

Supplementary Materials

Research

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Gymnemanol Modulates Lipid Metabolism and Adipogenic Gene Expression in 3T3-L1 Adipocytes

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Table S1: Sequences of primers

Gene	Primer ID	Primer sequence (5'.. .3')	
		Forward	Reverse
β -Actin	M_Actb_1	GATGTATGAAGGCTTTGGTC	TGTGCACTTTTATTGGTCTC
AdiQ	M_Adipoq_1	CCACTTTCTCCTCATTTCTG	CTAGCTCTTCAGTTGTAGTAAC
Ccl2	M_Ccl2_1	CAAGATGATCCCAATGAGTAG	TTGGTGACAAAACTACAGC
Cidea	M_Cidea_1	GTGTTAAGGAATCTGCTGAG	CTATAACAGAGAGCAGGGTC
Fasn	M_Fas_1	TGAATGCCTCAAATCTTAGC	TTTAGCTTCCTGGATTGTC
Lep	M_Lep_1	CTTTGGTCCTATCTGTCTTATG	TCTTGGACAAACTCAGAATG
Lipe	M_Lipe_1	AACTCCTTCCTGGAATAAG	CTTCTTCAAGGTATCTGTGC
Lpl	M_Lpl_1	GAGACTCAGAAAAAGGTCATC	GTCTTCAAAGAACTCAGATGC
Plin2	M_Plin2_1	ATAAGCTCTATGTCTCGTGG	GCCTGATCTTGAATGTTCTG
Pparg	M_Pparg_1	AAAGACAACGGACAAATCAC	GGGATATTTTTGGCATACTCTG
Scd1	M_Scd1_1	GTGGGGTAATTATTTGTGACC	TTTTTCCCAGACAGTACAAC

Table S2: Simple linear regression

Best-fit values	
Slope	-18.74
Y-intercept	106.2
X-intercept	5.668
1/slope	-0.05336
Std. Error	
Slope	1.126
Y-intercept	1.409
95% Confidence Intervals	
Slope	-21.10 to -16.38
Y-intercept	103.3 to 109.2
X-intercept	5.152 to 6.330
Goodness of Fit	
R squared	0.9358
Sy.x	3.108
Is slope significantly non-zero?	
F	277.0
DFn, DFd	1, 19
P value	<0.0001
Deviation from zero?	Significant
Equation	$Y = -18.74 \cdot X + 106.2$
Data	
Number of X values	21
Maximum number of Y replicates	3
Total number of values	24
Number of missing values	0

Table S3: Two-way ANOVA for TGs analysis

Two-way RM ANOVA		Matching: Across row			
Alpha		0.05			
Source of Variation	% of total variation	P value	P value summary	Significant?	
Row Factor x Column Factor	0.03574	0.0159	*	Yes	
Row Factor	0.3752	0.0005	***	Yes	
Column Factor	99.54	<0.0001	****	Yes	
Subject	0.03286	0.1227	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Row Factor x Column Factor	123.5	2	61.75	F (2, 6) = 8.939	P=0.0159
Row Factor	1296	2	648.2	F (2, 6) = 34.25	P=0.0005
Column Factor	344002	1	344002	F (1, 6) = 49799	P<0.0001
Subject	113.6	6	18.93	F (6, 6) = 2.740	P=0.1227
Residual	41.45	6	6.908		
Difference between column means					
Mean of 4th day	72.90				
Mean of 8th day	349.4				
Difference between means	-276.5				
SE of difference	1.239				
95% CI of difference	-279.5 to -273.5				
Data summary					
Number of columns (Column Factor)	2				
Number of rows (Row Factor)	3				
Number of subjects (Subject)	9				
Number of missing values	0				

Table S4: Two-way ANOVA multiple comparisons for TGs analysis

Alpha	0.05								
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value				
4th day									
Row 1 vs. Row 2	12.82	4.987 to 20.65	Yes	**	0.0024				
Row 1 vs. Row 3	27.16	19.33 to 34.99	Yes	****	<0.0001				
Row 2 vs. Row 3	14.34	6.511 to 22.17	Yes	**	0.0010				
8th day									
Row 1 vs. Row 2	7.803	-0.02586 to 15.63	No	ns	0.0508				
Row 1 vs. Row 3	14.42	6.591 to 22.25	Yes	***	0.0010				
Row 2 vs. Row 3	6.617	-1.213 to 14.45	No	ns	0.1018				
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF	
4th day									
Row 1 vs. Row 2	86.22	73.41	12.82	2.935	3	3	6.176	12.00	
Row 1 vs. Row 3	86.22	59.07	27.16	2.935	3	3	13.09	12.00	
Row 2 vs. Row 3	73.41	59.07	14.34	2.935	3	3	6.911	12.00	
8th day									
Row 1 vs. Row 2	356.8	349.0	7.803	2.935	3	3	3.760	12.00	
Row 1 vs. Row 3	356.8	342.4	14.42	2.935	3	3	6.949	12.00	
Row 2 vs. Row 3	349.0	342.4	6.617	2.935	3	3	3.189	12.00	

