

FISETIN AND CMS121 ATTENUATE NEURONAL LIPID DYSHOMEOSTASIS AND DEGENERATION UPON PALMITIC ACID EXPOSURE IN DIFFERENTIATED SH-SY5Y CELLS

Débora Mena^{1,2,3,4#}, Katarzyna Michalik^{1,2#}, Carla Sequeira^{1,2}, José Teixeira^{3,5,6}, Teresa Rodrigues^{2,3}, Paulo J. Oliveira^{3,5}, João O. Malva^{1,2,3}, Ana I. Duarte^{1,2,3,6*}

ePOSTER #9201

¹Institute of Pharmacology and Experimental Therapeutics, Faculty of Medicine. ²CiBR - Coimbra Institute for Clinical and Biomedical Research, Faculty of Medicine. ³CiBB - Center for Innovative Biomedicine and Biotechnology. ⁴Institute for Interdisciplinary Research (IIIUC) Doctoral Programme in Experimental Biology and Biomedicine (PDBEB). ⁵CNC-UC – Center for Neuroscience and Cell Biology. ⁶Life Sciences Department, Faculty of Sciences & Technology, University of Coimbra, Coimbra, Portugal. [#]Both authors contributed equally. ^{*}Presenting author.

Corresponding author: Ana I. Duarte, aimduarte@uc.pt.



