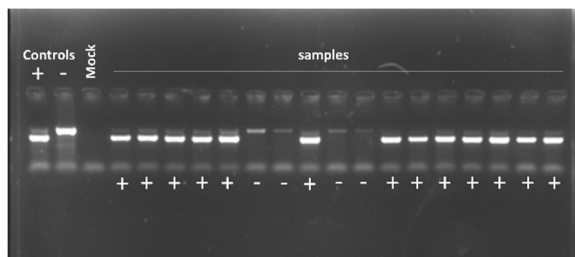


Supplementary Material

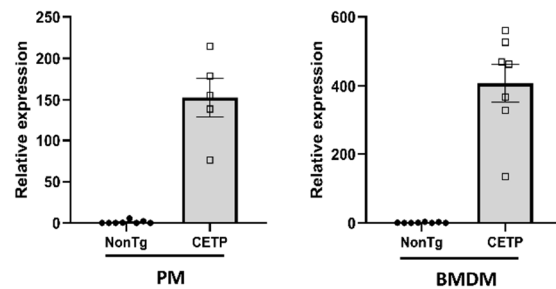
Novel Role of CETP in Macrophages: Reduction of Mitochondrial Oxidants Production and Modulation of Cell Immune-Metabolic Profile

Gabriel G. Dorighello, Leandro H. P. Assis, Thiago Rentz, Joseane Morari, Monique F. M. Santana, Marisa Passarelli, Neale D. Ridgway, Anibal E. Vercesi and Helena C. F. Oliveira

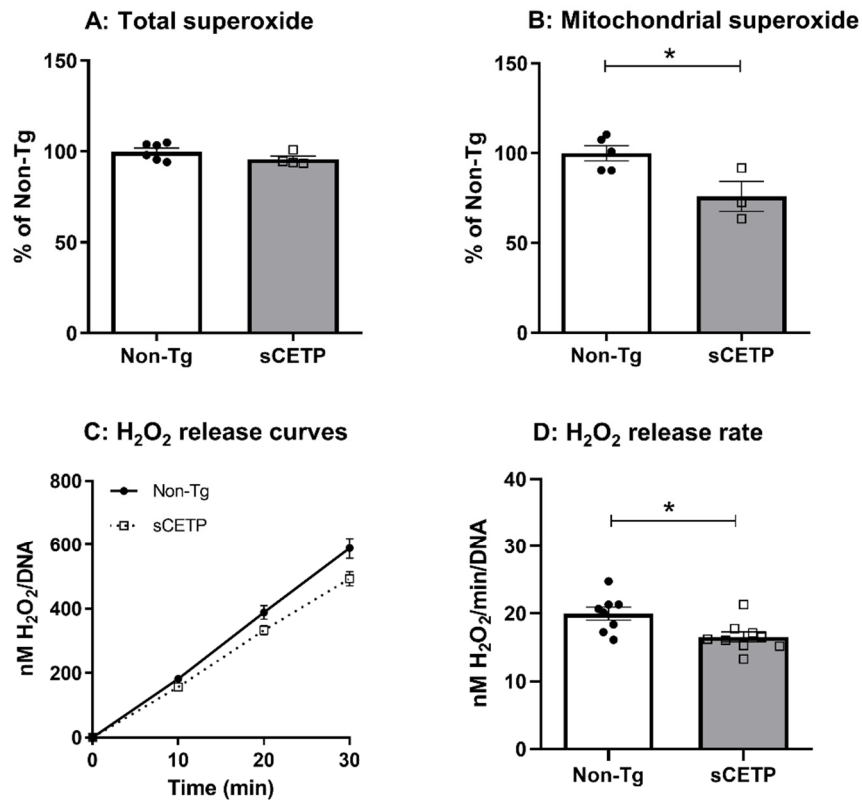
A: CETP genotyping: tail tip genomic DNA



