

Supplementary Information

Hepatic oleate regulates adipose tissue lipogenesis and fatty acid oxidation

Maggie S. Burhans¹, Matthew T. Flowers², Kristin R. Harrington², Laura M. Bond², Chang-An Guo², Rozalyn M. Anderson^{3,4}, and James M. Ntambi^{1,2,*}

¹Department of Nutritional Sciences University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706 USA

²Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706 USA

³Department of Medicine, University of Wisconsin-Madison, Madison, WI 53705 USA

⁴Geriatric Research, Education, and Clinical Center, VA Medical Center, Madison, Wisconsin 53705 USA

*Correspondence: James M. Ntambi, jmntambi@wisc.edu

A GKO



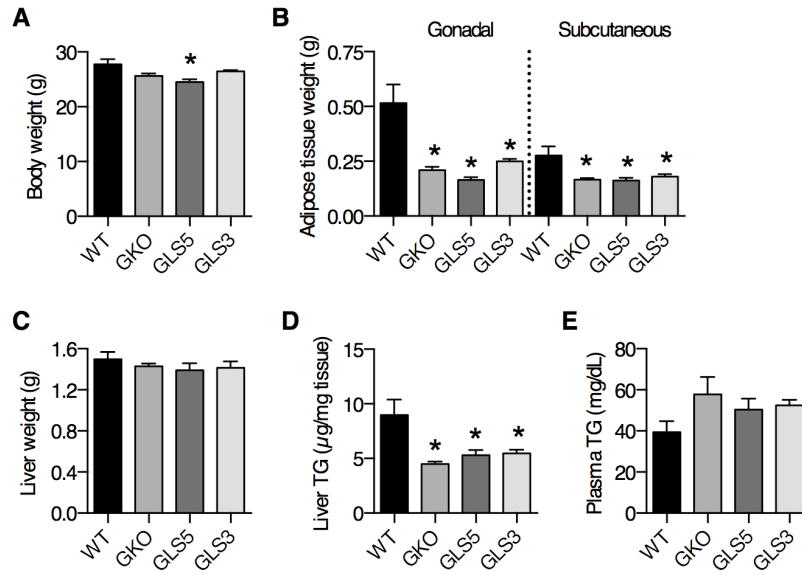
B GLS5



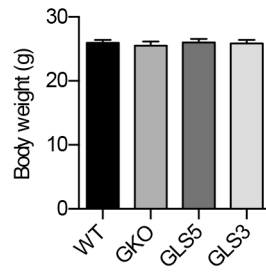
C GLS3



Supplemental Figure 1. GKO, GLS5 and GLS3 mice exhibit dry skin and alopecia.
(A) GKO, (B) GLS5 and (C) GLS3 adult males were fed a lipogenic diet diet for 10 days.



Supplemental Figure 2. Adiposity and liver TG do not differ among GKO, GLS5 and GLS3 chow-fed male mice. Chow-fed 10–12 week old male mice were 4 hour fasted (n=5–6/group). (A) Body weight, (B) gonadal and subcutaneous white adipose tissue weights and (C) liver tissue weight were determined. (D) Liver TG and (E) plasma TG were measured by enzymatic colorimetric assays. Values are mean \pm SEM, * $P < 0.05$ vs. WT, one-way ANOVA analysis with Tukey's post-hoc test.



Supplemental Figure 3. Body weight of male mice at start of lipogenic diet (LD) feeding. Body weight of Male mice (n=9–11/group) was determined at the start of the LD feeding study. Values are mean \pm SEM, one-way ANOVA analysis with Tukey's post-hoc test.

