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Adipose transplant for inborn errors of branched chain amino acid metabolism in mice

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ABSTRACT

Liver transplantation appears to be quite beneficial for treatment of maple syrup urine disease (MSUD, an inherited disorder of branched chain amino acid metabolism); however, there is a limited availability of donor livers worldwide and the first year costs of liver transplants are quite high. Recent studies have suggested that intact adipose tissue, already widely used in reconstructive surgery, may have an underappreciated high capacity for branched chain amino acid (BCAA) metabolism. Here we examined the potential for adipose tissue transplant to lower circulating BCAAs in two models of defective BCAA metabolism, BCATm and PP2Cm [branched chain keto acid dehydrogenase complex (BCKDC) phosphatase] knockout (KO) mice. After 1–2 g fat transplant, BCATm and PP2Cm KO mice gained or maintained body weight 3 weeks after surgery and consumed similar or more food/BCAAs the week before phlebotomy. Transplant of fat into the abdominal cavity led to a sterile inflammatory response and nonviable transplanted tissue. However when 1–2 g of fat was transplanted subcutaneously into the back, either as small (0.1–0.3 g) or finely minced pieces introduced with an 18-ga. needle, plasma BCAAs decreased compared to Sham operated mice. In two studies on BCATm KO mice and one study on PP2Cm KO mice, fat transplant led to 52–81% reductions in plasma BCAAs compared to baseline plasma BCAA concentrations of untreated WT type siblings. In PP2Cm KO mice, individual BCAAs in plasma were also significantly reduced by fat transplant, as were the alloisoleucine/Phe ratios. Therefore, subcutaneous fat transplantation may have merit as an adjunct to dietary treatment of MSUD. Additional studies are needed to further refine this approach.

1. Introduction

MSUD is an autosomal recessive inborn error of branched chain amino acid (BCAA) metabolism named for the burnt sugar smell of the urine due to accumulation of incompletely metabolized metabolites in the urine [1]. During their metabolism, BCAAs are transaminated to α-keto acids in a rapid and reversible reaction catalyzed by isoforms of branched chain amino acid transaminase (BCATc or BCATm), principally BCATm in most peripheral tissues. Subsequently, the α-keto acids are oxidized by the branched-chain alpha-keto acid dehydrogenase complex (BCKD). This step is irreversible and rate-controlled by phosphorylation. BCKD kinase inactivates the complex after phosphorylation of the BCKD E1-α subunit at Ser283 and this phosphorylation is reversed by the recently discovered BCKD phosphatase [PP2cm, PPM1K gene, see 2–12]. In humans, several forms of MSUD result from mutations in genes encoding the E1, E2 or E3 subunits of BCKD [see reviews: 13–18]. Dysfunction of this enzyme complex results in whole body accumulation of BCAAs and branched chain keto acids (BCKAs) along with potential depletion of essential amino acids in the brain through amino acid transport competition [19]. In a subset of patients, MSUD may also be caused by a mutation of BCKD phosphatase leading to inactivation and phosphorylation of the E1-α subunit of BCKD [6,20].

Loss of the ability to metabolize BCAAs can cause neurological symptoms and seizures through multiple mechanisms as first described by Menkes et al. [21]. At the molecular level these include competition for transport of other critical amino acids into the brain, increased reactive oxygen species generation, activation of several stress kinases, mitochondrial transition pore opening leading to Krebs cycle disruption, apoptosis and cell death in neurons and other cell types [4,6,19,22–25]. Intracellular accumulation of the keto acid of leucine, α-ketoisocaproate, has been linked to some of these toxicities, while the ketoacid of valine,

Abbreviations: BCAA, branched-chain amino acids; BCATc or BCATm, the cytosolic or mitochondrial isoform of branched-chain amino acid transaminase respectively (gene names: BCAT1 for BCATc, BCAT2 for BCATm); BCKA, branched chain keto acids; BCKD, branched-chain keto acid dehydrogenase complex; Exp, experiment; KO, knock out mouse; MSUD, maple syrup urine disease; PP2Cm, branched-chain keto acid dehydrogenase phosphatase (gene name: PPM1K); WT, wildtype sibling mice homozygous for wild type allele.

