Acta Med. Nagasaki 48 : 29−33

Changes of Branched Chain Amino Acids and Tyrosine Ratio (BTR) after Hepatectomy

Atsushi NANASHIMA, Hiroyuki YAMAGUCHI, Shinichi SHIBASAKI, Takafumi ABO, Shigeyuki MORINO, Megumi YOSHINAGA, Terumitsu SAWAI, Kenji TANAKA, Shigekazu HIDAKA, Takashi TSUJI, Tohru NAKAGOE, Hiroyoshi AYABE

First Department of Surgery, Nagasaki University School of Medicine

To clarify the clinical usefulness of measuring branched chain amino acids and tyrosine ratio (BTR), which is correlated with Fischer's ratio, we examined the serum BTR level in 33 patients with liver diseases. Serum levels of branched-chain amino acids (BCAAs) and tyrosine were measured by the new enzymatic method, which costs inexpensive and is immediate compared to measuring Fischer's ratio. BTR was calculated as ratio of concentration of BCAA to tyrosine. BTR was correlated with levels of albumin, transaminase and cholinesterase and was lower in patients with chronic viral hepatitis, Child B cirrhosis and portal hypertension. In 19 patients who underwent hepatic resection included 8 for major hepatectomy, resected volume, blood loss, operation time and background of liver diseases were not associated with changes of BTR after hepatectomy. In patients with prolonged jaundice, postoperative BTR level was significantly lower between day 1 and 7 after hepatectomy. We concluded that serum BTR level was correlated with poor liver function and monitoring of BTR levels after hepatectomy may be useful to evaluate degree of hepatic damage after liver surgery.

ACTA MEDICA NAGASAKIENSIA 48 : 29−33, 2003

Key Words: hepatectomy, BTR, BCAA, tyrosine, liver function

Introduction

Imbalance of Fischer's ratio, which is a molar ratio of branched-chain amino acids (valine, leucine and isoleucine; BCAAs) to aromatic amino acids (phenylalanine, and tyrosine; AAAs),[1] is a useful indicator of the severity of hepatic parenchymal injury in patients with hepatitis or cirrhosis.[1,2] In case of severe hepatic insufficiency, decrease of BCAAs and increase of AAAs result in decrease of Fischer's ratio, this leads to hepatic encephalopathy.[1] Fischer's ratio well correlates with other liver functional parameters, and decreases after hepatic resection.[3] Fischer's ratio is useful to evaluate the functional liver reserve in patients who undergo surgical resection of the liver. Fischer's ratio is usually obtained by high-performance liquid chromatography (HPLC).[1] While, recent reports described that BCAAs and tyrosine ratio (BTR) by the enzymatic method was closely correlated with Fischer's ratio.[4-7] Examination of BTR is inexpensive and data is provided immediately compared to HPLC method.[8] BTR also correlates any liver functions[6,7,9] and, furthermore, this ratio was correlated with resected hepatic volume and liver regeneration after hepatectomy.[10-12]

In the present study, we examined the BTR level before and after hepatectomy and analyzed the relationship with liver functions, anesthetic and surgical data and postoperative course.

Patients and Methods

Patients

The subjects were 33 patients with hepato-biliary diseases who were hospitalized in the First Department of Surgery, Nagasaki University School of Medicine between April 2001 and March 2002. They included 18 males and 15 females with a mean age of 66 ± 10 years old (range,41-81 years old). Hepatobiliary diseases included 14 hepatocellular carcinomas, 3 metastatic liver tumors originating from colorectal cancers, 2 cholangiocarcinomas, 3 gallbladder carcinomas, 7 bile duct carcinomas and 4 benign diseases. The background liver abnormality included chronic viral liver diseases in 15 (including those caused in 4 by hepatitis viral B and 11 by hepatitis viral C),

Address Correspondence: Atsushi Nanashima, M.D.
First Department of Surgery, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.
TEL: +81-95-849-7304, FAX: +81-95-849-7306
E-mail: a-nanasm@alpha.med.nagasaki-u.ac.jp

icteric livers in 6 and liver with normal function in 12 patients.

