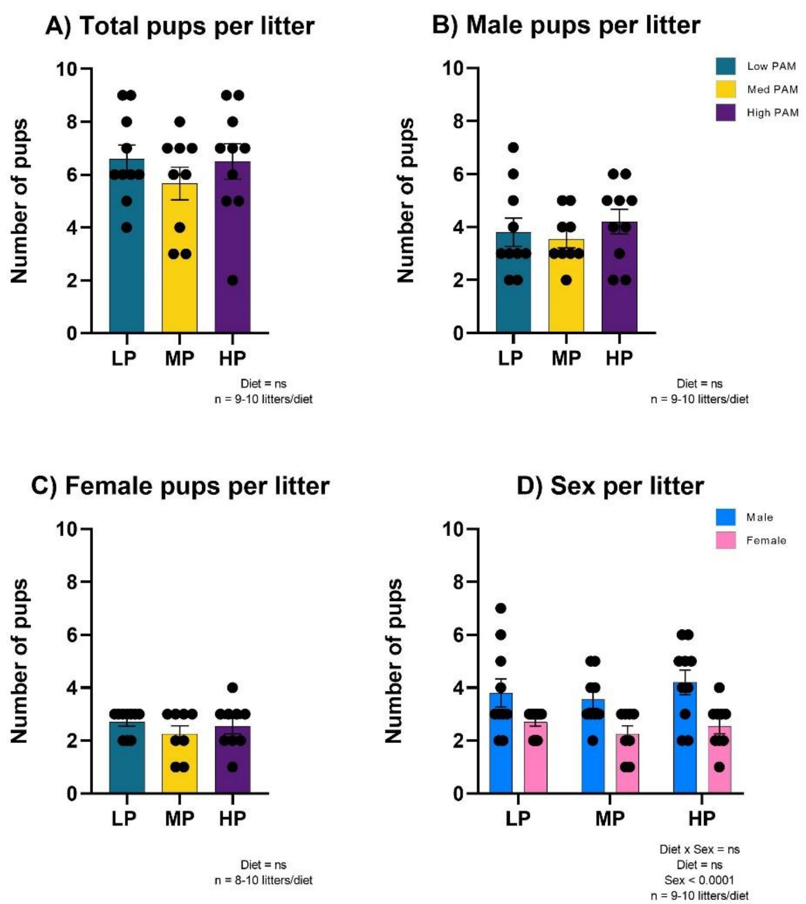
Supplemental Figure 11: Male Pup Brain Gene Dendrogram and Module Colours at Day 35. A network of 743 genes was divided into 8 modules by brain weighted gene co-expression network analysis (WGCNA). n = 5 male mice per diet (biological replicates). Soft threshold was set to 6 and a minimum module size of 20 was used.



