

**COMT-catalyzed palmitic acid methyl ester biosynthesis in perivascular adipose tissue
and its potential role against hypertension**

Chin-Hung Liu, Hao-Jen Hsu, Tzu-Ling Tseng, Tsung-Jen Lin, Wei-Hsiang Weng,

Mei-Fang Chen* and Tony Jer-Fu Lee*

Journal of Pharmacology and Experimental Therapeutics

Supplementary Figure 1. Ion chromatogram and mass spectra of [¹³C₁₆]-PAME in GC-MS analysis. (A) In the chromatogram, peak 1, which represents PAME, and peak 2, which represents [¹³C₁₆]-PAME, have identical retention time ($t_R = 8.33$ min). (B) The characteristic molecular ion ($[M]^+$) of PAME in the mass spectrum is 270 of m/z and of [¹³C₁₆]-PAME ($= [M]^+ + 16$) is 286 of m/z . Pentadecanoic acid methyl ester was used as internal standard (IS). PAME, palmitic acid methyl ester; PA, palmitic acid; GC-MS, gas chromatography-mass spectrometry.

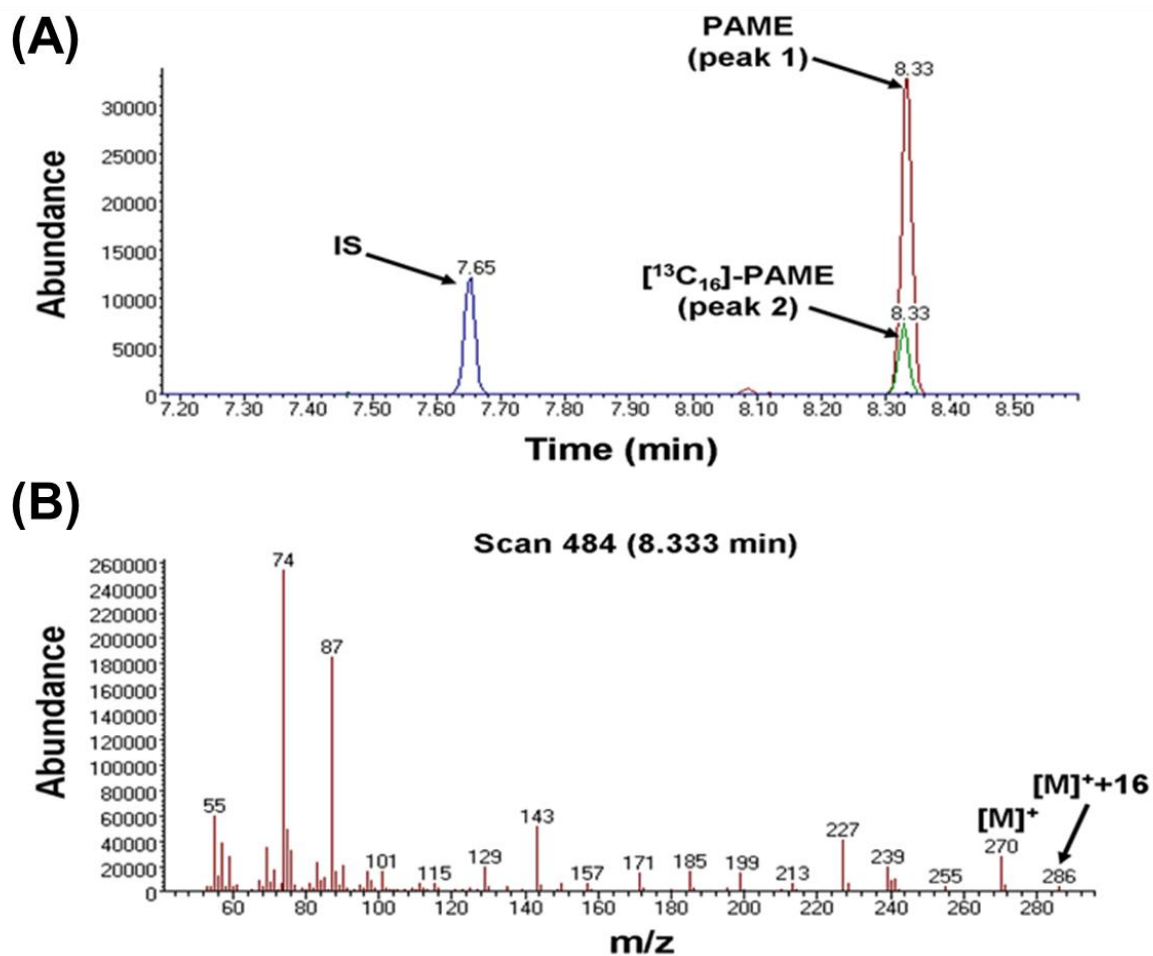
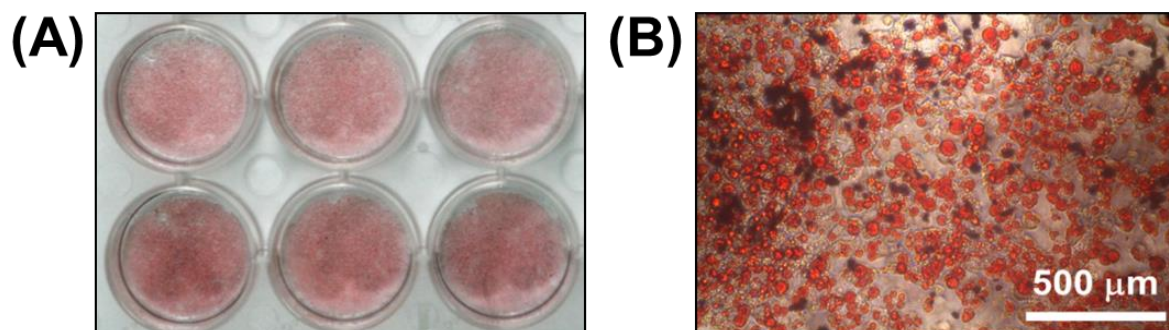
Supplementary Figure 2. Oil Red O staining of lipid droplets in differentiated 3T3-L1 adipocytes. (A) 3T3-L1 adipocytes were stained with Oil Red O, after the differentiation of 3T3-L1 cells were induced for 6 days and subsequently maintained for additional 4 days. The red color indicates lipid droplets in the adipocytes. (B) A enlarged image from plot A.