Hepatectomy was performed in 19 patients and hepatectomy exceeding 40% of the liver volume (major hepatectomy) was performed in 8 patients (42%). Postoperative complications included prolonged jaundice in 2 (representing total bilirubin >3mg/dl at postoperative day 7) and prolonged ascites in 3 (representing massive ascites even under treatment with diuretics for more than two weeks). However, hepatic failure could not been obviously observed in the present study. In patients who underwent major hepatectomy, 800 ml of BCAA-rich amino acid solution (Aminoleban; Otsuka Pharmaceutical Co.,Ltd, Tokyo) and 10 units of fresh frozen plasma (FFP) per day were administrated for a couple of days after hepatectomy in order to prevent hepatic failure. Otherwise, the regular amino acid solution was given in patients who had minor hepatectomy. Usual oral diet was started day 4 after hepatectomy in all patients.

Measurement of BTR

Peripheral blood samples were collected in the early morning from each patient at the following time intervals: when the patient was at a stable condition during hospitalization and 28 post-operative days after hepatic resection. The blood sample was immediately centrifuged at 3,000 rpm for 15 minutes, and 0.5 ml of serum was stored at -80℃. The concentration of BCAAs and tyrosine in 0.002ml of serum were measured with Diacolor-BTR by an automated biochemical analyzer (BML, Inc., Tokyo, Japan) using the enzymatic method (Ono Pharmaceutical, Osaka, Japan).[4,5] It took 10 minutes for analysis. Serum BTR was calculated by formula as follows:

the concentration of total BCAAs (μmol/l)/ the concentration of tyrosine (μmol/l).

The normal value of BTR was ranged between 5.82 and 8.64.[4,5]

The BTR level was compared with conventional liver function tests, Child Pugh-score,[13] operation associated factors including operative procedure, resected volume and blood loss, and the regeneration rate of the remnant liver at day 28 after major hepatectomy. The volume of the liver section to be resected excluding tumor volume is then measured by CT volumetry, in which serial liver scans at 1.0 cm intervals were taken and summed (cm^3). The regeneration rate of the remnant liver at day 28 was calculated in the present study as follows:

(Remnant liver volume after hepatectomy/estimated remnant liver volume before surgery) ×100-100 (%)

Statistical analysis

Data were expressed as mean ± SD. Data of different groups were compared using one way analysis of variance (ANOVA) and examined by Student's t-test or Dunnet's multiple comparison test. Differences of changes of data between groups were examined using repeated measure ANOVA and Scheffe's multiple comparison test. Correlations between two parameters were examined by calculating the Pearson's correlation coefficient. A two-tailed P value < 0.05 was considered significant. The StatView Software for Windows, Version 5.0 (SAS Institute, Inc., Cary, NC) was used for all statistical analyses.

Results

The mean BTR concentration in all patients was 5.57±1.62 (range, 2.88 -9.53). Age did not correlate with BYR level (data not shown). There was no significant difference between male and female (Table 1).

Table 1. Relationship between BTR and clinical data before and after hepatectomy

	Preoperative	POD 1	POD 28
Gender			
Male (n=16)	5.41+/-1.68		
Female (n=17)	5.85+/-1.56		
Background of the liver			
Normal (n=12)	6.15+/-1.61		5.24+/-0.12
Chronic hepatitis (n=7)	5.31+/-1.49		3.83+/-0.67 [c]
Cirrhosis (n=8)	4.98+/-1.51 [a]		4.34+/-1.69
Obstructive jaundice (n=6)	5.86+/-1.84		3.55+/-1.01
Child-Pugh classification			
A (n=31)	5.66+/-1.61		
B (n=2)	4.19+/-1.85		
Portal hypertension (>15mmHg)			
No (n=26)	6.05+/-1.61		
Yes (n=7)	4.44+/-0.74 [b]		
Major hepatectomy (>40%)			
No (n=8)		5.47+/-2.14	4.75+/-1.09
Yes (n=11)		4.14+/-3.37	4.11+/-0.88
Postoperative complication			
None (n=14)	5.72+/-1.18		4.63+/-0.39
Long-term ascites (n=3)	4.44+/-1.37		4.63+/-1.14
Long-term jaundice (n=2)	5.17+/-0.15		2.79+/-1.33

Data of BTR were shown as mean+/-SD. a; p=0.10 vs. normal liver, b; p=0.09, c; p=0.09 vs. normal liver.

Preoperative BTR in patients with cirrhosis or portal hypertension tended to be lower but not statistically significant (Table 1).

