

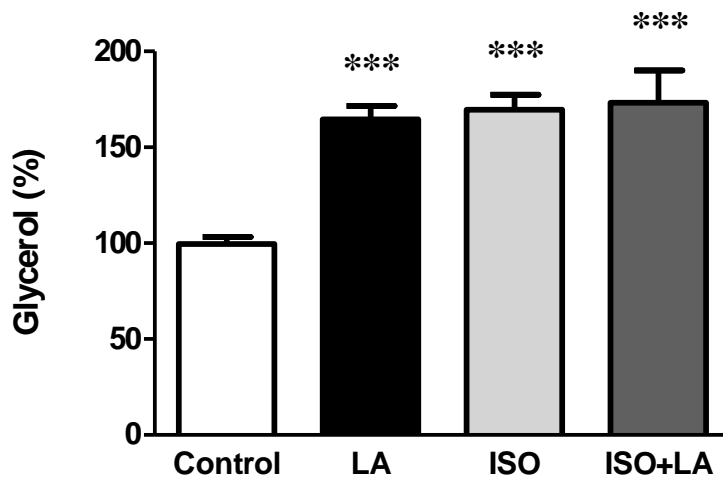
Supplemental data

Effects of lipoic acid on lipolysis in 3T3-L1 adipocytes

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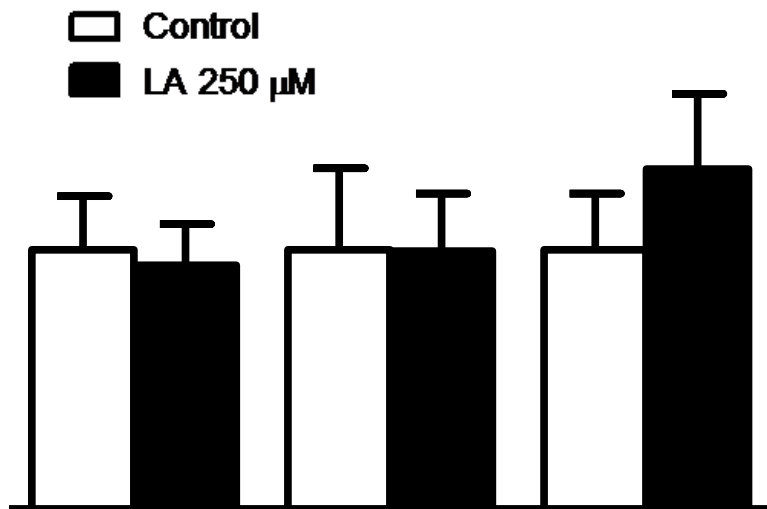
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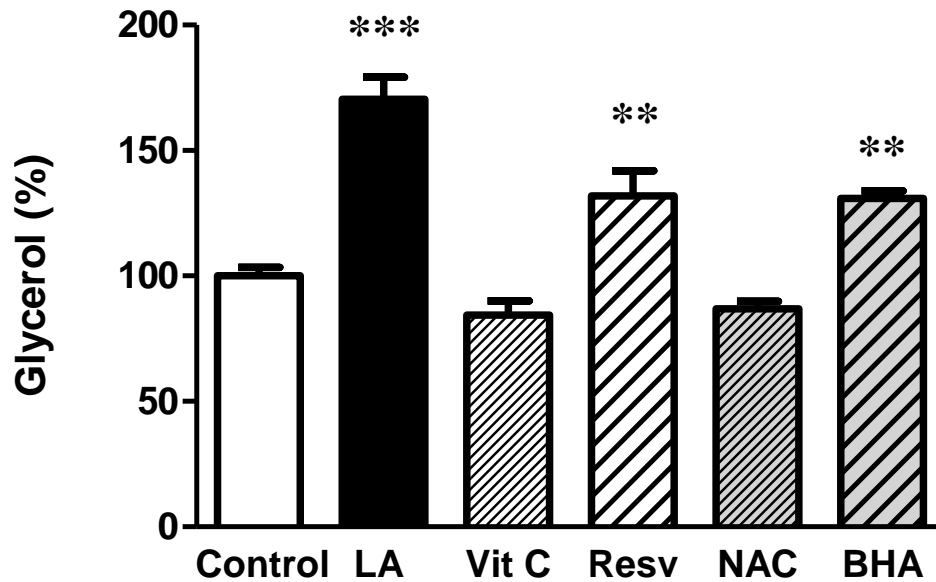
Supplementary Figure I. LA stimulates basal but not isoproterenol-induced lipolysis.

Differentiated 3T3-L1 adipocytes were treated with LA (250 μ M) in the absence or presence of isoproterenol (10^{-6} M) for 24 h. Lipolysis was estimated by measuring the amount of glycerol released into media. Data are means \pm SE of 4 independent experiments.

