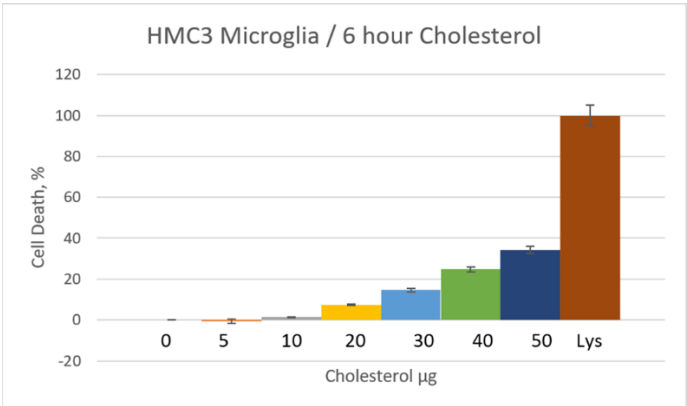# Cholesterol, amyloid beta, fructose and LPS influence ROS and ATP concentrations and the phagocytic capacity of HMC3 hu-man microglia cell line
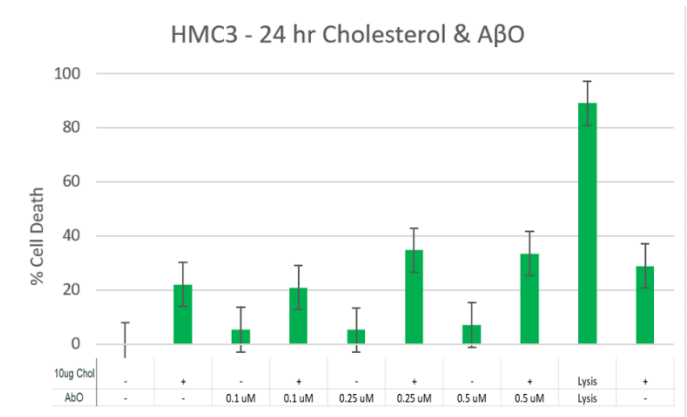
Oscar M. Muñoz Herrera[1], Brian V. Hong[1], Ulises Ruiz Mendiola[2], Izumi Maezawa[2], Lee-Way Jin[2], Carlito B. Lebrilla[3], Danielle J. Harvey[4], Angela M. Zivkovic[1*]

[1]Department of Nutrition, UC Davis; [2]Department of Pathology and Laboratory Medicine, UC Davis Medical Center; [3]Department of Chemistry, University of California; [4]Department of Public Health Sciences, UC Davis; Davis, California 95616 United States
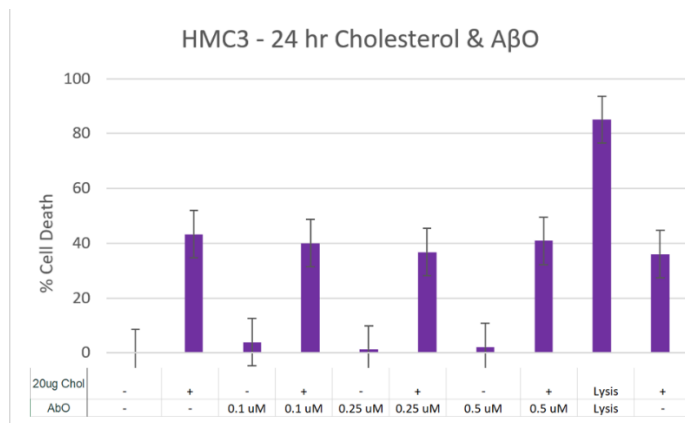
* Correspondence: amzivkovic@ucdavis.edu; Tel.: +1-530-752-3973

