

Supplementary data file

Title page

Title: Overexpressed amyloid precursor protein, and its C-terminal fragment, augment mitochondrial dysfunction in kidney and adipose tissues in a diet-induced obesity model

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Keywords: Amyloid precursor protein, overexpression, high fat diet, kidney tissue, adipose tissue

Non-normalised complex I, IV and CS activities

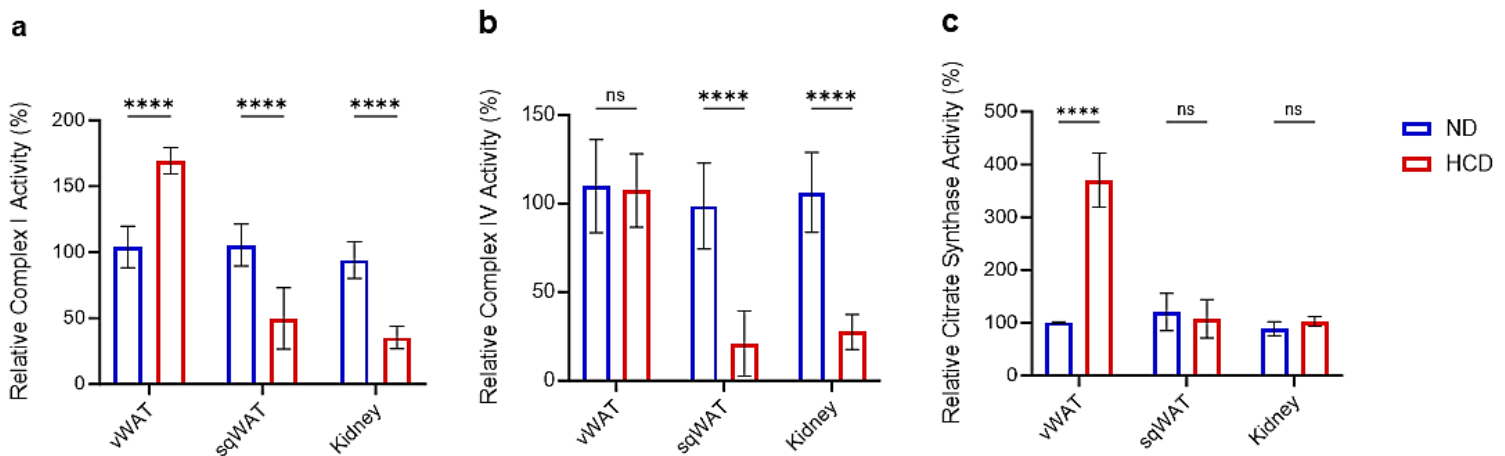


Figure S1. Non-normalised mitochondrial enzyme complex I (A), IV (B) and citrate synthase (C) activity in adipose and kidney tissue from mice fed a high calorie diet (HCD – red) as compared to mice fed a normal diet (ND – blue). Relative complex I, IV and citrate synthase enzyme activity as mean $\pm$ SD from visceral white adipose (vWAT), subcutaneous white adipose (sqWAT) and kidney mitochondrial subcellular fraction from mice fed a ND or a HCD. Each enzyme activity was normalised to total protein concentration. Relative activity was calculated with the assumption that the first ND sample was 100% (n=4). Statistical analysis was performed using a two-way ANOVA with Šidák multiple comparison test. **** p <0.0001 (α =0.05, CI 95%).

