Published in final edited form as:

Mol Genet Metab. 2010 ; 100(Suppl 1): S72–S76. doi:10.1016/j.ymgme.2010.02.019.

New Insights in Nutritional Management and Amino Acid Supplementation in Urea Cycle Disorders

Fernando Scaglia^{1,2}

¹Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030, USA

²Texas Children's Hospital, Houston, TX, 77030, USA

Abstract

Sodium phenylbutyrate is used in the pharmacological treatment of urea cycle disorders to create alternative pathways for nitrogen excretion. The primary metabolite, phenylacetate, conjugates glutamine in the liver and kidney to form phenylacetylglutamine that is readily excreted in the urine. Patients with urea cycle disorders taking sodium phenylbutyrate have a selective reduction in the plasma concentrations of branched chain amino acids despite adequate dietary protein intake. Moreover, this depletion is usually the harbinger of a metabolic crisis. Plasma branched chain amino acids and other essential amino acids were measured in control subjects, untreated ornithine transcarbamylase deficiency females, and treated patients with urea cycle disorders (ornithine transcarbamylase deficiency and argininosuccinate synthetase deficiency) in the absorptive state during the course of stable isotope studies. Branched chain amino acid levels were significantly lower in treated patients with urea cycle disorders when compared to untreated ornithine transcarbamylase deficiency females or control subjects. These results were replicated in control subjects who had low steady-state branched chain amino acid levels when treated with sodium phenylbutyrate. These studies suggested that alternative pathway therapy with sodium phenylbutyrate causes a substantial impact on the metabolism of branched chain amino acids in patients with urea cycle disorders, implying that better titration of protein restriction can be achieved with branched chain amino acid supplementation in these patients who are on alternative pathway therapy.

Keywords

branched chain amino acids; sodium phenylbutyrate; urea cycle disorders

Corresponding author: Fernando Scaglia, M.D., Department of Molecular and Human Genetics, Baylor College of Medicine, Texas Children's Hospital, Clinical Care Center, Suite 1560, 6621 Fannin Street, Mail Code CC1560, Houston, Texas, 77030, U.S.A., fscaglia@bcm.tmc.edu.

Conflict of Interest statement

The authors declare that there are no conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

The nutritional management of patients with urea cycle disorders (UCDs) includes restriction of dietary protein along with provision of adequate protein-free energy, essential amino acid supplements, and vitamins and minerals. In addition, the overall treatment involves the prevention or supportive management of catabolic stress, and the stimulation of alternative pathways of nitrogen excretion by the use of pharmacotherapy that promotes the excretion of waste nitrogen. The pharmacological intervention includes the use of sodium phenylacetate/benzoate (Ammonul) or sodium phenylbutyrate (Buphenyl) to reduce in plasma glutamine levels in clinical situations of metabolic decompensation accompanied by hyperammonemia and hyperglutaminemia (1). Sodium phenylbutyrate is β -oxidized to phenylacetate and the elimination of waste nitrogen is achieved by conjugating glutamine with phenylacetate in the liver and kidney to form phenylacetylglutamine (2). In the case of Ammonul, the action of sodium phenylacetate is complemented by the addition of sodium benzoate that conjugates glycine to promote the synthesis of hippuric acid. These conjugates are then eliminated in the urine, accounting for a variable fraction of the dose (1, 3).

It has been demonstrated that in adults fed a low protein diet, the oral administration of phenylacetate increases glutamine flux and lowers circulating glutamine concentrations (4). This finding has also been observed in infants with normal plasma glutamine levels by a similar mechanism (1). However, previously unknown secondary metabolites of phenylbutyrate have been recently identified in urine of normal human subjects and in perfused rat livers (5). These metabolites are derived from interaction between the metabolism of phenylbutyrate and that of carbohydrates and lipids, and comprise two categories, glucuronides and phenylbutyrate beta-oxidation side products. Low serum levels of branched chain amino acids (BCAA) during the administration of high doses of sodium phenylbutyrate were observed in a study conducted to evaluate long-term survival of patients with argininosuccinate synthetase deficiency (6). Moreover, we have identified a consistently selective and striking depletion of serum BCAA among patients with UCDs treated with sodium phenylbutyrate despite, being on an isocaloric and adequate dietary protein intake. This decrease of serum BCAA has often foreshadowed a metabolic crisis accompanied by hyperammonemia and hyperglutaminemia.

