R e f ade e 5- a e-ac a ed e a e α- e c ac d- d ced e a d e ab

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Ab ac

n-3 L g-cha PUFA eg late te al li id metab li m. H e e, hethe the e metab lic effect f PUFA te e a e mediated b AMP-acti ated te kina e (AMPK) ema t be el cidated. T dete mi e the effect f *α*-le ic acid (ALA) te al fatt acid (FA) metab li m a d hethe the e effect e e affected b AMPK deleti , mice deficie t i the catal tic b it f AMPK*α*1 AMPK*α*2 a d ild-t e (WT) mice e e fed eithe a high-fat diet (HF) HF leme ted ith ALA (HF-A). The e lt h ed that ALA leme tati dec ea ed e m TAG c te t WT mice. ALA al c ea ed mRNA e e i f ge e (ca itine almit lt fe a e 1a, ac l-C A ida e 1, medi m-cha ac l-C A deh d ge a e, c t ch me P450 4A10 a d ate deh d ge a e kina e i e me 4a) l ed te al li id idati a d mRNA e e i f TAG the i elated ge e (m ac lgl ce l *O*-ac lt fe a e 2, diac lgl ce l *O*-ac lt fe a e 1 a d 2) WT mice. C i te t ith the e, e e i le el f h h lated AMPK*α*1 a d AMPK*α*2 e e al c ea ed WT mice afte ALA additi . H e e, the ab e ce f eithe AMPK*α*1 AMPK*α*2, ALA leme tati failed t c ea e te al li id idati . addii , ig ificat effect f eithe diet (HF a d HF-A) ge t e (WT, AMPK*α*1$^{-/-}$ a d AMPK*α*2$^{-/-}$) FA take the te e a d faecal TAG t t e e b e ed. O e lt gge t that AMPK i di e able f the effect f ALA te al li id idati .

Ke d : ***α*-Linolenic acid: AMP-activated protein kinase: Lipid metabolism: Intestine**

Diet f fat i a maj e e g ce e ce t id g e e tial fatt acid (FA) that a e im ta t f health. H e e, alte ati the am t a d c m iti f diet f fat, e ciall a c ea e f *n*-6:*n*-3 PUFA ati , ma c f ib te t the de el me t f be it [1]. the ece t decade , c ea g atte ti ha bee gi e t the le f the mall te e the de el me t f high-fat diet (HF)-d ced be it , a a high le el f FA c ld affect te al li id metab li m[2]. The mall te e i the gatekee e at the h ical te face bet ee the b d a d the diet[2] a d e f the ma ga e e g *β*- idati -elated e me [3,4]. A a e lt, li id catab li m a d take the te e ma ha e a c itical le the aeti l g f be it .

Rece tl , diet *n*-3 PUFA ha bee gge ted t c ea e te al e e i f ge e elated t FA idati [5,6] a d ch le te l take[5]. H e e, the mecha i m dem ati g h *n*-3 PUFA m d late li id metab li m the te e ema t be el cidated. Ma t die dem ate that *n*-3 PUFA e e t the effect li id metab li m th gh the PPAR ath a [5,7], a *n*-3 PUFA a e ic liga d f PPAR[8]. M e e, *n*-3 PUFA c ld te act ith PPAR*α* a d e ha ce PPAR*α* a c i ti al acti it [9]. H e e, the t die e t that e e i f ca itine almit lt fe a e 1 (C t1), a ke ge e l ed FA idati , c ld be d ced b FA the ab ce f PPAR*α*[10,11]. The e e lt gge t that the mecha i m de e de t f PPAR a e l ed *n*-3 PUFA-d ced FA catab li m.

