

Supplemental Table 1. Diet formulations[†].

Ingredients	0.06% diet			0.2% diet			0.6% diet		
	L	10	M	L	10	M	L	10	M
	gm	gm	gm	gm	gm	gm	gm	gm	gm
Casein	189.6	189.6	189.6	189.6	189.6	189.6	189.6	189.6	189.6
L-Cystine	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Corn Starch	298.5	298.6	298.6	298.6	298.6	298.6	298.6	298.6	298.6
Maltodextrin 10	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2
Sucrose	331.7	331.8	331.8	331.8	331.8	331.8	331.8	331.8	331.8
Cellulose	47.4	47.4	47.4	47.4	47.4	47.4	47.4	47.4	47.4
Soybean Oil	23.1	23.1	23.1	21.7	21.7	21.7	17.7	17.7	17.7
Lard	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0
Linoleic acid	0.6	0.3	0	2.0	1.0	0	6.0	3.0	0
9,11 CLA	0	0	0.3	0	0	1.0	0	0	3.0
10,12 CLA	0	0.3	0.3	0	1.0	1.0	0	3.0	3.0
Mineral Mix S10026	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
DiCalcium Phosphate	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3
Calcium Carbonate	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
Potassium Citrate, 1 H ₂ O	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6
Vitamin Mix V10001	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
Choline Bitartrate	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000

[†]L, linoleic acid controls; 10, 10,12 CLA plus linoleic acid; M, 10,12 CLA plus 9,11 CLA.

Supplemental Table 2. Fat composition of the diet^{*}.

Ingredients	0.06% diet			0.2% diet			0.6% diet		
	L	10	M	L	10	M	L	10	M
	%	%	%	%	%	%	%	%	%
Soybean Oil	54.1	54.1	54.1	50.8	50.8	50.8	41.4	41.4	41.4
Lard	44.5	44.5	44.5	44.5	44.5	44.5	44.5	44.5	44.5
Linoleic acid	1.4	0.7	0	4.7	2.35	0	14.1	7.05	0
9,11 CLA	0	0	0.7	0	0	2.35	0	0	7.05
10,12 CLA	0	0.7	0.7	0	2.35	2.35	0	7.05	7.05

^{*} Data were expressed as a percentage of total fat. L, linoleic acid controls; 10, 10,12 CLA plus linoleic acid; M, 10,12 CLA plus 9,11 CLA.

Supplemental Table 3. Fatty acids analysis of the diets ^{††}.

	0.06% diet			0.2% diet			0.6% diet		
	<u>L</u>	<u>10</u>	<u>M</u>	<u>L</u>	<u>10</u>	<u>M</u>	<u>L</u>	<u>10</u>	<u>M</u>
C14:0	0.027	0.033	0.017	0.027	0.033	0.027	0.028	0.023	0.024
C16:0	0.509	0.607	0.359	0.525	0.605	0.502	0.460	0.468	0.462
C16:1	0.032	0.038	0.021	0.033	0.038	0.032	0.033	0.029	0.029
C18:0	0.171	0.226	0.157	0.201	0.231	0.188	0.152	0.174	0.188
C18:1	0.800	0.987	0.606	0.858	0.972	0.827	0.704	0.766	0.746
C18:2	1.521	1.822	1.023	1.652	1.787	1.515	1.685	1.452	1.228
C18:3 (n6)	0.004	0.006	0.005	0.004	0.006	0.005	0.004	0.004	0.004
C18:3 (n3)	0.198	0.243	0.137	0.188	0.232	0.206	0.158	0.136	0.150
9,11 CLA	0.005	0.005	0.016	0.008	0.006	0.069	0.005	0.013	0.225
10,12 CLA	0.003	0.022	0.015	0.006	0.069	0.067	0.004	0.220	0.220
C20:0	0.003	0.005	0.004	0.004	0.006	0.005	0.003	0.003	0.004
C20:1	0.007	0.010	0.008	0.009	0.011	0.009	0.007	0.008	0.009
C20:2	0.010	0.013	0.009	0.011	0.014	0.011	0.009	0.011	0.011
Fat%	3.369	4.096	2.410	3.637	4.069	3.517	3.309	3.300	3.364

[†]Data were expressed as fatty acid g / 100 g diet. L, linoleic acid controls; 10, 10,12 CLA plus linoleic acid; M, 10,12 CLA plus 9,11 CLA.