Table S1. Liver cholesteryl ester fatty acid composition in 10 d LD-fed mice

Fatty acid		WT	GKO	GLS5	GLS3
16:0	$\mu\text{g}/\text{mg}$	0.189 ± 0.029	0.258 ± 0.020	0.175 ± 0.023	0.217 ± 0.018
	% of total	20.7 ± 1.9	$38.1 \pm 1.3^*$	$30.4 \pm 1.7^{*\#}$	$34.2 \pm 2.1^*$
16:1n-7	$\mu\text{g}/\text{mg}$	0.115 ± 0.019	$0.005 \pm 0.001^*$	$0.007 \pm 0.001^*$	$0.025 \pm 0.004^{*\#\wedge}$
	% of total	12.5 ± 1.3	$0.7 \pm 0.1^*$	$1.3 \pm 0.1^{*\#}$	$4.0 \pm 0.9^{*\#\wedge}$
18:0	$\mu\text{g}/\text{mg}$	0.148 ± 0.030	$0.274 \pm 0.034^*$	0.208 ± 0.014	0.243 ± 0.024
	% of total	16.3 ± 2.6	$39.9 \pm 2.7^*$	$37.1 \pm 2.2^*$	$38.1 \pm 2.0^*$
18:1n-9	$\mu\text{g}/\text{mg}$	0.318 ± 0.070	$0.054 \pm 0.007^*$	$0.084 \pm 0.015^*$	$0.046 \pm 0.005^*$
	% of total	34.3 ± 4.0	$8.3 \pm 1.4^*$	$14.4 \pm 2.0^{*\#}$	$7.4 \pm 1.0^{*\wedge}$
18:1n-7	$\mu\text{g}/\text{mg}$	0.026 ± 0.012	ND	ND	0.008 ± 0.002
	% of total	2.6 ± 0.9			1.2 ± 0.4
18:2n-6	$\mu\text{g}/\text{mg}$	0.032 ± 0.004	0.030 ± 0.005	0.039 ± 0.003	0.041 ± 0.005
	% of total	3.6 ± 0.5	4.7 ± 1.2	7.0 ± 0.6	6.6 ± 1.0
20:4n-6	$\mu\text{g}/\text{mg}$	0.009 ± 0.001	0.009 ± 0.002	0.009 ± 0.001	0.011 ± 0.001
	% of total	1.0 ± 0.2	1.4 ± 0.4	1.7 ± 0.3	1.8 ± 0.3
20:5n-3	$\mu\text{g}/\text{mg}$	ND	ND	0.004 ± 0.001	$0.002 \pm 0.000^\wedge$
	% of total			0.6 ± 0.2	$0.3 \pm 0.0^\wedge$
22:6n-3	$\mu\text{g}/\text{mg}$	0.008 ± 0.000	0.004 ± 0.001	0.004 ± 0.001	0.007 ± 0.002
	% of total	0.8 ± 0.2	0.6 ± 0.1	0.8 ± 0.1	1.2 ± 0.3
18:1n-9/18:0	DI	2.37 ± 0.84	$0.22 \pm 0.06^*$	$0.41 \pm 0.10^*$	$0.20 \pm 0.04^*$
16:1n-7/16:0	DI	0.62 ± 0.12	$0.02 \pm 0.0^*$	$0.04 \pm 0.01^{*\#}$	$0.12 \pm 0.04^{*\#\wedge}$

10 week old male mice were fed a lipogenic diet (LD) for 10 days and fasted 4 hours prior to tissue collection. Hepatic lipids were extracted, lipid species were separated by TLC, extracted, transmethylated and analyzed by gas liquid chromatography. DI, desaturation index. Values are mean \pm SEM, (n=4–6/group). * $P < 0.5$ vs. WT, # $P < 0.05$ vs. GKO; $^\wedge P < 0.05$ vs. GLS5; one-way ANOVA analysis with Tukey's post-hoc test.

