Dietary nitrate attenuates high-fat diet-induced obesity via mechanisms involving higher adipocyte respiration and alterations in inflammatory status

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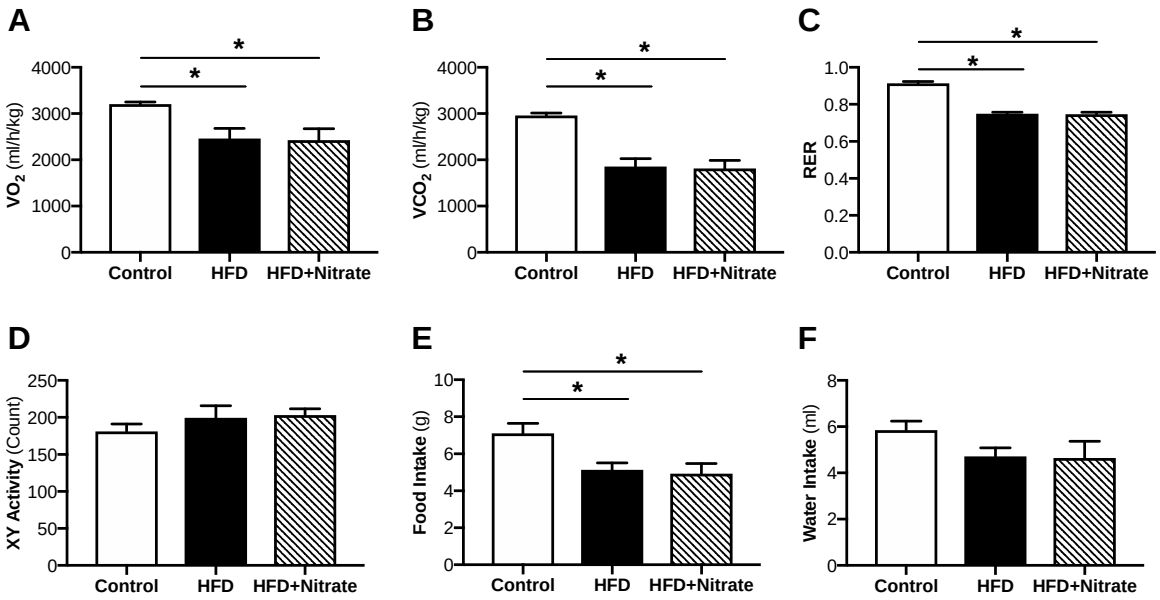
TABLE S1. *TAQMAN Hydrolysis probes used for the analysis of M1 & M2 markers.*

Mm03024075_m1	Hprt
Mm00475988_m1	Arg1
Mm00456650_m1	Egr2

TABLE S2. *Primer sequences used for the investigation of thermogenesis and creatine cycle gene expression.*

