

LEGENDS

Figure 1. Effect of fatty acids at different concentrations on endothelial cell viability. Viability of EAHy926 cells after incubation for 48 h with DMEM (0.1% of ethanol, CTL) or different concentrations (1 uM, 5uM, 10 uM, 20uM, 50uM, 100 uM, 200 uM and 500 uM) of palmitic acid, oleic acid or palmitoleic acid, followed by incubation with (+) or without (-) TNF α (1 ng/mL) for 24 h. Bars are mean \pm SEM of 7 samples performed in 2 experiments. Two-way ANOVA with Bonferroni post-hoc test. *p<0.05, **p<0.01 and ***p<0.001 vs. CTL.

Figure 2. Incubation with palmitic, oleic or palmitoleic acids results in their incorporation to endothelial cells, in a dose response dependent manner, and unaffected by TNF α . (A) Incorporation of palmitic acid (16:0); (B) oleic acid (18:1n-9) and (C) palmitoleic acid (16:1n-7) into EAHy926 cells incubated for 48 h with DMEM containing 0.1% ethanol (Control) or 20 uM and 50 uM of palmitic acid (PA), oleic acid (OA) or palmitoleic acid (POA), followed by incubation with (+) or without (-) TNF α (1 ng/mL) for 24 h. Bars are mean \pm SEM of 3 to 5 samples. Two way ANOVA with Bonferroni post-hoc test. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 vs. Control; # p<0.05, ##p<0.01 vs. fatty acids 20uM.

Figure 3. Palmitoleic acid reduced the production of several pro-inflammatory cytokines stimulated by TNF α (6 h). Production (pg/mL) of (A) MCP-1, (B) IL-6, (C) IL-8, (D) VEGF, (E) RANTES and (F) ICAM-1 by EAHy926 cells incubated for 48 h with DMEM containing 0.1% of ethanol (Control) or different concentrations (20 uM, and 50 uM) of palmitic acid, oleic acid or palmitoleic acid, followed by incubation with (+) or without (-) TNF α (1 ng/mL) for 6 h. Bars are mean \pm SEM of 3 samples performed in 2 experiments. Two way ANOVA with Bonferroni post test. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 vs. without TNF α ; #p<0.05, ##p<0.01 and ###p<0.001 vs. Control; \$p<0.05, \$\$p<0.01, \$\$\$p<0.0001 vs. fatty acids.

Figure 4. Palmitoleic acid reduced the production of several pro-inflammatory cytokines stimulated by TNF α (24 h). Production (pg/mL) of (A) MCP-1, (B) IL-6, (C) IL-8, (D) VEGF, (E) RANTES and (F) ICAM-1 by EAHy926 cells incubated for 48h with DMEM containing 0,1% of ethanol (Control) or different concentrations (20 uM, and 50 uM) of palmitic acid , oleic acid or palmitoleic acid, followed by incubation with (+) or without (-) TNF α (1ng/mL) for 24h. Bars are mean \pm SEM of 6 samples performed in 2 experiments. Two way ANOVA with Bonferroni post-hoc test. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 vs. without TNF α ; #p<0.05 and ##p<0.01 vs. Control; \$p<0.05, \$\$p<0.01 and \$\$\$p<0.0001 vs. fatty acids.

Figure 5. Palmitoleic acid reduced the expression of ICAM-1 on endothelial cells stimulated by TNF α (6 h). (A) Gates, (B and C) % of ICAM-1/CD54 positive cells and (D and E) Mean of Fluorescence Intensity (MFI) of ICAM-1/CD54. EAHy926 cells incubated for 48 h with DMEM containing 0.1% ethanol (Control) or different concentrations (20 uM, and 50 uM) of palmitic acid, oleic acid or palmitoleic acid, followed by incubation with (+) or without (-) TNF α (1 ng/mL) for 6 h. Bars are mean \pm SEM of 6 samples performed in 2 experiments. Two way ANOVA TWO WAY with Bonferroni post-hoc test. ***p<0.001, ****p<0.0001 vs. without TNF α ; #p<0.05, #####p<0.0001 vs. Control; \$p<0.05 vs. different fatty acids.

Figure 6. Palmitoleic reduced the expression of inflammatory genes induced by TNF α . Gene expression of (A) NF κ B, (B) COX-2, (C) MCP-1, (D) IL-6. EAHy926 cells were incubated for 48 h with DMEM containing 0.1% ethanol (Control) or 50 uM of palmitic acid, oleic acid or palmitoleic acid, followed by incubation with (+) or without (-) TNF α (1 ng/mL) for 6 h. All Ct values were normalized to B2M and β -actin. Bars are mean \pm SEM of 6 samples performed in 2 experiments. Two way ANOVA with Bonferroni post-hoc test. *p<0.05, **p<0.01, ****p<0.0001 vs. without TNF α ; #p<0.05, ##p<0.01, ####p<0.0001 vs. Control; \$p<0.05 \$\$p<0.01, \$\$\$p<0.0001 vs. different fatty acids.

Figure 7. Palmitoleic partially reversed the TNF α induced inhibition of PPAR α gene expression. Gene expression of PPAR α stimulated with TNF α for (A) 6 hours or (B) 24 hours. EAHy926 cells were incubated for 48 h with DMEM containing 0.1% of ethanol (Control) incubated with 50uM of palmitic acid, oleic acid or palmitoleic acid for 48h followed by TNF α

(1ng/mL) (+) or PBS (-). All Ct values were normalized to B2M. Bars are mean \pm SEM of 6 samples performed in 2 experiments. Two way ANOVA with Bonferroni post-hoc test. * $p < 0.05$ and **** $p < 0.0001$ vs. without TNF α ; # $p < 0.05$ vs. Control (+).