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Branched-chain amino acid ratios modulate lipid metabolism in adipose tissues of growing pigs

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ABSTRACT

The effects and roles of branched-chain amino acid (BCAAs) ratios in lipid metabolism in adipose tissues of pigs are still unkown. We used pigs (Large White × Landrace, 35 ± 2 d) to investigate the effects of varying BCAA ratios (Leu: Ile: Val = 1:1:1, 1:0.75:0.75, 1:0.51:0.63, 1:0.25:0.25) on growth, carcass traits, and fat metabolism in adipose tissues. Results showed that as the ratio declined, the weight of total fat mass reduced while the adiponectin concentrations increased ($P < .05$), with the lowest/highest values observed in the 1:0.25:0.25 group, respectively. Moreover, varying BCAA ratios modulated the expression of genes related to adipose tissue function ($P < .05$). Concomitant with these changes, the 1:0.25:0.25 group increased/decreased the phosphorylation of AMPKα/mTOR, respectively ($P < .05$). The mRNA abundance of PGC-1α and IL-15 were also increased in diets with BCAA ratios from 1:0.75:0.75 to 1:0.25:0.25. Our data suggest that dietary BCAA ratios in the adequate range, i.e. 1:0.75:0.75–1:0.25:0.25, modulate adipose tissue function including fatty acid synthesis, transport, and oxidation, lipolysis, and adipokine secretion. These effects are partly mediated by AMPK-mTOR pathway and associated with mitochondrial biogenesis, the AMPK-PGC-1α axis, and IL-15 secreted by muscle tissues.

1. Introduction

Adipose tissue is interspersed throughout the body. It has various forms, including white adipose tissue (WAT), brown adipose tissue (BAT), and beige adipose tissue (Ross, 2014). WAT not only serves as an energy storage site, but also plays key roles in glucose and lipid homeostasis via the release of bioenergetic substrates through lipolysis and the storage of excess nutrients in lipid droplets (Green et al., 2016). In addition, WAT can produce a wide range of adipokines and execute numerous functional roles through endocrine and paracrine signaling (Rosen & Spiegelman, 2014). The synthesis and release of lipids and adipokines affect fatty acid metabolism in other tissues such as the liver and skeletal muscle (Green et al., 2016; Yao et al., 2016). However, dysfunction in these pathways can promote the development of insulin resistance (Herman et al., 2012). Moreover, in humans, the increased adiposity is associated with obesity and diabetes, emphasizing the need to prevent and/or treat adipose tissue disturbance that associates with increased risks of developing metabolic disorders (Yao et al., 2016). In

Abbreviations: ACC, Acetyl-CoA carboxylase; AMPKα, AMP-activated protein kinase α; ASA, Abdominal subcutaneous adipose tissue; BAT, Brown adipose tissue; BCAA, Branched-chain amino acid; BM, Biceps femoris muscle; c/EBPα, CCAAT-enhancer-binding-protein α; DSA, Dorsal subcutaneous adipose tissue; FATP-1, Fatty acid transport protein 1; FABP-4, Fatty acid binding protein 4; HSL, Hormone-sensitive lipase; Ile, Isoleucine; L-CPT-1, Liver carnitine palmitoyl transferase-1; Leu, Leucine; LM, Longissimus dorsi muscle; LPL, Lipoprotein lipase; mTOR, Mammalian target of rapamycin; PGC-1α, Peroxisome proliferator-activated receptor gamma co-activator 1-alpha; PM, Psoas major muscle; PPARγ, Peroxisome proliferator-activated receptor γ; PRA, Perirenal adipose tissue; SAT, Subcutaneous adipose tissue; SIRT1, Silent information regulator transcript 1; TF, Transcription factor; UCP3, Uncoupling protein 3; Val, Valine; VAT, Visceral adipose tissue; WAT, White adipose tissue

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the meat industry, body fat content and distribution in growing pigs are of special interest for production efficiency and meat quality (Lebret & Mourot, 1998). Overall, these observations suggest that a better understanding of fat mass development is of great importance for both humans and animals and highlight the need to elucidate the molecular mechanisms underlying the metabolic regulation of adipose tissues.

