Effects of essential amino acids on lipid metabolism in mice and humans

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Abstract

Eight amino acids are considered essential for human nutrition, and three of them, including leucine, isoleucine and valine, are called as branched-chain amino acids (BCAAs). We recently discovered that dietary deficiency of any BCAA for 7 days rapidly reduces the abdominal fat mass in mice. The goal of this study was to investigate (1) whether dietary deficiency of the other five essential amino acids (EAAs), including phenylalanine, threonine, tryptophan, methionine and lysine, would produce similar effects and (2) whether an association between serum AAs and obesity was observed in humans in Chinese Han population. Similar to BCAAs deprivation, dietary deficiency of any of these five EAAs for 7 days significantly reduced abdominal fat mass, which is likely caused by increased energy expenditure. Expression of genes and proteins related to lipolysis, however, were differentially regulated by different EAAs. These results suggest a crucial role of EAAs deprivation on lipid metabolism in mice. Our human studies revealed that levels of four EAAs (leucine, isoleucine, valine and phenylalanine) were elevated in obese humans compared with those in lean controls in Chinese Han population. Based on the results obtained from mice, we speculate that these four EAAs might play important roles in human obesity.

Key Words

- ▶ amino acids
- ▶ deprivation
- ▶ lipid metabolism

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Introduction

Obesity has become a leading public health problem in many countries around the world. One of the important factors that contributes to obesity is dietary macronutrient including fat, glucose and amino acids (AAs) (Attie & Scherer 2009). The effects of fat and glucose on lipid metabolism regulation have been well known; however, the effects of AAs are poorly understood. AAs are the building blocks of proteins and have also been shown to regulate many cell signaling pathways (Nair & Short 2005). Eight amino acids, including phenylalanine (Phe), valine (Val), threonine (Thr), tryptophan (Trp), isoleucine (Ile), methionine (Met), leucine (Leu) and lysine (Lys),

are considered as essential amino acids (EAAs) for human nutrition, as they could not be synthesized by the body and should be obtained from diet or degradation from protein. Among all EAAs, Leu, Ile and Val are called as branched-chain amino acids (BCAAs), and all of them have non-linear aliphatic side chains. As EAA plays a major role in various physiological processes, such as reproduction, recovery and brain function, altered levels of any specific EAA might result in significant changes in whole body metabolism.

Increasing evidence has shown that BCAAs play important roles in lipid metabolism regulation

(Zhang *et al.* 2007, Nairizi *et al.* 2009, Nishimura *et al.* 2010). By feeding mice a diet deficient of any BCAA, we recently demonstrated that BCAA deprivation has a significant impact on decreasing abdominal fat mass and improving insulin sensitivity (Guo & Cavener 2007, Cheng *et al.* 2010, Xiao *et al.* 2011, Du *et al.* 2012). In addition, some of these effects are regulated by a pathway dependent on an AA sensor named general control non-depressible (GCN)2 (Guo & Cavener 2007, Xiao *et al.* 2011), which is activated in response to any EAA deficiency (Zhang *et al.* 2002). The effects of deprivation of the other five EAAs on lipid metabolism, however, remain to be explored. Furthermore, recent studies have indicated a different BCAA-related AA metabolic signature between obese and lean subjects in Western population (Newgard *et al.* 2009). As many aspects, including lifestyle and food resources contributing to AA metabolism and obesity, are different between Chinese and Western population, whether this AA metabolic signature exists in Chinese Han population is unknown. For these reasons, the aim of this study was to (1) investigate whether deficiency of the other five EAAs, including Phe, Thr, Trp, Met and Lys, would produce similar effects and (2) whether an association between serum AAs and obesity was observed in humans in Chinese Han population.

