

## RESEARCH

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# AMP-activated protein kinase is required for the anti-adipogenic effects of alpha-linolenic acid

Xihong Zhou, Weiche Wu, Jingqing Chen, Xinxia Wang and Yizhen Wang\*

## Abstract

**Background:** n-3 long chain polyunsaturated fatty acid (n-3 LC PUFA) increases adiponectin level and reduces body weight accumulation in adipocytes. The current study was conducted to determine whether alpha-linolenic acid (ALA) could also exert the above effects and how AMP-activated protein kinase (AMPK) is involved.

**Methods:** AMPKα1<sup>-/-</sup>, AMPKα2<sup>-/-</sup> mice and wild-type (WT) mice were fed a high-fat diet (HFD) with or without ALA. Body weight was recorded weekly and serum was collected. Adipocytes size and mitochondrial biogenesis and lipid oxidation were also measured.

**Results:** Our results showed an elevated serum adiponectin level and reduced body weight in ALA-fed HFD mice when compared with WT mice fed HFD. In addition, adipocytes size and mitochondrial biogenesis were also reduced in ALA-fed HFD mice. At protein level, mitochondrial carnitine palmitoyltransferase 1α [CPT1α] and nuclear respiratory factor 1 [NRF1] were increased by dietary ALA in epididymal fat of WT mice. Consistently, mitochondrial DNA copy numbers. Moreover, lipogenesis was repressed by dietary ALA through down-regulating fatty acid synthase (FAS), acetyl CoA carboxylase (ACC) and stearoyl-CoA desaturase 1 [SCD1]. These aforementioned effects were abolished in the AMPKα1 and AMPKα2 knockout mice.

**Conclusions:** Our results suggest that ALA could reduce body weight and its anti-adipogenic effects are dependent on AMPK.

**Keywords:** Alpha-linolenic acid, AMP-activated protein kinase, Mitochondrial biogenesis, Adipose tissue

## Introduction

Obesity is a global health problem, which is associated with a high risk of developing type 2 diabetes, cardiovascular disease, and certain cancers [1]. Adipose tissue is a specialized organ that stores energy in the form of triglycerides and secretes various adipokines, including adiponectin, which plays a crucial role in regulating metabolism and energy balance [2]. Adiponectin is a secreted protein that is produced by adipocytes and has been shown to have anti-inflammatory and anti-adipogenic effects [3].

Alpha-linolenic acid (ALA) is an n-3 long chain polyunsaturated fatty acid (n-3 LC PUFA) that is found in various plant-based oils, including flaxseed oil, chia seed oil, and hemp seed oil. ALA has been shown to have various health benefits, including reducing inflammation, improving insulin sensitivity, and reducing the risk of cardiovascular disease [4].

AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme complex that plays a central role in regulating energy balance and metabolism. AMPK is activated by a variety of stimuli, including low energy status, exercise, and certain drugs [5]. AMPK activation leads to the phosphorylation of various substrates, which in turn regulates the activity of these proteins and ultimately leads to the inhibition of anabolic metabolism and the promotion of catabolic metabolism [6].

Recent studies have shown that AMPK is involved in the regulation of adipogenesis and lipid metabolism. AMPK activation has been shown to inhibit the differentiation of pre-adipocytes into mature adipocytes and to reduce the accumulation of lipids in adipocytes [7].

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## Materials and methods

## Animals and diets

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### Plasma biochemical assays

### Measurement of AMPK activity

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### DNA content in adipose tissue and Mitochondrial (mt)

## DNA analysis

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### Histology and cell-size measurement

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## Statistical analysis

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## Results

### Effects of ALA on serum insulin, adiponectin and leptin concentration in WT, AMPK $\alpha$ 1<sup>-/-</sup> and AMPK $\alpha$ 2<sup>-/-</sup> mice

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### Effects of ALA on HFD-induced fat deposition in white adipose tissue required AMPK

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 AMPK $\alpha$ 1<sup>-/-</sup>  
 AMPK $\alpha$ 2<sup>-/-</sup> .

