

Supplemental material

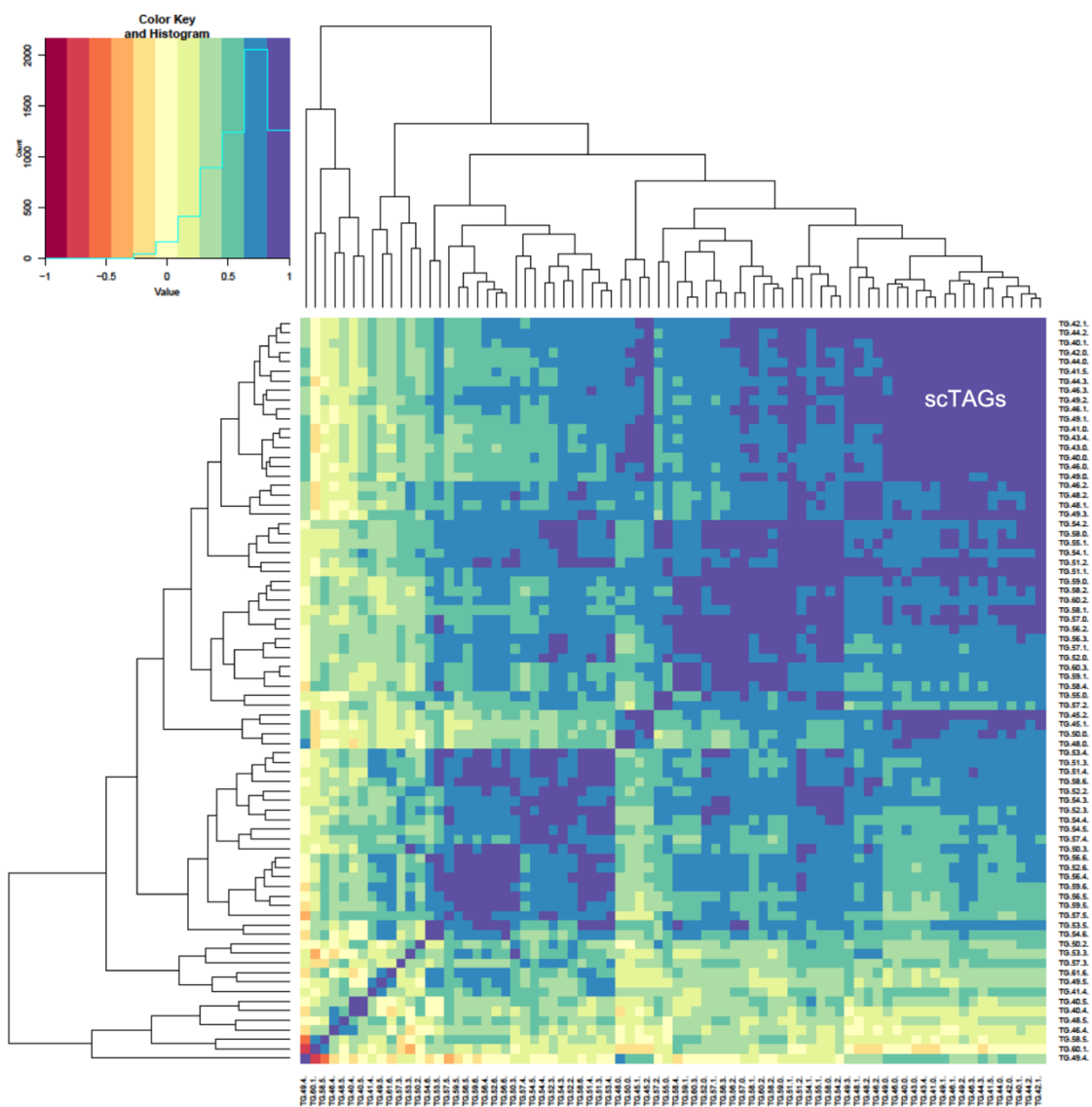


Figure 1. scTAGs form a distinct cluster. Hierarchical Cluster Analysis with Spearman correlation among the TAG profiles measured by LC-MS of all individuals after a HP meal; $n = 9$.