As observed for BCAAs deprivation, dietary deficiency of the other five EAAs significantly reduced abdominal fat mass, which was also likely caused by increased energy expenditure. In contrast, lipolysis-related genes and proteins were differentially regulated by different EAAs. These results suggest a crucial role of EAAs in lipid

metabolism regulation in mice. Consistent with these results, our human studies revealed that levels of four essential amino acids (Leu, Ile, Val and Phe) and eight non-essential amino acids were significantly different in obese humans compared with those in lean controls in Chinese Han population. Based on the results obtained from mice, we speculate that these four EAAs might play important roles in human obesity.

Materials and methods

Subjects

The population of this study consisted of 100 unrelated Shanghai residents (77 men and 23 women) from Shanghai Xuhui Central Hospital, Shanghai, China. All participants attended a physical examination during which standard anthropometric measurements and fasting blood samples were collected. Those with severe psychological disorders; physical disabilities; cancer; cardiovascular disease; Alzheimer's disease or currently diagnosed with tuberculosis, AIDS and other communicable diseases were excluded. All participants were recruited after written consent was approved by Shanghai Xuhui Central Hospital. Body weight and height were measured, and body mass index (BMI) was calculated as weight (kg)/height (m²). Overweight/obesity and normal weight were defined as BMI ≥ 24.0 kg/m² and $18.0 \leq \text{BMI} < 24.0$ kg/m², respectively, which was modified from recommendation by the Working Group on Obesity in China (Wu 2006).

Figure 1

The effects on body weight, fat weight and food intake by deficiency of Phe, Thr, Trp, Met and Lys. Mice were fed a control diet or diet deficient of Phe, Thr, Trp, Met or Lys for 7 days. Data are mean $\pm$ S.E.M. of at least two independent experiments with mice of each diet for each experiment ($n=5-6$ for each group). Statistical significance was determined by the two-tail Student's *t*-test for the effect of any deficient diet vs control diet ($*P<0.05$). (A) Body weight reduction; (B) food intake change; (C) abdominal fat in proportion to body weight and (D and E) body composition measured with NMR.

Table 1 Serum measurements in mice maintained on different diets. * $P < 0.05$.

	Control	(-) Phe	(-) Thr	(-) Trp	(-) Met	(-) Lys
Norepinephrine (ng/L)	140.31 ± 21.81	260.07 ± 14.52*	187.62 ± 14.48*	296.05 ± 14.02*	190.07 ± 10.50*	272.41 ± 14.51*
TG (mmol/L)	0.14 ± 0.15	0.08 ± 0.02*	0.10 ± 0.01*	0.08 ± 0.01*	0.09 ± 0.01*	0.09 ± 0.02*
Cholesterol (mmol/L)	2.66 ± 0.10	2.12 ± 0.06*	2.23 ± 0.06*	2.05 ± 0.06*	1.70 ± 0.09*	2.33 ± 0.03 ($P = 0.069$)

Animals and diets

Wild-type male C57BL/6J mice were obtained from Shanghai Laboratory Animal Co, Ltd. (SLAC, Shanghai, China). Eight- to ten-week-old mice were housed in laboratory cages under a 12 h light:12 h darkness cycle at 25°C and were maintained on a regular commercial rodent chow diet before experiments. Control (nutritionally complete amino acid), (-) Phe (phenylalanine deficient), (-) Thr (threonine deficient), (-) Trp (tryptophan deficient), (-) Met (methionine deficient) and (-) Lys (lysine deficient) diets were obtained from Research Diets, Inc (New Brunswick, NJ, USA). All diets were isocaloric and compositionally the same in terms of carbohydrate and lipid component. Animal studies were conducted with experimental procedures as described previously (Guo & Cavener 2007, Cheng *et al.* 2010, Du *et al.* 2012). Mice were fed a control diet or diet deficient for Phe, Thr, Trp, Met or Lys for 7 days. All the animals were killed at the same time of light cycle–1400 h. All animal

experiments were performed using protocols approved by the Institutional Animal Care and Use Committee of the Institute for Nutritional Sciences, Sibbs, CAS and in conformity with the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Indirect calorimetry

After 6 days of feeding with control, (-) Phe, (-) Thr, (-) Trp, (-) Met or (-) Lys diet, oxygen consumption and physical activity of mice were determined by a comprehensive lab animal monitoring system (Columbus Instruments, Columbus, OH, USA) for 24 h according to the manufacturer's instructions (Cheng *et al.* 2010, Du *et al.* 2012).