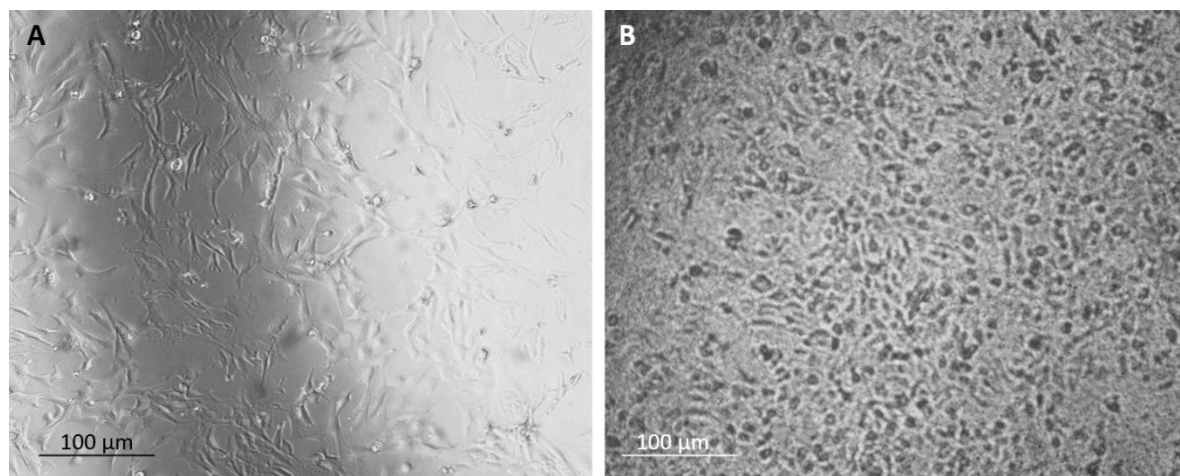**Supplemental Figure S1. HMC3 Cytotoxicity Assay**. Cholesterol loaded HMC3 microglia (varying doses), for 6 hours.



**Supplemental Figure S2**. HMC3 Cytotoxicity Assay. Cholesterol (10µg/mL) loaded and AβO treated HMC3 microglia, for 24 hours.

**Supplemental Figure S3**. HMC3 Cytotoxicity Assay. Cholesterol (20μg/mL) loaded and AβO treated HMC3 microglia, for 24 hours.
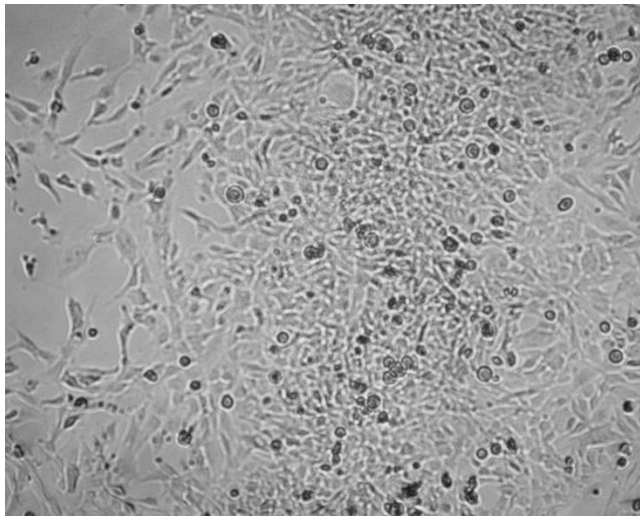


