

Branched-chain Amino Acids as Potential Diagnostic and Prognostic Disease Biomarkers

Federica Ianni, Lucia Pucciarini, Emidio Camaioni, Giulia Alunni, Roccaldo Sardella*, Benedetto Natalini

Department of Pharmaceutical Sciences, Section of Chemistry and Technology of Drugs, Via del Liceo 1, 06123 Perugia, Italy

Abstract

Recent evidences demonstrate that branched-chain amino acids (BCAAs) are associated with a number of pathologies encompassing insulin resistance, type 2 diabetes mellitus, chronic kidney disease as well as ischemic stroke, major depression and Huntington disease. Therefore, the measurement and monitoring of BCAAs levels in biological fluids and organs could represent a promising method for aiding in the clinical diagnosis and prognosis assessment for patients. However, too many questions are still open regarding the mechanisms at the basis of the relationship between BCAAs levels and disease process/severity. It is widely accepted that BCAAs levels in diagnosing, predicting or monitoring disease can be concretely useful in the presence of additional information that will place these levels in the context of the whole clinical picture.

Publication History:

Received: December 12, 2016

Accepted: March 13, 2017

Published: March 15, 2017

Keywords:

Branched-chain amino acids,
Diagnostic biomarkers, Prognostic
biomarkers

Introduction

Branched-chain amino acids (BCAAs), that are isoleucine (Ile), leucine (Leu) and valine (Val), are proteinogenic essential amino acids carrying an aliphatic-branched side chain. BCAAs not only play a pivotal role as key building blocks for peptide synthesis, but are recognized to be also important sources for the biosynthesis of sterols, ketone bodies and glucose. It has been estimated that the BCAAs pool accounts for about 21% of the total body protein content, while free BCAAs constitute an extremely minor proportion (272 to 556-fold less) of the total mass of BCAAs in the body [1]. In spite of this paucity, free BCAAs act however as important nutrient signals and metabolic regulators [2].

Mounting evidences clearly demonstrate that BCAAs are associated with several pathological states, including obesity [3], insulin resistance [4-7], type 2 diabetes mellitus (T2DM) [6, 8], cancer [9], cardiovascular [10] and liver diseases [11], chronic kidney disease (CKD) [12] and ischemic stroke [13]. Nonetheless, altered levels of BCAAs have been also found in patients with either major depression or Huntington Disease (HD)[14].

This body of evidence readily suggests the potential use of BCAAs as biomarkers of disease in clinical practice. Interestingly, it has been also demonstrated that, circulating levels of BCAAs could be valuable prognostic tools and predict populations, at risk for various pathological conditions, encompassing cardiometabolic disease [15] and T2DM [16].

For some pathology, the underlying biochemical mechanisms accounting for the altered levels of BCAAs is still unclear or rather speculative.

Some of these issues will be touched in the following sections.

Obesity, Type 2 Diabetes Mellitus and Cancer

In the last decades, obesity has become a global public problem indistinctly diffused in both developed and developing countries [17, 18]. The increasing number of obese people in industrialized countries is intimately related to various aspects of modern lifestyle, including inadequate nutrition and leisure time activities. The strong association of obesity with T2DM [19], hypertension [20], and metabolic

syndrome [21], highlights the urgent need to monitor its persistence in adults and to investigate its possible pathological developments.