Serum measurements

Serum was obtained as described previously (Guo & Cavener 2007, Cheng *et al.* 2010, Du *et al.* 2012).

Figure 2

The effects on energy expenditure by deficiency of Phe, Thr, Trp, Met and Lys. The energy expenditure was measured by indirect calorimetry in mice fed a control diet or diet deficient of Phe, Thr, Trp, Met or Lys for 7 days over 24–48 h after 6-h acclimation to the metabolic chamber. Data are mean ± S.E.M. of at least two independent experiments with mice of each diet for each experiment ($n = 5–6$ for each group). Statistical significance was determined by the two-tail Student's *t*-test for the effect of any deficient diet vs control diet (* $P < 0.05$). (A) 24-h oxygen consumption; (B) respiratory exchange ratio (RER) and (C) physical activity. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JME-16-0116>.

Figure 3

The effects on genes and proteins regulating lipolysis in WAT by deficiency of Phe, Thr, Trp, Met and Lys. Mice were fed a control diet or diet deficient of Phe, Thr, Trp, Met or Lys for 7 days. Data are mean $\pm$ S.E.M. of at least two independent real-time PCR experiments (A) or western blot (B) with mice of each diet for each experiment ($n=5-6$ for each). Statistical significance was determined by the two-tail Student's *t*-test for the effect of any deficient diet vs control diet (* $P<0.05$). (A) *Atgl* mRNA; (B) p-PKA substrate proteins and p-HSL (top western blot; bottom, p-HSL and p-PKA substrate proteins relative to actin).

Amino acid (AA) levels were determined by standard ion exchange chromatography using a Beckman 6300 automated amino acid analyzer. Serum triglyceride (TG), cholesterol and norepinephrine (NE) were determined using triglyceride kit (BTKH Clinical Reagents, Beijing, China), cholesterol kit (BTKH Clinical Reagents) and enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems), respectively. These assays were conducted according to manufacturer's instructions.

Western blot analysis

Western blot was performed as described previously (Cheng *et al.* 2010). Primary antibodies anti-p-HSL and anti-p-PKA substrate antibodies were purchased from Cell Signaling; anti-actin antibody was from Sigma. Band intensities were measured using Quantity One (Bio-Rad Laboratories) and normalized to actin.

RNA isolation and relative quantitative RT-PCR

Total RNA from mouse tissues was isolated as described previously (Cheng *et al.* 2010, Du *et al.* 2012). Real-time quantitative PCR was performed with SYBR Green I Master Mix reagent by ABI 7500 system (Applied Biosystems), using GAPDH as an internal control for normalization. The primers for *Atgl* were as follows: sense 5'-GTGAAGCAGGTGCCAACATTATTG-3', antisense 5'-AAACACGAGTCAGGGAGATGCC-3'.

Statistics

All data are expressed as mean $\pm$ S.E.M. Significant differences between (-) Phe, (-) Thr, (-) Trp, (-) Met or (-) Lys and control group were assessed using the two-tailed

Student's *t*-test. For the human profiling data, statistical analysis was carried out using STATA. The level of amino acid in obese and lean subjects was compared using multi-factor variance analysis (ANOVA) with or without adjustment for age and sex. $P<0.05$ was considered statistically significant.