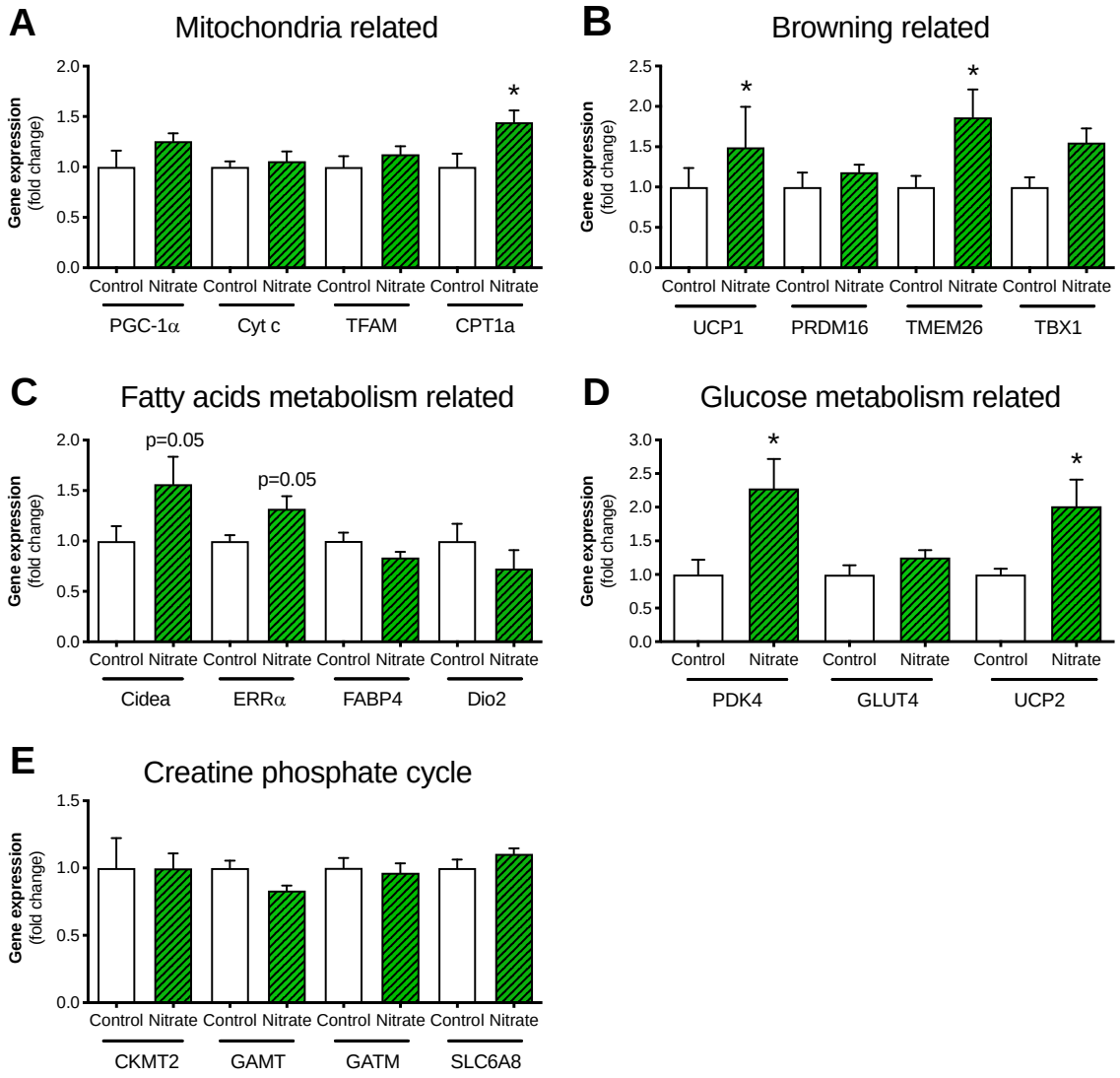
Primer	**Sequence**
Cidea FW	TGC TCT TCT GTA TCG CCC AGT
Cidea RV	GCC GTG TTA AGG AAT CTG CTG
Ckmt1 FW	TGA GGA GAC CTA TGA GGT ATT TGC
Ckmt1 RV	TCA TCA AAG TAG CCA GAA CGG A
Ckmt2 FW	CCA GTG CCT TCT CAA AGT TGC
Ckmt2 RV	AGT CCG CAC TTG GGG GAA AGA G
DIO2 FW	AAT TAT GCC TCG GAG AAG ACC G
DIO2 RV	GGC AGT TGC CTA GTG AAA GGT
Gamt FW	GCA GCC ACA TAA GGT TGT TCC
Gamt RV	CTC TTC AGA CAG CGG GTA CG
Gatm FW	GAC CTG GTC TTG TGC TCT CC
Gatm RV	GGG ATG ACT GGT GTT GGA GG
PGC-1a_Total FW	TGA TGT GAA TGA CTT GGA TAC AGA CA
PGC-1a_Total RV	GCT CAT TGT TGT ACT GGT TGG ATA TG
PRDM16 FW	CAG CAC GGT GAA GCC ATT C
PRDM16 RV	GCG TGC ATC CGC TTG TG
Slc6a8 FW	GTG TGG AGA TCT TCC GCC AT
Slc6a8 RV	CCC GTG GAG AGC CTC AAT AC
UCP1 FW	CAA TGA ACA CTG CCA CAC CTC
UCP1 RV	GGC ATT CAG AGG CAA ATC AGC T
HPRT FW	AGT CCC AGC GTC GTG ATT AG
HPRT RV	TTT CCA AAT CCT CGG CAT AAT GA
TFAM FW	GAGCGTGCTAAAAGCACTGG
TFAM RV	ACTTCGGAATACAGACAAGACTGA
CPT1a FW	CACTGCAGCTCGCACATTAC
CPT1a RV	CCAGCACAAAGTTGCAGGAC
PDK4 FW	AGG GAG GTC GAG CTG TTC TC
PDK4 RV	GGA GTG TTC ACT AAG CGG TCA
TMEM26 FW	ACCCTGTCATCCCACAGAG
TMEM26 RV	TGTTTGGTGGAGTCCTAAGGTC
TBX1 FW	GGCAGGCAGACGAATGTTC
TBX1 RV	TTGTCATCTACGGGCACAAAG
UCP2 FW	ATGGTTGGTTTCAAGGCCACA
UCP2 RV	CGGTATCCAGAGGGAAAGTGAT
ERRa FW	GGGGAGCATCGAGTACAGC
ERRa RV	AGACGCACACCCTCCTTGA
Cyt C FW	ACAAGAAGACTCAAATGTGTTTCAGTTT
Cyt C RV	TGCACTGTCAAGAATAGACAGTTGC
GLUT4 FW	AAAAGTGCCTGAAACCAGAG
GLUT4 RV	TCACCTCCTGCTCTAAAAGG
FABP4 FW	AAGGTGAAGAGCATCATAACCCT
FABP4 RV	TCACGCCTTTCATAACACATTCC

