Branched Chain Amino Acids Enhance the Maturation and Function of Myeloid Dendritic Cells *Ex Vivo* in Patients with Advanced Cirrhosis

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An imbalance of plasma amino acids is observed in patients with advanced cirrhosis. The aim of this study was to investigate the influence of the extracellular amino acid imbalance on the function of myeloid dendritic cells (DCs) in patients with advanced cirrhosis. We made a serum-free culture medium consistent with the average concentration of plasma amino acids from healthy controls (HC, n = 25) or patients with advanced cirrhosis (LC, n = 43) to reflect more closely the actual environment of the living body. We compared the phenotypical and biological functions of blood dendritic cells antigen-positive dendritic cells (BDCA+ DCs) and monocyte-derived dendritic cells (MoDCs) from LC and HC with these media. After adding stimulants, the CD83 and CD86 expressions of DCs from LC were lower than those from HC. In both HC and LC, both CD83 and CD86 expressions of DCs stimulated under the cirrhotic medium were lower than under the control medium. This phenomenon was accompanied by a suppression of the mammalian target of rapamycin (mTOR)/S6K-signaling pathways. The interleukin 12 (IL-12) production in the cirrhotic medium was significantly lower than in the control medium and increased when valine or leucine was added to the medium. In patients with advanced cirrhosis, peripheral blood mononuclear cells stimulated in the autologous plasma after oral administration of branched-chain amino acid (BCAA) granules had significantly increased interferon gamma production. *Conclusion:* In advanced cirrhosis, there is impairment of the function and maturation of DCs, which has been shown to be related to an imbalance in the extracellular amino acid profile. Elevating the extracellular concentration of BCAAs *ex vivo* in patients with advanced cirrhosis improved the function of DCs. (HEPATOLOGY 2009;50:1936-1945.)

Cirrhosis makes it increasingly difficult for the liver to carry out its essential functions, such as detoxifying harmful substances and manufacturing vital nutrients. Cirrhosis progresses to decompensated cirrhosis and ultimately liver failure because of a lack of suitable treatment. Not only hepatocellular carcinoma but also nosocomical infections, such as spontaneous bacterial peritonitis (SBP) or pneumonia, are frequent clinical complications in these immune-compromised patients.[1] In patients with advanced cirrhosis, various metabolic disorders involving glucose, protein-amino acids, lipids, vitamins, and minerals might appear. Furthermore, an imbalance of plasma amino acids, with decreased levels of branched-chain amino acids (BCAAs)

Abbreviations: AAA, aromatic amino acid; ACM, advanced cirrhotic media; APC, antigen-presenting cell; BCAA, branched-chain amino acid; BDCA, blood dendritic cells antigen; DC, dendritic cell; HCM, healthy control media; IFN-γ, interferon gamma; IL, interleukin; MLR, mixed lymphocytes reaction; MoDC, monocyte-derived dendritic cell; mTOR, mammalian target of rapamycin; NKT, natural killer T; PBMC, peripheral blood mononuclear cell; SBP, spontaneous bacterial peritonitis.

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and increased levels of aromatic amino acids (AAAs), is commonly seen in patients with advanced cirrhosis.[2] In clinical situations, long-term nutritional supplementation with oral BCAA has been shown to be useful to prevent progressive hepatic failure and to improve surrogate markers and the perceived health status.[3,4] Moreover, the oral administration of BCAA granules was reported to inhibit hepatic carcinogenesis in patients with compensated cirrhosis.[5,6]

On the one hand, it has become clear that amino acids are not only important as substrates for various metabolic pathways but also activate a nutrient-sensitive signaling pathway in synergy with insulin.[7-10] The mammalian target of rapamycin (mTOR) signaling pathway is one of the most representative pathways, and this pathway has been shown to act as a major effector of cell growth and proliferation by way of the regulation of protein synthesis.[7-9] The phosphorylation of downstream effectors of mTOR is inhibited by rapamycin and activated by BCAA, especially by leucine,[11-13] although little is known about the impact of changes in the extracellular amino acid levels on the immune system.[14] Recently, we have shown that extracellular BCAAs, especially valine, regulate the maturation and function of monocyte-derived dendritic cells (MoDCs).[15] Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that stimulate innate and adaptive immune reactions by priming other types of blood cells. Typically, immature DCs migrate to lymphoid tissues and present antigenic peptides to naive T cells.[16] The mature DCs, which characteristically express CD83,[17] can rapidly activate other innate immune cells including natural killer (NK) cells and natural killer T (NKT) cells through the production of immunomodulatory cytokines such as interleukin (IL)-10 and IL-12. Several studies have reported that the immunological abnormalities occurring in cirrhosis,[18,19] such as a depressed reticuloendothelial system, neutrophil dysfunction, reduced serum complement, and low bactericidal function, account for the increased susceptibility of patients with cirrhosis to bacterial seeding and diffusion, and for the impaired functions of DCs in patients with liver cirrhosis.[15,20,21] However, it is not clear why the responses of immune cells, particularly DCs, are suppressed in patients with cirrhosis.

Roswell Park Memorial Institute medium 1640 (RPMI 1640) with human or bovine serum is typically used to culture peripheral blood mononuclear cells (PBMCs) or DCs and examine the function. The concentrations of almost all the amino acids in RPMI 1640 are higher than those typically found in the plasma of healthy adult humans. Accordingly, there are large differences between the amino acids of living bodies and those of culture systems. The concentration of amino acids except BCAAs in the medium used in our previous study was higher than that of plasma *in vivo*.[15] Furthermore, various types of amino acid imbalance actually appear in the plasma of patients with advanced cirrhosis. The aim of the study, therefore, was to investigate the influence of the extracellular amino acid imbalance observed in patients with advanced cirrhosis on the function of DCs using a serum-free culture medium consistent with the average concentration of plasma amino acids from healthy volunteers (healthy control media, HCM) or patients with advanced cirrhosis (advanced cirrhotic media, ACM) to reflect more closely the actual environment of the living body. Furthermore, we investigated whether oral administration of BCAA granules could enhance the responses of immune cells in patients with advanced cirrhosis.

Patients and Methods

Serum-Free Culture Media. The concentrations of the plasma amino acids from fasting healthy volunteers (n = 25), chronic hepatitis (n = 14), and patients with cirrhosis (n = 60) were measured by high-performance liquid chromatography (HPLC) in the early morning (Table 1). Briefly, sulfosalicylic acid was added to plasma to a final concentration of 5%. The samples were then placed on ice for 15 minutes followed by centrifugation to remove precipitated proteins. The extracts were then analyzed for the amino acid content with a JLC-500/V (Japan Electron Optics Laboratories, Tokyo, Japan). Also, these patients with cirrhosis were classified according to the Child-Pugh classification. We defined as Child-Pugh grade B or C the patients with advanced cirrhosis (n = 43: hepatitis c virus [HCV] n = 22; primary biliary cirrhosis [PBC] n = 5; alcoholic n = 3; nonalcoholic steatohepatitis [NASH] n = 3; hepatitis b virus [HBV] n = 2; primary sclerosing cholangitis [PSC] n = 2; HCV+HBV n = 1; autoimmune hepatitis [AIH] n = 1; Wilson's disease n = 1; Budd-Chiari syndrome n = 1; cryptogenic n = 2). A serum-free culture medium consistent with the average concentration of plasma amino acids from healthy volunteers was defined as the HCM; whereas that from patients with advanced cirrhosis was defined as the ACM (Table 2). Other components except amino acids were identical among media. We verified that there was no difference between the theoretical value and actual value in HCM and ACM. We cultured PBMCs under the two media with stimulant for 48 hours and measured the amino acid concentrations of these media. There was no difference in the concentrations of amino acids before and after culture in these media. The viability of PBMCs was determined using Annexin V^{FITC}, with dead cells identi-

Table 1. Aminogram for the Plasma in Chronic Hepatitis Patients and Patients with Cirrhosis

	HC (n=25)	CH (n=14)	Child A (n=17)	Child B (n=19)	Child C (n=24)
Glycine	225	250	205	234	313
Alanine	391	400	311	317	339
Serine	119	135	139	137	169
Threonine	142	139	137	135	165
Cystine	38	54	63	62	73
Methionine	29	31	40	60	68
Glutamine	564	585	616	642	739
Asparagine	51	57	62	58	77*
Glutamic acid	42	70	62	65	47
Aspartic acid	3	3	5	4	3
Valine	249	243	222	195†	164†
Leucine	132	141	120	110	93†
Isoleucine	76	71	63	56	51†
Phenylalanine	63	70	80	89	99*
Tyrosine	65	81	111	112	151*
Tryptophan	62	52	52	43	47
Lysine	183	223	219	199	179
Arginine	78	79	94	93	100
Histidine	83	90	77	81	93
Proline	204	163	142	165	202
Fischer's ratio	3.57	3.01	2.36†	1.95†	1.27†

The concentrations of plasma amino acids from fasting healthy volunteers (n=25), chronic hepatitis (n=14) and patients with cirrhosis (n=60) were measured by HPLC in the early morning after fasting. Also, these patients with cirrhosis were classified according to the Child-Pugh classification. Amino acid concentrations are expressed in nmol/mL.

