Environ Health Perspect

DOI: 10.1289/EHP1318

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Supplemental Material

Adipogenic Effects and Gene Expression Profiling of Firemaster® 550 Components in Human Primary Preadipocytes

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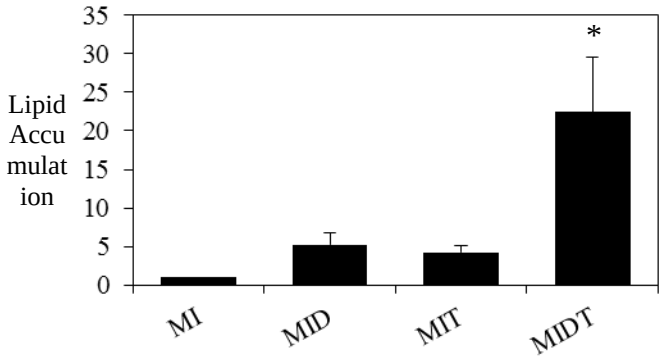
Figure S1. Lipid accumulation in differentiating human preadipocytes treated with MI, MID, MIT, and MIDT positive control conditions. Human primary preadipocytes were induced to differentiate for 14 days in the presence of MI, MI and 1 µM Dexamethasone (MID), MI and 5 µM troglitazone (MIT), or MID with and 5 µM troglitazone (MIDT). At day 14 of differentiation lipid accumulation was quantified by Nile red staining normalized to DAPI. Data from all treatments, normalized to MI control condition are graphically presented as mean ± S.E.M. of 4 separate donor samples. * denotes $p < 0.05$ compared to MI control, as assessed by one-way ANOVA with Tukey's post-hoc tests.

Figure S2. mRNA levels of transcriptional regulators of adipogenesis and adipogenic markers in differentiating human preadipocytes treated with MIDT positive control conditions. Human primary preadipocytes were induced to differentiate for 4, 6, 9, and 12 days in the presence of MID with 5µM troglitazone1 (MIDT). RNA was isolated and the mRNA levels of adipogenic markers, *PPARG* (A), *CEBPA* (B), *FABP4* (C), *LPL* (D), *PLIN1* (E), and *SREBF1* (F) were quantified by real-time qPCR. Levels were normalized to endogenous *ACTB* mRNA, and expressed as a fold over the control condition (MID) for each time point. Results from 5 separate donor samples are graphically presented as means ± S.E.M. * denotes $p<0.05$, ** denotes $p<0.01$, *** denotes $p<0.001$, and **** denotes $p<0.0001$ compared to control, as assessed by one-way ANOVA with Dunnett's post-hoc tests.

Figure S3. Select differentially expressed genes (DEGs) were validated by RT-qPCR in the MIDT positive control conditions. Human primary preadipocytes were induced to differentiate for 4, 6, 9, and 12 days in the presence of MID with 5µM troglitazone1 (MIDT). At day 6 of differentiation RNA was collected for RNA-seq analysis. The mRNA levels of select DEGs from RNA-seq analysis were quantified by RT-qPCR. Levels were normalized to endogenous *ACTB* mRNA, and expressed as a fold over the control condition (MID). Results from 5 separate donor samples are graphically presented as means ± S.E.M. * denotes $p<0.05$, ** denotes $p<0.01$, and ***denotes $p<0.001$ for TPP- and IPTP- treated samples compared to control, as assessed by student's *t*-test analysis.

Table S1. Primers Sequence for RT-qPCR.