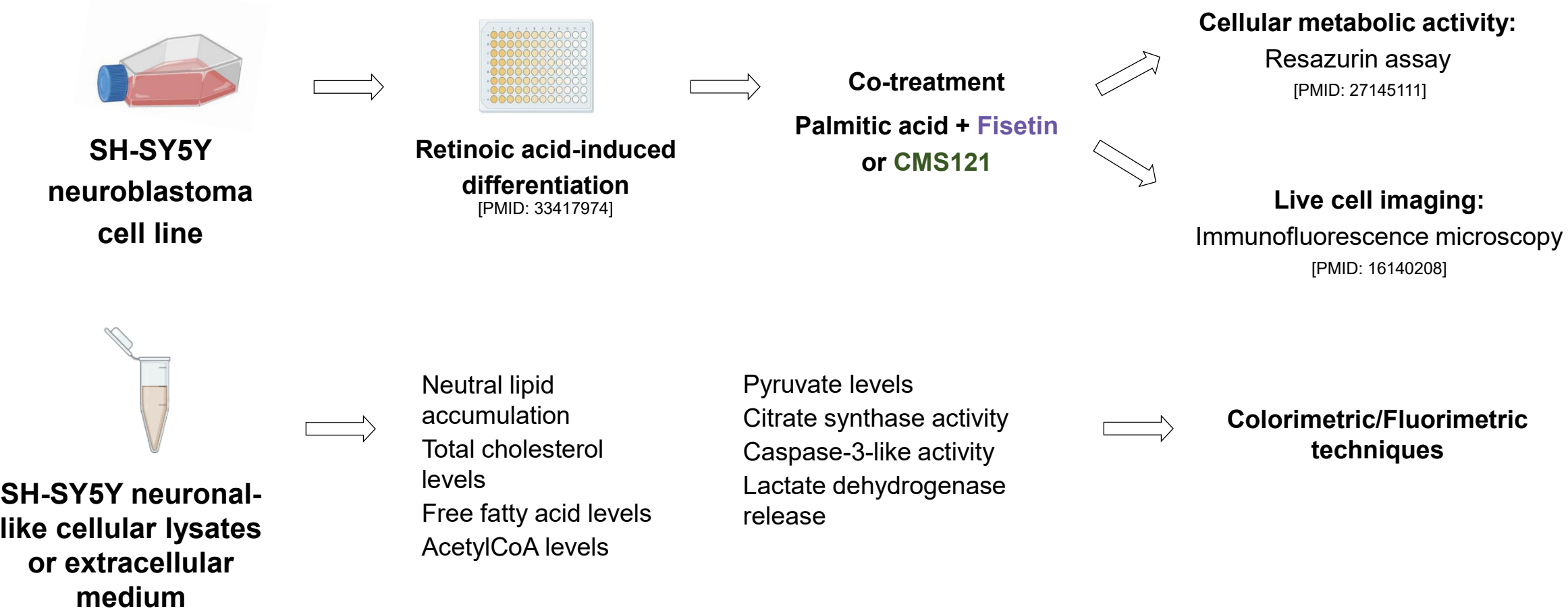
INTRODUCTION

Stroke is a leading cause of morbidity and mortality worldwide, frequently resulting in persistent physical and cognitive impairment [PMID: 36127392]. This burden is exacerbated by **cardiometabolic risk factors** - such as obesity, dyslipidemia, type 2 diabetes, and hypertension – which are often associated with unhealthy hypercaloric diets rich in saturated fats, particularly **palmitic acid** [PMID: 30355080]. This fatty acid has been shown to induce brain metabolic dysfunction, oxidative stress, and neuroinflammation [PMID: 37291105]; however, the underlying mechanisms remain poorly understood.

Healthier dietary patterns, such as the **Mediterranean diet (MedDiet)** – characterized by high intake of plant-based foods and olive oil, and low consumption of red meat and saturated fats - may counteract cardiometabolic-related brain damage, including that associated with stroke [PMIDs: 40657695, 40128348]. Among MedDiet-related bioactive compounds, the flavonol **Fisetin** [PMID: 39004102] and its synthetic derivative **CMS121** [PMID: 39422940] have been suggested to exert neuroprotective effects. However, their potential to mitigate brain injury under cardiometabolic risk (and stroke) conditions remains to be fully elucidated.

Hence, we **aimed** to investigate whether **Fisetin** and **CMS121** could protect against **palmitic acid**-induced lipid dysregulation, neurodegeneration and death in human-derived SH-SY5Y neuronal-like cells.