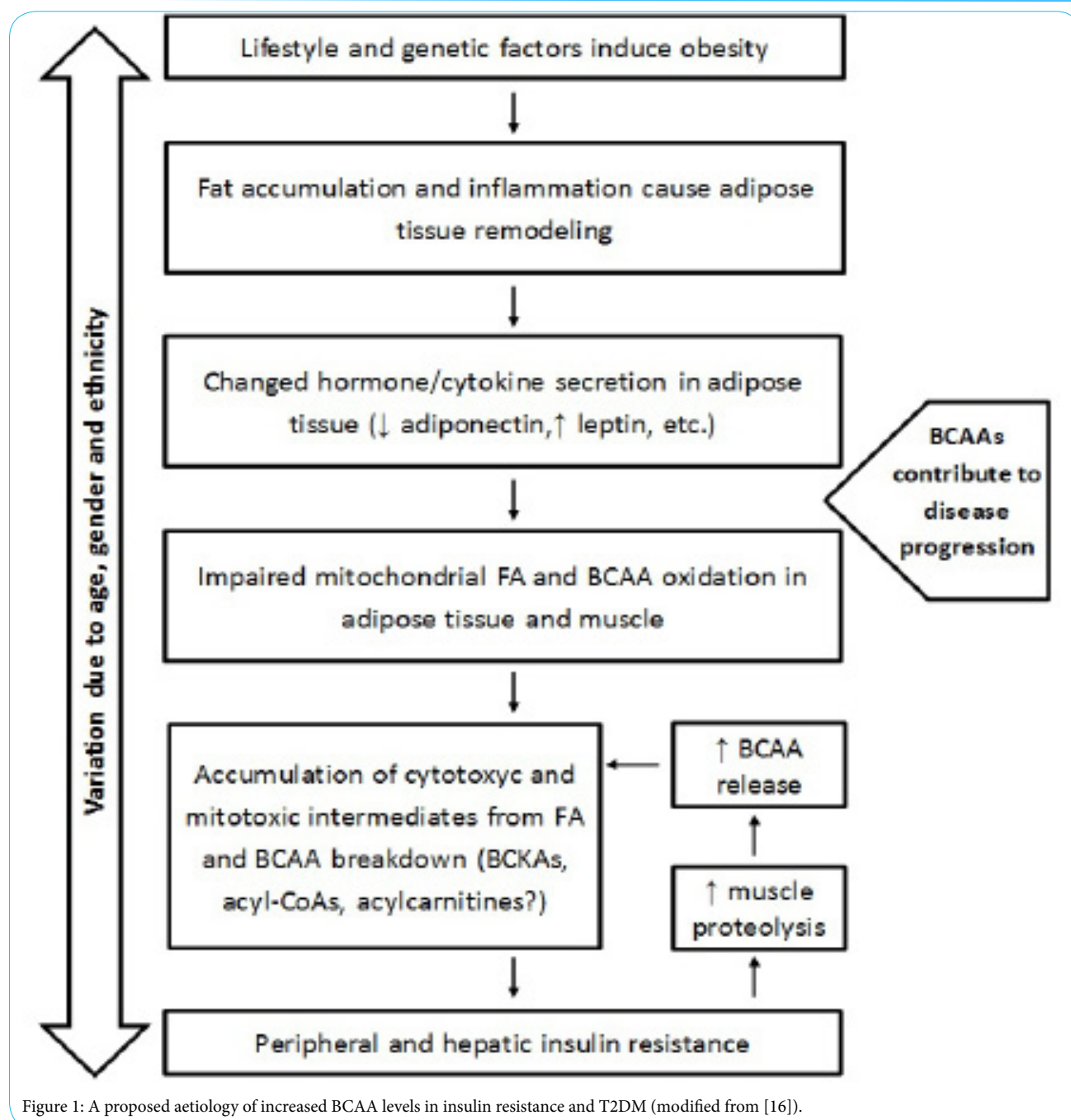
During the last decade, many scientific studies have repeatedly described increased plasma levels of BCAAs in overweight and obese adults and children [22]. Very interestingly from a diagnostic point of view, elevated levels of BCAAs have often been demonstrated to presage the development of T2DM well in advance of its actual occurrence. Thus, their quantification in plasma could be a useful indication of the occurrence of metabolic derangements on route to diabetes [6, 23, 24]. A large number of studies shows a strong correlation between circulating BCAAs levels and insulin sensitivity. Stronger effects have been observed in men, with evident age- and ethnicity-related variations. Many evidences coming from *in-vitro* and *in-vivo* studies have indisputably demonstrated that BCAAs may contribute to the development T2DM as well [7, 16, 25].

Impaired mitochondrial oxidation is thought to be the major cause for increases in BCAAs levels, independent of established insulin resistance. In obese individuals, the adipose tissue is characterized by an accumulation of fat, an increased proinflammatory state, and alterations in hormone and cytokine secretion. All of these factors can strongly affect the mitochondrial function in peripheral tissues. Indeed, incomplete oxidation of BCAAs results in the accumulation of toxic intermediates, such as branched-chain keto acids and branched-chain fatty acids, which interfere with insulin signalling and can induce insulin resistance. Impaired insulin signalling in muscle causes an increased proteolysis, leading to a further release of BCAAs in the circulation and a further supply of substrates for mitochondrial oxidation. This issue may be further complicated in case of high-protein diets. A proposed aetiology of increased BCAAs levels in insulin resistance and T2DM is shown in Figure 1 [16].

***Corresponding Author:** Dr. Roccaldo Sardella, Department of Pharmaceutical Sciences, University of Perugia, Section of Chemistry and Technology of Drugs, Via del Liceo 1, 06123 Perugia, Italy; E-mail: roccaldo.sardella@unipg.it

Citation: Ianni F, Pucciarini L, Camaioni E, Alunni G, Sardella R, et al. (2017) Branched-chain Amino Acids as Potential Diagnostic and Prognostic Disease Biomarkers. Int J Clin Res Trials 2: 112. doi: <https://doi.org/10.15344/2456-8007/2017/112>

Copyright: © 2017 Ianni et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.



A growing body of epidemiological studies has indicated that obese and diabetic individuals have a higher risk of getting some types cancer than lean, healthy people [26, 27]. Pancreas and liver cancer as well as endometrial, breast, colorectal, bladder and kidney cancers are among the types of cancers mostly associated with T2DM [28]. It is well known that amino acid levels are distinctly perturbed in obese and diabetic subjects [3, 29, 30]. Many types of cancer accompany with a sensitive alteration in amino acid metabolism due to the demands of the tumour and its interaction with the host [31, 32]. These perturbations are particularly evident for the group of BCAAs [6, 8, 33-38], whose significant alterations are found in the presence of cachectic wasting disorder [39, 40]. Cachexia is a disorder characterized by the massive loss of both adipose and skeletal muscle tissue and represents one of the most devastating sequela of many

types of cancers. The progression of cachexia appears to involve a net increase in protein catabolism along with an activation of proteolysis and a significant reduction in protein synthesis.

In a healthy state, BCAA oxidation in skeletal muscle provides around 10% of the energy needs. Instead, under a pathological state, that is, under highly catabolic circumstances, BCAA oxidation can contribute up to 20% [41]. Myofibrillar proteins are composed of approximately 20% BCAAs and therefore, breakdown of skeletal muscle can produce a noticeable increase in BCAAs [40].

A number of studies have noted widespread decreases in circulating amino acids in cachectic patients, with the most profound decreases being experienced by BCAAs [42, 43].