^{††}C20:3 (n6), C20:3 (n3), C20:4, C20:5, C22:0, C22:1, C22:2, C22:3, C22:4, C22:6, and C24:1 were underdetectable.

Supplemental Table 4. Fatty acid content of muscle.

mg/g tissue	0.06% diet			0.2% diet			0.6% diet		
	L	10	M	L	10	M	L	10	M
C12:0	0.03 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.04 ± 0.02	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
C14:0	0.63 ± 0.14 ^a	0.30 ± 0.02 ^{abc}	0.36 ± 0.04 ^{abc}	0.63 ± 0.19 ^a	0.48 ± 0.07 ^{ab}	0.67 ± 0.07 ^a	0.33 ± 0.05 ^{abc}	0.07 ± 0.01 ^c	0.12 ± 0.04 ^{bc}
C14:1	0.18 ± 0.05	0.08 ± 0.01	0.11 ± 0.02	0.15 ± 0.03	0.13 ± 0.02	0.18 ± 0.02	0.09 ± 0.02	0.01 ± 0.00	0.01 ± 0.00
C15:0	0.05 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
C16:0	6.0 ± 1.5 ^a	3.2 ± 0.2 ^{ab}	3.4 ± 0.3 ^{ab}	5.1 ± 0.9 ^a	3.8 ± 0.4 ^{ab}	4.7 ± 0.4 ^{ab}	3.4 ± 0.3 ^{ab}	1.6 ± 0.1 ^b	2.9 ± 1.1 ^{ab}
C16:1	5.1 ± 1.1	2.5 ± 0.2	3.1 ± 0.4	4.4 ± 0.8	3.8 ± 0.6	5.0 ± 0.5	2.8 ± 0.4	0.4 ± 0.1	0.8 ± 0.3
C17:1	0.08 ± 0.01	0.04 ± 0.00	0.05 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.01 ± 0.00	0.03 ± 0.01
C18:0	0.77 ± 0.23	0.8 ± 0.04	0.60 ± 0.03	0.68 ± 0.04	0.56 ± 0.05	0.59 ± 0.03	0.67 ± 0.03	0.54 ± 0.02	0.56 ± 0.09
C18:1c	8.1 ± 2.0	3.8 ± 0.3	4.5 ± 0.5	5.5 ± 0.7	5.4 ± 0.7	6.8 ± 0.8	4.3 ± 0.5	1.5 ± 0.1	3.5 ± 1.5
C18:2c	4.5 ± 1.1	2.0 ± 0.2	2.4 ± 0.3	3.1 ± 0.5	2.8 ± 0.4	3.6 ± 0.4	2.5 ± 0.4	0.7 ± 0.1	0.9 ± 0.2
C18:2t	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
9,11 CLA	0.07 ± 0.01^b	0.03 ± 0.00^b	0.05 ± 0.01^b	0.05 ± 0.01^b	0.05 ± 0.01^b	0.14 ± 0.03^a	0.03 ± 0.00^b	0.01 ± 0.00^b	0.06 ± 0.02^b
10,12 CLA	0.04 ± 0.01^b	0.02 ± 0.00^{ab}	0.03 ± 0.00^{ab}	0.02 ± 0.00^{ab}	0.06 ± 0.01^a	0.06 ± 0.01^a	0.02 ± 0.00^{ab}	0.03 ± 0.00^{ab}	0.04 ± 0.01^b
C18:3n3	0.33 ± 0.09	0.12 ± 0.01	0.15 ± 0.02	0.28 ± 0.09	0.17 ± 0.03	0.21 ± 0.03	0.13 ± 0.03	0.03 ± 0.01	0.04 ± 0.01
C18:3n6	0.08 ± 0.02	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.06 ± 0.02	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.01
C20:1	0.07 ± 0.02	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.00	0.03 ± 0.00	0.05 ± 0.02
C20:2	0.08 ± 0.03	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.01	0.09 ± 0.04	0.05 ± 0.00	0.04 ± 0.00	0.03 ± 0.01	0.04 ± 0.01
C20:3n6	0.08 ± 0.05	0.05 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.01
C20:4n6	0.80 ± 0.46	0.57 ± 0.03	0.38 ± 0.03	0.62 ± 0.06	0.37 ± 0.04	0.43 ± 0.02	0.58 ± 0.06	0.39 ± 0.02	0.42 ± 0.06
C22:5	0.13 ± 0.03	0.17 ± 0.01	0.12 ± 0.01	0.14 ± 0.03	0.12 ± 0.02	0.14 ± 0.01	0.21 ± 0.02	0.17 ± 0.02	0.16 ± 0.03
C22:6n3	0.62 ± 0.26	0.54 ± 0.04	0.39 ± 0.05	0.65 ± 0.07	0.33 ± 0.05	0.42 ± 0.33	0.59 ± 0.08	0.31 ± 0.02	0.32 ± 0.05
C24:0	0.04 ± 0.01	0.06 ± 0.01	0.04 ± 0.00	0.06 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.00	0.04 ± 0.01
SAT	7.5 ± 1.8	4.4 ± 0.2	4.5 ± 0.3	6.6 ± 1.1	5.0 ± 0.5	6.1 ± 0.5	4.5 ± 0.4	2.3 ± 0.1	3.6 ± 1.2
MUFA	13.4 ± 2.7	6.5 ± 0.5	7.9 ± 0.9	9.6 ± 1.2	9.4 ± 1.3	12.1 ± 1.3	7.2 ± 0.1	2.0 ± 0.2	4.4 ± 1.9
PUFA	6.8 ± 1.9	3.6 ± 0.3	3.6 ± 0.4	4.7 ± 0.6	4.1 ± 0.5	5.1 ± 0.5	4.2 ± 0.5	1.8 ± 0.1	2.1 ± 0.4