Nutritional Information

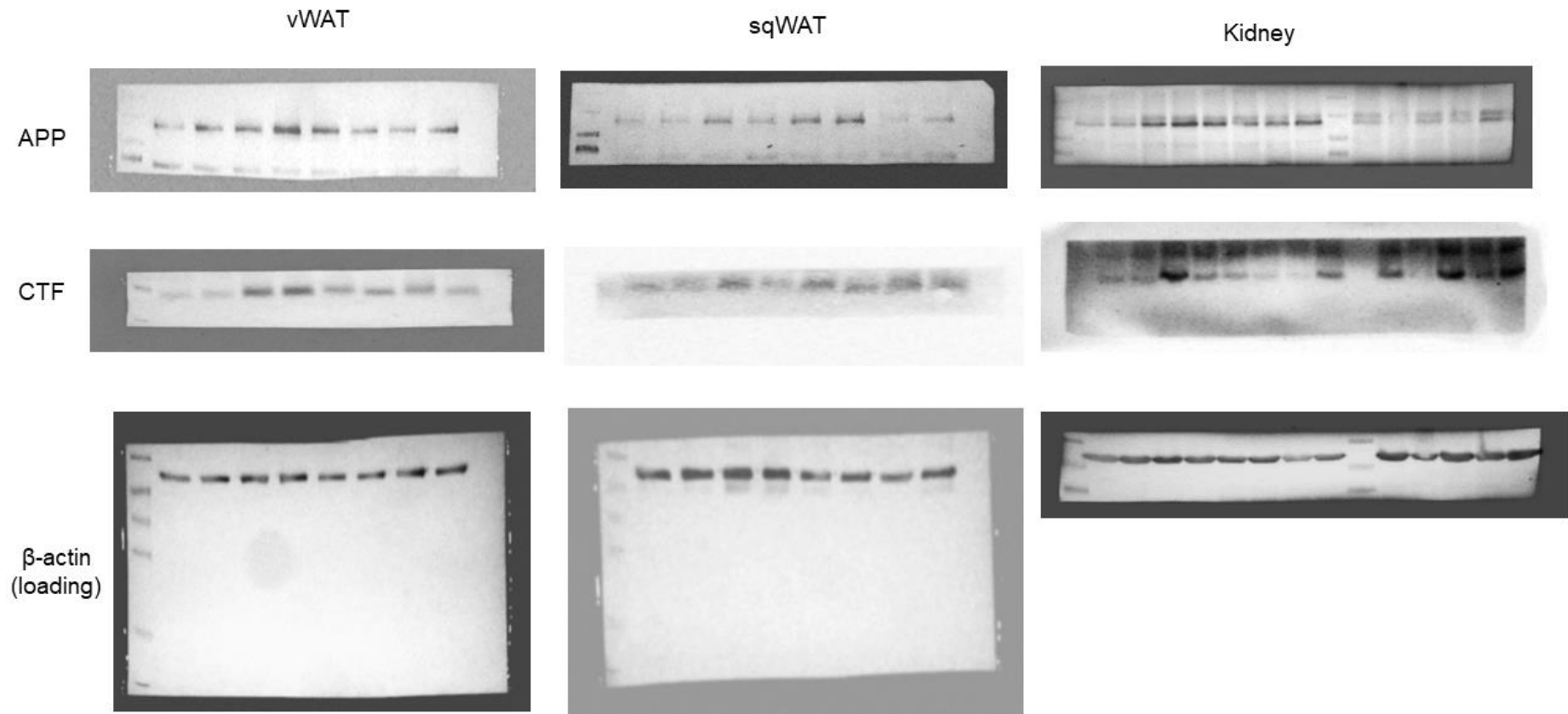
Table S1. Nutritional information for the high calorie supplement

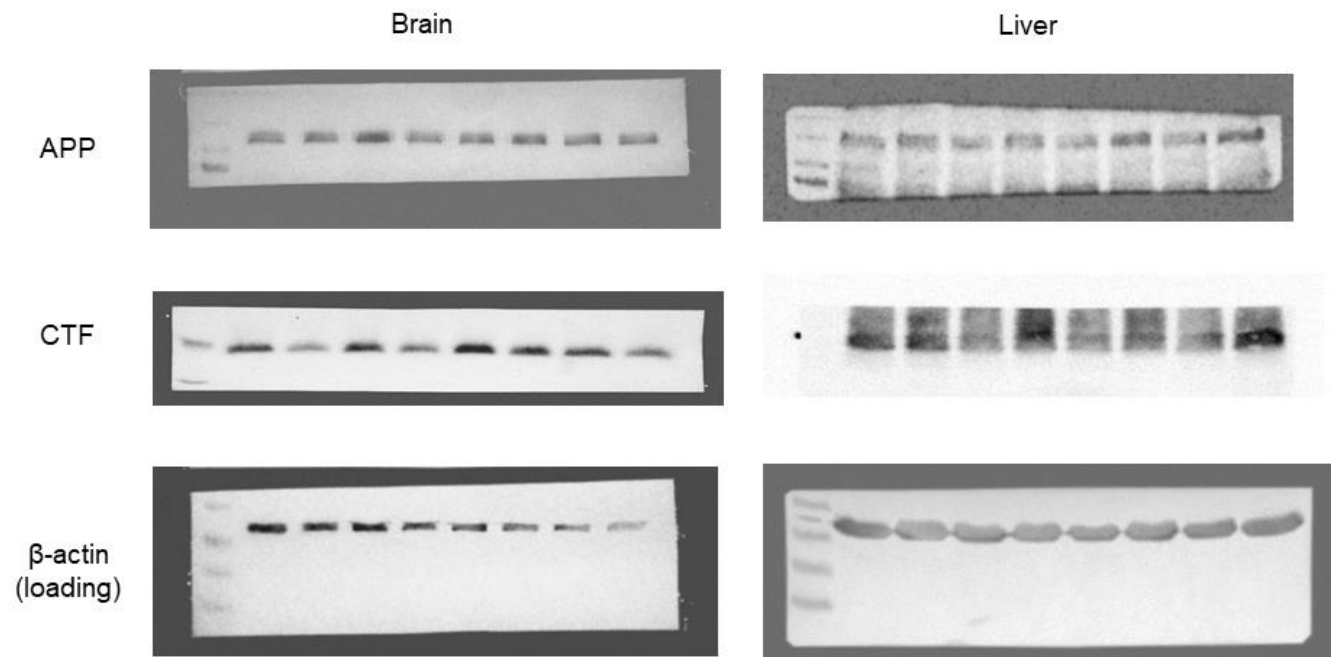
High Calorie Supplement ^a	
Protein	10.7%
Carbohydrates	13.0%
Sugar	8.75%
Fat	63.45%
Sodium	0.445%