Based on the above assumptions, it might be expected that the increased proteolysis would lead to an increase in the circulating BCAAs. Instead, this increase turns into an amplification of the oxidative pathway. Moreover, the tumour itself uses the catabolized BCAAs for the protein synthesis process. Both events justify the decrease of circulating BCAAs in cachectic patients.

It should be pointed out that the balance of catabolism, oxidation and protein synthesis may be quite different for different tumours and, therefore, the use of BCAAs as biomarkers of tumours could lead to erroneous conclusions without a greater metabolic and phenotypic profile.

Besides reducing the quality of life, cachexia often limits the efficacy of many chemotherapeutic interventions. Therefore, a rational way of monitoring the BCAAs levels in cancer patients may be of aid to improve the effect of pharmacological treatment with a selective supplementation of BCAAs[44].

Liver Diseases

Serum concentrations of BCAAs have been found to decrease in patients with advanced liver diseases. An opposite situation has been instead observed for the aromatic amino acids (AAAs) phenylalanine (Phe) and tyrosine (Tyr). This in turn results in a lower concentration ratio of BCAAs to AAAs, a ratio called the 'Fischer ratio' [45, 46], in comparison to patients with healthy liver conditions. The imbalance of amino acids becomes more pronounced with the progression of the liver pathology, and aminograms have demonstrated to be valuable prognostic tools for liver cirrhosis in patients with or without hepatocellular carcinoma [47, 48].

The amino acid metabolism in patients with hepatitis B virus HBV infection is markedly perturbed.

In the frame of a broad study, Yang and co-workers [49] measured increased peripheral blood plasma concentrations of Tyr and

decreased concentrations BCAAs in HBV positive patients. As a consequence, the BCAAs to Tyr ratio (an index currently indicated as BTR) resulted significantly lower in patients with HBV infection compared to healthy donors (i.e. the control group). Very interestingly, BTR correlated well with the severity of the various clinical types of HBV infection (Figure 2) [50,51]. Indeed, colorimetric determinations indicated that, when compared with the control group, the concentration of Tyr from patients with HBV infection showed significant differences decrease ($p < 0.05$ or 0.01) (Figure 2a).

A significant decrease ($p < 0.05$ or 0.01) was recorded when the BTR values from patients with various clinical stages of HBV infection namely, acute hepatitis B (AHB), moderate chronic hepatitis B (CHB-m), heavy chronic hepatitis B (CHB-h), liver cirrhosis (LC), and severe chronic hepatitis B (CHB-s) were compared with those of the healthy donor group (Figure 2b).

Based on these experimental outcomes, even though still not well explained from a biochemical standpoint, the measurement and monitoring of plasma levels of BCAAs and Try, could be fruitfully used for the typing and staging of HBV diagnosis. Furthermore, BTR value could help to evaluate the nutritional status of HBV patients, and to gain a better understanding of pathological changes in the liver.

Chronic Kidney Disease

The nutritional status of CKD patients is a predictor of prognosis during the first period of dialysis. Uremic malnutrition is highly diffused among CKD patients, and significantly affects the morbidity and mortality rate observed in this patient population. Plasma levels of BCAAs (especially Val and Leu) were found to be in strict correlation with nutritional status.

Patients with CKD usually show anomalies in terms of plasma AAs. However, the mechanisms behind these abnormalities are still rather unclear. It is widely accepted that inadequate nutritional intake and malnutrition contribute to plasma AA abnormalities.

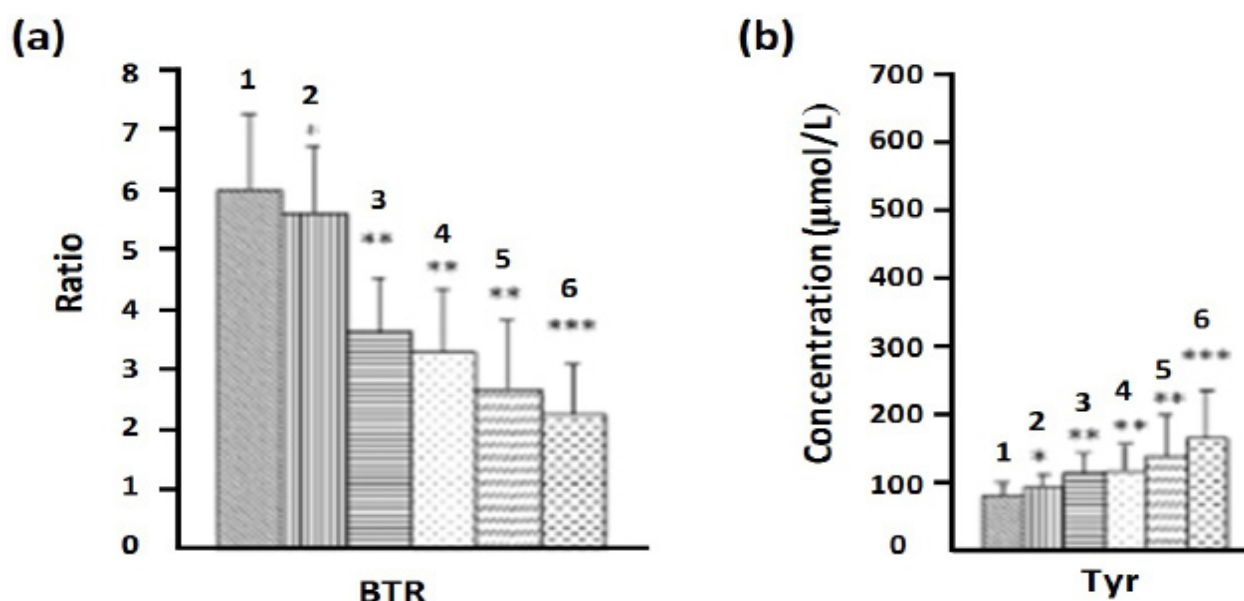


Figure 2: Amino acid profile from various conditions of patients with HBV infection. In each cluster, the columns indicate: 1, healthy donors; 2, acute hepatitis B (AHB); 3, moderate chronic hepatitis B (CHB-m); 4, heavy chronic hepatitis B (CHB-h); 5, liver cirrhosis (LC) (compensated or decompensated); 6, severe chronic hepatitis B (CHB-s). # $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs the healthy donor group (modified from [49]).

