Original article

MOLECULAR METABOLISM

Branched-chain amino acid restriction in Zucker-fatty rats improves muscle insulin sensitivity by enhancing efficiency of fatty acid oxidation and acyl-glycine export

Phillip J. White [1,3], Amanda L. Lapworth [2,3], Jie An [1], Liping Wang [1], Robert W. McGarrah [1], Robert D. Stevens [1], Olga Ilkayeva [1], Tabitha George [1], Michael J. Muehlbauer [1], James R. Bain [1], Jeff K. Trimmer [2], M. Julia Brosnan [2], Timothy P. Rolph [2], Christopher B. Newgard [1,*]

ABSTRACT

Objective: A branched-chain amino acid (BCAA)-related metabolic signature is strongly associated with insulin resistance and predictive of incident diabetes and intervention outcomes. To better understand the role that this metabolite cluster plays in obesity-related metabolic dysfunction, we studied the impact of BCAA restriction in a rodent model of obesity in which BCAA metabolism is perturbed in ways that mirror the human condition.

Methods: Zucker-lean rats (ZLR) and Zucker-fatty rats (ZFR) were fed either a custom control, low fat (LF) diet, or an isonitrogenous, isocaloric LF diet in which all three BCAA (Leu, Ile, Val) were reduced by 45% (LF-RES). We performed comprehensive metabolic and physiologic profiling to characterize the effects of BCAA restriction on energy balance, insulin sensitivity, and glucose, lipid and amino acid metabolism.

Results: LF-fed ZFR had higher levels of circulating BCAA and lower levels of glycine compared to LF-fed ZLR. Feeding ZFR with the LF-RES diet lowered circulating BCAA to levels found in LF-fed ZLR. Activity of the rate limiting enzyme in the BCAA catabolic pathway, branched chain keto acid dehydrogenase (BCKDH), was lower in liver but higher in skeletal muscle of ZFR compared to ZLR and was not responsive to diet in either tissue. BCAA restriction had very little impact on metabolites studied in liver of ZFR where BCAA content was low, and BCKDH activity was suppressed. However, in skeletal muscle of LF-fed ZFR compared to LF-fed ZLR, where BCAA content and BCKDH activity were increased, accumulation of fatty acyl CoAs was completely normalized by dietary BCAA restriction. BCAA restriction also normalized skeletal muscle glycine content and increased urinary acetyl glycine excretion in ZFR. These effects were accompanied by lower RER and improved skeletal muscle insulin sensitivity in LF-RES fed ZFR as measured by hyperinsulinemic-isoglycemic clamp.

Conclusions: Our data are consistent with a model wherein elevated circulating BCAA contribute to development of obesity-related insulin resistance by interfering with lipid oxidation in skeletal muscle. BCAA-dependent lowering of the skeletal muscle glycine pool appears to contribute to this effect by slowing acyl-glycine export to the urine.

Keywords Obesity; BCAA; Insulin sensitivity; Metabolism

1. INTRODUCTION

Aberrant amino acid metabolism has long been recognized as a feature of obesity and accompanying metabolic disease. In 1969 Felig, Marliss, and Cahill [1] made the seminal observation that obese persons have higher levels of the branched chain (BCAA; Leucine, isoleucine and valine) and aromatic (phenylalanine and tyrosine) amino acids and lower levels of glycine in blood compared to lean individuals. More recently, unbiased metabolic profiling studies performed by our group [2–4] and others [5] have revived interest in perturbed amino acid metabolism as a potential contributor to development of metabolic diseases by revealing that a cluster of circulating metabolites comprising these same branched-chain and aromatic amino acids, as well as glutamate/glutamine, methionine, alanine, and the C3 and C5 acylcarnitines is strongly associated with insulin sensitivity [2], cardiometabolic health [6], future diabetes risk [5], and metabolic outcomes of weight loss interventions [7,8].

[1]Sarah W. Stedman Nutrition and Metabolism Center, Duke Molecular Physiology Institute, Departments of Pharmacology & Cancer Biology and Medicine, Duke University Medical Center, Durham, NC, 27701, USA [2]CV and Metabolic Diseases Research Unit, Pfizer, Cambridge, MA, USA

[3] Phillip J. White and Amanda L. Lapworth contributed equally to this work.

*Corresponding author. Duke Molecular Physiology Institute, 300 North Duke Street, Durham, NC, 27701, USA. Tel.: +1 919 668 6059. E-mail: Chris.Newgard@duke.edu (C.B. Newgard).

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Current evidence suggests that the obesity-related rise in circulating BCAA is the product of multiple metabolic perturbations related to their synthesis and catabolism, rather than being driven by increased intake of these essential amino acids [4,9]. One potential contributing factor has emerged from studies of the microbiota from monozygotic twins discordant for obesity, which revealed that obesity-driven shifts in microbial communities results in higher production and lower catabolism of BCAA by the intestinal flora [10]. Indeed, transfer of gut microbiota from obese or lean twins to gnotobiotic mice is sufficient to raise circulating BCAA in animals that received the microbiota of the obese twin by a magnitude similar to that reported for obese versus lean humans.