Results

The effects on body weight and fat mass by deficiency of Phe, Thr, Trp, Met or Lys

We previously have shown that mice maintained on a BCAA-deficient diet undergo rapid loss of abdominal fat (Cheng *et al.* 2010, Du *et al.* 2012). The goal of this study was to investigate whether the other five EAAs deficiency produce similar effects in mice. For this purpose, mice were fed a control diet or diet deficient for Phe, Thr, Trp, Met or Lys for 7 days, a duration that is comparable with those during BCAAs deprivation. Deficiency of any of these five EAAs for 7 days resulted in significant reduction in body weight, to the smallest extent by Lys deprivation, compared with mice maintained on a control diet (Fig. 1A). Similar changes were observed for abdominal fat mass (Fig. 1C). Consistent with these results, total body fat was decreased significantly by the deprivation of Phe, Thr, Trp or Met in comparison with

Table 2 Baseline characteristics of the study population.

	Lean	Obese
Baseline characteristic	$n=20$	$n=80$
Age (years)	32.7 ± 1.63	33.1 ± 0.68
Male	4 (20%)	73 (91.25%)
Female	16 (80%)	7 (8.75%)
BMI	18.9 ± 0.27	29.6 ± 0.23

the control mice as measured by NMR (Fig. 1D). Although not statistically significant, total fat mass also showed decreased tendency in Lys-deprived mice compared with control mice (Fig. 1D). There was a small increase in the proportion of lean mass in Phe, Thr, Trp or Met-deprived mice compared with that in the control group (Fig. 1E). Deficiency of Phe, Thr, Trp and Met also significantly reduced the brown fat mass. Brown fat mass remained the same in Lys-deprived mice.

Similar to those observed during BCAA deprivation, we found that serum levels of triglyceride (TG) and cholesterol were decreased, and serum norepinephrine (NE) levels were increased, in mice maintained on these

five amino acids-deficient diet compared with control groups (Table 1).

The effects on food intake and energy expenditure by deficiency of Phe, Thr, Trp, Met or Lys

As decreased fat mass is caused by reduced food intake and/or increased energy expenditure, we compared the food intake among different groups. Deprivation of these 5 essential amino acids significantly decreased food intake, with the smallest extent by Lys deprivation, compared with mice maintained on the control diet (Fig. 1B).

Table 3 Amino acids profiles.

