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Role of adenosine 5'-monophosphate-activated protein kinase in α-linolenic acid-induced intestinal lipid metabolism

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Abstract

n-3 Long-chain PUFA up-regulate intestinal lipid metabolism. However, whether these metabolic effects of PUFA on intestine are mediated by AMP-activated protein kinase (AMPK) remains to be elucidated. To determine the effects of α-linolenic acid (ALA) on intestinal fatty acid (FA) metabolism and whether these effects were affected by AMPK deletion, mice deficient in the catalytic subunit of AMPKα1 or AMPKα2 and wild-type (WT) mice were fed either a high-fat diet (HF) or HF supplemented with ALA (HF-A). The results showed that ALA supplementation decreased serum TAG content in WT mice. ALA also increased mRNA expression of genes (carnitine palmitoyltransferase 1a, acyl-CoA oxidase 1, medium-chain acyl-CoA dehydrogenase, cytochrome P450 4A10 and pyruvate dehydrogenase kinase isoenzyme 4a) involved in intestinal lipid oxidation and mRNA expression of TAG synthesis-related genes (monoacylglycerol *O*-acyltransferase 2, diacylglycerol *O*-acyltransferases 1 and 2) in WT mice. Consistent with these, expression levels of phosphorylated AMPKα1 and AMPKα2 were also increased in WT mice after ALA addition. However, in the absence of either AMPKα1 or AMPKα2, ALA supplementation failed to increase intestinal lipid oxidation. In addition, no significant effects of either diet (HF and HF-A) or genotype (WT, AMPKα1$^{-/-}$ and AMPKα2$^{-/-}$) on FA uptake in the intestine and faecal TAG output were observed. Our results suggest that AMPK is indispensable for the effects of ALA on intestinal lipid oxidation.

Key words: **α-Linolenic acid: AMP-activated protein kinase: Lipid metabolism: Intestine**

Dietary fat is a major energy source except providing essential fatty acids (FA) that are important for health. However, alterations in the amount and composition of dietary fat, especially an increase of *n*-6:*n*-3 PUFA ratio, may contribute to the development of obesity[1]. In the recent decades, increasing attention has been given to the role of the small intestine in the development of high-fat diet (HF)-induced obesity, as a high level of FA could affect intestinal lipid metabolism[2]. The small intestine is the gatekeeper at the physical interface between the body and the diet[2] and one of the main organs expressing β-oxidation-related enzymes[3,4]. As a result, lipid catabolism and uptake in the intestine may have a critical role in the aetiology of obesity.

Recently, dietary *n*-3 PUFA has been suggested to increase intestinal expression of genes related to FA oxidation[5,6] and cholesterol uptake[5]. However, the mechanism demonstrating how *n*-3 PUFA modulates lipid metabolism in the intestine remains to be elucidated. Many studies demonstrate that *n*-3 PUFA exert their effects on lipid metabolism through the PPAR pathway[5,7], as *n*-3 PUFA are intrinsic ligands for PPAR[8]. Moreover, *n*-3 PUFA could interact with PPARα and enhance PPARα transcriptional activity[9]. However, other studies report that expression of carnitine palmitoyltransferase 1 (Cpt1), a key gene involved in FA oxidation, could be induced by FA in the absence of PPARα[10,11]. These results suggest that other mechanisms independent of PPAR are involved in *n*-3 PUFA-induced FA catabolism.

AMP-activated protein kinase (AMPK) is the key sensor of whole-body energy homeostasis, and it has a critical role in the regulation of lipid metabolism through modulating Cpt1[12]. Previous studies have reported that *n*-3 PUFA could beneficially affect lipid oxidation through phosphorylated AMPK (pAMPK) in the liver[13], adipose tissue and skeletal muscle[14]. In addition, long-chain *n*-3 PUFA supplementation could activate AMPK and improve glucose uptake in the intestine[15]. Nevertheless, these results could not lead to the conclusion that AMPK are indispensable for the effects of long-chain *n*-3 PUFA on FA oxidation. Moreover, no evidence has suggested any effects of *n*-3 PUFA on intestinal lipid metabolism through AMPK.

Another important point is that most of the previous studies were focused on DHA and EPA. However, the plant-derived α-linolenic acid (ALA) is more available than the marine-derived DHA and EPA, because of the cost and supply constraints of

Abbreviations: ALA, α-linolenic acid; AMPK, AMP-activated protein kinase; FA, fatty acid; HF, high-fat diet; HF-A, high-fat diet supplemented with ALA; WT, wild type.