**Supplemental Figure S4. Effects of treatments on HMC3 microglia morphology** A) Untreated HMC3 microglia at timepoint 0 hours, on 96-well plates. n=3, three biological replicates. Digital Microscope Camera was used for the picture. 10x magnification, scale bar 100 μm. B) HMC3 microglia following the 24-hour period treatment with AβO+Fru+Chol+LPS. n=3, three biological replicates. Digital Microscope Camera was used for the picture. Terms: Lipopolysaccharide (LPS), amyloid beta oligomer (AβO), cholesterol (Chol), fructose (Fru). 10x magnification, scale bar 100 μm.
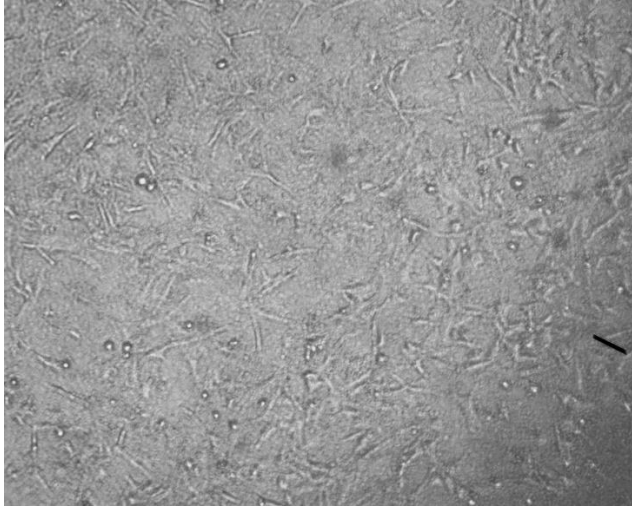
**Supplemental Figures S5. Effects of treatments on HMC3 microglia morphology, continued.**
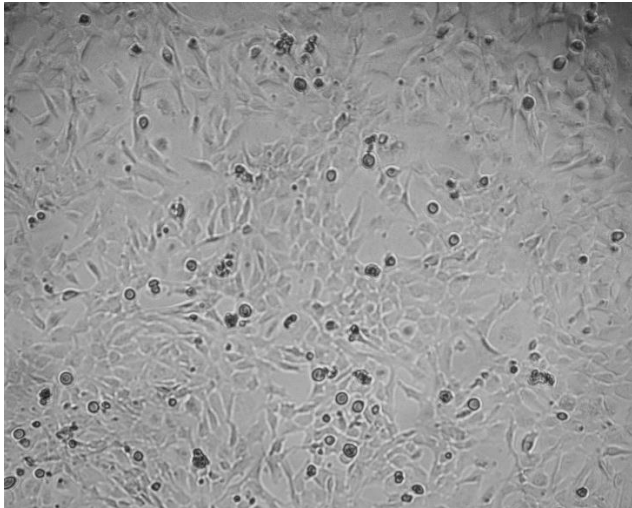


A) HMC3 microglia, at 24 hour time-point, following amyloid-beta oligomer (AβO) treatment. 10x magnification.



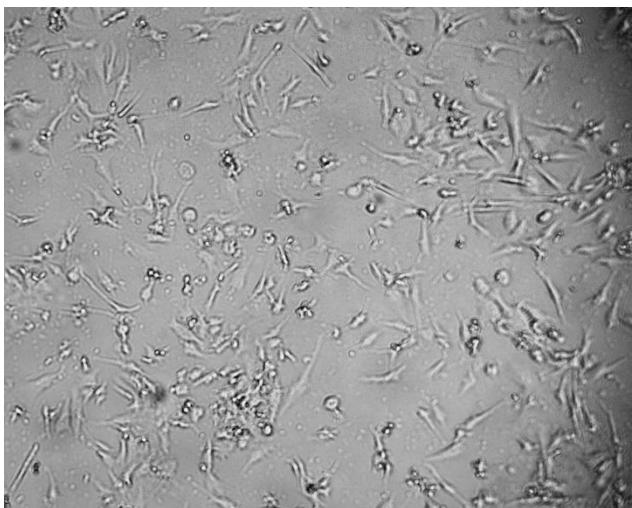B) HMC3 microglia, at 24 hour time-point, following fructose (Fru) treatment. 10x magnification.

C) HMC3 microglia, at 24 hour time-point, following cholesterol (Chol) treatment. 10x magnification.



D) HMC3 microglia, at 24 hour time-point, following Lipopolysaccharide (LPS) treatment. 10x magnification.



E) HMC3 microglia, at 24 hour time-point, following AβO+Fru treatment. 10x magnification.