METHODOLOGY



RESULTS

1. Effect of Fisetin and CMS121 in SH-SY5Y neuronal-like cellular metabolic activity under palmitic acid exposure

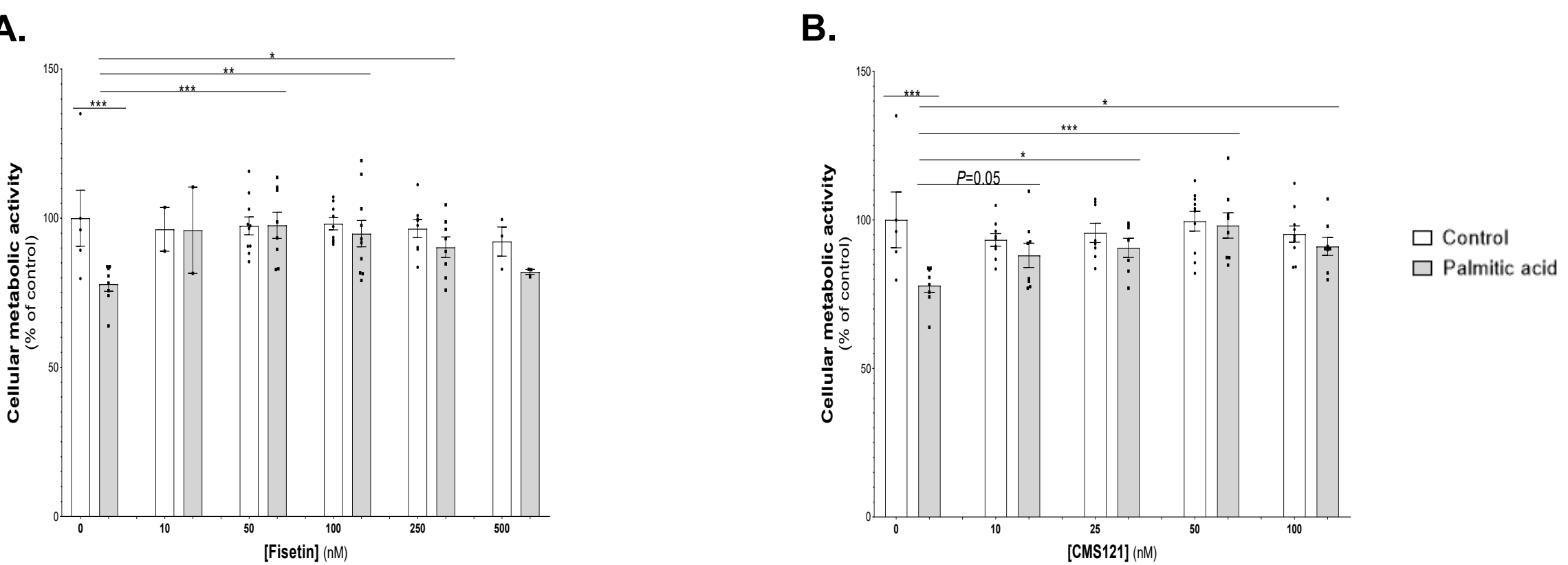


Figure 1. Effect of increasing concentrations of Fisetin and CMS121 on the cellular metabolic activity of differentiated SH-SY5Y neuronal-like cells treated with palmitic acid, as given by the resazurin assay. Results are presented as % of control (Control=100%). Data are mean $\pm$ SEM of 2-10 independent experiments. Statistical significance: *P<0.05, **P<0.01, ***P<0.001, by the one-way ANOVA test, followed by the DMS *post-hoc* test (Gaussian distribution).

2. Fisetin and CMS121 exposure attenuates lipid dyshomeostasis and improves late glycolytic and TCA cycle markers in SH-SY5Y neuronal-like cells under palmitic acid