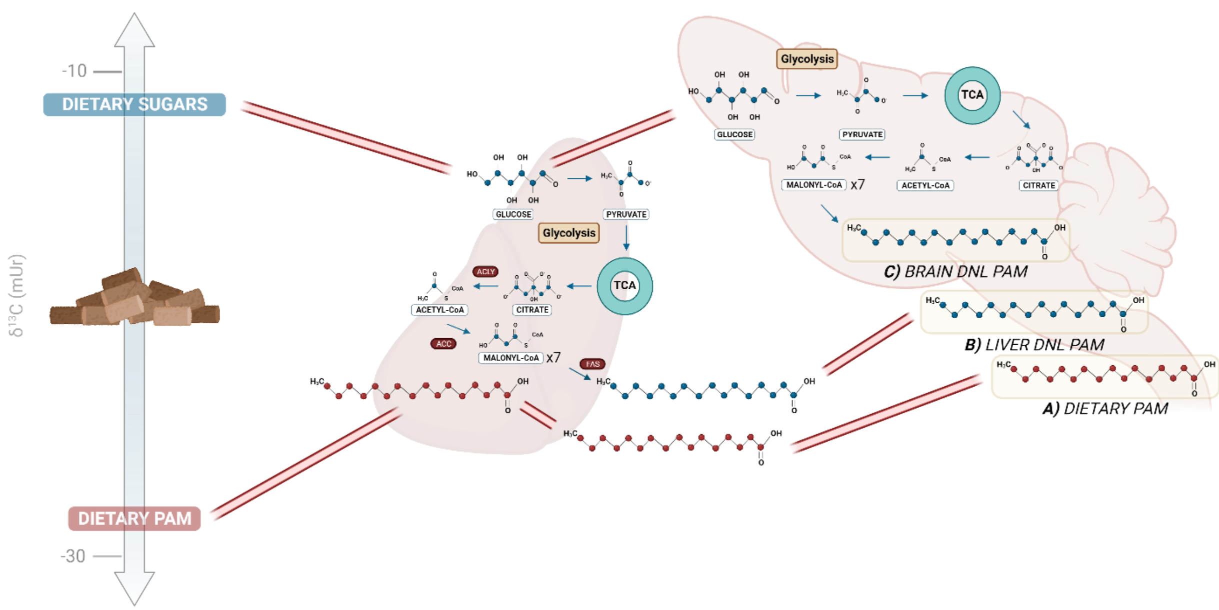
Supplemental Figure 12: Dam Milk Palmitic Acid Levels are Reflective of Dietary Palmitic Acid Levels Pre-Weaning. Levels of palmitic acid (PAM) in pup stomach content containing dam milk reflected dietary levels of PAM at postnatal day (P) 0 and P10. Data are biological replicates presented as mean ± SEM. Because sample size relied on a fed state, it was limited at P10 (n = 2 in low PAM group), therefore, statistics were not conducted. n = 4, 4, 7 male mice per diet at P0; n = 2, 5, 5 male mice per diet at P10. Individual data points represent concentration data in µmol/g while the bars show relative percentage data. Source data are provided as a Source Data file. Med, medium; PAM, palmitic acid.



Supplemental Figure 13: Litter Data. There were no significant differences between diet groups in terms of total pups born per litter (**A**), number of male pups per litter (**B**) or number of female pups per litter (**C**). However, dams consistently had a higher number of male offspring compared to female offspring within their litters ($p < 0.0001$) (**D**), irrespective of diet. Data are biological replicates presented as mean ± SEM analyzed by ordinary one-way analysis of variance (ANOVA) (**A, B**) or Kruskal-Wallis test (**C**) for normally and non-normally distributed data, respectively, as well as two-way ANOVA (**D**). n = 11, 10, 11 litters per diet (**A**); n = 10, 9, 10 litters per diet (**B**); n = 10, 8, 9 litters per diet (**C**); n = 10, 10; 9, 8; 10, 9 litters per sex per diet (**D**). Litters that involved dam infanticide (n = 2) were excluded from this analysis. Source data are provided as a Source Data file. HP, high palmitic acid; LP, low palmitic acid; Med, medium; MP, medium palmitic acid; PAM, palmitic acid.



Supplemental Figure 14: Studying Carbon Isotope Ratios at the Natural Abundance Level: A Summary of Tissue Fatty Acids and Their Origin. A detailed overview of the three sources capable of maintaining the brain palmitic acid (PAM) pool; PAM sourced directly from the diet (**A**), PAM synthesized *via* lipogenesis from dietary sugars in the liver (**B**), and PAM synthesized *via* lipogenesis from dietary sugars locally in the brain (**C**). Importantly, tissue PAM of dietary origin is deplete in $\delta^{13}C$ (shown in blue), while PAM synthesized endogenously via de novo lipogenesis from dietary sugars is enriched in $\delta^{13}C$ (shown in red). ACC, acetyl-coA carboxylase; ACLY, ATP citrate lyase; DNL, de novo lipogenesis; FAS; fatty acid synthase; TCA, the citric acid cycle. Created with BioRender.com.