F) HMC3 microglia, at 24 hour time-point, following AβO+Chol treatment. 10x magnification.



G) HMC3 microglia, at 24 hour time-point, following AβO+LPS treatment. 10x magnification.



H) HMC3 microglia, at 24 hour time-point, following Fru+Chol treatment. 10x magnification.

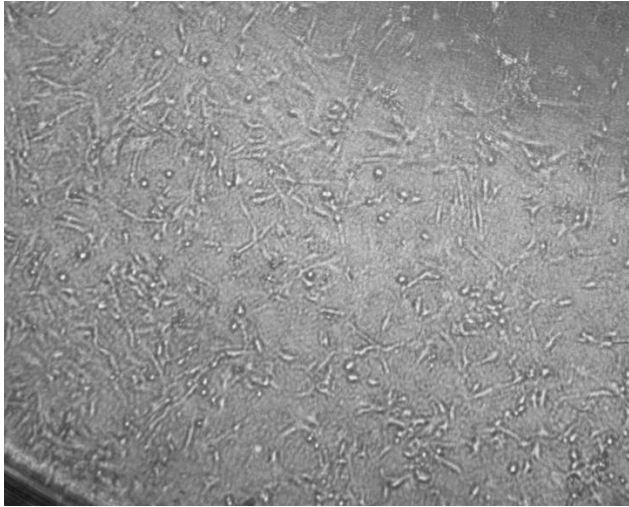I) HMC3 microglia, at 24 hour time-point, following Fru+LPS treatment. 10x magnification.


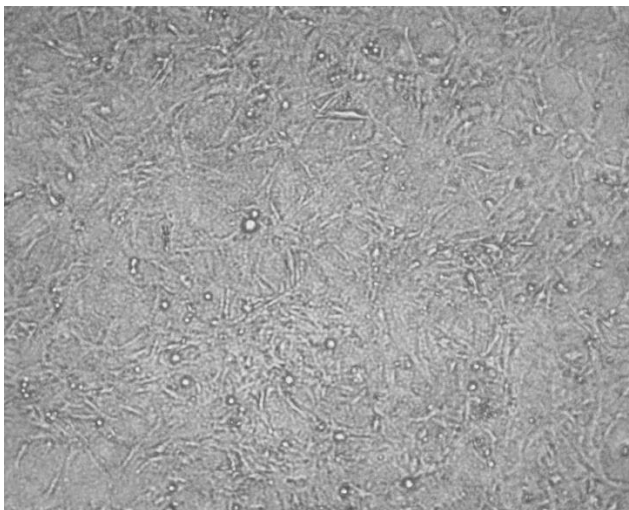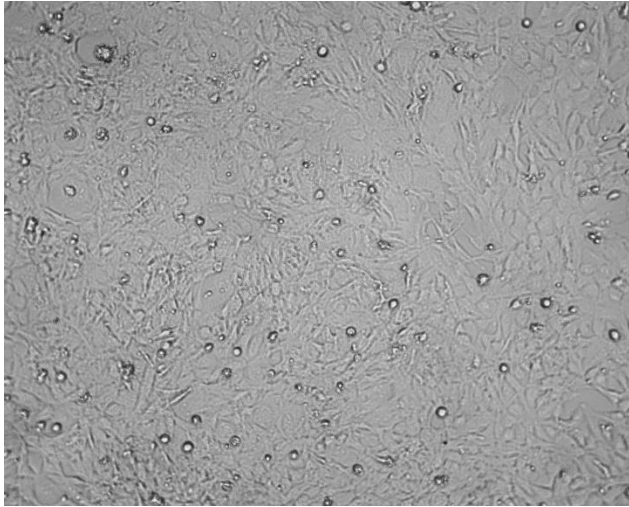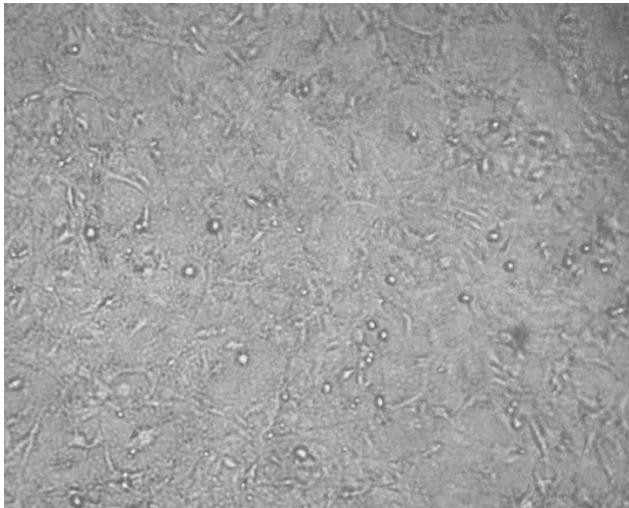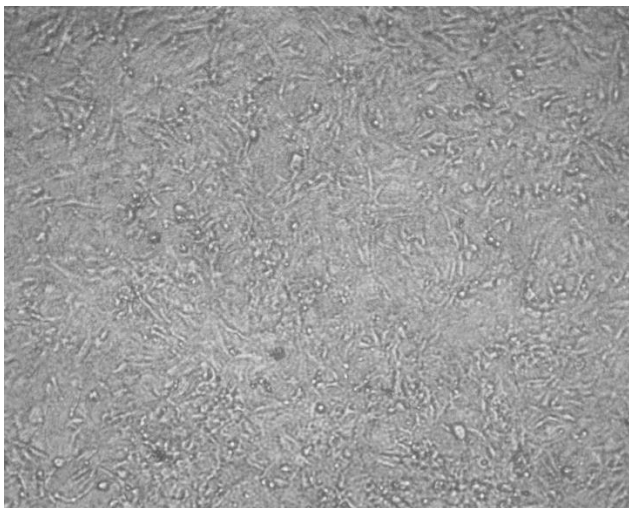J) HMC3 microglia, at 24 hour time-point, following Chol+LPS treatment. 10x magnification.