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seafood[16]. As a result, ALA is the most widely consumed *n*-3 PUFA in the Western diet[17]. It is also worth noting that fish oil (rich in DHA and EPA) may not completely reproduce the effects of ALA[18]. Therefore, the present study was conducted to determine whether ALA could increase intestinal lipid metabolism and whether AMPK are necessary for these effects of ALA by using mice deficient in AMPKα1 and AMPKα2.

Methods

Animals

All mice were housed individually and maintained at 21 ± 2°C, under a 12:12 h light–dark cycle with free access to water and food. To analyse the effects of ALA on intestinal lipid metabolism and to clarify whether AMPK was involved, we used AMPK$\alpha1^{-/-}$ and AMPK$\alpha2^{-/-}$ mice with a C57BL/6 genetic background. The AMPKα1 and AMPKα2 knockout mice were purchased from the Jackson laboratory and C57BL/6 mice were used as control.

Experimental design and sample collection

All mice at 8 weeks of age were maintained on a 45 % HF diet. The HF diet consisted of 45 % (kcal%) fat from lard and soyabean oil, 20 % protein and 35 % carbohydrate. FA composition of diet fat was presented in Table 1. C57BL/6, AMPK$\alpha1^{-/-}$ and AMPK$\alpha2^{-/-}$ mice (eight male mice per treatment) at 9 weeks of age were separately divided into two groups: either consuming a 45 % HF or 45 % HF containing 10 % ALA (Aladdin Ltd; maintaining the total amount of fat at 45 %) for 12 weeks. Food intake was measured throughout the study every 3 d, and body weight was recorded every week. On the last day of the experiment, mice were killed by cervical dislocation and blood was collected from the eyeballs. Next, the small intestine was removed and the jejunum and ileum were separated, thoroughly fluxed with sterile saline and immediately frozen in liquid N_2 and then stored at –80°C.

All experiments were approved by the Committee of Experimental Animal Care, Zhejiang University (Hangzhou, China).

Measurement of TAG and fatty acid composition in blood and faeces

TAG content was measured in dried faecal samples and presented as μg/d according to a previous study[7]. Briefly, the faeces were dried at 60°C overnight and lipids were extracted by the method of Folch *et al.*[19]. Next, TAG content in the lipid extracted from faeces and in serum were assayed using a TAG assay kit (GPO-POD; Applygen Technologies Inc.) as Zhang *et al.* did[20]. To analyse the FA composition of serum, lipid extraction and transesterification were processed according to a previous method[21]. FA methyl esters were measured by GC. The FA were identified by comparing the retention time of standard esters, and the composition of FA was calculated as a percentage of the total area.

Table 1. Fatty acid (FA) composition of dietary fat

FA (% of total fat)	HF	HF-A
C14	1·1	1·0
C16	24·71	22·24
C16 : 1	1·78	1·6
C18	13·9	12·51
C18 : 1	38·56	34·66
C18 : 2	17·0	15·3
C18 : 3	1·1	11·0
C20 : 3	0·58	0·52

HF, high-fat diet; HF-A, high-fat diet supplemented with *α*-linolenic acid.

Quantitative RT-PCR analysis

Total RNA was extracted with the TRIzol reagent (Invitrogen) and then complementary DNA was synthesised with RevertAid Reverse Transcriptase (Fermentas). Real-time PCR was performed according to the method used previously[22]. Briefly, the PCR system consisted of 10 μl of SYBR Premix Ex Taq (2×) mix (Roche), 0·4 μl of ROX (50×) (Roche), 1·0 μl of cDNA, 7·8 μl of double-distilled water and 0·4 μl of primer pairs (10 mM), all in a total volume of 20 μl. The PCR cycle involved an initial step at 95°C for 30 s, followed by 40 cycles of 5 s at 95°C and 34 s at 60°C. All primers were presented in Table 2. The comparative C_t value method was used to quantify mRNA expression relative to 18s rRNA. All samples were run in triplicate, and the average values were calculated.