Biochemical alterations occurring in the context of inflammation lead to an increase in the AA metabolism. Indeed, inflammation commonly affecting CKD patients has been shown to reduce their plasma amino acid concentrations [52,53].

The systemic inflammatory response enforces protein catabolism [54,55], while the release of AAs from muscle protein provides a substrate for the synthesis of acute phase proteins and proteins of the immune system [56]. This in turn results into a general decrease in plasma AA levels and, in particular, of BCAAs.

Plasma BCAAs concentrations were measured by Kumar and co-workers in 15 patients of early stages of CKD and 15 healthy controls. The quantitative estimation was carried out by using a reversed-phase high-performance liquid chromatography (RP-HPLC) system after pre-column derivatization with the O-phthalaldehyde-mercaptoethanol-based system [12,57]. A significant decrease in Val and Leu concentration was observed in CKD patients when compared with the controls ($p < 0.05$).

Muscle Mass and Knee Osteoarthritis

Identification of biochemical mechanisms associated to the maintenance of muscle and fat-free mass in older adults is of great importance for addressing the rising challenge that health care is already facing with physical disablement in the growing aging population. With this aim, Lustgarten and co-workers [58] identified serum metabolites displaying significant associations with thigh muscle cross-sectional area (CSA) and fat-free mass index (FFMI), with particular regard to functionally limited older adults. As a result of the HPLC-MS study, BCAAs were found to be significantly associated with both thigh muscle CSA and the FFMI.

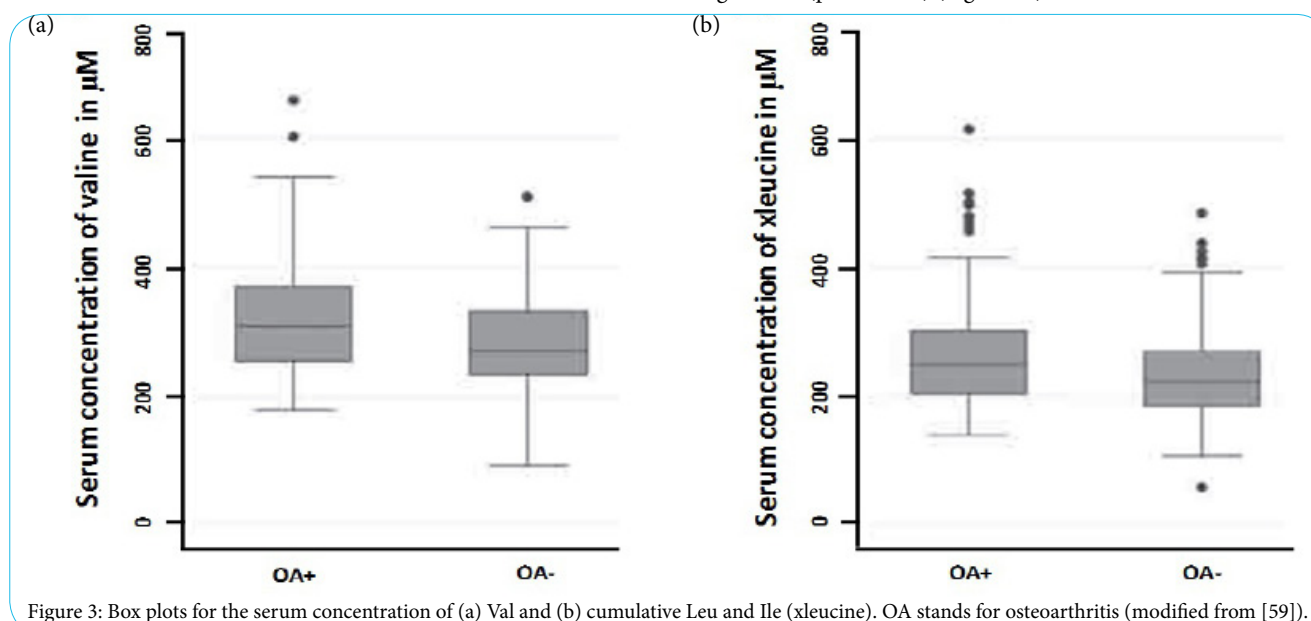
A targeted metabolomic study was carried out by Zhai and co-workers in response to the urgent need to identify and develop valuable biochemical markers useful for characterising the status, prognosis and measures of treatment response in knee osteoarthritis [59]. In all white unrelated women with the pathology, knee osteoarthritis was defined as either radiographic, physician-diagnosed clinical osteoarthritis, or total knee joint replacement due to primary osteoarthritis.