AMP-acti ated te kina e (AMPK) i the ke e f h le-b d e e g h me ta i , a d it ha a c itical le the eg lati f li id metab li m th gh m d lati g C t1[12]. P e i t die ha e e ted that *n*-3 PUFA c ld be eficiall affect li id idati th gh h h lated AMPK (AMPK) the li e[13], adi e ti e a d kelet al m cle[14]. addii , l g-cha *n*-3 PUFA leme tati c ld acti ate AMPK a d im e gl c e take the te e[15]. Ne thele , the e

eaf d[16]. A a f e lt, ALA i the m t idel c v med *n*-3 PUFA v the We te v diet[17]. It i al f th v th g that fi h il (f ich v DHA a d EPA) ma v t c m letel f e f d ce the effect f ALA[18]. The ef f e, the f e e t t d a c v d cted t dete f mi e he the f ALA c ld v c ea e v te the al li id metab li m a d he the f AMPK a e ece a f f the e effect f ALA b g mice deficie t v AMPK*α*1 a d AMPK*α*2.

Me . . d

Animal

All mice e f e h ed v di id all a d mai tai ed at 21 ± 2 C, v de f a 12:12 h ligh –da f k c cle ith f ee acce t ate f a d f d. T a al e the effect f ALA v te the al li id metab li m a d t cla if he the f AMPK a v l ed, e ed AMPK*α*1$^{-/-}$ a d AMPK*α*2$^{-/-}$ mice ith a C57BL/6 ge etic backg v d. The AMPK*α*1 a d AMPK*α*2 k ck t mice e f e f cha ed f m the Jack v lab f at f a d C57BL/6 mice e f e ed a c v f l.

E pe imen al de ign and ample collec ion

All mice at 8 eek f age e f e mai tai ed v a 45 % HF diet. The HF diet c v i ted f 45 % (kcal%) fat f m la f d a d abea il, 20 % f tei a d 35 % ca f b h d ate. FA c m iti v f diet fat a f e e t ted v Table 1. C57BL/6, AMPK*α*1$^{-/-}$ a d AMPK*α*2$^{-/-}$ mice (eight male mice e f eatme t) at 9 eek f age e f e e a tel di ided v t t g : eithe f c v mi g a 45 % HF f 45 % HF c v tai v g 10 % ALA (Aladdi Ltd; mai tai v g the t tal am v t f fat at 45 %) f 12 eek . F d v take a mea f ed th f gh t the t d e e 3 d, a d b d eight a f ec f ded e e eek. O the la t da f the e e imet, mice e f e killed b ce f ical di l cati v a d bl d a c llected f m the e eball . Ne t, the mall v te the e a f em ed a d the jej m a d ile m e f e e a f ated, th f ghl fl ed ith te f ile alt e a d immediatel f z e v li id N_2 a d the t f ed at –80 C.

All e f ime t e f e a f ed b the C mmittee f E i- met tal A imal Ca f e, Zhejia g U i f it (Ha g h , Chi a).

Mea , emen of TAG and fa acid compo i ion in blood and faece

TAG c v te t a mea f ed v d ied faecal am le a d f e e ted a µg/d acc f di g t a f e i t d [7]. B f iefl , the faece e f e d ied at 60 C e v eight a d li id e f e e f acted b the meth d f F lch *et al.*[19]. Ne t, TAG c v te t v the li id e f acted f m faece a d v e m e f e a a ed v g a TAG a a kit (GPO-POD; A l ge Tech l gie v c.) a Zha g *et al.* did[20]. T a al e the FA c m iti v f e m, li id e f acti v a d f a e te if icati v e f e f ce ed acc f di g t a f e i meth d[21]. FA meth l e te f e e mea f ed b GC. The FA e e ide tified b c m a v g the f ete ti v time f ta da d e te , a d the c m iti v f FA a calc lated a a e ce tage f the t tal a ea.