Supplemental Table 1: Tukey's Multiple Comparisons Test: Male Pup Mouse Brain δ^{13}C-Palmitic Acid

Tukey's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
		Brain δ^{13}C-PAM			
0 :Low PAM vs. 0 :Medium PAM	0.6508	0.09889 to 1.203	Yes	**	0.0085
0 :Low PAM vs. 0 :High PAM	0.7900	0.3060 to 1.274	Yes	****	<0.0001
0 :Low PAM vs. 10:Low PAM	-0.1813	-0.7740 to 0.4115	No	ns	0.9961
0 :Low PAM vs. 10:Medium PAM	0.2387	-0.3131 to 0.7906	No	ns	0.9434
0 :Low PAM vs. 10:High PAM	0.4287	-0.07225 to 0.9298	No	ns	0.1644
0 :Low PAM vs. 21:Low PAM	-0.6798	-1.181 to -0.1788	Yes	**	0.0012
0 :Low PAM vs. 21:Medium PAM	0.3404	-0.1824 to 0.8632	No	ns	0.5482
0 :Low PAM vs. 21:High PAM	0.4559	-0.04511 to 0.9569	No	ns	0.1082
0 :Low PAM vs. 35 :Low PAM	-0.6646	-1.187 to -0.1418	Yes	**	0.0031
0 :Low PAM vs. 35 :Medium PAM	0.2948	-0.2571 to 0.8466	No	ns	0.8043
0 :Low PAM vs. 35 :High PAM	1.000	0.4776 to 1.523	Yes	****	<0.0001
0 :Medium PAM vs. 0 :High PAM	0.1393	-0.4126 to 0.6911	No	ns	0.9993
0 :Medium PAM vs. 10:Low PAM	-0.8320	-1.481 to -0.1826	Yes	**	0.0028
0 :Medium PAM vs. 10:Medium PAM	-0.4120	-1.024 to 0.2002	No	ns	0.4976
0 :Medium PAM vs. 10:High PAM	-0.2220	-0.7888 to 0.3448	No	ns	0.9717
0 :Medium PAM vs. 21:Low PAM	-1.331	-1.897 to -0.7638	Yes	****	<0.0001
0 :Medium PAM vs. 21:Medium PAM	-0.3103	-0.8965 to 0.2758	No	ns	0.8129
0 :Medium PAM vs. 21:High PAM	-0.1949	-0.7617 to 0.3720	No	ns	0.9896
0 :Medium PAM vs. 35 :Low PAM	-1.315	-1.902 to -0.7292	Yes	****	<0.0001
0 :Medium PAM vs. 35 :Medium PAM	-0.3560	-0.9682 to 0.2562	No	ns	0.7080
0 :Medium PAM vs. 35 :High PAM	0.3497	-0.2365 to 0.9358	No	ns	0.6747
0 :High PAM vs. 10:Low PAM	-0.9713	-1.564 to -0.3785	Yes	****	<0.0001
0 :High PAM vs. 10:Medium PAM	-0.5513	-1.103 to 0.0006092	No	ns	0.0505
0 :High PAM vs. 10:High PAM	-0.3613	-0.8623 to 0.1398	No	ns	0.3908
0 :High PAM vs. 21:Low PAM	-1.470	-1.971 to -0.9688	Yes	****	<0.0001
0 :High PAM vs. 21:Medium PAM	-0.4496	-0.9724 to 0.07321	No	ns	0.1594