Western blotting analysis

Proteins were extracted and supernatants were resolved on 10 % SDS/PAGE gel, and then electro-blotted onto a polyvinylidene difluoride membrane. Primary antibodies against AMPKα1, AMPKα2, pAMPKα1 (Santa Cruz), pAMPKα2 (Abcam) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Boster) were applied overnight at 4°C. After incubating with the secondary antibody for 1 h at room temperature, the membrane was detected using the Enhanced Chemiluminescence Detection Kit for horseradish peroxidase (Biological Industries).

Table 2. Mouse primers used for RT-PCR analysis

Genes	Primer sequence (5'–3')
CPT1a	Forward: CAGTCGACTCACCTTTCCTG
	Reverse: CATCATGGCTTGTCTCAAGTG
ACOX1	Forward: CTCTCTATGGGATCAGCCAGAA
	Reverse: CCACTCAAACAAGTTTTCATACACA
ACADM	Forward: TGCTCGCAGAAATGGCGATGA
	Reverse: CAATGTGCTCACGAGCTATGA
Cyp4a10	Forward: GTACCATGGTCTCCAAAATCCAA
	Reverse: ACTCGTGTGAGGATTAATCAGGTAGA
pdk4	Forward: GATCAGGGCAGTGACTTTCACAG
	Reverse: TCAGAGCTGAAATTTCAATGGAAAC
MGAT2	Forward: GTGTGGGATTAGGGGGACTT
	Reverse: TCCCTGTTTGTCCTTTGGTC
DGAT1	Forward: TTCCGCCTCTGGGCATT
	Reverse: AGAATCGGCCCACAATCCA
DGAT2	Forward: AGTGGCAATGCTATCATCATCGT
	Reverse: TCTTCTGGACCCATCGGCCCCAGGA
CD36	Forward: GGGCTGTGATCGGAACT
	Reverse: CTTGGCTAGATAACGAACTCTG
FATP4	Forward: GTGATGCCTTTGTGGG
	Reverse: TGTCGTCTGCGGTGAT

CPT1a, carnitine palmitoyltransferase 1a; *ACOX1*, acyl-CoA oxidase 1; *ACADM*, medium-chain acyl-CoA dehydrogenase; *Cyp4a10*, cytochrome P450 4A10; *pdk4*, pyruvate dehydrogenase kinase isoenzyme 4; *MGAT2*, monoacylglycerol *O*-acyltransferase 2; *DGAT1*, diacylglycerol *O*-acyltransferase 1; *DGAT2*, diacylglycerol *O*-acyltransferase 2; *CD36*, cluster of differentiation 36; *FATP4*, fatty acid transport protein 4.

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Statistical analysis

Data were analysed as a 3×2 factorial, except that RT-PCR data were analysed as a 3×2×2 factorial using Proc Mixed of SAS (SAS Institute Inc.). The statistical model for body weight gain, food intake and serum parameters include diet, genotype and their interaction. The statistical model for gene expression includes diet, genotype, intestinal segment and their interaction. Treatment means were calculated using the LSMEANS statement, and means are separated using the PDIFF option of PROC MIXED. An α-value of 0·05 was used to assess significant differences among means.

Results

Body weight gain and food intake in wild-type, AMPKα1$^{-/-}$ and AMPKα2$^{-/-}$ mice

According to Table 3, there was no effect of genotypes (wild type (WT), AMPKα1$^{-/-}$ and AMPKα2$^{-/-}$) or type of diets (HF and HF diet supplemented with ALA (HF-A)) on food intake, and no interactions between genotypes and types of diet were observed. Moreover, no interactions between genotypes and types of diet were observed for body weight gain. When compared with mice fed an HF diet, mice fed an HF-A diet had less body weight gain (17.2±0.3 v. 13.2±1.1 g). However, there was no significant effect of genotypes on body weight gain.

TAG concentration in serum and faeces and serum fatty acid composition in wild-type, AMPKα1$^{-/-}$ and AMPKα2$^{-/-}$ mice

According to Table 4, there was a tendency for significant interactions between genotypes and types of diet for TAG concentration in serum. In fact, this tendency was reflected in WT mice only. In addition, there was no effect of genotypes or types of diet on faecal TAG output, and no interactions between genotypes and types of diet were observed. As no interactions between genotypes and types of diet were observed and the effect of the genotypes was never significant, the data of FA composition in serum were presented independently from the genotypes (Table 5). Compared with mice fed an HF diet, mice fed an HF-A diet had higher levels of ALA (C18 : 3), EPA (C20 : 5) and DHA (C22 : 6) in serum, whereas mice fed an HF-A diet had a lower level of C18 : 1.