	Lean	Obese	P value adjusted for			
Amino acids (μmol/L)			–	Age	Sex	Age and sex
Phosphoserine (pSer)	0.54±0.05	0.61±0.03	0.3353	0.4208	0.8248	0.8584
Phosphoethanolamine (pEtn)	2.11±0.47	2.58±8.91	0.4639	0.3834	0.4021	0.3663
Taurine (Tau)	145.39±12.09	136.06±8.91	0.6218	0.6073	0.5014	0.4730
Asparagine (Asn)	90.31±4.35	92.27±1.87	0.6523	0.7532	0.9168	0.8979
Serine (Ser)	215.54±6.04	200.73±5.69	0.2126	0.1611	0.1591	0.1379
Hydroxyproline (Hyp)	18.39±1.66	20.36±0.94	0.3395	0.3686	0.1772	0.1869
Glycine (Gly)	384.18±18.33	328.51±5.43	<0.001	<0.001	<0.001	<0.001
Glutamine (Gln)	830.08±37.07	858.31±10.89	0.3240	0.3916	0.1947	0.1616
Aspartic Acid (Asp)	23.38±1.21	29.10±0.98	0.0066	0.0103	0.0829	0.0936
Ethanolamine (Etn)	176.35±1.79	178.22±1.08	0.4248	0.4530	0.4105	0.4022
Histidine (His)	102.46±2.97	111.38±1.33	0.0043	0.0077	0.9887	0.9493
Threonine (Thr)	181.54±8.88	167.68±2.84	0.0566	0.0559	0.1049	0.0903
Citrulline (Cit)	35.98±1.56	35.60±0.91	0.8499	0.7997	0.0422	0.0351
Sarcosine (Sar)	2.28±0.13	2.38±0.08	0.5436	0.6007	0.4876	0.4907
β-Alanine (bAla)	16.93±2.05	22.16±0.99	0.0213	0.0218	0.0216	0.0156
Alanine (Ala)	527.63±28.01	653.31±14.25	<0.001	<0.001	0.0232	0.0246
Glutamate (Glu)	61.63±4.21	108.04±3.31	<0.001	<0.001	<0.001	<0.001
1-Methyl-histidine (1MHis)	10.30±1.74	12.85±0.59	0.0834	0.0824	0.9122	0.9019
3-Methyl-histidine (3MHis)	3.81±0.26	4.91±0.13	<0.001	<0.001	0.4728	0.4716
Argininosuccinate (Asa)	248.71±13.04	295.96±5.15	<0.001	<0.001	0.3933	0.4377
Carnosine (Car)	0.45±0.09	0.44±0.04	0.9733	0.8948	0.9982	0.9683
Anserine (Ans)	0.71±0.10	0.63±0.05	0.4714	0.4020	0.2901	0.2908
Homocitrulline (Hcit)	0.61±0.09	0.88±0.06	0.0357	0.6797	0.8580	0.8723
Arginine (Arg)	134.40±4.34	138.52±2.67	0.4764	0.5199	0.7003	0.6987
α-Aminoadipic acid (Aad)	1.70±0.08	2.79±0.07	<0.001	<0.001	<0.001	<0.001
β-Aminoisobutyric acid (bAib)	2.45±0.34	2.15±0.17	0.4342	0.5019	0.0675	0.0664
α-Aminobutyric acid (Abu)	26.90±2.46	28.38±0.89	0.4946	0.5705	0.7656	0.7074
δ-Hydroxylysine (Hyl)	1.24±0.15	1.26±0.09	0.9043	0.9955	0.1811	0.2043
Proline (Pro)	191.59±16.00	231.47±6.54	0.0106	0.0130	0.1104	0.1143
Ornithine (Orn)	112.22±9.49	134.35±4.48	0.0315	0.0280	0.9692	0.9049
Cystathionine (Cth)	3.44±0.34	2.95±0.25	0.3527	0.4314	0.5163	0.5074
Cystine (Cys)	24.16±1.73	37.32±1.20	<0.001	<0.001	0.0075	0.0072
Lysine (Lys)	244.15±11.70	292.55±4.71	<0.001	<0.001	0.4651	0.5232
Methionine (Met)	42.07±1.57	42.51±1.18	0.8603	0.9748	0.4948	0.4292
Valine (Val)	280.54±11.51	366.17±6.74	<0.001	<0.001	0.0189	0.0220
Tyrosine (Tyr)	77.08±2.82	95.38±1.71	<0.001	<0.001	<0.001	0.0011
Homocysteine (Hcy)	4.68±0.37	4.23±0.19	0.2891	0.3377	0.2365	0.2282
Isoleucine (Ile)	74.78±3.22	106.28±2.14	<0.001	<0.001	0.0047	0.0054
Leucine (Leu)	148.11±4.94	207.84±3.84	<0.001	<0.001	0.0028	0.0033
Phenylalanine (Phe)	82.86±2.24	99.79±1.57	<0.001	<0.001	0.0079	0.0093
Tryptophan (Trp)	59.50±2.36	64.57±1.04	0.0354	0.0391	0.3502	0.3680

We have previously shown that the decreased fat mass during BCAA deprivation is caused by increased energy expenditure (Cheng *et al.* 2010, Du *et al.* 2012). Therefore, we measured the energy expenditure by indirect calorimetry in mice under different diets. The total energy expenditure (24-h O₂ consumption, normalized to lean body mass) was markedly increased in all these five EAA-deprived mice compared with that in mice maintained on a control diet (Fig. 2A). The respiratory exchange ratio (RER, VCO₂/VO₂) was also lower in these mice during both darkness and light phases (Fig. 2B). Similar to BCAA deprivation (Cheng *et al.* 2010, Du *et al.* 2012), locomotor activity was not affected by deficiency of any of these five EAAs compared with control mice (Fig. 2C). By contrast, locomotor activity in z axis was significantly increased in Trp-deprived mice compared with that in control mice (Fig. 2C).