K) HMC3 microglia, at 24 hour time-point, following AβO+Chol+LPS treatment. 10x magnification.

**Table S1. Log2 Fold change of treatment on expression of pro-inflammatory cytokine genes and APOE in HMC3 microglia compared to control.**

| Treatment | IL1B | IL6 | TNFA | APOE |
|---|---|---|---|---|
| AβO | -1.76 | -0.27 | -0.59 | 0.52 |
| Cholesterol | -2.40 | 0.01 | 1.00 | -1.43 |
| Fructose | -0.72 | -0.11 | -0.54 | 0.94 |
| LPS | 2.97 | 3.93 | 3.08 | 0.54 |
| AβO_Cholesterol | -3.19 | -1.29 | 0.62 | 1.39 |
| AβO_Fructose | -1.55 | 0.09 | 0.63 | 1.68 |
| AβO_LPS | 1.49 | 3.61 | 3.39 | 1.46 |
| Cholesterol_LPS | 1.56 | 5.07 | 3.57 | 0.96 |
| Fructose_Cholesterol | -1.30 | 0.61 | 0.94 | 1.57 |
| Fructose_LPS | 2.90 | 3.90 | 2.61 | 0.36 |
| AβO_Cholesterol_LPS | -2.87 | 0.87 | 1.76 | 2.51 |
| AβO_Fru_Chol_LPS | -1.78 | 1.46 | 4.14 | 3.32 |

