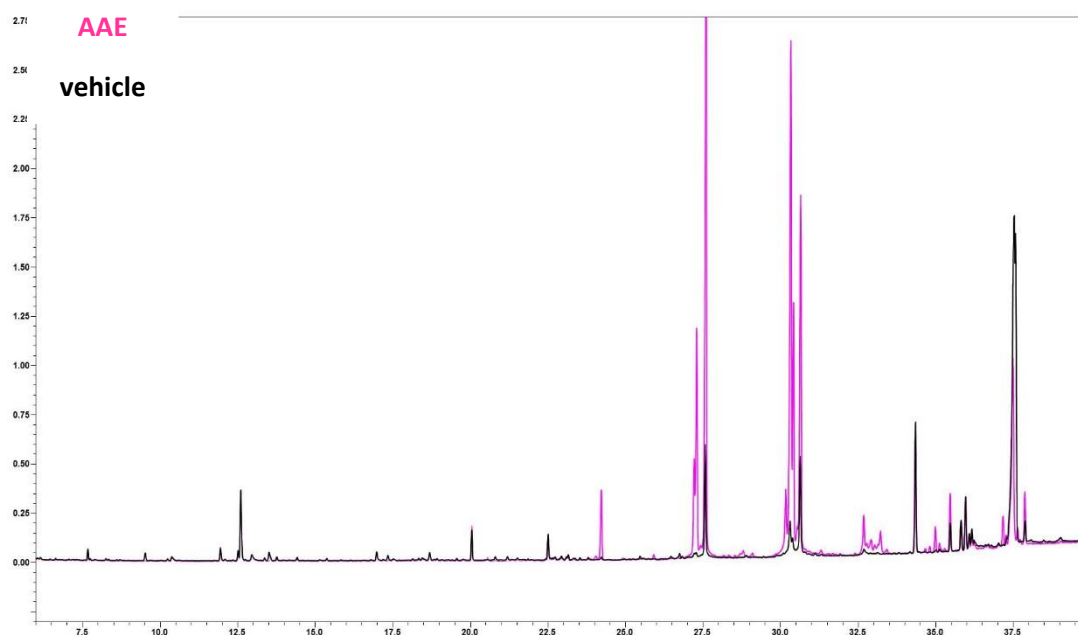


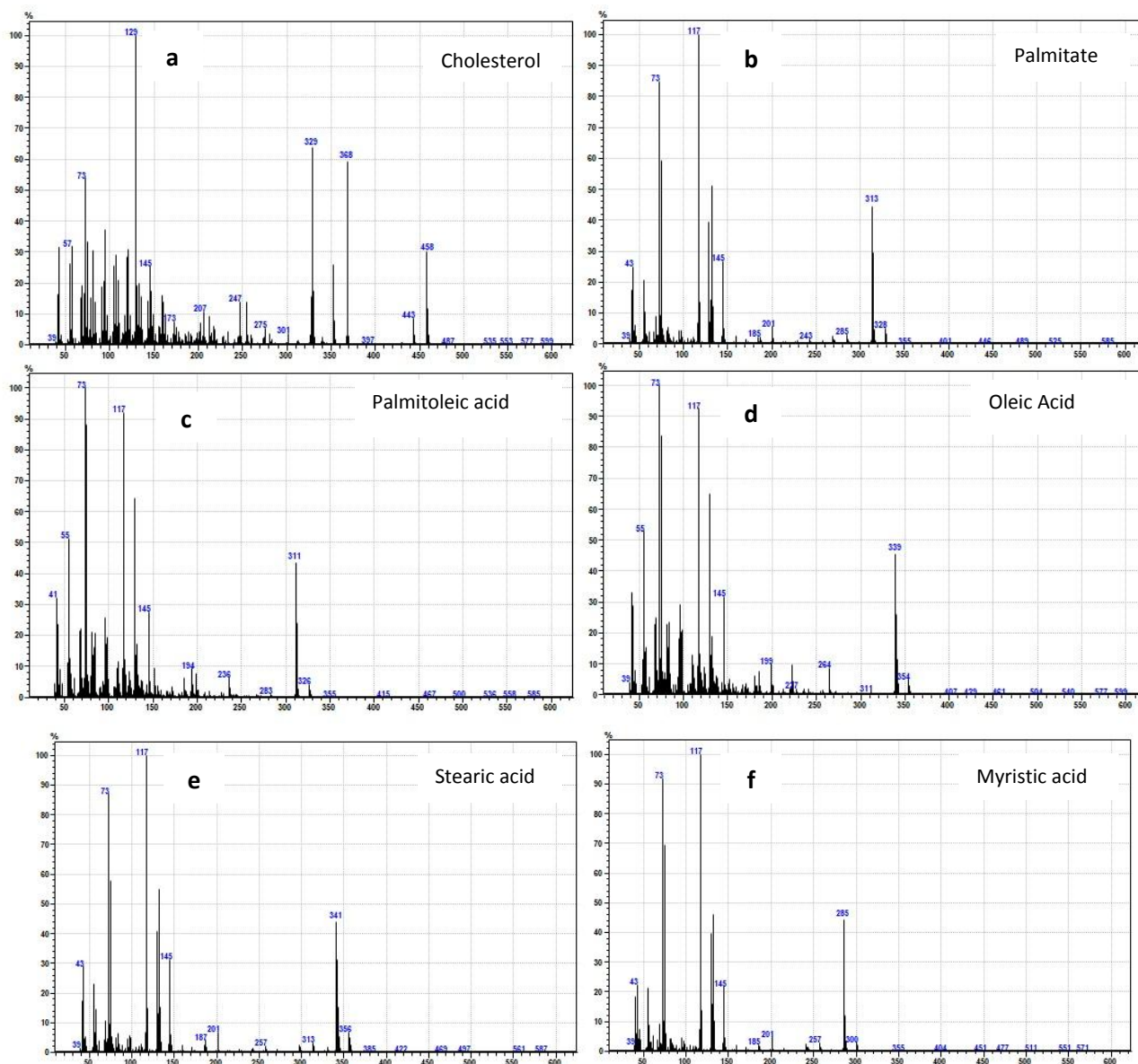
Supplementary material for the manuscript

# A Boost in Mitochondrial Activity Underpins the Cholesterol-Lowering Effect of Annurca Apple Polyphenols on