*$P < 0.01$ increased. †$P < 0.01$ decreased. Fischer's ratio means: Valine+Leucine+Isoleucine / Tyrosine+Phenylalanine †decrease *increase $P < 0.01$ vs. CH (the data were analyzed with ANOVA and Dunnett's post-hoc procedure).

fied by propidium iodide (PI) staining (Annexin V-FITC Apoptosis Detection Kit, BioVision, Mountain View, CA), according to the manufacturer's instructions. We confirmed the viability of PBMCs cultured in HCM and ACM equal to that of complete culture medium (CCM) and X-VIVO 10 (Cambrex Bio Science Walkersville, Walkersville, MD). The percentages of living cells were 78.7 ± 0.67, 77.7 ± 2.2, 71.7 ± 0.67, and 74.7 ± 0.33 for HCM, ACM, CCM, and X-VIVO10, respectively. The culture media, CCM, and other depleted media were made as described.[15]

Patients and Healthy Volunteers. We selected 15 patients with cirrhosis for *in vitro* or *ex vivo* studies (Table 3). All of these patients were inpatients. There were no significant differences on clinical and laboratory findings in this population compared to the 43 patients with advanced cirrhosis (Table 1): age 60.4 ± 12.8 versus 59.1 ± 11.3; aspartate aminotransferase (AST) 78.8 ± 45.4 IU/L versus 96.3 ± 65.0 IU/L; alanine aminotransferase (ALT) 47.6 ± 25.2 IU/L versus 54.3 ± 36.7 IU/L; total bilirubin 4.5 ± 5.36 mg/dL versus 3.94 ± 3.70 mg/dL; albumin 2.80 ± 0.51 g/dL versus 2.85 ± 0.55 g/dL; prothombin time / international normalized ratio (PT-INR) 1.54 ± 0.39 versus 1.37 ± 0.29; PLT 93.9 ± 68.7 × $10^3/\mu L$ versus 113.1 ± 54.2 × $10^3/\mu L$; Child Pugh score 9.0 ± 1.77 versus 8.6 ± 2.10; Model for End-Stage Liver Disease (MELD) score 11.9 ± 5.55 versus 11.2 ± 4.23; plasma Fischer's ratio 1.56 ± 0.77 versus 1.65 ± 0.57. The MELD score[22] was calculated by an online worksheet available on the Internet at www.mayoclinic.org/meld/mayomodel5.html. None of the patients had clinical or laboratory findings compatible with bacterial infection when we collected PBMCs from the patients. Written informed consent was obtained from each individual and the study protocol was approved by the Ethics Committee of Tohoku University School of Medicine (2003-326, 2008-337).

BDCA+ DCs Maturation and MoDCs Generation. PBMCs were separated from the peripheral blood of HC and LC by centrifugation on a density gradient. The blood dendritic cells antigen-positive dendritic cells (BDCA+ DCs) and the CD14-positive monocytes were isolated from PBMCs using magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). BDCA1+ DCs were cultured at a density of 2.5 × 10^5 cells/well in 96-well flat-bottom plates (Corning, NY) for 48 hours with 1,000 U/mL GM-CSF (PreproTech, London, UK), 500 U/mL (hu) IL-4 in each media. At 24 hours culture,

Table 2. Serum-Free Culture Media Used in This Study (nmol/mL)

	CCM	HCM	ACM
Glycine	400	225	280
L-Alanine	400	391	307
L-Serine	400	119	151
L-Threonine	800	142	138
L-Cystine 2HCl	200	38	67
L-Methionine	200	29	75
L-Glutamine	4000	564	689
L-Asparagine	400	51	64
L-Glutamic Acid	400	42	53
L-Aspartic Acid	400	3	4
L-Valine	800	249	175
L-Leucine	800	132	100
L-Isoleucine	800	76	53
L-Phenylalanine	400	63	99
L-Tyrosine	400	65	133
L-Tryptophan	80	62	45
L-Lysine-HCl	800	183	184
L-Arginine-HCl	400	78	92
L-Histidine HCl-H2O	200	83	85
L-Proline	400	204	176
Fischer's ratio	3.00	3.57	1.42

Complete culture medium (CCM) contains 20 amino acids that are relevant to the make-up of mammalian proteins. HCM (healthy control medium): consistent with the average concentration of plasma amino acids from healthy volunteers (n=25). ACM (advanced cirrhotic medium): consistent with the average concentration of plasma amino acids from patients with advanced cirrhosis (Child-Pugh grade B or C, n=43). The amino acid concentrations are expressed in nmol/mL. Fischer's ratio means: Valine+Leucine+Isoleucine / Tyrosine+Phenylalanine.

Table 3. Characteristics of Study Participants

Patient Number	Disease	Sex	Age (years)	AST/ALT	Total Bilirubin	Albumin	PT-INR	PLT	Child-Pugh Classification	MELD Score	Plasma Fischer's Ratio	BCAA Medication
1	LC-C	M	71	116/61	0.8	3.3	1.09	149	A	6	2.49	-
2	LC-C+HCC	M	70	73/46	1.5	2.3	1.15	75	B	6	2.26	-
3	LC-C+HCC	F	80	72/55	1.3	2.8	1.19	144	B	9	NA	+
4	LC-C	M	42	52/38	4.2	1.8	1.79	79	C	16	0.99	+
5	LC-C+HCC	F	61	238/98	6.3	2.9	1.65	76	B	18	2.74	+
6	PBC	F	43	241/144	12.3	2.8	1.32	152	C	18	1.57	-
7	LC-C	M	56	71/45	2.2	3.7	1.24	81	B	10	1.90	+
8	LC-C	M	48	111/109	1.6	3.7	1.08	81	A	8	NA	-
9	LC-C	F	60	25/5	11.6	3.2	2.05	83	C	15	0.88	+
10	LC-C+HCC	F	69	68/40	1.3	2.8	1.17	132	B	7	1.81	-
11	non B non C	F	44	28/18	2.4	2.6	1.54	122	C	8	1.31	+
12	PBC	F	62	130/49	6.8	2.0	1.33	120	C	8	1.43	+
13	PBC	F	62	83/30	2.3	2.5	1.11	207	B	13	1.29	+
14	Alcoholic	M	54	53/24	2.5	3.1	1.60	219	C	14	1.24	+
15	LC-C+HCC	M	65	83/53	2.0	3.2	1.29	96	B	12	1.52	+

LC-C, liver cirrhosis due to HCV; HCC, hepatocellular carcinoma; PBC, primary biliary cirrhosis; NASH, nonalcoholic steatohepatitis; NA, not available; PLT, platelet counts ($x10^3/\mu L$); PT-INR, prothrombin time-international normalized ratio; AST/ALT, aspartate aminotransferase / alanine aminotransferase (IU/L); total bilirubin (mg/dL); albumin (g/dL); Fischer's ratio: Valine+Leucine+Isoleucine / Tyrosine+Phenylalanine.

DCs were stimulated by 500 ng/mL lipopolysaccharide (LPS; *Escherichia coli* 026:B6; Sigma, St. Louis, MO) or polyinosinic:polycytidylic acid (polyI:C) (30 μg/mL). Monocytes were cultured at a density of 3.0×10^5 cells/well with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 for 6 days in CCM. On day 6 we changed the medium from CCM to HCM or ACM with poly(I:C) and the culture was continued for an additional 48 hours.

Surface Marker Analysis. DCs were harvested and labeled with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-labeled monoclonal antibodies (mAbs) (antihuman CD14, CD40, CD83, CD86, CD98, HLA-DR, or the relevant isotype controls; BD PharMingen, San Diego, CA) according to the manufacturer's instructions. Using a FACS Calibur (BD Immunocytometry Systems, San Diego, CA) flow cytometer, surface marker expressions were analyzed using the CellQuest (BD Immunocytometry Systems) program.

Phagocytosis Assay with Dextran. To evaluate the endocytosis potential of DCs, 1 mg/mL of FITC-dextran was supplied to 2.5×10^5 DCs that were then incubated for 30 minutes at 37°C. As a control, the DCs were given the same doses of FITC-dextran and stored for 30 minutes at 4°C. After the incubation the DCs were washed and subjected to FACS analysis.

Cytokine Analysis. BDCA1+ DCs were cultured at a density of 2.5×10^5 cells/well in 96-well flat-bottom plates for 48 hours with 1,000 U/mL GM-CSF, 500 U/mL (hu) IL-4 in each of the media. At 24 hours, 500 ng/mL LPS or poly(I:C) (30μg/mL) were added. The supernatants were collected after 48 hours and immediately IL-12 (p40+p70) and IL-10 were determined by specific cytokine enzyme-linked immunosorbent assay (ELISA) kits (Bender MedSystems) according to the manufacturer's instructions. For the interferon gamma (IFN-γ) production of PBMCs, PBMCs were cultured at a density of 2.5×10^5 cells/well in HCM or ACM for 48 hours, and at 5.0×10^5 cells/well in autologous plasma for 12 hours. IFN-γ was determined by specific cytokine ELISA kits (Bender MedSystems).

Mixed Lymphocytes Reaction (MLR). BDCA+ DCs were cultured at a density of 1.0×10^5 cells/well in 96-well round-bottom plates (Falcon) containing HCM or ACM with GM-CSF and IL-4 for 48 hours. At 24 hours culture, immature DCs were induced to mature using LPS or poly(I:C) for an additional 24 hours. The allostimulatory capacity of irradiated DCs (3,000 Rad) was tested in a one-way MLR with normal 2×10^5cells/well allogeneic CD4+ lymphocytes (isolated from PBMCs using magnetic beads) under CCM. Cocultured cells were maintained for 7 days and the proliferation rate of the cells was measured using an 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) Assay (CellTiter 96 aqueous one-solution cell proliferation assay; Promega, Madison, WI) according to the manufacturer's instructions. On carboxyfluorescein succinimidyl ester (CFSE) staining, cells were analyzed using a CellTrace CFSE Cell Proliferation Kit (Molecular Probes, Eugene, OR). The staining methods followed the manufacturer's protocol.

Immunoblotting. DCs were cultured at a density of 3.0×10^5 cells/well in 96-well flat-bottom plates (Corn-

Table 4. Phenotypic Difference of BDCA1+DCs Derived from Patients with Cirrhosis and Healthy Volunteers

			CD40	CD83	CD86	HLA-DR
Isolated DC	Healthy control (n=4)		5 ± 1.4	6 ± 2.2	14 ± 3.1	166 ± 52.2
	LC patients (n=4)		12 ± 16.1	4 ± 1.4	12 ± 3.4	195 ± 79.3
Mature DC	Healthy control (n=5)	HCM	131 ± 54	240 ± 25	201 ± 67	910 ± 121
		ACM	121 ± 37	*190 ± 33**	*170 ± 53**	783 ± 90
	LC patients (n=5)	HCM	139 ± 44	*154 ± 48†*	*169 ± 37†*	*691 ± 112†*
		ACM	124 ± 47	*125 ± 45‡*	*122 ± 11‡*	625 ± 160

The MFI are presented for each marker as the mean ± SD of healthy controls and patients with cirrhosis (isolated DC: Patients 6, 7, 8, 10 / mature DC: Patients 8, 9, 10, 11, 12).