^a Means ± SEM, not sharing a common letter within the same row differ (p<0.05). L, linoleic acid controls; 10, 10,12 CLA plus linoleic acid; M, 10,12 CLA plus 9,11 CLA.

Supplemental Table 5. The effects of CLA on body weight gain (BWG), food intake (FI), food conversion efficiency (FCE), fasting blood glucose (BG), glucose tolerance test (GTT) area under the curve (AUC), fasting insulin, and HOMA-IR index.

Diets	Total BWG (g)	Total FI (per cage, g)	FCE (per cage)	Fasting BG (mg/dL)	GTT AUC	Fasting insulin (ng/mL)	HOMA-IR index
Type	< 0.0001	0.0003	< 0.0001	0.3661	0.7136	0.7590	0.9649
Dose	< 0.0001	0.9844	< 0.0001	0.2580	0.5534	0.0640	0.0378
Interaction	< 0.0001	0.6104	0.0016	0.1085	0.5043	0.0412	0.0956
0.06 L	5.7 ± 0.1 ^{ab}	276 ± 3.0	25.1 ± 1.5 ^b	78.1 ± 3.8	23640 ± 808	0.51 ± 0.11 ^{ab}	2.35 ± 0.55
0.06 10	5.5 ± 0.1 ^{abc}	265 ± 3.9	24.2 ± 0.9 ^b	74.8 ± 2.6	23344 ± 919	0.39 ± 0.05 ^b	1.69 ± 0.21
0.06 M	5.0 ± 0.2 ^{abc}	269 ± 4.1	28.3 ± 2.4 ^b	73.4 ± 3.8	21418 ± 995	0.53 ± 0.09 ^{ab}	2.31 ± 0.48
0.2 L	6.2 ± 0.2 ^a	280 ± 2.4	22.8 ± 1.3 ^b	79.8 ± 2.1	23112 ± 1124	0.60 ± 0.11 ^{ab}	2.85 ± 0.54
0.2 10	3.9 ± 0.1 ^{cde}	261 ± 4.7	33.6 ± 1.4 ^b	75.6 ± 4.7	24082 ± 1000	0.44 ± 0.08 ^{ab}	1.96 ± 0.37
0.2 M	4.4 ± 0.1 ^{bcd}	270 ± 2.2	31.2 ± 1.6 ^b	78.5 ± 4.0	23230 ± 1084	0.47 ± 0.06 ^{ab}	2.22 ± 0.35
0.6 L	6.4 ± 0.1 ^a	282 ± 2.1	22.2 ± 0.8 ^b	74.6 ± 2.8	22193 ± 469	0.49 ± 0.09 ^{ab}	2.28 ± 0.50
0.6 10	2.8 ± 0.1 ^{de}	262 ± 2.5	52.1 ± 5.2 ^a	77.9 ± 4.8	22436 ± 1126	0.94 ± 0.21 ^a	4.20 ± 0.98
0.6 M	2.6 ± 0.1 ^e	264 ± 2.3	53.7 ± 4.7 ^a	89.5 ± 4.9	23194 ± 1350	0.60 ± 0.09 ^{ab}	3.18 ± 0.64