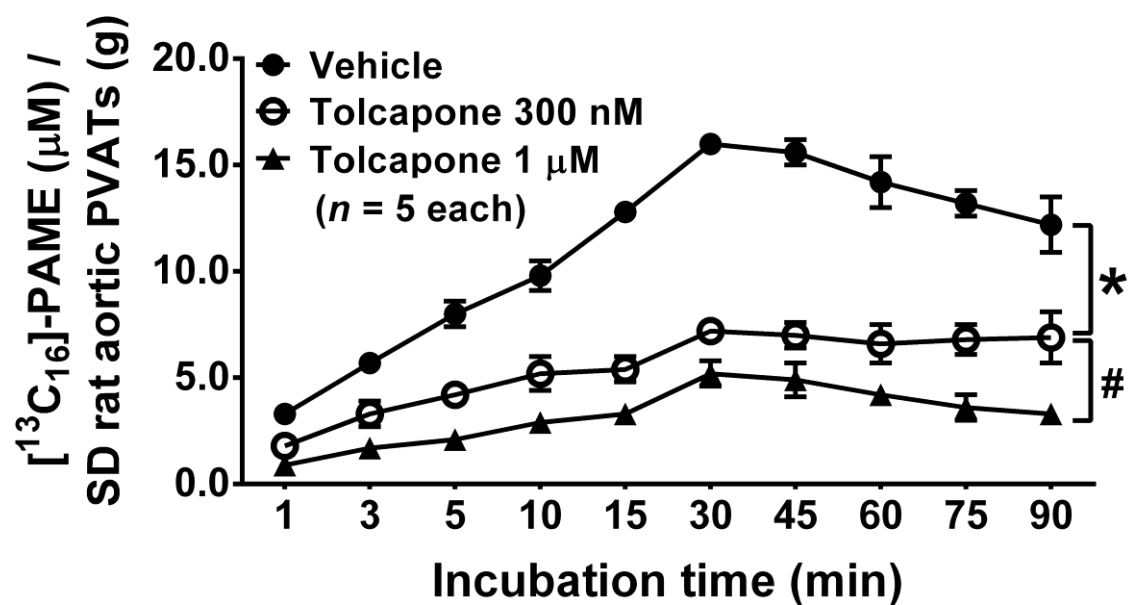
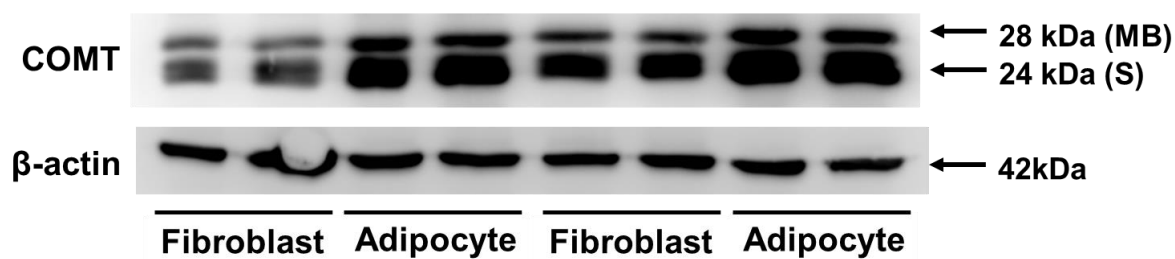
Supplementary Figure 3. Concentration-dependent decrease in PAME biosynthesis in isolated aortic PVAT of SD rats in the presence of 300 nM or 1 μ M tolcapone pre-incubation. PAME, palmitic acid methyl ester; SD, Sprague Dawley; PVAT, perivascular adipose tissue.

