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# Evaluating Response to Nutritional Therapy Using the Branched-Chain Amino Acid/Tyrosine Ratio in Patients With Chronic Liver Disease

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The branched-chain amino acid (BCAA)/tyrosine (Tyr) ratio (BTR) recently has been reported to be a good indicator of the severity of hepatic parenchymal injury in patients with chronic liver disease. In the present study, sequential changes of BTR after BCAA administration were determined in patients with chronic liver disease to evaluate the value of BTR as a marker of the clinical response to nutritional therapy in these patients. This study comprised 75 patients with chronic hepatitis and 96 with liver cirrhosis. BTR was significantly decreased in patients with cirrhosis and hepatitis compared with healthy subjects. BTR was significantly correlated with the Child-Pugh score and with other liver function tests. BCAA increased significantly 2 hr after BCAA administration and decreased gradually thereafter. Tyr significantly decreased 4 hr after BCAA administration. BTR significantly increased 2 and 4 hr after BCAA therapy. The increase in BTR 3 hr after BCAA administration was low in patients with decreased basal BTR. The results of this study showed that BTR is a good index of the hepatic parenchymal damage and that it may be a useful marker for monitoring response to nutritional therapy in patients with chronic liver disease. J. Clin. Lab. Anal. 13:31–34, 1999. © 1999 Wiley-Liss, Inc.

**Key Words:** chronic liver disease; branched-chain amino acid; tyrosine; nutritional therapy

## INTRODUCTION

The plasma levels of branched-chain amino acids (BCAAs; valine, isoleucine, leucine) are decreased and those of aromatic amino acids (AAAs; tyrosine, phenylalanine) are increased in patients with chronic liver disease, particularly in those with liver cirrhosis. In Japan, the ratio of the plasma levels of these two types of amino acids, termed "Fischer's ratio," is currently being determined to evaluate the degree of hepatic parenchymal damage in patients with chronic liver disease (1). However, measurement of the plasma levels of BCAAs and AAAs by conventional methods is time consuming and required the use of sophisticated instrumentations. Azuma and Shimizu reported a simple and rapid enzymatic method for measuring the plasma levels of BCAA and tyrosine (2,3); they reported that the BCAA/Tyr ratio (BTR) may be a good indicator of the severity of liver injury (2). To further expand the clinical application of BTR, in the present study, we evaluated the value of BTR as a marker for assessing the response to nutritional therapy in patients with chronic liver disease.

## PATIENTS AND METHODS

This study was comprised of 75 patients (male, 56; female, 19; mean age, 55 yrs) with chronic hepatitis and 96 (male, 62; female, 34; mean age, 62 yrs) with liver cirrhosis admitted to our clinical department from November 1995 through July 1997. Chronic hepatitis was due to hepatitis B virus in 13 patients and to hepatitis C virus in 57 patients; 5 patients had cryptogenic chronic hepatitis. Among these chronic hepatitis cases, 29 were of persistent type and 46 of active type. The average length of time from onset of hepatitis was $18.3 \pm 6.8$ years. The etiology of liver cirrhosis was as follows: viral hepatitis, 83 patients; alcoholic liver injury, 11; cryptogenic cirrhosis, 2. Forty-two cases of liver cirrhosis were associated with hepatocellular carcinoma. The diagnosis of each liver disease was based on clinical and laboratory data, histological findings, and imaging studies. BCAA and Tyr concentrations were determined using the Diacolor-BTR kit (Ono Pharmaceutical Co., Tokyo, Japan) following a method previously described (2). Measurement of BCAA is based on the oxidative deamination of these amino acids and on the formation of formazan after coupling the release of nicotinamide adenine dinucle-

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otide with the redox system; the absorbence of formazan so produced is then measured at 600 nm. Measurement of Tyr is based on the decarboxylation and conversion of this amino acid to tyramine whose subsequent oxidation produces hydrogen peroxide. In the presence of peroxidase and specific substrates, hydrogen peroxide is converted to a quinone dye; the absorbence of this dye is then measured at 546 nm. The intra-assay and the inter-assay variations were 0.3 and 1% for BCAA, and 0.9 and 2.4% for Tyr, respectively. The detection of limit of the BCAA and Tyr assays were 5 μmol/l and 4 μmol/l, respectively. The severity of LC was assessed using the Child-Pugh score (4). The relation of BTR with the serum concentrations of albumin, total bilirubin, hyaluronic acid, cholinesterase, with the values of prothrombin time, platelet count, hepaplastin (5), and 15-min plasma retention of indocyanin green was also evaluated.