Branched-chain amino acids (BCAAs), containing leucine (Leu), isoleucine (Ile), and valine (Val), are metabolic signature in obesity and diabetes, and function as a direct-acting nutrient signal and act directly on the adipocytes (the major cellular constituent of adipose tissue) to affect the fat metabolism, favoring adiposity reduction (McAllan, Cotter, Roche, Korpela, & Nilaweera, 2013; Yao et al., 2016). In support of this view, numerous studies have demonstrated that Leu is a promising candidate for the regulation of overall lipid balance (Bai, Greene, Li, Kidd, & Dridi, 2015; Bruckbauer et al., 2012; Chen & Reimer, 2009; Fu, Li et al., 2015; Fu, Bruckbauer et al., 2015; Sun & Zemel, 2007; Zhang et al., 2007). However, we note that most studies have mainly focused on effects of the increasing dietary Leu levels (Zhang et al., 2007). More importantly, some studies have indicated that increasing dietary Leu concentrations exert no effect on lipid metabolism (Nairizi, She, Vary, & Lynch, 2009). One possible explanation for this observation is that dietary BCAA imbalance occurs. Leu, Ile, and Val have similar chemical structures and compete for the same enzymes that catalyze the first two catabolic steps (Langer, Scislowski, Brown, Dewey, & Fuller, 2000). An excessive supply of Leu may increase the catabolism of all BCAAs and further enhance the nutritional needs for Ile and Val (Wiltafsky, Pfaffl, & Roth, 2010). Consequently, the imbalanced BCAA do not have the ability to affect lipid metabolism. Moreover, Val deficiency could induce the reduction of feed intake and growth, and a high level of Leu further aggravates the consequences of Val deficiency (Gloaguen et al., 2011). Therefore, balancing the three BCAA ratio in diets is of enormous nutritional importance. Previous studies used the indicator amino acid oxidation technique to test the ratio of BCAAs during enteral feeding and show that the Leu: Ile: Val ratio of 1.8:1:1.2 is appropriate in neonatal piglets weighing between 1 and 5 kg during enteral feeding since the percentage of phenylalanine oxidation was minimal (Elango, Goonewardene, Pencharz, & Ball, 2004). However, the optimum ratio of BCAA might be different for growing pigs, and this has not been investigated to the authors' knowledge.

Investigation into the effects of BCAA, especially Leu, on fatty acid metabolism has been facilitated by the development of animal models, particularly rats and mice, whereas studies in swine models to date are sparse (Fu, Bruckbauer et al., 2015; Guo, Yu, Hou, & Zhang, 2010; Li, Xu, Lee, He, & Xie, 2012; Zhang et al., 2007). Although rodents are small and thus useful for multivariable experiments, they differ from humans in metabolism and physiology (Arner, 2005; Davis, Cain, Banz, & Peterson, 2013). In contrast, pigs possess many anatomical and physiological similarities to humans, as well as a high sequence and chromosome structure homology (Groenen et al., 2012; Vamathevan et al., 2013). These peculiarities make the pig an interesting model for understanding the role of BCAA in the regulation of fat metabolism in adipose tissues.

Therefore in the present study, a pig model was used to: (1) compare and contrast the effects of dietary BCAA ratios on fat metabolism in different location of adipose tissues, and (2) unraveling the molecular mechanisms of BCAA ratio action of fat metabolism. It was hypothesized that the optimal dietary BCAA ratios could inhibit fatty acid synthesis and elevate fatty acid β-oxidation in the adipose tissue of growing pigs. Insights into the underlying molecular mechanisms of BCAA action of fat metabolism are of great interest not only in animal biology for feed efficiency improvement, but also in molecular nutrition and medicine for potential nutritional supplement optimization and therapeutic perspectives.