*Value of $P < 0.05$ vs. DCs of healthy control cultured under HCM (Wilcoxon t test).

†Value of $P < 0.05$ vs. DCs of healthy control cultured under HCM (Mann-Whitney U test).

‡Value of $P < 0.05$ vs. DCs of LC patients cultured under HCM (Wilcoxon t test).

ing) containing 200 μL medium supplemented with GM-CSF and IL-4 for 24 hours and the DCs were stimulated by poly(I:C) for 1 hour. The DCs were harvested and lysed using CelLyticTM-M Mammalian Cell Lysis/ Extraction Reagent (Sigma). The lysed cells were centrifuged to pellet the cellular debris. Thereafter, these protein concentrations were determined by a Modified Lowry Protein Assay Kit (Pierce, Rockford, IL). Equal amounts of protein were loaded onto sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransferred to PVDF (Immun-Blot PVDF Membrane; Bio-Rad, Hercules CA). After washing and blocking, immunostaining was performed with rabbit polyclonal primary antibody (PI3K, phospho-PI3K, mTOR, p70 S6K, phospho-p70 S6K; Cell Signaling Technology, Beverly, MA), followed by incubation with a secondary antibody conjugated to horseradish peroxidase (HRP) (Sigma). Immunoreactive proteins were revealed with an ECL reagent (ECL advance; Amersham Biosciences, Little Chalfont, UK).

Oral Administration of BCAA to Patients with Advanced Cirrhosis and* Ex Vivo *Cytokine Production Assay. In the early morning we measured the fasting concentration of the plasma amino acids before and after oral administration of BCAA granules (30, 60, 120, 180 minutes) from healthy volunteers and patients with advanced cirrhosis. The BCAA granules: LIVACT (Ajinomoto Pharma, Tokyo, Japan) were composed of a mixture of valine, 1.144 g, leucine, 1.904 g, and isoleucine, 0.952 g. The concentrations of the plasma amino acids were measured by HPLC. We stimulated PBMCs from patients for 12 hours by LPS or poly(I:C) under autologous plasma, which was collected both before and after oral administration. After 12 hours we recovered the plasma and measured the IFN-γ by ELISA.

Statistical Analysis. The data were analyzed with analysis of variance (ANOVA) and multiple comparisons were performed with Dunnett's post-hoc procedure for the plasma aminogram. When two groups were analyzed, the differences between media were analyzed by the Wilcoxon t test. Frequencies of BDCA1+ DCs were compared between patient groups by the Mann-Whitney U test. All statistical analyses were performed with standard statistical software (SPSS 13.0 for Windows, Chicago, IL).

Results

Amino Acid Concentrations Similar to Those in Plasma of Patients with Advanced Cirrhosis Impaired the Maturation of Myeloid DCs from Healthy Controls. First we measured the cytokine production from PBMCs both under HCM and ACM. The IFN-γ production of PBMCs stimulated by poly(I:C) under ACM was significantly impaired (28.1 ± 7.3 pg/mL versus 16.7 ± 3.9 pg/mL; $P = 0.04$). Next, we cultured the BDCA+ DCs (purity >90%) for 48 hours under HCM and ACM and evaluated the phenotypes of DCs by flow cytometry. In ACM, the CD83 and CD86 expression of DCs was significantly impaired compared to that in HCM (Table 4). The HLA-DR expression had a tendency to decrease in ACM. This phenomenon was observed in MoDCs (Supporting Fig. 1). Next, The IL-12 production of BDCA+ DCs stimulated under ACM was significantly impaired (110.7 ± 8.6 pg/mL versus 79.9 ± 12.5 pg/mL; $P = 0.04$), although the IL-10 production of DCs was not different between HCM and ACM (31.0 ± 4.0 versus 32.4 ± 8.2; $P = 0.59$). Flow cytometric analysis revealed that the amount of FITC-dextran taken up by BDCA+ DC and MoDC did not differ between HCM and ACM (data not shown). The allostimulatory capacity of BDCA+ DCs cultured under ACM was significantly decreased as shown by the MTS assay (1.00 ± 0.15 versus 0.82 ± 0.13; $P = 0.04$; absorbance 490 nm), and this tendency was also confirmed by the CFSE assay.

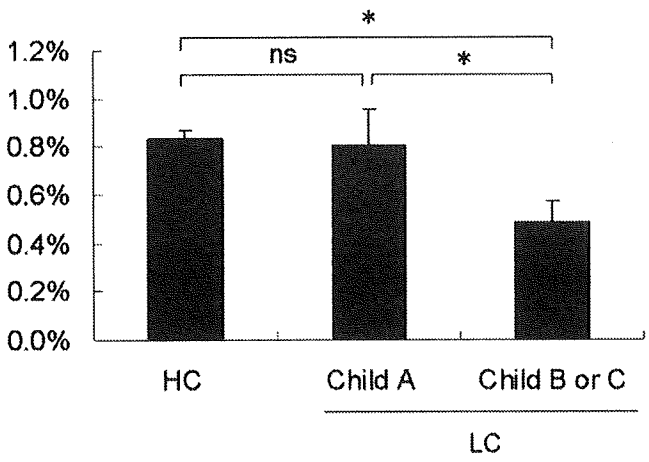

Fig. 1. The frequencies of DCs were significantly lower in the peripheral blood from patients with advanced cirrhosis compared with those from HC or early patients with cirrhosis. Percentages of BDCA+ DC in PBMCs were determined by flow cytometry. Significant differences in the percentages of DCs were observed between patients with advanced cirrhosis (Child-Pugh grade B or C: n = 10) and HC (n = 7). There was no difference between patients with Child-Pugh grade A (n = 7) and HC. Data are expressed as mean ± standard error of the mean (SEM).

Amino Acid Concentrations Similar to Those in Plasma of Patients with Advanced Cirrhosis Also Impaired the Maturation of Myeloid DCs from Patients with Cirrhosis. We first evaluated the frequency of BDCA+ DCs between HC and LC (Fig. 1). The frequencies of DCs were significantly lower in the peripheral blood from patients with advanced cirrhosis compared to those from HC or patients with early cirrhosis. Second, we determined the phenotype of BDCA1+ DCs from the LC before and after adding the stimulants. There was no difference regarding the mean fluorescence intensity (MFI) of isolated immature DCs expressing CD40, CD83, CD86, and HLA-DR between the HC and LC (Table 4). After adding the stimulants, the expressions of CD83 and HLA-DR by DCs from the LC were significantly decreased compared to those from the HC in both HCM and ACM (Table 4). The CD83 and CD86 expression of DCs was significantly impaired in ACM compared to that in HCM (Table 4).

Elevating the Concentration of BCAA Enhanced the IL-12 Production in BDCA+ DCs. As in the *in vivo* study, we confirmed that the plasma concentrations of BCAAs were significantly decreased and AAAs (except tryptophan) were increased along with the Child-Pugh grade (Table 1). Based on these data, to investigate which amino acid especially influenced the function of BDCA1+ DCs, we measured the cytokine production of DCs under HCM, ACM, and ACM supplemented with 800 nmol/mL of a single amino acid: valine, leucine, isoleucine, or AAAs. Interestingly, the IL-12 production of DCs stimulated under ACM plus valine or leucine was more increased than that under ACM, although there was no difference among ACM plus isoleucine, ACM plus AAAs, and ACM (Fig. 2A). Similar to the cytokine production, the allostimulatory capacity of DCs cultured under ACM plus valine or leucine had a tendency to be increased, as shown by the MTS assay (ACM: 0.71 ± 0.07, ACM plus valine: 0.88 ± 0.06; ACM plus leucine: 0.83 ± 0.03; absorbance 490 nm). Next, we determined the BDCA1+ DCs phenotype (CD14 and CD83) in CCM, BCAA-depleted, valine-depleted, leucine-depleted, and isoleucine-depleted media. In CCM, leucine-depleted and isoleucine-depleted media the DC phenotypes were similar (the percentages of CD83-positive cells were 33.7 ± 7.2%, 31.5 ± 5.4%, and 35.5 ± 7.9% for CCM, leucine-depleted, and isoleucine-depleted media, respectively). However, in BCAA-depleted and valine-depleted media, the CD83 expression of DCs was significantly impaired compared to that in CCM (BCAA-depleted media: 19.6 ± 3.0% and valine-depleted media 14.6 ± 1.8%; $P = 0.04$ versus CCM). After we cultured the DCs under depletion of valine for 2 days, we added valine to the medium and cultured the cells for an additional 24 hours. Then, the percentage of mature DCs was higher than that of valine-depleted media. Furthermore, to reflect more closely the actual environment of the living body, we induced DCs from LC to mature with either autologous plasma or autologous plasma supplemented with 100 nmol/mL valine for 12 hours. In all cases the DCs matured in the autologous plasma with valine had enhanced allostimulatory capacity and IL-12 production (Fig. 2B).