The healthy controls were defined as having none of these. The study performed with a QTRAP-LC-MS/MS system highlighted a statistically significant association between the serum ratios of Val to histidine (His) and the sum of Leu and Ile (here called xleucine) to His and knee osteoarthritis; p-values resulted respectively equal to 1.6×10^{-10} and 8.2×10^{-10} . Based on these results, the ratio of the BCAAs to His has potential in clinical use as osteoarthritis biomarker. Also interestingly, the absolute concentration of Val (Figure 3a) and xleucine (Figure 3b) themselves were significantly increased in knee osteoarthritis cases compared with controls.

Ischemic stroke

With the aim of establishing a metabolomic profile of ischemic stroke, Kimberly and co-workers first examined serial blood samples after filament occlusion in a rat model. Twenty animals were included in the analysis: 7 sham-operated animals, 6 animals with small infarction (stroke volume $9 \pm 5\%$), and 7 animals with large infarction (stroke volume $29 \pm 5\%$). By using a LC-MS/MS system, the authors revealed a significant decrease in the concentration of BCAAs in the large stroke group (p values equal to 0.003, 0.01, and 0.04, respectively) from baseline to 2 hours after stroke. Being BCAAs coordinately regulated, their levels change in conjunction with each other [13, 60]. Therefore, a composite measurement of the BCAAs levels was considered which showed a $16 \pm 6\%$ decrease in large stroke ($p = 1 \times 10^{-5}$) and a nonsignificant trend in small stroke (Figure 4a).

The same evaluation was done on cerebrospinal fluid (CSF) samples. Also in this case, a composite of BCAAs demonstrated a decrease of $23 \pm 9\%$ compared with sham CSF ($n = 5$ for each group; $p < 0.005$). The authors also evaluated the levels of BCAAs in human patients differently classifiable according to the National Institute of Health Stroke Scale (NIHSS). The set of individuals comprised a control group (patients with a diagnosis of transient ischemic attack or absence of stroke), a group with mild stroke (patients with a NIHSS 4-5), and a severe stroke group (NIHSS 15-19). Also in this case, all the three BCAAs were found to be decreased in stroke compared with control patients, and to a greater extent in large compared with small stroke ($p < 0.01$ for each). The composite BCAAs score demonstrated a $9 \pm 17\%$ decrease in small stroke ($p = 0.03$) and a $17 \pm 23\%$ decrease in large stroke ($p = 1.1 \times 10^{-5}$) (Figure 4b).



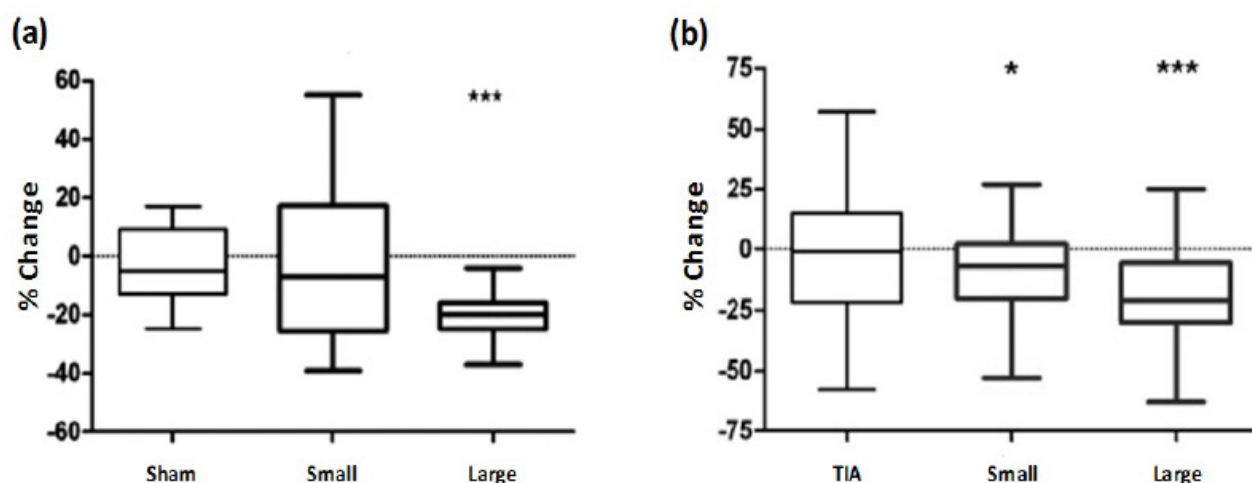


Figure 4: The concentration of BCAAs in (a) rat is diminished from baseline to 2 hours after stroke and (b) in patients with stroke when compared with control subjects at the time of acute presentation. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ (modified from [13]).

Among the proposed explanations for the reduction of BCAAs levels in blood or CSF, the triggering of a specific metabolic pathway leading to their consumption or sequestration in a different tissue compartment, is worth to be cited.

Major Depression and Huntington Disease

The proteinogenic BCAAs were found to play a role in the development of depression through the activation of the mammalian target of rapamycin (mTor) pathway. Accordingly, the results of a recent study authored by Baranyi and co-workers [61] has strongly evidenced that in patients with major depression, BCAAs might be appropriate biomarkers for the pathology. mTor is dysregulated during depressive episodes, and ketamine-induced activation of mTor is associated with a short-term decrease of depressive symptoms in patients suffering from major depression [61, 62]. The study was performed on plasma samples from 119 participants: 71 suffering from major depression while 48 were physically and mentally healthy and without a former history of psychiatric disorders. The BCAAs levels were chromatographically measured in a RP-LC system after derivatization with o-phthalaldehyde. The BCAAs pool was found to be significantly decreased in patients with major depression in comparison with healthy subjects. The decrease of BCAAs in depressive patients observed in the study could be explained on the basis of a dysregulation of the mTor pathway due to BCAA deficiency during depressive episodes.