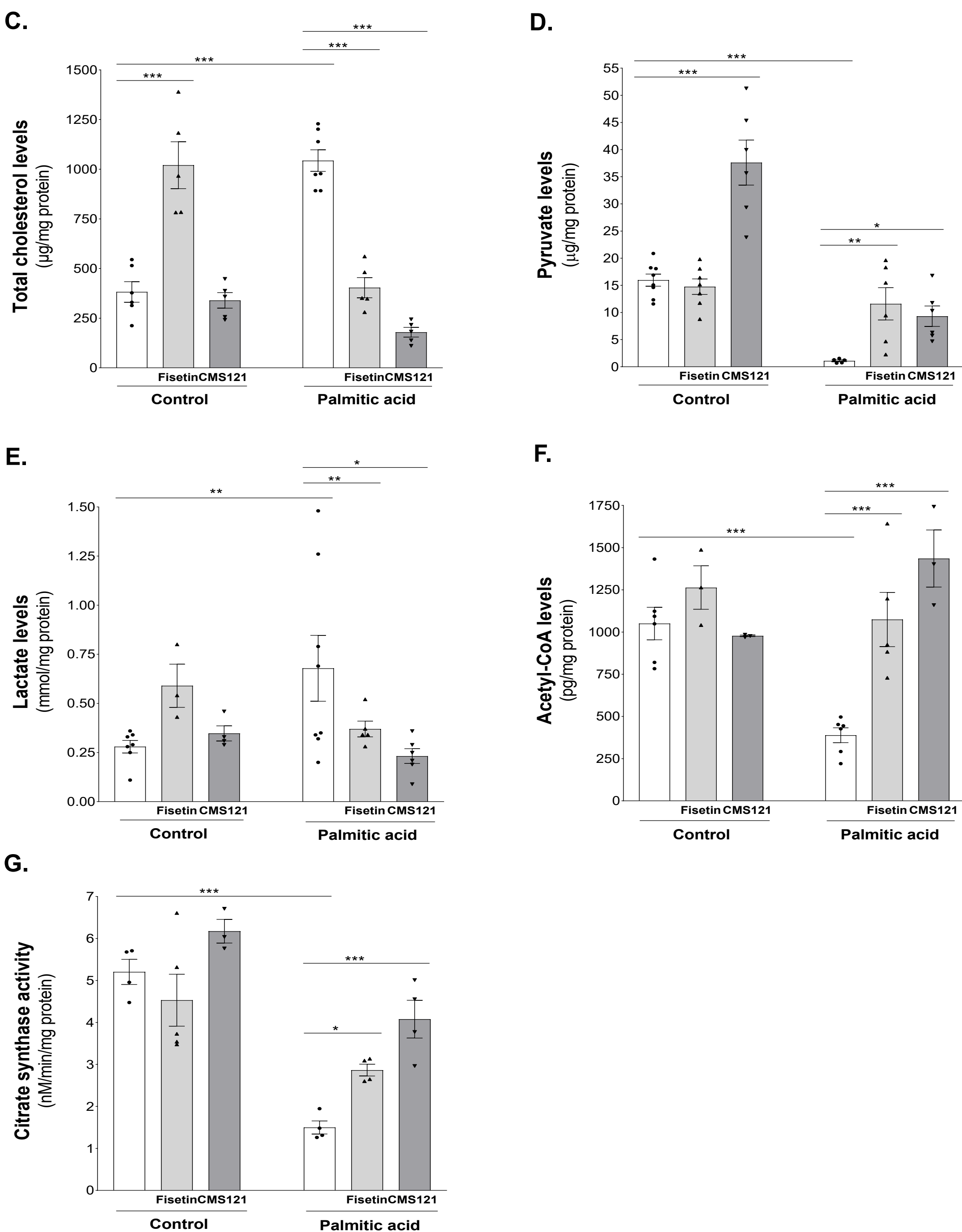
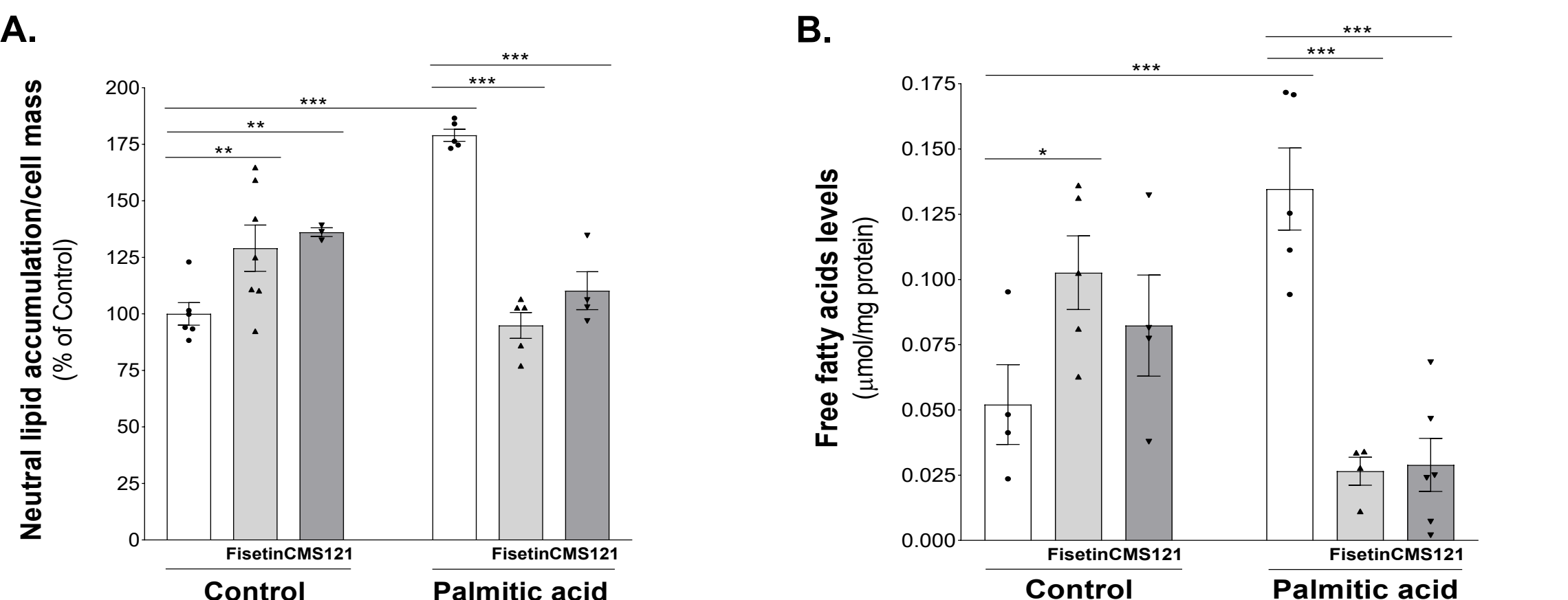