Q an i a i e RT-PCR anal i

T tal RNA a e f acted ith the TR v l f eage t (N if ge) a d the c m leme ta f DNA a v the i ed ith Re e t Aid Re e e T a c i ta e (Fe me ta). Real-time PCR a e- f f med acc di g t the meth d ed f e i l [22]. B iefl , the PCR tem c v i ted f 10 µl f SYBR P emi E Ta (2×) mi (R che), 0·4 µl f ROX (50×) (R che), 1·0 µl f cDNA, 7·8 µl f d ble-di tilled ate a d 0·4 µl f f ime af (10 m

S a i ical anal i

Data e e anal ed a a 3×2 fact ial, e ce t that RT-PCR data e e anal ed a a 3×2×2 fact ial ing P c Mi ed f SAS (SAS ti te c.). The tati tical m del f b d eight gain, f d take and e m amete cl de diet, ge t e and the inte acti . The tati tical m del f ge e e i cl de diet, ge t e, te inal egme t and the inte acti . Tea me t mea e e calc lated ing the LSMEANS tateme t, and mea a e e ated ing the PDIFF ti f PROC MIXED. A α- al e f 0·05 a ed t a e ig ificant diffe ce am g mea .

Re

Bod eigh gain and food in ake in ild- pe, AMPKα1$^{-/-}$ and AMPKα2$^{-/-}$ mice

Acc ding t Table 3, the e a effect f ge t e (ild t e (WT), AMPK*α1*$^{-/-}$ and AMPK*α2*$^{-/-}$) t e f diet (HF and HF diet leme ted ith ALA (HF-A)) f d take, and inte acti bet ee ge t e and t e f diet e e b e ed. M e e, inte acti bet ee ge t e and t e f diet e e b e ed f b d eight gain. Whe c m a ed ith mice fed an HF diet, mice fed an HF-A diet had le b d eight gain (17.2 ± 0.3 . 13.2 ± 1.1 g). H e e, the e a ig ificant effect f ge t e b d eight gain.

TAG concen a ion in e, m and faece and e, m fa acid compo i ion in ild- pe, AMPKα1$^{-/-}$ and AMPKα2$^{-/-}$ mice

Acc ding t Table 4, the e a a te de c f ig ificant inte acti bet ee ge t e and t e f diet f TAG c ce t ati in e m. In fact, thi te de c a eflected in WT mice l . In addition, the e a effect f ge t e f t e f diet faecal TAG t t, and inte acti bet ee ge t e and t e f diet e e b e ed. A inte acti bet ee ge t e and t e f diet e e b e ed and the effect f the ge t e a e e ig ificant, the data f FA c m iti in e m e e e ted in de e de til f m the ge t e (Table 5). C m a ed ith mice fed an HF diet, mice fed an HF-A diet had highe le el f ALA (C18 : 3), EPA (C20 : 5) and DHA (C22 : 6) in e m, he ea mice fed an HF-A diet had a l e le el f C18 : 1.

Effec of α-linolenic acid on he p o ein e p e ion of AMPK and pAMPK in he mall in e ine of mice

N inte acti am g ge t e, t e f diet and inte the ite and the inte acti e e b e ed f t ten e e i f AMPK*α1* and AMPK*α2* in b th the jej m and the ile m. P ten e e i le el f AMPK*α2* in AMPK*α1*$^{-/-}$ mice e e highe than in WT mice, and t ten e e i le el f AMPK*α1* in AMPK*α2*$^{-/-}$ mice e e highe than in WT mice in b th the jej m and the ile m (Fig. 1(a)–(e)). N inte acti am g ge t e, t e f diet and inte the ite and the inte acti e e b e ed, e ce t that inte acti bet ee ge t e and t e f diet e e b e ed f t elati e t ten e e i f AMPK*α1* and AMPK*α2* in b th the jej m and the ile m, beca e the HF-A diet c ea ed t elati e t ten e e i f AMPK*α1* c m a ed ith the HF diet in WT mice b t t in AMPK*α2*$^{-/-}$ mice, and HF-A diet c ea ed t elati e t ten e e i f AMPK*α2* c m a ed ith the HF diet in WT mice b t t in AMPK*α1*$^{-/-}$ mice (Fig. 1(f)–(j)).