Means ± SEM without a common letter in the same column differ (p<0.05). L, linoleic acid controls; 10, 10,12 CLA plus linoleic acid; M, 10,12 CLA plus 9,11 CLA.

Supplemental Table 6. Probability levels for the main effects of fatty acid type and dose and their interactions for the experimental outcomes.

Dependent variable	Type	Dose	Interaction	Dependent variable	Type	Dose	Interaction
Fig 1				Fig 7A			
EPI	< 0.0001	< 0.0001	< 0.0001	PPAR γ	< 0.0001	< 0.0001	< 0.0001
ING	< 0.0001	< 0.0001	< 0.0001	Perilipin	< 0.0001	0.0093	< 0.0001
MEN	< 0.0001	< 0.0001	0.0007	FABP4	< 0.0001	0.2678	< 0.0001
RET	< 0.0001	< 0.0001	0.0002	HSL	0.0004	< 0.0001	< 0.0001
Total WAT	< 0.0001	< 0.0001	< 0.0001	ATGL	0.0007	< 0.0001	< 0.0001
BAT	< 0.0001	< 0.0001	0.0002	Fig 7B			
Fig 2B				PPAR γ	0.8922	< 0.0001	0.0002
Liver Weight	< 0.0001	< 0.0001	< 0.0001	Perilipin	< 0.0001	< 0.0001	< 0.0001
Liver TG	< 0.0001	< 0.0001	< 0.0001	FABP4	0.0039	0.0173	< 0.0001
Fig 3				HSL	0.0398	< 0.0001	0.0012
MCP1	0.0142	< 0.0001	0.0044	ATGL	0.7346	< 0.0001	0.001
IL6	< 0.0001	0.001	0.0019	Fig 8A			
Serum TG	< 0.0001	< 0.0001	< 0.0001	GPR120	< 0.0001	0.313	< 0.0001
Fig 4A				GPR40	0.0443	0.3533	0.0132
UCP1	< 0.0001	< 0.0001	< 0.0001	Fig 8B			
Elov3	< 0.0001	0.0001	0.0706	GPR120	0.5264	< 0.0001	< 0.0001
Cidea	< 0.0001	< 0.0001	< 0.0001	GPR40	< 0.0001	< 0.0001	0.0433
CPT1b	< 0.0001	< 0.0001	< 0.0001	Suppl Fig 1			
Cox8b	< 0.0001	< 0.0001	< 0.0001	UCP1	0.8595	0.0022	0.1126
PPAR α	< 0.0001	< 0.0001	< 0.0001	Elov3	< 0.0001	< 0.0001	< 0.0001
COX2	< 0.0001	< 0.0001	< 0.0001	Cidea	0.0792	< 0.0001	< 0.0001
PGF2 α	< 0.0001	< 0.0001	< 0.0001	CPT1b	0.0012	< 0.0001	0.0001
synthase				Cox8b	< 0.0001	< 0.0001	< 0.0001
TMEM26	< 0.0001	< 0.0001	< 0.0001	PPAR α	0.0033	< 0.0001	< 0.0001
Fig 4B				COX2	0.0013	< 0.0001	< 0.0001
UCP1	0.0012	< 0.0001	< 0.0001	PGF2 α	< 0.0001	0.0011	0.0422
Elov3	0.0629	0.019	< 0.0001	synthase			
Cidea	0.2104	< 0.0001	< 0.0001	TMEM26	< 0.001	< 0.001	< 0.001
CPT1b	0.0002	< 0.0001	< 0.0001	Suppl Fig 2			