Figure 2. Effect of Fisetin and CMS121 on neutral lipid accumulation (A), and on the levels of the metabolic markers, free-fatty acids (B), total cholesterol (C), pyruvate (D), lactate (E), acetyl-CoA (F), alongside the activity of citrate synthase (G), after exposure of differentiated SH-SY5Y neuronal-like cells to palmitic acid, as given by colorimetric and fluorimetric assays. Data are mean $\pm$ SEM of 3-8 independent experiments. Statistical significance: *P<0.05, **P<0.01, ***P<0.001, by the one-way ANOVA test, followed by the DMS *post-hoc* test (Gaussian distribution).

3. Fisetin and CMS121 exposure protects against cell death markers in SH-SY5Y neuronal-like cells under palmitic acid

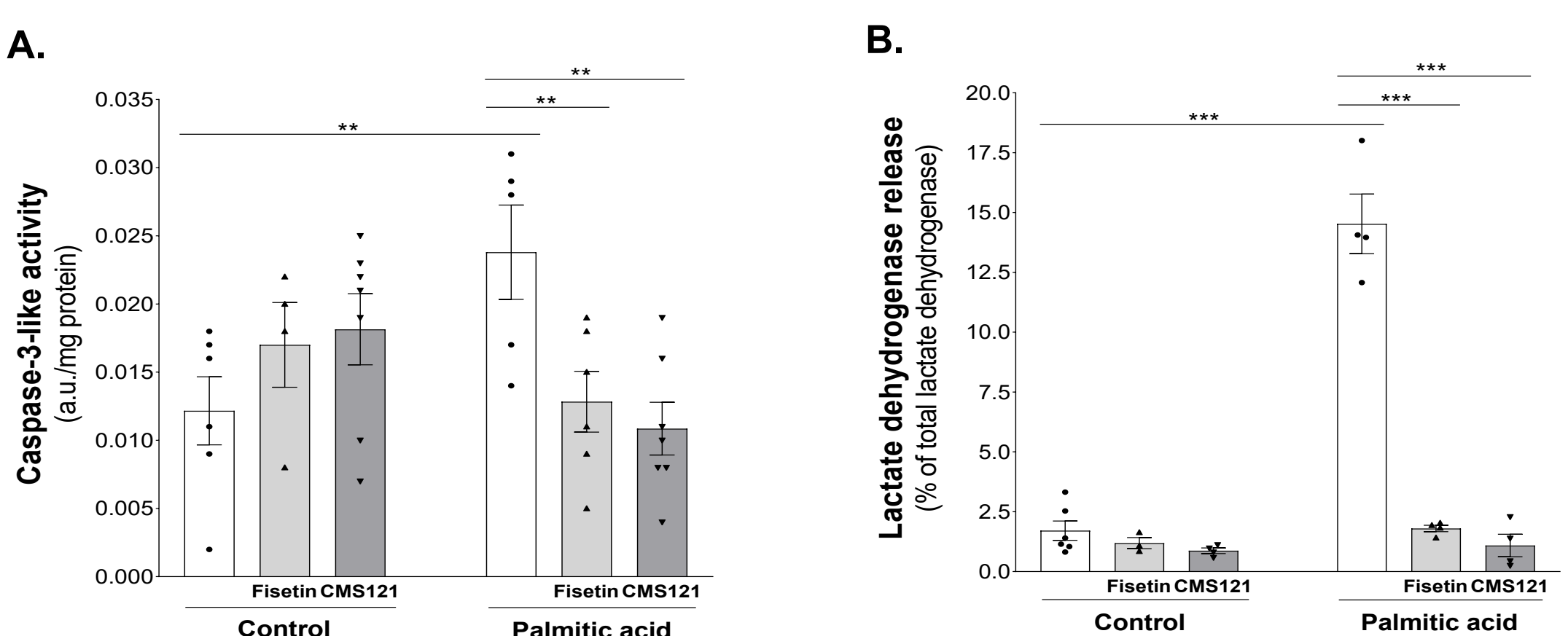


Table 1. Quantification of apoptotic and necrotic cells based on fluorescence intensity after double staining with Hoechst 33342 and propidium iodide.

	Control		Palmitic acid			
	(n=4)	(n=3)	Fisetin (n=3)	CMS121 (n=4)		
Live cells (% total cells)	93.4 $\pm$ 0.7	83.1 $\pm$ 0.6 ***	94.1 $\pm$ 0.2 ^{EEE}	93.9 $\pm$ 0.3 ^{EEE}		
Apoptotic cells (% total cells)	4.1 $\pm$ 0.3	7.9 $\pm$ 0.2 ***	3.4 $\pm$ 0.5 ^{EEE}	4.1 $\pm$ 0.3 ^{EEE}		
Necrotic cells (% total cells)	2.5 $\pm$ 0.4	9 $\pm$ 0.5 ***	2.5 $\pm$ 0.7 ^{EEE}	1.9 $\pm$ 0.1 ^{EEE}		