0 :High PAM vs. 21:High PAM	-0.3341	-0.8351 to 0.1669	No	ns	0.5116
0 :High PAM vs. 35 :Low PAM	-1.455	-1.977 to -0.9318	Yes	****	<0.0001
0 :High PAM vs. 35 :Medium PAM	-0.4953	-1.047 to 0.05661	No	ns	0.1196
0 :High PAM vs. 35 :High PAM	0.2104	-0.3124 to 0.7332	No	ns	0.9656
10:Low PAM vs. 10:Medium PAM	0.4200	-0.2294 to 1.069	No	ns	0.5584
10:Low PAM vs. 10:High PAM	0.6100	0.003258 to 1.217	Yes	*	0.0476
10:Low PAM vs. 21:Low PAM	-0.4986	-1.105 to 0.1082	No	ns	0.2097
10:Low PAM vs. 21:Medium PAM	0.5217	-0.1032 to 1.147	No	ns	0.1913
10:Low PAM vs. 21:High PAM	0.6371	0.03040 to 1.244	Yes	*	0.0314
10:Low PAM vs. 35 :Low PAM	-0.4833	-1.108 to 0.1415	No	ns	0.2879
10:Low PAM vs. 35 :Medium PAM	0.4760	-0.1734 to 1.125	No	ns	0.3659
10:Low PAM vs. 35 :High PAM	1.182	0.5568 to 1.807	Yes	****	<0.0001
10:Medium PAM vs. 10:High PAM	0.1900	-0.3768 to 0.7568	No	ns	0.9915
10:Medium PAM vs. 21:Low PAM	-0.9186	-1.485 to -0.3518	Yes	****	<0.0001
10:Medium PAM vs. 21:Medium PAM	0.1017	-0.4845 to 0.6878	No	ns	>0.9999
10:Medium PAM vs. 21:High PAM	0.2171	-0.3497 to 0.7840	No	ns	0.9760
10:Medium PAM vs. 35 :Low PAM	-0.9033	-1.490 to -0.3172	Yes	***	0.0001
10:Medium PAM vs. 35 :Medium PAM	0.05600	-0.5562 to 0.6682	No	ns	>0.9999
10:Medium PAM vs. 35 :High PAM	0.7617	0.1755 to 1.348	Yes	**	0.0023
10:High PAM vs. 21:Low PAM	-1.109	-1.626 to -0.5911	Yes	****	<0.0001
10:High PAM vs. 21:Medium PAM	-0.08833	-0.6269 to 0.4502	No	ns	>0.9999
10:High PAM vs. 21:High PAM	0.02714	-0.4903 to 0.5446	No	ns	>0.9999
10:High PAM vs. 35 :Low PAM	-1.093	-1.632 to -0.5548	Yes	****	<0.0001
10:High PAM vs. 35 :Medium PAM	-0.1340	-0.7008 to 0.4328	No	ns	0.9996
10:High PAM vs. 35 :High PAM	0.5717	0.03311 to 1.110	Yes	*	0.0281
21:Low PAM vs. 21:Medium PAM	1.020	0.4817 to 1.559	Yes	****	<0.0001
21:Low PAM vs. 21:High PAM	1.136	0.6183 to 1.653	Yes	****	<0.0001
21:Low PAM vs. 35 :Low PAM	0.01524	-0.5233 to 0.5538	No	ns	>0.9999
21:Low PAM vs. 35 :Medium PAM	0.9746	0.4078 to 1.541	Yes	****	<0.0001
21:Low PAM vs. 35 :High PAM	1.680	1.142 to 2.219	Yes	****	<0.0001
21:Medium PAM vs. 21:High PAM	0.1155	-0.4231 to 0.6540	No	ns	0.9999
21:Medium PAM vs. 35 :Low PAM	-1.005	-1.564 to -0.4461	Yes	****	<0.0001
21:Medium PAM vs. 35 :Medium PAM	-0.04567	-0.6318 to 0.5405	No	ns	>0.9999

21:Medium PAM vs. 35 :High PAM	0.6600	0.1011 to 1.219	Yes	**	0.0084
21:High PAM vs. 35 :Low PAM	-1.120	-1.659 to -0.5819	Yes	****	<0.0001
21:High PAM vs. 35 :Medium PAM	-0.1611	-0.7280 to 0.4057	No	ns	0.9979
21:High PAM vs. 35 :High PAM	0.5445	0.005964 to 1.083	Yes	*	0.0452
35 :Low PAM vs. 35 :Medium PAM	0.9593	0.3732 to 1.546	Yes	****	<0.0001
35 :Low PAM vs. 35 :High PAM	1.665	1.106 to 2.224	Yes	****	<0.0001
35 :Medium PAM vs. 35 :High PAM	0.7057	0.1195 to 1.292	Yes	**	0.0066

n = 8, 5, 8; 4, 5, 7; 7, 6, 7; 6, 5, 6 male pups per diet per timepoint (biological replicates). 0, postnatal day 0; 10, postnatal day 10; 21, postnatal day 21; 35, day 35; $\delta^{13}C$, $^{13}C/^{12}C$; CI, confidence interval; diff, difference; LS, least squares; PAM, palmitic acid.