Correlation between preoperative BTR level and other parameters of liver function were shown in Table 2. Preoperative BTR tended to be correlated with serum albumin and alanine aminotransferase level but not significant. BTR was significantly correlated with aspartate aminotransferase levels and international normalized ratio (Table 2).

Table 2. Relationship between BTR and other liver functions before and after hepatectomy

	Preoperative	POD 1#	POD 7	POD 28
Total bilirubin (mg/dl)	0.288*			-0.561
Albumin (g/dl)	0.947 [a]			0.692 [e]
AST (IU/l)†	-0.596 [b]			-0.698 [f]
ALT (IU/l)‡	-0.432 [c]			-0.739 [g]
Platelet counts (/mm^3)	-0.256			0.523
INR※	-0.963 [d]			-0.209
Cholinesterase (mg/dl)	0.394			
Total cholesterol (mg/dl)	0.248			0.836 [b]
Ammonium (μg/dl)	-0.210			0.660
ICG R15 (%)¶	-0.129			-0.451
LHL 15§	0.236			0.597
Hyaluronic acid (ng/ml)	-0.353			-0.227
Portal pressure (mmHg)	-0.038			
Resected volume (%)		-0.156	0.084	
Blood loss (ml)		0.135	0.130	
Operation time (hour)		-0.216	0.050	

#: POD: post-operative day. † : aspartate aminotransferase, ‡ : alanine aminotransferase, ※: international normalized ratio, ¶: indocyanine green retention rate at 15 minutes, §: liver activity at 15 minutes by technetium-99m galactosyl human serum albumin scintigraphy

*: R value by calculating the Pearson's correlation coefficient.

a; p=0.072, b; p<0.01, c; p=0.073, d; p<0.05, e; p=0.088, f; p=0.085, g; p=0.058

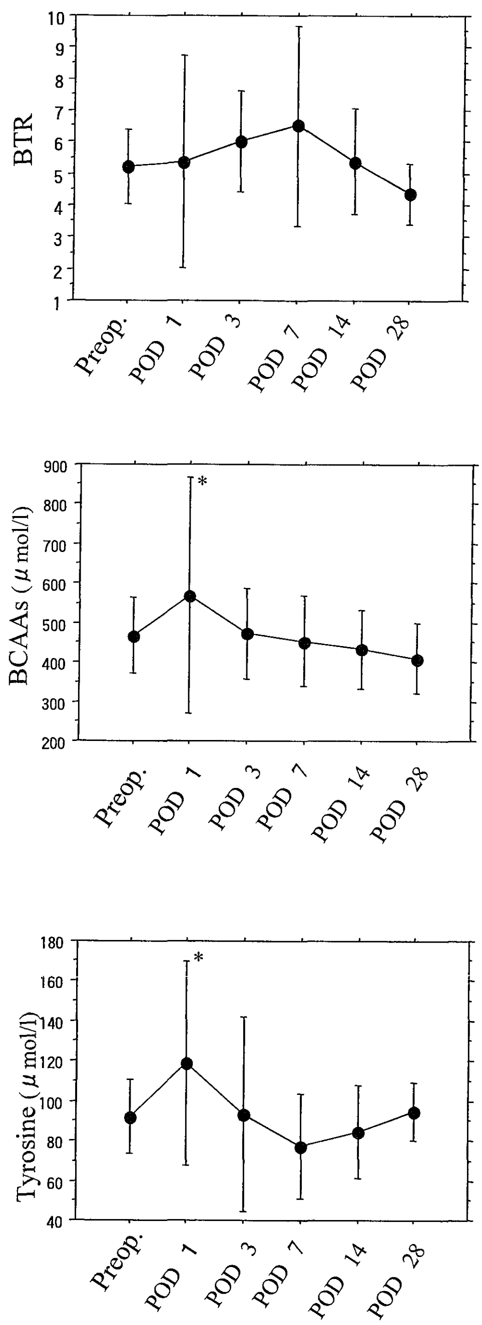

Figure 1. Changes of levels of BTR, BCAAs and tyrosine after hepatectomy. POD: post-operative day. *; p<0.05 vs preoperative value.