Six hypoproteinemic LC patients (mean Child-Pugh score: 7.0) were subjected to a therapeutic diet (1,600 Kcal, 30 g of protein). They were treated with Aminoleban EN® (210 kcal, 13.5 g of protein, 6 g of BCAA per package) from Otsuka Pharmaceutical Co. (Tokyo, Japan). Blood samples for measuring BCAA and Tyr were collected at 08:00, 10:00, 12:00, 18:00 20:00, 00:00, and 08:00. To evaluate whether the improvement in BTR depends on its pretreatment basal values, 13 LC patients underwent nutritional therapy and the BTR values before and after (3 hr) the therapy was evaluated. Data obtained in 20 healthy subjects were available for making comparison. Written informed consent was obtained from all subjects before the beginning of the study.

### Statistical Analysis

Data are expressed as the mean ± the standard deviation (SD). The difference between the mean of two variables was calculated by Student's *t*-test and that among the mean of three or more variables by analysis of variance. A $P < 0.05$ was considered as statistically significant.

## RESULTS

The plasma levels of BCAA were significantly reduced in LC patients compared with healthy controls. The plasma levels of Tyr were significantly increased in patients with LC and CH as compared to controls. As a result, BTR was significantly decreased in both LC and CH patients compared to healthy controls (Fig. 1). BTR values were not significantly different between LC and LC+HCC patients. BTR was significantly correlated with the Child-Pugh score (Fig. 2), with the serum concentrations of albumin, cholinesterase, total bilirubin, hyaluronic acid, with the values of hepaplastin and indocyanin green tests, and with the platelet count (Table 1).

BCAA significantly increased 2 hr after nutritional therapy and decreased rapidly thereafter (Fig. 3). Tyr slightly de-

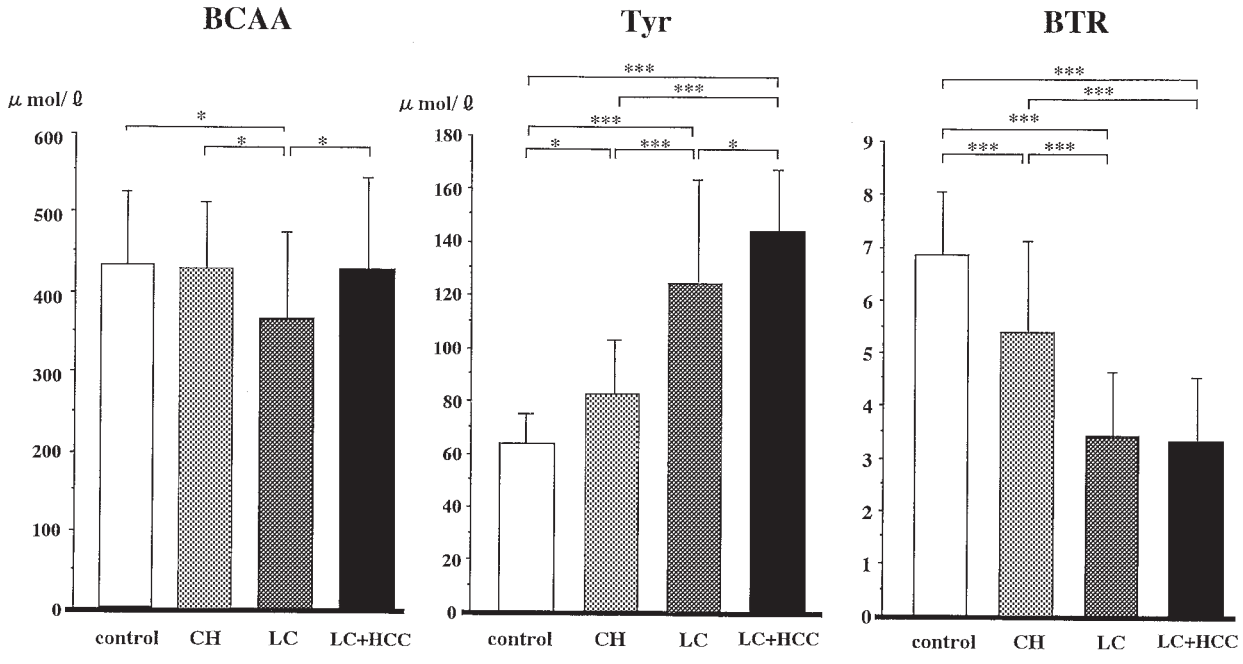


**Fig. 1.** Branched-chain amino acid (**BCAA**), tyrosine (**Tyr**), and the branched-chain amino acid/tyrosine ratio (**BTR**) in patients with chronic liver disease and healthy subjects. BCAA were significantly reduced in LC patients compared with healthy controls. The plasma levels of Tyr were significantly increased in patients with LC and CH as compared to controls. As a result, BTR was significantly decreased in both LC and CH patients compared to healthy controls. BTR values were not significantly different between LC and LC+HCC patients. CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