**Table S2. Mean ± SD of the effects of treatments on whole-cell total and esterified cholesterol concentrations, mitochondrial total cholesterol concentrations and ApoE secretion in HMC3 microglia.**

| Treatment | Total Cholesterol Mean (µM) | SD | Esterified Cholesterol Mean (µM) | SD | Mitochondrial Cholesterol Mean (µM) | SD | ApoE Mean (pg/mL) | SD |
|---|---|---|---|---|---|---|---|---|
| Control | 5.27 | 1.78 | 1.79 | 0.06 | 7.73 | 0.33 | 638.28 | 110.96 |
| AβO | 8.21 | 0.02 | 3.81 | 0.19 | 9.16 | 0.02 | 299.93 | 38.96 |
| Cholesterol | 8.05 | 0.26 | 3.14 | 0.24 | 10.45 | 0.25 | 541.90 | 81.65 |
| Fructose | 12.20 | 1.22 | 5.79 | 1.25 | 10.44 | 2.04 | 587.79 | 59.47 |
| LPS | 6.81 | 0.48 | 3.48 | 0.42 | 10.51 | 1.05 | 575.70 | 53.48 |
| AβO_Cholesterol | 8.70 | 0.18 | 3.36 | 0.04 | 11.50 | 2.98 | 341.23 | 40.86 |
| AβO_Fructose | 10.14 | 0.26 | 5.76 | 0.34 | 10.10 | 0.41 | 330.39 | 158.83 |
| AβO_LPS | 8.55 | 0.03 | 3.87 | 0.21 | 9.14 | 0.32 | 348.32 | 93.38 |
| Cholesterol_LPS | 9.33 | 1.68 | 3.67 | 0.53 | 11.08 | 1.00 | 503.94 | 66.78 |
| Fructose_Cholesterol | 12.45 | 2.01 | 4.34 | 0.11 | 10.20 | 0.29 | 441.36 | 70.09 |
| Fructose_LPS | 10.76 | 0.50 | 5.60 | 0.81 | 10.93 | 0.28 | 478.91 | 54.36 |
| AβO_Cholesterol_LPS | 11.90 | 0.05 | 5.24 | 1.06 | 11.04 | 0.40 | 365.01 | 48.45 |
| AβO_Fru_Chol_LPS | 11.04 | 0.26 | 6.42 | 0.27 | 14.10 | 3.36 | 326.21 | 80.64 |

**Table S3. Mean ± SD of the effects of treatments on HMC3 microglia ROS and ATP concentrations and phagocytic activity.**

| Treatment | ATP Mean (nmol/well) | SD | ROS Mean (Log2Fluorescence) | SD | Phagocytosis Mean (Log2Fluorescence) | SD |
|---|---|---|---|---|---|---|
| Control | 1.93 | 0.43 | 12.77 | 0.04 | 12.98 | 0.62 |
| AβO | 1.72 | 0.21 | 13.52 | 0.50 | 10.64 | 0.24 |
| Cholesterol | 1.66 | 0.21 | 13.35 | 0.46 | 11.89 | 0.20 |
| Fructose | 1.92 | 0.22 | 12.23 | 0.47 | 11.97 | 0.13 |
| LPS | 2.01 | 0.24 | 12.37 | 0.61 | 11.68 | 0.37 |
| AβO_Cholesterol | 1.54 | 0.26 | 13.42 | 0.44 | 11.89 | 0.08 |
| AβO_Fructose | 1.60 | 0.17 | 13.30 | 0.22 | 9.10 | 0.71 |
| AβO_LPS | 1.70 | 0.31 | 13.26 | 0.26 | 9.95 | 0.11 |
| Cholesterol_LPS | 1.80 | 0.29 | 13.05 | 0.33 | 13.14 | 0.37 |
| Fructose_Cholesterol | 1.68 | 0.17 | 12.21 | 0.46 | 12.31 | 0.60 |
| Fructose_LPS | 1.87 | 0.24 | 12.30 | 0.43 | 11.51 | 0.80 |
| AβO_Cholesterol_LPS | 1.53 | 0.22 | 13.07 | 0.24 | 11.97 | 0.59 |
| AβO_Fru_Chol_LPS | 1.27 | 0.13 | 15.51 | 0.45 | 10.73 | 0.67 |