Effects of α-linolenic acid on the protein expression of AMPK and pAMPK in the small intestine of mice

No interactions among genotypes, types of diet and intestine site and other interactions were observed for protein expression of AMPKα1 and AMPKα2 in both the jejunum and the ileum. Protein expression levels of AMPKα2 in AMPKα1$^{-/-}$ mice were higher than in WT mice, and protein expression levels of AMPKα1 in AMPKα2$^{-/-}$ mice were higher than in WT mice in both the jejunum and the ileum (Fig. 1(a)–(e)). No interactions among genotypes, types of diet and intestine site and other interactions were observed, except that interactions between genotypes and types of diet were observed for relative protein expression of pAMPKα1 and pAMPKα2 in both the jejunum and the ileum, because the HF-A diet increased relative protein expression of pAMPKα1 compared with the HF diet in WT mice but not in AMPKα2$^{-/-}$ mice, and HF-A diet increased relative protein expression of pAMPKα2 compared with the HF diet in WT mice but not in AMPKα1$^{-/-}$ mice (Fig. 1(f)–(j)).

Effects of α-linolenic acid on gene expression of fatty acid oxidation, fatty acid transportation and TAG synthesis-related genes in the small intestine of mice

According to Table 6, no interactions among genotypes, types of diet and intestine site and other interactions were detected, except that interactions between genotypes and types of diet

Table 3. Body weight gain (BWG) and food intake (FI) in wild-type mice (WT), AMPKα1 whole-body knockout mice (AMPKα1$^{-/-}$) and AMPKα2 whole-body knockout mice (AMPKα2$^{-/-}$)
(Mean values with their pooled standard errors)

	WT		AMPKα1$^{-/-}$		AMPKα2$^{-/-}$			*P*		
	HF	HF-A	HF	HF-A	HF	HF-A	SEM	Diet	Genotype	Diet × genotype
IBW (g)	26·6	25·9	26·2	26·1	26·5	26·2	0·32			
BWG (g)	16·0	7·7	16·9	15·9	19·0	18·0	0·71	0·04	0·22	0·16
FI (kJ/d)	64·5	67·0	63·0	66·5	64·0	65·0	2·29	0·70	0·61	0·31

AMPK, AMP-activated protein kinase; HF, high-fat diet; HF-A, high-fat diet supplemented with *α*-linolenic acid; IBW, initial body weight.

Table 4. TAG content in serum and faeces of wild-type mice (WT), AMPKα1 whole-body knockout mice (AMPKα1$^{-/-}$) and AMPKα2 whole-body knockout mice (AMPKα2$^{-/-}$)
(Mean values with their pooled standard errors)

	WT		AMPKα1$^{-/-}$		AMPKα2$^{-/-}$			*P*		
	HF	HF-A	HF	HF-A	HF	HF-A	SEM	Diet	Genotype	Diet × genotype
Serum TAG (mmol/l)	0·72^{b}	0·49^{c}	0·89^{a}	0·77^{a,b}	0·78^{a,b}	0·66^{b}	0·05	0·03	0·16	0·06
Faecal TAG output (μg/d)	154	159	147	167	151	160	12	0·61	0·90	0·66

AMPK, AMP-activated protein kinase; HF, high-fat diet; HF-A, high-fat diet supplemented with *α*-linolenic acid.
[a,b,c] Within a row, means with unlike superscript letters tended to be significantly different.

Table 5. Fatty acid composition in serum of wild-type mice (WT), AMPKα1 whole-body knockout mice (AMPK$\alpha1^{-/-}$) and AMPKα2 whole-body knockout mice (AMPK$\alpha2^{-/-}$)*
(Mean values with their pooled standard errors)

Fatty acid (% of total fat)	HF	HF-A	SEM	*P*
C16	28·6	26·5	1·05	0·09
C18	17·7	17·1	1·22	0·17
C18 : 1	37·6[a]	31·8[b]	1·52	0·03
C18 : 2	13·5	14·2	0·99	0·41
C18 : 3	0·2[b]	7·3[a]	0·61	<0·001
C20 : 5	0[b]	0·34[a]	0·03	<0·001
C22 : 6	0[b]	0·54[a]	0·05	<0·001

AMPK, AMP-activated protein kinase.
HF: WT, AMPK$\alpha1^{-/-}$ and AMPK$\alpha2^{-/-}$ mice fed a high-fat diet; HF-A: WT, AMPK$\alpha1^{-/-}$ and AMPK$\alpha2^{-/-}$ mice fed a high-fat diet supplemented with α-linolenic acid.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* As no interactions between genotypes and types of diet were observed and the effect of the genotypes is never significant, the data are presented independently from the genotype.