Cox8b	< 0.0001	0.0019	0.0003	MCP1	< 0.0001	< 0.0001	< 0.0001
PPAR α	< 0.0001	< 0.0001	< 0.0001	TNF α	< 0.0001	< 0.0001	< 0.0001
COX2	< 0.0001	0.0003	< 0.0001	F4/80	< 0.0001	< 0.0001	< 0.0001
PGF2 α	< 0.0001	< 0.0001	< 0.0001	IL6	< 0.0001	< 0.0001	< 0.0001
synthase				Arginase 1	< 0.0001	< 0.0001	< 0.0001
TMEM26	< 0.0001	< 0.0001	0.0175	Mrc 1	< 0.0001	< 0.0001	< 0.0001
Fig 6A				Clec 10	< 0.0001	0.036	0.0164
MCP1	< 0.0001	< 0.0001	< 0.0001	Suppl Fig 3			
TNF α	< 0.0001	< 0.0001	< 0.0001	PPAR γ	0.0466	< 0.0001	< 0.0001
F4/80	< 0.0001	< 0.0001	< 0.0001	Perilipin	< 0.0001	< 0.0001	< 0.0001
IL6	< 0.0001	< 0.0001	< 0.0001	FABP4	0.0003	< 0.0001	< 0.0001
Arginase 1	< 0.0001	< 0.0001	< 0.0001	HSL	0.0631	< 0.0001	0.0001
Mrc 1	< 0.0001	< 0.0001	< 0.0001	ATGL	0.0368	< 0.0001	0.0022
Clec 10	< 0.0001	< 0.0001	< 0.0001	Suppl Fig 4			
Fig 6B				GPR120	< 0.0001	< 0.0001	< 0.0001
MCP1	< 0.0001	< 0.0001	< 0.0001	GPR40	0.0044	0.0229	0.0635
TNF α	< 0.0001	< 0.0001	0.0001				
F4/80	< 0.0001	< 0.0001	< 0.0001				
IL6	< 0.0001	0.1794	0.0003				
Arginase 1	0.2374	0.2133	0.4565				
Mrc 1	< 0.0001	0.0535	< 0.0001				
Clec 10	< 0.0001	0.1012	0.0028				

Supplemental Fig. 1. Differential effects of CLA dose and treatments on markers of browning in retroperitoneal (RET) WAT. Panel A; direct markers of browning. Panel B; markers of prostaglandin production that are associated with the activation of browning. mRNA levels of markers of brown fat-like adipocytes were measured in RET WAT by real time qPCR. Means \pm SEM without a common letter differ ($P < 0.05$). L, linoleic acid; 10, 10,12 CLA plus linoleic acid; M, 10,12 CLA plus 9,11 CLA; UCP-1, uncoupling protein 1; Elovl3, elongation of very long chain fatty acids 3; Cidea, cell death-induced DNA fragmentation factor-a-like effector A; CPT-1b, carnitine palmitoyltransferase 1 b; Cox8b, cytochrome c oxidase subunit VIII b; PPAR α , proliferator-activated receptor alpha; TMEM26, transmembrane protein 26; COX-2, cyclooxygenase-2; PGF2 α , prostaglandin F2 alpha.