In the frame of a study aimed at evaluating the actual utility of BCAAs as biomarkers in Huntington Diseases (HD), Mochel and co-workers [63] measured fasting levels of plasma BCAAs as previously described in 16 presymptomatic HD gene carriers and 70 patients with HD at a mild, moderate, or severe stage of the disease. Instead, the control group was composed of 21 healthy individuals. Plasma levels of BCAAs were significantly lower in the HD group compared with controls. Among the three BCAAs, Leu was of particular interest because it was significantly reduced in patients at an early stage of the disease and, more remarkably, in presymptomatic individuals (Figure 5). Leu is a well-known activator of the mTor pathway [64] which regulates protein synthesis and when inhibited leads to an increased autophagic proteolysis [65]. Protein kinase B (PKB), also known as Akt, has been shown to be altered in HD rat brain and in peripheral blood

cells of patients with HD [66], and found to mediate the activity of mTor. Therefore, reduced Leu levels and altered Akt activation in HD may both result in mTor inhibition and exacerbation of proteolysis in HD [67].

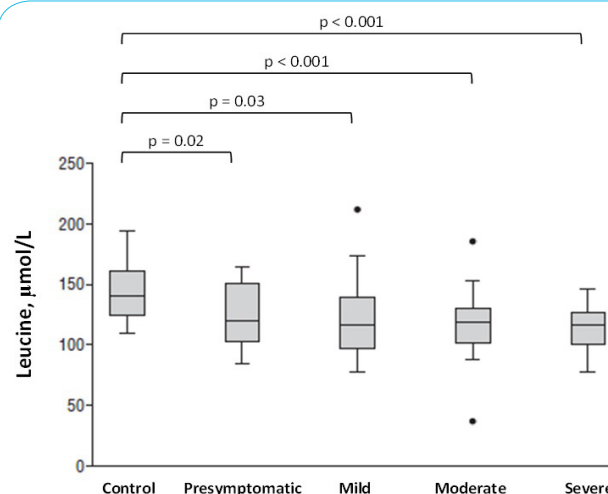


Figure 5: Plasma levels of Leu in presymptomatic individuals and patients at a mild, moderate or severe stage of disease compared with controls (modified from [63]).

Conclusions

Recent evidences demonstrate that BCAAs are associated with insulin resistance, type 2 diabetes, chronic kidney disease as well as ischemic stroke, major depression and Huntington disease. Circulating levels of BCAAs have been also demonstrated having the potential to predict populations at risk for several pathologies. Therefore, the measurement and monitoring of plasma amino acid profiles represents as a promising alternative method to aid in the clinical diagnosis, and prognosis assessment for patients.

Although the use of BCAAs pool as a biomarker in the clinical setting has great potential, many questions can be raised regarding the mechanisms at the basis of the relationship between BCAAs levels and disease process/severity. Therefore, the concrete utility for

BCAAs levels in diagnosing, predicting or monitoring disease strictly depends on the presence of additional information that will place these levels in the context of the entire phenotypic condition.

Competing Interests

The authors declare that they have no competing interests.