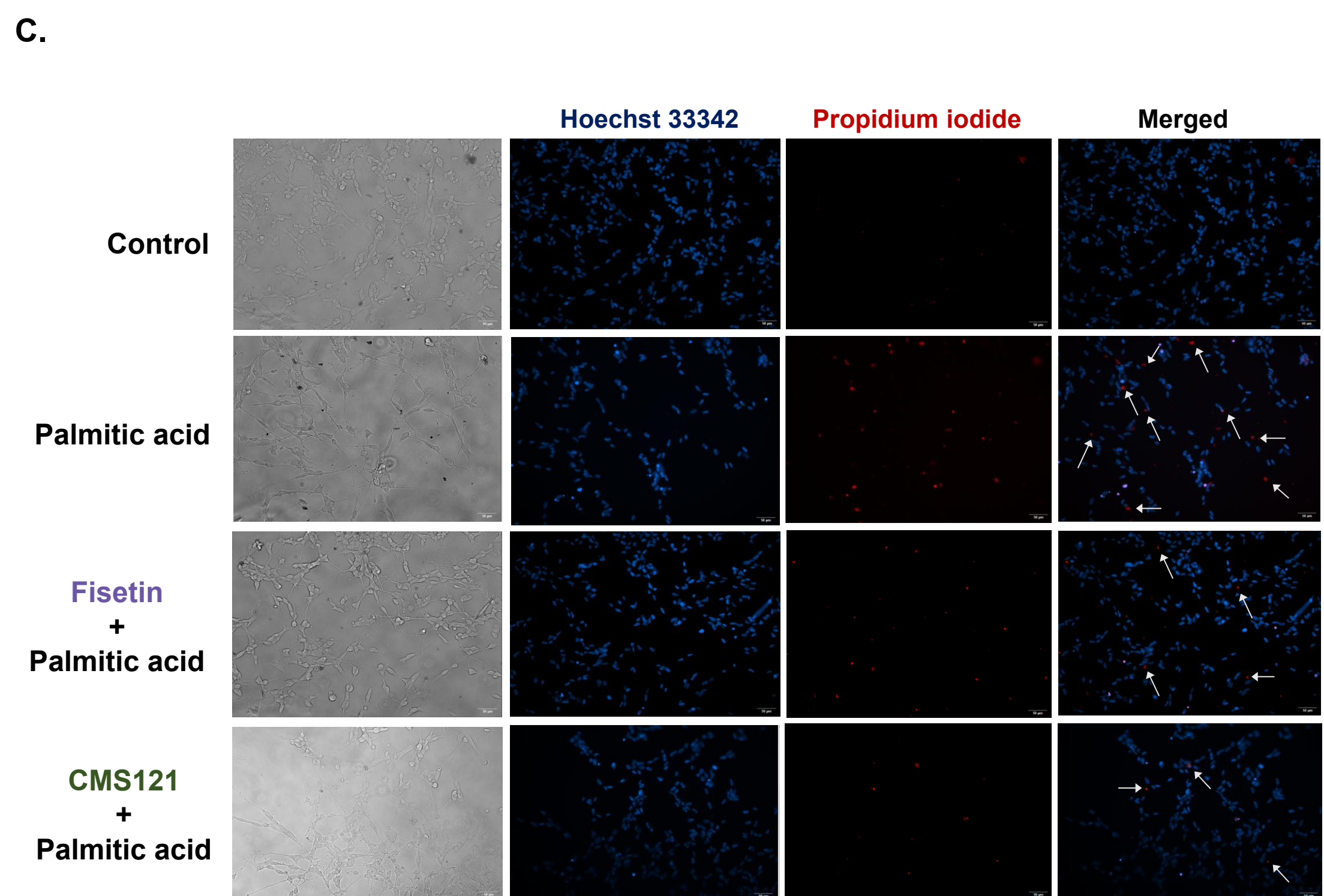
