



Characteristics of Nutritional Status and the Effect of Pre-transplant Branched-chain Amino Acid Administration in Patients Undergoing Living Donor Liver Transplantation

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Abstract

Background: Protein-energy malnutrition is common in patients with end-stage liver disease undergoing liver transplantation. We examined the characteristics of nutritional status and impact of pre-admission branched-chain-amino-acids treatment on skeletal muscle mass, nutritional/metabolic parameters and on post-transplant outcomes.

Methodology: Preoperative skeletal muscle mass and nutritional/metabolic parameter levels were compared in 129 patients undergoing adult-to-adult living donor liver transplantation whether received branched-chain-amino-acids treatment before admission or not. We examined relationships among these parameters, and risk factors for post-transplant bacteremia and early mortality after LT focusing on nutritional parameters.

Results: Pre-albumin and branched-chain-amino-acids-to-tyrosine ratio were significantly higher while tyrosine was lower in branched-chain-amino-acids-pre-supplemented than non-pre-supplemented group, while skeletal muscle mass, total lymphocyte count, zinc, branched-chain-amino-acids and ammonia levels were not significantly different. Skeletal muscle mass positively correlated with tyrosine ($r=0.437$, $P<0.001$) and branched-chain-amino-acids ($r=0.282$, $P=0.001$) and negatively with branched-chain-amino-acids-to-tyrosine-ratio ($r=-0.259$, $P=0.003$). Multivariate predictors of post-transplant bacteremia were: Child-Pugh class C ($P=0.012$), low preoperative total lymphocyte count ($P=0.027$), operative blood loss ≥ 10 L ($P=0.039$) and absence of pre-admission branched-chain-amino-acids treatment ($P=0.040$). Nutritional/metabolic parameters and pre-admission branched-chain-amino-acids treatment were not crucial for post-transplant early mortality.

Conclusion: Pre-admission branched-chain-amino-acid therapy could ameliorate preoperative amino acid imbalance and the incidence of post-transplant bacteremia.

Keywords: Branched-chain amino acids; Bioelectrical impedance analysis; Skeletal muscle mass; Sarcopenia; Zinc

Abbreviations

AAAs: Aromatic Amino Acids; BCAAs: Branched-Chain Amino Acids; BIA: Bioelectrical Impedance Analysis; BTR: Bcaas-To-Tyrosine Ratio; CP: Child-Pugh; DDLT: Deceased Donor Liver Transplantation; DSM-BIA: Direct Segmental BIA; HE: Hepatic Encephalopathy; LC: Liver Cirrhosis; LDLT: Living Donor Liver Transplantation; LT: Liver Transplantation; MELD: Model For End-Stage Liver Diseases; NH_3 : Ammonia; PA: Pre-Albumin; SMM: Skeletal Muscle Mass; TLC: Total Lymphocyte Count; TYR: Tyrosine; Zn: Zinc

Introduction

Derangements of various serum biochemical nutritional parameters including zinc, pre-albumin, branched-chain amino acids (BCAA), tyrosine, total lymphocyte count and the related metabolic parameters as the molar ratio of BCAA-to-tyrosine (BTR) and ammonia, are not uncommon in patients with end-stage liver disease undergoing liver transplantation (LT) due to hepatic debilitating pathology and its medical management [1-5].

Patients with decompensated liver cirrhosis frequently receive nutritional therapy with a nutrient mixture enriched with BCAA or BCAA nutrients. BCAA supplementation was reported to delay reduction of hepatic reserve in patients with end-stage liver disease [6]. However, the effect on patients undergoing living donor LT (LDLT) is unclear. Moreover, the nutritional status and relationships between

nutritional parameters including skeletal muscle mass and zinc in such patients are not well understood.

We recently reported that skeletal muscle mass had a significant negative correlation with BTR and low preoperative skeletal muscle mass to be closely involved with post-transplant mortality [7]. However, neither the impact of both pre-admission BCAA treatment and preoperative levels of nutritional/metabolic parameters on post-transplant outcomes nor the reason of the negative relationship is clear.

The aim of the present study was therefore to examine the characteristics of nutritional status and the impact of pre-admission BCAA treatment on both skeletal muscle mass and nutritional/metabolic parameter levels in LDLT candidates. Additionally, we assessed relationships among these parameters, especially with skeletal muscle mass and zinc levels, and examined risk factor for bacteremia

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and early mortality after LT focusing on nutritional factors.

Methodology

Patients

Two hundred and eight adult (age ≥ 18 years) patients underwent primary LDLT at Kyoto University Hospital between February 2008 and August 2012. Excluded from the study were 15 patients with acute liver failure as an indication for LT for the following reasons: First, the pathophysiology and nutritional status is different from patients with other end-stage liver diseases. Second, BCAA supplementation was not suitable for those patients and even regarded as a contraindication [8]. Third, they could not undergo preoperative bioelectrical impedance body composition analysis (BIA) examination due to emergent LT. Next, 64 patients who could not undergo BIA per the dietitians, limitations of dietitians' manpower or the hospital's circumstances were further excluded. The study thus comprised 129 patients. There were 63 males and 66 females. The median patient age was 53 years (range, 19-65 years). The study was approved by the Ethics Committee of Kyoto University and conducted in accordance with the Declaration of Helsinki of 1996.

The median Model for End-stage Liver Disease (MELD) score was 19 (range, 4-47). Thirty-seven patients were ABO incompatible, and 92 were identical or compatible. The Child-Pugh classifications were C, B, and A for 78, 42, and 9 patients, respectively. The indications for LT were hepatocellular carcinoma (n=36), followed by hepatocellular diseases such as hepatitis B or C virus-associated liver cirrhosis (n=32), progressive intrahepatic cholestatic diseases including primary biliary cirrhosis and primary sclerosing cholangitis (n=24), biliary atresia after the Kasai procedure (n=8), alcoholic liver cirrhosis (n=7), metabolic liver diseases (n=5), nonalcoholic steatohepatitis (n=4), autoimmune hepatitis (n=3) and other causes (n=10).

In February 2008, we introduced body composition measurements on admission for patients undergoing LDLT, using direct segmental multi-frequency BIA with eight tactile electrodes (InBody 720; Biospace, Tokyo, Japan). The BIA device used 6 frequencies and produced 30 impedance values for 5 body segments and takes direct impedance measurements from each, unlike conventional BIA used before [9,10] which takes only partial measurements and relies on formulas to estimate whole body composition. The BIA device was reported as an accurate substitute for the dual-energy X-ray absorptiometry in measurement of total and appendicular body composition [11]. Skeletal muscle mass was measured automatically by the InBody 720 and shown as a percent against standard skeletal muscle mass calculated by sex and height of each patient. The normal skeletal muscle mass ratio obtained by the InBody 720 ranges from 90% to 110% of the standard skeletal muscle mass.

Selection criteria for the recipients as well as surgical techniques for recipient operations have been described in detail elsewhere [12,13]. Immunosuppressive treatment usually consisted of tacrolimus or cyclosporine and low-dose steroids as described elsewhere [14,15]. All patients received intravenous antimicrobial prophylaxis with ampicillin (0.5 g) and cefotaxime (0.5 g) twice daily for 3 days starting 30 min before surgery.

Infections were defined using the criteria proposed by the Centers for Disease Control and Prevention and based on reports of liver transplant patients [16]. The isolation of bacteria other than common skin contaminants from a single blood culture in the presence of clinical symptoms or of an infection was considered bacteremia. When caused

by common skin contaminants, bacteremia was considered significant only if an organism was isolated from two blood cultures and clinical signs of infection were evident.

Subgroups assignment

Sixty-six patients out of 129 received BCAA treatment before admission to LDLT for a duration of at least a few months, either in the form of 1 to 3 packets of BCAA-enriched nutrient mixture (Aminoleban EN[®]; Otsuka Pharmaceutical Co., Tokyo, Japan) per day (50-150 g/day), (n=32) or 3 packets of BCAA granules (Livact[®]; Ajinomoto Pharma Co., Tokyo, Japan) per day (12.45 g/day), (n=34). The other 63 patients did not receive any BCAA supplements before admission. BCAA were introduced at the discretion of the attending physician before referral of a potential recipient for LDLT. After admission to LDLT, all patients received the same perioperative nutritional therapy as described before [7].

Analyzed parameters

Preoperative laboratory data of nutritional/metabolic parameters on admission were retrospectively reviewed from the clinical charts of the recipients. The standard reference intervals of these parameters at our institute were: zinc (65-110 $\mu\text{g/dL}$), prealbumin (20-40 mg/dL), total lymphocyte count (1200-3200/ μL), ammonia (20-60 $\mu\text{g/dL}$), BCAA (344-713 $\mu\text{mol/L}$), tyrosine (53-98 $\mu\text{mol/L}$) and the BTR (4.4-9.3).

Skeletal muscle mass and parameter levels on admission were examined. Correlations of skeletal muscle mass and all parameters and those of zinc with other parameters were examined. Because BCAA treatment before admission may affect BCAA blood level, we separately analyzed relationship between skeletal muscle mass and BCAA in groups with or without BCAA pretreatment. A transversal study was performed where preoperative nutritional/metabolic parameters and skeletal muscle mass, length of postoperative hospital stay, incidence of postoperative bacteremia, and biopsy-proven acute cellular rejection were examined in patients admitted to LDLT according to whether they received BCAA treatment before admission to LDLT (BCAA+, n=66) or not (BCAA-, n=63).

Moreover, we examined risk factors for post-transplant death within 90 days after LT and post-transplant bacteremia focusing on preoperative nutritional/metabolic parameter levels and pre-admission BCAA treatment [18].