One of the potential mechanisms leading to a catabolic state could be caused by the down-regulation of total body protein synthesis due to the BCAA depletion. Phenylbutyrate use in healthy subjects caused glutamine depletion and disrupted BCAA metabolism with increased leucine oxidation (7). Another study of UCD patients demonstrated that the use of phenylbutyrate had a marked effect on the metabolism of branched chain amino acids (8, 9). In addition, acute depletion of plasma glutamine and BCAA and increased leucine oxidation were observed in prednisone-treated healthy volunteers who received sodium phenylbutyrate over 24 hours (10).

The aim of this review is to summarize new observations of amino acid supplementation in UCD patients with a particular focus on the effect of alternative pathway therapy on BCAA metabolism and the function of leucine as a key regulator of protein synthesis.

Study subjects and methods

Subjects

The study was conducted at the Texas Children's Hospital's General Clinical Research Center. The research protocol received prior approval from the Institutional Review Boards for Human Subjects of Baylor College of Medicine. Appropriate informed consent was signed by the study subjects. The study of the role of BCAA in UCD subjects was carried out during admission for investigative studies of nitrogen flux to estimate *in vivo* urea cycle activity (4).

The study subjects who participated included eleven healthy adult control subjects, six asymptomatic and untreated ornithine transcarbamylase deficiency (OTCD) females, and five subjects with UCDs (two severe, neonatal-onset OTCD male patients and three patients with argininosuccinate synthetase deficiency (ASSD)). All controls subjects were initially evaluated on an isocaloric low natural protein intake [0.4g/(kg day)] diet for the three-day duration of the study. Five of these control subjects were randomly assigned to also receive sodium phenylbutyrate ($10\text{g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$). The asymptomatic OTCD females were all evaluated on an isocaloric low natural protein intake [0.4g/(kg day)] diet for the three-day duration of the initial part of the study. Following this, two of these subjects were also given sodium phenylbutyrate ($10\text{g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) in addition to the same low protein intake. Patients with ASSD (ages 9–13 years) were evaluated on an isocaloric low total protein and amino acid intake [0.4g/(kg day)] for the three-day duration of the study, with half of their intake from food protein and half derived from essential amino acids. During the three-day duration of the study, the two severe, neonatal-onset males with OTCD were studied on an isocaloric total protein and amino acid intake [1g/(kg day)] that was an integral part of their usual dietary management, with half of that intake derived from food protein and the other half from essential amino acids.

Five UCD patients with severe neonatal presentation (three OTCD patients and two patients with carbamoylphosphate synthetase I deficiency) who were being treated with sodium phenylbutyrate and two patients with partial OTCD being treated with sodium benzoate were followed retrospectively during a period of three months. All UCD patients continued with their previously assigned doses of pharmacological therapy throughout the study.

Clinical research protocol

Each study subject was started on the assigned level of protein intake and sodium phenylbutyrate (if indicated) at the time of admission. The estimation of protein intake was done by weighing food portions before and after each meal. On the third day of the study, following an overnight fast and a pre-infusion blood sampling for the analysis of baseline plasma amino acids, the subjects were given four small meals every two hours. Each meal supplied $\frac{1}{12}$ of the prior daily protein intake. A blood sample for plasma amino acids and ammonia was obtained at baseline (0 hr), and at 4, 6, and 7.5 hr during the infusion of the stable isotopes (sampling was done 1.5–2 hr after the intake of the small meals). Serum BCAA and other essential non-BCAA in these diverse groups were measured at the plateau phase of isotopic enrichments in the absorptive state during the course of the studies (8).

Results

All serum BCAA levels were significantly lower in severely deficient UCD patients treated with sodium phenylbutyrate when compared to normal subjects or untreated asymptomatic OTCD females. No significant differences were found in other essential non-BCAAs among the three groups (8). Fairly similar results were encountered when plasma amino acids were measured in a group of control subjects and in two asymptomatic OTCD females after stabilization on their assigned diet, compared with the same two groups while taking phenylbutyrate treatment and receiving the same low protein intake [0.4g/(kg day)]. Furthermore, a retrospective study in which serum leucine levels were followed in UCD subjects treated with either sodium phenylbutyrate or sodium benzoate demonstrated a decrease of serum leucine levels in the group receiving sodium phenylbutyrate. However, a similar effect was not observed in the group treated with sodium benzoate. The caloric intake and protein sufficiency in all of these subjects was adequate by evaluation of their growth, diet records, and biochemical markers including albumin and prealbumin levels (8).