Hepatic Cells

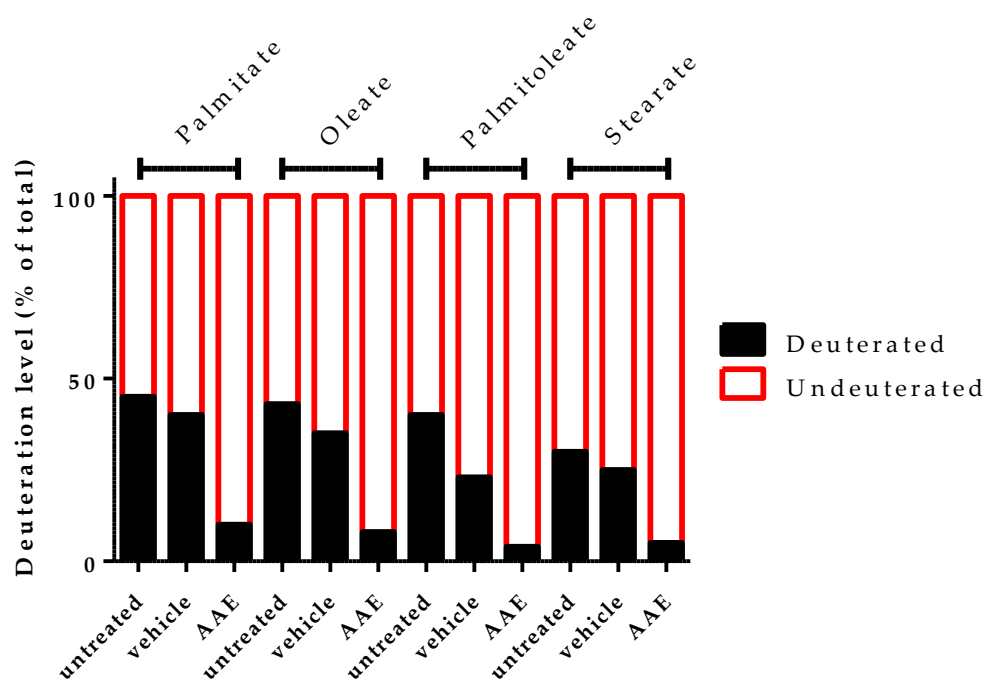
Eduardo Sommella, Nadia Badolati, Gennaro Riccio, Emanuela Salviati, Sara Bottone, Monica Dentice, Pietro Campiglia, Gian Carlo Tenore, Mariano Stornaiuolo and Ettore Novellino