Table 2 Effects of ALA on fat depots of WT, AMPK $\alpha 1^{-/-}$  and AMPK $\alpha 2^{-/-}$  mice fed HFD

	WT		AMPK $\alpha 1^{-/-}$		AMPK $\alpha 2^{-/-}$	
	HF	HF-A	HF	HF-A	HF	HF-A
Final BW (g)	42.6 ± 3.1	32.6 ± 1.8 <sup>ab</sup>	43.1 ± 3.0	42 ± 3.2	45.5 ± 3.4	44.2 ± 3.7
Epididymal						
weight (mg)	2270 ± 158	1224 ± 98 <sup>ab</sup>	2242 ± 201	2137 ± 198	2349 ± 178	2146 ± 184
DNA (μg/mg)	0.32 ± 0.04	0.67 ± 0.05 <sup>ab</sup>	0.29 ± 0.05	0.35 ± 0.06	0.29 ± 0.05	0.36 ± 0.07
Inguinal						
Weight (mg)	1090 ± 42	1037 ± 57	1097 ± 45	1058 ± 55	1102 ± 49	1072 ± 59
DNA (μg/mg)	0.42 ± 0.05	0.5 ± 0.06	0.36 ± 0.04	0.39 ± 0.06	0.38 ± 0.05	0.41 ± 0.07

Note: The values are means ± SE. <sup>a</sup>P < 0.05 for difference between different genotypes with HF-A diet; <sup>b</sup>P < 0.05 for difference between WT mice. BW, body weight. HF, mice fed high-fat diet; HF-A, mice fed high-fat diet with ALA.

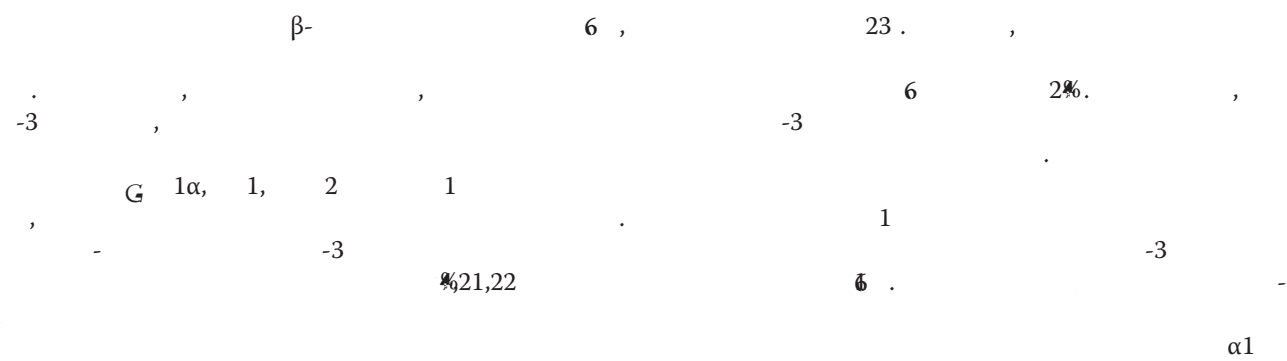


Figure 1 Adipocyte size in WT, AMPK $\alpha 1^{-/-}$  and AMPK $\alpha 2^{-/-}$  mice. **A, a**, WT mice fed HF diet; **B, b**, AMPK $\alpha 1^{-/-}$  mice fed HF diet; **C, c**, AMPK $\alpha 2^{-/-}$  mice fed HF diet; **D, d**, WT mice fed HF-A diet; **E, e**, AMPK $\alpha 1^{-/-}$  mice fed HF-A diet; **F, f**, AMPK $\alpha 2^{-/-}$  mice fed HF-A diet; **G, g**, mean adipocyte area in WT mice, AMPK $\alpha 1^{-/-}$  and AMPK $\alpha 2^{-/-}$  mice (uppercase represents epididymal fat; lowercase represents inguinal fat; cell size, μm<sup>2</sup>); HF, mice fed high-fat diet; HF-A, mice fed high-fat diet with ALA.