Amino Acid Concentration of Plasma in Patients with Advanced Cirrhosis Down-regulated the mTOR/S6K Signaling Pathway of BDCA1+ DCs. We hypothesized that the amino acid imbalance of the plasma in patients with advanced cirrhosis influence the mTOR/S6K signaling pathway of DCs and impaired their maturation. Under HCM with rapamycin, the percentage of CD14-/CD83+ mature DCs was higher than under HCM without rapamycin (Fig. 3A). BDCA+ DCs expressed similar levels of total PI3K, phospho-PI3K, mTOR, p70 S6K, and β-actin among all media. Interestingly, DCs cultured in ACM expressed lower levels of phospho-p70 S6K than those cultured in HCM (Fig. 3B). The expression of phospho-p70 S6K by DCs in ACM was partially recovered by adding 400 nmol/mL BCAA to the medium during stimulation. Isolated immature BDCA+ DCs expressed moderate levels of CD98 which modulate the amino acid transport functions and, after adding the stimulants, mature DC showed the up-regulation of CD98. There was no difference regarding

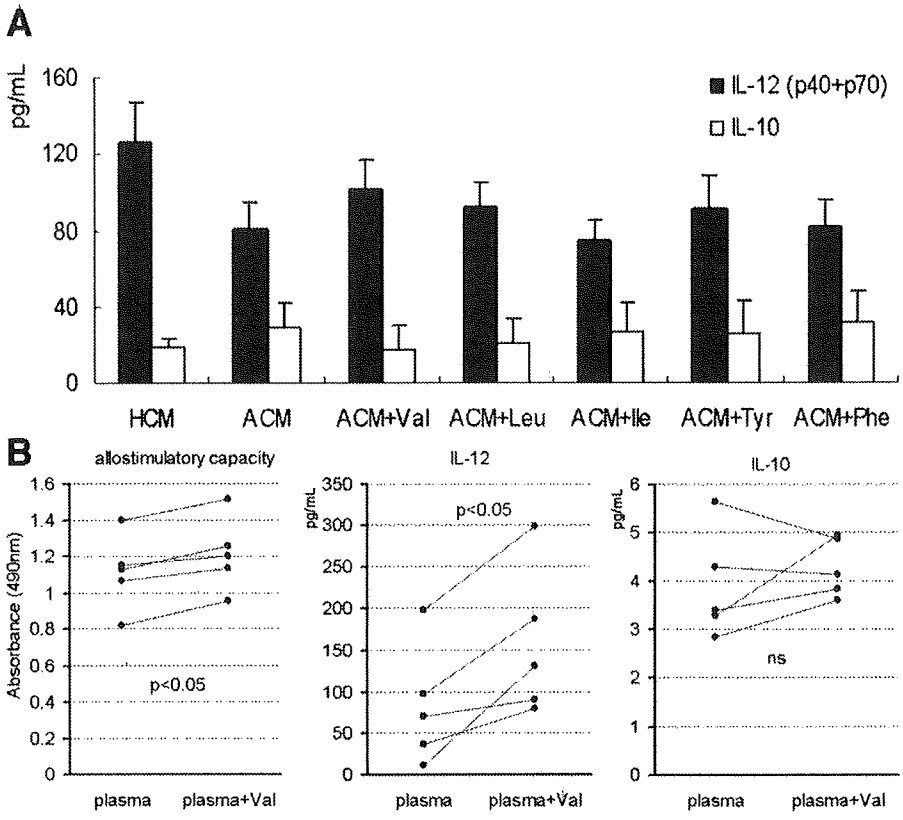

Fig. 2. Elevating the concentration of BCAAs enhanced the IL-12 production in BDCA1+ DCs. Isolated BDCA1+ DCs were cultured under HCM, ACM, and ACM supplemented with 800 nmol/mL single amino acid: valine, leucine, isoleucine, or AAAs. (A) After 48 hours the supernatants were assayed for cytokine concentrations. Mean ± SEM values from five different donors. (B) We induced BDCA1+ DCs from LC patients (Patients 1-5) to mature with either autologous plasma or autologous plasma supplemented with 100 nmol/mL valine for 12 hours. Supernatants were measured by ELISA. $P < 0.05$ (paired Student's t test, two-tailed).

the expression of CD98 between HCM and ACM (data not shown).

Oral Administration of BCAAs Enhanced the Production of IFN-γ by PBMCs from Patients with Advanced Cirrhosis Ex Vivo. Finally, we evaluated whether BCAAs have an effect on the immune response *ex vivo*. In healthy volunteers the concentration BCAAs of plasma was maximum 30 minutes after oral administration (Fig. 4A). Fischer's ratio increased from 4.78 ± 1.41 (standard deviation [SD]) to 13.39 ± 2.41 (SD). On the other hand, in the patients with advanced cirrhosis (Table 3: Patients 10-13), the concentration BCAAs of plasma was maximum 60 minutes after oral administration. Fischer's ratio increased from 1.37 ± 0.98 (SD) to 4.94 ± 0.99 (SD). AAAs decreased slowly during the following 3 hours. We stimulated PBMCs from the patients with advanced cirrhosis (Table 3: Patients 11-15) using either autologous plasma before and after 60 minutes oral administration. Interestingly, in all cases PBMCs stimulated by LPS in the latter had more IFN-γ production than the former (Fig. 4B).

Discussion

In this study we started by making two serum-free media (HCM and ACM) to be more representative of the human physiological environment and quantitatively measured the plasma amino acid profiles. First, we found that the amino acid imbalance of plasma in patients with advanced cirrhosis impaired the production of IFN-γ from PBMCs. IFN-γ is a dimerized soluble cytokine that is the only member of the type II class of interferons.[23] IFN-γ is secreted by Th1 cells, DCs, and NK cells. Although the commitment toward either the Th1 or the Th2 phenotype can be influenced by many signals active at the moment of naive Th cell priming, the levels of IL-12p70 (IL-12) produced by APC, especially DCs, are of major importance.[24,25] Therefore, we hypothesized that the impaired production of IFN-γ from PBMCs caused the dysfunction of DCs. Expectedly, the maturation and the IL-12 production of DCs were impaired in ACM. Furthermore, we confirmed that the allostimulatory capacity of DCs stimulated in ACM was impaired by MTS and CFSE assays. Previous studies have suggested an increase in IL-10 in cirrhosis and a potential link between high IL-10 and low HLA-DR expression in relation to immune dysfunction,[26] but in this study there was no difference in IL-10 secretion between DCs from ACM compared with HCM. Such differences were probably caused by (1) differences in the stimulation period of the immune cells (the former was *ex vivo*, this study was *in vitro*); (2) differences in the cell sources (the former was monocytes, this study was DCs); (3) other factors besides amino acids influence IL-10 production. Also in patients

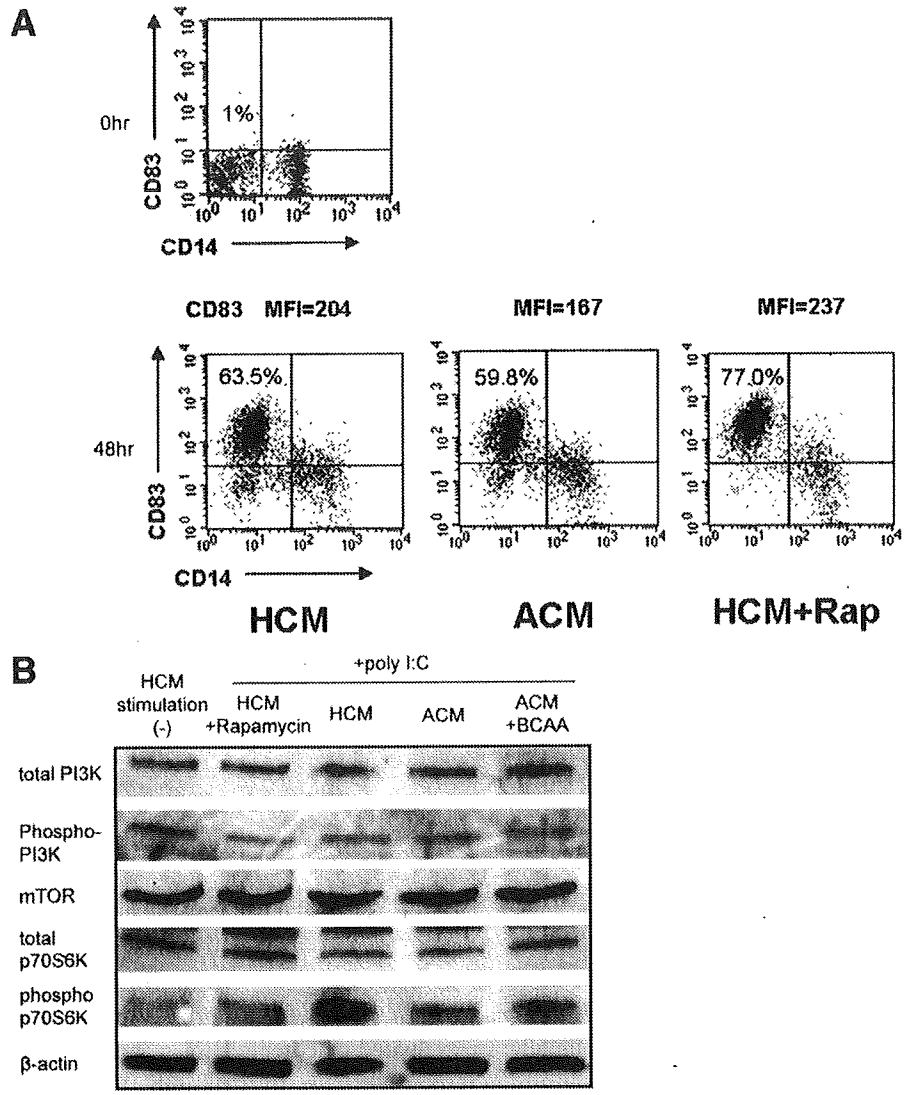

Fig. 3. Amino acid imbalance in plasma of patients with advanced cirrhosis down-regulated the mTOR/S6K signaling pathway of BDCA1+ DCs. (A) We stimulated BDCA1+ DCs under HCM, ACM, and HCM plus rapamycin (500 nM) for 24 hours with GM-CSF and IL-4, and exposed them to poly(I:C) for an additional 24 hours. We evaluated the phenotypes of DCs by flow cytometry. The percentages indicate the proportion of cells adopting the DC immunophenotype (CD14−/CD83+). (B) We cultured BDCA1+ DCs under HCM and ACM for 24 hours with GM-CSF and IL-4 and stimulated them with poly(I:C) for 1 hour. We also evaluated HCM plus rapamycin, and ACM plus BCAA. Equal amounts of protein were loaded and the levels of PI3K, phospho-PI3K, mTOR, p70 S6K, and phosho-p70 S6K were determined by Western blot analysis. (A,B) Data shown are representative of four independent experiments with cells from different donors.

with cirrhosis, the CD83 and CD86 expression of DCs stimulated under ACM was lower than that under HCM. When compared under the same medium, the CD83, CD86, and HLA-DR expressions of DCs from LC were lower than those from DCs of HC. To summarize these results, in advanced cirrhosis not only the DCs themselves but also the extracellular environments tend to impair the maturation of DCs.