Statistical analysis

Data was summarized as mean $\pm$ standard deviation (SD) for continuous variables. Categorical variables were compared using the χ^2 test or Fisher's exact test where appropriate. Continuous variables were non-parametrically analyzed using the Mann-Whitney U test with the sequential step-down Holm-Bonferroni method applied to adjust for multiple testing [17]. Correlation between two variables was analyzed using Spearman's rank correlation coefficient. Any variable identified as significant ($P < 0.05$) in univariate analysis using the above tests was considered a candidate for multivariate analysis using multiple logistic regression models. Survival rate was calculated using Kaplan-Meier methods with differences evaluated using log-rank testing. Two tailed $P < 0.05$ was considered significant. All statistical data were generated using JMP 5.0.1 (SAS Institute, Cary, NC) and Prism 6.02 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Pretransplant nutritional status

Marked hyperammonemia ($94.1 \pm 16.8 \mu\text{g/dL}$), hypertyrosinemia ($138.6 \pm 12.1 \mu\text{mol/L}$), hypozincemia ($44.4 \pm 12.6 \mu\text{g/dL}$), hypoprealbuminemia ($6.8 \pm 2.5 \text{ mg/dL}$) and a decreased total lymphocyte count ($855.3 \pm 207.3/\mu\text{L}$), were seen on admission before LDLT. Preoperative BCAA level ($397.2 \pm 56 \mu\text{mol/L}$) was low, yet still within reference range. Consequently, the BTR (3.1 ± 0.5) was subnormal. The median ratio of preoperative skeletal muscle mass was 92% (range 65%-32%) of the standard muscle mass.

Correlations between parameters

Correlations of preoperative skeletal muscle mass with both tyrosine ($r=0.437$, $P<0.001$; Figure 1A) and BCAA levels ($r=0.282$, $P=0.001$; Figure 1B) were significantly positive. Correlation of skeletal muscle mass with the BTR was significantly negative ($r=-0.259$, $P=0.003$; Figure 1C). Subgroup analysis showed that skeletal muscle mass remained positively correlated with BCAA in both BCAA-pre-supplemented group ($r=0.257$, $P=0.039$; Figure 1D) and non-pre-supplemented group ($r=0.259$, $P=0.040$; Figure 1E), although the correlations between these parameters were weak. No significant correlations were identified between skeletal muscle mass and zinc ($P=0.219$), pre-albumin ($P=0.143$), total lymphocyte count ($P=0.716$) or ammonia ($P=0.061$).

Correlations of preoperative zinc with pre-albumin ($r=0.472$, $P<0.001$; Figure 2A), BCAA ($r=0.189$, $P=0.011$; Figure 2B) and the BTR ($r=0.343$, $P<0.001$; Figure 2C) were significantly positive. While, correlations of zinc with tyrosine ($r=-0.224$, $P=0.002$, Figure 2D) and ammonia levels ($r=-0.178$, $P=0.016$; Figure 2E) were significantly negative, although the correlations between these parameters were weak. Significant correlations were not identified between zinc and the total lymphocyte count ($P=0.750$).

The effect of BCAA treatment before admission to LDLT

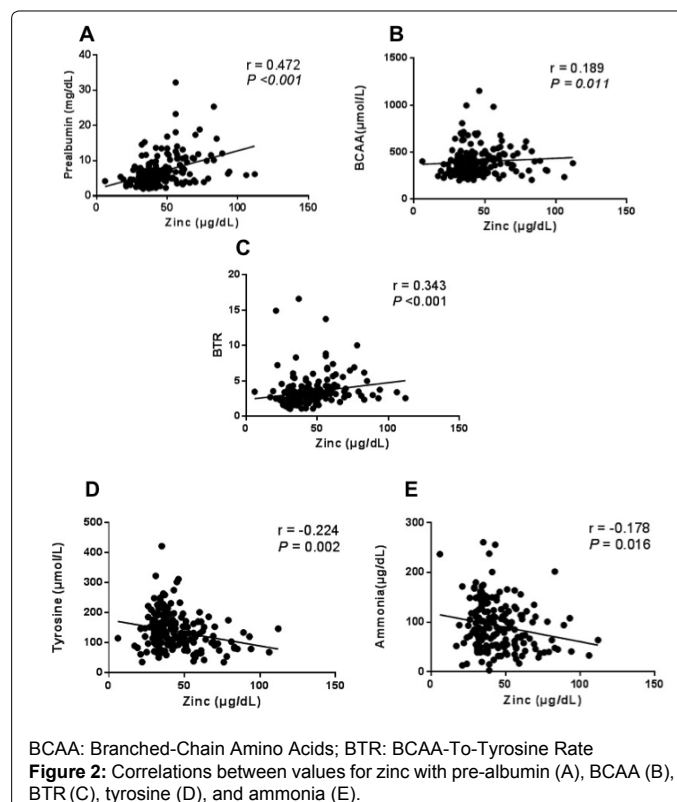
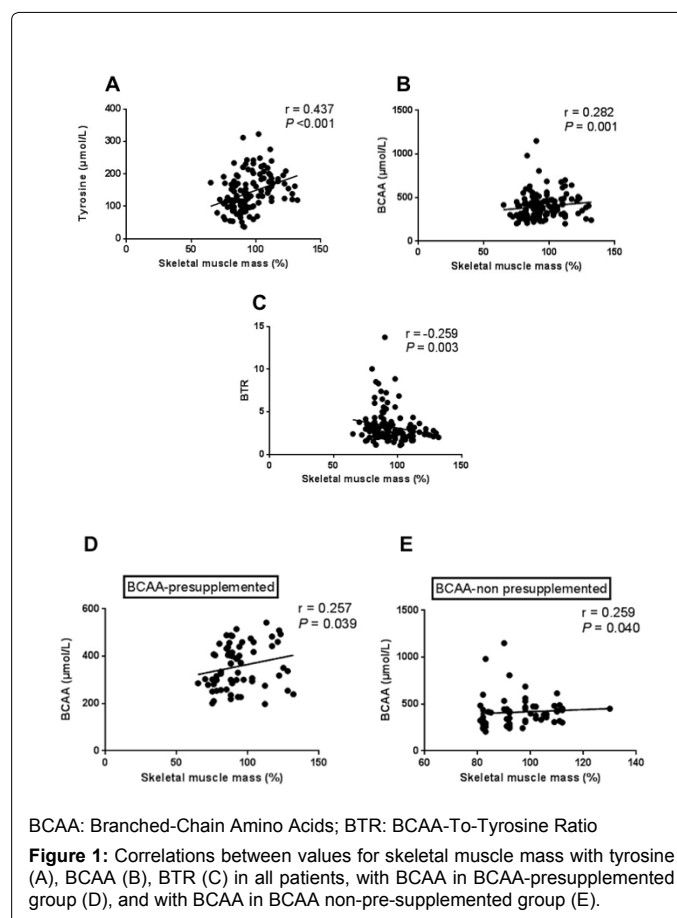
The clinical characteristics and surgical variables of the patients in BCAA+ and BCAA- groups on admission were compared in Table 1. There were no significant differences in age, sex, body mass index, Child-Pugh classification, MELD score, etiology of disease, number of ABO-incompatible grafts, donor age, operative blood loss and transfusion units (erythrocyte concentrates).

Pre-albumin level on admission was significantly higher, while tyrosine level was significantly lower in the BCAA+ than in BCAA- group ($P=0.023$ and $P<0.001$), respectively (Table 2). Zinc, BCAA, total lymphocyte count and ammonia levels did not significantly differ between both groups ($P=0.834$, $P=0.421$, $P=0.781$ and $P=0.560$), respectively. Consequently, the BTR was significantly higher in BCAA+ than in BCAA- group ($P=0.046$). Preoperative skeletal muscle mass did not significantly differ between both groups ($P=0.143$).

As for post-transplant outcomes, incidence of post-transplant bacteremia was significantly lower in BCAA+ than BCAA- group (30% versus 52%, $P=0.011$). However, the incidence of acute cellular rejection and the mean length of postoperative hospital stay were similar in the two groups ($P=0.279$, $P=0.576$, respectively).

Risk factor analysis for early posttransplant mortality

Univariate analysis revealed that neither pre-admission BCAA supplementation nor preoperative levels of nutritional/metabolic



Variable	BCAA+ (n=66)	BCAA- (n=63)	P
Donor age (years)	43.7 ± 10.6	43.7 ± 10.6	0.465
Recipient age at transplantation (years)	52.2 ± 11.1	47.0 ± 14.4	0.148
Gender (male/female)	33/33	30/33	0.861
Body mass index on admission (kg/m ²)	23.0 ± 4.3	21.6 ± 4.4	0.153
Underlying disease	-	-	-
HCC with viral hepatitis	22	14	0.168
Viral hepatitis B/C related cirrhosis	15	17	0.684
PBC/PSC	12	12	0.899
Biliary atresis post Kasai	3	5	0.486
Alcoholic cirrhosis	4	3	0.745
Metabolic disease	2	3	0.675
NASH	2	2	0.962
Autoimmune hepatitis	2	1	0.587
Others	4	6	0.534
ABO compatibility	-	-	0.057
Identical/compatible	42	50	-
incompatible	24	13	-
Child-Pugh classification (A, B/C)	20/46	31/32	0.366
MELD score	18.3 ± 6.9	20.5 ± 8.2	0.754
Graft type	-	-	0.259
Left lobe	27	32	-
Right lobe	39	31	-
Graft weight (g)	541.1 ± 153.5	493.5 ± 113.2	0.754
Surgical duration (min)	914 ± 127	957 ± 167	0.544
Intra-operative blood loss (ml)	9556 ± 3544	9256 ± 3334	0.964
Intra-operative erythrocyte transfusion (U)	20.9 ± 10.6	21.3 ± 10.5	0.132

*Including one domino graft

†BCAA: Branched-Chain Amino Acids; BCAA+: Received pre-admission BCAA treatment; BCAA-: Did not receive pre-admission BCAA treatment; HCC: Hepatocellular Carcinoma; PBC: Primary Biliary Cirrhosis; PSC: Primary Sclerosing Cholangitis; NASH: Non-Alcoholic Steatohepatitis; MELD: Model for End-Stage Liver Disease

Table 1: Patient characteristics according to branched chain amino acids treatment given before admission.