References

- Waterlow JC, Golden MH, Garlick PJ (1978) Protein turnover in man measured with ^{15}N : comparison of end products and dose regimes. *Am J Physiol* 235: E165-174.
- Bifari F, Nisoli E (2016) Branched-chain amino acids differently modulate catabolic and anabolic states in mammals: a pharmacological point of view. *Br J Pharmacol*.
- Felig P, Marliss E, Cahill GF Jr (1969) Plasma amino acid levels and insulin secretion in obesity. *N Engl J Med* 281: 811-816.
- Huffman KM, Shah SH, Stevens RD, Bain JR, Muehlbauer M, et al. (2009) Relationships between circulating metabolic intermediates and insulin action in overweight to obese, inactive men and women. *Diabetes Care* 32:1678-1683.
- Tai ES, Tan ML, Stevens RD, Low YL, Muehlbauer MJ, et al. (2010) Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. *Diabetologia* 53: 757-767.
- Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, et al. (2011) Metabolite profiles and the risk of developing diabetes. *Nat Med* 17: 448-453.
- Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, et al. (2009) A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab* 9: 311-326.
- Wurtz P, Soininen P, Kangas AJ, Ronnema T, Lehtimäki T, et al. (2013) Branched-chain and aromatic amino acids are predictors of insulin resistance in young adults. *Diabetes Care* 36: 648-655.
- Okamoto N, Miyagi Y, Chiba A, Akaike M, Shiozawa M, et al. (2009) Diagnostic modeling with differences in plasma amino acid profiles between non-cachectic colorectal/breast cancer patients and healthy individuals. *Int J Med MedSci* 1: 1-8.
- Magnusson M, Lewis GD, Ericson U, Orho-Melander M, Hedblad B, et al. (2013) A diabetes-predictive amino acid score and future cardiovascular disease. *Eur Heart J* 34: 1982-1989.
- Blonde-Cynober F, Aussel C, Cynober L (1999) Abnormalities in branched-chain amino acid metabolism in cirrhosis: influence of hormonal and nutritional factors and directions for future research. *Clin Nutr* 18: 5-13.
- Kumar MA, Bitla AR, Raju KV, Manohar SM, Kumar VS, et al. (2012) Branched chain amino acid profile in early chronic kidney disease. *Saudi J Kidney Dis Transpl* 23: 1202-1207.
- Kimberly WT, Wang Y, Pham L, Furie KL, Gerszten RE (2013) Metabolite Profiling Identifies a Branched Chain Amino Acid Signature in Acute Cardioembolic Stroke. *Stroke* 44: 1389-1395.
- Mochel F, Charles P, Seguin F, Barriault J, Coussieu C, et al. (2007) Early Energy Deficit in Huntington Disease: Identification of a Plasma Biomarker Traceable during Disease Progression. *Plos one* 2: e647.
- Batch BC, Hyland K, Svetkey LP (2014) Branch chain amino acids: biomarkers of health and disease. *Curr Opin Clin Nutr Metab Care* 17: 86-89.
- Giesbertz P, Daniel H (2016) Branched-chain amino acids as biomarkers in diabetes. *Curr Opin Clin Nutr Metab Care* 19: 48-54.
- Ebbeling CB, Pawlak DB, Ludwig DS (2002) Childhood obesity: public-health crisis, common sense cure. *Lancet* 360: 473-482.
- James PT, Rigby N, Leach R (2004) The obesity epidemic, metabolic syndrome and future prevention strategies. *Eur J Cardiovasc Prev Rehabil* 11: 3-8.
- Guilherme A, Virbasius JV, Puri V, Czech MP (2008) Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol* 9: 367-377.
- Sowers JR (2003) Obesity as a cardiovascular risk factor. *Am J Med* 115 Suppl 8A: 37S-41S.
- Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, et al. (2005) Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement - executive summary. *CritPathwCardiol* 4: 198-203.
- Rauschert S, Uhl O, Koletzko B, Hellmuth C (2014) Metabolomic biomarkers for obesity in humans: a short review. *Ann Nutr Metab* 64: 314-324.
- Yamakado M, Nagao K, Imaizumi A, Tania M, Toda A, et al. (2015) Plasma free amino acid profiles predict four-year risk of developing diabetes, metabolic syndrome, dyslipidemia, and hypertension in Japanese population. *Sci Rep* 5: 11918.
- McCormack SE, Shaham O, McCarthy MA, Deik AA, Wang TJ, et al. (2013) Circulating branched-chain amino acid concentrations are associated with obesity and future insulin resistance in children and adolescents. *Pediatr Obes* 8: 52-61.
- Xiao F, Yu J, Guo Y, Deng J, Li K, et al. (2014) Effects of individual branched-chain amino acids deprivation on insulin sensitivity and glucose metabolism in mice. *Metabolism* 63: 841-850.
- O'Connell TM (2013) The Complex Role of Branched Chain Amino Acids in diabetes and cancer. *Metabolites* 3: 931-945.
- Calle EE, Kaaks R (2004) Overweight, obesity and cancer: Epidemiological evidence and proposed mechanisms. *Nat Rev Cancer* 4: 579-591.
- Johnson JA, Carstensen B, Witte D, Bowker SL, Lipscombe L, et al. (2012) Diabetes and cancer: Evaluating the temporal relationship between type 2 diabetes and cancer incidence. *Diabetologia* 55: 1607-1618.
- Felig P, Wahren J, Hendler R, Brundin T (1974) Splanchnic glucose and amino acid metabolism in obesity. *J Clin Invest* 53: 582-590.
- Gougeon R, Morais JA, Chevalier S, Pereira S, Lamarche M, et al. (2008) Determinants of whole-body protein metabolism in subjects with and without type 2 diabetes. *Diabetes Care* 31: 128-133.
- Deberardinis RJ, Sayed N, Ditsworth D, Thompson CB (2008) Brick by brick: metabolism and tumor cell growth. *Curr Opin Genet Dev* 18: 54-61.
- Kroemer G, Pouyssegur J (2008) Tumor cell metabolism: cancer's Achilles' heel. *Cancer Cell* 13: 472-482.
- Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, et al. (2009) A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab* 9: 311-326.
- Cheng S, Rhee EP, Larson MG, Lewis GD, McCabe EL, et al. (2012) Metabolite profiling identifies pathways associated with metabolic risk in humans. *Circulation* 125: 2222-2231.
- Ferrannini E, Natali A, Camastra S, Nannipieri M, Mari A, et al. (2013) Early metabolic markers of the development of dysglycemia and type 2 diabetes and their physiological significance. *Diabetes* 62: 1730-1737.
- Floegel A, Stefan N, Yu Z, Mühlenbruch K, Drogan D, et al. (2013) Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomics approach. *Diabetes* 62: 639-648.
- McCormack SE, Shaham O, McCarthy MA, Deik AA, Wang TJ, et al. (2013) Circulating branched-chain amino acid concentrations are associated with obesity and future insulin resistance in children and adolescents. *Pediatr Obes* 8: 52-61.
- Menni C, Fauman E, Erte I, Perry JR, Kastenmuller G, et al. (2013) Biomarkers for type 2 diabetes and impaired fasting glucose using a non-targeted metabolomics approach. *Diabetes* 62: 4270-4276.
- Eley HL, Russell ST, Tisdale MJ (2007) Effect of branched-chain amino acids on muscle atrophy in cancer cachexia. *Biochem J* 407: 113-120.
- Baracos VE, Mackenzie ML (2006) Investigations of branched-chain amino acids and their metabolites in animal models of cancer. *J Nutr* 136: 237S-42S.
- Lam VW, Poon RT (2008) Role of branched-chain amino acids in management of cirrhosis and hepatocellular carcinoma. *Hepatol Res* 38 Suppl 1: S107-115.

