

SUPPLEMENTARY MATERIAL

FOR

Vitamin A regulates tissue-specific organ remodeling in diet-induced obesity independent of mitochondrial function

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Supplemental Table 1: Composition of mouse diets

Ingredient		Unit	NCD	HFD	VAD	VAD HFD
Carbohydrate		[% by calories]	67.9	23.2	67.8	22.5
Protein		[% by calories]	20.1	16.5	20.1	16.6
Fat		[% by calories]	13.0	60.1	13.0	60.8
Amino acids	Alanine	[g/kg]	2.53	11.68	2.53	11.76
	Arginine	[g/kg]	9.83	13.33	9.83	13.43
	Aspartic acid	[g/kg]	3.58	12.26	3.59	12.45
	Cystine	[g/kg]	3.20	1.41	3.20	1.43
	Glutamic acid	[g/kg]	23.67	29.68	23.69	30.18
	Glycine	[g/kg]	3.14	24.10	3.14	24.25
	Histidine	[g/kg]	5.28	3.42	5.28	3.48
	Isoleucine	[g/kg]	7.22	7.13	7.23	7.30
	Leucine	[g/kg]	14.76	7.48	14.77	7.52
	Lysine	[g/kg]	17.40	11.75	17.40	11.91
	Methionine	[g/kg]	7.22	4.32	10.69	4.41
	Phenylalanine	[g/kg]	7.17	7.04	7.18	7.16
	Proline	[g/kg]	12.76	20.46	12.77	20.67
	Serine	[g/kg]	5.27	8.12	5.27	8.25
	Threonine	[g/kg]	7.15	6.16	7.16	6.27
	Tryptophan	[g/kg]	1.98	1.25	1.98	1.29
	Tyrosine	[g/kg]	9.29	5.12	9.29	5.23
	Valine	[g/kg]	3.30	7.49	3.30	7.64
Fatty acids	C4, Butyric	[g/kg]		4.19		4.36
	C7, Caproic	[g/kg]		3.68		3.83
	C8, Caprylic	[g/kg]		1.27		1.32
	C10, Capric	[g/kg]		2.62		2.72
	C12, Lauric	[g/kg]	0.05	3.13	0.05	3.25
	C14, Myristic	[g/kg]	0.10	13.90	0.10	14.34
	C16, Palmitic	[g/kg]	2.50	56.56	2.50	56.67
	C17, Margaric	[g/kg]	0.10	0.43	0.10	0.43
	C18, Stearic	[g/kg]	1.35	37.17	1.35	37.49
	C20, Arachidic	[g/kg]	0.05	3.79	0.05	3.79
	C22, Behenic	[g/kg]	0.15		0.15	
	C24, Lignoceric	[g/kg]	0.25		0.25	
	C16:1, Palmitoleic	[g/kg]	0.15	8.90	0.15	8.99
	C18:1, Oleic	[g/kg]	13.50	111.56	13.50	112.45
	C-20:1, Eicosaenoic	[g/kg]	0.15	1.71	0.15	1.71
	C22:1, Erucic	[g/kg]	0.05	1.75	0.05	1.82
	C18:2, Linoleic	[g/kg]	28.50	22.12	28.50	22.17
	C18:3, Linolenic	[g/kg]	0.15	3.13	0.15	3.16

Ingredient		Unit	NCD	HFD	VAD	VAD HFD
Vitamins	Biotin	[mg/kg]	0.20	0.20	0.20	0.20
	Choline chloride	[g/kg]	1.01	1.00	1.01	1.00
	Folic acid	[mg/kg]	10.00	10.02	10.01	10.02
	Nicotinic acid	[mg/kg]	50.17	50.03	50.17	50.03
	Pantothenic acid	[mg/kg]	50.11	50.02	50.11	50.02
	Vitamin A	[I.E./kg]	15,000	15,000		
	Vitamin B1	[mg/kg]	20.04	20.01	20.04	20.01
	Vitamin B2	[mg/kg]	20.32	20.06	20.32	20.06
	Vitamin B6	[mg/kg]	15.23	15.21	15.03	15.01
	Vitamin B12	[mg/kg]	0.04	0.03	0.04	0.03
	Vitamin C	[mg/kg]	20.00	20.00	21.00	21.00
	Vitamin D3	[I.E./kg]	500.00	500.00	500.00	500.00
	Vitamin E	[mg/kg]	180.40	150.07	180.40	150.07
	Vitamin K3	[mg/kg]	10.20	10.00	10.20	10.00
Nutrients	Aluminum	[mg/kg]	3.71	2.37	3.74	2.45
	Calcium	[g/kg]	9.31	7.21	9.54	7.28
	Chlorine	[g/kg]	3.63	3.93	3.63	4.00
	Cobalt	[mg/kg]	0.15	0.09	0.15	0.09
	Copper	[mg/kg]	5.75	3.32	5.65	3.32
	Fluorine	[mg/kg]	4.17	3.03	4.17	3.03
	Iodine	[mg/kg]	0.51	0.27	0.45	0.27
	Iron	[mg/kg]	178.58	104.43	178.61	104.42
	Magnesium	[g/kg]	0.68	0.60	0.67	0.60
	Manganese	[mg/kg]	100.89	58.94	100.89	58.95
	Molybdenum	[mg/kg]	0.20	0.18	0.20	0.18
	Phosphorus (digested)	[g/kg]	7.20	4.45	7.21	4.49
	Phosphorus (total)	[g/kg]	7.52	5.35	7.53	5.41
	Potassium	[g/kg]	7.09	6.58	7.17	6.67
	Selenium	[mg/kg]	0.33	0.17	0.33	0.17
	Sodium	[g/kg]	2.49	1.67	2.50	1.67
	Sulfur	[g/kg]	2.79	1.42	2.79	0.68
	Zinc	[mg/kg]	29.30	15.24	29.31	15.24
Supplements	Cholesterol	[mg/kg]		211.85		211.85
	Chondroitin	[g/kg]		31.20		31.20
	Glucosamine	[g/kg]		66.00		66.00
	Inositol	[mg/kg]	111.00	101.93	111.00	101.93