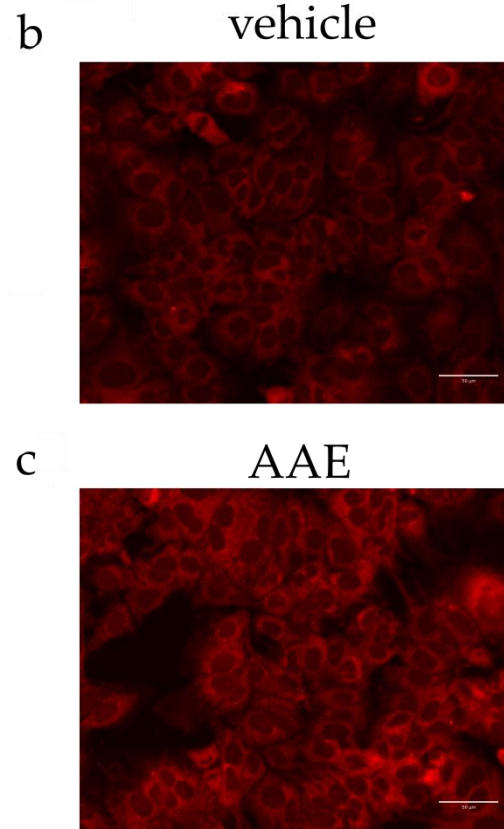
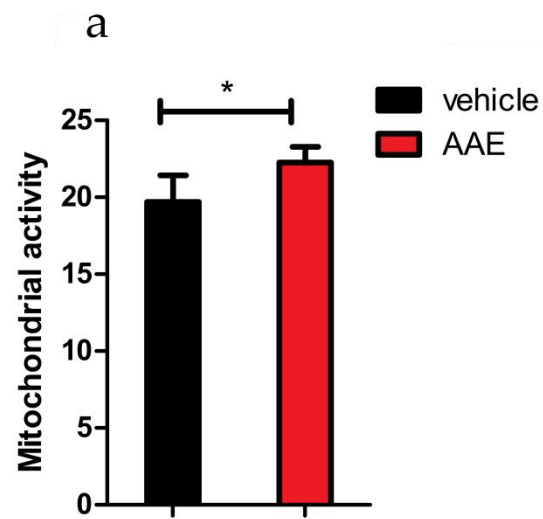
**Figure S1.** GC-MS total ion chromatogram (AAE (purple) vs Vehicle (black)).