^aMice in the high calorie diet group ate on average 1.13 ± 0.4 g/day of the supplement.

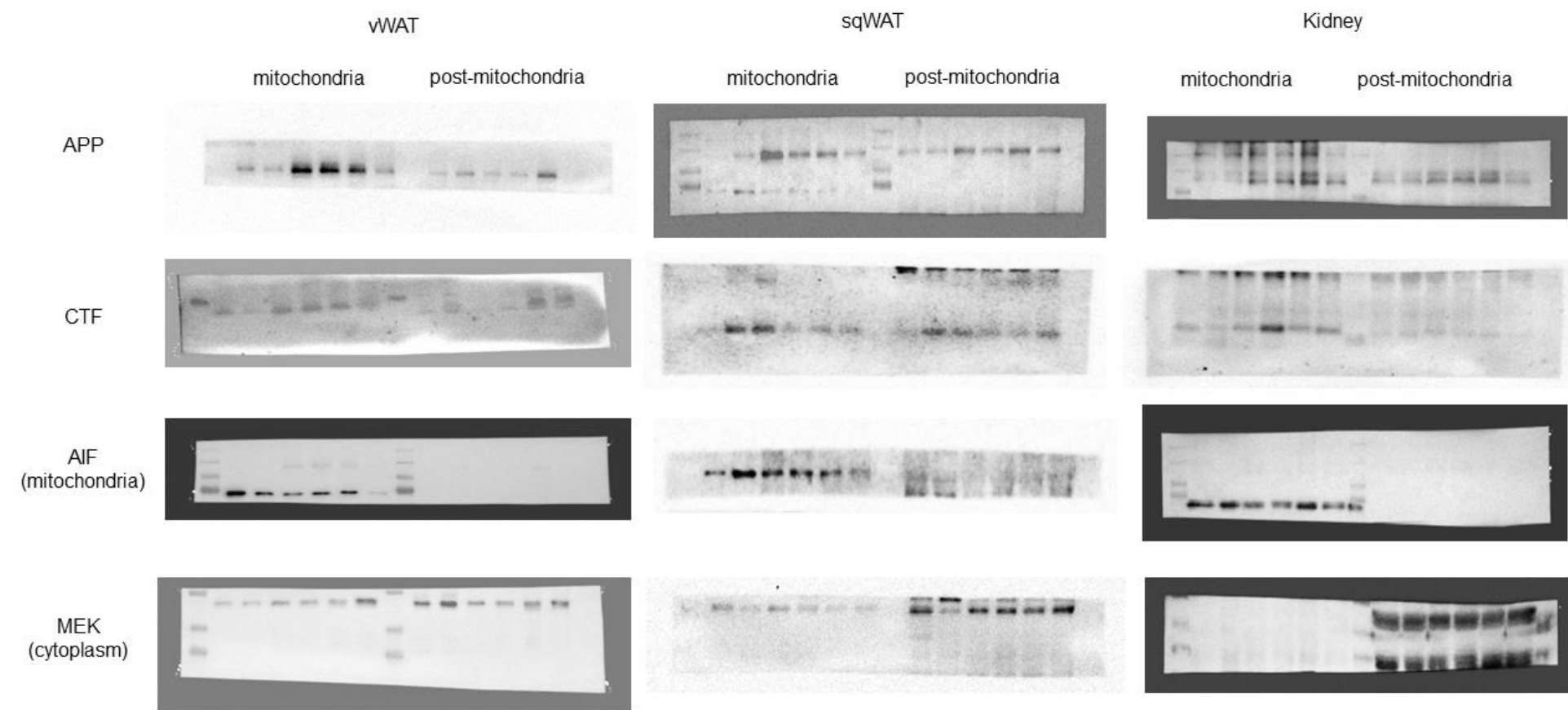
Raw data

Full-length Western Blots – Figure 2





Full-length Western Blots – Figure 3



APP and APP-βCTF level in fold from whole tissue homogenates as mean ± SD – Figure 2

Tissue	APP		APP-βCTF	
	ND	HCD	ND	HCD
vWAT	1.11 ± 0.14	3.66 ± 1.29	1.14 ± 0.16	4.25 ± 0.51
sqWAT	0.94 ± 0.27	3.72 ± 1.24	0.99 ± 0.09	1.66 ± 0.41
Kidney	0.83 ± 0.31	3.89 ± 0.88	0.69 ± 0.41	1.82 ± 0.88
Brain	0.89 ± 0.12	1.29 ± 0.34	0.96 ± 0.20	1.33 ± 0.31
Liver	0.98 ± 0.04	1.20 ± 0.09	1.00 ± 0.01	0.68 ± 0.04

APP, amyloid precursor protein; CTF, C-terminal fragment; ND, normal diet; HCD, high calorie diet; WAT, white adipose tissue; v, visceral; sq, subcutaneous.

APP and APP-βCTF level in fold from mitochondrial (m) and post mitochondrial (pm) fractions as mean ± SD – Figure 3

Sample	APP		APP-βCTF	
	ND	HCD	ND	HCD
vWAT m	0.98 ± 0.03	4.13 ± 0.74	0.99 ± 0.02	4.96 ± 0.12
vWAT pm	0.90 ± 0.13	1.13 ± 0.21	0.97 ± 0.05	1.03 ± 0.11
sqWAT m	0.89 ± 0.27	4.93 ± 2.63	0.76 ± 0.34	1.36 ± 0.68