Discussion

Glutamine as a positive regulator of protein biosynthesis

Glutamine comprises 60% of the free amino acids in muscle and is the most abundant amino acid in the body (11). It is a non-essential amino acid since it is synthesized *de novo* from glutamate and ammonia by the cytosolic enzyme glutamine synthetase (12). Although a non-essential amino acid, it plays an important role in protein homeostasis. Furthermore, since the capacity to produce appropriate amounts of glutamine can be overwhelmed by catabolic stress associated with illness, this amino acid may become “conditionally essential” as the observed protein wasting will be reflected on the depletion of skeletal muscle glutamine (12). Glutamine exerts a pivotal role in regulating protein homeostasis and inhibiting protein degradation (13). It has been shown that replenishment of glutamine leads to an improvement of nitrogen balance in periods of metabolic stress associated with protein catabolism (14). Glutamine administered enterally limits amino acid oxidation (including leucine oxidation) and has a sparing effect on whole-body amino acids in hypercatabolic dogs during the fed state (15). Two different ways of supplementing clinical nutrition products with glutamine, either with free glutamine or with a glutamine-rich protein source, were evaluated in hypercatabolic rats treated with glucocorticoids. Glutamine provided as dietary protein was extensively metabolized by the splanchnic tissues and did not influence peripheral glutamine status to the same extent as glutamine provided as the free amino (16). Glutamine depletion induced by inhibition of glutamine synthetase also led to a marked reduction of protein synthesis in cultured human enterocytes (17). Regardless of the route of supply, glutamine corrects some of the harmful effects of fasting in this model via deamination to glutamate (18). In the fasting state, the intestine uses glutamine as main energy fuel producing ammonia and α -ketoglutarate via the actions of intestinal glutaminase and glutamate dehydrogenase. In addition, a small fraction of the intestinal glutamine pool is converted into ornithine that can be used for the *de novo* synthesis of arginine via the hepatic urea cycle and citrulline (19). It has been demonstrated that total parenteral nutrition supplemented with glutamine enhances nitrogen balance in surgical patients (20, 21).

Enteral infusion of L-glutamine in healthy subjects led to an inhibition of leucine oxidation and an increase in whole body protein synthesis (22).

Supplementation of oral glutamine was found to decrease whole body protein breakdown in children with Duchenne muscular dystrophy (23). Obviously, it would not be feasible to use glutamine supplementation in UCD patients affected by a catabolic state as they exhibit decreased capacity for handling waste nitrogen.

Effect of sodium phenylbutyrate therapy on BCAA

Sodium phenylbutyrate given in a single dose has been shown to reduce not only plasma glutamine but, in addition, reduces plasma leucine with an increase in leucine oxidation in fasting healthy individuals (7). These changes were observed in conjunction with decreased whole body protein synthesis as estimated by NOLD (7). In a follow-up study using an experimental model of catabolism induced by glucocorticosteroids, administration of phenylbutyrate caused acute glutamine depletion accompanied by an increase in leucine oxidation (10). A study conducted to evaluate the effect of alternative pathway therapy on branched chain amino acid metabolism in UCD patients clearly demonstrated that use of phenylbutyrate had a marked effect on the metabolism of branched chain amino acids (8, 9). This observation was also replicated by a cross-sectional multi-center study of UCD patients in the United States, where plasma levels of BCAA were reduced in those patients treated with sodium phenylbutyrate (24).

Potential mechanisms for the observed depletion of branched chain amino acids associated with the use of sodium phenylbutyrate in UCD patients

The principal source of plasma glutamine is from muscle. Thus, it has been hypothesized that phenylbutyrate treatment may induce depletion of intracellular glutamine stores (10). Reduction of the intracellular glutamate pool would lead to increased BCAA oxidation and possibly increased transamination of BCAA with α -ketoglutarate, potentially offering an explanation for the observed decrease of plasma BCAA levels. Glutamate is synthesized from α -ketoglutarate using amino groups derived from BCAA (25) and hyperammonemia activates BCAA aminotransferases (26). Of interest, a decrease of plasma BCAA concentrations has been described in patients with liver cirrhosis and hyperammonemia (27). In addition, depletion of plasma BCAA levels has been noted in idiopathic portal hypertension, a clinical condition characterized by hyperammonemia and hyperglutaminemia (28, 29). These observations are consistent with studies performed in dogs and rats in which infusion of ammonium salts led to a decrease in plasma BCAA levels (30, 31). In the study conducted in rats, the decreased plasma levels of BCAA preceded the eventual decrease in the concentration of these amino acids in skeletal muscle (31). Concomitantly, increased intracellular concentrations of glutamine and decreased intracellular concentrations of glutamate and alanine were observed (31).