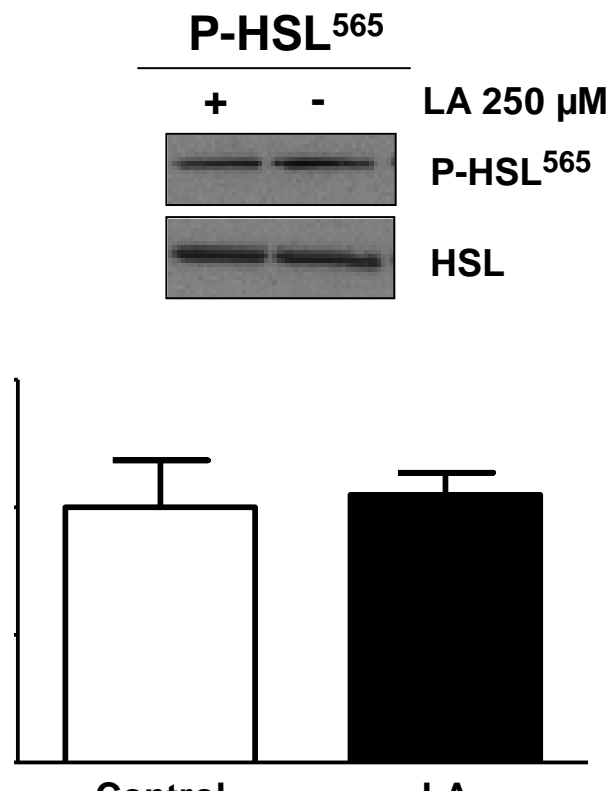
*** P <0.001 vs. Control (vehicle-treated cells).



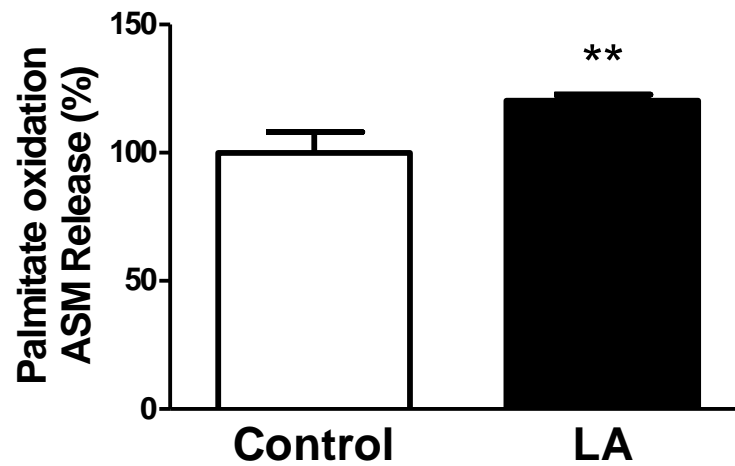
Supplementary Figure II. LA treatment does not modify adipocyte differentiation markers in mature 3T3-L1 adipocytes. Differentiated 3T3-L1 adipocytes were treated with LA (250 μ M) for 24 h. mRNA levels of several adipogenic factors (PPAR γ , C/EBP α and C/EBP β) were determined by RT-PCR. Data are means \pm SE of 5 independent experiments.



Supplementary Figure III. Differential effects on lipolysis of several antioxidants. The amount of glycerol released into media was determined in fully differentiated 3T3-L1 adipocytes treated with LA (250 μ M), Vit C (250 μ M), resveratrol (50 μ M), NAC (20 mM) and BHA (10 mM) during 24 h. Data are means \pm SE of at least 3 independent experiments. ** $P < 0.01$ and *** $P < 0.001$ vs. Control (vehicle-treated cells).



Supplementary Figure IV. LA does not phosphorylate HSL at Ser⁵⁶⁵. Representative Western blots for Ser⁵⁶⁵-phosphorylated HSL and total HSL in differentiated 3T3-L1 adipocytes treated with LA (250 μ M) for 1 h. Band intensities were normalized to total HSL.



Supplementary Figure V. LA increases palmitate oxidation to acid-soluble metabolites.

Fatty acid oxidation was estimated as ^{14}C -labeled palmitate oxidation to acid-soluble metabolites (ASM) in 3T3-L1 adipocytes treated for 6 h with or without LA (250 μM) in DMEM containing 2.5% BSA, 200 μM L-carnitine, 200 μM cold palmitic acid and 200 μM [$^{14}\text{C}(\text{U})$] palmitate (0.1 $\mu\text{Ci}/\text{mL}$). The value of a vehicle control was set at 100% and the relative value was presented as fold induction with respect to that of the vehicle control. Data are means \pm SE of 6 independent experiments. ** $P < 0.01$.