Changes of BTR, BCAAs and tyrosine level after hepatectomy were shown in Figure 1. Both BCAAs and tyrosine levels were significantly increased at day 1 after hepatectomy, which resulted in no changes of BTR peri-operatively. BTR levels at day 1 and 7 after hepatectomy were not correlated with operation time, the volume of bleeding, and resected volume of the liver (Table 1, 2 and Figure 2a). At day 28 after hepatectomy, BTR level was significantly correlated with cholinesterase levels (Table 2). BTR level tended to be correlated with albumin and transaminase levels but not significant (Table 2). Changes of BTR in each group of background liver disease were not significantly different after hepatectomy (Figure 2b). BTR level at day 28 in patients with chronic hepatitis and cirrhosis tended to be lower compared to that in patients with normal liver but not statistically significant (Table 1). BTR value in patients, who had complication of long-term jaundice (not obvious liver failure), was significantly lower between day 1 and 7 after hepatectomy compared to patients without complications or with ascites (Figure 2c).

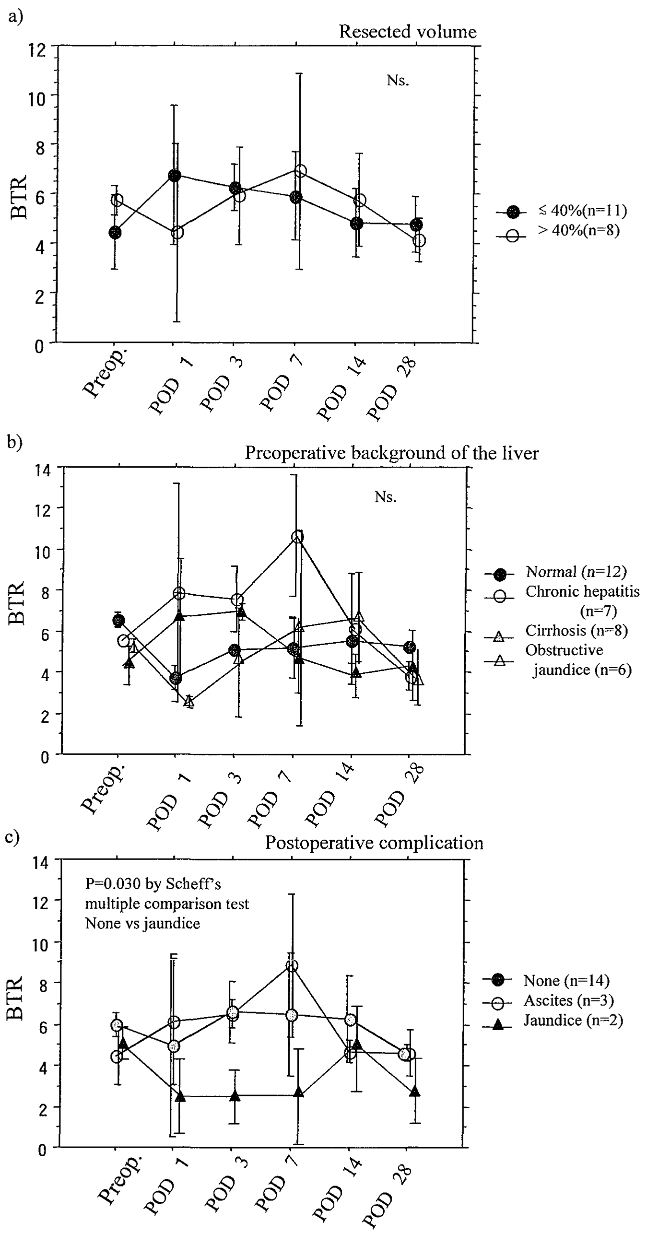

Figure 2. Changes of BTR levels after hepatectomy and relationship with resected volume of the liver, background of liver diseases, and postoperative complications. Differences of changes of data between groups were examined using repeated measure ANOVA and Scheffe's multiple comparison test.
POD: post-operative day.