However, despite all this indirect evidence, the hypothesis that depletion of plasma BCAA associated with the use of sodium phenylbutyrate is due depletion of intracellular glutamate and increased transamination activity has not been completely proven and remains controversial. Other alternatives may involve a direct effect of sodium phenylbutyrate on

essential metabolic pathways. It is known that the action of phenylbutyrate is not limited to the conjugation and excretion of glutamine. This compound also is known to inhibit DNA methylation, histone deacetylation, and protein isoprenylation (32). In addition, it is known to influence the growth and differentiation of a variety of neoplastic cells (33, 34). In addition, it has been shown that phenylbutyrate increases the expression of certain enzymes of the urea cycle, such as arginase I (35). It is possible that phenylbutyrate could directly or indirectly activate the branched chain α -keto acid dehydrogenase complex (BCKDC common to the three BCAA and the rate-limiting enzyme in their catabolism (36). This hypothesis could explain the increased rate of leucine oxidation observed in prednisone treated healthy volunteers who received phenylbutyrate for 24 hours (10). Upregulation of this enzyme activity could be related to intracellular glutamine depletion, since *in vitro* studies have documented that glutamine deprivation induced the upregulation of several genes, including the gene encoding the E1 α subunit of the BCKD complex in a lymphocytic cell line (37).

Role of leucine as a regulator of protein synthesis

Amino acids are involved as signaling molecules controlling signal-transduction pathways. The concentration of leucine in muscle cells may play a role in regulating the turnover of muscle proteins, influencing the transition to negative nitrogen balance during fasting, uncontrolled diabetes, and the posttraumatic state. These findings suggested that leucine may exert a key protein-sparing effect in skeletal muscle (38). Furthermore, other publications have shown that among the BCAA, leucine is the strongest regulator of mRNA translation initiation (39). The signaling mechanism of leucine is apparently mediated via a mammalian target of rapamycin (mTOR) sensitive pathway (40). TOR complex 1 (mTORC1) is sensitive to rapamycin, however TOR complex 2 (mTORC2) is not (41). mTORC1 is activated by leucine in synergy with insulin, and activation of this pathway inhibits autophagy (42). Because activation of mTORC1 pathway stimulates protein synthesis, it seems that protein synthesis and autophagic protein degradation are reciprocally controlled by the same pathway.

Stimulation of translation via mTORC1 by leucine seems to increase the phosphorylation of the translational repressor protein 4E-BP1 and the ribosomal protein S6 kinase (mTORC1 effectors). The phosphorylation of 4E-BP1 releases the eukaryotic translation initiation factor 4E (eIF4E), which is then able to associate with eukaryotic translation initiation factor 4G (eIF4G) to stimulate protein translation initiation (39, 43).

Conversely, a decreased concentration of leucine induces dephosphorylation of S6K1 and 4E-BP1 in a mTORC-1 dependent manner leading to inhibition of protein synthesis (44). Amino acids may activate mTORC1 via inhibition of the tuberous sclerosis proteins TSC1 (hamartin) and TSC2 (tuberin) or via stimulation of a small GTPase Ras homologue that is enriched in brain (Rheb) (45). Leucine regulates mTORC1 by controlling the ability of Rheb-GTP to activate mTORC1. Rheb binds directly to mTOR which is a required step in mTORC1 activation. In addition, Rheb-GTP stimulates phospholipase D1 to generate phosphatidic acid, a positive regulatory molecule of mTORC1 activation. Rheb-GTP also binds to the mTOR inhibitor FKBP38 to displace it from mTOR (46). Although leucine

influence on mTORC1 is initiated subsequently to leucine entry, it is not known whether this action is mediated by leucine itself, by a ligand, or a metabolically transformed product (46). The detailed mechanism by which amino acids and leucine in particular stimulate this signaling pathway and the connection with autophagic machinery remains unknown. Thus, the precise mechanism by which nutrients, and in particular leucine, signal to mTORC1 awaits further elucidation.