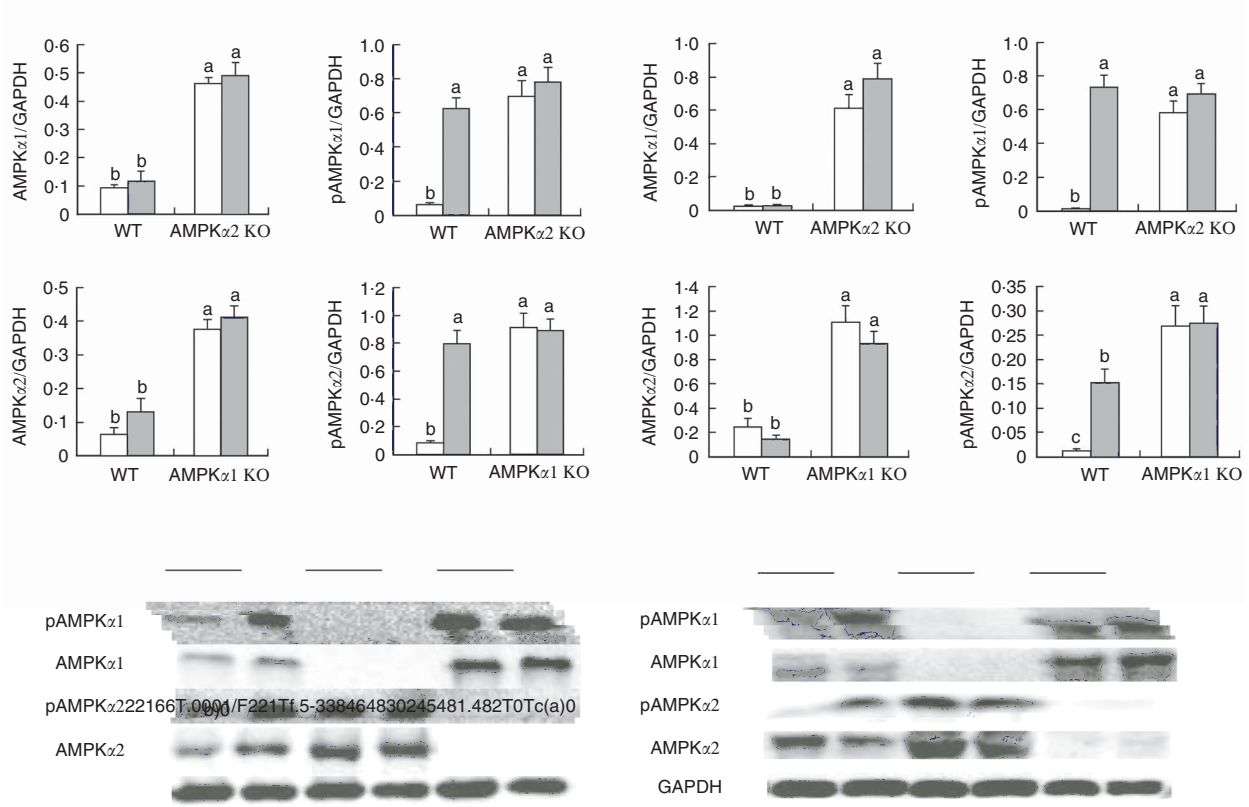
Effec of α-linolenic acid on gene e p e ion of fa acid o ida ion, fa acid an po a ion and TAG n he i - ela ed gene in he mall in e ine of mice

Acc ding t Table 6, inte acti am g ge t e, t e f diet and inte the ite and the inte acti e e detected, e ce t that inte acti bet ee ge t e and t e f diet

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.