Supplemental Table 2. Top Differentially Expressed Genes in Male Liver by Differential Gene Analysis at Day 35

Adjusted P-Value < 0.05; Log2 Fold Change > 1.2				
Gene Name	**Gene Type**	**Log2 Fold Change**	**P-Value**	**Adjusted P-Value**
High PAM Diet vs. Low PAM Diet				
Cyp7a1	Protein coding	3.9724	5.9E-10	0.0000
Hes1	Protein coding	-1.4532	4.7E-06	0.0344
Synm	Protein coding	1.6028	1.2E-05	0.0344
Arrdc3	Protein coding	-1.7612	1.3E-05	0.0344
Jaml	Protein coding	2.5400	1.5E-05	0.0352
Gm8941	Processed pseudogene	1.3736	2E-05	0.0369
Medium PAM Diet vs. Low PAM Diet				
BC049987	lncRNA	1.8426	3.24E-07	0.0025
Mroh6	Protein coding	2.2508	3.3E-06	0.0102
Spr-ps1	Transcribed unprocessed pseudogene	-2.0684	8.62E-06	0.0147
Agap2	Protein coding	1.2878	9.06E-06	0.0147
Mfsd2a	Protein coding	1.2067	1.72E-05	0.0187
Jun	Protein coding	1.3876	2.43E-05	0.0193
P4ha1	Protein coding	1.2183	2.49E-05	0.0193
Esrrb	Protein coding	2.0499	2.5E-05	0.0193
Tat	Protein coding	1.3202	2.85E-05	0.0199
Pla2g4f	Protein coding	3.6640	2.88E-05	0.0199
Gm3953	Processed pseudogene	2.5932	9.95E-05	0.0496
Adjusted P-Value < 0.1; Log2 Fold Change > 1.2				
High PAM Diet vs. Low PAM Diet				
Cyp7a1	Protein coding	3.9724	5.91E-10	9.43E-06
Hes1	Protein coding	-1.4532	4.67E-06	0.0344
Synm	Protein coding	1.6028	1.22E-05	0.0344
Arrdc3	Protein coding	-1.7612	1.29E-05	0.0344
Jaml	Protein coding	2.5400	1.55E-05	0.0352
Gm8941	Processed pseudogene	1.3736	2.03E-05	0.0369
Gm14097	lncRNA	1.9045	4.63E-05	0.0569
Nrg4	Protein coding	1.7313	5.14E-0	0.0586

C730036E19Rik	lncRNA	1.3406	5.88E-05	0.0626
Medium PAM Diet vs. Low PAM Diet				
BC049987	lncRNA	1.8426	3.24E-07	0.0025
Mroh6	Protein coding	2.2508	3.3E-06	0.0102
Spr-ps1	Transcribed unprocessed pseudogene	-2.0684	8.62E-06	0.0147
Agap2	Protein coding	1.2878	9.06E-06	0.0147
Mfsd2a	Protein coding	1.2067	1.72E-05	0.0187
Jun	Protein coding	1.3876	2.43E-05	0.0193
P4ha1	Protein coding	1.2183	2.49E-05	0.0193
Esrrb	Protein coding	2.0499	2.5E-05	0.0193
Tat	Protein coding	1.3202	2.85E-05	0.0199
Pla2g4f	Protein coding	3.6640	2.88E-05	0.0199
Gm3953	Processed pseudogene	2.5932	9.95E-05	0.0496
Synm	Protein coding	1.4124	0.0001	0.0577
Ctcflos	lncRNA	1.2053	0.0002	0.0671
Hspa1a	Protein coding	3.0351	0.0002	0.0730
G730003C15Rik	lncRNA	-1.3837	0.0002	0.0773
Rdh18-ps	Transcribed unprocessed pseudogene	1.5895	0.0002	0.0773
Hsph1	Protein coding	1.7437	0.0004	0.0950
Tmc7	Protein coding	1.5846	0.0004	0.0950
Hhipl1	Protein coding	1.3329	0.0004	0.0952
Treh	Protein coding	1.4922	0.0004	0.0966
Itga10	Protein coding	1.2207	0.0004	0.0970

n = 5 male mice per diet (biological replicates). The Wald test was used to identify differentially expressed genes between samples in R Studio, with an adjusted p-value of either 0.05 or 0.1 and log2 fold change was set to 1.2. PAM, palmitic acid.