Summary

There is enough evidence based on the review of the literature summarized in this article to conclude that a potential outcome of the use of therapeutic doses of phenylbutyrate in UCD patients is the depletion of plasma BCAA levels. It has been observed in the clinical setting that depletion of BCAA may be a harbinger of metabolic crises, followed by hyperammonemia and hyperglutaminemia. These metabolic abnormalities negatively impact total body protein synthesis, exacerbating negative nitrogen balance and increasing the metabolic nitrogen load on a metabolic patient who is already at high risk for hyperammonemia and metabolic decompensation.

It has been observed that supplementation with BCAA (10 mg.kg.day of each BCAA) to UCD subjects receiving sodium phenylbutyrate is metabolically and clinically beneficial, preventing body protein catabolism and increasing protein tolerance in the clinical management of UCDs. Due to their dietary protein restriction, UCD patients are at risk of limited intake of other essential amino acids resulting in potential deficiency (47). Therefore, in conjunction with BCAA supplementation, it is relevant in UCD patients to supply about 50% of their dietary protein in the form of an essential amino acid mixture high in BCAAs in order to offset essential amino acid imbalances and promote growth.

Either the depletion of intracellular glutamate pools followed by activation of BCAA aminotransferases with increased transamination of BCAAs or the activation of the BCKD complex by phenylbutyrate could lead to abnormal regulation of body protein turnover and a reduction of total body protein synthesis. However, the ultimate mechanism by which phenylbutyrate may exert this effect has not been completely elucidated and further studies in the clinical research arena are needed to dissect the mechanism of sodium phenylbutyrate on BCAA metabolism and total body protein turnover.

References

1. Brusilow SW. Phenylacetylglutamine may replace urea as a vehicle for waste nitrogen excretion. *Pediatr Res.* 1991 Feb; 29(2):147–50. [PubMed: 2014149]
2. James MO, Smith RL, Williams RT, Reidenberg M. The conjugation of phenylacetic acid in man, sub-human primates and some non-primate species. *Proc R Soc Lond B Biol Sci.* 1972 Jul 25; 182(66):25–35. [PubMed: 4403084]
3. Maestri NE, McGowan KD, Brusilow SW. Plasma glutamine concentration: a guide in the management of urea cycle disorders. *J Pediatr.* 1992 Aug; 121(2):259–61. [PubMed: 1640294]
4. Lee B, Yu H, Jahoor F, O'Brien W, Beaudet AL, Reeds P. In vivo urea cycle flux distinguishes and correlates with phenotypic severity in disorders of the urea cycle. *Proc Natl Acad Sci U S A.* 2000 Jul 5; 97(14):8021–6. [PubMed: 10869432]