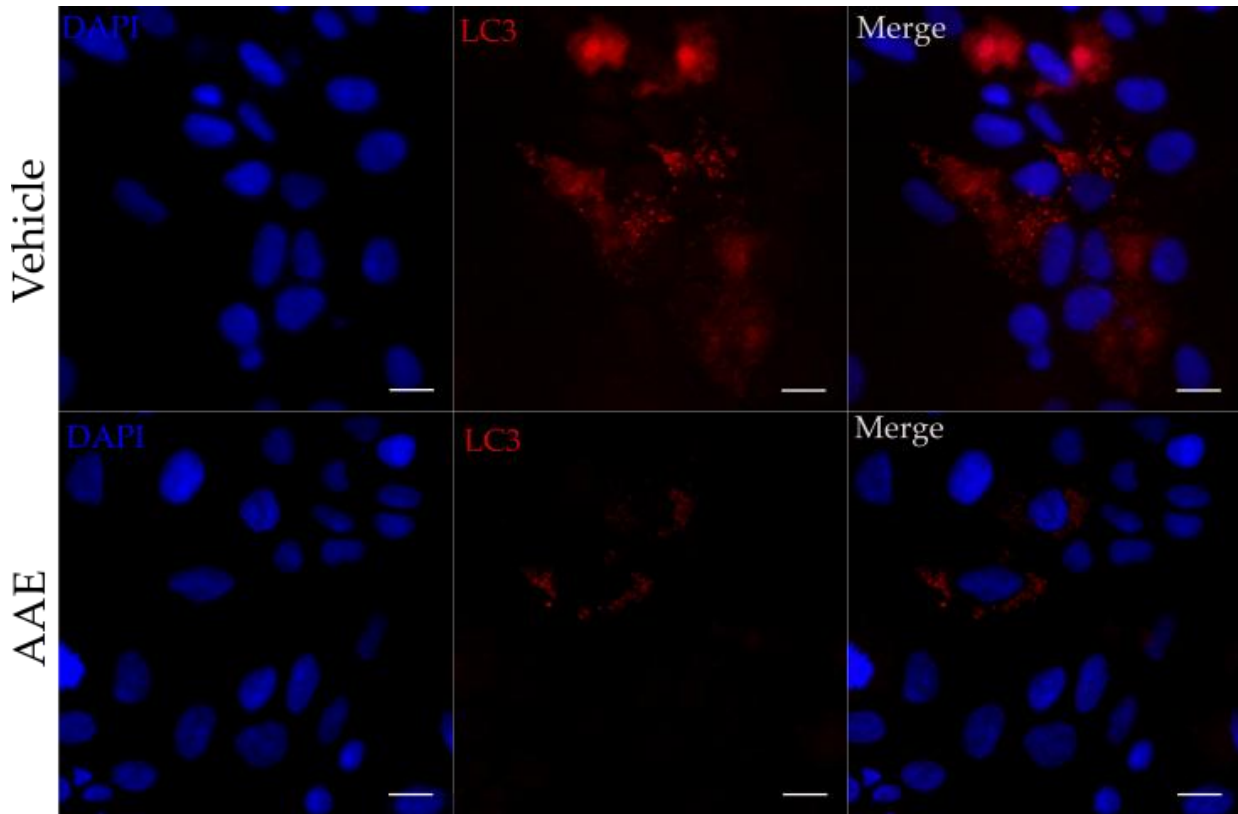
**Figure S2:** Mass spectra and retention time (RT) of: **a**) cholesterol ( RT 37.5 min, (m/z 458, 368, 329)), **b**) palmitate (RT 27.6 min, (m/z 313, 117, 129, 328)), **c**) palmitoleic acid ((RT 27.3 (m/z 311, 326, 129, 117 and 236)), **d**) oleic acid ((RT 30.3 min m/z 354,339,236,129,117)) **e**) stearic acid (RT 30.6, (m/z 356,341,132,117)) and **f**) myristic acid (RT 24.2 (m/z 285,117,132)).



**Figure S3.** AAE inhibits lipogenesis in HuH7 cells. HuH7 cells were grown in the presence of D<sub>2</sub>O and treated for 72 h in the presence 400 mg/L AAE, of the corresponding volume of DMSO (vehicle) or of water (untreated). Deuterium level (relative intensity (% of total)) of chemical species presenting masses heavier than those naturally occurring for the indicated FA species. See Figure 1 for further details.



