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Original research article

Supplementation of branched-chain amino acids in protein-restricted diets modulates the expression levels of amino acid transporters and energy metabolism associated regulators in the adipose tissue of growing pigs

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ABSTRACT

This experiment was conducted to investigate the effects of branched-chain amino acids (BCAA) supplemented in protein-restricted diets on the growth performance and the expression profile of amino acid transporters and energy metabolism related regulators in the white adipose tissue (WAT) of different regional depots including dorsal subcutaneous adipose (DSA) and abdominal subcutaneous adipose (ASA). A total of 24 crossbred barrows (7.40 ± 0.70 kg) were randomly divided into 4 groups and were fed the following isocaloric diets for 33 days: 1) a recommended adequate protein diet (AP, 20% CP, as a positive control); 2) a low protein diet (LP, 17% CP); 3) the LP diet supplemented with BCAA (LP + B, 17% CP) to reach the same level of the AP diet group; 4) the LP diet supplemented with 2 times the amount of BCAA (LP + 2B, 17% CP). The daily gain and daily feed intake of the LP diet group were the lowest among all the treatments ($P < 0.01$). The feed conversion was improved markedly in the group of LP + B compared with the LP diet group ($P < 0.05$). No significant difference was noted for the serum biochemical parameter concentrations of glucose, triglyceride, nonesterified fatty acid and insulin among the groups ($P > 0.05$). Moreover, BCAA supplementation down-regulated the expression levels of amino acid transporters including L-type amino acid transporter 1 and sodium-coupled neutral amino acid transporter 2 in DSA, but up-regulated the expression level of L-type amino acid transporter 4 in ASA ($P < 0.05$). Meanwhile, the energy sensor AMP-activated protein kinase α was activated in the DSA of pigs fed LP diet and in the ASA of the pigs fed AP or LP + 2B diets ($P < 0.05$). The mRNA expression profile of the selected mitochondrial component and mitochondrial biogenesis associated regulators in DSA and ASA also responded differently to dietary BCAA supplementation. These results suggested that the growth performance of growing pigs fed protein restricted diets supplemented with BCAA could catch up to that of the pigs fed AP diets. The results

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also partly demonstrated that the regulation mechanisms of BCAA are different in the adipose tissues of different depots.

1. Introduction

The overall metabolic profile of pigs is similar to that of humans, thus pigs may be an optimal animal model for investigating lipid metabolism and metabolic disorders (Douglas, 1972; Spurlock and NK, 2008). Currently, adipose tissue has been perceived predominantly as an active fuel reservoir in role of energy balance, instead of a metabolism inertness depot in the last decade (Valenzuela and Sanhueza, 2009). Sufficient *in vitro* and *in vivo* evidence has pointed out that adipose tissue is capable of metabolizing significant quantities of branch chain amino acids (BCAA), including leucine, isoleucine and valine (Rosenthal et al., 1974; Tischler and Goldberg, 1980; Layman, 2003; Herman et al., 2010). The critical roles of BCAA in protein synthesis and turnover have been widely documented, especially in skeletal muscle (Corporation HP, 2014). Now, the potential relationship between BCAA function and energy metabolism in adipose tissue is of great interest. It is beneficial for us to know how energy metabolism is regulated and coordinated by BCAA in white adipose tissue (WAT). The oxidation of BCAA seems to be advantageous to human metabolic health as their catabolism increases fatty acid oxidation as well as controls obesity (Corporation HP, 2014). Nishimura et al. (2010) have observed that isoleucine supplementation leads to a decrease in weight gain and a reduction in lipid mass. In a double-blind, placebo-control, crossover study on human volunteers, Gualano et al. (2011) noticed that BCAA supplementation increases lipid oxidation during exercise and helps to overcome fatigue. Qin et al. (2011) investigated on middle aged healthy adults and found that there is an inverse relation between BCAA intake and obesity. All of these findings suggest that BCAA have a large influence on energy metabolism.