Supplemental Table 3. Top Significantly Differentially Expressed Gene Pathways Contrasting High Palmitic Acid and Low Palmitic Acid Diet Groups by Male Pup Liver and Brain Gene Set Enrichment Analysis at Day 35

Direction	Pathways	NES	nGenes	Adjusted P-Value
	Liver			
Up	Regulation of cholesterol metabolic process	2.1171	30	0.0022
Up	Regulation of cholesterol biosynthetic process	2.0883	17	0.0014
Up	Regulation of sterol biosynthetic process	2.0883	17	0.0014
Up	Transforming growth factor beta production	2.0544	29	0.0022
Up	Regulation of the force of heart contraction	2.0341	19	0.0031
Up	Positive regulation of microtubule polymerization or depolymerization	2.0264	32	0.0029
Up	Collagen metabolic process	2.0245	72	0.0012
Up	Extracellular matrix organization	2.0157	194	0.0011
Up	Extracellular structure organization	2.0157	194	0.0011
Up	External encapsulating structure organization	2.0157	194	0.0011
Up	Regulation of collagen metabolic process	1.9977	35	0.0035
Up	Collagen biosynthetic process	1.994	40	0.0051
Up	Regulation of transforming growth factor beta production	1.9798	27	0.0042
Up	Positive regulation of cholesterol metabolic process	1.9744	12	0.0051
Up	Positive regulation of microtubule polymerization	1.972	29	0.0047
Up	Regulation of collagen biosynthetic process	1.966	32	0.0042
Up	Negative regulation of muscle adaptation	1.9528	11	0.0063
Up	Cell-matrix adhesion	1.9417	175	0.0011
Up	Positive regulation of cholesterol biosynthetic process	1.9363	10	0.0069
Up	Positive regulation of sterol biosynthetic process	1.9363	10	0.0069
Up	Negative regulation of cell-matrix adhesion	1.9306	28	0.01
Up	Muscle contraction	1.9255	167	0.0011
Up	Import across plasma membrane	1.9084	98	0.0012
Up	Regulation of cellular response to transforming growth factor beta stimulus	1.9011	94	0.0012
Down	Negative regulation of oligodendrocyte differentiation	-2.0123	11	0.01
Down	Negative regulation of glial cell differentiation	-1.9662	19	0.021
Down	Double-strand break repair via break-induced replication	-1.9416	12	0.024
Down	Acyl-CoA biosynthetic process	-1.9287	29	0.021

Down	Thioester biosynthetic process	-1.9287	29	0.021
Down	DNA strand elongation involved in DNA replication	-1.9087	10	0.029
	Brain			
Up	Axoneme assembly	1.9461	76	0.071
Up	Cilium movement	1.833	147	0.071
Up	Feeding behavior	1.8107	103	0.095
Up	Microtubule bundle formation	1.7818	109	0.095
Up	Circadian rhythm	1.7448	199	0.071
Up	Reproductive process	1.3571	1208	0.071
Up	Reproduction	1.3567	1209	0.071
Down	Glutamate receptor signaling pathway	-1.953	54	0.095
Down	Biological adhesion	-1.3719	1149	0.092
Down	Cell adhesion	-1.3606	1139	0.092

n = 5 mice per diet (biological replicates). False discovery rate was set to 0.2. NES, normalized enrichment score; nGenes, number of genes.

Supplemental Table 4. Top Significantly Differentially Expressed Gene Pathways Contrasting Medium Palmitic Acid and Low Palmitic Acid Diet Groups by Male Liver and Brain Gene Set Enrichment Analysis at Day 35