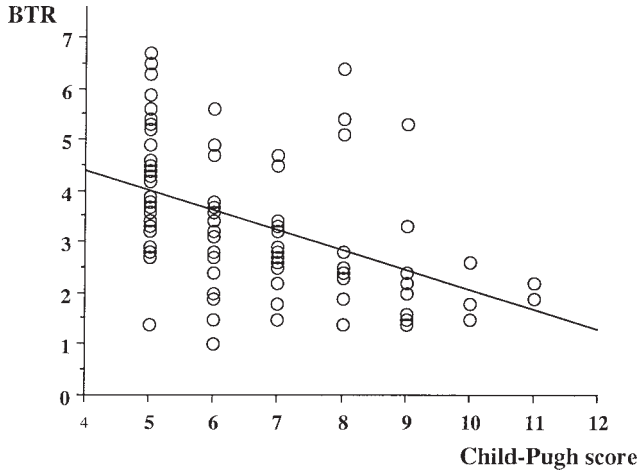


**Fig. 2.** Relation of BTR with the Child-Pugh score. BTR was significantly correlated (r = –0.58, $P < 0.001$, n = 74) with the Child-Pugh score.

creased 2 hr after BCAA administratrion and returned to basal values after 10 hr. BTR increased nearly twofold 2 and 4 hr after BCAA administration and decreased also to basal values after 10 hr. The increase of BTR (ΔBTR) in LC patients with low pretreatment basal values of BTR after BCAA administration was smaller than in those with high basal values of BTR.

A positive and significant correlation was observed between basal BTR values and ΔBTR (Fig. 4).

## DISCUSSION

AAAs are mainly metabolized in the liver because the enzymes phenylalanine hydroxilase and tyrosine aminotransferase are present specifically in hepatocytes. Thus, patients with liver dysfunction present increased plasma levels of AAAs. The metabolism of BCAAs, however, mainly occurs in skeletal muscles where they react with α-ketoglutaric acid and are converted to glutamine by transamination (6). In LC patients, the reduced ability of liver cells to dispose of ammonia is conpensated by its increased metabolism in skeletal muscles. This increased metabolism of BCAA in skeletal muscles causes a reduction in the

**TABLE 1. Correlation of BTR With Liver Function Tests**

| | n | r | *P* |
|---|---|---|---|
| Albumin | 171 | +0.67 | < 0.001 |
| Cholinesterase | 171 | +0.66 | < 0.001 |
| Hepaplastin test | 137 | +0.63 | < 0.001 |
| Platelet count | 171 | +0.40 | < 0.001 |
| Indocyanine green test 15[a] | 73 | –0.62 | < 0.001 |
| Prothrombin time | 171 | –0.40 | < 0.001 |
| Total bilirubin | 115 | –0.41 | < 0.001 |
| Hyaluronic acid | 78 | –0.62 | < 0.001 |

[a]15-min plasma retention of indocyanin green test.