Variable	BCAA+ (n=66)	BCAA- (n=63)	P	P*
Zinc (µg/dL)	40.5 ± 10.2	47.2 ± 11.2	0.834	-
Prealbumin (mg/dL)	7.9 ± 2.1	5.2 ± 2.1	0.0038	0.023
BCAA (µmol/L)	401.3 ± 48.2	392.7 ± 49.5	0.421	-
Tyrosine (µmol/L)	124.7 ± 12.4	149.2 ± 13.1	<0.001	<0.001
BTR	3.7 ± 0.3	3.0 ± 0.3	0.0066	0.046
Total lymphocyte count	893.3 ± 213.1	841.9 ± 215.6	0.781	-
Ammonia (µg/dL)	96.1 ± 17.3	93.5 ± 15.3	0.56	-
Skeletal muscle mass (%)	96.8 ± 13.4	92.7 ± 12.5	0.143	-

†BCAA: Branched-Chain Amino Acids; BCAA+: Patients who received pre-admission BCAA treatment; BCAA-: Patients who did not receive pre-admission BCAA treatment; BTR: BCAA-To-Tyrosine Ratio

Table 2: Levels of nutritional/metabolic parameters and skeletal muscle mass on admission according to pre-admission BCAA supplementation.

parameters on admission were significant risk factors for early post-transplant mortality (Table 3).

Risk factor analysis for post-transplant bacteremia

Fifty-three patients of 129 developed post-transplant bacteremia. Univariate analysis showed that incidence of post-transplant bacteremia was significantly higher in patients with Child-Pugh classification C ($P=0.001$), operative blood loss ≥ 10 L ($P=0.010$), preoperative total lymphocyte count $<700/\mu\text{L}$ ($P=0.004$), and absence of pre-admission BCAA treatment ($P=0.011$) (Table 4). Multivariate analysis demonstrated four independent adverse risk factors for posttransplant bacteremia: Child-Pugh classification C ($P=0.012$), preoperative total lymphocyte count $<700/\mu\text{L}$ ($P=0.027$), operative blood loss of ≥ 10 L ($P=0.039$) and absence of pre-admission BCAA treatment ($P=0.040$) (Table 5).

Discussion

This retrospective study is the first to collectively examine pre-transplant characteristics of nutritional/metabolic parameters and skeletal muscle mass among patients with end-stage liver disease undergoing LDLT and investigate the impact of preoperative BCAA treatment before admission. Moreover, BCAA treatment before admission improved nutritional status in LDLT candidates. BCAA treatment before admission and preoperative TLC level has potential impacts on the incidence of post-transplant bacteremia. Selberg et al. [18] conducted a prospective cohort study of nutritional and metabolic parameters in 150 patients with end-stage liver disease undergoing LT. They showed that a poor nutritional state as well as hypermetabolism was not only an important prognostic factor in the evaluation of the risks of patients but also adversely affected survival after LT, the concept of which is in line with our recent report showing

	Variable	90-days OS	P
Recipient Age (y)	<60 (n=88)	86%	0.283
	≥ 60 (n=41)	91%	
Donor Age (y)	<50 (n=93)	91%	0.335
	≥ 50 (n=36)	85%	
Sex	Male (n=63)	91%	0.143
	Female (n=66)	83%	
Original disease	HCC (n=36)	81%	0.144
	Non-HCC (n=93)	90%	
ABO blood type	Compatible (n=92)	90%	0.087
	Incompatible (n=37)	81%	
Child-Pugh classification	A, B (n=51)	91%	0.227
	C (n=78)	84%	
MELD score	<20 (n=69)	92%	0.069
	≥ 20 (n=50)	81%	
GRWR	<0.8% (n=36)		
	≥ 0.8% (n=93)	91%	
Graft	Right lobe (n=70)	88%	0.535
	Left lobe (n=59)	86%	
Operative time (h)	<12 (n=30)	89%	0.09
	≥ 12 (n=99)	83%	
Operative blood loss (L)	<10 (n=88)	92%	0.079
	≥ 10 (n=41)	81%	
Pre-transplant zinc level (µg/dL)	<39 (n=58)	84%	0.634
	≥ 39 (n=71)	88%	
Pre-transplant prealbumin level (mg/dL)	<5.4 (n=64)	83%	0.343
	≥ 5.4 (n=65)	90%	
Pre-transplant BCAA level (µmol/L)	<375.2 (n=62)	81%	0.476
	≥ 375.2 (n=67)	85%	
Pre-transplant BTR	<2.92 (n=60)	85%	0.786
	≥ 2.92 (n=69)	87%	
Pre-transplant tyrosine (µmol/L)	<131.7 (n=63)	89%	0.1
	≥ 131.7 (n=66)	85%	
Pre-transplant total lymphocyte count (/µL)	<700 (n=61)	85%	0.698
	≥ 700 (n=68)	87%	
Pre-transplant ammonia level (µg/dL)	<87 (n=61)	90%	0.5
	≥ 87 (n=68)	85%	
BCAA supplementation before admission	With (n=66)	91%	0.329
	Absent (n=63)	86%	

†OS: Overall Survival; BCAA: Branched-Chain Amino Acids; BTR: BCAA-To-Tyrosine Ratio; GRWR: Graft-To-Recipient Body Weight Ratio; HCC: Hepatocellular Carcinoma; MELD: Model for End-Stage Liver Disease; LDLT: Living Donor Liver Transplantation

Table 3: Univariate analysis of factors affecting post-transplant patient survival.

that low preoperative skeletal muscle mass was closely involved with post-transplant mortality [7]. In the present study, we revealed that preoperative BCAA treatment before admission could ameliorate the incidence of post-transplant bacteremia and improve post-transplant mortality.

We previously uncovered a significantly negative correlation between skeletal muscle mass and the BTR [7]. At that time, we speculated that since BCAA are mainly metabolized in the skeletal muscle of patients with cirrhosis, more skeletal muscle mass and more BCAA consumption would lead to a decrease in the BTR. However, the present study identified a significantly positive correlation between skeletal muscle mass and BCAA. This finding can be explained by the fact that BCAA is also released from skeletal muscle protein due to endogenous breakdown under hypercatabolic conditions in liver cirrhosis [19]. This represents another source of plasma BCAA beside dietary intake in patients with cirrhosis. Therefore, when skeletal muscle mass decreases in such patients, BCAA also decreases due to skeletal muscle mass depletion. Moreover, the correlation between skeletal muscle mass and tyrosine was notably significant and positive. Tyrosine was similarly reported to be released from muscle protein breakdown [20], however, it is solely metabolized by the liver without being metabolized nor consumed by the muscle, probably explaining the stronger correlation of pre-transplant skeletal muscle mass with tyrosine than with BCAA. Thus, the negative correlation between skeletal muscle mass and BTR would be mainly due to positive correlation between skeletal muscle mass and tyrosine.

We recently reported that Child-Pugh classification C and massive operative blood loss were independent risk factors for post-transplant bacteremia [21]. In the present study, in addition to these variables, low pre-operative total lymphocyte count and absence of pre-admission BCAA therapy were newly revealed to be independent risk factors for post-transplant bacteremia. This finding strongly suggests that pre-transplant nutritional status is closely involved with the onset of post-transplant bacteremia. We are now investigating immunological mechanism why pre-transplant nutritional treatment is beneficial to prevent post-transplant severe infection.

Some limitations must be borne in mind when considering this study. First, BCAA were introduced at the discretion of the attending physician before referral of a potential recipient for LDLT. In the present study, however, clinical background including Child-Pugh and MELD scores between both groups were comparable on admission. Therefore, general condition and selection bias might have been at minimum. Second, because of retrospective nature of this study, nutritional status before BCAA administration was unclear and the exact duration and amount of BCAA given within the preoperative nutritional therapy protocol were different patient by patient. Prospective randomized study to examine the effect of pre-admission BCAA treatment is thus needed to adjust these biases. Third, as this was a cross-sectional study, we have not completely verified a causal relationship between BCAA pre-supplementation and parameter levels on admission. Therefore, it will be necessary to perform a future cohort study. Lastly, a limitation was reported for BIA in assessment of body water components of cirrhotic patients with ascites [22]. Fürstenberg et al reported that BIA was highly correlated with other methods in measuring lean body mass even in over-hydrated subjects [23]. Moreover, we recently reported that the effect of over hydration on overestimation of BIA might be, if any, minimum in using this device [24]. At present, therefore, segmental multi-frequency BIA used in this study would be best modality to assess skeletal muscle mass.

Variable		Incidence of post-transplant bacteremia	P
Recipient Age (y)	<60 (n=88)	47%	0.063
	≥ 60 (n=41)	29%	
Donor Age (y)	<50 (n=93)	38%	0.2
	≥ 50 (n=36)	50%	
Sex	Male (n=63)	41%	0.967
	Female (n=66)	41%	
Original disease	HCC (n=36)	42%	0.933
	Non-HCC (n=93)	41%	
ABO blood type	Compatible (n=92)	38%	0.268
	Incompatible (n=37)	49%	
Child-Pugh	A, B (n=51)	24%	0.001
	C (n=78)	53%	
MELD score	<20 (n=69)	48%	0.397
	≥ 20 (n=50)	40%	
GRWR	<0.8% (n=36)	42%	0.933
	≥ 0.8% (n=93)	41%	
Graft	Right (n=70)	47%	0.128
	Left (n=59)	34%	
Operative time (h)	<12 (n=30)	43%	0.775
	≥ 12 (n=99)	40%	
Operative blood loss (L)	<10 (n=87)	33%	0.01
	≥ 10 (n=42)	57%	
Pre-transplant zinc level (µg/dL)	<39 (n=58)	41%	0.951
	≥ 39 (n=71)	41%	
Pre-transplant pre-albumin level (mg/dL)	<5.4 (n=64)	41%	0.916
	≥ 5.4 (n=65)	42%	
Pre-transplant BCAA level (µmol/L)	<375.2 (n=62)	42%	0.85
	≥ 375.2 (n=67)	40%	
Pre-transplant BTR	<2.92 (n=60)	42%	0.9
	≥ 2.92 (n=69)	41%	
Pre-transplant tyrosine (µmol/L)	<131.7 (n=63)	37%	0.229
	≥ 131.7 (n=66)	47%	
Pre-transplant total lymphocyte count (/µL)	<700 (n=61)	54%	0.004
	≥ 700 (n=68)	29%	
Pre-transplant ammonia level (µg/dL)	<87 (n=61)	33%	0.07
	≥ 87 (n=68)	49%	
BCAA supplementation before admission	With (n=66)	30%	0.011
	Absent (n=63)	52%	

†BCAA: Branched-Chain Amino Acids; BTR: BCAA-To-Tyrosine Ratio; GRWR: Graft-To-Recipient Body Weight Ratio; HCC: Hepatocellular Carcinoma; MELD: Model for End-Stage Liver Disease; LDLT: Living Donor Liver Transplantation

Table 4: Univariate analysis of factors affecting post-transplant bacteremia.