The effects on lipolysis in WAT by deficiency of Phe, Thr, Trp, Met or Lys

We have previously shown that deficiency of BCAAs stimulates lipolysis in WAT (Cheng *et al.* 2010, Du *et al.* 2012). To investigate whether this is a general effect of all EAAs, we examined the changes in genes and proteins related to lipolysis in WAT of mice maintained on a control diet or diet deficient for Phe, Thr, Trp, Met or Lys. mRNA levels of adipose triglyceride lipase (*Atgl*), the first enzyme regulating lipolysis (Schoenborn *et al.* 2006), were significantly increased by Phe, Thr, Trp and Met deprivation compared with those maintained on a control diet (Fig. 3A). Deprivation of these four EAAs also increased the levels of phosphorylated hormone-sensitive lipase (HSL), the key enzyme regulating lipolysis (Martin *et al.* 2009), in WAT compared with control mice (Fig. 3B). Consistent with increased levels of phosphorylated HSL, levels of phosphorylated substrate for PKA, the kinase that phosphorylates HSL (Yeaman 1990), were also elevated in WAT of these mice (Fig. 3B). Lys deprivation, however, has no effect on levels of *Atgl* mRNA and phosphorylation of HSL and PKA in WAT compared with control mice (Fig. 3A and B).

Amino acids profiling in Chinese population

Based on our animal studies that suggest a close relationship between AAs and lipid metabolism, we were interested in exploring whether this correlation exists in Chinese Han population. We therefore measured plasma levels of AAs in obese and lean subjects recruited from

Shanghai as examples for Chinese Han population. The characteristics of the 100 participants are presented in Table 2. Eighty obese and twenty lean subjects underwent baseline evaluation. The obese subjects comprised 91.25% men and 8.75% women, whereas the lean subjects were 20% men and 80% women. The average age of the obese subjects was 33.1 years, and their BMI was 29.6 kg/m², compared with 32.7 years and 18.9 kg/m² for the lean controls (Table 2). After adjustment for age and sex, among a total of 41 amino acids measured in the serum, levels of 10 amino acids were found to be dramatically elevated in obese compared with lean participants (β -alanine (bAla), alanine (Ala), glutamate (Glu), α -aminoadipic acid (Aad), cystine (Cys), tyrosine (Tyr), Val, Ile, Leu and Phe; $P \leq 0.05$), whereas citrulline (Cit) and glycine (Gly) levels were lower in obese subjects (Table 3).

Discussion

Although human studies have implicated an association between AAs and obesity, the causal link is not fully understood. Thus, animal studies have been conducted by providing animals with increased or decreased amounts of AAs in diet or drinking water. Regarding lipid metabolism regulation, the effects of BCAAs are the most noticed so far, although results are not always the same. For example, some studies showed that increased BCAAs prevent obesity and hyperglycemia in mice (Zhang *et al.* 2007, Nishimura *et al.* 2010), whereas others reported that increased BCAAs either have no effect or lead to obesity (Nairizi *et al.* 2009, Newgard *et al.* 2009). By feeding mice with a diet deficient for individual BCAA, we found that BCAA deficiency rapidly decreases fat mass (Cheng *et al.* 2010, Du *et al.* 2012) and improves insulin sensitivity (Xiao *et al.* 2011). In contrast to extensive studies about the effects of BCAAs on lipid metabolism, the effects of the other five EAAs, however, remain largely unknown. Here, we showed that dietary deficiency of Phe, Thr, Trp, Met or Lys also reduces fat mass, in a similar manner as observed during BCAAs deprivation. These results suggest an important role of EAAs on lipid metabolism regulation. The extent of fat loss, however, varies under deficiency of different EAAs, which could be due to the specific character of each AA.