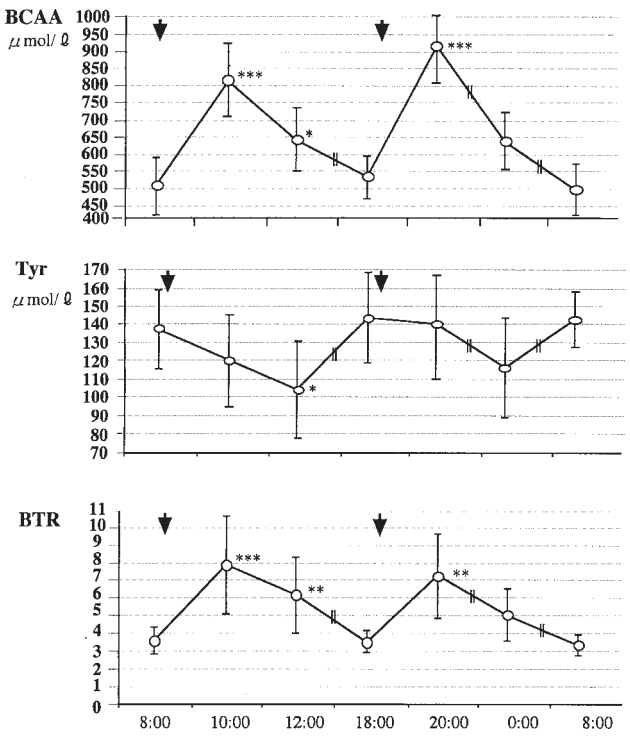


**Fig. 3.** Sequential changes of branched-chain amino acid (BCAA), tyrosine (Tyr), and BCAA/Tyr ratio (BTR) in patients with chronic liver disease during BCAA administration. BCAA significantly increased 2 hr after nutritional therapy and decreased rapidly thereafter. Tyr slightly decreased 2 hr after BCAA administration and returned to basal values after 10 hr. BTR increased nearly twofold 2 and 4 hr after BCAA administration and decreased also to basal values after 10 hr. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, compared with values obtained at 08:00.

plasma levels of these amino acids and in the BCAA/AAA ratio in patients with liver cirrhosis (7,8). Azuma et al. reported that the BCAA/AAA ratio is a useful method for assessing the grade of liver dysfunction in patients with chronic liver disease. In agreement with this, BTR in the present study was found to be significantly decreased in LC and CH patients compared with healthy subjects, and to be significantly correlated with several liver function tests and with the Child-Pugh score. These findings suggest that BTR may be a useful marker of the severity of liver dusfunction in patients with chronic liver disease.

In many clinical centers of Japan, long-term administration of BCAA preparations is being used to correct the imbalance of plasma amino acids and to improve the level of consciousness and the nutritional state in patients with hepatic encephalopathy (9). It was previously reported that AAA concentration decreases and that BCAA/AAA ratio increases significantly during the first and the second week of BCAA-rich diet (10). Therapy with BCAA increases the pool of valine and leucine, and decreases that of phenylalanine, Tyr, and tryptophan (11). The decrease in the plasma

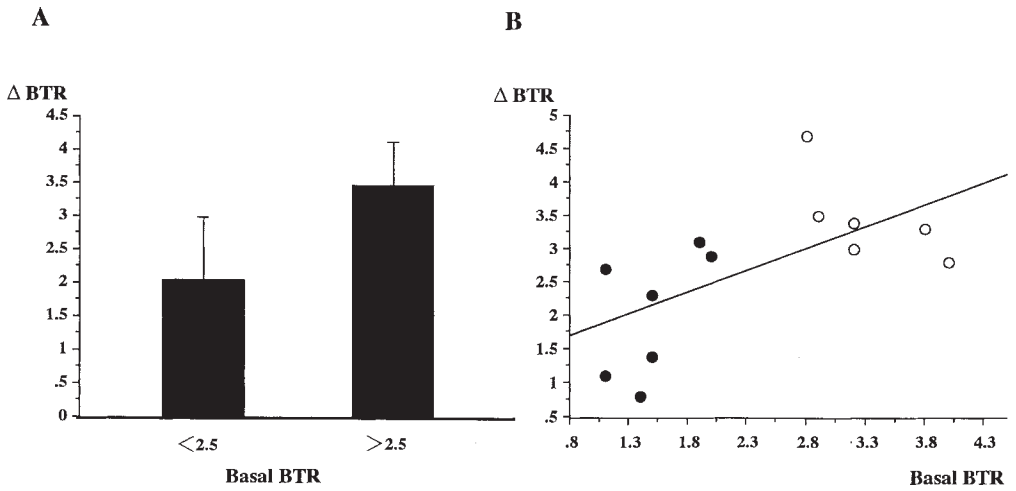


**Fig. 4.** Improvement in the branched-chain amino acid/tyrosine ratio (ΔBTR) in relation with the basal values of BTR. **A,** The increase in BTR (ΔBTR) in LC patients with low pretreatment basal values of BTR after BCAA administration was smaller than in those with high basal values of BTR ($P < 0.05$). **B,** A positive and significant correlation ($r = 0.64$, $P < 0.05$, $n = 13$) was observed between ΔBTR and basal BTR values.

levels of AAA is considered to be caused by an increase in the rate of protein synthesis in peripheral tissues (12). However, in the present study, the plasma levels of Tyr, an aromatic amino acid, markedly decreased during the administration BCAA-rich preparation. This reduction of Tyr may depend on a rapid increase in the rate of protein synthesis following therapy with BCAA. However, further studies must be carried out to clarify this point. On the other hand, the increase in BTR was lower in patients with decreased basal values of BTR than in those with relatively high BTR, suggesting the need of administering high doses of BCAA to patients with very low basal BTR.

In brief, the results of this study suggest that BTR may be a useful marker of the severity of liver parenchymal injury and for monitoring the response to nutritional therapy in patients with chronic liver disease.

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