Variable	Odds ratio	95% CI	P
Child-Pugh classification C	7.322	1.600-29.996	0.012
Preoperative low total lymphocyte count (<700/ μ L)	5.434	1.313-20.133	0.027
Operative blood loss $\geq$ 10 L	4.230	1.373-16.763	0.039
Absence of pre-admission BCAA treatment	2.942	1.644-8.643	0.040

†BCAA: Branched-Chain Amino Acids; CI: Confidence Interval

Table 5: Multivariate analysis of factors affecting post-transplant bacteremia.

In conclusion, BCAA therapy before admission could ameliorate the incidence of post-transplant bacteremia and could enhance improve disease-induced amino acid imbalance or protein status in LDLT candidates. Further randomized clinical trials are warranted to confirm our hypothesis.

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Perioperative Changes in Nutritional Parameters and Impact of Graft Size in Patients Undergoing Adult Living Donor Liver Transplantation

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Derangements of various serum biochemical nutritional/metabolic parameters are common in patients with end-stage liver disease who undergo liver transplantation (LT). The aim of this study was to explain the benefit of LT with respect to parameter changes and to examine the impact of the graft-to-recipient weight ratio (GRWR) on such changes. We investigated each parameter's course in 208 adult recipients for 1 year after living donor LT and analyzed changes in the parameters with a GRWR of 0.8% as the cutoff point. Bonferroni corrections were applied to account for multiple testing. Liver disease–induced high pretransplant ammonia and tyrosine levels and low branched-chain amino acids to tyrosine ratio (BTR) and zinc levels normalized within 2 weeks after transplantation, and the total lymphocyte count (TLC) normalized within 2 months, whereas low pretransplant prealbumin levels took 1 year to normalize. Branched-chain amino acids (BCAA), zinc, and TLC levels transiently dropped shortly after transplantation and then were corrected later. An accelerated recovery of ammonia and tyrosine levels and the BTR were found with larger grafts, especially early after transplantation, whereas zinc, prealbumin, BCAA, and TLC levels recovered regardless of the graft size. In conclusion, graft size had little effect on the recovery of nutritional/metabolic parameters except for ammonia and tyrosine levels. *Liver Transpl* 20:1486–1496, 2014.

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In patients with end-stage liver disease undergoing liver transplantation (LT), protein-energy malnutrition is common and negatively affects clinical outcomes in terms of posttransplant survival and complications.¹ Therefore, the instigation of specialized nutritional status measurements and interventions is required. Derangements of various serum biochemical nutritional parameters such as zinc, prealbumin, branched-chain amino acids (BCAA), tyrosine, and

total lymphocyte count (TLC) and related metabolic parameters such as the BCAA to tyrosine ratio (BTR) and ammonia are not uncommon in these patients as a result of the debilitating hepatic pathology and its medical management.^{2–6} These parameters could be good indicators of nutritional/metabolic status trends after LT. However, their posttransplant changes with respect to the preoperative levels remain unclear.

Abbreviations: ALF, acute liver failure; BCAA, branched-chain amino acids; BMI, body mass index; BTR, branched-chain amino acids to tyrosine ratio; CT, computed tomography; CTP, Child-Turcotte-Pugh; GRWR, graft-to-recipient weight ratio; HCC, hepatocellular carcinoma; LDLT, living donor liver transplantation; L/S, liver-to-spleen attenuation; LT, liver transplantation; MELD, Model for End-Stage Liver Disease; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cirrhosis; POD, postoperative day; PSC, primary sclerosing cholangitis; TLC, total lymphocyte count; w1, week 1; w2, week 2; w3, week 3; w4, week 4.

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Studies reporting amino acid levels after LT have been performed only in the early postoperative period⁷ or without a definite time course.⁸ It is, therefore, not clear whether levels of circulating amino acids are normalized in the clinically stable long-term course after LT. We hypothesize that successful LT would be sufficient to correct the disturbed amino acid metabolism found in liver cirrhosis.

In deceased donor LT, the graft size is sufficient for the recipient. In contrast, in living donor liver transplantation (LDLT), the graft size is small and is an important factor for posttransplant survival. However, there is relatively little information on the short- and long-term changes in nutritional/metabolic parameters after LDLT that could reflect the adequacy of the graft mass to provide sufficient metabolic and synthetic function, which is the key factor in the success of LDLT. Although partial liver grafts undergo a rapid regenerative response, with the largest changes in the liver volume occurring during the first week after transplantation, regeneration is suppressed in small-for-size grafts after LDLT. Thus, grafts with an inadequate graft-to-recipient weight ratio (GRWR) cannot meet the functional demand of the recipients.⁹ On the other hand, some have concluded that smaller grafts are capable of regeneration to a greater extent and that the regenerative liver response is proportional to the amount of liver transplanted.^{10,11} Yoshida et al.⁷ presumed improvements in some nutritional parameters shortly after LDLT to be derived from the GRWR discrepancy. Our hypothesis is that the posttransplant recovery of nutritional/metabolic parameters, especially in the early period after grafting, might be affected by GRWR. To obtain insight into these questions, the present retrospective longitudinal study was performed to clarify the short- and long-term courses of circulating levels of the aforementioned parameters after successful adult LDLT and to analyze the impact of GRWR on such posttransplant changes in LDLT recipients.

PATIENTS AND METHODS

Patients

The study subjects were 208 adult patients (age ≥ 18 years) who underwent primary LDLT at Kyoto University Hospital between February 2008 and August 2012. There were 98 males and 110 females, and the median patient age was 54 years (range = 18–69 years). The patients provided written informed consent before the start of the study, which was approved by the ethics committee of Kyoto University in accordance with the Declaration of Helsinki of 1996.

The median Model for End-Stage Liver Disease (MELD) score was 19 (range = 6–55). Sixty-eight patients were ABO-incompatible, and 140 were identical or compatible. The Child-Turcotte-Pugh (CTP) classifications were C, B, and A for 139, 55, and 14 patients, respectively. The indications for LT were hepatocellular carcinoma (HCC; $n = 52$), hepatocellular

diseases such as hepatitis B or C virus-associated liver cirrhosis ($n = 46$), progressive intrahepatic cholestatic diseases including primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC; $n = 34$), acute liver failure (ALF; $n = 15$), biliary atresia after the Kasai procedure ($n = 14$), alcoholic liver cirrhosis ($n = 11$), metabolic liver diseases ($n = 7$), non-alcoholic steatohepatitis (NASH; $n = 7$), autoimmune hepatitis ($n = 4$), and other causes ($n = 18$).

Donor and Graft Selection

The selection criteria for donors and grafts have been described elsewhere.^{12,13} Briefly, according to computed tomography (CT) scan volumetric analysis, the liver weight was calculated, and the graft type was selected. If necessary, portosystemic collateral ligation with splenectomy was performed for the prevention of the steal phenomenon after LT as well as the control of the portal venous pressure (≤ 15 mm Hg). This allowed the lower limit of GRWR to be safely reduced to 0.6% and a left lobe graft to be used whenever feasible.^{13,14} All grafts used had a liver-to-spleen attenuation (L/S) ratio on CT ≥ 1.1 to exclude grafts with hepatic steatosis $> 30\%$.¹⁵

Surgical Procedures and Immunosuppressive Treatments

The selection criteria for the recipients as well as surgical and back-table techniques for the donors and recipients have been described in detail elsewhere.^{16–18} Orthotopic adult LDLT was performed with a right lobe graft for 102 patients, with a left lobe graft for 100 patients, with a posterior segment graft for 5 patients, and with a whole liver graft as a domino graft from a patient with familial amyloid polyneuropathy for 1 patient. Each graft was perfused with cold histidine tryptophan ketoglutarate (0°C – 4°C ; Custodiol, Essential Pharmaceuticals, LLC, Newtown, PA). Immediately after the perfusion of the preservation solution, all resected liver grafts were measured. The actual graft weight was used for the calculation of GRWR. The median GRWR was 0.89% (range = 0.53%–1.50%). At the time of surgery in all recipients, a tube jejunostomy for enteral nutrition was placed in the proximal jejunum with a 9-Fr enteral tube.

The baseline immunosuppressive regimen consisted of tacrolimus or cyclosporine and low-dose steroids, as described elsewhere.¹⁹ Patients who were ABO-incompatible also underwent preoperative plasma exchange to reduce A/B antibodies to 1:8 or more and received 300 mg of intravenous rituximab (anti-CD20 monoclonal antibody) approximately 2 weeks before LT. A hepatic artery infusion of prostaglandin E1 and methylprednisolone was started at the time of the surgery and was continued for 21 and 7 days, respectively, and this was followed by oral mycophenolate mofetil (500 mg twice daily).²⁰ All patients received intravenous antimicrobial prophylaxis with

ampicillin (0.5 g) and cefotaxime (0.5 g) twice daily for 3 days; this started 30 minutes before surgery.