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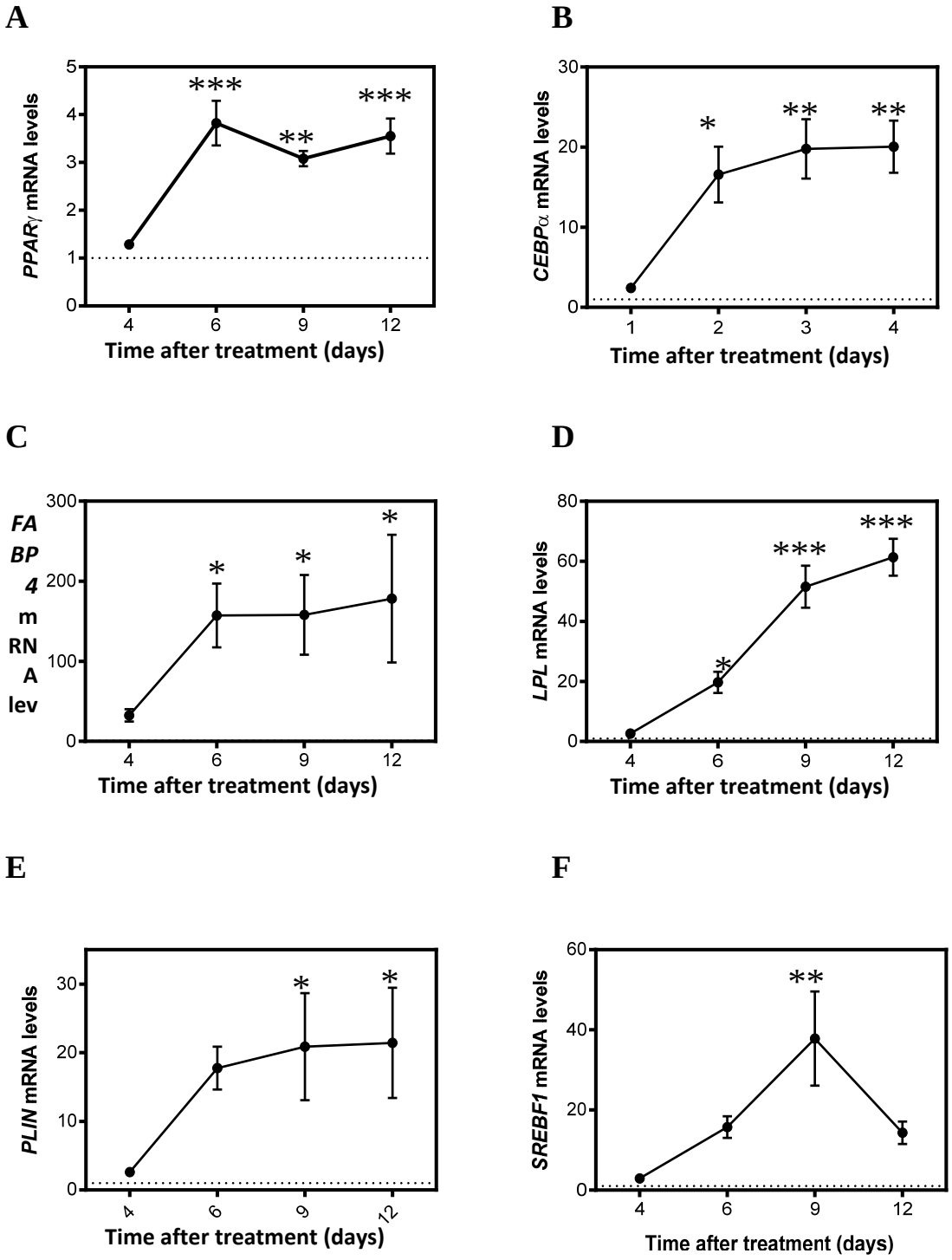

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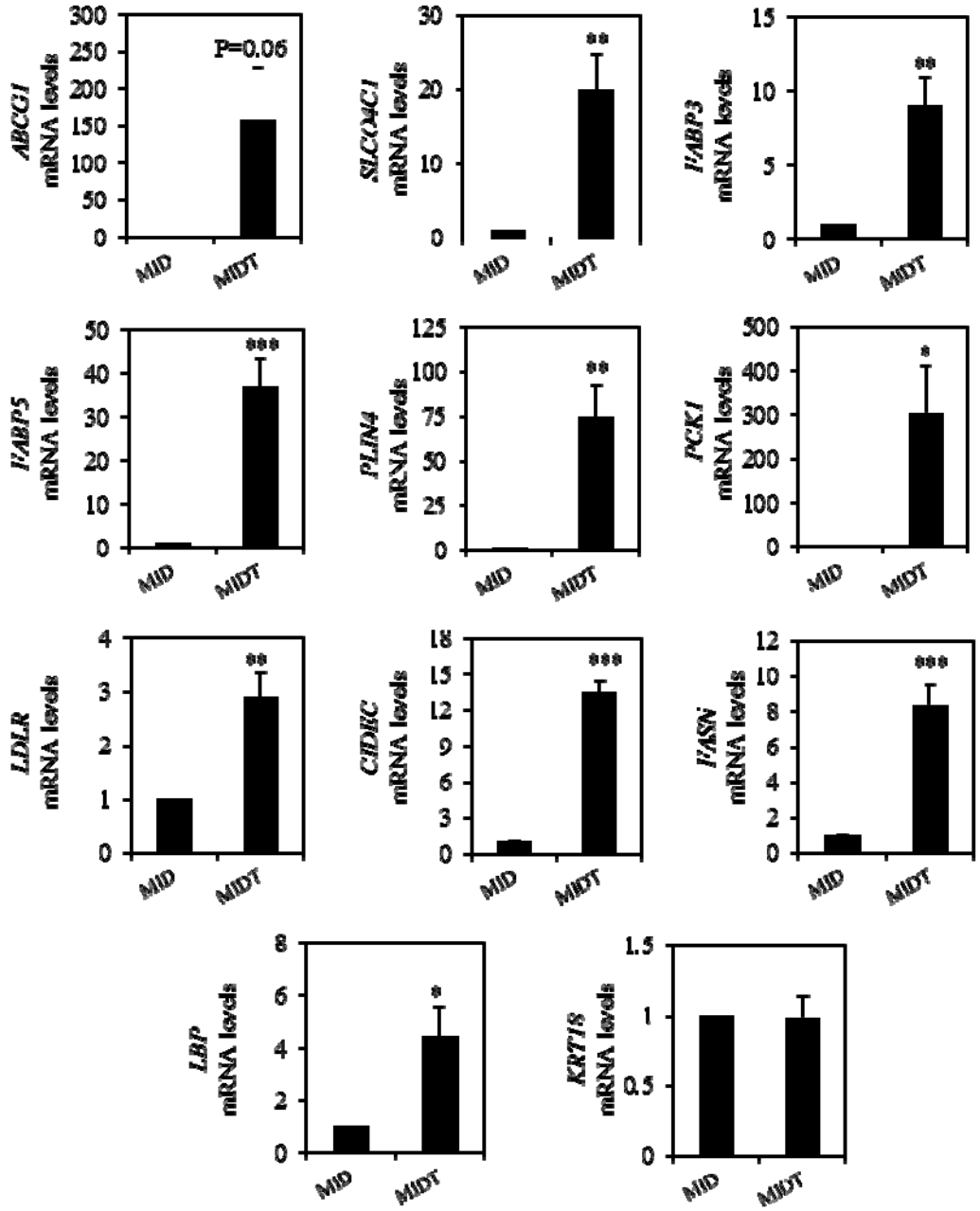