Supplemental Fig. 2. Intermediate and high dose of CLA treatments increase marker of low grade inflammation in retroperitoneal (RET) WAT. Panel A; markers of classically-activated M1 macrophage. Panel B; markers of alternatively-activated M2 macrophage. mRNA levels of markers of low grade inflammation were measured in RET WAT by real time qPCR. Means \pm SEM without a common letter differ ($P < 0.05$). L, linoleic acid; 10, 10,12 CLA plus linoleic acid; M, 10,12 CLA plus 9,11 CLA; COX-2, cyclooxygenase 2; MCP-1, monocyte chemoattractant protein 1; IL-6, interleukin 6; TNF α = tumor necrosis factor alpha

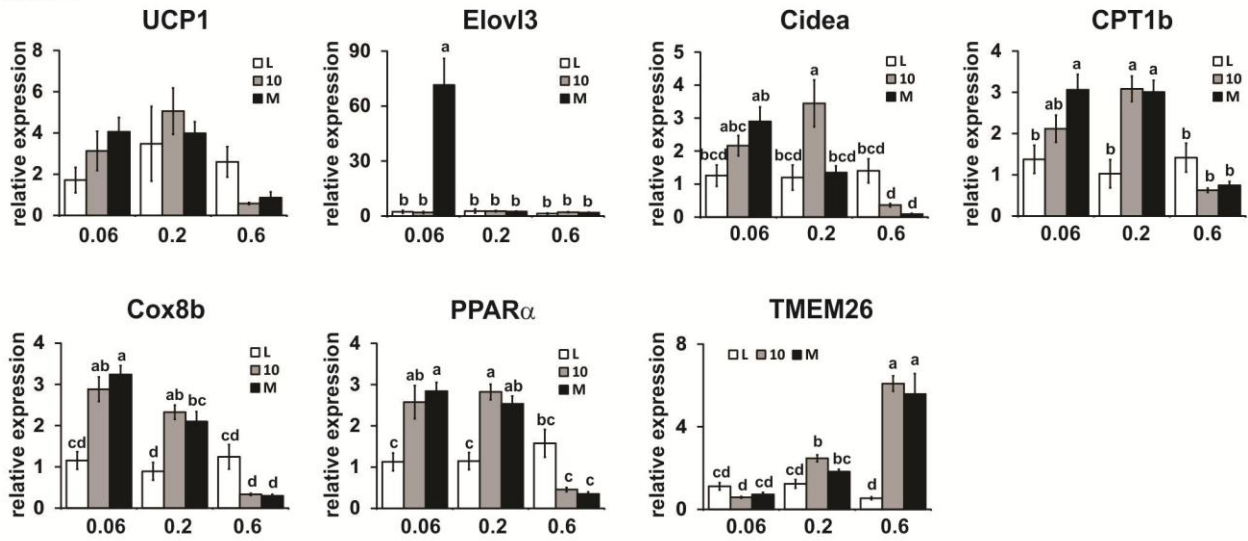
Supplemental Fig. 3. Differential effects of CLA dose and treatments on markers of lipogenesis and lipolysis in retroperitoneal (RET) WAT. mRNA levels of markers of lipogenesis (Panel A) and lipolysis (Panel B) were measured in RET WAT by real time qPCR. Means \pm SEM without a common letter differ ($P < 0.05$). L, linoleic acid; 10, 10,12 CLA plus linoleic acid; M, 10,12

CLA plus 9,11 CLA; PPAR α , peroxisome proliferator-activated receptor alpha; FABP4, fatty acid binding protein 4; HSL, hormone sensitive lipase; ATGL, adipose tissue TG lipase.