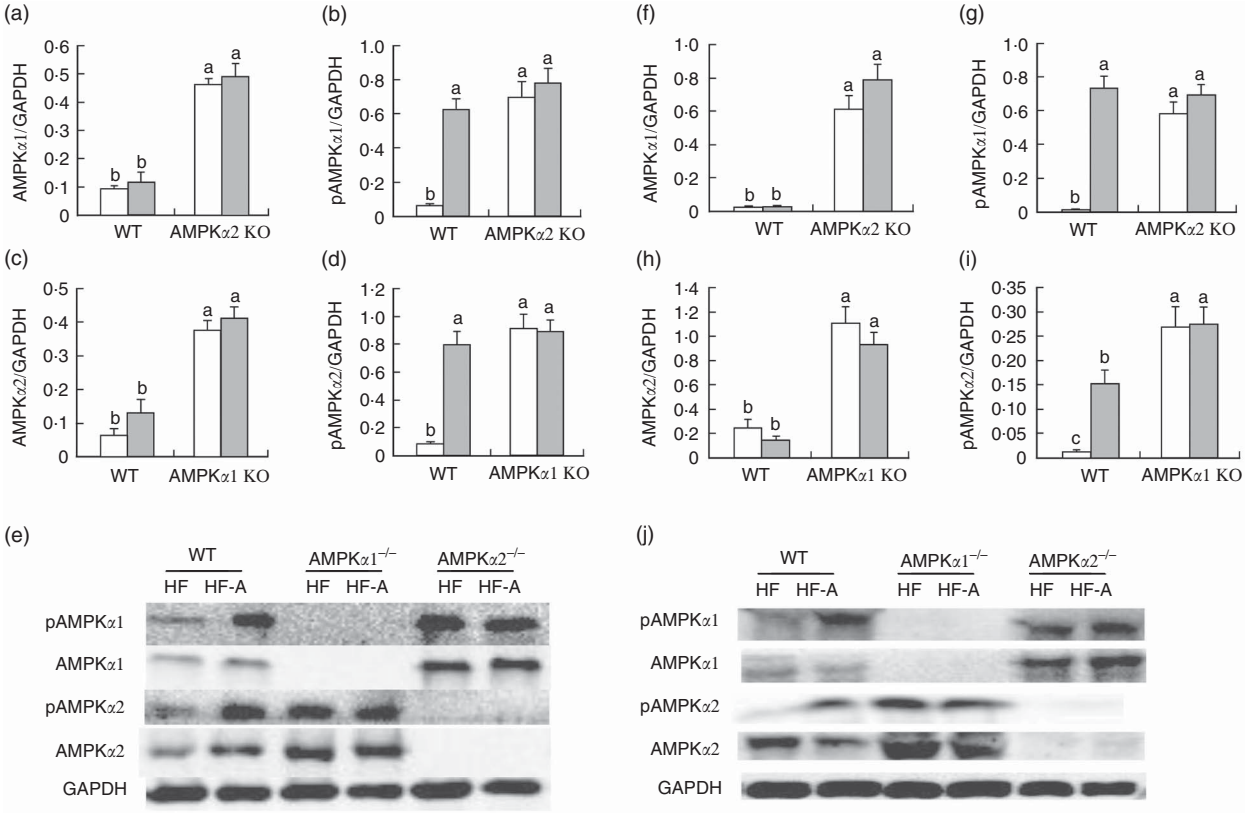

Fig. 1. Protein expression of AMP-activated protein kinase (AMPK) and phosphorylated AMPK (pAMPK) in the jejunum and ileum of mice. For subparts (a), (b), (c), (d), (f), (g), (h) and (i), relative protein expression was analysed by ImageJ. In subparts (e) and (j), Western blot analysis. AMPKα1 KO, AMPK$\alpha1^{-/-}$, AMPKα1 whole-body knockout mice; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; WT, wild type; AMPKα2 KO, AMPK$\alpha2^{-/-}$, AMPKα2 whole-body knockout mice; HF, high-fat diet (□); HF-A, high-fat diet supplemented with α-linolenic acid (■). [a,b,c] Mean values with unlike superscript letters were significantly different ($P<0·05$). No diet × genotype × site interactions and other interactions were detected ($P>0·05$) except that diet × genotype interactions were observed for relative protein expression of pAMPKα1 and pAMPKα2 in both the jejunum and ileum ($P<0·05$).