Consistent with our results of Met and Thr deficiency, other studies have shown that Met restriction decreases fat mass in rats (Malloy *et al.* 2006, Hasek *et al.* 2010) and Thr deficiency decreases lipid content in rat kidney (Singh 1979). A possible link between Trp and lipid metabolism has been implicated by a previous study showing that

lipids content in liver were significantly decreased as the levels of dietary Trp increased in growing broiler chicks (Rogers & Pesti 1990). In addition, very little has been known about the effect of Trp on lipid metabolism regulation. Therefore, this study has provided important direct evidence for an effect of Trp deficiency on lipid metabolism in adipose tissue. Furthermore, the decreased fat mass in Trp-deprived mice may also be stimulated by increased locomotor activity, which is a unique character distinguished from other EAAs deficiency. Consistent with these results, studies have shown that Trp deletion increased the total distance traveled in an animal model of schizophrenia by about 42% (Bubenikova *et al.* 2004). Phe is the other essential amino acid, which exhibited increased levels, in addition to BCAA, in obese people in Western populations (Newgard *et al.* 2009), indicating its possible strong association with lipid metabolism. Consistent with this possibility, we observed significant reduction of fat mass in mice under a diet deficient for Phe.

The extent of fat loss, however, is the smallest by Lys deprivation compared with deprivation of the other EAAs. It is not that surprising as some studies have shown that either thermogenesis or serum lipid profile was not changed by removing or addition of Lys from the diet (Smriga *et al.* 2000, Rathore *et al.* 2010, Hlais *et al.* 2012). There are several factors that may contribute to the smaller effect of Lys deprivation. It is shown that lipolysis and energy expenditure are stimulated by the activation of SNS and release of NE (Lowell & Bachman 2003). In addition, multiple hormones also play important roles in the regulation of lipolysis and energy expenditure and one of such examples is insulin (Zhang *et al.* 2005). In this study, we found that levels of serum NE and phosphorylated PKA substrates in white adipose tissue were increased in Phe, Thr, Trp and Met-deprived mice, suggesting increased SNS activity. Furthermore, based on our previous observation showing that serum insulin levels were reduced in leucine-deprived mice (Xiao *et al.* 2011), we speculated that deprivation of these EAAs may have similar effects. Therefore, the activation of SNS and decreased levels of serum insulin may both contribute to the increased lipolysis and energy expenditure in mice under deprivation of each EAA. In contrast to obviously stimulated lipolysis in WAT by deficiency of other EAAs, Lys deficiency has no effect on lipolysis as demonstrated by the unaltered levels of Atgl mRNA and HSL phosphorylation in WAT compared with control mice. Because energy expenditure and serum NE levels are increased by Lys deprivation, we speculated that SNS activity is also

increased in Lys-deprived mice. The increased SNS activity, however, did not lead to stimulated lipolysis, implicating that Lys might have a direct effect on lipolysis. Another reason may come from much less inhibitory effects on food intake by Lys deprivation compared with other EAAs, which is possibly caused by the differences in signaling pathways controlling food intake and time required to sense deficiency of different amino acids. It has been well established that the suppressed food intake by amino acid deficiency is regulated by GCN2-dependent pathway (Maurin *et al.* 2005). Additional signaling pathways might also be involved, for example, enzymatic pathways involved in the NO formation have been implicated in food intake regulation under Thr deprivation (Monda *et al.* 1998), which may account for the different extents in suppressing food intake by different EAAs. On the other hand, time required for sensing each EAA deficiency could also be different (Gietzen *et al.* 2007). It may take longer time for mice to sense the deficiency of Lys in the diet.