Table S2. Liver triacylglycerol fatty acid composition in 10 d LD-fed mice

Fatty acid		WT	GKO	GLS5	GLS3
16:0	$\mu\text{g}/\text{mg}$	4.74 \pm 0.92	0.95 \pm 0.13	8.43 \pm 1.62 [#]	3.00 \pm 0.90 [^]
	% of total	26.8 \pm 1.59	41.7 \pm 0.92 [*]	39.6 \pm 1.47 [*]	43.0 \pm 1.27 [*]
16:1n-7	$\mu\text{g}/\text{mg}$	1.50 \pm 0.35	0.01 \pm 0.00 [*]	0.08 \pm 0.02 ^{*#}	0.10 \pm 0.03 ^{*#}
	% of total	8.54 \pm 1.13	0.25 \pm 0.05 [*]	0.36 \pm 0.02 [*]	1.74 \pm 0.30 ^{*#^}
18:0	$\mu\text{g}/\text{mg}$	0.27 \pm 0.05	0.49 \pm 0.06	5.63 \pm 1.44 ^{*#}	1.71 \pm 0.43 [^]
	% of total	1.54 \pm 0.17	21.9 \pm 1.13 [*]	26.7 \pm 3.49 [*]	25.8 \pm 1.35 [*]
18:1n-9	$\mu\text{g}/\text{mg}$	7.77 \pm 0.87	0.29 \pm 0.05 [*]	3.80 \pm 1.02 ^{*#}	0.76 \pm 0.21 ^{*^}
	% of total	45.5 \pm 2.47	12.7 \pm 0.49 [*]	17.7 \pm 2.66 [*]	11.4 \pm 0.45 ^{*^}
18:1n-7	$\mu\text{g}/\text{mg}$	1.35 \pm 0.10	0.02 \pm 0.00 [*]	0.11 \pm 0.03 ^{*#}	0.12 \pm 0.03 ^{*#}
	% of total	8.02 \pm 0.66	0.75 \pm 0.04 [*]	0.53 \pm 0.07 [*]	2.09 \pm 0.34 ^{*#^}
18:2n-6	$\mu\text{g}/\text{mg}$	0.97 \pm 0.17	0.34 \pm 0.06	2.19 \pm 0.57 [#]	0.74 \pm 0.21 [^]
	% of total	5.63 \pm 0.68	14.9 \pm 0.96 [*]	9.87 \pm 1.27 [#]	10.9 \pm 0.87 ^{*#}
18:3n-3	$\mu\text{g}/\text{mg}$	0.02 \pm 0.01	0.00 \pm 0.00 [*]	0.02 \pm 0.00 [#]	0.01 \pm 0.00
	% of total	0.10 \pm 0.02	0.07 \pm 0.02	0.10 \pm 0.01	0.09 \pm 0.02
20:1n-9	$\mu\text{g}/\text{mg}$	0.17 \pm 0.02	0.01 \pm 0.00 [*]	0.11 \pm 0.4 [#]	0.01 \pm 0.00 ^{*^}
	% of total	1.05 \pm 0.15	0.25 \pm 0.02 [*]	0.50 \pm 0.13 [*]	0.20 \pm 0.03 ^{*^}
20:4n-6	$\mu\text{g}/\text{mg}$	0.04 \pm 0.01	0.05 \pm 0.01	0.12 \pm 0.05	0.05 \pm 0.01
	% of total	0.24 \pm 0.03	2.16 \pm 0.32 [*]	0.57 \pm 0.14 [#]	0.94 \pm 0.17 [#]
22:5n-3	$\mu\text{g}/\text{mg}$	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00 ^{*#}	0.01 \pm 0.00 [^]
	% of total	0.04 \pm 0.01	0.24 \pm 0.02 [*]	0.07 \pm 0.01 [#]	0.11 \pm 0.03 [#]
22:6n-3	$\mu\text{g}/\text{mg}$	0.05 \pm 0.01	0.05 \pm 0.01	0.18 \pm 0.05 ^{*#}	0.06 \pm 0.02
	% of total	0.29 \pm 0.06	2.31 \pm 0.30 [*]	0.83 \pm 0.15 [#]	1.03 \pm 0.16 [#]
18:1n-9/18:0	DI	30.1 \pm 2.13	0.59 \pm 0.06 [*]	0.79 \pm 0.25 [*]	0.45 \pm 0.04 [*]
16:1n-7/16:0	DI	0.32 \pm 0.03	0.01 \pm 0.00 [*]	0.01 \pm 0.00 [*]	0.04 \pm 0.01 ^{*#^}

10 week old male mice were fed a lipogenic diet (LD) for 10 days and fasted 4 hours prior to tissue collection. Hepatic lipids were extracted, lipid species were separated by TLC, extracted, transmethylated and analyzed by gas liquid chromatography. DI, desaturation index. Values are mean \pm SEM, (n=4–6/group). * P < 0.5 vs. WT, # P < 0.05 vs. GKO; ^ P < 0.05 vs. GLS5; one-way ANOVA analysis with Tukey's post-hoc test.