NCD, normal chow diet; HFD, high fat diet; VAD, vitamin A-deficient diet; VAD HFD, vitamin A-deficient high fat diet.

Supplemental Table 2: *Antibodies used for immunoblotting*

Antigen	Company	Catalog number
4-HNE	Abcam, Cambridge, MA, USA	46545
ATP5A	Abcam, Cambridge, MA, USA	14748
MnSOD	BD Biosciences, San Jose CA, USA	611580
NDUFA9	Abcam, Cambridge, MA, USA	14713
SDHA	Abcam, Cambridge, MA, USA	14715
UCP3	Abcam, Cambridge, MA, USA	180643
Vinculin	Santa Cruz Biotechnology, Santa Cruz, CA	7649
Anti-goat	Jackson ImmunoResearch, West Grove, PA, USA	305-035-045
Anti-mouse	GE HealthCare, Chicago, IL, USA	NA934V
Anti-rabbit	GE HealthCare, Chicago, IL, USA	NXA931V

Supplemental Table 3: Primers used for quantitative real-time PCR analysis

Gene Name	Gene Sequence of forward and reverse primers (5' → 3')	GenBank Accession Number
<i>Carnitine palmitoyltransferase 1b (Cpt1b)</i>	TGCCTTTACATCGTCTCCAA AGACCCCGTAGCCATCATC	NM_009948.2
<i>Citrate synthase (Cs)</i>	CTCACAGTGGGGTGCTGCT CCCAGTCTCCCATTTTACCC	NM_026444.4
<i>Collagen, type I, alpha 1 (Col1a1)</i>	TGGTGAACGTGGTGTACAAGGT CAGTATCACCCCTTGGCACCAT	NM_007742.4
<i>Collagen, type III, alpha 1 (Col3a1)</i>	AAGGCTGAAGGAAACAGCAA TGGGGTTTCAGAGAGTTTGG	NM_009930.2
<i>Hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha (Hadha)</i>	TCAGGAGGGCTCAAAGAATAA GAAAGCCAAGCCCAAAGAC	NM_178878.3
<i>Hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit beta (Hadhb)</i>	GCCAACAGACTGAGGAAGGA ACACTGGCAAGGCTGGATT	NM_001289798.1
<i>Hypoxanthine guanine phosphoribosyl transferase (Hprt)</i>	GGACCTCTCGAAGTGTTGGATAC GCTCATCTTAGGCTTTGTATTTGGCT	NM_013556.2
<i>Ribosomal protein S16 (Rps16)</i>	TGCTGGTGTGGATATTCGGG CCTTGAGATGGGCTTATCGG	NM_013647.2
<i>Tissue inhibitor of metalloproteinase 1 (Timp1)</i>	TCTGAGCCCTGCTCAGCAA AACAGGGAAACACTGTGCACAC	NM_001044384.1

Supplemental Table 4: Analysis of liver sections according to NAFLD Activity Score (NAS)

Endpoint	NCD	HFD	VAD	VAD HFD
Steatosis [Score] #, \$	0.20 ± 0.13	2.20 ± 0.25 *	0.00 ± 0.00	1.20 ± 0.39 *†
Lobular inflammation [Score] #	0.00 ± 0.00	0.50 ± 0.17	0.00 ± 0.00	0.50 ± 0.22
Hepatocyte ballooning [Score] #	0.30 ± 0.21	0.70 ± 0.21	0.11 ± 0.11	0.80 ± 0.13
NAS #	0.50 ± 0.31	3.40 ± 0.31 *	0.11 ± 0.11	2.50 ± 0.64 *

Data are reported as mean values ± SE, n=9-10. Two-way ANOVA was performed to analyze differences by HFD feeding and VitA. Results of post-hoc analyses for each comparison are summarized by symbols as defined: # p<0.05 for HFD feeding and \$ p<0.05 for VitA. No significant effect for the interaction between HFD feeding and VitA was determined. * p<0.05 vs. normocaloric diet same VitA availability, † p<0.05 vs. VitA sufficiency same caloric diet.