were observed for gene expression of CPT1a, acyl-CoA oxidase 1 (ACOX1), medium-chain acyl-CoA dehydrogenase (ACADM), cytochrome P450 4A10 (Cyp4a10), pyruvate dehydrogenase kinase isoenzyme 4 (pdk4), monoacylglycerol *O*-acyltransferase 2 (MGAT2), diacylglycerol *O*-acyltransferases 1 and 2 (DGAT1 and DGAT2) in both the jejunum and the ileum, because diet supplemented with ALA increased gene expression of *CPT1a, ACOX1, ACADM* (FA oxidation-related genes, which suggested an enhanced β-oxidation activity), Cyp4a10 (FA oxidation-related genes, which suggested an enhanced ω-oxidation activity), *pdk4* (strongly suggested a switch from glycolysis to fatty acid oxidation)[23], *MGAT2, DGAT1 and DGAT2* (TAG synthesis-related genes), but not in AMPK$\alpha2^{-/-}$ and AMPK$\alpha1^{-/-}$ mice. Gene expression levels of fatty acid transport protein 4 (FATP4) and the cluster of differentiation 36 (CD36) were higher in the jejunum than those in the ileum, whereas neither genotypes nor types of diet significantly affected gene expression of *FATP4* and *CD36*.

Table 6. Relative gene expression of fatty acid (FA) oxidation, transportation and TAG synthesis-related genes in the jejunum and the ileum of mice with different genotypes and diet treatment*
(Mean values with their pooled standard errors)

	Wild type		AMPK$\alpha1^{-/-}$		AMPK$\alpha2^{-/-}$			*P*		
Gene	HF	HF-A	HF	HF-A	HF	HF-A	SEM	Diet	Genotype	Diet × genotype
Jejunum										
CPT1a	1·00^{b}	2·74^{a}	1·31^{b}	1·13^{b}	1·14^{b}	1·04^{b}	0·24	0·16	0·08	0·01
ACOX1	1·00^{b}	2·47^{a}	0·96^{b}	1·23^{b}	1·12^{b}	1·26^{b}	0·30	0·03	0·11	0·01
ACADM	1·00^{b}	1·94^{a}	1·17^{b}	1·25^{b}	0·90^{b}	1·22^{b}	0·26	0·03	0·09	0·01
Cyp4a10	1·00^{b}	1·45^{a}	0·89^{b}	1·10^{b}	0·94^{b}	1·11^{b}	0·16	0·02	0·11	0·02
pdk4	1·00^{b}	1·99^{a}	0·92^{b}	1·06^{b}	1·05^{b}	1·14^{b}	0·25	0·02	0·31	0·02
CD36	1·00	1·33	0·94	1·28	0·87	1·11	0·23	0·17	0·18	0·12
FATP4	1·00	1·20	0·89	1·04	0·87	1·16	0·18	0·12	0·36	0·15
MGAT2	1·00^{a}	0·71^{b}	1·02^{a}	1·12^{a}	0·88^{a,b}	0·96^{a}	0·20	0·19	0·60	0·03
DGAT1	1·00^{a}	0·77^{b}	1·04^{a}	0·93^{a}	0·95^{a}	0·86^{a,b}	0·18	0·08	0·54	0·01
DGAT2	1·00^{a}	0·50^{b}	0·89^{a}	0·91^{a}	1·05^{a}	0·93^{a}	0·14	0·23	0·41	0·02
Ileum										
CPT1a	0·11^{b}	0·34^{a}	0·10^{b}	0·12^{b}	0·10^{b}	0·12^{b}	0·04	0·27	0·36	0·02
ACOX1	0·22^{b}	0·35^{a}	0·20^{b}	0·21^{b}	0·20^{b}	0·25^{b}	0·04	0·12	0·45	0·04
ACADM	0·21^{b}	0·37^{a}	0·20^{b}	0·24^{b}	0·20^{b}	0·25^{b}	0·03	0·11	0·27	0·04
Cyp4a10	0·27^{b}	0·78^{a}	0·25^{b}	0·29^{b}	0·24^{b}	0·27^{b}	0·05	0·15	0·37	0·03
pdk4	0·08^{b}	0·16^{a}	0·06^{b}	0·07^{b}	0·06^{b}	0·07^{b}	0·02	0·35	0·54	0·03
CD36	0·61	0·76	0·68	0·75	0·51	0·64	0·18	0·20	0·72	0·29
FATP4	0·51	0·60	0·46	0·53	0·50	0·53	0·14	0·79	0·61	0·48
MGAT2	0·53^{a}	0·32^{b}	0·52^{a}	0·46^{a}	0·60^{a}	0·52^{a}	0·06	0·12	0·16	0·01
DGAT1	0·27^{a}	0·16^{b}	0·30^{a}	0·24^{a}	0·28^{a}	0·27^{a}	0·04	0·11	0·58	0·03
DGAT2	0·62^{a}	0·35^{b}	0·64^{a}	0·58^{a}	0·67^{a}	0·53^{a}	0·10	0·17	0·54	0·01