Table S3. Liver free fatty acid composition in 10 d LD-fed mice

Fatty acid		WT	GKO	GLS5	GLS3
16:0	$\mu\text{g}/\text{mg}$	0.323 ± 0.025	0.268 ± 0.021	$0.412 \pm 0.035^{\#}$	$0.161 \pm 0.010^{*\#\wedge}$
	% of total	52.3 ± 1.43	58.2 ± 1.38	58.5 ± 2.90	$50.0 \pm 0.94^{\#\wedge}$
16:1n-7	$\mu\text{g}/\text{mg}$	0.016 ± 0.003	$0.003 \pm 0.000^*$	$0.005 \pm 0.001^*$	$0.004 \pm 0.000^*$
	% of total	2.50 ± 0.30	$0.71 \pm 0.08^*$	$0.73 \pm 0.08^*$	$1.32 \pm 0.12^{*\#\wedge}$
18:0	$\mu\text{g}/\text{mg}$	0.189 ± 0.011	0.140 ± 0.009	$0.224 \pm 0.026^{\#}$	$0.118 \pm 0.004^{*\wedge}$
	% of total	30.9 ± 3.07	30.5 ± 0.95	31.5 ± 1.61	$37.0 \pm 1.21^{\#}$
18:1n-9	$\mu\text{g}/\text{mg}$	0.046 ± 0.012	$0.014 \pm 0.001^*$	0.030 ± 0.008	$0.012 \pm 0.001^{*\wedge}$
	% of total	7.29 ± 1.42	$3.02 \pm 0.12^*$	$3.94 \pm 0.71^*$	$3.75 \pm 0.24^*$
18:2n-6	$\mu\text{g}/\text{mg}$	ND	0.006 ± 0.001	0.010 ± 0.006	0.003 ± 0.001
	% of total		1.26 ± 0.26	1.21 ± 0.61	0.88 ± 0.26
18:3n-3	$\mu\text{g}/\text{mg}$	0.001 ± 0.001	0.000 ± 0.000	0.001 ± 0.000	0.002 ± 0.001
	% of total	0.13 ± 0.09	0.08 ± 0.01	0.21 ± 0.07	0.54 ± 0.18
20:4n-6	$\mu\text{g}/\text{mg}$	0.008 ± 0.001	$0.004 \pm 0.000^*$	$0.005 \pm 0.000^*$	$0.005 \pm 0.001^*$
	% of total	1.25 ± 0.09	0.87 ± 0.06	0.71 ± 0.10	$1.58 \pm 0.25^{\wedge}$
22:6n-3	$\mu\text{g}/\text{mg}$	ND	0.005 ± 0.001	0.003 ± 0.001	0.003 ± 0.001
	% of total		1.11 ± 0.25	0.42 ± 0.11	0.97 ± 0.21
18:1n-9/18:0	DI	0.251 ± 0.076	$0.099 \pm 0.005^*$	$0.124 \pm 0.018^*$	$0.102 \pm 0.009^*$
16:1n-7/16:0	DI	0.048 ± 0.004	$0.012 \pm 0.002^*$	$0.013 \pm 0.002^*$	$0.026 \pm 0.002^{*\#\wedge}$

10 week old male mice were fed a lipogenic diet (LD) for 10 days and fasted 4 hours prior to tissue collection. Hepatic lipids were extracted, lipid species were separated by TLC, extracted, transmethylated and analyzed by gas liquid chromatography. DI, desaturation index. Values are mean \pm SEM, (n=4–6/group). * $P < 0.5$ vs. WT, $^{\#}P < 0.05$ vs. GKO; $^{\wedge}P < 0.05$ vs. GLS5; one-way ANOVA analysis with Tukey's post-hoc test.

Table S4. Liver phospholipid fatty acid composition in 10 d LD-fed mice

Fatty acid		WT	GKO	GLS5	GLS3
16:0	$\mu\text{g}/\text{mg}$	5.65 ± 0.11	6.69 ± 0.30	6.67 ± 0.55	6.38 ± 0.21
	% of total	22.3 ± 0.68	25.1 ± 0.21	23.5 ± 1.43	24.4 ± 0.59
16:1n-7	$\mu\text{g}/\text{mg}$	0.70 ± 0.01	$0.04 \pm 0.00^*$	$0.05 \pm 0.00^*$	$0.30 \pm 0.06^{*\#^{\wedge}}$
	% of total	2.77 ± 0.01	$0.16 \pm 0.01^*$	$0.17 \pm 0.01^*$	$1.17 \pm 0.24^{*\#^{\wedge}}$
18:0	$\mu\text{g}/\text{mg}$	3.57 ± 0.10	$5.19 \pm 0.22^*$	$5.86 \pm 0.22^*$	$5.29 \pm 0.27^*$
	% of total	14.1 ± 0.08	$19.5 \pm 0.21^*$	$20.8 \pm 0.87^*$	$20.2 \pm 0.59^*$
18:1n-9	$\mu\text{g}/\text{mg}$	3.16 ± 0.13	$1.36 \pm 0.04^*$	$1.99 \pm 0.18^{\#}$	$1.12 \pm 0.11^{\wedge}$
	% of total	12.5 ± 0.29	$5.12 \pm 0.13^*$	$7.02 \pm 0.58^{\#}$	$4.31 \pm 0.40^{\wedge}$
18:1n-7	$\mu\text{g}/\text{mg}$	1.11 ± 0.13	$0.16 \pm 0.00^*$	$0.15 \pm 0.01^*$	$0.41 \pm 0.05^{*\#^{\wedge}}$
	% of total	4.36 ± 0.44	$0.60 \pm 0.02^*$	$0.55 \pm 0.07^*$	$1.57 \pm 0.21^{*\#^{\wedge}}$
18:2n-6	$\mu\text{g}/\text{mg}$	2.81 ± 0.11	$3.80 \pm 0.16^*$	$3.99 \pm 0.32^*$	3.67 ± 0.20
	% of total	11.1 ± 0.28	$14.3 \pm 0.27^*$	$14.0 \pm 0.74^*$	$14.0 \pm 0.44^*$
20:4n-6	$\mu\text{g}/\text{mg}$	3.76 ± 0.15	$2.95 \pm 0.17^*$	$3.38 \pm 0.15^*$	$2.90 \pm 0.28^*$
	% of total	14.8 ± 0.32	11.1 ± 0.42	11.9 ± 0.34	11.1 ± 0.82
20:5n-3	$\mu\text{g}/\text{mg}$	0.12 ± 0.01	$0.07 \pm 0.00^*$	$0.11 \pm 0.01^{\#}$	0.09 ± 0.00
	% of total	0.49 ± 0.04	$0.27 \pm 0.01^*$	0.39 ± 0.05	$0.33 \pm 0.01^*$
22:6n-3	$\mu\text{g}/\text{mg}$	3.45 ± 0.10	$5.35 \pm 0.25^*$	$5.05 \pm 0.32^*$	$4.95 \pm 0.14^*$
	% of total	13.6 ± 0.57	$20.1 \pm 0.62^*$	$18.0 \pm 1.60^*$	$19.0 \pm 0.02^*$
18:1n-9/18:0	DI	0.88 ± 0.02	$0.26 \pm 0.01^*$	$0.34 \pm 0.04^*$	$0.22 \pm 0.02^{\wedge}$
16:1n-7/16:0	DI	0.12 ± 0.00	$0.01 \pm 0.00^*$	$0.01 \pm 0.00^*$	$0.05 \pm 0.01^{*\#^{\wedge}}$