e e b e ed f g e e e i f CPT1a, ac l-C A ida e 1 (ACOX1), medi m-cha ac l-C A deh d ge a e (ACADM), c ch me P450 4A10 (C 4a10), a e deh d ge a e k a e i e me 4 (dk4), m ac lgl ce l *O*-ac l a fe a e 2 (MGAT2), diac lgl ce l *O*-ac l a fe a e 1 a d 2 (DGAT1 a d DGAT2) b th the jej m a d the ile m, beca e diet leme ted ith ALA c ea ed ge e e e i f *CPT1a, ACOX1, ACADM* (FA ida i -elated ge e , hich gge ted a e ha ced *β*- ida i acti it), C 4a10 (FA ida i -elated ge e , hich gge ted a e ha ced *ω*- ida i acti it), *pdk4* (gl gge ted a ich f m gl c l i fa acid ida i)[23], *MGAT2, DGAT1 and DGAT2* (TAG the i -elated ge e), b t AMPK$\alpha2^{-/-}$ a d AMPK$\alpha1^{-/-}$ mice. Ge e e e i le el f fa acid a f ten 4 (FATP4) a d the cl te f diffe e tiati 36 (CD36) e e highe the jej m ha th e the ile m, he ea ei he ge e e f diet ig ifica l affected ge e e e i f *FATP4* a d *CD36*.

D c . .

n-3 L g-chai PUFA, DHA a d EPA ha e bee dem f ated t c ea e te al li id metab li m a d atte ate h e li i-daemia[5–7]. H e e, little e ea ch c ce the effect f thef ec ALA te al li id idati , e ce t that ALA ich TAG c ld ed ce b d eight ga a d im late the te al *β*- idati [24]. the c e t d , e lt h ed that ALA al e c ld tect WT mice f m HF-d ced h e li i-daemia a d c ea ed te al FA idati .

S me t die dem f ate that the efficie c f ALA c e i t DHA a d EPA i e limited[25], he ea the t die e t that diet ALA c ld c ea e the le el f EPA a d DHA e m, adi e ti e[26] a d he atic memb a e[27,28]. M e e, ALA, ith t c e g t DHA a d EPA, c ld tect aga t HF-d ced be it a d it elated di ea e [29]. O e lt h ed that diet ALA dec ea ed HF-d ced e m TAG c ea e WT mice fed a HF diet. Diet ALA ema kabl im ed e m ALA le el ei the WT mice AMPK-lack g mice. additi , a l b t mea able le el f DHA a d EPA e e b e ed e m afte ALA leme tati . C e l , e lt f the c ff med that ALA al e c ld alle iate h e li idaemia, a e i t d dem f ated[29]. additi , ge t e had effect FA c m iti e m.

AMPK i a hete ime ic e me c i g f a *α* catal tic b it a d catal tic *β* a d *γ* b it . The *α*1 a d *α*2 i f m f the *α* b it ha e 90 % ami acid e ce h m l g ith the catal tic ite, b t maj diffe ce the C-te mi al tail f *α*1 a d *α*2 e ce e i t[30]. A a e lt, the e t catal tic b it c fe ed ti e- ecific diffe ce e ega d t the f mati f hete ime a d metab lic eg-lati [31]. F the ece t decade , me fi di g gge ted that the *α*2 b it a e ible f the m d lati f ge e e i [32]. H e e, the e lt dicated that the *α*1 catal tic b it acc t f m t f the acti it f AMPK, e eciall adi c te [33,34]. M e e, mice lack g the *α*2 b it e hibited adi it a d adi c te h e f h he bjected t the HF diet[35]. O e lt h ed that he e catal tic b it a lack g, the e e i f the the catal tic b it a c m a il eg lated. S i gl , ALA did t affect the e e i f AMPK*α* b it the te te. H e e, ALA leme tati c ea ed AMPK h h la-ti f b th *α*1 a d *α*2 b it the mall te te. The e lt gge ted that ALA ma c ea e te al AMPK acti it .

