nutrients

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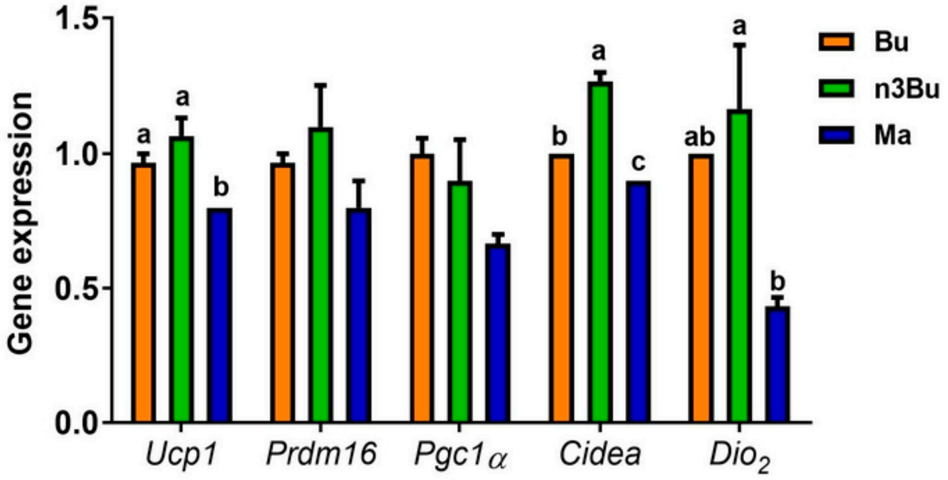

Figure S1. Supplementation with ALA-biofortified butter alters thermogenic genes expression in the BAT. mRNA expression levels of *Ucp1*, *Prdm16*, *Pgc1α*, *Cidea*, and *Dio2* from the pooled DNA (n=8 per group) with triplicated assay. All data represented as mean ± SEM. Treatments with different letters are significantly different from one another by one-way ANOVA (P<0.05).

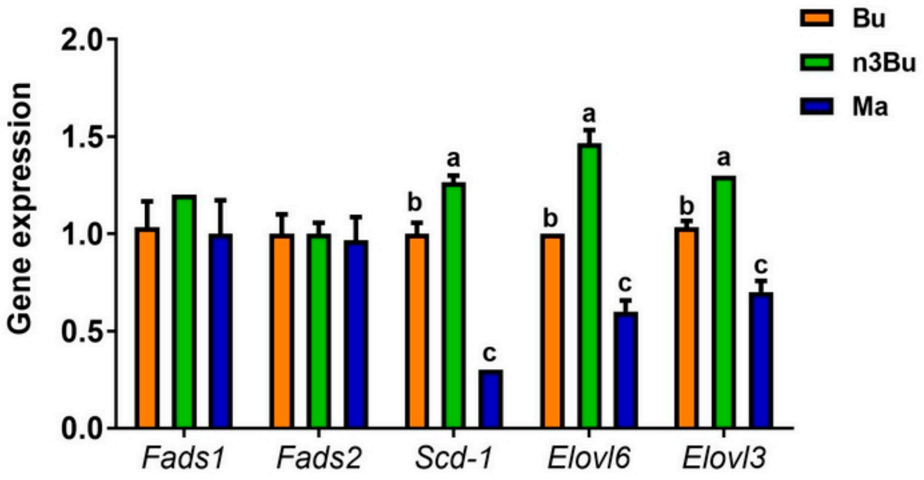

Figure S2. Supplementation with ALA-biofortified butter increased fatty acid denaturation and elongation related genes expression in the BAT. mRNA expression levels of *Fads1*, *Fads2*, *Scd-1*, *Elovl6*, and *Elovl3* from the pooled DNA (n=8 per group) with triplicated assay. All data represented as mean ± SEM. Treatments with different letters are significantly different from one another by one-way ANOVA (P<0.05).