Second, we examined which amino acids more strongly influences the function of DCs between HCM and ACM. We found that BCAA, especially valine and leucine, increased the BDCA+ DC allostimulatory capacity and IL-12 production. This confirms the findings of our previous study,[15] although the enhancement by a single amino acid was very subtle. To obtain greater enhancements, we may need to use combinations of other amino acids.

Concerning the mechanism that underlies these phenomena, we confirmed that the CD98 expression of DCs were not different between HCM and ACM. CD98 can regulate the expression and distribution of the light chains to modulate the amino acid transport functions. CD98hc is highly expressed on proliferating lymphocytes and on other rapidly growing cells.[27] Next, we examined whether the amino acid imbalance in the plasma of patients with advanced cirrhosis influenced the mTOR/S6K signaling pathway of the DCs. Recently, some studies reported the PI3K-mediated negative feedback regulation of IL-12 production in DCs,[28] and rapamycin-enhanced IL-12 production in LPS-stimulated DC.[29,30] In the present study, BDCA+ DCs stimulated in ACM impaired IL-12 production, even though the mTOR signaling was decreased. This paradox raises the possibility that the amino acid imbalance influences not only mTOR signaling but also other types of signaling such as GSK3 or NF-κB signaling. This issue should be evaluated in future studies.

Finally, we investigated whether elevating the level of plasma BCAAs enhances the immune response *ex vivo* in

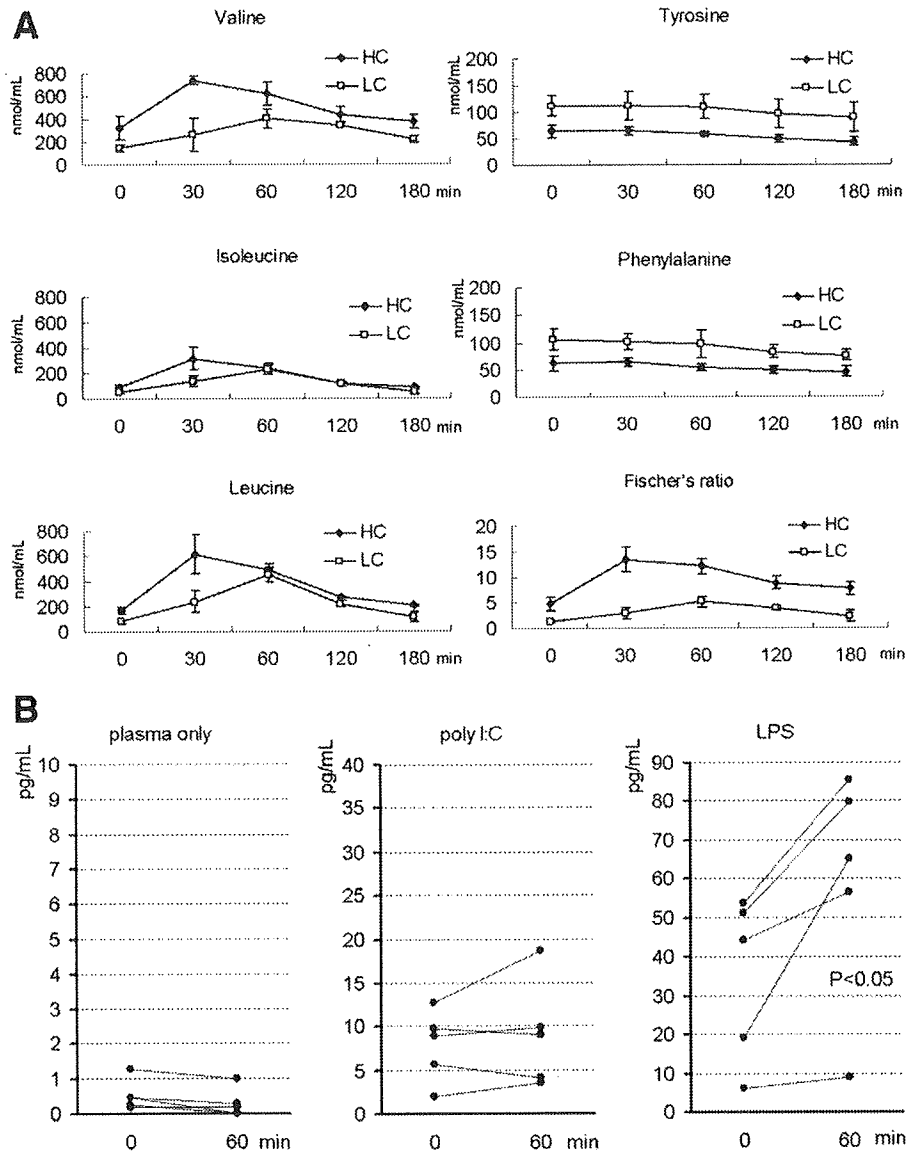

Fig. 4. Oral administration of BCAA granules enhanced the production of inflammatory cytokines from PBMCs stimulated by LPS *ex vivo*. (A) We analyzed the kinetics of the plasma amino acids after oral administration of BCAA granules. In the early morning while fasting, the concentrations of plasma amino acids were measured before and after oral administration of BCAA (30, 60, 120, 180 minutes). Mean ± SD values from three different HC and four patients with advanced cirrhosis (Patients 10-13). (B) We stimulated PBMCs from the patients using either autologous plasma before or after 60 minutes oral administration. After 12 hours we recovered the plasma and measured the IFN-γ by ELISA (Patients 11-15). $P < 0.05$ (paired Student's t test, two-tailed).

patients with advanced cirrhosis. BCAA granules have been used to effectively reverse the hypoalbuminemia and hepatic encephalopathy in patients with advanced cirrhosis.[31] In the preliminary investigation, we analyzed the kinetics of plasma amino acids after oral administration of BCAA granules. After oral administration, the BCAA concentration in plasma was maximal at 30 minutes in healthy volunteers. This was in contrast to patients with advanced cirrhosis, who had a slow increase in BCAA plasma concentrations that was maximal at 60 minutes. This difference was probably caused by the malabsorption of amino acids in the patients. In the *ex vivo* study, we could not use the medium to analyze the function of DCs of PBMCs because the concentration of the amino acids in medium influences the function. Thus, we stimulated cells in autologous plasma and analyzed the function over a short period of time. We found that oral administration of BCAAs enhanced the production of IFN-γ from PBMCs *ex vivo* in patients with advanced cirrhosis.

The results of this study still cannot be construed as conclusive evidence of a change in the functional clinical state in terms of lowering the risk of sepsis in cirrhosis or enabling consideration of such treatment for viral hepatitis. We need to perform a prospective, randomized, controlled trial in a well-characterized group of patients with appropriate immune mechanistic evaluation and determine the effects on the risk of sepsis in a longitudinal follow-up. In the present study we demonstrated at least that extracellular amino acids, especially BCAAs, influence the function of the immune system, and the amino acid imbalance in the plasma of patients with advanced cirrhosis impaired the maturation of DCs and the production of inflammatory cytokines from PBMCs or DCs.

In conclusion, the data from this study provide a rationale for future studies utilizing nutrition therapies that could be beneficial to immune function in patients with advanced cirrhosis.

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健康診断からみた学生の肥満の実態
－医科系大学入学生の体格とメタボリックシンドローム関連指標－

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要旨

本学学生の入学時の肥満の実態を明らかにし，メタボリックシンドローム（以下，Met S）との関連について探索的に検討した．対象は平成 19 年度入学時健康診断を受診した学生 333 名（平均年齢 19.2 歳）．男性：女性 =188：145．学生の健康白書 2005 を参照し肥満の割合を比較するとともに，BMI 区分別の血圧，血液生化学検査値について検討した．BMI の中央値は男性 22.4，女性 20.7．本学学生は全国の同年代（18 歳）の学生に比べ肥満の割合が男女ともに高く，特に歯学部において高率だった．BMI の増加に伴い収縮期血圧，拡張期血圧は高値を示した．BMI と尿酸，AST，ALT，γGTP，TC，TG は有意の正の相関を示し，BMI と HDL-C は負の相関を示した．また高度肥満者における ALT 異常者は 80%に達した．これらの結果は成人の検診データと同様の傾向だった．青年期の健康状態が将来の Met S 発症につながる可能性があるため，生活習慣病を視野に入れた健康管理体制の構築と入学早期からの食・生活習慣の改善が必要である．

Key words : metabolic syndrome, body mass index, BMI, obesity, freshman in university, medical examination

I. はじめに

近年，成人を対象とした住民健診や職域健診においてメタボリック・シンドローム（以下，Met S）の概念が広く認識され，保健指導の重要な対象となっている[1]．Met S は，生活習慣に起因する内臓脂肪の蓄積を背景として高血圧，高血糖，脂質異常症を呈する病態であるが，青年期の学生における肥満が注目され，生活指導介入の有効性も報告されている[2, 3]．また近年，小児，思春期における肥満は成人後の Met S 発症に関係するとの報告もある[4]．

本学では，学校保健法に基づき学生健康診断（身長，体重，血圧，視力測定および内科診察，耳鼻科診察，眼科診察）を年 1 回行っており，入学生には血液生化学検査，尿検査，胸部 X 線検査を実施している．そして健康診断の結果に基づき異常値を示した学生に対しては個別に健康支援を行っている．

以上の背景から，現在の本学学生の肥満の実態と Met S との関連が明らかになれば，将来，医療界において中心的役割を担っていく学生の育成を目標とした生活および栄養指導の重要性を提言する根拠の一つとなり，生活習慣病予防を視野に入れた健康管理体制の構築に繋がるものと期待される．そこで，本学学生の体格の特徴を明らかにし，Met S を構成する要因について体格との関連から探索的に検討した．

II. 対象と方法

1. 対象

平成 19 年度入学時健康診断を受診した学生 333 名（平均年齢 19.2 歳，18 ～ 30 歳）を

対象とした．内訳は，医学部80名（男性：女性＝56：24，平均年齢20.0歳），歯学部82名（男性：女性＝60：22，平均年齢19.7歳），薬学部171名（男性：女性＝72：99，平均年齢18.5歳）である．