Because food intake is decreased by EAAs deficiency, we could not ignore the possible contributions from the decreased food intake to the reduced fat mass, in addition to the effect of AA deficiency. The effects of reduced food intake, however, is unlikely to contribute significantly to the decreased fat mass in mice maintained on a diet deficient for any of these five EAAs, based on results obtained in our previous work by including a pair-fed (pf) group to compare the effects of leucine deprivation (Cheng *et al.* 2010). Consistent with this possibility, Virginia L. Malloy and coworkers showed that Met restriction significantly decreases visceral fat mass, whereas pf group exhibits comparable amounts of visceral fat to control diet-fed rats (Malloy *et al.* 2006).

Results from our studies (Guo & Cavener 2007, Cheng *et al.* 2010, Du *et al.* 2012) and by others (Zhang *et al.* 2007, Nairizi *et al.* 2009, Newgard *et al.* 2009, Nishimura *et al.* 2010, Noguchi *et al.* 2010) suggest a close relationship between AAs and lipid metabolism in mice. Recently, this relationship has also been confirmed in human subjects in Western population by the group of Christopher B. Newgard (Newgard *et al.* 2009). They used components analysis (PCA) to analyze the difference of metabolites between obese and lean subjects and found that the component showing the strongest differences was composed of a combination of AAs. An association between AAs and obesity in Chinese population, however, has never been tested. In this study, we found that levels of twelve serum amino acids, including four EAAs (Val, Ile, Leu and Phe) and eight non-EAAs (Gly, Cit, bAla, Ala, Glu, Aad, Cys and Tyr), were different between lean and

obese groups in Chinese Han population. These results suggest that a close relationship between AAs and BMI is also observed in Chinese Han population, though many different factors affecting AA metabolism and obesity exist between Western and Chinese population. We should also note that this is the first study measuring the most complete AA profile in humans.

In this study, we found levels of four EAAs (Val, Ile, Leu and Phe) are elevated in obese subjects compared with lean controls. Very importantly, elevated levels of the same set of EAAs are also observed in the Western population (Newgard *et al.* 2009). This is a very exciting finding due to the important roles that EAAs play in metabolic control. These results suggest that these four EAAs might play important roles in the occurrence of human obesity. Consistent with this possibility, deficiency of any of these four EAAs significantly reduces fat mass in mice. We did not see any difference in the levels of the other four EAAs (Thr, Trp, Met or Lys) between obese and lean Chinese. However, it does not necessarily mean that these EAAs do not contribute to obesity, as deficiency of any of these four EAAs also decreases fat mass in mice. Future studies will be required to clarify this issue.

In addition to EAAs, levels of non-EAAs were also compared between lean and obese subjects in Chinese Han population. No changes in levels of His, Met, Orn and Ser were detected between obese and lean groups in Chinese and Western population, indicating that these AAs are unlikely to be associated with obesity. Among eight non-EAAs showing difference between obese and lean humans in Chinese Han population, five of them are also found changed in obese subjects in Western population. These are Gly, Cit, Ala, Glu and Tyr. Therefore, these five non-EAAs might also be involved in human obesity. In addition, we found the levels of bAla, Aad and Cys were also different between these two groups in Chinese population. Besides, levels of Asp, Arg and Pro were not dramatically elevated in Chinese Han population as reported in Western population (Newgard *et al.* 2009), suggesting a possible unique AA profile between different populations, which might be caused by differences in dietary intake and genetic background.

Taken together, this study showed that the effects of BCAAs deprivation represent a general effect of EAAs deficiency in mice and demonstrated a close relationship between AAs and lipid metabolism in Chinese Han population. These observations provide important hints in understanding nutritional and genetic regulation of obesity and possible intervention strategy by manipulation of dietary amino acids.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

F Xiao researched data, wrote, reviewed and edited the manuscript. Y Du, Z Lv and S Chen researched data and contributed to the discussion. J Zhu and H Sheng provided research material. F Guo directed the project, contributed to discussion and wrote, reviewed and edited the manuscript.

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