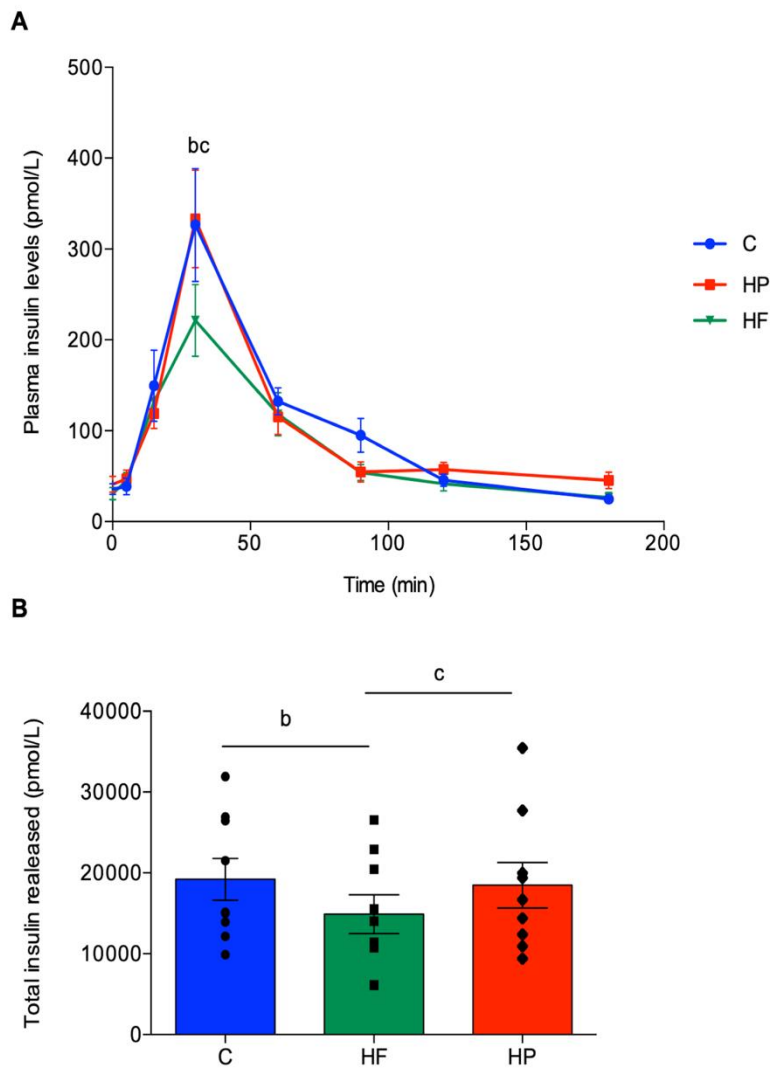


Figure 2. Plasma insulin levels. (A) Plasma insulin released after consuming a C (blue), HP (red) and a HF (green) meal. **(B)** Total plasma insulin released after consuming a C (blue), HP (red) and a HF (green) meal over 3 hours. Data are presented as mean \pm SEM and analysed by two-way ANOVA with post-hoc Sidak's multiple comparisons test; a,b,c = $p \leq 0.05$, $n = 9/\text{group}$. a = comparisons between C and HP; b = comparisons between C and HF; C = comparisons between HP and HF **(A)**; or by one-way ANOVA with post-hoc Dunnett's multiple comparisons test; * = $p \leq 0.05$, $n = 9/\text{group}$ **(B)**.