Table S5: One-way ANOVA analysis of lipase enzymes genes expression

ANOVA summary					
F	23.45				
P value	0.0015				
P value summary	**				
Significant diff. among means ($P < 0.05$)?	Yes				
R squared	0.8866				
Brown-Forsythe test					
F (DFn, DFd)	0.4468 (2, 6)				
P value	0.6593				
P value summary	ns				
Are SDs significantly different ($P < 0.05$)?	No				
Bartlett's test					
Bartlett's statistic (corrected)					
P value					
P value summary					
Are SDs significantly different ($P < 0.05$)?					
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	6.409	2	3.204	F (2, 6) = 23.45	P=0.0015
Residual (within columns)	0.8200	6	0.1367		
Total	7.229	8			
Data summary					
Number of treatments (columns)	3				
Number of values (total)	9				

Table S6: One-way ANOVA multiple comparisons for lipase enzymes genes expression

Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
FASN/Actin vs. Lipe/Actin	-1.000	-1.926 to -0.07385	Yes	*	0.0371			
FASN/Actin vs. Lpl/Actin	-2.067	-2.993 to -1.141	Yes	**	0.0012			
Lipe/Actin vs. Lpl/Actin	-1.067	-1.993 to -0.1405	Yes	*	0.0285			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
FASN/Actin vs. Lipe/Actin	1.367	2.367	-1.000	0.3018	3	3	4.685	6
FASN/Actin vs. Lpl/Actin	1.367	3.433	-2.067	0.3018	3	3	9.683	6
Lipe/Actin vs. Lpl/Actin	2.367	3.433	-1.067	0.3018	3	3	4.998	6

Table S7: One-way ANOVA analysis of Genes involved in lipogenesis

ANOVA summary					
F	113.2				
P value	<0.0001				
P value summary	****				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.9770				
Brown-Forsythe test					
F (DFn, DFd)	1.123 (3, 8)				
P value	0.3956				
P value summary	ns				
Are SDs significantly different (P < 0.05)?	No				
Bartlett's test					
Bartlett's statistic (corrected)					
P value					
P value summary					
Are SDs significantly different (P < 0.05)?					
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	2765	3	921.6	F (3, 8) = 113.2	P<0.0001
Residual (within columns)	65.12	8	8.140		
Total	2830	11			
Data summary					
Number of treatments (columns)	4				
Number of values (total)	12				

Table S8: One-way ANOVA multiple comparisons of Genes involved in lipogenesis

Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
PPARG/Actin vs. Plin/Actin	2.270	-5.190 to 9.730	No	ns	0.7673			
PPARG/Actin vs. Scd/Actin	-35.66	-43.12 to -28.20	Yes	****	<0.0001			
PPARG/Actin vs. Cidea/Actin	-6.447	-13.91 to 1.013	No	ns	0.0924			
Plin/Actin vs. Scd/Actin	-37.93	-45.39 to -30.47	Yes	****	<0.0001			
Plin/Actin vs. Cidea/Actin	-8.717	-16.18 to -1.257	Yes	*	0.0236			
Scd/Actin vs. Cidea/Actin	29.21	21.75 to 36.67	Yes	****	<0.0001			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
PPARG/Actin vs. Plin/Actin	8.967	6.697	2.270	2.329	3	3	1.378	8
PPARG/Actin vs. Scd/Actin	8.967	44.63	-35.66	2.329	3	3	21.65	8
PPARG/Actin vs. Cidea/Actin	8.967	15.41	-6.447	2.329	3	3	3.914	8
Plin/Actin vs. Scd/Actin	6.697	44.63	-37.93	2.329	3	3	23.03	8
Plin/Actin vs. Cidea/Actin	6.697	15.41	-8.717	2.329	3	3	5.292	8
Scd/Actin vs. Cidea/Actin	44.63	15.41	29.21	2.329	3	3	17.74	8

Table S9: One-way ANOVA analysis of expression of adipokines secreted by adipocytes

ANOVA summary					
F	7.729				
P value	0.0219				
P value summary	*				
Significant diff. among means ($P < 0.05$)?	Yes				
R squared	0.7204				
Brown-Forsythe test					
F (DFn, DFd)	0.1121 (2, 6)				
P value	0.8957				
P value summary	ns				
Are SDs significantly different ($P < 0.05$)?	No				
Bartlett's test					
Bartlett's statistic (corrected)					
P value					
P value summary					
Are SDs significantly different ($P < 0.05$)?					
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	13.60	2	6.798	F (2, 6) = 7.729	P=0.0219
Residual (within columns)	5.277	6	0.8795		
Total	18.87	8			
Data summary					
Number of treatments (columns)	3				
Number of values (total)	9				

Table S10: One-way ANOVA multiple comparisons of expression of adipokines secreted by adipocytes

Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
Adipoq/Actin vs. Lep/Actin	2.933	0.5838 to 5.283	Yes	*	0.0202			
Adipoq/Actin vs. Ccl2/Actin	2.053	-0.2962 to 4.403	No	ns	0.0811			
Lep/Actin vs. Ccl2/Actin	-0.8800	-3.229 to 1.469	No	ns	0.5220			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Adipoq/Actin vs. Lep/Actin	6.797	3.863	2.933	0.7657	3	3	5.417	6
Adipoq/Actin vs. Ccl2/Actin	6.797	4.743	2.053	0.7657	3	3	3.792	6
Lep/Actin vs. Ccl2/Actin	3.863	4.743	-0.8800	0.7657	3	3	1.625	6