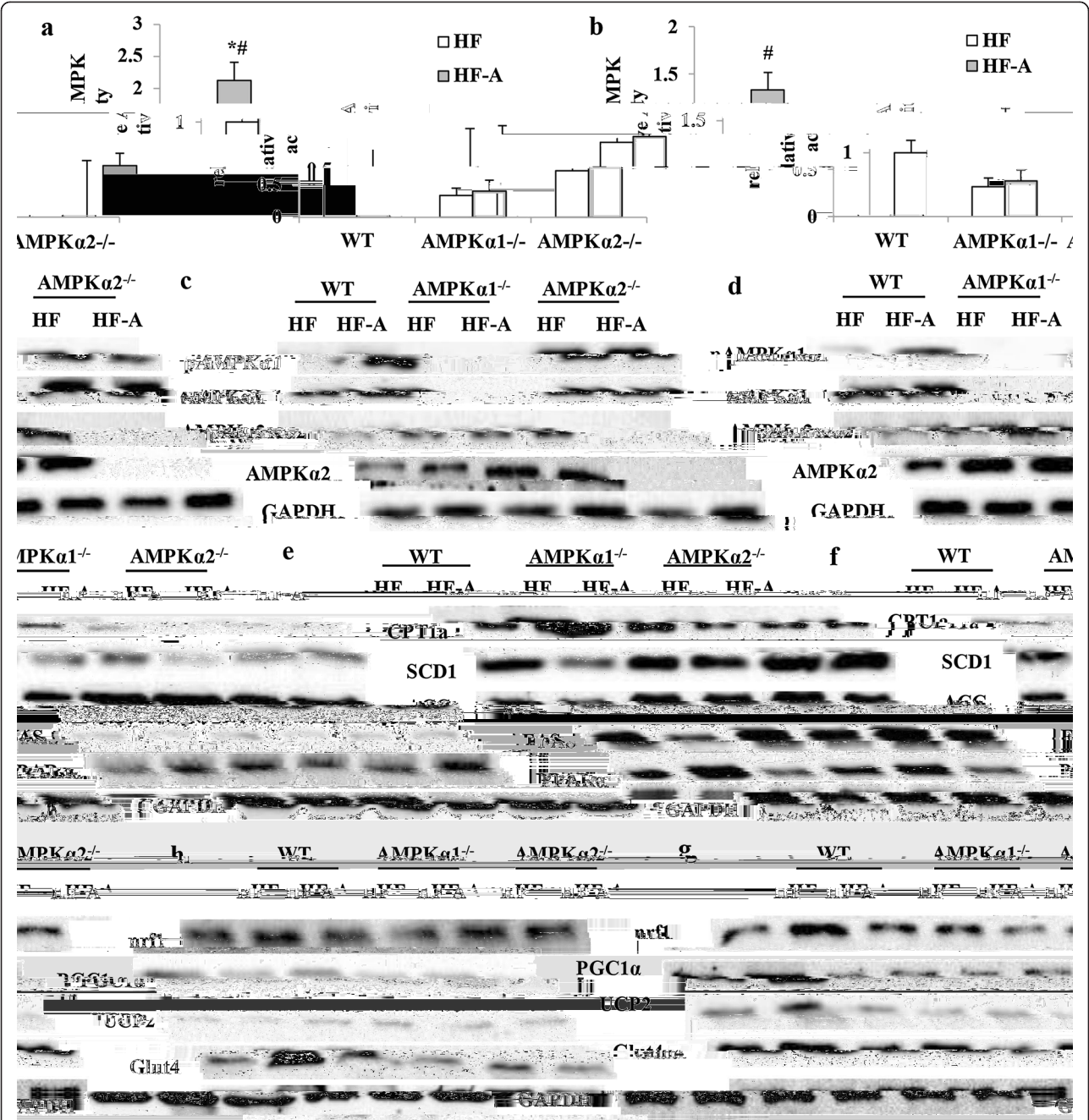


Figure 2 AMPK activity, protein expression of mitochondrial biogenesis and  $\beta$ -oxidation related genes in WT, AMPK $\alpha$ 1 $^{-/-}$  and AMPK $\alpha$ 2 $^{-/-}$  mice. HF, mice fed high-fat diet; HF-A, mice fed high-fat diet with ALA. (a, c, e, g, activity of AMPK and protein expressed in epididymal fat; b, d, f, h, activity of AMPK and protein expressed in inguinal fat). The values are means  $\pm$  SE. \*P < 0.05 for difference between different genotypes with HF-A diet; #P < 0.05 for difference between WT mice.

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carried out the experiments and collected the data; WYZ participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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