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| Title | Lorenzo's oil inhibits ELOVL1 and lowers the level of sphingomyelin with a saturated very long-chain fatty acid |
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Supplemental MATERIALS AND METHODS

**RT-PCR**

The total RNA was extracted from HeLa cells, untreated or treated with Lorenzo's oil, using NucleoSpin RNA II kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. *ELOVL1* and *GAPDH* cDNAs were amplified from the total RNA using SuperScript One-Step RT-PCR with Platinum *Taq* kit (Life Technologies, Carlsbad, CA) following the manufacturer's instructions. The primers for *ELOVL1* and *GAPDH* were described previously (1).

Supplemental REFERENCES

1. Ohno, Y., S. Suto, M. Yamanaka, Y. Mizutani, S. Mitsutake, Y. Igarashi, T. Sassa, and A. Kihara. 2010. ELOVL1 production of C24 acyl-CoAs is linked to C24 sphingolipid synthesis. *Proc. Natl. Acad. Sci. U. S. A.* **107:** 18439-18444.

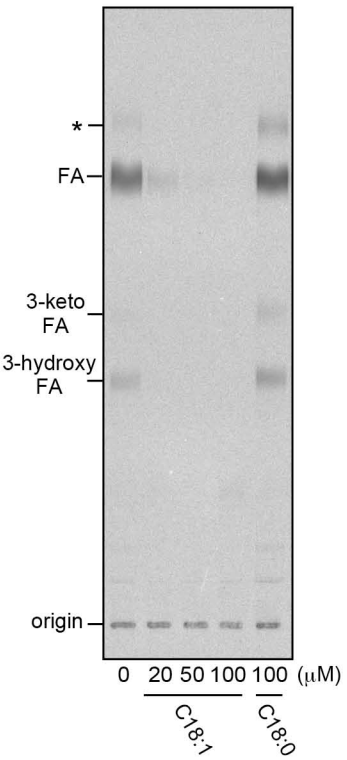



**Fig. S1. Stearic acid does not inhibit ELOVL1 activity.** The total membrane fraction (10 μg protein) of HEK 293T cells transiently expressing the WT 3xFLAG-ELOVL1 protein was incubated with 50 μM C22:0-CoA and 0.075 μCi (27.3 μM) [2-$^{14}$C]malonyl-CoA at 37 °C for 30 min in the presence of the indicated concentration of stearic acid (C18:0), oleic acid (C18:1), or ethanol alone (vehicle control). Each FA was dissoloved in 2 μl ethanol and used in the assay. After a sequential work-up (see Materials and Methods), lipids were separated by normal-phase TLC and detected by a bioimaging analyzer BAS-2500. *, a possible decarboxylation product of 3-keto-FA formed during saponification.

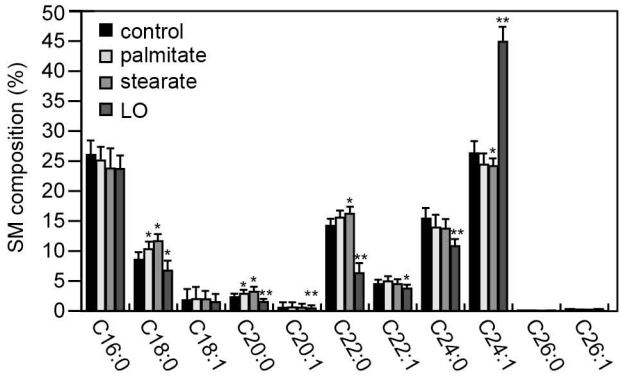



**Fig. S2. Neither palmitic nor stearic acid reduces the cellular level of SM with a saturated VLCFA.** HeLa-*ELOVL1* cells were treated with palmitic acid (C16:0), stearic acid (C18:0), or a 4:1 mixture of oleic (C18:1) and erucic (C22:1) acids at a concentration of 20 μM. A proper amount of FA or FA mixture was dissolved in 2 μl ethanol and added to the culture media. Control cells received an equal volume of ethanol only. Cells were cultured for 6 days, during which the media were replaced every 2 days with fresh media containing either the corresponding FA or ethanol only. Lipids were extracted from the cells and sphingomyelin (SM) composition was determined by UPLC-MS on a Xevo G2 QTof LC/MS system. The data were analyzed by MassLynx software. Each bar represents the percent of the amount of each SM species relative to the total amount of SM and the mean ± SD from three independent assays. Statistically significant differences from vehicle (ethanol)-treated control cells are indicated (*$p < 0.05$ and **$p < 0.01$, Student's t-test). LO, 4:1 mixture of oleic and erucic acids.

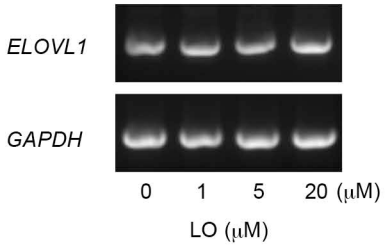



**Fig. S3. A 4:1 mixture of oleic and erucic acids does not alter the level of *ELOVL1* mRNA.** HeLa cells were treated with an ethanol solution of a 4:1 mixture of oleic and erucic acids in a total concentration of 1, 5, or 20 μM. Control cells received an equal volume of ethanol only. Cells were cultured for 6 days, during which the media were replaced every 2 days with fresh media containing either the 4:1 mixture in ethanol or ethanol only. The total RNA was prepared from the cells and subjected to RT-PCR using primers specific for either *ELOVL1* or *GAPDH*. LO, 4:1 mixture of oleic and erucic acids.