2．方法

1）評価項目

Body Mass Index（BMI），収縮期血圧・拡張期血圧，尿酸，AST，ALT，γGTP，総コレステロール（TC），HDLコレステロール（HDL-C），中性脂肪（TG），HbA_{1c}，血糖．

2）データ区分

①BMIは日本肥満学会の判定基準5）に従い18.5未満を低体重，18.5以上25.0未満を標準，25.0以上を肥満とし，特に30.0以上を高度肥満とした．

②血圧は日本高血圧学会のガイドライン2009[6]（以下，JSH2009）に従い，収縮期血圧140mmHg以上または拡張期血圧90mmHg以上を高血圧とした．

3）解析方法

①学部別，男女別に体格区分の割合を算出し，学生の健康白書2005[7]（以下，健康白書）に示されているデータと比較した．

②BMI区分別の収縮期および拡張期血圧，血液生化学検査値を算出し，肥満とそれぞれの平均値および異常値を示す割合を検討した．

③空腹時採血を行った例について，現行のMet S診断基準と照合し，Met S相当例の頻度について推察した．

④BMIと収縮期・拡張期血圧，血液生化学検査値の相関関係を検討した．

⑤データ解析は，統計解析ソフトStat Partner Ver4.5を用い有意水準が両側5%以下を有意と判定した．

III．結　果

1．学部別，男女別の体格について（図1，図2）

学部別のBMIの中央値（最小値～最大値）は，医学部21.2（17.2～45.8），歯学部22.0（16.0～35.0），薬学部21.7（16.1～40.7）であった．男女別では，男子22.4（16.0～45.8），女子20.7（16.1～40.7）であった．対象者全体の体格は，低体重が9.9%，標準が70.6%，肥満が19.5%であったが，ことに

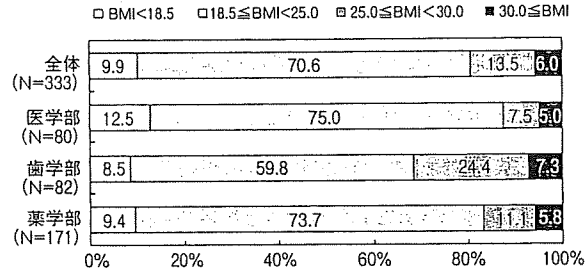


図1．学部別BMIの分布

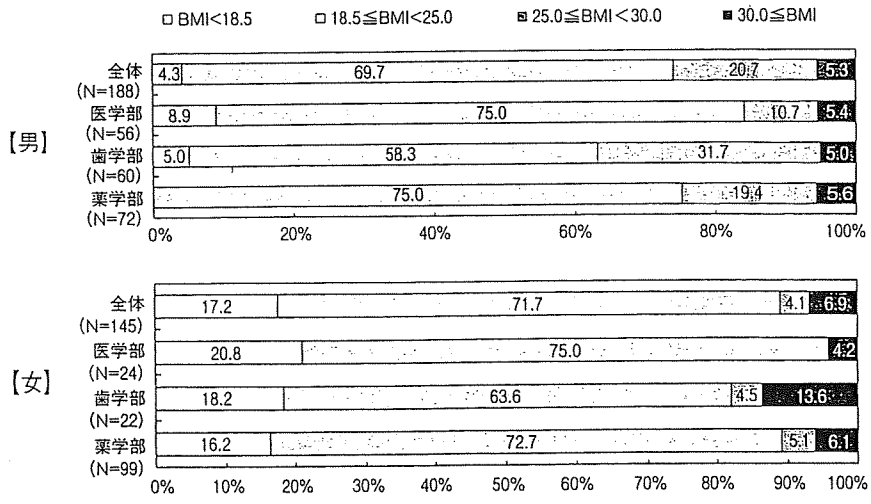


図2．性別学部別BMIの分布

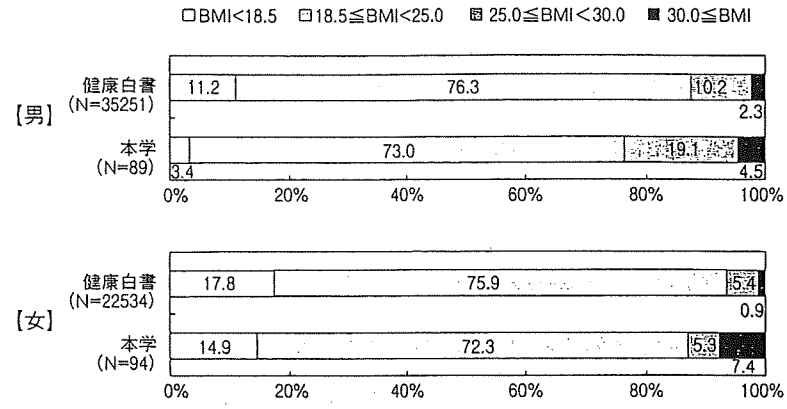


図3. 健康白書による同年代の学生と本学学生のBMIの比較

歯学部では男女ともに他学部より低体重の割合が低く，肥満の割合が高かった．男女別では，男子は女子に比べ低体重の割合が低く(4.3% vs. 17.2%)，肥満の割合が高かった(26.0% vs. 11.0%)．歯学部男子の肥満の割合は36.7%，歯学部女子の高度肥満の割合は13.6%と高率であった．

2. 健康白書との比較(18歳)(図3)

本学入学生は，男女とも同年代の学生に比べ低体重の割合が低く(男子：3.4% vs. 11.2%，／女子：14.9% vs. 17.8%)，肥満の割合が高かった(男子：23.6% vs. 12.5%，／女子：12.7% vs. 6.3%)が，この傾向は男子学生において顕著だった．

3. BMI区分別の収縮期，拡張期血圧値(図4)

高血圧は，9.9% (33/333)に認められ，そのうち肥満が54.4% (18/33)を占め，低体重には高血圧は認められなかった．またBMIの増加に伴い収縮期血圧，拡張期血圧は有意に増加し(収縮期血圧：順位相関係数=0.45，$p<0.001$／拡張期血圧：順位相関係数=0.33，$p<0.001$)，高度肥満における収縮期血圧の中央値は137 mmHg (110～168)と高値を示した．

4. 血液生化学検査で異常値を示した学生の割合(表1)

BMIの増加に伴い異常値を示す割合が増加した．高度肥満においてはALT異常者が80%に達し，尿酸異常者も40%を示した．

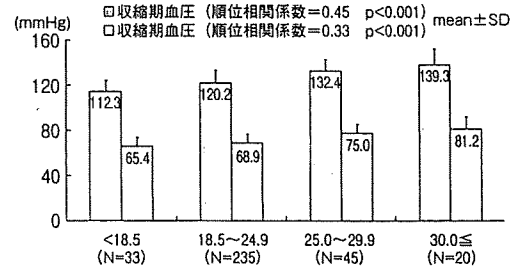
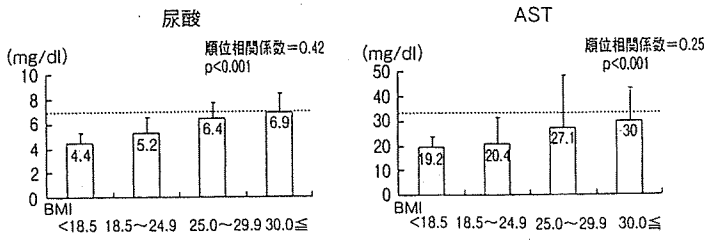


図4. BMI区分別の収縮期・拡張期血圧

表1. BMI区分別の生化学検査異常値の頻度

項目(基準値)		BMI			
		<18.5 (N=33)	18.5～24.9 (N=235)	25.0～29.9 (N=45)	>30.0 (N=20)
尿酸	(2～7 mg/dl)	0	11.1	31.1	40.0 (%)
AST	(10～32 IU/l)	0	4.3	17.8	25.0
ALT	(7～27 IU/l)	6.1	9.8	44.4	80.0
γGTP	(5～55 IU/l)	3.0	0.9	8.9	10.0
TC	(118～219 mg/dl)	3.0	5.5	17.8	0
HDL-C	(40 mg/dl以上)	0	0.9	4.4	15.0
TG	(34～170 mg/dl)	0	3.4	8.9	20.0
HbA_{1c}	(4～5.4 %)	0	0.4	2.2	15.0



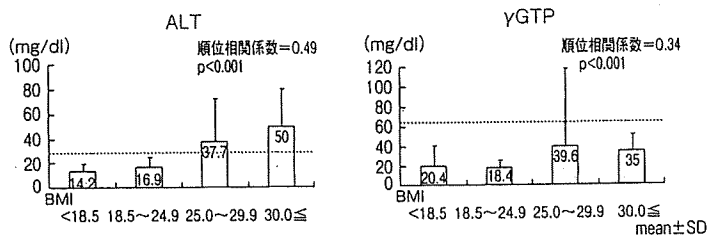
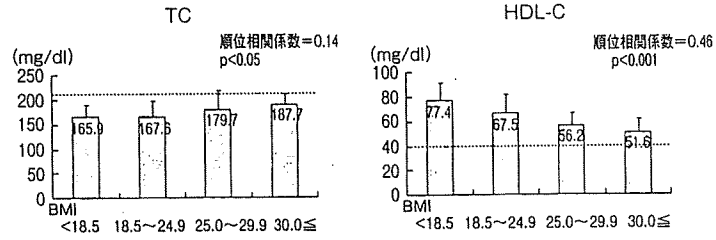


図5. BMI区分別の血液生化学検査値
(尿酸，AST，ALT，γGTP)



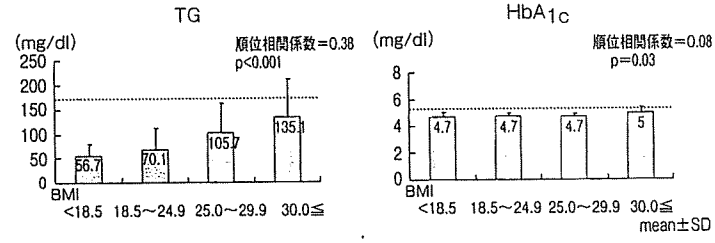


図6. BMI区分別の血液生化学検査値
(TC，HDL-C，TG，HbA_{1c})