Energy metabolism and mitochondrial biogenesis are inextricably linked. The AMP-activated protein kinase α (AMPKα) is a crucial metabolic fuel gauge and a signal transducer for maintaining energy homeostasis and regulating mitochondrial biogenesis. Notably, the expression of multiple genes, such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and silent information regulator 1 (SIRT1) involved in the regulation of energy metabolism, appears to be associated with mitochondrial biogenesis (Koves et al., 2005; Bastin et al., 2008). The impact of BCAA on energy balance and mitochondrial function in skeletal muscle has been widely studied (Scarpulla et al., 2012; Liang et al., 2014), while only several *in vitro* experiments have indicated that leucine mediates the energy metabolism of adipocyte partly through mitochondrial biogenesis (Sun and Zemel, 2009). The BCAA-specific transporters play critical roles in this process, which are present on membranes to sense amino acid availability and relay nutrient signals to the cell interior (Hundal and Taylor, 2009; Nicklin et al., 2009; Duan et al., 2015) Several predominant transporters that are recently reported to be directly or indirectly associated with BCAA have been studied (Evans, 2007).

In the present study, we attempted to address whether BCAA affect the growth performance and the expression levels of selected genes that are involved in AA transporters and energy metabolism in WAT including dorsal subcutaneous adipose (DSA) tissue and abdominal subcutaneous adipose (ASA) tissue *in vivo* using the pig as an animal model.

2. Materials and methods

2.1. Animals and experimental diets

All procedures outlined in this experiment were approved by the Animal Care and Use Committee of the Chinese Academy of Sciences (Fugui et al., 2010).

A total of 24 crossbred barrows (Landrace × Large White, 7.40 ± 0.70 kg BW) were randomly divided into 4 treatments. Each treatment had 6 replicates ($n = 6$). Pigs were housed individually in cages (Tan et al., 2011) and fed diets based on maize, soybean meal, fish meal and whey powder (Table 1). The dietary treatments were as follows: 1) a recommended adequate protein (AP) diet containing 20% CP, considered as the positive control group; 2) a low protein (LP) diet containing 17% CP, considered as the negative control group; 3) the LP diet supplemented with BCAA (LP + B) to contain the same level as that of the AP diet; 4) the LP diet supplemented with 2 times amount of the BCAA (LP + 2B). All experimental diets were formulated to be isocaloric, and the limiting AA, including lysine, methionine, threonine and tryptophan, were all designed to meet the National Research Council (NRC, 2012) recommendations. The pigs had *ad libitum* access to diets and drinking water throughout the study (Tan et al., 2009). All pigs were weighed at the start and the end of this 33-day experiment, and feed intakes were recorded on a daily basis to calculate final body weight (FBW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) (Yin et al., 2010).

2.2. Sample collection

Blood samples (about 5 mL from each pig) were collected into 10-mL tubes and centrifuged at 3,000 × *g* at 4 °C for 15 min. Then, the supernatants (serum) were collected and stored at −20 °C until required for analysis. Immediately after blood sampling, pigs were electrically stunned (250 V, 0.5 A, 5 or 6 s), exsanguinated and eviscerated in a slaughterhouse (Liu et al., 2012; Tan et al., 2011). Adipose tissue samples including DSA and ASA were rapidly excised from the right side of the carcasses. Samples were immediately frozen in liquid nitrogen and then stored at −80 °C until further analysis (Liu et al., 2015).

2.3. Serum chemical parameters

We determined the serum concentrations of glucose (Glu) and triacylglycerols (TG) using a Biochemical Analytical Instrument (Beckman CX4) and commercial kits (Sino-German Beijing Leadman Biotech Ltd., Beijing, China). We analyzed the nonesterified fatty acid (NEFA) concentration using colorimetric assays according to the manufacturer's instructions of the NEFA detection kit (Nanjing Jianchen Bioengineering Institute, China). Six samples of each group were measured.

2.4. RNA extraction and cDNA synthesis

We isolated the total RNA from DSA and ASA using the TRIZOL reagent (100 mg tissue per 1 mL Trizol; Invitrogen, Carlsbad, USA).