Supplementary Figure 4. Whole uncropped images of original western blots from Figure 2D ($n = 4$).

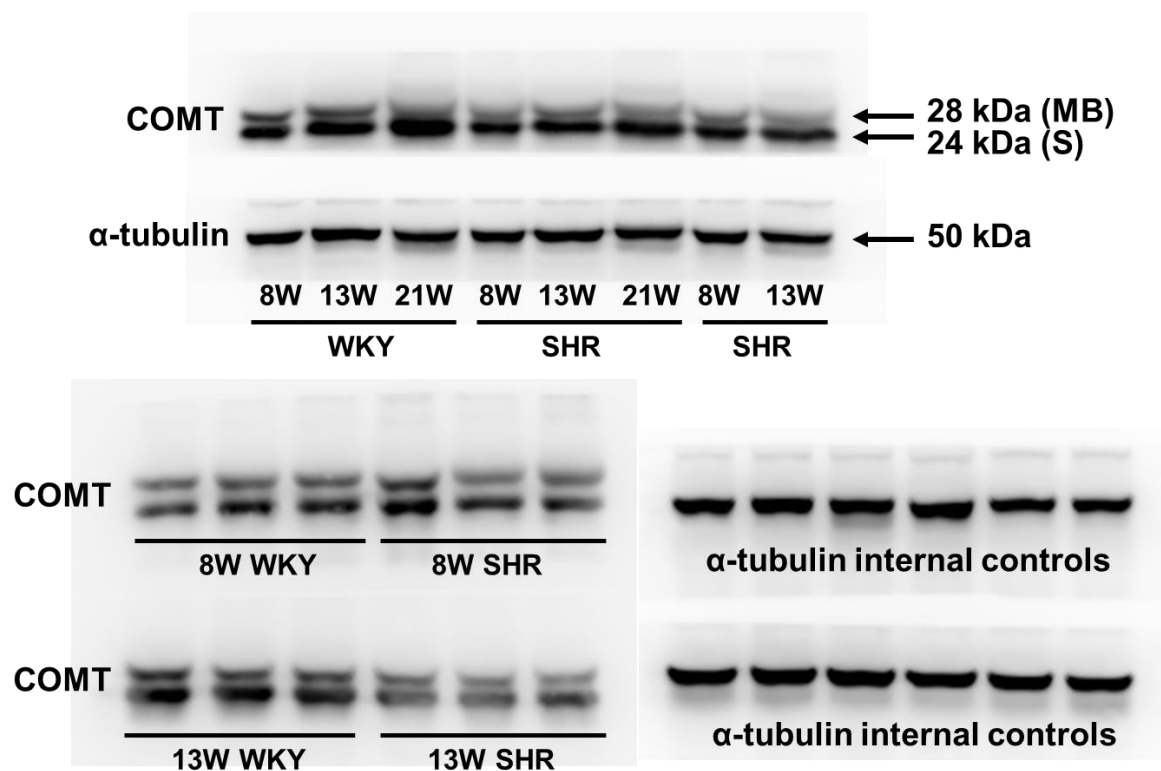
Supplementary Figure 5. Whole uncropped images of original western blots from Figure 5C ($n = 4-5$).

Supplementary Figure 6. Hematoxylin and eosin Y-stained cross-sections of aortic (A, C) and mesenteric PVATs (B, D). PVATs were harvested from a Sprague Dawley rat. The specimens were examined under a light microscope with magnification at 100X (A and B) and 400X (C and D). Scale bars indicate 200 μ m in (A and B), and 50 μ m in (C and D), respectively. PVAT, perivascular adipose tissue.

Supplementary Figure 1—Chin-Hung Liu *et al.*Supplementary Figure 2—Chin-Hung Liu *et al.*

Supplementary Figure 3—Chin-Hung Liu *et al.*Supplementary Figure 4—Chin-Hung Liu *et al.*

Supplementary Figure 5—Chin-Hung Liu *et al.*



Supplementary Figure 6—Chin-Hung Liu *et al.*