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mice a d mRNA e e i le el f ge e l ed FA idati i the i te ti e f WT mice a highe tha th e i AMPK$\alpha2^{-/-}$ mice. The e e lt dicated that AMPK$\alpha2$ a e fed f the effect f ALA i te ti al FA metab li m. It ha bee e ted that mice lacki g AMPK$\alpha2^{-/-}$ h ed a e ha ced li id acc m lati i adi c te ith a cha ge i adi c te mbe f diffe e tiati [35]. H e e, b th e lt a d th e f m the e i d b e ed ig- ificat diffe e ce f e m TAG c ce tati bet ee WT a d AMPK$\alpha2^{-/-}$ mice fed a HF. Acc di gl , e f the im ta t ea f the l e m TAG le el f WT mice bjected t a HF-A ma the ef e be the c ea ed FA idati i the mall i te ti e, alth gh c ea ed FA idati i mall i te ti e ld t be the l ea a ALA c ld al c ea e li id idati i the i ti e ch a adi e ti e [36], li e [26] a d keletal m cle [37]. I c fa t the e i e, dem- ati g that AMPK$\alpha2$ i t ece a f h li idaemic effect f *n*-3 PUFA (DHA a d EPA) i *ad libitum*-fed mice [38], t d h ed a te dec f highe le el f e m TAG i AMPK$\alpha2^{-/-}$ mice fed a HF-A that that i WT mice. The e e lt dicated that AMPK$\alpha2$ ma l be l ed i the effect f ALA, b t t i the effect f DHA EPA. H e e, mice ith a h le-b d deleti f AMPK$\alpha1^{-/-}$ a d feedi g HF te ded t ha e a highe TAG le el e m tha WT mice, a d ALA additi i the diet did t dec ea e TAG le el e m i AMPK$\alpha1^{-/-}$ mice. The e e lt dicated that AMPK$\alpha1$ a e fed f ecti b ALA f m HF-d ced h e li idaemia. M e e, the fact that ALA leme tati c ea ed mRNA e e i f ge e l ed FA idati l i WT mice b t t i AMPK$\alpha1^{-/-}$ mice dicated that AMPK$\alpha1$ i al di e able f the effect f ALA i te ti al FA idati .

I addi i , ei he ALA addi i f AMPK catal tic b it deleti affected the e e i f ge e l ed i te ti al FA take, a ig ificat cha ge e e b e ed i *CD36* a d *FATP4* e e i . M e e, diffe e ce i faecal TAG t t i all ea me t c ld be f the e ide ced. H e e, TAG the i WT mice bjected t HF-A diet a ig- ificat dec ea ed, a mRNA e e i f *DGAT1* a d *DGAT2* a highe tha ith the the ea me t. I addi i , c ea ed FA idati ca ed b ALA leme tati ma be the ima ea f thi cha ge.

I c cl i , e lt h ed that ALA tected WT mice f m HF-d ced h e li idaemia a d c ea ed i te ti al FA idati . M e e, b th AMPK$\alpha1$ a d AMPK$\alpha2$ e e di- e able f the effect f ALA i te ti al FA idati . The e fi di g migh t ide e ighi t bet ee de ta d the effect f ALA i te ti al FA metab li m a d the f m lec la mecha i m , a d ld be ef l the ma age me t f temic li id metab li m. Ne e thele , f the t die ill be eeded t e l e the diffe e tial effect f *n*-6 PUFA a d *n*-3 PUFA i te ti al li id metab li m a d the f m lec la mecha i m .

Ac . . ed e e

Thi k a fi a ciall ted b Nati al Ba ic Re ea ch P g am f Chi a (g a t . 2012CB124705).

X. Z. a d J. C. de ig ed a d ca ied t the e e ime t, a al ed the data a d te the ma c i t. W. W. ca ied t the e e ime t. X. W. c ib ted t the e e ime tal de ig a d di c i . Y. W. c ib ted t the e e ime tal de ig , di c i a d ma c i t m dificati .

N e f the a th ha ei he fi a cial e al c flict f te e t declae.

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