Discussion

Measurement of BTR showed the metabolic imbalance of amino acids caused by liver impairments as well as Fischer's ratio.[4,5,9,14] Measuring of BTR by the new enzymatic method using the biochemical auto-analyzer[4,5] is immediate (about 10 minutes) and sensitive, and a large number of serum specimens can be treated in a short time compared to measuring Fischer's ratio by HPLC method.[4,5,8] Therefore, BTR is a useful parameter to examine metabolic impairment of amino acids under the stressful condition to the liver. Some investigators showed the correlations between BTR and liver functional parameters such as levels of albumin,[6,7] and prothrombin activity,[6,7] indocyanine green retention rate at 15 minutes,[6,7,15] the hepatic uptake ratio of technetium-99m-diethylenetriaminepentaacetic acid-galactosyl-human serum albumin at 15 minutes (LHL15)[14] and Child-Pugh classification.[9,15] BTR is lower following deterioration of liver functions in patients with any chronic liver diseases[9,15,16] and is available to discriminate between chronic hepatitis and cirrhosis.[17] In the present study, we showed tendency of correlations with levels of albumin, transaminase and cholinesterase before and at day 28 after hepatectomy although these correlations were not statistically significant. Furthermore, BTR level was lower in patients with chronic viral hepatitis and cirrhosis, with portal hypertension. Therefore, BTR might reflect chronic liver dysfunctions in the present study as well.

BTR level immediately decreases after chemoembolization[18] or surgical stress.[8,10-12] The peak of BTR level after hepatectomy might be at day 7 and, however, significant changes of BTR level at each point after hepatectomy could not be observed in the present study. Niguma et al. described that BTR level became lower by decreases of BCAAs and increases of tyrosine at day 1 after major hepatectomy.[8] They also showed that infusion of BCAAs-rich solution might prevent the decrease of BTR after hepatectomy. In the present study, BCAAs level was conversely increased and resulted in no changes of BTR levels after hepatectomy. No correlation with resected volume, degree of bleeding and operation time could be observed, either. In all patients with major hepatectomy, infusion of a large amount of FFP and BCAAs-rich amino acid solutions started during operation and higher level of BTR exceeding 20 could be observed in a few patients who underwent major hepatectomy in our series. Therefore, decreases of BTR might be covered by in the present study. Previous reports showed that administration of BCAAs supported protein synthesis and regeneration of the remaining liver after major hepatectomy.[10-12,19,20] Compared to AAAs, administration of BCAAs is well utilized to generate the glucose-alanine cycle.[21] Long-term oral intake of BCAAs-rich granules (Livact, Ajinomoto Pharmaco., Ltd., Tokyo) also improve BTR level and amino acid imbalance in patients with chronic liver diseases.[22]

In the present study, BTR level was also decreased at the early phase after hepatectomy in two patients with long-term jaundice, who haven't had preoperative jaundice. On the other hand, preoperative BTR levels were not different in patients with or without postoperative complications. Therefore, monitoring of postoperative BTR value may be useful to predict prolongation of jaundice after hepatectomy by the present results although it is difficult to predict postoperative complications by measuring of preoperative BTR level.

In conclusion, measuring of serum BTR level might be an auxiliary hepatic parameter to evaluate liver dysfunction in patients with chronic liver diseases. Furthermore, monitoring of BTR levels after hepatectomy may be useful to evaluate prolongation of jaundice after liver surgery representing severe hepatic damage.