**Figure S4:** AAE increases mitochondrial membrane potential in HuH7. Cells treated with AAE or with vehicle were incubated with Mitotracker CMX-ROS. **a)** Quantitation of fluorescence intensity of Mitotracker in HuH7 treated with AAE or with vehicle. \*  $p < 0.05$ . **b-c)** Immunofluorescence analysis showing increased fluorescence emission of Mitotracker CMX-ROS in HuH7 cells treated with AAE. Scale bars in **b** and **c** correspond to 50  $\mu\text{m}$



**Figure S5.** AAE does not induce autophagy in HuH7 cells. Non-confocal immunofluorescence showing LC3B positive punctuated structure in HuH7 cells grown in presence of AAE (400 mg/L) or the corresponding volume of vehicle (DMSO) for 72h. Cells were immuno-stained with an anti-LC3 antibody (red channel) to identify autophagosomes and with DAPI to reveal nuclei ( blue channel). Scale bars correspond to 10  $\mu$ m.

**Table S1.** Metabolites of HuH7 cells identified by DI-FT-ICR-MS and discussed in this manuscript.

Metabolite	m/z	Ionization	Error (ppm)
Glucose		[M-H] <sup>-</sup>	-0.958
Lactate	113.02091	[M+Na] <sup>+</sup>	-0.176
Maltose	341.1089	[M-H] <sup>-</sup>	-0.894
Glucose 6-P	259.0222	[M-H] <sup>-</sup>	-0.857
Proline	138.05254	[M+Na] <sup>+</sup>	-0.936
Threonine	142.04746	[M+Na] <sup>+</sup>	-0.801
Glutamine	169.05836	[M+H] <sup>-</sup>	-0.845
Lysine	147.11280	[M+H] <sup>+</sup>	-0.708
Histidine	156.07675	[M+H] <sup>+</sup>	-0.980
Cysteine	120.01247	[M-H] <sup>-</sup>	-0.774
Tryptophan	203.08260	[M-H] <sup>-</sup>	-0.695
Taurine	124.00738	[M+H] <sup>-</sup>	-0.948
Creatine	154.0586	[M+Na] <sup>+</sup>	-0.890
Glutamic Acid	170.04237	[M+Na] <sup>+</sup>	-0.931
Leucine	132.10190	[M+H] <sup>+</sup>	-0.947
Tyrosine	180.06631	[M+H] <sup>-</sup>	-0.703
Phenylalanine	188.06819	[M+Na] <sup>+</sup>	-0.953
Aspartic Acid	132.03023	[M-H] <sup>-</sup>	-0.918
$\alpha$ - GPC	257.23931	[M-H] <sup>-</sup>	0.987
Ribose 5-P	229.01187	[M-H] <sup>-</sup>	-0.905
Sedoheptulose	233.0631735	[M+Na] <sup>+</sup>	-0.958
Sedoheptulose 7-P	289.0327	[M-H] <sup>-</sup>	-0.934
NADP	663.1049	[M-H] <sup>-</sup>	-0.832
Xanthine	153.04010	[M+H] <sup>+</sup>	0.975
Adenosine	290.08597	[M+Na] <sup>+</sup>	-0.981
Cytidine	282.04867	[M+K] <sup>+</sup>	-0.957
Guanosine	322.05482	[M+K] <sup>+</sup>	-0.956
Inosine	291.06999	[M+Na] <sup>+</sup>	-0.962
Deoxy-Inosine	290.08570	[M+K] <sup>+</sup>	0.968
GSH	306.07652	[M-H] <sup>-</sup>	-0.783
Propionylcarnitine	218.13868	[M+H] <sup>+</sup>	-0.983
Butyrylcarnitine	232.15433	[M+H] <sup>+</sup>	-0.924
Valerylcarnitine	246.16998	[M+H] <sup>+</sup>	-0.709
Citrate	191.0197	[M-H] <sup>-</sup>	-0.974
Fumarate	115.00368	[M-H] <sup>-</sup>	-0.801
Malate	133.01424	[M-H] <sup>-</sup>	-0.878