表2. BMI区分別の血液生化学検査値（中央値）

項目（基準値）	BMI			
	<18.5 (N=33)	18.5～24.9 (N=235)	25.0～29.9 (N=45)	＞30.0 (N=20)
尿酸 (2～7 mg/dl)	4.3 (3.9-4.9)	5.2 (4.25-6.15)	6.3 (5.6-7.3)	6.8 (5.85-7.9)
AST (10～32 IU/l)	18 (16-20)	18 (16-22)	19 (17-27)	26.5 (23-32)
ALT (7～27 IU/l)	13 (10-16)	14 (11.5-19)	26 (18-45)	44.5 (30.5-61)
γGTP (5～55 IU/l)	17 (13-22)	17 (14-21)	24 (17-32)	28.5 (24-46.5)
TC (118～219 mg/dl)	165 (147-178)	164 (147.5-183)	172 (159-203)	188 (183-204)
HDL-C (40 mg/dl 以上)	78 (69-87)	65 (57-77)	56 (50-60)	51 (45-56)
TG (34～170 mg/dl)	49 (40-67)	58 (44.5-82.5)	94 (72-119)	103.5 (80-177.5)
$aHbA_{1c}$ (4～5.4 %)	4.7 (4.5-4.9)	4.7 (4.5-4.8)	4.7 (4.6-4.8)	5.1 (4.7-5.2)

中央値（25-75%）

5. BMIと血液生化学検査値との関連(図5，図6，表2)

BMIと尿酸，AST，ALT，γGTP，TCは有意の正の相関を認め，BMIとHDL-Cの間には有意の負の相関が認められた．BMIとHbA_{1c}の間には明らかな相関関係はみられなかった．これらはBMI区分別にみてもほぼ同様の傾向を示していた．TGに関しては，採血条件（空腹時，随時）が異なるため今回の検討からは除外した．

表3. Met S 存在の推定　(%)

異常項目の数	BMI	
	＞25.0 (N＝26)	≦25.0 (N＝7)
0	21 (80.8)	1 (14.3)
1	5 (19.2)	4 (57.1)
2	0	2 (28.6)
3	0	0

①空腹時血糖：110 mg/dl 以上
②血圧：130 mmHg 以上，
　かつ／または，85 mmHg 以上
③脂質：TG150mg/dl 以上，
　かつ／または，HDL-C40mg/dl 未満

6. Met S 相当例の頻度の推計（表3）

空腹時採血を行った33名の学生をBMI<25（N=26）と25≦BMI（N=7）に分け，Met S の診断基準である臨床検査値，①収縮期血圧130 mmHg 以上，かつ／または，拡張期血圧85 mmHg 以上，②空腹時血糖110 mg/dl 以上，③TG150 mg/dl 以上，かつ／または，HDL-C40 mg/dl 未満，の3項目中，該当する項目数を示した．BMI<25ではMet S に相当する可能性がある2項目以上に該当する学生は存在しなかったが，25≦BMI では7名中2名存在した．

IV. 考　察

本学入学生は同年代の学生（18歳）に比べて低体重が少なく肥満が多かった．この傾向は特に男子学生において顕著であり，中でも歯学部男子では36.7%が肥満であった．今回の調査は入学時のものであるため，それまでの成育環境が影響している可能性も考えられるが，全体的に肥満にシフトしている傾向が認められた．

平成19年度学校基本調査によると，平成19年度に国公私立大学に入学した学生は合計613,613名で，そのうち現役生は508,292名（82.8%）であった[8]．同年度の本学入学生は，現役生が約50%と他大学に比べ浪人生が多いことから，受験勉強による不規則な生活や食習慣，運動不足といったものが肥満者の増加につながった可能性が考えられる．その他，家庭環境や東北出身者が多い（医学部：42.5%／歯学部：42.7%／薬学部：84.8%）といった地域性も考えられる．ちなみに本県の学校保健統計によると，本県の児童は全国平均に比し肥満の割合が高いことが報告されているが，いずれも今回の調査のみでは明確な理由を明らかにすることはできなかった．ことに，歯学部に肥満が多い理由も不明であり，これらの点について今後さらに背景の詳細を検討し明らかにしていく必要があると考えられる．

近年，小児・思春期の高血圧や肥満は成人期に移行することが報告されており[9,10]，Met S の発症予防には小児期からの食生活をはじめとする生活習慣への介入が重要であると言われている．青年期初期に相当する大学生は，これまでの生活習慣がある程度すでに確立されていることが多いため，ライフスタイルを変えることは容易ではなく，また本人のモチベーションが高くないことも考えられる．しかし，入学生については初めて親元を離れて自立する学生が多く，生活環境が変わる時期にあたるため，生活習慣に対して比較的介入しやすく，行動変容が得られやすいものと推定される．従って，入学時における健康診断をこれまでの生活習慣を見つめなおす機会と捉え，生活支援が必要な学生を早期に発見し，積極的に関わっていくことが重要と考えられる．

成人における肥満と生活習慣病に関する臨床検査項目の異常値については多くの報告がなされているが[11-13]，大学生における報告は少ない．これは，学校基本法では健康診断における血液生化学検査が必須項目ではなく，各大学の裁量に任せられている現状によるためと考えられる．本学では入学時のみに血液生化学検査を実施しているが，今回の調査においても成人の健診データと同様の傾向があることが明らかとなった．血圧については，

収縮期および拡張期血圧ともBMIの増加に伴い高値を示し，特に高度肥満の血圧の中央値は，JSH2009基準の正常高値血圧に該当した．さらに，BMIと尿酸，AST，ALT，γGTP，TC，HDL-Cの間に正または負の相関があることも明らかになった．すなわち肥満が高度になるほど，血液生化学検査値で異常を示す割合が高くなり，特に高度肥満学生においてそれが顕著であり，ALTの中央値は44.5 IU/Lと異常値を示していた．ALT異常者については，近年，肥満人口の増加とともに脂肪肝の頻度の増加が指摘されており，その大半は脂肪肝が基本病変と推定されていることから[13]，本学学生にも脂肪肝を有する学生が相当数存在すると推測される．

肥満の判定基準には従来BMIが用いられ，健康障害との関連で論じられてきたが，近年新たに内臓肥満に着目したMet Sという概念が導入された[1]．Met Sの診断基準には，腹囲の測定が必須で，BMIは含まれていない．今回の健診では腹囲を測定していないので，本学学生における正確なMet Sの頻度の集計は出来なかったが，従来，肥満の基準として用いられてきたBMIを用いて，おおよそのMet Sの頻度を推計した．25≦BMIでは，Met Sの診断に該当する臨床検査値のうち2項目以上異常の学生が28.6%（2/7）存在した．また肥満学生は，非肥満学生に比し血液生化学検査で異常値を示す割合が高く，かつMet Sのリスク要因を満たしている学生が存在すると推察される．このことは，青年期の健康状態が将来のMet S発症につながる可能性を示唆し，肥満学生には入学早期からの生活習慣病を視野に入れた生活支援が重要であると考えられる．

V. 結　語

本学入学生は肥満の割合が高く，Met S予備群である可能性があることから，生活習慣病を視野に入れた健康管理体制の構築と入学早期からの積極的かつ継続的な生活改善支援が必要である．

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Clinical survey of obesity in freshmen of medical university : association between physique and metabolic syndrome

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Abstract

To clarify the clinical association between obesity and metabolic syndrome, we investigated the frequency of obese freshman students at a university. Our study enrolled 333 students (average age 19.2 years), including 80 medical (56 men, 24 women), 82 dentistry (60 men, 22 women), and 171 pharmacy students (72 men, 99 women). Participants were divided into four groups according to BMI : lean (<18.5), standard (≧ 18.5, 25.0>), obese (≧ 25.0, 30.0>), and excessively obese (≧ 30.0). In the present study, more students were found to be obese compared with previous reports for students in the same age group in the Healthy White Paper 2005. The proportion of students with hypertension tended to increase with BMI. A positive correlation was also seen between BMI and uric acid, AST, ALT, γGTP, TC, whereas a negative correlation was seen between BMI and HDL-C. Further, 80% of excessively obese students were noted to have abnormal ALT values (median 44.5 IU/l). Taken together, these observations suggest a close association between obesity and the possible future development of metabolic syndrome, and emphasize the importance of ongoing active intervention to improve the lifestyle of obese students on entering the university

慢性肝不全の病態と栄養治療

鈴木一幸* 遠藤龍人 黒田英克
及川寛太 柿坂啓介

要 旨

・肝不全とは，急性または慢性に起こる高度の肝細胞機能障害により，意識障害(肝性脳症)，黄疸，腹水，消化管出血，出血傾向などの予後不良な重篤な症状が生じる症候群を意味する。
・慢性肝不全の病態には，肝細胞障害と門脈・大循環短絡の二つの因子が相互に関連し，臨床病型としては大きく肝細胞障害型(末期昏睡型)と門脈・大循環短絡型(シャント型)の二つに分類されるが，これ以外に特殊型(先天性尿素サイクル異常症など)がまれに存在する。
・慢性肝不全の栄養代謝異常を的確に把握することが栄養治療を行ううえで極めて重要であり，その評価には身体計測，血液・尿生化学検査，間接熱量測定などを行う。
・間接熱量測定によるエネルギー代謝動態では，糖質と脂肪の燃焼比率の逆転，非蛋白呼吸商の低下がみられ，これらの異常は肝の重症度とともに顕著となる。
・栄養治療では，脳症時と非脳症時に分けて，基本的にはESPENのガイドラインに準じて行うが，個々の病態に応じて投与エネルギー量，蛋白量を決定することが重要である。
・蛋白・エネルギー代謝異常(PEM)を認める例では，分岐鎖アミノ酸(BCAA)製剤(顆粒製剤，経腸栄養剤)の併用を加味した栄養治療を行うが，分割食(LES)は耐糖能の異常およびQOLの改善に有効である。