5. Kasumov T, Brunengraber LL, Comte B, Puchowicz MA, Jobbins K, Thomas K, et al. New secondary metabolites of phenylbutyrate in humans and rats. *Drug Metab Dispos.* 2004 Jan; 32(1): 10–9. [PubMed: 14709615]
6. Maestri NE, Clissold DB, Brusilow SW. Long-term survival of patients with argininosuccinate synthetase deficiency. *J Pediatr.* 1995 Dec; 127(6):929–35. [PubMed: 8523191]
7. Darmaun D, Welch S, Rini A, Sager BK, Altomare A, Haymond MW. Phenylbutyrate-induced glutamine depletion in humans: effect on leucine metabolism. *Am J Physiol.* 1998 May; 274(5 Pt 1):E801–7. [PubMed: 9612237]
8. Scaglia F, Carter S, O'Brien WE, Lee B. Effect of alternative pathway therapy on branched chain amino acid metabolism in urea cycle disorder patients. *Mol Genet Metab.* 2004 Apr; 81(Suppl 1):S79–85. [PubMed: 15050979]
9. Scaglia, F.; Lanpher, B.; Marini, J.; Lee, B., editors. Role of branched-chain amino acids in patients with urea cycle disorder; International Symposium 2006; Fulda. 2006; Fulda, Germany: SPS Verlagsgesellschaft; 2007.
10. Le Bacquer O, Mauras N, Welch S, Haymond M, Darmaun D. Acute depletion of plasma glutamine increases leucine oxidation in prednisone-treated humans. *Clin Nutr.* 2007 Apr; 26(2): 231–8. [PubMed: 17097772]
11. Bergstrom J, Furst P, Noree LO, Vinnars E. Intracellular free amino acid concentration in human muscle tissue. *J Appl Physiol.* 1974 Jun; 36(6):693–7. [PubMed: 4829908]
12. Lacey JM, Wilmore DW. Is glutamine a conditionally essential amino acid? *Nutr Rev.* 1990 Aug; 48(8):297–309. [PubMed: 2080048]
13. Hankard RG, Haymond MW, Darmaun D. Effect of glutamine on leucine metabolism in humans. *Am J Physiol.* 1996 Oct; 271(4 Pt 1):E748–54. [PubMed: 8897864]
14. Wernerman J, Hammarqvist F, Vinnars E. Alpha-ketoglutarate and postoperative muscle catabolism. *Lancet.* 1990 Mar 24; 335(8691):701–3. [PubMed: 1969067]
15. Humbert B, Nguyen P, Dumon H, Deschamps JY, Darmaun D. Does enteral glutamine modulate whole-body leucine kinetics in hypercatabolic dogs in a fed state? *Metabolism.* 2002 May; 51(5): 628–35. [PubMed: 11979397]
16. Boza JJ, Turini M, Moennoz D, Montigon F, Vuichoud J, Gueissaz N, et al. Effect of glutamine supplementation of the diet on tissue protein synthesis rate of glucocorticoid-treated rats. *Nutrition.* 2001 Jan; 17(1):35–40. [PubMed: 11165886]
17. Le Bacquer O, Nazih H, Blottiere H, Meynial-Denis D, Labois C, Darmaun D. Effects of glutamine deprivation on protein synthesis in a model of human enterocytes in culture. *Am J Physiol Gastrointest Liver Physiol.* 2001 Dec; 281(6):G1340–7. [PubMed: 11705738]
18. Le Bacquer O, Labois C, Darmaun D. Glutamine preserves protein synthesis and paracellular permeability in Caco-2 cells submitted to “luminal fasting”. *Am J Physiol Gastrointest Liver Physiol.* 2003 Jul; 285(1):G128–36. [PubMed: 12799310]
19. Rabier, D., editor. Ammonia production and detoxification; International Symposium 2006; Fulda. 2006; Fulda: SPS Verlagsgesellschaft; 2007.
20. Stehle P, Zander J, Mertes N, Albers S, Puchstein C, Lawin P, et al. Effect of parenteral glutamine peptide supplements on muscle glutamine loss and nitrogen balance after major surgery. *Lancet.* 1989 Feb 4; 1(8632):231–3. [PubMed: 2563409]
21. Morlion BJ, Stehle P, Wachtler P, Siedhoff HP, Koller M, König W, et al. Total parenteral nutrition with glutamine dipeptide after major abdominal surgery: a randomized, double-blind, controlled study. *Ann Surg.* 1998 Feb; 227(2):302–8. [PubMed: 9488531]
22. Hankard RG, Haymond MW, Darmaun D. ESPEN research fellows symposia—presented at ESPEN 1995. Effect of enteral glutamine on glutamine and leucine metabolism in humans. *Clin Nutr.* 1996 Apr; 15(2):84–5. [PubMed: 16844004]
23. Hankard RG, Hammond D, Haymond MW, Darmaun D. Oral glutamine slows down whole body protein breakdown in Duchenne muscular dystrophy. *Pediatr Res.* 1998 Feb; 43(2):222–6. [PubMed: 9475288]
24. Tuchman M, Lee B, Lichter-Konecki U, Summar ML, Yudkoff M, Cederbaum SD, et al. Cross-sectional multicenter study of patients with urea cycle disorders in the United States. *Mol Genet Metab.* 2008 Aug; 94(4):397–402. [PubMed: 18562231]