FIGURE S1



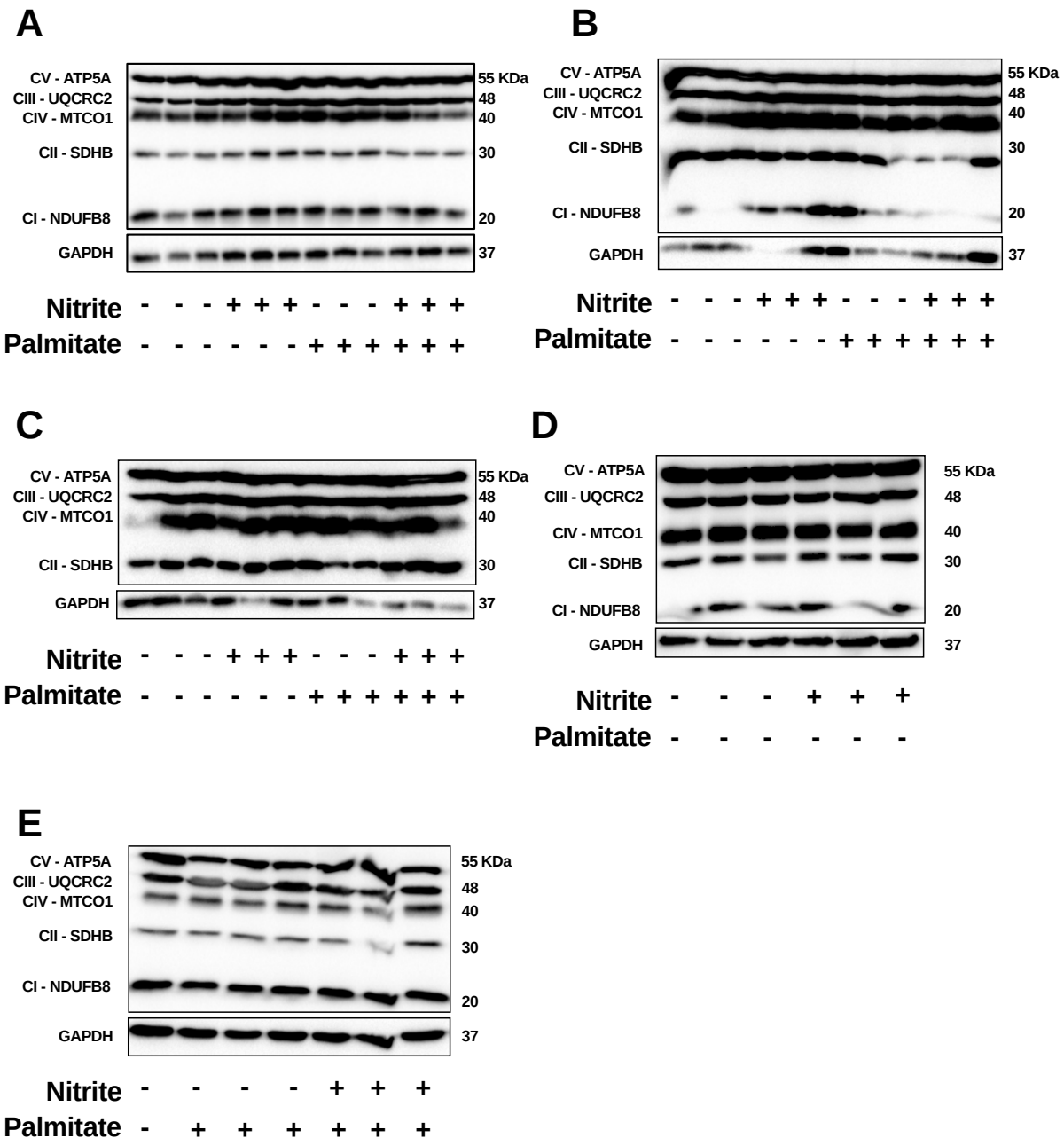
Measurement of the VO_2-oxygen consumption (A), VCO_2-carbon dioxide production (B), RER-Respiratory Exchange Ratio (C), physical activity in the X and Y axis (total number of counts), food (E) and water (F) intake, in conscious mice using metabolic cages. The mice acclimatized in the new environment (i.e. metabolic cages) for 24 h, followed by subsequent measurements for the next 48 h. Averaged data are presented as Mean±SEM, n=6/group, *p<0.05.