Perioperative Nutritional Therapy

Preoperative nutritional therapy was administered for approximately 2 weeks before LDLT and consisted of the following components: a nutrient mixture enriched with BCAA (Aminoleban EN, Otsuka Pharmaceutical Co., Tokyo, Japan) or BCAA nutrients (Livact, Ajinomoto Pharmaceuticals Co., Ltd., Tokyo, Japan) as a late-evening snack, synbiotics with a supplementation product (GFO, Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) 3 times daily, a lactic fermented beverage once per day, and polaprezinc (Promac D, Zeria Pharmaceutical Co., Ltd., Tokyo, Japan) for patients with low zinc, as described previously.²¹ Dietitians adjusted the type and amount of food for each patient to maintain a total daily caloric intake of 35 to 40 kcal/kg and a protein intake of 1.2 to 1.5 g/kg, including BCAA nutrients, according to the guidelines of the European Society of Parenteral and Enteral Nutrition.²² We could not perform preoperative nutritional therapy for patients with ALF due to emergent LT, and also BCAA supplementation was unsuitable for those patients already having elevated plasma amino acids.²³

Formulas containing BCAA and polaprezinc were discontinued after LDLT, and early postoperative enteral nutrition was started within the first 24 hours after surgery through the tube jejunostomy with an immune-modulating enteral diet enriched with hydrolyzed whey peptide (MEIN, Meiji Dairies Co., Tokyo, Japan); its composition²¹ and its administration protocol^{24,25} have been previously described. Oral nutrition was started after the ability to swallow was regained, usually on approximately postoperative day (POD) 5. Dietitians calculated the daily amounts of protein and carbohydrates required for each recipient and the speed of the enteral nutrition accordingly. Enteral feeding was stopped when adequate oral intake containing solid food was tolerated. For synbiotics, all patients received the aforementioned supplementation product 3 times daily and a lactic fermented beverage once per day via the feeding tube or orally until discharge.

Analyzed Parameters

Preoperative and postoperative laboratory parameters were retrospectively reviewed from the clinical charts of the recipients. The standard reference intervals for these parameters at our institute were as follows: zinc, 65 to 110 µg/dL; prealbumin, 20 to 40 mg/dL; TLC, 1200 to 3200/µL; ammonia, 20 to 60 µg/dL; BCAA, 344 to 713 µmol/L; tyrosine, 53 to 98 µmol/L; and BTR, 4.41 to 9.3. A longitudinal study was performed to examine peritransplant changes in the aforementioned parameters at the following serial time points: before the operation at admission (baseline); PODs 2, 3, and 5; week 1 (w1), week 2 (w2), week 3 (w3), and week 4 (w4); months 2, 3, and 6;

and year 1 after transplantation. The baseline pretransplant level of each parameter was statistically compared with its counterparts at each time point in the posttransplant observation (follow-up) period, and the degree of significance of each comparison was plotted on graphs.

A statistical comparison was performed for the level of each parameter at each of the assigned peritransplant time points between recipients of grafts with a GRWR < 0.8% (n = 67) and recipients of grafts with a GRWR ≥ 0.8% (n = 141). There were 31 patients who received grafts with a GRWR < 0.7% and 36 patients who received grafts with a GRWR between 0.7% and 0.8%. We also compared peritransplant parameters among recipients who were stratified as follows: those with a GRWR < 0.7% (n = 31), those with a GRWR between 0.7% and 0.8% (n = 36), and those with a GRWR ≥ 0.8% (n = 141).

Perioperative changes in parameters were compared among patients with preoperative CTP classification A (n = 14), B (n = 55), or C (n = 139) at various time points and also among patients with diseases of other etiologies who received preoperative nutritional therapy (n = 193) and those with ALF (n = 15) who did not. We also compared nutritional recovery between ABO-incompatible recipients (n = 68) and ABO-compatible recipients (n = 140). Moreover, we examined these preoperative nutritional/metabolic parameters as risk factors for posttransplant mortality.

Statistical Analysis

Data were summarized as means and standard deviations for continuous variables. Continuous variables (or parameters) were nonparametrically analyzed with the Wilcoxon signed-rank test to assess postoperative changes from the preoperative state, whereas other comparisons, including those of groups with GRWR < 0.8% and GRWR ≥ 0.8% at each time point, were compared with the Mann-Whitney U test or the one-way analysis of variance as appropriate. Two-tailed *P* values were corrected for multiple testing with the Bonferroni method²⁶ (with the statistical significance set at *P* < 0.0045, where 0.0045 = 0.05/11) for 11 tests within each independent family of comparisons of each single parameter performed at the designated 11 peritransplant time points. Only the adjusted *P* values are presented here. Categorical variables were compared with the χ^2 test or Fisher's exact test as appropriate. The survival rate was calculated via Kaplan-Meier methods, with differences evaluated by log-rank testing. Any variable identified as significant (*P* < 0.05) in the univariate analysis was considered a candidate for the multivariate analysis using multiple logistic regression models. All statistical data were generated in JMP 5.0.1 (SAS Institute, Cary, NC) and Prism 6.02 (GraphPad Software, Inc., La Jolla, CA).

RESULTS

The 1-, 2-, 3-, 4-, and 5-year cumulative survival rates of the whole cohort were 72.6%, 69.2%, 68.1%,

67.3%, and 66.4%, respectively. Overall survival rates and graft survival, defined as the time from LT to death or retransplantation, did not significantly differ between recipients with GRWR $\geq 0.8\%$ and those with

GRWR $< 0.8\%$ [$P = 0.79$ (Fig. 1) and $P = 0.87$, respectively].

Baseline Assessments of the Whole Cohort

Marked decreases in zinc ($45.4 \pm 12.1 \mu\text{g/dL}$), prealbumin ($7.0 \pm 2.4 \text{ mg/dL}$), and TLC ($863.3 \pm 207.3/\mu\text{L}$) and marked increases in ammonia ($98.1 \pm 17.8 \mu\text{g/dL}$) and tyrosine ($138.8 \pm 12.0 \mu\text{mol/L}$) were seen before LDLT. The pretransplant BCAA level ($395.2 \pm 51.0 \mu\text{mol/L}$) was low but still within the reference range. Consequently, the BTR (3.0 ± 0.5) was subnormal.

Peritransplant Changes in the Parameters

The low pretransplant zinc level steeply dropped for 2 to 3 days after LDLT and subsequently increased to reach the pretransplant level at about POD 5, continued to increase until it was normalized during w2, and gradually improved thereafter (Fig. 2A). The low pretransplant prealbumin level increased gradually after LDLT and took up to 1 year to normalize (Fig. 2B). The high pretransplant ammonia level notably declined immediately after LDLT to normalize within

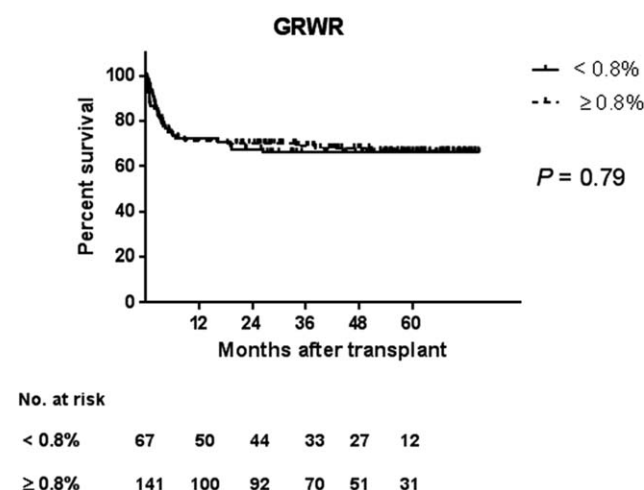


Figure 1. Overall survival rates according to GRWR.

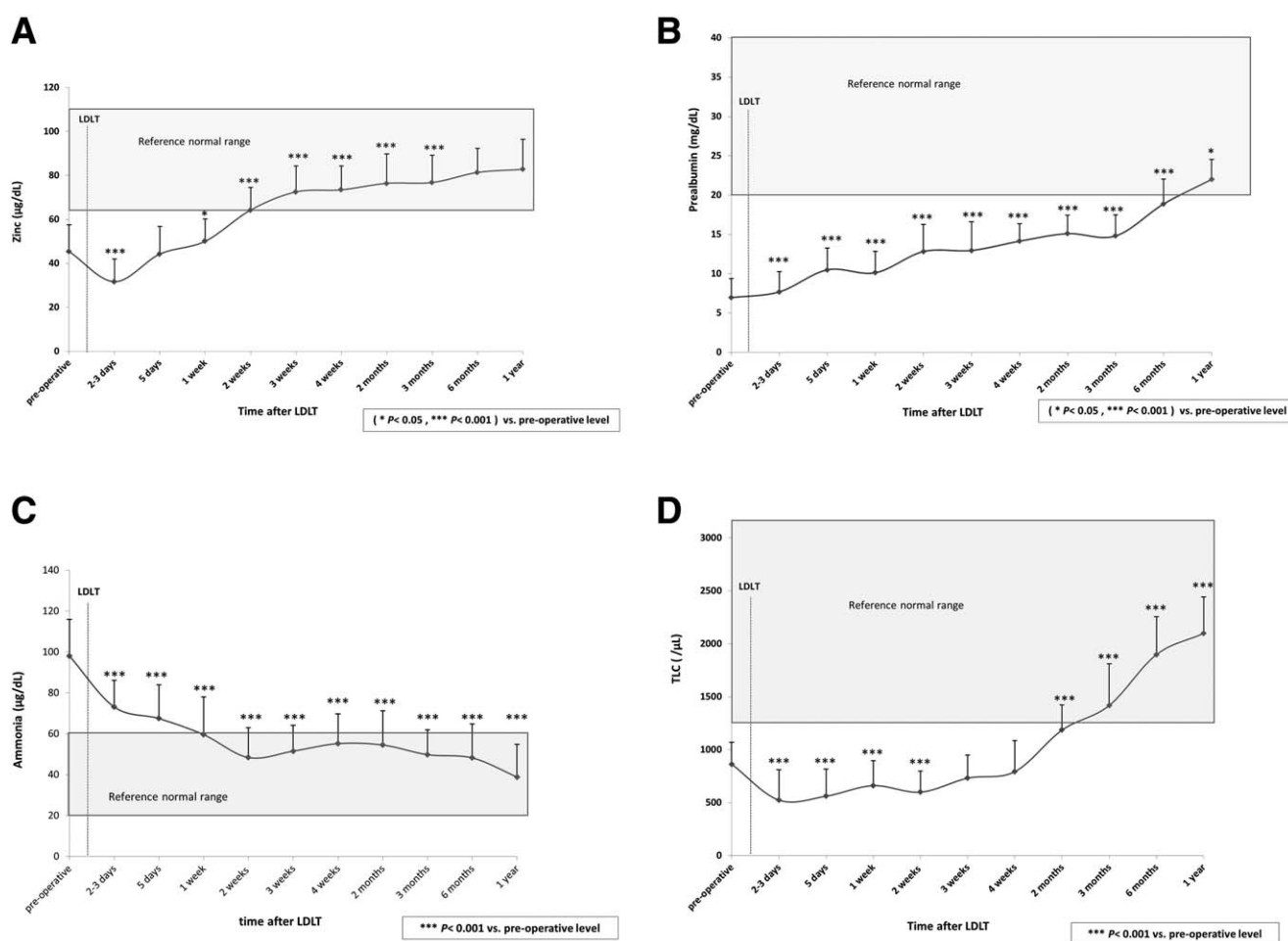


Figure 2. Time course of peritransplant changes of parameters: (A) zinc, (B) prealbumin, (C) TLC, (D) BCAA, (E) ammonia, (F) tyrosine, and (G) BTR.