10 week old male mice were fed a lipogenic diet (LD) for 10 days and fasted 4 hours prior to tissue collection. Hepatic lipids were extracted, lipid species were separated by TLC, extracted, transmethylated and analyzed by gas liquid chromatography. DI, desaturation index. Values are mean \pm SEM, (n=4–6/group). * $P < 0.5$ vs. WT, # $P < 0.05$ vs. GKO; $\wedge P < 0.05$ vs. GLS5; one-way ANOVA analysis with Tukey's post-hoc test.

Table S5. Plasma cholesteryl ester fatty acid composition in 10 d LD-fed mice

Fatty acid		WT	GKO	GLS5	GLS3
16:0	$\mu\text{g}/100 \mu\text{l}$	5.61 ± 0.26	20.6 ± 1.93	17.0 ± 6.99	14.2 ± 2.96
	% of total	6.84 ± 0.15	$29.9 \pm 2.06^*$	$18.4 \pm 3.72^{*\#}$	$19.0 \pm 2.07^{*\#}$
16:1n-7	$\mu\text{g}/100 \mu\text{l}$	16.3 ± 0.88	$1.01 \pm 0.12^*$	$1.68 \pm 0.36^*$	10.9 ± 4.15
	% of total	19.8 ± 1.03	$1.47 \pm 0.16^*$	$1.94 \pm 0.26^*$	$11.4 \pm 2.54^{*\#\wedge}$
18:0	$\mu\text{g}/100 \mu\text{l}$	1.23 ± 0.21	$18.2 \pm 2.20^*$	12.0 ± 4.94	13.1 ± 3.15
	% of total	1.51 ± 0.27	$26.2 \pm 1.75^*$	$12.4 \pm 2.49^{*\#}$	$16.4 \pm 2.03^{*\#}$
18:1n-9	$\mu\text{g}/100 \mu\text{l}$	21.9 ± 2.26	11.1 ± 1.70	17.0 ± 4.18	$7.70 \pm 1.57^*$
	% of total	26.4 ± 1.23	$15.7 \pm 0.60^*$	$19.4 \pm 0.86^{*\#}$	$10.3 \pm 0.89^{*\#\wedge}$
18:1n-7	$\mu\text{g}/100 \mu\text{l}$	2.21 ± 0.48	0.94 ± 0.16	0.74 ± 0.16	2.49 ± 0.87
	% of total	2.61 ± 0.40	1.44 ± 0.34	$0.85 \pm 0.05^*$	$2.67 \pm 0.50^\wedge$
18:2n-6	$\mu\text{g}/100 \mu\text{l}$	32.0 ± 1.49	15.9 ± 4.36	34.3 ± 8.22	26.1 ± 6.30
	% of total	38.9 ± 0.85	21.5 ± 3.20	$40.4 \pm 7.05^\#$	33.9 ± 3.76
18:1n-9/18:0	DI	19.1 ± 3.05	$0.61 \pm 0.05^*$	$1.70 \pm 0.18^{*\#}$	$0.65 \pm 0.06^{*\wedge}$
16:1n-7/16:0	DI	2.90 ± 0.15	$0.05 \pm 0.01^*$	$0.12 \pm 0.03^*$	$0.68 \pm 0.19^{*\#\wedge}$