Table 1
Composition and nutrient levels of the diets (air-dried basis, %).

Ingredients (%)	Leu:Ile:Val			
	1:1:1	1:0.75:0.75	1:0.51:0.63	1:0.25:0.25
Corn	70.26	70.26	70.26	70.26
Soybean meal	12.40	12.40	12.40	12.40
Whey powder	4.30	4.30	4.30	4.30
Fish meal	4.00	4.00	4.00	4.00
Soybean oil	2.80	2.80	2.80	2.80
L-Lysine HCl	0.80	0.80	0.80	0.80
DL-Methionine	0.25	0.25	0.25	0.25
L-Threonine	0.29	0.29	0.29	0.29
L-Tryptophan	0.08	0.08	0.08	0.08
L-Leucine	0.09	0.34	0.60	1.34
L-Isoleucine	0.76	0.64	0.40	0.14
L-Valine	0.70	0.57	0.55	0.07
Dicalcium phosphate	0.74	0.74	0.74	0.74
Limestone	0.70	0.70	0.70	0.70
Salt	0.30	0.30	0.30	0.30
Premix[a]	1.00	1.00	1.00	1.00
Nutritional content, %				
Digestible energy (MJ/kg)[b]	14.23	14.23	14.23	14.23
Ether extract	5.06	5.01	5.13	4.92
Crude protein	16.91	16.88	17.01	17.05
Lysine	1.05	1.00	1.10	1.01
Methionine	0.41	0.41	0.37	0.39
Threonine	0.73	0.77	0.76	0.74
Tryptophan	0.21	0.20	0.22	0.23
Leucine	1.21	1.44	1.65	2.35
Isoleucine	1.15	1.12	0.81	0.56
Valine	1.29	1.07	0.99	0.59
Leu:Ile:Val	1:0.95:1.06	1:0.78:0.74	1:0.49:0.60	1:0.24:0.25

[a] Supplied per kg of diet: $CuSO_4 \cdot 5H_2O$ 19.8 mg; KI 0.20 mg; $FeSO_4 \cdot 7H_2O$ 400 mg; $NaSeO_3$ 0.56 mg; $ZnSO_4 \cdot 7H_2O$ 359 mg; $MnSO_4 \cdot H_2O$ 10.2 mg; Vitamin K (menadione) 5 mg; Vitamin B_1 2 mg; Vitamin B_2 15 mg; Vitamin B_{12} 30 μg; Vitamin A 5400 IU; Vitamin D_3 110 IU; Vitamin E 18 IU; choline chloride 80 mg; antioxidants 20 mg; fungicide 100 mg.

[b] Digestible energy was calculated values.

2. Materials and methods

2.1. Animals and experimental diets

The present study was performed following the Chinese guidelines for experimental protocols and animal welfare, and approved by the committee on animal care of the Institute of Subtropical Agriculture, the Chinese Academy of Sciences.

A total of 32 pigs (Large White × Landrace, 35 ± 2 d, barrow) with a mean initial weight of 9.85 ± 0.35 kg were chosen and divided into four groups using a randomized complete block design based on body weight, with eight pigs per group. The pigs in the four groups were fed one of the four isoenergetic diets with Leu: Ile: Val ratios of 1:1:1, 1:0.75:0.75, 1:0.51:0.63, and 1:0.25:0.25. The diet composition is presented in Table 1. All the experimental diets meet the nutritional requirements for growing pigs. The experiment lasted for 45 d. The clean water and food were freely available during the whole experiment period.

2.2. Sample collection

Pigs were weighed at the beginning and the end of the experiment respectively, and feed consumption was recorded on a daily basis. Blood samples were collected into 10 ml tubes from the jugular vein puncture and centrifuged at 3000*g* and 4 °C for 15 min. Then, the serum supernatants were stored at −80 °C until analysis. At the end of the experiment, pigs were slaughtered by electrically stunning (250 V, 0.5 A, for 5–6 s) and exsanguinating after blood sampling. Perirenal fat