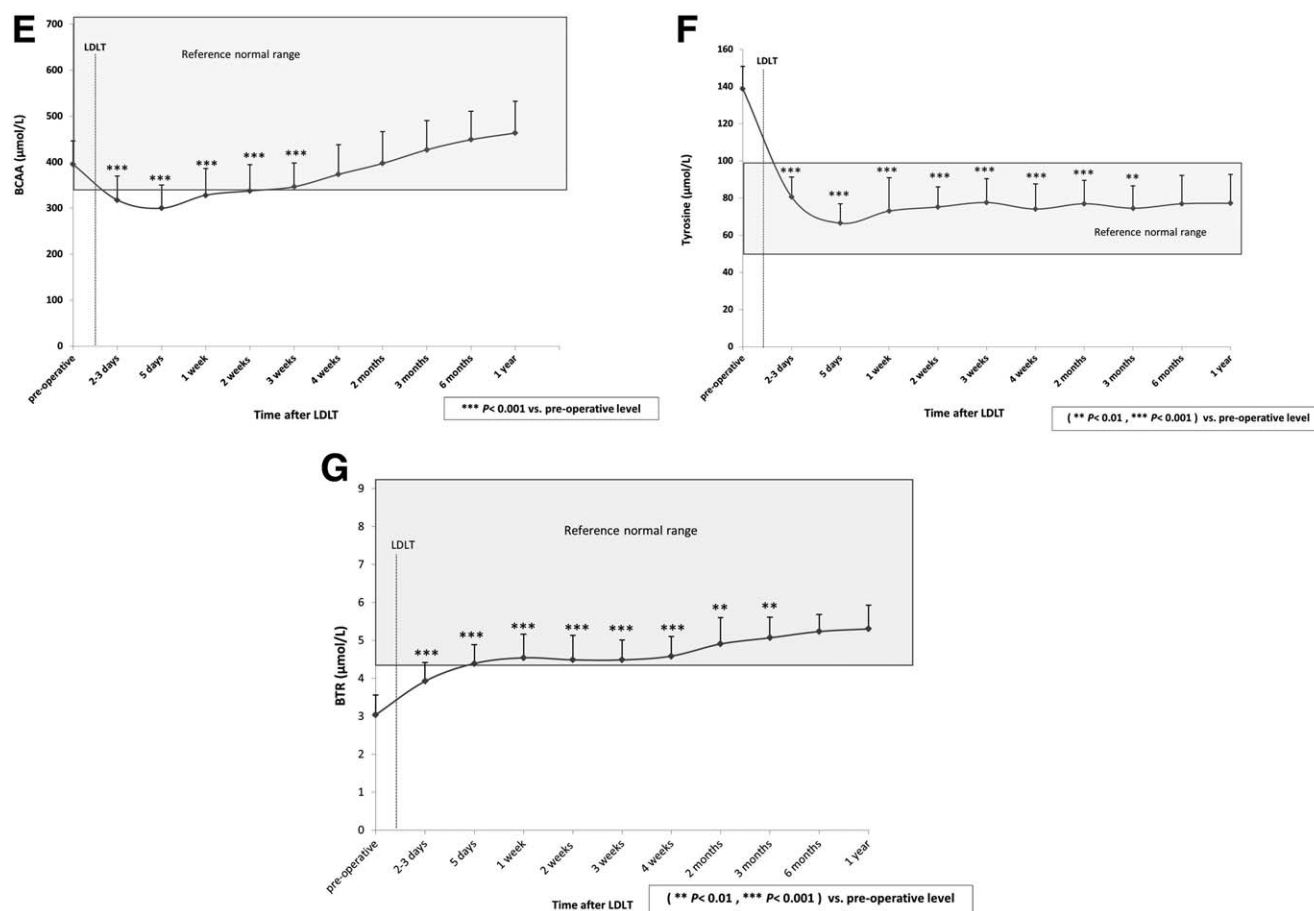


Figure 2. Continued.

w1 and continued to decrease slightly thereafter (Fig. 2C). The TLC level dropped shortly after LDLT, then gradually recovered to the normal level within 2 months after transplantation, and continued to increase thereafter (Fig. 2D). The BCAA level decreased over the first 5 days after LDLT to a sub-normal level, then gradually increased until it normalized in w2, and further improved thereafter (Fig. 2E). The high pretransplant serum tyrosine level rapidly declined immediately after LDLT to return within the normal range by POD 2/3, then further decreased until POD 5, and remained relatively stable thereafter (Fig. 2F). Consequently, the BTR rose rapidly to normalize on POD 5, remained stationary for the next 3 weeks, and gradually improved thereafter (Fig. 2G).

Peritransplant Changes in the Parameters According to the GRWR: <0.8% Versus ≥0.8%

The backgrounds and peritransplant characteristics of the recipients with GRWR < 0.8% and those with GRWR ≥ 0.8% are given in Tables 1 and 2. There were no significant differences between the groups in age, sex, body mass index (BMI), CTP, MELD scores, etiology of disease, number of ABO-incompatible grafts, donor age, preoperative levels of the parameters

examined, operative blood loss and transfusion units (erythrocyte concentrates), or cold and warm ischemia times.

There were no significant differences between the 2 groups with respect to zinc, prealbumin, TLC, or BCAA levels at any time point (Fig. 3A-D). Although the prealbumin and BCAA levels were close between the groups early after surgery, at a longer time period after surgery, the GRWR ≥ 0.8% group showed somewhat persistent yet insignificant increases in prealbumin and BCAA levels in comparison with the levels in the GRWR < 0.8% group.

Ammonia and tyrosine levels declined faster and normalized earlier in the GRWR ≥ 0.8% group. The ammonia level significantly dropped from 98.6 ± 15.5 and 97.0 ± 17.7 μg/dL before LDLT to 46.0 ± 13.1 and 48.8 ± 17.1 μg/dL at w2 in the GRWR ≥ 0.8% group and the GRWR < 0.8% group ($P < 0.001$ and $P < 0.001$), respectively, with a significantly greater mean ratio of reduction in the GRWR ≥ 0.8% group versus the GRWR < 0.8% group ($P = 0.01$). The ammonia level was significantly lower in the GRWR ≥ 0.8% group versus the GRWR < 0.8% group on PODs 2 and 3 ($P = 0.001$) and remained decreased, although nonsignificantly, thereafter (Fig. 3E). The tyrosine level significantly dropped in the GRWR ≥ 0.8% group and

TABLE 1. Patient Characteristics

	GRWR $\geq$ 0.8% (n = 141)	GRWR < 0.8% (n = 67)	P Value
Donor age (years)	43.4 $\pm$ 11.9	41.0 $\pm$ 10.9	0.21
Recipient age at transplantation (years)	51.3 $\pm$ 12.5	48.9 $\pm$ 14.6	0.25
Sex: male/female (n/n)	65/76	33/34	0.77
BMI on admission (kg/m ²)	22.9 $\pm$ 4.3	23.8 $\pm$ 4.7	0.17
Underlying disease (n)			
HCC on top of viral hepatitis B or C	35	17	0.93
Viral hepatitis B/C-related cirrhosis	34	12	0.44
PBC/PSC	23	11	0.99
ALF	9	6	0.50
Biliary atresia after Kasai operation	8	6	0.38
Alcoholic cirrhosis	7	4	0.76
Metabolic diseases	4	3	0.54
NASH	5	2	0.83
Autoimmune hepatitis	3	1	0.76
Others	13	5	0.59
ABO compatibility (n)			0.35
Identical/compatible	98	42	
Incompatible	43	25	
Preoperative CTP classification: A or B/C	48/93	21/46	0.75
Preoperative MELD score	19.8 $\pm$ 8.5	21.7 $\pm$ 10.5	0.17
Baseline levels of parameters			
Zinc (μ g/dL)	44.9 $\pm$ 10.5	46.4 $\pm$ 12.4	0.59
Prealbumin (mg/dL)	6.8 $\pm$ 2.3	7.3 $\pm$ 2.9	0.54
BCAA (μ mol/L)	388.7 $\pm$ 49.9	402.7 $\pm$ 60.2	0.38
Tyrosine (μ mol/L)	145.3 $\pm$ 13.3	151.5 $\pm$ 12.6	0.60
BTR	3.4 $\pm$ 0.5	3.5 $\pm$ 0.4	0.96
TLC (/ μ L)	852.3 $\pm$ 216.6	886.6 $\pm$ 213.0	0.78
Ammonia (μ g/dL)	98.6 $\pm$ 15.5	97.0 $\pm$ 17.7	0.85

TABLE 2. Surgical Variables

Variable	GRWR $\geq$ 0.8% (n = 141)	GRWR < 0.8% (n = 67)	P Value
Graft type			<0.001
Left lobe	48	52	
Right lobe, including a posterior segment graft	93*	15	
Graft weight (g)	571.1 $\pm$ 163.6	427.5 $\pm$ 114.5	<0.001
Surgical duration (minutes)	919 $\pm$ 138	987 $\pm$ 181	0.35
Blood loss (mL)	9598 $\pm$ 3155	9769 $\pm$ 3190	0.92
Intraoperative erythrocyte transfusion (U)	20.9 $\pm$ 10.6	20.8 $\pm$ 12.6	0.74
Cold ischemia time (minutes)	57.3 $\pm$ 17.2	82.4 $\pm$ 13.1	0.64
Warm ischemia time (minutes)	39.9 $\pm$ 12.1	46.9 $\pm$ 17.3	0.39

*Including 1 domino graft.

the GRWR < 0.8% group from 135.3 $\pm$ 13.3 and 141.5 $\pm$ 12.6 μ mol/L before LDLT to 59.7 $\pm$ 13.3 and 75.5 $\pm$ 13.7 μ mol/L on POD 5 (P < 0.001 and P < 0.001), respectively, with a significantly higher mean ratio of reduction in the GRWR $\geq$ 0.8% group (P = 0.02). The tyrosine level was significantly lower in the GRWR $\geq$ 0.8% group versus the GRWR < 0.8% group on PODs 2 and 3 (P = 0.001) and remained decreased, although nonsignificantly, thereafter (Fig. 3F).