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α-ketoisovalerate, may cause seizures. On the other hand, BCAAs have been linked to neuronal degeneration or growth restriction [for reviews see, 19,22]. However, further studies are needed to definitively determine the relative contributions of BCAAs versus their rapidly formed metabolites, the α-ketoacids, to these toxicities.

If untreated, MSUD can precipitate seizures, coma, and early death usually before 3 months of age [14,19,25]. Standard treatment for MSUD patients involves nutritional interventions designed at monitoring plasma BCAAs and adjusting dietary intake. Under normal conditions, plasma BCAA concentrations are derived both from the diet and from whole body protein turnover, and their levels are relatively steady in plasma. Unfortunately, rates of protein catabolism can increase significantly in stress situations such as infection, psychosocial stress, trauma, food deprivation, etc. Thus, even with excellent dietary control, stress can precipitate episodic rises in BCAAs leading to acute "metabolic crises" in MSUD patients. Reoccurrences of these episodes and/or poor nutritional control over time can accelerate the rate of progressive neurological damage [for reviews see 15,26–29]. Thus while dietary intervention is lifesaving, its efficacy can be diminished during stress or infection through the normal activation of protein catabolism and/or diminished rates of protein synthesis.

A new experimental treatment for MSUD involves liver transplantation [14,17,30–36]. The liver is an important site for BCAA metabolism that expresses higher concentrations of BCKD and lower concentrations of the inhibitory BCKD kinase compared to other tissues like muscle [37]. Thus, the total activity and percent of the enzyme that is active is higher in liver than in muscle. Experimental treatment with liver transplant has been described in case reports and a recent clinical trial [17,30,31,33,34,36]. The results of these transplants have been quite encouraging with experiences ranging from greatly decreased incidences of metabolic crises to subjects being able to resume normal diets. Despite this success, there may be two obstacles for the broad adoption of this procedure. One is that demand for donor livers exceeds availability in the U.S. and worldwide — roughly 17,000 people were waiting for liver transplants in the U.S. alone last year. Fortunately, livers from MSUD patients may sometimes be donated to patients with liver disease in a so-called domino transplant. However, another potential barrier may be the cost of a liver transplant. Some estimates of U.S. billed charges including admission, surgery and one-year post-transplant treatment range from around four to six hundred thousand dollars per transplant [38,39].

Another tissue that expresses enzymes for BCAA metabolism is fat. During white and brown adipocyte differentiation BCKD is induced [40,41] and recent studies have suggested that human and rodent adipose tissues may possess an under-appreciated capacity for catalyzing the first steps in BCAA metabolism [42,43]. Thus while adipose tissue lysates have very low BCKD activity in vitro, Western blots suggest that BCATm and BCKD are expressed at comparable concentrations as found in other tissues. Consistent with Western blot findings, adipose tissue explants possess higher activity than muscle [43]. Several aspects of adipose tissue biology facilitate its use in reconstructive surgery and thereby make it amenable for use in transplantation [e.g., 44–50]. First, adipocytes exhibit a high capacity for neovascularization and low oxygen requirements. Second, approaches for repair of adipose precursors in vitro, coupled with in vitro or in vivo production of adipose tissue from those cells are available using current technologies. This could open the door for autogenic transplant after repair of the affected BCKD complex gene or genes. Third, recent studies are focused on approaches to convert white fat into so-called beige fat that has higher numbers of mitochondria, the organelle where BCAA oxidation occurs [51–60]. Approaches to stably initiate such conversion before transplant might be available in the future. Finally, if used for MSUD, finding adipose tissue donors could be easier than finding liver donors and the first year costs of adipose tissue transplantation might also be significantly lower.