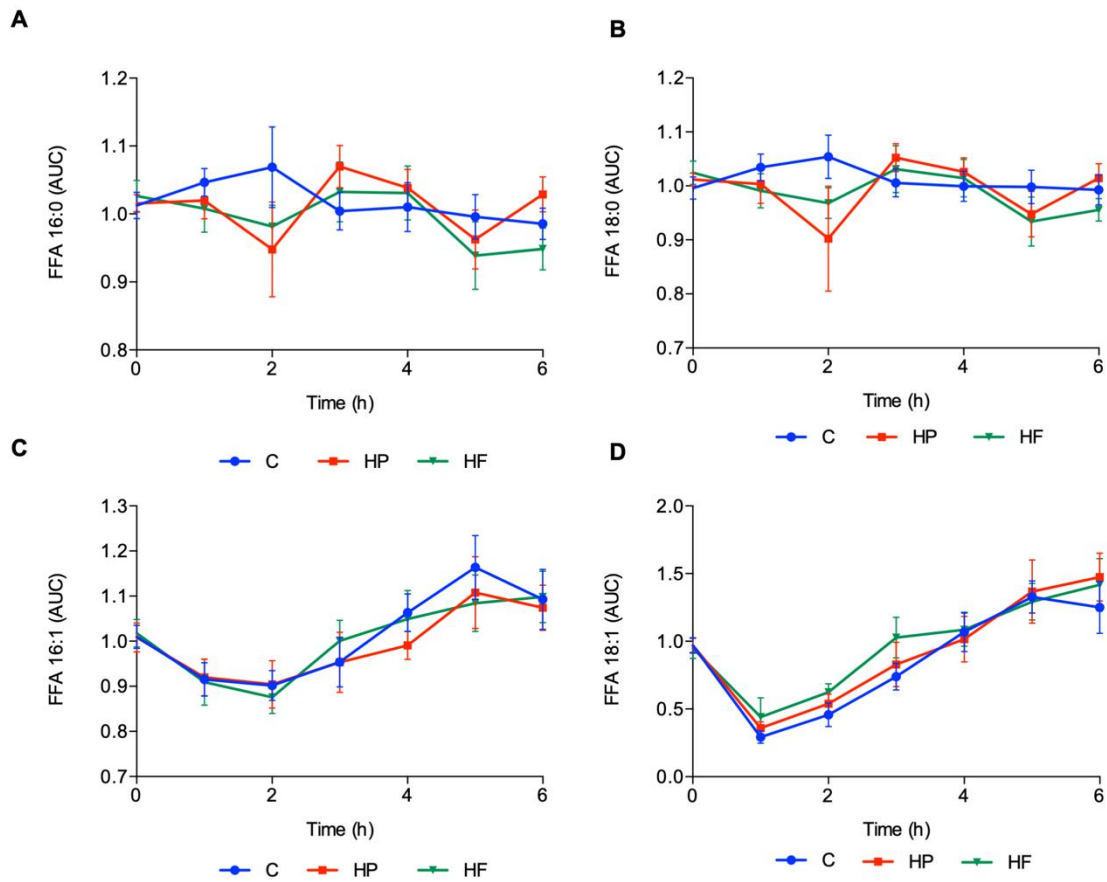


Figure 3. Plasma FFA content is not different between the groups. (A) FFA 16:0 levels measured by LC-MS over 6 hours. **(B)** FFA 18:0 levels measured by LC-MS over 6 hours. **(C)** FFA 16:1 levels measured by LC-MS over 6 hours. **(D)** FFA 18:1 levels measured by LC-MS over 6 hours. Data are presented as mean \pm SEM and analysed by two-way repeated measures ANOVA with post-hoc Sidak's multiple comparisons test; * = $p \leq 0.05$, $n = 9/\text{group}$.

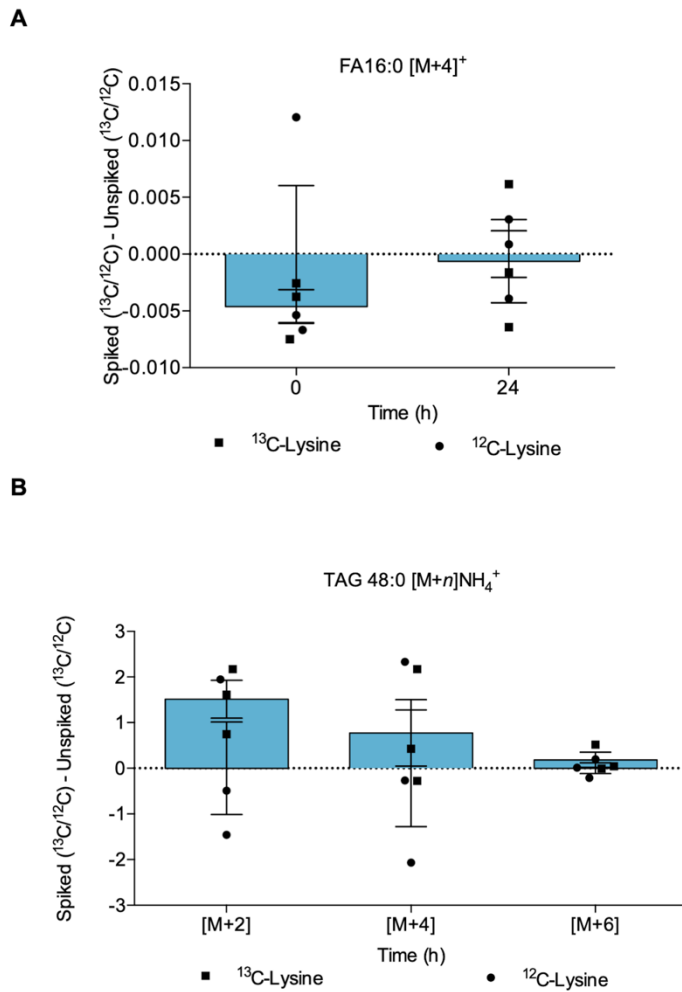


Figure 4. Carbon from ¹³C₆-labelled lysine is not incorporated into triacylglycerols or DNL-derived palmitate in AML 12 hepatocytes. (A) The [M+4]⁺ ion of the picolynyl-ester of palmitate (FA16:0) detected by LC-MS was not labelled by lysine over 24 h post-supplementation. **(B)** The [M+2]NH₄⁺, [M+4]NH₄⁺ and [M+6]NH₄⁺ ions of glyceryl tripalmitate (TAG 48:0) in cells detected by LC-MS were not labelled by lysine after 24 h. Data are presented as mean ± SEM and analysed by two-way ANOVA with post-hoc Sidak's multiple comparisons test; * = *p* ≤ 0.05, *n* = 3/group.