Consequently, the BTR increased faster and normalized earlier in the GRWR $\geq$ 0.8% group versus the

GRWR < 0.8% group: it increased significantly from 3.0 $\pm$ 0.5 and 3.1 $\pm$ 0.4 before LDLT to 4.8 $\pm$ 0.5 and 3.9 $\pm$ 0.5 on POD 5 (P < 0.001 and P = 0.02), respectively, with a significantly greater mean ratio of increase (P = 0.1) in the GRWR $\geq$ 0.8% group. The BTR remained significantly higher in the GRWR $\geq$ 0.8% group versus the GRWR < 0.8% group during the first postoperative month [on PODs 2/3 and 5 and in w1, w2, and w3 (P < 0.001) and in w4 (P = 0.002)] and remained increased, although nonsignificantly, thereafter (Fig. 3G).

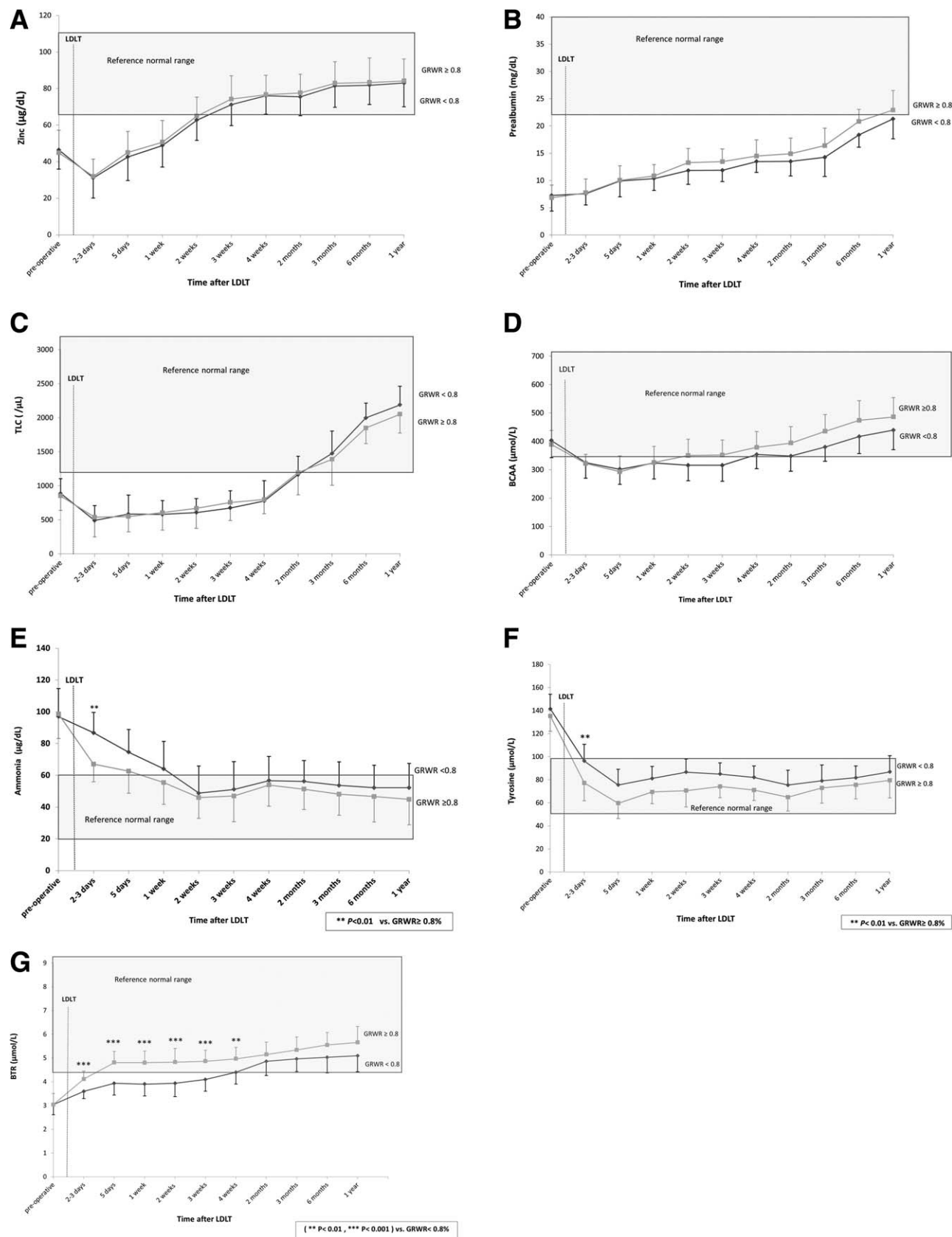


Figure 3. Time course of peritransplant changes of parameters according to GRWR (<0.8% versus $\geq 0.8\%$): (A) zinc, (B) prealbumin, (C) TLC, (D) BCAA, (E) ammonia, (F) tyrosine, and (G) BTR.

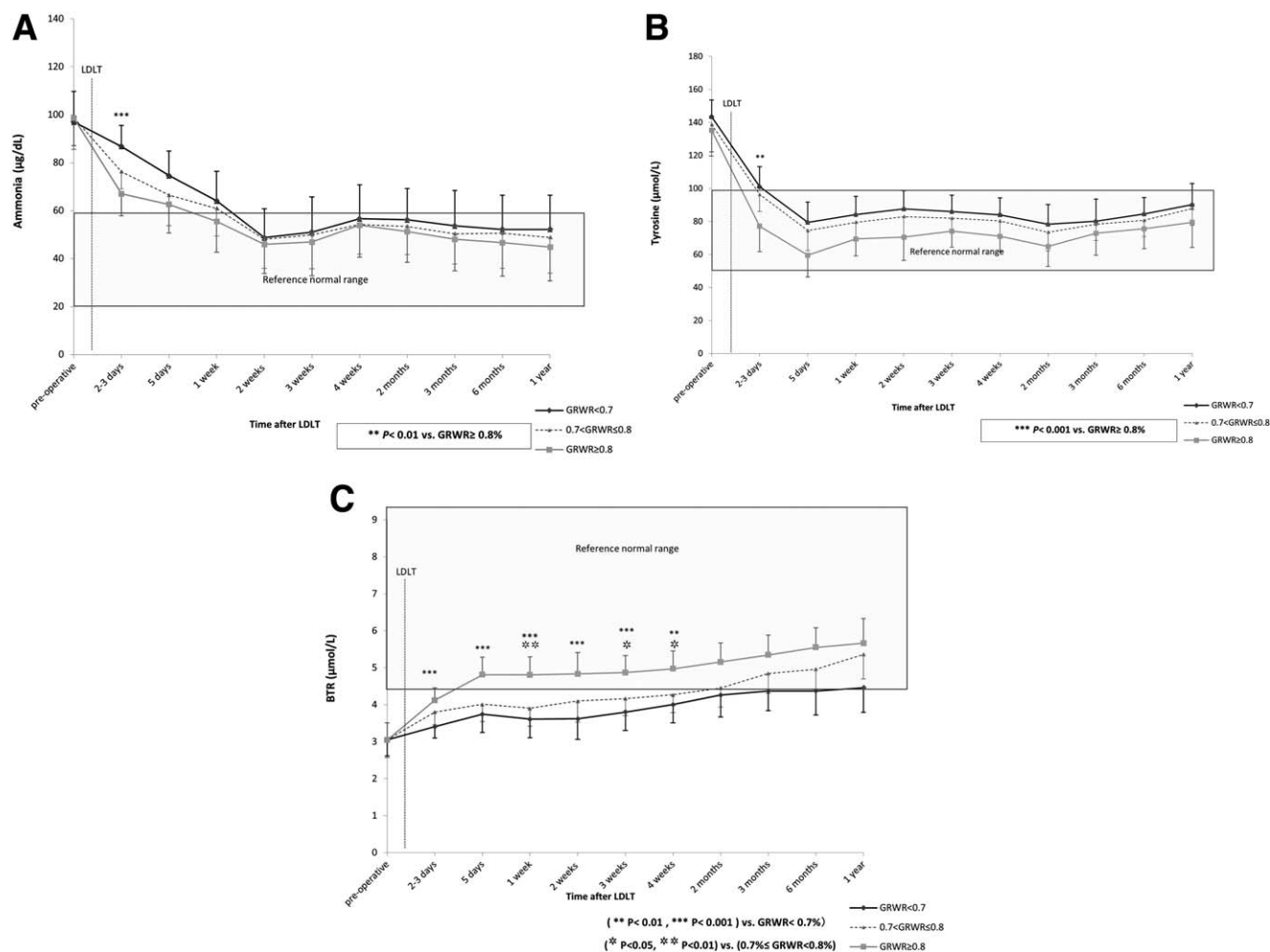


Figure 4. Comparison of time course of peritransplant levels of (A) ammonia, (B) tyrosine, and (C) BTR among patients with GRWR < 0.7%, between 0.7% and 0.8%, and $\geq 0.8\%$.