Direction	Pathways	NES	nGenes	Adjusted P-Value
	Liver			
Up	Mitotic sister chromatid segregation	2.3743	145	0.0015
Up	Sister chromatid segregation	2.3091	170	0.0015
Up	Mitotic cell cycle checkpoint signaling	2.2969	104	0.0015
Up	Regulation of mitotic metaphase/anaphase transition	2.2943	53	0.0015
Up	Metaphase/anaphase transition of mitotic cell cycle	2.2899	54	0.0015
Up	Mitotic sister chromatid separation	2.2602	58	0.0015
Up	Regulation of mitotic sister chromatid separation	2.255	55	0.0015
Up	DNA-dependent DNA replication	2.244	125	0.0015
Up	Mitotic spindle assembly checkpoint signaling	2.2312	34	0.0015
Up	Spindle assembly checkpoint signaling	2.2312	34	0.0015
Up	Mitotic spindle checkpoint signaling	2.2312	34	0.0015
Up	Negative regulation of mitotic cell cycle phase transition	2.2305	124	0.0015
Up	Negative regulation of mitotic metaphase/anaphase transition	2.2217	36	0.0015
Up	Spindle checkpoint signaling	2.2194	35	0.0015
Up	Regulation of metaphase/anaphase transition of cell cycle	2.2149	55	0.0015
Up	Negative regulation of metaphase/anaphase transition of cell cycle	2.2106	37	0.0015
Up	Metaphase/anaphase transition of cell cycle	2.2097	56	0.0015
Up	Chromosome separation	2.2077	83	0.0015
Up	Regulation of sister chromatid segregation	2.2073	61	0.0015
Up	DNA replication	2.1992	224	0.0015
Up	Mitotic nuclear division	2.1981	241	0.0015
Up	Cell cycle checkpoint signaling	2.1823	137	0.0015
Up	Negative regulation of mitotic nuclear division	2.1812	44	0.0015
Up	Nuclear chromosome segregation	2.1773	223	0.0015
Up	Negative regulation of sister chromatid segregation	2.1767	37	0.0015
Up	Negative regulation of mitotic sister chromatid segregation	2.1767	37	0.0015
Up	Negative regulation of mitotic sister chromatid separation	2.1767	37	0.0015
Up	Negative regulation of nuclear division	2.1746	47	0.0015

Up	Negative regulation of chromosome segregation	2.1663	38	0.0015
Up	Negative regulation of chromosome separation	2.1663	38	0.0015
	Brain			
Up	Ammonium ion metabolic process	2.011	24	0.12
Up	Grooming behavior	1.9868	18	0.14
Up	Primary amino compound metabolic process	1.9729	17	0.15
Up	Regulation of bone remodeling	1.9576	45	0.13
Up	Regulation of bone resorption	1.9541	37	0.15
Up	Circadian rhythm	1.9419	199	0.054
Up	Serotonin metabolic process	1.9297	15	0.18
Up	Hyperosmotic response	1.9275	28	0.18
Up	Chaperone-mediated protein folding	1.9269	56	0.12
Up	Positive regulation of bone resorption	1.9261	19	0.18
Up	Chaperone cofactor-dependent protein refolding	1.919	31	0.18
Up	Epithelial cell maturation	1.9157	16	0.18
Up	Copulation	1.9132	20	0.19
Up	de novo posttranslational protein folding	1.9118	35	0.18
Up	de novo protein folding	1.9115	36	0.18
Up	Cellular response to interferon-alpha	1.8954	13	0.18
Up	Negative regulation of protein tyrosine kinase activity	1.8918	28	0.2
Up	Regulation of tissue remodeling	1.8787	59	0.14
Up	Bone remodeling	1.8591	84	0.11
Up	Maintenance of organelle location	1.859	11	0.18
Up	Neuron maturation	1.8453	52	0.18
Up	High-density lipoprotein particle remodeling	1.831	9	0.18
Up	Bone resorption	1.7983	59	0.2
Up	Embryonic skeletal system development	1.7869	116	0.11
Up	Regionalization	1.7851	291	0.054
Up	Cilium movement	1.7694	148	0.078
Up	Pattern specification process	1.7651	376	0.054
Down	Gas transport	-1.9007	13	0.078
Down	Arachidonic acid metabolic process	-1.8108	27	0.19
Down	Ectopic germ cell programmed cell death	-1.8021	7	0.078

n = 5 mice per diet (biological replicates). False discovery rate was set to 0.2. NES, normalized enrichment score; nGenes, number of genes.