AMPK$\alpha1^{-/-}$, AMPK$\alpha1$ whole-body knockout mice; AMPK$\alpha2^{-/-}$, AMPK$\alpha2$ whole-body knockout mice; HF, high-fat diet; HF-A, high-fat diet supplemented with α-linolenic acid. *CPT1a* (carnitine palmitoyltransferase 1a), *ACOX1* (acyl-CoA oxidase 1) and *ACADM* (medium-chain acyl-CoA dehydrogenase) are involved in β-oxidation activity; *Cyp4a10* (cytochrome P450 4A10) is involved in ω-oxidation activity; high expression of *pdk4* (pyruvate dehydrogenase kinase isoenzyme 4) suggests a switch from glycolysis to FA oxidation; *CD36* (the cluster of differentiation 36) and *FATP4* (fatty acid transport protein 4) are involved in fatty acid transportation; *MGAT2* (monoacylglycerol *O*-acyltransferase 2), *DGAT1* and *DGAT2* (diacylglycerol *O*-acyltransferase 1 and 2) are involved in TAG synthesis.

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).

* No diet × genotype × site interactions and other interactions were detected ($P > 0·05$), except that diet × genotype interactions were observed for gene expression of *CPT1a, ACOX1, ACADM, Cyp4a10, pdk4a, MGAT2, DGAT1 and DGAT2* in both the jejunum and ileum ($P < 0·05$).

Discussion

n-3 Long-chain PUFA, DHA and EPA have been demonstrated to increase intestinal lipid metabolism and attenuate hyperlipidaemia[5–7]. However, little research concern the effects of their precursor ALA on intestinal lipid oxidation, except that ALA-rich TAG could reduce body weight gain and stimulate the intestinal β-oxidation[24]. In the current study, our results showed that ALA alone could protect WT mice from HF-induced hyperlipidaemia and increased intestinal FA oxidation.

Some studies demonstrate that the efficiency of ALA conversion to DHA and EPA is very limited[25], whereas other studies report that dietary ALA could increase the levels of EPA and DHA in serum, adipose tissue[26] and hepatic membrane[27,28]. Moreover, ALA, without converting to DHA and EPA, could protect against HF-induced obesity and its related diseases[29]. Our results showed that dietary ALA decreased HF-induced serum TAG increase in WT mice fed an HF diet. Dietary ALA remarkably improved serum ALA level in either WT mice or AMPK-lacking mice. In addition, a low but measurable level of DHA and EPA were observed in serum after ALA supplementation. Consequently, our results further confirmed that ALA alone could alleviate hyperlipidaemia, as previous study demonstrated[29]. In addition, genotypes had no effects on FA composition in serum.