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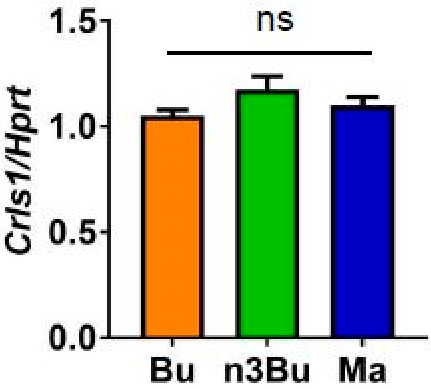

Figure S3. Supplementation with ALA-biofortified butter did not alter cardiolipin (CL) synthase (*Crls1*) expression in the BAT. mRNA expression *Crls1* in the BAT (n=4 of individual animals per group). All data represented as mean ± SEM. Treatments with different letters are significantly different from one another by one-way ANOVA (P<0.05).

Table S1. Diet composition of HF diet

Ingredients (g)	Diet		
	Bu	**n3Bu**	**Ma**
Casein	235	235	235
L-cystine	5	5	5
Corn starch	210	210	210
Maltodextrin	100	100	100
Sucrose	100	100	100
Cholesterol	2	2	2
Cellulose	50	50	50
Mineral mix	35	35	35
Calcium carbonate	30	30	30
Vitamin mix	10	10	10
Choline bitartrate	2.5	2.5	2.5
Solid Fat			
Hiland Butter	240	-	-
Sunseo Omega Butter		240	-
Land O Lake Margarine	-	-	240
% calories from Fat	45.4	45.4	45.4
% calories from CHO	34.4	34.4	34.4
% calories from Protein	20.2	20.2	20.2
Kcal/g diet	4.67	4.67	4.67

Table S2. Primer sequences for qPCR.

Primer	Primer sequence
Ucp1	F: 5′-AGGCTTCCAGTACCATTAGGT-3′ R: 5′-CTGAGTGAGGCAAAGCTGATTT-3′
Prdm16	F: 5′-CAG CAC GGT GAA GCC ATT C-3′ R: 5′-GCG TGC ATC CGC TTG TG-3′
Pgc1α	F: 5′-CCCTGCCATTGTTAAGACC-3′ R: 5′-TGCTGCTGTTCCTGTTTTC-3′
Cidea	F: 5′-TGCTCTTCTGTATCGCCCAGT-3′ R: 5′-GCCGTGTTAAGGAATCTGCTG-3′
Dio2	F: 5′-CAGTGTGGTGCACGTCTCCAATC-3′ R: 5′-TGAACCAAAGTTGACCACCAG-3′
Fads1	F: 5′-TCAGTCTTTGGCACCTCGAC-3′ R: 5′-TCCTTGCGGAAGCAGTTAGG-3′
Fads2	F: 5′-TCCTGTCCCACATCATCGTCATGG-3′ R: 5′-GCTTGGGCCTGAGAGGTAGCGA-3′
Scd-1	F: 5′-GGGACAGATATGGTGTGAAACTATG-3′ R: 5′-TTACAGACACTGCCCCTCAAC-3′
Elovl6	F: 5′-CGTAGCGACTCCGAAGATCAGCC-3′ R: 5′-AGCGTACAGCGCAGAAAACAGGA-3′
Elovl3	F: 5′-CTTCGAGACGTTTCAGGACTTAAG-3′ R: 5′-TCTGGCCAACAACGATGAG-3′
16S rRNA	F: 5′-CCGCAAGGGAAAGATGAAAGAC-3′ R: 5′-TCGTTTGGTTTCGGGGTTTC-3′
Hexokinase	F: 5′-GCCAGCCTCTCCTGATTTTAGTGT-3′ R: 5′-GGGAACACAAAAGACCTCTTCTGG-3′

Table S3. List of primary antibodies

Antibody	Host	Dilution	Company	Catalog no.
UCP1	Rabbit	1:1000	Abcam	Ab155117
PRDM16	Mouse	1:1000	Santa Cruz	Sc130243
CD11c	Rabbit	1:1000	Cell Signaling	97585
F4/80	Rabbit	1:1000	Abcam	Ab6640
SCD1	Rabbit	1:1000	Cell Signaling	2794
Elovl6	Rabbit	1:1000	Abcam	Ab69857
VDAC1	Rabbit	1:1000	Cell Signaling	4661
PDH	Rabbit	1:1000	Cell Signaling	3205
OxPhos	Mouse	1:250	Abcam	ab110413
SIRT3	Rabbit	1:1000	Cell Signaling	5490
β-actin	Rabbit	1:1000	Cell Signaling	4967