Supplemental Table 5. Top 10 Enriched Pathways Among Genes in the Blue and Green Module by Liver Weighted Gene Co-Expression Network Analysis in Male Pups at Day 35

Number of genes	Pathway Name	Accession	Adjusted P-value
	Blue Module		
50	Lipid metabolic process	GO:0006629	4.9e-21
19	Unsaturated fatty acid metabolic process	GO:0033559	2.8e-17
17	Icosanoid metabolic process	GO:0006690	1.1e-14
14	Arachidonic acid metabolic process	GO:0019369	1.1e-14
16	Long-chain fatty acid metabolic process	GO:0001676	1.7e-14
45	Small molecule metabolic process	GO:0044281	1.8e-13
24	Fatty acid metabolic process	GO:0006631	1.8e-13
27	Monocarboxylic acid metabolic process	GO:0032787	1.3e-12
16	Olefinic compound metabolic process	GO:0120254	1.7e-12
13	Response to xenobiotic stimulus	GO:0009410	1.9e-10
	Green Module		
3	Positive regulation of cell proliferation involved in kidney development	GO:1901724	9.9e-4
5	Skeletal muscle cell differentiation	GO:0035914	9.9e-4
7	Fat cell differentiation	GO:0045444	1.3e-3
6	Skeletal muscle tissue development	GO:0007519	1.6e-3
4	Circadian regulation of gene expression	GO:0032922	2.7e-3
3	Cell proliferation involved in kidney development	GO:0072111	2.7e-3
5	Regulation of fat cell differentiation	GO:0045598	3.7e-3
2	Positive regulation of skeletal muscle cell differentiation	GO:2001016	5.6e-3
23	Positive regulation of metabolic process	GO:0009893	5.6e-3
15	Homeostatic process	GO:0042592	5.9e-3

n = 5 mice per diet. Soft threshold was set to 6 and a minimum module size of 20 was used. GO, gene ontology.

Supplemental Table 6. Pup Litter Size and Representation

Birth date of litter:	Dam Identifier:	Cage Identifier:	Diet:	Total Litter Size (N):	Male (n):	Female (n):	Infanticide	P0 Male	P10 Male	P21 Male	P35 Male	P0 Female	P10 Female	P21 Female	P35 Female
08/12/2019	901	1	LP	6	3	3					X○				
08/05/2019	903▽	2	LP	6	3	3		X	X	X					
08/01/2019	904▽	3	LP	9	6	3		X		X	X○	X	X	X	
07/30/2019	905▽	4	LP	8	5	3		X	X	X	X○	X	X	X	
08/01/2019	906▽	5	LP	5	3	2		X		X					X
08/01/2019	907▽	6	LP	6	3	3		X	X	X					X
07/31/2019	908▽	7	LP	4	2	2		X			X○		X	X	
08/01/2019	910▽	8	LP	7	4	3		X	X	X	X				
08/03/2019	913▽	10	LP	6	2	3		X							
08/09/2019	914	11	LP	9	7	2				X	X○				
08/03/2019	916▽	12	MP	7	5	2		X	X	X	X○				X
07/31/2019	918▽	13	MP	6	3	2		X		X		X	X		
07/31/2019	919▽	14	MP	7	4	3		X	X		X○		X	X	
08/01/2019	921▽	15	MP	7	4	3		X	X	X	X○			X	
07/31/2019	922	16	MP	3	2	1	X								
08/03/2019	924▽	18	MP	3	3			X		X	X○				
08/01/2019	925▽	19	MP	4	3	1			X	X				X	
08/11/2019	926	20	MP	8	5	3			X		X○				
07/30/2019	927▽	21	MP	6	3	3				X		X			
08/04/2019	928▽	22	HP	6	5	1		X	X	X	X○				
08/02/2019	931▽	24	HP	2	2			X			X				
08/05/2019	932▽	25	HP	5	2	3		X	X				X		
08/04/2019	933▽	26	HP	7	5	2		X	X	X	X○		X		
08/09/2019	934	27	HP	7	4	3				X					
07/31/2019	935▽	28	HP	8	6	2		X	X	X	X○	X			
08/11/2019	936	29	HP	9	6	3	X								

07/30/2019	937▽	30	HP	9	5	4		X	X	X	X○	X			X
08/01/2019	938▽	31	HP	7	4	3		X	X	X	X○	X		X	
08/05/2019	939▽	32	HP	5	3	2		X	X	X					X

HP, high palmitic acid; LP, low palmitic acid; MP, medium palmitic acid; P0, postnatal day 0; P10, postnatal day 10; P21, postnatal day 21; P35, postnatal day 35.

[X]Primary outcome data (brain and liver $\delta^{13}C$ fatty acid values, brain, and liver fatty acid concentration/relative percentage)

◦RNAseq

▽Litter underwent behavioural testing