AMPK is a heterotrimeric enzyme consisting of an α catalytic subunit and noncatalytic β and γ subunits. The $\alpha1$ and $\alpha2$ isoforms of the α subunit share 90 % amino acid sequence homology within the catalytic site, but major differences in the C-terminal tails of $\alpha1$ and $\alpha2$ sequences exist[30]. As a result, these two catalytic subunits conferred tissue-specific difference in regard to the formation of heterotrimers and metabolic regulation[31]. For the recent decades, some findings suggested that the $\alpha2$ subunit was responsible for the modulation of gene expression[32]. However, other results indicated that the $\alpha1$ catalytic subunit accounts for most of the activity of AMPK, especially in adipocytes[33,34]. Moreover, mice lacking the $\alpha2$ subunit exhibited adiposity and adipocyte hypertrophy when subjected to the HF diet[35]. Our results showed that when one catalytic subunit was lacking, the expression of the other catalytic subunit was compensatorily up-regulated. Surprisingly, ALA did not affect the expression of AMPKα subunit in the intestine. However, ALA supplementation increased AMPK phosphorylation of both $\alpha1$ and $\alpha2$ subunit in the small intestine. These results suggested that ALA may increase intestinal AMPK activity.

Furthermore, mice with a whole-body deletion of AMPK$\alpha2^{-/-}$ and feeding on HF had the same serum TAG level as WT mice, as previously described[35]. In our results, the observation that AMPK$\alpha2^{-/-}$ mice also had the same expression of genes involved in FA oxidation in the intestine as WT mice when fed an HF diet indicated a compensatory effect in the AMPK catalytic subunit. However, when subjected to an HF-A, WT mice tended to have a lower level of serum TAG than AMPK$\alpha2^{-/-}$

mice and mRNA expression levels of genes involved in FA oxidation in the intestine of WT mice was higher than those in AMPK$\alpha 2^{-/-}$ mice. These results indicated that AMPKα2 was required for the effects of ALA on intestinal FA metabolism. It has been reported that mice lacking AMPK$\alpha 2^{-/-}$ showed an enhanced lipid accumulation in adipocytes without any changes in adipocytes number or differentiation[35]. However, both our results and those from the previous study observed no significant difference of serum TAG concentration between WT and AMPK$\alpha 2^{-/-}$ mice fed an HF. Accordingly, one of the important reasons for the low serum TAG level of WT mice subjected to an HF-A may therefore be the increased FA oxidation in the small intestine, although increased FA oxidation in small intestine would not be the only reason as ALA could also increase lipid oxidation in other tissues such as adipose tissue[36], liver[26] and skeletal muscle[37]. In contrast to the previous report, demonstrating that AMPKα2 is not necessary for hypolipidaemic effect of *n*-3 PUFA (DHA and EPA) in *ad libitum*-fed mice[38], our study showed a tendency of higher level of serum TAG in AMPK$\alpha 2^{-/-}$ mice fed an HF-A than that in WT mice. These results indicated that AMPKα2 may only be involved in the effects of ALA, but not in the effects of DHA or EPA. However, mice with a whole-body deletion of AMPK$\alpha 1^{-/-}$ and feeding on HF tended to have a higher TAG level in serum than WT mice, and ALA addition in the diet did not decrease TAG level in serum in AMPK$\alpha 1^{-/-}$ mice. These results indicated that AMPKα1 was required for protection by ALA from HF-induced hyperlipidaemia. Moreover, the fact that ALA supplementation increased mRNA expression of genes involved in FA oxidation only in WT mice but not in AMPK$\alpha 1^{-/-}$ mice indicated that AMPKα1 is also indispensable for the effects of ALA on intestinal FA oxidation.

In addition, neither ALA addition nor AMPK catalytic subunit deletion affected the expression of genes involved in intestinal FA uptake, as no significant changes were observed in *CD36* and *FATP4* expression. Moreover, no difference in faecal TAG output in all treatments could be further evidenced. However, TAG synthesis in WT mice subjected to HF-A diet was significantly decreased, as mRNA expression of *DGAT1* and *DGAT2* was higher than with the other treatment. In addition, increased FA oxidation caused by ALA supplementation may be the primary reason for this change.

In conclusion, our results showed that ALA protected WT mice from HF-induced hyperlipidaemia and increased intestinal FA oxidation. Moreover, both AMPKα1 and AMPKα2 were indispensable for the effects of ALA on intestinal FA oxidation. These findings might provide new sights to better understand the effects of ALA on intestinal FA metabolism and their molecular mechanisms, and would be useful in the management of systemic lipid metabolism. Nevertheless, further studies will be needed to explore the differential effects of *n*-6 PUFA and *n*-3 PUFA on intestinal lipid metabolism and their molecular mechanisms.

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