Peritransplant Changes in the Parameters According to the GRWR: <0.7% Versus 0.7% to 0.8% Versus $\geq 0.8\%$

Ammonia and tyrosine levels were significantly lower in the GRWR $\geq 0.8\%$ group versus the GRWR < 0.7% group on POD 2/3 ($P < 0.001$ and $P = 0.001$, respectively) and remained decreased, although the difference did not reach significance (Fig. 4A,B). Levels of BTR were significantly higher in the group with GRWR $\geq 0.8\%$ versus the group with GRWR < 0.7% during the first postoperative month [PODs 2/3 and 5 and w1, w2, and w3 ($P < 0.001$) and w4 ($P = 0.001$)] and was also significantly higher than that in the group with GRWR between 0.7% and 0.8% at w1, w3, and w4 ($P = 0.002$, $P = 0.01$, and $P = 0.04$, respectively; Fig. 4C). No other parameters differed significantly among the 3 groups at any of the time points analyzed.

Peritransplant Changes in the Parameters According to Preoperative CTP

Baseline preoperative zinc and prealbumin levels were significantly higher, whereas those of tyrosine and

ammonia were significantly lower, in the group with CTP class A versus the group with class C ($P < 0.001$, $P < 0.001$, $P = 0.04$, and $P = 0.03$, respectively). Preoperative zinc and prealbumin levels were significantly higher in the group with class A versus the group with class B ($P = 0.04$ and $P < 0.001$, respectively). In contrast, none of the other parameters significantly differed among the 3 groups at any posttransplant time point examined.

Peritransplant Changes in the Parameters in ALF Recipients and Other Recipients

Preoperative baseline levels of ammonia, tyrosine, and prealbumin were significantly higher, whereas that of BTR was significantly lower, in the group with ALF versus the group without ALF ($P = 0.03$, $P = 0.01$, $P = 0.002$, and $P < 0.001$, respectively). Otherwise, preoperative levels of all other parameters did not significantly differ between the groups at any posttransplant time point analyzed.

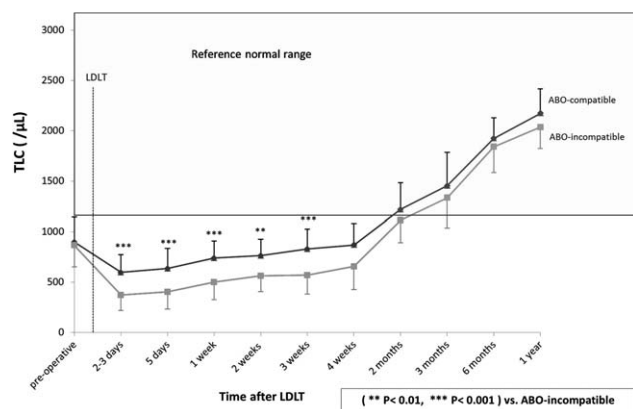


Figure 5. Time course of peritransplant changes of TLC in ABO-compatible and ABO-incompatible groups.

Peritransplant Changes in the Parameters in ABO-Incompatible and ABO-Compatible Recipients

Levels of zinc, prealbumin, BCAA, tyrosine, BTR, and ammonia did not significantly differ between the ABO-incompatible and ABO-compatible groups at any time point. However, TLC was significantly lower in the ABO-incompatible group versus the compatible group during the first 3 postoperative weeks [PODs 2/3 and 5, w1 and w3 ($P < 0.001$) and w2 ($P = 0.006$)] and remained low, although the difference was not significant (Fig. 5).

Risk Factor Analysis for Post-LT Survival

Univariate analysis revealed that none of the preoperative nutritional/metabolic parameters was a significant risk factor for posttransplant mortality (Table 3).

DISCUSSION

This is the first study to provide long-term collective profiling of nutritional/metabolic parameters in LT recipients. LDLT recipients tend to fall into a severe posttransplant catabolic phase because of the invasiveness of the operative procedure and the necessity for regeneration of the partial liver graft.⁷ This might explain the temporarily decreased zinc and BCAA levels during the early postoperative period. Significance and increased utilization of zinc and BCAA for liver regeneration have been reported,^{2,27} with the levels recovering later in the patient's course after surgery, regardless of the graft size, with the shift to an anabolic state. Presumably, the improved zinc level also followed the improvement in its absorption and decrease in its diuretic-induced urinary excretion after LT.

In the present study, the TLC level showed a prolonged decline during the initial posttransplant catabolic phase, presumably as a result of immunosuppressive therapy, and this may indicate the importance of an early, immunomodulating

TABLE 3. Univariate Analysis of Factors Affecting Posttransplant Patient Survival

Variable	Overall Survival (%)	<i>P</i> Value
Recipient age		
<60 years (n = 147)	86	0.38
≥60 years (n = 61)	92	
Donor age		
<50 years (n = 147)	91	0.48
≥50 years (n = 61)	84	
Sex		
Male (n = 98)	91	0.57
Female (n = 110)	84	
Original disease		
HCC (n = 52)	82	0.37
Non-HCC (n = 156)	90	
ABO blood type		
Compatible (n = 140)	91	0.24
Incompatible (n = 68)	82	
CTP score		
A or B (n = 69)	92	0.57
C (n = 139)	83	
MELD score		
<20 (n = 123)	91	0.77
≥20 (n = 85)	82	
GRWR		
<0.8% (n = 67)	88	0.79
≥0.8% (n = 141)	90	
Graft		
Right (n = 108)	89	0.55
Left (n = 100)	85	
Operative time		
<12 hours (n = 51)	89	0.08
≥12 hours (n = 157)	84	
Operative blood loss		
<10 L (n = 138)	92	0.09
≥10 L (n = 70)	82	
Pretransplant zinc level		
<40.5 μg/dL (n = 99)	84	0.73
≥40.5 μg/dL (n = 109)	89	
Pretransplant prealbumin level		
<5.6 mg/dL (n = 97)	83	0.34
≥5.6 mg/dL (n = 111)	91	
Pretransplant BCAA level		
<372.2 μmol/L (n = 99)	81	0.58
≥372.2 μmol/L (n = 109)	86	
Pretransplant BTR		
<2.8 (n = 100)	81	0.76
≥2.8 (n = 108)	86	
Pretransplant tyrosine		
<132 μmol/L (n = 100)	88	0.10
≥132 μmol/L (n = 108)	84	
Pretransplant TLC		
<700 μL (n = 100)	86	0.68
≥700 μL (n = 108)	86	
Pretransplant ammonia level		
<89 μg/dL (n = 104)	91	0.51
≥89 μg/dL (n = 104)	85	

enteral diet. Then, TLC gradually improved in parallel with an increase in lymphocyte proliferation upon recovery from protein-energy malnutrition and tapering of the immunosuppressive therapy.⁴ The comparatively suppressed TLC in ABO-incompatible recipients during the first 3 weeks after transplantation might be attributable to the addition of immunosuppressants such as rituximab approximately 2 weeks before LT and complete B-cell elimination approximately 3 weeks after administration.²⁸

Although CTP classification is one of the best tools for predicting mortality in patients with cirrhosis, one of its main limitations is the lack of an assessment of the nutritional and functional status.²⁹ Recovery of nutritional/metabolic parameters after successful LDLT occurred regardless of the pretransplant CTP class.

BCAA escape hepatic extraction with primary muscle uptake.⁸ Thus, BCAA recovery regardless of graft size after the initial posttransplant decline might be attributed mainly to an improvement in cirrhosis-induced disturbances of muscular amino acid metabolism, namely, the hyperammonemia-activated glutamine synthesis, with a subsequent decrease in BCAA utilization and catabolism in skeletal muscle.³⁰ However, an influence of preoperative BCAA supplementation on baseline BCAA levels and levels in the early postoperative period should be also considered.

Tyrosine and ammonia levels decreased immediately after LDLT, presumably because of improvements in the visceral uptake capacity and hepatic metabolism. However, possible influences of preoperative malnutrition, preexisting extrahepatic shunts, or enteral nutrition cannot be excluded. Consistently, tyrosine clearance significantly improved 2 to 3 days after transplantation,³¹ and ammonia levels improved within the first month after LDLT.³² The tyrosine level was stabilized in the long term after LDLT, probably because of the improved graft hemodynamics and decreased basal proteolysis. The significant drop in ammonia and tyrosine levels seen with GRWR > 0.8% might be expected because of the greater liver mass for detoxifying or metabolizing these substrates.

Some limitations must be borne in mind. First, this was a retrospective, single-center study with an observational protocol. We gradually improved the perioperative nutritional regimen on the basis of our most recent findings to include preoperative BCAA and early post-LDLT enteral nutrition with an immunomodulating enteral diet enriched with hydrolyzed whey peptide. Therefore, a prospective analysis is needed to confirm the present findings. However, the study scale would be sufficient for follow-up of 208 recipients with diverse indications and under homogeneous immunosuppression regimens. Second, we were not able to collect all data at all time points. The exact duration and the amount of BCAA given in the preoperative nutritional therapy protocol varied among the individual patients, but because this was a retrospective study, these factors could not be adjusted. Third, graft function is dependent not only

on size but also on graft quality. However, all grafts used had an L/S ratio ≥ 1.1 , and both donor age and ischemia times were insignificantly different; therefore, large and small grafts were of nearly the same quality. Moreover, the baseline pretransplant parameter levels were insignificantly different. Fourth, we could not assess liver regeneration rates because neither CT nor magnetic resonance scanning was routinely performed at designated posttransplant follow-up visits. Fifth, many therapeutic interventions may have short-term effects on the functional development of hepatocytes or graft regeneration or directly alter the course of the parameters. However, we calculated the slopes within the first year after LT, when most patients received similar traditional therapies and the same perioperative nutritional therapy.

In conclusion, graft size had little impact on the recovery of nutritional parameters except for the ammonia and tyrosine levels. Further prospective studies are warranted to elucidate the role of nutritional parameters in the assessment of graft function and survival.

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