Supplementary Table 1. Fatty acid composition of meals. The fatty acid compositions were calculated using the FoodWorks database (59), which performed a nutrient analysis of the meals.

Fatty acid (grams)	Control C	High Fat HF	High Protein HP
FA 4:0	0.3419	0.6838	0.3945
FA 6:0	0.20241	0.40482	0.23355
FA 8:0	0.1200527	0.237832727	0.137036364
FA 10:0	0.2666927	0.531112727	0.306236364
FA 12:0	0.2982827	0.594292727	0.342686364
FA 13:0	0	0	0
FA 14:0	1.0945009	2.154910909	1.240595455
FA 15:0	0.0045455	0.004545455	0.002272727
FA 16:0	4.994	7.763	4.3075
FA 17:0	0.0159091	0.015909091	0.007954545
FA 18:0	2.0618609	3.339630909	1.866395455
FA 20:0	0.0090909	0.009090909	0.004545455
FA 22:0	0.0113636	0.011363636	0.005681818
FA 24:0	0.0022727	0.002272727	0.001136364
FA 14:1	0.0090909	0.009090909	0.004545455
FA 15:1	0	0	0
FA 16:1	0.5338073	0.769887273	0.421263636
FA 17:1	0	0	0
FA 18:1	6.1247273	8.776727273	4.796363636
FA 20:1	0.0272727	0.027272727	0.013636364
FA 22:1	0.0022727	0.002272727	0.001136364
FA 18:2	1.3858873	1.624047273	0.848663636
FA 20:2	0	0	0
FA 18:3	0.1874909	0.340890909	0.194045455
FA 18:4	0	0	0
FA 20:4	0.1409091	0.140909091	0.070454545
FA 20:5	0.0045455	0.004545455	0.002272727
FA 22:5	0	0	0
FA 22:6	0.0363636	0.036363636	0.018181818

Supplementary Table 2. Amino acid composition of the meals. The amino acid compositions were calculated using the FoodWorks database (59), which performed a nutrient analysis of the meals.

Amino acid (grams)	Control C	High Fat HF	High Protein HP
Alanine	0.8576	0.8857	1.7382
Arginine	0.9029	0.9299	2.3174
Aspartic acid	1.5410	1.5921	3.8066
Cystine	0.3629	0.3748	0.6504
Glutamic acid	2.0204	2.0995	5.6847
Glycine	0.5189	0.5360	1.3386
Histidine	0.3599	0.3723	0.8495
Isoleucine	0.8429	0.8733	1.8245
Leucine	1.3075	1.3539	2.9243
Lysine	1.0924	1.1297	2.3081
Methionine	0.4874	0.5046	0.7902
Phenylalanine	0.8289	0.8588	1.8981
Proline	0.6148	0.6418	1.5943
Serine	1.1246	1.1597	2.1439
Threonine	0.7294	0.7535	1.4775
Tryptophan	0.1877	0.1945	0.4392
Tyrosine	0.6208	0.6425	1.3628
Valine	0.9429	0.9771	1.9829

Supplementary Table 3. scTAG masses and their corresponding inter-batch and intra-batch CVs. These TAGs are associated with the main fatty acid products from DNL including palmitate, palmitoleic acid, stearic acid and oleic acid. These TAGs have been shown to increase when DNL is raised in humans (9, 19 & 20). Samples across this study were run in two batches of roughly equal size. The technical replicates were pool samples from batch 1, the inter-batch CV was calculated as STDEV (all pools for batch X)/Mean of all pools across batch X *100%. The inter-batch CV is calculated across the two batches.

Triglyceride	Product ion ([M+NH₄]¹⁺)	Intra-batch RSD/CV (%) Batch 1; Batch2	Inter-batch RSD/CV (%)
TG 46:2	792.7076	9.65; 6.31	23.6
TG 46:1	794.7232	13.1; 11.4	19.2
TG 46:0	796.7389	12.7; 14.5	15.5
TG 48:2	820.7389	12.0; 7.80	26.5
TG 48:1	822.7545	9.68; 9.55	15.9
TG 48:0	824.7702	34.3; 12.0	33.8
TG 49:2	834.7545	9.38; 9.25	22.1
TG 49:1	836.7702	12.7; 12.6	31.3
TG 49:0	838.7858	5.48; 12.3	34.0