10 week old male mice were fed a lipogenic diet (LD) for 10 days and fasted 4 hours prior to tissue collection. Plasma lipids were extracted, lipid species were separated by TLC, extracted, transmethylated and analyzed by gas liquid chromatography. DI, desaturation index. Values are mean \pm SEM, (n=4–6/group). * $P < 0.5$ vs. WT, # $P < 0.05$ vs. GKO; $^\wedge P < 0.05$ vs. GLS5; one-way ANOVA analysis with Tukey's post-hoc test.

Table S6. Plasma triglyceride fatty acid composition in 10 d LD-fed mice

Fatty acid		WT	GKO	GLS5	GLS3
16:0	$\mu\text{g}/100 \mu\text{l}$	17.8 \pm 3.23	10.4 \pm 0.48	16.5 \pm 3.28	13.0 \pm 1.80
	% of total	21.5 \pm 1.45	37.2 \pm 0.83*	35.0 \pm 1.22*	34.7 \pm 1.67*
16:1n-7	$\mu\text{g}/100 \mu\text{l}$	5.80 \pm 0.98	0.06 \pm 0.03*	0.10 \pm 0.03*	1.09 \pm 0.28* [#] [^]
	% of total	6.99 \pm 0.25	0.23 \pm 0.09*	0.19 \pm 0.05*	2.58 \pm 0.40* [#] [^]
18:0	$\mu\text{g}/100 \mu\text{l}$	1.35 \pm 0.24	8.00 \pm 0.37	12.0 \pm 2.65*	10.1 \pm 1.31*
	% of total	1.65 \pm 0.17	28.8 \pm 0.75*	24.9 \pm 1.66*	27.0 \pm 1.51*
18:1n-9	$\mu\text{g}/100 \mu\text{l}$	40.4 \pm 7.68	4.50 \pm 0.33*	11.3 \pm 1.97* [#]	6.38 \pm 1.32*
	% of total	47.7 \pm 1.88	16.1 \pm 0.40*	25.0 \pm 1.99* [#]	16.0 \pm 0.57* [^]
18:1n-7	$\mu\text{g}/100 \mu\text{l}$	6.57 \pm 1.25	0.04 \pm 0.02*	0.15 \pm 0.06*	1.49 \pm 0.42* [#] [^]
	% of total	7.80 \pm 0.56	0.14 \pm 0.05*	0.27 \pm 0.07*	3.45 \pm 0.74* [#] [^]
18:2n-6	$\mu\text{g}/100 \mu\text{l}$	10.3 \pm 3.53	3.91 \pm 0.32	5.55 \pm 1.27	6.06 \pm 1.84
	% of total	11.3 \pm 1.75	14.0 \pm 0.78	11.6 \pm 0.67	14.2 \pm 2.14
18:1n-9/18:0	DI	29.9 \pm 3.45	0.56 \pm 0.02*	1.05 \pm 0.17* [#]	0.61 \pm 0.05* [^]
16:1n-7/16:0	DI	0.33 \pm 0.02	0.01 \pm 0.00*	0.01 \pm 0.00*	0.08 \pm 0.01* [#] [^]

10 week old male mice were fed a lipogenic diet (LD) for 10 days and fasted 4 hours prior to tissue collection. Plasma lipids were extracted, lipid species were separated by TLC, extracted, transmethylated and analyzed by gas liquid chromatography. DI, desaturation index. Values are mean \pm SEM, (n=4–6/group). * P < 0.5 vs. WT, # P < 0.05 vs. GKO; ^ P < 0.05 vs. GLS5; one-way ANOVA analysis with Tukey's post-hoc test.