FIGURE S2



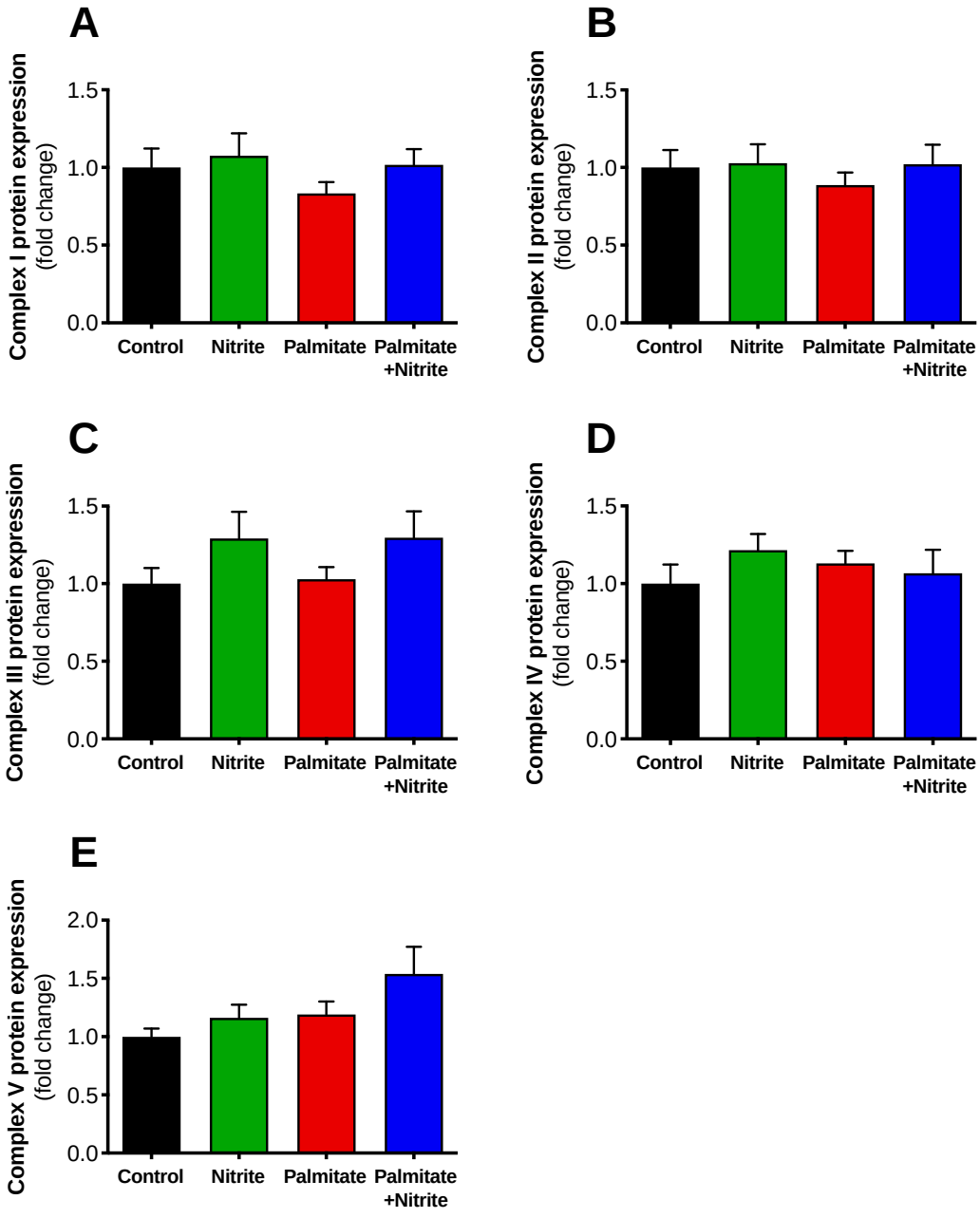
Gene expression in subcutaneous fat obtained from Control or Nitrate treated mice as described in the method section. (A) mRNA expression of mitochondria related genes: PGC-1α (Peroxisome proliferator-activated receptor gamma coactivator 1-α); Cyt c (cytochrome c); TFAM (Transcription Factor A, Mitochondrial); CPT1a (Carnitine palmitoyltransferase 1a). (B) mRNA expression of browning related genes: UCP1 (uncoupling protein 1); PRDM16 (PR domain containing 16); TMEM26 (Transmembrane Protein 26); TBX1 (T-box transcription factor 1). (C) mRNA expression of fatty acids metabolism related genes: Cidea (Cell death activator); ERRα (Estrogen-related receptor α); FABP4 (Fatty Acid-Binding Protein 4); Dio2 (Deiodinase, Iodothyronine Type II). (D) mRNA expression of glucose metabolism related genes: PDK4 (Pyruvate dehydrogenase lipoamide kinase isozyme 4); GLUT4 (Glucose transporter type 4); UCP2 (uncoupling protein 2). (E) mRNA expression of creatine phosphate cycle related genes: CKMT2 (Creatine Kinase, Mitochondrial 2); GAMT (Guanidinoacetate methyltransferase); GATM (Glycine Amidinotransferase); SLC6A8 (Solute Carrier Family 6 Member 8). Values are shown as mean±SEM, n=6/group. *p<0.05 compared with Control.