はじめに

肝不全とは，急性または慢性に起こる高度の肝細胞機能障害により，意識障害(肝性脳症)，黄疸，腹水，消化管出血，出血傾向などの予後不良な重篤な症状が生じる症候群を意味するが，これらの臨床徴候の中で肝性脳症がもっとも重篤であることから，肝性脳症の発現している状態をもって肝不全と呼ぶのが通例である。急性肝不全をきたす成因は極めて多岐にわたるが，欧米では成因にかかわらず急性肝不全と呼び，わが国ではウイルス，薬剤，自己免疫による例を劇症肝炎，他の成因による例を急性肝不全と呼んで区別している[1,2]。一方，慢性肝不全をきたす代表的な疾患は肝硬変(肝癌合併例を含む)であるが，慢性肝疾患の存在を認めない門脈・大循環短絡形成による例や，先天性尿素サイクル異常症などによる肝不全例もまれにみられる[3]。

肝臓は糖質，蛋白質，脂質の三大栄養素のみならずビタミン，ミネラル，微量元素などすべての栄養素の代謝の中心的臓器である。したがって，肝不全では，これらの栄養代謝に何らかの異常を高頻度に認めることは容易に想像できる。栄養療

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法は，肝不全の原疾患に対する根本的な治療法ではないが，肝性脳症の改善のみならず肝病態の進展抑制や合併症などの病態改善，QOL の改善，さらには肝移植における周術期管理のうえで有用な治療法と考えられる[4]。

本稿では，とくに慢性肝不全における栄養代謝異常を含めた肝病態と栄養治療について解説する。

病　態

慢性肝不全の病態形成には肝細胞機能障害と門脈・大循環短絡の二つの要因が相互に密接に関連しており，臨床的には肝細胞障害の強いタイプ(肝細胞障害型あるいは末期昏睡型)と門脈・大循環短絡(シャント)の因子が強いタイプ(シャント型あるいは慢性再発型)に分類される[3]。最近，意識状態が一見正常と判断される例において定量的精神神経機能検査を行うと，少なからず異常を認める例が存在し，このような病態を潜在性肝性脳症と呼ぶことが提唱されており，わが国でもその診断法，病態解析が進められている[5,6]。潜在性肝性脳症と診断された例については治療の対象と考えられる[7]。

肝性脳症の発生機序として，アンモニアを中心とした多因子説，アミノ酸代謝異常説，偽性神経伝達物質説，γアミノ酪酸(GABA)／ベンゾジアゼピン受容体複合体異常説などの説があるが，いずれの説も単一では明確に肝性脳症の発生機序を説明できない[8]。

栄養代謝異常

肝不全に対する栄養療法を実施するにあたっては，栄養代謝異常をできるだけ的確に把握することが極めて重要である。実際には，図に示すように主観的包括的評価(subjective global assessment；SGA)を行い，血液・尿などの生化学検査で肝の重症度 (Child-Pugh 分類[9]による重症度スコア)を判定することが重要である。また，合併症の有無を確認し，肝移植を考慮する例ではMELD(The Model for End-Stage Liver Disease)スコア[10]も把握する。一般的に，MELD スコアが 15 点以上の場合には肝移植の適応となる。

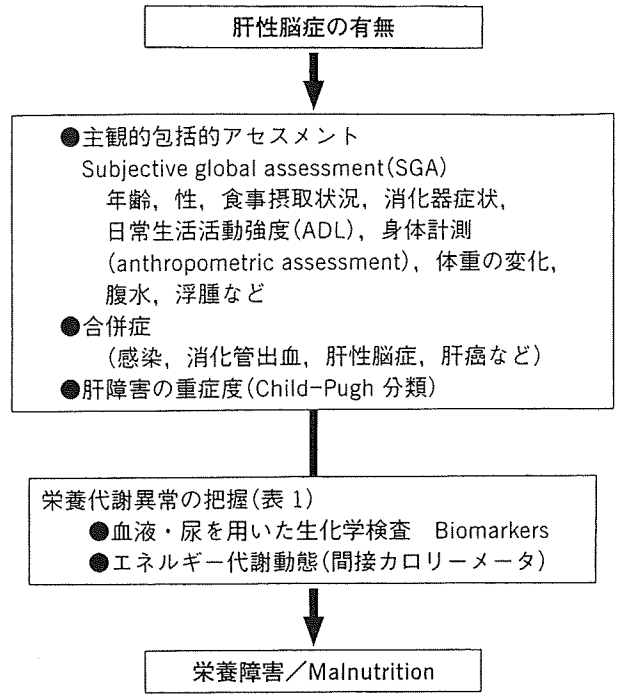

図　慢性肝不全患者の栄養代謝異常・病態の把握

各種血液・尿生化学検査では，**表 1** に示した検査項目を目的に応じて測定するが，間接熱量測定法(indirect calorimetry；IC)を用いたエネルギー代謝動態の測定により正確な動的栄養代謝動態の把握が可能となる。間接熱量測定法とは，糖質，蛋白質，脂質のエネルギー消費量をリアルタイムに測定する方法であり，開放式と閉鎖式があるが，通常は前者が用いられている。

呼気分析より炭酸ガス生成量(Vco2)と酸素消費量(Vo2)を算出し，同時に採取測定した尿中尿素窒素値(g/日)より，三大栄養素基質の燃焼比率，安静時エネルギー消費量(resting energy expenditure；REE)，非蛋白呼吸商(nonprotein respiratory quotient；npRQ)などを求める。正確な測定を行うためには測定条件を一定とすることが重要であり，また測定機種により測定誤差が生じることを認識する必要がある。最近は，REE のみを測定する機器(メタバイン)も開発され，日常診療での使用機会が増加しつつある。

肝硬変では重症度とともに，身体計測では筋肉

表1　慢性肝不全患者の病態・栄養指標

血液・尿を用いた生化学検査（バイオマーカー）
- 末梢血
  - WBC, RBC, Hb, Ht, PLT
- 肝の障害度・予備能
  - T-Bil, AST, ALT, ALP, γ-GTP, ChE, プロトロンビン時間(PT), ICG_{R15}
- 蛋白・アミノ酸代謝
  - TP, Alb, NH_3, BTR(Fischer 比), 血漿遊離アミノ酸分析(アミノグラム), トランスサイレチン, レチノール結合蛋白, トランスフェリン
- 糖代謝
  - FBS, HbA_{1c}, IRI, HOMA-R, 血糖日内変動, 尿糖, 尿蛋白, 尿ケトン, 尿中 C-ペプチド
- 脂質代謝
  - T-Chol, TG, LDL-C, HDL-C, FFA
- 微量元素, 免疫能など
  - Fe, Zn, フェリチン, 総リンパ球数, 免疫グロブリン, ほか
- 尿
  - クレアチニン排泄量, クレアチニン身長指数, 尿中総窒素, 尿中尿素窒素, 窒素平衡(出納), 尿中 3-メチルヒスチジン(3-Met-His)

エネルギー代謝動態(間接カロリーメータを用いた測定)
- 安静時エネルギー消費量(REE), 基礎エネルギー消費量(BEE), REE/BEE, 呼吸商(RQ)または非蛋白呼吸商(npRQ)

BTR：branched-chain amino acid and tyrosine molar ratio, REE：resting energy expenditure, BEE：basal energy expenditure, RQ：respiratory quotient, npRQ：nonprotein respiratory quotient

量や皮下脂肪厚の減少，血液生化学検査では血清アルブミン，コレステロール，コリンエステラーゼの低下，プロトロンビン時間の延長，Fischer 比の低下〔分岐鎖アミノ酸(branched chain amino acid；BCAA)であるバリン，ロイシン，イソロイシンと芳香族アミノ酸(aromatic amino acids；AAA)であるフェニルアラニン(Phe)，チロシン(Tyr)のモル比〕などがみられる。また，進行した肝硬変(肝不全)では，蛋白・エネルギー栄養障害(protein-energy malnutrition；PEN)を認めることが特徴であり，REE は健常者と比べて増加していることが多い。IC における各栄養素(糖質，脂質，蛋白質)の燃焼比率は，糖質よりも内因性脂肪の利用率が増加し，npRQ は低下しており，これらの異常の程度は肝の重症度と相関する[11~14]。

慢性肝不全の臨床病型別にみると，肝細胞障害型では，糖質代謝では栄養素の摂取量の減少とともに実質肝細胞におけるグリコーゲン貯蔵量の減少や糖新生系の障害，インスリン抵抗性などにより血糖の恒常的維持が高度に破綻しており，しばしば低血糖あるいは高血糖が認められる。脂質代謝では，脂肪酸合成能の低下を反映しコレステロール，リン脂質の血中濃度の低下がみられ，レシチンコレステロールアシルトランスフェラーゼ活性の低下によりコレステーロル・エステル比も低下する。また，リポ蛋白の合成・分泌障害もきたし，HDL-コレステロールは低下し，一方，脂肪分解の亢進や脂肪酸酸化の遅延により遊離脂肪酸は増加している。蛋白・アミノ酸代謝ではアンモニア処理能の低下，尿素合成能の低下がみられ，Fischer 比の低下あるいは BCAA/Tyr 比の低下が特徴的である。微量元素では，とくに亜鉛の低下が著しく，血清銅/亜鉛比は肝重症度と相関して低下している。

一方，シャント型あるいは慢性再発型では比較的肝機能は良好であるため，血液アンモニア濃度の上昇や Fischer 比または BCAA/Tyr 比の低下をみるが，上記に述べた各栄養素の代謝異常は軽度であることが多い。先天性尿素サイクル代謝異常症では，血液アンモニア濃度の著明な増加がみられ，それぞれの病型に応じた特徴的な血漿遊離アミノ酸濃度の変動が観察される。

治　療

1．基本方針

肝性脳症の誘因や増悪因子，臨床病型および合併症の有無を把握して，治療方針を決定する。代表的な誘因として食事蛋白量の過剰摂取，消化管出血，便秘，感染症，鎮静薬・鎮痛薬の過剰投与，利尿薬の過剰投与による電解質異常などがあり，慢性型では約 70％の例に何らかの誘因を認める。近年は消化管出血による肝性脳症例は減少し，誘因不明例が増加している。また，肝性脳症の増悪因子として低酸素血症，循環不全，低血糖，低血圧，血清電解質異常(とくにナトリウム，カリウム，マグネシウム)，血漿蛋白(アルブミン)減少などがある。