Consequently, we explored the use of adipose transplant in mitigating elevated BCAAs in two mouse models. Mouse models of MSUD present additional challenges compared to human MSUD. For example, a formula feeding intervention with a purified amino acid formula, as might be used in patients with classic MSUD, would be difficult to achieve with newborn mice. Furthermore, blood volume is too limited in neonatal mice to allow frequent monitoring of blood metabolites and formula adjustment. For transplant studies a mouse model needs to be sufficiently robust not only to survive to adulthood without formula intervention, but also as adults they must be sufficiently healthy to survive a surgical intervention.

While there are no recorded human cases of MSUD resulting from BCAT2 mutation, BCAT2 deletion has nevertheless been proposed as an MSUD model [16,61]. BCATm catalyzes the transamination of BCAAs in most peripheral tissues and glia; the KO of this enzyme in mice leads to elevated circulating BCAAs but normal BCKAs. These mice survive to weaning at which time a dietary intervention can be used to lower BCAAs [62].

PP2Cm KO mice are another recently described MSUD model. These mice display greatly increased phosphorylation on the BCKD E1-α subunit (pSer293) leading to inactivation of BCKD, along with elevated plasma concentrations of BCAAs and BCKAs [2–6]. PP2Cm KOs and patients with homozygous for PPm1K mutation display similar toxicities at the cellular level and have elevations of the pathognomonic plasma biomarker of MSUD, alloisoleucine [2–6,25].

Three studies were conducted. In the first experiment, fat was transplanted into a subcutaneous pocket on the back and into the peritoneum of BCATm KO mice. As peritoneal fat did not become vascularized in the first study, the second experiment entailed finely minced subcutaneous adipose tissue that was deposited with a wide gauge needle only along the back. In the final experiment, this second approach was repeated in PP2Cm KO mice. In all cases plasma BCAA concentrations were significantly reduced in mice with a fat transplant compared to those who received sham surgery treatment.

2. Materials and methods

2.1. Mice

All procedures were conducted after review and approval by the Penn State Hershey Institutional Animal Care and Use Committee (IACUC). The Animal Resource Program, operated by the Department of Comparative Medicine is accredited by AAALAC, International. All animal living conditions are consistent with standards laid forth in the *Guide for the Care and Use of Laboratory Animals*, 8th edition, published by the National Research Council.

BCATm KO and PP2Cm KOs lines were maintained by heterozygote breeding strategies, backcrossed to C57BL/6J more than 10 times and maintained by backcrossing to new mice from Jackson Laboratories approximately every 3–4 generations after that. The creation and genotyping strategy for BCATm (aka BCAT2 gene) KOs from the floxed allele (MGI: 3772355, $BCAT2^{tm1.1Clyn}$) has been previously described [62]. The metabolic phenotype and genotyping strategy of the second mouse strain used, PP2Cm KO (a generous gift from Yibin Wang, UCLA), has also been previously described [2]. This strain arises from KO of the Ppm1K allele (MGI: 3850971, $Ppm1k^{tm1Yiwa}$) the gene that codes for PP2Cm, the intramitochondrial BCKD phosphatase.

Animals for these studies were housed in open-top, solid-bottom polycarbonate cages (Max75, Alternative Design, Siloam Springs, AR) with wire bar lids and corncob bedding (Teklad 7097 Corn Cob Bedding, Harlan Laboratories, Frederick, MD). Lighting was controlled with a 12 h:12 h light: dark cycle with lights on at 0700 h and off at 1900 h. Water and food were available ad libitum unless otherwise noted for study purposes. Prior to surgery and afterwards to facilitate regular perioperative and postoperative monitoring, they were moved to a satellite animal facility next to the main lab. Food intake was determined at 24 h intervals and Leu intake was calculated from information provided by the diet manufacturer on the Leu content of the diets. Elevated BCAA concentrations were verified in the animals used for these studies by

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