42. Beck SA, Tisdale MJ (1989) Nitrogen excretion in cancer cachexia and its modification by a high fat diet in mice. *Cancer Res* 49: 3800-3804.
43. Norton JA, Gorschboth CM, Wesley RA, Burt ME, Brennan MF (1985) Fasting plasma amino acid levels in cancer patients. *Cancer* 56: 1181-1186.
44. Laviano A, Muscaritoli M, Cascino A, Preziosa I, Inui A, et al. (2005) Branched-chain amino acids: the best compromise to achieve anabolism? *Curr Opin Clin Nutr Metab Care* 8: 408-414.
45. Tajiri K, Shimizu Y (2013) Branched-chain amino acids in liver diseases. *World J Gastroenterol* 19: 7620-7629.
46. Campollo O, Sprengers D, McIntyre N (1992) The BCAA/AAA ratio of plasma amino acids in three different groups of cirrhotics. *Rev Invest Clin* 44: 513-518.
47. Steigmann F, Szanto PB, Poulos A, Lim PE, Dubin A (1984) Significance of serum aminograms in diagnosis and prognosis of liver diseases. *J Clin Gastroenterol* 6: 453-460.
48. Watanabe A, Higashi T, Sakata T, Nagashima H (1984) Serum amino acid levels in patients with hepatocellular carcinoma. *Cancer* 54: 1875-1882.
49. Yang J, He J, Cao H, Zhao X, Fu S, et al. (2012) Correlation between plasma amino acid profiles and the various stages of hepatitis B infection. *Eur J Clin Microbiol Infect Dis* 31: 2045-2052.
50. Ninomiya T, Yoon S, Sugano M, Kumon Y, Seo Y, et al. (1999) Improvement of molar ratio of branched-chain amino acids to tyrosine (BTR) associated with liver fibrosis in chronic hepatitis C patients treated with interferon-alpha. *Dig Dis Sci* 44: 1027-1033.
51. Kawamura-Yasui N, Kaito M, Nakagawa N, Fujita N, Ikoma J, et al. (1999) Evaluating response to nutritional therapy using the branched-chain amino acid/tyrosine ratio in patients with chronic liver disease. *J Clin Lab Anal* 13: 31-34.
52. Suliman ME, A Rashid Qureshi, Stenvinkel P, Pecoits-Filho R, Bárány P, et al. (2005) Inflammation contributes to low plasma amino acid concentrations in patients with chronic kidney disease. *Am J Clin Nutr* August 82: 342-349.
53. Reeds PJ, Fjeld CR, Jahoor F (1994) Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? *J Nutr* 124: 906-910.
54. Jahoor F, Desai M, Herndon DN, Wolfe RR (1988) Dynamics of the protein metabolic response to burn injury. *Metabolism* 37: 330-337.
55. Karlstad MD, Sayeed MM (1987) Effect of endotoxic shock on skeletal muscle intracellular electrolytes and amino acid transport. *Am J Physiol* 252: R674-680.
56. Grimble RF (2001) Nutritional modulation of immune function. *Proc Nutr Soc* 60: 389-397.
57. Rajendra W (1987) High performance liquid chromatographic determination of aminoacids in biological samples by precolumn derivatization with O-Phthaldialdehyde. *J Liq Chromatogr* 10: 941-955.
58. Lustgarten MS, Price LL, Chale A, Phillips EM, Fielding RA (2014) Branched Chain Amino Acids Are Associated With Muscle Mass in Functionally Limited Older Adults. *J Gerontol A Biol Sci Med Sci* 69: 717-724.
59. Zhai G, Wang-Sattler R, Hart DJ, Arden NK, Hakim AJ, et al. (2010) Serum branched-chain amino acid to histidine ratio: a novel metabolomic biomarker of knee osteoarthritis. *Ann Rheum Dis* 69: 1227-1231.
60. Brosnan JT, Brosnan ME (2006) Branched-chain amino acids: enzyme and substrate regulation. *J Nutr* 136: 207S-11S.
61. Baranyi A, Amouzadeh-Ghadikolai O, Von Lewinski D, Rothenhäusler HB, Theokas S, et al. (2016) Branched-Chain Amino Acids as New Biomarkers of Major Depression - A Novel Neurobiology of Mood Disorder. *Plos one* 11: e0160542.
62. Aquilani R, Boselli M, Boschi F, Viglio S, Iadarola P, et al. (2008) Branched-chain amino acids may improve recovery from a vegetative or minimally conscious state in patients with traumatic brain injury: a pilot study. *Arch Phys Med Rehabil* 89:1642-1647.
63. Mochel F, Benaich S, Rabier D, Durr A (2011) Validation of plasma branched chain amino acids as biomarkers in Huntington disease. *Arch Neurol* 68: 265-267.
64. Flier JS (2006) Neuroscience. Regulating energy balance: the substrate strikes back. *Science* 312: 861-864.
65. Hay N, Sonenberg N (2004) Upstream and downstream of mTOR. *Genes Dev* 18: 1926-1945.
66. Colin E, Régulier E, Perrin V, Dürr A, Brice A, et al. (2005) Akt is altered in an animal model of Huntington's disease and in patients. *Eur J Neurosci* 21: 1478-1488.
67. Heng MY, Duong DK, Albin RL, Tallaksen-Greene SJ, Hunter JM, et al. (2010) Early autophagic response in a novel knock-in model of Huntington disease. *Hum Mol Genet* 19: 3702-3720.