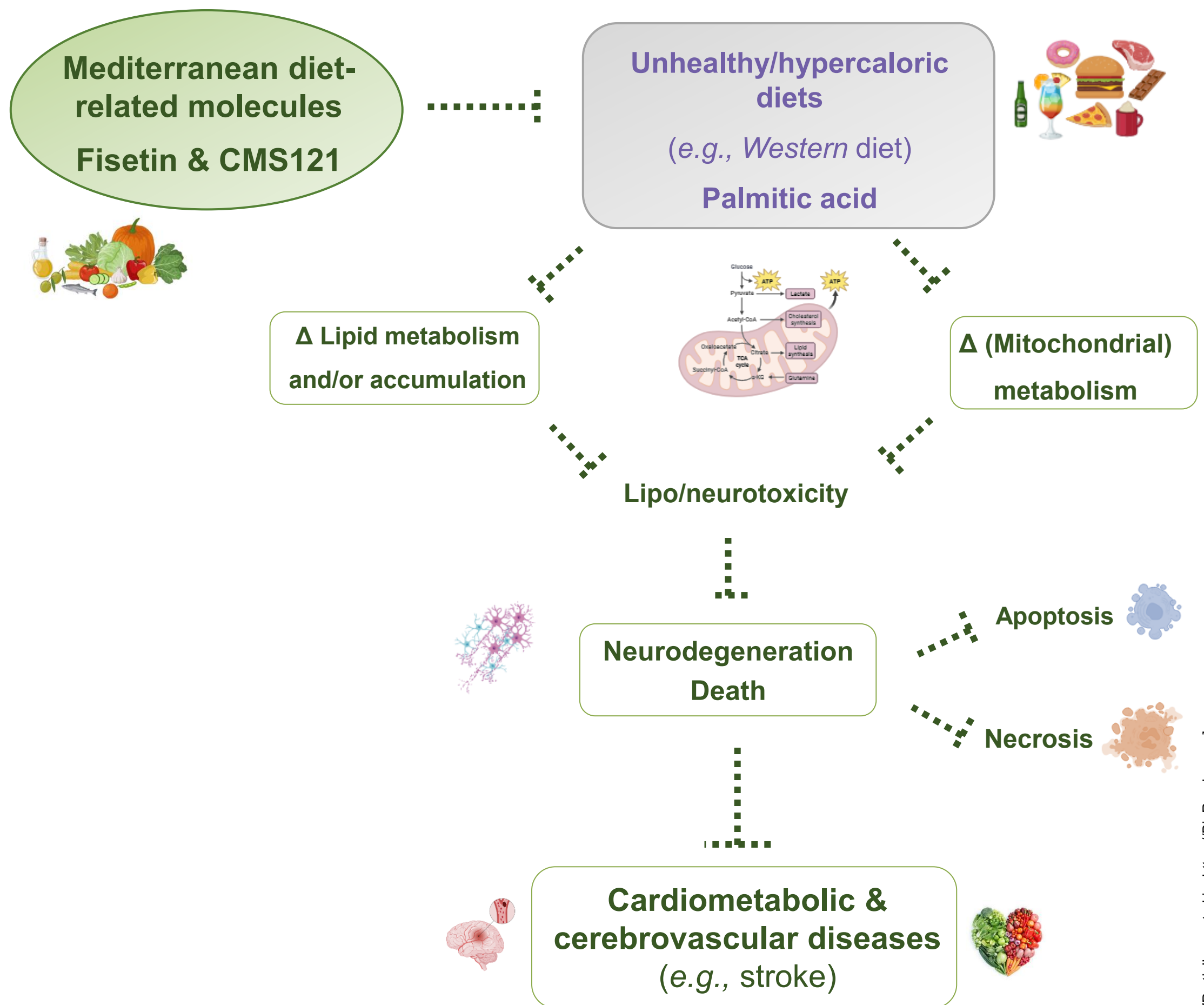