FIGURE S3



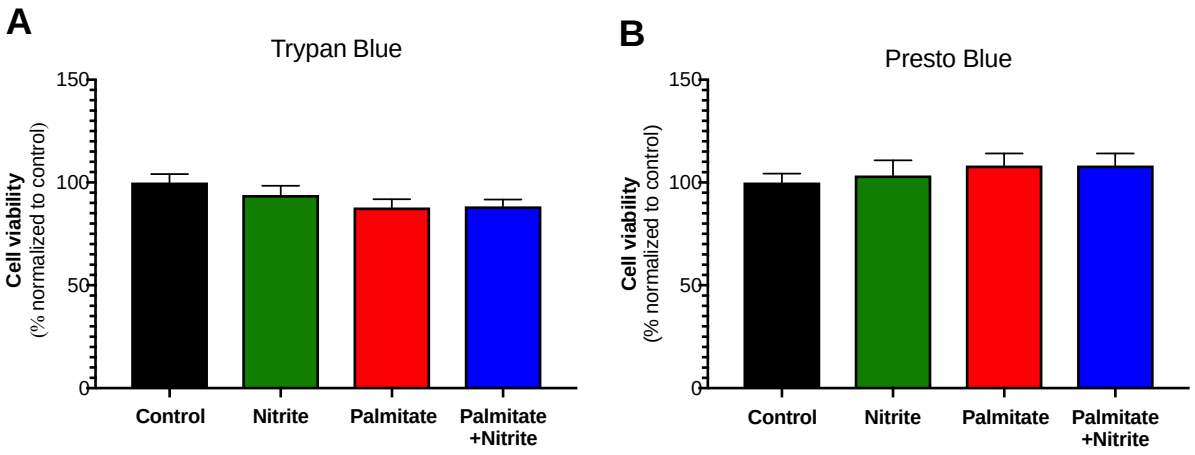
Uncropped gels for Western blot analysis of mitochondrial complexes in mouse primary white adipocytes. Five independent experiments for control cells or cells treated with Nitrite (10 μM), Palmitate (50 mM) or Palmitate+Nitrite for 24 hours. n = 12 *per* group

FIGURE S4



Mitochondrial complexes protein expression in mouse primary white adipocytes. Cells without any treatment (Control, n=13) or cells treated with Nitrite (10 μM, n=12), Palmitate (50 mM n=12) or Palmitate+Nitrite (n=12) for 24 hours. Values are shown as mean±SEM.

FIGURE S5



Measurement of cell viability in the experimental conditions used for mouse primary white adipocytes. Cell viability was estimated with the Trypan Blue (A) and Presto Blue (B) methods. The methods were performed in a blinded fashion where the person performing the methods was not aware of the different treatment groups. The Trypan Blue measurements (A) were done by cell counting on a haematocytometer whereas the Presto Blue method (B) is based on measuring the fluorescence intensity emitted from viable cells exposed to resazurin. Values are presented as Mean±SEM, n=12/group.