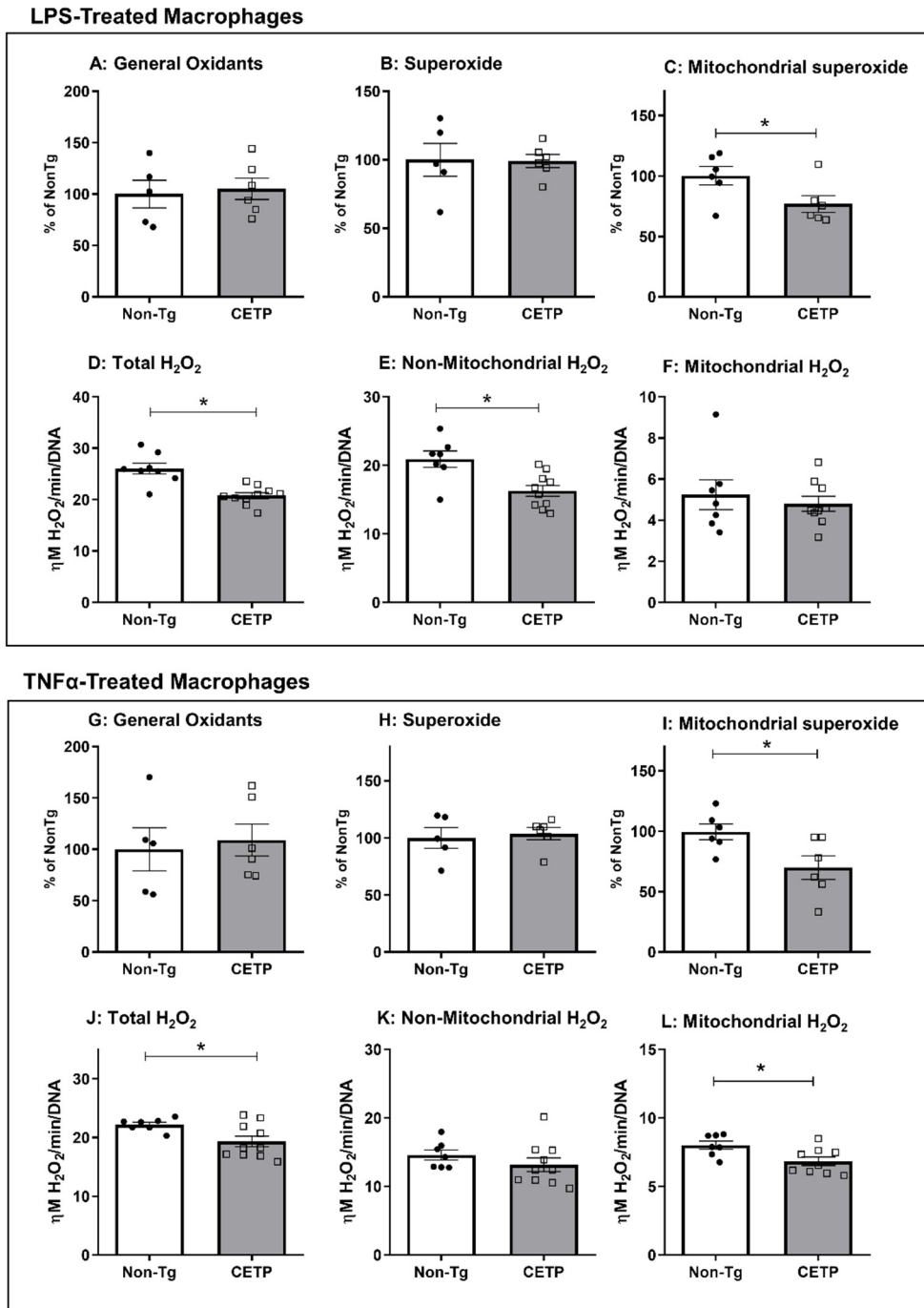
B: CETP mRNA



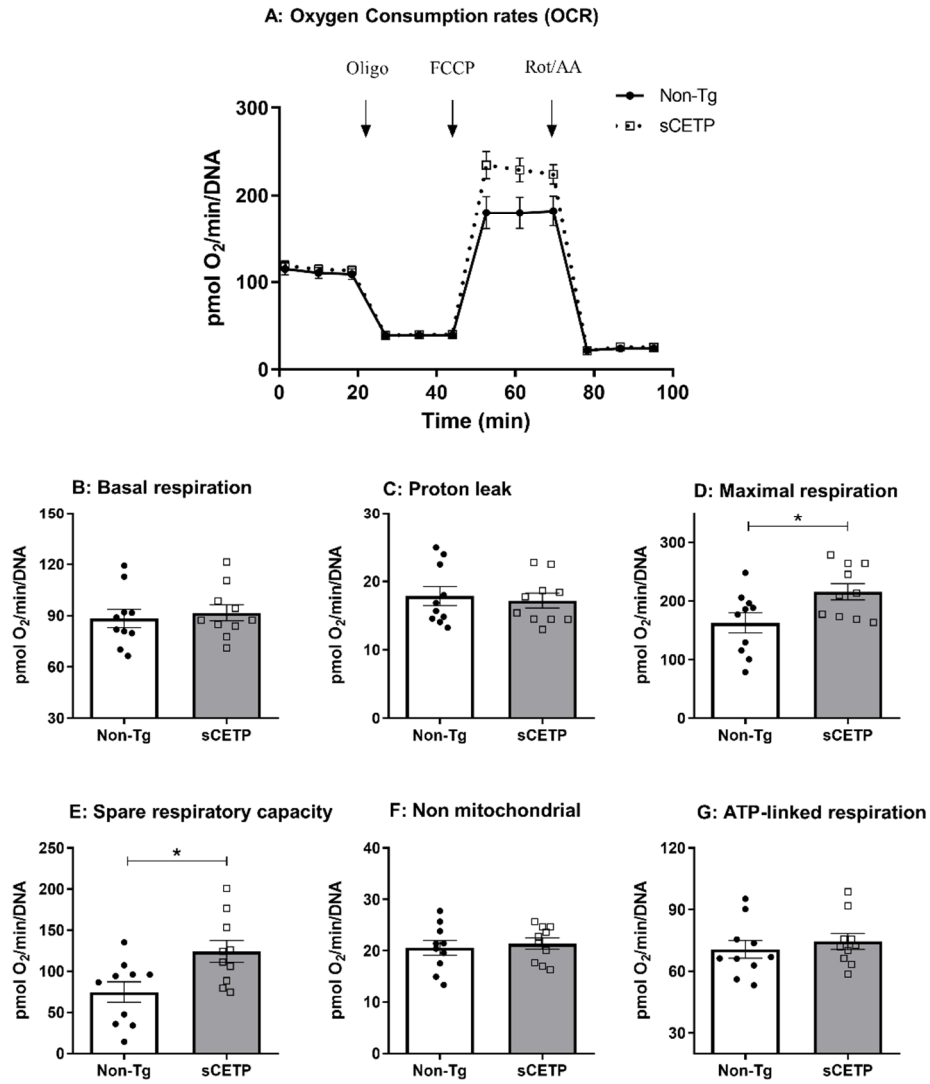
Supplementary Figure S1: CETP genotyping (A) by PCR of genomic DNA and mRNA expression (B) in peritoneal macrophage (PM) and bone marrow derived macrophage (BMDM).



Supplementary Figure S2: Oxidant production in thioglycolate-elicited peritoneal macrophages from simian CETP transgenic mice and non-transgenic mice (Non-Tg). Total cell superoxide quantified with DHE (A), mitochondrial superoxide quantified with Mitosox® (B), total hydrogen peroxide release rates (C-D) quantified with Amplex® red and HRP. A: n=4-6 for cell superoxide; B: n=3-5 for mitochondrial superoxide; C-D: n=8-9 for H₂O₂ release; *p<0.05 vs. Non-Tg.



Supplementary Figure S3: Oxidant generation in thioglycolate-elicited peritoneal macrophages from human CETP transgenic mice and non-transgenic mice (Non-Tg) after activation with LPS (100 nM) (**A-F**) or TNF α (5 U/mL) (**G-L**). General oxidants quantified with H₂DCFDA (**A, G**), total cell superoxide quantified with DHE (**B, H**), mitochondrial superoxide