References

1. Fischer JE, Rosen HM, Ebeid AM, Janes H, keane JM, soeters PB. The effect of normalization of plasma amino acids on hepatic encephalopathy in man. *Surgery* 80: 77-91, 1976
2. Morgan MY, Marshall AW, Milsom JP, Sherlock S. Plasma amino-acids patterns in liver disease. *Gut* 23: 362-370, 1982
3. Joyeux H, Matias J, Saint-Aubert B, Astre C, Gouttebel MC, Vedrenne JB, Deneux L. Serum marker of the functional hepatic mass after expensive hepatectomy. The branched/aromatic amino acid ratio. Experimental and clinical studies. *Chirurgie* 120: 283-288, 1994
4. Azuma Y, Maekawa M, Kawabara Y, Nakajima T, Taniguchi K, Kanno T. Determination of branched-chain amino acids and tyrosine in serum of patients with various hepatic diseases, and its clinical usefulness. *Clin Chem* 35: 1399-1403, 1989
5. Shimizu H, Taniguchi K, Sugiyama M, Kanno T. Rapid enzymatic analysis of plasma for tyrosine. *Clin Chem* 36: 32-35, 1990
6. Suzuki K, Naito Y, Kasuga Y, Shimada N, Ishikawa T, Karasawa T, Okubo I, Niiya M, Fujise K, Kobayashi Y. Transition of branched-chain amino acids and tyrosine ratio (BTR) in the blood of acute hepatitis patients. (in Japanese and English abstract) *Rinsho Byori* 47: 1075-1078, 1999
7. Nakamura T, Mori M, Yoshida T, Murakami N, Kato T, Sugihara J, Saito K, Moriwaki H, Tomita E, Muto Y. Enzymatic determination of a molar ratio of free branched-chain amino acids to tyrosine (BTR) and its clinical significance in plasma of patients with various liver diseases. (in Japanese and English abstract) *Rinsho Byori* 37: 911-917, 1989
8. Niguma T, Yumura M, Yamashita Y, Maeda K, Kimura T, Yamamura M, Kodani J. Ratio of branched chain amino acids to tyrosine after hepatectomy. *Surg Today* 29: 825-827, 1999
9. Kawamura N, Kaito M, Nakagawa N, Fujita N, Ikoma J, Gabazza EC, Watanabe S, Adachi Y. 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, 1999
10. Nagasue N, Kanashima R, Inokuchi K. Alteration in plasma amino acid concentrations following subtotal hepatectomy in dogs. *Ann Chir Gyaecol* 70: 50-55, 1981
11. Nagasue N, Yukasa H, Sasaki Y, Ogawa Y, Hirosue S. Infusion of branched chain amino acids after partial hepatectomy in man. *Nutr Cancer* 6: 32-39, 1984
12. The San-in group of liver surgery. Long-term oral administration of branched chain amino acids after curative resection of hepatocellular acrcinoma:a prospective randomized trial. *Br J Surg* 84: 1525-1531, 1997
13. Pugh RNH, Murray-Lyon IM, Dawson JL, et al. Transection of oesophagus for bleeding oesophageal varices. *Eur J Surg* 60: 646-9, 1973
14. Mori T, Ohta M, Sakai M, Horii T, Fujino H, Inada Y, Okanoue T, Kashima K. Determination of BTR on patients with liver cirrhosis and its clinical usefulness-correlation to ^{99m}Tc-GSA-. (in Japanese and English abstract) *Kazou* 39: 325-330, 1998
15. Suzuki H. Assessment of liver injury use of the moler ratio of branched-chain amino acids to tyrosine (BTR). (in Japanese) 13: 54-57, 1998
16. Suzuki H, Mamata Y, Mizuno H, Tominaga T, Suga M, Suemori S, Sato A, Suzuki M. Influence of alcohol on branched-chain amino acid/tyrosine molar ratio in patients with cirrhosis. *Alcohol Clin Exp Res* 3 (suppl): 137-140, 1998
17. Sugiyama M, Kanno T, Ohkubo A, Muto Y, Murata K, Ueno Y. The clinical usefulness of the molar ratio of ranched-chiain amino acids to tyrosine (BTR) in discriminating stage of chronic liver diseases. (in Japanese and English abstract) *Rinsho Byori* 40: 673-678, 1992
18. Inoue H, Siraki K, Sugimoto K, Sakai T, Oomori S, Takase K, Nakano T. Effect of segmental transcatheter arterial chemoembolization on branched chain amino acids and tyrosine ratio in patients with hepatocellular carcinoma. *Int J Oncol* 17: 977-980, 2000
19. Cerra FB, Upson D, Angelico R, Wiles III C, Lyons J, Faulkenbach L, Paysinger J. Branched chains support postoperative protein synthesis. *Surgery* 32: 192-199, 1982
20. Freund HR, James JH, Fischer JE. Nitrogen-sparing mechanisms of singly administered branched-chain amino acids in the injured rat. *Surgery* 90: 237-243, 1981
21. Joyeux H, Matias J, Saint-Aubert B, Astre C, Gouttebel MC, Vedrenne JB, Deneux L. Serum marker of the functional hepatic mass after extensive hepatectomy. The branched/aromatic amino acid ratio. Experimental and clinical studies. *Chirurgie* 120: 283-288, 1994
22. Ninomiya T, Yoon S, Seo Y, Kumon Y, Nagano H, Yano Y, Nakaji M, Kasuga M. Long-term oral supplementation of branched-chain amino acid granules' is effective for maintaining serum level of albumin in viral liver cirrhosis. (in Japanese) *JJPEN* 23: 221-224, 2001