Figure 3. Effect of Fisetin and CMS121 on neuronal-like viability and death, after exposure of differentiated SH-SY5Y neuronal-like cells to palmitic acid, as given by the activity of the apoptosis-related effector caspase-3 (A), and the released lactate dehydrogenase (B). Table 1 corresponds to the quantification of viable, apoptotic and necrotic cells after measurement of Hoechst 33342 and propidium iodide fluorescence intensity. Figure 3C corresponds to representative immunofluorescence images showing Hoechst 33342 (blue) and propidium iodide (red) staining, obtained after visualization with a 20x objective, in a fluorescence microscope (Carl Zeiss Microscope Primovert, Zeiss, Oberkochen, Germany) with a coupled Zeiss AxiCam Erc 5s camera. Scale bar: 50 µm. Results are represented as mean $\pm$ SEM of 3-7 independent experiments (A,B), or n=6-15 images from 3-4 independent cultures (C, Table 1). Statistical significance of Figures A and B: *P<0.01, **P<0.001, by the one-way ANOVA test, followed by the DMS *post-hoc* test (Gaussian distribution). Statistical significance of Table 1: ***P<0.001 vs. Control, ^{EEE}P<0.001 vs. Palmitic acid, by the one-way ANOVA test, followed by the DMS *post-hoc* test (Gaussian distribution).

CONCLUSIONS



ACKNOWLEDGEMENTS

Funded by EU Horizon Europe CHANGing - Excellence Hubs-HORIZON WIDERA-2022-ACCESS-04-01 (grant agreement #101087071); the Spanish Fundación Mapfre – Ignacio H. de Larramendi Research Grant 2025; the EU Recovery and Resilience Facility and Portuguese national funds via Fundação para a Ciência e a Tecnologia (FCT, I.P.): 2024.13687.PEX [DOI: <https://doi.org/10.54499/2024.13687.PEX>], 2021.04707.BD [DOI: <https://doi.org/10.54499/2021.04707.BD>] (DM), FCT-Tenure Program [2023.15623.TENURE.091/CP00067/CT00031] via PRR, FCT and EU – Next Generation EU (AID), CiBB Strategic Project [DOI: <https://doi.org/10.54499/UID/04539/2025>].

Visit us



Funded by the European Union



CHANGING EXCELLENCE HUBS

1 2 9 0



UNIVERSIDADE DE COIMBRA