Table S7. Plasma free fatty acid composition in 10 d LD-fed mice

Fatty acid		WT	GKO	GLS5	GLS3
16:0	$\mu\text{g}/100 \mu\text{l}$	4.45 \pm 0.50	4.64 \pm 0.78	5.09 \pm 0.49	4.02 \pm 0.63
	% of total	35.2 \pm 0.70	37.6 \pm 1.23	39.1 \pm 1.16	39.8 \pm 0.71*
16:1n-7	$\mu\text{g}/100 \mu\text{l}$	1.72 \pm 0.24	0.05 \pm 0.01*	0.05 \pm 0.01*	0.09 \pm 0.02*
	% of total	13.5 \pm 0.40	0.41 \pm 0.03*	0.38 \pm 0.03*	0.81 \pm 0.12* [#] \wedge
18:0	$\mu\text{g}/100 \mu\text{l}$	0.87 \pm 0.07	4.83 \pm 0.63*	4.81 \pm 0.40*	3.98 \pm 0.63*
	% of total	7.02 \pm 0.56	40.0 \pm 1.22*	37.1 \pm 1.43*	39.7 \pm 1.66*
18:1n-9	$\mu\text{g}/100 \mu\text{l}$	3.21 \pm 0.42	0.85 \pm 0.15*	1.07 \pm 0.08*	0.63 \pm 0.11*
	% of total	25.2 \pm 0.68	6.90 \pm 0.70*	8.31 \pm 0.32*	6.31 \pm 0.65*
18:1n-7	$\mu\text{g}/100 \mu\text{l}$	0.44 \pm 0.04	0.05 \pm 0.01*	0.03 \pm 0.00*	0.09 \pm 0.02* \wedge
	% of total	3.61 \pm 0.44	0.38 \pm 0.10*	0.26 \pm 0.00*	0.73 \pm 0.14* [#] \wedge
18:2n-6	$\mu\text{g}/100 \mu\text{l}$	0.88 \pm 0.19	1.00 \pm 0.21	0.89 \pm 0.09	0.69 \pm 0.12
	% of total	6.72 \pm 0.84	7.90 \pm 0.98	6.85 \pm 0.18	6.28 \pm 0.72
20:4n-6	$\mu\text{g}/100 \mu\text{l}$	ND	0.01 \pm 0.00	0.00 \pm 0.00	0.01 \pm 0.00
	% of total		0.10 \pm 0.03	0.03 \pm 0.00	0.10 \pm 0.06
18:1n-9/18:0	DI	3.68 \pm 0.38	0.17 \pm 0.02*	0.23 \pm 0.01*	0.16 \pm 0.01*
16:1n-7/16:0	DI	0.38 \pm 0.02	0.01 \pm 0.00*	0.01 \pm 0.00*	0.02 \pm 0.00* [#] \wedge

10 week old male mice were fed a lipogenic diet (LD) for 10 days and fasted 4 hours prior to tissue collection. Plasma lipids were extracted, lipid species were separated by TLC, extracted, transmethylated and analyzed by gas liquid chromatography. DI, desaturation index. Values are mean \pm SEM, (n=4–6/group). * P < 0.5 vs. WT, [#] P < 0.05 vs. GKO; \wedge P < 0.05 vs. GLS5; one-way ANOVA analysis with Tukey's post-hoc test.

Table S8. Plasma phospholipid composition in 10 d LD-fed mice

Fatty acid		WT	GKO	GLS5	GLS3
16:0	% of total	34.4 ± 1.23	47.9 ± 1.25*	35.0 ± 3.31 [#]	35.2 ± 2.28 [#]
16:1n-7	% of total	2.00 ± 0.11	0.23 ± 0.03*	0.15 ± 0.03*	1.06 ± 0.24 ^{#^}
18:0	% of total	18.9 ± 1.05	18.1 ± 0.59	20.4 ± 1.48	20.0 ± 1.26
18:1n-9	% of total	22.9 ± 0.80	13.9 ± 0.74*	14.6 ± 2.19 [#]	8.30 ± 1.10 ^{*^}
18:1n-7	% of total	4.83 ± 0.60	0.50 ± 0.01*	0.28 ± 0.03*	1.46 ± 0.26 ^{*#^}
18:2n-6	% of total	10.3 ± 0.60	14.6 ± 1.77	18.1 ± 2.26*	21.6 ± 1.17 ^{*#}
20:4n-6	% of total	3.03 ± 0.13	1.90 ± 0.35	5.04 ± 1.59	5.01 ± 0.96
22:6n-3	% of total	2.39 ± 0.08	1.56 ± 0.22	4.04 ± 0.99	4.66 ± 0.80 [#]
18:1n-9/18:0	DI	1.23 ± 0.11	0.77 ± 0.04*	0.74 ± 0.13*	0.43 ± 0.07*
16:1n-7/16:0	DI	0.06 ± 0.00	0.00 ± 0.00*	0.00 ± 0.00*	0.03 ± 0.01 ^{*#^}

10 week old male mice were fed a lipogenic diet (LD) for 10 days and fasted 4 hours prior to tissue collection. Plasma lipids were extracted, lipid species were separated by TLC, extracted, transmethylated and analyzed by gas liquid chromatography. DI, desaturation index. Values are mean ± SEM, (n=4–6/group). **P* < 0.05 vs. WT, [#]*P* < 0.05 vs. GKO; [^]*P* < 0.05 vs. GLS5; one-way ANOVA analysis with Tukey's post-hoc test.