quantified with Mitosox[®] (**C, I**), total H₂O₂ (**D, J**), non-mitochondrial H₂O₂ (**E, K**) and mitochondrial H₂O₂ (**F, L**) with Amplex[®] red and HRP. Non-mitochondria H₂O₂ contribution was determined in cells treated with FCCP (1 μ M) and mitochondrial derived H₂O₂ was calculated as [total - non-mitochondrial H₂O₂]. N=5-6 for global oxidants and superoxide; n=7-10 for H₂O₂ release; *p<0.05 vs. Non-Tg.



Supplementary Figure S4: Oxygen consumption rates (OCR) in thioglycolate-elicited peritoneal macrophages from simian CETP (sCETP) transgenic and non-transgenic mice (Non-Tg). The OCR were evaluated using a Seahorse® - XF24 Extracellular Flux Analyzer (Agilent, USA). **A:** Average curves of OCR with sequential additions of oligomycin (1 μ M), FCCP (1 μ M), and rotenone/antimycin A (1 μ M); **B:** Basal respiration; **C:** Proton leak; **D:** Maximal respiration; **E:** Spare respiratory capacity; **F:** Non-mitochondrial respiration; **G:** ATP linked respiration; n= 10; *p<0.05 vs. Non-Tg.

Supplementary Table S1. Oligonucleotides used to assess gene expression.