In addition, hepatic activity of the branched chain keto acid dehydrogenase (BCKDH) complex, which is responsible for the first irreversible and rate limiting step in BCAA metabolism, is low in obese and insulin resistant animals [11,12]. This is due to increased expression of the BCKDH kinase, BDK, and decreased expression of the BCKDH phosphatase, PPM1K, causing BCKDH to be in a hyperphosphorylated and inhibited state. Since liver is considered the primary site for catabolism of branched chain keto acids (BCKA) this represents a considerable systemic impairment. Demonstrating the control strength of this reaction, hypothalamic insulin and leptin lower circulating BCAA by up to 50% by reducing the phosphorylation state of hepatic BCKDH [13]. Furthermore, expression of BDK and PPM1K is regulated by adiponectin through an AMPK-dependent signal, and adiponectin knockout mice have lower PPM1K expression in liver accompanied by higher circulating BCAA [12]. Together, these data suggest that obesity-related changes in the hormonal milieu likely drive the inactivation of hepatic BCKDH by influencing the balance of the BDK/PPM1K regulatory node.

In adipose tissue, decreased BCAA metabolism in response to obesity appears to occur via global regulation of multiple enzymes in the catabolic pathway at a transcriptional level, rather than by post-translational modification as in liver [14,15]. Interestingly, interventions that reverse obesity-associated metabolic dysregulation, including bariatric surgery or treatment with thiazolidinedione drugs, restore expression of the BCAA catabolic enzymes in adipose tissue in concert with improved glucose homeostasis [11,15]. Remarkably, obesity-regulated post-translational or transcriptional regulation of the BCAA catabolic pathway has not been reported in skeletal muscle. Taken together, these findings highlight tissue-specific differences in BCAA catabolism in response to obesity.

Although our understanding of factors regulating the levels of circulating BCAA in obesity has evolved as described, the role of these metabolites in obesity-associated metabolic disorders remains to be defined [16]. In healthy humans, acute infusion of a complete amino acid mixture results in skeletal muscle and hepatic insulin resistance accompanied by activation of the mammalian target of rapamycin/S6 kinase 1 pathway [17–19]. However, various rodent studies involving specific supplementation of BCAA have yielded diverse findings, probably due in part to differences in study design. These have ranged from supplementation of all three BCAA in HF diets, resulting in exacerbation of insulin resistance [2], to addition of leucine alone in the drinking water, which is reported to have either no effect [20] or a beneficial effect on glucose homeostasis [21,22]. Less studied is the impact of BCAA restriction on metabolic control in models in which endogenous BCAA metabolism is perturbed in ways that mirror the human condition.

Herein we describe the results of a nutritional intervention study in which we restricted BCAA dietary supply by 45% in Zucker-lean (ZLR) and Zucker-fatty rats (ZFR), the latter being a model of insulin resistance and impaired BCAA metabolism [23]. We performed comprehensive metabolic and physiologic profiling to characterize the role of BCAA restriction on energy balance, insulin sensitivity, and glucose, lipid, and amino acid metabolism. Our findings demonstrate a clear cause and effect relationship between BCAA supply and insulin sensitivity and highlight underlying biochemical mechanisms.

2. MATERIALS & METHODS

2.1. Animals and diets

Six week-old male Zucker-lean (ZLR) and Zucker-fatty rats (ZFR) from Charles River Laboratories were placed on either a custom control low fat (LF) diet (A11072001, Research Diets, New Brunswick, NJ) or a LF BCAA restricted diet (LF-RES; A11072002, Research Diets, New Brunswick, NJ) in which 45% of the BCAA component of the LF diet was removed and replaced by a small increment in all other amino acids (except phenylalanine and tyrosine) such that nitrogen content was equal in both diets (Table 1). Rats were individually housed in a 12 h light:dark cycle with ad libitum access to water and food for up to 15 weeks. Food intake and weight gain were monitored weekly. A non-fasting blood sample was taken after 9 weeks on the diets at 9AM to determine the genotype and dietary effect on circulating amino acid concentrations. Plasma and tissue samples used for biochemical analysis were derived from rats that were euthanized in the fed state at week 15 by exsanguination following intraperitoneal administration of Nembutal (80 mg/kg). Tissues were rapidly excised, weighed, and freeze clamped in liquid nitrogen and then stored at −80 °C until further analysis. All animal procedures were approved and carried out in accordance with the directions of the Duke University Institutional Animal Care and Use Committee.

Table 1 — Diet amino acid content.

%	LF: Research Diets A11072001		LF-RES: Research Diets A11072002	
	grams	kcal	grams	kcal
Macronutrients				
Protein	33.6	33	33.6	33
Carbohydrate	49.3	49	49.3	49
Fat	8.0	18	8.0	18
Fiber	4.0	0	4.0	0
Total	90.9	100	90.9	100
kcal/gm	4.03		4.03	
Amino acids				
Isoleucine	17.88	72	9.83	39
Leucine	31.63	127	17.40	70
Valine	23.38	94	12.86	51
Phenylalanine	17.88	72	17.88	72
Tyrosine	12.38	50	12.38	50
Cysteine	5.50	22	6.29	25
Lysine	24.75	99	28.28	113
Methionine	6.88	28	7.86	31
Tryptophan	5.50	22	5.50	22
Histidine	8.25	33	9.43	38
Alanine	12.38	50	14.15	57
Arginine	27.50	110	31.43	126
Aspartic acid	20.63	83	23.58	94
Glutamic acid	37.13	149	42.43	170
Glycine	38.50	154	44.00	176
Proline	17.88	72	20.43	82
Serine	15.13	61	17.29	69