Table S9. Gonadal white adipose tissue triacylglycerol fatty acid composition in LD-fed mice

Fatty acid		WT	GKO	GLS5	GLS3
16:0	$\mu\text{g}/\text{mg}$	265.6 \pm 8.58	163.8 \pm 8.46	246.6 \pm 2.05	235.1 \pm 9.68
	% of total	24.4 \pm 0.49	33.4 \pm 1.11*	32.5 \pm 0.72*	33.0 \pm 1.67*
16:1n-7	$\mu\text{g}/\text{mg}$	123.4 \pm 5.93	2.38 \pm 0.22*	4.64 \pm 0.40* [#]	11.3 \pm 1.04* ^{#^}
	% of total	11.3 \pm 0.40	0.48 \pm 0.03*	0.61 \pm 0.05*	1.56 \pm 0.09* ^{#^}
18:0	$\mu\text{g}/\text{mg}$	23.4 \pm 0.78	139.0 \pm 9.15*	179.1 \pm 7.83* [#]	182.0 \pm 8.40* [#]
	% of total	2.15 \pm 0.07	28.4 \pm 1.68*	23.7 \pm 1.39*	25.6 \pm 1.51*
18:1n-9	$\mu\text{g}/\text{mg}$	414.6 \pm 16.6	84.8 \pm 8.06*	164.2 \pm 11.9* [#]	125.0 \pm 15.6*
	% of total	38.0 \pm 0.36	17.2 \pm 1.17*	21.5 \pm 1.11*	17.1 \pm 1.43* [^]
18:1n-7	$\mu\text{g}/\text{mg}$	41.9 \pm 1.95	5.23 \pm 0.59*	7.96 \pm 0.67*	14.0 \pm 1.31* ^{#^}
	% of total	3.84 \pm 0.09	1.06 \pm 0.09*	1.04 \pm 0.07*	1.93 \pm 0.10* ^{#^}
18:2n-6	$\mu\text{g}/\text{mg}$	177.5 \pm 8.10	77.5 \pm 7.94*	129.1 \pm 9.13* [#]	124.1 \pm 15.0* [#]
	% of total	16.3 \pm 0.61	15.7 \pm 1.11	16.9 \pm 0.89	17.0 \pm 1.33
18:3n-3	$\mu\text{g}/\text{mg}$	6.30 \pm 0.44	0.83 \pm 0.17*	2.62 \pm 0.43*	2.52 \pm 0.65*
	% of total	0.58 \pm 0.04	0.17 \pm 0.03*	0.34 \pm 0.05*	0.33 \pm 0.07*
20:4n-6	$\mu\text{g}/\text{mg}$	3.06 \pm 0.26	0.65 \pm 0.09*	0.93 \pm 0.14*	0.89 \pm 0.17*
	% of total	0.28 \pm 0.02	0.13 \pm 0.02*	0.12 \pm 0.02*	0.12 \pm 0.02*
22:6n-3	$\mu\text{g}/\text{mg}$	2.31 \pm 0.26	0.66 \pm 0.12	0.74 \pm 0.15*	0.78 \pm 0.22*
	% of total	0.21 \pm 0.02	0.13 \pm 0.02	0.10 \pm 0.02*	0.10 \pm 0.03*
18:1n-9/18:0	DI	17.8 \pm 0.73	0.62 \pm 0.09*	0.93 \pm 0.09*	0.70 \pm 0.11*
16:1n-7/16:0	DI	0.46 \pm 0.01	0.01 \pm 0.00*	0.02 \pm 0.00*	0.05 \pm 0.00* ^{#^}

10 week old male mice were fed a lipogenic diet (LD) for 10 days and fasted 4 hours prior to tissue collection. Gonadal white adipose lipids were extracted, lipid species were separated by TLC, TG band was extracted, transmethylated and analyzed by gas liquid chromatography. DI, desaturation index. Values are mean \pm SEM, (n=4–6/group). * P < 0.5 vs. WT, # P < 0.05 vs. GKO; ^ P < 0.05 vs. GLS5; one-way ANOVA analysis with Tukey's post-hoc test.