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Target		Primer Sequence
FABP5	Forward	5' CATGAAGGAGCTAGGAGTGGG 3'
	Reverse	5' GCTCTCAGTTTTTATGGTGAGGT 3'
PLIN4	Forward	5' CATTTGAACACGCGGTGAGC 3'
	Reverse	5' GCTGGGCCTTTTCAATCAGC 3'
ACTB	Forward	5' GACTTCGAGCAAGAGATGGC 3'
	Reverse	5' CCAGACAGCACTGTGTTGGC 3'
CEBPA	Forward	5' TGGACAAGAACAGCAACGAG 3'
	Reverse	5' CCATGGCCTTGACCAAGGAG 3
PPARG	Forward	5' TCCGAGGGCCAAGGCTTCAT 3'
	Reverse	5' GCAAACCTGGGCGGTCTCCA 3'
FABP4	Forward	5' CATCAGTGTGAATGGGGATG 3'
	Reverse	5' GTGGAAGTGACGCCTTTCAT 3'
PCK1	Forward	5' ATCCACATCTGTGACGGCTC 3'
	Reverse	5' GGGTCAGTGAGAGCCAACC 3'
LPL	Forward	5' CAGGATGTGGCCCGGTTTAT
	Reverse	5' CGGGGCTTCTGCATACTCAA
PLIN1	Forward	5' CCCAGGAGTGACAGGAATTGT 3'
	Reverse	5' AGGCCTTTGTTGACTGCCAT 3'
SREBF1	Forward	5' GAGCCATGGATTGCACTTTCG 3'
	Reverse	5' AAGAGAGGAGCTCAATGTGGC 3'
CIDEC	Forward	5' AACGCAGTCCAGCTGACAAG 3'
	Reverse	5' CACTGACACATGCCTGGAGA 3'
FASN	Forward	5' CTTCAAGGAGCAAGGCGTGA 3'
	Reverse	5' ACTGGTACAACGAGCGGATG 3'
ABCG1	Forward	5' CTGTCTGATGGCCGCTTTCT 3'
	Reverse	5' CAGGAGGGTCTTGTATCCTTTCT 3'
FABP3	Forward	5' TGGAAGCTAGTGGACAGCAAG 3'
	Reverse	5' GGGTGAGTGTCAGGATGAGT 3'
SLCO4C1	Forward	5' TTGGCTTTTGTCCCCTCCAG 3'
	Reverse	5' TACAATACCTTGCGTGACGG 3'
KRT18	Forward	5' AAATCCGGGAGCACTTGGAG 3'
	Reverse	5' TTTGCGAAGATCTGAGCCCT 3'
LBP	Forward	5' TTCGGTCAACCTCCTGTTGG 3'
	Reverse	5' CGCAAATCCTGCTCTCCAGT 3'
LDL-R	Forward	5' GCTTGTCTGTCACCTGCAAA 3'
	Reverse	5' AACTGCCGAGAGATGCACTT 3'

Table S1: Primers Sequence used for RT-qPCR