Gene	Oligonucleotides sequences	
ACTB	forward	5'-AGAAGCTGTGCTATGTTGCTCTA-3'
	reverse	5'-TCAGGCAGCTCATAGCTCTTC-3'
hCETP	forward	5'-GGCCAAGTCAAGTATGGGTTG-3'
	reverse	5'-ACAGACACGTTCTGAATGGAGA-3'
ARG1	forward	5'-CTCCAAGCCAAAGTCCTTAGAG-3'
	reverse	5'-AGGAGCTGTCATTAGGGACATC-3'
CD36	forward	5'-GGAAGTGTGGGCTCATTGC-3'
	reverse	5'-CATGAGAATGCCTCCAAACAC-3'
IL-10	forward	5'-GCTCTTACTGACTGGCATGAG-3'
	reverse	5'-CGCAGCTCTAGGAGCATGTG-3'
IL-1 β	forward	5'-CCTTCCAGGATGAGGACATGA-3'
	reverse	5'-TGAGTCACAGAGGATGGGCTC-3'
IL-4	forward	5'-CCAAACGTCCTCACAGCAAC-3'
	reverse	5'-AAGCCCGAAAGAGTCTCTGC-3'
IL-6	forward	5'-CACGGCCTTCCCTACTTCAC-3'
	reverse	5'-GGTCTGTTGGGAGTGGTATC-3'
iNOS	forward	5'-GTTCTCAGCCCAACAATAACAAGA-3'
	reverse	5'-GTGGACGGGTCGATGTCAC-3'
MFN-2	forward	5'-TGCACCGCCATATAGAGGAAG-3'
	reverse	5'-TCTGCAGTGAAGTGGCAATG-3'
TNF- α	forward	5'-CCCTCCTGGCCAACGGCATG-3'
	reverse	5'-TCGGGGCAGCCTTGTCCCTT-3'
MFN1	forward	5'-GCAGACAGCACATGGAGAGA-3'
	reverse	5'-GATCCGATTCCGAGCTTCCG-3'
OPA1	forward	5'-ACCTTGCCAGTTTAGCTCCC-3'
	reverse	5'-TTGGGACCTGCAGTGAAGAA-3'
DRP1	forward	5'-ATGCCAGCAAGTCCACAGAA-3'
	reverse	5'-TGTTCTCGGGCAGACAGTTT-3'
FIS1	forward	5'-CAAAGAGGAACAGCGGGACT-3'
	reverse	5'-ACAGCCCTCGCACATACTTT-3'
SREBP1	forward	5'-GATGTGCGAACTGGACACAG-3'
	reverse	5'-CATAGGGGGCGTCAAACAG-3'
SRBP2	forward	5'-GCAGCAACGGGACCATTCT-3'
	reverse	5'-CCCCATGACTAAGTCCTTCAACT-3'
LDLR	forward	5'-TGACTCAGACGAACAAGGCTG-3'
	reverse	5'-ATCTAGGCAATCTCGGTCTCC-3'
HMGCR	forward	5'-TGTTACCGGCAACAACAAGA-3'
	reverse	5'-CCGCGTTATCGTCAGGATGA-3'
SRA	forward	5'-TCAGACTGAAGGACTGGGAACA-3'
	reverse	5'-GGAGGCCCTTGAATGAAGGT-3'
SRB1	forward	5'-TGTAAGTGCCTAACATCTTGGTCC-3'
	reverse	5'-ACTGTGCGGTTCAAAAAGCA-3'
ABCG1	forward	5'-TCACCCAGTTCTGCATCCTCT-3'
	reverse	5'-GCAGATGTGTCAGGACCGAGT-3'
ABCA1	forward	5'-CGTTTCCGGGAAGTGTCTTA-3'
	reverse	5'-GCTAGAGATGACAAGGAGGATGGA-3'
hCETP-THP-1	forward	5'-TCAGTCCATTGACTTCGAGATCG-3'
	reverse	5'-GCATCGGTCCGCACTCTAC-3'
hTNF- α -THP-1	forward	5'-TCAGCAAGGACAGCAGAGG-3'
	reverse	5'-CAGTATGTGAGAGGAAGAGAACC-3'
hMFN-2-THP-1	forward	5'-CACATGGAGCGTTGTACCAG-3'

Gene	Oligonucleotides sequences	
hCD36-THP-1	reverse	5'-TTGAGCACCTCCTTAGCAGAC-3'
	forward	5'-GGCTGTGACCGGAACTGTG-3'
	reverse	5'-AGGTCTCCAAGTGGCATTAGAA-3'
hGAPDH-THP-1	forward	5'-CTGGGCTACACTGAGCACC-3'
	reverse	5'-AAGTGGTCGTTGAGGGCAATG-3'
hPGK1-THP-1	forward	5'-GACCTAATGTCCAAAGCTGAGAA-3'
	reverse	5'-CAGCAGGTATGCCAGAAGCC-3'