sqWAT pm	1.32 ± 0.59	3.16 ± 0.48	1.80 ± 0.93	2.14 ± 1.51
Kidney m	1.08 ± 0.33	4.82 ± 0.45	1.10 ± 0.71	3.56 ± 2.90
Kidney pm	1.04 ± 0.08	1.82 ± 0.58	0.86 ± 0.17	1.39 ± 0.38

APP, amyloid precursor protein; CTF, C-terminal fragment; ND, normal diet; HCD, high calorie diet; WAT, white adipose tissue; v, visceral; sq, subcutaneous.

Relative complex I and IV activities as mean ± SD – Figure 4

Tissue	Complex I		Complex IV	
	ND	HCD	ND	HCD
vWAT	104.0 ± 15.7 %	46.7 ± 2.8 %	109.8 ± 26.3 %	29.6 ± 5.7 %
sqWAT	105.6 ± 15.9 %	43.6 ± 20.3 %	109.4 ± 16.5 %	9.9 ± 4.1 %
Kidney	96.8 ± 12.5 %	22.2 ± 5.4 %	104.9 ± 22.3 %	27.1 ± 9.8 %

APP, amyloid precursor protein; CTF, C-terminal fragment; ND, normal diet; HCD, high calorie diet; WAT, white adipose tissue; v, visceral; sq, subcutaneous.

Relative loss of complex I and IV activity in HCD-fed mice (100%-HCD activity) as mean ± SD – Figure 4

Tissue	Complex I	Complex IV
vWAT	53.3 ± 2.8 %	70.4 ± 5.7 %
sqWAT	67.3 ± 27.4 %	90.1 ± 4.1 %
Kidney	77.7 ± 5.4 %	78.7 ± 14.3 %

APP, amyloid precursor protein; CTF, C-terminal fragment; HCD, high calorie diet; WAT, white adipose tissue; v, visceral; sq, subcutaneous.