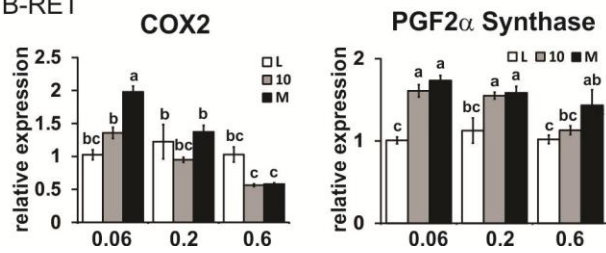
Supplemental Fig. 4. FFA receptors GPR120 and 40 in retroperitoneal (RET) WAT are differentially regulated by CLA. mRNA levels of markers of GPR 120 and 40 were in RET WAT measured by real time qPCR. Means \pm SEM without a common letter differ (P<0.05). L, linoleic acid; 10, 10,12 CLA plus linoleic acid; M, 10,12 CLA plus 9,11 CLA; GPR, G protein receptor

Supplemental Fig 1.

A-RET

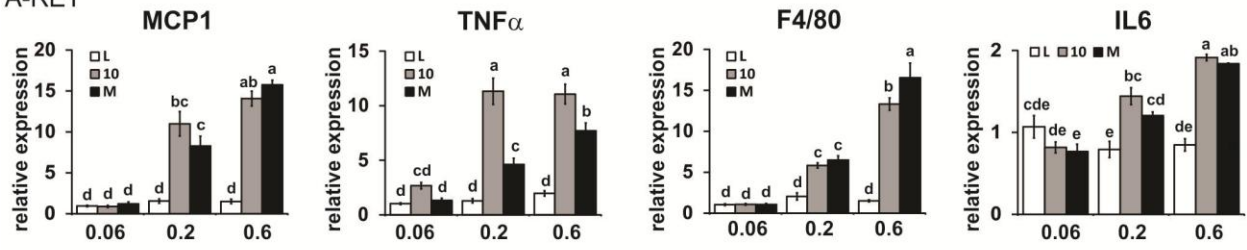


B-RET

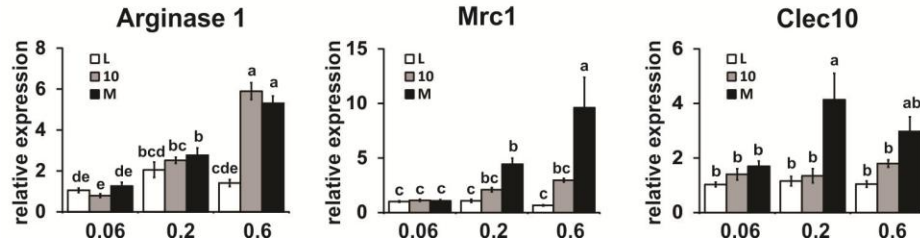


Supplemental Fig 2.

A-RET

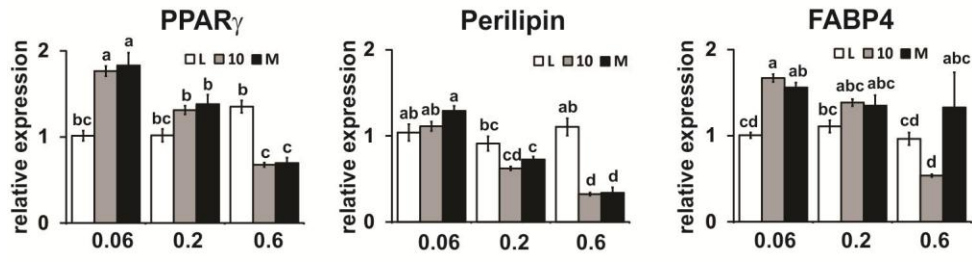


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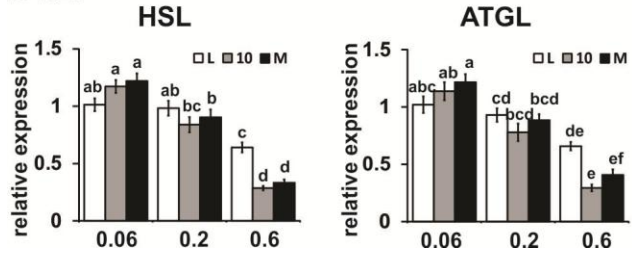


Supplemental Fig 3.

A-RET



B-RET



Supplemental Fig 4.

RET