25. Odessey R, Khairallah EA, Goldberg AL. Origin and possible significance of alanine production by skeletal muscle. *J Biol Chem.* 1974 Dec 10; 249(23):7623–9. [PubMed: 4436328]
26. Hod G, Chaouat M, Haskel Y, Lernau OZ, Nissan S, Mayer M. Ammonia uptake by skeletal muscle in the hyperammonaemic rat. *Eur J Clin Invest.* 1982 Dec; 12(6):445–50. [PubMed: 6129977]
27. Limberg B, Kommerell B. Why decreased serum branched-chain amino acids in cirrhosis. *Gastroenterology.* 1981 Jan; 80(1):211–2. [PubMed: 6108893]
28. Iwasaki Y, Sato H, Ohkubo A, Sanjo T, Futagawa S, Sugiura M, et al. Effect of spontaneous portal-systemic shunting on plasma insulin and amino acid concentrations. *Gastroenterology.* 1980 Apr; 78(4):677–83. [PubMed: 6986317]
29. Matsubara S, Ouchi K, Okabe K, Suzuki M, Matsuno S. Clinical outcome of non-shunting operation for oesophageal varices in patients with liver cirrhosis. *J Gastroenterol Hepatol.* 1989; 4(Suppl 1):235–8. [PubMed: 2519064]
30. Strombeck DR, Rogers QR, Stern JS. Effects of ammonia infusion on plasma glucagon, insulin, and amino acids in intact, pancreatectomized, and adrenalectomized dogs. *Am J Vet Res.* 1981 May; 42(5):810–8. [PubMed: 7020500]
31. Leweling H, Breitreutz R, Behne F, Staedt U, Striebel JP, Holm E. Hyperammonemia-induced depletion of glutamate and branched-chain amino acids in muscle and plasma. *J Hepatol.* 1996 Nov; 25(5):756–62. [PubMed: 8938556]
32. Gore SD, Samid D, Weng LJ. Impact of the putative differentiating agents sodium phenylbutyrate and sodium phenylacetate on proliferation, differentiation, and apoptosis of primary neoplastic myeloid cells. *Clin Cancer Res.* 1997 Oct; 3(10):1755–62. [PubMed: 9815560]
33. Waxman S. Differentiation therapy in acute myelogenous leukemia (non-APL). *Leukemia.* 2000 Mar; 14(3):491–6. [PubMed: 10720148]
34. Andratschke N, Grosu AL, Molls M, Nieder C. Perspectives in the treatment of malignant gliomas in adults. *Anticancer Res.* 2001 Sep-Oct; 21(5):3541–50. [PubMed: 11848521]
35. Kern RM, Yang Z, Kim PS, Grody WW, Iyer RK, Cederbaum SD. Arginase induction by sodium phenylbutyrate in mouse tissues and human cell lines. *Mol Genet Metab.* 2007 Jan; 90(1):37–41. [PubMed: 16935537]
36. Yeaman SJ. The 2-oxo acid dehydrogenase complexes: recent advances. *Biochem J.* 1989 Feb 1; 257(3):625–32. [PubMed: 2649080]
37. Peng T, Golub TR, Sabatini DM. The immunosuppressant rapamycin mimics a starvation-like signal distinct from amino acid and glucose deprivation. *Mol Cell Biol.* 2002 Aug; 22(15):5575–84. [PubMed: 12101249]
38. Buse MG, Reid SS. Leucine. A possible regulator of protein turnover in muscle. *J Clin Invest.* 1975 Nov; 56(5):1250–61. [PubMed: 1237498]
39. Anthony JC, Anthony TG, Kimball SR, Vary TC, Jefferson LS. Orally administered leucine stimulates protein synthesis in skeletal muscle of postabsorptive rats in association with increased eIF4F formation. *J Nutr.* 2000 Feb; 130(2):139–45. [PubMed: 10720160]
40. Anthony JC, Yoshizawa F, Anthony TG, Vary TC, Jefferson LS, Kimball SR. Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. *J Nutr.* 2000 Oct; 130(10):2413–9. [PubMed: 11015466]
41. Wullschlegel S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell.* 2006 Feb 10; 124(3):471–84. [PubMed: 16469695]
42. Meijer AJ. Amino acid regulation of autophagosome formation. *Methods Mol Biol.* 2008; 445:89–109. [PubMed: 18425444]
43. Greiwe JS, Kwon G, McDaniel ML, Semenkovich CF. Leucine and insulin activate p70 S6 kinase through different pathways in human skeletal muscle. *Am J Physiol Endocrinol Metab.* 2001 Sep; 281(3):E466–71. [PubMed: 11500301]
44. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev.* 2004 Aug 15; 18(16):1926–45. [PubMed: 15314020]
45. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, et al. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell.* 2002 Jul 26; 110(2):163–75. [PubMed: 12150925]

46. Avruch J, Long X, Ortiz-Vega S, Rapley J, Papageorgiou A, Dai N. Amino acid regulation of TOR complex 1. *Am J Physiol Endocrinol Metab*. 2009 Apr; 296(4):E592–602. [PubMed: 18765678]
47. Bachmann, C., editor. *Hyperammonemia: Review of current treatment strategies*; International Symposium 2006; Fulda. 2006; Fulda: SPS Verlagsgesellschaft; 2007.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript