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Effects of individual branched-chain amino acids deprivation on insulin sensitivity and glucose metabolism in mice

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ABSTRACT

Objective. We recently discovered that leucine deprivation increases hepatic insulin sensitivity via general control nondepressible (GCN) 2/mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) pathways. The goal of the present study was to investigate whether the above effects were leucine specific or were also induced by deficiency of other branched chain amino acids including valine and isoleucine.

Methods. Following depletion of BCAAs, changes in metabolic parameters and the expression of genes and proteins involved in regulation of insulin sensitivity and glucose metabolism were analyzed in mice and cell lines including human HepG2 cells, primary mouse hepatocytes and a mouse myoblast cell line C2C12.

Results. Valine or isoleucine deprivation for 7 days has similar effect on improving insulin sensitivity as leucine, in wild type and insulin-resistant mice models. These effects are possibly mediated by decreased mTOR/S6K1 and increased AMPK signaling pathways, in a GCN2-dependent manner. Similar observations were obtained in in vitro studies. In contrast to leucine withdrawal, valine or isoleucine deprivation for 7 days significantly decreased fed blood glucose levels, possibly due to reduced expression of a key gluconeogenesis gene, glucose-6-phosphatase. Finally, insulin sensitivity was rapidly improved in mice 1 day following maintenance on a diet deficient for any individual BCAAs.

Conclusions. Our results show that while improvement on insulin sensitivity is a general feature of BCAAs depletion, individual BCAAs have specific effects on metabolic pathways, including those that regulate glucose level. These observations provide a conceptual framework for delineating the molecular mechanisms that underlie amino acid regulation of insulin sensitivity.

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Abbreviations: AKT, protein kinases B; AMPK, AMP-activated protein kinase; BCAAs, Branched-chain amino acids; GCN2, General Control Nonderepressible 2; IR, insulin receptor; mTOR: mammalian Target of Rapamycin; S6K1, p70 ribosomal protein S6 Kinase 1.

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1. Introduction

Diabetes is one of the major causes of morbidity and mortality worldwide. A common feature of type 2 diabetes is insulin resistance, which is characterized by reduced glucose uptake in muscle and adipose tissue, and increased glucose production in liver [1,2]. One of the important factors that contribute to insulin resistance is the unbalance of dietary macronutrients including fat, glucose and amino acids [3]. Aside from their role as the building blocks of proteins, amino acids are also critical mediators of intracellular signaling [4]. Branched-chain amino acids (BCAAs) that have non-linear aliphatic side-chains include leucine, valine and isoleucine, and are the most studied essential amino acids. Increased leucine concentration is reported to either improve or have no effect on glucose metabolism in mice [5,6]; however, there is increasing evidence of a correlation between increased levels of BCAAs and insulin resistance [7–9]. For example, metabolomic profiling of obese *versus* lean humans reveals a BCAAs-related metabolite signature that is suggestive of increased catabolism of BCAAs and correlated with insulin resistance [9]. Furthermore, Wang and colleagues found that quantification of BCAAs serum levels facilitates risk assessment for onset t of type 2 diabetes [10]. Though progress has been made in understanding the effect of BCAAs on insulin sensitivity and glucose metabolism, the effect of each individual BCAA remains largely unknown.

In contrast to other studies examining relationships between increased levels of BCAAs and insulin sensitivity, our studies have focused on investigating the effects of elimination of dietary leucine and previously shown that leucine deprivation improves hepatic insulin sensitivity *in vivo* and *in vitro* [11]. It remains unclear, however, whether deficiency of other BCAAs, including valine and isoleucine, would have similar effects. A previous study showed that mice deleted for the *BCATm* gene encoding the enzyme catalyzing the first step in peripheral BCAAs metabolism, which would be expected to have low leucine, valine or isoleucine utilization, exhibited increased insulin sensitivity [12]. These results suggest that deficiency of valine or isoleucine may also modulate insulin sensitivity. The aim of our current study was to investigate these possibilities and elucidate underlying mechanisms. This study will help in understanding the unique feature of each individual BCAA. These observations are also important for understanding the molecular mechanisms underlying amino acid regulation of insulin sensitivity.

2. Methods

2.1. Animals and treatments

Male C57BL/6 J mice were obtained from Shanghai Laboratory Animal (SLAC, Shanghai, China). GCN2 knockout (*Gcn2*$^{-/-}$) and leptin receptor-mutated (*db/db*) mice were kindly provided by Dr. Douglas Cavener, Penn State University, USA and Dr. Xiang Gao, Nanjing University, China, respectively. Eight- to ten-week-old mice were maintained on a 12-h light/dark cycle at 25 °C. Control, (–) leu (leucine-deficient), (–) val (valine-deficient) and (–) ile (isoleucine- deficient) chow were obtained from Research Diets (New Brunswick, NJ, USA). All diets were isocaloric and compositionally the same in terms of carbohydrate and lipid components. At the start of the feeding experiments, mice were acclimated to a control diet for 7 days and then randomly divided into control, (–) val or (–) ile diet group, with free access to each diet for 7 days. To determine the possible influences of reduced food intake in the BCAAs-deprived group, pair-fed (pf) groups were included. Mice in the pf group were provided with 18%, 30% or 40% less food, as determined in our preliminary experiments, compared to mice in the control group. A subset of mice underwent glucose tolerance test (GTT)/insulin tolerance test (ITT) prior to being killed by CO_2 inhalation. As many things can change blood glucose quickly and significantly, we included corresponding control groups during every experiment. These experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Institute for Nutritional Sciences, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences (CAS).

2.2. Cell culture and treatments

HepG2 cells were maintained in DMEM (Gibco, Grand Island, NY, USA) with 25 mmol/L glucose, 10% FBS, 50 μg/ml penicillin and streptomycin at 37 °C, 5% CO_2–95% air. C2C12 myoblasts were obtained from the CAS Cell Bank of Type Culture Collection. Maintenance and induction of differentiation were performed as previously described [13]. Primary hepatocyte isolation was achieved by collagenase perfusion as described previously [14]. Control, (–) val or (–) ile medium were prepared by adding all the components of regular DMEM or lacking the corresponding amino acid.

2.3. Blood glucose, serum insulin, GTT, ITT and HOMA-IR index

Blood glucose levels were measured using a Glucometer Elite monitor. Serum insulin levels were measured using the Mercodia Ultrasensitive Rat Insulin ELISA kit (Catalog Number: 80-INSRTU-E01, ALPCO Diagnostic, Salem, NH, USA). GTT and ITT were performed by IP injection of 2 g/kg glucose after overnight fasting and 0.75 U/kg or 0.375 U/kg insulin after 4 h fasting, respectively. The HOMA-IR index was calculated according to the formula: [Fasting glucose levels (mmol/L)] × [Fasting serum insulin (μU/ml)]/22.5.

2.4. In vivo insulin signaling assay

Mice maintained on different diets were fasted for 6 h prior to insulin injection as previously described [14]. Sections of liver and soleus muscle were excised from anesthetized mice and snap-frozen, as untreated controls. Three or five minutes after injection with 2 U/kg of insulin via the portal vein, pieces of tissue section were excised and snap-frozen for western blot analysis.

2.5. Western blot analysis

Western blot analysis was performed as previously described [15]. Protein concentrations were assayed using BCA Kit (Pierce, Rockford, USA). Primary antibodies [anti-p-insulin receptor (Tyr1150/1151), anti-insulin receptor, anti-p-AKT (Ser473), anti-AKT, anti-p-mTOR (Ser2448), anti-mTOR, anti-p-p70 S6K1

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